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ORIGINAL RESEARCH

Biological Response after 14-Day Cannabidiol and Propylene Glycol Inhalation in Sprague–Dawley Rats

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Abstract

Objective: Cannabidiol (CBD), a phytocannabinoid of increasing interest for its purported therapeutic effects, is primarily consumed *via* ingestion and inhalation. While the toxicology of orally administered CBD has been reported, little is known about the effects of CBD inhalation. Doses selected for the present analysis allowed for evaluation of dose-response at concentrations >100-fold higher than typical human consumption levels.

Materials and Methods: CBD (98.89% pure) was formulated in propylene glycol (PG) and aerosolized by nebulization to evaluate biological response after nose-only inhalation. Sprague Dawley rats (n = 35 males, 30 females) were exposed to 1.0 and 1.3 mg/L nominal concentrations of CBD and PG, respectively, for 12–180 min. Resulting average daily presented dose ranges were 8.9–138.5 mg/kg CBD and 11.3–176.0 mg/kg PG. Aerosols of 1.4 μm median diameter were achieved. Biological response indicators included clinical signs, clinical chemistry, hematology, body/organ weights, and pulmonary/systemic histopathology.

Results: Inflammatory and necrotic responses were observed in the nose at the highest doses of CBD. Limited findings in the larynx and lung were mainly observed at higher doses. There were no histological findings in extrapulmonary organs. Dosimetry modeling differentiated the no observable adverse effect level between the nasal region and lungs to be 2.8 and 10.6 mg/kg CBD, respectively.

Conclusions: Dose-depending findings of histological changes in the respiratory tract are observed at high doses. At lower doses consistent with typical over-the-counter vape products there appears to be substantial safety margin in the present study (93- and 353-fold lower for nose and lung, respectively).

Keywords: vaping; cannabidiol; CBD; propylene glycol; nose-only inhalation

Introduction

Cannabidiol (CBD), the major non-euphorigenic phytocannabinoid derived from cannabis, is of increasing interest for its therapeutic properties, including the treatment of seizure disorders, inflammation, depression, anxiety, and for tobacco smoking cessation.[1–7] The main routes for human cannabinoid consumption are ingestion and inhalation; inhalation may improve cannabinoid delivery into systemic circulation by circumventing the pharmacokinetic variability associated with gastrointestinal absorption and first-pass hepatic metabolism, thereby improving time to onset of effects.[8] Vaporization of cannabinoid products, including CBD, has increased in popularity as an alternative to smoking combustible products.[9,10]

While the safety of oral CBD administration has been evaluated in animals and humans,[11,12] few publications have explored toxicological endpoints following CBD inhalation. *In vitro*, exposure of epithelial cells to CBD aerosols resulted in differential effects on inflammatory markers.[13] In rats, CBD inhalation was shown to significantly reduce body temperature (100 or 400 mg/mL CBD) and locomotor activity (400 mg/mL CBD).[14,15] In humans, inhalation of a dry-powder

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inhaler CBD formulation (2.1 mg CBD) resulted in a transient, dry, throat-clearing cough in 3 of 12 study participants, but no clinically meaningful changes in physical examinations, clinical hematology, urinalysis, electrocardiogram, or vital signs.[16] To date, no *in vivo* repeat dose inhalation toxicology studies including pathology endpoints have been reported.

The present study assessed the safety of CBD inhalation across a range of doses in Sprague–Dawley rats. CBD was formulated in propylene glycol (PG), a thinning agent often included in vaping pens for efficient product delivery. The toxicology of PG has been extensively investigated, and its hazards, even at high doses for long durations (e.g., 90 days), have been deemed largely insignificant.[17,18] We have previously reported on the comparative safety of PG over 14 days at the doses used in the present study.[19] CBD doses selected for the present analysis allowed for the evaluation of effects at doses >100-fold higher than typical human consumption levels in over the counter products.

