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August 18, 2023

VIA EMAIL

House Energy and Commerce Committee - CBD@mail.house.gov
Senate Health, Education, Labor, and Pensions Committee - CBD@help.senate.gov

Re: Response to Bicameral Request for Information Regarding Food and Drug Administration (FDA) Regulation of Hemp-Derived Cannabidiol, Issued July 27, 2023

Dear Committee Members and Staff:

Thank you for the opportunity to assist Congress in its work with the U.S. Food and Drug Administration ("FDA") to craft a legislative approach to the regulation of hemp-derived products. This comment is submitted on behalf of the undersigned Hemp-Derived Product industry leaders by Dentons U.S. Cannabis and Hemp Group, serving as experts in the U.S. cannabinoid market since 2014. This comment provides Congress with requested data and a proposed policy solution which both prioritizes consumer safety and provides certainty to consumers and producers of quality hemp-derived products, meeting the U.S. market where it exists in 2023.

In summary, given the safety profile of nonintoxicating (or non-impairing) hemp-derived cannabinoids (including hemp-derived cannabidiol ("CBD")), no justification exists to regulate Hemp-Derived Products (as defined at Appendix A) through a "new regulatory pathway" under the Food, Drug, and Cosmetic Act ("FD&C Act"), or otherwise. Precedent treatment of analogous dietary supplements, with known and unknown risks, supports that the FDA has more than sufficient "risk management tools" under the FD&C Act's existing regulatory framework for dietary supplements including, without limitation, requirements regarding: (l) premarket notice; (2) labeling and packaging; (3) disclaimer and warning statements; and (4) manufacturing and testing. *See* 21 C.F.R. §101.3(a); 21 C.F.R. §101.105(a); 21 C.F.R. §101.36; 21 C.F.R. §101.4(a)(1); and 21 C.F.R. §101.5 (Labeling, Packaging, Disclaimer, and Warning Requirements); 21 C.F.R. §111 and 21 U.S.C. §350(b) (Manufacturing, Testing and Notification Requirements).

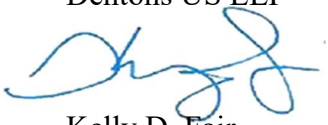
This comment proposes a policy solution (the "Proposed Policy Solution"), which is presented as stand-alone legislation, requiring the FDA to regulate nonintoxicating Hemp-Derived Products (as defined therein) as dietary supplements under the FD&C Act, with the addition of *two* hemp-specific risk management tools to address FDA's stated concerns: (1) authority to promulgate regulations to age gate certain Hemp-Derived Products to individuals aged 18 and over; and (2) authority to require a clear warning statement and product disclosures for Hemp-Derived Products containing 100 milligrams or less of hemp-derived cannabinoids and less than 1.5 milligrams of tetrahydrocannabinol ("THC") per serving ("Low-Dose Hemp-Derived Products"), which science supports are non-impairing and considered by credible studies as generally well-tolerated for consumption by healthy adults. *See* full legislative text of Proposed Policy Solution appended at **Appendix A**.

This Proposed Policy Solution strikes a balance between FDA concerns and industry stability. It does so by leveraging the existing FD&C Act infrastructure for dietary supplements to safely regulate Hemp-Derived Products; providing FDA two additional risk management tools to mitigate against all stated concerns; providing certainty to farmers and Hemp-Derived Product consumers and producers; and meeting the U.S. hemp market where it is in 2023, after a nearly five-year delay in receiving the benefit of federal oversight through regulation and enforcement.

Thank you for your consideration of this RFI response and Proposed Policy Solution. We will be happy to provide Congressional Leadership with any additional information as needed.

Very truly yours,

Dentons US LLP



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CHARLOTTE'S WEB
STANLEY BROTHERS

By: _____
JARED STANLEY
Co-Founder & Chief Operating Officer
CHARLOTTE'S WEB

OPEN BOOK EXTRACTS

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OSCAR HACKETT
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OPEN BOOK EXTRACTS

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PULAK SHARMA, MBA
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Enclosure

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RESPONSE TO REQUEST FOR INFORMATION

Current Market Dynamics

1. ***What does the current market for CBD products look like? Please describe the types and forms of products available, manufacturing practices within the industry, market supply chain, how products are marketed and sold, the types of cannabinoids used in products, the marketed effects of CBD products, and the range of CBD doses currently found in the market.***

As the Committees know, Public Law 115-334, the Agriculture Improvement Act of 2018 (the "2018 Farm Bill") removed hemp from the definition of marijuana under the Controlled Substances Act ("CSA") and expanded the definition of hemp to include "all derivatives, extracts, cannabinoids, isomers, acids, salts, and salts of isomers," containing no more than 0.3% concentration of delta-9 THC,[1] thus descheduling hemp-derived cannabinoids. The 2018 Farm Bill preserved the authority of the FDA to oversee hemp cannabinoids, including CBD, in FDA-regulated products.[2]

Since late 2018, the FDA has maintained that because CBD (and THC) is an active ingredient in a drug product that has been approved under section 505 of the FD&C Act, 21 U.S.C. § 355, products containing CBD cannot be marketed as a dietary supplement. While FDA also has the authority under 21 U.S.C. § 321(ff)(3)(B) to issue regulations "finding that the article would be lawful under this chapter," it has refused to do so based on its concerns with the safety of such products. Since 2019, the FDA has received hundreds if not thousands of stakeholders' oral and written submissions supporting the safety profile of consuming hemp cannabinoids at doses appropriate for human dietary supplements and pet supplements.

In the nearly five years since FDA began its inquiry and delay in federal regulation of hemp-derived products, the U.S. hemp-derived product market has suffered negative consequences through constrained distribution channels (with largest retailers restricting Hemp-Derived Product

[1] P.L. 115-334 §10113

[2] 7 U.S.C. § 1639r

sales waiting for FDA guidance); declining hemp commodity prices, which disproportionately harms to farmers and rural communities; divestment from well-capitalized hemp investors and operators; job losses; and a proliferation of intoxicating and unsafe hemp cannabinoid products in the U.S. market to a consumer base that demands and will continue to demand Hemp-Derived Products.

To assist Congress in understanding market trends, the following provides a data overview of U.S. hemp market historically and as it exists for consumers and producers in 2023, including a view on the impact of federal oversight on the market.

A. Current CBD Market Data[3]

The U.S. market for CBD is projected to reach $6.9 billion in sales by 2025.[4] As outlined below, the future projections of market size and scope depends heavily on federal oversight. In 2018, hemp legalization catalyzed farmers across the country to invest in growing high cannabinoid yielding crops. Best-in-class producers invested in manufacturing and marketing quality dietary supplement hemp-derived products. Finally, consumers of all ages have grown to rely on these products, in particular CBD products to support general health and well-being as part of daily wellness routines. This industry, as envisioned by the 2018 Farm Bill, has the potential to support public health, bring jobs and economic growth to communities, including rural communities, and increase tax revenues.

However, in the absence of federal regulation and enforcement, this market has realized only a fraction of this potential. The absence of regulation and enforcement from FDA has created a landscape where producers of high-quality CBD products are denied broad product distribution via access to retail channels who are waiting for clear FDA approval and regulation of these products. Farmers have consequently suffered from constrained retail product distribution due to a shrinking demand for the high cannabinoid hemp crop commodity. Worst, consumers have

[3] All market data presented in response to RFI Question 1 is supplied by the Brightfield Group data analytics 2022-2023 insights reports. The full reports are available by login at https://www.brightfieldgroup.com/ and are as follows:

- General Market Insights:
  - Brightfield, "US CBD Consumer Insights, 2022-23"
- Product Insights:
  - Brightfield, "US CBD Introduction & Overview"

[4] https://hempindustrydaily.com/supply-chain-custody-the-key-to-success-in-the-cbd-category/

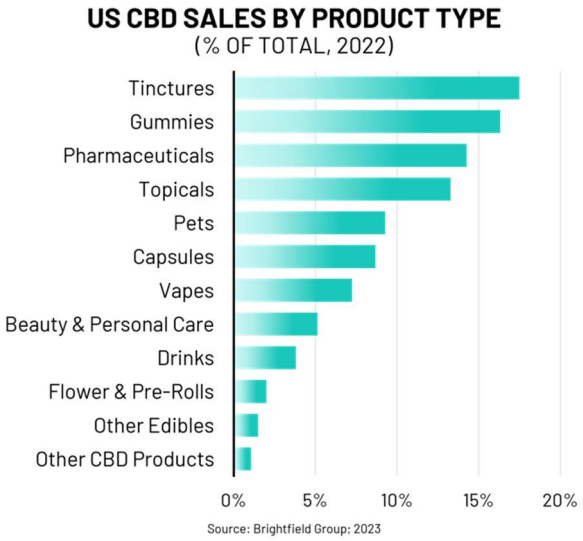
suffered by exposure to a market of unregulated products, which includes products noncompliant with dietary supplements standards, including GMP manufacturing and transparent product labeling. Finally, communities have been denied the economic growth, including jobs and tax revenues, promised by the 2018 Farm Bill, as producers are forced to downsize or halt production, given the constrained product distribution landscape.

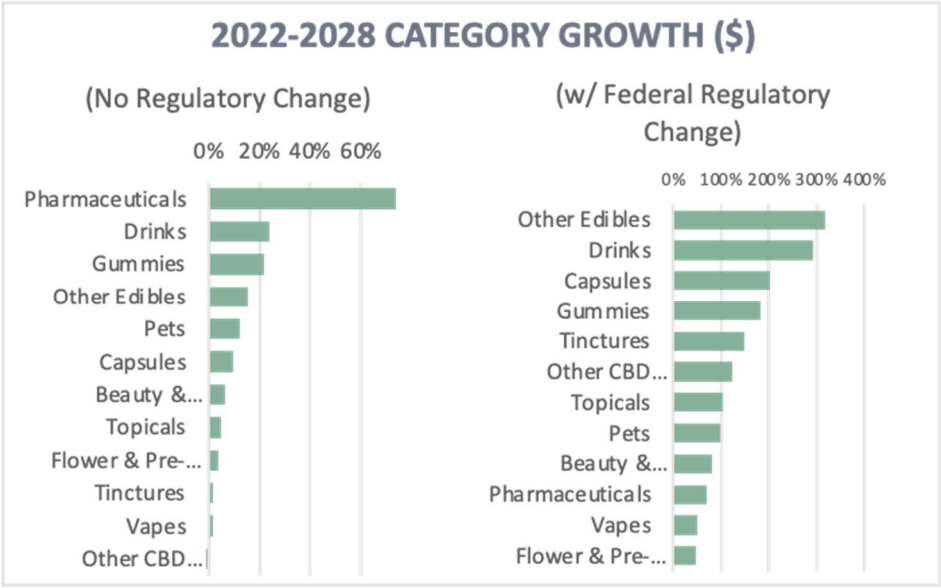
To address the problems caused by more than four years of regulation and enforcement, any policy solution from Congress must strike a balance between policy considerations and righting the existing market *i.e.*, meeting the market of producers and consumers where they are, within reason, while imposing federal standards to ensure compliance with existing standards for dietary supplements, and reducing consumer exposure to illicit, noncompliant, products through federal enforcement. In doing so, Congress would bring the remaining roughly 75% of the market into a legal framework to preserve the interests of regulators, consumers, producers, and enforcement agents.

i. Hemp-Derived Product Form-Factor Landscape

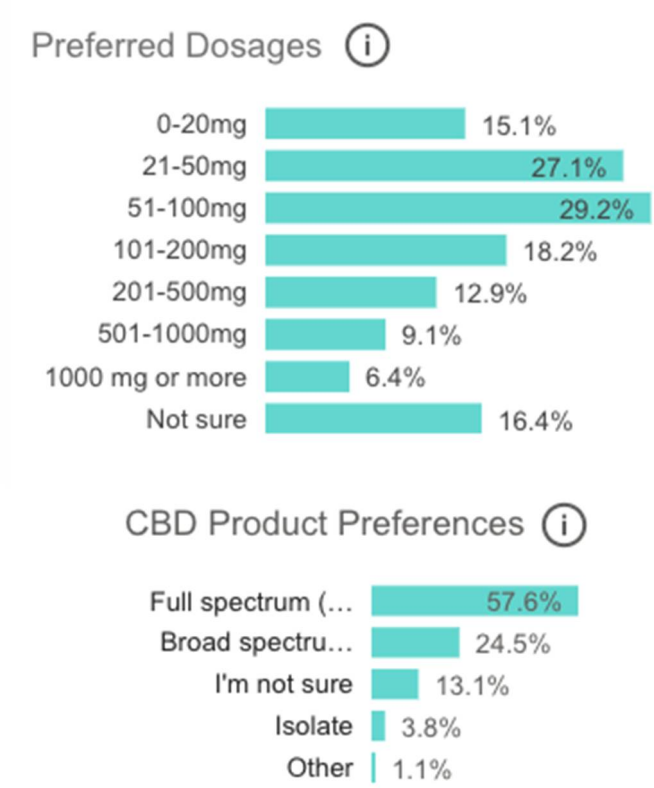
The current U.S. market for CBD includes the following form-factors: Ingestibles (Tinctures, Gummies, Capsules), Pharmaceuticals, Topicals (Therapeutic and Cosmetic/Beauty), Pet, Smokeables (Vapes, Flower, Pre-Rolls) and Drinks. According to Brightfield data, in 2022, Tinctures remained the largest product segment in the CBD market (17.5% of total CBD sales), followed by gummies (16.3% market share) and pharmaceuticals (14.3%).

With federal regulatory reform and clarity, Ingestibles are projected by data analysts to outpace the overall market by 2028. Within the ingestible category, Gummies are forecasted to overtake tinctures by 2028, reaching 19.9% market share. Product share is expected to decrease in the more niche Smokeables category. Without federal regulatory guidance, Brightfield does not expect the top product categories to shift significantly.





Regarding cannabinoid content, 57.6% of consumers polled prefer "Full spectrum CBD" Products (defined as containing all the cannabinoids plus <0.3% THC), followed by 24.5% who prefer Broad Spectrum (all the cannabinoids, with THC removed). Thirteen point 1% of consumers surveyed by Brightfield voiced no preference. More than half (56%) of consumers prefer 21-100mg of CBD per serving, though a notable amount of consumers report being unsure (16.4%) of their preferred dosage.



With regard to product innovation, as the Hemp-Derived Product industry continues to mature consumers are no longer seeking products that simply contain hemp cannabinoids but are seeking products that can make a noticeable difference in their lives. Hemp-Derived Product producers have responded by shifting increasingly towards producing "need state-oriented" products that incorporate an array of functional ingredients, appealing to both the general public and niche customer groups such as pet owners, professional and weekend athletes, and women's health consumers.[5] For many, hemp cannabinoids have become a part of their daily routines and companies are responding with inventive and original products that can be integrated into their wellness routines. Products formulated for weight loss, restful sleep, appetite management and focus are gaining consumer traction for both new and long-term CBD users as CBD continues to carve its own path in the wider cannabinoid, supplement, and functional product spaces. The inclusion of functional ingredients helps to boost the products' intended effects, allows brands to

[5] https://www.health.harvard.edu/blog/why-are-women-using-cbd-products-and-do-they-work-2019111818317#:~:text=In%202017%E2%80%932018%2C%20counterfeit%20CBD,CBD%20shown%20on%20the%20label.

make some benefits claims on those functional ingredients with an FDA disclaimer, and capitalizes on existing consumer knowledge about supplements and adaptogens. Finally, many Hemp-Derived Product brands have also taken to creating goods built around consumer use cases, with the inclusion of functional ingredients such as cannabinol ("CBN") to assist with sleep, caffeine to boost energy, and L-theanine to help consumers focus.

The Pet Product category is also a growing category for Hemp-Derived Products. In 2022, the American Pet Products Association estimated that more than $135 billion was spent on pet products and CBD is continuing to carve its niche within that space. While the pet CBD market is primarily centered around infused treats, especially dog treats, brands have found ways to differentiate themselves. Many companies have taken to creating goods centered around specific use occasions, such as reducing anxiety, boosting mental acuity, or helping to relieve joint pain. Others have created more niche goods such as paw balms, infused shampoos, and equine pastes designed to appeal strongly to specific segments of pet owners.

Finally, topical products are a popular consumer category. Compared to other topical CBD categories, balms and creams are primarily designed for consumers in search of relief from pain or physical discomfort. However, this does not mean that all potential niches have been filled. Products in the category are often positioned very differently, with some goods designed to serve as an all-purpose solution for any sort of ache or irritation while others are designed for specific use cases, like soothing tired feet or helping to ease sore muscles after exercise. Brands have also taken to using additional ingredients such as arnica and vitamin E to help boost efficacy while others have experimented with their product formulations to modulate onset time and the length of products' effects.

ii. Overview of Current Hemp-Derived Product Manufacturing Practices

Following the 2018 Farm Bill and as the CBD category matures, consumer demands have shifted from products that simply contain CBD to products that "make a noticeable difference in their lives." This includes the inclusion of other functional ingredients in both human and pet products. Manufacturers of CBD Tinctures, Gummies and Capsules often add dietary supplement ingredients (*i.e.*, melatonin, ginseng, Chinese skullcap, and acacia) to serve consumer demand for

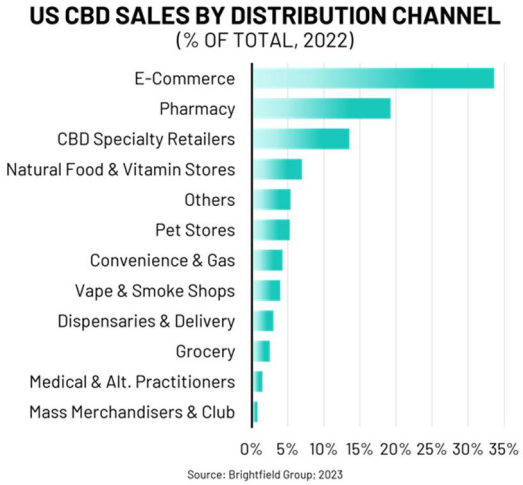
functional products. Manufacturers are increasingly looking to minor cannabinoids, such as CBN, purported as a sleep aid, to further this product innovation.

The top CBD product manufacturers are using high quality traceable materials to produce their products, operate facilities using qualifications demanded of other dietary supplement manufacturers (*i.e.*, cGMP-certified, FDA-registered facilities), and manage customer complaints with full recall capabilities. This self-regulation by the top CBD manufacturers is valuable, but too much of the market is being supplied by CBD manufacturers that are not self-regulating or manufacturing to FDA standards. Without regulatory oversight, CBD product consumers cannot be assured their products are safe.

iii. Hemp-Derived Product Distribution Landscape

As of 2023, the largest retailers in the country do not sell Hemp-Derived Products (or will sell only topical products) due to FDA's failure to regulate the products. Hemp-Derived Products are marketed and sold via e-commerce (33.6% of U.S. CBD Distribution), pharmacies (19.2%), and CBD specialty retailers (13.5%). Brightfield expects federal guidance to increase sales dramatically through mass merchandisers, club, and grocery stores. The majority of the top Hemp-Derived Product producers' market ingestible products using guidance found in the Dietary Supplement Health and Education Act of 1994 ("DSHEA") and include the statement, "These statements have not been evaluated by the Food and Drug Administration. This product is not intended to diagnose, treat, cure, or prevent any disease." on packaging and in marketing materials. Topical products are marketed under OTC Drug Monographs for therapeutic products or the FD&C Act for cosmetic topicals.

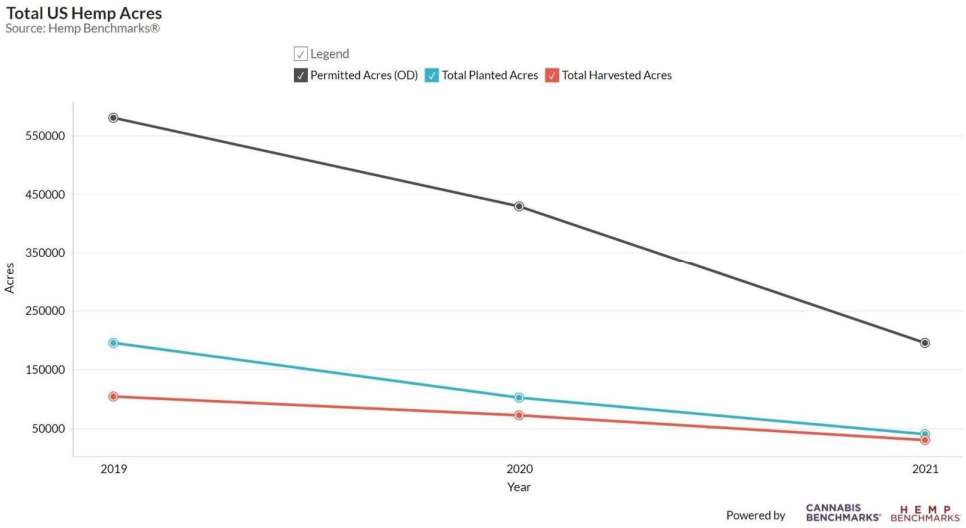
In the absence of federal regulatory clarity, the top CBD product categories are unlikely to shift significantly because of constrained distribution. In 2022, 33.6% of CBD sales took place via e-commerce, the largest distribution channel, followed by pharmacies with 19.2% and CBD specialty retailers with 13.5% of sales. Though e-commerce is expected to remain the largest channel overall, its market share of e-commerce is expected to drop over the forecast period (to 29.3% with regulatory reform versus 30.1% without) as consumers take advantage of in-person retail options. Assuming no reform, drinks and gummies are expected to make the largest gains through 2028, with these categories growing in consumer product market share by 13.3% and 11.2%, respectively, benefitting from both increased interest in Ingestibles and state-level regulations that have allowed for such goods to be sold in some large regional chains. On the other hand, other CBD products (-10.8%) and vapes (-7.0%) see the largest declines in market share. While consumer preferences are shifting over time, without new retail channels opening their doors to CBD, the choices consumers face on the shelf are unlikely to shift significantly.



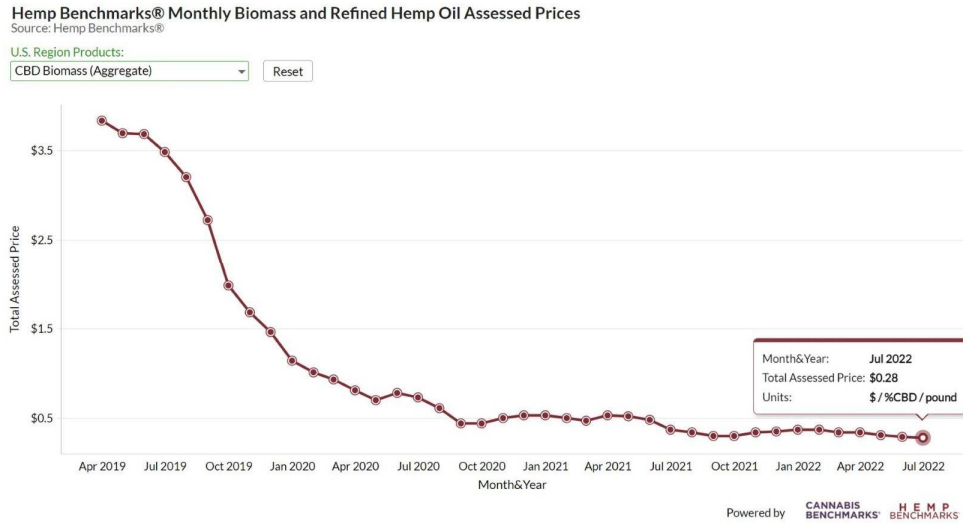
With regulatory reform, growth of the Hemp-Derived Product market is projected to be dramatic, favoring ingestible goods and supplements – product categories that have typically had more difficulty gaining mainstream distribution compared to Topicals. Regulatory clarity would open the floodgates for widespread distribution of such products. Federal guidance will open Hemp-Derived Product sales through mainstream retail channels such as mass merchandisers and club as well as grocery are set to massively increase, with compound annual growth rates ("CAGRs") of 51.7% and 37.9%, respectively. Under this scenario, the edibles category is expected to grow in share by 72.9%, while drinks will experience similarly large gains, increasing by 62.3%. The growth in the share of Ingestible products is expected to come primarily at the expense of more niche categories such as vapes and flower & pre-rolls.

2. How has the market changed since the passage of the 2018 Farm Bill?

Again, the passage of the 2018 Farm Bill opened the floodgates to farmers to produce CBD biomass but with lack of FDA regulation, farmers were left stranded with hemp biomass inventory and reaching new all-time low's each year the FDA did not regulate CBD. Nearly 200,000 acres of hemp were planted in 2019 compared to <50,000 acres of hemp planted in 2021, and even less in 2022 and 2023.



Due to lack of FDA regulation, prices of hemp biomass fell to an all-time low in July of 2023 and were down 94% compared to July of 2019.



The anticipation of FDA regulation since the 2018 Farm Bill fueled growth in the CBD market coupled with consumer demand. After legalization, Hemp-Derived Products exploded as a wellness trend in media with just over 25% of Americans having bought a CBD product. With CBD in the national spotlight, numerous other nonintoxicating hemp-derived cannabinoids have emerged, each with their own unique wellness benefits. For example, CBN, known for its potential as a sleep aid, cannabigerol ("CBG") for sports recovery, tetrahydrocannabivarin ("THCV") for energy and focus, and cannabidivarin ("CBDV") for appetite management. With such a diverse

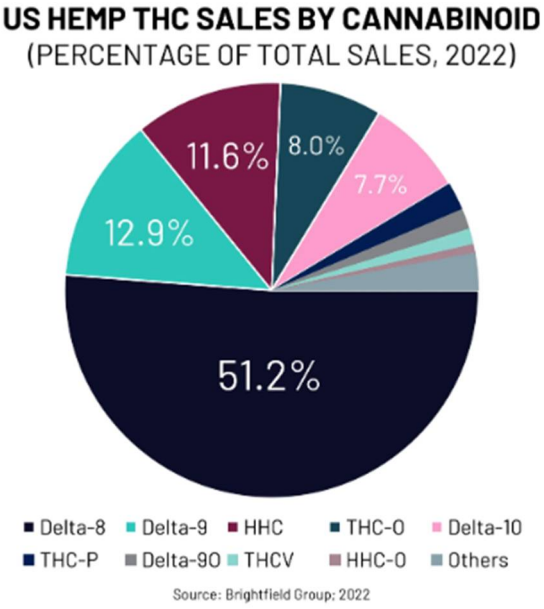
array of cannabinoids available, brands are increasingly pairing CBD with other hemp compounds to fully leverage their potential. Hemp-Derived Products showed up in mainstream retailers such as grocery chains, mass merchandisers, the natural products channel, and large e-commerce platforms such as Amazon and Sephora. This explosion led to a massive influx of over 3,500 brands into the marketplace. However, with the lack of regulation, in 2022, the number of nonintoxicating brands continues to decline, now well under 2,000, approximately half of the peak.

The cost of Hemp-Derived Products has decreased significantly since the 2018 Farm Bill due to the proliferation of products and price compression caused by producers with non-GMP manufacturing quality standards and cheaply made products that do not meet FDA standards for dietary supplements for transparent labels and ingredient standards. The lack of standardization across the industry has discouraged the consumer base seeking quality Hemp-Derived Products.

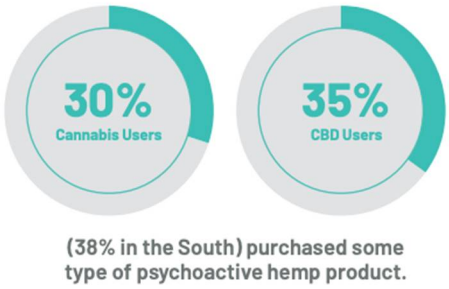
The emergence of intoxicating hemp-derived products has also surged in the void of FDA regulations. The popularity of delta-8 THC ("delta-8") has surged as a cost-effective and easily accessible option for experiencing psychoactive effects. Consumers can legally obtain delta-8 through authorized channels or via mail delivery, avoiding the potential legal risks associated with purchasing delta-9 THC illicitly. Remarkably, states with more stringent cannabis restrictions, particularly in the South, are witnessing the most dynamic and active delta-8 markets. With the enactment of the 2018 Farm Bill, the delta-8 and emerging cannabinoid market found its footing as the legislation permitted the commercialization of hemp products, provided they contained less than 0.3% delta-9 THC by dry weight, which some players swiftly recognized opened the door to alternative cannabinoids with psychoactive properties. This has opened the door to marketing intoxicating products as hemp-derived, leading to a bifurcation of the market. One segment caters to the health and wellness needs of millions of CBD consumers. The other segment offers intoxicating, often low quality and untested products that mislead consumers. By the end of 2020, delta-8 products became prevalent and started gaining significant attention among consumers and that market segment has grown to $2B in the last two years, now representing close to half of the "hemp-derived" market.

Delta 8 and Emerging Cannabinoid Market Forecast (Brightfield)

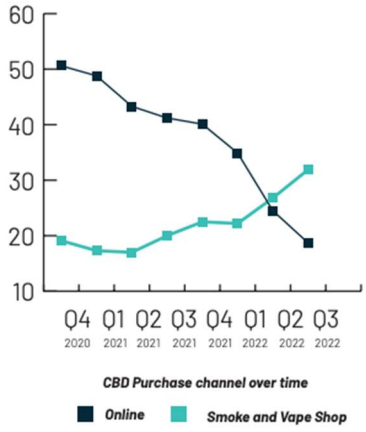
Product Type	2021	2022	2023	2024	2025	2026	2027
Vapes	$852,410,118	$1,070,239,033	$1,455,444,257	$1,741,648,005	$1,956,033,577	$2,098,492,192	$2,192,647,776
Gummies	$284,208,504	$632,834,975	$901,447,775	$1,119,027,070	$1,281,626,740	$1,389,166,998	$1,458,091,374
Flower & Pre-Rolls	$111,408,143	$208,344,046	$265,354,656	$296,054,782	$313,835,224	$322,070,669	$324,443,745
Concentrates	$88,200,744	$155,293,408	$197,035,136	$216,582,223	$225,411,639	$228,415,281	$227,882,581
Other Edibles	$36,824,505	$87,835,337	$126,678,832	$158,072,324	$181,492,488	$197,368,845	$207,453,013
Tinctures	$61,937,130	$109,874,657	$129,624,306	$138,518,277	$141,603,153	$142,445,065	$141,933,420
Drinks	$15,870,510	$40,038,037	$60,744,256	$77,847,437	$91,905,634	$102,029,482	$108,798,115
Capsules	$15,772,882	$33,338,411	$38,982,646	$41,501,977	$42,357,767	$42,452,519	$42,173,391
Beauty & Topicals	$4,203,967	$11,228,816	$12,983,706	$13,821,483	$14,155,979	$14,196,464	$14,125,234
Total	**$1,470,836,503**	**$2,349,026,719**	**$3,188,295,569**	**$3,803,073,579**	**$4,248,422,202**	**$4,536,637,513**	**$4,717,548,649**



Analysts report that 35% of existing CBD users have made purchases within the intoxicating hemp-derived product category in the past six months, highlighting the growing availability for these impairing alternatives. Today, 11.6% of American consumers report using delta-8, delta-10 THC, tetra cannabinoid-O-acetate ("THC-O"), and/or hexahydrocannabinol ("HHC") as of Q3 2022– approximately the same number of Americans that consume hard seltzer.



Although consumer demand has grown significantly since the 2018 Farm Bill, consumer surveys cite that while CBD products are available at tens of thousands of outlets, the lack of convenience in superstores, big box retailers and large chain retailers where millions of Americans shop, limits consumer access to CBD in the absence of FDA regulation. Recent data shows that the Hemp-Derived Product consumer behavior is changing, suggesting the influence of the emergence of unregulated delta-8 products. Online CBD purchases have been steadily declining since their peak in November 2020, and the decline has become more pronounced since November 2021, witnessing a significant 53% drop in online purchases from Q4 2021 to Q3 2022. Consequently, vape and smoke shops have experienced a notable uptick of 42% in purchasers during the same period suggesting sale of delta-8 products.



Another negative post-2018 Farm Bill trend due to lack of FDA oversight relates to reputable Hemp-Derived Product producers divesting hemp-related assets and reducing production of high-quality products due to lack of federal regulation and a challenging path to gain mainstream distribution and/or evolving their market strategy to include psychoactive

cannabinoids. Examples include the Molson Coors exiting CBD drink joint venture with Hexo, Inc. and Truss USA; Cronos Group exiting all U.S. CBD operations, including Kristen Bells' Happy Dance CBD line; Columbia Care scaling back from N2P CBD products; and Canopy Growth Corporation discontinuing SurityPro CBD pet-wellness product line and scaling back all CBD product operations, including divesting U.S. manufacturing facilities. The exit of well-capitalized operators from the U.S. CBD market denies consumers quality products, and eliminates potential for economic growth, including jobs and tax revenue, in the communities where investments were made.

Finally, since the 2018 Farm Bill legalized hemp, in the absence of a cohesive federal regulatory framework to regulate Hemp-Derived Products, the hemp-based THC market is predominantly shaped by state-level initiatives, including manufacturing, packaging, labeling, THC limits, and delta-8 product frameworks. This patchwork model exists with no federal floor to level set standards.

STATE-LEVEL HEMP THC REGULATORY STATUS

Unregulated, Not banned	Alabama, Arkansas, Florida, Georgia, Hawaii, Indiana, Maine, Missouri, Nebraska, New Hampshire, New Jersey, New Mexico, North Carolina, Oklahoma, Pennsylvania, Tennessee, Virginia, West Virginia, Wisconsin, Wyoming
Unregulated, Temporarily Legal:	Kentucky, Texas
Unregulated, Ban Unclear	Illinois, Kansas, Massachusetts, South Carolina
Regulated	Louisiana, Minnesota, South Dakota
Prohibited Outside Cannabis Channels	Arizona, California, Connecticut, Maryland, Michigan, Nevada, Utah
Regulated within Cannabis Channels	Ohio
Fully Prohibited:	Alaska, Colorado, Delaware, Idaho, Iowa, Mississippi, Montana, New York, North Dakota, Oregon, Rhode Island, Vermont, Washington

Source: Brightfield Group 2022

3. How is the lack of national standards for CBD products affecting the market?

The lack of national standards for CBD products has wide-ranging effects on the market, including issues of inconsistent product quality, consumer safety concerns, regulatory complexities, limited advancement in scientific research, degrading consumer trust, degrading trust in the FDA, and stunting industry growth.

- **Product Quality Inconsistencies**: Without standardized regulations, the CBD market suffers from inconsistent product quality among various brands and manufacturers. The absence of uniform testing and quality control measures means that consumers may encounter CBD products with varying levels of potency and purity, resulting in uncertainty and potential dissatisfaction with the product's effectiveness.
    - A 2017 JAMA publication[6] published a University of Pennsylvania study by top researcher Dr. Marcel Bonn-Miller, Ph.D., with over 20 years in cannabis research made an alarming discovery: nearly 70% of all CBD products available for purchase online are inaccurately labeled, posing potential serious risks to consumers. Dr. Bonn-Miller attributes this mislabeling issue to the lack of sufficient regulation and oversight in the CBD industry. The comprehensive study involved the purchase and analysis of 84 CBD products from 31 different companies. Troublingly, the findings revealed that more than 42% of the products were under-labeled, with higher CBD concentrations than indicated on the labels. An additional 26% of the products were over-labeled, containing lower CBD amounts than stated. Surprisingly, only 30% of the purchased CBD products had an accurate CBD content within 10% of what was listed. This variability across products could hinder consumers from obtaining a reliable benefit and raise concerns about potentially diminished benefits due to inadequate or excessive CBD levels.
- **Consumer Safety Concerns**: The lack of clear national standards opens the door for substandard and potentially hazardous CBD products to flood the market. Consumers may

[6] Marcel O. Bonn-Miller, PhD; Mallory J. E. Loflin, PhD; Brian F. Thomas, PhD; et al
https://jamanetwork.com/journals/jama/fullarticle/2661569

be at risk of using products contaminated with harmful substances or containing higher THC levels than advertised. This lack of oversight impairs consumers' ability to make informed choices about the CBD products they buy and use.

- In a survey by the Consumer Brands Association, the majority of Americans believe a federal agency, like the FDA, should have regulatory control over CBD to guarantee safety (72%), protect public health (64%) and ensure consistent regulations across states (55%).[7]
- As reported by JAMA and Harvard Health in 2017–2018, counterfeit CBD oil was found that contained synthetic cannabinoids and led to a poisoning outbreak in Utah.[8] Five samples of products labeled as CBD collected from sickened individuals in Utah contained 4-cyano CUMYL-BUTINACA, a form of synthetic cannabinoid, and no CBD. Those sickened reported taking the products sublingually (17.6%) or by vaping (72.5%).[9] In 60% of these cases, symptoms including altered mental status, vomiting, seizures, or shaking preceded hospitalization.[10] Many users (66.7%) reported purchasing the fake CBD products from tobacco stores.[11]

- **Regulatory Complexities**: The absence of consistent national guidelines creates a complex landscape of state and local regulations that CBD businesses must navigate. This regulatory confusion poses challenges for companies trying to operate across state lines or expand their market presence. Compliance requirements varying significantly from one jurisdiction to another can burden businesses administratively, hampering market growth and development.
- **Limited Advancement in Scientific Research**: The absence of national standards hinders research and development endeavors in the CBD industry. The lack of standardized protocols and regulations makes conducting robust clinical trials and studies more

[7] http://chrome-extension/efaidnbmnnnibpcajpcglclefindmkaj/https:/consumerbrandsassociation.org/wp-content/uploads/2019/11/ConsumerBrands_CBD_Clarity.pdf

[8] https://jamanetwork.com/journals/jama/article-abstract/2684620

[9] *Id.*

[10] *Id*.

[11] *Ibid.*

challenging. Consequently, there might be limited conclusive evidence regarding the safety and efficacy of CBD products, impeding further investments and innovation in the market.

- **Degrading Consumer Trust**: The lack of national standards may lead to skepticism among consumers regarding the legitimacy and credibility of CBD products. Without a central authority to ensure consistency and safety, some consumers might shy away from using CBD altogether or be hesitant to try new products, resulting in restricted market expansion and growth.
- **Stunted Industry Growth**: The absence of clear national standards can hinder the overall growth and prospects of the CBD market. Uncertainty stemming from the lack of regulations may deter businesses from investing, while consumers may refrain from exploring CBD products due to concerns about quality and safety. Consequently, the industry's development and its potential contributions to the economy may suffer setbacks.

A Consumer Brands Association survey asked consumers if they would consider a CBD product if a trusted and recognizable brand sold such a product. A combined 70 percent of American consumers said they would (45%) or might (25%) consider purchasing it.[12] The majority of Americans (70%) were clear that they would be more confident in the safety of CBD products if they were manufactured by a large, well-known brand, because more than half of consumers believe large, well-known brands have more safety controls in place (55%), employ higher manufacturing standards (54%) and have more experience in making high quality, consistent products (53%).[13]

To address these challenges and negative consequences of FDA inaction and underregulation, comprehensive and uniform national regulations are essential to ensure consumer safety, promote product quality, and foster a flourishing and responsible Hemp-Derived Product market. Congress has an opportunity to set a baseline of regulation for Hemp-Derived products

[12] http://chrome-extension/efaidnbmnnnibpcajpcglclefindmkaj/https:/consumerbrandsassociation.org/wp-content/uploads/2019/11/ConsumerBrands_CBD_Clarity.pdf

[13] *Id*.

by requiring FDA to regulate the products under the existing framework for dietary supplements, while providing FDA additional risk management tools for Low Dose Hemp-Derived Products.

Pathway

4. Please comment on the concerns FDA has raised with regard to regulating most CBD products through existing pathways (i.e., conventional foods, dietary supplements, and cosmetics), and FDA's view that there is a need for a new regulatory pathway for CBD products. If existing regulatory pathways are sufficient for regulating CBD products, please explain how these existing pathways can be used to address the concerns raised by FDA, as appropriate.

A. Proposed Policy Solution Position Summary

Given the safety profile of nonintoxicating or non-impairing hemp-derived cannabinoids (including hemp-derived CBD), no justification exists to regulate Hemp-Derived Products through a "new regulatory pathway" under the FD&C Act, or otherwise. FDA can look to its precedent treatment of analogous dietary supplements with known risks to safely regulate Hemp-Derived Products, including using risk management regulations regarding: (l) premarket notice; (2) labeling and packaging; (3) disclaimer and warning statements; and (4) manufacturing and testing. *See* 21 C.F.R. §101.3(a); 21 C.F.R. §101.105(a); 21 C.F.R. §101.36; 21 C.F.R. §101.4(a)(1); and 21 C.F.R. §101.5 (Labeling, Packaging, Disclaimer, and Warning Requirements); 21 C.F.R. §111 and 21 U.S.C. §350(b) (Manufacturing, Testing and Notification Requirements).

The Proposed Policy Solution eliminates the need for any additional FDA infrastructure to regulate Hemp-Derived Products, and proposes additional oversight regulations to address FDA's stated concerns: (1) authority to promulgate regulations to age-gate certain Hemp-Derived Products to individuals aged 18 and over to address vulnerable population concerns; and (2) authority to require clear warning statement and product disclosures on Low-Dose Hemp-Derived Products containing 100 milligrams or less of hemp-derived cannabinoids and less than 1.5 milligrams of THC per serving. Given that Low-Dose Hemp-Derived Products are supported by science to be non-impairing and considered by credible toxicology data as well-tolerated for healthy adults, the Proposed Policy Solution proposes regulating these products as dietary supplements, though without requiring a further premarket safety submission to the FDA, so long

as the products include a dose-dependent warning statement. *See* Appendix A; and Appendix B, Exhibits A-F.

The following outlines a key safety consideration for Congress in assessing the Proposed Policy Solution as well as addresses how the Proposed Policy Solution addresses each of the FDA's stated concerns.

B. The Proposed Policy Solution Addresses All FDA Concerns and Provides Requested Risk-Management Tools for Low-Dose Hemp-Derived Products

i. Summary of FDA's Concerns and Request for "Risk Management Tools"

Since late 2018, the FDA has maintained that because CBD (and THC) is an active ingredient in a drug product that has been approved under section 505 of the FD&C Act, 21 U.S.C. § 355, products containing CBD cannot be marketed as a dietary supplement. While FDA also has the authority under 21 U.S.C. § 321(ff)(3)(B) to issue regulations "finding that the article would be lawful under this chapter," it has refused to do so based on its concerns with the safety of such products. In 2019, the FDA announced it would evaluate CBD's safety profile further to assess whether the ingredient was appropriate for human consumption as a dietary supplement and/or food, notwithstanding its IND Preclusion position. From 2019 to January 2023, the FDA accepted comments and submissions from all stakeholders regarding the U.S. CBD market, consumer and patient impact, and all available clinical studies related to CBD safety, including addressing the FDA's stated concerns related to liver toxicity, reproductive toxicity, drug interaction risk and risks to vulnerable populations including minors and pregnant and lactating women. *See* compendium of most relevant clinical studies submitted to FDA at Appendix B, Exhibits A-C.

The FDA, however, has remained dissatisfied with submitted clinical evidence supporting safe dosage levels for CBD consumption, especially for long term use and in the realm of liver toxicity; reproductive toxicity; drug-drug interactions; and potential sedative effects; and impacts to vulnerable populations, *i.e.*, children, and pregnant and lactating women.

In January 2023, the FDA affirmed that it will not create new standards to regulate cannabis or cannabinoid compounds and products, and particularly CBD, within existing dietary supplement

and food product regulatory frameworks, citing its inability to properly assess the safety of such products. The announcement states: "Given the available evidence, it is not apparent how CBD products could meet safety standards for dietary supplements or food additives. For example, we have not found adequate evidence to determine how much CBD can be consumed, and for how long, before causing harm." The FDA also recognized as a potential solution the use of ***"risk management tools" including "clear labels, prevention of contaminants, CBD content limits, and measures, such as minimum purchase age, to mitigate the risk of ingestion by children."*** To move the industry forward, we agree that Congress needs to act and implement by law those risk management tools it has available under the FD&C Act regulatory framework for dietary supplements.

ii. FDA Fails to Address Toxicity Data Supporting Safe Use of Hemp-Derived Products

Since 2019, the FDA's stated concerns regarding regulating Hemp-Derived Products have related to liver toxicity, reproductive toxicity, drug-drug interaction risk, risks to vulnerable populations including minors and pregnant and lactating women, and dosing.

However, since 2019, the FDA has not substantively publicly addressed any of the hundreds of stakeholder safety and toxicology submissions supporting the safety of hemp-derived CBD for human and animal consumption. Several available studies in humans demonstrate that CBD at much higher consumption levels is well-tolerated. Serious adverse events in these studies are generally limited to elevated liver enzyme levels (as an indicator of potential liver toxicity), which has been observed primarily at very high pharmacological doses of CBD and in individuals taking concomitant medications. In fact, a recent systematic review and meta-analysis[14] of 28 clinical trials concluded that CBD exposure was only statistically significantly associated with elevated enzyme levels at dose levels ≥1,000 mg/day; all but one clinical trial reporting this effect administered CBD at doses of 20 mg/kg/day *(i.e.*, 400 mg/day in adults) or higher. In addition, 76.10% of participants with elevated liver enzyme levels across all studies were also taking concomitant medications. *See* Appendix B, Exhibit D. The studies support that cannabinoids are

[14] Lo LA, Christiansen A, Eadie L, Strickland JC, Kim DD, Boivin M, Barr AM, MacCallum CA. 2023. Cannabidiol-associated hepatotoxicity: A systematic review and meta-analysis. J Intern Med. 13. doi: https://doi.org/10.1111/joim.13627.

safe and well-tolerated for human consumption, which is consistent with the fact that cannabinoids have been consumed by humans for hundreds of years without a public record of significant adverse events or harm to consumer health.

For CBD specifically, a robust science-based assessment of currently available published preclinical and clinical data has identified safe levels for consumption at 100 milligrams or less, where 70 mg/day is considered safe for consumption by all healthy adults, and 100 mg/day is considered safe for most healthy adults (excluding those who are trying to conceive, pregnant, and/or lactating).

The Proposed Policy Solution follows a 2023 study conducted and led by Rayetta G. Henderson, Ph.D., Senior Managing Scientist, ToxStrategies, LLC, who was called as an expert witness in the House Committee on Oversight and Accountability Subcommittee on Health Care and Financial Services' July investigation into the FDA's failure to regulate CBD products. This assessment applied a systematic approach to review all publicly available preclinical toxicity studies in animal models and clinical studies in humans on hemp-derived CBD isolate. The studies determined to be of the highest quality and relevance were selected and used to derive possible safe levels of CBD consumption. The study's conclusion supports safe CBD consumption of 70 mg daily for healthy adults, including pregnant or lactating adults; 100 mg daily for healthy adults not currently trying to conceive, pregnant or lactating based on liver effects; and up to 160 mg daily for healthy adults not currently trying to conceive, pregnant or lactating and excluding populations at risk for liver injury. The definition of "healthy adults" in this study excluded those treated for or diagnosed with any medical condition, or currently taking any medications. *See* Appendix B, Exhibits A-C.

The Proposed Policy Solution further relies on credible science regarding THC impairment to set a threshold for "Low-Dose Hemp-Derived Products." The preliminary data of a recent unpublished study conducted by Johns Hopkins University Professor, renowned cannabinoid researcher, and Scientific Advisor to Charlotte's Web, Ryan Vandrey, Ph.D. concluded, based on aggregate data, that human consumption of products containing 3.7 milligrams of THC and 100 milligrams of CBD combined were not impairing, *i.e.*, did not present abuse liability risk or cognitive impairment, including measures of psychomotor ability, working memory, divided

attention, and higher order cognitive functioning.[15] *See* Appendix B, Exhibit F. Low-Dose Hemp Derived Products defined under the Proposed Policy Solution set a threshold of 1.5 milligrams of THC, well below the impairment levels supported by Dr. Vandrey's conclusions.

Conversely, the FDA has cited to no dispositive studies clearly supporting its position that Hemp-Derived Products are unsafe for consumption at dosing consistent with dietary supplement use.

iii. Existing Regulatory Framework For Dietary Supplement Provide FDA Risk-Management Tools It Needs To Regulate Hemp-Derived Products

Notwithstanding the safety profile of Hemp-Derived Products and even if the risks identified by the FDA were entirely substantiated by science, under existing regulations for dietary supplements and conventional foods, the FDA possesses tools to regulate Hemp-Derived Products. FDA has the authority to protect vulnerable populations and inform consumers about any known or unknown risks (including drug interactions) for such products. *See* 21 C.F.R. §101.3(a); 21 C.F.R. §101.105(a); 21 C.F.R. §101.36; 21 C.F.R. §101.4(a)(1); and 21 C.F.R. §101.5 (Labeling, Packaging, Disclaimer, and Warning Requirements); 21 C.F.R. §111 and 21 U.S.C. §350(b) (Manufacturing, Testing and Notification Requirements). FDA has fully acknowledged such supplement-drug interactions exist for dietary supplements already in the marketplace and generally handles the issue by providing guidance to consumers to talk with their health care professional.[16]

Indeed, precedent supports that the FDA and the market has applied existing regulations and guidelines to other marketable and regulated dietary ingredients and dietary supplements products with safety concerns in specific subpopulations to effectively manage risk through labeling and disclosure regulation, for example:

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[15] The preliminary data from this study was presented last year at the annual scientific meeting of the College on Problems of Drug Dependence (June 11-15, 2022: 84th CPDD Scientific Meeting, Minneapolis, MN). The Poster Presentation is attached at Appendix B, Exhibit F.

[16] https://www.fda.gov/consumers/consumer-updates/mixing-medications-and-dietary-supplements-can-endanger-your-health

- Saint John's Wort – significant drug interactions;[17]
- Iron – liver damage, systemic toxicity, death;[18]
- Aconite – nausea, vomiting, low blood pressure and heart rhythm disorders;[19]
- Kava – Liver damage;[20]
- Yohimbe – rapid heartbeat, kidney failure, seizure, and heart attack;[21]
- Grapefruit – drug-drug interactions;[22]
- Melatonin – drowsiness and operating machinery;[23]

The Proposed Policy Solution leverages existing risk-management tools under FD&C Act infrastructure for dietary supplement regulation, sets a reasonable non-impairing limit on federally legal THC within the product servings, and adds two additional hemp-specific measures addressing the concerns of the FDA through clear warning statements and limiting purchases of such products to individuals aged 18 and over to address vulnerable population concerns. With this approach, there will be no need for new FDA infrastructure, i.e., a "center," or a novel pathway to regulate these products.

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[17] Natural Medicines, 2023 https://naturalmedicines.therapeuticresearch.com/databases/food,-herbs-supplements/professional.aspx?productid=329; *and see*, EMA, 2022 https://www.ema.europa.eu/en/documents/herbal-monograph/final-european-union-herbal-monograph-hypericum-perforatum-l-herba-revision-1_en.pdf

[18] 21 C.F.R. 101.17

[19] Natural Medicines, 2023 https://naturalmedicines.therapeuticresearch.com/databases/food,-herbs-supplements/professional.aspx?productid=609; *and see*, Zhou G, Tang L, Zhou X, Wang T, Kou Z, Wang Z. (2015). A review on phytochemistry and pharmacological activities of the processed lateral root of Aconitum carmichaelii Debeaux. J Ethnopharmacol 160: 173-93. doi: 10.1016/j.jep.2014.11.043.; *and see*, Chan TY. Aconite poisoning. Clin Toxicol (Phila). 2009 Apr;47(4):279-85. doi: 10.1080/15563650902904407.

[20] Natural Medicines, 2023 https://naturalmedicines.therapeuticresearch.com/databases/food,-herbs-supplements/professional.aspx?productid=872; *and see*, EMA, 2017 https://www.ema.europa.eu/en/documents/herbal-report/final-assessment-report-piper-methysticum-g-forst-rhizoma_en.pdf

[21] Natural Medicines, 2023 https://naturalmedicines.therapeuticresearch.com/databases/food,-herbs-supplements/professional.aspx?productid=759; *and see*, EFSA ANS Panel (EFSA Panel on Food Additives and Nutrient Sources Added to Food), 2013. Scientific Opinion on the evaluation of the safety in use of Yohimbe (Pausinystalia yohimbe (K. Schum.) Pierre ex Beille). EFSA Journal 2013; 11(7):3302, 46 pp. doi:10.2903/j.efsa.2013.3302

[22] Natural Medicines, 2022 https://naturalmedicines.therapeuticresearch.com/databases/food,-herbs-supplements/professional.aspx?productid=946; *and see*, FDA, 2021 https://www.fda.gov/consumers/consumer-updates/grapefruit-juice-and-some-drugs-dont-mix; *and see*, Dahan A, Altman H. Food-drug interaction: grapefruit juice augments drug bioavailability--mechanism, extent, and relevance. Eur J Clin Nutr. 2004 Jan;58(1):1-9. doi: 10.1038/sj.ejcn.1601736; *and see*, Bressler R. Grapefruit juice and drug interactions. Exploring mechanisms of this interaction and potential toxicity for certain drugs. Geriatrics. 2006 Nov;61(11):12-8. PMID: 17112309.

