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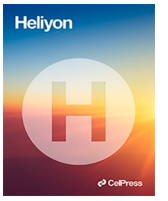

Toxicological safety assessment of HempChoice® hemp oil extract; a proprietary extract consisting of a high concentration of cannabidiol (CBD) in addition to other phytocannabinoids and terpenes derived from *Cannabis sativa*L

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A R T I C L E I N F O

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A B S T R A C T

HempChoice® Hemp Oil Extract (Geocann, LLC) is an extract of the aerial parts of hemp (*Cannabis sativa* L.) primarily comprised of 55–75% cannabidiol (CBD), 1–15% other phytocannabinoids and 1–15% terpenes. The results of multiple safety studies demonstrated that it was non-mutagenic in an Ames and mammalian cell micronucleus.

test and was well tolerated in a 14-day range-finding study at dose levels up to 96.03.

mg/kg BW/day. In the 90-day study, no HempChoice® Hemp Oil Extract-related significant changes were noted in weekly BW, daily BW gain, food consumption, functional observational battery or motor activity assessment. In addition, no HempChoice® Hemp Oil Extract related mortalities, abnormal clinical observations and ophthalmological changes were reported. Some HempChoice® Hemp Oil Extract-related changes were reported in the hematology and clinical chemistry parameters evaluated. These changes were not outside the normal range and were considered reversible during the 28-day recovery period. No macroscopic findings were reported, and histopathological changes related to HempChoice® Hemp Oil Extract exposure were limited to adaptive changes in the liver which were not observed in the recovery group animals. The no observed adverse effect level (NOAEL) for HempChoice® Hemp Oil Extract was determined to be 185.90 mg/kg BW/day in male and female Sprague-Dawley rats.

1. Introduction

The plant *Cannabis sativa* L. (hemp) comprises a wide variety of phytocannabinoid and terpene compounds, including the constituent cannabidiol (CBD) [1]. In recent years, CBD has gained increasing interest due to its potential health benefits [2–5]. While considerable research has been undertaken to identify and characterize the compounds in hemp as well as identify their potential health benefits, analyzing human pharmacokinetics, and developing a variety of hemp products, there is a clear need for determining the safety of these compounds [6–8].

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In recognition of the health interests in these compounds, the US Congress passed the Hemp Production Act [9], often referred to as the 2018 Farm Bill, which included changes to the production and marketing of hemp and derivatives of cannabis that have less than 0.3% delta-9-tetrahydrocannabinol (THC) [9]. This regulatory change has resulted in the increase of availability of products containing CBD and other phytocannabinoids and terpenes, many without known safety data.

THC and synthetic cannabinoids have direct affinity for cannabinoid receptors (CBR1 and CBR2), while CBD does not have affinity for these receptors [6,10,11]. Epidiolex (Greenwich Biosciences, Carlsbad, CA) is a CBD drug that is approved by the US FDA as a treatment for childhood seizures associated with Lennox-Gastaut syndrome and Dravet syndrome [12]. With the increasing interest in using hemp extract products containing CBD in humans for an assortment of health benefits, it is essential to fully evaluate the safety of CBD-containing hemp extracts [13–16]. The current laboratory animal and human data provide substantial evidence that these extracts are safe in humans [13–17].

Reviews and research reports in humans and laboratory animals have described the *in vivo* and *in vitro* toxicologic effects of CBD-containing hemp extracts at various dosage levels [13,18–22]. The authors concluded that these studies generally support the conclusion that CBD-containing hemp extracts are safe for humans inclusive of intermittent chronic use. However, since there are variations in the methods used to manufacture hemp extracts, additional studies are needed to evaluate the safety of specific hemp extract preparations.

The objective of the current studies was to assess the genotoxicity and preclinical safety of a proprietary hemp extract (HempChoice® Hemp Oil Extract). There are no acceptable alternatives to the use of live animals to accomplish the objective of this study because of the current state of scientific knowledge. The results of these multiple safety studies are reported here, including a bacterial reverse mutation (Ames) assay and a micronucleus assay conducted in human lymphocytes, as well as a 14-day range-finding study and a GLP compliant 90-day repeat dose study with a 28-day recovery period, both in male and female Sprague-Dawley rats.

2. Material and methods

2.1. GLP, OECD compliances

A GLP compliant bacterial reverse mutation assay and micronucleus assay were conducted with HempChoice® Hemp Oil Extract. In addition, the same HempChoice® Hemp Oil Extract was used in two preclinical animal studies: a 14-day range-finding study (14-day study) and a 90-day study with a 28-day recovery period (90-day study). All studies were conducted in GLP compliant facilities. The Ames test and the micronucleus assay were compliant with the OECD Guidelines for the Testing of Chemicals, Section 4, Test No 471 and 487, respectively [23,24]. The 14-day study was compliant with the OECD Guidelines for Testing of Chemicals, Section 4, Test No. 407, the US FDA Toxicological Principles for the Safety Assessment of Food Ingredients (Chapter IV.C. 4. a.) Animals [25,26]. The 90-day study was also compliant with the OECD Guidelines for Testing of Chemicals, Section 4, Test No. 408 and the US CFR Title 21 Part 58 Good Laboratory Practice for Nonclinical Laboratory Studies [27]. Both studies were compliant with the NRC Guide for the Care and Use of Laboratory Animals [28]. The in-life procedures and tissue harvests for the 14-day and 90-day repeat dose studies were performed at Product Safety Labs' (PSL) test facility in Dayton, New Jersey which is accredited by the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC). The use of animals in the described studies were reviewed and unanimously approved by the PSL Institutional Animal Care and Use Committee under Animal Use Protocols P710 and P713.

2.2. Test material

The test article was provided by Geocann (320 E Vine Drive, Suite 207, Fort Collins, CO 80524). HempChoice® Hemp Oil Extract is produced from the aerial parts of the *Cannabis sativa* L plant using supercritical CO_2 extraction manufacturing compliant with current US Good Manufacturing Practices (CGMP). Geocann has established specifications for HempChoice® Hemp Oil Extract and each lot manufactured must meet the established specifications. These specifications require that each lot contain between 55 and 75% cannabidiol (CBD) alone, 1–15% other phytocannabinoids, $\leq$0.3% tetrahydrocannabinol (THC) and 1–15% terpenes. Therefore, approximately 100% of the constituents of this proprietary hemp extract are accounted for. HempChoice® Hemp Oil Extract complies

Table 1
Specifications for HempChoice® hemp oil extract.

Parameter	Specification	Testing Method
Identification		
Appearance	Clear, amber oil	Visual
Color	Amber	Visual
Odor	Characteristic	Olfactory
Phytochemicals		
CBD (%)	55–75	HPLC/UPLC
Total Other Phytocannabinoids (%)	1–15	HPLC/UPLC
Terpenes (%)	1–15	GC

CBD – cannabidiol; HPLC/UPLC = High Performance Liquid Chromatography/Ultra High-Performance Liquid Chromatography; GC = Gas chromatography.

with the Federal requirements for hemp products because it contains less than 0.3% THC as specified under the 2018 Agriculture Improvement Act. The three different lots of HempChoice® Hemp Oil Extracts used in the studies met the specifications which are outlined in Table 1 and the composition information of each of these three lots can be found in Table 2 and HPLC chromatograms for each lot are shown in Fig. 1. HempChoice® Hemp Oil Extract is manufactured from a botanical source and some variation in the phytochemical composition is expected. The composition of the lots used in the studies, as found in Table 2 and Fig. 1, show that the raw material selection and manufacturing processes are well controlled so that there is consistency between lots and that they remain within the specifications which Geocann has set (Table 1). In addition to the parameters outlined in Tables 1 and 2, HempChoice® Hemp Oil Extract meets or exceeds the specification limits set by Geocann for solvents, heavy metals, and pesticides and does not exceed the limits specified for microbiology.

2.2.1. Test material preparation

For the bacterial reverse mutation (Ames) assay, the test article was prepared as a solution in dimethyl sulfoxide (DMSO) at concentrations of 0.00531, 0.01593, 0.0210, 0.0531, 0.0664, 0.15934, 0.210, 0.5311, 0.664, 2.1, 6.64, 21 and 66.4 mg test article/mL to provide final dose levels of up to 6640 μg/plate. The solutions were then vortexed prior to use. In the micronucleus test, the HempChoice® Hemp Oil Extract was dissolved in DMSO at a 100-fold increased concentration and then diluted in cell culture medium to obtain the required final test material concentrations. The final concentration of DMSO in the samples was 1% (v/v).

For both the 14-day and 90-day studies, the test article was mixed weight to volume (w/v) in corn oil (Sigma Aldrich, Lot #MKCK641) to obtain the required concentrations, and fresh formulations were prepared daily. For the 14-day study, formulations containing 8.23, 13.71, and 19.20 mg HempChoice® Hemp Oil Extract/mL were used, and for the 90-day study, formulations containing 7.9, 18.59 and 37.18 mg/mL of HempChoice® Hemp Oil Extract were used. The formulations were stirred at ambient temperature to achieve a homogenous mixture. Given that the dose preparations were prepared daily, maintained on a stir plate during dose administration, and used within approximately 2 h, the test substance in the preparations was considered to be stable.

2.3. In vitro study - bacterial reverse mutation (Ames) assay

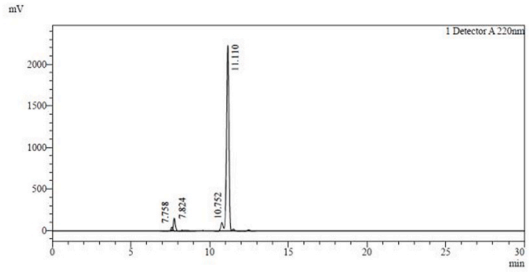
The mutagenic potential of the test article was examined using a bacterial reverse mutation assay, and was carried out in accordance with the most recent versions of the following guidelines: OECD Guidelines for Testing of Chemicals, Section 4 (No. 471): "Bacterial Reverse Mutation Test" [24]; U.S. FDA Toxicological Principles for the Safety Assessment of Food Ingredients, Redbook, 2000, IV.C.1.a. "Bacterial Reverse Mutation Test" [29]; ICH S2 (R1) Guidance on Genotoxicity Testing and Data Interpretation for Pharmaceuticals Intended for Human Use (2011) [30]. The study was also conducted in compliance with U.S.FDA GLP (21 CFR Part 58, 1987) which are compatible with OECD Principles of GLP (as revised in 1997): ENV/MC/CHEM(98)17 [27,31]. The study was carried out using *Salmonella typhimurium* (strains TA98, TA100, TA1535 and TA1537) and *Escherichia coli* (WP2 uvrA) as previously described [32]. Positive controls were included and used both with and without a metabolic activation system. Sodium azide, ICR 191 acridine, daunomycin and methyl methanesulfonate were used as positive controls for *S. typhimurium* strains TA100 and TA1535, TA1537, TA98 and *E. coli* WP2 uvrA, respectively in the absence of metabolic activation, and 2-aminoanthracene was used as the positive control for all strains in the presence of metabolic activation. The test article was prepared in dimethyl sulfoxide (DMSO) to provide final dose levels of up to 6640 μg/plate and the solutions were vortexed prior to use.

Table 2
HempChoice® hemp oil extract lot information.

Parameter	Lot #*		
	GEO113020	GEO420.01312020	GEO420.1111.2019
Appearance	Clear, amber oil	Clear, amber oil	Clear, amber oil
Color	Amber	Amber	Amber
Odor	Characteristic	Characteristic	Characteristic
CBD (%)	74.3	75.31	72.899
CBDV (%)	0.508	10.311	10.799
CBDVA (%)	ND	ND	NR
CBDA (%)	ND	0.443	0.450
CBG (%)	1.55	0.814	0.892
CBGA (%)	ND	ND	0.029
CBN (%)	ND	0.026	0.020
CBC (%)	2.53	ND	ND
Total Other Phytocannabinoids (%)	4.588	11.594	12.19
THC	Not detected	<LOQ	<LOQ
THC - A	Not detected	<LOQ	<LOQ
Terpenes (%)	2.49	6.8	13.8
Fatty Acids/Fatty Aldehydes/Wax Esters	NR	8.12	12.65

CBD – cannabidiol; CBDV – cannabidivarin; CBDVA – cannabidivarinic acid; CBDA – cannabidiolic acid; CBC – cannabichromene; CBG – cannabigerol; LOQ – limit of quantification; ND – not detected; NR -not reported; THC – tetrahydrocannabinol; THC-A – tetrahyrdocannabinolic acid. Lot GEO113020 was used for the *in vitro* micronucleus study, lot GEO42001312020 was used for the Ames and 90-day studies and lot GEO420.111.2019 was used for the 14-day range finding study.

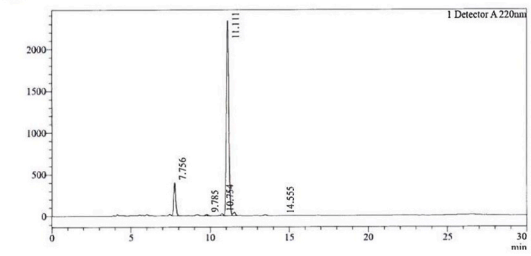
A



HempChoice Lot GEO113020

Name	Conc.	Unit	Ret. Time
CBDV	0.508	%	7.758
CBC	2.53	%	7.824
CBG	1.55	%	10.752
CBD	74.3	%	11.110

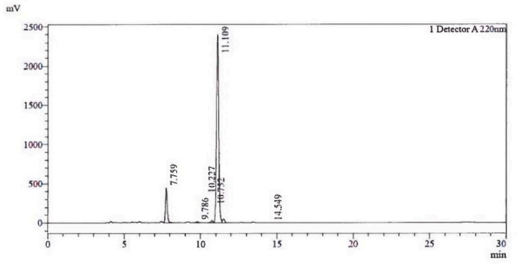
B



HempChoice Lot GEO420.01312020

Name	Conc.	Unit	Ret. Time
CBDV	10.311	%	7.756
CBDA	0.443	%	9.785
CBG	0.814	%	10.754
CBD	75.310	%	11.111
CBN	0.026	%	14.555

C



HempChoice Lot GEO420.1111.2019

Name	Conc.	Unit	Ret. Time
CBDV	10.799	%	7.759
CBDA	0.450	%	9.786
CBGA	0.029	%	10.227
CBG	0.892	%	10.752
CBD	72.899	%	11.109
CBN	0.020	%	14.549

Fig. 1. HPLC chromatograms of HempChoice® Hemp Oil Extracts (A) Lot GEO113020, (B) Lot GEO420.01312020, and (C) Lot GEO420.1111.2019. Lot GEO113020 was used for the *in vitro* micronucleus study, Lot GEO42001312020 was used for the Ames and 90-day studies, and Lot GEO420.111.2019 was used for the 14-day range finding study.