Material and Methods

Experimental design

Six (6) groups of animals ($n = 5$ males and 5 females per group) were exposed to filtered air or test article at a target aerosol concentration of 1.0 mg/L CBD and 1.3 mg/L PG *via* nose-only inhalation for 14 consecutive days. Filtered air (control) exposures were 180 min per day, CBD/PG exposures were 12, 23, 45, 90, or 180 min per day. Animals were monitored for clinical signs and body weight changes and were euthanized on study day (SD) 15, 1 day following the final exposure. Blood was collected for clinical pathology endpoints. Gross necropsy was performed, organ weights were obtained, and tissue was collected for histopathology.

The corresponding pulmonary doses were approximately 1–20 mg/kg in rats. These doses were selected to provide a significant margin of safety when compared to common clinical doses (e.g., 0.02 mg/kg/puff, 70 kg human).[21] Detailed methods can be found in the Supplementary Data S1.[22,24–28]

All animal work complied with the Final Rules of the Animal Welfare Act regulations (9 CFR Parts 1, 2, and 3) and the Guide for the Care and Use of Laboratory Animals.[20]

Results

Inhalation exposure and pulmonary doses

The average aerosol concentration for CBD and PG was 1.0 and 1.3 mg/L, respectively (Table 1), resulting in average daily presented doses ranging from 8.9 to 138.5 mg/kg CBD and 11.3 to 176.0 mg/kg PG. Deposited doses were calculated for nasal (tracheobronchial) and lung (pulmonary) deposition, and ranged from 2.8 to 44.1 mg/kg CBD for nose and 1.4 to 21.8 mg/kg CBD for lung, and 3.6 to 56.1 mg/kg PG for nose and 1.8 to 27.7 mg/kg PG for lung. The MMAD (GSD) for aerosol was 1.4 (2.1) μm.

Clinical observations and survival

Four animals in Group 6 (21.8/27.7 mg/kg CBD/PG lung deposited dose) had exposure related clinical signs in the form of respiratory distress and labored breathing and were euthanized as moribund or subsequently found dead, with deaths occurring on SD 5 (1 male), SD 11 (1 male and 1 female), and SD 15 (1 male). Remaining animals survived until scheduled

Table 1. Daily Concentration, Presented Dose, and Nasal and Lung Deposition of CBD and PG in Sprague Dawley Rats

Group	*N* (sex)	Exposure duration (min)	Concentration (mg/L)*			Presented dose (mg/kg)†		Nose deposited dose (mg/kg)*†		Lung deposited dose (mg/kg)*†	
			Gravimetric	CBD	PG	CBD	PG	CBD	PG	CBD	PG
1	10 (5M: 5F)	180	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
2	10 (5M: 5F)	12	2.9 ± 0.3	1.0 ± 0.1	1.3 ± 0.2	8.9	11.3	2.8 ± 0.1	3.6 ± 0.1	1.4 ± 0.0	1.8 ± 0.0
3	10 (5M: 5F)	23				17.0	21.6	5.4 ± 0.1	6.9 ± 0.2	2.7 ± 0.1	3.4 ± 0.1
4	10 (5M: 5F)	45				34.6	44.0	11.0 ± 0.2	14.0 ± 0.3	5.5 ± 0.1	6.9 ± 0.1
5	15 (10M: 5F)	90				67.0	86.2	21.4 ± 0.5	27.5 ± 0.6	10.6 ± 0.2	13.6 ± 0.3
6	10 (5M: 5F)	180				138.5	176.0	44.1 ± 1.0	56.1 ± 1.3	21.8 ± 0.5	27.7 ± 0.7

*mean ± SD.

†presented and deposited doses are calculated using the following formula: Dose = (C × RMV × T × DF)/BW; where C = mean total aerosol concentration (mg/L), RMV, respiratory minute volume = $0.608 \times BW^{0.852}$,[22] T, exposure time (min), DF, deposition fraction, assumed to be 100% for the presented dose, 32% for nose and 16% for lung deposited dose, based on calculations using Multipath Particle Dosimetry (MPPD) model software)[23] and BW, body weight (kg).

CBD, cannabidiol; F, female; M, male; MPPD, multipath particle dosimetry; PG, propylene glycol; SD, standard deviation.

necropsy and did not present with any persistent clinical observations.