[23] CRN Guidelines, https://www.crnusa.org/newsroom/crn-issues-recommended-guidelines-melatonin-supplements-focus-marketing-formulation-sleep

Scope

5. How should CBD and/or cannabinoid-containing products be defined? What compounds should be included and excluded from a regulatory framework?

The Proposed Policy Solution defines "Hemp-Derived Product" to exclusively encompass nonintoxicating hemp-derived finished products, inclusive of naturally occurring cannabinoids but excluding naturally occurring THC that has been isolated or concentrated in the manufacturing process or THC or its isomers/analogs that have been produced through conversion or chemical synthesis. This approach follows the spirit of the 2018 Farm Bill, which recognizes and permits a percentage of naturally occurring delta-9 THC in nonintoxicating hemp crops. However, the Proposed Policy Solution goes a step further to protect public health in finished products by defining Hemp-Derived Product to clarify what constitutes a nonintoxicating vs. intoxicating finished product and to mitigate against isolation of naturally occurring THC and production of THC and its isomers/analogs through conversion or chemical synthesis to produce federally illegal intoxicating products.

See Appendix A, Definition of certain Cannabinoids and Cannabinoid Ingredients Section 2 (a) (3)-(6) (reproduced below):

> (a) Cannabinoid definitions:
>
> > (1) "Cannabidiol" or "CBD" means the compound $C_{21}H_{30}O_2$ (PubChem CID: 644019) whether found in *Cannabis sativa L.* or Hemp, or from any other source.
> >
> > (2) "Cannabinoid" means any chemical compound that naturally occurs in the plant *Cannabis sativa L.*, or is physically, chemically, and optically identical to any chemical compound that naturally occurs in the plant *Cannabis sativa L.*, and acts as an agonist or antagonist on human cannabinoid receptors. These chemical compounds include endocannabinoids, phytocannabinoids, biosynthesized cannabinoids, and chemically

synthesized cannabinoids, but do not include terpenes.

(3) “Hemp Cannabinoid” means those Cannabinoids naturally occurring in hemp, whether extracted from hemp or from any other legal source. For the avoidance of doubt, Hemp Cannabinoid does not include cannabinoids that are not naturally occurring in the plant, for example, O-acetyl delta-9 tetrahydrocannabinol.

(4) “Hemp Cannabinoid Ingredient” means a substance that includes only whole hemp extracts containing Hemp Cannabinoids, purified hemp extracts containing one or more Hemp Cannabinoids, or one or more biosynthesized or chemically synthesized Hemp Cannabinoids. A Hemp Cannabinoid Ingredient shall not include any THC Ingredient.

(5) “Tetrahydrocannabinol” or “THC” means any tetrahydrocannabinol, including delta-6 tetrahydrocannabinol, delta-8 tetrahydrocannabinol, delta-9 tetrahydrocannabinol, delta-10 tetrahydrocannabinol, delta-12 tetrahydrocannabinol, hexahydrocannabinol (HHC), O-acetyl delta-9 tetrahydrocannabinol (THCO), tetrahydrocannabiphorol, tetrahydrocannabinolic acid, or any other Cannabinoid, at a similar or greater potency that the Secretary of Health and Human Services in consultation with the Attorney General determines to have a psychological and cognitive profile associated with intoxication or euphoria similar to tetrahydrocannabinol and has a potential for abuse.

(6) “THC Ingredient” means any Tetrahydrocannabinol that has been isolated from a Whole Hemp Extract, concentrated by the removal of non-THC Cannabinoids from a Whole Hemp Extract, or that has been produced by any other means, whether through conversion, chemical synthesis, biosynthesis, or otherwise.

> (7) "Whole Hemp Extract" means an extract of Hemp produced through an extraction process intended to preserve the naturally occurring Cannabinoid profile of the Hemp biomass.

By these definitions, the Proposed Policy Solution mitigates against including concentrated naturally occurring THC or THC or its isomers/analogs produced through conversion or chemical synthesis in Hemp-Derived Products (*e.g.*, delta-8). While the 2018 Farm Bill defines hemp as a crop having a delta-9 THC level below 0.3 percent, it does not define a hemp-derived finished product or otherwise expressly limit the THC levels in those products. This left the market open to products which technically comply with the 2018 Farm Bill's crop requirements but include elevated doses of THC and its isomers/analogs.

Many stakeholders and states have grappled with setting THC serving limits to ensure hemp products are non-impairing. The preliminary data of a recent unpublished study conducted by Johns Hopkins University Professor, renowned cannabinoid researcher, and Scientific Advisor to Charlotte's Web, Ryan Vandrey, Ph.D. concluded, based on aggregate data, that human consumption of products containing 3.7 milligrams of THC and 100 milligrams of CBD combined were not impairing, *i.e.*, did not present abuse liability risk or cognitive impairment, including measures of psychomotor ability, working memory, divided attention, and higher order cognitive functioning.[24] *See* Appendix B, Exhibit F. States regulating this issue permit a range of THC content in hemp-derived products based on impairment levels from 0 to 5 milligrams. Minnesota, for example, set a THC cap of 5 milligrams per serving; New York mandates not more than 1 milligram per serving; and Colorado caps THC content at 1.75 milligrams per serving for age-gated hemp-derived products sold within the state.[25]

The Proposed Policy Solution is silent on a THC product cap, believing that the state legislators and regulators are in the best position to exercise their rights to set these limits within their respective jurisdictions based on constituent and public health needs. However, the Proposed

[24] The preliminary data from this study was presented last year at the annual scientific meeting of the College on Problems of Drug Dependence (June 11-15, 2022: 84th CPDD Scientific Meeting, Minneapolis, MN). The Poster Presentation is attached at Appendix B, Exhibit F.

[25] MN ST § 342.46(6)(1) (Minnesota law addressing delta-9 THC level for "lower-potency hemp edibles"); 9 NY ADC 114.8(b)(1)(i) (New York Regulation stating "cannabinoid hemp products" orally consumed shall contain no more than 1 milligram total THC per serving); CO ST § 25-5-427(2)(d)(V) (Colorado law defining "hemp product" to contain no more than 1.75 mgs of THC per serving).

Policy Solution does propose that products with above 1.5 milligrams of THC per serving do undergo FDA premarket notification safety submissions to ensure a federal baseline for ingredient safety for Hemp-Derived Products. *See* Appendix A, Definition of "Hemp-Derived Product" and "Low-Dose Hemp-Derived Product," Section 2(c) and (d). Products defined as "Low-Dose Hemp-Derived Products," with less than 1.5 milligrams of THC and 100 milligrams of hemp-cannabinoids (presumed non-impairing by a large safety margin compared to Dr. Vandrey's Study and well-tolerated levels of hemp-derived CBD), can remain in market without further safety submissions to FDA with appropriate dose-based warning labels, product notice and age-gating. *Id*.

In summary, the Proposed Policy Solution solves major policy concerns regarding impairing vs. non-impairing products and mitigates against synthetic and elevated-THC products being sold in federally legal channels. The Solution accomplishes this definitionally, without creating a need for either additional FDA infrastructure or a novel regulatory framework for nonintoxicating products.

a. Should Congress or FDA limit the amount of intoxicating or potentially intoxicating substances produced by Cannabis sativa L. in food and dietary supplements? Which substances, if any, warrant greater concern? How should these substances of concern be addressed? What products, if any, should not be allowed on the market?

In the wake of ambiguity and manipulation of the 2018 Farm Bill's definition of "hemp" as applied to finished goods, a great need exists for a baseline level of what is a legally permissible nonintoxicating Hemp-Derived Product. The Proposed Policy Solution's intent is to regulate nonintoxicating (or non-impairing) products. To accomplish this, the Policy Solution definitionally excludes synthetic intoxicating cannabinoids (*e.g.*, delta-8) and concentrated or isolated delta-9 THC from the definition of nonintoxicating Hemp-Derived Product. Thus, under the Proposed Policy Solution, no hemp cannabinoid can be manipulated or synthesized to create synthetic intoxicating cannabinoids (*e.g.*, delta-8) and no concentration or isolation of THC is permissible. Further, the Proposed Policy Solution requires finished products with more than 1.5 milligrams of THC to be evaluated by the FDA in premarket review to accomplish: (1) regulating only non-impairing products; and (2) ensuring safety review of Hemp-Derived Products, while

meeting the market where it is in 2023 for Low-Dose Hemp-Derived Products. *See* Appendix A, Sections 2(a)(3)-(4) and (c) and (d).

Finally, with regard to limiting the amount of naturally occurring THC within a Hemp-Derived Product, the Proposed Policy Solution is silent and gives deference to the states to resolve this issue in alignment with the needs of the respective jurisdictions. Many states now have THC cap regulations, ranging from 0 to 5 milligrams of THC per serving, within the existing state hemp-derived product regulatory frameworks promulgated in the five-year gap of federal oversight of these products.

b. How should Congress or FDA identify appropriate limits for THC and other cannabinoids in finished products? Relatedly, how should a framework account for "total THC," including tetrahydrocannabinol acid (THCA), in FDA's regulation of intermediate and finished products?

See supra, Response to RFI Question 5(a).

c. Should FDA regulate the manufacture and sale of "semisynthetic derivatives," or "biosynthetic cannabinoids," which are still scheduled under the CSA?

Any substance scheduled as a controlled substance under the Controlled Substances Act is under the jurisdiction of the Drug Enforcement Administration within the Department of Justice for the purpose of combating drug trafficking and diversion. While the FDA does assume a role for certain of these substances in so far as (1) the 2018 Farm Bill descheduled cannabinoids naturally occurring in hemp (including delta-9 THC) or (2) the FD&C Act traditional pathway for products investigated and approved as a new pharmaceutical drug; until such substances are rescheduled entirely, or sold legally as pharmaceutical drugs in interstate commerce, it would be an inappropriate expansion of the FDA's jurisdiction to generally protect consumers from trafficking of these substances.

6. Other non-cannabinoid products are available on the market that have raised safety concerns among some individuals, which FDA has regulated without a substance-specific regulatory framework (e.g., kratom, caffeine, etc.). How has FDA dealt with products containing those substances? How might these products be implicated by a CBD-specific product framework?

The FDA has a long history of effectively managing the risks associated with certain dietary supplement products within its existing regulatory frameworks. Many currently marketed dietary supplements products have safety concerns in specific subpopulations that are effectively mitigated through warning statements, public education, including by FDA, and at the health care provider level. Key examples include:

- Saint John's Wort – significant drug interactions;[26]
- Iron – liver damage, systemic toxicity, death;[27]
- Aconite – nausea, vomiting, low blood pressure and heart rhythm disorders;[28]
- Kava – Liver damage;[29]
- Yohimbe – rapid heartbeat, kidney failure, seizure, and heart attack;[30]
- Grapefruit – drug-drug interactions;[31]
- Melatonin – drowsiness and operating machinery;[32]

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[26] Natural Medicines, 2023 https://naturalmedicines.therapeuticresearch.com/databases/food,-herbs-supplements/professional.aspx?productid=329; *and see*, EMA, 2022 https://www.ema.europa.eu/en/documents/herbal-monograph/final-european-union-herbal-monograph-hypericum-perforatum-l-herba-revision-1_en.pdf

[27] 21 C.F.R. 101.17

[28] Natural Medicines, 2023 https://naturalmedicines.therapeuticresearch.com/databases/food,-herbs-supplements/professional.aspx?productid=609; *and see*, Zhou G, Tang L, Zhou X, Wang T, Kou Z, Wang Z. (2015). A review on phytochemistry and pharmacological activities of the processed lateral root of Aconitum carmichaelii Debeaux. J Ethnopharmacol 160: 173-93. doi: 10.1016/j.jep.2014.11.043; *and see*, Chan TY. Aconite poisoning. Clin Toxicol (Phila). 2009 Apr;47(4):279-85. doi: 10.1080/15563650902904407.

[29] Natural Medicines, 2023 https://naturalmedicines.therapeuticresearch.com/databases/food,-herbs-supplements/professional.aspx?productid=872; *and see*, EMA, 2017 https://www.ema.europa.eu/en/documents/herbal-report/final-assessment-report-piper-methysticum-g-forst-rhizoma_en.pdf

[30] Natural Medicines, 2023 https://naturalmedicines.therapeuticresearch.com/databases/food,-herbs-supplements/professional.aspx?productid=759; *and see*, EFSA ANS Panel (EFSA Panel on Food Additives and Nutrient Sources Added to Food), 2013. Scientific Opinion on the evaluation of the safety in use of Yohimbe (Pausinystalia yohimbe (K. Schum.) Pierre ex Beille). EFSA Journal 2013; 11(7):3302, 46 pp. doi:10.2903/j.efsa.2013.3302

[31] Natural Medicines, 2022 https://naturalmedicines.therapeuticresearch.com/databases/food,-herbs-supplements/professional.aspx?productid=946; *and see*, FDA, 2021 https://www.fda.gov/consumers/consumer-updates/grapefruit-juice-and-some-drugs-dont-mix; *and see*, Dahan A, Altman H. Food-drug interaction: grapefruit juice augments drug bioavailability--mechanism, extent and relevance. Eur J Clin Nutr. 2004 Jan;58(1):1-9. doi: 10.1038/sj.ejcn.1601736; *and see*, Bressler R. Grapefruit juice and drug interactions. Exploring mechanisms of this interaction and potential toxicity for certain drugs. Geriatrics. 2006 Nov;61(11):12-8. PMID: 17112309.

[32] CRN Guidelines, https://www.crnusa.org/newsroom/crn-issues-recommended-guidelines-melatonin-supplements-focus-marketing-formulation-sleep

Despite known risks, none of these supplements have required substance-specific regulatory pathways and associated risks are mitigated through existing FD&C Act frameworks.

However, given the FDA's publicly stated concerns about known and unknown risks related to hemp cannabinoid consumption, and desire to assume a harm-reduction approach, the Proposed Policy Solution proposes a compromised approach, leveraging existing FD&C Act dependent warning statement requirements for Low-Dose Hemp-Derived Products to address specific risk concerns, mandating the following warning statements to be clearly displayed on any Low-Dose Hemp-Derived Product label:

> **"WARNING: The Food and Drug Administration has not evaluated this product for safety, or any statements made in connection with this product. Effects of acute and chronic use of this product are unknown and may cause adverse health events, including liver and reproductive toxicity. This product is not intended to diagnose, treat, cure, or prevent any disease. Consult a physician prior to use, especially if you are pregnant, may become pregnant, are breastfeeding, or taking prescription or over-the-counter medication(s). Keep out of reach of children."**

See Appendix A, Section 5(d)(1)(A). The Proposed Policy Solution requires the above warning statement to be prominently displayed on Low-Dose Hemp-Derived Products, with one exception. Consistent with the 2023 study led by Rayetta G. Henderson, Ph.D., Senior Managing Scientist, ToxStrategies, LLC, which recognizes safety in daily consumption of up to 70 milligrams of hemp-derived CBD for healthy adults, including pregnant or lactating adults, the Proposed Policy Solution exempts Hemp-Derived Products containing less than or equal to 70 milligrams of Hemp Cannabinoids from warning against reproductive toxicity or warnings targeted to pregnant or lactating adults. *See* Appendix A, Section 5(d)(1)(A)(i) and (B)(i). This approach is backed by science and promotes truthful disclosures to consumers regarding known and unknown risks.

In sum, using appropriate warning statements as a tool to manage risk is consistent with FDA's treatment of other dietary supplement ingredients that also present but ultimately are approved. FDA can also mitigate risks as it does for other dietary products through transparent labeling and product education mandated under the FD&C Act.

7. How has the absence of federal regulation over CBD created a market for intoxicating, synthetically produced compounds, such as Delta-8 THC, THC-O, THC-B, HHC-P, and others?33

Ambiguity in the 2018 Farm Bill with neither follow-up clarifying legislation nor FDA regulatory action has left consumers exposed to products which technically comply with the 2018 Farm Bill's crop requirements but include elevated delta-9 THC and synthetic cannabinoids, including delta-8. These intoxicating products should be deemed federally illegal and regulated under state cannabis programs. Instead, many such products are sold as unregulated (*i.e.*, untested and improperly labeled; improperly taxed; and/or non-age gated) products in retail channels. Lack of regulation of these products rapidly created a large and arguably illicit market. Indeed, the delta-8 products market is estimated to have generated $2 billion in sales since 2021.[34]

The popularity of delta-8 has surged as a cost-effective and easily accessible option for experiencing psychoactive effects. Consumers can obtain delta-8 through retail channels or via mail delivery, avoiding the potential legal risks associated with purchasing delta-9 THC illicitly. Statistically, it is states with more stringent cannabis restrictions, particularly in the South that are witnessing the most dynamic and active delta-8 markets. With the enactment of the 2018 Farm Bill, the delta-8 and emerging cannabinoid market found its footing on the basis that the legislation permitted the commercialization of hemp products, provided they contained less than 0.3% delta-9 THC by dry weight. Certain players swiftly recognized the potential of alternative cannabinoids with psychoactive properties. This has opened the door to marketing intoxicating products as hemp-derived, leading to a bifurcation of the market. One segment caters to the health and wellness needs of millions of CBD consumers. The other segment generally offers intoxicating, subpar, and untested products that mislead consumers. By the end of 2020, delta-8 products became prevalent and started gaining significant attention among consumers and have grown $2B in the last two years.

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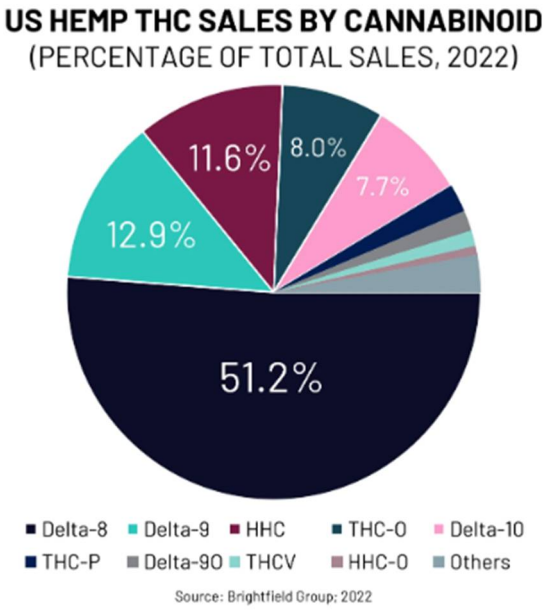
[33] All market data presented in response to RFI Question 7 is supplied by the Brightfield Group data analytics 2022-2023 insights reports. The full reports are available by login at https://www.brightfieldgroup.com/ and are as follows:

- Brightfield, "Delta-8 & Emerging Cannabinoids Introduction & Overview" and "How Big of a Threat is Delta-8?"

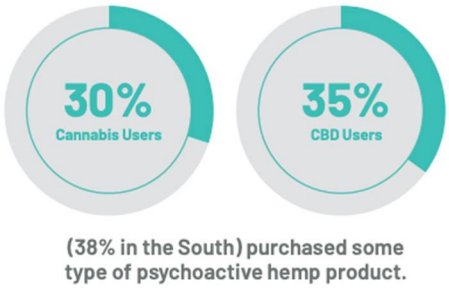
[34] Delta-8 THC Generated $2 Billion In Revenue In Two Years, Report Finds, Forces (Jan. 16, 2023), https://www.forbes.com/sites/dariosabaghi/2023/01/16/delta-8-thc-generated-2-billion-in-revenue-in-2-years-report-finds/?sh=2137ab624a62

Delta 8 and Emerging Cannabinoid Market Forecast (Brightfield)

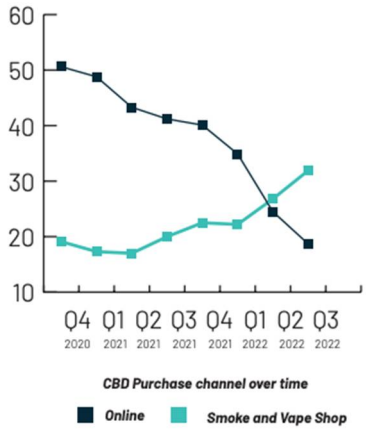
Product Type	2021	2022	2023	2024	2025	2026	2027
Vapes	$852,410,118	$1,070,239,033	$1,455,444,257	$1,741,648,005	$1,956,033,577	$2,098,492,192	$2,192,647,776
Gummies	$284,208,504	$632,834,975	$901,447,775	$1,119,027,070	$1,281,626,740	$1,389,166,998	$1,458,091,374
Flower & Pre-Rolls	$111,408,143	$208,344,046	$265,354,656	$296,054,782	$313,835,224	$322,070,669	$324,443,745
Concentrates	$88,200,744	$155,293,408	$197,035,136	$216,582,223	$225,411,639	$228,415,281	$227,882,581
Other Edibles	$36,824,505	$87,835,337	$126,678,832	$158,072,324	$181,492,488	$197,368,845	$207,453,013
Tinctures	$61,937,130	$109,874,657	$129,624,306	$138,518,277	$141,603,153	$142,445,065	$141,933,420
Drinks	$15,870,510	$40,038,037	$60,744,256	$77,847,437	$91,905,634	$102,029,482	$108,798,115
Capsules	$15,772,882	$33,338,411	$38,982,646	$41,501,977	$42,357,767	$42,452,519	$42,173,391
Beauty & Topicals	$4,203,967	$11,228,816	$12,983,706	$13,821,483	$14,155,979	$14,196,464	$14,125,234
Total	**$1,470,836,503**	**$2,349,026,719**	**$3,188,295,569**	**$3,803,073,579**	**$4,248,422,202**	**$4,536,637,513**	**$4,717,548,649**



Analysts report that 35% of existing CBD users have made purchases within the intoxicating hemp-derived product category in the past six months, highlighting the growing availability for these impairing alternatives. Today, 11.6% of American consumers report using delta-8, delta-10, THC-O, and/or HHC as of Q3 2022–approximately the same number of Americans that consume hard seltzer.



Although consumer demand has grown significantly since the 2018 Farm Bill, and consumer surveys suggest that Hemp-Derived Products are available at tens of thousands of outlets, the lack of access in superstores, club, and big box retailers and large chain retailers where millions of Americans shop will continue to remain limited without FDA regulation. Recently data shows that consumer behavior in these categories is changing, suggesting the influence of the emergence of unregulated delta-8 products. Online CBD purchases have been steadily declining since their peak in November 2020, and the decline has become more pronounced since November 2021, witnessing a significant 53% drop in online purchases from Q4 2021 to Q3 2022. Consequently, vape and smoke shops have experienced a notable uptick of 42% in purchasers during the same period suggesting sale of delta-8 or other intoxicating cannabinoid products.



a. What is the public health impact of these novel compounds?

The public health impact of a proliferation of intoxicating, unregulated and technically federally legal products is profound, insofar as there is minor access to these intoxicating products,

and that consumers are trending toward consuming the products, which have little to no testing or manufacturing standards. State legislators and regulators have tackled regulating or banning these products in accordance with the needs of their jurisdictions. In the absence of federal oversight and potential scheduling of these substances under the CSA, which is beyond the scope of regulating nonintoxicating Hemp-Derived Products, this comment suggests that these policy determinations to regulate or ban these substances be left to the legislators and regulators of the states.

b. How have FDA and state regulators enforced against products containing these compounds?

The FDA has not regulated these products which the federal courts have now recognized as legal. In *AK Futures, LLC vs. Boyd Street Distro, LLC,* D.C. No. 8:21-cv-01027- JVS-ADS, the Ninth Circuit affirmed the District Court granting preliminary injunction in favor of AK Futures, a delta-8 vaporizer device manufacturer, rejecting the alleged counterfeiter's argument that AK Futures could not own a valid trademark in connection with these products because federal law forbids possession and sale of delta-8. The Ninth Circuit rejected this argument, holding that the 2018 Farm Bill legalized the company's delta-8 products. The Court opined: "If Boyd Street is correct, and Congress inadvertently created a loophole legalizing vaping products containing delta-8, then it is for Congress to fix its mistake**.** Boyd Street's intent-based argument is thus unsuccessful." *Id*. The FDA has exclusively enforced against producers of CBD operators making egregious heath claims[35] but has not enforced against intoxicating delta-8 products (with the exception of one warning letter issued to Koi CBD).[36] Again, state legislators and regulators have tackled regulating or banning these products in accordance with the needs of their jurisdictions. In the absence of federal oversight and potential scheduling of these substances under the CSA, which is beyond the scope of regulating nonintoxicating Hemp-Derived Products, this comment suggests that these policy determinations to regulate or ban these substances be left to the legislators and regulators of the states.

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[35] *See* FDA, Warning Letters and Test Results for Cannabidiol-Related Products, https://www.fda.gov/news-events/public-health-focus/warning-letters-and-test-results-cannabidiol-related-products.

[36] https://www.fda.gov/inspections-compliance-enforcement-and-criminal-investigations/warning-letters/koi-cbd-llc-651252-07182023

c. How should Congress consider the inclusion of these products in a regulatory framework for cannabinoid hemp products, if at all

The Proposed Policy Solution definitionally delineates between nonintoxicating vs. intoxicating hemp products. To permit the nonintoxicating Hemp-Derived Products to thrive in appropriate channels, Congress should address the products separately, and regulate nonintoxicating products through the FD&C Act framework for dietary supplements with additional hemp-specific regulations; and intoxicating hemp products through existing and future regulatory pathways for intoxicating cannabis.

8. CBD products are not limited to just ingestible routes of administration—some are interested in products with alternative routes of administration (e.g., inhalable, topical, ophthalmic drops, etc.). a. For which non-ingestible routes of administration are consumers interested in consuming CBD products? b. How should a regulatory framework for cannabinoid products account for non-ingestible routes of administration

The Proposed Policy Solution intends to regulate only certain form factors "intended for ingestion" in a form described in section 350(c)(1)(B)(i) of the Federal Food, Drug, And Cosmetic Act (21 U.S.C. §§ 301-392). Other form factors and methods to administer hemp-derived products, such as inhalation, injection, etc., should remain under applicable regulatory pathways as determined by Congress or the FDA and the state legislators and regulators.

<u>Federal-State Interaction</u>

9. In the absence of federal regulation or enforcement over CBD products, many states have established state regulatory programs to safeguard public health and create market certainty for industry participants.

a. Which product standards relating to warning labels, minimum age of sale, manufacturing and testing, ingredient prohibitions, adverse event reporting, and others, have states adopted to protect consumer safety?

b. Which such standards, if any, should Congress look to as models?

In the gap of federal regulation, many states have imposed regulatory standards for Hemp-Derived Products that mirror those of the FD&C Act for dietary supplement and conventional food manufacturing, *e.g.*, Montana, Rhode Island, and New York. In fact, immediately after passage of

the 2018 Farm Bill, many states directly cross-referenced compliance to the federal FD&C Act as a requisite to market Hemp-Derived Products in intrastate commerce. For example, on June 20, 2019, New York State Senate and Assembly passed legislation (S6184A/A7680A) directing promulgation of further regulations related to hemp manufacturing to be based on FDA regulation of dietary supplements, providing:

> All hemp extract products shall be extracted and manufactured in accordance with good manufacturing processes, pursuant to Part 111 or 117 of Title 21 of the Code of Federal Regulations as may be modified and decided upon by the commissioner in regulation.

As state regulations have evolved to address public health issues related to intoxicating cannabinoids in accordance with the needs of the respective states, FD&C Act standards for manufacturing, adverse event reporting, and general consumer safety are still prevalent. For example, the Colorado Department of Public Health and Education has expanded testing requirements to ensure contaminant testing is completed on all retail products; and Colorado companies are audited annually by a third party, Eurofins, to ensure 21 C.F.R. §111 compliance.[37]

The Proposed Policy Solution is aligned with state best practices that look to the existing regulatory frameworks for dietary supplements within the FD&C Act. This comment advocates that Congress looks no further than to federal standards to set a federal baseline for quality and product safety in Hemp-Derived Products related to: (l) premarket notice; (2) labeling and packaging; (3) disclaimer and warning statements; and (4) manufacturing and testing are more than sufficient to manage quality and safety in Hemp-Derive Products in the U.S. market. *See* 21 C.F.R. §101.3(a); 21 C.F.R. §101.105(a); 21 C.F.R. §101.36; 21 C.F.R. §101.4(a)(1); and 21 C.F.R. §101.5 (Labeling, Packaging, Disclaimer, and Warning Requirements); 21 C.F.R. §111 and 21 U.S.C. §350(b) (Manufacturing, Testing and Notification Requirements).

[37] See https://www.eurofinsus.com/food-testing/resources/press-release-eurofins-us-food-expands-cdphe-hemp-testing-laboratory-certification-to-colorado-location

10. How should Congress consider federal preemption as it works towards a regulatory pathway? Should states be able to continue to build upon federal regulation of CBD products?

The safety profile of CBD supports Congress looking to the FD&C Act regulatory pathway for dietary supplements as the appropriate general model for CBD regulations. Again, several states, like Montana, Rhode Island, and New York, initially modeled their hemp-product regulations against the FD&C Act frameworks for dietary supplements, conventional foods, and cosmetics.

While the Proposed Policy Solution recognizes that the existing FD&C Act regulatory pathway for dietary supplements is adequate to regulate Hemp-Derived Products with some hemp-specific regulations related to age-gating and dose-based warning labels for Low-Dose Hemp-Derived Products, the Solution also recognizes that state regulatory frameworks play a role in regulating hemp-derived products, including THC product serving limits, which Congress should consider. The industry is looking to federal regulation to end uncertainty to manufacturers created by a state-patchwork regulatory model framework but recognizes that the state legislators and regulators are in the best position to regulate certain aspects to meet public health needs within their jurisdictions. The Solution specifically preserves the rights of state and local authorities to exceed some of the requirements for hemp and cannabis products sold within their jurisdictions, while setting a baseline and preempting differing regulations in other respects:

> (a) In general.
>
> (1) Preservation. Except as provided in subsection (2)(A), nothing in this Act, or rules promulgated thereunder, shall be construed to limit the authority of a Federal agency (including the Armed Forces), a State or political subdivision of a State, or the government of an Indian tribe to enact, adopt, promulgate, and enforce any law, rule, regulation, or other measure with respect to Hemp-Derived Supplements that is in addition to, or more stringent than, requirements established under this subchapter, including a law, rule, regulation, or other measure relating to or prohibiting the sale, distribution, possession, exposure to, access to, advertising and promotion of, or use of Hemp-Derived Supplements by individuals of any age, or information reporting to the State. No provision of this subchapter shall limit or otherwise

affect any State, tribal, or local taxation of Hemp-Derived Supplements.

(2) Preemption of certain State and local requirements.

(A) No State or political subdivision of a State may establish or continue in effect with respect to a Hemp-Derived Supplement any requirement which is different from, or in addition to, any requirement under the provisions of this subchapter relating to Hemp-Derived Supplement product standards, notification, adulteration, misbranding, labeling, registration, or good manufacturing standards.

(B) Exception. Subsection (A) does not apply to requirements relating to the sale, distribution, possession, information reporting to the State, exposure to, access to, the advertising and promotion of, or use of, Hemp-Derived Supplements by individuals of any age.

(b) Rule of construction regarding product liability. No provision of this subchapter relating to a Hemp-Derived Supplements shall be construed to modify or otherwise affect any action or the liability of any person under the product liability law of any State.

(c) Rule of construction regarding Marihuana. No provision of this subchapter relating to a Hemp-Derived Supplements shall be construed to modify or otherwise affect the legality, regulation or enforcement policies relating to marihuana (as that term is defined in section 102 of the Controlled Substances Act (21 U.S.C. § 802)) under federal law.

See Appendix A, Section 6.

Safety

11. What is currently known about the safety and risk-benefit profile of CBD and other hemp-derived cannabinoids? What safety and toxicity data are available to support this knowledge. Please include in your answer any relevant information about safety with regard to specific populations, such as children and pregnant individuals.

Several available public studies submitted to the FDA support that hemp-derived CBD is well-tolerated at consumption levels suitable for use as a dietary supplement.

A 2023 study has been conducted and led by Rayetta G. Henderson, Ph.D., Senior Managing Scientist, ToxStrategies, LLC, who was called as an expert witness in the House Committee on Oversight and Accountability Subcommittee on Health Care and Financial Services' July investigation into the FDA's failure to regulate CBD products. This assessment applied a systematic approach to review all publicly available preclinical toxicity studies in animal models and clinical studies in humans on hemp-derived CBD isolate. The studies determined to be of the highest quality and relevance were selected and used to derive safe levels of CBD consumption, including: 70 mg daily for healthy adults, including pregnant or lactating adults; 100 mg daily for healthy adults not currently trying to conceive, pregnant or lactating based on liver effects; and up to 160 mg daily for healthy adults not currently trying to conceive, pregnant or lactating and excluding populations at risk for liver injury. The definition of "healthy adults" in this study excluded those treated for or diagnosed with any medical condition, or currently taking any medications. *See* Appendix B, Exhibits A-C.

Additionally, available studies in humans demonstrate that CBD at much higher consumption levels is well-tolerated. Serious adverse events in these studies are generally limited to elevated liver enzyme levels (as an indicator of potential liver toxicity), which has been observed primarily at very high pharmacological doses of CBD and in individuals taking concomitant medications. In fact, a recent systematic review and meta-analysis[38] of 28 clinical trials concluded that CBD exposure was only statistically significantly associated with elevated enzyme levels at dose levels ≥1,000 mg/day; all but one clinical trial reporting this effect administered CBD at doses

[38] Lo LA, Christiansen A, Eadie L, Strickland JC, Kim DD, Boivin M, Barr AM, MacCallum CA. 2023. Cannabidiol-associated hepatotoxicity: A systematic review and meta-analysis. J Intern Med. 13. doi: https://doi.org/10.1111/joim.13627.

of 20 mg/kg/day (*i.e.*, 400 mg/day in adults) or higher. In addition, 76.10% of participants with elevated liver enzyme levels across all studies were also taking concomitant medications. *See* Appendix B, Exhibit D. Hemp-Derived Products marketed as dietary supplements do not include hemp cannabinoid doses even approaching these high, yet well-tolerated, pharmacological doses. Thus, these data further support the safety of regulating Hemp-Derived Products under the existing framework for dietary supplements, including the FDA's warning statement risk-management tool.

Another 2023 study related to "broad spectrum CBD" human consumption supports safe consumption at 100 milligrams of hemp cannabinoids per day for a healthy 70 kg adult. *See* Appendix B, Exhibit E.

Regarding pregnant or lactating individuals, newly published reproductive toxicity studies[39] conducted in animal models according to gold-standard, guideline compliant methods are now available on CBD isolate. In addition to guideline studies already available in the EPIDIOLEX® non-clinical safety review, these studies provide critical information regarding the potential adverse effects of CBD to individuals trying to conceive, pregnant, and/or lactating. Data from these studies can be used to identify safe levels of intake for this population, such as was conducted in the study above.

While the risks, known and unknown, related to CBD consumption have been cited by the FDA as concerns, warning statements have been a perfectly sufficient risk management tool for other marketable and regulated dietary ingredients through existing regulation, *i.e.*, warning statements, which have been effective with the following ingredients:

- Saint John's Wort – significant drug interactions[40]
- Iron – liver damage, systemic toxicity, death[41]
- Aconite – nausea, vomiting, low blood pressure and heart rhythm disorders[42]

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[39] Henderson RG, Welsh BT, Rogers JM, Borghoff SJ, Trexler KR, Bonn-Miller MO, Lefever TW. 2023. Reproductive and developmental toxicity evaluation of cannabidiol. Food Chem Toxicol. 176:113786. https://doi.org/10.1016/j.fct.2023.113778.

[40] Natural Medicines, 2023 https://naturalmedicines.therapeuticresearch.com/databases/food,-herbs-supplements/professional.aspx?productid=329; *and see*, EMA, 2022 https://www.ema.europa.eu/en/documents/herbal-monograph/final-european-union-herbal-monograph-hypericum-perforatum-l-herba-revision-1_en.pdf

[41] 21 C.F.R. 101.17

[42] Natural Medicines, 2023 https://naturalmedicines.therapeuticresearch.com/databases/food,-herbs-supplements/professional.aspx?productid=609; *and see,* Zhou G, Tang L, Zhou X, Wang T, Kou Z, Wang Z. (2015). A review on phytochemistry and pharmacological activities of the processed lateral root of Aconitum carmichaelii Debeaux. J

- Kava – Liver damage[43]
- Yohimbe – rapid heartbeat, kidney failure, seizure, and heart attack[44]
- Grapefruit – drug-drug interactions[45]

Ethnopharmacol 160: 173-93. doi: 10.1016/j.jep.2014.11.043; *and see*, Chan TY. Aconite poisoning. Clin Toxicol (Phila). 2009 Apr;47(4):279-85. doi: 10.1080/15563650902904407.

[43] Natural Medicines, 2023 https://naturalmedicines.therapeuticresearch.com/databases/food,-herbs-supplements/professional.aspx?productid=872; and see EMA, 2017 https://www.ema.europa.eu/en/documents/herbal-report/final-assessment-report-piper-methysticum-g-forst-rhizoma_en.pdf

[44] Natural Medicines, 2023 https://naturalmedicines.therapeuticresearch.com/databases/food,-herbs-supplements/professional.aspx?productid=759; *and see,* EFSA ANS Panel (EFSA Panel on Food Additives and Nutrient Sources Added to Food), 2013. Scientific Opinion on the evaluation of the safety in use of Yohimbe (Pausinystalia yohimbe (K. Schum.) Pierre ex Beille). EFSA Journal 2013; 11(7):3302, 46 pp. doi:10.2903/j.efsa.2013.3302

[45] Natural Medicines, 2022 https://naturalmedicines.therapeuticresearch.com/databases/food,-herbs-supplements/professional.aspx?productid=946; FDA, 2021 https://www.fda.gov/consumers/consumer-updates/grapefruit-juice-and-some-drugs-dont-mix; *and see*: Dahan A, Altman H. Food-drug interaction: grapefruit juice augments drug bioavailability--mechanism, extent and relevance. Eur J Clin Nutr. 2004 Jan;58(1):1-9. doi: 10.1038/sj.ejcn.160173; and Bressler R. Grapefruit juice and drug interactions. Exploring mechanisms of this interaction and potential toxicity for certain drugs. Geriatrics. 2006 Nov;61(11):12-8. PMID: 17112309.

The FDA's stated concerns related to Hemp-Derived Products specifically relate to:

- Liver Toxicity Risk;
- Drug Interaction Risk;
- Vulnerable Population Risk;
- Reproductive Toxicity Risk; and
- Risks to pregnant and lactating women.

Given the data supporting the safety profile of hemp cannabinoids, the precedent highlighted above supports that the FDA's identified risks can be managed through existing regulation requiring transparent marketing, education, and warning statements. The Proposed Policy Solution goes a step further and proposes requiring dose-dependent hemp-specific warning statements to address FDA stated concerns and safeguard public health as follows for Low-Dose Hemp-Derived Products containing more than 70 milligrams of total Hemp Cannabinoids:

> **"WARNING: The Food and Drug Administration has not evaluated this product for safety, or any statements made in connection with this product. Effects of acute and chronic use of this product are unknown and may cause adverse health events, including liver and reproductive toxicity. This product is not intended to diagnose, treat, cure, or prevent any disease. Consult a physician prior to use, especially if you are pregnant, may become pregnant, are breastfeeding, or are taking prescription or over-the-counter medication(s). Keep out of reach of children."**

See Appendix A, Section 5(d)(1)(A)

These recommendations are consistent with the 2023 study led by Rayetta G. Henderson, Ph.D., Senior Managing Scientist, ToxStrategies, LLC, which again recognizes safety in daily consumption of up to 70 milligrams of hemp-derived CBD for healthy adults, including pregnant or lactating adults, the Proposed Policy solution exempts Hemp-Derived Products containing less than or equal to 70 milligrams of Hemp Cannabinoids from warning against reproductive toxicity or warnings targeted to pregnant or lactating adults. *See* Appendix A, Section 5(d)(1)(A)(i).

12. What actions, if any, should the Federal government take to better understand the potential benefits or harms of CBD products and other cannabinoids?

Hemp-Derived Products include hemp extracts with variable cannabinoid composition and/or cannabinoid isolates other than CBD, which may impart differences in their respective safety profiles. As such, safety data on the specific material within the hemp plant should be generated and prioritized, where relevant, and the toxicological profile of individual constituents should be considered.

13. How should a new framework for CBD products balance consumer safety with consumer access?

Ideal Congressional action would provide access and continuity in the consumer marketplace, including regulation for testing, transparent labeling and warning statements on which consumers can rely in making purchase decisions. Adult consumers who rely on Hemp-Derived Products are owed assurances that universally applicable safety standards are implemented (*e.g.*, products are not adulterated or misbranded) so that they may be empowered to exercise their own personal risk/benefit analysis for products they consume.

The Proposed Policy Solution provides a comprehensive solution through both leveraging existing FD&C Act regulatory frameworks for dietary and authorizing FDA to add two additional hemp-specific measures addressing the concerns of the FDA through clear dose-based warning statements and limiting purchases of such products to individuals aged 18 and over to address vulnerable population concerns.

See Appendix A, Section 5(d)(A)-(B).

14. Some stakeholders have raised concerns that CBD products have inherent risks. What are those inherent risks, and at what levels of CBD do those risks present themselves? What data and other evidence support the existence of such risks, and from which products are such data and evidence derived?

See supra, Response to RFI Question 11.

15. FDA approved Epidiolex, a drug containing CBD, based in part on a data package that included preclinical data from rodent safety models, as well as clinical trials. FDA has received safety data on CBD products from several manufacturers also based on rodent models. How should FDA consider data submitted for a CBD-containing drug as evidence to support that CBD is safe for human consumption in non-drug products, recognizing the inherent differences in the intended uses of such products?

Rodent models are the standard for evaluating safety and risk of dietary ingredients recommended by the FDA,[46] including ingredients in dietary supplements, food, and/or beverages. Modern day preclinical toxicity studies are generally designed according to the same guidelines (*e.g.*, OECD), regardless of the intended use of the product; as such, the OECD-compliant toxicity studies conducted in rodents used to support EPIDIOLEX® safety are directly relevant to the safety evaluation of CBD as a dietary ingredient. In all cases, preclinical toxicology studies are intentionally designed to be conducted at high enough exposure levels to enable identification of adverse effects – the exposure levels associated with such effects in these studies are then used to determine safe levels of exposure. The process of calculating safe levels based on these studies differs depending on the intended application; for example, use as a drug would include a risk/benefit analysis, whereas uses as a dietary ingredient would not.

In addition to the preclinical studies in animal models reviewed by FDA as part of the EPIDIOLEX® package, several new guideline-compliant toxicology studies[47] have been published that expand the available data on CBD and address some of the gaps identified by the agency (*e.g.*, reproductive toxicity). Toxicity studies in animal models are an integral part of evaluating the potential toxicity of substances for human consumption, as they enable dosing at

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[46] Draft Guidance for Industry: New Dietary Ingredient Notifications and Related Issues (August 2016). Last updated 4 October 2016. FDA website. https://www.fda.gov/regulatory-information/search-fda-guidance-documents/draft-guidance-industry-new-dietary-ingredient-notifications-and-related-issues; *and see*, Redbook (food) ref: FDA (U.S. Food and Drug Administration). 2007. Guidance for industry and other stakeholders; Toxicological principles for the safety assessment of food ingredients. In: Redbook 2000. Office of Food Additive Safety in the Center for Food Safety and Applied Nutrition.

[47] Henderson RG, Welsh BT, Trexler KR, Bonn-Miller MO, Lefever TW. 2023. Genotoxicity evaluation of cannabidiol. Regul Toxicol Pharmacol 142:105425; doi: 10.1016/j.yrtph.2023.105425.

Henderson RG, Lefever TW, Heintz MM, Trexler KR, Borghoff SJ, Bonn-Miller MO. 2023. Oral toxicity evaluation of cannabidiol. Food Chem Toxicol 176:113778; https://doi.org/10.1016/j.fct.2023.113786.

Henderson RG, Welsh BT, Rogers JM, Borghoff SJ, Trexler KR, Bonn-Miller MO, Lefever TW. 2023. Reproductive and developmental toxicity evaluation of cannabidiol. Food Chem Toxicol. 176:113786. https://doi.org/10.1016/j.fct.2023.113778

high levels to understand any potential endpoints of concern, as well as investigation in vulnerable populations, such as during pregnancy.

Clinical trials in humans were also studied in the context of approval of the drug EPIDIOLEX® for treatment of seizure disorders in children with highly compromised health, many of which were taking concomitant anti-epileptic drugs. Other studies in humans are also available on various oral CBD preparations and are available in the published literature. These studies primarily include individuals of varying disease states; however, more recent publications focus on healthy populations as well. Similarly, most available clinical trials with CBD are conducted using very high, pharmacological dose levels with fewer available studies on lower doses, especially those relevant to potential dietary supplement use.

High-quality studies in humans provide valuable information for consideration in a safety evaluation of CBD for consumer use as a dietary ingredient. Importantly, the available studies have evaluated the potential adverse effects of CBD across a broad range of populations, including age groups, sex, race, etc. The relevance of the dose levels used should be carefully considered when using these studies to conduct risk calculations. Combined with studies in animal models, the available data are sufficient to perform a robust safety assessment for use of CBD as a dietary supplement.

16. Should there be limits on the amount of CBD in foods, dietary supplements, tobacco, or cosmetics? If so:

a. Should Congress or FDA set such limits, recognizing the time it can take to complete the legislative process and the regulatory process at FDA?

b. How should that amount be determined? What should the amount be?

c. Should such limits be applied on the amount per serving, and/or per package?

d. Could FDA set such limits under its current statutory regulatory authorities for foods and dietary supplements to potentially address safety concerns, notwithstanding exclusionary clause issues?

e. How should the experience of states inform the setting of limits on amounts of CBD in products?

Several available public studies submitted to the FDA support CBD's safety profile and consumption levels suitable for use as a dietary supplement. The 2023 study conducted and led by Rayetta G. Henderson, Ph.D. applied a systematic approach to review all publicly available preclinical toxicity studies in animal models and clinical studies in humans on hemp-derived CBD isolate. The studies determined to be of the highest quality and relevance were selected and used to derive potential safe levels of CBD consumption, including: 70 mg daily for healthy adults, including pregnant or lactating adults; 100 mg daily for healthy adults not currently trying to conceive, pregnant or lactating based on liver effects; and up to 160 mg daily for healthy adults not currently trying to conceive, pregnant or lactating and excluding populations at risk for liver injury. The definition of "healthy adults" in this study excluded those treated for or diagnosed with any medical condition, or currently taking any medications. *See* Appendix B, Exhibits A-C. These safety guidelines would permit well-tolerated daily CBD consumption at 160 mg daily for healthy adults not currently trying to conceive, pregnant or lactating and excluding populations at risk for liver injury. The Proposed Policy Solution requires that products with more than 100 milligrams of hemp-cannabinoids per serving submit to premarket safety submission. Within this process, if safety data proves and justifies a daily hemp cannabinoid limit or recommended daily dose, FDA should promulgate regulations or publish guidance accordingly.

17. How should a regulatory framework account for CBD products marketed in combination with other substances that may alter or enhance the effects of CBD (e.g., caffeine, melatonin, etc.)?

Under the FD&C Act, the FDA has existing authority to restrict certain substances from being combined in food or dietary supplements. The Proposed Policy Solution does not amend or disrupt the FDA's power to regulate adding certain ingredients, for example, melatonin to dietary supplements. *See supra*, Response to RFI Question 10, *and see* Appendix A, Sections 5 (a):

> (a) Generally Regulated as Dietary Supplements. Except as expressly indicated by this Act, a Hemp-Derived Supplement shall be deemed to be a dietary supplement within the meaning of the Federal Food, Drug, and Cosmetics Act and its implementing regulations.

Specifically with regard to caffeine, the first human safety and effects study examining the combination of CBD and caffeine was presented at the 2023 annual meeting of the College on Problems of Drug Dependence in June. The placebo-controlled study examined the interaction between 200mg caffeine and CBD doses ranging from 25mg to 240mg. The study found no interaction between CBD and caffeine in terms of subjective effects and no safety concerns.[48] *See* Appendix B, Exhibit G.

18. What precedent is there for FDA restricting certain otherwise allowable ingredients in legally marketed products? What amount and type of evidence has been required/demonstrated to support any such restrictions?

Caffeine is an interesting example of FDA restrictions on an ingredient found both in the general food supply and as a dietary supplement. Specific to pure caffeine, guidance was issued by FDA in 2018[49] on pure or highly concentrated caffeine sold directly to the consumer wherein the FDA found that these products were "sufficiently dangerous such that a warning cannot remedy the adulteration" citing adulteration under section 402(f)(1)(A). Since caffeine can be highly toxic at doses greater than 1.2g, with death possible at typically >10g, the FDA issued guidance that,

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[48] Ferretti, M. L., Gustin, N. D., Zindler, R. E., Sokol, C. M., Knowlton, A., Porter, K., Carmack, O., Hoffman, E., Feldner, M. T., Bonn-Miller, M. O., & Irons, J. G. (2023, June). Investigating the simultaneous effects of cannabidiol and caffeine. *See* Poster presented at the 85th annual meeting of the College on Problems of Drug Dependence (CPDD), Denver, CO, at Appendix B, Exhibit F.

[49] Highly Concentrated Caffeine in Dietary Supplements Guidance for Industry (fda.gov)
https://pubmed.ncbi.nlm.nih.gov/28438661/

even if the label of a bulk product suggests an appropriate serving size, there was still an unreasonable risk of illness or injury. Thus, FDA restricted bulk sale but did not restrict caffeine sold in other forms, finding that these products eliminated the need for consumers to judge appropriate amounts for consumption to mitigate the risk identified.

Caffeine is codified for very low use in cola beverages as a flavor and food manufacturers can use caffeine for additional specific intended uses under their own self-affirmed Generally Recognized as Safe ("GRAS") documentation. In addition, caffeine can frequently be found in dietary supplements. Much controversy has existed over caffeine and the varying safe levels associated with sensitive population types. Although not formal restrictions, congressional pressure led to an Institute of Medicine ("IOM") workshop on the safety of caffeine. As an outcome of this workshop, ToxStrategies conducted the largest systematic review on caffeine[50] that is often cited as support that there are unique levels for different populations that should be considered when ingesting caffeine. The FDA and other authoritative agencies often cite 200mg/day for pregnant women, 400mg/day for healthy adults, and 2.5mg/kg-bw/d for children. This is an important example of a situation whereby toxicity is recognized to occur, but the ingredient is still allowed in the food supply, and there is a long history of safe use.

While FDA does not restrict certain vitamins and minerals in foods and supplements, many have daily upper limits based on adverse effects derived by the National Institutes of Health ("NIH"). For example, on August 12, 2022, the NIH updated its guidance[51] regarding the daily upper limits for preformed vitamin A including intakes from all sources—food, beverages, and supplements. The basis for this recommendation is due to exposure to higher levels of preformed vitamin A (usually from supplements or certain medicines) being associated with severe headache, blurred vision, nausea, dizziness, muscle aches, and problems with coordination; in severe cases, even coma and death.

The Proposed Policy Solution does not amend or disrupt the FDA's power to regulate adding certain functional ingredients, for example, melatonin or caffeine to dietary supplements in alignment with this precedent.

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[50] Systematic review of the potential adverse effects of caffeine consumption in healthy adults, pregnant women, adolescents, and children - PubMed (nih.gov) https://pubmed.ncbi.nlm.nih.gov/28438661/

[51] https://ods.od.nih.gov/factsheets/VitaminA-Consumer/

19. What functional ingredients combined with cannabinoids raise safety concerns?

If combining functional ingredients with hemp cannabinoids prove to present health and safety risks in the future (no current studies of which we are aware support such safety concerns), the FDA has precedent for regulating and managing against such risks as outlined above. *See supra* Response to RFI Questions 17 and 18. Again, the Proposed Policy Solution does not amend or disrupt the FDA's power to regulate adding certain ingredients to dietary supplements. *See* Appendix A, Sections 5 (a).

Quality

20. How should Congress create an FDA-implemented framework to ensure that manufacturers provide appropriate consumer protections and quality controls?

a. How should such a framework compare to the current Good Manufacturing Practice (GMP) requirements that apply to food, dietary supplements, and cosmetics?

The FD&C Act's existing regulatory framework for dietary supplements related to (l) premarket notice; (2) labeling and packaging; (3) disclaimer and warning statements; and (4) anufacturing and testing are more than sufficient to manage quality and safety in Hemp-Derived Products in the U.S. market. *See* 21 C.F.R. §101.3(a); 21 C.F.R. §101.105(a); 21 C.F.R. §101.36; 21 C.F.R. §101.4(a)(1); and 21 C.F.R. §101.5 (Labeling, Packaging, Disclaimer, and Warning Requirements); 21 C.F.R. §111 and 21 U.S.C. §350(b) (Manufacturing, Testing and Notification Requirements). The Proposed Policy Solution expressly references these requirements, including GMP requirements for regulating Hemp-Derived Products.

