CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

210365Orig1s000

NON-CLINICAL REVIEW(S)

Tertiary Pharmacology Review

By: Paul C. Brown, Ph.D., ODE Associate Director for Pharmacology and Toxicology, OND IO
NDA: 210365
Submission date: 6/23/2017 (initial submission with nonclinical information)
Drug: cannabidiol
Applicant: GW Therapeutics
Indication: adjunctive treatment of seizures in patients with Lennox Gastaut or Dravet Syndrome

Reviewing Division: Division of Neurology Products

Discussion:
The pharmacology/toxicology reviewer and supervisor conducted a thorough evaluation of the nonclinical information submitted in support of this NDA. A major human metabolite (7-COOH-CBD) that may account for the majority of drug-related exposure was not adequately assessed in the nonclinical studies. This is a significant deficiency and was cited by the reviewer as the reason that the application cannot be considered approvable. The pharm/tox supervisor agreed that 7-COOH-CBD was not adequately assessed but concluded that nonclinical studies to characterize the toxicity of 7-COOH-CBD could be conducted post-marketing if the product was approved based on adequate clinical data for this serious unmet medical need. Recommended studies of the metabolite include an embryofetal development study in one species (preferably rat), a pre- and postnatal development study in rat, a juvenile animal toxicology study in rat, and a carcinogenicity study in one species.

Conclusions:
I agree that the lack of lack of nonclinical data on 7-COOH-CBD is a significant deficiency that should be addressed. I agree that if the clinical data warrant approval for the serious unmet medical need, then the nonclinical studies to characterize 7-COOH-CBD can be conducted post-marketing.

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/s/

PAUL C BROWN
06/14/2018

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: June 13, 2018

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

Subject: NDA 210-365 (cannabidiol, Epidiolex)

NDA 210-365 was submitted by the sponsor (GW Research Ltd.) on October 27, 2017, for cannabidiol for the adjunctive treatment of seizures in patients (≥2 years of age) with Lennox Gastaut or Dravet Syndrome. The proposed dose is 20 mg/kg/day (given in equal doses BID). Clinical development for these indications was conducted by the sponsor under IND 120055. The NDA was rolling submission, with the complete nonclinical package provided in the initial submission (June 23, 2017). The final portion was submitted on October 26, 2017, and the NDA was filed (Filing Communication, December 20, 2017). One nonclinical filing issue was identified in the December 20, 2017, letter:

> The major circulating metabolite of CBD in humans, 7-COOH-CBD, does not appear to have been adequately evaluated in the pivotal nonclinical studies. The lack of information regarding this metabolite is a significant review issue that will be closely examined during our review.

The following nonclinical studies of purified CBD (b) (4) or CBD-OS (clinical formulation containing CBD (b) (4) were submitted to support the NDA:

- Pharmacology
- PK/ADME
- Toxicology
  - Mouse: 13-week dose-ranging studies
  - Rat: 26-week toxicity study
  - Dog: 39-week toxicity study
- Reproductive and Developmental Toxicology
  - Fertility and early embryonic development study in rat
  - Embryofetal development studies in rat and rabbit
  - Pre- and postnatal development study in rat
- Genetic Toxicology
  - Ames assay, in vivo rat micronucleus assay, in vivo COMET assay
- Carcinogenicity
  - 2-year carcinogenicity study in rat

Additional nonclinical studies were conducted using different routes (IV, SC) and with other drug products (e.g., CBD-BDS [66-72% CBD]). These were not considered pivotal to support the NDA.

The nonclinical data were reviewed by Dr. Fisher (Pharmacology/Toxicology Review and Evaluation, NDA 210365, May 25, 2018). Based on that review, Dr. Fisher has concluded that the nonclinical data do not support approval because of the lack of an adequate assessment of 7-COOH-CBD, a major human metabolite that circulates in humans at levels (b) (4) times those of the parent. Dr. Fisher also notes the inadequacy of the 2-year rat carcinogenicity study, which he concludes "…may…partially be addressed by the mouse study that is currently underway." Dr. Fisher makes no specific recommendations for post-marketing studies.

The following provides a brief summary of the pivotal oral nonclinical studies of purified CBD and CBD-OS, with a focus on the deficiencies identified by Dr. Fisher. A detailed presentation and discussion of the data are available in Dr. Fisher's review.

Summary

Purified CBD (the API in the clinical formulation, CBD-OS) is extracted from the *Cannabis Savita L.* plant, to a purity of (b) (4) %. According to the sponsor, CBD exerts therapeutic effects in Dravet and LGS patients through interaction with multiple systems, including G-protein-coupled receptor 55 and modulation of adenosine signaling. However, at this time, there is not a clear understanding of the mechanism(s) responsible for therapeutic effects in these populations.

Metabolite 7-OH-COOH exhibited anticonvulsant effects similar to CBD in the Maximum Electroconvulsive Shock (MES) model in mouse. Metabolite 7-COOH-CBD was inactive in this model (acute doses up to 200 mg/kg IP) and also exhibited minimal binding affinity (concentrations up to $\leq$10 μM) in a limited in vitro binding/target panel, which included CB_1 and CB_2 receptors, the voltage gated Na channel, and monoamine transporters.

The PK/ADME of CBD was minimally characterized in studies, primarily in rat and dog, conducted by the sponsor. Oral bioavailability was reported only for female Sprague Dawley rat (2.8% after a single 10 mg/kg dose); $t_{1/2}$ after a single oral dose was reported for CBD in rat (4-5 hrs; 120 mg/kg) and for total radioactivity in rat (11 hrs; 15 mg/kg) and dog (18 hrs; 5 mg/kg). Understanding of the in vivo metabolic profile of CBD in animals and humans evolved over the course of clinical development. With identification of major human metabolites, analytical assays were updated and validated for CBD and metabolites, 6-OH-CBD, 7-OH-CBD, and 7-COOH-CBD. Based on steady-state PK data in humans, only metabolites 7-OH-CBD and 7-COOH-CBD are major circulating metabolites. Stability data were collected for rat and dog plasma to support reanalysis of samples for metabolites. The sponsor states there are no human-specific metabolites; however, there are interspecies differences in plasma exposure to the metabolites, as discussed by Dr. Fisher. In addition, as Dr. Fisher also noted, the sponsor reports methodological problems with quantitation of 7-COOH-CBD in animal plasma.

The pivotal oral toxicity studies (26-week in Wistar rat, 39-week in Beagle dog) were conducted using the clinical formulation (CBD-OS). In the 26-week (gavage) study (0, 15, 50, or 150

mg/kg QD; 15/sex/group + 10/sex/group for C and HD recovery), no dose-limiting toxicity was observed. The primary target organ was liver, with hepatocellular hypertrophy, accompanied by slight (1.2-1.4 fold) increases in ALT and ALP, observed at the MD and HD in males and females. No effects were observed on sperm parameters; interstitial cell hyperplasia in ovary was observed at the MD and HD. In the 39-week study (0, 10, 50, or 100 mg/kg QD; 4/sex/group + 2/sex for C and HD recovery), there were no deaths; the only clinical sign was soft/liquid/mucoid feces. Decreases in absolute body weight (compared to C) were observed at all doses in males (5, 15, and 12% at LD, MD, and HD, respectively) and females (22, 29, and 32% at LD, MD, and HD, respectively). As in rat, the primary target organ was liver, with hepatocellular hypertrophy detected at all doses (dose-related only in males), accompanied by increases in ALT (slight) and ALP (up to 8-fold).

The toxicokinetic (TK) data at the highest doses tested in rat and dog and at the maximum recommended dose in humans are summarized in the following table.

SPECIES	DOSE (mg/kg)	SEX	CBD		7-OH-CBD		7-COOH-CBD	
			C_{max} (ng/mL)	$AUC_{(0-24h)}$ (ng*hr/mL)	C_{max} (ng/mL)	$AUC_{(0-24h)}$ (ng*hr/mL)	C_{max} (ng/mL)	$AUC_{(0-24h)}$ (ng*hr/mL)
Rat	150	M	6160	60000	334	2560	4180	37100
		F	7530	67500	625	6730	2710	40500
Dog	100	M	2570	20500	134	1380	82.1	994
		F	2660	22400	117	1090	137	1560
Human*	10 BID	M/F	--	2790	--	1562	--	137886

*Data were extrapolated (by the sponsor) from data in humans at 750 mg BID, following the first daily dose (Study GWEP1544).

A full battery of oral reproductive and developmental studies was conducted using Purified CBD. Fertility and early embryonic development, embryofetal development (EFD), and pre- and postnatal development studies in Wistar rat were conducted using dose of 0, 75, 150, and 250 mg/kg. According to Dr. Fisher, "minimal toxicity," expected at a high dose in these studies (ICH S5(R2), November 2005), was not observed in females; males exhibited a statistically significant body weight effect at the high dose. Drug-related effects were observed in the EFD study (total litter loss at the HD) and in the pre- and postnatal development study (including adverse effects on body weight, attainment of developmental landmarks, learning and memory, and reproductive structure and, possibly, function, primarily at the MD and HD). In the EFD study in New Zealand White rabbit (0, 50, 80, and 125 mg/kg), adverse fetal effects (reduced body weight and increased variations) were observed at the HD, associated with maternal toxicity (body weight loss). In rat, TK data were collected only in the EFD study. The GD17 data are summarized in the following table.

COMPOUND	DOSES (mg/kg)					
	75		150		250	
	C_{max} (ng/mL)	$AUC_{(0-24h)}$ (ng*hr/mL)	C_{max} (ng/mL)	$AUC_{(0-24h)}$ (ng*hr/mL)	C_{max} (ng/mL)	$AUC_{(0-24h)}$ (ng*hr/mL)
CBD	9000	86300	12800	149000	13200	170000
7-OH-CBD	657	8730	770	11200	918	12600
7-COOH-CBD	5020	41500	6140	100000	9550	155000

In the rabbit study, CBD, 6-OH-CBD, 7-OH-CBD, and 7-COOH-CBD were quantitated in plasma on Gestation Days (GD) 7 and 19. The GD19 data for all but 6-OH-CBD are summarized in the following table.

COMPOUND	DOSES (mg/kg)					
	50		80		125	
	C_{max} (ng/mL)	$AUC_{(0-24h)}$ (ng*hr/mL)	C_{max} (ng/mL)	$AUC_{(0-24h)}$ (ng*hr/mL)	C_{max} (ng/mL)	$AUC_{(0-24h)}$ (ng*hr/mL)
CBD	89.9	1010	220	2030	406	4630
7-OH-CBD	4602	400	72.6	652	149	2040
7-COOH-CBD	3240	31700	7780	63700	7470	106000

A juvenile animal toxicology study was conducted in Wistar rats, with dosing of Purified CBD initiated on PND 4. Dosing during PNDs 4-6 was by subcutaneous (SC) injection (control and 3 treated groups, all receiving 15 mg/kg), followed by oral (gavage) administration on PNDs 7-77 (0, 100, 150, and 250 mg/kg). Doses for the pivotal study were based on the results of a dose-ranging study in which the high dose of 15 mg/kg SC on PNDs 4-6, followed by 400 mg/kg PO on PNDs 7-25, exceeded the maximum tolerated dose, as evidenced by clinical signs and death. In the pivotal study, there were deaths in HDM, preceded by reduced body weight gain. According to Dr. Fisher, developmental findings included neurobehavioral deficits at the HD and delayed sexual maturation in males at all doses. TK data were provided for CBD, 6-OH-CBD, 7-OH-CBD, and 7-COOH-CBD on PNDs 4, 7, and 70; the data for all but 6-OH-CBD are summarized in the following table.

SEX	PND	DOSES (mg/kg)					
		15/100		15/150		15/250	
		C_{max} (ng/mL)	$AUC_{(0-24h)}$ (ng*hr/mL)	C_{max} (ng/mL)	$AUC_{(0-24h)}$ (ng*hr/mL)	C_{max} (ng/mL)	$AUC_{(0-24h)}$ (ng*hr/mL)
CBD							
pooled	4	1640	12600	1370	9440	1530	12300
	7	13000	160000	11400	178000	17000	296000
M	70	11600	81400	11200	109000	11200	128000
F		12500	82400	11900	127000	14600	180000
7-OH-CBD							
pooled	4	39.5	508	35.0	425	32.2	465
	7	145	2600	180	3150	171	3640
M	70	400	3860	443	5300	448	6150
F		479	6080	537	6830	497	6920
7-COOH-CBD							
pooled	4	43.9	885	51.8	956	59.0	1120
	7	501	8950	548	10500	896	13800
M	70	6010	67100	8600	101000	13900	171000
F		5580	68000	7950	103000	13700	189000

A standard battery of genetic toxicology studies was conducted on CBD (Purified CBD: Ames assay, in vivo micronucleus assay in rat; CBD-OS: in vivo alkaline COMET assay); CBD was negative for mutagenicity and clastogenicity in adequately conducted assays.

The carcinogenic potential of CBD was assessed in a 104-week dietary carcinogenicity study in Wistar rat using CBD BDS, which contains ~58-67% CBD. Doses (expressed as CBD) were 0, 5, 15, and 50 mg/kg. No drug-related neoplastic findings were identified. However, Dr. Fisher considered the study inadequate because of multiple deficiencies, including use of a relatively impure drug substance, excessive body weight effects, and uncertain documentation of plasma exposures to CBD and major human metabolites (7-OH-CBD and 7-COOH-CBD). Clearly the use of a complex drug substance (characterized as a "high cannabidiol-yielding strain of cannabis"), containing ~30-40% compounds other than CBD potentially confounds the study design (e.g. dose selection) and data interpretation. Plasma levels of CBD, THC, and 11-OH-THC were quantitated; however, TK parameters were not calculated for these compounds and the major human metabolites were not quantitated in plasma. TK data following dietary administration were provided in 14-day and 13-week dose-ranging studies of CBD BDS in Wistar rat, but the metabolites were not quantitated in plasma in either study. In the 14-day study (50, 150, and 500 mg/kg CBD in diet), the plasma CBD AUCs at 50 mg/kg on Day 14 were 3847.51 and 4502.26 ng*hr/mL in males and females, respectively; in the 13-week study (25, 75, and 225 mg/kg CBD in diet), TK data were not available for doses similar to those used in the carcinogenicity study. For comparison, in the 26-week study in rat (Purified CBD by oral gavage), the plasma AUCs for CBD at 50 mg/kg were 36700-39000 ng*hr/mL.

A 104-week carcinogenicity study in mouse is ongoing. The results of two 13-week dose-ranging studies in mouse and the protocol were reviewed by the division and the Executive CAC under IND 120055 as an SPA (Meeting Minutes, January 21, 2016, and November 8, 2017). The division found it acceptable for the study to be conducted post-approval.

Conclusions and Recommendations

The nonclinical studies of CBD (as Purified CBD or CBD-OS) were adequately conducted to characterize the effects of CBD in adult and juvenile animals, except for the assessment of carcinogenic potential. The 2-year carcinogenicity study in rat was inadequate by design and no carcinogenicity study was conducted in mouse. However, considering the indications for which CBD is intended and the division's prior agreement that the mouse carcinogenicity study could be conducted post-approval, a 2-year carcinogenicity study of Purified CBD (or CBD-OS) in rat may be conducted post-approval.

A more serious deficiency is the lack of an adequate assessment of metabolite, 7-COOH-CBD, (b) (4) The lack of this assessment is the basis for Dr. Fisher's conclusion that "The application cannot be considered approvable from a pharmacology/toxicology standpoint…" Comparison of the plasma 7-COOH-CBD exposures achieved in the pivotal nonclinical studies with those in humans at the recommended human dose of 10 mg/kg BID indicates, as Dr. Fisher noted, that exposures in animals were substantially less than that in humans at the RHD. According to the sponsor, plasma 7-COOH-CBD exposure (AUC) in humans is approximately (b) (4) hr/mL; however, the clinical pharmacology team has calculated a plasma AUC of approximately 250000 ng*hr/mL, based on data from single-dose food effect phase of clinical study GWEP1544. Because labeling is to state that patients may take Epidiolex in either the fed or fasted state, the clinical team has concluded that the higher plasma

exposure is the more relevant and recommends its use for interspecies comparisons. In none of the pivotal nonclinical studies for which data are available was similar plasma exposures achieved.

To address this deficiency, the sponsor will need to assess the effects of 7-COOH-CBD in a battery of nonclinical studies, specifically in an embryofetal development study in one species (preferably rat), a pre- and postnatal development study in rat, a juvenile animal toxicology study in rat, and a carcinogenicity study in one species. If feasible, 7-COOH-CBD and CBD may be tested in the same 2-year carcinogenicity study in rat, which would also provide an assessment of the chronic toxicity of 7-COOH-CBD. Based on the pharmacology data suggesting 7-COOH-CBD is an inactive metabolite, nonclinical studies in one species is considered sufficient. However, justification for the species selected should be provided in final study reports.

As Dr. Fisher stated in his review, the nonclinical studies did not adequately assess the potential toxicity of the major human circulating metabolite, 7-COOH-CBD. However, because of the seriousness of the indications and the unmet medical need, if the clinical team concludes that the clinical data are sufficient to support approval, the nonclinical studies needed to address the inadequate assessment of the major human metabolite, 7-COOH-CBD, may be conducted as post-marketing requirements.

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LOIS M FREED
06/13/2018

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

PHARMACOLOGY/TOXICOLOGY REVIEW AND EVALUATION

NDA NUMBER(S):	210365
SERIAL NUMBER:	000
DATE RECEIVED BY CENTER:	12/26/17
PRODUCT:	(b) (4) (cannabidiol) 100 mg/mL oral solution
INDICATION:	Dravet and Lennox Gastaut syndromes
SPONSOR:	GW Therapeutics
REVIEW DIVISION:	Division of Neurology Products (DNP)
PHARM/TOX REVIEWER:	Ed Fisher
PHARM/TOX SUPERVISOR:	Lois Freed
DIVISION DIRECTOR:	Billy Dunn
PROJECT MANAGER:	Stephanie Parncutt

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

I. EXECUTIVE SUMMARY

A. Drug

Trade name: [redacted] (previously Epidiolex)

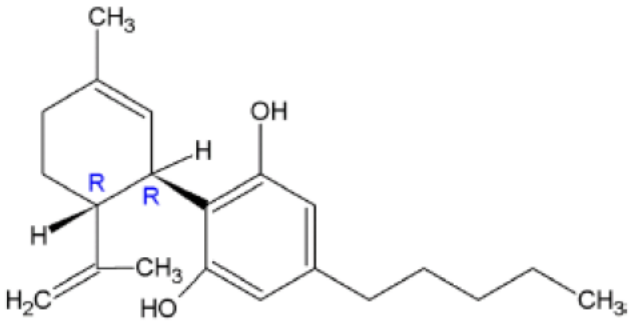
Generic name: Cannabidiol Oral Solution (CBD-OS)

Code names: [redacted], Purified CBD, GWP42003

Chemical name: 2-[(1*R*,6*R*)-3-Methyl-6-(1-methylethenyl)-2-cyclohexen-1-yl]-5-pentyl-1,3-benzenediol

CAS registry number: 13956-29-1

Structure:



Molecular Formula: $C_{21}H_{30}O_2$

Molecular Weight: 314.[redacted]

Drug class: cannabinoid

Indication: Dravet and Lennox Gastaut syndromes

Clinical dose: 20 mg/kg

Dosage forms: oral solution

Relevant IND: 120055

B. Background and brief discussion of nonclinical findings

Pharmacology - Cannabidiol (CBD) drug substance is derived from cultivated *Cannabis sativa* L plants that are processed [redacted]

Purified CBD showed anticonvulsant effects in the maximal electroshock (MES) and audiogenic seizure models in mice (100-200 mg/kg ip) and in the pentylenetetrazole (PTZ)-induced seizure model in rats (100 mg/kg ip). CBD BDS demonstrated comparable anticonvulsant efficacy in the

PTZ rat model at an ip dose of 150 mg/kg CBD. In the MES model in mice, anticonvulsant effects were observed following ip doses of 150 and 200 mg/kg of the major human metabolite 7-OH-CBD. In a separate study, a second major human metabolite, 7-COOH-CBD (up to 200 mg/kg ip), exhibited no anticonvulsant activity in the mouse MES test. The mechanism of anticonvulsant action remains unclear. While it has been shown to interact with numerous classical ion channels, receptors, transporters, and enzymes, the molecular targets thought to be most likely involved in the anticonvulsant activity of CBD include the voltage-dependent anion channel 1 (VDAC1), voltage-gated calcium channel (CaV3.x), serotonin receptor (5-HT1A), glycine receptor (GlyR), G protein-coupled receptor 55 (GPR55), and ENT1 (modulating adenosine transport). CBD has minimal affinity at both CB_1Rs and CB_2Rs.

In safety pharmacology studies, Purified CBD decreased rat locomotor activity (≥ 60 mg/kg ip) and CBD as CBD BDS (100 mg/kg ip) reduced locomotor behavior in ICR mice. CBD BDS inhibited hERG currents in a concentration-dependent manner (25, 50, and 75% inhibition at 64, 130, and 250 ng/mL CBD, respectively). CBD BDS (10, 50, and 100 mg/kg po) decreased heart rate (≥ 50 mg/kg po; 4 hrs post-dose) and increased systolic blood pressure and R-R, R-H, QRS, and QT intervals (100 mg/kg po) in conscious, telemeterized dogs. CBD as CBD BDS (10, 50, or 100 mg/kg po) had no biologically significant effects on respiratory parameters in conscious rats.

ADME - Following absorption after oral administration (with low bioavailability), CBD is mostly eliminated by metabolism. The main routes of CBD metabolism appear to be direct glucuronidation and oxidation of CBD to form 7-hydroxy-cannabidiol (7-OH-CBD), which circulates in human plasma at levels of approximately 50% those of parent, making it a major human metabolite. 7-OH-CBD is metabolized by conjugation with glucuronic acid or further oxidation to 7-carboxy-cannabidiol (7-COOH-CBD). This metabolite circulates at levels far exceeding (up to 50X) those of parent in humans, representing at least 90% of all drug-related material measured in plasma, and is clearly a major human metabolite. 6-hydroxy-cannabidiol (6-OH-CBD) is formed in in vitro systems utilizing human enzymes, but circulating levels in humans are low (<10% of parent). 7-OH-CBD demonstrated anticonvulsant activity in a mouse model and was approximately equipotent to CBD. 7-COOH-CBD exhibited no anticonvulsant effects in the mouse and did not bind to any of a variety of molecular targets. Compared to humans, the toxicology species do not produce the two major human metabolites to a comparable extent, and there is inadequate coverage for 7-COOH-CBD in all three. In addition, questions about the reliability of toxicokinetic data for the metabolites have arisen due to irregularities in the bioanalytical procedures as reported in the sponsor's response to the Division's 74-day letter in which it was stated that the bioanalytical and toxicokinetic phases of most of the pivotal toxicity studies are no longer considered GLP compliant.

Toxicology - Pure CBD (as OS [formulated in (b) (4) sucralose, strawberry flavoring, and sesame oil]) was evaluated in repeat-dose general toxicity studies in mice, rats, and dogs by oral (gavage) administration for up to 13, 26, or 39 weeks, respectively. The drug presented a relatively benign toxicity profile in the rat and dog studies, with adaptive liver changes being the primary findings at CBD exposures up to 24- and 8-fold, respectively, those expected clinically. More significant toxic effects, including mortality and renal histopathology, were seen in mice, but only at CBD exposures >13 times those anticipated in humans.

In the 13-week (CD-1) mouse study (0, 400, 550, or 700/625 mg/kg/day), there were 7 early deaths at the high dose (HD) that were attributed to drug-induced nephropathy. Clinical signs at the HD included convulsions and tremor. The HD was subsequently lowered to 625 mg/kg/day beginning at Week 6. Clinical chemistry changes included increased ALT (up to 2.5X), creatinine (up to 50%), cholesterol, total protein, and globulin. At necropsy, liver weights were increased in both sexes at all doses and large liver was noted macroscopically in some animals from all drug-treated groups. A dose-related increase in the incidence and/or severity of nephropathy was observed microscopically in drug groups and was considered adverse in MD and HD males and in HD females due to the severity and extent of the lesion. Based on the clinical observations,

early deaths, and nephropathy noted in MD and HD males and HD females, the NOAEL was 400 mg/kg/day for males (Cmax and AUC values of 6420 ng/mL and 37800 ng·h/mL) and 550 mg/kg/day for females (Cmax and AUC values of 8440 ng/mL and 41200 ng·h/mL).

In the 26-week (Wistar) rat study (0, 15, 50, or 150 mg/kg/day), there were no drug-related deaths. Changes in liver were characterized by centrilobular hypertrophy at the MD and HD associated with increased ALT and ALP, increased liver weight, and macroscopic enlargement at the HD. These findings were not considered adverse and demonstrated a tendency for reversal at the end of the recovery period. No other toxicity findings were reported. The NOAEL (150 mg/kg/day) was associated with Cmax and AUC values for CBD of 6160 ng/mL and 60000 ng·h/mL in males and 7530 ng/mL and 67500 ng·h/mL in females, respectively.

In the 39-week study in Beagle dogs (0, 10, 50, or 100 mg/kg/day), there were no deaths, but body weight was reduced over the study in MD and HD males and in females at all doses. There were consistent decreases in heart rate in HD males but no drug-related cardiac rhythm disturbances. Drug-related liver changes characterized by hepatocyte hypertrophy associated with increased liver weight, macroscopic enlargement, and marked increases in ALP (up to 8-fold compared to controls) were seen at all doses. These were considered adaptive and demonstrated a tendency for reversal at the end of the recovery period. No other toxicological effects were observed. The NOAEL (100 mg/kg/day) was associated with CBD exposures (AUC) of 20500 ng·h/mL in males and 22400 ng·h/mL in females.

Genotoxicity - Purified CBD and CBD BDS were negative in the Ames test in vitro at up to 5000 µg/plate, with or without metabolic activation (S-9). In micronucleus assays, Purified CBD and CBD BDS did not induce micronuclei (MN) in the polychromatic erythrocytes (PCE) of the bone marrow of rats at oral (gavage) doses up to 500 or 350 mg/kg/day, respectively. CBD-OS did not induce DNA damage in the liver of rats at oral (gavage) doses of up to 500 mg/kg/day in the alkaline COMET assay.

Carcinogenicity - A 104-week dietary carcinogenicity study was conducted in Wistar rats with CBD BDS (58–67% CBD) at concentrations intended to achieve doses of 5, 15, or 50 mg/kg/day (expressed in terms of CBD). There were no apparent effects of treatment on survival and no toxicity findings considered to be of toxicological significance. A dose-related reduction in overall body weight gain and food consumption was seen at the MD and HD that resulted in a greater than 10% reduction in body weight in both sexes at the HD (20 and 24% in M & F). There was no apparent increase in the incidence of neoplasia, alteration in the time of tumor onset, or induction of rare tumors. Non-neoplastic findings included an increased incidence of centrilobular hypertrophy in the liver of both sexes at the HD. TK data from this study were limited to plasma measurements of CBD, THC, and 11-OH THC at 2 timepoints during the day (morning and evening), so animal to human exposure comparisons for CBD and its metabolites are not possible, but the highest administered dose was approximately 0.4 times the MRHD on a mg/m2 basis. An agreement was made with the sponsor that an oral gavage mouse carcinogenicity study of CBD-OS could be submitted postmarketing, and this study is ongoing.

Reproductive toxicity - In an oral (gavage) fertility and early embryonic development toxicity study of Purified CBD (b) (4) in Wistar rats (0, 75, 150, or 250 mg/kg/day; HD associated with decreased body weight gain) there were no apparent drug-related effects on male or female reproductive indices, male reproductive organ weights, female estrus cycling, or any cesarean-section parameters.

Developmental toxicity - In an oral (gavage) rat (Wistar) embryofetal development (EFD) study of Purified CBD (0, 75, 150, or 250 mg/kg/day; HD associated with maternal BW reductions), total litter loss in 2 of 20 dams at the HD was considered drug-related. There were no drug-related effects on fetal weights or fetal abnormalities. The MD was considered the NOAEL for

embryofetal toxicity based on embryolethality at the HD. Maternal exposure (AUC) at this dose was 149000 ng·h/mL.