Body weight

In males, body weights of Group 5 (10.6/13.6 mg/kg CBD/PG lung deposited dose) and Group 6 (21.8/27.7 CBD/PG lung deposited dose) animals were significantly reduced starting at SD 8 or SD 3, respectively, as compared with the air control (Fig. 1). These groups displayed an overall body weight loss of 3.33 and 17.79%, respectively, as compared with pre-exposure body weight. In females, no difference was detected between CBD/PG treated and control animals.

Gross pathology and organ weight

No CBD/PG exposure related gross lesions were observed, but some significant changes in organ weight were noted (Table 2). Changes were attributed to high dose male animals and all but the changes in kidney and liver weights were dose responsive. Group 5 females (6.4 mg/kg CBD) showed a significantly increased lung to body weight ratio.

Clinical pathology

Significant dose-responsive changes in several clinical pathology parameters were observed following inhalation of CBD/PG *vs.* air control. In males, lymphocytes, white blood cells, blood urea nitrogen, and blood urea nitrogen/creatinine ratio were significantly decreased. In females, mean corpuscular hemoglobin concentration, albumin/globulin ratio, albumin and total protein were significantly decreased, while red cell distribution width was increased. Changes were of small magnitude and, although dose-responsive, are not considered CBD/PG exposure related.

Microscopic observations

CBD/PG exposed groups demonstrated prominent, dose-responsive changes within the nose/turbinates at higher dose levels (Fig. 2). Changes were present in a much more limited fashion in some other respiratory tract tissues including larynx, trachea, and lung. There were no remarkable changes within tissues examined in the control group.

In Group 2 (1.4/1.8 mg/kg CBD/PG lung deposited dose), minimal to mild degenerative changes were observed in most animals, focused primarily on the olfactory epithelium of the caudal nose/turbinates (levels 3 and 4). Minimal alteration of laryngeal epithelium at the base of the epiglottis was also present in most animals (characterized by minor increases in cell layers and slight flattening), which has been observed in rodent larynx as a precursor to squamous metaplasia. Lungs demonstrated only minimal sporadic changes in scattered animals typical of background findings.

In Group 3 (2.7/3.4 mg/kg CBD/PG lung deposited dose), increased nasal changes were observed, with most animals demonstrating minimal to mild mixed inflammation, squamous metaplasia of respiratory epithelium, and degeneration of olfactory epithelium at increased prevalence in both the rostral and caudal levels of the nose/turbinates. Minimal

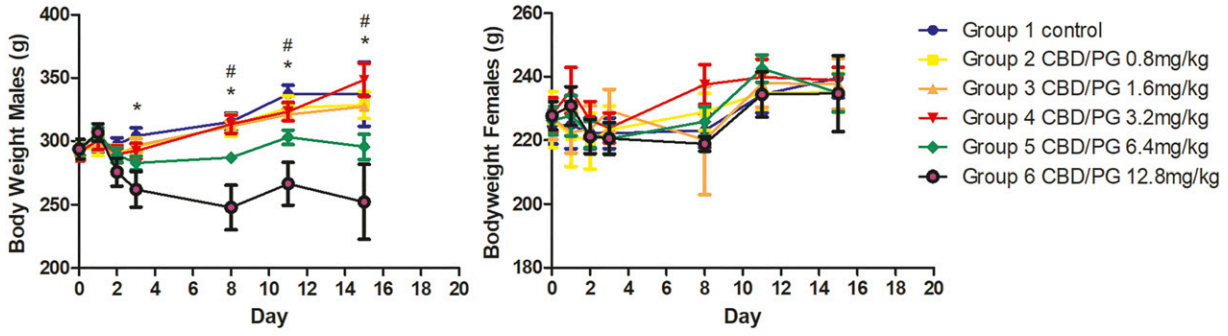

FIG. 1. Body weight following 14-day CBD/PG inhalation in **(A)**: male and **(B)**: female Sprague–Dawley rats ($n = 5$ per sex per group; $n = 10$ [Group 5, days 0–11]). Values are presented as mean ± SD. Day 0 values represent pre-exposure body weight (Day −1 or 2). Significant differences (adjusted *p-value* ≤ 0.05) between air control (group 1) and treatment groups 5 (*) and 6 (#) are indicated. CBD, Cannabidiol; PG, propylene glycol.