In the gap of regulation, reputable Hemp-Derived Product producers have been successfully producing Hemp-Derived Products in compliance with dietary supplement regulations for manufacturing under 21 C.F.R. §111. In fact, many states, like Colorado, already impose GMP standards for manufacturing Hemp-Derived Products. For example, the Colorado Department of Public Health and Education has expanded testing requirements to ensure contaminant testing is completed on all retail products; and Colorado companies are audited

annually by a third party, Eurofins, to ensure 21 C.F.R. §111compliance.[52] Congress now has the opportunity to set a federal baseline for requiring GMP manufacturing standards for manufacturing Hemp-Derived Products nationwide ensuring consistent, safe, and quality products to consumers.

b. Are those food, dietary supplement, and cosmetics GMP frameworks adequate for regulating quality in CBD? Why or why not?

Existing FDA standards governing manufacturing of dietary supplements provide ample authority for FDA to ensure safe issues related to manufacturing, process, and storing of Hemp-Derived Products. Specifically, FDA's expansive manufacturing requirements for dietary ingredients and finished dietary supplements products are entirely sufficient to ensure that the appropriate controls are in place throughout the manufacturing process and the supply chain. Any facility that manufactures hemp cannabinoid ingredients supplement use would be required to register with FDA. Further 21 C.F.R. §111 requires manufacturing controls for supplement components, quality, strength, weight, concentrations, packaging, and labeling, all of which would apply to the manufacture of Hemp Derived Products under the Proposed Policy Solution. Dietary supplement manufacturers must also report serious adverse events to FDA and maintain records of all adverse event reports they receive and must ensure that dietary ingredients are safe for their intended uses.

Given the safety profile of hemp cannabinoids, the regulatory framework for dietary supplement manufacturing provides ample risk management and enforcement tools to protect consumers while still allowing them the desired access to a wide variety of affordable, high quality, safe and beneficial Hemp-Derived Products. Responsible hemp companies like the undersigned have fully supported being regulated under this existing dietary supplement regulatory framework in order to create a level playing field for companies across the board and help increase consumer confidence in the quality and safety of these products.

[52] *See* https://www.eurofinsus.com/food-testing/resources/press-release-eurofins-us-food-expands-cdphe-hemp-testing-laboratory-certification-to-colorado-location

21. What are alternative quality approaches that Congress should consider for CBD products? For example, how should third parties be leveraged for the creation and auditing of manufacturing and testing requirements

While the Proposed Policy Solution recognizes that the existing FD&C Act regulatory pathway for dietary supplements is adequate to regulate Hemp-Derived Products with some hemp-specific regulations, the Proposed Policy Solution also recognizes that state regulatory frameworks play a role in regulating Hemp-Derived Products, including required third-party testing, which Congress should consider while setting a baseline for how these products should be regulated. While the industry is looking to federal regulation to end uncertainty to manufacturers created by a state-patchwork regulatory model framework, the Proposed Policy Solution specifically preserves the rights of state and local authorities to exceed some of the requirements for hemp and cannabis products sold within their jurisdictions, while setting a baseline and preempting differing regulations in other respects:

> (d) In general.
>
> (1) Preservation. Except as provided in subsection (2)(A), nothing in this Act, or rules promulgated thereunder, shall be construed to limit the authority of a Federal agency (including the Armed Forces), a State or political subdivision of a State, or the government of an Indian tribe to enact, adopt, promulgate, and enforce any law, rule, regulation, or other measure with respect to Hemp-Derived Supplements that is in addition to, or more stringent than, requirements established under this subchapter, including a law, rule, regulation, or other measure relating to or prohibiting the sale, distribution, possession, exposure to, access to, advertising and promotion of, or use of Hemp-Derived Supplements by individuals of any age, or information reporting to the State. No provision of this subchapter shall limit or otherwise affect any State, tribal, or local taxation of Hemp-Derived Supplements.
>
> (2) Preemption of certain State and local requirements.
>
> (A) No State or political subdivision of a State may establish or continue in effect with respect to a Hemp-Derived Supplement any requirement which is different from, or in addition to, any requirement

> under the provisions of this subchapter relating to Hemp-Derived Supplement product standards, notification, adulteration, misbranding, labeling, registration, or good manufacturing standards.
>
> (B) Exception. Subsection (A) does not apply to requirements relating to the sale, distribution, possession, information reporting to the State, exposure to, access to, the advertising and promotion of, or use of, Hemp-Derived Supplements by individuals of any age.

> (e) Rule of construction regarding product liability. No provision of this subchapter relating to a Hemp-Derived Supplements shall be construed to modify or otherwise affect any action or the liability of any person under the product liability law of any State.
>
> (f) Rule of construction regarding Marihuana. No provision of this subchapter relating to a Hemp-Derived Supplements shall be construed to modify or otherwise affect the legality, regulation or enforcement policies relating to marihuana (as that term is defined in section 102 of the Controlled Substances Act (21 U.S.C. § 802)) under federal law.

See Appendix A, Section 6.

For example, states have crafted frameworks mandating robust testing requirements which should continue to be leveraged, like Colorado's 6 C.C.R. 1010-21, which sets strict regulations for finished product testing to ensure microbials, mycotoxins, heavy metals, intoxicating cannabinoids (THC), common process solvents, and pesticides found in hemp are free from retail products. Further, this comment recommends third party accreditation bodies, certified through the FDA, be used to conduct third party compliance audits for Hemp-Derived Product manufacturers. Finally, states will be critical for product tracking and compliance audits.

Form, Packaging, Accessibility, and Labeling

22. What types of claims should product manufacturers be permitted to make about CBD products? Please reference how such permitted claims compare to the types of claims that may be made about drugs, foods, dietary supplements, and cosmetics.

The Proposed Policy Solution disallows marketing Hemp-Derived Products with health claims, which is consistent with existing regulations for dietary supplements under the FD&C Act. *See* Section 5(d). Consistent with these regulations, marketing for Hemp-Derived Products should be focused on general wellbeing and structure-function claims if supported by valid evidence in compliance with the FD&C Act.

23. What is the evidence regarding the potential benefits of including a symbol or other marking on product labeling to provide clarity for consumers who would purchase products that contain CBD

The Proposed Policy Solution includes a Hemp-Derived Product warning statement to address FDA concerns and ensure transparent labeling and marketing to allow for consumers to make informed purchasing and health decisions. Section 5(c)(1) and (2)(a)) requires the following to accomplish these goals:

> (1) Low-Dose Hemp-Derived Products will carry the following warning statements on the package:
>
> > (A) Human Ingestion. Low-Dose Hemp-Derived Products intended for human ingestion must contain the following statements placed prominently on the information panel located on the product's immediate container:
>
> **"WARNING: The Food and Drug Administration has not evaluated this product for safety, or any statements made in connection with this product. Effects of acute and chronic future use of this product are unknown and may cause adverse health events, including liver and reproductive toxicity. This product is not intended to diagnose, treat, cure, or prevent any disease. Consult a physician prior to use, especially if you are pregnant, may become pregnant, are breastfeeding, or taking prescription or over-the-counter medication(s). Keep out of reach of children."**
>
> > > (i) Notwithstanding the foregoing, Low-Dose Hemp-Derived Products intended for human ingestion containing a total Hemp Cannabinoid content of equal to or less than 70 milligrams per serving are exempt from including any reproductive toxicity warnings and warnings targeted to those who are pregnant, may become pregnant or are lactating.
>
> > (B) Animal Ingestion. Low-Dose Hemp-Derived Products intended for animal ingestion must contain following statements placed prominently on the information panel located on the product's immediate container:
>
> **"WARNING: The Food and Drug Administration has not evaluated this product for safety. Effects of acute and chronic use of this product are unknown and may cause adverse health events, including liver and reproductive toxicity. This product is not**

intended to diagnose, treat, cure, or prevent any disease. Consult a veterinarian before administering this product to any animal."

(i) Notwithstanding the foregoing, Low-Dose Hemp-Derived Products intended for animal ingestion containing a total Hemp Cannabinoid content of equal to or less than 70 milligrams per serving are exempt from including any reproductive toxicity warnings.

These clear warning statements, coupled with transparent labeling, including all THC and hemp cannabinoid content, as well as other existing mandates under the existing framework for dietary supplement is sufficient to inform consumers and mitigate against risk.

24. What are the potential benefits or drawbacks of an additional or substitute standardized label panel for CBD products, compared to the current Nutrition Facts Label and Supplements Label

The benefits of the additional information regarding cannabinoid content and CBD-specific additional warning statement and labeling requirements serve to inform the public of any known and unknown risks to address the FDA's harm-reduction policy concerns. Transparent marketing is what the consumers who rely on Hemp-Derived Products deserve in making informed consumer wellness purchases and health decisions. *See* full proposed labeling framework at *supra*, Response to RFI Question 23. In fact, consumers in this category are accustomed to active cannabinoid ingredient product disclosures, and the Proposed Policy Solution's labeling requirements adhere to these consumer expectations.

The drawback of a hemp cannabinoid-specific warning statement is that this may create confusion for consumers since the traditional nutrition/supplement facts are well known and understood. Another drawback for additional warnings is that many Hemp-Derived Products currently in the market are in smaller containers, so adding more label requirements would be difficult from a marketing perspective. While the current supplement facts panel label requirements are reasonable for Hemp-Derived Products, the Proposed Policy Solution requires Hemp-Specific warning statements as a compromise to address the FDA's stated concerns. Another option would be requiring a URL/QR code pointing the customer either to a product Certificate of Analysis or additional cannabinoid information; such requirements have been adopted by many states and proven effective to educate customers

25. What precedent exists in foods, dietary supplements, tobacco, and cosmetics for requirements of labeling to present risks to special populations in labeling (e.g., children, pregnant and lactating women, consumers taking certain drugs, etc.)? What amount and type of evidence has been required to support such requirements

Extensive precedent exists for the FDA using warning statements to protect consumers including vulnerable populations has highlighted above:

- Saint John's Wort – significant drug interactions[53]
- Iron – liver damage, systemic toxicity, death[54]
- Aconite – nausea, vomiting, low blood pressure and heart rhythm disorders[55]
- Kava – Liver damage[56]
- Yohimbe – rapid heartbeat, kidney failure, seizure, and heart attack[57]
- Grapefruit – drug-drug interactions[58]

With regard to dietary supplement risk to children, the FDA currently mandates warning statements to specifically protect children: 21 C.F.R. §310.518(a) mandates the following warning for iron supplements:

> **WARNING: Accidental overdose of iron-containing products is a leading cause of fatal poisoning in children under 6. Keep this product out of reach of children. In case of accidental overdose, call a doctor or poison control center immediately.**

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[53] Natural Medicines, 2023 https://naturalmedicines.therapeuticresearch.com/databases/food,-herbs-supplements/professional.aspx?productid=329; and see, EMA, 2022 https://www.ema.europa.eu/en/documents/herbal-monograph/final-european-union-herbal-monograph-hypericum-perforatum-l-herba-revision-1_en.pdf

[54] 21 C.F.R. 101.17

[55] Natural Medicines, 2023 https://naturalmedicines.therapeuticresearch.com/databases/food,-herbs-supplements/professional.aspx?productid=609; *and see,* Zhou G, Tang L, Zhou X, Wang T, Kou Z, Wang Z. (2015). A review on phytochemistry and pharmacological activities of the processed lateral root of Aconitum carmichaelii Debeaux. J Ethnopharmacol 160: 173-93. doi: 10.1016/j.jep.2014.11.043; *and see*, Chan TY. Aconite poisoning. Clin Toxicol (Phila). 2009 Apr;47(4):279-85. doi: 10.1080/15563650902904407.

[56] Natural Medicines, 2023 https://naturalmedicines.therapeuticresearch.com/databases/food,-herbs-supplements/professional.aspx?productid=872; and see EMA, 2017 https://www.ema.europa.eu/en/documents/herbal-report/final-assessment-report-piper-methysticum-g-forst-rhizoma_en.pdf

[57] Natural Medicines, 2023 https://naturalmedicines.therapeuticresearch.com/databases/food,-herbs-supplements/professional.aspx?productid=759; *and see,* EFSA ANS Panel (EFSA Panel on Food Additives and Nutrient Sources Added to Food), 2013. Scientific Opinion on the evaluation of the safety in use of Yohimbe (Pausinystalia yohimbe (K. Schum.) Pierre ex Beille). EFSA Journal 2013; 11(7):3302, 46 pp. doi:10.2903/j.efsa.2013.3302

[58] Natural Medicines, 2022 https://naturalmedicines.therapeuticresearch.com/databases/food,-herbs-supplements/professional.aspx?productid=946; FDA, 2021 https://www.fda.gov/consumers/consumer-updates/grapefruit-juice-and-some-drugs-dont-mix; *and see*: Dahan A, Altman H. Food-drug interaction: grapefruit juice augments drug bioavailability--mechanism, extent and relevance. Eur J Clin Nutr. 2004 Jan;58(1):1-9. doi: 10.1038/sj.ejcn.160173; and Bressler R. Grapefruit juice and drug interactions. Exploring mechanisms of this interaction and potential toxicity for certain drugs. Geriatrics. 2006 Nov;61(11):12-8. PMID: 17112309.

Given the data supporting the safety profile of hemp cannabinoids, the precedent highlighted above supports that the FDA's risks related to vulnerable populations identified can be managed through existing regulation requirement transparent marketing, education, and warning statement. The Proposed Policy Solution goes a step further and proposes a hemp-specific dose-dependent warning statements to target FDA stated concerns and safeguard public health.

26. Some suggest requiring labels for CBD products to include "potential THC content." Would THC content be unknown in a particular product? Is there precedent for such a labeling requirement

The Proposed Policy Solution advocates for transparent labeling of THC content pursuant to the existing regulations requiring dietary supplement facts panel to include all acting ingredient contents. For Hemp-Derived Products, this includes disclosure of THC content. *See* full proposed labeling framework at *supra*, Response to RFI Question 23.

27. How should access to CBD products by children be regulated? For example, would it be appropriate to have an age restriction on the purchase of CBD products? If so, what is an appropriate age limit

The Proposed Policy Solution addresses this issue in three ways: (1) limiting purchases of Hemp-Derived Products with more than 500 micrograms of THC to individuals over 18; (2) including warning statement that products should be kept out of reach by children; and (3) allowing for FDA rulemaking around products to ensure products are not appealing to children. Section 5(b) provides:

> (b) Minimum age of sale. It shall be unlawful for any retailer to sell a Hemp-Derived Product containing a total Tetrahydrocannabinol content equal to or exceeding 500 micrograms per serving to any person younger than 18 years of age.
>
> > (1) Sale and Distribution. Within six months after the effective date, the Secretary shall promulgate regulations regarding the sale and distribution of Hemp-Derived Products containing a total Tetrahydrocannabinol content equal to or exceeding 500 micrograms per serving that occur through means other than a direct, face-to-face exchange between a retailer and a consumer in order to prevent the sale and distribution of these products to individuals who have not attained the minimum age established by

applicable law for the purchase of such products, including requirements for age verification;

(2) Promotion and Marketing. Within six months after the effective date, the Secretary shall issue regulations to address the promotion and marketing of Hemp-Derived Products containing a total Tetrahydrocannabinol content equal to or exceeding 500 micrograms per serving that are sold or distributed through means other than a direct, face-to-face exchange between a retailer and a consumer in order to protect individuals who have not attained the minimum age established by applicable law for the purchase of such products, except that any regulation shall not completely or de facto restrict sales of Hemp-Derived Products direct, face-to-face exchange between a retailer and a consumer; and

(3) Not later than six months after the effective date of regulations adopted pursuant to subsection, the Secretary shall develop and publish an action plan to enforce these restrictions.

28. What specific additional restrictions should apply to CBD products regarding their appeal to or use by children with regard to marketing, packaging, and labeling? Is there precedent in the food, dietary supplement, tobacco, or cosmetics space for restricting certain product features that would make products appealing to children? Please describe

See supra, Response to RFI Question 27.

29. Some suggest requiring packages with multiple servings to be easily divisible into single servings. Does a framework like this exist today for any other product or substance?

Requiring clearly divisible products is seen in state regulatory frameworks for cannabis to ensure that consumers understand dosing and to require homogenized dosing of a cannabinoid active ingredient. Given Hemp-Derived Products generally are marketed as edible dietary supplements, these products typically already come in an easily divisible form, *e.g.,* tinctures or tablets. With formal imposition of dietary supplement regulations on Hemp-Derived Products, producers will be incentivized to ensure products are easily divisible so that the required supplement facts panel and servings per package are not misleading to consumers.

APPENDIX A

H. R.

To provide for the regulation of “Hemp-Derived Products,” to amend the Federal Food, Drug, And Cosmetic Act consistent with that regulation, to promote consumer protection, to address the existing hemp-derived cannabinoid market and for other purposes. The purpose of this bill is to regulate Hemp-Derived Products under the existing regulatory framework for “dietary supplements” within the Federal Food, Drug, And Cosmetic Act.

SECTION 1. SHORT TITLE.

This Act may be cited as the “Hemp-Derived Product Act.”

SECTION 2. DEFINITIONS.

As used in this Act:

(a) Cannabinoid definitions:

(1) "Cannabidiol” or “CBD” means the compound $C_{21}H_{30}O_2$ (PubChem CID: 644019) whether found in *Cannabis sativa L.* or Hemp, or from any other source.

(2) “Cannabinoid” means any chemical compound that naturally occurs in the plant *Cannabis sativa L.*, or is physically, chemically, and optically identical to any chemical compound that naturally occurs in the plant *Cannabis sativa L.*, and acts as an agonist or antagonist on human cannabinoid receptors. These chemical compounds include endocannabinoids, phytocannabinoids, biosynthesized cannabinoids, and chemically synthesized cannabinoids, but do not include terpenes.

(3) “Hemp Cannabinoid” means those Cannabinoids naturally occurring in hemp, whether extracted from hemp or from any other legal source, without conversion, chemical synthesis, biosynthesis or otherwise. For the avoidance of doubt, Hemp Cannabinoid does not include cannabinoids that are not naturally occurring in the plant, for example, O-acetyl delta-9 tetrahydrocannabinol.

(4) “Hemp Cannabinoid Ingredient” means a substance that includes Hemp Cannabinoids, purified hemp extracts containing one or more Hemp Cannabinoids, or one or more biosynthesized or chemically synthesized Hemp Cannabinoids. A Hemp Cannabinoid Ingredient shall not include any THC Ingredient.

(5) “Tetrahydrocannabinol” or “THC” means any tetrahydrocannabinol, including delta-6 tetrahydrocannabinol, delta-8 tetrahydrocannabinol, delta-9 tetrahydrocannabinol, delta-10 tetrahydrocannabinol, delta-12 tetrahydrocannabinol, hexahydrocannabinol (HHC), O-acetyl delta-9 tetrahydrocannabinol (THCO), tetrahydrocannabiphorol, tetrahydrocannabinolic acid, or any other Cannabinoid, at a similar or greater potency that the Secretary of Health and Human Services in consultation with the Attorney General determines to have a psychological and cognitive profile associated with intoxication or euphoria similar to tetrahydrocannabinol and has a potential for abuse. For the avoidance of doubt, “Tetrahydrocannabinol” or “THC” does not include tetrahydrocannabivarin (THCV).

(6) “THC Ingredient” means any Tetrahydrocannabinol that has been isolated from a Whole Hemp Extract, concentrated by the removal of non-THC Cannabinoids from a

Whole Hemp Extract, or that has been produced by any other means, whether through conversion, chemical synthesis, biosynthesis, or otherwise.

(7) "Whole Hemp Extract" means an extract of Hemp produced through an extraction process intended to preserve the naturally occurring Cannabinoid profile of the Hemp biomass.

(b) "Hemp" has the same meaning as "Hemp" under the Agriculture Improvement Act of 2018, SEC. 10113, as amended or superseded.

(c) "Hemp-Derived Product" means

(1) a product that is intended to support general wellbeing and that satisfies the following:

(A) has one or more Hemp Cannabinoid Ingredients;

(B) is intended for ingestion in a form described in section 350(c)(1)(B)(i) of the Federal Food, Drug, And Cosmetic Act (21 U.S.C. 301-392);

(C) has a total Hemp Cannabinoid content of more than 100 milligrams per serving or contains a Hemp Cannabinoid Ingredient that is not a Hemp Cannabinoid;

(D) is labeled as a Dietary Supplement;

(E) is not represented for use as a conventional food, as a sole item of a meal or the diet, or a drug;

(F) is not packaged, labeled or advertised in such a form or in such a manner, that indicates an intent to produce intoxicating effects; and

(G) is manufactured consistent with this Act.

(d) "Low-Dose Hemp-Derived Product" means a Hemp-Derived Product that has a total Hemp Cannabinoid content of equal to or less than 100 milligrams per serving; and does not contain a Hemp Cannabinoid Ingredient that is not a Hemp Cannabinoid; and has a total Tetrahydrocannabinol content, if any, of not more than 1.5 milligrams per serving.

(e) "Indian Tribe" has the meaning given the term in section 4 of the Indian Self-Determination and Education Assistance Act (25 U.S.C. 5304).

(f) "Secretary" means the Secretary of Health and Human Services.

(g) "State" means (1) a State; (2) the District of Columbia; (3) the Commonwealth of Puerto Rico; and (4) any other Territory or possession of the United States.

SECTION 3. SETTING AGENCY FUNCTIONS WITH REGARD TO HEMP-DERIVED PRODUCTS

Comptroller General Review of Laws and Regulations. The Comptroller General shall conduct a review of Federal laws, regulations, and policies to determine if any changes are desirable in the light of the purposes and provisions of this Act. Not later than one year after the date of the

enactment of this Act, the Comptroller General shall make to Congress and the relevant agencies such recommendations relating to the results of that review as the Comptroller General deems appropriate.

SECTION 4. AMENDMENTS TO THE FEDERAL FOOD, DRUG, AND COSMETIC ACT.

(a) Inclusion In Definition Of Dietary Supplement. Section 201(ff)(3)(B) of the Federal Food, Drug, and Cosmetic Act (21 U.S.C. 321(ff)(3)(B)) is amended in each of clauses (i) and (ii) by inserting "(other than Hemp-Derived Products)" after "an article".

(b) Modification of "Prohibited Acts". Section 21 USC 331(ll) is amended in the matter preceding subparagraph (1), by inserting "(other than Hemp-Derived Products)" after "made public".

SECTION 5. REGULATION OF HEMP-DERIVED PRODUCTS

(a) Generally Regulated as Dietary Supplements. Except as expressly indicated by this Act, a Hemp-Derived Product shall be deemed to be a dietary supplement within the meaning of the Federal Food, Drug, and Cosmetics Act and its implementing regulations.

(b) Minimum age of sale. It shall be unlawful for any retailer to sell a Hemp-Derived Product containing a total Tetrahydrocannabinol content equal to or exceeding 500 micrograms per serving to any person younger than 18 years of age.

(1) Sale and Distribution. Within 6 months after the effective date, the Secretary shall promulgate regulations regarding the sale and distribution of Hemp-Derived Products containing a total Tetrahydrocannabinol content equal to or exceeding 500 micrograms per serving that occur through means other than a direct, face-to-face exchange between a retailer and a consumer in order to prevent the sale and distribution of these products to individuals who have not attained the minimum age established by applicable law for the purchase of such products, including requirements for age verification;

(2) Promotion and Marketing. Within 6 months after the effective date, the Secretary shall issue regulations to address the promotion and marketing of Hemp-Derived Products containing a total Tetrahydrocannabinol content equal to or exceeding 500 micrograms per serving that are sold or distributed through means other than a direct, face-to-face exchange between a retailer and a consumer in order to protect individuals who have not attained the minimum age established by applicable law for the purchase of such products, except that any regulation shall not completely or de facto restrict sales of Hemp-Derived Products direct, face-to-face exchange between a retailer and a consumer; and

(3) Not later than 6 months after the effective date of regulations adopted pursuant to subsection, the Secretary shall develop and publish an action plan to enforce these restrictions.

(c) Supplement Facts Panel. Hemp-Derived Products will conform with the requirements of 21 C.F.R. 101.3(a); 21 C.F.R. 101.105(a); 21 C.F.R. 101.36; 21 C.F.R. 101.4(a)(1); and 21 C.F.R. 101.5 except as follows:

(1) Hemp-Derived Products shall include, following other dietary ingredients:

(A) In bold typeface total tetrahydrocannabinol content by weight;

(B) In bold typeface total tetrahydrocannabinol concentration in milligrams per serving;

(C) In bold typeface cannabidiol content by weight; and

(D) In bold typeface the content by weight of any Hemp Cannabinoid Ingredient which is over 5% of the total weight or volume.

(d) Warning Statements. Hemp-Derived Products will conform with the requirements of 21 U.S.C. 343(r)(6)(C) and 21 C.F.R. 101.93(b)–(d); and 21 C.F.R. 101.17, as applicable, except as follows:

(1) Low-Dose Hemp-Derived Products will carry the following warning statements on the package:

(A) Human Ingestion. Low-Dose Hemp-Derived Products intended for human ingestion must contain the following statements placed prominently on the information panel located on the product's immediate container:

"WARNING: The Food and Drug Administration has not evaluated this product for safety or any statements made in connection with this product. Effects of acute and chronic use of this product are unknown and may cause adverse health events, including liver and reproductive toxicity. This product is not intended to diagnose, treat, cure or prevent any disease. Consult a physician prior to use, especially if you are pregnant, may become pregnant, are breastfeeding, or taking prescription or over-the-counter medication(s). Keep out of reach of children."

(i) Notwithstanding the foregoing, Low-Dose Hemp-Derived Products intended for human ingestion containing a total Hemp Cannabinoid content of equal to or less than 70 milligrams per serving are exempt from including any reproductive toxicity warnings and warnings targeted to those who are pregnant, may become pregnant or are lactating.

(B) Animal Ingestion. Low-Dose Hemp-Derived Products intended for animal ingestion must contain following statements placed prominently on the information panel located on the product's immediate container:

"WARNING: The Food and Drug Administration has not evaluated this product for safety. Effects of acute and chronic use of this product are unknown and may cause adverse health events, including liver and reproductive toxicity. This product is not intended to diagnose, treat, cure or prevent any disease. Consult a veterinarian before administering this product to any animal."

(i) Notwithstanding the foregoing, Low-Dose Hemp-Derived Products intended for animal ingestion containing a total Hemp Cannabinoid content of equal to or less than 70 milligrams per serving are exempt from including any reproductive toxicity warnings.

(2) The Food and Drug Administration may update such warnings through rulemaking, including (i) requiring warnings for dosing limits applicable to the general population and vulnerable populations and (ii) warnings for co-administration with other bioactive compounds, as deemed necessary by Good Evidence of adverse reactions. As used in this subsection, "Good Evidence" means evidence that consists of results from multiple published peer reviewed studies of strong design for answering the question addressed and which are generally accepted in the scientific community. The results must be both clinically important and consistent, with only minor exceptions at most, and shall be free of any significant doubts about generalizability, bias, and flaws in research design. Studies with negative results must have sufficiently large sample sizes to have adequate statistical power and must be reviewed by a panel of experts outside of the Food and Drug Administration.

(e) Testing and Notification Procedures. Hemp-Derived Products will conform with the requirements of 21 C.F.R. 111; 21 C.F.R. 190.6; and 21 U.S.C. 350(b), except that Low-Dose Hemp-Derived Products shall not be subject to premarket notification to or approval by the Food and Drug Administration.

(1) The Food and Drug Administration shall not reject any premarket notification submissions related to Hemp-Derived Products submitted pursuant to 21 C.F.R. 190.6, or deem any product adulterated under 21 U.S.C. 350(b), solely on the basis of the presence of delta-9 tetrahydrocannabinol or an isomer or analogue within the product, provided that the delta-9 tetrahydrocannabinol meets the definition of Hemp Cannabinoid. Notwithstanding the forgoing, nothing shall prevent the Food and Drug Administration from considering delta-9 tetrahydrocannabinol or an isomer or analogue within a product, in the context of conducting or recommending an assessment of abuse potential pursuant to the 2017 Guidance for Assessment of Abuse Potential of Drugs. This provision shall not apply to Low-Dose Hemp-Derived Products.

(2) Notwithstanding the foregoing, at least 60 days following the date that any Low-Dose Hemp-Derived Product is introduced or delivered for introduction into interstate commerce, a person must submit to the Food and Drug Administration:

(A) Company or individual name and complete address;

(B) The name of the Hemp-Derived Product;

(C) A description of the Hemp-Derived Product;

(D) Images of all relevant labels, including the applicable warning;

(E) Certificates of Analysis from an ISO 17025 accredited independent testing laboratory, or other applicable ISO certification for the relevant laboratory testing, showing:

(a) all cannabinoids tested for and present in the Hemp-Derived Product, the limits of detection, limits of quantitation, and results reflected in percentage and weight in milligrams per gram; and

(b) testing for the following harmful substances to ensure the product contains an acceptable level of such substances: (i) Microbiological impurities such as mycotoxins; (ii) residual solvents or pesticides; (iii) filth and foreign material, and (iv) heavy metals.

(F) An attestation confirming the product is in compliance with the Act.

(G) The signature of a person you designate who is responsible for the content of the notification and can be contacted for questions.

(f) Existing Products.

(1) Following the date that is 90 days following the effective date of this Act, no product containing Hemp Cannabinoid Ingredients or THC Ingredients that are intended for human or animal ingestion may be sold in interstate commerce other than Hemp-Derived Products in compliance with this Act.

(2) Within 120 days of the effective date of this Act, the Food and Drug Administration shall publish and maintain a public database that contains copies of any and all enforcement actions issued related to any products containing Hemp Cannabinoid Ingredients or THC Ingredients sold in violation of this Act or the rules and regulations promulgated thereunder.

(g) Notwithstanding anything to the contrary:

(1) The Food and Drug Administration or Federal Trade Commission may initiate enforcement against products failing to comply with this Act and the rules and regulations promulgated thereunder consistent with their authority for other consumer foods and dietary supplements.

(2) A company marketing a Low-Dose Hemp-Derived Product may make statements pursuant to and in compliance with 21 U.S.C. 343(r)(6).

(3) The Food and Drug Administration will continue to regulate any product containing Hemp Cannabinoid Ingredients or THC Ingredients intended for use as a drug, as that term is defined in 21 U.S.C. 321(g), under its existing authorities in the Federal Food, Drug, and Cosmetic Act and its implementing regulations.

(h) Amendment to the Federal Food, Drug, and Cosmetic Act. This Section amends the Food, Drug, and Cosmetic Act (21 U.S.C. 301 *et seq*) consistent with the provisions of this subsection.

SECTION 6. Preservation of State and Local Authority

(a) In general.

(1) Preservation. Except as provided in subsection (2)(A), nothing in this Act, or rules promulgated thereunder, shall be construed to limit the authority of a Federal agency (including the Armed Forces), a State or political subdivision of a State, or the government of an Indian tribe to enact, adopt, promulgate, and enforce any law, rule, regulation, or other measure with respect to Hemp-Derived Products that is in addition to, or more stringent than, requirements established under this subchapter, including a law, rule, regulation, or other measure relating to or prohibiting the sale, distribution, possession, exposure to, access to, advertising and promotion of, or use of Hemp-Derived Products by individuals of any age, or information reporting to the State. No provision of this subchapter shall limit or otherwise affect any State, tribal, or local taxation of Hemp-Derived Products.

(2) Preemption of certain State and local requirements.

(A) No State or political subdivision of a State may establish or continue in effect with respect to Hemp-Derived Products any requirement which is different from, or in addition to, any requirement under the provisions of this subchapter relating to Hemp-Derived Product standards, notification, adulteration, misbranding, labeling, registration, or good manufacturing standards.

(B) Exception. Subsection (A) does not apply to requirements relating to the sale, distribution, possession, information reporting to the State, exposure to, access to, the advertising and promotion of, or use of, Hemp-Derived Products by individuals of any age.

(b) Rule of construction regarding product liability. No provision of this subchapter relating to Hemp-Derived Products shall be construed to modify or otherwise affect any action or the liability of any person under the product liability law of any State.

(c) Rule of construction regarding Marihuana. No provision of this subchapter relating to a Hemp-Derived Products shall be construed to modify or otherwise affect the legality, regulation or enforcement policies relating to marihuana (as that term is defined in section 102 of the Controlled Substances Act (21 U.S.C. 802)) under federal law.

APPENDIX B

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EXHIBIT A

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Genotoxicity evaluation of cannabidiol

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ABSTRACT

Consumer use of cannabidiol (CBD) for personal wellness purposes has garnered much public interest. However, safety-related data on CBD in the public domain are limited, including a lack of quality studies evaluating its genotoxic potential. The quality of available studies is limited due to the test material used (e.g., low CBD purity) and/or study design, leading some global regulatory agencies to highlight genotoxicity as an important data gap for CBD. To address this gap, the genotoxic potential of a pure CBD isolate was investigated in a battery of three genotoxicity assays conducted according to OECD testing guidelines. In an *in vitro* microbial reverse mutation assay, CBD up to 5000 μg/plate was negative in *Salmonella typhimurium* strains TA98, TA100, TA1535, and TA1537, and *Escherichia coli* strain WP2 *uvrA*, with and without metabolic activation. Testing in an *in vitro* micronucleus assay was negative in human TK6 cells up to 10–11 μg/mL, with and without metabolic activation. Finally, an *in vivo* micronucleus assay conducted in male and female rats was negative for genotoxicity up to 1000 mg/kg-bw/d. Bioanalysis of CBD and its primary metabolite, 7-carboxy CBD, confirmed a dose-related increase in plasma exposure. Together, these assays indicate that CBD is unlikely to pose a genotoxic hazard.

1. Introduction

Cannabidiol (CBD) is a naturally occurring cannabinoid, and the dominant cannabinoid found in industrial hemp (*Cannabis sativa* containing <0.3% tetrahydrocannabinol [THC] w/w) (Mechoulam et al., 2007; Pertwee, 2014; VanDolah et al., 2019). Although *C. sativa* plants and preparations thereof have been used for industrial, medicinal, and recreational purposes for thousands of years, the public and medical communities have recently become particularly interested in CBD for its therapeutic potential, following the Hemp Farming Act in the United States (US), part of the Agricultural Improvement Act of 2018 (aka, "2018 Farm Bill") (Rupasinghe et al., 2020). CBD is proposed to have anticonvulsive, analgesic, anti-anxiety, neuroprotective, antioxidant, and antimicrobial properties (Small and Marcus, 2002; Pertwee, 2004; Billakota et al., 2019; Devinsky et al., 2018). Epidiolex® (active ingredient CBD isolate) has been approved by the US Food and Drug Administration (FDA) for the treatment of seizures associated with Lennox-Gastaut syndrome and Dravet syndrome in pediatric patients (Jazz Pharmaceuticals, 2023). In addition, Sativex® (CBD and THC combination), is approved in other countries for the treatment of moderate to severe spasticity due to multiple sclerosis (Jazz Pharmaceuticals, 2023).

Interest in hemp-derived CBD consumer products, however, has outpaced the development of a legal pathway for CBD use in foods and dietary supplements. While the FDA has not established tolerable daily intake levels associated with consumer use, an overview of the agency's activities related to evaluating the safe use of CBD in food and dietary supplement products can be found on its website (FDA). The United Kingdom (UK) Food Safety Authority (United Kingdom Food Safety Authority, 2022), Health Canada (2022), and the Australian Therapeutic Goods Administration (Therapeutic Goods Administration, 2021) have conducted safety evaluations resulting in recommended established recommended maximum upper intake levels of CBD by healthy adults (except those planning to be or currently pregnant or breastfeeding). However, these agencies continue to highlight safety data gaps. Specifically, in regard to the potential genotoxicity of CBD, the European Food Safety Authority (EFSA) and the UK FSA (2022) have concluded the available data to be insufficient. While CBD is one of the most well-studied phytocannabinoids, there exist limited safety-related data on CBD in the public domain, including a lack of quality studies conducted according to regulatory test guidelines to evaluate its genotoxic

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Abbreviations

2AA	2-aminoanthracene
2NF	2-nitrofluorene
ANOVA	analysis of variance
API	atmospheric pressure ionization
bw	body weight
CBD	cannabidiol
CP	cyclophosphamide monohydrate
CRL	Charles River Laboratories
CRO	Contract Research Organization
DMSO	dimethyl sulfoxide
EFSA	European Food Safety Authority
FDA	US Food and Drug Administration
FSA	UK Food Safety Authority
GLP	Good Laboratory Practice
cGMP	current Good Manufacturing Practice
ICH	International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use
ICR	ICR-191 acridine
LC-MS/MS	liquid chromatography with tandem mass spectrometry
MHLW	Ministry of Health, Labour and Welfare of Japan
MMC	mitomycin C
MN	micronucleus
MTD	maximum tolerated dose
NAAZ	sodium azide
NCE	normochromatic erythrocyte
NQNO	4-nitroquinoline-N-oxide
OECD	Organisation for Economic Co-operation and Development
PCE	polychromatic erythrocyte
QC	quality control
SCGE	single cell gel electrophoresis
SD	standard deviation
SOP	standard operating procedure
TE	total erythrocytes
TGA	Therapeutic Goods Administration
THC	tetrahydrocannabinol
UK	United Kingdom
US	United States
VIN	vinblastine sulfate

potential. Publicly available *in vivo* genotoxicity tests also present limitations with respect to the test material and/or study design (Russo et al., 2019; Zimmerman and Raj, 1980; Marx et al., 2018; Dziwenka et al., 2020, 2021; Carvalho et al., 2022). Studies summarized by the FDA as part of its review of the non-clinical safety data package for Epidiolex provide information on the genotoxic potential of CBD, however, no publications or study reports are available for public review (CDER, 2018).

While the data from non-guideline-compliant studies and those studies using test materials with lower CBD contents can provide corroborative evidence for the safe use of CBD, no publicly available studies on CBD isolate conducted according to regulatory test guidelines have been identified that evaluate genotoxicity, repeated oral toxicity, or reproductive and developmental toxicity endpoints. Here we present the findings from three genotoxicity studies (i.e., Ames, *in vitro* micronucleus, and *in vivo* micronucleus assays) that were conducted according to U.S. Food and Drug Administration Good Laboratory Practice (GLP) and OECD guidelines. These studies were conducted as part of a larger program to investigate the safety of CBD isolate (Henderson et al., 2023a, 2023b).

2. Materials and methods

2.1. Test material

Hemp-derived CBD isolate (99.08–101.46%; CAS No. 13956-29-1) provided by Canopy Growth USA (Evergreen, Colorado) was produced by an ethanol extraction method and subsequent crystallization under current Good Manufacturing Practices (CGMP). The test material was stored at Charles River Laboratories (CRL) protected from light with desiccant at room temperature (19 °C–25 °C), and under nitrogen. The test article Certificate of Analysis (Botancor Laboratories, Denver, CO) demonstrated that the test article was 99.62% CBD. Based on the demonstrated purity, a correction factor of 1.004 was used in the preparation of dose formulations.

2.2. Genotoxicity studies

All genotoxicity studies were conducted in accordance with U.S. FDA (21 CFR Part 58): Good Laboratory Practice for Nonclinical Laboratory Studies, and as accepted by Regulatory Authorities throughout the European Union (OECD Principles of Good Laboratory Practice) and Japan (MHLW), except for the characterization analyses of the test article, which were conducted to GMP standards.

2.2.1. Microbial reverse mutation assay

The assay design was based on OECD Guideline 471 (OECD, 2020). *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537 and *Escherichia coli* strain WP2 *uvrA* were originally obtained from Molecular Toxicology, Inc. (Boone, NC).

2.2.1.1. Test article and positive controls preparation. CBD isolate was tested in an initial dose range-finding plate incorporation assay with a limited number of strains, followed by the full mutagenicity assay with all strains, performed under identical conditions (Ames et al., 1975; Maron and Ames, 1983). On the day of the range-finding and mutagenicity assays, CBD isolate was prepared as a formulation in dimethyl sulfoxide (DMSO) at a stock concentration up to 50 mg/mL. On the day of the repeat mutagenicity assay (i.e., TA100, without metabolic activation), CBD isolate was prepared at 1.00 mg/mL. The plate incorporation method was conducted using molten agar (2.0 mL) as the medium for transference of the test and control articles, bacterial culture (0.1 mL), control or test article (0.10 mL), and saline or Aroclor™ 1254-induced rat liver S9 fraction metabolic activation system (0.5 mL; Molecular Toxicology, Inc.), allowing colony growth.

The dose range-finding assay included CBD doses of 1.0, 5.0, 10.0, 50, 100, 500, 1000, or 5000 μg/plate with and without S9 (one plate per dose) using the tester strains TA100 and WP2 *uvrA*. Based on the results, the definitive mutagenicity assay evaluated CBD doses of 0.25, 0.5, 1.0, 2.5, 5, 10, 25, 50, 100, 250, 500, 1000, 2500, or 5000 μg/plate with and without S9. Three test plates per strain per treatment condition were used. Positive controls used in the absence of metabolic activation were as follows: 2-nitrofluorene (2NF; Sigma-Aldrich) at 2.5 μg/plate with TA98, sodium azide (NAAZ; Sigma-Aldrich) at 1.0 μg/plate with TA100 and TA1535, ICR-191 acridine (Sigma-Aldrich) at 0.5 μg/plate with TA1537, and 4-nitroquinoline-N-oxide (Acros Organics) at 2.0 μg/plate with *E. coli* WP2 *uvrA*. With metabolic activation, 2-aminoanthracene (2AA; Sigma-Aldrich) was used as a positive control for all bacterial strains (2.5 μg/plate), and 10 μg/plate for *E. coli* WP2 *uvrA*. The vehicle control used in the assay was dimethyl sulfoxide (DMSO; Sigma-Aldrich).

2.2.1.2. Experimental design. The following procedures were used in both the dose range-finding and the definitive mutagenicity assays.

Study number, tester strain, treatment group, concentration, and the presence or absence of metabolic activation were identified on each test plate. A stock solution of CBD isolate was prepared in DMSO on the day of the assay at a concentration of up to 50 mg/mL and above, while lower concentrations were prepared by serial dilution with DMSO. The dosing volume for all assays was 100 μL per plate.

Briefly, sterile 12 × 75 mm test tubes were placed in heating blocks set to approximately 46 °C, and the relevant items were added stepwise for each concentration of test or control article. After addition of the required components, the mixture was gently mixed and overlaid onto minimal glucose plates and incubated for 2 day at 36–38 °C. All cultures gave acceptable absorbance readings (in the range of 0.2–0.5) prior to each assay. Bacterial background lawn was evaluated macroscopically for test-article precipitate and microscopically for indications of cytotoxicity (i.e., thinning). Evidence of cytotoxicity was scored (by hand or automatic colony counter) relative to the vehicle control plate and recorded along with the revertant counts for all plates at that dose level. Assay acceptance was determined by comparing the vehicle and positive control plates against historical data of revertant count ranges (CRL, 2020). All plates had confluent background lawn; however, cytotoxicity (i.e., reduction in the background lawn and/or mean number of revertant colonies) was observed at ≥ 10 μg/plate in strain TA1537 without metabolic activation, ≥250 μg/plate in strain TA100 with metabolic activation and, ≥1000 μg/plate in strain TA1537 with metabolic activation.

2.2.2. *In vitro micronucleus assay*

The assay design was based on OECD Guideline 487 (OECD, 2016b). Human lymphoblast TK6 cells were originally obtained from Pfizer Global Research (Groton, CT) and subcloned at Charles River (Skokie). All cells used for this assay were free of mycoplasma contamination. The passage number of the cells was 22 for the range-finding assay and 10 for the micronucleus assay.

2.2.2.1. Test article and positive controls preparation. CBD (200 mg/mL in DMSO) isolate was tested in an initial dose range-finding cytotoxicity assay, as a stock solution in DMSO at a target concentration of 200 mg/mL, to determine the highest soluble concentration in the vehicle, followed by the micronucleus assay, which used a CBD isolate concentration of 2.20 mg/mL. The metabolic activation system used was Aroclor™ 1254-induced rat liver S9 fraction (Molecular Toxicology, Inc.). Positive controls were vinblastine sulfate (VIN; Sigma-Aldrich, target dose levels 0.003 and 0.0025 μg/mL) for the 27-h treatments without metabolic activation, cyclophosphamide monohydrate (CP; Sigma-Aldrich, target dose levels 11.9 and 4.7 μg/mL) for the 4-h treatments with metabolic activation, and mitomycin C (MMC, Sigma-Aldrich, target dose levels 0.125 and 0.0625 μg/mL) for the 4-h treatments without metabolic activation. Each culture flask was labeled with the study number, assay date, treatment group, concentration, length of treatment, and the presence or absence of metabolic activation. The vehicle control was dimethyl sulfoxide (DMSO; Sigma-Aldrich).

2.2.2.2. Experimental design. The dose range-finding cytotoxicity assay evaluated target concentrations of CBD isolate: 3.91, 7.81, 15.6, 31.3, 62.5, 125, 250, 500, 1000, or 2000 μg/mL; with and without metabolic activation. Cytotoxicity was assessed using cell-count data obtained from Coulter counts and an appropriate calculation of cytotoxicity (i.e., relative population doubling); cultures exhibiting ≥60% cytotoxicity were not processed and analyzed. Based on the results of the range-finding assay, target concentrations of CBD used during the micronucleus assay ranged from 0.100 to 22.0 μg/mL for the 4-h treatments with and without metabolic activation and for the 27-h treatment without metabolic activation, as shown in Table 1.

The test system was treated with the test article, positive control, or vehicle in the presence and absence of metabolic activation for short incubations (4 h) and in the absence of activation for the long incubation (27 h). The metabolic activation mixture was adjusted and added as appropriate, equal to the volume (mL) of cell culture in ICM at the adjusted cell density multiplied by 0.02. A harvest time of approximately 27 h was used for the 27-h exposure without S9 with no recovery period. A harvest time of approximately 44 h was used for the 4-h exposures with and without metabolic activation, with a 40-h recovery period. Cultures were resuspended at harvest, and an aliquot was removed for counting via Coulter counter and for micronucleus evaluation by flow cytometry. Micronucleus frequencies were analyzed from the processed cultures in at least 20,000 nucleated events (approximately 10,000 nucleated events per culture). All test-article concentrations up to the cytotoxic limit, along with the vehicle control and two concentrations of the positive control, were scored for micronuclei in each treatment condition.

Table 1
Treatment conditions and test material concentrations processed for micronuclei in the main *in vitro* micronucleus study.

Group	Treatment Conditions and Concentrations		
	~4 h without S9	~4 h with S9	~27 h without S9
DMSO (%)	1.0	1.0	1.0
Vinblastine (μg/mL)	–	–	0.0025 and 0.003
Cyclophosphamide (μg/mL)	–	4.7 and 11.9	–
Mitomycin C (μg/mL)	0.0625 and 0.125	–	–
CBD isolate (μg/mL)	0.100, 0.250, 0.500, 1.00, 2.00, 4.00, 6.00, 8.00, 9.00, 10.0, and 11.0	0.100, 0.250, 0.500, 1.00, 2.00, 4.00, 6.00, 8.00, 9.00, and 10.0	0.100, 0.250, 0.500, 1.00, 2.00, 4.00, and 6.00

Cultures for micronucleus evaluation were processed according to the manufacturer's instructions for the *in vitro* MicroFlow kit (Litron Labs, Rochester, NY), and the final samples were analyzed after ≥30 min (and up to 24 h) at ambient temperature, protected from light. Alternatively, samples were stored refrigerated for up to 80 h, prior to analysis.

2.2.2.3. Micronuclei analysis. Data acquisition and analysis was conducted using a FACSCanto II (or equivalent) with FACSDiva Software following CRL Standard Operating Procedures (SOPs). The test article was considered positive for micronuclei induction if a significant increase (z'≥0.6) in percentage of multinucleated cells was observed at one or more concentrations (Wojciechowski et al., 2016), and any observed dose-response was defined as a statistically significant Cochran-Armitage test ($p \leq 0.05$). The test article was considered negative for inducing micronuclei if the positive response criteria were not met and results were not comparable to the historical control range of the vehicle control. Cases that did not clearly fit either criteria were judged equivocal.

2.2.3. *In vivo micronucleus assay*

The assay design was based on OECD Guideline 474 (OECD, 2016a) and the International Council for Harmonisation (ICH) Harmonized Tripartite Guideline S2 (R1).

2.2.3.1. Test article and vehicle control preparations. Oral gavage dose formulations were prepared fresh daily by mixing appropriate concentrations of CBD in olive oil (vehicle control; Spectrum, New Brunswick, NJ) and heating at 35 ± 5 °C for 30 min. Preparations were dispensed prior to dosing and stored at controlled room temperature while stirring to maintain homogeneity.

2.2.3.2. Bioanalysis. Bioanalysis was conducted using a validated method (Charles River Testing Facility Study No. 3281–011) to determine the concentrations of CBD and 77-carboxy-CBD (7-COOH-CBD) in 50 μL of standard, quality control (QC), or rat plasma samples using a SCIEX API [atmospheric pressure ionization] 5000 triple quadrupole LC-MS/MS (liquid chromatography with tandem mass spectrometry) system. The calibration range of the assay was 20.0 to 20,000 ng/mL. Certified reference materials from Cerilliant Corporation (Round Rock, TX) were used as internal standards: cannabidiol-D_3 (99.6%) and 7-carboxy cannabidiol-D_3 (99.1%). The biological matrix used was Sprague-Dawley rat plasma with K_2EDTA obtained from BioIVT (Hicksville, New York).

2.2.3.3. Animals. Male and female Sprague Dawley, CD® [Crl:CD® (SD)] rats, approximately 7–7.5 weeks of age, were obtained from CRL (Raleigh, NC, or Stone Ridge, NY) and randomized into test groups. Animals were housed single sex, three per cage, in solid-bottom cages with nonaromatic bedding and environmental enrichment in a room that maintained temperatures of 20–26.1 °C, relative humidity of 30–70%, and a 12-h light/dark cycle. Rats were fed Lab Diet® (Certified Rodent Diet #5002, PMI Nutrition International, Inc.) *ad libitum*. Following a 7-day acclimation period, the animals weighed between 152 and 284 g at initiation of dosing. Animals were cared for according to the published National Research Council guidelines.

2.2.3.4. Experimental design. The following in-life assessments were performed for all animals at least daily: mortality/cageside observations, clinical/post-dose observations, detailed clinical observations, individual body weights, and food consumption.

Doses were selected based on dose range-finding toxicity studies in rats and on limit dose recommendations (ICH, 2012). For the range-finding study, animals (three/sex/group) were administered 500, 1000, or 2000 mg/kg-bw/d CBD by oral gavage once daily for two consecutive days. Based on observed toxicity at the 2000-mg/kg-bw/d dose, 1000 mg/kg-bw/d was selected at the maximum tolerated dose (MTD) in the main study, and subsequent doses were based on 50% of the next-highest dose. In the main study, six animals/sex/group were administered 0 (vehicle control), 250, 500, or 1000 mg/kg-bw/d CBD once daily on two consecutive days. Blood samples (approximately 0.5 mL) were collected from non-fasted, anesthetized animals via cardiac puncture prior to terminal necropsy and processed to plasma for determination of plasma CBD and 7-COOH-CBD concentrations. On Day 3, animals were euthanized, and bone marrow was collected from animals (5/group) for micronucleus evaluation. Slides were prepared and maintained at controlled room temperature and shipped to CRL (Skokie, IL) for analysis. To verify scorer proficiency, positive control reference slides from a historical experiment in which 60 mg/kg cyclophosphamide was administered via oral gavage were used (CRL, 2016).

2.2.3.5. Micronuclei analysis. Coded slides were stained with acridine orange solution prior to analysis. Two separate counts were made for each animal: 1) ≥ 500 total erythrocytes (TE; equals polychromatic erythrocytes [PCEs] + normochromatic erythrocytes [NCEs]) were counted and the PCE:TE ratio was determined; and 2) the number of micronucleated PCEs (MN-PCEs) in a total of 4000 PCEs scored. The % MN PCE and PCE:TE ratio results were compared between the test article and vehicle control groups, and between the positive and vehicle control groups, using analysis of variance (ANOVA). The MN-PCE frequencies were analyzed using a one-tailed test; PCE:TE ratios were analyzed using a two-tailed test. The Cochran-Armitage test was used to evaluate dose-response. Statistical significance was determined at a 95% confidence level ($p \leq 0.05$).

3. Results

3.1. Analytical verification of CBD dose formulation

For the bacterial reverse mutagenicity assay, CBD formulations of 0.5 and 50 mg/mL quantified at 96.2% and 96.6% of the nominal concentration, respectively. For the repeat mutagenicity assay, CBD formulations of 0.05 and 1.00 mg/mL quantified at 99.1% and 105% of the nominal concentration, respectively. These concentrations met the acceptance criterion of ≥90% of nominal. The lowest concentration (0.0025 mg/mL) from the mutagenicity and repeat mutagenicity assays was collected but not reported because this concentration was outside the validated range. For the *in vitro* micronucleus assay, CBD formulations of 0.0100, 1.00, and 2.20 mg/mL quantified at 96.8%, 103% and 103% of the nominal concentration, respectively, meeting the acceptance criterion of ≥90% of nominal.