Initially the plate incorporation method was used, and the following materials were mixed and added to a minimal agar plate: 100 μL of the test substance, negative control, or positive control substance, 500 μL of S9 mix or substitution buffer, 100 μL of bacterial suspension, and 2000 μL of overlay agar which was maintained at 45 °C. Triplicate plates were prepared and incubated at 37 °C until the growth was sufficient for enumeration. The confirmatory test was then carried out using the pre-incubation method. Bacterial suspensions were incubated with the test or control substances and S9 mix or substitution buffer under agitation for approximately 30 min at 37 °C, prior to mixing with overlay agar and adding to the minimal agar plates, and continuing as described above for the initial test. The bacterial strains and dose levels used were the same in both the initial and confirmatory assays. The final doses of test article corresponded to 2.1, 6.64, 21, 66.4, 210, 664, 2100, and 6640 μg/plate. A supplemental test was carried out at additional dose levels to clarify the results using final doses of 1.593, 5.311, 15.934 and 53.11 μg/plate for TA98 and TA1535 and 0.531, 1.593, 5.311 and 15.934 μg/plate for TA100 and TA1537, following the plate incorporation and pre-incubation methods described above.

The number of colonies on each plate was counted manually and/or with the aid of a plate counter, and the mean and standard deviation was determined for each set of triplicate plates. The following validity criteria were used: the background lawn for the vehicle control plates was normal, the mean revertant colony counts for each strain treated with vehicle was close to or within the expected laboratory historical control range or published values, and the positive controls should produce substantial increases in revertant colony numbers for the appropriate bacterial strain. An evaluation for cytotoxicity was also conducted on all plates.

2.4. *In vitro micronucleus assay*

An *in vitro* micronucleus assay was carried out to evaluate the chromosome damaging potential of HempChoice® Hemp Oil Extract, and was conducted following the procedures outlined in OECD Guidelines for Testing of Chemicals, Section 4, No. 487, "In Vitro Mammalian Cell Micronucleus Test', adopted 29 July 2016, and Commission Regulation (EU) 2017/735 B.49 "In Vitro Mammalian Cell Micronucleus Test", dated February 14, 2017 and in compliance with GLP [23,33]. Cell culture medium was used as the negative control and methylmethanesulfonate (MMS), cyclophosphamide (CPA), and colchicine were used as positive controls. Human peripheral blood lymphocytes from healthy, non-smoking donors were used in the study. S9 liver microsomal fraction prepared from male Sprague Dawley rats induced with phenobarbital and β-naphthoflavone was utilized in the assay. A pre-test was conducted to determine the toxicity of the test article. For the main study, the following concentrations were selected for microscopic analysis: 1.0, 5.0, 7.5, and 10 μg/mL without metabolic activation and 40, 50, and 60 μg/mL with metabolic activation. Whole blood samples were treated with heparin and precultured for 44–48 h in the presence of phytohemagglutinin (PHA) prior to exposure to HempChoice® Hemp Oil Extract. The cells were then incubated with the HempChoice® Hemp Oil Extract for an additional 4 h in the presence or absence of metabolic activation. The cells were washed and then incubated in complete culture medium and cytochalasin B for 40–42 h at 37 °C and 5% CO_2. A second experiment was conducted using a continuous treatment without metabolic activation. The whole blood was first pre-cultured in the presence of PHA for 44–48 h prior to HempChoice® Hemp Oil Extract exposure. HempChoice® Hemp Oil Extract was then added and 1 h later, cytochalasin B was added followed by another 43-h incubation at 37 °C. Duplicate cultures were carried out at each concentration level. At the end of incubation period for both experiments, the culture medium was removed, and the cells were prepared and stained with acridine orange solution. The slides were then analyzed for micronuclei and at least 2000 binucleated cells per concentration level were evaluated for micronuclei. A cytokinesis block proliferation index (CBPI) was determined from 500 cells as an assessment of cytotoxicity, and the CBPI was then used to calculate % cytostasis. The acceptance criteria were as follows: concurrent negative or solvent control was acceptable as compared to historical controls, concurrent positive controls induced a response that was comparable to historical positive controls, cell proliferation criteria for the negative or solvent control were fulfilled, all experimental conditions were tested, and an adequate number of cells and concentrations were analysable.

2.5. *In vivo studies*

2.5.1. *Animals and husbandry*

Male and female Sprague-Dawley rats (Charles River CD® IGS; Raleigh, NC) were used in the 14-day dose range finding study (14-day study) and the 90-day subchronic study with a 28-day recovery period (90-day study) (Tables 3 and 4). The objective of the14-day study was to determine tolerable doses for the 90-day study (Table 4). For both studies, the rats were 8–9 weeks of age at initiation. Animal husbandry was compliant with the NRC Guide for the Care and Use of Laboratory Animals [28]. Filtered tap water and feed (2016 Certified Envigo Teklad Global Rodent Diet, www.envigo.com/teklad) were provided *ad libitum*. There were no reasonably

Table 3
Rat treatment groups for the dose range finding 14-day study.

Group No.	Males/Females[a]	Dose[b] of HempChoice® Hemp Oil Extract (mg/kg bw/day)	Sacrifice Day Male/Female
1	5/5	0	16/16
2	5/5	41.5	16/16
3	5/5	68.59	16/16
4	5/5	96.03	16/16

[a] Each sex 8–9 wk of age.
[b] Dose is mg HempChoice® Hemp Oil Extract/kg BW/day for 14 consecutive days and the dose concentrations of HempChoice® Hemp Oil Extract were 8.23, 13.71 and 19.20 mg/ml. A standard volume of 5 mL was administered.

Table 4
Treatment groups for the 90-day study with a 28-day recovery.

Group No.	Males/Females[a]	Dose of HempChoice® Hemp Oil Extract (mg/kg BW/day)[b]	Sacrifice Day Male/Female
1	10/10	0.00	93/94
2	10/10	39.84	93/94
3	10/10	92.95	93/94
4	10/10	185.9	93/94
5	5/5	0.00	121/121
6	5/5	39.84	121/121
7	5/5	92.95	121/121
8	5/5	185.9	121/121

[a] Each sex is 8–9 wk of age.
[b] Dose is mg HempChoice® Hemp Oil Extract/kg BW/day for 90 consecutive days and the dose concentrations of HempChoice® Hemp Oil Extract were 7.97, 18.59, and 37.18 mg/ml. A standard volume of 5 mL was given.

expected contaminants in the food and water which would interfere with the results of the studies. The acclimation period was 6 days and 13 days for the 14-day and 90-day studies, respectively. Following the acclimation period, only animals that were free of clinical signs of injury or disease and had a BW range within ±25% and ±20% of the mean within each sex for the 14-day study and the 90-day study, respectively, were selected. For the 14-day study, 20 male rats weighing 235–368 g and 20 female rats weighing 174–225 g were distributed to treatment groups (5/sex/group) as shown in Table 3. One hundred and twenty rats (60 animals/sex) were used in the 90-day study, with weights ranging from 239 to 351 g and 186–283 g for the males and females, respectively. Before the start of the studies, the animals were allocated to treatment groups according to stratification by body weight to ensure there were no statistically significant differences between mean group body weights within a sex. The rats were housed two to three rats of the same sex per cage for both studies, and in the 90-day study, sentinel rats were kept in the animal rooms. Serum from each sentinel rat was screened (IDEXX BioAnalytics, Columbia, MO) for common rat pathogens (rat parvovirus, Toolan's H-1 virus, Kilham rat virus, rat minute virus, parvovirus NS-1, rat coronavirus, rat Theilovirus, and *Pneumocystis carinii*). All serums were negative for evidence of infection by these organisms.

2.5.2. Treatments

The HempChoice® Hemp Oil Extract was mixed (w/v) in corn oil and the concentrations were adjusted for administration in a 5 mL volume. Control and treated rats were orally administered (gavage) corn oil vehicle or HempChoice® Hemp Oil Extract in corn oil, respectively. The dose preparations were prepared daily, and the dose was calculated using the past weekly BW. Dosing was started on study day 1. For the 14-day study, the doses of HempChoice® Hemp Oil Extract were administered daily for 14 days (Table 3). All rats in the 90-day study received HempChoice® Hemp Oil Extract or vehicle for at least 90 consecutive days (Table 4). For both studies, the doses of HempChoice® Hemp Oil Extract or vehicle were administered at the same time of day ±2 h. After dosing ceased in the 90-day study, rats in the recovery groups (Groups 5 to 8) remained on study for an additional 28 days.

2.5.3. Body weights, feed consumption, clinical and neurobehavioral exams

The animals in the 14-day and the 90-day studies were observed daily for clinical evidence of ill health and given weekly physical exams corresponding to BW and feed consumption determinations. For both studies, feed consumption was determined daily per cage by calculating the amount of feed consumed/day/cage. Animals were examined daily for changes in skin, fur, eyes, and mucous membranes, occurrence of secretions and excretions and autonomic activity (e.g., lacrimation, piloerection, pupil size and unusual respiratory pattern). Changes in gait, posture, and response to handling, as well as the presence of clonic or tonic movements, stereotypies (e.g., excessive grooming, repetitive circling), or bizarre behavior (e.g., self-mutilation, walking backwards), and all abnormal observations were also recorded. Animals in the 90-day study were subjected to a Functional Observational Battery during week 12 to test for excitability, autonomic function, gait and sensorimotor coordination (open field and manipulative evaluations), reactivity and sensitivity (elicited behavior) and other abnormal clinical signs including, but not limited to convulsions, tremors, unusual or bizarre behavior, emaciation, dehydration, and general appearance. During week 12, rats in the 90-day study were also subjected to a Motor Activity Assessment using a Photobeam Activity System (San Diego Instruments Inc, San Diego, CA) following recommended procedures. Investigators performing the physical examinations, Functional Observation Battery, and Motor Activity Assessment were blinded to the treatments the animals were receiving.

2.5.4. Ophthalmology

In the 90-day study, ophthalmic examinations were carried out on all rats in Groups 1–8 by a Board-Certified veterinary ophthalmologist. During the pretrial period, the evaluations for superficial and interocular pathology were performed once using indirect ophthalmoscopy. On study Day 87, the eyes were examined in-life for pathology by focal illumination and slit lamp biomicroscopy.

2.5.5. Pathological methods

2.5.5.1. Hematology and clinical chemistry. On Day 16 of the 14-day study, rats were fasted overnight, anesthetized with isoflurane and

blood was collected from the inferior vena cava. Only clinical chemistry parameters were evaluated. In the 90-day study, blood was collected for hematology and clinical chemistry on study Days 93 and 94 for males and females, respectively, in Groups 1 to 4 (90-day sacrifice) and on study Day 121 for Groups 5 to 8 (after 28 days of recovery before sacrifice). Blood samples were collected by sublingual bleeding after animals were anesthetized with isoflurane for hematology (except coagulation samples) and clinical chemistry. Approximately 500 μL of blood was collected in a pre-calibrated tube containing potassium ethylenediaminetetraacetic acid (EDTA) anticoagulant for analysis of hematologic parameters, and 1 mL of whole blood was collected in tubes (no anticoagulant) for analysis of clinical chemistry parameters. Whole blood samples were kept refrigerated until analysis using standard hematology methods. For clinical chemistry analysis, blood samples were allowed to coagulate and then centrifuged in a refrigerated centrifuge. The serum supernatant was collected, transferred to cryotubes and stored at −80 °C until thawed and assayed. Approximately 2 mL of whole blood was collected, frozen, and stored at −80 °C for assessment of thyroid function by an ELISA method. Hematology analysis was carried out using an ADVIA 120 Hematology System (Siemens, Erlangen, Germany) and clinical chemistry analysis was carried out using a COBAS C311 autoanalyzer (Roche, Rotkreuz, Switzerland). Blood samples were collected immediately before terminal sacrifice by venipuncture of the inferior vena cava during anesthesia with isoflurane to determine the prothrombin time and activated partial thromboplastin time. Approximately 1.8 mL of blood was collected in pre-calibrated tubes containing an anticoagulant (3.2% sodium citrate) and centrifuged in a refrigerated centrifuge. Plasma was collected, frozen, and stored at −80 °C until analysis using a Sysmex CA620 (Siemens, Erlangen, Germany). On the day before sample collection for clinical chemistry analysis, animals were placed into metabolism cages, food was withheld for at least 15 h before blood collection, and voided urine was collected from each animal. Urine samples were refrigerated until analysis. Urine volume was measured, the appearance was recorded, chemical parameters were measured by Multistix® 10 SG Reagent Strips (Siemens, Erlangen, Germany) and urine sediment was evaluated by light microscopy.

2.5.5.2. Necropsy and histopathology (14-day and 90-day studies). A full necropsy was performed on each study animal including animals removed from both the 14-day and 90-day studies before scheduled terminations. The complete necropsy included examination of the external body surface, body orifices, and the thoracic, abdominal, and cranial cavities inclusive of contents for abnormally appearing organs and tissues. All surviving animals were weighed, anesthetized with isoflurane, exsanguinated from the abdominal aorta and necropsy examination was carried out. Any gross lesions were noted. Absolute and normalized organ weights (organ weight/BW ratio and, in the 90-day study, organ/brain weight ratio) were recorded for selected tissues as indicated in Table 5. The eyes, optic nerve, epididymides, and testes were fixed in modified Davidson's fixative and stored in ethanol, and all other tissues were fixed in 10% neutral buffered formalin. Tissues as specified in Table 5 were embedded in wax, and thin sections were cut and stained with hematoxylin and eosin, followed by examination by light microscopy for histopathology. For the 14-day study, only liver and adrenal glands from animals in Groups 1 and 4 were examined by histopathology. For the 90-day study, all tissues from Groups 1, 4, 5 and 8 and the livers from Groups 2 and 3 and groups 6 to 8 were examined for histopathologic changes by light microscopy (Table 5). All gross lesions observed were described and representative tissues were collected and examined by histopathology. All necropsy procedures were performed under the supervision of a veterinarian and histopathology was carried out by a Board-Certified veterinary pathologist.

Table 5
Tissues collected at necropsy - 90-day study.