Table 2. Summary of Significant Organ Weight Changes following 14-Day CBD/PG Inhalation in Sprague Dawley Rats

	CBD dose					
Organ	0.8 mg/kg (group 2)	1.6 mg/kg (group 3)	3.2 mg/kg (group 4)	6.4 mg/kg (group 5)	12.8 mg/kg (group 6)	Dose response
Adrenal / BW				↑ (M)		↑ (M)
Brain / BW				↑ (M)	↑ (M)	↑ (M)
Kidney				↓ (M)	↓ (M)	
Liver	↓ (M)	↓ (M)		↓ (M)	↓ (M)	
Liver / brain weight	↓ (M)			↓ (M)	↓ (M)	
Lung / BW				↑ (F)	↑ (M)	↑ (M)
Spleen				↓ (M)	↓ (M)	↓ (M)
Spleen / brain weight				↓ (M)	↓ (M)	↓ (M)
Spleen / BW					↓ (M)	↓ (M)
Testis / BW				↑ (M)	↑ (M)	↑ (M)
Thymus				↓ (M)	↓ (M)	↓ (M)
Thymus / brain weight				↓ (M)	↓ (M)	↓ (M)
Thymus / BW						↓ (M)

$n = 5$ males and 5 females (groups 2, 3, 4 and 6); $n = 10$ males and 5 females (group 5).
↓significant decrease ($p \leq 0.05$) compared with the air control; ↑ significant increase ($p \leq 0.05$) compared with the air control.
BW, body weight; CBD, cannabidiol; F, female; M, male; PG, propylene glycol.

alteration of laryngeal epithelium at the base of the epiglottis was present in all animals.

In Group 4 (5.5/6.9 mg/kg CBD/PG lung deposited dose), nasal changes continued to be increased in distribution and severity, with all animals demonstrating minimal to marked inflammatory and degenerative changes including mixed inflammation, squamous metaplasia of respiratory epithelium, and degeneration of olfactory epithelium in both the rostral and caudal levels of the nose/turbinates, and minimal alteration of laryngeal epithelium at the base of the epiglottis.

Group 5 (10.6/13.6 mg/kg CBD/PG lung deposited dose) displayed similar but more severe nasal changes. Degenerative changes had progressed to degeneration/loss of the epithelium in several animals. This is a more severe change than other degenerative changes, which leave the epithelial layer intact. Epithelial loss may prevent further characterization of the other degenerative changes and thus should be regarded as essentially "substituting" for them in some cases (that is, changes in the next higher dose group to a lower incidence or severity may be ignored when this change is present). Larynges demonstrated minimal to mild inflammation and/or squamous metaplasia in most animals.

Highest dose exposure (Group 6; 21.8/27.7 mg/kg CBD/PG lung deposited dose) caused the most severe nasal changes with widespread moderate to severe inflammatory and degenerative changes. There was an increase in the incidence and severity of degeneration/loss of nasal epithelium which was marked severe in one or more nasal sections of almost half the animals. The larynx demonstrated changes including mild to moderate inflammation and/or squamous metaplasia in most animals.

Minor findings in the nose/turbinates and larynx of Group 2 animals are judged likely to recover readily and are considered non-adverse. Findings in all other dose groups are considered adverse based on the severity of their necrosis and inflammation.

In Groups 2 to 5 (1.4–21.8 mg/kg CBD lung deposited dose), lungs demonstrated only minimal sporadic changes in scattered animals typical of background findings. Lungs of half of the animals in Group 6 (21.8/27.7 mg/kg CBD/PG lung deposited dose) had minimal to moderate mixed inflammation in centriacinar areas. There were no histological findings in extrapulmonary organs.