For the *in vivo* micronucleus assay, CBD formulations of 50, 100, and 200 mg/mL quantified at 99%, 95.7% and 95.9% of the nominal concentration, respectively, meeting the acceptance criterion of ±15% of nominal. CBD was not detected in vehicle control samples from any of these studies.

3.2. Bacterial reverse mutagenicity assay

In the range-finding assay, precipitates were observed in both strains (TA100 and WP2 *urvA*), at ≥ 500 μg/plate without metabolic activation and at ≥ 1000 μg/plate with metabolic activation. Cytotoxicity was observed at ≥ 50 μg/plate in strain TA100 without metabolic activation and ≥500 μg/plate in strain TA100 with metabolic activation.

In the definitive assay, precipitates were observed in the following conditions: ≥250 μg/plate in strain TA1535 without metabolic activation and in strains TA98 and TA100 with metabolic activation; at ≥ 500 μg/plate in strains TA98, TA1537, and WP2 *uvrA* without metabolic activation and in strains TA1535 and TA1537 with metabolic activation; and at ≥ 1000 μg/plate in strain WP2 *uvrA* with metabolic activation. Cytotoxicity was observed at ≥ 10 μg/plate in strain TA1537 without metabolic activation, ≥250 μg/plate in strain TA100 with metabolic activation, and ≥1000 μg/plate in strain TA1537 with metabolic activation. A reduction in the background lawn was observed at 500 μg/plate in strain TA98 without metabolic activation and in strain WP2 *uvrA* with and without metabolic activation, and at 250 μg/plate in strain TA1535 without metabolic activation. However, the concentrations higher and lower than these doses showed mean revertant counts comparable to vehicle control. Therefore, this was not a dose-dependent response and determined not to be biologically relevant. Additionally, the highest concentration evaluated was as per the OECD guidelines and was limited by solubility, indicating that the test article was tested up to the maximum feasible limits in the present assay.

Under the conditions of the study, CBD did not cause an increase in the number of histidine revertants (*Salmonella* strains) or tryptophan revertants (*E. coli*) per plate in the presence or absence of S9 microsomal enzymes (Table 2). Positive controls produced the expected (or greater) increase in mutation frequency and all criteria for a valid study were met.

3.3. In vitro micronucleus assay

Precipitates were observed in the range-finding assay at ≥ 125 μg/mL in the 4-h treatment with metabolic activation and ≥500 μg/mL 27-h treatment without metabolic activation and in the 4-h treatment without metabolic activation at the end of CBD treatment. Excessive cytotoxicity was observed at ≥ 8 μg/mL in the 27-h treatment without metabolic activation; at ≥ 12 μg/mL in the 4-h treatment without metabolic activation; and at ≥ 11 μg/mL in the 4-h treatment with metabolic activation. Changes in the pH were not observed in any treatment at the end of test article treatment.

Table 2
Mean (±SD) revertant colonies per plate in main bacterial reverse mutation assay with CBD.

Treatment Group	μg/plate	TA98		TA100[B]		TA1535		TA1537		WP2 *uvrA*	
		-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9
DMSO	100 μL	9 ± 2	11 ± 5	103 ± 6	112 ± 9	7 ± 1	7 ± 5	3 ± 1	6 ± 2	68 ± 10	69 ± 14
CBD	0.25	9 ± 6	16 ± 3	107 ± 20	125 ± 14	6 ± 1	10 ± 2	3 ± 2	4 ± 4	70 ± 11	63 ± 3
	0.5	12 ± 7	16 ± 7	117 ± 9	141 ± 18	8 ± 3	7 ± 1	4 ± 1	5 ± 2	66 ± 15	72 ± 8
	1	14 ± 3	10 ± 3	116 ± 12	204 ± 11	10 ± 3	5 ± 1	2 ± 1	2 ± 1	68 ± 21	70 ± 14
	2.5	9 ± 2	11 ± 3	112 ± 4	125 ± 13	9 ± 1	8 ± 2	2 ± 1	4 ± 1	66 ± 5	68 ± 14
	5	11 ± 3	19 ± 4	104 ± 32	104 ± 11	8 ± 4	6 ± 2	3[S] ± 1	3 ± 1	65 ± 13	56 ± 11
	10	8 ± 3	11 ± 7	89 ± 9	97 ± 10	7 ± 3	6 ± 3	–[R]	3 ± 2	85 ± 10	65 ± 9
	25	9 ± 1	13 ± 6	73[S] ± 9	85[S] ± 4	6 ± 3	7 ± 3	–[R]	3 ± 3	60 ± 9	61 ± 8
	50	9[S] ± 1	17 ± 3	74[S] ± 7	91[S] ± 12	6 ± 3	5 ± 4	–[R]	3 ± 2	49 ± 7	65 ± 18
	100	6[S] ± 2	12 ± 3	–[R]	93[S] ± 13	8 ± 1	7 ± 2	–[R]	6 ± 1	63 ± 2	67 ± 5
	250	6[S] ± 2	14[P] ± 2	NT	–[PR]	–[PR]	8 ± 1	–[R]	2[S] ± 2	62 ± 3	54 ± 18
	500	–[PR]	14[P] ± 3	NT	–[PR]	10[PS] ± 3	7[P] ± 2	–[PR]	3[PS] ± 1	–[PR]	–[R]
	1000	8[P] ± 3	8[P] ± 2	NT	–[PR]	6[P] ± 2	9[P] ± 3	–[PR]	–[PR]	37[P] ± 3	43[P] ± 7
	2500	8[P] ± 3	10[P] ± 3	NT	–[PR]	7[P] ± 3	9[P] ± 3	–[PR]	–[PR]	33[P] ± 9	29[P] ± 2
	5000	13[PS] ± 8	12[P] ± 3	NT	–[PR]	15[P] ± 2	9[P] ± 1	–[PR]	–[PR]	42[P] ± 6	52[P] ± 9
2AA	2.5	NA	2207 ± 1311[C]	NA	1153 ± 156[C]	NA	322 ± 40[C]	NA	134 ± 14[C]	NA	NA
	10.0	NA	NA	NA	NA	NA	NA	NA	NA	NA	335 ± 80[C]
2NF	2.5	1126 ± 296[C]	NA	NA	NA	NA	NA	NA	NA	NA	NA
NAAZ	1.0	NA	NA	417 ± 35[C]	NA	536 ± 139[C]	NA	NA	NA	NA	NA
ICR	0.5	NA	NA	NA	NA	NA	NA	151 ± 15[C]	NA	NA	NA
NQNO	2.0	NA	NA	NA	NA	NA	NA	NA	NA	641 ± 80[C]	NA

2AA – 2-Aminoanthracene; 2NF – 2-Nitrofluorene; CBD – cannabidiol; ICR – ICR-191 acridine; NAAZ – sodium azide; NQNO – 4-nitroquinoline-N-oxide; SD – standard deviation; DMSO – dimethyl sulfoxide; NA – not applicable; NT – not tested.
Note: All plates had confluent background lawn, unless otherwise noted.
[A] Calculated from triplicate plates.
[B] Data from repeat assay for TA100 without activation; data from vehicle control for strain TA100 without metabolic activation in original assay was outside the historical control data.
[C] Protocol criteria for a positive response met.
[P] Precipitates present.
[S] Slightly reduced background lawn.
[R] Cytotoxicity: Reduced background lawn, plates not counted.

Table 3
Cytotoxicity and micronucleus summary data from *in vitro* micronucleus study with CBD.

		Cytotoxicity (%)	Mean MN (%)	z'	Cytotoxicity (%)	Mean MN (%)	z'	Cytotoxicity (%)	Mean MN (%)	z'
Treatment Group	**μg/mL**	4-Hour Treatment without Metabolic Activation			27-Hour Treatment without Metabolic Activation			4-Hour Treatment with Metabolic Activation		
DMSO	1%	0.00	0.12	NA	0.00	0.21	NA	0.00	0.33	NA
CBD	0.100	0.77	0.21	<0	6.83	0.15	<0	2.59	0.32	<0
	0.250	1.41	0.28	<0	5.30	0.10	<0	4.15	0.31	<0
	0.500	0.40	0.26	<0	3.18	0.13	<0	3.55	0.26	<0
	1.00	1.40	0.21	<0	6.36	0.10	<0	5.62	0.29	<0
	2.00	1.54	0.28	<0	12.76	0.07	<0	5.43	0.30	<0
	4.00	7.01	0.24	<0	23.03	0.14	<0	10.64	0.32	<0
	6.00	11.20	0.34	0.10	57.09	0.55	0.24	17.75	0.28	<0
	8.00	18.79	0.29	<0	NA	NA	NA	35.38	0.47	<0
	9.00	18.08	0.30	<0	NA	NA	NA	48.11	0.47	<0
	10.0	29.97	0.34	0.10	NA	NA	NA	40.86	0.52	<0
	11.0	45.09	0.43	0.31	NA	NA	NA	NA	NA	NA
MMC	0.0625	19.28	1.56	0.77[a]	NA	NA	NA	NA	NA	NA
	0.125	36.58	4.18	0.88[a]	NA	NA	NA	NA	NA	NA
VIN	2.5	NA	NA	NA	54.09	3.09	0.84[a]	NA	NA	NA
	1.0	NA	NA	NA	56.24	3.33	0.85[a]	NA	NA	NA
CP	4.7	NA	NA	NA	NA	NA	NA	26.20	2.55	0.80[a]
	11.9	NA	NA	NA	NA	NA	NA	59.78	7.17	0.90[a]

No data are shown for CBD concentrations that exhibited excessive cytotoxicity (designated as NA), i.e., at ≥ 8 μg/mL in the 27-h treatment without metabolic activation; at ≥ 12 μg/mL in the 4-h treatment without metabolic activation; and at ≥ 11 μg/mL in the 4-h treatment with metabolic activation.
CBD – cannabidiol; CP – Cyclophosphamide monohydrate; DMSO – Dimethyl sulfoxide; MMC – Mitomycin C; NA – Not Applicable; VIN – Vinblastine sulfate.
MN – Micronucleated cells.
[a] z' ≥ 0.6.

In the definitive assay, precipitates were not observed in any treatment with or without activation, up to 22.0 μg/mL. Excessive cytotoxicity was observed at ≥ 8 μg/mL in the 27-h treatment without metabolic activation; at ≥ 12 μg/mL in the 4-h treatment without metabolic activation; and at ≥ 11 μg/mL in the 4-h treatment with metabolic activation. The vehicle and positive control data were comparable to the relevant historical control values.

Cannabidiol was considered negative for inducing micronuclei in TK6 cells in the 27-h treatment without metabolic activation and in the 4-h treatments with and without metabolic activation under the conditions of this test system (Table 3).

3.4. *In vivo* micronucleus assay

3.4.1. *In-life and clinical observations*

In the preliminary dose range-finding experiment, absolute body weights were similar between groups (data not shown). Average food consumption per animal decreased in an apparent dose-proportional manner in males; however, females in the 2000 mg/kg-bw/day group had increased consumption on Day 2 (data not shown). Overall, the lowest dose (500 mg/kg-bw/d CBD) was generally well-tolerated; all animals showed only mild incoordination and three animals decreased activity following dosing. At the mid dose (1000 mg/kg-bw/d CBD), all males and females were observed with pronounced piloerection, partially closed eyes, moderate to severe incoordination, and decreased activity. Similar or more severe signs were noted at the highest dose (2000 mg/kg-bw/d CBD), along with observations of shallow breathing, intermittent tremoring, and cold to touch in all animals and low carriage in all males. A few animals were observed convulsing. Based on these findings and the severity of the toxic effects, all animals in the 2000 mg/kg-bw/d dose group were humanely euthanized late on Day 2 and the functional MTD was considered to be 1000 mg/kg-bw/d CBD.

In the main micronucleus experiment, no difference in absolute body weights was reported in animals administered up to 500 mg/kg-bw/d CBD compared to concurrent control animals. However, at 1000 mg/kg-bw/d CBD, average body weights on Day 3 were statistically significantly decreased in males, along with a non-significant decrease observed in females. Dose-dependent decreases in the average rate of body weight gain relative to concurrent controls was observed in animals of the 500 and 1000 mg/kg-bw/d CBD groups; however, this finding was only statistically significant in males. Average food consumption per animal decreased in a dose proportional manner in males and females. There were no notable observations recorded throughout the study for animals receiving 250 mg/kg-bw/d CBD and animals receiving 500 mg/kg-bw/d CBD were minimally affected, with a single male and female each presenting with wet fur on their ventral surface and a single male presenting with decreased activity. Animals administered 1000 mg/kg-bw/d CBD were more noticeably affected, as they were observed with hunched posture (one male, two females), incoordination (one per sex), and decreased activity (four per sex) after 2 days of dosing. One female at this high dose was also observed with low carriage and abnormal gait.

3.4.2. *Bioanalysis*

Plasma samples (n = 36) from the main experiment were analyzed for CBD and 7-COOH-CBD. Administration of CBD to male and female animals resulted in significant, dose-related exposure to both CBD and 7-COOH-CBD at all dose levels (Table 4).

3.4.3. *Micronucleus analysis*

There was no statistically significant or dose-dependent increase in the %MN PCEs in male or female rats at any CBD dose level as compared to the vehicle control group (Table 5). No evidence of bone marrow cytotoxicity (decreases in PCE:TE ratio) was found in any animal at any CBD dose level. Group mean values for %MN-PCEs and PCE:TE ratios for the vehicle and positive controls were within 95% of the historical control intervals obtained by CRL (Skokie, IL), demonstrating the acceptability of the assay (Table 5) (CRL, 2016). Therefore, CBD was negative for clastogenic activity and/or disruption of the mitotic apparatus under the conditions of this assay.

Table 4
Average CBD and 7-COOH-CBD rat plasma concentrations on Day 3 following CBD administration (*in vivo* micronucleus assay).

CBD Dose Group (mg/kg-bw/d)	Gender	CBD Plasma Concentration (ng/mL) ± SD	7-COOH-CBD Plasma Concentration (ng/mL) ± SD
250	M	677 ± 338	2025 ± 1589
	F	911 ± 724	3736 ± 4734
500	M	4969 ± 3192	16305 ± 10888
	F	4800 ± 4138	12288 ± 8943
1000	M	26250 ± 15642	43333 ± 8815
	F	16800 ± 2990	39483 ± 11788

CBD – cannabidiol; M – male; F – female; SD – Standard deviation.

Table 5
Summary of micronucleus assay data for Sprague Dawley rats administered CBD for two consecutive days.

Treatment Group (mg/kg-bw/d)	Sex	% MN-PCEs ± SD	PCE:TE Ratio ± SD
CBD: 0 (vehicle control)	M	0.06 ± 0.05	0.58 ± 0.06
	F	0.09 ± 0.03	0.54 ± 0.10
CBD: 250	M	0.10 ± 0.02	0.53 ± 0.07
	F	0.05 ± 0.04	0.53 ± 0.08
CBD: 500	M	0.10 ± 0.04	0.60 ± 0.04
	F	0.05 ± 0.04	0.55 ± 0.06
CBD: 1000	M	0.09 ± 0.03	0.55 ± 0.07
	F	0.07 ± 0.05	0.49 ± 0.10
CP: 60 (positive control)	M	1.63 ± 0.46*	0.45 ± 0.14
	F	0.97 ± 0.13*	0.28 ± 0.05*

CBD – pure cannabidiol isolate; CP – cyclophosphamide monohydrate; MN – micronucleated; PCE – polychromatic erythrocyte; SD – standard deviation; vehicle control – olive oil; *statistically different from vehicle control $p \leq 0.01$.

4. Discussion

The commercial availability of hemp-derived products in the US has increased dramatically since the passage of the Hemp Farming Act, part of the 2018 Farm Bill. Despite the increase in consumer use of hemp-derived CBD in the US and in other countries globally, few high-quality, guideline-based genotoxicity studies have been conducted or published on CBD itself. In addition, EFSA and UK FSA (2022) have highlighted this endpoint as a data gap, concluding the currently available studies to be insufficient for reaching a conclusion regarding genotoxic potential. Interpretations of the results of previously published genotoxicity assays using CBD and CBD-containing mixtures have been inconsistent and complicated by issues of purity and potency of the test article and/or limitations in the study design. For example, an early investigation of CBD's genotoxic potential found evidence of micronucleus induction in bone marrow cells following intraperitoneal injection of 10 mg CBD/kg-bw in (C57BL x C3H)F1 mice for 5 consecutive days, resulting in structural and numerical chromosomal aberrations (Zimmerman and Raj, 1980). However, no abnormal effects on sperm morphology were observed with CBD exposure for 5 days followed by a 35-day recovery as reported (Zimmerman et al., 1979). More recently, the Epidiolex non-clinical safety review (CDER, 2018) describes negative results from an *in vivo* micronucleus study with a pure CBD test material. However, the public summary of this report does not describe the justification for dose selection or why the doses used were limited to a top dose of 500 mg/kg, nor does it provide any indication that CBD was confirmed to reach the bone marrow compartment. Results from *in vitro*

comet assays have been inconsistent. The CDER (2018) review summarizes a study in which CBD did not induce DNA damage in the liver of rats at doses up to 500 mg/kg-bw/day in the alkaline comet assay. Carvalho et al. (2022) reported significantly increased DNA damage in sperm, but not leukocytes in comet assays. Whereas, Russo et al. (2019) reported CBD-induced DNA damage in single cell gel electrophoresis (SCGE) experiments in a human liver cell line (HepG2) and in buccal-derived cells (TR146) In addition, results of an *in vitro* micronucleus assay using HepG2 cells, found CBD to be positive for induction of micronuclei (Russo et al., 2019).

To date, three publications have explored the genotoxic potential of CBD-containing hemp extracts following OECD guidelines. Marx et al. (2018) conducted a guideline-compliant study using a battery of genotoxicity assays (i.e., an *in vitro* reverse mutation Ames assay [OECD 471], an *in vitro* micronucleus assay [OECD 473], and *in vivo* mouse micronucleus assay) [OECD 474] on a hemp extract (~25% CBD). Although genotoxicity results were negative from all assays, extrapolation of results to pure CBD is difficult. Slight reduction in polychromatic erythrocytes was observed *in vivo* (evidence of bone marrow toxicity), but was not considered biologically significant (Marx et al., 2018). Results from this assay cannot be considered definitively negative, due to a failure to demonstrate the presence of CBD in the blood or toxicity to bone marrow. Dziwenka et al. (2020, 2021) have also conducted OECD guideline studies on hemp extracts (~7% CBD), although negative results were obtained, the low CBD content in the test articles decreases the utility of the data for understanding the genotoxicity of pure CBD. Given the absence of high-quality, guideline-compliant genotoxicity assessments on pure CBD, additional research to fully assess the safety of this compound is warranted.

In the present study, CBD isolate (>99%) did not produce an increase in the number of revertants in the presence or absence of S9 microsomal enzymes in the Ames assay. This negative result aligns with previously conducted Ames assays using hemp extracts containing ~7–25% CBD (Marx et al., 2018; Dziwenka et al., 2020, 2021), as well as unpublished data reviewed by CDER (2018) in which CBD was negative in an Ames assay up to 5000 μg/plate, with and without metabolic activation. Results from the *in vitro* micronucleus assay indicate that CBD was negative for inducing micronuclei in TK6 cells in both the 27-h treatment without metabolic activation and the 4-h treatments with and without metabolic activation. Notably, this finding contrasts with other published work that used a pure CBD test material; however, our study was conducted in the human TK6 cell line, which is considered by the OECD 487 guideline to be validated more extensively for this assay than the HepG2 cell line previously reported (Zhang et al., 1995; OECD, 2016b; Russo et al., 2019). Finally, the *in vivo* micronucleus study provides additional support for a lack of genotoxicity of CBD. There was no significant or dose-dependent increase in the %MN PCEs in male or female Sprague Dawley rats for any CBD dose level tested, up to 1000 mg/kg-bw/d. This result is similar to the negative findings obtained at up to 2000 mg/kg-bw/d in a mouse micronucleus study using a hemp extract containing approximately 25% CBD, as well as up to 500 mg/kg-bw/d in rats using CBD isolate in the Epidiolex non-clinical review package (Marx et al., 2018; CDER, 2018).

Finally, the conclusions from the studies presented here are supported by the results of a 2-year cancer bioassay reviewed by the FDA (CDER, 2018) in which a CBD Botanical Drug Substance (containing 57.5–67.2% CBD) was administered in the diet up to 50 mg/kg-bw/d and demonstrated no treatment-related increase in tumor incidence. However, test-article impurities and the dietary route of exposure were concerns highlighted by the FDA for this study, which limit its relevance to the present assessment.

In conclusion, three GLP- and OECD guideline-compliant mutagenicity and genotoxicity studies were performed to test the ability of CBD to induce mutation or cause chromosomal damage. The results from this testing battery indicate that pure CBD isolate was nonmutagenic, nonclastogenic, and nongenotoxic under the study conditions. These studies are the first to be published using guideline-compliant methods on a pure CBD isolate, and together, they provide information critical to assessing the safe consumer use of CBD in food and dietary supplements.

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CRediT authorship contribution statement

Rayetta G. Henderson: Conceptualization, Writing – original draft, Writing – review & editing. **Brian T. Welsh:** Study monitoring, Writing – original draft, Writing – review & editing. **Kristen R. Trexler:** Conceptualization, Writing – review & editing. **Marcel O. Bonn-Miller:** Conceptualization, Supervision. **Timothy W. Lefever:** Conceptualization, Writing – review & editing.

Declaration of competing interest

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Data availability

Data will be made available on request.

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EXHIBIT B

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Reproductive and developmental toxicity evaluation of cannabidiol

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ABSTRACT

An important data gap in determining a safe level of cannabidiol (CBD) intake for consumer use is determination of CBD's potential to cause reproductive or developmental toxicity. We conducted an OECD Test Guideline 421 GLP-compliant study in rats, with extended postnatal dosing and hormone analysis, where hemp-derived CBD isolate (0, 30, 100, or 300 mg/kg-bw/d) was administered orally. Treatment-related mortality, moribundity, and decreased body weight and food consumption were observed in high-dose F_0 adult animals, consistent with severe maternal toxicity. No effects were observed on testosterone concentrations, F_0 reproductive performance, or reproductive organs. Hepatocellular hypertrophy in the 100- and 300 mg/kg-bw/day groups correlated with hypertrophy/hyperplasia in the thyroid gland and changes in mean thyroid hormone concentrations in F_0 animals. Mean gestation length was unaffected; however, total litter loss for two females and dystocia for two additional females in the high-dose group occurred. Other developmental effects were limited to lower mean pup weights in the 300 mg/kg-bw/d group compared to those of concurrent controls. The following NOAELs were identified for CBD isolate based on this study: 100 mg/kg-bw/d for F_0 systemic toxicity and female reproductive toxicity, 300 mg/kg-bw/d for F_0 male reproductive toxicity, and 100 mg/kg-bw/d for F_1 neonatal and F_1 generation toxicity.

1. Introduction

The implementation of the Hemp Farming Act—part of the Agricultural Improvement Act of 2018 (aka, "2018 Farm Bill")—has led to greater market availability and public interest in consumer products containing hemp-derived cannabidiol (CBD) in the United States (US). While various forms of cannabis have been used globally for medicinal and recreational purposes for thousands of years, only recently has a CBD drug (Epidiolex®) been approved by the US Food and Drug Administration (FDA) for the treatment of seizures associated with Lennox-Gastaut syndrome and Dravet syndrome in patients 2 years of age and older (Jazz Pharmaceuticals, 2023). In addition, Sativex®, a combination of CBD and delta-9 tetrahydrocannabinol (THC), is approved in other countries for the treatment of moderate to severe spasticity due to multiple sclerosis (Jazz Pharmaceuticals, 2023). CBD is also proposed to have analgesic, anxiolytic, neuroprotective, antioxidant, and antimicrobial properties (Small and Marcus, 2002; Pertwee, 2004; Billakota et al., 2019; Devinsky et al., 2018).

The FDA has not identified a suitable regulatory pathway for use of CBD in food or dietary supplements, nor has the agency established tolerable daily intake levels associated with consumer use. An overview of the FDA's activities related to evaluating the safe use of CBD in food and dietary supplement products can be found on its website (FDA, 2023). However, based on recent evaluations of the available safety data, the United Kingdom (UK) Food Safety Authority (FSA, 2022), Health Canada (2022), and the Australian Therapeutic Goods Administration (TGA, 2021) have established recommended maximum upper intake levels of CBD by healthy adults, except those planning to be or currently pregnant or breastfeeding. In addition, recent literature reviews, including a systematic mapping study, have been published summarizing the available CBD toxicity data and knowledge gaps (Henderson et al., 2023a; Li et al., 2021). While limited safety-related data on CBD are available in the public domain, regulatory agencies continue to highlight data gaps in the understanding of CBD toxicology. Specifically, no publicly available non-clinical studies on CBD isolate have been conducted according to regulatory test guidelines to evaluate genotoxicity, repeated oral toxicity, or reproductive and developmental

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Abbreviations

AGD	anogenital distance
ANOVA	analysis of variance
BSA	bovine serum albumin
CASA	computer-aided sperm analysis
CBD	cannabidiol
CDER	Center for Drug Evaluation and Research
CFR	US Code of Federal Regulations
FDA	US Food and Drug Administration
FSA	UK Food Safety Authority
GD	gestational day
GLP	Good Laboratory Practice
HPLC	high-performance liquid chromatography
IACUC	institutional animal care and use committee
LD	lactation day
NOAEL	no-observed-adverse-effect level
OECD	Organisation for Economic Co-operation and Development
PND	postnatal day
POD	point of departure
SD	standard deviation
T3	triiodothyronine
T4	total thyroxine
TGA	Therapeutic Goods Administration
THC	delta-9-tetrahydrocannabinol
TSH	thyroid stimulating hormone
UDPGT	uridine diphosphate glucuronosyltransferase
UK	United Kingdom
US	United States of America

toxicity endpoints. Understanding potential effects of CBD on reproduction and/or offspring development is critical in determining a safe CBD intake level for consumer use (e.g., in dietary supplements, foods, and/or beverages).

Using studies reviewed by CDER (2018a), as well as other, published studies, to review CBD safety, Li et al. (2021) summarized the reproductive and developmental toxicity findings in rats, mice, and rabbits: "A full battery of assessments was conducted including litter size, body weight, physical and functional development, sexual milestones, auditory startle, motor activity, and learning and memory. Adverse effects of CBD treatment have been observed primarily in the dose groups of 150 or 250 mg/kg-bw/day including decreased pup body weights, delays in achieving developmental landmarks (eye opening, pupillary reflex, and sexual maturation in male and female), neurobehavioral changes (decreased locomotor activity), and adverse effects on reproductive system structure (small testis) and possibly function." Studies reviewed by the FDA as part of the Epidiolex non-clinical package provide data that can be incorporated into an overall assessment of the potential reproductive and developmental toxicity of CBD (CDER, 2018a). However, none of these studies conducted on CBD isolate included dosing in both sexes starting prior to mating and continuing through weaning, and thus have been deemed insufficient by some regulatory agencies for evaluation of CBD for consumer use. Similarly, published *in vitro* and *in vivo* studies evaluating the developmental and reproductive toxicity of CBD are diverse and include acute and repeated dosing, different species (from mammals to invertebrates), and various dose levels and routes of exposure but do not address the key data gaps identified by regulatory agencies needed to evaluate safety for use in food and dietary supplements (e.g., Carvalho et al., 2018a,b and 2022; Rosenkrantz et al., 1981; Rosenkrantz and Esber, 1980; Dalterio et al., 1982, 1984a,b; Patra and Wadsworth, 1991). Other investigators have hypothesized mechanisms of action for some of the reproductive effects observed with CBD. For example, a recent review article by Carvalho et al. (2020) provides an extensive overview of the available data regarding the potential effects of CBD on the male reproductive system. While these studies contribute to the overall information on CBD safety, none provide sufficient data from which to derive a point of departure (POD) for human health risk assessment. Furthermore, some potential adverse reproductive effects have been observed inconsistently across studies, such as effects on testosterone concentrations and sperm parameters in males (Carvalho et al., 2018a; Dalterio et al., 1982; Marx et al., 2018).

In response to this need for developmental and reproductive toxicity data on CBD, the present study evaluated the effects of repeat oral dosing of pure (>99%) hemp-derived CBD on male and female reproductive performance and offspring development in rats. Testing was performed according to the Organization for Economic Co-operation and Development (OECD) Test Guideline No. 421 (OECD, 2016) with extended offspring evaluation through postnatal day (PND) 42. This study was conducted as part of a larger program to investigate the safety of CBD isolate (Henderson et al., 2023b)

2. Materials and methods

2.1. Test material and vehicle

Hemp-derived CBD isolate (99.08–101.46%; CAS No. 13956-29-1) was provided by Canopy Growth USA (Evergreen, Colorado). CBD was stored, protected from light and with desiccant, at room temperature (19 °C–25 °C) under nitrogen. Third-party analysis (Botanacor Laboratories, Denver, CO) by high-performance liquid chromatography (HPLC) with UV absorbance detection, certified the isolate to be 99.62% CBD and 0.16% cannabidivarin; all other cannabinoids tested were below the limit of quantification (Botanacor, Denver, CO).

The CBD was mixed into an olive oil vehicle (Spectrum, New Brunswick, NJ), which was also used for dosing the control animals. Based on the measured purity, a correction factor of 1.004 was used for dose formulations. Dose formulations for oral gavage were prepared approximately weekly, and all preparations were dispensed into daily aliquots, stored at controlled room temperature (18–24 °C) and protected from light until use. CBD formulations were confirmed to be stable when stored refrigerated (5 °C) and at room temperature for 8 days. On the day of dosing, preparations were heated to 35 ± 5 °C for at least 30 min, followed by continuous stirring at room temperature while dosing to maintain homogeneity. Concentration analyses of the first and last dose preparations confirmed that the dosing formulations contained 94.5%–100.4% of the target concentrations and were within the protocol-specified ranges. CBD was not detected in vehicle control formulations.

2.2. Animals

Sprague Dawley, CD® [Crl:CD®] rats were obtained from Charles River Laboratories (Raleigh, North Carolina) at approximately 10–11 weeks of age. Following a 7-day acclimation period, animals were assigned to test groups using a stratified randomization procedure. Females not exhibiting a normal 4- to 5-day estrous cycle were not assigned to groups. Females and males weighed 198–261 g and 274–407 g, respectively, at initiation of dosing. Animals were housed in solid-bottom cages with nonaromatic bedding and environmental enrichment in a room that maintained temperatures of 68–78 °F, relative humidity of 30%–70%, and a 12-h light/dark cycle. During the acclimation and pre-mating period, animals were housed 2–3 per cage (single sex), and then, during the cohabitation period for mating, the females were paired 1:1 with a male in the male's home cage. On

successful mating or at the end of the mating period, all adult males remained individually housed until termination. Following positive signs of mating or the end of the mating period, females were housed individually and remained in their cages with their litters until termination. On PND 4, eight pups per litter (four/sex) were selected where possible, and remaining pups were euthanized by intraperitoneal sodium pentobarbital after collecting blood for thyroid hormone assessment. Standardization of litters was not done for litters of fewer than eight pups. All offspring selected after weaning for the F_1 generation were housed in groups of 2–3 by sex. Rats were provided treats and cage enrichment and had access to municipal tap water treated by reverse osmosis and UV irradiation, and were given food [Lab Diet® (Certified Rodent Diet #5002, PMI Nutrition International, Inc.)] *ad libitum*. Animals were cared for according to the published National Research Council guidelines.

2.3. Reproductive toxicology

The *in vivo* reproductive toxicology study was conducted in accordance with US Code of Federal Regulations (CFR) Title 40, Parts 160 and 792: Good Laboratory Practice (GLP) Standards. The protocol was reviewed and approved by an institutional animal care and use committee (IACUC). The study design was based on the OECD Guideline for the Testing of Chemicals, Guideline 421, Reproduction/Development Toxicity Screening Test, July 2016 (Modified) and is summarized in Table 1.

2.3.1. Experimental design

For the main study, the control group and three CBD dose groups (30, 100, 300 mg/kg-bw/d) each consisted of 10 animals per sex. The oral route was chosen, because it is the most likely route of exposure for humans. As described in the OECD (2016) guidelines, dose levels were selected based on results from existing reproductive toxicity studies conducted with CBD isolate. The high dose of 300 mg/kg-bw/day was not expected to cause death or severe suffering and was selected based on the highest dose tested of 250 mg/kg-bw/day in the most relevant available study, in which rats were exposed to CBD for two weeks prior to mating and until gestation day (GD) 6 (reviewed by CDER, 2018a; study number GW1456[1]). In that study, decreased weight gain was observed in parental males and females and slight decreases in fertility indices were observed in the mid- and high-dose groups. In a separate pre- and postnatal study (GD 6 to postnatal day [PND] 21), some reproductive and developmental effects were also noted at doses up to 250 mg/kg bw/d (reviewed by CDER, 2018a; study number GWTX1532[2]). Based on these two studies reviewed by CDER (2018a), and taking into consideration other available studies reviewed by FDA (CDER, 2018a) and findings from a male reproductive study conducted in monkeys (Rosenkrantz et al., 1981), reproductive effects were expected at the selected high dose of 300 mg/kg bw/d and the low- and mid-dose levels were selected to derive a graded dose-response for any toxicity effects observed.

Animals were dosed via oral gavage once daily at a dosing volume of 5 mL/kg. F_0 males assigned to the main study were dosed for 14 days prior to mating and continuing through one day prior to euthanasia. F_0 females assigned to the main study were dosed for 14 days prior to mating and continuing through lactation day (LD) 20. Offspring selected as the F_1 generation were dosed by oral gavage from weaning on PND 21 through PND 42 (any prior exposure *in utero* or via nursing was not assessed).

Estrous cyclicity was determined in all F_0 females by daily vaginal lavage for 14 days prior to randomization and through the mating period until mating was confirmed. Stage of estrous was determined by microscopic examination of vaginal cells, and cycle length was calculated over the period of observation.

The following in-life assessments were performed at least daily for all F_0 animals: mortality/cage-side observations, detailed clinical observations prior to and approximately 2 h after dosing, and individual body weights. On the day of parturition, females were observed three times per day for completion of delivery or signs of dystocia or other difficulties, and live pups were counted. Food consumption was measured twice weekly until cohabitation and in females on gestation days (GDs) 0, 4, 7, 11, 14, 17, and 20, and on LDs 1, 4, 7, 10, 14, 17, 20, and 21.

The F_0 generation was necropsied with anatomic histopathology (gross lesions [all groups] and microscopic evaluations [high- and low-dose groups only]) and sperm collection for quantitation and morphological evaluation. Blood samples were collected for thyroid hormones and testosterone analyses, as described below.

F_1 litters were observed twice daily for general health, mortality, and morbidity. Detailed clinical observations and body weights were collected twice weekly from PND 1 through PND 21. Pups were sexed individually on PNDs 0, 4, 14, and 21. Anogenital distance of all pups was measured on PND 1, and areola/nipple anlagen retention was evaluated in all male pups on PND 13. One pup/sex/litter (same as those used for thyroid hormone assessment) was terminated on PND 21 and underwent necropsy, tissue collection, and recording of thyroid weight (after fixation). Remaining F1 pups were terminated on PND 43 and underwent necropsy, tissue collection, and recording of organ weights.

2.4. Sampling and quantification of hormones

Blood samples for thyroid hormone analyses were collected from a jugular vein around the same time of day (before noon), to reduce variability due to normal diurnal variation. F_0 males and females were sampled at euthanasia (Study Day 28 for males, LD 21 for females), and F_1 pups were sampled on PND 4 (pooled by litter; at least two per litter) and PND 21 (one per sex per litter). Samples were processed to serum and analyzed for triiodothyronine (T3) and/or total thyroxine (T4) using validated ultra-high performance liquid chromatography with dual mass spectroscopy (UHPLC/MS/MS) assays (Lucarell, 2017).

Blood samples for testosterone analyses were collected from F_0 males on Study Day 28, and from F_1 males on PND 43, and processed to serum. Electrochemiluminescence detection of testosterone was performed using a COBAS E411 system (Roche, Indianapolis, Indiana) using appropriate methods; the upper limit of quantification for the assay was 1501 ng/dL.

2.5. Sperm evaluations

Immediately after euthanasia on Study Day 28, the reproductive tract of each male was exposed, and the right cauda epididymis was excised and weighed. An incision was made in the distal region of the right cauda epididymis, and it was then placed in Dulbecco's phosphate buffered saline with 10 mg/mL bovine serum albumin (BSA) at approximately 37 °C. After a minimum 10-min incubation, a sample of sperm was loaded onto a slide for determination of sperm motility at a constant 37 °C. Analysis of at least 200 motile and nonmotile spermatozoa per animal (if possible) was performed to determine percent motile sperm. The right epididymis was then placed in modified Davidson's solution for microscopic examination. Sperm morphology was evaluated by light microscopy via a modification of the wet-mount evaluation technique (Linder et al., 1992). Abnormal forms (double heads, double tails, microcephalic, or megacephalic, etc.) from a differential count of 200 spermatozoa per animal, if possible, were recorded.

[1] Cited in CDER (2018a) as *Epidiolex (Purified CBD): Oral (Gavage) Study of Fertility and Early Embryonic Development in Male and Female Rats (GW Report No. GWTX1456; dated 30/9/16; conducted by [redacted]; GLP)*.

[2] Cited in CDER (2018a) as *Purified CBD: Oral (Gavage) Study of Pre- and Postnatal Development in the Rat (GW Report #: GWTX1532; conducted by [redacted] report dated 4/21/17; GLP)*.

Table 1
Modified OECD 421 multidose reproductive toxicology study in Sprague Dawley rats.

Species	Study Dosing Duration(daily)	Groups	Oral Gavage Dose		Clinical Pathology Sampling[a]	Reproductive, Developmental, and Specialized Endpoints
			Levels (mg/kg-bw/d)	Total Doses		
SD Rat, F_0 generation	M: 14 days prior to mating and throughout mating for a minimum of 28 days F: 14 days prior to mating and throughout mating, gestation, and lactation	10/sex/group	0, 30, 100, 300	28+ 28	Thyroid Hormone M: T4 at termination F: T3 and T4 on LD 21 (at termination) Testosterone M: At termination F: N/A	Pregnancy, mating, and fertility indices; number of estrous cycles and cycle length; hormone analysis (testosterone and thyroid); sperm assessment (count, motility/viability, morphology)
SD Rat, F_1 generation	PND 21 – PND 42[b]	10/sex/group	0, 30, 100, 300	22	Thyroid Hormone M/F: T3 and T4 on PND 4 (culling); T4 on PND 21 (non-selected pup[c] termination) Testosterone M: PND 43 (termination) F: N/A	Litter observations, continuous (e.g., sex ratio - males, mean litter body weights); live birth, viability, and lactation index; anogenital distance; areolae/nipple anlagen

Abbreviations: F, female; FOB, functional observational battery; GLP, good laboratory practices; LD, lactation day; M, male; N/A, not applicable; PND, postnatal day; SD, Sprague Dawley (CD®).
[a] Serum samples from adult males and from pups terminated on PND 21 were analyzed for serum levels of Total T4; serum samples from adult females and culled pups (PND 4) were analyzed for serum levels of Total T3 and T4.
[b] Potentially also exposed to CBD *in utero* and through nursing during lactation.
[c] "Non-selected pups" were those culled but retained for thyroid hormone analysis.

The left testis and cauda epididymis from each male was weighed and stored frozen. The left cauda epididymis was homogenized and evaluated for sperm numbers using the Hamilton Thorne computer-aided sperm analysis (CASA) system (Beverly, Massachusetts) on a minimum of 200 cells, if possible.

2.6. Statistical analyses

Indices were calculated as follows.

- Female mating index = Number of Females with Evidence of Mating (or no confirmed mating date and pregnant)/Number of Females Paired
- Female fertility index = Number of Pregnant Females/Number of Females with Evidence of Mating (or no confirmed mating date and pregnant)
- Female pregnancy index = Number of Pregnant Females/Number of Females Paired
- Male mating index = Number of Males with Evidence of Mating (or female partner confirmed pregnant)/Number of Males Paired
- Male fertility index = Number of Males Impregnating a Female/Number of Males with Evidence of Mating (or female partner confirmed pregnant)
- Male pregnancy index = Number of Males Impregnating a Female/Number of Males Paired
- Live birth index = (Number of Live Newborn Pups x 100)/Number of Newborn Pups
- Viability index = (Number of Live Pups on Day 4 Postpartum x 100)/Number of Liveborn Pups
- Lactation index = (Number of Live Pups on Day 21 Postpartum x 100)/Number of Live Pups on Day 4 Postpartum
- Post-implantation loss/litter = Number of Implants – Number of Newborn Pups (total).

The litter was the unit of comparison for all F_1 litter data through culling on PND 4. Levene's test was used to assess the homogeneity of group variances. Groups were compared using an overall one-way analysis of variance (ANOVA) F-test if Levene's test was not significant, or the Kruskal-Wallis test if it was significant. If the overall F-test or Kruskal-Wallis test was found to be significant, then pairwise comparisons were conducted using Dunnett's or Dunn's test, respectively. For incidence data, Fisher's exact test was used for pairwise group comparisons.

3. Results

3.1. F_0 generation clinical observations, body weights, and food consumption

3.1.1. Mortality and clinical observations

CBD-related mortality and moribundity were noted in F_0 animals at 300 mg/kg-bw/d. One male exhibited marked body-weight loss (11.5%) from Study Days 21 through 23, salivation, and stained and wet fur, and the animal was found dead on Study Day 24. Also, at the 300 mg/kg-bw/d dose, a total of seven females were euthanized during the study. In general, these animals exhibited erect, stained, and wet fur; skin pallor; and hunched posture, and/or were thin. Pups from these dams were cold to the touch and had no milk band. One female euthanized on Day 25 was nongravid and therefore not included in any further calculations. Two females in the 300 mg/kg-bw/d group exhibited severe maternal toxicity and these females were euthanized *in extremis*, one each on LDs 0 and 2. These dams had severe clinical observations prior to delivery consistent with toxicity observed in other animals, had retained fetuses and/or late resorptions *in utero* at necropsy, and exhibited a lack of maternal care (e.g., not nursing). Based on the pre-existing toxicity, these two litters were excluded from

calculations of Live Birth Index, Live Pups/Litter, and Post Implantation Loss/Litter (Table 4). The other four females euthanized in the 300 mg/kg-bw/d group were included in PND 1 parameters (one *in extremis* due to poor clinical condition (LD 1), two due to total litter losses (LDs 1 and 3), and one with all early resorptions (Day 25)). For parameters calculated starting on PND 4, three total litters were remaining in the 300 mg/kg-bw/d group. A single total litter loss occurred on LD 1 in the control group. The other F_0 animals survived to the scheduled necropsies, except for two females from the 300 mg/kg-bw/d group that were euthanized 25 days after mating—one had all early resorptions,

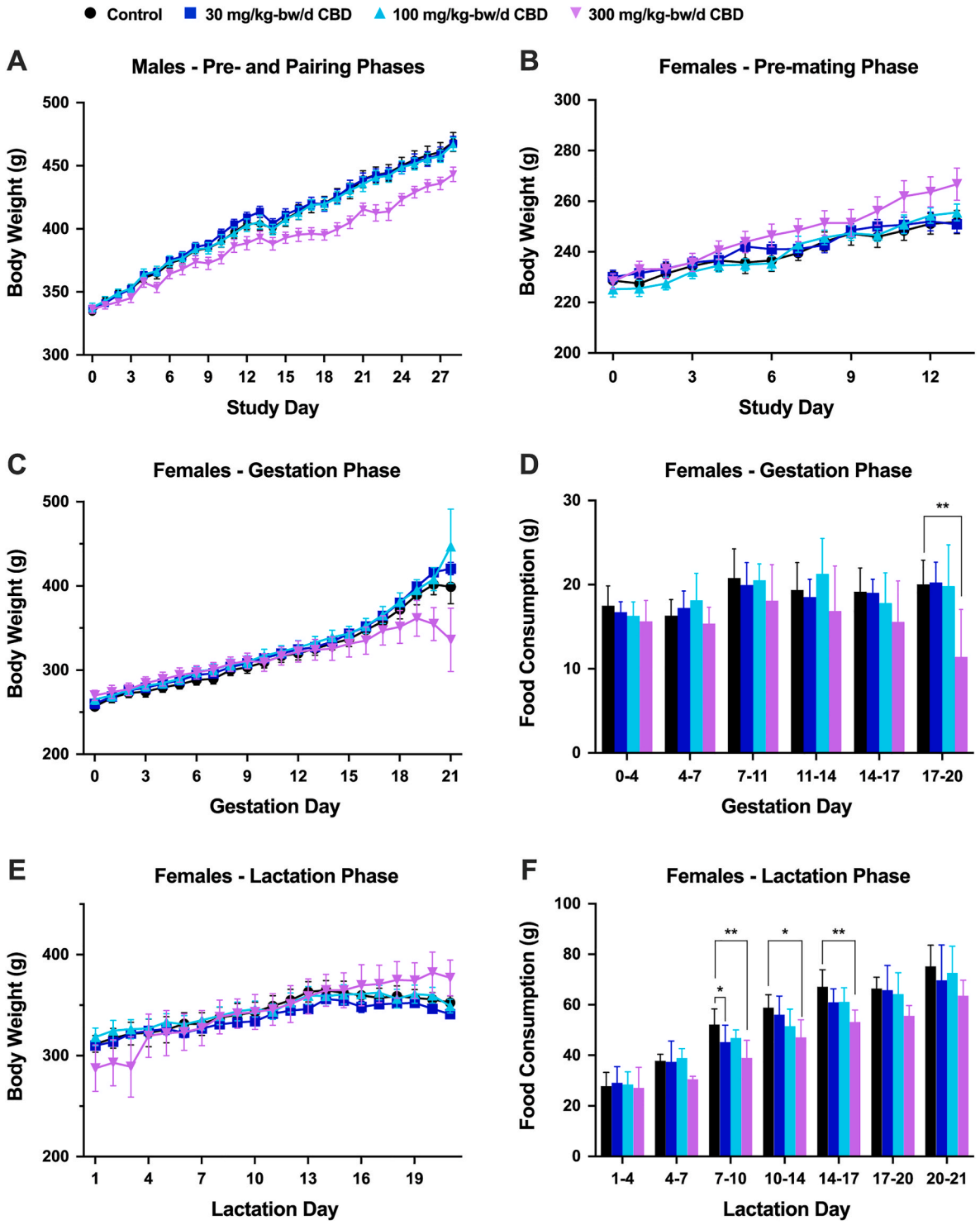

Fig. 1. Body weights and food consumption for F_0 animals. (A) Mean body weights per group for F_0 males; data shown for pre-mating phase through Day 13 and for the pairing/mating phase from Days 14 through 28 (B) Mean body weights per group for F_0 females during the pre-mating phase. (C) Mean body weights per group for F_0 females during the gestation. (D) Mean food consumption per group for F_0 females during the gestation phase (E) Mean body weights per group for F_0 females during the lactation phase. (F) Mean food consumption per group for F_0 females during the lactation phase. All means are shown ±SD. Food consumption is shown as the mean food/animal/d and reported per interval. ANOVA & Dunnett: * = $p \leq 0.05$; ** = $p \leq 0.01$. N = 10/sex/group except for female control group during lactation (n = 9), 300 mg/kg-bw/d males (n = 9), and 300 mg/kg-bw/d females during gestion (n = 9) and lactation (n = 3).

and the other was nongravid. Similar CBD-related adverse clinical observations (erect, stained, and wet fur; skin pallor; hunched posture; and/or thinness) were observed in two females in the 300 mg/kg-bw/d group during late gestation (GDs 12–24) and early lactation (LD 9). Throughout the dosing period, at approximately 2 h following dosing, an increased incidence of salivation and wet fur were noted in the 100- and 300 mg/kg-bw/d group males and females. These observations generally did not persist to the daily examinations and were sporadic at 100 mg/kg-bw/d.

3.1.2. Body weight and food consumption

Prior to pairing and during mating, there was a statistically significant decrease in body weights ($p \leq 0.01$, or 0.05; Fig. 1A) in the 300 mg/kg-bw/d males from Study Days 17–28 compared to those of concurrent contorls; however, these changes were small in magnitude and correlated with a significant decrease in food consumption (Supplementary Table 1A). Body weights and food consumption in males were similar to controls in the 30- and 100 mg/kg-bw/d groups. There was no effect of CBD on female body weights at any dose prior to pairing (Fig. 1B), despite a transient lower mean food consumption at 300 mg/kg-bw/d (Supplementary Table 1B). During gestation, females dosed with 300 mg/kg-bw/d had lower body weights (non-significant; Fig. 1C) and overall, significantly lower food consumption ($p \leq 0.01$; Fig. 1D) than controls from GDs 0–20. Mean body weights and body-weight gains in the 30- and 100 mg/kg-bw/d groups were unaffected by CBD administration during gestation (Fig. 4D; Supplementary Table 2). During lactation (LDs 1–21), there was a nonsignificant increase in mean body weight (Fig. 1E) and significantly lower food consumption (Fig. 1F) in dams at 300 mg/kg-bw/d. However, only three females remained in the highest dosage group after LD 3.

3.2. F_0 reproductive indices, gestation, and parturition

3.2.1. Male and female reproductive indices

No CBD-related effects were observed on precoital interval, estrous cycle length, mating, fertility, or pregnancy indices at any dosage level (Table 2). One mating pair in the 300 mg/kg-bw/d group did not produce a litter.

3.2.2. Gestation length and parturition

Mean gestation lengths in all CBD-treated groups were similar to those in the control group. There were no significant differences in the mean number of implantation sites or proportions of postimplantation loss in the CBD-treated groups compared to controls. As discussed in Section 3.1.1, two females in the 300¬mg/kg-bw/d group that exhibited severe maternal toxicity (with possible dystocia) were euthanized, one each on LDs 0 and 2. There were no effects on parturition or clinical condition of the dams during delivery in the 30- and 100 mg/kg-bw/d groups. Adverse clinical findings were noted for two other females in the 300 mg/kg-bw/d group during early lactation.

Table 2
F_0 male and female reproductive performance parameters.

Parameter	Dose (mg/kg-bw/d)			
	0	30	100	300
Male Mating Index (%)	100.0	100.0	100.0	100.0
Female Mating Index (%)	100.0	100.0	100.0	100.0
Male Fertility Index (%)	100.0	100.0	100.0	90.0
Female Fertility Index (%)	100.0	100.0	100.0	90.0
Male Pregnancy Index (%)	100.0	100.0	100.0	90.0
Female Pregnancy Index (%)	100.0	100.0	100.0	90.0
Estrous Cycle Length (days)	4.00 ± 0.24	4.40 ± 0.52	4.87 ± 1.93	4.38 ± 0.90
Pre-Coital Interval (days)	1.9 ± 1.1	3.0 ± 1.9	2.9 ± 4.0	2.9 ± 2.8

Average parameters are shown as mean ± standard deviation, derived from n = 10 females per group.
See Methods section for detailed description of parameters.

Table 3
F_0 male testosterone and sperm motility, concentration, and morphology.

Parameter	Dose (mg/kg-bw/d)			
	0	30	100	300
Testosterone (ng/dL)	451.9 ± 190.8	326.9 ± 106.8	431.9 ± 175.8	719.6 ± 512.4[a]
Caudal Epididymis, Weight (g)	0.24 ± 0.027	0.22 ± 0.031	0.21 ± 0.038	0.25 ± 0.042
Sperm Concentration (millions/g)	493.8 ± 108.90	464.3 ± 118.16	458.1 ± 144.61	424.3 ± 75.49
Motility (%)	67 ± 20.1	65 ± 16.8	77 ± 10.0	72 ± 8.2
Normal (%)	99.3 ± 0.89	99.6 ± 1.26	99.9 ± 0.17	99.8 ± 0.36
Normally Shaped Head Separated from Flagellum (%)	0.2 ± 0.37	0.4 ± 0.94	0.0 ± 0.00	1.1 ± 0.22
Head Absent with Normal Flagellum (%)	0.6 ± 0.73	0.1 ± 0.32	0.1 ± 0.17	0.1 ± 0.22
Abnormal Head (%)	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Abnormal Flagellum (%)	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Other (%)	0 ± 0	0 ± 0	0 ± 0	0 ± 0

[a] The high nominal value (not significantly different from controls) and variability due to two males with values above the upper limit of quantitation. Parameters are shown as mean ± standard deviation, derived from n = 8–10 males per group. All parameters were measured at termination following the end of the mating period and at least 28 days of CBD administration.

3.3. F_0 male testosterone, caudal epididymis weight, and sperm evaluation

There were no statistically significant differences in serum testosterone between control and CBD-treated F_0 males of the low- and mid-dose groups (Table 3). The high nominal value (not significantly different from controls) and variability for testosterone in the 300 mg/kg-bw/d group was due to two males with values above the upper limit of quantitation. Caudal epididymis weight was similar in controls and all CBD-treated groups. All measured sperm parameters were similar between control and CBD-treated males. There were low incidences in all groups of normal sperm heads separated from flagella and normal flagella with heads missing; however, no abnormal sperm heads or sperm flagella were observed in controls or any CBD-treated group.