Tissues Collected and Preserved in Fixative and Selected Tissues for Histopathology	
Adrenals[a,b,c]	Lymph nodes (mandibular, mesenteric)[c]
Brain (medulla/pons, cerebellum, cerebral cortex)[a,c]	Sternum[c]
Spinal cord (cervical, mid-thoracic, lumbar)[c], sciatic nerve[c]	Femur (bone)[c]
Epididymies[a,b,c]	Bone marrow (femur and sternum)[c]
Testes[a,b,c]	Pituitary gland[c]
Prostate[c]	Thyroid[c]
Seminal vesicles[c]	Parathyroid gland[c]
Ovary and oviducts[a,b,c]	Nose[c]
Vagina[c], uterus[c,d], cervix[c]	Nasal turbinates[c]
Mammary gland[c]	Pharynx[c]
Heart[a,c]	Larynx[c]
Aorta[c]	Trachea[c]
Kidneys[a,b,c]	Lungs[c]
Urinary bladder[c]	Eyes[c]
Pancreas[c]	Skeletal muscle[c]
Liver[a,c,d]	Skin[c]
Esophagus[c], stomach[c], duodenum[c], ileum with Peyer's patches[c], jejunum[c,5], colon[c], cecum[c], rectum[c]	Harderian gland[c]
Salivary glands (sublingual, submandibular, parotid)[c]	Optic nerve[c]
Spleen[a,c]	Uterus[a,c]
Thymus[a,c]	Gross lesions[a]

[a] Wet organ weight determined.
[b] Combined weight.
[c] Histopathology done for Groups 1 and 4.
[d] Histopathology for Groups 1 to 8.

2.6. Statistical analysis

2.6.1. Bacterial reverse mutation (Ames) assay

The mean and standard deviation were calculated for all quantitative data.

2.6.2. In vitro micronucleus assay

A nonparametric c^2 Test was performed to determine if there was a concentration-related increase in the micronucleated cell frequency.

2.6.3. 14-Day and 90-day studies

For the 14-day and 90-day studies, mean and standard deviations were calculated for all quantitative data. Comparison of treatment and control groups was carried out using a two-way analysis of variance (ANOVA) for all in-life endpoints identified as multiple measurements of continuous data over time (e.g., BW, BW gain, food consumption, and food efficiency). The effects of both treatment and time were analyzed as described previously [32]. Pristima® version 7 (Statistical Analysis, Xybion Corporation, Lawrenceville, NJ), Instem LSS (Staffordshire, UK), Provantis version 9 (Tables and Statistics, Instem LSS, Staffordshire UK.), and Prism Biostatistics (GraphPad Software, San Diego, CA) were used for statistical analysis. Data from male and female rats were analyzed separately A probability value of $p < 0.05$ was considered significant. Clinicopathology data was analyzed as previously described [32] in a sequential manner. A probability value of $p < 0.05$ was used to reject the null hypothesis. In the case that an individual observation was reported as less than a specific value, e.g., below the lower limit of quantitation, one-half of the reported value was used to carry out the calculations. For example, if bilirubin was reported as <0.1 or ≤0.1, then a value of 0.05 was used for all calculations performed with that data. On the other hand, if an individual observation was reported as being greater than a specific value, e.g., above the upper limit

Table 6
Reverse mutation assay of HempChoice® Hemp Oil Extract in *Salmonella typhimurium* and *E. coli*.

Concentration (μg/plate)	TA98		TA100		TA1535		TA1537		*E. coli* WP2 uvrA	
	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9
Experiment 1										
0[a]	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
2.1	1.00	0.86	1.00	0.95	1.33	0.87	0.83	0.83	0.90	1.48
6.64	1.00	0.96	1.02	0.90	0.83	0.73	1.08	1.00	1.05	1.64
21	0.95	1.04	1.07	0.97	0.92	0.80	0.67	0.92	0.93	1.42
66.4	1.29	0.89	0.86	0.79	1.00	0.73	0.50	0.92	0.88	1.48
210	1.05	0.89	0.49	0.80	0.83	0.60	0.25	0.67	0.78	1.30
664	1.14	0.96	0.39	0.74	0.67	0.60	0.17	0.83	0.83	1.45
2100	1.05	1.00	0.34	0.37	0.67	0.60	0.25	0.67	0.78	1.30
6640	1.10	0.93	0.28	0.30	0.67	0.80	0.08	0.42	0.78	1.00
Positive control	Daunomycin 45.43	2-AA 74.39	Sodium Azide 6.31	2-AA 26.06	Sodium Azide 57.92	2-AA 30.80	ICR 191 Acridine 71.00	2-AA 34.58	MMS 9.39	2-AA 7.88
Experiment 2										
0[a]	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
2.1	0.96	1.07	1.05	1.05	1.08	1.08	1.20	1.00	0.89	0.96
6.64	1.00	1.07	0.96	0.96	1.00	0.58	0.80	1.00	1.08	1.00
21	0.91	1.00	1.01	1.01	0.69	1.25	0.60	1.00	0.89	0.85
66.4	0.91	1.00	0.92	0.92	0.62	0.83	0.50	0.67	0.89	0.94
210	0.83	1.11	0.74	0.74	0.69	0.75	0.10	0.25	0.92	0.98
664	0.87	0.89	0.64	0.64	0.77	0.67	0.20	0.42	0.92	1.00
2100	0.91	0.89	0.46	0.46	0.85	0.83	0.20	0.17	1.08	0.83
6640	0.87	0.85	0.46	0.46	0.62	0.75	0.10	0.25	0.75	0.89
Positive control	Daunomycin 39.00	2-AA 79.00	Sodium Azide 5.94	2-AA 25.14	Sodium Azide 56.46	2-AA[b] 38.83	ICR 191 Acridine 450.90	2-AA 33.42	MMS 11.08	2-AA 4.21
0[a]	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	NT	NT
1.593	1.10	0.80	1.05	1.02	0.77	1.00	0.53	0.71	NT	NT
5.311	1.25	1.16	1.05	1.10	1.08	0.91	0.53	0.64	NT	NT
15.934	1.10	1.04	1.02	1.01	1.00	1.00	0.40	0.57	NT	NT
53.11	1.20	1.00	1.06	0.98	0.92	1.00	0.67	0.50	NT	NT
Positive control	Daunomycin 70.05	2-AA 105.28	Sodium Azide 6.44	2-AA 23.73	Sodium Azide 44.23	2-AA 38.00	ICR 191 Acridine 39.60	2-AA 39.71	NT	NT

Substance was tested using the standardized plate incorporation assay (Experiment 1) and the pre-incubation method (Experiment 2). A supplemental test (Experiment 3) was conducted to add additional dose levels to clarify results. Results are means of three replicates per test condition displayed as mean ± standard deviation.

[a] DMSO vehicle.

[b] 2-aminoanthracene.

of quantitation, then the greater value was used in place of the reported value. For example, if specific gravity was reported as >1.100 or ≥1.100, then a value of 1.100 was used for all calculations performed using that specific gravity value.

3. Results

3.1. Concentration verification

The target doses of HempChoice® Hemp Oil Extract given to the rats in the 14-day and 90-day studies met or exceeded the required target levels. For the 14-day study, the stability analysis results were 100.2% (Day 1) to 100.2% (Day 15), and in the 90-day study, the neat HempChoice® Hemp Oil Extract was 98.9% of the target concentration on Day 1 (initial), and 99.2% on Day 93 (final).

3.2. Bacterial Reverse Mutation Test

No substantial concentration-related or test material related increases in the number of revertant colonies were observed with any of the strains tested, either in the absence or presence of metabolic activation (Table 6). Precipitate was observed for all strains at doses ≥2100 μg/plate in both the plate incorporation and the pre-incubation method. Some signs of toxicity with evidence of incomplete background lawn were observed but did not interfere with the conclusions of the study. Based on the findings of the study, HempChoice® Hemp Oil Extract was not mutagenic to bacteria.

3.3. In vitro micronucleus study

3.3.1. Cytostasis

No precipitation was observed in the test material in any of the cultures. In the first experiment without metabolic activation, no increase above 30% cytostasis was noted at concentrations up to 7.5 μg/mL, and at 10 μg/mL, 59% cytostasis was observed. In the first experiment with metabolic activation, no increase of cytostasis above 30% was seen at concentrations up to 40 μg/mL, however 38% cytostasis was noted at 50 μg/ml, and at 60 μg/mL, 51% cytostasis was observed (Table 7).

Table 7
In vitro micronucleus assay of HempChoice® Hemp Oil Extract in human lymphocytes.

Concentration (μg/ml)	Cytostasis (%)	Relative Cell Growth	Frequency of Micronucleated Cells (%)
Experiment 1 – without metabolic activation			
Negative control[a]	0*	103	0.30
Solvent control[b]	0	100	0.25
1.0	20	80	0.15
5.0	14	86	0.20
7.5	30	70	0.40
10	59	41	0.30
Positive control[c]	18	82	1.60
Positive control[d]	69	31	1.30
Experiment 1 – with metabolic activation			
Negative control[a]	0*	105	0.60
Solvent control[b]	0	100	0.75
40	7	93	0.40
50	38	62	0.25
60	51	49	0.50
12.5	4	96	2.85
Experiment 2 – without metabolic activation			
Negative control[a]	0*	124	0.40
Solvent control[b]	0	100	0.35
5.0	0*	102	0.20
7.5	18	82	0.25
10	35	65	0.20
12.5	59	41	0.58
Positive control[e]	0	134	2.50
Positive control[d]	5	95	1.50

Experiment 1: Cells were treated for 4 h and harvested at 44 h.
Experiment 2: Continuous 44-h treatment followed by harvest.
[a] Culture medium.
[b] DMSO.
[c] Methylmethanesulfonate.
[d] Colchicine.
[e] Cyclophosphamide.

Table 8
Effect of 14-day oral administration of HempChoice® Hemp Oil Extract on clinical chemistry parameters in male and female rats.

Parameter (Normal Range)	Units	G1 (0) n = 5/sex	G2 (41.15) n = 5/sex	G3 (68.59) n = 5/sex	G4 (96.03) n = 5/sex
Males					
Na 127–155	mmol/L	143.2 ± 1.30	142.2 ± 2.59	143.2 ± 2.59	141.6 ± 2.70
K 3.73–6.90	mmol/L	7.702 ± 0.7582	6.270 ± 0.5123	7.478 ± 1.5525	6.758 ± 1.2547
Ca 6.5–12.1	mg/dL	10.50 ± 0.787	10.22 ± 0.444	10.56 ± 0.493	10.24 ± 0.709
Cl 87.5–110.3	mmol/L	100.42 ± 1.731	100.56 ± 1.467	101.52 ± 1.291	100.82 ± 2.007
PHOS 4.6–10.0	mg/dL	10.04 ± 0.733	9.14 ± 0.684	9.48 ± 1.057	8.80 ± 0.644
BUN 8–20	mg/dL	10.6 ± 0.89	11.0 ± 1.87	10.8 ± 0.84	11.2 ± 2.49
CREAT 0.01–0.27	mg/dL	0.196 ± 0.0305	0.214 ± 0.0329	0.214 ± 0.0279	0.196 ± 0.0404
AST 39–205	U/L	90.8 ± 10.35	74.2 ± 6.53[a]	75.0 ± 10.34[a]	71.0 ± 6.12**
ALT 15–139	U/L	43.600 ± 5.7706	31.000 ± 6.8191	39.000 ± 5.0498	39.400 ± 9.5026
ALKP 46–230	U/L	152.4 ± 44.21	174.0 ± 14.12	192.4 ± 30.51	176.2 ± 28.73
SDH 0.2–39.2	U/L	21.34 ± 9.955	7.54 ± 1.853***	11.04 ± 2.669***	7.28 ± 1.582***
TP 3.4–7.7	g/dL	5.46 ± 0.270	5.46 ± 0.152	5.70 ± 0.187	5.42 ± 0.409
ALB 2.2–4.6	g/dL	3.70 ± 0.200	3.62 ± 0.164	3.92 ± 0.130	3.66 ± 0.351
GLOB 1.2–3.5	g/dL	1.76 ± 0.195	1.84 ± 0.182	1.78 ± 0.084	1.76 ± 0.219
CHOL 39–163	mg/dL	53.2 ± 4.92	56.8 ± 14.32	61.2 ± 4.38	52.2 ± 6.34
TRIG 20–376	mg/dL	49.6 ± 9.84	31.2 ± 5.07	46.2 ± 15.90	50.4 ± 15.03
GLU 76–183	mg/dL	96.0 ± 42.31	112.4 ± 27.84	110.6 ± 34.61	136.0 ± 28.43
TBIL 0.03–0.9	mg/dL	0.066 ± 0.0089	0.056 ± 0.0089	0.058 ± 0.0148	0.042 ± 0.0130
Females					
Na 132–159	mmol/L	141.2 ± 2.17	139.8 ± 1.10	140.2 ± 2.49	140.2 ± 3.27
K 3.49–5.98	mmol/L	6.544 ± 1.4542	6.498 ± 1.5644	6.680 ± 1.6839	7.264 ± 1.6824
Ca 7.7–15.5	mg/dL	11.08 ± 0.657	11.20 ± 0.339	10.66 ± 0.288	10.72 ± 0.130
Cl 93.3–114.6	mmol/L	101.48 ± 1.863	99.92 ± 1.318	102.30 ± 2.545	102.62 ± 3.760
PHOS 2.4–8.0	mg/dL	8.44 ± 1.071	8.30 ± 0.596	8.88 ± 1.099	8.86 ± 1.119
BUN 8–22	mg/dL	13.8 ± 1.30	13.2 ± 2.05	14.4 ± 2.79	13.6 ± 2.97
CREAT 0.12–0.40	mg/dL	0.200 ± 0.0292	0.186 ± 0.0276	0.180 ± 0.0274	0.214 ± 0.0498
AST 43–301	U/L	66.0 ± 10.12	61.8 ± 5.76	68.0 ± 9.70	63.0 ± 3.08
ALT 13–182	U/L	29.800 ± 5.1672	32.800 ± 11.1893	24.600 ± 4.7223	25.600 ± 6.5038
ALKP 15–115	U/L	99.0 ± 33.90	102.6 ± 38.62	82.2 ± 15.32	89.2 ± 18.21
SDH 0.2–38.6	U/L	6.90 ± 0.464	8.88 ± 1.420	7.62 ± 3.470	8.62 ± 3.234
TP 5.2–9.0	g/dL	6.00 ± 0.367	6.16 ± 0.336	5.64 ± 0.089	5.86 ± 0.358
ALB 2.6–6.4	g/dL	4.28 ± 0.277	4.40 ± 0.245	3.90 ± 0.122[a]	4.04 ± 0.230
GLOB 1.1–3.6	g/dL	1.72 ± 0.164	1.76 ± 0.195	1.74 ± 0.089	1.82 ± 0.192
CHOL 43–249	mg/dL	64.8 ± 13.27	73.8 ± 11.69	71.2 ± 16.69	66.0 ± 15.05

(*continued on next page*)

Table 8 (*continued*)

Parameter (Normal Range)	Units	G1 (0) n = 5/sex	G2 (41.15) n = 5/sex	G3 (68.59) n = 5/sex	G4 (96.03) n = 5/sex
Males					
TRIG 25–934	mg/dL	40.0 ± 3.16	48.6 ± 13.28	31.6 ± 7.23	28.8 ± 11.39
GLU 87–193	mg/dL	129.6 ± 33.86	128.0 ± 31.87	105.0 ± 13.73	127.8 ± 26.13
TBIL 0.04–0.25	mg/dL	0.082 ± 0.0268	0.068 ± 0.0409	0.058 ± 0.0148	0.056 ± 0.0152

[a] = p 0.05; ** = p 0.01; ***p 0.001. Values are mean ± standard deviation. ALB = albumin; ALKP = alkaline phosphatase; ALT = alanine aminotransferase; AST = aspartate aminotransferase; BUN = urea nitrogen; CA = calcium; CHOL = cholesterol; Cl = chloride; CREAT = creatinine; GLOB = globulin; GLU = glucose; K = potassium; Na = sodium; PHOS = inorganic phosphorous; SDH = sorbitol dehydrogenase; TBIL = total bilirubin; TP = total protein; TRIG = triglycerides.