Determination of NOAEL

Regional dosimetry modeling allowed for improved extrapolation of regional risk in the nose and lungs of rodents. Using regional specific information and results from this study (with particular focus on the histopathology observations), we defined the no observable adverse effect level (NOAEL) to be 2.8 mg/kg CBD and 3.6 mg/kg PG. Since moderate mixed inflammation in centriacinar areas of the lung were found in highest dose animals only and findings in other groups were sporadic, the NOAEL for lung exposure was considered 10.6 mg/kg CBD and 13.6 mg/kg PG.

Discussion

The present study evaluated the toxicology of inhaled CBD across a range of doses (8.9–138.5 mg/kg CBD presented dose) over 14 days in rats. Dose levels were

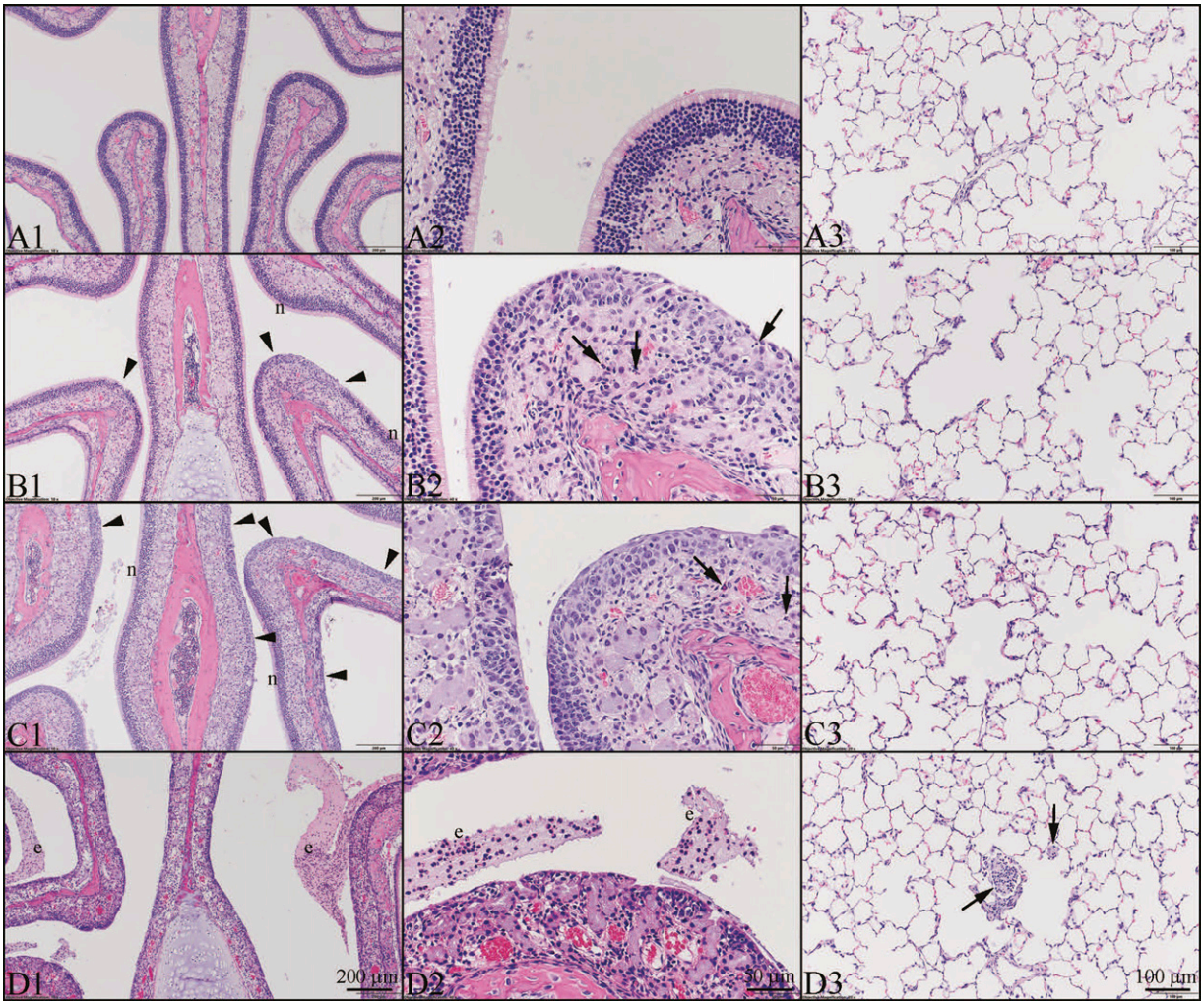

FIG. 2. Illustrative photomicrographs demonstrating nasal and lung changes in Sprague–Dawley rats following 14-day CBD/PG inhalation. Magnifications: column 1 *via* 10X objective, bar = 200 μm; column 2 *via* 40X objective, bar = 50 μm; column 3 *via* 20X objective; bar = 100 μm. A1-A3; Air Control. A1, A2: Normal nasal mucosa consisting of olfactory epithelium in the caudal nasal cavity (level 3 of 4 shown) overlying the ethmoid turbinates. A1: Low power view *via* 10X objective. A2: High power view *via* 40X objective demonstrating tip of one of the ventral/inferior turbinates (5th ethmoturbinate). A3: Normal lung; medium power view *via* 20X objective. B1-B3; CBD/PG 12-min daily exposure for 14 days. B1, B2: Minimal foci of degeneration of the olfactory epithelium (arrowheads). Most of the nasal epithelium is within normal limits (examples denoted by "n"). Note loss of normal orderly architecture along with mixed inflammation (arrows) with macrophages, lymphocytes and neutrophils and some necrotic cellular debris. B3: Lung is within normal limits. C1-C3; CBD/PG 23-min daily exposure for 14 days. C1, C2: Areas of epithelial degeneration (arrowheads) and inflammation (arrows) similar to those above are more extensive and affect more of the olfactory epithelium (arrowheads). Areas of essentially normal epithelium persist ("n"). C3: Lung is within normal limits. D1-D3; CBD/PG 90-min daily exposure for 14 days. D1, D2: Areas