3.4. F_0 organ weights and histopathology

Mean absolute liver weights (Fig. 2A) and liver weight relative to body or brain weights (Supplementary Tables 1A and 1B) were higher than controls in the 100- and 300 mg/kg-bw/d group F_0 males and females. Mean adrenal gland weight (Fig. 2B) and adrenal gland weight relative to body or brain weight were higher than controls in the 100- and 300 mg/kg-bw/d group males and 300 mg/kg-bw/d group females (Supplementary Tables 1A and 1B). Higher liver weights correlated with noted liver enlargement and microscopic findings of hepatocellular hypertrophy. Higher adrenal gland weights correlated with microscopic findings of adrenal cortical hypertrophy, noted adrenal gland enlargement, and/or pale discoloration. Thyroid plus parathyroid weights (after fixation) were not different between controls and CBD-treated groups (Fig. 2C), although minimal to moderate epithelial hypertrophy/hyperplasia was noted in the 100- and 300 mg/kg-bw/d groups. No other CBD-related organ-weight changes were noted in F_0 animals. Other sporadic organ-weight differences observed were considered incidental and not treatment-related.

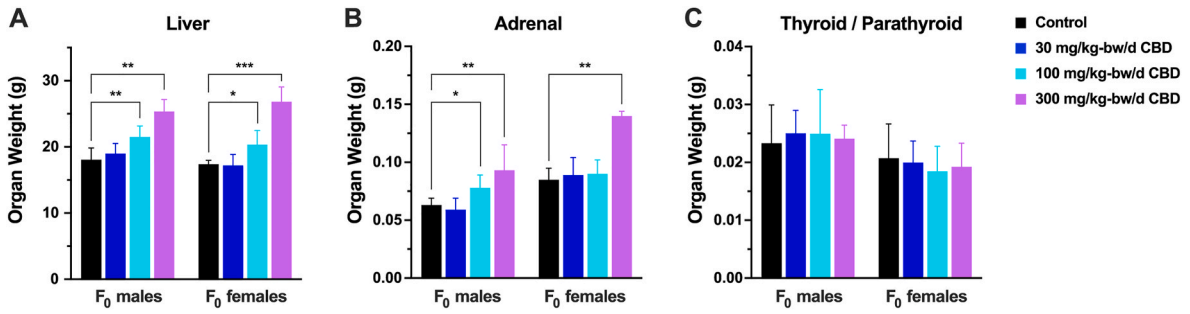

Fig. 2. Selected organ weights for F_0 animals. (A) Mean liver weights by group for F0 males and females. (B) Mean adrenal weights by group for F_0 males and females. (C) Mean thyroid/parathyroid weights by group for F_0 males and females. All means are shown ±SD. Dunnett's test: * = ≤0.05; ** = ≤0.01.

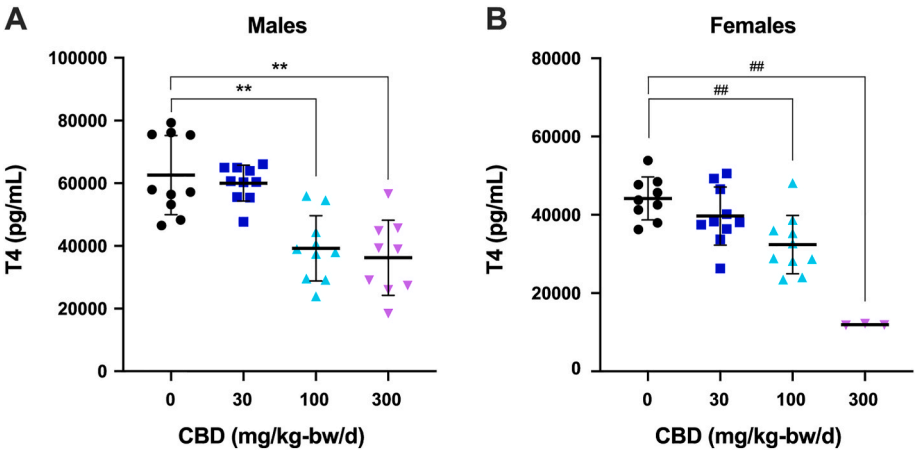

Fig. 3. F_0 Thyroxine (T4) concentrations. (A) Mean T4 concentrations measured in F_0 males on Day 28. (B) Mean T4 concentrations measured in F_0 females on LD 21. All means are shown ±SD. Kruskal-Wallis & Dunn: ** = $p \leq 0.01$; ANOVA & Dunnett: # = $p \leq 0.05$; ## = $p \leq 0.01$.

3.5. F_0 thyroid hormones

Mean T4 concentrations were significantly lower than controls in F_0 males of the 100- and 300¬mg/kg-bw/d groups (Fig. 3A), although these T4 values were within the range of Charles River Ashland (2020) historical control data. Mean T4 concentration in the 30 mg/kg-bw/d group F_0 males was not significantly different from the control group.

In F_0 females, mean T3 concentration on LD 21 was significantly lower than control levels in the 300¬mg/kg-bw/d group (180.3 ± 16.7 pg/mL vs. 316.6 ± 39.3 pg/mL in controls). Mean T4 concentrations on LD 21 in the 100- and 300¬mg/kg-bw/d groups (32,410.0 ± 7460.9 pg/mL and 11,966.7 ± 208.2 pg/mL, respectively) were significantly lower than concurrent control levels (44,200.0 ± 5466.5 pg/mL) (Fig. 3B). The mean T4 concentration in F_0 females at 300 mg/kg-bw/d (11,966.7 ± 208.2 pg/mL), but not at 100 mg/kg-bw/d, was below the minimum mean value in the Charles River Ashland (2020) historical control data (27,770.00 pg/mL). Mean T3 concentrations in the 30- and 100¬mg/kg-bw/d groups F_0 females, and T4 concentrations in the 30 mg/kg-bw/d group F_0 females, were similar to the control group (Supplementary Table 4).

4. F_1 litter data

4.1. PND 0 litter data and postnatal survival

4.1.1. Litter outcomes

There were no significant differences in live birth index, post-implantation loss, or average number of pups (male and/or female) per litter. Mean birth weight was similar across all groups for male pups, while female pups in the 300 mg/kg-bw/d had a significantly lower birth weight than controls (Table 4).

4.1.2. Postnatal survival

Neonatal survival to PND 4 in the in the 300 mg/kg-bw/d group (45.05%) was significantly lower than in the control group (88.89%) (Table 4). These differences were due to two F_0 females in the high-dose group that were euthanized on LDs 0 and 3 following total litter losses, and three females that were euthanized *in extremis* between LD 0 and 2, along with their remaining pups that were pale, cold to the touch, had no milk band, and/or had labored breathing. Survival of the remaining pups from PND 4 to weaning on PND 21 in the 300 mg/kg-bw/d group was comparable to the control group. Postnatal survival to weaning was unaffected by CBD administration in the 30- and 100 mg/kg-bw/d groups. The mean number of pups born and the percentage of males at birth in the 30-, 100-, and 300 mg/kg-bw/d groups were similar to the control-group values. Two pups (from two litters), nine (from four litters), four (from three litters), and sixty (from seven litters) in the control, 30-, 100-, and 300¬mg/kg-bw/d groups, respectively, were found dead or were euthanized *in extremis*. Two pups (from one litter) and one pup each in the 100- and 300¬mg/kg-bw/d groups, respectively, were missing, and five pups (from one litter) in the 300 mg/kg-bw/d group were euthanized due to death of the dam.

4.1.3. Offspring body weights

Male and female pup mean birth weights (PND 1) in the 300 mg/kg-bw/d group were lower (14.39% and 22.06%, respectively) than the control group; the difference was statistically significant for females (Table 4). F_1 male and female pup body-weight gains in this group were lower than the control group throughout the pre-weaning period and mean absolute body weights for males and females were up to 36%

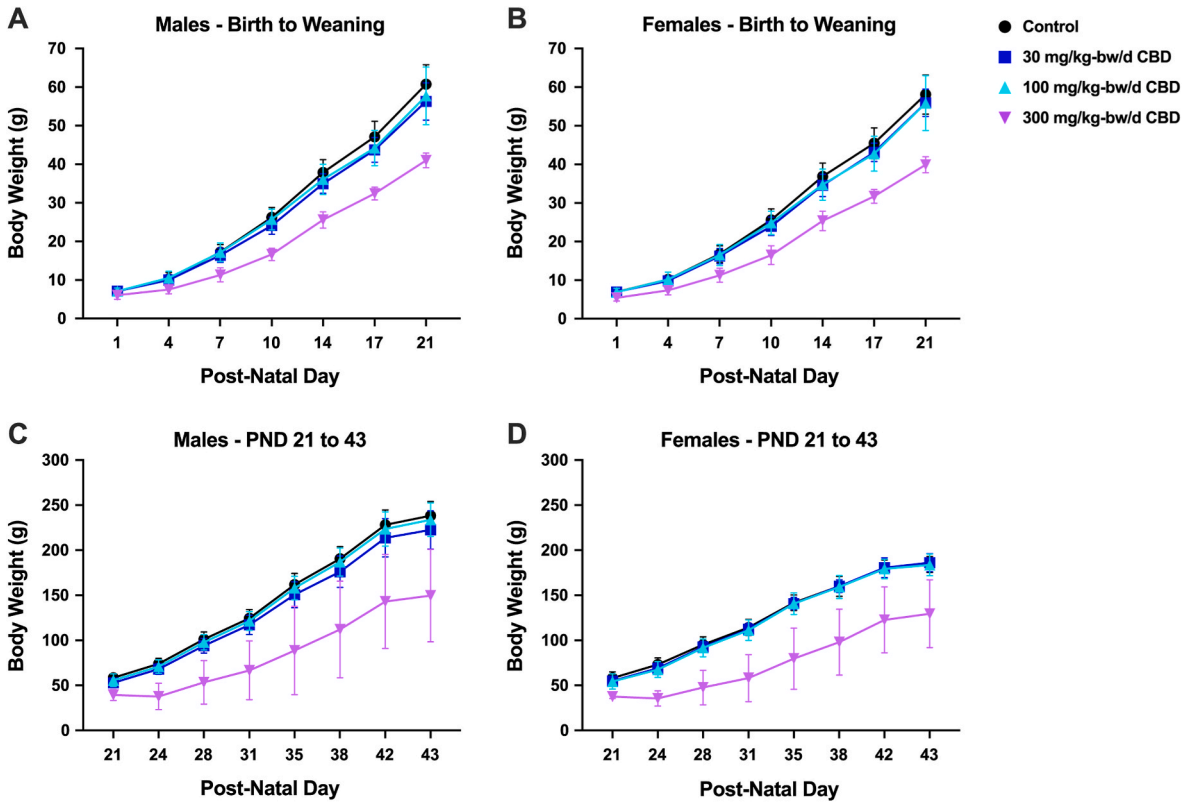

Fig. 4. F_1 Offspring body weight. (A) Mean body weights per group for F_1 males from birth to weaning. (B) Mean body weights per group for F_1 females from birth to weaning. (C) Mean body weights per group for F_1 males post-weaning from post-natal day 21 through 43. (D) Mean body weights per group for F_1 females post-weaning from postnatal day 21 through 43. All means are shown ±SD; statistics calculated using ANOVA and Dunnett test. Starting on PND 21, the number of pups representing a total of 5 litters each were: 9/sex (control), 10 males and 9 female (30 mg/kg-bw/d), and 10/sex (100 mg/kg-bw/d). In the 300 mg/kg-bw/d group, 3 pups/sex represented three litters.

Table 4
F_1 litter outcomes and postnatal survival.

Parameter	Dose (mg/kg-bw/d)			
	0	30	100	300[a]
Live Birth Index	100 ± 0	100 ± 0	100 ± 0	100 ± 0
Post-Implantation Loss/Litter	0.8 ± 0.9	0.5 ± 0.7	1.1 ± 1.7	0.2 ± 0.4
Mean Number of Live Pups/Litter (Day 1)				
Males	5.1 ± 3.2	7.9 ± 2.2	6.2 ± 2.7	4.0 ± 3.4
Females	7.1 ± 3.9	7.0 ± 2.1	6.5 ± 2.8	4.8 ± 2.0
Mean Pup Birth Weight (g)				
Males	7.16 ± 0.80	7.21 ± 0.96	7.20 ± 0.84	6.13 ± 1.06
Females	6.94 ± 0.90	6.98 ± 0.88	6.90 ± 1.04	5.41 ± 0.80*
Viability Index (PND 1–4)	88.89 ± 31.43	95.25 ± 7.71	96.21 ± 5.47	45.04 ± 49.91#
Lactation Index (PND 4–21)	100 ± 0	97.5 ± 7.91	100 ± 0	95.83 ± 7.22

Parameters are shown as mean ± standard deviation, derived from n = 9–10 for 0, 30, and 100 mg/kg-bw/d groups. For the 300-mg/kg-bw/d group, n = 6 except for male Mean Pup Birth Weight (n = 5) and Lactation index (n = 3). Lactation Index calculated post-culling. ANOVA and Dunnett: * = $p \leq 0.05$. Kruskal-Wallis and Dunn: # = $p \leq 0.05$.

[a] Two females were euthanized *in extremis* due to severe maternal toxicity on Lactation Day 0 and 2, respectively, with conceptuses retained *in utero*. Therefore, the Total Number Newborn Pups and Number Live Newborn Pups for these females were excluded from the calculations. As a result, the following parameters were not calculated for these litters: Live Birth Index, Live Pups/Litter, and Post-Implantation Loss/Litter. See Section 3.1.1 for additional details.

lower in the 300 mg/kg-bw/d group than in the control group during the pre-weaning period (Fig. 4A and B). Mean body weights for males in the 300 mg/kg/d group were statistically significantly decreased ($p \leq 0.01$, or 0.05) from postnatal day 4 through 21 (Fig. 4A). Mean body weights for females in the 300-mg/kg/d group were statistically significantly decreased ($p \leq 0.01$, or 0.05) on postnatal day 1 and from postnatal day 4 through 21, relative to controls (Fig. 4B). Mean F_1 male and female body weights and body-weight changes in the 30- and 100 mg/kg-bw/d groups during the preweaning period were similar to controls (Fig. 4A and B). Mean body weights for males in the 300¬mg/kg/d group were statistically significantly decreased ($p \leq 0.01$, or 0.05) from postnatal day 21 through 43 (Fig. 4C). Mean body weights for females in the 300-mg/kg/d group were statistically significantly decreased ($p \leq 0.01$, or 0.05) from postnatal day 21 through 43 (Fig. 4D).

4.1.4. Anogenital distance (AGD) and areolae/nipple anlagen retention

The AGD (absolute and relative to the cube root of pup body weight) in the 30-, 100-, and 300¬mg/kg-bw/d groups were similar to the control-group values (Supplementary Tables 5A and 5B). Areola/nipple anlagen in the F_1 male pups were evaluated on PND 13, and no areolae or nipples were noted.

4.1.5. Serum thyroid hormone concentrations on PNDs 4 and 21

Mean T3 and T4 concentrations in F_1 culled pups (pooled by litter) on PND 4 were lower in the 100¬mg/kg-bw/d (166.2 ± 27.1 and 20,377.8 ± 3347 pg/mL, respectively) and 300 mg/kg-bw/d (126.0 ± 9.8 and 16,933.3 ± 4046 pg/mL, respectively) groups compared to the control group (192.1 ± 20.2 and 25,425.0 ± 4422.3 pg/mL, respectively); differences were statistically significant at 300 mg/kg-bw/

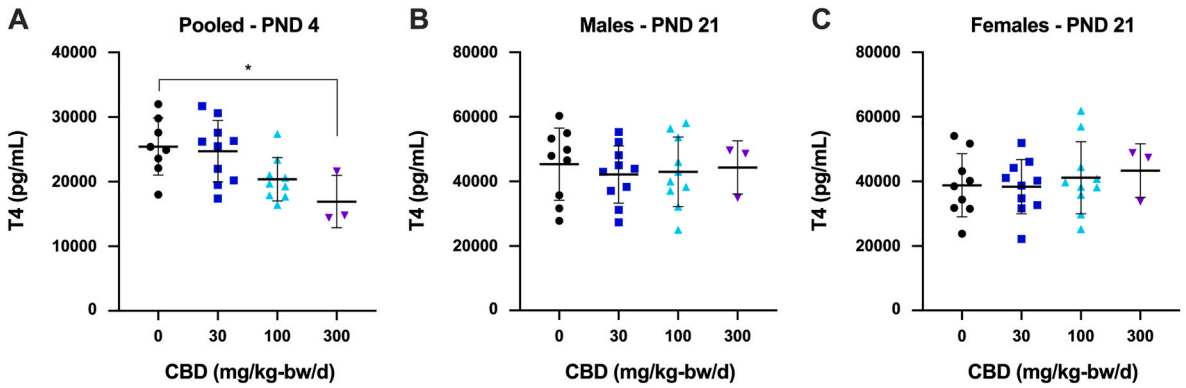

Fig. 5. F_1 Thyroxine (T4) concentrations. (A) T4 concentration of F1 litters pooled from animals culled per litter on PND 4. (B) Mean T4 concentrations of F1 males on PND 21. (C) Mean T4 concentrations of F1 females on PND 21. ANOVA and Dunnett: * = $p \leq 0.05$. On PND 21, the number of pups representing a total of 5 litters each were: 9/sex (control), 10 males and 9 females (30 mg/kg-bw/d), and 10/sex (100 mg/kg-bw/d). In the 300 mg/kg-bw/d group, 3 pups/sex represented three litters.

d (Fig. 5A; Supplementary Table 6). On PND 4, the mean T3 concentration at 300 mg/kg-bw/d, but not 100 mg/kg-bw/d, was below the minimum mean value in the Charles River Ashland (2020) historical control database (158.3 pg/mL). Mean T3 and T4 concentrations in the 30 mg/kg-bw/d group PND 4 culled pups were similar to the control group. There were no CBD-related effects on thyroid hormone concentrations in the F_1 males and females on PND 21 at any maternal dosage level (Fig. 5B and C).

4.1.6. Organ weights

In the 300 mg/kg-bw/d group, significant decreases in absolute epididymides (left and right), testis (right), and ovary/oviduct occurred (data not shown). Of note, only slightly lower mean absolute and higher mean relative (to final body weight) thyroid/parathyroid weights were noted in F_1 males and females in the 300¬mg/kg-bw/d group on PND 21, but these differences were not statistically significant.

4.2. F_1 generation post-weaning

4.2.1. F_1 post-weaning mortality and moribundity

There were no CBD-related effects on mortality or moribundity in the F_1 generation at any post-weaning dosage level; however, due to mortality at 300 mg/kg-bw/d during the preweaning period, only six animals (three/sex, representing three litters) were available for evaluation. In the 300¬mg/kg-bw/d group F_1 males and females, clinical findings similar to those seen in the F_0 generation were noted, including skin pallor, thinness, and partially closed eyes. No CBD-related clinical findings were noted for F_1 generation males and females in the 30-and 100¬mg/kg-bw/d groups.

4.2.2. F_1 post-weaning body weights

After weaning on PND 21, offspring were dosed by oral gavage from PND 21 through 42 at the same dosages administered to the F_0 parental males and females. In the 300 mg/kg-bw/d group, mean body-weight losses or lower mean body-weight gains were noted for F_1 males and females generally throughout the dosing period, resulting in lower mean body-weight gains when the entire post-weaning period (PNDs 21–43) was evaluated (Supplementary Tables 7A and 7B). Mean absolute body weights, but not body-weight gains, for males and females were statistically significantly lower (up to 48% and 50%, respectively), than the control group (Fig. 4C and D). No test-substance-related effects on mean body weight or body-weight gain were noted in F_1 males or females in the 30- and 100 mg/kg-bw/d groups.

4.2.3. F_1 male testosterone analysis

There were no statistically significant differences in testosterone concentrations between controls and CBD-treated F_1 males at any dose level (Supplementary Table 8).

4.2.4. F_1 organ weights

The 300 mg/kg-bw/d group had significantly lower mean absolute epididymides and right testis weights, higher mean relative (to body weight) brain and liver weights, and lower mean relative (to brain weight) epididymides and testis weights. Females in this group had significantly lower mean absolute and relative (to brain weight) ovary/oviduct weights and higher mean (relative to body weight) liver weights compared to controls. There were no CBD-related effects on organ weights in the 30- and 100 mg/kg-bw/d groups (Supplementary Tables 9A and 9B)).

5. Discussion

With increasing availability and public interest in CBD-containing products, it is critical that CBD safety be well investigated, with results widely disseminated in peer-reviewed publications. The present study addresses a critical gap in CBD research–the potential adverse effects on male and female reproduction and offspring development. This research was conducted within the scope of a modified screening study and is the first published standard, guideline-compliant reproductive toxicity study on pure hemp-derived CBD. In this study, OECD Test Guideline No. 421 (OECD, 2016) was modified to include extended postnatal dosing through PND 42 and hormone analysis (testosterone and thyroid hormones). It should be noted that the major circulating metabolite after CBD ingestion in humans is 7-COOH-CBD, whereas CBD is the primary compound measured in rats, followed by 7-COOH-CBD (Deabold et al., 2019; Harvey et al., 1991; CDER, 2018a). Despite these differences, rats appear to be the most appropriate non-primate model for investigating toxicological effects of CBD, as studies in dogs show that 7-COOH-CBD is not a prominant metabolite (CDER, 2018a; Vaughn et al., 2020). Nevertheless, these differences in circulating metabolites should be considered when applying the findings of our study in rats for human health risk assessment purposes.

Dose selection for the current study was based on existing pre- and postnatal toxicity studies conducted with CBD and other CBD-containing test materials. The high dose of 300 mg/kg-bw/d was chosen based on the doses of up to 250–300 mg/kg-bw/d tested in the most relevant available studies (reviewed by CDER, 2018a; study numbers GWTX1456[1] and GWTX1532[2]). Overall, litter parameters and postnatal effects following CBD exposure have only previously been investigated in these and other studies reviewed by FDA (CDER, 2018a), therefore these studies serve as the primary basis for discussion for such effects in our study. Where relevant, data from other study paradigms are also

included for these parameters and are discussed extensively in the context of male reproductive effects.

Treatment-related mortality and moribundity were observed in F_0 animals receiving 300 mg/kg-bw/d, including severe maternal toxicity during pregnancy and lactation. This finding provides important information regarding systemic toxicity in parental animals, in that the previous reproductive toxicity studies reviewed by (CDER, 2018a) that served as the primary basis for dose selection in the current study reported adverse effects, but not severe toxicity, at doses up to 250 mg/kg bw/d.

Treatment-related effects on organ weights and histopathology of the F_0 males and females in this study are concluded to be nonadverse. The constellation of liver changes (e.g., liver enlargement, increased liver weights, and hepatocellular hypertrophy) in F_0 male and female rats at both 100 and 300 mg/kg-bw/d suggests induction of both phase 1 and phase 2 metabolic enzymes involved in thyroid hormone elimination (Papineni et al., 2015; Noyes et al., 2019). Similar changes were noted in a recent 90-day repeat-dose study conducted in male and female rats in which centrilobular hepatocellular hypertrophy was observed and found to be fully resolved following a 28-day recovery period (reported in our companion paper—Henderson et al., 2023b). Hepatocellular hypertrophy without histopathological or other changes indicative of liver toxicity, as is the case with CBD both the present study and in the aforementioned companion paper, is considered adaptive and non-adverse, as described in a review by Hall et al. (2012).

Administration of 100 or 300 mg/kg-bw/d CBD to F_0 male and female rats also resulted in minimum to moderate thyroid hyperplasia/hypertrophy. Although thyroid weights were not changed, these thyroid lesions correlated with significant decreases in serum T4 (male and female at 100 and 300 mg/kg-bw/d) and T3 (females only at 300 mg/kg-bw/d) concentrations; however, only T4 concentrations in females of the high-dose group were below historical control values (Charles River Ashland, 2020). Changes in thyroid hormone levels were considered to be secondary to the adaptive liver changes observed in this study. One possible pathway for the effects of CBD on thyroid hormones may be hepatic microsomal enzyme induction (as evidenced by centrilobular hepatocellular hypertrophy and increased liver weights), with a corresponding increase in thyroid hormone clearance and thyroid follicular cell hypertrophy, a hypothesis previously considered in the review of Epidiolex clinical data (CDER, 2018b). There are several pathways by which chemicals can produce antithyroid effects by perturbing thyroid-pituitary homeostasis and reducing circulating thyroid hormones, increasing thyroid stimulating hormone (TSH) levels, and inducing thyroid hyperplasia/hypertrophy (Hurley et al., 1998; Zabke et al., 2011; Noyes et al., 2019; Huisinga et al., 2020). One pathway involves chemical induction of thyroid hormone conjugation to glucuronic acid by uridine diphosphate glucuronosyltransferase (UDPGT), resulting in increased elimination and decreased serum concentrations of T3 and T4 (Papineni et al., 2015; Noyes et al., 2019). The pattern of liver changes observed in these CBD studies may reflect hepatic microsomal enzyme induction, including UDPGT activity. Also of critical importance is that this liver induction is adaptive; this is reflected in the resolution of the liver lesions when CBD exposure ends, as demonstrated in our 90-day study (Henderson et al., 2023b).

Although T3 and T4 levels in F_1 culled pups (pooled) at PND 4 were lower in the two highest dose groups compared to the controls, differences were only statistically significant at 300 mg/kg-bw/d CBD. These decreased levels observed in the high-dose group may be related to the persistent maternal toxicity and corresponding reduced pup weights observed in this group. In addition, thyroid hormone concentrations were similar across all F_1 groups on PND 21. Changes in thyroid hormone levels on PND 4 were not considered toxicologically significant; such changes have been suggested to indicate slight disturbances of normal homeostasis and therefore may not be biologically significant (Beekhuijzen et al., 2019). Absence of significant developmental neurobehavioral changes in other studies further reduces concern about the limited changes in thyroid hormones. For example, in a pre- and postnatal study conducted in Wistar rats, while thyroid hormone levels were not assessed, doses up to 250 mg/kg-bw/d CBD did not cause any consistent effects on learning or memory on PND 65 (reviewed by CDER, 2018a).

Some previously observed effects were replicated in the current study, such as increased pup mortality and lower pup weight in the high-dose group (reviewed in CDER, 2018a). Lower postnatal survival in the high-dose group was observed, including the two litters with total litter loss and three litters euthanized *in extremis*. Mean pup weights in this group were lower than those of controls, which correlated with decreases in some organ weights. For surviving litters, there were no effects on other developmental parameters, including anogenital distance and areola/nipple retention. However, many findings reported elsewhere, including decreased testis weight, changes in preimplantation loss, and developmental delays (e.g., as reviewed by CDER, 2018a; Dalterio et al., 1984b; Rosenkrantz et al., 1981), were not observed in the present study, even at the high dose of 300 mg/kg-bw/d. Studies reporting these effects did not follow standard guidelines, and in some cases are more than 40 years old; as such, limitations in study design may account for inconsistencies in results. In the current OECD guideline compliant study, no CBD treatment-related effects were observed on F_0 male or female reproductive performance at any dose, and mean gestation lengths were similar between control and CBD-treated groups.

A limitation of this study is that it was designed as a screening study and not a generational reproductive toxicity study (e.g., two-generation or extended one-generation). However, as described by Beekhuijzen et al. (2014), key differences between the current OECD 421 (2016) screening study protocol and generational studies are primarily a lack of a second generation and a limited postnatal period. These authors concluded that only 3% (4 of 134) reproductive toxicity screening studies failed to provide definitive results. The shorter postnatal period is partially addressed in the current study, which extended postnatal dosing out to PND 42. Moreover, Piersma et al. (2011) found that second-generation mating and F_2 offspring data rarely provide additional critical information. In this retrospective analysis of 498 multi-generational studies, no critical differences in sensitivities between generations were found, supporting reliance on the one-generation study. Guidance Document 117 on the Current Implementation of Internal Triggers in Test Guideline 443 for an Extended One Generation Reproductive Toxicity Study, in the United States and Canada (OECD, 2011), presents trigger criteria for needing a second generation, including effects on adults (fertility and estrous cycle) and offspring (litter parameters, developmental landmarks, survival, malformations, live birth index, and body weight). According to these criteria, none of the findings in the current study would have triggered a second generation, because the affected endpoints were driven by severe maternal toxicity. F_1 visceral and skeletal malformations were not analyzed in this study; however, studies reviewed as part of the Epidiolex submission inconsistently found increased fetal variations across gestational exposure studies with CBD at doses up to 250 mg/kg-bw/d (CDER, 2018a).

The male reproductive NOAEL of 300 mg/kg-bw/d under the conditions of this study is an important finding, given that much of the extant research has focused on the male as being critical to understanding the reproductive toxicity of CBD. In a recent narrative review published by Carvalho et al. (2020), the authors concluded that CBD caused male reproductive toxicity, including impaired sexual behavior, reduced testosterone levels, testicular cell degeneration, and decreased fertilization rates. However, the authors acknowledged that data are "still limited, and additional research is required to fully elucidate the mechanisms of action, as well as the reversibility of CBD effects on the reproductive system." In addition to these data gaps, understanding the exposure levels associated with adverse effects is critical to determining a safe level of CBD exposure in consumers. Decreased testosterone in males has been reported in various studies, most involving short-term

exposures (e.g., single day; Dalterio et al., 1984a) and none being standard toxicology assessments. Testosterone levels were decreased in mice receiving oral CBD for 34 days at 30 mg/kg-bw/d but not at 15 mg/kg-bw/d in one study published by Carvalho et al. (2018a) but not in a more recent study published by the same laboratory using the same dosing regimen (Carvalho et al., 2022). In addition, no changes in testosterone concentrations were observed in mice given 50 mg/kg-bw/d CBD orally for 5 weeks (Dalterio et al., 1982). Conversely, testosterone levels were decreased in monkeys receiving oral CBD for 90 days at 300 mg/kg-bw/d but not at 30 or 100 mg/kg-bw/d (Rosenkrantz and Esber, 1980). Carvalho et al. (2018b) reported that exposure of male mice to 15 mg/kg-bw/d CBD for 34 days impaired sexual performance, but exposure to 30 mg/kg-bw/d improved sexual performance. This contrasts with the study by Dalterio et al. (1982), in which 50 mg/kg-bw/d CBD for 50 days in males was associated with reduced impregnation of females.

In the current guideline study, no treatment-related effects on testosterone levels or the testes were seen in F_0 or F_1 males. This finding agrees with available repeat-dose studies in mice, in which CBD doses up to 30–50 mg/kg-bw/d did not affect testes weights (Dalterio et al., 1982; Patra and Wadsworth, 1991; Carvalho et al., 2018b, 2022), whereas other studies reported a decrease in testis weight (Rosenkrantz et al., 1981; Dalterio et al., 1984b). In the current study, sperm analysis was added to further investigate and compare against the findings of Rosenkrantz et al. (1981). Changes in sperm quality and spermatogenesis were reported previously in mice treated with CBD up to 30 and 50 mg/kg-bw/d for 34–35 days (Patra and Wadsworth, 1991; Carvalho et al., 2018a, 2022). In a recent OECD guideline compliant study, Marx et al. (2018) performed a series of studies on the effects of an orally dosed, supercritical fluid extract of the aerial parts of *Cannabis sativa* (26% phytocannabinoids [96% CBD, <1% THC]) in rats. Total sperm count, sperm morphology, and percentage of motile and immotile sperm were found to be similar between control and high-dose males. The findings of Marx et al. (2018) are similar to those reported here, including no changes in sperm motility, viability, morphology, or enumeration in rats dosed with up to 300 mg/kg-bw/d for up to 42 days in the F_0 generation. While no effects on spermatogenesis were observed in the current study, the duration of our study did not encompass a full spermatogenic cycle. As such, and per the OECD (2016) guidelines, these data do "not provide evidence for definite claims of no effects" on sperm parameters. No impact of up to 300 mg/kg-bw/d CBD was observed on reproductive performance in rats, including fertility, in the current study.

6. Conclusion

To aid in the determination of a safe level of CBD intake for consumers, we investigated the potential adverse effects of CBD on male and female reproduction and offspring development in a modified screening study. Exposure to 300 mg/kg-bw/d CBD resulted in treatment-related mortality and decreased body weight in the parental generation. Hepatocellular hypertrophy in the F_0 100 and 300 mg/kg-bw/d groups correlated with thyroid hypertrophy/hyperplasia, as well as hormone changes at the high dose. Body weights were also decreased in F_1 pups in this group; however, no other developmental parameters were adversely affected by CBD administration. While maternal toxicity was associated with adverse reproductive measures in the high-dose group, no effects on male reproductive toxicity were found. However, definitive conclusions regarding effects on sperm parameters could not be made due to limitations in study design. Based on the endpoints evaluated in this study, the following NOAELs were identified for CBD isolate: 100 mg/kg-bw/d for F_0 male and female systemic toxicity and female reproductive toxicity, 300 mg/kg-bw/d for F_0 male reproductive toxicity, and 100 mg/kg-bw/d for F_1 neonatal and F_1 generation toxicity.

CRediT authorship contribution statement

Rayetta G. Henderson: Conceptualization, Methodology, Writing – original draft, Writing – review & editing. **Brian T. Welsh:** Methodology, and monitoring, Writing – original draft, Writing – review & editing. **John M. Rogers:** Writing – original draft, Writing – review & editing. **Susan J. Borghoff:** Methodology, Writing – review & editing. **Kristen R. Trexler:** Conceptualization, Writing – review & editing. **Marcel O. Bonn-Miller:** Conceptualization, Supervision. **Timothy W. Lefever:** Conceptualization, Writing – review & editing.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: This work was funded by Canopy Growth Corporation. Authors KRT, TWL, and MOB-M were employees of Canopy Growth Corporation during the conduct and drafting of this study; during their employment, they received stock options. ToxStrategies, a private consulting firm providing services on toxicology and risk assessment issues, received funds for conducting this work. Authors RGH, JMR, and SJB are employees of ToxStrategies; and author BTW was an employee of ToxStrategies during the conduct and drafting of this study.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fct.2023.113786.

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EXHIBIT C

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Oral toxicity evaluation of cannabidiol

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ABSTRACT

Use of cannabidiol (CBD) in humans has increased considerably in recent years. While currently available studies suggest that CBD is relatively safe for human consumption, data from publicly available studies on CBD conducted according to modern testing guidelines are lacking. In the current study, the potential for toxicity following repeated oral exposure to hemp-derived CBD isolate was evaluated in male and female Sprague Dawley rats. No adverse treatment-related effects were observed following administration of CBD via oral gavage for 14 and 90 days at concentrations up to 150 and 140 mg/kg-bw/d, respectively. Microscopic liver and adrenal gland changes observed in the 90-day study were determined to be resolved after a 28-day recovery period. CBD was well tolerated at these dose levels, and the results of this study are comparable to findings reported in unpublished studies conducted with other CBD isolates. The current studies were conducted as part of a broader research program to examine the safety of CBD.

1. Introduction

Cannabis sativa L. and cannabis-derived products in various forms have been used widely throughout the world for thousands of years for medicinal and recreational purposes (Bergamaschi et al., 2011; Rupasinghe et al., 2020). While delta-9-tetrahydrocannabinol (THC), the primary psychoactive component of cannabis, has historically been the primary focus of much research, attention has also turned to other phytocannabinoids and terpenes. In particular, cannabidiol (CBD), a non-intoxicating phytocannabinoid, has received much recent attention from both the general public and the scientific community for its purported anticonvulsive, analgesic, anti-anxiety, neuroprotective, antioxidant, and antimicrobial properties (Small and Marcus, 2002; Pertwee, 2004; Billakota et al., 2019). Epidiolex® (active ingredient CBD isolate) has been approved by the United States (US) Food and Drug Administration (FDA) for the treatment of seizures associated with Lennox-Gastaut syndrome and Dravet syndrome in patients 2 years of age and older (Jazz Pharmaceuticals, 2023). In addition, Sativex®, a combination of CBD and THC, is approved in other countries for the treatment of moderate to severe spasticity due to multiple sclerosis (Jazz Pharmaceuticals, 2023).

Cannabidiolic acid (CBDa), typically the most common phytocannabinoid in fiber (hemp) plants, is converted to CBD through decarboxylation (Formato et al., 2020; Rupasinghe et al., 2020). CBD and its metabolites identified in human plasma have been shown to possess low affinity and lack appreciable functional activity at classical cannabinoid receptors 1 and 2 (CB1 and CB2; CDER, 2018a). A substantial body of data exists that describes the different pharmacodynamic properties of CBD and its modulation of targets unrelated to the endocannabinoid system (ECS), such as serotonin 1a (5HT1a) (Russo et al., 2005; Gomes et al., 2011). CBD has the ability to interact with multiple 7-transmembrane receptor systems, ion channels, transporters, and enzymes (Small and Marcus, 2002; Pertwee, 2004). Although a number of other targets have been identified *in vitro*, their potential physiological implications are currently theoretical.

Following implementation of the Hemp Farming Act, part of the Agricultural Improvement Act of 2018 (aka, "2018 Farm Bill"), interest in hemp-derived products, especially CBD, has outpaced the development of a legal pathway for their use in foods and dietary supplements in the US. Data submitted to FDA as part of the nonclinical and clinical packages for Epidiolex® (CDER, 2018a, b) are key to understanding CBD consumer safety; however, only summaries of such studies are available to the public. While the FDA has not established tolerable daily intake

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Abbreviations

ALB	albumin
ALP	alkaline phosphatase
ALT	alanine aminotransferase
ANOVA	analysis of variance
AST	aspartate aminotransferase
BUN	blood urea nitrogen
Ca	calcium
CB1, CB2	cannabinoid receptors 1 and 2
CBD	cannabidiol
CHOL	total cholesterol
Cl	chloride
ECS	endocannabinoid receptor system
FDA	US Food and Drug Administration
FOB	functional observational battery
FSA	UK Food Safety Authority
GLOB	globulin
GLP	Good Laboratory Practice
GLU	fasting glucose
H&E	hematoxylin and eosin
HDL	high-density lipoprotein
K	potassium
LDL	low-density lipoprotein
Na	sodium
NOAEL	no-observed-adverse-effect level
OECD	Organization for Economic Co-operation and Development
PHOS	inorganic phosphorus
SD	standard deviation
SDH	sorbitol dehydrogenase
TAG	triglycerides
TBIL	total bilirubin
TGA	Therapeutic Goods Administration
THC	delta-9-tetrahydrocannabinol
TP	total serum protein
TSH	thyroid stimulation hormone
UK	United Kingdom
US	United States

levels associated with consumer use, an overview of the agency's activities related to evaluating the safe use of CBD in food and dietary supplement products can be found on its website (FDA, 2023). In addition, the United Kingdom (UK) Food Safety Authority (UK FSA, 2022), Health Canada (2022), and the Australian Therapeutic Goods Administration (TGA, 2021) have established recommended maximum upper intake levels of CBD by healthy adults, except those planning to be or currently pregnant or breastfeeding. While some limited safety-related data on CBD are available in the public domain, these regulatory agencies continue to highlight gaps in available toxicology and other related data. In addition, recent literature reviews, including a systematic mapping study, have been published summarizing the available CBD toxicity data and knowledge gaps (Henderson et al., 2023a; Li et al., 2021). Specifically, no publicly available studies on CBD conducted according to regulatory test guidelines are identified to evaluate genotoxicity, repeated oral toxicity, or reproductive and developmental toxicity endpoints.

Given that consumer use of CBD has increased drastically in recent years, it is essential to continue to generate data on which to evaluate its safety. Additional research is needed to fill the aforementioned data gaps and, subsequently, to enable calculation of a margin of safety/exposure. The present study was conducted to investigate the potential for toxicity following repeated exposure to oral CBD in male and female Sprague Dawley rats. The current studies were conducted as part of a larger program to investigate the safety and potential for toxicity of CBD isolates (Henderson et al., 2023b).

2. Materials and methods

2.1. Test material

Hemp-derived CBD isolate (99.08–101.46%; CAS No. 13956-29-1) was provided by Canopy Growth USA (Evergreen, Colorado). The test substance was stored under ambient conditions and remained stable through the duration of the study, as demonstrated by analysis on samples of the test substance (neat) collected at the beginning, middle, and end of the in-life phase (data not shown).

2.2. Animals

Seven or eight-week-old CRL Sprague Dawley CD® IGS rats (20/sex) were obtained from Charles River Laboratories (Raleigh, North Carolina). The animals were housed individually in single polycarbonate cages in temperature-controlled and humidity-monitored rooms with a 12-h light/dark cycle. Test animals were provided filtered tap water and 2016 Certified Envigo Teklad Global Rodent Diet® *ad libitum* throughout the study. Animals were cared for according to the published National Research Council guidelines. The testing laboratory is Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC) accredited.

2.3. 14-day oral toxicity study in rats

2.3.1. Dose selection and test article preparation

Dose levels of 0 (vehicle control; olive oil), 30, 70, or 150 mg/kg-bw/d of the CBD were selected. The high dose was selected based on available data from studies submitted as part of the Epidiolex non-clinical data package reviewed by FDA, including a 26-week study in rats (CDER, 2018a; Study number GWTX1412). The low- and mid-dose levels were selected to derive a dose-response for observed effects. Fresh preparations containing 20, 46.7, and 100 mg/mL of the test substance mixed in olive oil (w/v) were prepared daily using a dosing volume of 1.5 ml/kg. Individual doses were calculated weekly and adjusted based on current body weights. Samples from each dose were collected and tested to verify homogeneity and concentration. Analytical chemistry results can be found in Supplementary Table 1A.

2.3.2. Experimental design

The design was conducted following the principles of FDA Toxicological Principles for the Safety Assessment of Food Ingredients (FDA, 2007) and Organization for Economic Co-operation and Development Test Guideline 407 (OECD, 2008). All animals were acclimated for 5 days prior to testing. Rats were distributed into four groups (one vehicle control and three test substance groups; n = 5/sex). Dose levels of 0 (vehicle control), 30, 70, or 150 mg/kg-bw/d of CBD were administered once daily via oral gavage for 14 days. Throughout the study, animals were observed daily for signs of gross toxicity and behavioral changes, and weekly for a battery of detailed observations. Body weight and food consumption were recorded weekly. Animals were fasted overnight prior to sacrifice on day 16. Necropsies were performed on all study animals, and any gross observations, including lesions, were recorded. Wet weights of the liver, kidneys (combined), and adrenal glands (combined) from each animal were recorded, and tissues were fixed in 10% neutral buffered formalin for histopathological examination.

2.3.3. Serum chemistry

Blood samples were collected from the inferior vena cava in all animals at terminal sacrifice. Serum from each sample was separated via refrigerated centrifugation, transferred to a fresh tube, and stored at −80 °C. Clinical chemistry parameters evaluated on a Cobas C 311 Analyzer (Roche Diagnostics) included aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), sorbitol dehydrogenase (SDH), total bilirubin (TBIL), blood urea nitrogen (BUN), creatinine, total cholesterol (CHOL), triglycerides (TAG), fasting glucose (GLU), total serum protein (TP), albumin (ALB), globulin (GLOB), calcium (Ca), inorganic phosphorus (PHOS), sodium (Na), potassium (K), and chloride (Cl).

2.3.4. Histopathology

During necropsy, selected organs (liver, kidneys, and adrenal glands) from the control and high-dose animals were placed in 10% formalin. Fixed tissues were paraffin embedded, sectioned, and stained with hematoxylin and eosin (H&E). Slides were prepared and evaluated by a board-certified veterinary pathologist at Histo-Scientific Research Laboratories (HSRL).

2.4. 90-day oral toxicity study in rats

2.4.1. Dose selection and test article preparation

Dose levels of 0 (vehicle control; olive oil), 50, 80, 120, or 140 mg/kg-bw/d of CBD were selected for this study. As with the previous 14-day oral toxicity study, the high dose was selected based on available data from studies submitted as part of the Epidiolex non-clinical data package reviewed by FDA (CDER, 2018a). One 26-week study in rats demonstrated no toxicologically significant effects of pure CBD at doses up to 150 mg/kg-bw/day (Study number GWTX1412); however, other studies reviewed by CDER (2018a) reported adverse effects following similar exposure levels to mixtures containing high concentrations of CBD (e.g., 50–65.6% CBD; Study numbers GWTX10124 and JJG0001). As such, the doses in the current study were selected to confirm the findings of the 26-week study with pure CBD. Fresh preparations containing 50, 80, 120, and 140 mg/mL of the test substance mixed in olive oil (w/v) were prepared daily using a dosing volume of 1 ml/kg. Individual doses were calculated weekly and adjusted based on current body weights. Samples from each dose were collected and tested to verify homogeneity and concentration at the beginning, middle, and end of the study. Analytical chemistry results can be found in Supplementary Table 1B.

2.4.2. Experimental design

The study was conducted in compliance with FDA (21 CFR Part 58) and the OECD Principles of Good Laboratory Practice (GLP) ENV/MC/CHEM (98)17. The study design followed FDA Toxicological Principles for the Safety Assessment of Food Ingredients (FDA, 2007) and OECD Test Guideline 408 (OECD, 2018). All animals were acclimated for 5–6 days prior to testing. Rats were distributed into five main groups (one vehicle control and four treatment groups; n = 10/sex). An additional five recovery groups (n = 5/sex) also received the same dose levels as the main test group for 90 days, followed by a 28-day recovery period. CBD was administered daily via oral gavage 92 days (males) and 93 days (females). Ophthalmologic evaluations were conducted once during the acclimation period and again on dosing Day 87 for all study animals. Animals were observed twice daily for viability, signs of gross toxicity, and behavioral changes, in addition to weekly detailed clinical observations. Body weight and food consumption were recorded weekly. All rats were fasted overnight prior to terminal sacrifice. Necropsies were performed on all study animals, and any gross observations, including lesions, were recorded. Wet weights of the liver, kidneys (combined), adrenal glands (combined), brain, heart, spleen, thymus, epididymides (combined), testes (combined), uterus, and ovaries with oviducts (combined) were recorded for all animals.

2.4.3. Functional observational battery

During week 12 of the study, a functional observational battery (FOB) was performed on all main-test animals using a validated protocol (Product Safety Labs Standard Operating Procedure, issue date 04/05/18). Each rat was evaluated for the following: excitability, autonomic function, gait and sensorimotor coordination, reactivity and sensitivity, and other abnormal clinical signs. The observer was blind to treatment groups, and all animals were observed in random order. In addition, duplicate measurements of foot splay and triplicate measurements of grip strength of forelimb and hindlimb (Dillon GS Series Digital Force Gage, Fairmont, Minnesota) were recorded for each animal, and the corresponding mean was calculated.

2.4.4. Motor activity

During week 12 of the study, motor activity was evaluated on all main-test animals. Activity was monitored using an automated Photobeam Activity System®, San Diego Instruments, Inc. Each rat was placed into a polycarbonate solid-bottom cage and evaluated for 1 h in a quiet, darkened room. Photobeam counts accumulated over six 10-min intervals.

2.4.5. Clinical pathology

Blood samples for hematology (except those for coagulation analyses) and thyroid hormone assessment were collected following an overnight fast from main-test animals on days 93 (male) and 94 (female) and from recovery animals on Day 122. The day prior, animals were placed in metabolism cages, and urine was collected from all animals after at least 15 h of fasting. At terminal sacrifice, blood was sampled for clinical chemistry, as well as determination of prothrombin time and activated partial thromboplastin time. Additional selected hematology analyses were determined with an ADVIA 120 Hematology System (Siemens Healthineers) and included white blood cell count (WBC) and differential leukocyte count, red blood cell count (RBC), red cell distribution width, hematocrit (Hct), hemoglobin concentration (Hgb), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC; calculated), reticulocyte count, and platelet count. Coagulation times were determined on a Siemens Sysmex CA620 (Siemens Healthineers) automated system. Thyroid hormone analysis was done only for main-test animals; ELISA was used to measure triiodothyronine (T3), thyroxine (T4), and thyroid stimulation hormone (TSH). Clinical chemistry parameters determined on a COBAS C311 (Roche Diagnostics) analyzer included: AST, ALT, ALP, SDH, TBIL, BUN, creatinine, CHOL, high-density lipoprotein (HDL), low-density lipoprotein (LDL), TAG, GLU, TP, ALB, GLOB, Ca, PHOS, Na, K, and Cl. Urinalysis (CLINITEK Advantus urinalysis analyzer, Siemens Healthineers) included quality, color, clarity, volume, pH, glucose, specific gravity, total protein, ketone, bilirubin, blood, urobilinogen, and microscopic urine sediment.

2.4.6. Histopathology

Tissues and organs were collected and stored in 10% buffered formalin and included prostate and seminal vesicles, adrenals, ileum with Peyer's patches, rectum, jejunum, salivary glands, kidneys, larynx, aorta, liver, skeletal muscle, bone (femur), lungs, skin, bone marrow (femur and sternum), mandibular and mesenteric lymph nodes, spinal cord (cervical, mid-thoracic, and lumbar), brain (medulla/pons, cerebellar, and cerebral cortex), mammary gland, nasal turbinates, spleen, nose, sternum, cecum, ovaries, stomach, cervix, oviducts, thymus, colon, pancreas, thyroid, duodenum, parathyroid, trachea, esophagus, peripheral nerve (sciatic), urinary bladder, Harderian gland, pharynx, uterus, heart, pituitary gland, vagina, and all gross lesions. Eyes, epididymides, optic nerve, and testes samples from the main-test group were preserved in Davidson's fixative and stored in ethanol.

Histological examination was performed on all samples from the control and high-dose groups. In addition, samples of adrenal gland and liver from all main-test animals in the 50-, 80-, and 120¬mg/kg-bw/

d groups were processed. Fixed tissues were paraffin embedded, sectioned, and stained with H&E. Slides were prepared and assessed by a board-certified veterinary pathologist.

2.5. Statistical analysis

Statistical analysis was conducted using GraphPad Prism 9 software (San Diego, CA).

Mean and standard deviations (SDs) were calculated for all quantitative data. In both studies, in-life data from treatment and control groups were compared using a two-way analysis of variance (ANOVA; Motulsky, 2014) and tested for time effect, group effect, and time/group interaction effect. Repeated measures were accounted for in one independent variable (i.e., time). Dunnett's test (Dunnett, 1964, 1980) was used as the post hoc multiple comparisons test to compare individual treatment groups to the control group within each time variable. Endpoints with single measurements of continuous data within groups (e.g., organ weight, clinical pathology) were evaluated for homogeneity of variances (Bartlett, 1937) and normality (Shapiro and Wilk, 1965). One-way ANOVA was subsequently used between treatment and control groups where homogeneous variances and normal distribution were observed. If one-way ANOVA was significant, treated groups were compared to controls using a multiple comparisons test (e.g., Dunnett's test). If variances were considered significantly different, groups were compared using a non-parametric method (e.g., Kruskal-Wallis non-parametric ANOVA; Kruskal and Wallis, 1952). If non-parametric ANOVA was significant, treated groups were compared to control using Dunn's test (Dunn, 1964).

For clinical pathology data (90-day study), when variances were considered significantly different, data were log transformed to achieve variance homogeneity and normality. If log transformation failed, a non-parametric method (e.g., Kruskal-Wallis non-parametric ANOVA) was used. When variance was significant, a comparison of treated groups to control was performed (e.g., Dunn's test). One outlier value was identified in the control group of the main 90-day study in males for the hematology parameter reticulocytes. This outlier was identified using the ROUT test method, a method combining regression and outlier removal, with a cutoff Q value set to 0.1% (Motulsky and Brown, 2006). This value was removed prior to performing statistical analysis.

For histopathology of terminal sacrifice animals, Fishers exact test was used to compare the incidence of each microscopic finding between control and the high-dose group animals, and between each group and controls, where specific findings were noted. The extended Mantel-Haenszel (MH) test was also used. Statistical analysis of microscopic findings in recovery animals was not performed due to a lack of sample size and associated power.

3. Results

3.1. 14-Day oral toxicity study

No treatment-related deaths or clinical signs were observed throughout the study. Mean body weights (Table 1; Suppl. Table 2) and food consumption (Suppl. Table 3) of male and female rats administered CBD for 14 days were similar to that of control groups. Mean relative liver weights increased ($p < 0.05$) in the males of the high-CDB-dose group (150 mg/kg-bw/d), while mean absolute and relative liver weights were increased ($p < 0.001$–0.05) in females in the two highest CBD dose groups (70 and 150 mg/kg-bw/d) compared to control groups. Of note, all mean absolute liver weights were within the laboratory's historical control range for this parameter (Product Safety Labs, 2022). No other changes in weights of organs evaluated were found (Table 1). In general, significant differences in serum chemistry parameters were observed in a non-dose-dependent manner and were within range of biological variation and/or lab historical ranges, and therefore, were considered to be not toxicologically relevant (Suppl. Table 4; Product Safety Labs, 2022). The only treatment-related adverse change observed in serum parameters was elevated total cholesterol levels in female rats administered 150 mg/kg-bw/d CBD; however, the mean value was well within the laboratory's historical control range for this parameter (Product Safety Labs, 2022). No treatment-related macroscopic observations were observed. However, liver histopathology (Table 2) revealed a dose-dependent increase in the mean severity of centrilobular hepatocellular hypertrophy, with increased cytoplasmic volume in the hypertrophic cells in both male and female rats, correlating with an increase in liver weights in the higher dose groups. Under the conditions of this 14-day study and the endpoints evaluated, Sprague Dawley rats tolerated an oral dose of 150 mg/kg-bw/d CBD.