In an oral (gavage) rabbit (NZW) EFD study of Purified CBD (0, 50, 80, or 125 mg/kg/day), fetal BWs were decreased (10%) and fetal variations (unossified metacarpal, bulging eyes, and nonerupted incisors) increased at the HD, which was also associated with evidence of maternal toxicity. The NOAEL for embryofetal developmental toxicity (80 mg/kg) was associated with a maternal exposure of 2030 ng·h/mL (i.e., much lower exposures than in rats).

In an oral (gavage) rat (Wistar) prenatal and postnatal development (PPND) study of Purified CBD (0, 75, 150, or 250 mg/kg/day), pup BW was reduced at birth and throughout lactation at the MD and HD and was associated with delays in achieving developmental landmarks (pinna unfolding, eye opening, pupillary reflex) including landmarks of male and female sexual maturation. Neurobehavioral changes (decreased locomotor activity) were observed in offspring tested after weaning and there was a dose-related increased number of males with small testis at all doses when animals were necropsied as adults. The LOAEL for developmental toxicity (75 mg/kg) was associated with maternal CBD exposures of approximately 25900 and 86300 ng.h/mL on GDs 6 and 17, respectively (based on TK in the rat EFD study, since plasma levels were not collected in the PPND study).

Juvenile animal toxicity - In a 10-week neonatal/juvenile toxicity study in Wistar rats, Purified CBD (0 or 15 mg/kg/day) was given subcutaneously on PND 4 to 6 followed by oral (gavage) doses of 0, 100, 150, or 250 mg/kg/day CBD-OS from PND 7 to 77. Drug-related findings included 3 HD deaths, dose-dependent increases in body weight gain, neurobehavioral deficits (decreased locomotor activity and startle habituation, both persisting after recovery period at the HD), delayed sexual maturation in males (all doses), transient increases in plasma calcium in males and increased cholesterol, total protein, and globulin in females, a reversible increase in bone mineral density in males (HD), and hepatocyte hypertrophy and vacuolation (all doses) that remained after the recovery period at the MD and HD. Vacuolation was not reported in toxicity studies conducted in adult rats. The LOAEL (15 SC/100 PO mg/kg/day) was associated with a PND 70 exposure to CBD of 82000 ng·h/mL (sexes combined).

Qualification of impurities - Analysis of CBD revealed 4 impurities (b) (4) at levels greater than the ICH qualification thresholds, and the proposed limits for these impurities are ~(b) (4) higher than the actual levels in the nonclinical batches. Each impurity was therefore qualified in a battery of studies including in vitro and in vivo genotoxicity tests,13 or 26-week oral (gavage) toxicity studies in the rat, and rat embryofetal development studies. (b) (4) was (b) (4) in CBD toxicity studies. All other studies were performed on purified materials. In the Sativex study, toxicities (convulsions and reproductive system histopathology) which had not previously been observed in studies of Purified CBD were seen; however, these occurred at (b) (4) much greater than those that would be associated with administration of Purified CBC at the proposed impurity limits.

C. Recommendations

The application cannot be considered approvable from a pharmacology/toxicology standpoint due to the inadequate nonclinical safety assessment of the major human metabolite 7-COOH-CBD. Bioanalytical data quality issues regarding quantitation of metabolites in the pivotal toxicology studies contribute to this nonclinical deficiency. An inadequate carcinogenicity assessment in which only the (b) (4) CBD BDS was administered in the diet, resulting in uncertain exposures, potential interactions with impurities, and excessive BW effects in the single species tested is also an important deficiency. This may at least partially be addressed by the mouse study that is currently underway. The toxicity evaluation of the parent compound can otherwise be considered adequate.

II. PHARMACOLOGY

A. Brief summary

CBD (25-200 mg/kg ip, purity not specified) showed limited efficacy in the maximal electroshock (MES) model of generalized seizures, only reducing the hindlimb extension component of MES-induced seizure, and was much less potent than cannabidivarin (CBDV, Table II.A.1). However, Pure CBD showed similar efficacy and potency compared to Pure CBV in the audiogenic seizure test in mice (Table II.A.2).

Table II.A.1 Effect of CBD and CBDV on MES-induced seizure in mice

Compound (Dose; mg/kg; i.p.)		Incidence of seizure		Severity
		Forelimb extension	Hindlimb extension	
Vehicle		10/10	10/10	2 ± 0
CBD	(25)	10/10	9/10	1.9 ± 0.1
	(50)	9/10	4/10*	1.3 ± 0.2
	(100)	10/10	4/10*	1.4 ± 0.2
	(200)	10/10	3/10**	1.3 ± 0.2*
CBDV	(25)	8/10	6/10	1.4 ± 0.3
	(50)	4/10*	1/10**	0.6 ± 0.2**
	(100)	4/10*	0/10**	0.4 ± 0.2**
	(200)	1/10**	0/10**	0.1 ± 0.2**

Table 2: Effects of Pure CBD and Pure CBDV in the Audiogenic Seizure Test in the DBA/2 mouse (10 mice per group)

Frequencies

TREATMENT (mg/kg) i.p. -60 min	WILD RUNNING			CLONIC CONVULSION			TONIC CONVULSION			DEATH		
	number of mice		% change	number of mice		% change	number of mice		% change	number of mice		% change
Vehicle	10			10			3			3		
Pure CBD (10)	10	NS	0%	8	NS	-20%	0	NS	-100%	0	NS	-100%
Pure CBD (20)	10	NS	0%	6	NS	-40%	2	NS	-33%	0	NS	-33%
Pure CBD (40)	10	NS	0%	8	NS	-20%	0	NS	-100%	0	NS	-100%
Pure CBD (65)	9	NS	-10%	6	NS	-40%	0	NS	-100%	0	NS	-100%
Pure CBD (100)	6	NS	-40%	4	+	-60%	0	NS	-100%	0	NS	-100%
Pure CBD (200)	2	+++	-80%	1	+++	-90%	0	NS	-100%	0	NS	-100%
Pure CBDV (10)	10	NS	0%	10	NS	0%	2	NS	-33%	1	NS	-67%
Pure CBDV (20)	9	NS	-10%	5	+	-50%	0	NS	-100%	0	NS	-100%
Pure CBDV (40)	9	NS	-10%	6	NS	-40%	0	NS	-100%	0	NS	-100%
Pure CBDV (65)	10	NS	0%	10	NS	0%	1	NS	-67%	1	NS	-67%
Pure CBDV (100)	8	NS	-20%	4	+	-60%	0	NS	-100%	0	NS	-100%
Pure CBDV (200)	3	++	-70%	2	+++	-80%	0	NS	-100%	0	NS	-100%

Fisher's Exact test: NS = Not Significant; + = $p < 0.05$; ++ = $p < 0.01$; +++ = $p < 0.001$.

Pure CBD also demonstrated anticonvulsant activity in the chemically-induced PTZ model of generalized seizures at the highest dose of 100 mg/kg ip (Fig II.A.1).

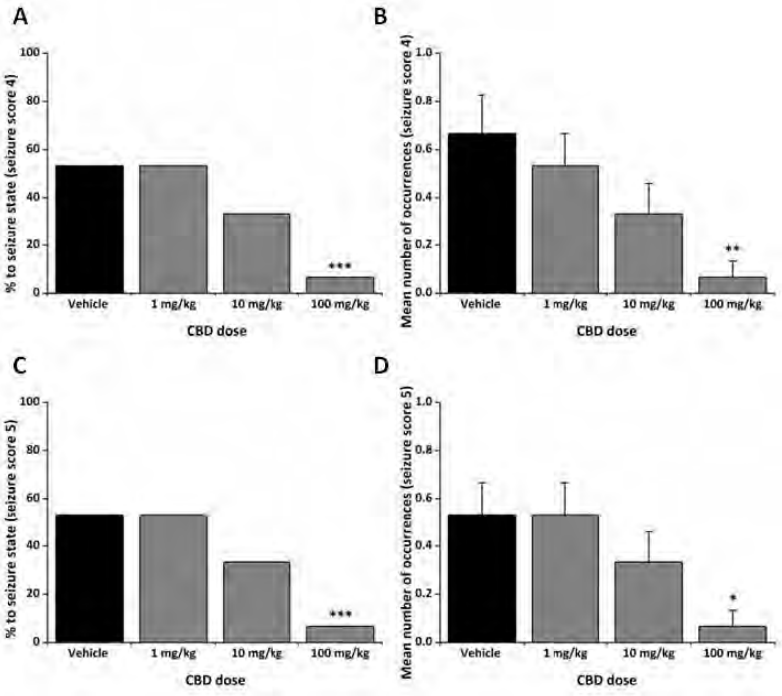


Figure II.A.1. Effect of Pure CBD on PTZ-induced seizures

Other models in which efficacy was demonstrated included the pilocarpine-induced model of temporal lobe seizure, in which only moderate activity was seen at ip doses of up to 100 mg/kg, and penicillin-induced model of partial seizure in which greater efficacy was seen at ip doses of 1 to 100 mg/kg Purified CBD.

When the MES mouse model was used to evaluate the major CBD metabolites, anticonvulsant effects were observed following dosing of 150 and 200 mg/kg 7-OH-CBD, while 7-COOH-CBD (50, 100, 150, or 200 mg/kg) exhibited no significant anticonvulsant effects (Table II.A.3).

Table II.A.3. Effects on tonic convulsion in mouse MES test

Treatment	Number of animals showing tonic convulsion			
	Number	Compared with Vehicle (i.p.)		
Vehicle (i.p.)	11			
7-COOH CBD (50 mg/kg i.p.)	12	NS	1.0000	+9%
7-COOH CBD (100 mg/kg i.p.)	10	NS	1.0000	-9%
7-COOH CBD (150 mg/kg i.p.)	9	NS	0.5901	-18%
7-COOH CBD (200 mg/kg i.p.)	9	NS	0.5901	-18%
CBD (200 mg/kg i.p.)	1	***	0.0001	-91%
Diazepam (2 mg/kg i.p.)	2	***	0.0006	-82%

Fisher's Exact test: NS = Not Significant; * = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$.

In a [redacted] (b) (4) screen, no high affinity binding of Purified CBD or its major metabolites 7-OH-CBD and 7-COOH-CBD was observed (Table II.A.4). Based on a literature review of receptor, ion channel, and transporter targets of CBD (Ibeas Bih et al, Neurotherapeutics 12:699-730, 2015), the molecular targets thought to be most likely to be involved in the anticonvulsant activity of CBD include the voltage-dependent anion channel 1 (VDAC1), voltage-gated calcium channel (CaV3.x), serotonin receptor (5-HT1A), glycine receptor (GlyR), G protein-coupled receptor 55 (GPR55), and ENT1 (modulating adenosine transport). In contrast to Δ9-THC, CBD has a very low (micromolar) affinity and shows little agonist activity at the G protein-coupled endocannabinoid system (ECS) receptors, CB1R and CB2R.

Table II.A.4. [redacted] (b) (4) results showing an inhibition or stimulation higher than 50% and calculable IC50 and EC50 values

Pure CBD

Assay	1.0E-05 M	1.0E-06 M	1.0E-07 M	1.0E-08 M
5-HT_{2A}*(h)* (agonist radioligand)	88.3%			
5-HT_{2B}*(h)* (agonist radioligand)	91.6%			
α_{2A}*(h)* (antagonist radioligand)	62.5%			
α_{2B}*(h)* (antagonist radioligand)	68.1%			
α_{2C}*(h)* (antagonist radioligand)	80.1%			
CB_1*(h)* (agonist radioligand)	70.7%			
CB_2*(h)* (agonist radioligand)	67.8%			
Cl^- channel (GABA-gated) (antagonist radioligand)	90.6%			
D_1*(h)* (antagonist radioligand)	88.2%			
δ_2 (DOP) *(h)* (agonist radioligand)	53%			
dopamine transporter*(h)* (antagonist radioligand)	73.5%			
GR *(h)* (agonist radioligand)	53.8%			
κ (KOP) (agonist radioligand)	73%			
M_3*(h)* (antagonist radioligand)	83.8%			
μ (MOP) *(h)* (agonist radioligand)	96.6%			
Na^+ channel (site 2) (antagonist radioligand)	93.2%			
norepinephrine transporter*(h)* (antagonist radioligand)	90.3%			
OX_1*(h)* (agonist radioligand)	91.3%			

Assay	IC_{50}	K_I	K_B	EC_{50}	nH
5-HT_{2A}*(h)* (antagonist effect)	1.0E-05 M		1.4E-06 M		
5-HT_{2B}*(h)* (antagonist effect)	2.4E-05 M		3.7E-06 M		
CB_1*(h)* (agonist effect)				4.6E-05 M	
δ_2 (DOP) (antagonist effect)	3.4E-05 M		5.9E-06 M		
M_3*(h)* (antagonist effect)	1.1E-05 M		1.0E-06 M		
OX_1*(h)* (antagonist effect)	2.7E-05 M		5.6E-06 M		

7-OH CBD

Assay	1.0E-05 M	1.0E-06 M	1.0E-07 M	1.0E-08 M
5-HT_{2A}*(h)* (agonist radioligand)	55.2%			
5-HT_{2B}*(h)* (agonist radioligand)	70.2%			
CB_1*(h)* (agonist radioligand)	69.3%			
CB_2*(h)* (agonist radioligand)	64.4%			
Cl^- channel (GABA-gated) (antagonist radioligand)	85.8%			
GR *(h)* (agonist radioligand)	59%			
N neuronal α7 *(h)* (antagonist radioligand)	61.3%			
Na^+ channel (site 2) (antagonist radioligand)	95.1%			
OX_1*(h)* (agonist radioligand)	83%			

Assay	IC_{50}	K_I	K_B	EC_{50}	nH
5-HT_{2A}*(h)* (antagonist effect)	9.2E-06 M		1.3E-06 M		
5-HT_{2B}*(h)* (antagonist effect)	4.5E-06 M		6.9E-07 M		
CB_1*(h)* (agonist effect)				8.0E-05 M	
OX_1*(h)* (antagonist effect)	4.6E-06 M		9.8E-07 M		

7-COOH CBD

Assay	1.0E-05 M	1.0E-06 M	1.0E-07 M	1.0E-08 M
N neuronal α7 *(h)* (antagonist radioligand)	73.4%			

B. Safety Pharmacology

1. CNS

Pure CBD (120 mg/kg, ip, 30 min prior to test) had no effects on basal locomotor activity and muscle strength in male Swiss mice. However, an ip dose of 100 mg/kg CBD BDS reduced locomotor behavior in male ICR mice and Pure CBD (120 mg/kg, ip, 30 min prior to test) decreased locomotor activity in male Wistar and Lister Hooded rats. CBD BDS (up to 100 mg/kg, po by gavage) had no significant effects on behavioral and physiological measures or body temperature in the rat Irwin test.

2. Cardiovascular

CBD BDS inhibited hERG tail current in a concentration-dependent manner (25, 50, and 75% inhibition at concentrations of 64, 130, and 250 ng/mL CBD, respectively).

When CBD BDS (0, 10, 50, and 100 mg/kg) was administered by oral gavage to conscious, telemeterized dogs (Study # GWOR10111, conducted by (b) (4), report dated 2/2/11, GLP), a dose-related decrease in heart rate (≥50 mg/kg), increased systolic blood pressure (100 mg/kg, 5 hrs postdose, SS), and dose-related increases in R-R, R-H, QRS, and QT (but not QTc) intervals (SS at 100 mg/kg, 4 hr) were observed (Table II.B.1). According to the study report, the effect on heart rate was biologically significant but the other changes were not due to their low magnitude. The NOAEL was 10 mg/kg CBD BDS.

Table II.B.1.

Group mean change from pre-dose
Time = 300 minutes

Variable Statistics	Dose 1	Dose 2	Dose 3	Dose 4	
SYS A	-8	-4	7	19*	DR*(I)
DIA A	-6	-5	-6	4	
MEAN A	-7	-5	-3	8	
HR A	-3	12	-11	-10	
RRI A	-8	-91	157	199	DR*(I)
RH A	0.2	0.3	0.3	0.7	
QRS A	3	1	1	7	
PR A	2	-6	-3	-8	
QT A	2	-8	20	20	DR*(I)
QTC A	-0	0	5	-11	

* P<0.05
** P<0.01
*** P<0.001
DR=significant dose response test
(I)=increasing dose response

A=ANOVA, dose response and Dunnett's on the change from pre-dose

3. Respiratory

CBD BDS (0, 10, 50, or 100 mg/kg, oral [gavage]) had no biologically significant effects on respiratory parameters in conscious rats evaluated using whole body plethysmography. The NOEL was 100 mg/kg.

III. PHARMACOKINETICS

A. Absorption

The absorption of Pure CBD in an earlier Solutol-based formulation was investigated in female Sprague Dawley rats following 3 routes of administration of 10 mg/kg. Cmax values after iv, ip, and po administration were 10556, 549, and 295 ng/mL, at 5 min, 15-30 min, and 1 hr, respectively. Other PK parameters were not calculated. In the only evaluation of bioavailability provided by the sponsor, the oral bioavailability of CBD in the clinical sesame oil-based formulation was 15% in the minipig. In a literature report, the PK of CBD (purity not specified) was studied in dogs after the administration of two iv doses (45 and 90 mg) and one oral dose (180 mg). After iv administration, terminal half-life was 9 hr, total body clearance of CBD was 17 L/hr (after the 45-mg dose) and 16 L/hr (after the 90-mg dose), volume of distribution approximately 100 L, and oral bioavailability ranged from 13 to 19%.

B. Distribution

Concentrations of radioactivity in plasma and tissues were determined following single oral administrations of either [14C]-THC or [14C]-CBD to male albino (Sprague-Dawley) and partially pigmented (Long Evans) rats at 15 mg/kg or 1:1 mixture of [14C]-THC:cold CBD or 1:1 mixture of [14C]-CBD:cold THC to albino and partially pigmented rats at 15 mg/kg (i.e.7.5 mg/kg CBD:7.5 mg/kg THC). Concentrations of radioactivity in plasma peaked between 4 and 8 hours after dosing with [14C]-CBD (Table III.B.1).

Table III.B.1. Plasma PK of [14C]-CBD and [14C]-THC in male rats

Parameter	Plasma			
	[^{14}C]-CBD dose 15 mg/kg	1:1 mixture of [^{14}C]-CBD:cold THC dose 7.5:7.5 mg/kg	[^{14}C]-THC dose[A] 15 mg/kg	1:1 mixture of [^{14}C]-THC: cold CBD dose 7.5:7.5 mg/kg
C_{max} (µg equiv./mL)	3.60	2.20	0.53	0.43
T_{max} (h)	8	6	24	8
T½ (h)	14.0	7.2	87.6	11.6
AUC_{0-t} (µg equiv.h/mL)	77.1	33.5	22.0	6.8
AUC_{0-Inf} (µg.equiv.h/mL)	87.0	34.0	69.1	7.4
Elimination rate constant (h^{-1})	0.05	0.10	0.01	0.06

[A] = Values generated from data points with similar concentrations and therefore should be considered as an estimate only.

Tissue distribution is shown in Table III.B.2-3. There were no significant differences between albino and pigmented rats, so the data were combined.

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Table III.B.2. Tissue concentrations of [14C]-CBD and [14C]-THC in male rats

Tissue	Concentrations of Radioactivity µg equivalents CBD/gram of tissue				Concentrations of Radioactivity µg equivalents THC/gram of tissue			
	6 hours	12 hours	24 hours	168 hours	6 hours	12 hours	24 hours	168 hours
Adrenal gland	3.27	2.38	0.198	BLQ	1.26	2.40	2.16	0.251
Brain	0.229	0.308	BLQ	BLQ	0.311	0.727	0.459	BLQ
Epididymis	0.795	1.52	0.190	0.158	0.488	0.817	0.755	BLQ
Eye: whole	0.217	0.215	BLQ	BLQ	BLQ	0.154	0.198	BLQ
Fat: brown	3.08	4.69	0.973	BLQ	1.75	3.99	2.99	0.076
Fat: white	0.879	1.61	1.20	0.272	0.435	1.53	1.18	0.134
Harderian gland	1.86	2.57	0.454	BLQ	1.27	3.43	2.25	BLQ
Heart blood	2.08	2.76	0.279	BLQ	0.305	0.686	0.568	BLQ
Kidney: whole	2.85	2.29	0.479	BLQ	0.950	1.81	1.25	BLQ
Liver	7.76	8.20	2.18	0.294	2.86	4.41	3.10	0.097
Lung	1.83	2.58	0.235	BLQ	0.491	0.997	0.648	BLQ
Myocardium	1.31	1.74	0.110	BLQ	0.931	1.68	1.50	0.087
Spinal cord	0.230	0.381	BLQ	BLQ	0.314	0.879	0.471	BLQ
Spleen	0.891	0.805	0.082	BLQ	0.482	1.28	0.536	0.114
Testis	0.559	0.889	0.100	BLQ	0.169	0.497	0.186	BLQ
Thyroid gland	1.15	1.93	0.565	BLQ	0.846	1.81	1.44	BLQ
GI tract (range)	1.35 - 109	1.28 - 276†	0.125 – 17.4	BLQ – 0.196	1.02 - 180†	1.41 - 341†	1.00 - 149†	BLQ – 0.158
Remaining tissues (range)	BLQ – 18.6	0.190 – 2.40	BLQ – 0.354	BLQ	BLQ – 1.13	BLQ – 0.603	BLQ – 0.755	BLQ

BLQ Below limit of accurate quantification (<0.077 (for CDB)/0.079 (for THC) µg equivalents/g)
NS No sample – tissue not sectioned
† Above limit of accurate quantification (>132 (for CDB)/135 (for THC) µg equivalents/g) – extrapolated value reported

Table III.B.3.Tissue concentrations of [14C]-CBD:cold THC and [14C]-THC:cold CBD in male rats

Tissue	Concentrations of Radioactivity µg equivalents CBD/gram of tissue				Concentrations of Radioactivity µg equivalents THC/gram of tissue			
	6 hours	12 hours	24 hours	168 hours	6 hours	12 hours	24 hours	168 hours
Adrenal gland	1.43	1.83	0.712	BLQ	1.53	1.99	3.26	0.164
Brain	0.138	0.225	0.080	BLQ	0.357	0.487	0.508	BLQ
Epididymis	0.532	0.398	0.287	BLQ	0.445	NS	0.293	BLQ
Eye: whole	0.096	0.093	0.079	BLQ	0.105	0.130	0.193	BLQ
Fat: brown	1.02	1.71	1.37	BLQ	2.61	3.17	4.25	0.107
Fat: white	0.210	0.246	0.742	BLQ	0.572	1.26	3.96	0.209
Harderian gland	0.781	1.52	0.922	BLQ	1.37	2.63	4.40	BLQ
Heart blood	1.53	1.74	1.15	BLQ	0.252	0.411	0.659	0.112
Kidney: whole	2.82	3.75	2.46	BLQ	1.08	1.38	1.84	0.119
Liver	4.63	4.81	4.02	0.116	2.06	2.90	2.75	0.111
Lung	1.28	1.58	1.07	BLQ	0.372	0.759	0.794	0.077
Myocardium	0.749	1.09	0.480	BLQ	0.882	1.29	1.49	0.111
Spinal cord	0.132	0.221	0.096	BLQ	0.379	0.554	0.470	0.083
Spleen	0.601	0.853	0.279	BLQ	0.504	0.757	0.745	BLQ
Testis	0.387	0.420	0.306	BLQ	0.217	0.335	0.293	BLQ
Thyroid gland	0.710	0.998	0.601	BLQ	0.824	0.818	1.10	BLQ
GI tract (range)	0.225 – 155†	0.887 – 152†	0.598 – 66.8	BLQ – 0.127	0.364 – 120	1.10 – 52.4	1.61 – 81.2	BLQ – 0.221
Remaining tissues (range)	BLQ – 24.5	BLQ – 11.4	BLQ – 0.532	BLQ	BLQ – 7.13	BLQ – 1.19	0.133 – 4.36	BLQ

BLQ Below limit of accurate quantification (<0.078 (for CDB)/0.079 (for THC) µg equivalents/g)
NS No sample – tissue not sectioned
† Above limit of accurate quantification (> 134 µg equivalents/g) – extrapolated value reported

Purified CBD was highly protein bound in rat, dog, and human plasma (> 94%) but less protein bound in mouse and rabbit plasma (83 and 65%, respectively) (Table III.B.4). Binding was determined to be independent of concentration. The major CBD metabolites, 6-OH-CBD, 7-OH-CBD, and 7-COOH-CBD, showed high to very high binding to mouse, rat, rabbit, dog, and human plasma (98.8 - >99.0%).

Table III.B.4. In vitro binding of CBD to rat, dog, and human plasma

Substrate	Binding Methodology	% Bound				
		Mouse	Rat	Rabbit	Dog	Human
CBD	Equilibrium Dialysis	Not examined	> 99.8[a]	Not examined	> 99.8[a]	> 99.8[a]
^{14}C-CBD	Ultra-centrifugation	83.0 ± 3.4[c]	94.8 ± 1.2[b]	65.1 ± 10.6[c]	95.5 ± 1.2[b]	94.0 ± 1.2[b]

C. Metabolism

When the metabolism of CBD was examined in rat, dog, and human liver microsomes and hepatocytes in vitro, the major phase I metabolites were 7-OH-CBD, 7-COOH-CBD, and 6-OH-CBD (Figure III.C.1). These were subsequently found to be present in rat, rabbit, dog, and human plasma.

Hepatocyte incubations with CBD also indicated that direct glucuronide conjugation of CBD and conjugation following hydroxylation were important metabolic routes. This study showed that the acid metabolite was produced at relatively high levels. However, the acyl-glucuronide (glucuronide of the acid group of 7-COOH-CBD) was not detected following incubation periods of up to 4 hours. This suggested that conjugates of 7-COOH-CBD may occur in vivo, but whether acyl-glucuronides are present is unknown. According to the sponsor, “it is therefore important to determine the nature of these conjugates since the glucuronide metabolite has shown some instability as it undergoes acyl migration.” But no additional data examining this possibility were submitted.

In vivo studies of 14C-CBD in rat (15 mg/kg po) or dog (5 mg/kg po) identified a major acid metabolite that was confirmed to be 7-COOH-CBD. This was consistent with the human clinical PK data, which showed that 7-COOH-CBD was the predominant metabolite in the plasma, circulating at levels far in excess of parent in human, representing at least 90% of all drug-related material in plasma.

A clinical study (GWEP1544) of CBD-OS provided the most thorough PK assessment of CBD and its metabolites in healthy volunteers (Table III.C.1). The sponsor asserted that exposures in patients and healthy volunteers are comparable.