of degeneration with epithelial thinning, disorganization and loss are widespread, occupying the entire field of view in this image. Inflammatory cells within the submucosa are prominent and exudates ("e") of inflammatory cells, mucus, protein, and debris are often present within nasal passages. D3: Scattered centriacinar areas of the lung (areas at the junction between conducting airways and gas exchange regions) demonstrate minimal mixed inflammatory infiltrates in some animals. CBD, Cannabidiol; PG, propylene glycol.

regulated by exposure duration, from 12 min (low level of exposure) to 180 min (highest feasible level of exposure), which was in line with Organisation for Economic Co-operation and Development (OECD) recommendations for repeated dose inhalation studies.[29] Nose-only inhalation of a CBD/PG formulation resulted in no exposure related gross lesions, no histological findings in extrapulmonary organs, small changes to select clinical pathology measures, and minimal limited microscopic findings in the larynx and lung. We did observe significant changes in certain organ weights and inflammatory and necrotic responses in the nose, which were dose responsive. Male animals showed a significant loss in body weight at higher dose levels, which was not seen in female animals. However, microscopical evaluation did not indicate male animals being more sensitive than females and potential sex differences could not be confirmed. Dosimetry modeling differentiated the NOAEL between the nasal region (2.8 mg/kg CBD) and lungs (10.6 mg/kg CBD).

The present study did not include a control group exposed to PG, the thinning agent used in the present analysis, without CBD. However, the toxicology of PG has been extensively investigated and deemed to be insignificant, even at high doses for extended durations.[17,18] In a previous 14-day inhalation study from our group, PG alone did not cause adverse effects in Sprague–Dawley rats at presented doses up to 1151.7 mg/kg/day.[19] Given that maximum PG presented doses in the present study were 176.0 mg/kg/day (i.e., >6.5-fold lower than the highest dose in our previous investigation), the observed effects in the present study are most likely attributable to CBD.