Table 1
Absolute and relative organ weights of male (A) and female (B) rats administered 0, 30, 70, or 150 mg/kg-bw/day CBD isolate for 14 days.

A				
Terminal	Treatment group (mg/kg-bw/day)			
Weights	0	30	70	150
Mean organ weights (g)				
Body Weight	314.80 ± 19.52	320.20 ± 22.80	315.20 ± 19.06	315.40 ± 16.85
Liver	10.72 ± 1.87	10.92 ± 1.10	12.01 ± 1.61	13.24 ± 1.48
Adrenal Glands	0.07 ± 0.01	0.06 ± 0.01	0.06 ± 0.01	0.06 ± 0.01
Kidneys	2.56 ± 0.30	2.66 ± 0.30	2.67 ± 0.18	2.70 ± 0.27
Mean organ-to-body weight (g/kg)				
Liver	33.90 ± 4.10	34.13 ± 2.46	38.18 ± 5.37	42.04 ± 4.84*
Adrenal Glands	0.22 ± 0.02	0.19 ± 0.02	0.20 ± 0.03	0.19 ± 0.04
Kidneys	8.15 ± 0.88	8.32 ± 1.05	8.49 ± 0.52	8.54 ± 0.58
B				
Terminal	Treatment group (mg/kg-bw/day)			
Weights	0	30	70	150
Mean organ weights (g)				
Body Weight	196.80 ± 12.56	190.20 ± 13.03	202.20 ± 10.76	201.20 ± 11.12
Liver	7.96 ± 1.51	9.01 ± 0.76	10.06 ± 0.68*	11.82 ± 1.14***
Adrenal Glands	0.07 ± 0.01	0.07 ± 0.01	0.08 ± 0.01	0.08 ± 0.01*
Kidneys	1.65 ± 0.15	1.71 ± 0.10	1.75 ± 0.08	1.84 ± 0.15
Mean organ-to-body weight (g/kg)				
Liver	40.41 ± 7.01	47.35 ± 1.79	49.78 ± 3.21*	58.73 ± 3.83***
Adrenal Glands	0.33 ± 0.04	0.38 ± 0.05	0.38 ± 0.06	0.41 ± 0.05
Kidneys	8.40 ± 0.66	9.03 ± 0.58	8.67 ± 0.10	9.13 ± 0.49

Data are presented as mean ± SD. Statistical significance was determined by one-way ANOVA followed by Dunnett's test (n = 5). * indicates a p-value <0.05, ** indicates a p-value <0.01, and *** indicates a p-value <0.001 compared to control group.

3.2. 90-Day oral toxicity study

3.2.1. Survival and clinical observations

No treatment-related mortality was observed throughout the study. One female control rat was euthanized for humane reasons on Day 22 due to moderate visible swelling in the chest, later confirmed during necropsy to be caused by a dosing error. No clinical observations throughout the study were attributed to administration of the CBD. In male rats, incidental clinical signs included unilateral red ocular discharge, slight hypersalivation, slight to moderate alopecia on forelimb/hindlimb or head, superficial eschar on the head or tail, and slight to moderate visible swelling on the right ear. Incidental findings for females included slight to extreme hypersalivation, slight alopecia on the head or forelimb, slight moist rales, abnormal gait, a damaged left hindlimb, eschar on the head, and slight swelling in the right ear. These

Table 2
Histopathology results for male (A) and female (B) rats administered 0, 30, 70, or 150 mg/kg-bw/day CBD isolate for 14 days.

A

Treatment group (mg/kg-bw/day)	Liver: Hypertrophy	Adrenal Glands	Kidneys: Chronic Progressive Nephropathy	Kidneys: Dilation
0	No remarkable findings	No remarkable findings	1 ≥ 4	No remarkable findings
30	1 ≥ 2 2 ≥ 1 3 ≥ 2	–	–	–
70	3 ≥ 3 4 ≥ 2	–	–	–
150	3 ≥ 2 4 ≥ 3	No remarkable findings	1 ≥ 3	1 ≥ 1

B

Treatment group (mg/kg-bw/day)	Liver: Hypertrophy	Adrenal Glands	Kidneys: Chronic Progressive Nephropathy
0	No remarkable findings	No remarkable findings	1 ≥ 1
30	1 ≥ 2 2 ≥ 3	–	–
70	3 ≥ 3 4 ≥ 2	–	–
150	3 ≥ 1 4 ≥ 4	No remarkable findings	No remarkable findings

– = no data; histopathology severity scores: 1 ≥ Minimal, 2 ≥ Mild, 3 ≥ Moderate, 4 ≥ Marked, 5 ≥ Severe; (n = 5).

findings were sporadic among controls and treatment groups, and therefore, were considered unrelated to the CBD test material.

3.2.2. Body weight and food consumption

Body weight and body weight gain for all treatment groups were comparable to that of the control group through the 28-day recovery period (Fig. 1; Suppl. Table 5). Additionally, there were no significant changes in food consumption or food efficiency in any of the treatment groups during both the main toxicity test and recovery period (Suppl. Table 6).

3.2.3. Ophthalmologic examinations, functional observation battery, and motor activity assessment

Ophthalmologic examinations revealed no abnormalities in any of the treatment or control groups at either time point. Similarly, functional observations showed no treatment-related findings. Mean quantitative measurements for forelimb/hindlimb grip strength and hindlimb foot splay were comparable between animals in the control and CBD-treated groups (Suppl. Table 7). Overall, motor activity measurements (i.e., mean total movements) for CBD-treated groups were considered comparable to those of the control group. Mean total movements were statistically significantly higher in males in the rats administered 120 mg/kg-bw/d (time intervals 2 and 3 only) and 140 mg/kg-bw/d (time intervals 1 and 3 only), compared to concurrent controls, with mean total movements similar to controls for the remainder of the time intervals. For females, all groups exhibited a similar level of movement over all intervals, with the single exception of females in the 80 mg/kg-bw/d group during the sixth time interval (Suppl. Table 8).

3.2.4. Clinical chemistry and pathology

CBD-treated females in some groups exhibited a significant increase in total cholesterol (140 mg/kg-bw/d), HDL (≥120 mg/kg-bw/d), and LDL (≥120 mg/kg-bw/d) compared to the female control group after the 90-day study (Table 3). However, these observed increases in CHOL,

Table 3
Serum clinical chemistry parameters for male (A) and female (B) rats administered 0, 50, 80, 120, or 140 mg/kg-bw/day CBD isolate for 90 days.

A

Serum Parameters	Treatment Group (mg/kg-bw/day) 0	50	80	120	140
AST (U/L)	91.90 ± 48.14	76.60 ± 18.14	75.80 ± 11.41	82.50 ± 17.60	79.80 ± 24.42
ALT (U/L)	37.90 ± 17.63	31.50 ± 6.62	28.70 ± 5.56	30.20 ± 7.07	38.70 ± 23.92
ALKP (U/L)	77.50 ± 17.67	76.60 ± 10.95	78.90 ± 21.95	79.90 ± 13.53	83.60 ± 16.52
BUN (mg/dL)	15.30 ± 2.21	15.90 ± 1.79	15.70 ± 2.00	16.90 ± 2.33	15.10 ± 2.23
Ca (mg/dL)	11.42 ± 0.42	11.15 ± 0.77	11.11 ± 0.88	11.31 ± 0.43	11.31 ± 0.75
Cl (mmol/L)	100.70 ± 2.73	101.30 ± 3.08	101.30 ± 2.08	101.80 ± 2.54	100.60 ± 3.36
Na (mmol/L)	143.80 ± 4.29	143.90 ± 4.33	144.10 ± 4.33	145.30 ± 4.03	144.90 ± 4.56
K (mmol/L)	8.25 ± 1.46	7.60 ± 1.26	7.34 ± 1.54	7.61 ± 0.68	7.50 ± 1.73
CHOL (mg/dL)	63.50 ± 16.98	58.40 ± 7.76	56.60 ± 7.34	64.30 ± 15.87	66.10 ± 12.85
LDL (mmol/L)	0.26 ± 0.12	0.27 ± 0.07	0.18 ± 0.07	0.28 ± 0.08	0.27 ± 0.08
HDL (mmol/L)	1.03 ± 0.25	0.91 ± 0.16	0.92 ± 0.11	1.02 ± 0.18	1.02 ± 0.21
GLU (mg/dL)	258.90 ± 59.10	248.00 ± 69.58	226.60 ± 41.74	233.60 ± 51.41	210.80 ± 47.15
CREAT (mg/dL)	0.31 ± 0.05	0.29 ± 0.05	0.29 ± 0.04	0.33 ± 0.05	0.31 ± 0.03
PHOS (mg/dL)	9.25 ± 0.70	8.48 ± 1.04	8.43 ± 1.03	8.60 ± 0.54	8.82 ± 0.88
TBIL (mg/dL)	0.07 ± 0.02	0.05 ± 0.01*	0.04 ± 0.02**	0.04 ± 0.02**	0.04 ± 0.02*
TAG (mg/dL)	69.10 ± 26.92	72.50 ± 20.91	115.30 ± 56.83	65.00 ± 27.00	68.50 ± 34.78
SDH (U/L)	20.01 ± 5.91	22.22 ± 11.54	20.43 ± 6.12	26.39 ± 13.23	28.08 ± 13.04
TP (g/dL)	6.33 ± 0.25	6.31 ± 0.39	6.42 ± 0.47	6.55 ± 0.33	6.61 ± 0.39
ALB (g/dL)	4.07 ± 0.13	3.98 ± 0.25	4.01 ± 0.23	4.12 ± 0.20	4.19 ± 0.28
GLOB (g/dL)	2.26 ± 0.28	2.33 ± 0.23	2.41 ± 0.31	2.43 ± 0.28	2.42 ± 0.25

B

Serum Parameters	Treatment Group (mg/kg-bw/day) 0	50	80	120	140
AST (U/L)	69.33 ± 8.78	72.80 ± 18.12	94.40 ± 80.44	64.70 ± 13.12	81.50 ± 38.59
ALT (U/L)	25.78 ± 3.99	28.30 ± 11.70	28.70 ± 18.75	25.20 ± 3.58	36.20 ± 16.80
ALP (U/L)	43.44 ± 13.47	32.70 ± 9.88	32.40 ± 6.50	37.70 ± 14.23	36.90 ± 10.74
BUN (mg/dL)	18.67 ± 4.47	20.50 ± 2.27	18.80 ± 3.52	17.80 ± 2.53	17.60 ± 4.06
Ca (mg/dL)	11.92 ± 0.47	11.66 ± 0.70	11.65 ± 1.35	11.50 ± 082	11.92 ± 0.86
Cl (mmol/L)	101.80 ± 2.54	99.15 ± 1.89*	100.80 ± 1.14	100.40 ± 1.82	99.89 ± 1.62
Na (mmol/L)	144.80 ± 3.31	140.90 ± 2.96	143.00 ± 2.63	143.00 ± 2.83	143.30 ± 2.79
K (mmol/L)	7.39 ± 1.54	7.69 ± 0.62	6.97 ± 0.85	7.18 ± 2.11	6.45 ± 0.94
CHOL (mg/dL)	94.44 ± 12.76	92.70 ± 17.16	100.50 ± 29.90	120.90 ± 16.35	141.40 ± 45.81*
LDL (mmol/L)	0.24 ± 0.07	0.24 ± 0.06	0.29 ± 0.13	0.40 ± 0.13*	0.44 ± 0.18**
HDL (mmol/L)	1.83 ± 0.21	1.88 ± 0.29	1.93 ± 0.43	2.27 ± 0.27*	2.62 ± 0.58***
GLU (mg/dL)	246.00 ± 66.35	214.70 ± 78.20	227.10 ± 68.64	218.10 ± 58.76	227.80 ± 42.93
CREAT (mg/dL)	0.43 ± 0.09	0.36 ± 0.05	0.42 ± 0.07	0.40 ± 0.06	0.37 ± 0.08

(continued on next page)

Table 3 (*continued*)

PHOS (mg/dL)	8.50 ± 1.10	9.17 ± 1.59	8.88 ± 1.43	8.46 ± 1.50	8.17 ± 1.35
TBIL (mg/dL)	0.07 ± 0.03	0.04 ± 0.02**	0.05 ± 0.02	0.04 ± 0.01*	0.04 ± 0.02*
TAG (mg/dL)	80.44 ± 19.07	67.90 ± 41.30	67.50 ± 29.07	66.10 ± 16.78	68.40 ± 39.91
SDH (U/L)	13.36 ± 1.85	15.59 ± 4.82	20.68 ± 15.03	14.72 ± 2.54	24.43 ± 20.07
TP (g/dL)	7.21 ± 0.40	7.38 ± 0.74	7.35 ± 0.58	7.45 ± 0.52	7.77 ± 0.73
ALB (g/dL)	5.29 ± 0.33	5.14 ± 0.69	5.05 ± 0.54	5.20 ± 0.51	5.38 ± 0.46
GLOB (g/dL)	1.92 ± 0.21	2.24 ± 0.32	2.30 ± 0.28*	2.25 ± 0.31	2.39 ± 0.41**

Data are presented as mean ± SD. Statistical significance was determined by one-way ANOVA followed by Dunnett's test or Dunn's test if data failed tests for normality or homogeneity (n = 10). * indicates a p-value <0.05, ** indicates a p-value <0.01 and *** indicates a p-value <0.001 compared to control group.

HDL, and LDL were not associated with lesions that reflect alterations in lipid metabolism in the liver (Section 3.2.6); in addition, all CHOL and HDL levels were within the laboratory's historical control values (Product Safety Labs, 2022). All levels were back to control levels at the end of the 28-day recovery period (Suppl. Table 10). Total bilirubin was significantly higher in males (all doses) and females (50, 120, and 140 mg/kg-bw/d); this finding was considered test substance related but not adverse, as it was not dose dependent, returned to control levels after the recovery period, and correlated with adaptative changes in the liver (Hall et al., 2012). All other differences in clinical chemistry parameters between treatment groups and controls were minimal and random and were determined to occur as a result of biological variation among rats (Table 3; Suppl. Table 10).

The serum levels of thyroid hormones T3 and T4 did not change with treatment. TSH levels increased significantly in male and female rats administered 80–140 mg/kg-bw/d CBD when compared to their respective control groups following the 90-day exposure (Suppl. Table 11).

Absolute reticulocyte levels were significantly reduced in males in the 50, 120, and 140 mg/kg-bw/d groups compared to control animals; this finding was considered nonadverse, as it was not dose dependent, was within the historical control range for this parameter (Product Safety Labs, 2022), and returned to control levels following the recovery time period. In addition, this finding was not accompanied by changes in red blood cells or lesion in the bone marrow (Suppl. Tables 12 and 13). The few other observed differences in hematology parameters were considered a result of biological variation among rats and appeared to have occurred sporadically (Suppl. Tables 12 and 13). Additionally, no treatment-related changes in coagulation or urinalysis parameters were observed (Suppl. Tables 14 and 15).

3.2.5. Organ weights and gross pathology

At terminal sacrifice on Day 93/94 and recovery sacrifice on Day 122, all gross findings were determined to be incidental or commonly found in Sprague Dawley rats; findings were of similar incidence in both control and treatment groups and were therefore not related to CBD administration.

In comparison to control groups at terminal sacrifice, mean absolute and relative liver weights were increased significantly in male rats administered 80–140 mg/kg-bw/d CBD, as well as in female rats administered 120 and 140 mg/kg-bw/d CBD. Mean relative kidney weights were increased significantly in male rats at the highest dose, with female rats in the two highest dose groups (120 and 140 mg/kg-bw/d) having significantly increased absolute and kidney weights compared to control rats. Weights of adrenal glands were increased significantly in male rats treated with 120 mg/kg-bw/d CBD (relative only), and in female rats that received 80–140 mg/kg-bw/d CBD

Table 4
Absolute and relative organ weights of male (A) and female (B) rats administered 0, 50, 80, 120, or 140 mg/kg-bw/day CBD isolate for 90 days.

A

Terminal Weights	Treatment Group (mg/kg-bw/day) 0	50	80	120	140
Mean organ weights (g)					
Body Weight	573.20 ± 49.53	524.70 ± 58.36	590.20 ± 51.05	543.60 ± 51.39	546.20 ± 45.02
Adrenal Glands	0.06 ± 0.01	0.06 ± 0.01	0.06 ± 0.01	0.07 ± 0.01	0.07 ± 0.01
Brain	2.33 ± 0.15	2.29 ± 0.08	2.34 ± 0.15	2.36 ± 0.13	2.29 ± 0.06
Epididymis	1.52 ± 0.17	1.41 ± 0.16	1.51 ± 0.28	1.49 ± 0.20	1.44 ± 0.16
Heart	1.56 ± 0.11	1.45 ± 0.18	1.59 ± 0.26	1.45 ± 0.18	1.48 ± 0.14
Kidneys	3.53 ± 0.37	3.44 ± 0.42	3.67 ± 0.32	3.61 ± 0.45	3.74 ± 0.24
Liver	15.08 ± 1.90	14.72 ± 1.61	18.16 ± 2.22**	18.18 ± 1.96**	18.69 ± 2.57**
Spleen	0.97 ± 0.09	0.85 ± 0.17*	0.91 ± 0.12	0.82 ± 0.12*	0.78 ± 0.08**
Testes	3.92 ± 0.36	3.74 ± 0.42	3.43 ± 0.91	3.67 ± 0.31	3.85 ± 0.26
Thymus	0.28 ± 0.07	0.33 ± 0.06	0.27 ± 0.12	0.22 ± 0.05	0.23 ± 0.07
Mean organ-to-body weight (g/kg)					
Adrenal Glands	0.11 ± 0.02	0.13 ± 0.02	0.11 ± 0.01	0.14 ± 0.02*	0.13 ± 0.02
Brain	4.09 ± 0.49	4.43 ± 0.57	4.00 ± 0.46	4.36 ± 0.31	4.22 ± 0.33
Epididymis	2.65 ± 0.16	2.75 ± 0.27	2.59 ± 0.57	2.76 ± 0.39	2.65 ± 0.26
Heart	2.72 ± 0.19	2.79 ± 0.32	2.69 ± 0.34	2.67 ± 0.13	2.71 ± 0.29
Kidneys	6.16 ± 0.38	6.67 ± 0.71	6.24 ± 0.57	6.64 ± 0.62	6.88 ± 0.54*
Liver	26.24 ± 1.37	28.16 ± 1.67	30.69 ± 1.68***	33.45 ± 1.93***	34.14 ± 3.02***
Spleen	1.69 ± 0.16	1.63 ± 0.29	1.54 ± 0.19	1.51 ± 0.16	1.43 ± 0.14*
Testes	6.86 ± 0.67	7.25 ± 0.65	5.89 ± 1.67	6.79 ± 0.73	7.11 ± 0.96
Thymus	0.49 ± 0.10	0.61 ± 0.13	0.46 ± 0.21	0.41 ± 0.10	0.42 ± 0.14

B

Terminal Weights	Treatment Group (mg/kg-bw/day) 0	50	80	120	140
Mean organ weights (g)					
Body Weight	300.33 ± 29.43	285.90 ± 22.12	315.70 ± 31.57	299.80 ± 25.53	289.80 ± 18.21
Adrenal Glands	0.07 ± 0.01	0.07 ± 0.01	0.09 ± 0.01*	0.09 ± 0.01***	0.10 ± 0.02***
Brain	2.07 ± 0.07	2.09 ± 0.09	2.07 ± 0.06	2.09 ± 0.10	2.05 ± 0.11
Heart	1.01 ± 0.11	0.93 ± 0.10	0.98 ± 0.07	0.94 ± 0.07	0.99 ± 0.08
Kidneys	1.86 ± 0.10	1.93 ± 0.15	2.05 ± 0.13	2.15 ± 0.31*	2.23 ± 0.21**
Liver	8.52 ± 0.84	8.71 ± 1.13	10.64 ± 1.22	12.13 ± 1.16 ***	12.98 ± 2.05 ***
Spleen	0.51 ± 0.08	0.51 ± 0.06	0.60 ± 0.07	0.53 ± 0.09	0.53 ± 0.08
Ovaries w/ Oviducts	0.13 ± 0.01	0.13 ± 0.02	0.13 ± 0.02	0.13 ± 0.03	0.12 ± 0.01
Thymus	0.24 ± 0.07	0.23 ± 0.04	0.25 ± 0.04	0.23 ± 0.09	0.21 ± 0.05
Uterus	0.70 ± 0.21	0.64 ± 0.22	0.88 ± 0.70	0.83 ± 0.32	0.71 ± 0.06

(*continued on next page*)

Table 4 (*continued*)

Mean organ-to-body weight (g/kg)					
Adrenal Glands	0.23 ± 0.04	0.25 ± 0.03	0.27 ± 0.04	0.31 ± 0.03***	0.33 ± 0.06***
Brain	6.94 ± 0.61	7.37 ± 0.67	6.63 ± 0.72	7.01 ± 0.59	7.10 ± 0.52
Heart	3.37 ± 0.23	3.24 ± 0.19	3.11 ± 0.22	3.15 ± 0.34	3.42 ± 0.24
Kidneys	6.24 ± 0.46	6.76 ± 0.38	6.54 ± 0.62	7.17 ± 0.84**	7.70 ± 0.47***
Liver	28.40 ± 1.15	30.44 ± 2.91	33.91 ± 4.33	40.51 ± 2.87***	44.68 ± 5.12***
Spleen	1.72 ± 0.30	1.80 ± 0.24	1.90 ± 0.30	1.77 ± 0.26	1.81 ± 0.23
Ovaries w/ Oviducts	0.43 ± 0.05	0.45 ± 0.06	0.42 ± 0.05	0.44 ± 0.07	0.41 ± 0.05
Thymus	0.81 ± 0.25	0.81 ± 0.13	0.78 ± 0.09	0.77 ± 0.32	0.72 ± 0.20
Uterus	2.35 ± 0.76	2.25 ± 0.81	2.91 ± 2.67	2.78 ± 1.12	2.44 ± 0.25

Data are presented as mean ± SD. Statistical significance was determined by one-way ANOVA followed by Dunnett's test or Dunn's test if data failed tests for normality or homogeneity (n = 10). * indicates a p-value <0.05, ** indicates a p-value <0.01, and *** indicates a p-value <0.001 compared to control group.

(absolute: 80–140 mg/kg-bw/d, relative: 120–140 mg/kg-bw/d). Furthermore, spleen weights decreased significantly only in males treated with 50 and 120 mg/kg-bw/d (absolute) or 140 mg/kg-bw/d CBD (absolute and relative) compared to the concurrent control group. All other organ weights for male and female treatment groups were similar to controls after the 90-day exposure (Table 4).

Fewer differences in organ weights were observed following 28 days of recovery in the group of animals sacrificed on Day 122. Mean relative kidney weights were increased significantly in males receiving 120 mg/kg-bw/d, compared to controls. Mean absolute liver weights were significantly higher in females treated with 80 mg/kg-bw/d. Mean absolute and relative ovaries (with oviducts) weights were significantly higher in females treated with 140 mg/kg-bw/d CBD, when compared to the female control group. All other reported organ weights for male and female rats in the recovery group were similar across groups, including spleen weights (Suppl. Table 9).

3.2.6. *Histopathology*

CBD-related histopathological changes were found in the livers of male and female rats, as well as in the adrenal glands of males, following the 90-day exposure (Table 5). Increases in incidence of liver hypertrophy observed in both sexes were found to be statistically significant starting at 80 mg/kg-bw/d; however, hepatocellular hypertrophy fully resolved in both male and female rats following the 28-day recovery period (Table 5). Of note, the incidence and severity of hepatocellular hypertrophy correlated with the dose-dependent increase in liver weights (Table 4). Increased adrenal gland vacuolation observed in males was found to be statistically significant in the two highest dose groups at the end of dosing (Fig. 2). The vacuolation of male adrenal glands decreased after 28 days of recovery, with only minimal (<1) histopathology severity scores in one animal (0, 50, and 120 mg/kg-bw/d), two animals (140 mg/kg-bw/d), or no animals (80 mg/kg-bw/d). Notably, two male rats from the control group also received a minimal severity score for adrenal cortical vacuolation after either Day 90 or Day 122 of the study (Table 5). No other tissues showed remarkable changes due to CBD administration on histopathologic examination, including in the spleens of animals in all dose groups.

Under the conditions of this 90-day study oral toxicity study (followed by 28-day recovery period) and the toxicological endpoints evaluated, the NOAEL for the oral CBD administration was determined to be 140 mg/kg-bw/d for male and female Sprague Dawley rats.

4. Discussion

Consumer interest in and use of foods and dietary supplements

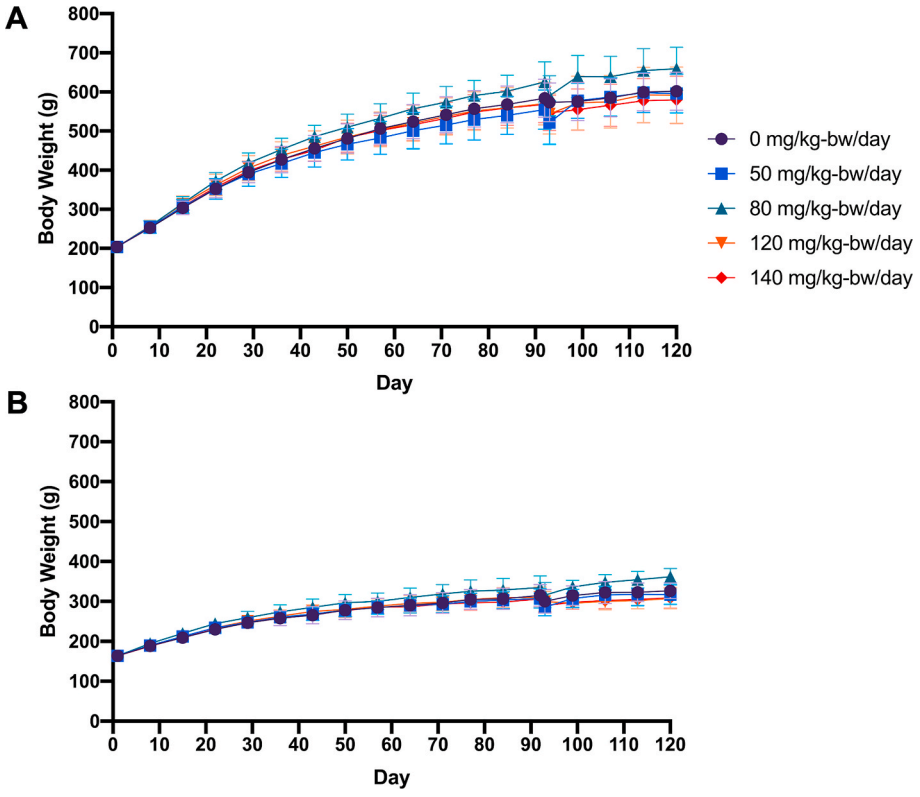

Fig. 1. Mean body-weight data for male (1A) and female (1B) rats administered CBD isolate by oral gavage for 90 days.

Table 5
Histopathology results for male (A) and female (B) rats administered 0, 50, 80, 120, or 140 mg/kg-bw/day CBD isolate for 90 days followed by a 28-day recovery period.

A

Treatment group (mg/kg-bw/day)	90-day Toxicity Study (Day 93/94)		28-day Recovery Period (Day 122)	
	Liver: Hypertrophy	Adrenal Glands: Vacuolation	Liver: Hypertrophy	Adrenal Glands: Vacuolation
0	No remarkable findings	1 ≥ 1	No remarkable findings	1 ≥ 1
50	1 ≥ 3	1 ≥ 4	No remarkable findings	1 ≥ 2
80	1 ≥ 8* 2 ≥ 1	1 ≥ 5	No remarkable findings	No remarkable findings
120	1 ≥ 7* 2 ≥ 3	1 ≥ 3* 2 ≥ 6	No remarkable findings	1 ≥ 1
140	1 ≥ 3* 2 ≥ 7	1 ≥ 3* 2 ≥ 6	No remarkable findings	1 ≥ 2

B

Treatment group (mg/kg-bw/day)	90-day Toxicity Study (Day 93/94)		28-day Recovery Period (Day 122)	
	Liver: Hypertrophy	Adrenal Glands: Vacuolation	Liver: Hypertrophy	Adrenal Glands: Vacuolation
0	No remarkable findings	No remarkable findings	No remarkable findings	No remarkable findings
50	No remarkable findings	No remarkable findings	No remarkable findings	No remarkable findings
80	1 ≥ 8*	No remarkable findings	No remarkable findings	No remarkable findings
120	1 ≥ 2* 2 ≥ 7	No remarkable findings	No remarkable findings	No remarkable findings
140	1 ≥ 1* 2 ≥ 8	No remarkable findings	No remarkable findings	No remarkable findings

* indicates a p-value <0.05 for dose group.
Histopathology severity scores: 1 ≥ Minimal, 2 ≥ Mild, 3 ≥ Moderate, 4 ≥ Severe; (n = 5¬10).

containing hemp-derived CBD is increasing. As a result, it is critical that CBD safety be demonstrated using validated, guideline-compliant methods, and that data supporting the derivation of safe levels be widely distributed in peer-reviewed publications. These are the first guideline-compliant repeat-dose toxicity studies on a hemp-derived CBD isolate to be made available in a scientific journal. In 14-day subacute and 90-day subchronic toxicity studies, administration of CBD at concentrations up to 150 and 140 mg/kg-bw/d, respectively, by oral gavage did not produce any significant toxic effects. CBD was well tolerated at these dose levels, as evidenced by the absence of major treatment-related changes in the general condition and appearance of the rats, as well as growth, feed and water intake, ophthalmoscopic examinations, routine hematology and clinical chemistry parameters, urinalysis, necropsy, and histopathological findings.

The NOAEL in this 90-day study was determined to be the highest dose tested—140 mg/kg-bw/d—in male and female Sprague Dawley rats. The results of this study are comparable to findings reported in studies used for safety support that were included as part of the Epidiolex Non-Clinical data package reviewed by FDA (CEER, 2018a,b), but not published in the peer-reviewed literature. In one such study, rats were administered CBD for 26 weeks followed by a 28-day recovery period (Study number GWTX1412). In a second study, dogs were administered CBD for 39 weeks followed by a 28-day recovery period (Study number GWTX1413). In each study, the NOAEL was identified as the highest dose tested of 150 and 100 mg/kg bw/day, respectively; the main findings of liver effects were determined by CDER (2018a) not to be toxicologically significant based on reversibility.

The transient changes in motor activity observed in males of the 90-day study measured at this single timepoint were determined to be non-adverse; changes were not dose dependent, habituation was similar to that of controls, and no changes in functional observations were seen. In addition, hyperactivity is inconsistent with other repeat dose studies administering oral CBD, in which motor activity was either unchanged or decreased (CDER, 2018a).

With regard to histopathological findings, microscopic liver and adrenal gland changes observed in the 90-day study were resolved after a 28-day recovery period, and the incidences of microscopic changes were deemed comparable to controls at this time point. The observed hepatocellular hypertrophy fully resolved in both male and female rats following the recovery period. The treatment-related effects on liver weights and histopathology in this study are concluded to be non-adverse as they indicate induction of both phase 1 and phase 2 metabolic enzymes; with phase 2 enzymes critical in the elimination of thyroid hormones (Papineni et al., 2015; Noyes et al., 2019). Similar changes were noted in a recent reproductive toxicity study conducted with CBD in male and female rats (reported in our companion paper (Henderson et al., 2023b). Hepatocellular hypertrophy without other changes in histopathology or clinical chemistry measures indicative of liver toxicity, as is the case with CBD both in this study, and in the aforementioned reproductive toxicity study, is considered adaptive and non-adverse, as described in a review by Hall et al. (2012).

There are several pathways by which chemicals can produce anti-thyroid effects by perturbing thyroid-pituitary homeostasis, e.g., reduction of circulating thyroid hormones (T3 and T4) with increase TSH levels resulting in thyroid hyperplasia/hypertrophy (Hurley et al., 1998; Zabke et al., 2011; Noyes et al., 2019; Huisinga et al., 2020). One pathway involves chemical induction of liver enzymes that conjugate glucuronic acid to T3 and T4 via uridine diphosphate glucuronosyltransferase (UDPGT), which leads to increased T3 and T4 elimination and decreased serum concentrations of these hormones that trigger an increased synthesis of TSH (Papineni et al., 2015; Noyes et al., 2019). Serum levels of TSH were significantly increased in male and female rats at 80 mg/kg-bw/d CBD treatment and above compared to concurrent controls, without any change in T3 and T4 levels. The small change in serum TSH was determined not to be test article specific as no dose response was observed, values were within the laboratory's historical control range (Product Safety Labs, 2022), and the change did not coincide with a change in thyroid weight or histopathologic changes in the thyroid glands of rats in the high-dose groups. In addition, the small change observed in TSH levels across dose groups may also represent a lack of specificity of the immunoassay due to cross-reactivity of antibodies to other molecules (Li et al., 2019). Although the pattern of liver changes observed in these CBD studies may reflect hepatic microsomal enzyme induction, including UDPGT activity, the thyroid pathway was not perturbed in this study. Also of critical importance is that this liver induction is adaptive; this is reflected in the resolution of the liver lesions when CBD exposure ends, as demonstrated in the current study.

CBD administration resulted in an increase in the incidence and severity of cytoplasmic vacuolation of cells within the adrenal zona fasciculata in male rats of the two highest dose groups (120 and 140 mg/kg-bw/d). However, these lesions were resolved at the recovery time point, and the increased incidence of vacuolation in male adrenal glands in treated groups (0–2 per group) were not considered meaningfully different from the incidence in controls (1 per group). In addition, in the present 14- and 90-day studies, clinical pathology changes did not support the histopathology changes in the adrenal gland of male rats with changes in cholesterol (14- and 90-day study) and HDL (90-day

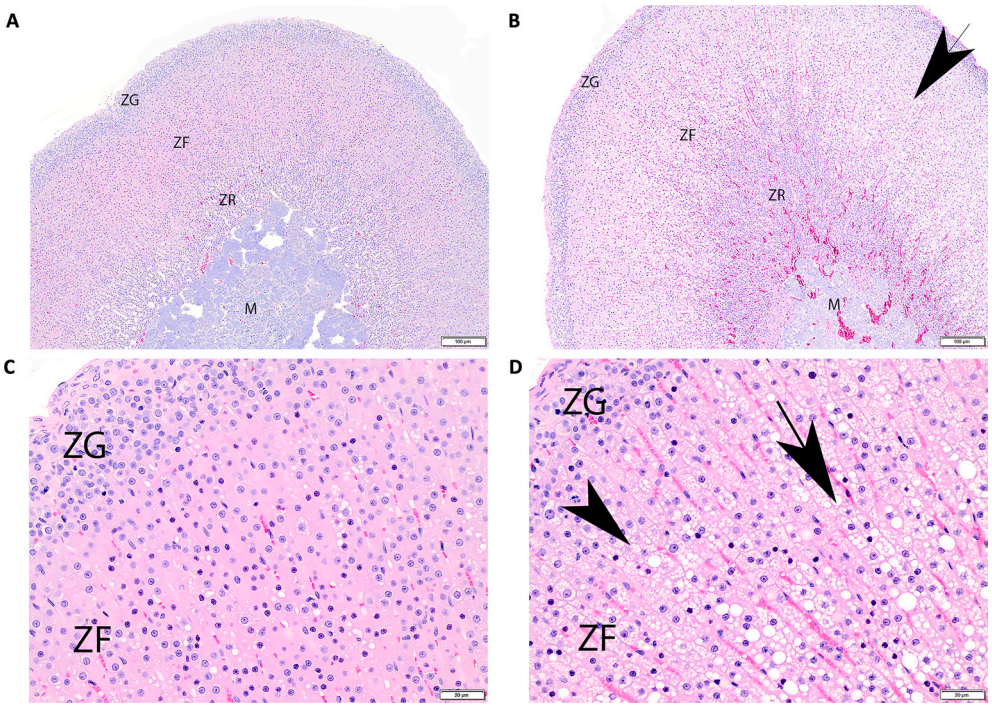

Fig. 2. Adrenal gland sections (H&E stained) from male Sprague-Dawley rats following oral gavage with daily doses of CBD Isolate for 90 days. A. Adrenal gland from a vehicle control male rat (Group 1 Animal 7006×, 40× magnification) with little cytoplasmic vacuolization noted in the cells of the zona fasciculata (ZF), below the minimum threshold for recording as a microscopic abnormality in this animal. B. Adrenal gland from a male rat administered 140 mg/kg-bw/d (Group 5 Animal 7122; 40× magnification) with cells of the ZF expanded by clear cytoplasmic vacuoles (arrow). This degree of cytoplasmic vacuolization was considered mild (Grade 2 severity). C. Adrenal gland from a vehicle control male rat (Group 1 Animal 7006×, 200× magnification) with little cytoplasmic vacuolization was noted in the cells of the ZF. D. Adrenal gland from a male rat administered 140 mg/kg-bw/d (Group 5 Animal 7122; 200× magnification) showing cytoplasmic microvacuolization (arrowhead) and macrovacuolization (arrow) that is appreciable in the cells of the ZF. ZG = zona glomerulosa; ZR = zona reticularis; M = medulla.

study) occurring only in females. The adrenal gland is a common target organ for chemical toxicity, and at the same time, it is not uncommon to observe non-specific cytotoxic effects in the adrenal cortex following administration of high dose levels of various test substances (Rosol et al., 2001). In addition, adrenal cortical vacuolization is considered a background lesion in laboratory rats (Laast et al., 2014) and is proposed to represent the accumulation of cholesterol and other steroid precursors. This vacuolization has been noted to be increased by xenobiotics that interfere with steroid synthesis (Brändli-Baliocco et al., 2018) and has been reported previously in laboratory rats administered cannabinoids (Dziwenka et al., 2020). For this reason, the fact that only male rats had an increase in the adrenal lesion, which was resolved when exposure stopped, combined with the knowledge that this is typically a background lesion in rats, support the conclusion that this lesion does not represent an adverse effect associated with administration of CBD.

5. Conclusion

No adverse treatment-related effects were observed following up to 90 days of treatment with a pure hemp-derived CBD isolate at any dose level tested. The oral NOAEL was therefore determined to be 150 and 140 mg/kg-bw/d in 14- and 90-day toxicity studies, respectively. These findings fill an important research gap in publicly available data on the safety profile of CBD, thus providing key data to support its safe use in foods and dietary supplements. Future studies testing higher doses of CBD will help to further elucidate any potential toxicity associated with repeat consumer ingestion.

CRediT authorship contribution statement

Rayetta G. Henderson: Conceptualization, Investigation, Writing – original draft. **Timothy W. Lefever:** Conceptualization, Writing – review & editing. **Melissa M. Heintz:** Writing – original draft. **Kristen R. Trexler:** Writing – review & editing. **Susan J. Borghoff:** Writing – review & editing. **Marcel O. Bonn-Miller:** Conceptualization, Supervision.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

This work was funded by Canopy Growth Corporation. Authors KRT, TWL, and MOB-M were employees of Canopy Growth Corporation during the conduct and drafting of this study; during their employment, they received stock options. ToxStrategies, a private consulting firm providing services on toxicology and risk assessment issues, received funds for conducting this work. Authors RGH, SJB, and MMH are employees of ToxStrategies.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fct.2023.113778.

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EXHIBIT D

JIM Original Article

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Cannabidiol-associated hepatotoxicity: A systematic review and meta-analysis

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Abstract. Lo LA, Christiansen A, Eadie L, Strickland JC, Kim DD, Boivin M, et al. Cannabidiol-associated hepatotoxicity: A systematic review and meta-analysis. *J Intern Med.* 2023;**00**:1–29.

Background. Findings of liver enzyme elevations in recent cannabidiol studies have raised concerns over liver safety. This study aimed to determine the association between cannabidiol use, liver enzyme elevation, and drug-induced liver injury (DILI).

Methods. In this systematic review and meta-analysis, a search of EMBASE, CENTRAL, CINAHL, Clinicaltrials.gov, Medline, medRxiv, and Web of Science of records up to February 2022 was conducted. Clinical trials initiating daily cannabidiol treatment with serial liver enzyme measures were included. The proportion of liver enzyme elevations and DILI were independently extracted from published reports. Pooled proportions and probability meta-analyses were conducted.

Results. Cannabidiol use was associated with an increased probability of liver enzyme elevation (N = 12 trials, n = 1229; OR = 5.85 95% CI = 3.84–8.92, $p < 0.001$) and DILI (N = 12 trials, n = 1229; OR = 4.82 95% CI = 2.46–9.45, $p < 0.001$) compared to placebo controls. In participants taking cannabidiol (N = 28 trials, n = 1533), the pooled proportion of liver enzyme elevations was 0.074 (95% CI 0.0448–0.1212), and DILI was 0.0296 (95% CI 0.0136–0.0631). High-dose CBD (≥1000 mg/day or ≥20 mg/kg/day) and concomitant antiepileptic drug use were identified as risk factors. No cases were reported in adults using cannabidiol doses <300 mg/day. No cases of severe DILI were reported.

Conclusions. Cannabidiol-associated liver enzyme elevations and DILI meet the criteria of common adverse drug events. Clinicians are encouraged to screen for cannabidiol use and monitor liver function in patients at increased risk.

Keywords: adverse events, cannabidiol, CBD, drug-induced liver injury, liver

Introduction

Cannabidiol (CBD) use has increased in recent years [1]. There is a belief that CBD is safer compared to its counterpart Δ9-tetrahydrocannabinol (THC), likely contributing to its popularity [1–3]. Although current evidence supports a more favorable safety profile for CBD compared to THC, there remains a need to investigate potential CBD harms [1, 4, 5].

Studies using CBD to prevent seizures in children and adults with epilepsy have noted incidental findings of liver enzyme (LE) elevation, predominantly the transaminases alanine transaminase (ALT) and aspartate aminotransferase (AST) [6–10]. These findings have been reported more frequently in individuals receiving high doses of CBD (e.g., ≥20 mg/kg/day) and taking valproate (VPA) or its derivative formulations concomitantly [4, 7–9, 11]. As such, the risk of elevated LEs

has been hypothesized to be associated with the use of concomitant hepatotoxic drugs (e.g., antiepileptic drugs [AEDs]) and high-dose CBD. Newer studies in healthy adults consuming the same mg/kg/day dose of CBD found 31% of participants also experienced significant LE elevation, consistent with drug-induced liver injury (DILI) [12]. Elevated cholestatic LEs, alkaline phosphatase (ALP), and gamma-glutamyltransferase were also seen in a study exploring CBD for symptom relief in Parkinson's disease, with one participant showing evidence of a DILI [13].

Despite LE elevations being an often harmless and reversible phenomenon associated with the administration of many drugs [12], significant LE elevation paired with abnormal liver function tests (e.g., elevated bilirubin) can be evidence of a severe DILI. In a multicenter observational study using data from the DILI Network, subjects with significant elevations in ALT (>5× upper limit of normal [ULN]), ALP (>2× ULN), and a total bilirubin >2.5 mg/dL or an INR >1.5 on 2 consecutive blood draws had a 9.4% risk of death or liver transplantation [14–16]. Severe DILI is the number one cause of acute liver failure in the United States [16]. There has yet to be a synthesis of information on liver enzyme elevations with CBD use. This potential risk is unknown to most clinicians, especially outside of pediatric epilepsy populations. Currently, there is no information on the magnitude of risk or how this risk may differ between populations or with other factors (e.g., dosing regimens). As access to CBD increases, it is important to evaluate this potential adverse drug event in a range of populations. Given this, a systematic review and meta-analysis were conducted to determine the association between CBD use, elevated LE, and DILI. Through this review, we sought to answer three key questions: (1) What is the proportion of CBD-induced liver injury?; (2) What are the outcomes of patients with elevated LEs?; and (3) What factors are associated with CBD-induced liver injury?

Methods

Search strategy and selection criteria

This review is registered on PROSPERO (the International Prospective Register of Systematic Reviews) (CRD42021249553) and reported in accordance with PRISMA guidelines (See eMethods for checklist) [17]. No ethical approval was necessary as this study retrieved and synthesized data from already published studies in which informed consent has already been obtained by trial investigators. A systematic search in EMBASE, CENTRAL, CINAHL, Clinicaltrials.gov, Medline, MedRxiv, and Web of Science was completed in August 2021 and updated on February 20th, 2022 (see eTable 1 for search strategies).

For inclusion, studies had to meet the following criteria: (1) involved human participants; (2) study design was a clinical trial or drug safety and tolerability study; (3) initiated daily CBD treatment with a reported dose, product type, and dosing schedule; (4) maximum 6 months duration of CBD treatment (to capture acute liver injury); and (5) completed serial LE measurements from baseline to the end of CBD treatment. See eTable 2 for the PICOS statement and eMethods for additional details on study selection. Three authors (LL, LE, and AC) assessed study eligibility and quality blinded and resolved any disagreement by consensus.

Data extraction and outcome measures

Data was blindly extracted by LL and AC (See eMethods additional for extraction details). The primary outcomes were the proportion of elevated LEs (ALT or AST >3× ULN or ALP >2× ULN) and DILI. DILI was defined as meeting one or more of the following criteria: (1) ALT or AST >5× ULN without symptoms; (2) rise of ALP >2× ULN, or the rise of bilirubin >2× ULN with any rise of ALT and AST; and (3) rise of ALT or AST <5× ULN with symptoms associated with liver injury, based on the American Association for the Study of Liver Disease (AASLD) and the American College of Gastroenterology's (ACG) guidelines.[15, 16, 18–20] Specifics on ULN can be found in eTable 3. Secondary prespecified measures included patient characteristics of those with LE elevations and DILI, time to detection and resolution, dose, and concomitant drugs.

Data synthesis and analysis

Data analysis was carried out in *R* using the *meta* [21] and *metafor* packages [22]. A descriptive summary was first carried out for study findings. Pooled proportions for elevated LEs and DILI were calculated using the metaprop function, which uses a mixed effects regression model to estimate the overall proportion from studies [21, 23]. Proportions meta-analyses used a generalized linear mixed model approach, logit transformation, and the Hartung–Knapp correction for confidence intervals. The pooled proportions analysis represents the estimated proportion of individuals

with elevated LEs or DILI among all participants taking CBD. In trials with multiple CBD-dosing arms, arms were collapsed into one. This analysis does not include a control intervention group. Using the analytic approach described above, additional moderation analyses were conducted for relevant drug- and population-related factors, including (1) CBD dose, (2) seizure disorder present, (3) age (child, adult, or mixed), (4) trial duration (continuous in weeks), and (5) concomitant VPA use. Dose was categorized as high-dose versus low-to-moderate dose, with high dose being a 1000 mg/day or ≥20 mg/kg/day cut-point, inline with the definition for a high dose used in other studies [12, 24]. Sensitivity tests using a trichotomized dose range (<300 mg/day vs. 300–999 mg/day vs. ≥1000 mg/day) and a continuous dose factor revealed similar findings (eTable 4). VPA was compared within studies in which the counts of participants with VPA use and elevated LEs and DILI by VPA use could be determined using Peto's method for fixed-effect pooled odds ratio as described below.

Effect estimates between CBD and placebo groups for elevated LEs and DILI were calculated using Peto's method for fixed-effect pooled odds ratios (OR). This approach was used due to rare baseline event rates. Peto's method has been reported as the preferred and less biased approach over traditional 0.5 cell correction methods when baseline event rates are rare [25, 26]. Only parallel RCTs with an intervention and placebo group were included in this analysis. The experimental intervention is made up of participants receiving the CBD intervention (inclusive of all dosing groups). The control intervention is made up of participants receiving the placebo intervention. Appropriateness of a fixed-effects model was assessed using the Q test statistic for heterogeneity. The proportion of total variability due to between-study heterogeneity was evaluated with the Higgins I^2 statistic.

Results

Figure 1 shows the PRISMA flowchart of study selection [17]. A total of 3116 records were identified from database searches. After the removal of duplicates, 2647 records were screened, from which 140 full-text documents were reviewed, and 28 papers were included [6–10, 12, 13, 27–47]. The pooled proportions analysis included 1533 participants on CBD across all 28 studies. The pooled OR analysis included 1229 participants from 12 randomized controlled trials (RCTs).

Study characteristics of the 28 studies are presented in Table 1. In total, there were 1533 participants on CBD and 494 on placebo. Of the study populations, eight were pediatric participants with seizure disorders (n = 633) [7, 9, 30, 37–39, 42, 47], two were adult participants with seizure disorders (n = 54) [6, 46], six were pediatric and adult participants with seizure disorders (n = 910) [8, 10, 29, 33, 35, 45], six were healthy adult participants (n = 263) [12, 28, 32, 40, 43, 44], two were adult participants with neurodegenerative disorders (n = 28) [13, 27], two were pediatric participants with developmental disorders (n = 45) [31, 36], and two were adult participants with inflammatory bowel disease (n = 79) [34, 41].

The majority of studies (n = 25, 89.29%) administered CBD through an oral solution [6–10, 12, 27–30, 33, 39, 40, 42–47], two studies (7.14%) used sublingual administration [32, 41], and one study (3.57%) used transdermal administration [31].

The pooled proportion of elevated LEs (ALT or AST >3× ULN or ALP >2× ULN) in participants taking CBD was 0.074 [95% CI 0.0448–0.1212] (Fig. 2). There were 159 cases in 1514 participants across 27 studies, representing a raw proportion of 10.50%. Within the 494 participants receiving a placebo control across the 12 RCTs, there were two cases (0.40%) of elevated LEs reported. The number of LE elevations could not be determined for 19 participants on CBD in one study based on the available data [37].

The pooled proportion for the subset of participants taking CBD who also met criteria for DILI was 0.0296 [95% CI 0.0136–0.0631] (Fig. 2). There were 57 cases of DILI in 1286 participants across 25 studies, representing a raw proportion of 4.43%. One of 494 participants (0.20%) receiving placebo had LE elevations that met criteria for DILI [7]. For three of the studies (n = 247), containing 26 cases of elevated LEs, the proportion of participants with DILI could not be determined based on the data provided [30, 33, 35]. No participants met the criteria for severe DILI, as determined by Hy's law (ALT or AST >3× ULN and total bilirubin >2× ULN) [20, 48]. A summary of results is presented in Table 1.