Figure III.C.1 Metabolic pathways of CBD in human liver microsomes

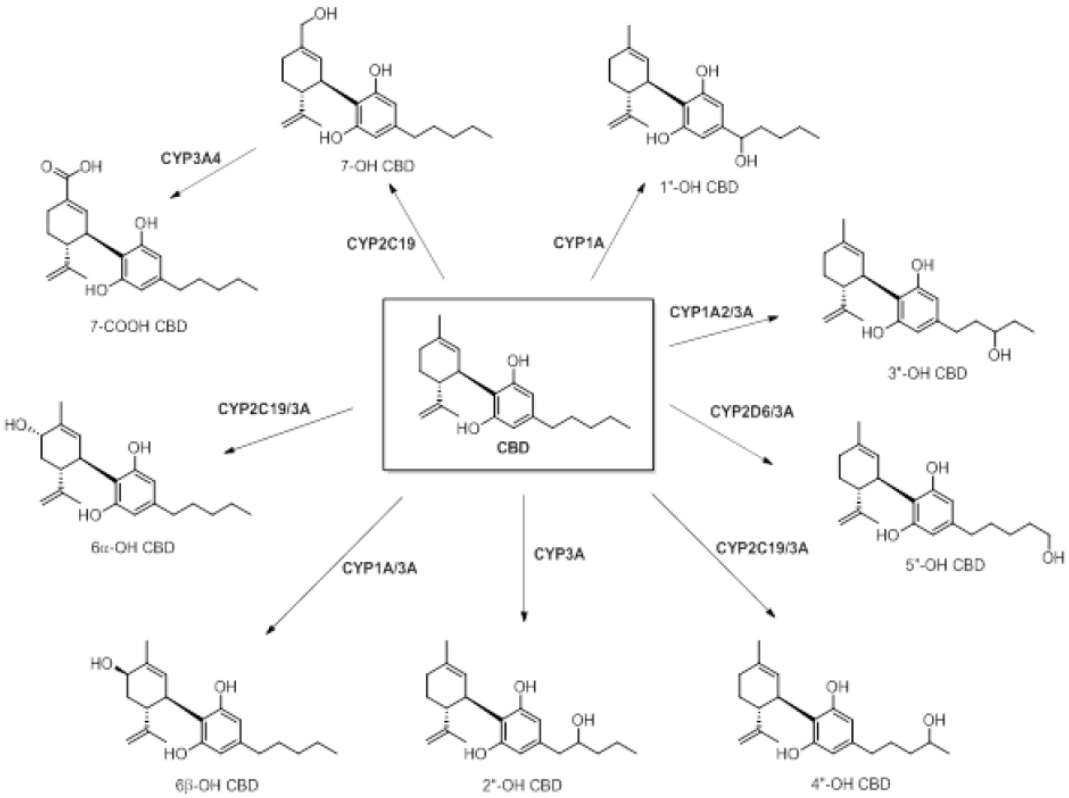


Table III.C.1 CBD and metabolite exposures in healthy volunteers

Dose of CBD-OS (mg)	Day	$AUC_{(0–tau)}$ ng.h/mL[a] n=9			
		CBD	6-OH-CBD	7-COOH-CBD	7-OH-CBD
750[b]	1 (morning)	1070 (74.6) (n=9)	47.60 (34.4) (n=4)	20807 (60.5) (n=9)	699.0 (59.6) (n=4)
	1 (evening)	2683 (33.4) (n=8)	71.90 (28.2) (n=7)	41656 (60.8) (n=4)	958.7 (33.3) (n=7)
	7 (morning)	1745 (38.4) (n=9)	93.35 (38.6) (n=8)	86179 (60.0) (n=9)	976.3 (27.9) (n=9)
1500	1 (morning)	1444 (101.4) (n=9)	30.50 (187.3) (n=5)	20526 (135.7) (n=9)	457.4 (207.8) (n=5)
	1 (evening)	9819 (32.3) (n=2)	96.41 (104.5) (n=2)	86076 (74.2) (n=6)	NC (n=0)
	7 (morning)	3236 (44.0) (n=9)	104.3 (82.3) (n=9)	151336 (66.4) (n=9)	1246 (58.8) (n=9)

$AUC_{(0–tau)}$ = area under the plasma concentration−time curve over a dosing interval, where tau is the dosing interval; NC = Not calculated.

[a] Geometric mean and geometric CV%.

[b] Equivalent to 20 mg/kg (10 mg/kg b.i.d) for a 75 Kg human and representative of pharmacologically relevant dose in Phase 3pivotal trials.

Source: GWEP1544 CSR, Section 8.4.2.3.3.2

The most abundant CBD metabolite was 7-COOH-CBD, present in concentrations up to (b) (4) times the parent compound. The next most abundant metabolite was 7-OH-CBD, present in concentrations up to (b) (4) % of parent. 6-OH-CBD was a minor metabolite (b) (4) % of parent).

7-OH-CBD demonstrated anticonvulsant activity in a mouse model and was approximately equipotent to CBD. 7-COOH-CBD exhibited no anticonvulsant effects in the mouse and did not bind to any of a variety of targets in a (b) (4) panel, inhibit reuptake of monoamines, or interact with sodium (Nav) channels at physiologically relevant concentrations. 7-COOH-CBD was, however, found to be a substrate of the human P-glycoprotein (P-gp) transporter and to inhibit the bile salt export pump (BCRP) and breast cancer resistance protein (BCRP) at clinically-relevant concentrations in vitro.

Metabolite determinations from pivotal toxicity studies showed marginal coverage for 7-OH-CBD in the rat and dog and inadequate coverage for 7-COOH in rodents and dog (Sponsor Tables 8-4 and 8-5; also Sponsor Table 1.1.1.1.2.2-1 on page 17). For mouse and rat studies:

Table 8-4 Summary of PK Parameters for 7-OH-CBD Following Multiple Oral Doses of CBD-OS

	GWEP1544 (Human)	GWTX1503 (Mouse)		GWTX1412 (Rat)	
	21 mg/kg/day	300 mg/kg/day		150 mg/kg/day	
PK parameter		**Male**	**Female**	**Male**	**Female**
C_{max} (ng/mL)	376	2590	2730	334	625
$AUC_{(0-t)}$ (ng.h/mL)	2492	19800	35700	2560	6730

Table 8-5 Summary of PK Parameters for 7-COOH-CBD Following Multiple Oral Doses of CBD-OS

	GWEP1544 (Human)	GWTX1503 (Mouse)		GWTX1412 (Rat)	
	21 mg/kg/day	300 mg/kg/day		150 mg/kg/day	
PK parameter		**Male**	**Female**	**Male**	**Female**
C_{max} (ng/mL)	32612	2330	4640	4180	2710
$AUC_{(0-t)}$ (ng.h/mL)	302672	30100	38800	37100	40500

The highest 7-COOH-CBD exposures were measured in the 10-week oral juvenile rat study. Following repeated oral (gavage) administration of Purified CBD-OS, the maximum 7-COOH-CBD plasma concentrations (Cmax) of 6010, 8600, and 13900 ng/mL in males and 5580, 7950 and 13700 ng/mL in females; and mean 7-COOH-CBD AUC0-t values of 67100, 101000 and 171000 ng.h/mL in males and 68000, 103000 and 189000 ng.h/mL in females were observed at doses of 100, 150, and 250 mg/kg/day, respectively.

The sponsor stated the following regarding 7-COOH-CBD coverage in the nonclinical toxicity species:

> While GW accepts that human exposure levels are around 10 fold greater than animal exposure, there have been no safety signals in the clinical trials related to 7-COOH-CBD.

D. Excretion

Excretion of radioactivity after oral administration of [14C]-CBD, [14C]-THC, [14C]-CBD:cold THC, or [14C]-THC:cold CBD to male partially pigmented rats was predominantly via the fecal route, with the majority of radioactivity recovered during the first 48 hours after dosing. Urine accounted for a smaller proportion of radioactivity (~3-5% for each animal) with remaining concentrations of radioactivity measured in the cage washings. The mean total recovery of radioactivity ranged from ~91 to 102% (n = 12).

Table III.D.1. Recovery of administered radioactivity in male rats

Sample	Mean % of dose administered			
	[^{14}C]-CBD	[^{14}C]-THC	1:1 mixture of [^{14}C]-CBD:THC	1:1 mixture of [^{14}C]-THC:CBD
Urine	3.40	2.59	3.91	5.12
Faeces	89.22	99.18	86.60	91.13
Cage Wash	0.38	0.36	0.29	0.64
Total	92.99	102.13	90.80	96.89

Excretion of radioactivity after oral administration of [14C]-THC, [14C]-CBD, 1:1 mixture of [14C]-THC:cold CBD, or [14C]-CBD:cold THC to male beagle dogs was predominantly via the fecal route, with the majority of radioactivity recovered during the first 48 hours after dosing. Urine accounted for a smaller proportion of radioactivity (*ca.* 3-9% for each animal) with remaining concentrations of radioactivity measured in the cage washings. The mean total recovery of radioactivity ranged from ~75 to 94%.

Table III.D.2. Recovery of administered radioactivity in male dogs

Sample	Mean % of dose administered			
	[^{14}C]-THC (Dog 2M only)	[^{14}C]- CBD (Dog 4M only)	1:1 mixture of [^{14}C]-THC:CBD [n=2]	1:1 mixture of [^{14}C]-CBD:THC [n=2]
Urine	4.62	8.18	5.16	9.39
Faeces	61.10	84.45	84.67	76.84
Cage Wash	9.25	1.56	1.19	2.75
Total	74.97	94.19	91.02	88.98

E. Exposure comparisons

Exposures of CBD observed in pediatric and adult Dravet syndrome (DS) and Lennox-Gastaut syndrome (LGS) patients at therapeutic doses are shown in the table below and are comparable to exposures in healthy volunteers in which similar doses were employed.

Table III.E.1. CBD Exposures (Cmax and AUCs) in Healthy Subjects and Patients

Parameter	Healthy Subjects, Day 7 (750 mg CBD-OS b.i.d; ~21.4 mg/kg/day)	DS Patients, Day 22 (20 mg/kg/day CBD-OS given as 10 mg/kg b.i.d.)	LGS Patients, 3 months (20 mg/kg/day CBD-OS given as 10 mg/kg b.i.d.)
C_{max} (ng/mL)	330	243	219
$AUC_{(tau)}$ (ng·h/mL)	1745	2220	2412

Comparison of exposures to CBD and its major metabolites in animals and humans is shown below (Sponsor Table 1.1.1.1.2.2-1).

Table 1.1.1.1.2.2-1 Margin of Safety for CBD, 7-COOH-CBD and 7-OH-CBD in Studied Populations

Species	NOAEL (mg/kg)	CBD		7-COOH-CBD		7-OH-CBD		Study Reference
		$AUC_{(0-24)}$ at NOAEL (ng·h/mL)	Margin of Safety[a,b] calculated using AUC	$AUC_{(0-24)}$ at NOAEL (ng·h/mL)	Margin of Safety[a,c] calculated using AUC	$AUC_{(0-24)}$ at NOAEL (ng·h/mL)	Margin of Safety[a,d] calculated using AUC	
Mouse (male)	300	44300	15.9	30100	0.22	19800	12.7	GWTX1503 (13 week)
Mouse (female)	300	46400	16.6	38800	0.28	35700	22.9	
Rat (male)	150	60000	21.5	37100[e]	0.27	2560	1.6	GWTX1412 (26 week)
Rat (female)	150	67500	24.2	40500[e]	0.29	6730	4.3	
Dog (male)	100	20500	7.3	994	0.01	1380	0.88	GWTX1413 (39 week)
Dog (female)	100	22400	8.0	1560	0.01	1090	0.70	
Rat (maternal)	250	170000	60.9	155000	1.12	12600	8.1	GWTX1454 (Embryo-Fetal Development)

NOAEL = No-observed-adverse-effect level

[a] From Clinical Study GWEP1544, $AUC_{(0-t)}$ at steady state following multiple oral doses of a clinical dose of 750 mg CBD twice daily; value used was Day 7 morning dose of 750 mg CBD. Margin of safety calculations were done using an AUC that was extrapolated for 20 mg/kg per dose. Human AUC was doubled to match the preclinical dosing interval.

[b] Adjusted human $AUC_{(0-24)}$ for CBD is 2790 ng.h/mL.

[c] Adjusted human $AUC_{(0-24)}$ for 7-COOH-CBD is 137,886 ng.h/mL.

[d] Adjusted human $AUC_{(0-24)}$ for 7-OH-CBD is 1,562 ng.h/mL.

[e] Anticipated under representation; degree of bias could be between 30% and 50% from the actual value.

IV. TOXICOLOGY

A. SUBCHRONIC TOXICITY

1. Purified CBD: 13 Week Oral (Gavage) Administration Range-finding Study in the Mouse (GW Study Number: GWTX1688, conducted by (b) (4), report dated 8/11/17, GLP)

a. Methods

CD-1 (Crl:CD1(ICR)) mice (12/sex/grp + 18/sex/grp TK) were administered Pure CBD (as CBD-OS [formulated in (b) (4) sucralose, strawberry flavoring, and sesame oil]; batch # LMP01/100) by oral gavage (10 mL/kg) at doses of 0 (vehicle: placebo oral solution), 400, 550, or 700/625 mg/kg/day for 13 weeks. Assessments included mortality, clinical signs, body weight, food consumption, and clinical and anatomic pathology evaluations. Complete necropsies with examination for macroscopic abnormalities were performed on all animals. Microscopic examinations were only conducted on a limited number of tissues (not including brain) in C and HD groups. Only liver, kidney, spleen, and gross lesions were examined in the LD and MD groups. Blood was collected for TK on Day 1 and Week 13 predose, and at 1, 2, 4, 6, and 24 hours postdose. This was a dose range-finding study for the mouse carcinogenicity study which was ongoing at the time of NDA submission. A previous 13-week dose range-finding study of Purified CBD produced no significant toxicity at the highest dose (see below).

Dose justification: Doses were based on the results of a 14-day oral (gavage) dose range-finding study of Purified CBD in CD-1 mice (b) (4) Study 8349347; Sponsor # GWTX1665) in which the HD (1000 mg/kg/day) group was terminated early (Day 7/8) due to deaths (6 male TK animals) during the first week and the MD (750 mg/kg/day) resulted in swollen/hard abdomen and necropsy findings of distended stomach and large liver, with 4 male deaths in the TK subgroup. The LD (400 mg/kg/day) was well tolerated with no deaths or adverse effects.

Prior to that, a 13-week dose range-finding study (GWTX1503) in CD-1 mice was conducted using oral (gavage) doses of 0 (vehicle: sesame oil containing 7.9% w/v (b) (4) 100, 150, and 300 mg/kg/day Purified CBD (submitted as draft report). There were no drug-related deaths, clinical observations, or body weight effects. Clinical pathology findings considered drug treatment-related (TR) consisted of mild increases in ALT and serum cholesterol at 7 and/or 13 weeks. Increases in liver weights, macroscopic observation of large liver, and centrilobular hepatocellular hypertrophy were the only TR terminal findings. TK data were not provided. An SPA for a mouse carcinogenicity study using this original 13-week study was considered inadequate by the Exec-CAC due to failure to identify an MTD or other basis for dose selection (minutes dated 1/21/16 under IND 120055).

b. Results

i. Mortality and Clinical Observations

There were 11 early deaths, 7 of which were considered TR. The cause of death in these 7 HD animals (6 males and 1 female) was identified as a renal lesion similar to that found at the terminal necropsy (Tables 2-5). Six HD males (3 main study, 3 TK) were terminated early during the first 5 weeks of dosing. Dosing was stopped for the surviving HD males on Days 31-35 and restarted at 625 mg/kg/day beginning at Week 6. A convulsive episode was observed in 1 HD main study female prior to dosing on Day 71; on Day 84, this animal was pale, staggering, thin, hunched, and subdued, with irregular respiration, and was sacrificed; the cause of death was identified as a drug-induced renal lesion.

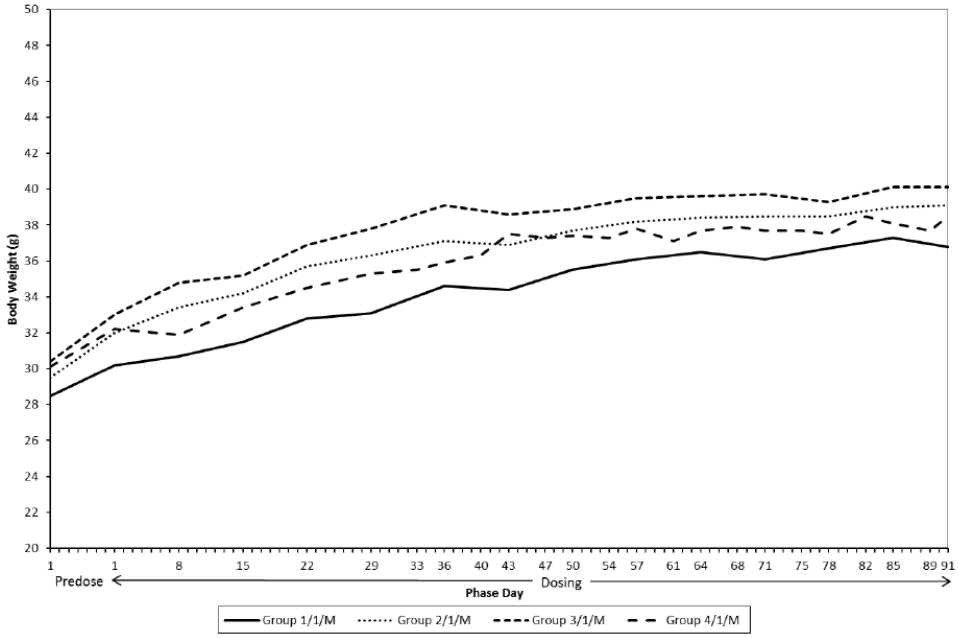
In addition to the 7 deaths attributed to drug-induced renal histopathology, 1 HD TK female died following a convulsive episode immediately after dosing on Day 33, but the cause of

death was not determined. Tremors were also observed in 2 HD males. Swollen abdomen (minimal to moderate) was noted in males at all doses and in MD and HD females with a dose-dependent increase in frequency.

ii. Body Weight

There were no effects on body weight gain, body weight, or food consumption in males (Figure IV.A.1.1, Table IV.A.1.1). Body weight gain was increased in drug-treated females compared to controls, but not in a dose-dependent manner (42, 79, and 56% greater than controls in LD, MD, and HD females, respectively). In the absence of any dose relationship or correlating effect on food consumption, this was not considered toxicologically significant by the sponsor.

Figure IV.A.1.1. Body weights in male and female mice



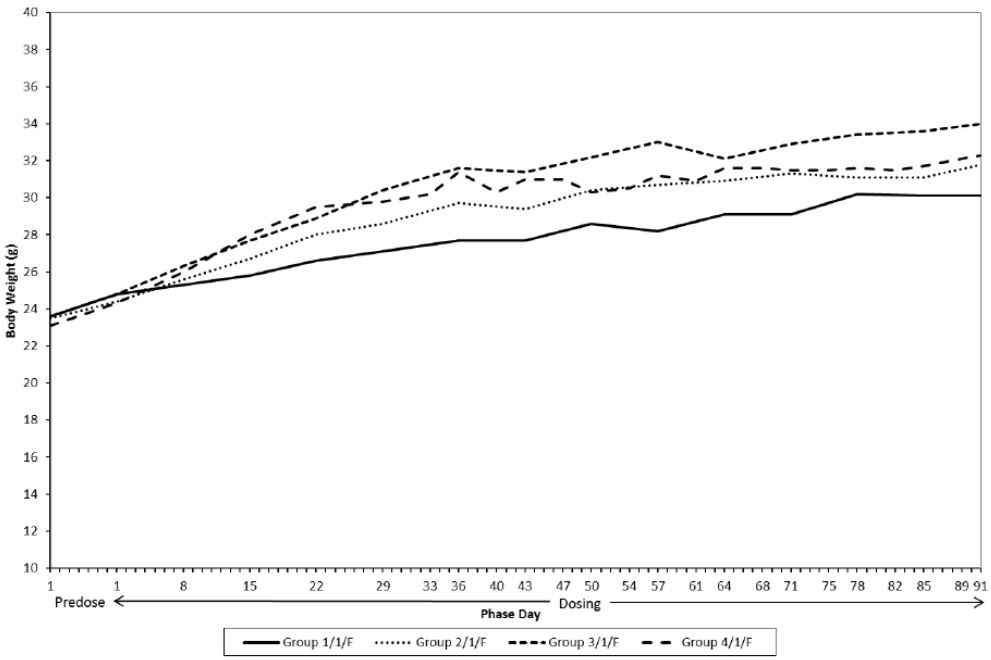


Table IV.A.1.1. Body weight change in mice

Group		1	2	3	4
Dose level (mg/kg/day)		0	400	550	700/625
Group/ Subgroup/ Sex	Phase Day	Data Presented in "g" DSNG 85 - 91		Interval X through X DSNG 1 - 91	
1/1/M	Mean	-0.5		6.9	
	SD	1.09		1.69	
	N	11		11	
2/1/M	Mean	0.2		7.1	
	SD	0.57		1.55	
	N	12		12	
3/1/M	Mean	0.0		7.1	
	SD	0.54		2.33	
	N	11		11	
4/1/M	Mean	0.4		6.7	
	SD	0.73		2.38	
	N	9		9	
	Statistics	A		A	

Group/ Subgroup/ Sex	Phase Day	Data Presented in "g" Interval X through X DSNG 85 - 91	DSNG 1 - 91
1/1/F	Mean	0.0	5.2
	SD	1.66	2.47
	N	12	12
2/1/F	Mean	0.7	7.4*
	SD	1.14	1.29
	N	12	12
3/1/F	Mean	0.5	9.3***
	SD	0.65	1.68
	N	12	12
4/1/F	Mean	0.6	8.1**
	SD	0.99	1.63
	N	11	11
	Statistics	AT	A

* P<=0.05
** P<=0.01
*** P<=0.001
A = ANOVA and Dunnett's
T = Rank-transformed data

iii. Clinical Pathology

Clinical chemistry changes consisted of increased ALT (up to 2.5X, all drug-treated groups and both sexes), inorganic phosphate (HD males, females in all treatment groups), creatinine (up to 50% in HD males), calcium (males in all drug-treated groups), cholesterol (all drug-treated groups, both sexes), total protein (MD and HD males), and globulin (all drug-treated groups, both sexes).

iv. Necropsy

Macroscopic

At necropsy, liver weights were increased in both sexes at all doses and large liver was noted macroscopically in some animals from all drug-treated groups. In the kidney, irregular surface was observed grossly in MD and HD males and in 1 HD female. Stomach distension was noted 1 HD male.

Microscopic

Microscopically, a dose-related increase in the incidence and severity of hepatocyte hypertrophy was observed at all doses in both sexes (Tables IV.A.1.2-4). In the kidney, a dose-related increase in incidence and/or severity of nephropathy was recorded in males from all drug-treatment groups (Table IV.A.1.2-5). Nephropathy was also recorded in 1 main study and 1 TK female at the HD. According to the pathology report, nephropathy was characterized by “various proportions of interstitial inflammatory response, hyaline casts, and tubular dilation, associated with a continuum of lesions ranging from degeneration to regeneration of renal tubules and/or scarring of the kidney” and was considered adverse in MD and HD males and in HD females due to the severity and extent of the lesion. Renal lesions were not reported at lower doses (up to 300 mg/kg) in the previous 13-week study.

Table IV.A.1.2. Liver and kidney findings in main study decedents

		Males				Females			
		1M	2M	3M	4M	1F	2F	3F	4F
Tissue and finding	Level (mg/kg/day)	0	400	550	625/700	0	400	550	625/700
Liver	No. examined:	1	0	1	3	0	0	0	1
hepatocyte hypertrophy	Grade -	1	0	0	0	0	0	0	0
	1	0	0	0	3	0	0	0	0
	2	0	0	1	0	0	0	0	0
	3	0	0	0	0	0	0	0	1
Kidney	No. examined:	1	0	1	3	0	0	0	1
nephropathy	Grade -	1	0	1	0	0	0	0	0
	1	0	0	0	0	0	0	0	0
	2	0	0	0	0	0	0	0	0
	3	0	0	0	1	0	0	0	0
	4	0	0	0	2	0	0	0	1

- = Finding not present; 1 = Minimal; 2 = Slight; 3 = Moderate; 4 = Marked; F = Female; M = Male.

Table IV.A.1.3. Liver and kidney findings in TK decedents

		Males				Females			
		1M	2M	3M	4M	1F	2F	3F	4F
Tissue and finding	Level (mg/kg/day)	0	400	550	625/700	0	400	550	625/700
Liver	No. examined:	0	0	1	3	0	0	0	1
hepatocyte hypertrophy	Grade -	0	0	0	2	0	0	0	0
	1	0	0	1	1	0	0	0	1
Kidney	No. examined:	0	0	1	3	0	0	0	1
nephropathy	Grade -	0	0	1	0	0	0	0	1
	1	0	0	0	0	0	0	0	0
	2	0	0	0	0	0	0	0	0
	3	0	0	0	2	0	0	0	0
	4	0	0	0	1	0	0	0	0

- = Finding not present; 1 = Minimal; 2 = Slight; 3 = Moderate; 4 = Marked; F = Female; M = Male.

Table IV.A.1.4. Liver findings at terminal necropsy

		Males				Females			
		1M	2M	3M	4M	1F	2F	3F	4F
Tissue and finding	Level (mg/kg/day)	0	400	550	625/700	0	400	550	625/700
Liver	No. examined:	11	12	11	9	12	12	12	11
hepatocyte hypertrophy	Grade -	11	3	0	0	12	4	0	0
	1	0	9	9	1	0	7	6	6
	2	0	0	2	6	0	1	6	4
	3	0	0	0	2	0	0	0	1

- = Finding not present; 1 = Minimal; 2 = Slight; 3 = Moderate; F = Female; M = Male.

Table IV.A.1.5. Kidney findings at terminal necropsy

		Males				Females			
		1M	2M	3M	4M	1F	2F	3F	4F
Tissue and finding	Level (mg/kg/day)	0	400	550	625/700	0	400	550	625/700
Kidney	No. examined:	11	12	11	9	12	12	12	11
nephropathy	Grade -	11	7	7	2	12	12	12	9
	1	0	5	0	2	0	0	0	1
	2	0	0	3	1	0	0	0	0
	3	0	0	1	3	0	0	0	0
	4	0	0	0	1	0	0	0	1

- = Finding not present; 1 = Minimal; 2 = Slight; 3 = Moderate; 4 = Marked; F = Female; M = Male.

v. Toxicokinetics

Generally, CBD exposures in males were less than those in females (Table IV.A.1.6); however, the sex difference in exposure was not consistent across doses as was also seen in the TK data from the previous 13-week study (Table IV.A.1.7). CBD exposures in the repeat study were similar across groups and similar to those in the previous 13-week study at 300 mg/kg. Based on an AUC(0-24h) of 2790 ng.h/mL at the MRHD of 20 mg/kg, the HD recommended by the Exec-CAC for the mouse carcinogenicity study (300 mg/kg) would provide an exposure margin of ~13X.

Table IV.A.1.6. TK parameters of CBD in plasma in repeat 13-week study in mice on Day 85 (b) (4) # 8354968)

Dose group	2		3		4	
Dose level (mg/kg/day)	400		550		625	700
Gender	Male	Female	Male	Female	Male	Female
C_{max} (ng/mL)	6420	8740	7050	8440	7020	8110
t_{max} (h)	4	6	2	2	2	2
$t_{½}$ (h)	NR	NR	3.51	3.73	4.13	3.56
$AUC_{(0-t)}$ (h.ng/mL)	37800	69200	31800	41200	41000	47200
$AUC_{(0-24)}$ (h.ng/mL)	37800	69200	31800	41200	41000	47200
$AUC_{(0-\infty)}$ (h.ng/mL)	NR	NR	32100	41700	41900	47900
C_{max}/D	16.0	21.8	12.8	15.4	11.2	11.6
$AUC_{(0-t)}/D$	94.6	173	57.8	74.9	65.5	67.5
RA C_{max}/D	0.658	1.16	0.837	0.603	0.677	0.515
RA $AUC_{(0-t)}/D$	0.591	0.960	0.487	0.346	0.484	0.566

NR = no result calculable

Table IV.A.1.7. TK parameters of CBD in plasma in original 13-week study in mice [redacted] (b) (4) # 8315321)

Study number	GWTX1503					
Dose Group	2		3		4	
Dose level	100		150		300	
Gender	Male	Female	Male	Female	Male	Female
C_{max} (mg/mL)	5120	3630	3700	3410	9810	5770
t_{max} (h)	2.0	4.0	4.0	2.0	2.0	1.0
$AUC_{(0-t)}$ (h.ng/mL)	21400	22400	37000	29500	44300	46400

On Day 85, 7-OH-CBD exposures (AUC0-t) were 12400, 10400, and 14500 ng.h/mL in males and 25900, 22000, and 27600 ng.h/mL in females and 7-COOH-CBD exposures were 18300, 16200, and 28500 in males and 51900, 59700, and 54900 ng.h/mL in females, at the LD, MD, and HD, respectively.

c. Conclusions

Oral (gavage) administrations of Purified CBD (400, 550, or 700/625 mg/kg/day) to CD-1 mice for 13 weeks resulted in early deaths and nephropathy in MD and HD males and HD females. The NOAEL was 400 mg/kg/day for males (CBD exposure 37800 ng·h/mL) and 550 mg/kg/day for females (CBD exposure 41200 ng·h/mL). Based on the results of this and the previous 13-week mouse study, the Exec-CAC recommended a HD of 300 mg/kg for both sexes in the 2-year mouse carcinogenicity study that is currently ongoing (minutes dated 11/8/17 under IND 120055).