Previous preclinical safety studies have largely relied on oral, intraperitoneal or subcutaneous CBD administration,[11] which does not reflect the pharmacokinetic profile or toxicological risk of CBD vapor inhalation. Previous studies have indicated that CBD/PG inhalation (30 min) resulted in a significant reduction in body temperature and locomotor activity in male rats.[14,15] We failed to observe any changes in activity following CBD inhalation, possibly owing to methodological differences between studies (e.g., timing of observations, nose-only *vs.* whole chamber inhalation apparatus, resultant CBD dose) or differences in the sensitivity of Wistar versus Sprague–Dawley rats to the locomotor effects of CBD inhalation. Of note, previously reported plasma CBD concentrations from animals in the highest exposure group in the present study (maximum concentration [C_{max}] following a single dose = 2400 and 3300 ng/mL in males and females, respectively)[8] were ~6- to 24-fold higher than plasma concentrations associated with locomotor effects in Wistar rats.[15] Body temperature was not evaluated in the present study.

The CBD doses selected for the present toxicological evaluation inform on a significant margin of safety for human extrapolation. Given that PG-formulated CBD vape cartridges contain ~200 to 600 mg CBD[31], and the number of puffs available per cartridge is typically 100, the resultant inhaled dose is 2–6 mg CBD per puff. For a 70 kg human, assuming 100% of product inhaled remains in the body (a significant portion would be exhaled), the resultant deposited dose is therefore ~0.03–0.09 mg/kg CBD per puff. As such, this study evaluated doses that are at least 300-fold higher than those typically consumed by humans.

The present research indicates rodent inhalation of a CBD/PG aerosol showed dose dependent toxicity in the nasal cavity of rodents, but limited response in the lung and extrapulmonary organs. A single puff in a human is considered to be 0.03 mg/kg, or 353 times lower than the reported NOAEL for lung in this study and 93 times lower for the nose. These early data suggest that CBD inhalation does result in adverse findings at extreme doses, but the dose-response to lower levels shows the effects subside at levels that are substantially above reported human doses.

The discovery of marked dose-dependent findings in the nasal region but not in the lung following CBD inhalation was unexpected. We first hypothesized that there was poor dose delivery to the lung, which could occur with a non-ideal aerosol characteristic. However, the particle MMAD of 1.4 μm (GSD: 2.1) suggests particle size was small enough to reach the deep lung.[30] Due to our region-specific findings in the respiratory tract, and challenges in benchmarking these results to oral inhalation in humans, the effect levels were determined separately for nasal and lung regions. This approach considers the challenge in risk extrapolation from rodents, who are obligate nasal breathers, to human oral inhalation.[23] In humans, the CBD dose would generally bypass the nose; it is unclear if findings from the rat nose reflect changes that may occur in human lungs that have significantly increased unit dose/surface area and different anatomy/physiology. Moreover, the nasal anatomy differs between species[32,33]. The cannabinoid receptors CB1 and CB2 have been identified in mouse

Table 3. Estimated Human Daily Nasal and Lung Deposition of CBD and PG

Human user (inhalation route)	Exposure duration (min)	Deposition fraction (%)		Nose deposited dose (mg/kg)*		Lung deposited dose (mg/kg)*	
		Nose	Lung	CBD	PG	CBD	PG
Active (oral)	12	3	28	0.12	1.1	0.15	1.4
Passive (oronasal)	12	29	20	1.1	1.4	0.76	1.0

*deposited dose is calculated using the following formula: Dose = (C ×RMV × T × DF)/BW; where C, mean total aerosol concentration (mg/L); RMV, respiratory minute volume ($0.608 \times BW^{0.852}$)[22], T, exposure time (min); DF, deposition fraction (assumed to be 32% for nose and 16% for lung deposited dose based on calculations using Multipath Particle Dosimetry (MPPD) model software)[23], and BW, body weight (kg).

CBD, cannabidiol; MPPD, multipath particle dosimetry; PG, propylene glycol.

olfactory epithelium;[34] the pathology observed in the upper respiratory tract in the present study may relate to CBD activity at these receptors. In addition, mechanistical research is needed to better understand the mechanistic aspects of the findings we outline in our study. Regardless, our findings in the rat nose reflect an adverse response to high doses of inhaled CBD in a living organism, and thus cannot be ignored.