Pooled analysis of 12 RCTs showed that the CBD group had significantly higher odds of LE elevation

Table 1. *Study characteristics*

Study	Study design	Population	Treatment arms, target dose	CBD administration protocol	Liver enzyme elevation (ALT or AST >3× ULN)	Subset meeting criteria for drug-induced liver injury	Pattern of elevation	Time course
Ben-Menachem et al. [6]	Double-blind–parallel RCT	Adult epilepsy (unspecified) Mean age: 30 (range: 17–55)	Oral CBD solution, 20 mg/kg/day + VPA, 1015 (200–1950) mg/day ($N = 16$) VPA, 1450 (900–2500) mg/day + placebo ($N = 4$) Oral CBD solution, 20 mg/kg/day + Stiripentol, 625 (500–2000) mg/day ($N = 12$) Stiripentol, 1250 (500–2000) mg/day + placebo ($N = 2$)	24-day CBD treatment period: 10-day dose titration, 14-day maintenance 10-day taper, 4-week follow-up	2/28 ppt on CBD Ppt 1: ALT 5.5× ULN (205 U/L) Ppt 2: ALT 365 U/L 12.2× ULN, AST 205 U/L 5.4× ULN 0 ppt on VPA + placebo 0 ppt on Stiripentol + placebo	2/2	Hepatocellular ($n = 2$)	Detection: Ppt 1: Day 20 Ppt 2: Day 28 Cases Resolved: 2/2 with discontinuation of CBD Time to resolution: Ppt 1: 20 days after CBD discontinuation Ppt 2: 31 days after CBD discontinuation
Consroe et al. [27] *No participant specific information available*	Double-blind–crossover RCT	Adult neurodegenerative disorder (Huntington's disease) Mean age: 48 (range: 17–66)	Oral CBD solution, 10 mg/kg/day → placebo ($N = 6$) placebo → Oral CBD solution, 10 mg/kg/day ($N = 9$)	6-week CBD treatment period 1-week washout 6-week placebo treatment 1-week follow-up	0/15 ppt while on CBD 0 ppt while on placebo	–	–	–

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Table 1. *(Continued)*

Study	Study design	Population	Treatment arms, target dose	CBD administration protocol	Liver enzyme elevation (ALT or AST >3× ULN)	Subset meeting criteria for drug-induced liver injury	Pattern of elevation	Time course
Crippa et al. [28]	Open-label–parallel RCT	Healthy adults Mean age: 33	Standard care + Oral CBD solution, 300 mg/day ($N = 59$) Standard care ($N = 59$)	4-week CBD treatment period	4/59 ppt on CBD Ppt 1: AST 3× ULN (AST 110) Ppt 2: AST 6.25× ULN (AST 200) Ppt 3: ALT 15.7× ULN (ALT 489), AST 5.4× ULN (AST 174) Ppt 4: ALT 3× ULN (ALT 105) 0 ppt on standard of care	2/4 (Ppt 70, Ppt 77)	Hepatocellular ($n = 4$)	Detection: Ppt 1, 2, 4: day 28 Ppt 3: day 21 Cases resolved: 4/4 with discontinuation of CBD Time to resolution: 4/4 resolved 7 days after CBD discontinuation
Devinsky et al. [29] *No participant specific information available*	Open-label–single-arm trial	Epilepsy–pediatric and young adult (treatment-resistant epilepsy) Mean age: 11 (range: 1–26)	Oral CBD solution, 25 mg/kg/day ($N = 114$) Oral CBD solution, >25–50 mg/kg/day ($N = 48$)	12-week CBD treatment period: Starting dose 2.5 mg/kg/day, titrate 2–5 mg/kg/day of CBD once per week until target dose reached	11/162 ppt on CBD	1/11	Unknown	Detection: Unknown within 3 months Cases resolved: Unknown Time to resolution: Unknown

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Table 1. *(Continued)*

Study	Study design	Population	Treatment arms, target dose	CBD administration protocol	Liver enzyme elevation (ALT or AST >3× ULN)	Subset meeting criteria for drug-induced liver injury	Pattern of elevation	Time course
Devinsky et al. [8] (GWPCARE1, Part A)	Double-blind–parallel RCT	Pediatric epilepsy (Dravet Syndrome) Mean age: 8 (range: 4–11)	Oral CBD solution, 5 mg/kg/day ($N = 10$) Oral CBD solution, 10 mg/kg/d ($N = 8$) Oral CBD solution, 20 mg/kg/day ($N = 9$) placebo ($N = 7$)	3-week CBD treatment period: Starting dose 2.5 mg/kg/day, titrate 2.5–5 mg/kg every other day until target dose reached 10-day taper, 4-week follow-up	6/27 ppt on CBD Ppt 1: AST 3× ULN (20 mg/kg/day of CBD) Ppt 2: AST 3× ULN, GGT 8× ULN - eosinophilia and vomiting (10 mg/kg/day of CBD) Ppt 3: AST >6× ULN, ALT >4× ULN–with fatigue (20 mg/kg/day of CBD) Ppt 4: AST > 6 × ULN, ALT >7× ULN (20 mg/kg/day of CBD) Ppt 5: ALT > 17 × ULN, AST > 8× ULN–with rash (20 mg/kg/day of CBD) Ppt 6: ALT >5×ULN, AST >3× ULN (5 mg/kg/day of CBD) 0 ppt on placebo	5/6 (Ppt 2, Ppt 3, Ppt 4, Ppt 5, Ppt 6)	Hepatocellular ($n = 5$) Mixed ($n = 1$)	Detection: 6/6 Between days 16–22 Cases resolved: 5/6 with discontinuation of CBD Ppt 6 ALT/AST returned to normal, GGT remained high at day 40 with discontinuation of CBD Time to resolution: Ppt 1: 13 days after CBD discontinuation Ppt 2: ∼1 month after CBD discontinuation Ppt 3: 24 days after CBD discontinuation Ppt 4: 10 days after detection following a taper of CBD Ppt 5: ∼1 month after CBD discontinuation Ppt 6: ALT/AST elevation resolved 40 days after CBD discontinuation

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Table 1. *(Continued)*

Study	Study design	Population	Treatment arms, target dose	CBD administration protocol	Liver enzyme elevation (ALT or AST >3× ULN)	Subset meeting criteria for drug-induced liver injury	Pattern of elevation	Time course
Devinsky et al. [7] (GWPCARE1, Part B) *No participant specific information available*	Double-blind–parallel RCT	Pediatric epilepsy (Dravet Syndrome) Mean age: 10 (range: 2–18)	Oral CBD solution, 20 mg/kg/day ($N = 61$) placebo ($N = 59$)	14-week CBD treatment period: 2-week titration, 12-week maintenance 10-day taper, 4-week follow-up	12/61 ppt on CBD 1/59 ppt on placebo	3/12 on CBD 1/1 ppt on placebo	Hepatocellular ($n = 12$ CBD, $n = 1$ placebo)	Detection: Unknown, within 4 months Cases resolved: 9/12 on CBD resolved spontaneously while continuing CBD 3/12 on CBD resolution unclear 1/1 on placebo resolution unclear Time to resolution: Unknown
Devinsky et al. [9] (GWPCARE3) *No participant specific information available*	Double-blind–parallel RCT	Epilepsy–pediatric and adult (Lennox-Gastaut syndrome) Mean age: 16 (range: 3–48)	Oral CBD solution, 20 mg/kg/day ($N = 76$) Oral CBD solution, 10 mg/kg/day ($N = 73$) placebo ($N = 76$)	14-week CBD treatment period: Starting dose 2.5 mg/kg/day, 2-week titration, 12-week maintenance 10-day taper, 4-week follow-up	14/149 ppt on CBD 20 mg/kg/day ($n = 11$) 10 mg/kg/day ($n = 3$) 0 ppt on placebo	5/14	Hepatocellular ($n = 13$) Mixed ($n = 1$)	Detection: Unknown, within 4 months Cases resolved: 14/14 cases resolved: Spontaneously while continuing CBD ($n = 3$) Discontinue or reduce dose of CBD or reduce dose of antiepileptic drugs ($n = 11$) Time to resolution: Unknown, within 4 months

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Table 1. *(Continued)*

Study	Study design	Population	Treatment arms, target dose	CBD administration protocol	Liver enzyme elevation (ALT or AST >3× ULN)	Subset meeting criteria for drug-induced liver injury	Pattern of elevation	Time course
D'Onofrio et al. [30] *No participant specific information available*	Open-label–single-arm trial	Pediatric epilepsy (treatment-resistant Epilepsy) Mean age: 9 (range: 6–14)	Oral CBD solution, 20 mg/kg/day (N = 119)	26-week CBD treatment period: Starting dose 2.5 mg/kg/day, 4-week titration to 10 mg/kg/day, gradual titration 2.5 mg/kg/day per week until target dose reached	11/119 ppt on CBD	Unknown	Hepatocellular (n = 11)	Detection: Unclear, within 6 months Cases resolved: 6/11 cases resolved: Decrease CBD dose (n = 1) Reduce dose of antiepileptic drugs (n = 5) Unknown if resolved (n = 5) Time to resolution: Unknown
Heussler et al. [31]	Open-label–single-arm trial	Pediatric developmental disorder (Fragile X syndrome) Mean age: 10 (range: 6–17)	Transdermal CBD gel, 50 mg/day (N = 1) Transdermal CBD gel, 100 mg/day (N = 3) Transdermal CBD gel, 250 mg/day (N = 16)	12-week CBD treatment period: 6-week titration, 6-week maintenance 1- or 2-week taper (100–250 mg/day group), or discontinuation (50 mg/day group), or enter open label extension	0/20 ppt on CBD (250 mg/day)	–	–	–

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Table 1. *(Continued)*

Study	Study design	Population	Treatment arms, target dose	CBD administration protocol	Liver enzyme elevation (ALT or AST >3× ULN)	Subset meeting criteria for drug-induced liver injury	Pattern of elevation	Time course
Hosseini et al. [32]	Double-blind–parallel RCT	Healthy adults Mean age: 30 (range: 27–34)	Sublingual CBD wafer, 100 mg/day ($N = 4$) placebo ($N = 2$)	5-day CBD treatment period	0/6 ppt on CBD (100 mg/day) 0 ppt on placebo	–	–	–
Iannone et al. [33] *No participant specific information available*	Open-label–single-arm trial	Epilepsy–pediatric and adult (Dravet and Lennox-Gastaut syndromes) Mean age: 21 (range: 3–56)	Oral CBD solution, 18–25 mg/kg/day ($N = 93$)	26-week CBD treatment period: Starting dose 2–5 mg/kg/day, individualized titration	10/93 ppt on CBD 0–10 mg/kg/day ($n = 4$) 11–15 mg/kg/day ($n = 3$) 16–25 mg/kg/day ($n = 3$))	Unknown	Hepatocellular ($n = 10$)	Detection: Unknown Cases resolved: Unknown Time to resolution: Unknown
Irving et al. [34]	Double-blind–parallel RCT	Adult inflammatory bowel disease (Ulcerative Colitis) Mean age: 44	Oral 50 mg CBD-rich (≤4.7% THC) capsules, 250 mg/day ($N = 29$) placebo ($N = 31$)	10-week CBD treatment period: Starting dose 50 mg/day, 2-week titration, 8-week maintenance 1-week follow-up	0/29 ppt on CBD 0 ppt on placebo	–	–	–

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Table 1. *(Continued)*

Study	Study design	Population	Treatment arms, target dose	CBD administration protocol	Liver enzyme elevation (ALT or AST >3× ULN)	Subset meeting criteria for drug-induced liver injury	Pattern of elevation	Time course
Klotz et al. [35] *No participant specific information available*	Open-label–single-arm trial	Epilepsy–pediatric and adult (treatment-resistant epilepsy) Mean age child: 9 Mean age adult: 32	Oral CBD solution, 18–20 mg/kg/day ($N = 35$)	13-week CBD treatment period: Starting dose 5 mg/kg/day, 2–3-week titration to 18–20 mg/kg/day, further titration to max. 50 mg/kg/day if needed	5/35 ppt on CBD at least 1/5 GGT >3× ULN	Unknown	unknown	Detection: Unknown Cases resolved: 5/5 cases resolved: Spontaneously while continuing CBD ($n = 4$) Discontinuation of CBD ($n = 1$) Time to resolution: Unknown
Leehey et al. [13]	Open-label–single-arm trial	Adult neurodegenerative disorder (Parkinson's disease) Mean age: 68 (range: 56–75)	Oral CBD solution, 20 mg/kg/day ($N = 13$)	approx. 3-week CBD treatment period: Starting dose 5 mg/kg/day, 1-week titration, 10–15-day maintenance 2-week follow-up	2/13 ppt on CBD Ppt 1: ALT 2.9× ULN (150 U/L), AST 1.5× ULN (58 U/L), GGT 3.2× ULN (206 U/L), ALP 4.3× ULN (503 U/L), bili 1.4 (norm 0.1–1.3) Symptomatic, classified as hepatitis Ppt 2: AST 2× ULN (80 U/L; GGT 3.6× ULN (229 U/L); ALP 2.7× ULN (317 U/L)–asymptomatic	2/13	Mixed ($n = 2$)	Detection: Day 27 Cases resolved: 2/2 cases resolved with discontinuation of CBD Time to resolution: 2/2 cases resolved 14 days after discontinuation

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Table 1. *(Continued)*

Study	Study design	Population	Treatment arms, target dose	CBD administration protocol	Liver enzyme elevation (ALT or AST >3× ULN)	Subset meeting criteria for drug-induced liver injury	Pattern of elevation	Time course
Libzon et al. [36] *No participant specific information available*	Open-label–single-arm trial	Pediatric developmental disorder (complex motor disorders) Mean age: 6 (range: 1–12)	Oral 20:1 CBD:THC solution, mean CBD dose 91.75 mg/day ($N = 11$) Oral 6:1 CBD:THC solution, mean CBD dose 38 mg/day ($N = 14$)	22-week CBD treatment period: individualized titration periods	0/25 ppt on CBD	–	–	–
McCoy et al. [37] *No participant specific information available*	Open-label–single-arm trial	Pediatric epilepsy (Dravet Syndrome) Mean age: 10 (range: 2–18)	Oral 50:1 CBD:THC solution, 16 mg/kg/day CBD: 0.32 mg/kg/day THC ($N = 19$)	20-week CBD treatment period: Starting dose 2 mg/kg/day, titrate 2 mg/kg/day CBD every 7 days until target or best-tolerated dose	Unknown 8/19 liver enzyme abnormalities but unknown how many >3× ULN	Unknown	Unknown	Detection: Unknown Cases resolved: Unknown Time to resolution: Unknown

(*Continued*)

Table 1. *(Continued)*

Study	Study design	Population	Treatment arms, target dose	CBD administration protocol	Liver enzyme elevation (ALT or AST >3× ULN)	Subset meeting criteria for drug-induced liver injury	Pattern of elevation	Time course
Miller et al. [38] (GWPCARE2)	Double-blind–parallel RCT	Pediatric epilepsy (Dravet syndrome) Mean age: 9 (range: 2–19)	Oral CBD solution, 20 mg/kg/day ($N = 67$) Oral CBD solution, 10 mg/kg/day ($N = 66$) placebo ($N = 65$)	14-week CBD treatment period: Starting dose 2.5 mg/kg/day, 2-week titration, 12-week maintenance 10-day taper, 4-week follow-up	16/133 ppt on CBD 10 mg/kg/day ($n = 3$) 20 mg/kg/day ($n = 13$) 0 ppt on placebo	2/16 ppt	Hepatocellular ($n = 16$)	Detection: Unknown Cases resolved: 16/16 resolved: Discontinuation of CBD ($n = 8$) Spontaneously while continuing CBD ($n = 4$) Reduce CBD dose ($n = 1$) Reduce AED dose ($n = 3$) Time to resolution: Unknown
Mitelpunkt et al. [39] *No participant specific information available*	Open-label–single-arm trial	Pediatric epilepsy (treatment-resistant epilepsy[1]) Mean age: 9	Oral 50 mg CBD capsules, 25 mg/kg/day or 450 mg/day (lower of the two was target dose) ($N = 16$)	12-week CBD treatment period: Starting dose: 50 mg/day, 2-week titration, 10-week maintenance 1-week taper, 1-week follow-up	0/16 ppt on CBD	–	–	–

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Table 1. *(Continued)*

Study	Study design	Population	Treatment arms, target dose	CBD administration protocol	Liver enzyme elevation (ALT or AST >3× ULN)	Subset meeting criteria for drug-induced liver injury	Pattern of elevation	Time course
Morrison et al. [40]	Open-label–single-arm trial	Healthy adults Mean age range: 30	CLB 10 mg/day + Oral CBD solution 1500 mg/day (N = 12) Oral CBD solution 1500 mg/day + CLB 10 mg/day (N = 15) Stiripentol 1500 mg/day + Oral CBD solution 1500 mg/day (N = 12) Oral CBD solution 1500 mg/day + Stiripentol 1500 mg/day (N = 12) VPA 1000 mg/day + Oral CBD solution 1500 mg/day (N = 12) Oral CBD solution 1500 mg/day + VPA 1000 mg/day (N = 14)	~1–2-week CBD treatment period CLB±CBD 1-day CLB titration, 0 or 10-day CBD titration CBD±CLB 0 or 10-day CBD titration, No CLB titration Stiripentol ±CBD 1-day Stiripentol titration, 3 or 10-day CBD titration CBD± Stiripentol 0 or 3-day CBD titration, No Stiripentol titration VPA±CBD 5-day VPA titration, 3 or 10-day CBD titration CBD±VPA 3 or 10-day CBD titration, 5-day VPA titration	0/78 ppt on CBD	0	–	–

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Table 1. *(Continued)*

Study	Study design	Population	Treatment arms, target dose	CBD administration protocol	Liver enzyme elevation (ALT or AST >3× ULN)	Subset meeting criteria for drug-induced liver injury	Pattern of elevation	Time course
Naftali et al. [41]	Double-blind–parallel RCT	Adult inflammatory bowel disease (Crohn's disease) Mean age CBD group: 45 (range: 18–75)	Sublingual CBD solution, 20 mg/day ($N = 10$) placebo ($N = 9$)	8-week CBD treatment period 2-week follow-up	0/10 ppt on CBD 0 ppt on placebo	–	–	
Neubauer et al. [42]	Retrospective Chart Review	Pediatric epilepsy (treatment-resistant epilepsy[1]) Mean age: 8 (range: 1–23)	Oral CBD solution, 16 mg/kg/day ($N = 66$)	26-week CBD treatment period: Starting dose 1–3 mg/kg/day, individual titration until at least 8 mg/kg/day, or target or best-tolerated dose	1/66 ppt on CBD Ppt 1: ALT 4.5× ULN, GGT 16.5× ULN–stomach pain (250 mg/day CBD)	1/1	Mixed ($n = 1$)	Detection: Week 5 Cases resolved: 1/1 with CBD dose decrease Time to resolution: 1 month after dose decrease

(*Continued*)

Table 1. *(Continued)*

Study	Study design	Population	Treatment arms, target dose	CBD administration protocol	Liver enzyme elevation (ALT or AST >3× ULN)	Subset meeting criteria for drug-induced liver injury	Pattern of elevation	Time course
Taylor et al. [43]	Open-label–single-arm trial Part 2: Double-blind Randomized Placebo-controlled (not used due to lack of abstinence period)	Healthy adults Mean age: 25	Oral CBD solution, 1500 mg/day ($N = 30$) placebo ($N = 12$)	4-week CBD treatment period	2/30 ppt on CBD Ppt 1: ALP 1.8× ULN, ALT 4.1× ULN–severe eosinophilia, headache, mild chest discomfort Ppt 2: ALT 7.7× ULN–mild epigastric discomfort, regurgitation, esophageal discomfort	1/1/2	Hepatocellular ($n = 1$) Mixed ($n = 1$)	Detection: Ppt 1: day 28 Ppt 2: day 21 Cases resolved: 2/2 with discontinuation of CBD Time to resolution: Ppt 1: 14 days after discontinuation Ppt 2: 28 days after discontinuation

(*Continued*)

Table 1. *(Continued)*

Study	Study design	Population	Treatment arms, target dose	CBD administration protocol	Liver enzyme elevation (ALT or AST >3× ULN)	Subset meeting criteria for drug-induced liver injury	Pattern of elevation	Time course
Thai et al. [44]	Open-label–single-arm trial	Healthy adults Mean age: 33	Oral CBD solution, 1500 mg/day + CYP1A2 caffeine probe ($N = 16$)	25-day CBD treatment period: Day 1: 200 mg caffeine + CBD-matched placebo, Day 3: 250 mg/day CBD, Day 4–5: 500 mg/day CBD, Day 6–7: 750 mg/day CBD, Day 8–9: 1000 mg/day CBD, Day 10–11: 1250 mg/day CBD, Day 12–27: 1500 mg/day CBD, Day 26: 200 mg caffeine + 1000 mg/day CBD	6/16 ppt on CBD Ppt 1, 2, 3: ALT and AST >3× ULN Ppt 4: ALT and AST >3× ULN and eosinophilia Ppt 5: ALT >3× ULN–abdominal discomfort, vomiting, eosinophilia Ppt 6: ALT and AST >3× ULN-nausea, syncope 2/6 also had ALP >2× ULN and 5/6 had GGT >3× ULN but unclear which participants	6/6	Hepatocellular and mixed, exact numbers unclear	Detection: 6/6 cases Day 23–28 Cases resolved: 5/6 cases resolved with discontinuation CBD 1/6 case unresolved Time to resolution: 5/6 cases Day 9–49 post-CBD discontinuation, most 10–20-day post-CBD discontinuation

(*Continued*)

Table 1. *(Continued)*

Study	Study design	Population	Treatment arms, target dose	CBD administration protocol	Liver enzyme elevation (ALT or AST >3× ULN)	Subset meeting criteria for drug-induced liver injury	Pattern of elevation	Time course
Thiele et al. [10] (GWPCARE4) *No participant specific information available*	Double-blind–parallel RCT	Epilepsy–pediatric and adult (Lennox-Gastaut syndrome) Mean age: 15 (range: 3–45)	Oral CBD solution, 20 mg/kg/day ($N = 86$) placebo ($N = 85$)	14-week CBD treatment period: Starting dose 2.5 mg/kg/day, 2-week titration, 12-week maintenance 10-day taper or enter open label extension, 4-week follow-up	20/86 ppt on CBD 3/20 ppt on CBD also had GGT >3× ULN 1/85 ppt on placebo	6/20	Hepatocellular ($n = 17$) Mixed ($n = 3$)	Detection: Unknown Cases resolved: 20/20 resolved: Spontaneous while continuing CBD at same dose ($n = 8$) Discontinue or reduce dose of CBD ($n = 9$) Reduce AED dose ($n = 3$) Time to resolution: Unknown
Thiele et al. [45] (GWPCARE6) *No participant specific information available*	Double-blind–parallel RCT	Epilepsy–pediatric and adult (tuberous sclerosis complex) Mean age: 11 (range: 1–57)	Oral CBD solution, 25 mg/kg/day ($N = 75$) Oral CBD solution, 50 mg/kg/day ($N = 73$) placebo ($N = 76$)	16-week CBD treatment period: Starting dose 5 mg/kg/day, 4-week titration, 12-week maintenance 10-day taper or enter open label extension, 4-week follow-up	28/148 ppt on CBD 25 mg/kg/day ($n = 9$) 50 mg/kg/day ($n = 19$) 0 ppt on placebo	14/28	Mixed ($n = 22$) Hepatocellular ($n = 6$)	Detection: 20/28 cases within 30 days of CBD initiation Cases resolved: 28/28 resolved: Spontaneous while continuing CBD at same dose ($n = 13$) Discontinuation of CBD ($n = 5$) Reduce dose of AED ($n = 10$) Time to resolution: Unknown

(*Continued*)

Table 1. *(Continued)*

Study	Study design	Population	Treatment arms, target dose	CBD administration protocol	Liver enzyme elevation (ALT or AST >3× ULN)	Subset meeting criteria for drug-induced liver injury	Pattern of elevation	Time course
VanLandingham et al. [46]	Double-blind–parallel RCT	Adult epilepsy (Unspecified) Mean age: 37	Oral CBD solution 20 mg/kg/day + CLB 10–20 mg/day ($N = 16$) CLB 5–20 mg/day + placebo ($N = 4$)	31-day CBD treatment period: Day 1: stable CLB dose, Day 2–11: 10-day titration of CBD until target dose, Day 12–32: 3-week maintenance Taper or enter open label extension	2/16 ppt on CBD Ppt 1: ALT 8.2× ULN, AST and GGT >3× ULN Ppt 2: ALT 5× ULN 0 ppt on placebo	2/2	Hepatocellular ($n = 1$) Mixed ($n = 1$)	Detection: 2/2 within 30 days Cases resolved: 2/2 with discontinuation of CBD Time to resolution: Ppt 1: 12-day post-CBD discontinuation Ppt 2: 22-day post-CBD discontinuation

(*Continued*)

Table 1. *(Continued)*

Study	Study design	Population	Treatment arms, target dose	CBD administration protocol	Liver enzyme elevation (ALT or AST >3× ULN)	Subset meeting criteria for drug-induced liver injury	Pattern of elevation	Time course
Watkins et al. [12]	Open-label–single-arm trial	Healthy adults Median age: 29	Oral CBD solution, 1500 mg/day + CYP1A2 caffeine probe ($N = 16$)	25-day CBD treatment period: Day 1: 200 mg caffeine + CBD-matched placebo, Day 3: 250 mg/day CBD, Day 4–5: 500 mg/day CBD, Day 6–7: 750 mg/day CBD, Day 8–9: 1000 mg/day CBD, Day 10–11: 1250 mg/day CBD, Day 12–27: 1500 mg/day CBD, Day 26: 200 mg caffeine + 1000 mg/day CBD 2-week follow-up	6/16 ppt on CBD Ppt 1: ALT 5× ULN GGT 3× ULN ALP 4× ULN Ppt 3: GGT 4× ULN ALT and AST >7× ULN Ppt 4: AST >9× ULN ALT 8× ULN GGT 7× ULN Ppt 5: ALT and AST 6× ULN GGT 3× ULN Ppt 13: ALT 6× ULN AST 5× ULN GGT 5× ULN Ppt x: ALT >3× ULN	5/6	Mixed ($n = 5$) Unclear ($n = 1$)	Detection: 6/6 day 23–27 Cases resolved: 6/6 with discontinuation of CBD Time to resolution: 6/6 approximately 10 days after CBD discontinuation
Wheless et al. [47] *No participant specific information available*	Open-label–single-arm trial	Pediatric epilepsy (treatment-resistant epilepsy) Mean age: 8	Oral CBD solution, 10 mg/kg/day ($N = 20$) Oral CBD solution, 20 mg/kg/day ($N = 20$) Oral CBD solution, 40 mg/kg/day ($N = 21$)	1-week CBD treatment period: Day 1 single dose 5, 10, or 20 mg/kg, Day 2–3 no CBD, Day 4–10: 10, 20, or 40 mg/kg/day 1-week follow-up	1/61 ppt on 40 mg/kg/day CBD	0	Hepatocellular ($n = 1$)	Detection: Day 8 Cases resolved: 1/1 with discontinuation of CBD Time to resolution: 3 days after CBD discontinuation

Abbreviations: AED, antiepileptic drug; ALP, alkaline phosphatase; ALT, alanine transaminase; AST, aspartate aminotransferase; GGT, gamma-glutamyltransferase.

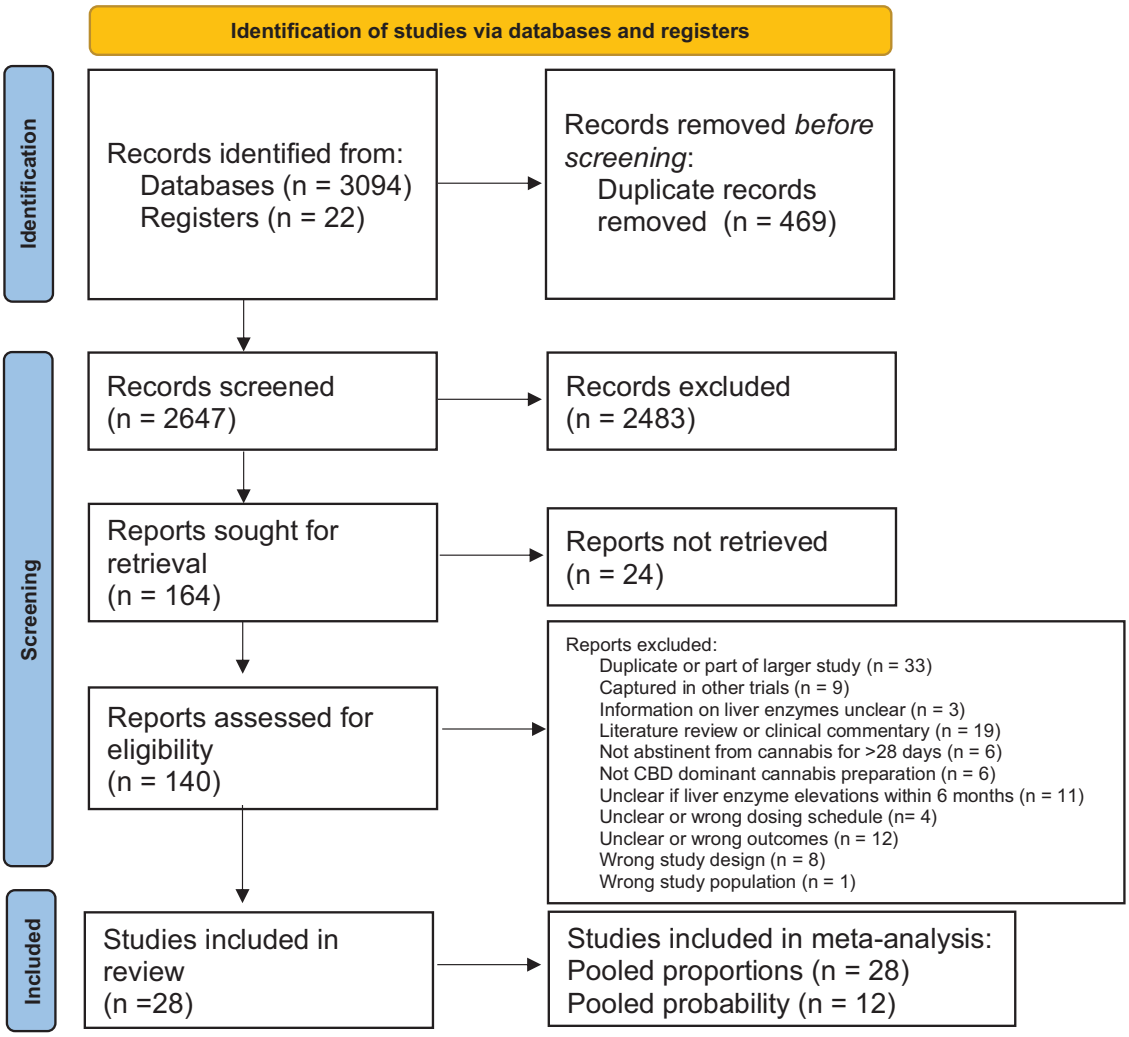

Fig. 1 *PRISMA flow diagram for review on cannabidiol and hepatotoxicity.*

than the control group (N = 12 trials, n = 1229; OR = 5.85 95% CI = 3.84–8.92, p< 0.001; Fig. 3). The Q test statistic for heterogeneity provided strong evidence against heterogeneity—Q(df = 8) = 0.81, p = 0.99—supporting the use of a fixed-effects model.

The CBD group had significantly higher odds of DILI than the control group (N = 12 trials, n = 1229; OR = 4.82 95% CI = 2.46–9.45, p < 0.001; Fig. 3). The Q test statistic for heterogeneity provided strong evidence against heterogeneity—Q(df = 8) = 0.82, p = 0.99—supporting the use of a fixed-effects model.

LE elevations and DILI were observed in both children and adults, and in a range of populations, including healthy adults. Additional participant details are presented in eResults.

In 123/159 cases (77.36%) of LE elevations, participants were receiving high doses of CBD (≥1000 mg/day or ≥20 mg/kg/day). A similar trend was seen for DILI, with 51/57 cases (89.47%) being in participants on high doses of CBD (≥1000 mg/day or ≥20 mg/kg/day). DILI at lower doses of CBD was rare, with only two cases reported in healthy adults at a dose of 300 mg/day [28] and three cases reported in pediatric

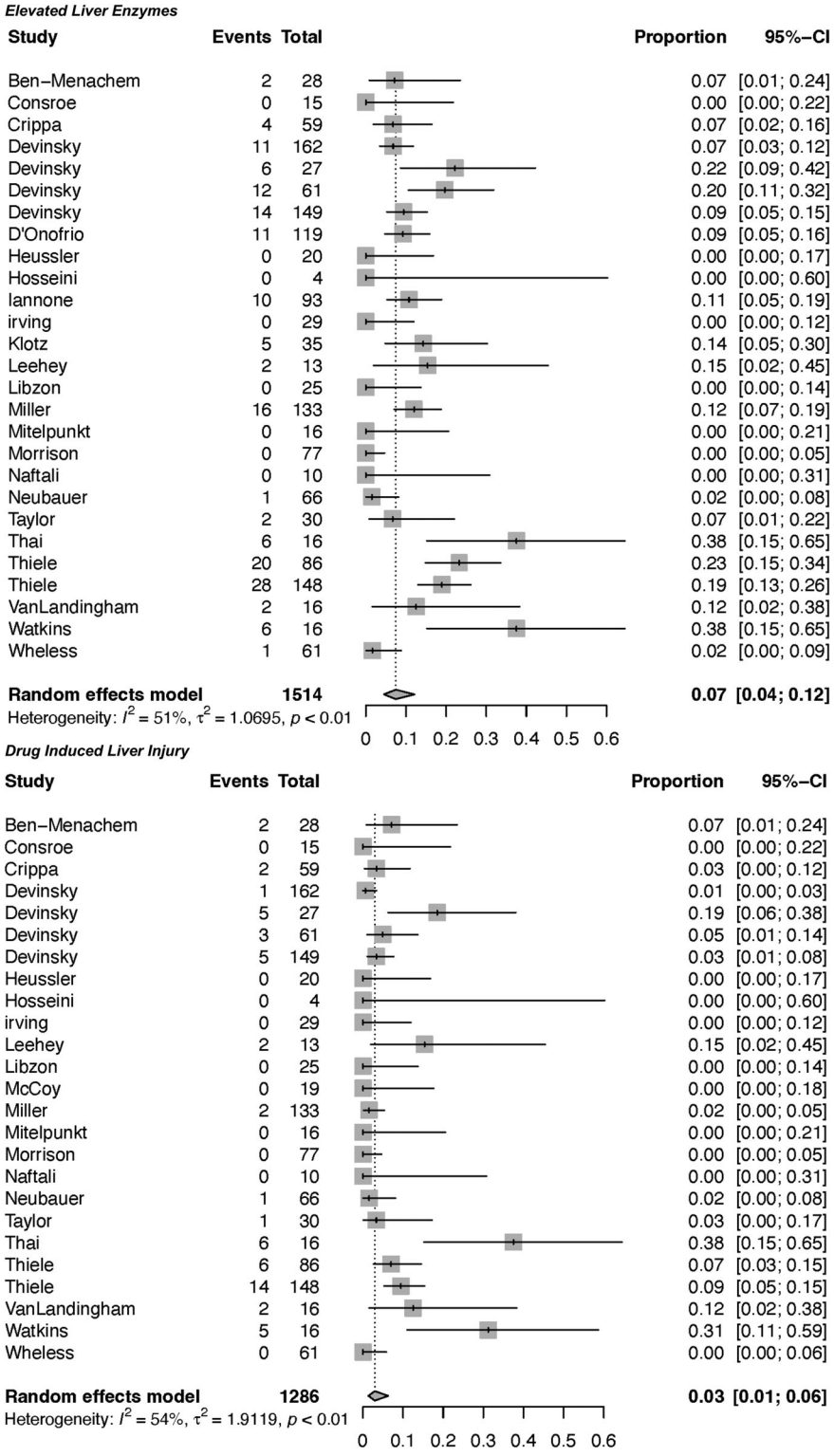

Fig. 2 *Pooled proportions of elevated liver enzymes and drug-induced liver injury for participants taking cannabidiol.*

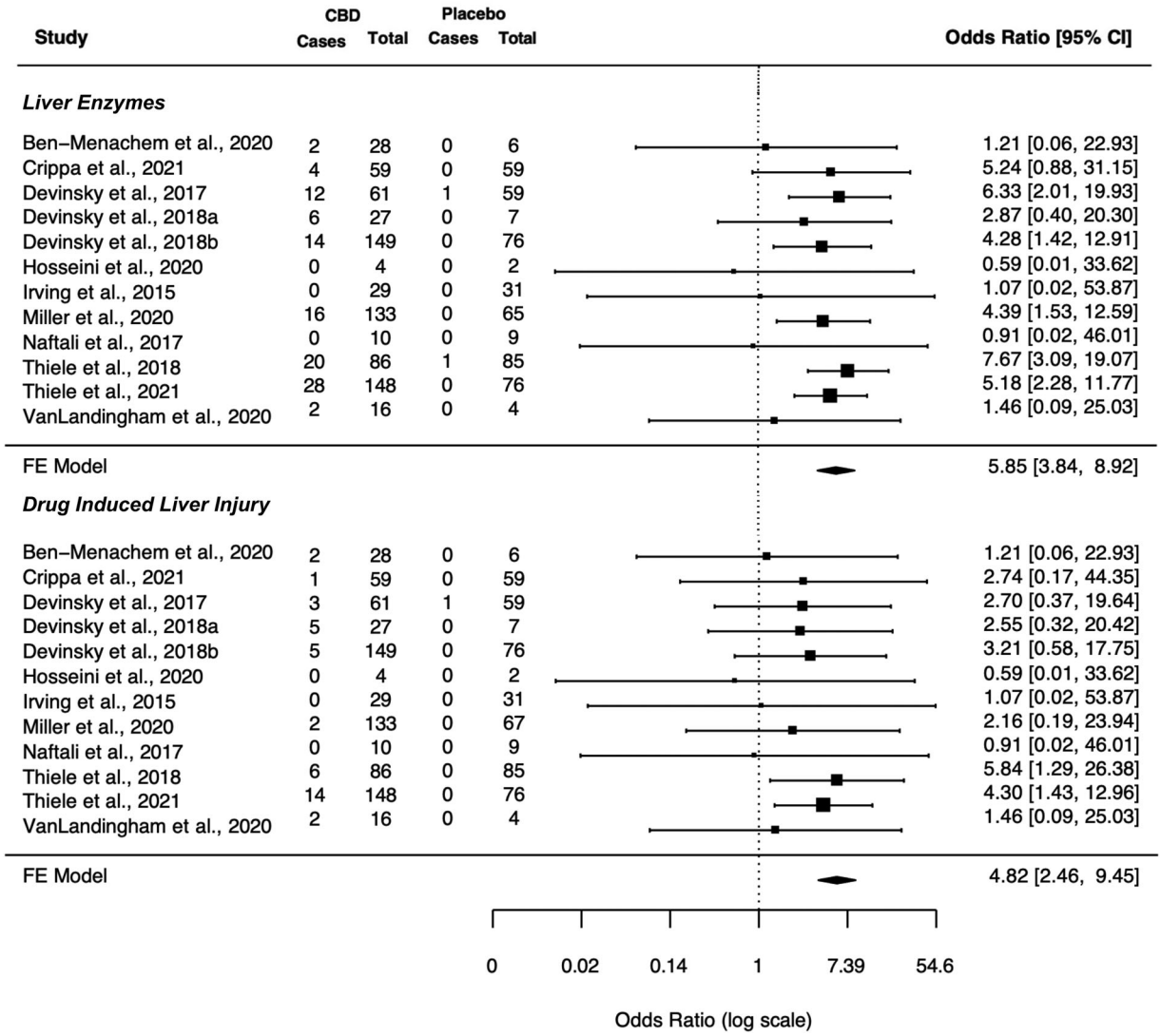

Fig. 3 *Odds ratio of increased liver enzymes and drug-induced liver injury in randomized clinical trials comparing CBD and placebo.*

participants with epilepsy at a dose of 5 mg/kg/day ($n = 1$) and 10 mg/kg/day ($n = 2$) [8, 9]. No reported cases were below these doses.

The majority of participants with LE elevation were taking concomitant medications. In 121/159 cases (76.10%) of LE elevation, there was concomitant use of VPA. Reporting was less clear for the subset of DILI cases due to a lack of patient-level data. It was determined that at least 24/57 DILI cases (42.11%) were on VPA [6–9, 29, 38, 42, 45, 46]. At least 7/57 cases (12.28%) of DILI participants were on concomitant clobazam [9, 46]. Three trials allowed the concomitant use of acetaminophen/paracetamol (ACET) [12, 13, 44]. ACET use was formally reported in 4/57 (7.02%) participants with DILI, with two of these participants only taking one dose (500 mg) of ACET [12]. Three studies in healthy participants allowed the use of hormonal birth control and was a concomitant medication taken by at least 2/57 (3.51%) of the participants with a hepatocellular pattern of DILI [28]. There was one confirmed case of DILI in a participant not taking any concomitant

medications [43]. However, participant-level concomitant medication use was not reported in all studies.

Eleven studies containing 60/159 cases (37.74%) of LE elevations reported time of detection. Of these, 49/60 cases (81.67%) were detected within 30 days of CBD initiation, with one case being detected as early as day 8 [47]. Eleven out of 159 cases (18.33%) were detected later than 30-day post-CBD initiation [12, 42, 45].

Most cases of LE elevation were transient, with 128/159 cases (80.50%) reported as resolved. Of the resolved cases, 64/128 (50.00%) resolved following cessation of CBD use, 32/128 cases (25.00%) resolved spontaneously with continued CBD use, 21/128 cases (16.40%) resolved with a dose reduction of other AEDs, 2/128 cases (1.56%) resolved with dose reduction of CBD, and 9/128 cases (7.03%) resolved with unclear reasons (either cessation or dose reduction of CBD, or an AED [8]). Seven of the 159 cases (4.40%) were reported as unresolved at the time monitoring ended. The outcome was unclear for 24/159 cases (15.09%) [7, 29, 33].

Among the subset of 57 cases that met criteria for DILI, 51/57 cases (89.47%) were resolved after the discontinuation of CBD. Two of the 57 cases (3.45%) were reported as unresolved at the time monitoring ended. The outcome was unclear for 5/57 cases (6.90%).

Moderator analyses of LE elevation within CBD treatment groups indicated a significant association of dose ($p = 0.003$ dose effect) such that the proportion of LE elevation was higher among participants receiving ≥1000 mg/day of CBD ($k = 14$; 11.4% predicted proportion) than participants receiving <1000 mg/day of CBD ($k = 10$; 2.0% predicted proportion). Concomitant use of VPA was also significantly associated with greater odds of LE elevation ($N = 12$ trials, $n = 1057$; OR = 6.92, 95% CI = 4.74–10.09, $p < 0.001$; eFigure 1). No other moderator variables were significantly associated with LE elevation (Seizure Disorder Population $p = .175$; Age $p = 0.193$; Trial Duration $p = 0.792$).

Moderator analyses of DILI within CBD treatment groups indicated a significant association of dose ($p = 0.026$ dose effect) such that the proportion of DILI was higher among participants receiving more than 1000 mg/day of CBD ($k = 13$; 4.8% predicted proportion) than participants receiving less than 1000 mg/day of CBD ($k = 10$; 0.5% predicted proportion). Concomitant use of VPA was also significantly associated with a greater odds of DILI ($N = 7$ trials, $n = 578$; OR = 5.05, 95% CI = 2.20–11.60, $p < 0.001$; eFigure 1). No other moderation variables were significantly associated with DILI (Seizure Disorder Population $p = 0.990$; Age $p = 0.298$; Trial Duration $p = 0.138$).

Quality of available evidence was moderate-to-low, primarily due to 50% of studies being open-label, non-randomized trials, and some studies having unclear metrics for DILI criteria. Among the 12 RCTs, 6/12 (50%) were assessed as having the high risk of bias primarily due to potential missing outcome data [7, 8, 10, 34, 38, 45]. Egger's test for funnel plot asymmetry provided weak evidence of publication bias for the primary outcome of DILI. Sensitivity analysis revealed similar results with the removal of these trials. Details on the risk of bias assessments (eFigures 2 and 3), sensitivity analysis, and publication bias (eFigure 4) are presented in eResults.

Discussion

The results of this systematic review and meta-analysis indicate that CBD is associated with increased odds of acute LE elevation and DILI compared to placebo ($OR_{LEelevation} = 5.85$; $OR_{DILI} = 4.82$). DILI was not reported in adults using CBD doses <300 mg/day. This is important for clinical practice as the vast majority of people using CBD for medical purposes take doses below this threshold [49–51]. The pooled proportion of elevated LEs in participants taking CBD was 0.074 (95% CI 0.0448–0.1212), whereas the pooled proportion of those with DILI was 0.0296 (95% CI 0.0136–0.0631). This proportion of events (LE elevation = 7.4%; DILI = 2.96%) suggests that LE elevation and DILI meet the CIOMS classification as a common adverse drug event [52]. The proportion of CBD-associated DILI is similar or greater to those found in other common hepatotoxic drugs such as statins (atorvastatin 1%–3%, fluvastatin 1%–5%, pravastatin 3%–7%) and fluoroquinolones (1%–3%) while being slightly lower than VPA (5%–10%) [53]. No cases of severe DILI were found, as determined by Hy's law [20, 48]. Several factors were associated with higher odds of LE elevations and DILI (Figure 4). High doses of CBD (>1000 mg/day or ≥20 mg/kg/day)

Fig. 4 *Potential risk factors for Cannabidiol (CBD)-associated drug-induced liver injury (DILI) based on findings from the current meta-analysis.*

were significantly associated with the proportion of LE elevations ($p = 0.003$) and DILI ($p = 0.026$). Concomitant use of VPA was also associated with LE elevation ($p < 0.001$) and DILI ($p < 0.001$).

The underlying pathophysiology of liver injury associated with CBD use remains unknown. Both high-dose CBD use and the use of concomitant medications, such as AEDs, appear to be risk factors. Preclinical evidence has shown that high-dose CBD impairs glutathione resynthesis, which may lead to liver injury [54]. There also appears to be a genetic component. A study on pharmacogenetic predictors of CBD response and tolerability in epilepsy observed the genetic variant ABCC5 rs3749442 was associated with a lower likelihood of abnormal liver function tests [55]. Further, CBD upregulates at least 50 genes and modulates many cytochrome p450 enzymes. These could be associated with genotoxic effects and DNA damage, which have been noted in human liver cell lines treated with CBD [56].

Other conditions associated with liver injury (e.g., chronic alcohol use, hepatitis B/C virus [HBV/HVC]) may also warrant investigation as risk factors of CBD-associated hepatotoxicity. None of the cases of LE elevations or DILI in this review occurred in patients using alcohol or with HBV/HCV. Interestingly, preclinical research has shown CBD may actually be protective against alcohol-induced hepatotoxicity [57]. Regardless, given the commonality of both factors and known potential for liver injury, studies assessing liver enzymes during CBD use in these populations (and other populations with increased liver injury risk) are warranted to better understand the full spectrum of risk factors.

CBD-related liver safety concerns were first described following the pivotal GW Pharma RCTs in childhood epilepsy [8–10, 38, 45]. A review of these trials by the FDA reported LE elevations in 14% of the participants taking CBD [58]. The current review expanded results beyond pediatric epilepsy trials and found a slightly lower raw proportion of LE elevations at 10.50%, with a pooled proportion of 7.4%. This may be due to the inclusion of trials using lower doses of CBD and participants not using as many concomitant medications, specifically AEDs. Chesney (2020) found a similar proportion of elevated LEs and noted the majority of cases occurred at higher doses. However, they reported a higher OR of 11.19 (vs. OR = 5.86 in this analysis). This may be due to a difference in inclusion criteria, dosing, final sample size, and meta-analytic method. Dos Santos (2020) also reviewed adverse effects of CBD and noted a similar association of VPA with elevated LEs. They did not report any cases of DILI, as they used different DILI criteria requiring >2× ULN bilirubin.

The current review supports a potentially concerning discrepancy between clinical trial definitions and real-world clinical definitions of DILI, as has previously been noted by the FDA [58]. Many trials classified DILI as ALT or AST >3× ULN with total bilirubin >2× ULN and thus reported no cases. This definition is at odds with criteria put forth by the AASLD and ACG in which DILI is defined as ALT or AST >5× ULN without symptoms, the rise of ALP >2× ULN, the rise of bilirubin >2× ULN with any rise of AST and ALT, or the rise of AST or ALT <5× ULN with symptoms [15, 16, 18, 19]. Based on these criteria, the pooled proportion of DILI was 2.96% among the included studies. Although most cases were resolved, it is important to note that participants with elevated LEs often had their CBD stopped or dose reduced. It is unknown if these cases may have progressed to acute liver failure if CBD were continued at the same dose. Watkins (2021) similarly noted that routine monitoring may have resulted in the early detection of abnormally elevated LEs, leading to an early discontinuation of CBD, which may have prevented liver injury progression.

Clinical guidance

Based on current evidence, clinicians are encouraged to monitor for signs of elevated liver enzymes and liver dysfunction in patients taking CBD who have risk factors for liver dysfunction, taking moderate-to-high doses of CBD (>300 mg/day), or using CBD with antiepileptic medications. Serum transaminases and total bilirubin levels are recommended to be assessed at baseline, 1, 3, and 6 months after CBD initiation [59]. Clinicians should actively inquire about CBD use, especially in those with idiopathic LE elevations, as access for both medical and nonmedical use is increasing worldwide. A slow-dose titration to facilitate early detection of LE elevations is recommended. If a patient has elevated transaminases, repeat blood work, separated by 48–72 h over 15 days, should be conducted in order to assess if elevations are sustained or transient [59]. If LE elevations are sustained, the reduction of CBD dosage or

adjustment in concomitant drugs should occur [59]. CBD should be discontinued in any patient with DILI.

Limitations

Several limitations were present in this review. Missing information from some studies on exact LE measures, time to detection and resolution, and concomitant medications limited our ability to complete some of the planned investigations (See eMethods for protocol deviations). Only 8/28 (28.57%) studies were able to provide individual participant data. The absence of clear DILI criteria required extrapolation of some cases based on patient withdrawal criteria. Finally, a clinical causality assessment for DILI was not possible with available data. As such, it is possible that other drugs could have caused elevated LEs. However, control groups had similar medication regimens, particularly antiepileptic regimens, and there were only two cases of elevated LEs. Further, there were cases of DILI in participants using no concomitant medications. This provides convincing evidence that elevations were caused by CBD, not other medications. Despite these limitations, the evidence indicates important liver-safety considerations. The limitations of this review highlight a need for more research specifically focused on evaluating CBD and liver safety.

Conclusion

This meta-analysis suggests elevated LEs and DILI are common adverse drug reactions associated with CBD use at moderate-to-high doses. CBD hepatotoxicity was strongly associated with daily high-dose CBD and concomitant use of AEDs, particularly VPA. Most cases of elevated LEs and DILI resolved upon CBD discontinuation. Although more investigation is needed, there also appears to be an increased risk of clinically significant transaminase elevations following CBD administration in individuals with baseline LE elevations. Clinicians are encouraged to actively inquire about CBD use and monitor for signs of elevated liver enzymes and liver dysfunction during CBD dose titration. At doses of <300 mg CBD/day, the risk of DILI is likely low. There is a great need for additional research and better reporting standards to determine the true proportion of elevated LEs and DILI across a broad group of patients taking CBD, assess risk factors and outcomes, and determine optimal management.

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Author contributions

All authors had full access to all the data in the study and take responsibility for the submission of this manuscript. Caroline A. MacCallum and Alasdair M. Barr were responsible for the concept and design. All authors partook in the acquisition, analysis, and interpretation of data. Lindsay A. Lo, Justin C. Strickland, and David D. Kim did the statistical analysis. Lindsay A. Lo, Lauren Eadie, and April Christiansen wrote the first draft of the manuscript. All authors partook in the critical revision of the manuscript. Caroline A. MacCallum and Alasdair M. Barr provided overall supervision of the study.

Conflicts of interest statement

Authors LAL, AC, LE, and DDK report no conflicts of interests. Outside of the submitted work, JCS reports receiving research funding from Canopy Growth Corporation and the Cure Addiction Now foundation. MB reports financial support as a speaker and consultant for CHE activities from Teva, Pfizer, Novo Nordisk, Khiron, Tilray, mdBriefcase, J&J, Abbvie, Ascensia, Astra Zeneca, Biosynt, and Emergent BioSolutions. AB reports research grants and contracts from Canneveert Therapeutics, Emerald Health Therapeutics, Entourage Biosciences, and Vitality Biopharma. CM is the Medical Director of Greenleaf Medical Clinic and Chief Medical Officer for Translational Life Sciences. She is on the Board of Directors for The Green Organic Dutchman. She is an advisor to PreveCeutical, Pinnacle Care, Africanna, EO Care, Andira Medicine, Active Patch Technologies, and Dosist. Additionally, she has provided medical consultation and/or received support for industry-sponsored continuing medical education from: Aleafia, Aurora, Canopy, Spectrum, Tilray, Emerald Health, and Syqe Medical.