3.3.2. Occurrences of micronucleated cells

In both experiments, with and without metabolic activation, no biologically relevant increase in the micronucleus frequency was observed following exposure to the test material (Table 7). Based on the findings of this study, HempChoice® Hemp Oil Extract did not induce structural and/or numerical chromosomal damage in human lymphocytes when used at concentrations up to 10 μg/mL without metabolic activation and up to 60 μg/mL with metabolic activation, under the experimental conditions used. The results of this assay indicate that HempChoice® Hemp Oil Extract did not induce aneugenic or clastogenic damage to human peripheral blood lymphocytes.

3.4. In vivo toxicity studies

3.4.1. Clinical signs and mortalities

No clinical signs or mortalities related to administration of HempChoice® Hemp Oil Extract occurred in the 14-day and the 90-day studies.

3.4.2. Feed consumption, body weight gains and body weights

For male and female rats in the 14-day study, no significant differences ($p > 0.05$) in weekly BW and feed consumption between treatment and control animals were observed. Mean food consumption for 90-day sacrifice male rats in Groups 2–4 was not significantly different from Group 1 rats ($p > 0.05$). The 28-day recovery male rats in Groups 6–8 were generally comparable to control Group 5 throughout the dosing and recovery periods with the exception of statistically significant decreases ($p < 0.05$) in food consumption for Group 7 animals on study days 1–8 and Group 6 animals on study days 22–29, 43–64, and 85–92. Significant increases ($p < 0.05$) in food consumption were also observed for Group 7 animals on study days 8–15, 22–29, and 99–120 and for Group 8 animals on Days 22–29. Mean weekly BW and daily BW gain for the 90-day sacrifice male rats in Groups 2–4 were generally comparable to Group 1 throughout the dosing phase except for statistically significant increases ($p < 0.05$) in mean daily BW gain for Group 3 animals on Days 15–22 and Group 2 on Days 78–85 when compared to means for Group 1 rats. Mean weekly BW and daily BW gain for the 28-day recovery male rats in Groups 2–4 were generally comparable to control Groups 1 and 5 throughout the dosing and recovery periods with the exception of statistically significant decreases ($p < 0.05$) in mean daily BW gain for Group 6 animals on Days 1–8 and statistically significant increases ($p < 0.05$) in mean daily BW weight gain for Group 7 on Days 8–29 and for Group 8 animals on Days 22–29.

For the 90-day female rats in Groups 2–4, mean food consumption was generally comparable to control Group 1 throughout the dosing phase with the exception of statistically significant decreases ($p < 0.05$) in food consumption for Group 4 animals on study days 1–8 and statistically significant increases ($p < 0.05$) in food consumption for Group 3 animals on study days 36–50 and Groups 3 and 4 on study days 78–85 when compared to control Group 1. Mean food consumption in the 28-day recovery female rats in Groups 6 and 8 was generally comparable to control Group 5 throughout the dosing and recovery periods with the exception of statistically significant increases ($p < 0.05$) in food consumption for Group 6 animals on study days 28–36 and 50–64 and for Group 8 animals on study days 29–36, 85–106, and 113–120. Statistically significant increases ($p < 0.05$) in food consumption were also noted for Group 7 animals on study days 15–64, 78–92, and during the recovery period. For the female rats, the mean weekly BW and daily BW gain for rats in Groups 2–4 were generally comparable to control Group 1 throughout the dosing phase with the exception of statistically significant decreases ($p < 0.05$) in mean daily BW gain in Group 3 and Group 4 animals on study days 64–71 and statistically significant increases ($p < 0.05$) in Group 2, Group 3, and Group 4 animals on Days 85–92 when compared to controls (Group 1). The mean weekly BW and daily BW for recovery female rats in Groups 6–8 were generally comparable to control Group 5 throughout the dosing and recovery periods with the exception of statistically significant increases ($p < 0.05$) in mean daily BW decreases ($p < 0.05$) in mean daily BW gain for Groups 7 and 8 animals on study days 92–99.

3.4.3. Ophthalmology

No abnormal ophthalmological changes, across all treatment groups in the 90-day study, were observed in rats of both sexes.

Table 9
Effect of 90-day oral administration of test article on hematological parameters (in male and female rats (n = 60/sex).

Parameter (Historical Value)	Units	Group and Dose (mg/kg BW/day)							
		G1 (0) n = 10	G2 (39.84) n = 10	G3 (92.95) n = 10	G4 (185.9) n = 10	G5 (0) n = 5	G6 (39.84 n = 5	G7 (92.95) n = 5	G8 (185.9) n = 5
Males									
RCB	×10^6/	8.670 ±	9.105 ±	8.850 ±	8.916 ±	8.738 ±	8.292 ±	8.370 ±	8.576 ±
7.73–10.08	μL	0.8966	0.2563	0.4917	0.3313	0.4658	0.2933	0.3492	0.3093
HGB	g/dL	15.49 ±	15.42 ±	15.22 ±	15.29 ±	15.42 ±	14.76 ±	13.62 ±	14.94 ± 0.559
13.7–19.1		0.784	0.424	0.492	0.556	0.550	0.527	2.918	
HCT	%	50.21 ±	51.67 ±	50.69 ±	50.57 ±	49.60 ±	47.46 ±	47.90 ±	48.64 ± 2.134
43.9–55.3		4.978	1.289	2.724	1.943	2.169	2.049	2.120	
MCV	fL	57.92 ±	57.06 ±	57.30 ±	56.72 ±	56.82 ±	57.26 ±	57.24 ±	56.72 ± 2.344
50.4–62.4		1.540	1.141	1.616	1.062	2.102	1.592	0.770	
MCH	pg	18.05 ±	16.97 ±	17.23 ±	17.14 ±	17.66 ±	17.80 ±	16.26 ±	17.40 ± 0.628
15.4–21.6		2.175	0.542	0.957	0.568	0.799	0.480	3.334	
RDW	%	12.82 ±	12.26 ±	12.12 ±	12.19 ±	13.52 ±	13.66 ±	14.32 ±	13.92 ± 0.606
10.9–17.2		1.118	0.406	0.326	0.599	1.141	1.238	1.083	
PLT	×10^3/	981.3 ±	1015.2 ±	1000.4 ±	1044.4 ±	1087.4 ±	1115.8 ±	1021.4 ±	1149.8 ±
650–1517	μL	223.81	86.88	116.84	84.03	191.57	59.21	233.01	112.39
WBC	×10^3/	12.084 ±	10.330 ±	9.575 ±	12.180 ±	10.990 ±	8.246 ±	9.628 ±	11.238 ±
5.66–24.27	μL	2.0446	2.5022	1.9707	2.8802	1.6022	1.3441	1.8992	3.3695
ANEU	×10^3/	1.656 ±	1.730 ±	1.746 ±	1.772 ±	1.678 ±	1.606 ±	2.054 ±	1.872 ±
	μL	0.4739	0.6365	0.7440	0.3064	0.6997	0.6986	0.8290	1.2169
ALYM	×10^3/	9.717 ±	7.906 ±	7.261 ±	9.703 ±	8.718 ±	6.04 ±	7.038 ±	8.908v2.2121
	μL	2.1130	1.9068	1.6121	2.7463	1.3834	1.2932	1.7770	
AMON	×10^3/	0.22 ±	0.24 ±	0.21 ±	0.27 ±	0.28 ±	0.22 ±	0.20 ±	0.21 ± 0.040
	μL	0.059	0.066	0.042	0.046	0.088	0.062	0.078	
AEOS	×10^3/	0.118 ±	0.084 ±	0.071 ±	0.088 ±	0.084 ±	0.064 ±	0.092 ±	0.090 ±
	μL	0.0557	0.0284	0.0285	0.0286	0.0351	0.0167	0.0363	0.0354
ABAS	×10^3/	0.126 ±	0.141 ±	0.127 ±	0.141 ±	0.044 ±	0.022 ±	0.036 ±	0.044 ±
	μL	0.0755	0.0626	0.0704	0.0754	0.0261	0.00884	0.0114	0.0114
ALUC	×10^3/	0.181 ±	0.231 ±	0.156 ±	0.200 ±	0.186 ±	0.128 ±	0.206 ±	0.110 ±
	μL	0.0543	0.1460	0.0403	0.0552	0.0607	0.0482	2606	0.0738
ARET	^10^3/μL	193.6 ±	178.9 ±	161.5 ±	169.1 ±	179.2 ±	196.3 ±	207.7 ±	213.0 ± 40.91
		65.41	30.33	21.72	25.57	60.67	27.36	37.40	
APTT	sec	15.9 ±	15.7 ± 1.77	15.5 ± 1.36	16.0 ± 1.56	17.4 ±	17.8 ±	17.4 ±	17.3 ± 1.83
		1.59				3.12	1.72	2.97	
PT	sec	10.0 ±	10.0 ± 0.23	9.9 ± 0.22	10.1 ± 0.22	9.9 ± 0.40	9.7 ± 0.13	10.0 ±	9.8 ± 0.16
		0.25						0.28	
MCHC	g/dL	31.7 ±	29.9 ± 1.08	30.1 ± 1.36	30.3 ± 0.88	31.3 ±	31.1 ±	28.4 ±	30.7 ± 0.38
		4.10				0.41	0.37	5.95	
Females									
RBC	×10^6/	8.128 ±	7.974 ±	7.940 ±	7.888 ±	7.788 ±	7.876 ±	7.658 ±	7.632 ±
	μL	0.3181	0.3891	0.2735	0.5082	0.1594	0.4643	0.3964	0.3574
HGB	g/dL	14.91 ±	14.40 ±	14.23 ±	13.60 ±	14.24 ±	14.76 ±	14.52 ±	14.12 ± 0.487
		0.563	0.663	0.688	0.685***	0.270	0.635	0.618	
HCT	%	47.96 ±	46.17 ±	45.74 ±	44.73 ±	43.90 ±	46.40 ±	44.66 ±	44.50 ± 1.518
		1.483	2.241	1.876**	2.248**	0.904	1.991	2.188	
MCV	fL	59.03 ±	57.90 ±	57.90 ±	56.78 ±	56.42 ±	58.98 ±	58.32 ±	58.42 ±
		1.462	1.471	1.310	1.515**	1.117	1.585**	0.614*	0.950*
MCH	pg	18.36 ±	18.06 ±	17.94 ±	17.29 ±	18.26 ±	18.76 ±	19.00 ±	18.52 ± 0.259
		0.599	0.570***	0.682***	0.739***	0.503	0.568	0.748	
RDW	%	11.03 ±	11.58 ±	11.32 ±	11.37 ±	12.38 ±	11.58 ±	11.98 ±	12.80 ± 0.418
		0.320	1.204	0.426	0.343	0.963	0.363	0.476	
PLT	×10^3/	974.8 ±	1009.1 ±	902.0 ±	943.1 ±	1040.8 ±	1035.2 ±	993.0 ±	967.6 ± 44.05
	μL	110.46	172.17	202.08	149.64	135.95	97.65	142.02	
WBC	×10^3/	5.419 ±	6.861 ±	4.799 ±	5.080 ±	6.082 ±	5.672 ±	6.310 ±	5.170 ±
	μL	1.9324	4.0676	1.6769	1.4983	1.5986	2.9765	1.4776	2.3538
ANEU	×10^3/	0.908 ±	1.400 ±	0.984 ±	0.838 ±	1.398 ±	1.012 ±	1.166 ±	0.828 ±
	μL	0.4930	1.3145	0.4601	0.3667	0.7454	0.5531	0.3240	0.2347
ALYM	×10^3/	4.230 ±	5.037 ±	3.547 ±	3.919 ±	4.336 ±	4.364 ±	4.758 ±	4.040 ±
	μL	1.4749	2.6600	1.4087	1.2853	1.6249	2.7894	1.2257	2.2195
AMON	×10^3/	0.12 ±	0.20 ±	0.13 ±	0.16 ±	0.19 ±	0.12 ±	0.20 ±	0.16 ± 0.033
	μL	0.039	0.075**	0.045	0.041	0.093	0.071	0.098	
AEOS	×10^3/	0.077 ±	0.106 ±	0.059 ±	0.055 ±	0.070 ±	0.066 ±	0.078 ±	0.054 ±
	μL	0.0343	0.0624	0.0393	0.0255	0.0394	0.0391	0.0268	0.0182
ABAS	×10^3/	0.018 ±	0.028 ±	0.019 ±	0.014 ±	0.010 ±	0.010 ±	0.022 ±	0.016 ±
	μL	0.0063	0.0187	0.0110	0.0070	0.0071	0.0071	0.0164	0.0134

(*continued on next page*)

Table 9 (*continued*)

Parameter (Historical Value)	Units	Group and Dose (mg/kg BW/day)							
		G1 (0) n = 10	G2 (39.84) n = 10	G3 (92.95) n = 10	G4 (185.9) n = 10	G5 (0) n = 5	G6 (39.84 n = 5	G7 (92.95) n = 5	G8 (185.9) n = 5
Males									
ALUC	$\times 10^3$/ µL	0.067 ± 0.0323	0.094 ± 0.0502	0.069 ± 0.0341	0.093 ± 0.0403	0.076 ± 0.0219	0.092 ± 0.0602	0.082 ± 0.0311	0.072 ± 0.0390
ARET	ˆ10^3/µL	143.0 ± 30.91	134.9 ± 29.82	152.2 ± 30.46	139.3 ± 10.75	167.9 ± 47.39	159.4 ± 30.13	154.8 ± 37.54	167.2 ± 12.53
APTT	sec	14.9 ± 1.82	13.5 ± 1.07	14.9 ± 1.40	16.8 ± 4.38	13.6 ± 1.16	15.3 ± 2.24	13.4 ± 1.44	14.5 ± 1.68
PT	sec	9.9 ± 0.32	9.8 ± 0.35	9.8 ± 0.21	10.3 ± 1.34	8.9 ± 0.40	8.9 ± 0.23	9.0 ± 0.20	8.9 ± 0.19
MCHC	g/dL	31.1 ± 0.39	31.2 ± 0.49	31.1 ± 0.59	30.4*D ±0.53	32.4 ± 0.56	31.8 ± 0.23	32.6 ± 0.94	32.1 ± 0.61

* = p 0.05; ** = p 0.01; ***p 0.001. Values are mean ± standard deviation. ABAS = absolute basophil; AEOS = absolute eosinophil; ALUC = absolute large unstained cell; ALYM = absolute lymphocyte; AMON = absolute monocyte; ANEU = absolute neutrophil (all forms); ARET = absolute reticulocyte; HCT = hematocrit; HGB = hemoglobin; MCH = mean corpuscular hemoglobin; MCHC = mean corpuscular hemoglobin concentration; MCV = mean corpuscular volume; PLT = platelet count; RBC = red blood cell count; RDW = red cell distribution width; WBC = white blood cell count.