B. CHRONIC TOXICITY

1. Epidiolex (Purified CBD): 26 Week Oral (Gavage) Administration Toxicity Study in the Rat Followed by a 4 Week Treatment-free Period [redacted] Study Number: 8302923, GW Study Number: GWTX1412, conducted by [redacted], report dated 5/8/17, GLP)

a. Methods

Wistar (HsdHan:WIST) rats (15/sex/group main, 10/sex/grp C & HD recovery, 3-6/sex/grp TK) were administered Purified CBD as the OS (Batch #s MG02/058 & JG01/040) at doses of 0 (OF vehicle),15, 50, or 150 mg/kg (1.5 mL/kg) by oral gavage once daily for 26 weeks.

			Number of Animals in Group			
		Dose level	Toxicity (Subgroup 1)		Treatment-free (Subgroup 1)	
Group	Description	(mg/kg/day)	Male	Female	Male	Female
1	Control	0	15	15	10	10
2	Low	15	15	15	-	-
3	Intermediate	50	15	15	-	-
4	High	150	15	15	10	10

			Number of Animals in Group	
		Dose level	Toxicokinetics[a] (Subgroup 2)	
Group	Description	(mg/kg/day)	Male	Female
1	Control	0	3	3
2	Low	15	6	6
3	Intermediate	50	6	6
4	High	150	6	6

a for toxicokinetic investigations only; no other experimental observation data from these animals has been reported

Observations included clinical signs, body weight, ophthalmology, clinical pathology, and gross and microscopic pathology evaluations. Microscopic examinations were conducted on a full panel of tissues in C and HD main groups and on liver, thyroid, lung, adrenal (males only), ovary and gross lesions only in LD and MD main and all recovery groups. Blood was collected for TK on Day 1 and during Weeks 13, 20, and 26.

Dose Justification:

The high dose was selected on the basis of a 14-day TK study of CBD-BDS (65.6% CBD) comparing oral gavage (10 mL/kg) and dietary dosing in rats at doses of 15, 50, and 150 mg/kg/day (expressed in terms of CBD) in the gavage arm and 50, 150, and 500 mg/kg/day in the dietary arm, with no control groups (Sponsor Ref: JJG0001). Dose-related clinical signs consisted of post dose salivation, hyperactivity followed by hypoactivity, increased response to stimuli, and vocalization. Body weight gain and food consumption decreased dose-dependently, but there were no controls for comparison. The dose adjusted exposure to CBD was equivalent in the LD gavage and diet groups and 2-fold greater after gavage administration in the MD and HD groups than that after dietary administration.

b. Results

i. Mortality and Clinical Observations

There were three deaths during the study, but none was considered drug-related.

There were no drug-related clinical signs.

ii. Body Weight

There were no effects on food consumption, body weight, or body weight gain during the treatment or recovery periods.

iii. Ophthalmoscopy

There were no drug-related effects.

iv. Clinical Pathology

There were no clear hematologic effects.

ALP (both sexes) and ALT (females) were slightly increased (up to 1.2 and 1.4X, respectively) at the HD. Slight increases (~1.3X) in inorganic phosphate (P) were seen MD and HD males.

There were no effects on urinalysis test results.

v. Necropsy

Dose-related increases in liver, thyroid, adrenal, and thymus weights (absolute and relative) were seen in both sexes at the MD and HD.

Macroscopic -

Large liver, pale focus in lung, and pale area in the adrenal were seen, primarily at the HD. At the end of the recovery period, large liver and pale focus/area were still noted in a few treated animals.

Microscopic (pathology report signed and dated) -

Centrilobular hepatocellular hypertrophy was seen with a dose-related incidence and severity at the MD and HD in both sexes (Table IV.B.1.1).

Table IV.B.1.1 Incidence of centrilobular hypertrophy at terminal sacrifice

		Males				Females			
		1M	2M	3M	4M	1F	2F	3F	4F
Tissue and finding	Level (mg/kg/day)	0	15	50	150	0	15	50	150
Liver	No. examined:	15	15	15	15	14	15	14	15
centrilobular hypertrophy	Grade -	15	15	7	2	14	15	3	0
	1	0	0	8	6	0	0	7	0
	2	0	0	0	5	0	0	4	12
	3	0	0	0	2	0	0	0	3

Key: "-" = finding not present, 1 = minimal, 2 = slight, 3 = moderate.

Thyroid follicular cell hypertrophy, foamy macrophages in the lung, cortical vacuolation of the adrenal, and interstitial cell hyperplasia of the ovary were also seen with dose-related incidence and severity, primarily at the MD and HD.

At the end of the recovery period, there was a tendency for reversal of drug-related findings, with reductions in incidence and severity (Table IV.B.1.2).

Table IV.B.1.2 Incidence of selected microscopic findings - Recovery

		Males		Females	
		1M	4M	1F	4F
Tissue and finding	Level (mg/kg/day)	0	150	0	150
Liver	No. examined:	10	9	10	10
centrilobular hypertrophy	Grade -	10	6	10	9
	1	0	3	0	1
Thyroid	No. examined:	10	9	10	10
follicular cell hypertrophy	Grade -	10	5	10	8
	1	0	4	0	2
Lung	No. examined:	10	9	10	10
foamy macrophages	Grade -	7	2	7	6
	1	3	6	3	4
	2	0	1	0	0
Adrenal	No. examined:	10	9	1	0
cortical vacuolation	Grade -	9	6	1	0
	1	1	3	0	0
Ovary	No. examined:	-	-	10	10
interstitial cell hyperplasia	Grade -	-	-	10	8
	1	-	-	0	2*

Key: "-" = finding not present, 1 = minimal, 2 = slight; * = one unilateral.

No effects on sperm count, motility, velocity or abnormal sperm were observed.

vi. Toxicokinetics

TK data for CBD are shown in Table IV.B.1.3. At Week 26, 7-OH-CBD exposures (AUC0-t) were 186, 1660, and 2560 ng.h/mL in males, and 1300, 4110, and 6730 ng.h/mL in females; 7-COOH-CBD exposures were 1450, 17300, and 37100 ng.h/mL in males, and 4960, 22700, and 40500 ng.h/mL in females; and THC exposure were 5.63, 24.6, and 40.3 ng.h/mL in males, and 10.7, 45.6, and 68.4 ng.h/mL in females, at the LD, MD, and HD, respectively.

It was noted by the sponsor that a potential bias in the assessment of 7-COOH-CBD was identified during a review of the stability data generated for 7-COOH-CBD in the ongoing rat plasma validation study (b) (4) 276174QB02. According to the report:

> The data from the validation long term stability assessments suggested that the calibrators and QC samples were incorrectly prepared with a greater concentration of 7-COOH-CBD being added which would result in an under representation for the amount of 7-COOH-CBD present in study samples. The degree of bias on the study samples was assessed by review of the data from the validation and showed that the amount of 7-COOH-CBD could be biased by between 30% and 50% from the actual value. This bias was based on an assessment of the respective stability timepoints analysed alongside freshly prepared calibrators. Due to the passage of time and limited sample volume further analysis of samples to more accurately assess the bias could not be performed. Consequently the results for 7-COOH-CBD should be interpreted with a degree of caution.

Table IV.B.1.3. Plasma TK parameters for CBD at 26 weeks

Group / Dose (CBD mg/kg/day)	C_{max} (ng/mL)	t_{max} (h)	$t_{1/2}$ (h)	AUC_{0-t} (ng.h/mL)	AUC_{0-inf} (ng.h/mL)	AUC_{ex} (%)	Cl/F (mL/min/kg)	V_z/F (L/kg)
2M / 15	1400	4.0	NR	8700	NR	NR	NR	NR
3M / 50	5240	2.0	n.d.	36700	n.d.	n.d.	n.d.	n.d.
4M / 150	6160	6.0	n.d.	60000	n.d.	n.d.	n.d.	n.d.
2F / 15	2070	2.0	NR	10800	NR	NR	NR	NR
3F / 50	3750	2.0	n.d.	39000	n.d.	n.d.	n.d.	n.d.
4F / 150	7530	4.0	4.5	67500	70100	3.7	35.7	13.8

NR Not reportable; n.d. Not determined; C_{max} Maximum plasma drug concentration

c. Conclusions

Oral (gavage) administration of Purified CBD (15, 50, or 150 mg/kg/day) to Wistar rats for 26 weeks produced no toxicologically significant effects. Centrilobular hypertrophy in the liver was the main finding, correlating with liver weight increase, macroscopic enlargement, and associated clinical chemistry changes (increases in ALP and ALT). Dose range-finding was clearly inadequate and dose selection inappropriate. However, CBD exposures were approximately 20X those expected in humans. Metabolite safety margins are considerably lower (7-OH-CBD) or nonexistent (7-COOH-CBD).

2. Epidiolex (Purified CBD): 39 Week Oral (Gavage) Administration Toxicity Study in the Dog Followed by a 4 Week Treatment-free Period ((b) (4) Study Number: 8302924, GW Study Number: GWTX1413, conducted by (b) (4), report dated 5/8/17, GLP)

a. Methods

Beagle dogs (4/sex/group main, 2/sex/grp C & HD recovery) were administered Purified CBD as the OS (Batch #s MG02/058, JG01/040, and MG02/102) at doses of 0 (OF vehicle), 10, 50 and 100 mg/kg/day) by oral gavage (1 mL/kg) once daily for 39 weeks.

Clinical signs, body weight, food consumption, ophthalmology, and clinical pathology evaluations were performed. Complete necropsies were performed on all animals with organ weight measurements and macroscopic and microscopic examinations of all tissues. Blood was collected for TK evaluations on Day 1 and during Week 13, 26, and 39 pre-dose and at 1, 2, 4, 6, 8, 12, and 24 hours post-dose. Additional samples were collected on Day 20 at pre-dose, 2, 4, and 6 hours post-dose.

			Number of Animals in Group			
			Toxicity		Treatment-free	
Group	Description	Dose level (mg/kg/day)	Male	Female	Male	Female
1	Control	0	4	4	2	2
2	Low	10	4	4		
3	Intermediate	50	4	4		
4	High	100	4	4	2	2

No range-finding study was conducted. The sponsor stated that the HD was based on the results of the cardiovascular safety pharmacology study in the dog (CBD BDS doses of 10, 50 and 100 mg CBD/kg given twice) and 4- (THC:CBD doses of 5:5, 30:30, and 100:100 mg/kg/day) and 52-week dog studies of Sativex (THC:CBD doses of 2.7:2.5, 13.5:12.5, or 27:25 mg/kg/day). In the 4-week Sativex study, dogs exhibited clinical signs of subdued behavior, tremors, vomiting, liquid feces, poor food consumption, body weight loss, and anorexia associated with poor food consumption that resulted in the reduction of the HD from 200 to 100 mg ACTIVE(THC:CBD combined doses)/kg/day. Similar clinical signs were observed in the chronic study of Sativex in which the NOAEL was considered to be 2.7:2.5 THC:CBD mg/kg/day.

b. Results

i. Mortality and Clinical Observations

There were no drug-related deaths and clinical signs were limited to soft/liquid/mucoid feces at all doses.

ii. Body Weight

Body weight (BW) gain was dose-dependently decreased in males and females at all doses (Table IV.B.2.1). At the end of the dosing period, BWs were 12 and 32% below C in HD males and females, respectively.

Table IV.B.2.1. Body weight gain in 39-week dog toxicity study

Group /sex	Dose (mg/kg day)	Dosing Interval (Week 1 to Week 40) Week 1	Week 40	Overall Change (kg)	Dosing Interval (Week 40) % Body weight Change from control
1M	0	9.94	11.82	+1.9 (+20%)	-
2M	10	10.13	11.25	+1.1 (+11%)	-4.8
3M	50	9.92	10.01	+0.1 (+1%)	-15.3
4M	100	9.78	10.46	+0.7 (+7%)	-11.5
1F	0	9.12	12.81	+3.7 (+41%)	-
2F	10	8.47	10.04	+1.6 (+19%)	-21.6
3F	50	8.77	9.06	+0.3 (+3%)	-29.3
4F	100	8.53	8.72	+0.2 (+2%)	-31.9

iii. Ophthalmoscopy

There were no drug-related changes in ophthalmic findings.

iv. ECG

There were no drug-related effects on PR, QRS, QT, or QTc(F) intervals in males or females in ECGs measured during Weeks 25 and 38 at approximately 4 hours post-dose. Decreased heart rate was seen in HD males at both times (31 and 46 bpm lower than pre-treatment, 25-28 bpm lower than C, at 25 and 38 weeks, respectively). The decreases in HR were reflected in increased RR intervals. There were no findings in recovery males or in females during the treatment or recovery periods.

v. Clinical Pathology

There were no apparent drug-related effects on hematology or urinalysis values, but increases in ALT (up to 1.5X) were seen in MD and HD males and HD females and marked increases in ALP (up to 8X) were seen in both sexes at all doses (Table IV.B.2.2). ALP remained elevated at the HD after recovery.

Table IV.B.2.2. Clinical chemistry changes in 39-week dog toxicity study

Group						
Dose level (mg/kg/day)						
			HALP IU/L			
Group/ Sex	Phase	Predose	Dosing			Recovery
	Wk	2	13	26	39	4
1/M	Mean	94	63	44	46	42
	SD	18.1	17.3	13.9	19.8	15.6
	N	6	6	6	6	2
2/M	Mean	101	179	215	389	-
	SD	28.2	101.8	132.5	290.2	-
	N	4	4	4	4	-
3/M	Mean	123	244	295	334	-
	SD	32.4	174.1	158.6	197.5	-
	N	4	4	4	4	-
4/M	Mean	102	270	273	380	60
	SD	13.5	144.6	166.1	257.1	19.1
	N	6	6	6	6	2

1/F	Mean	124	73	48	55	43
	SD	45.2	19.0	11.6	28.6	11.3
	N	6	6	6	6	2
2/F	Mean	88	136	120	167	-
	SD	20.1	53.4	78.9	87.7	-
	N	4	4	4	4	-
3/F	Mean	87	294	366	392	-
	SD	22.4	91.1	274.7	143.0	-
	N	4	4	4	4	-
4/F	Mean	115	282	218	302	71
	SD	51.0	197.7	174.8	98.2	24.7
	N	6	6	6	6	2

vi. Necropsy

Macroscopic -

Increased liver weights (all doses in both sexes) and observation of large liver (1 MD female) were the only drug-related findings noted at the end of the dosing period. These were no longer prominent after the recovery period.

Microscopic (full panel of tissues examined; signed and dated pathology report included) -

On microscopic examination, hepatocyte hypertrophy was noted in both sexes and all dose groups (Table IV.B.2.3). This finding generally correlated with large liver and increased liver weight. There were no drug-related microscopic findings in the recovery groups.

Table IV.B.2.3. Microscopic findings in 39-week dog toxicity study

		Males				Females			
		1M	2M	3M	4M	1F	2F	3F	4F
Tissue and finding	Level (mg/kg/day)	0	10	50	100	0	10	50	100
Liver	No. examined:	4	4	4	4	4	4	4	4
Hepatocype hypertrophy	Grade -	4	1	0	0	3	2	1	0
	1	-	3	3	2	1	1	1	3
	2	-	-	1	2	-	1	2	1

Key: "-" = finding not present, 1 = minimal, 2 = slight

vii. Toxicokinetics

At the end of the dosing period (Wk 39) CBD exposures (AUC0-t) were 12500, 26900, and 20500 ng.h/mL in males and 16500, 35900, and 22400 ng.h/mL in females; 7-OH-CBD exposures were 406, 1510, and 1380 ng.h/mL in males, and 1010, 2400, and 1090 ng.h/mL in females; 7-COOH-CBD exposures were 782, 936, and 994 ng.h/mL in males, and 1650, 2310, and 1560 ng.h/mL in females; and THC exposures were 1.15, 2.09, and 2.19 ng.h/mL in males, and 1.64, 5.11, and 3.01 ng.h/mL in females, at the LD, MD, and HD, respectively.

c. Conclusions

Oral (gavage) administration of Purified CBD (0, 10, 50 and 100 mg/kg/day) resulted in decreases in BW gain and BW (MD and HD males, females at all doses), decreases in heart rate (HD males), increased liver enzymes (ALT up to 1.5X in MD and HD males and in HD females, ALP up to 8X at all doses in both sexes), increased liver weights, and increased incidences of hepatocellular hypertrophy (all doses in both sexes). CBD exposures (Week 39) at the HD were 20500 ng.h/mL in males and 22400 ng.h/mL in females.

C. GENOTOXICITY

1. Reverse mutation in five histidine-requiring strains of Salmonella typhimurium (Study No. GWOR0910, conducted by (b) (4), report dated 6/18/09, GLP)

Pure CBD (Batch # 30301) was tested in the Ames assay using tester Salmonella strains TA98, TA100, TA1535, TA1537, and TA102 in the presence and absence of Aroclor 1254-induced rat liver S9 using the plate incorporation method at concentrations up to 5000 ug/plate. No positive mutagenic responses were observed with any of the tester strains in either the presence or absence of S9 activation. Under the conditions of the study, Pure CBD was negative in the Ames assay.

2. Induction of micronuclei in the bone marrow of treated rats (Study No. GWOR0903, conducted by (b) (4), report dated 2/09/10, GLP)

Pure CBD (Batch # 30301) was evaluated for its potential to increase the incidence of micronucleated polychromatic erythrocytes (MNPCEs) in rat bone marrow cells. Male Sprague Dawley rats (6/grp) received 2 oral gavage (20 mL/kg) doses of 0 (sesame oil), 125, 250, or 500 mg/kg/day. The positive control group was dosed once with cyclophosphamide (CPA 20 mg/kg) on the second day of dosing. Bone marrow smears were prepared from sacrificed animals approximately 24 hours following the final administration on Day 3. In addition to the micronucleus animals, two groups of satellite animals were dosed with vehicle and 500 mg/kg/day Pure CBD for confirmation of exposure (but not TK).

Clinical signs (lethargy, ataxia, piloerection, anogenital soiling, and unkempt appearance) generally first became apparent on Day 3. CBD-treated rats exhibited group mean MNPCE frequencies similar to those for the vehicle control group and which also fell within the laboratory's historical vehicle control range. A small increase (NS) was observed at the LD, but was attributable to a single animal (number 556) that exhibited an elevated number of MNPCEs (11 MNPCE/2000 PCE analyzed), exceeding historical control values. However, since all other CBD-treated animals in this group (and all others) demonstrated MNPCE frequencies consistent with concurrent and historical vehicle controls, this isolated increase appeared to be and was considered in the report to be spurious. Negative (vehicle) and positive controls performed as expected. Thus, it can be concluded that under the conditions of the study Pure CBD was negative in the rat bone marrow micronucleus assay.

3. Epidiolex (Purified CBD): Rat Alkaline Comet Assay (Study No. GWTX1510, conducted by (b) (4) report dated 8/26/15, GLP)

Male Sprague Dawley rats (6/group) received single oral (gavage) doses of 0 (sesame oil), 125, 250, or 500 mg/kg/day CBD-OS. Liver samples were obtained at 24 hours after the first dose. No clinical signs of toxicity were observed at any dose. There were no microscopic pathology findings attributed to CBD-OS. There was no dose-related increase in % hedgehogs in liver cells. There was an increase in group mean % tail intensity at the HD (0.78 compared to vehicle control mean of 0.29); however, the increase was due to a single animal (#19) having an elevated % tail intensity. None of the increases were found to be statistically significant and all animals fell well within the ranges determined from the laboratory historical control data. It can be concluded that CBD-OS was negative in the rat Comet assay.

D. CARCINOGENICITY

1. 104 Week Oral (Dietary) Carcinogenicity Study in the Rat (Study no.: JJG0003, conducted by [redacted]^(b) (4), report dated 9/15/05, GLP)

a. Methods

CBD BDS (Cannabidiol Botanical Drug Substance; batch numbers 05001 & 03602, 57.5–64.4 & 64.4–67.2% CBD, respectively) was administered orally, in the diet, to Wistar (HsdBrlHan:WIST) rats (50/sex/grp + 5/sex/grp TK) at doses of 0 (stock diet), 5, 15, or 50 mg/kg/day for 104 weeks. Observations consisted of mortality, clinical signs, body weight, hematology (no clinical chemistry), and gross and microscopic pathology. A full panel of tissues from C and HD animals was examined microscopically. In addition, gross lesions and livers were examined for animals from the LD and MD groups and thyroids were examined from LD and MD males.

Dose selection was based on the results of a 13-week toxicity study conducted in Han Wistar rats (10/sex/group) given CBD BDS (expressed in terms of CBD) in the diet to achieve doses of 0, 25, 75, or 225 mg/kg/day (Study no. JJG0002). There was no mortality or clinical signs; body weight gain and food consumption were reduced in a dose-related manner. There were no changes in sperm motility, concentration, or morphology. Changes in liver were characterized by centrilobular hepatocyte hypertrophy in males and one female given ≥ 75 mg/kg/day and in females given 225 mg/kg/day. Vacuolation of the adrenal cortex was observed in males given ≥ 75 mg/kg/day. At 225 mg/kg, the respective AUC and Cmax values after 13 weeks were 24103 ng·h/mL and 1464 ng/mL for males and 32718 ng·h/mL and 2451 ng/mL for females. In the report, it was concluded that 50 mg/kg/day would be a suitable HD for the carcinogenicity study. This dose was expected to provide a CBD exposure more than 25X that anticipated in humans. It was also anticipated that this dose would produce a 10-20% reduction in bodyweight gain in females. (Protocol was not reviewed by the Exec-CAC.)

b. Results

i. Mortality and Clinical Observations

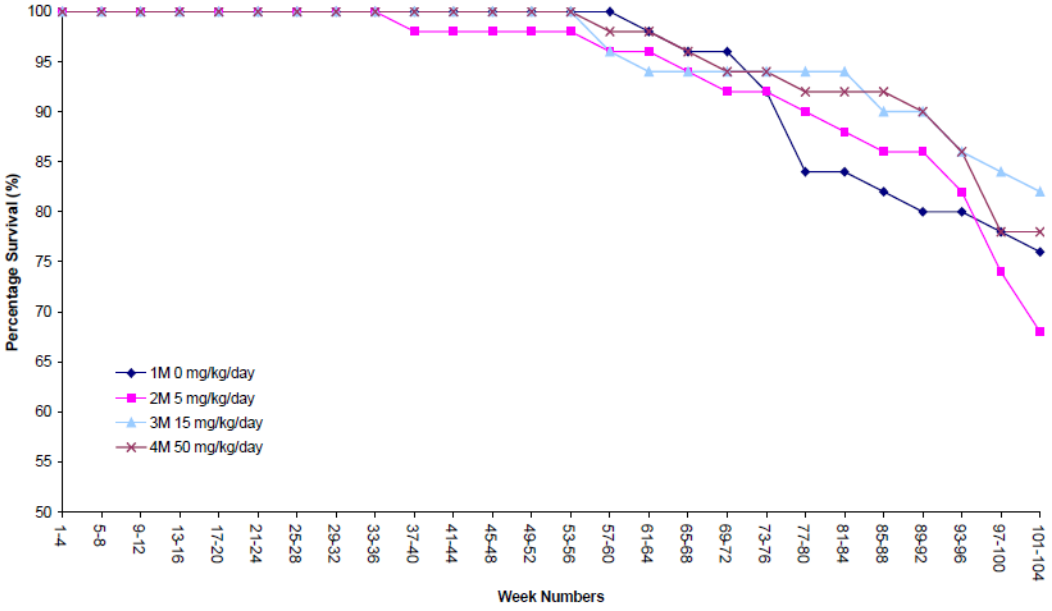
There were no apparent drug-related effects on survival (Table IV.D.1.1, Figure IV.D.1.1), clinical signs, or incidence or time of onset of palpable masses.

Table IV.D.1.1. Percent survival

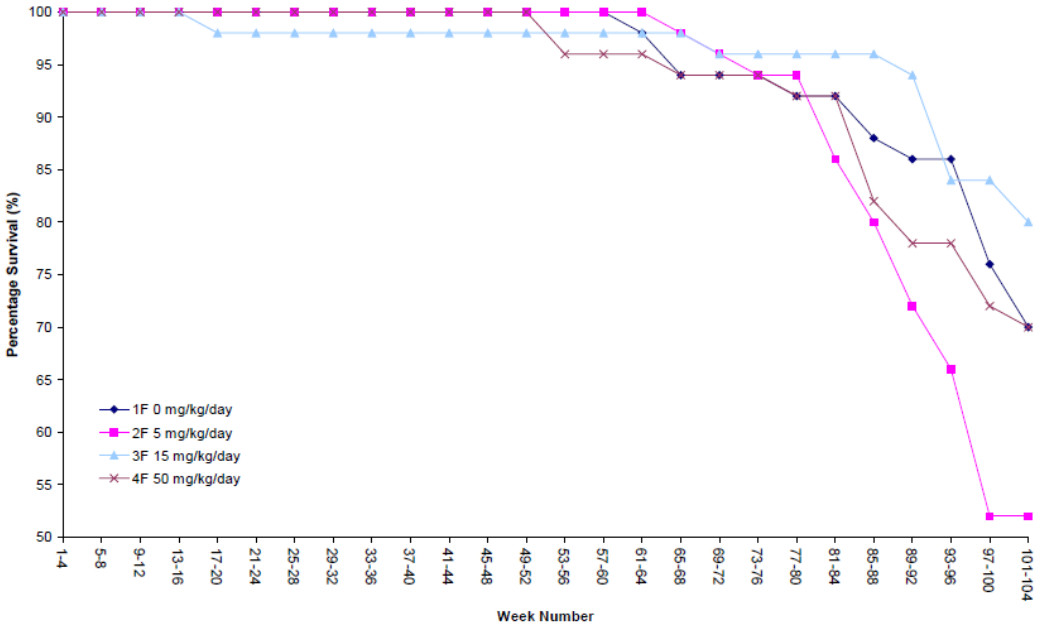
Group and Sex	Dose Level CBD (mg/kg/day)	Percentage survival at selected weeks				
		13	26	52	78	104
1M	0	100	100	100	88	76
2M	5	100	100	96	90	68
3M	15	100	100	100	94	82
4M	50	100	100	100	92	78
1F	0	100	100	100	94	70
2F	5	100	100	100	94	52
3F	15	100	98	98	96	80
4F	50	100	100	100	94	70

Figure IV.D.1.1. Survival in 2-year rat carcinogenicity study of CBD BDS

Males



Females



ii. Body Weight

Dose-related decreases in food consumption and bodyweight gain (Weeks 1–104) were seen at the MD and HD in both sexes (Table IV.D.1.2). At the HD, males had a 26% reduction and females had a 35% reduction in bodyweight gain compared to Controls (17 and 16% below C over first 13 weeks). Body weights at the end of the treatment period were 20 and 24% below C in HD males and females, respectively (Table IV.D.1.3).