In order to extrapolate the present findings to human exposure, we must differentiate between active users (those who orally inhale CBD, bypassing the nose), and passive users ("bystanders", who inhale through both nose and mouth). Multipath particle dosimetry modeling of human exposure at the aerosol concentration given to the rat (2.92 mg/L) resulted in deposition fractions of 3 and 28% for nasal and lung exposure, respectively, for active users, and 29 and 20% for nasal and lung exposure, respectively, for passive users (Table 3). A 12-min exposure (NOAEL for nasal effects in the rats) revealed CBD nasal and lung deposited doses of 0.12 and 0.15 mg/kg for active users, doses far below those associated with adverse effects in the rat. Since nasal effects are negligible for active users, the risk of adverse reactions in humans using CBD vaping products is likely low. The NOAEL estimated in the present study is roughly equivalent to constant CBD inhalation of over 2 h per day in humans. In contrast, passive oronasal exposure equivalent to the estimated NOAEL suggests deposited doses of 1.1 and 0.76 mg/kg for nose and lung tissue, respectively, achieved within 30 min in humans. However, given that passive users are exposed to only a small fraction of the CBD inhaled by an active user, passive CBD inhalation is not expected to result in adverse effects.

Several study limitations warrant discussion. While interspecies comparisons for human risk assessment are state of the art for toxicology, rats are obligate nose breathers,[32] whereas human real-world dosing occurs *via* oral inhalation. Moreover, human dosing involves inhalation of a CBD bolus over a few seconds; testing various dose levels in rats necessitates altering exposure time (12 to 180 min in the present study), which may inadvertently alter toxicological observations. Finally, while the present study evaluated a wide range of CBD doses across repeated administrations, the study duration was only 14 days. It is unknown whether longer-term chronic dosing would yield different results in lower dose groups.

Conclusions

Our findings in rodents suggest that the doses currently available in most over-the-counter CBD products (i.e., ≤200 mg CBD per cartridge) are well below an observable effect level (93- and 353-fold lower for nose and lung, respectively), and that doses more closely aligned with human consumption patterns are not associated with adverse findings in the toxicological endpoints assessed. They do, however, suggest that at doses substantially higher than typical over the counter use should be approached with caution, especially if targeting the nose.

Acknowledgments

This study was conducted at Lovelace Biomedical (New Mexico, USA).

Authors' Contributions

D.S.: Conducted experiments, wrote or contributed to writing of the article. J.K.: Wrote or contributed to writing of the article. A.G.: Performed Data Analysis. W.D.: Performed Data Analysis. K.T.: Wrote or contributed to writing of the article. H.I.: Conducted experiments, wrote or contributed to writing of the article. T.L.: Conceptualization (supporting). M.W.: Conceptualization (supporting). M.B.-M.: Conceptualization (supporting). J.M.-D.: Conducted experiments, wrote or contributed to writing of the article (lead).

Author Disclosure Statement

The study was commissioned by Canopy Growth USA, LLC (CG). Lovelace Biomedical designed and conducted the experiments based on the general principles of OECD guidance. Lovelace Biomedical collected data and interpreted the results of the study. At the time of research conduct, authors D.S., A.G., H.I., W.D., and J.M. were employees of Lovelace Biomedical, which has received fees from Canopy Growth Corporation for research services. Authors J.K., K.T., T.L., M. A.W., M.B-M. were employees of CG, which provided the funding for this research.

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Supplementary Material

Supplementary Data S1

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Abbreviations Used

μm	= Micrometer
BW	= Body Weight
C	= Concentration
CBD	= Cannabidiol
CFR	= Code of Federal Regulations
DF	= Deposition Fraction
F	= Female
GSD	= Geometric Standard Deviation
Kg	= Kilogram
L	= Liter
M	= Male
mg	= Milligram
min	= Minute(s)
MMAD	= Mass Median Aerodynamic Diameter
N	= Number
NOAEL	= No Observable Adverse Effect Level
PG	= propylene glycol
RMV	= Respiratory Minute Volume
SD	= Study Day or Standard Deviation
T	= Time