3.4.4. Functional observation battery

The male rats treated with HempChoice® Hemp Oil Extract did not exhibit significant ($p > 0.05$) deficiencies in functional or motor activity. Compared to Group 1 female rats, there was a significant increase ($p < 0.05$) in the forelimb grip strength in the Group 4 females. The mean forelimb grip for Group 4 females was significantly increased ($p < 0.05$) compared to the female control rats.

3.4.5. Clinicopathology

Clinicopathology parameters for rats of both sexes in the 14-day study were all within the clinically expected normal range (data not shown for hematology parameters). Specific changes were a significant decrease ($p < 0.05$) in serum aspartate aminotransferase (AST) in Group 4 males, and for sorbitol dehydrogenase (SDH) in Group 2 and 3 males (Table 8).

The hematology and coagulation results for the 90-day study the are shown in Table 9. There were no differences ($p > 0.05$) between group means for hematological parameters for the male rats terminated on study days 93 and 121 (28-day recovery). For the female rats terminated at study day 94, significant decreases ($p < 0.05$) in hemoglobin (HGB) for Group 4 and hematocrit (HCT) for Groups 2 and 3 were observed, while no significant differences ($p > 0.05$) were noted in these parameters between Groups 5–8. For female rats terminated on study day 93 and 121, there were no significant differences ($p > 0.05$) observed between treatment and controls for white blood cell numbers (WBC) and the white cell differential counts, with the exception of a significant increase ($p < 0.05$) in absolute monocyte counts for the rats in Group 2. For the coagulation parameters for both males and female rats, there were no differences ($p > 0.05$) observed between the groups. All changes in the hematological parameters for the female rats were considered within the normal range.

Clinical chemistry parameters are shown in Table 10. When the means of all treated male and female rats were compared to their respective controls, there were no significant increases ($p > 0.05$) in the activity of serum enzymes (AST, ALT, ALKP, SDH), and levels of serum calcium, sodium, potassium, phosphorus, glucose, and creatine. Male rats in Groups 2–4 had significantly decreased ($p < 0.05$) serum cholesterol (CHOL) compared to males in Group 1 and this decrease in CHOL was not observed in comparisons ($p > 0.05$) of Groups 6–8 with Group 5. Serum low-density lipoprotein cholesterol (LDL) was significantly decreased ($p < 0.05$) in male rats in Groups 2 and 4 compared to the means for male rats in Group 1, and serum high-density lipoprotein cholesterol (HDL) was significantly decreased ($p < 0.05$) for male rats in Groups 2–4 compared to mean HDL for controls (Group 1). No differences ($p > 0.05$) in LDL or HDL were observed between groups for male rats in Groups 5–8. When compared to females in Group 1, female rats in Groups 2 and 4 had a significant increase ($p < 0.05$) in the means for CHOL, while for Groups 5–8, there were no differences ($p > 0.05$) observed in the means for CHOL. For the female rats in all study groups, there were no differences ($p > 0.05$) in the means for LDL and HDL. For male rats in all study groups, there were no differences ($p > 0.05$) in the means between treated and controls for serum chloride. However, in the female rats, the mean serum chloride for Group 2 was significantly decreased ($p < 0.05$) when compared to the mean for Group 1, while the serum chloride means for Groups 6–8 were not significantly ($p > 0.05$) different from Group 5. The mean blood urea nitrogen (BUN) for males in Group 3 was significantly lower ($p < 0.05$) than the mean BUN for males in Group 1, and there were no differences ($p > 0.05$) in the BUN means for Groups 5–8. In females, there were no differences ($p > 0.05$) in the BUN means in Groups 1–8. The group means for total bilirubin (TBIL) were significantly ($p < 0.05$) decreased for male rats in Groups 2–4 and females in Groups 3 and 4 when compared to the mean serum TBIL for Group 1 for each sex, respectively. There were no differences ($p > 0.05$) in the TBIL means for male and female rats in Groups 5–8. For serum proteins, the means for total serum protein (TP) and globulin (GLOB) in male rats were significantly lower ($p < 0.05$) in Groups 2–4 than the mean for Group 1, and this change was not observed ($p > 0.05$) in the mean TP and GLOB for Groups 5–8. There were no differences ($p > 0.05$) in TP and albumin for female rats in Groups 1–8. For the female rats in Group 4, GLOB was significantly ($p < 0.05$) increased compared to controls. There were no differences ($p > 0.05$) in GLOB for Groups 5 to 8. For both sexes, there were no differences $p > 0.05$) between the treatment and control means for urinary parameters (Table 11).

A comparison of group means of thyroid test results in male rats showed that TSH levels were significantly increased ($p < 0.05$) and

Table 10
Effect of 90-day oral administration of test article on clinical chemistry parameters in male and female rats (n = 60/sex).

Parameter	Units	Group and Dose (mg/kg BW/day)							
		G1 (0) n = 10	G2 (39.84) n = 10	G3 (92.95) n = 10	G4 (185.9) n = 10	G5 (0) n = 5	G6 (39.84 n = 5	G7 (92.95) n = 5	G8 (185.9) n = 5
Males									
Na	mmol/L	141.5 ± 4.33	140.5 ± 2.68	139.9 ± 2.88	139.7 ± 6.63	143.0 ± 2.24	141.0 ± 1.22	141.2 ± 1.30	141.2 ± 1.64
K	mmol/L	7.280 ± 1.3702	7.691 ± 1.7985	7.140 ± 1.1106	7.903 ± 1.2044	7.356 ± 1.1076	6.982 ± 0.6374	7.546 ± 1.3946	8.154 ± 1.0435
Ca	mg/dL	10.94 ± 0.888	10.36 ± 0.814	10.28 ± 0.663	10.76 ± 0.658	10.90 ± 0.442	10.68 ± 0.377	10.56 ± 0.404	10.68 ± 0.432
Cl	mmol/L	98.70 ± 3.121	98.77 ± 2.258	98.38 ± 2.789	97.39 ± 4.687	100.62 ± 2.036	99.64 ± 0.879	99.98 ± 1.064	100.30 ± 0.957
PHOS	mg/dL	8.42 ± 0.757	8.55 ± 1.571	8.02 ± 0.705	8.64 ± 0.686	8.50 ± 0.897	7.98 ± 0.179	8.78 ± 1.415	8.78 ± 1.035
BUN	mg/dL	11.5 ± 1.58	11.9 ± 2.08	9.4d ± 0.97*	11.4 ± 1.24	14.8 ± 1.92	15.4 ± 2.51	13.0 ± 1.41	14.2 ± 0.84
CREAT	mg/dL	0.226 ± 0.0427	0.222 ± 0.0543	0.210 ± 0.0374	0.239 ± 0.0580	0.266 ± 0.0422	0.272 ± 0.0522	0.264 ± 0.0351	0.266 ± 0.0230
AST	U/L	82.7 ± 23.21	75.6 ± 21.47	85.5 ± 22.90	69.2 ± 11.86	108.6 ± 51.91	92.8 ± 12.70	86.8 ± 24.77	80.8 ± 11.56
ALT	U/L	34.100 ± 15.8216	28.000 ± 8.5114	28.400 ± 7.6041	25.111 ± 3.2956	48.400 ± 34.6165	31.600 ± 4.5607	32.200 ± 5.9330	31.800 ± 2.3875
ALKP	U/L	86.4 ± 16.81	77.2 ± 17.54	68.9 ± 10.86	75.9 ± 14.22	58.2 ± 14.02	52.6 ± 10.31	53.2 ± 3.27	54.6 ± 9.40
SDH	U/L	7.96 ± 10.569	11.11 ± 15.075	9.79 ± 8.138	4.14 ± 3.365	5.14 ± 10.224	1.86 ± 1.727	5.30 ± 5.049	2.36 ± 2.560
TP	g/dL	5.77 ± 0.517	5.568D ±0.554***	5.40D ±0.419***	6.10D ±0.587***	6.42 ± 0.286	6.22 ± 0.409	6.06 ± 0.251	6.36 ± 0.152
ALB	g/dL	3.70 ± 0.283	3.60 ± 0.383	3.56 ± 0.196	3.83 ± 0.350	3.86 ± 0.114	3.82 ± 0.192	3.78 ± 0.164	3.90 ± 0.071
GLOB	g/dL	2.07 ± 0.291	1.968 ± 0.222***	1.84 ± 0.317***	2.27 ± 0.292***	2.56 ± 0.195	2.40 ± 0.308	2.28 ± 0.164	2.46 ± 0.182
CHOL	mg/dL	61.8 ± 8.95	45.0D ±5.06***	46.2D ±8.22***	46.1D ±8.37***	62.8 ± 5.63	69.2 ± 12.48	67.4 ± 13.15	63.2 ± 4.49
LDL	mmol/L	0.206 ± 0.0580	0.128+ ±0.0274	0.165 ± 0.0384	0.134d ± 0.0381*	0.292 ± 0.0377	0.358 ± 0.1108	0.276 ± 0.0956	0.0258 ± 0.0554
HDL	mmol/L	0.897 ± 0.1365	0.650 ± 0.0873***	0.628 ± 0.1305***	0.662 ± 0.1410***	1.014 ± 0.1081	1.050 ± 0.2077	1.088 ± 0.1869	1.000 ± 0.0758
TRIG	mg/dL	93.8 ± 37.19	96.3 ± 43.99	72.7 ± 13.57	78.3 ± 41.85	75.2 ± 27.66	64.0 ± 23.59	77.2 ± 22.95	68.8 ± 21.81
GLU	mg/dL	222.1 ± 65.80	192.8 ± 60.53	174.7 ± 29.27	192.4 ± 30.37	193.2 ± 16.60	195.6 ± 16.04	222.6 ± 43.33	214.6 ± 37.21
TBIL	mg/dL	0.104 ± 0.0107	0.075d ± 0.0172**	0.078d ± 0.0123**	0.079d ± 0.0154**	0.090 ± 0.0245	0.088 ± 0.0192	0.084 ± 0.0167	0.096 ± 0.0114
Females									
Na	mmol/L	140.4 ± 4.43	138.7 ± 2.26	137.5 ± 2.27	138.7 ± 2.71	140.2 ± 1.64	141.8 ± 1.64	140.4 ± 0.89	140.0 ± 0.00
K	mmol/L	6.717 ± 0.9426	6.472 ± 0.6784	6.608 ± 1.0701	5.797 ± 0.7369	6.040 ± 0.7864	6.898 ± 0.7084	5.980 ± 0.4597	5.676 ± 0.5643
Ca	mg/dL	10.78 ± 0.598	11.24 ± 0.690	10.69 ± 0.592	10.88 ± 0.705	11.82 ± 0.981	11.34 ± 0.546	11.32 ± 0.487	11.76 ± 0.615
Cl	mmol/L	100.66 ± 2.758	97.45+d ±2.987	98.27 ± 1.574	98.69 ± 1.703	97.48 ± 2.028	100.06 ± 2.279	97.42 ± 1.066	98.38 ± 1.625
PHOS	mg/dL	7.25 ± 0.947	7.23 ± 0.693	7.27 ± 0.763	6.50 ± 0.704	7.56 ± 0.773	7.96 ± 0.541	7.02 ± 0.476	6.90 ± 0.632
BUN	mg/dL	15.2 ± 2.94	13.8 ± 3.01	13.3 ± 1.77	12.3 ± 2.63	17.4 ± 1.67	16.8 ± 2.59	16.2 ± 2.28	16.6 ± 1.95
CREAT	mg/dL	0.266 ± 0.0310	0.243 ± 0.0263	0.274 ± 0.0562	0.292 ± 0.0469	0.318 ± 0.0497	0.286 ± 0.0456	0.286 ± 0.0336	0.250 ± 0.0374
AST	U/L	107.9 ± 20.53	106.5 ± 27.36***	92.8 ± 24.90***	72.2 ± 16.86***	299.8 ± 453.71	78.6 ± 20.60	120.4 ± 50.44	108.2 ± 88.06
ALT	U/L	28.300 ± 9.6385	27.400 ± 7.2296	24.200 ± 8.8669	22.900 ± 4.4833	130.400 ± 217.0744	20.000 ± 3.8079	35.000 ± 17.8606	50.200 ± 54.5683
ALKP	U/L	31.5 ± 12.95	26.9 ± 21.43	22.9 ± 6.12	22.3 ± 7.83	21.6 ± 4.93	22.8 ± 4.92	16.4 ± 3.85	18.8 ± 3.96
SDH	U/L	6.97 ± 4.578	8.64 ± 6.573	10.56 ± 7.100	9.66 ± 7.619	32.20 ± 59.504	3.18 ± 4.440	14.54 ± 16.432	20.54 ± 29.639

(*continued on next page*)

Table 10 (*continued*)