Table IV.D.1.2. Body weight gain in 2-year rat carcinogenicity study of CBD BDS

Group/Sex	1M	2M	3M	4M	1F	2F	3F	4F
Dose level CBD (mg/kg/day)	0	5	15	50	0	5	15	50
Weeks -1 to 13	218.9	215.2	200.8	181.7	94.0	90.7	87.5	79.4
% of Control		**98**	**92**	**83**		**96**	**93**	**84**
Weeks -1 to 27	284.4	281.6	260.4	232.6	118.7	114.2	111.9	98
% of Control		**99**	**92**	**82**		**96**	**94**	**83**
Weeks -1 to 51	355.9	352.3	324.6	287.9	166.1	164.5	147.0	125.7
% of Control		**99**	**91**	**81**		**99**	**89**	**76**
Weeks 51 - 104	94	91.5	66.8	46.3	93.5	78.1	67.3	42.8
% of Control		**97**	**71**	**49**		**84**	**72**	**46**
Weeks -1- 104	449.9	443.8	391.4	334.2	259.6	242.6	214.3	168.5
% of Control		**99**	**87**	**74**		**93**	**83**	**65**

Table IV.D.1.3. Body weight in 2-year rat carcinogenicity study of CBD BDS

Group : 1 2 3 4

Test article : Control CBMR-G5

Dose (mg/kg/day) : 0 5 15 50

Group sex		Week number 83	87	91	95	99	103	104	105
1M	Mean	578.4	582.3	597.3	603.4	599.7	611.7	608.3	611.7
	S.D.	73.2	78.2	77.0	79.3	78.3	83.4	85.9	88.9
	N	42	42	40	39	39	38	38	18
2M	Mean	576.3	582.3	593.4	595.1	599.4	598.9	601.3	617.6
	S.D.	83.2	89.4	85.4	88.2	89.0	91.7	89.8	79.8
	N	45	44	43	41	38	36	34	19
3M	Mean	543.9	547.0	547.5	549.6	551.1	552.1	548.1	554.6
	S.D.	64.0	60.1	61.8	67.0	64.3	61.8	60.9	80.1
	N	47	46	45	45	42	41	41	20
4M	Mean	488.6	489.9	495.8	497.4	496.2	498.2	493.1	487.9
	S.D.	59.2	59.1	58.5	60.7	61.3	62.4	61.0	68.9
	N	46	46	45	44	41	39	39	19
1F	Mean	373.2	377.1	386.3	385.8	385.3	390.7	390.1	395.2
	S.D.	52.9	53.2	53.6	51.3	50.8	53.7	55.0	52.1
	N	46	45	43	43	37	35	35	21
2F	Mean	362.8	370.8	374.3	375.8	370.6	380.2	372.8	357.1
	S.D.	59.4	57.3	55.0	52.2	55.1	55.6	57.4	52.4
	N	46	40	36	33	29	26	26	16
3F	Mean	342.2	344.5	352.7	353.9	349.7	349.6	345.1	347.9
	S.D.	54.1	52.2	54.5	54.8	52.4	50.8	50.0	52.3
	N	48	48	47	44	42	41	40	21
4F	Mean	301.2	301.0	303.4	304.2	299.4	304.3	299.5	301.0
	S.D.	35.0	37.5	33.2	36.0	36.4	39.8	38.4	34.4
	N	46	42	40	39	36	35	35	24

- statistically analysed

iii. Hematology

There were no drug-related effects on hematological parameters during Weeks 52 or 78. During Week 103 only, white blood cell counts were statistically significantly decreased in MD and HD males compared to C. There was no evidence of an increased incidence of leukemia in treatment groups.

iv. Necropsy

Macroscopic -

There was an apparent drug-related increase in the incidence of abnormal size of the thyroid glands in males. There was a reduction in the number of skin masses recorded in both males and females at the HD and in the number of findings recorded in the pituitary gland and mammary tissue in HD females. In association with the reduced number of findings in the pituitary gland there was a reduction in the number of ventral depressions in the brain that are generally caused by pituitary enlargement.

Microscopic (no signed pathology report) **-**

There was no indication of drug-related increases in tumor incidences. There was an apparent reduction in the incidences of some tumors thought to be associated with hormonally-mediated neoplasia in ageing animals (pituitary adenoma or adenocarcinoma when sexes were combined, mammary fibroadenomas in the skin or subcutaneous fat in females) at the HD. Non-neoplastic findings considered to be associated with treatment included an increased incidence of centrilobular hypertrophy in the liver MD males and HD males and females. The FDA statistical analysis found no tumor types or combined tumor types with a SS positive dose response relationship (see statistical review by Stephen Thompson).

v. Toxicokinetics

Concentrations of CBD, THC, and 11-OH THC were determined in plasma samples taken at 8 AM and 8 PM (Table IV.D.1.5). There were no sex differences in levels at these times. CBD levels gradually increased with repeated dosing, with maximal values of 841.6 and 722.9 ng/mL in males and females, respectively, at 105 weeks.

Table IV.D.1.5. Plasma CBD, THC, and 11-OH THC levels

Group/ Sex	Dose CBD (mg/kg/ day)	Week of Study	Clock time	CBD (ng/mL)		THC (ng/mL)		11-OH THC (ng/mL)	
				Mean	SD	Mean	SD	Mean	SD
5F	5	14	08:00	6.40	1.75	<5.0	<5.0	<5.0	<5.0
5F	5	14	20:00	<5.0	<5.0	<5.0	<5.0	<5.0	<5.0
5F	5	27	08:00	5.75	0.68	<5.0	<5.0	<5.0	<5.0
5F	5	27	20:00	6.84	<5.0	<5.0	<5.0	<5.0	<5.0
5F	5	52	08:00	7.06	0.55	4.52	0.29	2.16	0.28
5F	5	52	20:00	7.69	5.10	4.49	2.18	1.95	0.47
5F	5	105	08:00	10.74	4.60	6.01	2.41	2.87	0.80
5F	5	105	20:00	13.57	6.08	6.02	1.26	2.96	0.77
5M	5	14	08:00	5.75	0.90	<5.0	<5.0	<5.0	<5.0
5M	5	14	20:00	5.07	<5.0	<5.0	<5.0	<5.0	<5.0
5M	5	27	08:00	9.83	2.42	<5.0	<5.0	<5.0	<5.0
5M	5	27	20:00	7.64	1.81	<5.0	<5.0	<5.0	<5.0
5M	5	52	08:00	8.03	1.88	3.30	0.66	<1.0	<1.0
5M	5	52	20:00	7.59	2.22	3.07	0.94	<1.0	<1.0
5M	5	105	08:00	16.29	6.50	5.99	3.18	1.87	0.91
5M	5	105	20:00	13.41	6.66	5.19	2.43	1.74	0.55
6F	15	14	08:00	47.00	16.21	18.06	6.41	<5.0	<5.0
6F	15	14	20:00	43.26	20.60	16.91	7.32	5.08	<5.0
6F	15	27	08:00	45.96	15.69	19.38	5.49	<5.0	<5.0
6F	15	27	20:00	34.10	13.78	17.12	6.52	<5.0	<5.0
6F	15	52	08:00	81.14	11.46	34.17	5.29	4.97	0.42
6F	15	52	20:00	62.05	13.25	27.53	5.18	4.64	0.39
6F/3F	15	105	08:00	108.42	59.81	40.30	17.96	6.77	1.22
6F/3F	15	105	20:00	139.61	87.88	49.51	27.00	6.89	1.68
6M	15	14	08:00	38.98	4.40	11.20	2.26	<5.0	<5.0
6M	15	14	20:00	38.77	18.85	12.49	5.13	<5.0	<5.0
6M	15	27	08:00	46.40	4.74	14.84	3.63	<5.0	<5.0
6M	15	27	20:00	45.62	21.80	13.62	5.74	<5.0	<5.0
6M	15	52	08:00	61.54	7.57	20.23	3.96	1.47	0.27
6M	15	52	20:00	49.46	9.69	15.24	2.21	1.24	0.29
6M/3M	15	105	08:00	119.35	17.00	47.53	18.03	4.25	2.46
6M/3M	15	105	20:00	118.96	26.70	43.17	15.89	3.90	2.43
7F	50	14	08:00	327.94	69.51	59.70	4.58	5.12	<5.0
7F	50	14	20:00	209.83	70.22	45.90	15.07	<5.0	<5.0
7F	50	27	08:00	372.89	126.48	75.47	28.65	5.25	0.13
7F	50	27	20:00	270.49	115.50	61.48	24.11	5.02	<5.0
7F	50	52	08:00	456.84	123.18	83.69	10.56	5.24	0.31
7F	50	52	20:00	451.73	99.54	93.83	24.88	4.85	0.58
7F/4F	50	105	08:00	722.94	133.42	118.60	25.58	6.34	1.46
7F/4F	50	105	20:00	659.67	236.48	117.17	36.05	6.31	0.83
7M	50	14	08:00	276.74	78.47	47.16	13.58	<5.0	<5.0
7M	50	14	20:00	206.04	80.46	36.81	11.38	<5.0	<5.0
7M	50	27	08:00	390.13	78.40	66.63	15.64	<5.0	<5.0
7M	50	27	20:00	309.49	66.37	57.08	14.19	<5.0	<5.0
7M	50	52	08:00	573.84	197.41	89.80	32.53	2.38	0.44
7M	50	52	20:00	381.45	74.48	66.29	16.91	1.82	0.43
7M	50	105	08:00	841.60	609.16	122.99	89.21	4.41	2.21
7M	50	105	20:00	682.71	353.60	102.34	50.90	3.82	1.51

c. Conclusions

Administration of CBD BDS in the diet to Wistar rats for 104 weeks resulted in a greater than 10% reduction in body weight in both sexes (20 and 24% in M & F) but no other evidence of toxicity. There was no evidence of a drug-related increase in tumor incidences. There was some evidence of drug-related decreases in some commonly seen hormone-mediated tumors.

E. REPRODUCTIVE AND DEVELOPMENTAL TOXICOLOGY

1. Epidiolex (Purified CBD): Oral (Gavage) Study of Fertility and Early Embryonic Development in Male and Female Rats (GW Report No. GWTX1456; dated 30/9/16; conducted by (b) (4); GLP)

a. Methods

Male and female Wistar (Crl:WI(Han)) rats (20/sex/grp) were administered Purified CBD (batch number: TM04/171) orally (by gavage, 5 mL/kg) at daily doses of 0 (sesame oil), 75, 150, or 250 mg/kg for 2 weeks prior to mating (up to 10 days) and until GD 6 for females. Males were dosed until the day prior to necropsy (after review of female pregnancy data). Females were sacrificed on GD 13 and numbers of corpora lutea, implantations, and live or dead embryos were recorded. Males were sacrificed following evaluation of the outcome of the female necropsies and reproductive organ weights were measured. No TK data were collected.

The high dose was selected on the basis of findings from the EFD dose range-finding study (GWTX1455) in which 250 mg/kg/day was associated with transient decreases in body weight/body weight gain that had resolved by GD 11.

b. Results

i. Mortality and Clinical Observations

Two early deaths (1 C male, 1 MD female) were not considered treatment-related. Arched gait, high stepping gait, raised tail, and head shaking were sporadically observed after dosing in the MD and HD groups, primarily on pairing day 13 and post-pairing days 4 and 11.

ii. Body Weight

During the post-pairing phase, there were drug-related lower overall bodyweight gains in MD and HD males compared to C (21 and 25%, respectively; SS). During the pre-pairing phase, there were drug-related increases in bodyweight gains in MD and HD females compared to C (48 and 54%, respectively; SS). There were slight dose-related decreases in BW gain in females during gestation (2, 4, and 5%, respectively, in LD, MD, and HD; NS).

iii. Male and female fertility and reproductive indices

Estrous cyclicity did not differ among groups. Fertility and reproductive performance did not appear to be affected by drug treatment, although there were slight decreases in fertility indices at the MD and HD due to the failure of 1 mating pair at each dose (Table IV.E1.1).

Table IV.E1.1. Fertility and Reproductive Performance

Males

Treatment Group	Control	75 mg/kg	150 mg/kg	250 mg/kg
Total males	21≠	20	20	20
Unscheduled Deaths Prior to Cohabitation	1	0	0	0
Males Cohabitated	20	20	20	20
Unscheduled Deaths During Cohabitation	0	0	0	0
Males mating with at least 1 female	20	20	20	20
Males impregnating at least 1 female	20	20	19	19
Mating Index (%)	100	100	100	100
Fecundity Index (%)	100	100	95	95
Fertility Index (%)	100	100	95	95

Mating index % = (Number of males mating with at least 1 female / Number of males cohabitated with at least 1 female) x 100
Fecundity index % = (Number of males impregnating at least 1 female / Number of males mating with at least 1 female) x 100
Fertility Index % = (Number of males impregnating at least 1 female / Number of males cohabitated with at least 1 female) x 100
≠ = Total includes untreated spare male 0081 who replaced 0009

Females

Treatment Group	Control	75 mg/kg	150 mg/kg	250 mg/kg
Total Females	20	20	20	20
Unscheduled Deaths Prior to Cohabitation	0	0	0	0
Females Cohabited	20	20	20	20
Unscheduled Deaths During Cohabitation	0	0	0	0
Females Mated	20	20	20	20
Pregnant Females	20	20	19	19
Non Pregnant Females	0	0	1	1
Matings Per Day Periods Of Cohabitation				
Day 1	5	4	3	10
Day 2	2	3	4	2
Day 3	7	6	3	5
Day 4	5	7	8	2
Day 6	1	0	0	1
Day 11	0	0	1	0
Mating Index %	100	100	100	100
Fecundity Index %	100	100	95	95
Fertility Index %	100	100	95	95

Mating index % = Mated females/females cohabited (excluding females sacrificed during Cohabitation) x 100
Fecundity Index % = Pregnant females/mated females (excluding females with an undetermined pregnancy status) x 100
Fertility Index % = Pregnant females/females cohabited (excluding females sacrificed during Cohabitation or with an undetermined pregnancy status) x 100

iv. C-section parameters

There were no clearly drug-related differences in C-section parameters (IV.E.1.2). Pre-implantation loss was slightly increased in treated groups (8.2, 14.1, and 11.1 in LD, MD, and HD groups, respectively, compared to 7.2% in C), but the differences were not strictly dose-dependent and were well within the historical control range.

Table IV.E.1.2. Caesarean section data

		Group			
		Control	75 mg/kg	150 mg/kg	250 mg/kg
Number of females pregnant at caesarean section	N	20	20	19	19
Corpora Lutea	N	20	20	19	19
	Mean	12.8	12.7	12.9	12.1
	SD	1.77	1.30	1.73	1.59
Implantation Sites	N	20	20	19	19
	Mean	11.8	11.7	11.1	10.8
	SD	1.68	1.84	3.39	2.83
Pre-implantation Loss	N	20	20	19	19
	Mean	1.0	1.1	1.8	1.3
	SD	1.45	1.47	3.20	1.91
Pre-implantation Loss (%)	N	20	20	19	19
	Mean	7.2	8.2	14.1	11.1
	SD	10.33	11.51	23.84	16.20
Early Resorptions	N	20	20	19	19
	Mean	0.8	0.5	0.9	0.8
	SD	1.36	0.69	1.08	1.08
Late Resorptions	N	20	20	19	19
	Mean	0.0	0.0	0.0	0.0
	SD	0.00	0.00	0.00	0.00
Post-implantation Loss	N	20	20	19	19
	Mean	0.8	0.5	0.9	0.8
	SD	1.36	0.69	1.08	1.08
Post-implantation Loss (%)	N	20	20	19	19
	Mean	7.7	4.3	8.5	7.8
	SD	13.58	5.93	9.85	12.17
Live Foetuses	N	20	20	19	19
	Mean	11.0	11.2	10.1	10.1
	SD	2.63	1.90	3.46	3.08

v. Necropsy

Terminal body weights for males were 2, 4, and 8% below C at the LD, MD, and HD, respectively (SS at HD). Prostate weights were 7, 10, and 15% below C; seminal vesicle weights were 6, 5, and 18% lower, and testis/epididymis weights were 2, 5, and 4% lower, at the LD, MD, and HD, respectively (all NS).

c. Conclusions

Oral (gavage) administration of Purified CBD (0, 75, 150, or 250 mg/kg/day) to male and female Wistar rats prior and during mating and early pregnancy (through GD 6) did not result in any clear effects on fertility and reproductive performance. The HD did not meet the usual criterion of minimal toxicity in females but did produce SS BW gain reduction in males.

2. Purified CBD: Oral (Gavage) Study of Embryo-Foetal Development in the Rat (GW Report No. GWTX1454; dated 5/5/17; conducted by (b) (4) GLP)

a. Methods

Mated female Wistar (Han) rats (20/group) received oral (gavage, 5 ml/kg) doses of 0 (sesame oil vehicle), 75, 150, or 250 mg/kg/day Purified CBD (batch # JG01/055) from GDs 6 through 17. Blood samples were taken for TK evaluation pre-dose and 1, 2, 4, 6, and 24 hours after dosing on GDs 6 and 17. Clinical signs, body weights, and food consumption were recorded in dams. Animals were killed on GD 21 and pregnancy outcome was evaluated. Fetuses were evaluated for external (all), visceral (half), and skeletal (half) malformations and variations.

The HD was selected based on the results of an EFD dose range-finding study (GWTX1455) in which body weight loss at 300 mg/kg/day resulted in the early termination of one female. At 250 mg/kg/day, transiently decreased body weight gain had resolved by GD 11.

b. Results

i. Maternal observations

There were no deaths and no drug-related clinical signs. Overall BW gain during the dosing period was decreased (8% compared to C, NS) at the HD, but there were no differences in BWs at the end of treatment or of gestation.

CBD exposures (AUC0−t) were 25900, 44300, and 83900 h*ng/mL on GD 6 and 86300, 149000, and 170000 h*ng/mL on GD 17; 7-OH-CBD exposures were 8270, 10900, and 19300 h*ng/mL on GD 6 and 8730, 11200, and 12600 h*ng/mL on GD 17; 7-COOH-CBD exposures were 17100, 36300, and 84200 h*ng/mL on GD 6 and 41500, 100000, and 155000 h*ng/mL on GD 17; and THC exposures were 54.7, 86.2, and 150 h*ng/mL on GD6 and 139, 233, and 249 h*ng/mL on GD 17; at the LD, MD, and HD, respectively.

ii. Litter parameters and fetal evaluations

Two HD females had total litter losses in utero, which were considered drug-related (Table IV.E.2.1). The total litter loss in these 2 dams resulted in increased post-implantation loss for this group, but there were no other group differences in the Caesarean data (Table IV.E.2.2).

Table IV.E.2.1. Pregnancy data in rat embryofetal development study

Treatment Group	Control	75 mg/kg	150 mg/kg	250 mg/kg
Total Females	20	20	20	20
Pregnant Females	20	19	20	20
Non Pregnant Females	0	1	0	0
Pregnant with Total Litter Loss	0	0	0	2
Females with Live Foetuses	20	19	20	18

Table IV.E.2.2. Caesarean-section observations

Group		Control	75 mg/kg	150 mg/kg	250 mg/kg
Summary of Caesarean Section Data					
Number of females pregnant at caesarean section	(n)	20	19	20	20
Corpora Lutea	(n)	20	19	20	20
	Mean	12.2	11.5	12.2	13.1
	SD	1.60	1.31	1.70	4.10
Implantation Sites	(n)	20	19	20	20
	Mean	11.1	10.2	10.4	11.0
	SD	1.43	2.22	3.10	2.21
Pre-implantation Loss	(n)	20	19	20	20
	Mean	1.1	1.4	1.9	2.1
	SD	1.21	1.61	2.50	3.19
Pre-implantation Loss (%)	(n)	20	19	20	20
	Mean	8.6	12.2	15.6	13.3
	SD	8.50	15.08	21.84	15.73
Early Resorptions	(n)	20	19	20	20
	Mean	0.9	0.5	0.4	1.8
	SD	1.50	0.90	0.68	3.96
Late Resorptions	(n)	20	19	20	20
	Mean	0.0	0.1	0.0	0.0
	SD	0.00	0.46	0.00	0.00
Total Resorptions	(n)	20	19	20	20
	Mean	0.9	0.6	0.4	1.8
	SD	1.50	0.96	0.68	3.96
Dead Foetuses	(n)	20	19	20	20
	Mean	0.0	0.0	0.0	0.0
	SD	0.00	0.00	0.00	0.00
Post-implantation Loss	(n)	20	19	20	20
	Mean	0.9	0.6	0.4	1.8
	SD	1.50	0.96	0.68	3.96
Post-implantation Loss (%)	(n)	20	19	20	20
	Mean	7.7	6.1	3.8	14.2
	SD	13.47	9.73	6.29	30.02
Live Foetuses	(n)	20	19	20	20
	Mean	10.2	9.5	10.0	9.2
	SD	2.04	2.32	3.05	3.78

Mean Foetal Weight (g)	(n)	20	19	20	18@
	Mean	5.21	5.33	5.52	5.36
	SD	0.331	0.372	0.460	0.454
Mean Weight - Male Foetuses (g)	(n)	20	19	20	18@
	Mean	5.33	5.52	5.64	5.45
	Adj Mean	5.35	5.48	5.64	5.47
	SD	0.329	0.404	0.463	0.408
Mean Weight - Female Foetuses (g)	(n)	20	19	20	18@
	Mean	5.08	5.18	5.40	5.26
	Adj Mean	5.10	5.14	5.40*H	5.28
	SD	0.333	0.389	0.434	0.470

*H = Dunnett Exact Homogeneous Test Significant: 0.05 level
@ = Number examined reduced due to excluded data

There were no clear effects on embryofetal development based on the pattern and incidences of fetal structural abnormalities (Table IV.E.2.3) and lack of effect on fetal BWs. Malpositioned esophagus was found in 3 fetuses from 1 HD litter (66 L3, L5, R7), with fetus R7 also having a large sutural bone in the skull. While this is a malformation, the fact that it was seen in 3 fetuses from a single dam was thought by the sponsor to indicate a genetic abnormality rather than a drug-related effect.

There were also 2 variations that achieved statistical significance compared to C: supernumerary liver lobe at the HD (11, 9, 9, and 20% fetal incidence, in C, LD, MD, and HD, respectively) and abnormal iliac alignment at the MD and HD (0.00, 2.5, 7.5, and 6.8% in C, LD, MD, and HD, respectively). These findings were both considered to be incidental by the sponsor in the absence of other effects.

Table IV.E.2.3. Fetal abnormalities in rat embryofetal development study

Summary of Foetal Observations

Malformation in Foetal Fresh Visceral - Live

		Dosage Group:	Control	2	3	4
		Number of Dams/Foetus:	20/204	20/181	20/199	18/184
		Number Examined Litter/Foetus:	20/102	19/91	20/100	18/92
Oesophagus	Number Examined Litter/Foetus:		20/102	19/91	20/100	18/92
M-oesophagus - malpositioned			0/0	0/0	0/0	1/3
		%Litter:	0	0	0	6
		%Foetal:	0.00	0.00	0.00	3.33
M= Malformation						
Liver	Number Examined Litter/Foetus:		20/102	19/90	20/99	18/92
V-liver lobe - supernumerary.			6/12	6/7	6/8	12/19
		%Litter:	30	32	30	67*
		%Foetal:	11.00	8.95	9.08	19.77
Pelvic Girdle	Number Examined Litter/Foetus:		20/102	19/91	20/100	18/92
V-iliac alignment - abnormal.			0/0	2/3	5/7	4/7
		%Litter:	0	11	25*	22*
		%Foetal:	0.00	2.51	7.50	6.79

* = Fisher 1-tail Ascending Test significant at the 0.05 level
V= Variation

c. Conclusions

Oral (gavage) administration of Pure CBD to pregnant Wistar rats throughout organogenesis (GDs 6-17) at doses up to 250 mg/kg/day resulted in total litter loss in 2 HD dams, but did not produce any other clear evidence of embryofetal toxicity.

3. Purified CBD: Oral (Gavage) Study of Embryo-Foetal Development in the Rabbit (GW Report No. GWTX1452; dated 10/31/16; conducted by [redacted](b) (4))

a. Methods

Mated female NZW rabbits (22/group) received oral (gavage, 1 ml/kg) doses of 0 (sesame oil vehicle), 50, 80, or 125 mg/kg/day Purified CBD (batch # JT01/050) from GDs 7 through 19. Blood samples were taken for TK evaluation pre-dose and 1, 2, 6, and 24 hours after dosing on GDs 7 and 19. Clinical signs, body weights, and food consumption were recorded in dams. Animals were killed on GD 29 and pregnancy outcome was evaluated. Fetuses were evaluated for external (all), visceral (all except head by fresh dissection; head in 50% by Wilson section), and skeletal (half) malformations and variations.

The HD was selected based on the results of an EFD dose range-finding study in NZW rabbits (GWTX1453) in which reduced body weight gain and food consumption were seen at all doses and fetal weights were slightly reduced at 125 mg/kg/day, which was the HD in the study.

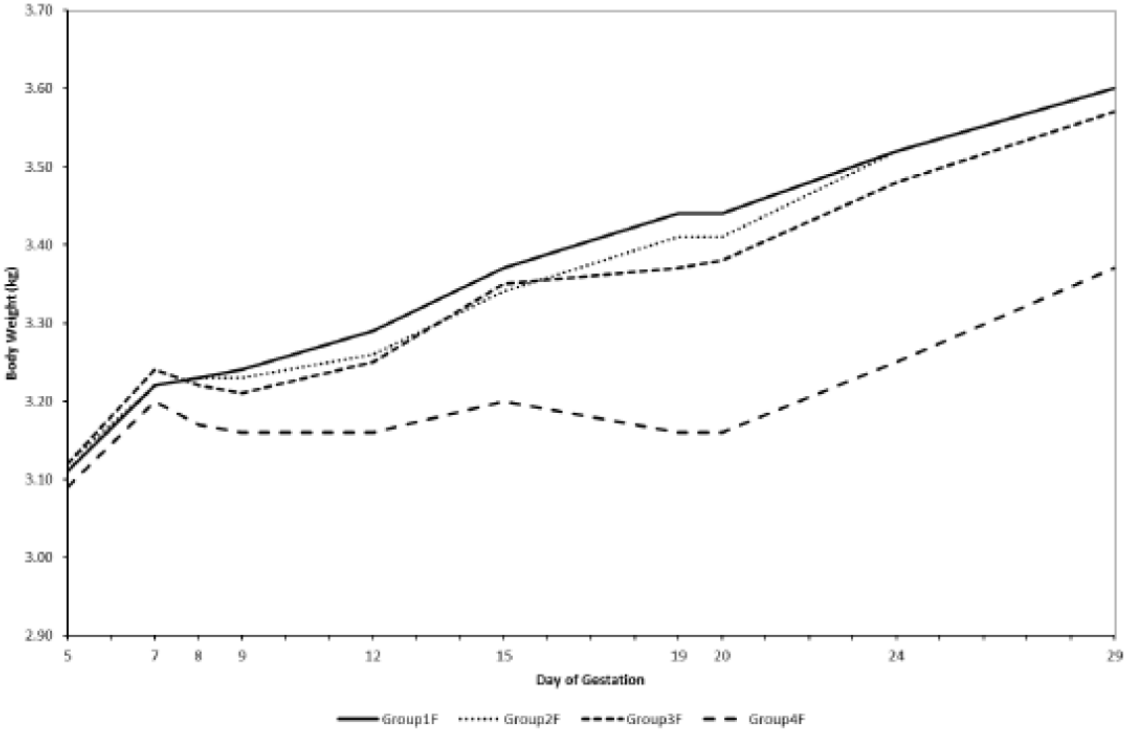
b. Results

i. Maternal observations

There were three abortions, in 1 LD and 2 HD. Decreased food consumption was noted in both HD animals. There were no drug-related clinical signs.

Reduced food consumption and BW gain were observed throughout the dosing period at the MD and HD (both SS; GDs 7-20 BW gain 0.22, 0.20, 0.15. and -0.02 kg in C, LD, MD, and HD), resulting in a net BW loss and SS difference in BW at the end of treatment and gestation in the HD group (Figure IV.E.3.1, Tables IV.E.3.1 and 2).

Figure IV.E.3.1 Maternal body weight in rabbit embryofetal development study



Maternal CBD exposures (AUC0−t) were 401, 931, and 1110 ng.h/mL on GD 7 and 1010, 2030, and 4630 ng.h/mL on GD 19; 7-COOH-CBD exposures were 23200, 81400, and 105000 ng.h/mL on GD 7 and 31700, 63700, and 106000 ng.h/mL on GD 19; 7-OH-CBD exposures were 46.0, 327, and 615 ng.h/mL on GDs 7 and 400, 652, and 2040 ng.h/mL on GD 19; at the LD, MD, and HD, respectively.

Table IV.E.3.1. Maternal body weight gain during treatment period (GDs 7-20)

		Females		
Group #		GD:24-GD:29	GD:7-GD:20	GD:20-GD:29
Control	(n)	22	22	22
	Means	0.07	0.22	0.16
	SD	0.067	0.102	0.117
2	(n)	21	22	21
	Means	0.07	0.20	0.17
	SD	0.092	0.137	0.097
3	(n)	22	22	22
	Means	0.09	0.15+r	0.19
	SD	0.081	0.103	0.104
4	(n)	20	21	20
	Means	0.08	-0.02#r	0.18
	SD	0.152	0.183	0.172

GD - Gestation
+r = Wilcoxon Rank Sum Test Significant at 0.01 level. #r = Wilcoxon rank Sum Test Significant at 0.001 level.