Parameter	Units	Group and Dose (mg/kg BW/day)							
		G1 (0) n = 10	G2 (39.84) n = 10	G3 (92.95) n = 10	G4 (185.9) n = 10	G5 (0) n = 5	G6 (39.84 n = 5	G7 (92.95) n = 5	G8 (185.9) n = 5
Males									
TP	g/dL	6.51 ± 0.651	7.07 ± 0.611	6.88 ± 0.520	7.09 ± 0.603	7.48 ± 0.792	7.06 ± 0.598	7.40 ± 0.604	7.34 ± 0.841
ALB	g/dL	4.73 ± 0.510	5.32 ± 0.535	5.02 ± 0.374	5.05 ± 0.502	5.18 ± 0.642	4.86 ± 0.439	5.28 ± 0.497	5.32 ± 0.691
GLOB	g/dL	1.78 ± 0.215	1.75 ± 0.227	1.86 ± 0.222	2.04 ± 0.207*	2.30 ± 0.412	2.20 ± 0.235	2.12 ± 0.217	2.02 ± 0.259
CHOL	mg/dL	66.7 ± 15.00	84.9 ± 18.47*	77.2 ± 13.85	101.2 ± 14.48***	109.4 ± 38.06	83.8 ± 19.15	97.8 ± 33.09	97.2 ± 31.54
LDL	mmol/L	0.130 ± 0.0442	0.157 ± 0.0427	0.146 ± 0.0347	0.215 ± 0.1581	0.326 ± 0.1305	0.198 ± 0.0642	0.262 ± 0.1023	0.202 ± 0.0476
HDL	mmol/L	1.289 ± 0.2872	1.631 ± 0.3505*	1.501 ± 0.2936	1.882 ± 0.2837***	1.968 ± 0.5734	1.566 ± 0.3658	1.850 ± 0.5897	1.824 ± 0.5131
TRIG	mg/dL	78.5 ± 31.34	63.7 ± 11.15	53.3 ± 16.03	65.4 ± 21.44	71.0 ± 40.54	66.6 ± 33.34	50.2 ± 20.56	76.0 ± 52.91
GLU	mg/dL	167.2 ± 28.35	179.9 ± 35.02	179.5 ± 30.15	163.0 ± 34.98	203.2 ± 33.63	199.4 ± 49.34	191.8 ± 9.04	206.0 ± 32.10
TBIL	mg/dL	0.101 ± 0.0233	0.085 ± 0.0151	0.075 ± 0.0135*	0.060 ± 0.0216**	0.112 ± 0.0342	0.090 ± 0.0173	0.118 ± 0.0286	0.096 ± 0.0207

See Table 5 for the Normal Range, * = p 0.05; ** = p 0.01; *** = p 0.001. Values are mean ± standard deviation. ALB = albumin; ALKP = alkaline phosphatase; ALT = alanine aminotransferase; AST = aspartate aminotransferase; BUN = urea nitrogen; CA = calcium; CHOL = cholesterol; Cl = chloride; CREAT = creatinine; GLOB = globulin; GLU = glucose; K = potassium; NA = sodium; PHOS = inorganic phosphorous; SDH = sorbitol dehydrogenase; TBIL = total bilirubin; TP = total protein; TRIG = triglycerides.

T3 and T4 were significantly decreased ($p < 0.05$) in Groups 3 & 4 when compared to Group 1. For female rats, the means for TSH were significantly increased ($p < 0.05$) for Groups 3 and 4, and the mean T4 was significantly decreased ($p < 0.05$) in Group 4 when compared to means for Group 1. Results for thyroid parameters are presented in Table 12.

3.4.6. Pathology

3.4.6.1. Necropsy observations. There were no macroscopic lesions observed in the 14-day and 90-day studies that were linked to the administration of the test article. In the 90-day study, one female in Group 2 developed a tumor that was consistent with the

Table 11
Urinalysis data for the 90-day study – Main study and recovery animals.

Parameter	Control	39.84 mg/kg bw/day	92.95 mg/kg bw/day	185.9 mg/kg bw/day	Recovery Controls	Recovery 39.84 mg/kg bw/day	Recovery 92.95 mg/kg bw/day	Recovery 185.9 mg/kg bw/day
Males								
pH	5.78 ± 0.667	6.67*d ±0.661	6.50 ± 0.316	6.00 ± 0.433	6.80 ± 0.274	6.70 ± 0.274	7.10 ± 0.548	6.90 ± 0.224
Specific Gravity	1.0289 ± 0.00333	1.0267 ± 0.00500	1.0242 ± 0.00736	1.0283 ± 0.00500	1.0240 ± 0.00548	1.0230 ± 0.00570	1.0210 ± 0.00652	1.0210 ± 0.00548
Protein (mg/dL)	59.4 ± 38.77	62.2 ± 45.42	29.2 ± 37.20	38.9 ± 36.12	27.0 ± 6.71	81.0 ± 122.60	21.0 ± 8.22	12.0 ± 12.55
Urobilinogen (EU/dL)	0.56 ± 0.422	0.38 ± 0.353	0.33 ± 0.327	0.29 ± 0.267	0.20 ± 0.000	0.36 ± 0.358	0.20 ± 0.000	0.20 ± 0.000
Urine Volume (mL)	4.06 ± 3.377	5.44 ± 4.391	9.25 ± 6.114	5.94 ± 3.893	7.70 ± 1.304	6.60 ± 3.190	11.90 ± 4.879	13.30 ± 4.009
Females								
pH	6.29 ± 0.488	5.93 ± 0.189	6.06 ± 0.300	6.15 ± 0.337	6.38 ± 0.750	6.25 ± 0.500	6.50 ± 0.354	6.80 ± 0.274
Specific Gravity	1.0286 ± 0.00244	1.0279 ± 0.00393	1.0267 ± 0.00559	1.0255 ± 0.00685	1.0275 ± 0.00289	1.0275 ± 0.00289	1.0270 ± 0.00274	1.0250 ± 0.00500
Protein (mg/dL)	45.7 ± 38.67	72.1 ± 105.27	16.7 ± 13.92	32.0 ± 37.21	22.5 ± 8.66	18.8 ± 7.50	78.0 ± 124.78	129.0 ± 156.46548
Urobilinogen (EU/dL)	0.66 ± 0.428	0.66 ± 0.428	0.47 ± 0.400	0.44 ± 0.386	0.20 ± 0.000	0.20 ± 0.000	0.20 ± 0.000	0.20 ± 0.000
Urine Volume (mL)	2.50 ± 1.826	2.64 ± 2.282	4.83 ± 6.240	5.30 ± 5.784	3.63 ± 0.479	3.63 ± 0.479	4.30 ± 1.754	5.90 ± 2.329

n = 10/sex/group (recovery n = 5/sex/group). [1]n = 9 for males. Data are presented as mean ± standard deviation (SD). * Indicates $p < 0.05$ when compared with the vehicle control group; dL = deciliter; EU = urobilinogen; mL = milliliters.

Table 12
Thyroid parameters for the 90-day study.

Parameter (unit)	G1 (0) n = 10	G2 (39.84) n = 10	G3 (92.95) n = 10	G4 (185.9) n = 10
Male				
TSH (ng/mL)	2.8 ± 0.18	2.9 ± 0.27	3.3 ± 0.19**	3.3 ± 0.29**
T4 (ng/mL)	20.6 ± 1.84	19.3 ± 1.50	18.1 ± 1.41*	17.9 ± 0.15*
T3 (ng/	0.93 ± 0.09	1.0 ± 0.11	0.74 ± 0.06**	0.71 ± 0.15**
Female				
TSH (ng/mL)	2.8 ± 0.18	2.9 ± 0.27	3.3 ± 0.19**	3.3 ± 0.29**
T4 (ng/mL)	19.3 ± 1.51	18.9 ± 1.56	20.8 ± 1.97	16.9 ± 1.90*
T3 (ng/mL)	1.2 ± 0.13	1.3 ± 0.26	1.2 ± 0.13	1.1 ± 0.20

* Different from control at ($p < 0.05$); * Different from control at ($p < 0.051$).

histopathology of mammary gland adenocarcinoma. No other tumors or neoplasms were observed in any of the study animals. Seven female rats in the 90-day study were observed to have fluid in the uterus. These were: two females in Group 1, one female in Group 2, one female in Group 3, three females in Group 4, one female in group 5, one female in group 6, and one female in Group 7.

3.4.6.2. Organ weights. In the 14-day study, there were no statistical differences ($p < 0.05$) between treatment group means for

Table 13
Organ weight (g) data for the 90-day study – Main study and recovery animals.

Examined Organ	Control	39.84 mg/kg	92.95 mg/kg	185.9 mg/kg	Recovery Controls	Recovery 39.84 mg/kg	Recovery 92.95 mg/kg	Recovery 185.9 mg/kg
Males – 93 Days Relative to Start Date/121 Days Relative to Start Date								
Terminal Body Weight	584.1 ± 58.9	608.3 ± 66.6	590.4 ± 81.0	574.6 ± 55.0	626.8 ± 112.2	607.2 ± 56.5	698.8 ± 59.2	626.2 ± 89.5
Adrenal glands	0.0679 ± 0.0176	0.0574 ± 0.0107	0.0673 ± 0.0292	0.0748 ± 0.0151	0.0608 ± 0.0083	0.0616 ± 0.0136	0.0644 ± 0.0137	0.0722 ± 0.0114
Brain	2.288 ± 0.129	2.296 ± 0.078	2.202 ± 0.093	2.291 ± 0.086	2.312 ± 0.139	2.292 ± 0.067	2.408 ± 0.114	2.366 ± 0.220
Epididymides	1.6123 ± 0.1296	1.6119 ± 0.1363	1.5239 ± 0.1964	1.6404 ± 0.1930	1.5530 ± 0.0608	1.7246 ± 0.1710	1.6054 ± 0.0394	1.6730 ± 0.1932
Heart	1.635 ± 0.216	1.708 ± 0.166	1.553 ± 0.206	1.537 ± 0.202	1.632 ± 0.197	1.802 ± 0.192	1.920 ± 0.113	1.788 ± 0.262
Kidneys	3.477 ± 0.584	3.657 ± 0.571	3.590 ± 0.570	3.844 ± 0.422	3.508 ± 0.369	3.548 ± 0.313	4.194 ± 0.334	3.926 ± 0.630
Liver	14.683 ± 2.122	17.179 ± 2.657	19.299** ± 3.161	20.580*** ± 3.066	16.100 ± 4.192	14.866 ± 2.148	19.930 ± 2.623	17.266 ± 3.736
Spleen	0.910 ± 0.182	0.882 ± 0.111	0.894 ± 0.209	1.026 ± 0.270	0.980 ± 0.296	0.908 ± 0.147	1.062 ± 0.199	1.080 ± 0.268
Testes	4.026 ± 0.406	3.922 ± 0.261	3.785 ± 0.471	3.891 ± 0.318	3.658 ± 0.297	4.108 ± 0.309	3.724 ± 0.393	3.990 ± 0.324
Thymus	0.2738 ± 0.0557	0.2863 ± 0.0553	0.2487 ± 0.0499	0.2917 ± 0.0773	0.2810 ± 0.0381	0.2666 ± 0.0184	0.2118* ± 0.0290	0.2728 ± 0.0449
Females – 94 Days Relative to Start Date/121 Days Relative to Start Date								
Terminal Body Weight	325.0 ± 44.9	327.1 ± 30.4	319.3 ± 25.0	301.1 ± 28.8	317.8 ± 15.0	319.4 ± 20.0	326.4 ± 3.4	328.4 ± 38.2
Adrenal glands	0.0758 ± 0.0117	0.0832 ± 0.0148	0.0890 ± 0.0121	0.1062*** ± 0.0252	0.0716 ± 0.0119	0.0784 ± 0.0143	0.0834 ± 0.0096	0.0826 ± 0.0158
Brain	2.042 ± 0.067	2.055 ± 0.106	2.051 ± 0.083	2.047 ± 0.068	2.104 ± 0.066	2.024 ± 0.135	2.078 ± 0.094	2.046 ± 0.056
Heart	1.012 ± 0.094	1.092 ± 0.101	1.054 ± 0.100	1.030 ± 0.102	1.032 ± 0.058	0.996 ± 0.134	1.134 ± 0.145	1.190 ± 0.090
Kidneys	1.935 ± 0.135	2.175* ± 0.147	2.141* ± 0.209	2.130 ± 0.234	1.984 ± 0.134	2.106 ± 0.204	2.302 ± 0.219	2.468** ± 0.269
Liver	8.472 ± 0.572	11.087* ± 1.475	11.465** ± 0.943	14.071*** ± 1.914	8.758 ± 0.828	9.292 ± 1.192	10.124 ± 0.692	10.600 ± 1.759
Ovaries with Oviducts	0.1288 ± 0.0165	0.1225 ± 0.0294	0.1484 ± 0.0264	0.1250 ± 0.0251	0.1162 ± 0.0398	0.1504 ± 0.0190	0.1214 ± 0.0313	0.1416 ± 0.0373
Spleen	0.554 ± 0.072	0.638 ± 0.165	0.576 ± 0.097	0.544 ± 0.090	0.524 ± 0.052	0.604 ± 0.074	0.576 ± 0.040	0.582 ± 0.036
Thymus	0.2475 ± 0.0690	0.2586 ± 0.0632	0.2669 ± 0.0802	0.2374 ± 0.0486	0.2072 ± 0.0566	0.2354 ± 0.0365	0.2112 ± 0.0484	0.2434
Uterus	0.762 ± 0.402	0.681 ± 0.186	0.829 ± 0.313	0.842 ± 0.325	0.742 ± 0.317	0.748 ± 0.352	0.814 ± 0.371	0.732 ± 0.135

n = 10/sex/group (recovery n = 5/sex/group). [1]n = 9 for males. Data are presented as mean ± standard deviation (SD). * Statistically significant difference with p ≤ 0.05; ** Statistically significant difference with p < 0.01; *** Statistically significant difference with p < 0.001; g - grams

absolute and relative organ weights. For the 90-day study, there were significant differences ($p < 0.05$) in the mean absolute and relative liver weights (Tables 13–15). For male rats in Groups 3 and 4, a statistically significant increase ($p < 0.05$) in absolute liver weight and an increase in relative liver-to-brain weights was observed. The mean liver-to-BW ratios of rats in Groups 2, 3, and 4 were significantly increased ($p < 0.05$) compared to the mean for Group 1. The mean thymus weights for male rats in Groups 6–8 were significantly increased ($p < 0.05$) compared to the mean for Group 5. The mean thymus-to-brain and thymus-to-BW ratios in Group 7 rats were also significantly increased ($p < 0.05$) compared to the means for Group 5. For the female rats in Groups 2–4, the mean absolute liver weights, mean ratios for relative liver-to-brain and liver-to-BW were significantly increased ($p < 0.05$) compared to means for Group 1 females. The mean absolute kidney weights for Group 2 and 4 females were significantly increased ($p < 0.05$) compared to the mean absolute kidney weights for Group 1. The mean ratios for relative kidney-to-brain or kidney-to-BW for Groups 2–4 females were significantly increased ($p < 0.05$). The mean absolute adrenal gland weight, and adrenal gland weight relative to BW or brain weight for Group 4 females were significantly increased ($p < 0.05$). Compared to Group 5, the means for relative spleen-to-brain and spleen-to-BW ratios for Group 6 females were also significantly increased ($p < 0.05$).