Table IV.E.3.2. Corrected maternal body weight and body weight change at C-section

	Group	Control	50 mg/kg	80 mg/kg	125 mg/kg
Summary of Caesarean Section Data					
Gravid Uterine Weight (g)	(n)	22	21@	22	20@
	Mean	472.4	502.9	474.6	436.4
	SD	84.29	100.36	86.22	103.30
Corrected Body Weight (Carcass Weight) (g)	(n)	22	21@	22	20@
	Mean	3119.6	3081.5	3094.5	2938.0*H
	SD	222.19	194.70	188.02	250.65
Corrected Weight Change Days 5-29 (g)	(n)	22	21@	22	20@
	Mean	14.2	-50.9	-23.7	-153.0
	SD	163.93	202.46	152.60	180.53
Total Weight Change (Weight Change) Days 5-29 (g)	(n)	22	21@	22	20@
	Mean	486.6	452.1	450.9	283.3
	SD	166.25	137.50	155.45	203.93

*H = Dunnett Exact Homogeneous Test Significant: 0.05 level
@ Number examined reduced due to excluded data

ii. Litter parameters and fetal evaluations

Fetal weights were decreased (10%, SS for males and females) at the HD compared to C, but there were no other clearly drug-related group differences in litter data (Table IV.E.3.3)

Table IV.E.3.3 Caesarean-section observations

Group		Control	50 mg/kg	80 mg/kg	125 mg/kg
Summary of Caesarean Section Data					
Number of females pregnant at Caesarean section	(n)	22	21@	22	20@
Corpora Lutea	(n)	22	21@	22	20@
	Mean	10.6	10.9	10.3	10.6
	SD	1.65	1.87	1.78	2.37
Implantation Sites	(n)	22	21@	22	20@
	Mean	9.1	9.6	8.9	9.6
	SD	1.63	2.06	2.02	1.98
Pre-implantation Loss	(n)	22	21@	22	20@
	Mean	1.5	1.3	1.4	1.0
	SD	1.54	1.39	2.26	1.17
Pre-implantation Loss (%)	(n)	22	21@	22	20@
	Mean	13.6	12.1	12.1	8.9
	SD	13.17	11.92	16.81	10.03
Early Resorptions	(n)	22	21@	22	20@
	Mean	0.5	0.5	0.3	0.3
	SD	0.91	0.75	0.57	0.73
Late Resorptions	(n)	22	21@	22	20@
	Mean	0.0	0.0	0.0	0.7
	SD	0.00	0.22	0.21	1.49
Total Resorptions	(n)	22	21@	22	20@
	Mean	0.5	0.6	0.4	1.0
	SD	0.91	0.75	0.58	1.56
Dead Foetuses	(n)	22	21@	22	20@
	Mean	0.0	0.0	0.0	0.0
	SD	0.00	0.00	0.00	0.00
Post-implantation Loss	(n)	22	21@	22	20@
	Mean	0.5	0.6	0.4	1.0
	SD	0.91	0.75	0.58	1.56
Post-implantation Loss (%)	(n)	22	21@	22	20@
	Mean	5.3	6.3	4.2	9.4
	SD	9.49	8.18	7.19	14.06
Live Foetuses	(n)	22	21@	22	20@
	Mean	8.6	9.0	8.5	8.6
	SD	1.74	2.21	2.02	1.96
Mean Foetal Weight (g)	(n)	22	21@	22	20@
	Mean	38.5	39.1	39.4	34.6
	SD	3.61	3.38	4.78	4.16
Mean Weight - Male Foetuses (g)	(n)	22	21@	22	20@
	Mean	38.9	39.8	39.8	35.1
	Adj Mean	38.8	40.1	39.7	35.0+H
	SD	3.98	2.76	5.15	4.15
Mean Weight - Female Foetuses (g)	(n)	22	21@	22	19@
	Mean	38.2	38.6	39.0	34.6
	Adj Mean	38.1	38.9	38.8	34.5*H
	SD	3.66	4.07	4.64	4.60

+H = Dunnett Exact Homogeneous Test Significant: 0.01 level *H = Dunnett Exact Homogeneous Test Significant: 0.05 level
@ Number examined reduced due to excluded data

There were no apparent drug-related increases in malformation incidences. Increases in several variations indicative of delayed or retarded development and consistent with the fetal BW reductions were observed at the HD. These were considered drug-related by the sponsor (Table IV.E.3.4).

Table IV.E.3.4. Fetal variations

	Percentage of affected litters (number of litters/foetuses)			
Variations	Group 1 (Control)	Group 2	Group 3	Group 4
Eye: bulge - large	0	0	**5** (1/1)	**10** (2/2)
Mouth: incisor - not erupted	32 (7/9)	43 (9/16)	**45** (10/23)	**55** (11/23)
Blood Vessel: subclavian artery - branching	55 (12/21)	62 (13/20)	41 (9/18)	**30** (6/9)
Stomach: Distention	0	5 (1/2)	**9** (2/2)	**10** (2/2)
Brain: Pituitary cyst	5 (1/2)	19 (4/4)	9 (2/2)	**25** (5/5)
Skull: zygomatic arch - ossification bridge	5 (1/1)	14 (3/4)	9 (2/4)	**20** (4/5)
Forelimb: metacarpal (pollex) - unossified	23 (5/7)	24 (5/9)	23 (5/16)	**70**** (14/32)

** $p<0.01$ compared with controls
Numbers in **bold** indicate possible treatment-related differences

c. Conclusions

Oral (gavage) administration of Purified CBD to pregnant rabbits throughout organogenesis (GDs 7-19) at doses of 0, 50, 80, or 125 mg/kg/day resulted in drug-related maternal (decreased BW gain) and developmental (decreased fetal BW and increased variations) toxicity at the HD. Plasma CBD exposure (AUC) at the NOAEL (80 mg/kg) was 2030 ng.h/mL on GD 19.

4. Purified CBD: Oral (Gavage) Study of Pre- and Postnatal Development in the Rat (GW Report #: GWTX1532; conducted by (b) (4) report dated 4/21/17; GLP)

a. Methods

Female Wistar (Han) rats (22/grp) were given oral (gavage) doses of Purified CBD (0 (sesame oil vehicle), 75, 150, or 250 mg/kg/day; 5 mL/kg) from GD 6 to PND 21. Clinical observations, body weights, and food consumption were recorded. Animals were allowed to litter and litter size, pup sex, pup body weight, and clinical observations were recorded on PNDs 1, 4, 7, 14, and 21. Pup physical (pinna unfolding, incisor eruption, and eye opening) and functional (surface and air righting) development was assessed preweaning. Following weaning, offspring were assessed for sexual milestones, auditory startle, motor activity, and learning and memory (Morris water maze). At approximately 10 weeks of age animals were mated and the outcome assessed.

b. Results

i. Maternal mortality, clinical observations, and body weight

There was no maternal mortality during the gestational dosing period. During lactation there were four total litter deaths: 1 C (F15) on PND 4, 2 LD (F37 and F38) on PND 1, and 1 HD (F78) on PND 4. After the litter deaths, the dams were sacrificed. These were not considered drug-related.

There were no drug-related maternal clinical observations.

Gestational and lactational maternal BW gain and maternal BWs at the end of gestation and lactation were similar among groups (Figures IV.E.4.1 and 2).

Figure IV.E.4.1. Maternal body weight during gestation

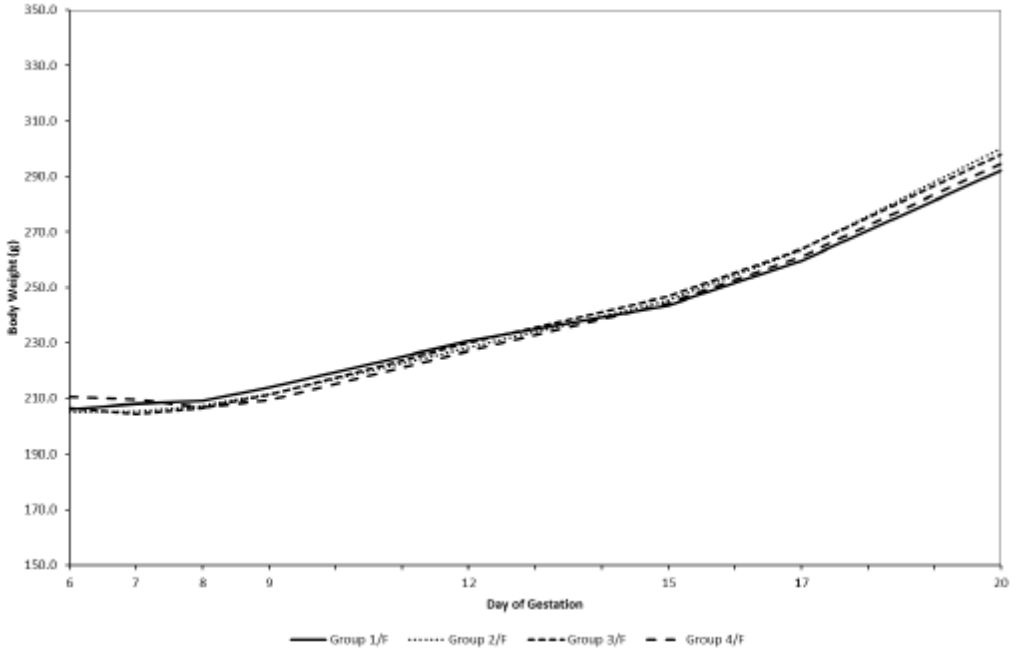
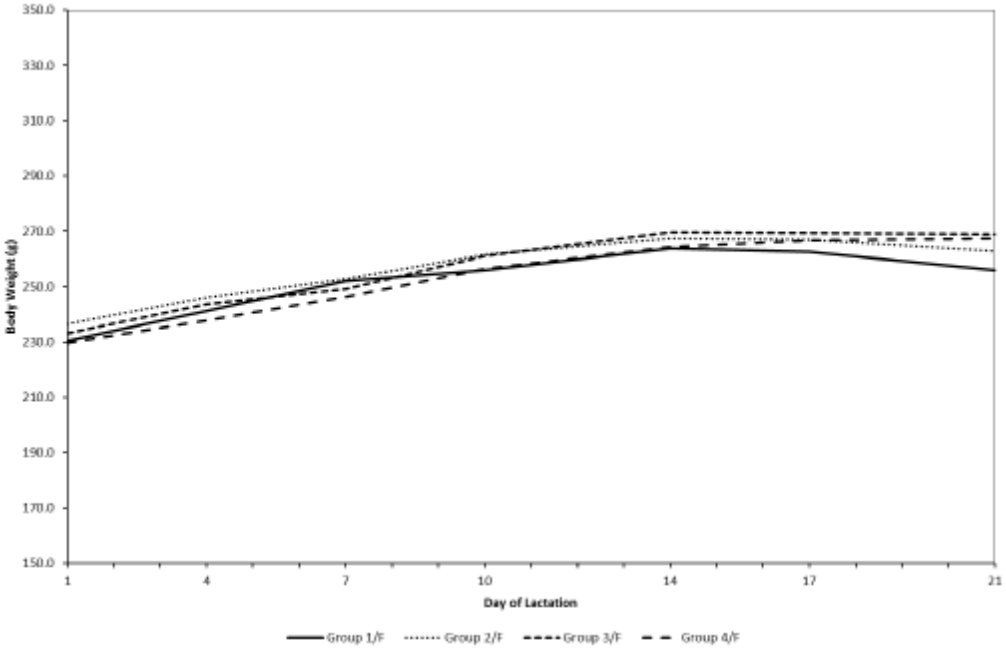


Figure IV.E.4.2. Maternal body weight during lactation



ii. Litter and preweaning offspring parameters

The mean duration of gestation was decreased (approximately 0.5 days, SS) in MD and HD dams. This was considered by the sponsor to be drug-related but not adverse since it was within the historical control range. There were no effects on the number of implantation sites, number of pups born, or pup survival during the lactation period. Clinical observations considered to drug-related consisted of increased incidences of pups with umbilical cord

attached, small size, and noisy respiration, at the MD and HD, and fur staining in the urogenital area at all doses.

Drug-related decreases in pup BWs at birth (PND 1) were seen in MD and HD litters (6 and 13%, respectively, SS; Table IV.E.4.1), which persisted throughout lactation until PND 21 (9 and 22%, at MD and HD, respectively, compared to C; both SS).

Table IV.E.4.1. Pup weights

			Group 1	Group 2	Group 3	Group 4	Statistics
Mean weight (g) Day 1:	Male	Adjusted	7.3	7.1	6.7*	6.2***	C
		Unadjusted	(7.3)	(7.1)	(6.8)	(6.2)	
	Female	Adjusted	6.9	7.0	6.5	6.0***	C
		Unadjusted	(7.1)	(6.9)	(6.6)	(5.9)	
	Combined	Adjusted	7.0	7.0	6.6*	6.1***	C
		Unadjusted	(7.2)	(6.9)	(6.6)	(6.0)	
Mean weight (g) Day 4:	Male	Adjusted	11.3	10.9	9.9***	8.7***	C
		Unadjusted	(11.4)	(10.8)	(10.0)	(8.7)	
	Female	Adjusted	11.0	10.8	9.7***	8.3***	C
		Unadjusted	(11.2)	(10.7)	(9.7)	(8.3)	
	Combined	Adjusted	11.1	10.8	9.8***	8.5***	C
		Unadjusted	(11.3)	(10.7)	(9.8)	(8.5)	
Mean weight (g) Day 7:	Male	Adjusted	17.3	16.6	15.1***	12.4***	C
		Unadjusted	(17.4)	(16.6)	(15.1)	(12.4)	
	Female	Adjusted	17.2	16.4	14.9***	12.1***	C
		Unadjusted	(17.3)	(16.3)	(14.9)	(12.1)	
	Combined	Adjusted	17.3	16.4	15.0***	12.3***	C
		Unadjusted	(17.4)	(16.3)	(15.0)	(12.2)	
Mean weight (g) Day 14:	Male	Adjusted	33.1	32.2	29.9**	25.0***	C
		Unadjusted	(33.1)	(32.2)	(29.9)	(24.9)	
	Female	Adjusted	33.0	32.0	29.4***	24.7***	C
		Unadjusted	(33.1)	(32.0)	(29.4)	(24.6)	
	Combined	Adjusted	33.2	32.0	29.7***	24.7***	C
		Unadjusted	(33.2)	(32.0)	(29.7)	(24.7)	
Mean weight (g) Day 21:	Male	Adjusted	53.2	52.1	49.3*	41.9***	C
		Unadjusted	(53.2)	(52.1)	(49.3)	(41.9)	
	Female	Adjusted	52.9	52.0	48.0**	41.3***	C
		Unadjusted	(52.9)	(51.9)	(48.0)	(41.3)	
	Combined	Adjusted	53.1	51.9	48.5**	41.5***	C
		Unadjusted	(53.1)	(51.9)	(48.5)	(41.5)	

* P≤0.05
** P≤0.01
*** P≤0.001

C = ANCOVA and Dunnett's

Physical development (pinna unfolding and eye opening) was delayed (2-3 days) at the MD and HD (SS at both doses). There were no apparent effects on the functional development (surface and air righting) of the pups.

iii. Postweaning offspring parameters

No drug-related mortality or clinical observations were reported during the postweaning period.

At the start of the postweaning period, BW was dose-dependently decreased at the MD (6-7%, SS in females) and HD (20%, SS in both sexes) compared to C. BW was still reduced at the HD in both sexes at the time of mating during Postnatal Weeks 11-12 (4-6%, SS in females). However, there were no group differences in BW gain during the postweaning period or in females during gestation.

There was a 1-day delay in the attainment of balanopreputial separation in MD and HD males and a 2-day delay in vaginal opening in HD females; these delays were associated with lower BW (Table IV.E.4.2).

Table IV.E.4.2. Offspring sexual maturation

Balano Pre-putial separation

Group and sex	Number of animals with complete development on Day *post-partum*																	Mean day *post-partum* for complete development	Mean body weight at completion (g)
	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56		
1M	4		5	3	4	2	1			1								43.0	187.0
2M	2	3	2	4	3	4	1	1										43.2	183.0
3M	1		3	5	1	5	4				1							44.2	186.8
4M		2	2	5	1	4	4	1	1									44.2	175.0
Statistics																		K	A

A = ANOVA and Dunnett's
K = Kruskal-Wallis and Wilcoxon

Vaginal Opening

Group and sex	Number of animals with complete development on Day *post-partum*												Mean day *post-partum* for complete development	Mean body weight at completion g
	30	31	32	33	34	35	36	37	38	39	40	41		
1F	2	3	2	3	5	3	2						33.2	109.0
2F	1		3	8	7	1							33.2	112.6
3F	2	2	4	2	2	5	2	1					33.4	108.6
4F	1		2		4	3	6	4					35.0**	105.4
Statistics													K	A

* P≤0.05
** P≤0.01
*** P≤0.001

A = ANOVA and Dunnett's
K = Kruskal-Wallis and Wilcoxon

When reflex development was assessed on PND 28 (Table IV.E.4.3), 3 HD males failed to show a pupillary response (M161, 162, and 166). The animals were not littermates. This finding was considered (in the report) to be associated with the smaller weight of the animals in this group (59.4, 78.4, and 80.7 g compared to C range of 80.7-100.8 g) and to represent a developmental delay.

Table IV.E.4.3. Reflex tests

Day 28 ± 3 *post-partum*		Group 1	Group 2	Group 3	Group 4	Statistics
Auditory Startle:	Male	2.0	2.0	2.0	2.0	X
	Female	2.0	2.0	2.0	2.0	X
Pupillary reflex:	Male	2.0	2.0	2.0	1.7	K
	Female	2.0	2.0	2.0	2.0	X

K = Kruskal-Wallis and Wilcoxon
X = No analysis performed

iv. Neurobehavioral testing

When locomotor activity was assessed on PND 35, apparent drug-related decreases in total activity counts were seen in MD and HD groups of both sexes (SS in males at both doses and in MD females), although the effect was not strictly dose-related (Table IV.E.4.4).

There were no consistent effects on learning and memory as assessed in the Morris water maze beginning on PND 65.

Table IV.E.4.4 Locomotor Activity - Total Activity

Group/ Sex	Phase	TA13 Maturation	TA14 Maturation	TA15 Maturation	TA Total Maturation
	Day	14	14	14	14
1/M	Mean	78	68	60	1062
	SD	53.3	50.4	49.4	438.6
	N	20	20	20	20
2/M	Mean	47	47	46	1053
	SD	46.3	39.3	41.2	541.1
	N	20	20	20	20
3/M	Mean	25**	24**	23*	663**
	SD	26.5	27.4	32.8	251.9
	N	20	20	20	20
4/M	Mean	28**	33*	40	741*
	SD	26.9	39.8	37.0	287.3
	N	20	20	20	20
	Statistics	AT	A	A	A
1/F	Mean	44	45	38	852
	SD	41.6	39.0	32.5	312.5
	N	20	20	20	20
2/F	Mean	53	52	49	850
	SD	49.3	51.2	50.6	532.0
	N	20	20	20	19
3/F	Mean	15	14**	14*	464***
	SD	24.8	21.7	19.7	194.5
	N	19	20	20	19
4/F	Mean	27	27	28	719
	SD	30.1	31.8	33.4	337.2
	N	20	20	20	20
	Statistics	AT	AT	AT	AT

* P<=0.05
** P<=0.01
*** P<=0.001
A = ANOVA and Dunnett's
T = Rank-transformed data

v. Reproductive function

The majority of paired animals mated within 1 estrus cycle. There was a single female in each of the MD and HD groups that was not pregnant (Table IV.E.4.5). There was no effect on C-section data (corpora lutea, implantations, pre- and post-implantation losses, or number of live fetuses).

Table IV.E.4.5. Fertility and Reproductive Performance – F1 Generation

	Males			
Treatment Group	Control	75 mg/kg	150 mg/kg	250 mg/kg
Total males	20	20	20	20
Unscheduled Deaths prior to Cohabitation	0	0	0	0
Males Cohabitated	20	20	20	20
Unscheduled Deaths during Cohabitation	0	0	0	0
Males mating with at least 1 female	19	20	19	20
Males impregnating at least 1 female	19	20	19	19
Mating Index (%)	95	100	95	100
Fecundity Index (%)	100	100	100	95
Fertility Index (%)	95	100	95	95

Mating index % = (Number of males mating with at least 1 female / Number of males cohabitated with at least 1 female) x 100
Fecundity index % = (Number of males impregnating at least 1 female / Number of males mating with at least 1 female) x 100
Fertility Index % = (Number of males impregnating at least 1 female / Number of males cohabitated with at least 1 female) x 100

	Females			
Treatment Group	Control	75 mg/kg	150 mg/kg	250 mg/kg
Total Females	20	20	20	20
Unscheduled Deaths Prior to Cohabitation	0	0	0	0
Females Cohabited	20	20	20	20
Unscheduled Deaths During Cohabitation	0	0	0	0
Females Mated	20	20	19	20
Pregnant Females	20	20	19	19
Non Pregnant Females	0	0	1	1
Matings Per Day Periods Of Cohabitation				
Day 1	2	4	3	3
Day 2	6	6	7	3
Day 3	6	6	4	8
Day 4	5	3	5	6
Day 5	0	1	0	0
Day 12	1	0	0	0
Mating Index %	100	100	95	100
Fecundity Index %	100	100	100	95
Fertility Index %	100	100	95	95

Mating index % = Mated females/females cohabited (excluding females sacrificed during Cohabitation) x 100
Fecundity Index % = Pregnant females/mated females (excluding females with an undetermined pregnancy status) x 100
Fertility Index % = Pregnant females/females cohabited (excluding females sacrificed during Cohabitation or with an undetermined pregnancy status) x 100

vi. Macroscopic examination

There was an increased number of males with small testis in the CBD-dosed groups at all doses: 1 LD (M133), 2 MD (M154 and 160), and 3 HD (M171, M173 and M174). None of the males were littermates. Females paired with M160 and M174 were not pregnant; the other 4 males successfully impregnated the female they were paired with. In addition, for 2 HD males, small prostate (M164) and small seminal vesicle (M180) was observed.

c. Conclusions

Oral (gavage) administration of Purified CBD (0, 75, 150, or 250 mg/kg/day) to female Wistar rats from GD 6 through PND 21 resulted in developmental toxicity at doses that were not maternally toxic. Developmental effects consisted of decreased pup body weights at birth and throughout lactation, delays in achieving developmental landmarks (pinna unfolding, eye opening, pupillary reflex, male and female sexual maturation), neurobehavioral changes (decreased locomotor activity), and adverse effects on reproductive system structure (small testis) and possibly function. These effects were primarily seen at the MD and HD, but small testis was also found in a single animal at the LD.

5. Epidiolex (Purified CBD): 10 Week Subcutaneous and Oral (Gavage) Administration Toxicity Study in the Juvenile Rat Followed by a 6 Week Treatment-free Period ((b) (4) Study Number: 8302481, GW Study Number: GWTX1408, conducted by (b) (4), report dated 9 May 2017, GLP)

a. Methods

Purified CBD (Batch nos. GWPb002, K14221, and K14233) was administered once daily to neonatal/juvenile rats (Wistar; 50/sex/grp main study + 3-30/sex/grp TK), subcutaneously from PNDs 4 to 6 (0, 15, 15, or 15 mg/kg/day) and orally (by gavage) from PNDs 7 to 77 (0, 100, 150, or 250 mg/kg/day). Of the 50/sex/group, 10/sex/group (Subset 1) were sacrificed at the end of the dosing period; 20/sex/group (Subset 2) were used to assess locomotor activity at the end of treatment and after a 3-week recovery period and auditory startle and learning and memory during weeks 5-7 of the recovery period; 10/sex/grp were used to evaluate general toxicity parameters (clinical pathology, gross and histopathology) and bone length and density at the end of recovery Week 7; and 20/sex/group (Subset 3) were used to evaluate learning and memory at the end of the treatment period and reproductive performance after mating on ~PND 90.

Doses were based on the results of a dose range-finding study in which once daily sc (2, 10, or 15 mg/kg/day on PND 4, 5, and 6) and oral (gavage) administration (5, 15, 50, 150, 250, or 400 mg/kg/day on PND 7 to 25) of Purified CBD to juvenile (Wistar) rats resulted in clinical signs of acute neurotoxicity, 3 deaths, and decreased body weight gain at the HD. Dark kidneys were found macroscopically in 3/12 HD females that were terminated on PND 12. Dark bladder was also seen in the same 3 females and in 1/12 HD males. The PND 7 CBD exposure (AUC0-t) reported at the HD that resulted in deaths and possible macroscopic evidence of nephrotoxicity was 306000 ng.h/mL. The NOAEL (MD) was associated with CBD exposures of 232000 ng.h/mL on PND 7 after the first oral dose and 50800 ng.h/mL on PND 25.

b. Results

i. Mortality and Clinical Observations

There were 17 deaths during this study, but of these, only 3 HD male deaths on PNDs 12, 15, and 26 were considered drug-related because decreased BW gain occurred prior to death.

There were no drug-related clinical signs during the study.

ii. Body Weight

In all drug-groups/subgroups, a trend toward increased body weight gain and mean body weights was observed throughout the dosing phase, reaching statistical significance in LD and HD females (Table IV.E.5.1). This was considered drug-related, although a clear dose relationship was not seen. There were no differences among groups at the end of the 6-week recovery period.

iii. Landmarks of development

Mean age at completion of balanopreputial separation was increased by 1 day in all drug-treated groups. Body weight at completion of balanopreputial separation was comparable among groups. For females, mean day of completion of vaginal opening and body weights on the day of completion of vaginal opening were comparable among groups.

Table IV.E.5.1.

Summary of Body Weight
Toxicity animals

Test Article		Control	CBD			Control	CBD		
Group		1	2	3	4	5	6	7	8
Subcut Dose level (mg/kg/day)		0	15	15	15	0	15	15	15
Oral Dose level (mg/kg/day)		0	100	150	250	0	100	150	250

Data Presented in "g"

Group/ Subgroup/ Sex	Phase Wk:Day	DSNG 10:67	DSNG 10:70	DSNG 11:74	DSNG 11:77
1/1/M	Mean	270.0	278.2	289.7	291.3
	SD	31.87	34.49	35.16	35.35
	N	10	10	10	10
2/1/M	Mean	294.5	303.8	314.2	318.3
	SD	31.34	33.47	35.46	37.37
	N	9	9	9	9
3/1/M	Mean	278.4	288.6	298.3	301.2
	SD	27.50	31.18	34.64	36.40
	N	10	10	10	10
4/1/M	Mean	304.4	314.8	326.4	326.0
	SD	35.59	37.29	39.35	41.16
	N	9	9	9	9
	Statistics	A	A	A	A

A = ANOVA and Dunnett's

Data Presented in "g"

Group/ Subgroup/ Sex	Phase Wk:Day	DSNG 10:67	DSNG 10:70	DSNG 11:74	DSNG 11:77
1/1/F	Mean	177.0	178.4	183.1	181.9
	SD	13.31	13.67	12.75	10.74
	N	10	10	10	10
2/1/F	Mean	197.8*	205.5**	208.6**	206.6**
	SD	16.75	19.49	20.32	18.99
	N	10	10	10	10
3/1/F	Mean	188.9	194.1	197.3	195.4
	SD	16.33	13.79	13.97	15.43
	N	9	9	9	9
4/1/F	Mean	201.6**	206.8***	211.0***	208.2**
	SD	13.97	13.58	14.26	13.63
	N	10	10	10	10
	Statistics	A	A	A	A

* P<=0.05
** P<=0.01
*** P<=0.001
A = ANOVA and Dunnett's

iv. Ophthalmoscopy

There were no drug-related effects.

v. Neurobehavioral testing

Locomotor activity was assessed using an automated photocell activity recorder for 30 minutes in Subset 2 during Weeks 9 and 10 of the treatment (dosing) period and during Week 3 of the treatment-free (recovery) period.

At the end of the treatment period, locomotor total activity (TA) and mobile counts (TM) were decreased toward the second half of the observation interval of 30 minutes, and total counts were dose-dependently decreased (primarily at MD and HD but SS at all doses during some intervals; Table IV.E.5.2). The same pattern was seen in HD males during the recovery period (Table IV.E.5.3), indicating a persistent, developmental effect.

Table IV.E.5.2.