3.4.6.3. Histopathology. There were no dose-dependent histopathological observations in tissues examined from female and male rats at the time of termination for the 14-day study. For rats in the 90-day study sacrificed on days 93 and 94, the histopathologic changes were limited to liver tissues collected from both sexes of rats in Groups 2–4 (Table 16). The dose dependent lesions were minimal to mild periportal hepatocellular vacuolization, and centrilobular hepatocellular hypertrophy was observed in essentially all hepatic lobules. The enlarged liver cells were most prevalent in the centrilobular location and were also observed in the midzonal location. In both locations the hepatocytes had homogenous to granular cytoplasm with increased pallor, and there was sinusoidal compression and limited hepatocellular degeneration. These histopathologic changes were considered to increase with the increase in liver weights. The periportal vacuolization was multifocal to diffuse in occurrence, cytoplasmic in location, variable in size, clear in appearance,

Table 14
Organ-to-body weight ratio (g) data – Main study and recovery animals.

Examined Organ	Control	39.84 mg/kg	92.95 mg/kg	185.90 mg/kg	Recovery Controls	Recovery 39.84 mg/kg	Recovery 92.95 mg/kg	Recovery 185.90 mg/kg
Males – 93 Days Relative to Start Date/121 Days Relative to Start Date								
Heart	2.799 ± 0.256	2.843 ± 0.475	2.638 ± 0.184	2.675 ± 0.229	2.633 ± 0.261	2.968 ± 0.160	2.769 ± 0.359	2.856 ± 0.134
Liver	25.101 ± 1.977	28.141* ± 2.055	32.625*** ± 1.786	35.756*** ± 3.1439	25.453 ± 2.508	24.399 ± 1.459	28.511 ± 2.818	27.391 ± 2.323
Spleen	1.550 ± 0.191	1.453 ± 0.134	1.510 ± 0.246	1.772 ± 0.360	1.552 ± 0.301	1.489 ± 0.151	1.518 ± 0.234	1.705 ± 0.185
Kidneys	5.939 ± 0.656	6.015 ± 0.720	6.088 ± 0.537	6.714 ± 0.675	5.691 ± 0.845	5.859 ± 0.438	6.013 ± 0.357	6.274 ± 0.475
Thymus	0.4749 ± 0.1182	0.4795 ± 0.1257	0.4284 ± 0.1055	0.5064 ± 0.1218	0.4611 ± 0.1015	0.4417 ± 0.0449	0.3037* ± 0.0391	0.4423 ± 0.0906
Adrenal Glands	0.1155 ± 0.0229	0.0939 ± 0.0122	0.1139 ± 0.0409	0.1304 ± 0.0240	0.0981 ± 0.0119	0.1015 ± 0.0197	0.0916 ± 0.0154	0.1155 ± 0.0093
Testes	6.917 ± 0.597	6.502 ± 0.657	6.456 ± 0.709	6.815 ± 0.729	5.997 ± 1.279	6.814 ± 0.838	5.355 ± 0.666	6.430 ± 0.606
Epididymides	2.7715 ± 0.1844	2.6674 ± 0.2605	2.6011 ± 0.3131	2.8613 ± 0.26949	2.5522 ± 0.5347	2.8458 ± 0.2176	2.3095 ± 0.1853	2.6900 ± 0.2571
Brain	3.949 ± 0.436	3.811 ± 0.391	3.787 ± 0.492	4.013 ± 0.321	3.762 ± 0.527	3.803 ± 0.401	3.456 ± 0.171	3.807 ± 0.320
Females – 94 Days Relative to Start Date/121 Days Relative to Start Date								
Heart	3.149 ± 0.381	3.345 ± 0.201	3.311 ± 0.322	3.427 ± 0.229	3.248 ± 0.141	3.110 ± 0.256	3.476 ± 0.463	3.650 ± 0.358
Liver	26.374 ± 2.882	34.004*** ± 4.198	35.949*** ± 2.112	46.688*** ± 3.638	27.554 ± 2.247	29.022 ± 2.334	31.020 ± 2.151	32.226* ± 2.958
Spleen	1.719 ± 0.233	1.953 ± 0.483	1.800 ± 0.238	1.802 ± 0.206	1.651 ± 0.181	1.895 ± 0.245	1.765 ± 0.136	1.784 ± 0.142
Kidneys	6.032 ± 0.736	6.675 ± 0.429	6.718 ± 0.577	7.069*** ± 0.256	6.242 ± 0.279	6.589 ± 0.379	7.054* ± 0.693	7.536** ± 0.488
Thymus	0.7597 ± 0.1809	0.7930 ± 0.1981	0.8328 ± 0.2229	0.7859 ± 0.1217	0.6493 ± 0.1591	0.7394 ± 0.1273	0.6472 ± 0.1493	0.7489 ± 0.2879
Adrenal Glands	0.2369 ± 0.0455	0.2570 ± 0.0570	0.2797 ± 0.0392	0.3512*** ± 0.0689	0.2251 ± 0.0346	0.2454 ± 0.0420	0.2555 ± 0.0294	0.2513 ± 0.0329
Uterus	2.399 ± 1.295	2.124 ± 0.736	2.593 ± 0.937	2.814 ± 1.104	2.343 ± 1.046	2.325 ± 1.031	2.494 ± 1.140	2.273 ± 0.592
Ovaries with Oviducts	0.4008 ± 0.0606	0.3760 ± 0.0893	0.4680 ± 0.0975	0.4139 ± 0.0678	0.3645 ± 0.1204	0.4716 ± 0.0575	0.3725 ± 0.0976	0.4365 ± 0.1197
Brain	6.397 ± 0.959	6.338 ± 0.732	6.462 ± 0.607	6.846 ± 0.582	6.627 ± 0.224	6.355 ± 0.562	6.367 ± 0.300	6.308 ± 0.861

n = 10/sex/group (recovery n = 5/sex/group). [1]n = 9 for males. Data are presented as mean ± standard deviation (SD). * Statistically significant difference with $p \leq 0.05$; ** Statistically significant difference with $p < 0.01$; *** Statistically significant difference with $p < 0.001$; g - grams.

Table 15
Organ-to-brain weight ratio (g) data – Main study and recovery animals.

Examined Organ	Control	39.84 mg/kg	92.95 mg/kg	185.90 mg/kg	Recovery Controls	Recovery 39.84 mg/kg	Recovery 92.95 mg/kg	Recovery 185.90 mg/kg
Males – 93 Days Relative to Start Date/121 Days Relative to Start Date								
Heart	0.715 ± 0.091	0.745 ± 0.087	0.705 ± 0.085	0.670 ± 0.073	0.706 ± 0.079	0.787 ± 0.091	0.800 ± 0.084	0.755 ± 0.085
Liver	6.428 ± 0.926	7.478 ± 1.110	8.748*** ± 1.246	8.969*** ± 1.177	6.931 ± 1.616	6.488 ± 0.920	8.268 ± 0.933	7.268 ± 1.204
Spleen	0.398 ± 0.074	0.384 ± 0.041	0.405 ± 0.085	0.447 ± 0.115	0.422 ± 0.115	0.397 ± 0.065	0.440 ± 0.072	0.453 ± 0.080
Kidneys	1.520 ± 0.247	1.591 ± 0.237	1.627 ± 0.216	1.678 ± 0.167	1.522 ± 0.185	1.547 ± 0.111	1.743 ± 0.124	1.658 ± 0.193
Thymus	0.1202 ± 0.0273	0.1250 ± 0.0257	0.1134 ± 0.0253	0.1269 ± 0.0321	0.1215 ± 0.0140	0.1164 ± 0.0085	0.0881* ± 0.0129	0.1168 ± 0.0259
Adrenal Glands	0.0298 ± 0.0079	0.0250 ± 0.0045	0.0304 ± 0.0124	0.0326 ± 0.0061	0.0263 ± 0.0028	0.0270 ± 0.0063	0.0266 ± 0.0050	0.0305 ± 0.0039
Testes	1.760 ± 0.152	1.709 ± 0.109	1.716 ± 0.170	1.699 ± 0.135	1.589 ± 0.193	1.791 ± 0.091	1.550 ± 0.181	1.692 ± 0.135
Epididymides	0.7064 ± 0.0601	0.7015 ± 0.0464	0.6905 ± 0.0681	0.7151 ± 0.0689	0.6745 ± 0.0629	0.7519 ± 0.0636	0.6677 ± 0.0289	0.7098 ± 0.0820
Females – 94 Days Relative to Start Date/121 Days Relative to Start Date								
Heart	0.497 ± 0.056	0.533 ± 0.059	0.515 ± 0.055	0.503 ± 0.050	0.491 ± 0.032	0.492 ± 0.056	0.548 ± 0.087	0.582 ± 0.044
Liver	4.151 ± 0.280	5.400* ± 0.708	5.592** ± 0.436	6.869*** ± 0.879	4.168 ± 0.447	4.618 ± 0.758	4.882 ± 0.435	5.189 ± 0.901
Spleen	0.272 ± 0.036	0.309 ± 0.072	0.281 ± 0.044	0.265 ± 0.037	0.249 ± 0.024	0.298* ± 0.030	0.278 ± 0.023	0.285 ± 0.021
Kidneys	0.948 ± 0.064	1.061* ± 0.087	1.044 ± 0.090	1.040 ± 0.104	0.943 ± 0.056	1.043 ± 0.105	1.110 ± 0.119	1.208** ± 0.141
Thymus	0.1211 ± 0.0336	0.1261 ± 0.0306	0.1300 ± 0.0379	0.1161 ± 0.0243	0.0986 ± 0.0272	0.1173 ± 0.0234	0.1011 ± 0.0196	0.1182 ± 0.0410
Adrenal Glands	0.0371 ± 0.0058	0.0406 ± 0.0072	0.0433 ± 0.0046	0.0518** ± 0.0120	0.0340 ± 0.0050	0.0392 ± 0.0099	0.0401 ± 0.0032	0.0404 ± 0.0076
Uterus	0.374 ± 0.203	0.332 ± 0.090	0.407 ± 0.162	0.410 ± 0.155	0.353 ± 0.152	0.369 ± 0.169	0.396 ± 0.190	0.358 ± 0.070
Ovaries with Oviducts	0.0632 ± 0.0087	0.0593 ± 0.0129	0.0721 ± 0.0109	0.0610 ± 0.0119	0.0550 ± 0.0179	0.0746 ± 0.0107	0.0582 ± 0.0137	0.0694 ± 0.0190

n = 10/sex/group (recovery n = 5/sex/group). [1]n = 9 for males. Data are presented as mean ± standard deviation (SD). * Statistically significant difference with $p \leq 0.05$; ** Statistically significant difference with $p < 0.01$; *** Statistically significant difference with $p < 0.001$; g = grams.

discrete and resembled areas void of lipid. The periportal vacuolization is considered linked to HempChoice® Hemp Oil Extract. Random multifocal hepatocellular vacuolation (variably resembling lipid- or glycogen-type vacuolation) and focal hepatocellular vacuolation (resembling focal fatty change) occurred sporadically across all treatment groups. The periportal hepatocellular vacuolization and centrilobular hepatocellular hypertrophy were not observed in male and female rats in Groups 6–8. All other histopathological observations were considered incidental because they commonly occur in this age and strain of rats and/or had a similar incidence across control and treatment groups.

4. Discussion

There is increasing interest in hemp extracts and in particular, the safety related to ingestion of these extracts which continues to be evaluated. It is well recognized that the composition of hemp extracts can vary considerably [34–36] and it is important that the results of well designed, OECD and GLP compliant safety studies conducted with hemp extracts be published for the scientific community to review. Given the variability which may be present in hemp extracts, evaluation of different hemp extracts using the same well recognized Guideline compliant studies allows for comparison of the safety of these extracts.

The proprietary hemp extract evaluated here contains CBD and other phytochemicals obtained by supercritical CO_2 extraction from the aerial parts of the *Cannabis sativa* L plant. In this study, multiple *in vitro* and *in vivo* parameters were used to evaluate the toxicology of HempChoice® Hemp Oil Extract. HempChoice® Hemp Oil Extract was not mutagenic in a bacterial reverse mutation assay conducted with four strains of *Salmonella typhimurium* and one strain of *Escherichia coli*, both with and without metabolic activation. An *in vitro* micronucleus study was also conducted, both with and without metabolic activation, using human lymphocytes. No biologically relevant increase in micronucleus frequency was noted following exposure to HempChoice® Hemp Oil Extract. The extract was therefore considered to be non-clastogenic in an *in vitro* mammalian cell micronucleus study using human lymphocytes. These results are consistent with the results from other studies with CBD-containing hemp extracts [13,20,37]. A previous study by Marx et al. [20] included an *in vivo* mouse micronucleus test in addition to the bacterial reverse mutation assay and the *in vitro* mammalian cell micronucleus study.

Table 16
Summary of histopathology findings for the 90-day study.