Summary of Locomotor Activity
Total Activity

Test Article		Control	CBD			Control	CBD		
Group		1	2	3	4	5	6	7	8
Subcut Dose level (mg/kg/day)		0	15	15	15	0	15	15	15
Oral Dose level (mg/kg/day)		0	100	150	250	0	100	150	250
Group/ Subgroup/ Sex	Phase Wk	TA13 Dosing 9-10	TA14 Dosing 9-10	TA15 Dosing 9-10	TA Total Dosing 9-10				
1/2/M	Mean	87	92	84	1458				
	SD	38.3	34.7	40.5	335.4				
	N	20	20	20	20				
2/2/M	Mean	86	81	95	1471				
	SD	41.7	40.5	46.5	401.0				
	N	20	20	20	20				
3/2/M	Mean	77	68	67	1414				
	SD	52.9	51.0	59.8	419.0				
	N	20	20	20	20				
4/2/M	Mean	66	77	69	1312				
	SD	58.2	70.3	70.5	463.8				
	N	20	20	20	20				
	Statistics	A	AT	AT	A				
Group/ Subgroup/ Sex	Phase Wk	TA13 Dosing 9-10	TA14 Dosing 9-10	TA15 Dosing 9-10	TA Total Dosing 9-10				
1/2/F	Mean	53	68	70	1108				
	SD	35.6	35.7	28.3	223.7				
	N	20	20	20	20				
2/2/F	Mean	47	33**	33**	1009				
	SD	42.9	41.5	35.8	228.4				
	N	20	20	20	20				
3/2/F	Mean	25*	25***	26***	953				
	SD	33.2	27.5	33.7	231.2				
	N	20	20	20	20				
4/2/F	Mean	27	40*	37**	947				
	SD	29.5	36.5	40.7	311.4				
	N	20	20	20	20				
	Statistics	A	A	A	A				

* P<=0.05
** P<=0.01
*** P<=0.001
A = ANOVA and Dunnett's

Table IV.E.5.3.

Summary of Locomotor Activity
Total Activity

Test Article	Control		CBD		Control		CBD	
Group	1	2	3	4	5	6	7	8
Subcut Dose level (mg/kg/day)	0	15	15	15	0	15	15	15
Oral Dose level (mg/kg/day)	0	100	150	250	0	100	150	250

Group/ Subgroup/ Sex	Phase	TA13 Recovery	TA14 Recovery	TA15 Recovery	TA Total Recovery
	Wk	3	3	3	3
1/2/M	Mean	96	95	89	1501
	SD	32.2	25.5	35.0	275.0
	N	20	20	20	20
2/2/M	Mean	81	67	82	1465
	SD	47.4	39.3	42.7	385.8
	N	20	20	20	20
3/2/M	Mean	76	74	71	1454
	SD	45.8	41.0	46.7	347.7
	N	20	20	20	20
4/2/M	Mean	66	57**	44**	1298
	SD	55.5	58.4	51.3	445.2
	N	20	20	20	20
	Statistics	A	AT	A	A

* P<=0.05
** P<=0.01
*** P<=0.001
A = ANOVA and Dunnett's
T = Rank-transformed data

Total Mobile Counts

Test Article	Control		CBD		Control		CBD	
Group	1	2	3	4	5	6	7	8
Subcut Dose level (mg/kg/day)	0	15	15	15	0	15	15	15
Oral Dose level (mg/kg/day)	0	100	150	250	0	100	150	250

Group/ Subgroup/ Sex	Phase	TM13 Recovery	TM14 Recovery	TM15 Recovery	TM Total Recovery
	Wk	3	3	3	3
1/2/M	Mean	15	12	10	299
	SD	11.5	10.1	10.0	90.7
	N	20	20	20	20
2/2/M	Mean	10	6	10	302
	SD	12.3	7.1	10.3	106.7
	N	20	20	20	20
3/2/M	Mean	9	10	8	265
	SD	9.4	14.7	12.6	94.7
	N	20	20	20	20
4/2/M	Mean	6	5	2***	228
	SD	9.2	10.0	6.4	105.7
	N	20	20	20	20
	Statistics	A	A	AT	A

* P<=0.05
** P<=0.01
*** P<=0.001
A = ANOVA and Dunnett's
T = Rank-transformed data

Auditory startle habituation and prepulse inhibition were evaluated in Subset 3 during Weeks 9 and 10 of the treatment period and in Subset 2 during Weeks 5 and 6 of the treatment-free period using a Kinder Auditory Startle Chamber.

In the recovery phase, CBD-treated males and females showed decreased (LD and MD males) or an absence of (HD males and females) habituation (Table IV.E.5.4). Percent prepulse inhibition also tended to be decreased in treated groups at the 78 and/or 86 dB, although statistical significance was not achieved (Table IV.E.5.5).

Table IV.E.5.4. Auditory Startle Habituation - Treatment-free Subgroup Recovery Phase

Data presented is Mean Maximum Amplitude (Ne)							
			Block				
Group/Sex	Dose Level (mg/kg/day)		1	2	3	4	5
1M	0	Mean	7.070	6.193	5.643	5.593	5.200
		Std	2.6001	2.5658	1.8572	1.8275	1.7824
		N	20	20	20	20	20
2M	100	Mean	6.292	5.482	5.547	5.965	6.051
		Std	2.1942	2.4297	2.3446	2.6248	2.2257
		N	20	20	20	20	20
3M	150	Mean	6.601	6.164	6.137	6.580	6.575
		Std	2.4399	2.5955	1.9692	2.1232	2.4940
		N	20	20	20	20	20
4M	250	Mean	5.884	5.915	6.212	6.460	6.128
		Std	1.9866	2.5324	2.4240	3.0128	2.7600
		N	20	20	20	20	20
1F	0	Mean	4.560	4.201	4.338	4.444	4.315
		Std	2.0071	1.5463	1.4553	1.7251	1.4992
		N	20	20	20	20	20
2F	100	Mean	4.968	4.404	4.628	4.652	4.809
		Std	2.3405	1.9637	2.1650	2.1223	1.9703
		N	20	20	20	20	20
3F	150	Mean	5.087	4.844	4.214	4.689	4.668
		Std	1.8914	2.1073	1.2129	1.8066	1.8527
		N	20	20	20	20	20
4F	250	Mean	4.117	4.163	4.138	4.670	4.407
		Std	1.6722	1.9957	1.7833	1.8962	1.4315
		N	20	20	20	20	20

Table IV.E.5.5. Auditory Startle Prepulse Inhibition (%) - Recovery group

Group/Sex	Dose Level (mg/kg/day)		dbLevel 74	78	86
1M	0	Mean	8	39	69
		Std	29.2	21.2	14.4
		N	20	20	20
2M	100	Mean	17	34	65
		Std	21.7	15.5	16.6
		N	20	20	20
3M	150	Mean	11	38	66
		Std	22.3	17.6	13.6
		N	20	20	20
4M	250	Mean	17	37	63
		Std	29.5	22.3	13.5
		N	20	20	20

Group/Sex	Dose Level (mg/kg/day)		dbLevel 74	78	86
1F	0	Mean	10	40	64
		Std	17.9	22.5	19.3
		N	20	20	20
2F	100	Mean	17	44	63
		Std	18.2	17.1	13.4
		N	20	20	20
3F	150	Mean	11	35	64
		Std	30.5	28.3	16.9
		N	20	20	20
4F	250	Mean	10	36	60
		Std	25.2	21.8	16.0
		N	20	20	20

Learning and Memory testing was conducted in Subset 3 during Weeks 8 to 10 of the treatment period and in Subset 2 during Weeks 5 and 6 and into Week 7 of the treatment-free period using the Morris water maze.

In the learning phase, values for latency to platform, path length, and swim speed were similar among groups. There was no effect on reference memory in the probe trial; time spent in the platform quadrant and entries into the platform zone were similar among groups. There was no effect on cued learning; mean latency to the platform was similar among groups. Therefore, there was no apparent effect on spatial learning and memory.

vi. Clinical pathology

There were no toxicologically significant changes in hematology parameters.

At the end of the dosing period, dose-related increases in plasma calcium (all doses), cholesterol, total protein and globulin, inorganic phosphate, and ALT and decreases in chloride were considered drug-related; the calcium, cholesterol, protein, and globulin findings were considered toxicologically significant based on historical values. At the end of the treatment-free period, these findings had resolved. Slightly elevated plasma urea and AST (HD males) and decreased creatinine (MD and HD males) seen in treated animals after the recovery period were considered drug-related but not toxicologically significant.

There were no changes in urinalysis parameters considered to be toxicologically significant; slight increases in urine volume in treated females were not associated with other evidence of renal dysfunction.

vii. Necropsy

Dose-related increases (absolute and relative) in liver (both sexes at all doses), thyroid/parathyroid (both sexes at all doses), and adrenal gland weights (males at all doses) were seen at the end of the dosing period. These had all reversed at the end of the recovery period.

At the end of the treatment period (Subset 1), hepatocyte hypertrophy (characterized by hepatocytes with increased amounts of eosinophilic, glassy cytoplasm causing an overall increase in the size of the organ) was seen in all treated males and females (Table IV.E.5.6). This finding was diffuse in males but more centrilobular in females and correlated with the increased organ weights and macroscopic enlargement. There was also treatment-related hepatocyte vacuolation (characterized by hepatocytes with small- to moderate-sized intracytoplasmic vacuoles, primarily within centrilobular regions) in males and females from all dose groups. Vacuolation was not reported in toxicity studies conducted in adult rats.

Table IV.E.5.6. Incidence of hepatocyte hypertrophy and hepatocyte vacuolation in Subset 1

		Males				Females			
		1M	2M	3M	4M	1F	2F	3F	4F
Tissue and finding	Level (mg/kg/day)	0	15/100	15/150	15/250	0	15/100	15/150	15/250
Liver	No. examined:	10	9	10	9	10	10	9	10
hepatocyte hypertrophy	Grade -	10	0	0	0	10	0	0	0
	1	0	3	1	0	0	5	1	0
	2	0	6	6	4	0	4	6	4
	3	0	0	3	5	0	1	2	6
hepatocyte vacuolation	Grade -	10	8	8	2	10	8	5	2
	1	0	1	2	7	0	2	3	7
	2	0	0	0	0	0	0	1	1

Key: "-" = finding not present, 1 = minimal, 2 = slight, 3 = moderate

Thyroid follicular cell hypertrophy was also seen in Subset 1 animals at all doses in both sexes, and an increase in cortical cell vacuolation in the adrenal gland was seen in males from all drug groups.

After the recovery period, all but the liver findings had reversed. Hepatocyte hypertrophy and vacuolation remained in MD and HD animals (Table IV.E.5.7).

Table IV.E.5.7. Incidence of hepatocyte hypertrophy and vacuolation in recovery group

		Males				Females			
		1M	2M	3M	4M	1F	2F	3F	4F
Tissue and finding	Level (mg/kg/day)	0	15/ 100	15/ 150	15/ 250	0	15/ 100	15/ 150	15/ 250
Liver	No. examined:	10	10	10	10	10	10	10	10
hepatocyte hypertrophy	Grade -	10	8	10	8	10	10	10	10
	1	0	2	0	2	0	0	0	0
hepatocyte vacuolation	Grade -	10	10	8	5	10	10	10	8
	1	0	0	2	5	0	0	0	2

Key: "-" = finding not present, 1 = minimal

viii. Bone Length and Density

At the end of the treatment period (Subset 1), no effect on femur length was observed; however, a statistically significant increase in bone mineral density was noted in HD males and was thought to be drug-related (Table IV.E.5.8). The finding was absent after the treatment-free period. No correlative anatomic pathology changes were noted. A possible relationship with the observed increase in plasma calcium was noted.

Table IV.E.5.8. DEXA parameters - terminal necropsy

Males

Test Article		Control	CBD		
Group		1	2	3	4
Subcut Dose level (mg/kg/day)		0	15	15	15
Oral Dose level (mg/kg/day)		0	100	150	250

Group	Dose Level (mg/kg/day)		Bone Area (cm^2)	Bone Mineral Content (g)	Bone Mineral Density (g/cm^2)
1	0	Mean	1.50	0.31	0.204
		SD	0.087	0.029	0.0088
		N	10	10	10
2	15/100	Mean	1.58	0.32	0.204
		SD	0.080	0.023	0.0063
		N	9	9	9
3	15/150	Mean	1.55	0.32	0.206
		SD	0.106	0.031	0.0090
		N	10	10	10
4	15/250	Mean	1.61	0.35*	0.215*
		SD	0.115	0.041	0.0105
		N	9	9	9

* = Statistically significant from Group 1 at $P \leq 0.05$.

ix. Reproductive

There were no effects on estrous cycle, mating and fertility, or pregnancy parameters.

x. TK

Plasma CBD levels on PNDs 4, 7, and 70 are shown in Table IV.E.5.9.

Table IV.E.5.9. TK parameters for CBD during juvenile rat toxicity study

PND 4 (Subcutaneous), Males and Females (pooled)

Group / Epidiolex Dose (mg/kg/day)	C_{max} (ng/mL)	C_{max} / D (kg.ng/mL/mg)	t_{max} (h)	$t_{1/2}$ (h)	AUC_{0-t} (h.ng/mL)
6 / 15	1640	109	2.0	6.7	12600
7 / 15	1370	91.1	2.0	11	9440
8 / 15	1530	102	2.0	7.4	12300

PND 7 (Oral Gavage), Males and Females (pooled)

Group / Epidiolex Dose (mg/kg/day)	C_{max} (ng/mL)	C_{max} / D (kg.ng/mL/mg)	t_{max} (h)	$t_{1/2}$ (h)	AUC_{0-t} (h.ng/mL)
6 / 100	13000	130	2.0	9.3	160000
7 / 150	11400	75.8	6.0	ND	178000
8 / 250	17000	67.9	4.0	ND	296000

PND 70 (Oral Gavage), Males

Group / Epidiolex Dose (mg/kg/day)	C_{max} (ng/mL)	C_{max} / D (kg.ng/mL/mg)	t_{max} (h)	$t_{1/2}$ (h)	AUC_{0-t} (h.ng/mL)
6 / 100	11600	116	4.0	ND	81400
7 / 150	11200	74.9	2.0	4.7	109000
8 / 250	11200	44.7	2.0	5.3	128000

PND 70 (Oral Gavage), Females

Group / Epidiolex Dose (mg/kg/day)	C_{max} (ng/mL)	C_{max} / D (kg.ng/mL/mg)	t_{max} (h)	$t_{1/2}$ (h)	AUC_{0-t} (h.ng/mL)
6 / 100	12500	125	2.0	4.9	82400
7 / 150	11900	79.1	4.0	ND	127000
8 / 250	14600	58.5	2.0	4.5	180000

ND = not determined due to insufficient data points post C_{max} to characterise the terminal slope Tables of the toxicokinetic parameters associated with the analytes of CBD can be found in the Bioanalytical and Toxicokinetic Report.

On PND 70, 7-OH-CBD AUC0-t values were 3860, 5300, and 6150 in males and 6080, 6830 and 6920 ng.h/mL in females; 7-COOH-CBD AUCs were 67100, 101000, and 171000 in males and 68000, 103000, and 189000 ng.h/mL in females; and THC AUCs were 75.2, 87.9, and 113 in males and 92.5, 130, and 201 ng.h/mL in females, at the LD, MD, and HD, respectively.

c. Conclusions

Daily sc (PNDs 4, 5, and 6) and oral (gavage) administration (PNDs 7-77) of Purified CBD (0, 15, 15, or 15 mg/kg sc; 0, 100, 150, or 250 mg/kg po) to juvenile rats for 10 weeks resulted in 3 HD deaths, dose-dependent increases in body weight gain, neurobehavioral deficits (decreased locomotor activity and startle habituation, both persisting after recovery period at HD), delayed sexual maturation in males (all doses), transient increases in plasma calcium in males and cholesterol, total protein, and globulin in females, a reversible increase in bone mineral density in males (HD), and hepatocyte hypertrophy and vacuolation (all doses) that remained after the recovery period at the MD and HD. The LOAEL for apparent unique effects (15 sc/100 po mg/kg/day) was associated with a PND 70 CBD exposure of 82000 ng·h/mL (sexes combined).

F. Other Studies – Qualification of impurities

Toxicology

1. [redacted]: 26 Week Oral (Gavage) Administration Toxicity Study in the Rat Followed by a 4 Week Treatment-free Period (Study Number: GWTX1429, conducted by [redacted] report dated 5/8/17, GLP)

a. Methods

Wistar (HsdHan:WIST) rats (15/sex/group main, 10/sex/grp C & HD recovery, 3-6/sex/grp TK) were administered [redacted] (Batch #s JG01/010 & MG02/077) at doses of 0 (sesame oil vehicle),10, 40, or 80 mg/kg (1.5 mL/kg) by oral gavage once daily for 26 weeks. Clinical signs, body weight, food consumption, ophthalmology, clinical pathology, and macroscopic and microscopic evaluations were performed. Blood was collected for TK evaluations on Days 1/2 and during Weeks 13 and 26. The HD was selected based on the results of a 13-week study of [redacted] in which liver (hepatocellular hypertrophy), thyroid (follicular cell hypertrophy), and adrenal effects were seen at the HD of 200 mg/kg and reduced BW was seen at 80 mg/kg or greater.

b. Results

There were no drug-related deaths or clinical signs and no consistent trends in body weight or body weight gain. There were no ophthalmology or toxicologically significant clinical pathology changes. Dose-related increases in liver weights and microscopic findings of liver centrilobular hypertrophy, thyroid follicular cell hypertrophy, and increased foamy macrophages in lung were seen in both sexes at the MD and HD. Additional findings at these doses were adrenocortical vacuolation in males and increased ovarian interstitial cell hyperplasia in females. These adaptive changes showed reversibility. Week 26 HD exposures (AUC(0-t)) were 35000 ng.h/mL in males and 69500 ng.h/mL in females for [redacted] 6150 ng.h/mL in males and 4270 ng.h/mL in females for [redacted]; and 5020 ng.h/mL in males and 12500 ng.h/mL in females fo [redacted].

2. [redacted] 13 Week Oral (Gavage) Administration Toxicity Study in the Rat with a 4 Week Interim Phase (Study Number: GWTX1599, conducted by [redacted], report dated 2/21/17, GLP)

a. Methods

Wistar (HsdHan:WIST) rats (10/sex/grp main, 10/sex/grp interim, 3-6/sex/grp TK) were administered [redacted] (Lot # JT01/124) at doses of 0 (sesame oil vehicle), 1, 10, or 100 mg/kg (1.5 mL/kg) by oral gavage once daily for 13 weeks with an interim sacrifice at 4 weeks. Clinical signs, body weight, food consumption, ophthalmology, clinical pathology, and macroscopic and microscopic anatomic pathology evaluations were performed. Blood was collected for TK evaluations on Day 1 and during Weeks 4 and 13. The HD was selected based on the results of a 14-day oral (gavage and dietary) study of CBD (BDS) in which decreased BW and clinical signs (postdose salivation, hyperactivity followed by hypoactivity, increased response to stimuli) were seen at the HD of 150 mg/kg.

b. Results

There were no drug-related deaths, clinical signs, body weight differences, ophthalmology, or clinical pathology changes. Large livers with correlating organ weight increases and centrilobular hypertrophy were observed at 4- and 13-weeks of treatment, mostly at the HD.

Thyroid follicular cell hypertrophy was also seen and considered secondary to the liver hypertrophy. Week 13 HD exposures (AUC(0-24h)) were 40600/48100 ng.h/mL (males/females).

3. (b) (4)): 13 Week Oral (Gavage) Administration Toxicity Study in the Rat with a 4 Week Interim Phase (Study Number: GWTX1637, conducted by (b) (4), report dated 9/7/17, GLP)

a. Methods

Wistar (HsdHan:WIST) rats (10/sex/grp main, 10/sex/grp interim, 3-6/sex/grp TK) were administered (b) (4) (Batch # 16/20/58FP) at doses of 0 (sesame oil vehicle),1, 10, or 100 mg/kg (1.5 mL/kg) by oral gavage once daily for 13 weeks with an interim sacrifice at 4 weeks. Clinical signs, body weight, food consumption, ophthalmology, clinical pathology, and macroscopic and microscopic anatomic pathology evaluations were performed. Blood was collected for TK evaluations on Day 1 and during Weeks 4 and 13. The HD was selected based on the results of the 14-day oral (gavage and dietary) study of CBD (BDS) and the 13-week study of (b) (4) (see above) and to provide an adequate safety margin to human exposures.

b. Results

Clinical signs of mouth rubbing, salivation, and paddling seen at the MD and HD were considered likely due to the unpalatability of the test material/vehicle. There were no drug-related effect on body weight, food consumption, or ophthalmoscopy; and no deaths. Hepatocyte and follicular cell hypertrophy were seen in the majority of HD animals at 4 and 13 weeks. An increased incidence of the diestrus/metestrus phases of cycle was present in the reproductive tract of females from all drug-treated groups compared to C, although not in a strictly dose-related manner (Table IV.F.3.1). A relationship of these findings to (b) (4) could not be excluded, according to the sponsor. MD (b) (4) exposures (AUC(0-24h)) at 13 weeks were 421/306 ng.h/mL (males/female). LD exposures were not calculable because there were less than 3 quantifiable concentrations above the LLOQ.

Table IV.F.3.1. Incidence of Uterine Finding

		Females			
		1F	2F	3F	4F
Tissue and finding	Level (mg/kg/day)	0	1	10	100
Uterus	No. examined:	10	10	10	10
Dioestrus	Grade -	10	9	9	9
	1	0	1	1	1
	No. examined:	10	10	10	10
Metoestrus	Grade -	7	5	7	4
	1	3	5	3	6
	No. examined:	10	10	10	10
Oestrus	Grade -	8	7	5	9
	1	2	3	5	1
	No. examined:	10	10	10	10
Pro- oestrus	Grade -	5	9	9	8
	1	5	1	1	2

F = Females.
- = Finding not present; 1 = Present.

4. [redacted]: 13 Week Oral (Gavage) Administration Range-finding Study of the constituent Botanical Drug Substances [redacted] present in Sativex Botanical Drug Product (BDP) in the Rat (Study no. GWTX10124, conducted by [redacted], report dated 8/10/12, GLP)

a. Methods

Wistar (HsdHan:WIST) rats (10/sex/group main, 3-6/sex/grp TK) were administered [redacted] (Sativex) (Batch # TM04/107) at doses of 0 (sesame oil vehicle),15, 50, or 150 mg/kg/day (30, 100, or 300 mg/kg active) by oral gavage (1.5 mL/kg) once daily for 13 weeks with an interim sacrifice. Clinical signs, body weight, food consumption, ophthalmology, clinical pathology, and macroscopic and microscopic anatomic pathology evaluations were performed. Blood was collected for TK evaluations on Day 1 and during Weeks 4 and 13. The HD was selected based on the results of the 6-week dietary study of [redacted] at doses of up to 100 mg/kg/day.

b. Results

Mortality was increased in treated groups, mainly at the MD and HD, secondary to convulsions (Table IV.F.4.1).

Table 1. Mortality in rat study of [redacted]

Group/sex	Males				Female			
Dose level (mg ACTIVE/kg/day)	0	15:15	50:50	150:150	0	15:15	50:50	150:150
Main study								
Group size	10	10	10	10	10	10	10	10
Decedents	0	0	0	2	0	1	5	0
Satellites								
Group size	3	9	9	9	3	9	9	9
Decedents	0	0	2	0	0	0	2	1

From Week 2 to the end of the study, MD and HD animals presented with observations up to ~4 hrs post-dose that included decreased activity, mouth rubbing, paddling and salivation. Sporadic incidences of hunched posture, prone, and piloerection were also observed in these animals. From Week 9 to Week 13, a total number of 5 main study animals (4 MD females. 1 HD male) and 5 TK animals (2 male and 2 females at MD and 1 HD female) had what were described as 4 "convulsive–type episodes" before or shortly after dosing and were subsequently terminated. An additional 4 main study (1 LD, 1 MD, and 2 HD females) and 2 TK (1 MD female and 1 HD male) animals had up to 3 convulsive-type episodes before or shortly after dosing during the same period, but survived to the end of the study. According to the report, these episodes were "transient, brief (lasting from approximately 15 seconds to approximately 5 minutes in duration) and were characterized by lateral recumbency followed by forelimb paddling. The affected animals made an immediate and complete recovery and were otherwise unaffected by the episode. There were no signs of body rigidity, body tremors or opisthotonus and the affected animals were eating well and gaining body weight." [redacted] is known to have pro-convulsive activity.

Dose-related decreases in BW gain were seen (37, 48, and 84% less than C in males; 24, 37 and 51% less than C in females, at the LD, MD, and HD, respectively; all SS).

AST was increased (1.3-1.5X) in HD males and MD and HD females and ALP was increased (1.2-3.4X) in MD and HD males and females. CHOL was increased in HD

males (1.4-fold) and in females at all doses (1.5, 1.8, and 1.7X). Bilirubin was increased in HD males (2.8X) and females (1.6X).

Adrenal, kidney, and liver weights were increased and hepatocyte hypertrophy, lymphoid and thymic atrophy, foamy macrophages in the lung, adrenal cortical hypertrophy and vacuolation, seminal vesicle and prostate contraction/atrophy, abnormal/persistent corpora lutea and increased interstitial cells in the ovary, and abnormal/persistent diestrus in the uterus were seen at the MD and HD.

c. Conclusions

Oral (gavage) administration of [redacted] (b) (4) (30, 100, or 300 mg ACTIVE/kg/day) to Wistar rats resulted in decreased body weight gain, convulsions, clinical chemistry changes, and drug-related histopathology findings in liver, spleen, thymus, lung, lymphoid system, adrenal, and reproductive system, primarily at the MD and HD. Plasma levels of [redacted] (b) (4) and CBD measured in this study are shown in Tables IV.F.4.2 and 3 below.

Table IV.F.4.2. TK parameters for [redacted] (b) (4) after administration of [redacted] (b) (4)

Parameter [a]	Period	Male (n=3)		
		Dose of [redacted] (b) (4) (mg ACTIVE/kg/day)		
		30	100	300
AUC_{0-24} (ng.h/mL)	Day 1	3070	12500	101000
	Day 91 (Wk13)	14300	35400	51700
Cmax (ng/mL)	Day 1	207	820	9060
	Day 91 (Wk13)	1720	4260	5670
Tmax (h)	Day 1	24	24	24
	Day 91 (Wk13)	6	6	6

a. Results are reported as mean unless stated otherwise

Parameter [a]	Period	Female (n=3)		
		Dose of [redacted] (b) (4) (mg ACTIVE/kg/day)		
		30	100	300
AUC_{0-24} (ng.h/mL)	Day 1	5630	17300	68700
	Day 91 (Wk13)	14800	27800	43900
Cmax (ng/mL)	Day 1	438	1610	4290
	Day 91 (Wk13)	1690	3090	4580
Tmax (h)	Day 1	6	24	24
	Day 91 (Wk13)	6	4	6

a - Results are reported as mean unless stated otherwise

Table IV.F.4.3. TK parameters for CBD after administration of [redacted] (b) (4)

Parameter [a]	Period	Male (n=3)		
		Dose of [redacted] (b) (4) (mg ACTIVE/kg/day)		
		30	100	300
AUC_{0-24} (ng.h/mL)	Day 1	1050	5590	48700
	Day 91 (Wk13)	9610	24600	28800
Cmax (ng/mL)	Day 1	73.0	412	4530
	Day 91 (Wk13)	1140	2860	3410
Tmax (h)	Day 1	24	24	24
	Day 91 (Wk13)	6.0	6.0	2.0

a. Results are reported as mean unless stated otherwise

Parameter [a]	Period	Female (n=3)		
		Dose of [(b) (4)] (mg ACTIVE/kg/day)		
		30	100	300
AUC_{0-24} (ng.h/mL)	Day 1	2120	6360	29900
	Day 91 (Wk13)	8110	16500	22200
Cmax (ng/mL)	Day 1	161	623	1950
	Day 91 (Wk13)	913	1850	2460
Tmax (h)	Day 1	6	24	24
	Day 91 (Wk13)	6.0	4.0	2.0

Genotoxicity

1. [(b) (4)] was negative in an Ames assay at up to 5000 µg/plate with or without S-9 (GWOR0963) and in a rat micronucleus assay at doses up to 750 mg/kg/day (GWOR0965); and did not induce DNA damage in the liver of rats treated up to 750 mg/kg/day in the COMET assay in vivo (GWOR1206).

2. [(b) (4)] was negative in an Ames assay at concentrations up to 5000 µg/plate with or without S-9 (GWTX1597) and did not induce an increase in micronucleated PCE in the bone marrow of rats or DNA damage in the liver of rats at doses up to 500 mg/kg/day in a combined alkaline COMET and micronucleus assay in rats (GWTX1598).

3. [(b) (4)] was negative in an Ames assay at concentrations up to 5000 µg/plate with or without S-9 (GWTX1625), and in a combined alkaline COMET and micronucleus assay in rats at doses up to 500 mg/kg/day (GWTX1624).

4. [(b) (4)] was negative in an Ames assay at concentrations up to 2700 µg/plate with or without S-9 (GWTX0603), and in a combined alkaline COMET and micronucleus assays in rats at given aged Sativex (expressed in terms of [(b) (4)] at calculated doses up to 73 mg/kg/day (GWTX1355).

Developmental toxicity

1. [(b) (4)]: Oral (Gavage) Embryo-Foetal Development Study in the Rat (GWTX1462)

Administration of [(b) (4)] (0, 80, 125, or 200 mg/kg/day) to Wistar rats by oral gavage on GDs 6-17 produced minimal maternal toxicity (slight reduction in body weight gain at HD) but no adverse effects on pregnancy outcome or embryofetal development.

2. [(b) (4)] Oral (Gavage) Study of Embryo-Foetal Development in the Rat (GWTX15109)

Administration of [(b) (4)] (0, 1, 10, or 100 mg/kg/day) to Wistar rats by oral gavage on GDs 6-17 produced a transient decrease in maternal BW gain at the HD but no adverse effects on pregnancy outcome or embryofetal development.

3. [redacted] Oral (Gavage) Embryo-Foetal Development Study in the Rat (GWTX1622)

Administration of [redacted] (0, 1, 10, or 100 mg/kg/day) to Wistar rats by oral gavage on GDs 6-17 produced a transient decrease in maternal BW gain at the HD but no adverse effects on pregnancy outcome or embryofetal development.

4. Oral (Gavage) Developmental Toxicity Study [of [redacted]] in the Rat (Study Number: JJG0015, conducted by [redacted], report dated 4/13/05, GLP)

a. Methods

Mated female SD rats (24/group) received oral (gavage, 5 ml/kg) doses of 0 (sesame oil vehicle), 1, 5, or 25 mg/kg/day [redacted] (batch # PN1-140) from GD 6 through GD 17. Blood samples were taken for TK evaluation pre-dose and 1, 2, 4, 8, and 24 hours after dosing on GDs 6 and 17. Clinical signs, body weights, and food consumption were recorded in dams. Animals were killed on GD 20 and pregnancy outcome was evaluated. Fetuses were evaluated for external (all), visceral (half fresh dissection, half Bouins' sectioning), and skeletal (half) malformations and variations. Doses were based on the results of a dose range-finding study.

b. Results

i. Maternal observations

There were no drug-related deaths. Drug-related clinical signs at the MD and HD included decreased activity, piloerection, rapid breathing, and partially closed eyes. BW gain during the dosing period was decreased (29 and 46% compared to C, both SS) at the MD and HD, and BWs were decreased (8 and 9%, both SS) in these groups at the end of gestation.

GD 17 maternal CBD exposures (AUC0−t) were 15.8, 1834, and 18171 ng.h/mL and [redacted] exposures were 68, 4657, and 40680 ng.h/ml at the LD, MD, and HD, respectively.

ii. Litter parameters and fetal evaluations

Fetal body weights were dose-dependently decreased at the MD and HD (mean fetal BWs 4.01, 4.01, 3.91, and 3.76 gm in C, LD, MD, and HD, respectively). There were no effects on other pregnancy or litter parameters. There were no drug-related increases in malformation incidences. Higher incidences of fetal variations (called minor abnormalities in study report) generally associated with decreased fetal body weight and developmental delay (incomplete or non-ossification of the skeleton) were seen at the MD and HD.

c. Conclusions:

Oral (gavage) administration of [redacted] (1, 5, or 25 mg ACTIVE/kg/day) to pregnant SD rats resulted in maternal (body weight gain) and developmental toxicity (decreased fetal body weights and increased fetal variations) at the MD and HD.

V. SUMMARY AND EVALUATION

Pharmacology

Cannabidiol (CBD) is a major component of cannabis. While cannabis and Δ^9-THC (the major psychoactive component) have been reported to be proconvulsant in some animal models, CBD has generally been shown to have anticonvulsant activity in various acute animal seizure models (MES and GABA-inhibition-based models, audiogenic seizures, electrical kindling), with a profile, therapeutic index, and potency comparable to established anticonvulsant drugs such as phenytoin, phenobarbital, and carbamazepine (Consroe and Wolkin, J Pharmacol Exp Ther 201:26-32, 1977). The antiepileptic mechanisms of CBD are not known, but unlike Δ^9-THC, CBD does not activate CB_1 or CB_2 receptors. At low micromolar to sub-micromolar concentrations, CBD has been shown to inhibit the equilibrative nucleoside transporter (ENT), orphan G-protein-coupled receptor GPR55, and the transient receptor potential of melastatin type 8 (TRPM8) channel; enhance the activity of 5-HT_{1a} receptors, α3 and α1 glycine receptors, the transient receptor potential of ankyrin type 1 (TRPA1) channel; and to have a bidirectional effect on intracellular calcium. The sponsor emphasized the effects on calcium channels. It is thought that CBD may reduce neuronal excitability by modulating intracellular calcium through its interactions with targets such as TRP channels, GPR55, or VDAC1.

CBD BDS inhibited hERG tail currents at therapeutic concentrations (IC_{50} of 130 ng/mL CBD; clinical Cmax 200-300 ng/mL at 20 mg/kg dose). In the cardiovascular safety pharmacology study in the dog, oral CBD BDS decreased heart rate and increased systolic blood pressure and R-R, R-H, QRS, and QT (but not corrected QT) intervals. Decreased heart rate, but without any rhythm disturbances, was also seen in the chronic dog toxicity study of Purified CBD. There were no other safety pharmacology signals; however, there were no CV safety pharmacology studies conducted with purified CBD.

ADME

Bioavailability of CBD after oral administration appears to be very low, in part due to significant first-pass metabolism in the liver; however, the sponsor did not provide actual F values (or other PK parameters) for the toxicology species. The sponsor determined the oral bioavailability of CBD in the clinical sesame oil-based formulation to be 15% in the minipig, and a literature study of CBD in the dog reported an oral bioavailability of 13-19% (Samara et al, Drug Metab Dispos 16:469-472, 1988).

Tissue distribution of CBD was extensive, presumably due to its high lipophilicity, and the drug was highly protein bound (>94% in rat, dog, and human).

Following absorption, CBD is mostly eliminated by metabolism. Based on literature and studies conducted by the sponsor, the main routes of CBD metabolism appear to be direct glucuronidation and oxidation to form 7-OH-CBD, which is further oxidized to 7-COOH-CBD. This acidic metabolite circulates at levels far in excess of parent in human, representing at least 90% of all drug-related material measured in plasma. 7-OH-CBD, which appears to have pharmacological activity based on animal seizure model testing, circulates in human plasma at levels of approximately 50% those of parent. Based on limited animal seizure model testing and binding analysis, 7-COOH-CBD is thought to be pharmacologically inactive (although it showed some transporter interactions). Hepatocyte incubations with CBD also indicated that direct glucuronide conjugation of CBD and conjugation following hydroxylation were important metabolic routes. This study also showed that the acid metabolite was produced at relatively high levels. However, the acyl glucuronide (glucuronide of the acid group of 7-COOH-CBD) was not detected in incubation periods of up to 4 hours. This suggested that conjugates of 7-COOH-CBD may occur in vivo, but whether acyl glucuronides are present is unknown. According to the sponsor, “it is therefore important to determine the nature of these conjugates since the glucuronide

metabolite has shown some instability as it undergoes acyl migration." But no additional data examining this possibility were submitted.

In rats, metabolites were excreted in the feces and, to a much lesser extent, in the urine. The terminal half-life of CBD in humans is estimated at 18–32 hours.

Safety Margin

The safety margins for CBD and its major human metabolites in the oral gavage 13-week mouse, chronic rat and dog toxicity, and rat embryofetal development studies of Purified CBD are shown in the sponsor's Table 1.1.1.1.2.2-1 below.

Table 1.1.1.1.2.2-1 Margin of Safety for CBD, 7-COOH-CBD and 7-OH-CBD in Studied Populations

Species	NOAEL (mg/kg)	CBD		7-COOH-CBD		7-OH-CBD		Study Reference
		$AUC_{(0-24)}$ at NOAEL (ng·h/mL)	Margin of Safety[a,b] calculated using AUC	$AUC_{(0-24)}$ at NOAEL (ng·h/mL)	Margin of Safety[a,c] calculated using AUC	$AUC_{(0-24)}$ at NOAEL (ng·h/mL)	Margin of Safety[a,d] calculated using AUC	
Mouse (male)	300	44300	15.9	30100	0.22	19800	12.7	GWTX1503 (13 week)
Mouse (female)	300	46400	16.6	38800	0.28	35700	22.9	
Rat (male)	150	60000	21.5	37100[e]	0.27	2560	1.6	GWTX1412 (26 week)
Rat (female)	150	67500	24.2	40500[e]	0.29	6730	4.3	
Dog (male)	100	20500	7.3	994	0.01	1380	0.88	GWTX1413 (39 week)
Dog (female)	100	22400	8.0	1560	0.01	1090	0.70	
Rat (maternal)	250	170000	60.9	155000	1.12	12600	8.1	GWTX1454 (Embryo-Fetal Development)

NOAEL = No-observed-adverse-effect level

[a] From Clinical Study GWEP1544, $AUC_{(0-t)}$ at steady state following multiple oral doses of a clinical dose of 750 mg CBD twice daily; value used was Day 7 morning dose of 750 mg CBD. Margin of safety calculations were done using an AUC that was extrapolated for 20 mg/kg per dose. Human AUC was doubled to match the preclinical dosing interval.

[b] Adjusted human $AUC_{(0-24)}$ for CBD is 2790 ng.h/mL.

[c] Adjusted human $AUC_{(0-24)}$ for 7-COOH-CBD is 137,886 ng.h/mL.

[d] Adjusted human $AUC_{(0-24)}$ for 7-OH-CBD is 1,562 ng.h/mL.

[e] Anticipated under representation; degree of bias could be between 30% and 50% from the actual value.

As shown in the sponsor's table above, margins for the major human metabolites are minimal (7-OH-CBD) or non-existent (7-COOH-CBD) in both rat and dog. The highest exposures to 7-COOH-CBD in oral studies conducted with Purified CBD were measured in the juvenile rat study, where PND 70 exposures were 171000 and 189000 ng.h/mL in males and females, respectively, at the HD of 250 mg/kg. According the FDA clinical pharmacology reviewer (Jagan Parepally), the plasma AUC for 7-COOH-CBD at a 1500 mg dose in a multiple dose study was 151336 ng.h/mL, and exposures were 2- to 3-fold higher in food effect studies.

As indicated in the above table, there was uncertainty about some metabolite levels measured in the pivotal oral toxicity studies due to questions about the bioanalytical procedures. In the chronic rat study report, it was noted by the sponsor that a potential bias in the assessment of 7-COOH-CBD was identified during a review of the stability data generated for 7-COOH-CBD in the rat plasma validation study (b) (4) 276174QB02 (see sponsor's statement on page 28 above).

In the response to the 74-day letter (dated 1/18/18), the sponsor indicated that irregularities in the bioanalysis procedures went beyond what had initially been described in the chronic rat toxicity study report, such that the TK sections of the reports were no longer considered to conform to GLP. These bioanalysis issues potentially impact the accuracy of several metabolite determinations including those for both 7-OH-CBD and 7-COOH-CBD.

> The Sponsor would like to inform FDA of an ongoing Good Laboratory Practice (GLP) compliance investigation and communication received from Medicines and Healthcare products Regulatory Agency (MHRA) to the Sponsor on 19 December 2017 pertaining to

several near-final nonclinical reports included in the NDA. (b) (4)

(b) (4)

In all studies, no issues of scientific validity were ever identified for the data obtained for Parent CBD. Therefore, adequate, scientifically valid, toxicokinetic data has been obtained to underwrite the toxicological assessment of CBD. Moreover, while some compromised data have been generated in individual studies for certain analytes, our assessment of the data suggests that it remains semi-quantitatively indicative of the exposure to metabolite and, where it could be assessed, was within a margin of error (e.g. <50% for preparation bias). As a consequence of these events, the Sponsor has initiated an expert, independent review of the scientific validity of the bioanalytical phase of the study reports. The Sponsor will use the findings of this independent review to further facilitate discussion with regulators, inform the initiation and execution of additional studies where necessary to mitigate against the consequences of this incident and further inform our drug development program.

Thus, the TK data for CBD metabolites from most of the pivotal toxicity studies should be considered suspect. This is a major deficiency that impacts the adequacy of the nonclinical evaluation, particularly with respect to coverage of the major active human metabolite, 7-OH-CBD, as well as the predominant drug-related species in humans, 7-COOH-CBD.

Aside from the bioanalytical issues, the clear species differences in CBD metabolism raise serious questions about the adequacy of the nonclinical evaluation. The failure of the sponsor to adequately evaluate the toxicity of 7-COOH-CBD was noted as a serious deficiency in the 74-day letter (dated 12/20/17). In response, the sponsor proposed to conduct additional studies with the synthesized metabolite, including genotoxicity testing (Derek for 7-COOH-CBD was negative, but this is obviously not an adequate assessment for a major metabolite). However, the sponsor's arguments for the adequacy of the toxicity studies are not supported by the available information. They state: "While GW accepts that human exposure levels are around 10 fold greater than animal exposure, there has been no safety signals in the clinical trials related to 7-COOH-CBD." But the clinical safety data obviously would not be adequate to rule out long-term effects due to the metabolite. They erroneously referenced the M3(R2) ICH guideline Q&A ("characterization of metabolite toxicity would generally be considered adequate when animal exposure is at least 50% the exposure seen in humans") and the Safety Testing of Drug Metabolites Guidance, which states that "If at least one animal test species forms this drug metabolite at adequate exposure levels (approximately equal to or greater than human exposure), as determined during toxicology testing of the parent drug, it can be assumed that the metabolite's contribution to the overall toxicity assessment has been established." These clearly do not apply to the current situation in which the metabolite in question is present at levels many times greater than those of the parent. And there were only 2 studies in which exposure to the 7-COOH-CBD metabolite approached that in humans (juvenile rat and rat EFD studies). The sponsor also argues that since the toxicological findings were similar between the juvenile rat study, in which the highest 7-COOH-

CBD exposures were measured, and other studies, including those in dogs, in which exposures to this metabolite were low, the toxicological effects were not driven by 7-COOH-CBD exposure. The sponsor stated:

> Specifically, it is notable that when the toxicological consequences and relative exposures of 7-COOH-CBD are compared between the adult animals of the two major nonclinical species examined (rat and dog), target organ pathology remained consistent (hepatic) despite a ~40 fold difference in exposure. By comparison, differences in exposure for CBD and 7-OH-CBD were at least an order of magnitude lower (~2-3 fold) between these species. Therefore, in summary, it does not appear that 7-COOH-CBD contributes significantly to the toxicological outcomes observed within and between the species investigated.

But while it may be reasonable to conclude that 7-COOH-CBD did not contribute significantly to the hepatic effects seen in the rat and dog toxicity studies, the metabolite could have contributed to the more serious effects (including deaths and renal toxicity) seen in the juvenile rat and repeat (GWTX1688 not GWTX1503 in the table above) 13-week mouse toxicity studies in which levels of the metabolite were higher than those measured in the chronic toxicity studies. However, males were generally more affected than females in the repeat 13-week mouse toxicity study despite much lower 7-COOH-CBD exposure, and it seems more likely that the higher plasma levels of 7-OH-CBD in the mouse would be responsible for the apparent species difference in response between mouse and rat. Males were also generally more affected in the juvenile rat study, in which there was no sex difference in exposure to 7-COOH-CBD. The fact remains, however, that the toxic (including carcinogenic) potential of 7-COOH-CBD exposures comparable to those expected in humans has not been evaluated.

Toxicology

Purified CBD (formulated in (b) (4) sucralose, strawberry flavoring, and sesame oil as in the clinical OS) was evaluated in repeat-dose general toxicity studies in mice, rats, and dogs by oral (gavage) administration for up to 13, 26, or 39 weeks, respectively. The representative clinical plasma CBD exposure (AUC_{0-24}) for comparison with TK data from the toxicity studies is approximately 2800 ng.h/mL. (Metabolite exposure comparisons are discussed above.) In general, CBD exhibited a relatively benign toxicity profile in the chronic toxicity studies, although the adequacy of dose selection was an issue in both of these studies; adaptive liver changes were the primary findings in rat and dog. However, more serious nephrotoxicity resulting in deaths was seen in the mouse, and adverse developmental effects were observed in the PPND and juvenile rat studies.

In the 13-week (CD-1) mouse study (0, 400, 550, or 700/625 mg/kg/day), there were 7 early deaths (6 males) at the high dose (HD) that were attributed to drug-induced nephropathy. Clinical signs at the HD included convulsions (1 HD female) and tremor. The HD was subsequently lowered to 625 mg/kg/day beginning at Week 6. Clinical chemistry changes included increased ALT (up to 2.5X), creatinine (up to 50%), cholesterol, total protein, and globulin. At necropsy, liver weights were increased in both sexes at all doses and large liver was noted macroscopically in some animals from all drug-treated groups. These correlated with dose-related increases in the incidence and severity of hepatocyte hypertrophy seen at all doses in both sexes. A dose-related increase in the incidence and/or severity of nephropathy was also observed microscopically and was considered adverse in MD and HD males and in HD females due to the severity and extent of the lesion. Based on the clinical observations, early deaths, and nephropathy noted in MD and HD males and HD females, the NOAEL was considered to be 400 mg/kg/day for males (CBD exposure 37800 ng·h/mL) and 550 mg/kg/day for females (41200 ng·h/mL).

In the 26-week (Wistar) rat study (0, 15, 50, or 150 mg/kg/day), there were no drug-related deaths. Centrilobular hypertrophy at the MD and HD was associated with increased ALT and

ALP, increased liver weight, and macroscopic liver enlargement at the HD. These findings demonstrated reversibility and were not considered toxicologically significant. The NOAEL (150 mg/kg) was associated with CBD exposures of 60000 and 67500 ng·h/mL in males and females. Dose range-finding was clearly inadequate and dose selection inappropriate, since the HD was based on a 14-day TK study of CBD-BDS (65.6% CBD) comparing oral gavage and dietary dosing in rats. However, CBD exposures in the chronic study were >20X those expected in humans. Major metabolite levels were of course much lower relative to clinical.

No range-finding study was conducted for the chronic dog study of Purified CBD. The sponsor stated that the HD was based on the results of the cardiovascular safety pharmacology study of CBD BDS and 4- and 52-week dog studies of Sativex (1:1 THC:CBD). However, the doses used in the 39-week dog study of Purified CBD (0, 10, 50, or 100 mg/kg/day) reduced BW gain over the course of the study in MD and HD males and in females at all doses such that the final BWs were decreased 12 and 32% in HD males and females. There were consistent decreases in heart rate in HD males but no drug-related cardiac rhythm disturbances. Drug-related liver changes consisted of hepatocyte hypertrophy associated with increased liver weight, macroscopic enlargement, and marked increases in ALP (up to 8-fold compared to controls), which were seen at all doses. These were considered adaptive and demonstrated reversibility. The NOAEL (100 mg/kg) was associated with CBD exposures of 20500 and 22400 ng·h/mL in males and females, which are lower than those seen in rats but still 7- to 8-fold expected human exposures. Exposure to the major active human metabolite, 7-OH-CBD, in dogs was less than (0.7-0.9X) that seen clinically, and exposure to 7-COOH-CBD was 1/100th that in humans.

Purified CBD and CBD BDS were negative in the Ames test at concentrations up to 5000 µg/plate, with or without metabolic activation (S-9). In in vivo micronucleus assays, Purified CBD and CBD BDS did not induce micronuclei (MN) in the polychromatic erythrocytes (PCE) of the bone marrow of rats at oral (gavage) doses up to 500 or 350 mg/kg/day, respectively. CBD-OS did not induce DNA damage in the liver of rats at oral (gavage) doses of up to 500 mg/kg/day in the alkaline COMET assay.

A 104-week dietary carcinogenicity study was conducted in Wistar rats with CBD BDS (58 – 67% CBD) at concentrations intended to achieve doses of 5, 15, or 50 mg/kg/day (expressed in terms of CBD). No SPA was submitted for this study. There were no apparent effects of treatment on survival and no toxicity findings considered to be of toxicological significance. A dose-related reduction in overall body weight gain and food consumption was seen at the MD and HD that resulted in a greater than 10% reduction in body weight in both sexes at the HD (20 and 24% in M & F). There was no apparent increase in the incidence of neoplasia, alteration in the time of tumor onset, or induction of rare tumors (see statistical review by Stephen Thompson). Non-neoplastic findings included an increased incidence of centrilobular hypertrophy in the liver of both sexes at the HD. TK data from this study were limited to plasma measurements of CBD, THC, and 11-OH THC at 2 timepoints during the day (morning and evening).

An agreement was made with the sponsor that an oral gavage mouse carcinogenicity study of CBD-OS could be submitted postmarketing. However, the current rat study is considered inadequate, due to use of the (b) (4) BDS form of CBD, containing a relatively low percentage of CBD and a significant amount of (b) (4) and other impurities that could interact with the compound of interest, and dietary administration, which provided an uncertain but apparently low exposure to CBD (maximum measured levels of 841.6 and 722.9 ng/mL in males and females compared to a Cmax of approximately 300 ng/mL at a therapeutic dose in patients and 6-7000 ng/mL in, for example, the chronic rat toxicity study of Purified CBD). The HD was approximately 0.4 times the MRHD on a mg/m2 basis.

In the oral (gavage) fertility and early embryonic development toxicity study of Purified CBD (b) (4) (b) (4) (b) (4) in Wistar rats (0, 75, 150, or 250 mg/kg/day), there were no apparent drug-related effects on male or female reproductive indices, male reproductive organ

weights, female estrus cycling, or any cesarean-section parameters. HD selection was questionable, however, as it only decreased body weight gain in males during the post-pairing phase (21 and 25% at MD and HD, respectively; SS). During the pre-pairing phase, there were drug-related increases in bodyweight gains in MD and HD females compared to C (48 and 54%, respectively; SS). There were slight dose-related decreases (up to 5%) in BW gain in females during gestation. TK was not assessed in this study, but based on maternal exposure in the rat EFD study (170000 ng.h/mL on GD 17), CBD exposures at the HD would be expected to have been large multiples (>50) of the human exposure.

In an oral (gavage) rat (Wistar) embryofetal development (EFD) study of Purified CBD (0, 75, 150, or 250 mg/kg/day; HD associated with maternal BW reductions), total litter loss in 2 of 20 dams at the HD was considered drug-related. There were no drug-related effects on fetal weights or fetal abnormalities. The MD was considered the NOAEL for embryofetal toxicity based on embryolethality at the HD. Maternal exposure (AUC) at this dose was 149000 ng·h/mL on GD17.

In an oral (gavage) rabbit (NZW) EFD study of Purified CBD (0, 50, 80, or 125 mg/kg/day), fetal BWs were decreased (10%) and fetal variations (unossified metacarpal, bulging eyes, and nonerupted incisors) increased at the HD, which was also associated with evidence of maternal toxicity. The NOAEL for embryofetal developmental toxicity (80 mg/kg) was associated with a GD 19 maternal exposure of 2030 ng·h/mL (i.e., much lower exposures than in rats).

In an oral (gavage) rat (Wistar) prenatal and postnatal development (PPND) study of Purified CBD (0, 75, 150, or 250 mg/kg/day), developmental toxicity was seen at doses that were not maternally toxic. Developmental effects consisted of decreased pup body weights at birth and throughout lactation, delays in achieving developmental landmarks (pinna unfolding, eye opening, pupillary reflex, male and female sexual maturation), neurobehavioral changes (decreased locomotor activity), and adverse effects on reproductive system structure (small testis) and function. These effects were primarily seen at the MD and HD, but small testis was also found at the LD. The LOAEL for developmental toxicity (75 mg/kg) was associated with maternal CBD exposures of approximately 25900 and 86300 ng.h/mL on GDs 6 and 17, respectively (based on TK in the rat EFD study, since plasma levels were not collected in the PPND study).

In a 10-week neonatal/juvenile toxicity study in Wistar rats, Purified CBD (0 or 15 mg/kg/day) was given subcutaneously on PND 4 to 6 followed by oral (gavage) doses of 0, 100, 150, or 250 mg/kg/day Purified CBD-OS from PND 7 to 77. Drug-related findings included 3 HD deaths (there were 17 deaths during the study, but only 3 HD male deaths on PNDs 12, 15, and 26 were considered drug-related), dose-dependent increases in body weight gain, neurobehavioral deficits (decreased locomotor activity and startle habituation, both persisting after recovery period; but no effect on learning and memory in Morris water maze), delayed sexual maturation in males, transient increases in plasma calcium in males and increased cholesterol, total protein, and globulin in females, a reversible increase in bone mineral density in males (HD), and hepatocyte hypertrophy and vacuolation that remained after the recovery period at the MD and HD. While adaptive liver changes were seen in adult rats as well as mice and dogs, hepatocellular vacuolation had not been reported. Whereas increases in hepatocyte hypertrophy and liver weight with no other effects are generally considered to be adaptive and not treated as adverse effects, histopathological findings of hepatocellular vacuolation (and necrosis, which was not seen in this study) are considered adverse. The results of this study indicated CBD effects on CNS and reproductive system development, in agreement with findings in the PPND study, as well as effects on bone development that appeared to be associated with changes in calcium homostasis. The LOAEL (15 SC/100 PO mg/kg/day) was associated with a PND 70 exposure to CBD of 82000 ng·h/mL (sexes combined). Exposure to the major active metabolite, 7-OH-CBD, was about 4-5X that expected clinically at the HD (6150 and 6920 ng.h/mL in males and females). Exposure to the major (putatively) inactive metabolite, 7-COOH-CBD was somewhat higher than that expected in humans (without the food effect) at the HD (171000 and 189000 ng.h/mL in males and females).

Analysis of CBD (API) revealed [redacted]) at levels greater than the ICH qualification threshold, and the specified limits for these impurities are [redacted] higher than the actual levels in the nonclinical batches. Therefore, each of these impurities was qualified in a battery of studies including in vitro and in vivo genotoxicity tests, 13 or 26-week oral (gavage) toxicity studies in rat; and rat embryofetal development studies. [redacted] was qualified [redacted] in CBD toxicity studies. All other studies were performed on purified materials.

New toxicities not seen with Purified CBD were identified in the 13-week rat toxicity studies of [redacted] and Sativex [redacted] In the [redacted] study, an increased incidence of the diestrus/metestrus phases of cycle was present in the reproductive tract of females from all drug-treated groups compared to C, although not in a strictly dose-related manner. A relationship of these findings to [redacted] could not be excluded, according to the sponsor. In the Sativex study, convulsions and reproductive system histopathology (seminal vesicle and prostate contraction/atrophy, abnormal/persistent corpora lutea and increased interstitial cells in the ovary, and abnormal/persistent diestrus in the uterus) were observed. Convulsions or convulsive-type episodes are consistent with those reported in toxicology studies of [redacted] (5-500 mg/kg) in rats and mice conducted by [redacted] Changes in the reproductive tract were also thought (by the sponsor) to be consistent with the pharmacological action of [redacted] are expressed in the ovary and are thought to mediate inhibition of follicular steroidogenesis. The increased incidence of diestrous seen in the uterus was thought to be consistent with cessation of cycling and perturbation of sex hormone levels. And perturbation of sex hormone levels in the rodent is frequently accompanied by ovarian interstitial cell hyperplasia. The plasma levels of [redacted] and [redacted] associated with these effects were much greater than those expected clinically.

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