	Main Study				Recovery			
	Control (n = 10)	39.84 mg/kg (n = 10)	92.95 mg/kg (n = 10)	185.90 mg/kg (n = 9 ♂; n = 10 ♀)	Control (n = 5)	39.84 mg/kg (n = 5)	92.95 mg/kg (n = 5)	185.90 mg/kg (n = 5)
Males								
Adrenal gland – cortex vacuolation	1 (mild) 1 (minimal)	NE	NE	1 (mild) 1 (minimal)	0	0	0	0
Prostate – mononuclear cell infiltration	1 (mild) 4 (minimal)	NE	NE	2 (minimal)	0	0	0	0
Prostate – acute inflammation	2 (minimal)	NE	NE	1 (minimal)	0	0	0	0
Kidney – chronic progressive nephropathy	5 (minimal)	NE	NE	6 (minimal)	0	0	0	0
Kidney – mononuclear cell infiltration	3 (minimal)	NE	NE	1 (minimal)	0	0	0	0
Liver – hepatocellular, centrilobular hypertrophy	0	0	8 (mild) 2 (minimal)	8 (mild) 1 (minimal)	0	0	0	0
Liver – mononuclear cell infiltration	7 (minimal)	8 (minimal)	7 (minimal)	7 (minimal)	5 (minimal)	5 (minimal)	5 (minimal)	5 (minimal)
Liver – hepatocellular necrosis	2 (minimal) 2 (single cell, minimal)	1 (single cell, minimal)	0	0	1 (minimal)	0	0	0
Liver – hepatocellular vacuolation	2 (minimal)	0	0	0	0	1 (minimal)	0	2 (minimal)
Liver – periportal, hepatocellular vacuolation	0	1 (mild) 3 (minimal)	1 (mild) 6 (minimal)	3 (mild) 2 (minimal)	0	0	0	0
Females								
Thyroid gland – ectopic tissue	1 (minimal)	NE	NE	0	NE	NE	NE	NE
Thyroid gland – mononuclear cell infiltration	0	NE	NE	1 (minimal)	NE	NE	NE	NE
Kidney – chronic progressive nephropathy	0	NE	NE	1 (mild) 2 (minimal)	NE	NE	NE	NE
Kidney – mononuclear cell infiltration	5 (minimal)	NE	NE	2 (minimal)	NE	NE	NE	NE
Kidney – tubule mineralization	1 (minimal)	NE	NE	0	NE	NE	NE	NE
Liver – hepatocellular, centrilobular hypertrophy	0	2 (mild) 2 (minimal)	7 (mild) 3 (minimal)	9 (mild) 1 (minimal)	0	0	0	0
Liver – mononuclear cell infiltration	7 (minimal)	7 (minimal)	9 (minimal)	9 (minimal)	4 (minimal)	4 (minimal)	3 (minimal)	4 (minimal)
Liver – hepatocellular necrosis	0	0	0	1 (minimal)	2 (minimal)	1 (minimal)	0	0
Liver – periportal, hepatocellular vacuolation	0	2 (minimal)	1 (mild) 2 (minimal)	1 (mild) 1 (mild)	0	0	0	0

NE – not examined

There were no HempChoice® Hemp Oil Extract exposure-related mortalities, abnormal clinical observations or ophthalmological changes. There were also no abnormal observations in the functional observational battery or motor activity assessment which were linked to HempChoice® Hemp Oil Extract exposure. In the 90-day and 28-day recovery study animals, there was no consistent effect on weekly body weight, daily body weight gain and food consumption related to HempChoice® Hemp Oil Extract exposure. These findings are consistent with the findings of previous studies, including Marx et al. [20] who used doses as high as 720 mg of CBD-containing hemp extract/kg BW. Similarly, Dziwenka et al. [13] reported using a dose-range similar to those used in the present study, and Dziwenka et al. [37] reported doses of up to 324 mg/kg BW/day of extract.

There were statistically significant changes in some of the hematological parameters evaluated in both the main and recovery males and females; however, none were considered adverse. The mean values for hematological parameters were within the expected normal range and considered reversible during the 28-day recovery period. The significant decrease in total serum cholesterol noted in all HempChoice® Hemp Oil Extract-treated main study males, as compared to main study controls, was not observed in the recovery males. Both LDL and HDL were significantly decreased in the main study males as well, but not in the recovery males. These changes

were not noted in the females. The changes in LDL and HDL are not considered to have toxicological significance. The changes in serum chloride levels noted in main study females were not dose-dependent and were not present in the males. None of the serum chloride means for the female rats were outside the expected normal range. This change is not considered a toxicological response. The decrease in BUN in the mid-dose males was also not a dose-dependent response and was not observed in females. These mean values were not outside of the expected normal range and the decrease in BUN is not considered of pathological and toxicologic significance. Total bilirubin was significantly decreased in all HempChoice® Hemp Oil Extract treated main study males and the mid- and high-dose females, as compared to their respective main study controls. This change was not observed in the recovery groups. Decreases in total bilirubin may be linked to the test article influencing hepatic-gut microbiome signaling [38]. Increased hepatic conjugation rates and secretion of bilirubin due to the induction of hepatic enzymes may be another possible mechanism for the reduced total bilirubin [39,40]. Decreases in ALT have been associated with CYP induction and small decreases in ALT/AST generally may be disregarded as non-adverse [40]. All other changes in clinical chemistry parameters were considered to be unrelated to HempChoice® Hemp Oil Extract exposure as they occurred sporadically and were considered to be due to biological variance.

The necropsy observations in the 90-day study included one female rat with a tumor (mammary adenocarcinoma) and increases in uterine size due to increased luminal fluid observed in some females across all study groups. Both necropsy observations were not considered to be a toxic effect of HempChoice® Hemp Oil Extract. Mammary adenocarcinomas spontaneously occur in female rats at 12-weeks of age and is a common spontaneous neoplasia in female Sprague-Dawley rats [41–43]. The mammary adenocarcinoma observed in this study occurred in only one rat and is considered a spontaneous occurrence. Fluid accumulation in the lumen causing distention of the uterus without histopathology is a sign of normally occurring estrus in the rat and is a physiological phenomenon and was not impeded by treatment with HempChoice® Hemp Oil Extract [44,45].

Changes in weights of some organs occurred in the study. Because organ weights may change as a result of changes in body weight, it is useful to determine the relative weight of organs respective to body weight to normalize the effect of body weight on organ weight. Further, it is useful to express organ weights relative to brain weight if brain weight is not affected by the test material. The changes reported in the weights of the thymus, kidneys and spleen were considered to be incidental as there were either no correlating histopathological findings, they occurred in the controls as well, were seen in only one sex, or were not dose dependent. Moreover, the increases in kidney weights were not with changes in the urinalysis parameters evaluated. An increase in adrenal gland weight occurred in Group 4 female rats (dosed with 185.9 mg/kg BW/day of HempChoice® Hemp Oil Extract and terminated at 93/94 days) regardless of method of expression. No change in adrenal weight was reported in the lower dose groups and no correlating abnormal histopathological findings were observed. Similar findings have been observed in 90-day studies in rats with other CBD-containing hemp extracts. Dziwenka et al. [13] reported a statistically significant increase in relative adrenal gland weight to body weight in female rats administered 800 mg hemp extract/kg BW/day (but not at 400 mg/kg BW/day) that did not correlate with any adrenal histopathology. In a toxicity study in Sprague-Dawley rats by Marx et al. [20], using a supercritical CO_2 hemp extract containing 96% CBD and <1% THC, vacuolization of cortical cells in the zona fasciculata and zona reticularis were observed in the rats dosed with 720 mg of extract/kg BW/day (6/10 males and 8/10 females), but not at 360 or 100 mg/kg BW/day. In males, increases in adrenal weights were observed at 720 or 360 mg/kg BW/day (regardless of method of expression), but not at 100 mg/kg BW/day. In females, this was true for the higher dose but not at lower doses (only relative adrenal weight to BW was increased at 360 mg/kg BW/day). These results suggest that the effects of hemp extracts on the adrenal glands are dose dependent, with increases in adrenal weights occurring prior to histopathological changes. It is important to note that the changes that have occurred in the adrenal glands of rats administered hemp extracts are reversible. The reason for the increased adrenal weight of female rats administered 185.9 mg/kg BW/day of HempChoice® Hemp Oil Extract is unclear but may be a secondary response to stress [46].

In the study being reported, a dose-dependent increase in liver weights occurred (regardless of method of expression) which correlated with increases in minimal to mild centrilobular hepatocellular hypertrophy and minimal to mild periportal hepatocellular vacuolation. The increase in liver weights observed in laboratory animals dosed with CBD-containing hemp extracts has been reported and similar histological changes may or may not be observed, depending on extract dose [13,20,37]. The liver responses noted are likely adaptive to a large metabolic load. Cannabinoids are extensively metabolized by CYP, and the highest level of expression of CYP is in the liver and intestine [47]. Kutanzi et al. [48] studied the molecular changes induced by CBD-rich extracts (5.1% CBD and 0.2% THC) in male mice (C57BL6/J), concurrently given methylsulfonylmethane (MSM). In the mice receiving MSM in drinking water that were also administered daily doses of the CBD-rich extract, increases in hepatic mRNA for CYP1a2, CYP2b10, CYP2c29, CYP3a4, CYP3a11, CYP2c65, and CYP2c66 were observed and the concurrent administration of MSM was considered to have no effect on these parameters. Using Sprague Dawley rats in a vitamin D_3 deficiency model, Trivedi et al. [49] showed that hepatic CYP-mRNA was likely up- and down-regulated by CBD. The vitamin D_3 deficient rats received vitamin D_3 plus CBD (15, 30 or 60 mg/kg BW) for 8 weeks. Hepatic CYP2R1-mRNA was up regulated 38.4% in the group receiving 60 mg CBD/kg BW compared to control.

The histopathological observations in Groups 2–4 related to HempChoice® Hemp Oil Extract administration were limited to the liver and included a dose-dependent increase in minimal to mild centrilobular hepatocellular hypertrophy and minimal to mild periportal hepatocellular vacuolation. These histopathological observations were not observed in any of the treated rats in Groups 5–8 (recovery groups). A summary of the histopathological findings from select tissues is shown in Table 16. There was no increase in liver-specific enzymes in serum indicating there was no necrosis. Liver histopathology linked to dosing with CBD-containing hemp extracts has been reported in rodents [13,20,37]; however, as in the current study, liver cell necrosis was not observed. Dziwenka et al. [37] reported increases in liver weights as well as fatty changes in the livers of animals dosed with 324 mg/kg BW/day of hemp extract which were reversible and did not have correlating changes in related clinical pathology parameters. The authors did not consider these changes to be adverse. Dziwenka et al. [13] in a study on a proprietary CBD containing hemp extract, described centrilobular hepatocellular hypertrophy occurring in a dose-dependent manner in both sexes of Sprague-Dawley rats and this histopathology was

also correlated with increased liver in weight. Dziwenka et al. [37] did not observe periportal hepatocellular vacuolation. The hemp extract was administered at doses of 200, 400, and 800 mg/kg/day for 90 days and the NOAEL was determined to be 800 mg/kg BW/day for the females and 400 mg/kg BW/day for the males based on the decrease in body weight which was >10% and still evident at the end of the recovery period, not the reversible changes related to the liver. In a study in which Hsd.Han Wistar rats were dosed with 0, 100, 360, and 720 mg CBD-containing hemp extract/kg/day for 90-days, the authors did not observe liver histopathology, however, cytoplasmic vacuolization of hepatocytes was noted in rats dosed with ≥1000 mg/kg BW/day of the same extract for 14 days [20].

Up-regulation of the CYP enzymes in rodents typically causes centrilobular to midzonal pattern of hepatocellular hypertrophy with the effect being more localized in the centrilobular region. This phenomenon is also accompanied by increased liver weight parameters. This histopathologic observation is reversible with cessation of treatment [50]. In the present study, the periportal hepatocellular vacuolation is a putative fatty, glycogen deposition and/or CYP induction change, based on location and appearance; however, special stains were not utilized for lipids and glycogen, or hepatic CYP enzyme parameters measured. Periportal hepatocellular vacuolation containing lipid can be a spontaneous occurrence in rats and generally occurs in older rats [40,50,51]. In this study, periportal hepatocellular vacuolation was not observed in control rats (Groups 1 and 5) and occurred in the treated rats (Groups 2–4) in a dose-dependent manner. This histopathology was not observed in livers of rats from Groups 6–8 (recovery groups) showing periportal hepatocellular vacuolation was reversible. The increase in relative liver weights, the minimal to mild centrilobular hepatocellular hypertrophy, and minimal to mild periportal hepatocellular vacuolation are considered to be non-adverse, adaptive and fully reversible changes given the decreases, rather than increases, in serum ALT and AST and no obvious adverse clinical effects overall. It is generally regarded that liver weight increases through hepatocyte enzyme induction, in the absence of histopathologically demonstrated degenerative or necrotic changes and without significant changes in hepatic derived plasma enzymes, are not considered adverse and would have little relevance to humans in terms of risk assessment [40].

Thyroid hormones were significantly changed by administration of HempChoice® Hemp Oil Extract. For male rats, a comparison of Group means showed that TSH levels were significantly increased ($p < 0.05$) and T3 and T4 were significantly decreased ($p < 0.05$) in the mid and high-dose groups when compared to the main study controls. For female rats, the means for TSH were significantly increased ($p < 0.05$) for the mid and high-dose groups, and the mean T4 was significantly decreased ($p < 0.05$) in the high-dose group when compared to means for the main study controls. It is well known that induction of hepatic xenobiotic metabolizing enzymes is linked with increased catabolism of T4 and T3 [52]. Decreases in plasma T4 and T3 are also associated with increased liver weights and hepatocellular hypertrophy [53]. There was no histopathology observed in the thyroid and pituitary glands. The changes in TSH, T4 and T3 observed in the study being reported are considered a secondary effect due to increased removal of T4 and T3 from plasma by the liver [53]. The increase in TSH is considered a physiological response to the decrease in serum T4 and T3 because it occurred without concurrent histological changes in the pituitary and thyroid glands [54].

5. Conclusion

The results of a bacterial reverse mutation assay and an *in vitro* micronucleus assay show that HempChoice® Hemp Oil Extract is non-genotoxic. The results of a 90-day repeat-dose oral toxicity study reported a no observed adverse effect level (NOAEL) for HempChoice® Hemp Oil Extract of 185.90 mg/kg BW/day for both male and female Sprague-Dawley rats, which was the highest dose evaluated.

Author contribution statement

Margitta Dziwenka, Robert Coppock: Analyzed and interpreted the data; Wrote the paper.

Michael H. Davidson, Marc A. Weder: Conceived and designed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data.

Data availability statement

The data that has been used is confidential.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Abbreviations

ALKP – alkaline phosphatase, ALT – alanine aminotransferase, AST - aspartate aminotransferase, BUN-blood urea nitrogen, BW – body weight, CBD – cannabidiol, CO2 – carbon dioxide, CYP – cytochrome P450, DMSO – dimethyl sulfoxide, GLOB – globulin, HDL-high-density lipoprotein cholesterol, LDL - low-density lipoprotein cholesterol, PHA – phytohemagglutinin, SDH – sorbitol dehydrogenase, TBIL - total bilirubin, THC - delta-9-tetrahydrocannabinol, TP – total protein, Funding details; No outside funding was used to support this work.

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