Data availability statement

Data related to this manuscript will be made available upon request.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

eTable 1. Search Strategies.
eTable 2. PICOS breakdown of study eligibility criteria.
eTable 3. General upper limit of normal ranges.
eTable 4. Comparison of predicted probability between dichotomous and trichotomous dose categories for elevated liver enzymes.
eFigure 1. Odds ratio of increased liver enzymes and drug induced liver injury in randomized clinical trials comparing cannabidiol patients with and without concomitant valproate use.
eFigure 2. RoB2 assessment.
eFigure 3. MINORS Bias assessment.
eFigure 4. Funnel plots for outcomes of elevated liver enzymes (a) and drug induced liver injury (b). ■

Online-only Supplemental Materials

1. eTable 1 Search Strategies

eTable 1. Search Strategies

Line	Search term
EMBASE	
1	cannabidiol/
2	CBD.mp. [mp=title, abstract, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword heading word, floating subheading word, candidate term word]
3	Cannabidiol.mp. [mp=title, abstract, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword heading word, floating subheading word, candidate term word]
4	Epidiolex.mp. [mp=title, abstract, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword heading word, floating subheading word, candidate term word]
5	Epidyolex.mp. [mp=title, abstract, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword heading word, floating subheading word, candidate term word]
6	hemp.mp. [mp=title, abstract, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword heading word, floating subheading word, candidate term word]
7	1 or 2 or 3 or 4 or 5 or 6
8	aminotransferase/ or alanine aminotransferase/ or aspartate aminotransferase/
9	alkaline phosphatase/
10	gamma glutamyltransferase/
11	bilirubin/
12	exp bilirubin blood level/
13	exp alkaline phosphatase blood level/
14	exp alanine aminotransferase blood level/
15	exp aspartate aminotransferase blood level/
16	albuminoid/ or albumin/ or serum albumin/
17	liver disease/ or liver cell damage/
18	toxic hepatitis/ or hepatitis/ or liver toxicity/
19	nonalcoholic fatty liver/
20	liver failure/ or liver dysfunction/ or acute liver failure/
21	liver/
22	exp liver function test/
23	international normalized ratio/
24	drug monitoring/
25	exp pharmacovigilance/
26	drug surveillance program/ or postmarketing surveillance/
27	transaminases.mp. [mp=title, abstract, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword heading word, floating subheading word, candidate term word]
28	aspartate aminotransferases.mp. [mp=title, abstract, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword heading word, floating subheading word, candidate term word]
29	alanine aminotransferase.mp. [mp=title, abstract, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword heading word, floating subheading word, candidate term word]
30	alkaline phosphatase.mp. [mp=title, abstract, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword heading word, floating subheading word, candidate term word]
31	gamma-Glutamyltransferase.mp. [mp=title, abstract, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword heading word, floating subheading word, candidate term word]
32	ALT.mp. [mp=title, abstract, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword heading word, floating subheading word, candidate term word]

33	AST.mp. [mp=title, abstract, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword heading word, floating subheading word, candidate term word]
34	ALP.mp. [mp=title, abstract, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword heading word, floating subheading word, candidate term word]
35	GGT.mp. [mp=title, abstract, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword heading word, floating subheading word, candidate term word]
36	DILI.mp. [mp=title, abstract, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword heading word, floating subheading word, candidate term word]
37	Bilirubin.mp. [mp=title, abstract, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword heading word, floating subheading word, candidate term word]
38	Albumin.mp. [mp=title, abstract, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword heading word, floating subheading word, candidate term word]
39	INR.mp. [mp=title, abstract, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword heading word, floating subheading word, candidate term word]
40	International Normalized Ratio.mp. [mp=title, abstract, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword heading word, floating subheading word, candidate term word]
41	Drug-Induced Livery Injury.mp. [mp=title, abstract, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword heading word, floating subheading word, candidate term word]
42	LFT.mp. [mp=title, abstract, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword heading word, floating subheading word, candidate term word]
43	Liver.mp. [mp=title, abstract, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword heading word, floating subheading word, candidate term word]
44	hepatotoxicity.mp. [mp=title, abstract, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword heading word, floating subheading word, candidate term word]
45	Hy's Law.mp. [mp=title, abstract, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword heading word, floating subheading word, candidate term word]
46	safety.mp. [mp=title, abstract, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword heading word, floating subheading word, candidate term word]
47	or/8-46
48	7 and 47
49	limit 48 to "review"
50	48 not 49
51	exp bile duct/
52	bile duct.mp. [mp=title, abstract, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword heading word, floating subheading word, candidate term word]
53	51 or 52
54	50 not 53
Medline	
1	Cannabidiol/
2	CBD.mp. [mp=title, abstract, original title, name of substance word, subject heading word, floating sub-heading word, keyword heading word, organism supplementary concept word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier, synonyms]
3	Cannabidiol.mp. [mp=title, abstract, original title, name of substance word, subject heading word, floating sub-heading word, keyword heading word, organism supplementary concept word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier, synonyms]
4	Epidiolex.mp. [mp=title, abstract, original title, name of substance word, subject heading word, floating sub-heading word, keyword heading word, organism supplementary concept word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier, synonyms]

5	Epidyolex.mp. [mp=title, abstract, original title, name of substance word, subject heading word, floating sub-heading word, keyword heading word, organism supplementary concept word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier, synonyms]
6	1 or 2 or 3 or 4 or 5
7	transaminases/ or alanine transaminase/ or aspartate aminotransferases/
8	Alkaline Phosphatase/
9	gamma-Glutamyltransferase/
10	Bilirubin/
11	Albumins/
12	liver diseases/ or "chemical and drug induced liver injury"/ or non-alcoholic fatty liver disease/ or hepatic insufficiency/ or exp liver failure/ or hepatitis/ or cytochrome p-450 enzyme inhibitors/ or cytochrome p-450 cyp3a inhibitors/ or cannabinoid receptor agonists/ or cytochrome p-450 cyp2c19 inhibitors/ or cannabinoid receptor modulators/
13	Liver Function Tests/
14	International Normalized Ratio/
15	"drug-related side effects and adverse reactions"/
16	exp Product Surveillance, Postmarketing/
17	Drug Monitoring/
18	transaminases.mp. [mp=title, abstract, original title, name of substance word, subject heading word, floating sub-heading word, keyword heading word, organism supplementary concept word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier, synonyms]
19	alanine transaminase.mp. [mp=title, abstract, original title, name of substance word, subject heading word, floating sub-heading word, keyword heading word, organism supplementary concept word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier, synonyms]
20	aspartate transaminase.mp. [mp=title, abstract, original title, name of substance word, subject heading word, floating sub-heading word, keyword heading word, organism supplementary concept word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier, synonyms]
21	alkaline phosphatase.mp. [mp=title, abstract, original title, name of substance word, subject heading word, floating sub-heading word, keyword heading word, organism supplementary concept word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier, synonyms]
22	gamma-Glutamyltransferase.mp. [mp=title, abstract, original title, name of substance word, subject heading word, floating sub-heading word, keyword heading word, organism supplementary concept word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier, synonyms]
23	ALT.mp. [mp=title, abstract, original title, name of substance word, subject heading word, floating sub-heading word, keyword heading word, organism supplementary concept word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier, synonyms]
24	AST.mp. [mp=title, abstract, original title, name of substance word, subject heading word, floating sub-heading word, keyword heading word, organism supplementary concept word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier, synonyms]
25	ALP.mp. [mp=title, abstract, original title, name of substance word, subject heading word, floating sub-heading word, keyword heading word, organism supplementary concept word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier, synonyms]
26	GGT.mp. [mp=title, abstract, original title, name of substance word, subject heading word, floating sub-heading word, keyword heading word, organism supplementary concept word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier, synonyms]
27	DILI.mp. [mp=title, abstract, original title, name of substance word, subject heading word, floating sub-heading word, keyword heading word, organism supplementary concept word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier, synonyms]

28	Bilirubin.mp. [mp=title, abstract, original title, name of substance word, subject heading word, floating sub-heading word, keyword heading word, organism supplementary concept word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier, synonyms]
29	Albumin.mp. [mp=title, abstract, original title, name of substance word, subject heading word, floating sub-heading word, keyword heading word, organism supplementary concept word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier, synonyms]
30	INR.mp. [mp=title, abstract, original title, name of substance word, subject heading word, floating sub-heading word, keyword heading word, organism supplementary concept word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier, synonyms]
31	International Normalized Ratio.mp. [mp=title, abstract, original title, name of substance word, subject heading word, floating sub-heading word, keyword heading word, organism supplementary concept word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier, synonyms]
32	LFT.mp. [mp=title, abstract, original title, name of substance word, subject heading word, floating sub-heading word, keyword heading word, organism supplementary concept word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier, synonyms]
33	"liver injury".mp. [mp=title, abstract, original title, name of substance word, subject heading word, floating sub-heading word, keyword heading word, organism supplementary concept word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier, synonyms]
34	"liver enzymes".mp. [mp=title, abstract, original title, name of substance word, subject heading word, floating sub-heading word, keyword heading word, organism supplementary concept word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier, synonyms]
35	hepatotoxicity.mp. [mp=title, abstract, original title, name of substance word, subject heading word, floating sub-heading word, keyword heading word, organism supplementary concept word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier, synonyms]
36	"Hy's Law".mp. [mp=title, abstract, original title, name of substance word, subject heading word, floating sub-heading word, keyword heading word, organism supplementary concept word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier, synonyms]
37	"Liver Function Test".mp. [mp=title, abstract, original title, name of substance word, subject heading word, floating sub-heading word, keyword heading word, organism supplementary concept word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier, synonyms]
38	"drug induced liver injury".mp. [mp=title, abstract, original title, name of substance word, subject heading word, floating sub-heading word, keyword heading word, organism supplementary concept word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier, synonyms]
39	or/7-38
40	6 and 39
CENTRAL (Cochrane controlled trials)	
1	Cannabidiol/
2	CBD.mp. [mp=title, original title, abstract, mesh headings, heading words, keyword]
3	Cannabidiol.mp. [mp=title, original title, abstract, mesh headings, heading words, keyword]
4	Epidiolex.mp. [mp=title, original title, abstract, mesh headings, heading words, keyword]
5	Epidyolex.mp. [mp=title, original title, abstract, mesh headings, heading words, keyword]
6	1 or 2 or 3 or 4 or 5
7	transaminases/ or alanine transaminase/ or aspartate aminotransferases/
8	Alkaline Phosphatase/
9	gamma-Glutamyltransferase/
10	Bilirubin/
11	Albumins/

12	liver diseases/ or "chemical and drug induced liver injury"/ or non-alcoholic fatty liver disease/ or hepatic insufficiency/ or exp liver failure/ or hepatitis/ or cytochrome p-450 enzyme inhibitors/ or cytochrome p-450 cyp3a inhibitors/ or cannabinoid receptor agonists/ or cytochrome p-450 cyp2c19 inhibitors/ or cannabinoid receptor modulators/
13	Liver Function Tests/
14	International Normalized Ratio/
15	"drug-related side effects and adverse reactions"/ or "chemical and drug induced liver injury"/
16	exp Product Surveillance, Postmarketing/
17	Drug Monitoring/
18	transaminases.mp. [mp=title, original title, abstract, mesh headings, heading words, keyword]
19	alanine transaminase.mp. [mp=title, original title, abstract, mesh headings, heading words, keyword]
20	aspartate aminotransferases.mp. [mp=title, original title, abstract, mesh headings, heading words, keyword]
21	Alkaline Phosphatase.mp. [mp=title, original title, abstract, mesh headings, heading words, keyword]
22	gamma-Glutamyltransferase.mp. [mp=title, original title, abstract, mesh headings, heading words, keyword]
23	ALT.mp. [mp=title, original title, abstract, mesh headings, heading words, keyword]
24	AST.mp. [mp=title, original title, abstract, mesh headings, heading words, keyword]
25	ALP.mp. [mp=title, original title, abstract, mesh headings, heading words, keyword]
26	GGT.mp. [mp=title, original title, abstract, mesh headings, heading words, keyword]
27	DILI.mp. [mp=title, original title, abstract, mesh headings, heading words, keyword]
28	Bilirubin.mp. [mp=title, original title, abstract, mesh headings, heading words, keyword]
29	Albumin.mp. [mp=title, original title, abstract, mesh headings, heading words, keyword]
30	INR.mp. [mp=title, original title, abstract, mesh headings, heading words, keyword]
31	International Normalized Ratio.mp. [mp=title, original title, abstract, mesh headings, heading words, keyword]
32	LFT.mp. [mp=title, original title, abstract, mesh headings, heading words, keyword]
33	"liver injury".mp. [mp=title, original title, abstract, mesh headings, heading words, keyword]
34	"liver enzymes".mp. [mp=title, original title, abstract, mesh headings, heading words, keyword]
35	hepatotoxicity.mp. [mp=title, original title, abstract, mesh headings, heading words, keyword]
36	"Hy's Law".mp. [mp=title, original title, abstract, mesh headings, heading words, keyword]
37	"Liver Function Test".mp. [mp=title, original title, abstract, mesh headings, heading words, keyword]
38	7 or 8 or 9 or 10 or 11 or 12 or 13 or 14 or 15 or 16 or 17 or 18 or 19 or 20 or 21 or 22 or 23 or 24 or 25 or 26 or 27 or 28 or 29 or 30 or 31 or 32 or 33 or 34 or 35 or 36 or 37
39	6 and 38
40	hemp.mp. [mp=title, original title, abstract, mesh headings, heading words, keyword]
41	6 or 40
42	38 and 41
CINAHL	
S43	S6 AND S42
S42	S7 OR S8 OR S9 OR S10 OR S11 OR S12 OR S13 OR S14 OR S15 OR S16 OR S17 OR S18 OR S19 OR S20 OR S21 OR S22 OR S23 OR S24 OR S25 OR S26 OR S27 OR S28 OR S29 OR S30 OR S31 OR S32 OR S33 OR S34 OR S35 OR S36 OR S37 OR S38 OR S39 OR S40 OR S41
S41	liver
S40	hepatotoxicity
S39	Hy's law
S38	liver enzymes or liver function test
S37	liver injury
S36	LFT
S35	international normalized ratio
S34	inr
S33	albumin

S32	bilirubin
S31	"DILI"
S30	"ggt"
S29	ALP
S28	"AST"
S27	"ALT"
S26	gamma-glutamyl transferase
S25	alkaline phosphatase
S24	aspartate aminotransferase
S23	alanine transaminase
S22	alanine transaminase
S21	(MH "Product Surveillance")
S20	(MH "Drug Monitoring")
S19	(MH "Pharmacovigilance")
S18	(MH "International Normalized Ratio")
S17	(MH "Liver Function Tests")
S16	(MH "Enzyme Tests") OR (MH "Cytochrome P-450 Enzyme System")
S15	(MH "Hepatotoxicity")
S14	(MH "Hepatitis")
S13	(MH "Liver Failure, Acute") OR (MH "Liver Failure")
S12	(MH "Nonalcoholic Fatty Liver Disease") OR (MH "Liver Diseases")
S11	(MH "Serum Albumin")
S10	(MH "Bilirubin")
S9	(MH "Gamma-Glutamyltransferase")
S8	(MH "Alkaline Phosphatase")
S7	(MH "Alanine Aminotransferase") OR (MH "Aspartate Aminotransferase") OR (MH "Liver Failure") OR "transaminase"
S6	S1 OR S2 OR S3 OR S4 OR S5
S5	hemp
S4	Cannabidiol
S3	CBD
S2	Epidiolex
S1	(MH "Cannabidiol")
Web of Science	
1	(ALL=(Cannabidiol OR CBD OR Epidiolex OR Epidyolex))
2	ALL=(transaminase* OR "alanine aminotransferase" OR "aspartate aminotransferase" OR "alkaline phosphatase" OR "gamma-Glutamyltransferase" OR Bilirubin OR Albumin OR ALT OR AST OR GGT) OR ALL=("drug induced liver injury" OR DILI OR "liver failure" OR "liver dysfunction" OR "liver toxicity" OR "hepatitis" OR "liver function test" OR LFT OR "international normalized ratio" OR INR OR "drug monitoring" OR pharmacovigilance OR hepatotoxicity OR "Hy's law")
3	#2 AND #1
4	ALL=("common bile duct")
5	#3 NOT #4
MedRxiv	
1	Cannabidiol AND liver
2	Clinicaltrials.gov
3	Cannabidiol OR CBD OR epidiolex and liver OR hepatic OR liver dysfunction OR liver enzymes

2. eTable 2 PICOS

eTable 2. PICOS breakdown of study eligibility criteria.

Component	Criteria
P (Population)	Participants of any age, gender and ethnicity with or without a medical condition
I (Intervention)	Daily CBD use
C (Comparison)	Placebo controls or within subject baseline measures
O (Outcome)	Liver enzyme levels and drug-induced liver injury
S (Study type selected)	Clinical trials or drug safety & tolerability studies

3. eMethods

PRISMA Checklist

Section and Topic	Item #	Checklist item	Location where item is reported
TITLE			
Title	1	Identify the report as a systematic review.	Title page
ABSTRACT			
Abstract	2	See the PRISMA 2020 for Abstracts checklist.	Abstract page
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of existing knowledge.	Introduction p 5
Objectives	4	Provide an explicit statement of the objective(s) or question(s) the review addresses.	Introduction p 5
METHODS			
Eligibility criteria	5	Specify the inclusion and exclusion criteria for the review and how studies were grouped for the syntheses.	Method p 6; eMethods
Information sources	6	Specify all databases, registers, websites, organisations, reference lists and other sources searched or consulted to identify studies. Specify thedate when each source was last searched or consulted.	Methods p 6
Search strategy	7	Present the full search strategies for all databases, registers and websites, including any filters and limits used.	eMethods
Selection process	8	Specify the methods used to decide whether a study met the inclusion criteria of the review, including how many reviewers screened each recordand each report retrieved, whether they worked independently, and if applicable, details of automation tools used in the process.	Methods p6

Data collection process	9	Specify the methods used to collect data from reports, including how many reviewers collected data from each report, whether they worked independently, any processes for obtaining or confirming data from study investigators, and if applicable, details of automation tools used in theprocess.	Methods p7
Data items	10a	List and define all outcomes for which data were sought. Specify whether all results that were compatible with each outcome domain in eachstudy were sought (e.g. for all measures, time points, analyses), and if not, the methods used to decide which results to collect.	Methods p7/8
	10b	List and define all other variables for which data were sought (e.g. participant and intervention characteristics, funding sources). Describe any assumptions made about any missing or unclear information.	Methods p7/8, eMethods
Study risk of bias assessment	11	Specify the methods used to assess risk of bias in the included studies, including details of the tool(s) used, how many reviewers assessed each study and whether they worked independently, and if applicable, details of automation tools used in the process.	eResults
Effect measures	12	Specify for each outcome the effect measure(s) (e.g. risk ratio, mean difference) used in the synthesis or presentation of results.	Methods p7/8
Synthesis methods	13a	Describe the processes used to decide which studies were eligible for each synthesis (e.g. tabulating the study intervention characteristics andcomparing against the planned groups for each synthesis (item #5)).	Methods p5, eMethods
	13b	Describe any methods required to prepare the data for presentation or synthesis, such as handling of missing summary statistics, or data conversions.	Methods p7/8
	13c	Describe any methods used to tabulate or visually display results of individual studies and syntheses.	Methods p7
	13d	Describe any methods used to synthesize results and provide a rationale for the choice(s). If meta-analysis was performed, describe themodel(s), method(s) to identify the presence and extent of statistical heterogeneity, and software package(s) used.	Methods p7/8
	13e	Describe any methods used to explore possible causes of heterogeneity among study results (e.g. subgroup analysis, meta-regression).	Methods p7/8
	13f	Describe any sensitivity analyses conducted to assess robustness of the synthesized results.	Methods p7/8; eResults
Reporting bias assessment	14	Describe any methods used to assess risk of bias due to missing results in a synthesis (arising from reporting biases).	eResults
Certainty assessment	15	Describe any methods used to assess certainty (or confidence) in the body of evidence for an outcome.	eResults

Data Sources and Search Strategy

The search strategy was developed with assistance from an academic librarian at Queen's University. Cannabidiol MeSH terms and keywords were combined using Boolean logic with liver enzyme and liver injury MeSH terms and keywords. No restrictions on language or publication date were applied. Search terms were reviewed by two internal medicine doctors, both with expertise in cannabinoid medicine. Reference lists of relevant reviews and candidate studies were manually searched for additional studies.

Study Selection

For findings published only in abstract form, we contacted the investigators to determine if the results were still considered to be valid. No abstracts met inclusion criteria. Studies were excluded if: 1) cannabis was used in the 28 days prior to study initiation, 2) moderate to heavy alcohol or substance use was permitted during the trial, 3) participants had a known history of liver or biliary disease, 4) the investigational medical product (IMP) was THC-dominant, and 5) they were extensions of other trials already included in our review and analysis. Study selection was completed in Covidence (Veritas Health Innovation Inc).

Thirteen studies nearly satisfied inclusion criteria, but were excluded due to them either having participants in multiple trials (n=2, [1,2]), being unclear if participants had a 28 day abstinence period from cannabis (n = 4, [3–6]) or it being unclear if LE elevations occurred within the first six months of treatment (n = 7, [7–13]).

Data extraction and outcome measures

Data was independently extracted by LL and AC using a standardized pre-designed data collection form. Discrepancies in study details were discussed to reach a consensus decision by LL and AC, with additional input from LE when needed. Data extracted from individual studies included the geographical location, study design, participant characteristics (e.g. age, sex, study population, sample size etc.), CBD dosing regimen, LFT schedule, proportion of participants with abnormal LEs, and proportion of participants with DILI. In studies reporting LE elevation, an attempt to gain patient specific information on specific transaminase levels, baseline elevations, time of detection, time of resolution, CBD dose, and concomitant medications was made. Authors from all 28 studies were contacted for further information, additional data was received for 15/28 studies. Data, code, and other materials are available upon request.

Risk of Bias

We used the RoB 2.0 and MINORs tools to assess risk of bias for each of the included studies (Figure S2, Figure S3). Publication bias was assessed using Egger's test for funnel plot asymmetry.

Deviations from PROSPERO

Due to limited available data, adjustments to pre-specified analyses were required. Standardized mean differences for continuous liver enzyme levels could not be estimated with the available data. Instead, the primary analyses included a pooled proportions and pooled probability analysis. To supplement the pooled proportions analysis, a secondary moderation analysis of age, dose, population, and concomitant valproic acid use, in line with pre-planned sub-group analyses, was carried out. Given the rare baseline event rate of the outcomes, odds ratios were computed instead of risk ratios. The other pre-specified analyses of time-to-event and sub-group analyses of cannabinoid product ratio and on-treatment versus intention-to-treat could not be carried out due to lack of data or variability between studies.

4. eTable 3 Upper limit of normal ranges

eTable3. General upper limit of normal ranges

Biochemistry	Sex	Age (Years)	Normal Range
Alanine Aminotransferase (ALT)	Male	1-3 4-6 7-9 10-17 18+	5-30 U/L 5-20 U/L 5-25 U/L 5-30 U/L 0-44 U/L
	Female	1-3 4-6 7-9 10-17 18+	5-30 U/L 5-25 U/L 5-25 U/L 5-30 U/L 0-33 U/L
Aspartate Aminotransferase (AST)	Male	1-3 4-6 7-9 10-12 13-15 16-17 18+	0-55 U/L 0-47 U/L 0-41 U/L 0-37 U/L 0-38 U/L 0-38 U/L 14-39 U/L
	Female	1-3 4-6 7-9 10-12 13-15 16-17 18+	0-68 U/L 0-58 U/L 0-40 U/L 0-36 U/L 0-31 U/L 0-29 U/L 14-34 U/L

Alkaline Phosphatase (ALP)	Male	1-3 4-6 7-9 10-12 13-15 16-17 18+	104-345 U/L 93-309 U/L 86-315 U/L 43-362 U/L 74-390 U/L 52-171 U/L 53-129 U/L
	Female	1-3 4-6 7-9 10-12 13-15 16-17 18+	108-317 U/L 96-297 U/L 69-325 U/L 51-332 U/L 50-162 U/L 47-119 U/L 42-98 U/L
Gamma-glutamyl Transferase (GST)	Male	1-12 13-17 18+	3-22 U/L 2-42 U/L 0-54 U/L
	Female	1-12 13-17 18+	4-22 U/L 4-24 U/L 0-37 U/L

5. eResults

Participant characteristics with liver enzyme elevations

Only five studies reported baseline liver enzymes. Out of the 27 participants with LE elevations in these five studies, 13/27 (48.15%) cases had elevated baseline liver enzymes between 1-3x ULN prior to initiating CBD [14–18]. 14/27 cases (51.85%) occurred in participants with confirmed normal baseline LEs [14,15,19–21].

Participants with LE elevations were reported to be asymptomatic in 48/159 cases (30.19%). 14/159 cases (8.81%) were noted to be symptomatic [15,17–20,22,23]. Out of these 14 participants, 5/14 (35.71%) had abdominal pain, 1/14 (7.14%) had diarrhea, 5/14 (35.71%) had eosinophilia, 2/14 (14.29%) had fatigue, 6/14 (42.85%) had nausea or vomiting, 2/14 (14.29%) had a rash, and 1/14 (7.14%) had somnolence. In 100/159 cases (62.89%) there was missing data regarding the proportion of symptomatic vs asymptotic cases .

The reported pattern of liver injury was hepatocellular for 100/159 cases (62.89%)**,** and mixed hepatocellular and cholestatic for 36/159 cases (22.64%). The pattern of injury could not be determined for 23/159 cases (14.47%) [17,23,24].

Risk of Bias

Quality of available evidence was low (Figure S2, S3), primarily due to the majority of studies being open-label, non-randomized trials (53.57%). Additionally, LEs were a secondary measure in most studies (96.42%), few trials reported details on how they defined DILI and the general lack of consensus for DILI criteria in the broader literature may have increased bias for the outcome. This was further complicated as some studies defined DILI at significantly higher LE elevation cut-off values than the clinical criteria used to define DILI in this review.

Among the 12 RCTs included in the meta-analysis, 6/12 (50%) were assessed as high risk of bias due to concerns in the domains of missing outcome data, selection of reported results, and non-adherence [25–30]. The primary source of bias was the potential of missing outcome data (33.33%) [25–30]. The MINORS assessment for non-randomized trials included in the descriptive synthesis [17–20,22–24,31–36] revealed low global scores overall. This was primarily due to unclear aims (93.33%) and endpoints (93.33%) with respect to the outcome of DILI, lack of blinding (100%), and lack of sample size calculations (93.33%).

Two studies were excluded from assessment as they could not be appropriately assessed with RoB 2.0 or MINORS, neither were included in the statistical analysis [22,37].

Sensitivity Analysis

Sensitivity analyses were performed to assess the influence on effect estimates when excluding studies with high risk of bias. Six RCT's were excluded due to high risk of bias [25–30]. The OR for LE >3x ULN between CBD and control groups decreased from 5.85 to 5.29. The OR for DILI between CBD and control groups decreased from

4.82 to 4.70. Due to only marginal shifts in effect estimates and because the trials scored low risk of bias in most other domains, including the randomization process, they were included in the meta-analysis.

Publication bias

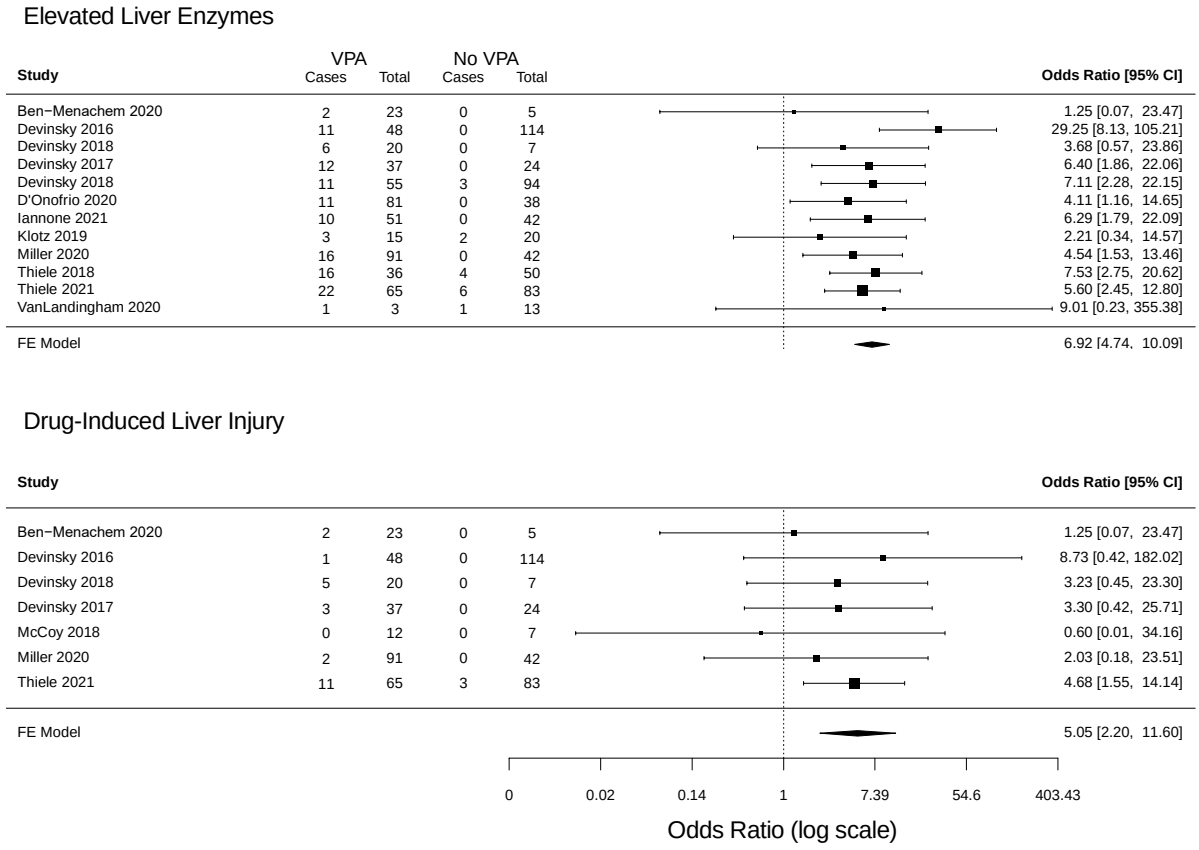
Egger's test for funnel plot asymmetry provided weak evidence of publication bias for primary outcome of DILI (z = -1.61, p = 0.107, Figure S4a). However, Egger's test revealed moderate evidence of asymmetry for cases of elevated LEs, indicating the presence of publication bias (z = -2.05, *p* = 0.04, Figure S4b). A limited pool of literature and exclusion of studies with insufficient details to confirm inclusion criteria may have contributed to the moderate observed evidence of publication bias.

Dosing sensitivity analysis

eTable 4. Comparison of predicted probability between dichotomous and trichotomous dose categories for elevated liver enzymes

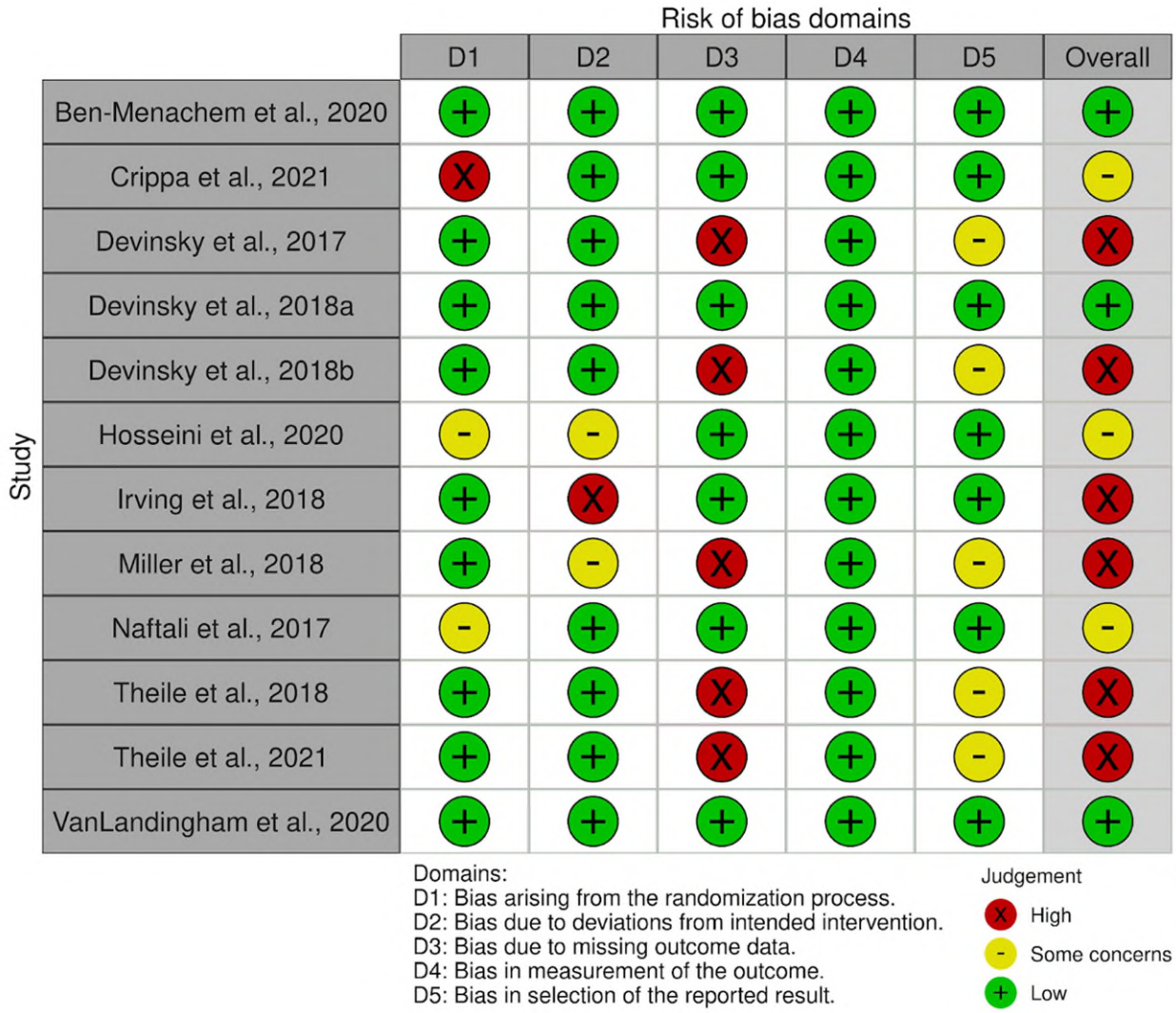
Dose variable	*k*	Predicted probability	*p*
Dichotomous			0.003
≥1000 mg	14	11.4%	
<1000 mg	10	2.0%	
Trichotomous			0.0164
≥1000 mg	14	11.6%	
300-999 mg	4	4.7%	
<300 mg	6	0.4%	
Continuous (per mg)	17	-	0.0208

6. eFigure 1. Moderation with concomitant valproate use



eFigure 1. Odds ratio of increased liver enzymes and drug induced liver injury in randomized clinical trials comparing cannabidiol patients with and without concomitant valproate use.

7. eFigure 2 RoB2 assessment



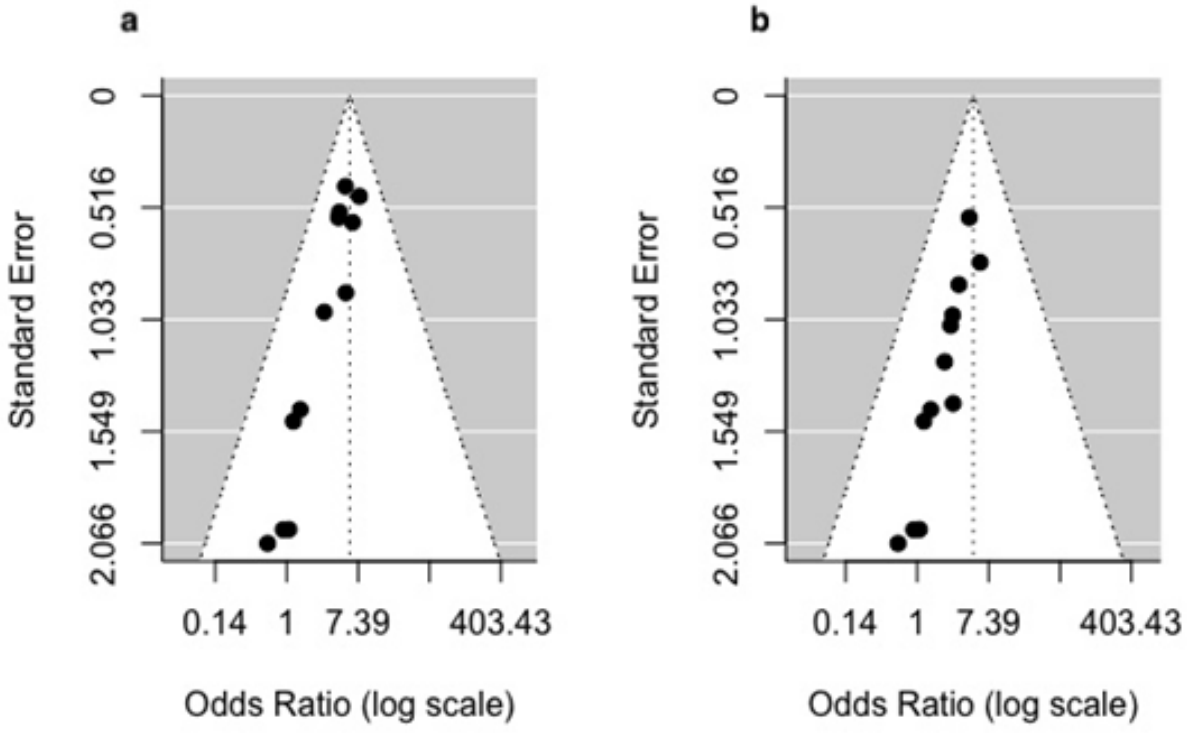
eFigure 2. RoB2 assessment

8. **eFigure 3 MINORS assessment**

Study	1	2	3	4	5	6	7	8	9	10	11	12	Global Score
D'Onofrio et al., 2020	1	1	2	0	0	2	2	0	-	-	-	-	8
Devinsky et al., 2016	1	1	2	0	0	2	1	0	-	-	-	-	7
Heussler et al., 2019	1	1	2	0	0	2	2	0	-	-	-	-	8
Iannone et al., 2021	1	1	2	0	0	2	2	0	-	-	-	-	8
Klotz et al., 2019	1	1	2	0	0	2	2	0	-	-	-	-	8
Leehey et al., 2020	1	2	2	1	0	1	2	0	-	-	-	-	9
Libzon et al., 2018	1	1	2	0	0	2	2	0	-	-	-	-	8
McCoy et al., 2018	1	1	2	1	0	2	2	0	-	-	-	-	9
Mitelpunkt et al., 2019	1	1	2	0	0	2	2	0	-	-	-	-	8
Morrison et al., 2019	1	2	2	1	0	1	2	1	1	2	1	2	16
Neubauer et al., 2018	1	1	0	1	0	2	2	0	-	-	-	-	7
Taylor et al., 2020	1	1	2	1	0	2	2	0	-	-	-	-	9
Thai et al., 2021	1	2	2	1	0	2	2	0	-	-	-	-	10
Watkins et al., 2020	2	1	2	2	0	2	2	0	-	-	-	-	11
Wheless et al., 2019	1	2	2	1	0	1	2	0	-	-	-	-	9

eFigure 3. MINORS Bias assessment

9. eFigure 4 Funnel plots



eFigure 4. Funnel plots for outcomes of elevated liver enzymes (a) and drug induced liver injury (b).

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EXHIBIT E

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RESEARCH ARTICLE

An evaluation of the genotoxicity and 90-day repeated-dose toxicity of a CBD-rich hemp oil

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Abstract

Currently, there is much interest in the sales and study of consumable *Cannabis sativa* L. products that contain relatively high levels of cannabidiol (CBD) and low levels of Δ-9-tetrahydrocannabinol. While there are published safety evaluations for extracts containing low concentrations of CBD, toxicological assessments for those with higher concentrations are still scant in the public domain. In this paper, genotoxicity tests and a 90-day repeated-dose toxicity study of an ethanolic extract of *C. sativa* containing ~85% CBD were performed following relevant OECD guidelines. No increased gene mutations were observed in a bacterial reverse mutation assay compared to controls up to the maximum recommended concentration of the guideline. An in vitro chromosomal aberration assay showed no positive findings in the short-term (3 h) treatment assays. Long-term treatment (20 h) showed an increased number of cells containing aberrations at the highest dose of 2 μg/mL, which was outside of historical control levels, but not statistically significantly different from the controls. An in vivo micronucleus study showed no genotoxic potential of the test item in mice. A 90-day repeated-dose gavage study using 0, 75, 125, and 175 mg/kg bw/day showed several slight findings that were considered likely to be related to an adaptive response to consumption of the extract by the animals but were not considered toxicologically relevant. These included increases in liver and adrenal weights compared to controls. The NOAEL was determined as 175 mg/kg bw/day, the highest dose tested (equivalent to approximately 150 mg/kg bw/day of CBD).

KEYWORDS
geno, toxicity, cannabidiol, CBD, hemp, NOAEL, safety, toxicity

Abbreviations: 2AA, 2-aminoanthracene; 9AA, 9-aminoacridine; ANOVA, analysis of variance; CBD, cannabidiol; CO_2, carbon dioxide; DME, Dulbecco's Modified Eagle's; DMSO, dimethyl sulfoxide; EMS, ethyl methanesulfonate; FBS, fetal bovine serum; FT4, free thyroxine; FOB, functional observation battery; GLP, good laboratory practice; KCl, potassium chloride; MMS, methyl methanesulfonate; MPCE, micronucleated polychromatic erythrocyte; NOAEL, no-observed-adverse-effect-level; NOEL, no-observed-effect-level; NPD, 4-nitro−1,2-phenylenediamine; OECD, Organisation for Economic Co-operation and Development; OPPTS, Office of Prevention, Pesticides and Toxic Substances; Pas, periodic acid-Schiff reagent; PCE, polychromatic erythrocyte; RICC, relative increase in cell counts; SAZ, sodium azide; SOPs, standard operating procedures; SPF, specific pathogen free; THC, Δ-9-tetrahydrocannabinol.

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1 | INTRODUCTION

The safety and efficacy of oral *Cannabis sativa* L. (common name, hemp) extracts containing high levels of the phytocannabinoid cannabidiol (CBD) and low levels of Δ-9-tetrahydrocannabinol (THC) components are currently of high interest to consumers around the globe, as can be deduced by the large number of oral hemp-CBD products currently available in the marketplace. According to reports by the American Botanical Council, in the United States, CBD was both the top selling herbal dietary supplement ingredient in 2018 and 2019, and the fastest growing ingredient in 2018, in the "natural channel" (Smith et al., 2019, 2020). The number of recent scientific articles being published on CBD is also rapidly expanding. A search of the U.S. National Library of Medicine PubMed database retrieved 1089 publications for the year 2022 that included the term "cannabidiol," as compared to 185 for the year 2015. Products high in CBD versus THC lack the intoxicating/psychotropic effects of products that are higher in the latter compound, and CBD does not exhibit effects suggestive of abuse or dependence potential (WHO & Expert Committee on Drug Dependence, 2017). *C. sativa* plant extracts also generally include various non-cannabinoid constituents such as terpenes and flavonoids (Baron, 2018; ElSohly et al., 2017; Elsohly & Slade, 2005; Radwan et al., 2008).

The U.S. Agriculture Improvement Act of 2018 (i.e., the "Farm Bill") legalized industrial hemp, which is defined as *C. sativa* containing less than 0.3% THC ("Agriculture Improvement Act of 2018, H.R. 2, 115th Cong., 2nd Sess.," 2018). Since that time, there has been an explosion in the number of hemp products being sold in the U.S. marketplace. Although the *C. sativa* plant contains numerous non-THC phytocannabinoid compounds, CBD currently has garnered the most research interest. CBD has generally low bioavailability, although it is increased during the fed state, especially when consumed with a high-fat meal (Perucca & Bialer, 2020). It undergoes significant first-pass metabolism in the liver, and metabolites are eventually excreted via the kidneys (Millar et al., 2018; Perucca & Bialer, 2020). The U.S. FDA has expressed potential concern related to the safety of long-term use of CBD products (FDA, 2023), yet this is in part related to data from the CBD approved drug Epidiolex, which is prescribed at significantly higher levels (5–20 mg/kg bw/day of CBD, equivalent to 350–1400 mg/day for a 70 kg person) (Greenwich Biosciences & FDA, 2021) as compared to recommended use levels found on the labeling of products in the general supplement marketplace, (typically 10–30 mg/day according to a review of the National Institutes of Health Dietary Supplement Label Database) (Saldanha et al., 2021). Human interventional studies suggest that CBD is generally well-tolerated when taken orally at various doses, with the most common reported side effects being minor and related to the gastrointestinal system, appetite, and drowsiness, as well as reversible increases in transaminases, especially if CBD is given at higher doses and co-administered with certain medications (Bergamaschi et al., 2011; Iffland & Grotenhermen, 2017; Lattanzi et al., 2018; Millar et al., 2019; WHO & Expert Committee on Drug Dependence, 2017). Post-marketing surveillance of a hemp extract supplement containing 15 mg CBD over a 2-year period during which approximately five million product units were sold showed that the most common reported adverse events were gastrointestinal related, and 99.8% of the events were considered non-serious (Schmitz, Lopez, & Marinotti, 2020). The two serious events that were reported (hallucinations and a hypersensitivity reaction) were considered unlikely to be due to intake of the supplement.

Toxicological assessments in rats of individual hemp extract products have been recently published, including two articles by Dziwenka et al. (2020 and 2021) and one by Marx et al. (2018) (Dziwenka et al., 2020, 2021; Marx et al., 2018). The test items in these studies included a 6% isopropanol CBD extract product (Dziwenka et al., 2020), and two 25% CBD supercritical CO_2 extracts (Dziwenka et al., 2021; Marx et al., 2018). The no observed adverse effect levels (NOAELs) determined in all three published 90-day repeated-dose rat studies were nearly identical with regard to total CBD exposure, equating to CBD constituent NOAELs of 23–25 mg/kg bw/day. The test item used in the current toxicological evaluation differs from those previously studied in that it is more concentrated in CBD. The batches used herein contained 84–87% CBD. The test item also differs in that it is manufactured using ethanol as the main solvent for the extraction method. Levels of THC and Δ-9-tetrahydrocannabinolic acid were extremely low in the test item, present at a maximum of 0.031% in the batches used in the current studies. It was of interest to perform a toxicological assessment on this product considering its differences compared to previously studied products, and these studies add to the totality of published data on CBD and hemp products for human consumption.

2 | MATERIAL AND METHODS

2.1 | Test item

The test item utilized in this study, called by the trade name Imperial Oil®, was a dark amber wax-like viscous liquid containing 86–89% total cannabinoids and 84–87% CBD, depending on the batch utilized in the particular study. THC was measured as non-detectable in the batch utilized for the genotoxicity studies, and at 0.031% in the batch used for the 90-day repeated-dose study (limit of detection equal to 0.003%). Total terpenes were measured at approximately 1.5%. Batches were otherwise confirmed to be of suitable chemical purity when provided by the sponsor (Kazmira™, Watkins, CO, USA), including parameters such as residual solvents, heavy metals, pesticides, and microbial contaminants. The extract was manufactured using industrial hemp biomass raw material, and denatured ethanol was utilized as the main solvent. Necessary quantities were provided for each study together with batch analyses confirming the identity, quality, purity, and safety specifications of the test item.

2.2 | Good laboratory practice and test item formulation

The toxicological studies described in this report were all conducted in compliance with OECD Good Laboratory Practices (GLP) (OECD, 1998). In the case of the genotoxicity studies, only preliminary tests were performed using non-GLP standards, whereas all main/final studies were GLP compliant.

2.3 | Bacterial reverse mutation test

The bacterial reverse mutation test followed the OECD Guidelines for Testing of Chemicals No. 471 (adopted 21 July, 1997 and corrected 26 June, 2020) (OECD, 1997, 2020). It additionally followed the standard operating procedures (SOPs) of the laboratory, which were developed with reference to published methods of Ames et al. (1975), Kier et al. (1986), Maron and Ames (1983), and Venitt and Parry (1984).

The phenotypically confirmed bacterial tester strains and the activated rat liver S9 fraction used in the assay were manufactured by Moltox Inc. and were obtained through Trinova Biochem GmbH. They were histidine-requiring auxotroph *Salmonella typhimurium* strains TA98, TA100, TA1535, and TA1537, and the tryptophan-requiring auxotroph *Escherichia coli* strain WP2 *uvrA*. All strains were studied in the presence and absence of a metabolic activation system (i.e., S9 mixture) prepared in the laboratory using the rat liver S9 fraction.

The study included a non-GLP preliminary solubility test, a non-GLP concentration range finding test (using the plate incorporation method), a GLP initial plate incorporation test, and a GLP confirmatory pre-incubation test. For the solubility test, the test item was dissolved and further diluted to appropriate concentrations in dimethyl sulfoxide (DMSO) (1.6, 5, 16, and 50 mg/mL, to allow for 160, 500, 1600, and 5000 μg/tube). Top agar and phosphate buffer was added, and the solutions were examined in a test tube without bacteria for behavior in the vehicle.

The concentration range finding test used strains TA98 and TA100 ± S9. Based on the solubility test results, a stock solution of 50 mg/mL was prepared in DMSO. Revertant colony numbers and inhibition of background lawn were determined at test item concentrations of 5, 16, 50, 160, 500, 1600, and 5000 μg/plate, along with positive, negative, and untreated controls, using the plate incorporation method. An effect on background lawn growth was the indicator for an inhibitory effect of the test item (reduced or slightly reduced background lawn and/or decreased revertant colony counts below the corresponding historical control data and/or actual vehicle control ranges).

Based on the preliminary tests, DMSO was used as the vehicle for the test item (this vehicle allowed for adequate solubility of the test item and is compatible with the survival of the bacteria and S9, and the laboratory has available historical data on this solvent). Positive controls in the tests without metabolic activation were 4-Nitro−1,2-phenylenediamine (NPD; 4 μg/plate) for strain TA98, sodium azide (SAZ; 2 μg/plate) for strains TA100 and TA1535, 9-aminoacridine (9AA; 50 μg/plate) for strain TA 1537, and methyl methanesulfonate (MMS; 2 μL/plate) for strain WP2 *uvrA*. In tests with metabolic activation, 2-aminoanthracene (2AA) was used for all strains (2 μg/plate for *S. typhimurium* strains, and 50 μg/plate for the *Escherichia coli* strain). The sensitivity, reliability, and promutagen activation potential of the S9 that was utilized was verified by the supplier using ethidium bromide, cyclophosphamide, benzo(a)pyrene, and 2AA. DMSO was used as the vehicle for NPD, 9AA, and 2AA controls, whereas ultrapure water was used for SAZ and MMS. The S9 fraction was prepared from the livers of phenobarbital/β-naphthoflavone treated rats. The DMSO, 9AA, NPD, and SAZ controls were obtained from Merck KGaA, whereas MMS and 2AA were from Sigma-Aldrich. An untreated control group was also utilized in the study.

Test item concentrations for the main tests were prepared based on the preliminary tests and according to OECD recommendations and were as follows: 5, 16, 50, 160, 500, 1600, and 5000 μg/plate (the latter being the recommended maximum concentration for soluble non-cytotoxic substances per the OECD guideline), at a test volume of 100 μL/plate, to be used with and without S9.

Frozen bacterial cultures were thawed and a 200 μL inoculum was used for each 50 mL of nutrient broth (Oxoid Ltd.) for overnight cultures incubated at 37°C. A standard plate incorporation method was used as the initial mutation test, with each condition performed in triplicate. The test item concentrations were prepared fresh, and 100 μL was added to tubes with 2 mL top agar (prepared in laboratory), 100 μL tester strain from overnight culture (approximately 109 cells per mL), and 500 μL phosphate buffer or S9 mixture. The tubes were poured over minimal agar plates (VWR International) and incubated at 37°C for approximately 48 h. For the pre-incubation procedure, the bacterial cultures and each test item concentration were added to tubes to allow direct contact for 20 min in a shaking incubator, prior to the remaining steps described above.

Resulting colonies were manually counted using both the unaided eye and a microscope at 40X magnification. The mutation rate was calculated by dividing the mean number of revertant colonies for a given experimental condition by the mean number of revertant colonies for the vehicle control. A test item was considered mutagenic if a concentration-related increase in the number of revertants occurred, and/or a reproducible biologically relevant positive response was noted for at least one concentration in at least one strain. A biologically relevant response was considered as at least twice as high of a reversion rate as the concurrent vehicle control for strains TA98, TA100, and WP2 *uvrA*, and at least three times the reversion rate as the concurrent vehicle control for strains TA1535 and TA1537. If the above criteria were not met, the test was considered negative for mutagenicity.

2.4 | In vitro mammalian chromosomal aberration test

The in vitro mammalian chromosomal aberration test followed the OECD Guidelines for Testing Chemicals No. 473 (adopted 29 July, 2016) (OECD, 2016a). Male Chinese hamster lung V79 cell line (supplied by the European Collection of Cell Cultures and checked for mycoplasma infections), which has low background aberration production, was used for the assay. Cells were grown in Dulbecco's Modified Eagle's (DME) medium supplemented with L-glutamine (2 mM; Biowest SAS, France), 1% of antibiotic–antimycotic solution, and 10% fetal bovine serum (FBS [Sigma-Aldrich Chemie GmbH]; reduced to 5% during test item treatments). The test item was dissolved in 10 mg/mL DMSO, and concentrations used in the study were obtained from this solution using serial dilutions with DME ± S9 (Trinova Biochem GmbH). The negative control was the DMSO vehicle. The positive control without metabolic activation was the clastogenic compound ethyl methanesulfonate (EMS; 0.4 and 1.0 µL/mL for short and long-term treatments), and the positive control with metabolic activation was cyclophosphamide monohydrate (Sigma-Aldrich Chemie GmbH, 5 µg/mL). Note that while EMS is not listed in the current OECD guidance, it was listed in the previous guidance and was used here because of historical control data availability. Controls were diluted in DME to get appropriate concentrations. All test concentration cultures were prepared in duplicate. The EMS, cyclophosphamide monohydrate, FBS, and antibiotic–antimycotic solution were all obtained from Sigma-Aldrich Chemie GmbH.

A preliminary cytotoxicity test was conducted to determine the test item concentrations for the main study. The 5×10^5 cells were seeded into sterile tissue culture dishes and were incubated in 10 mL of DME containing 10% FBS. After 24 h, the cells were treated using increasing concentrations of test item in the absence or presence of S9 mix (50 mg/mL) and were incubated at 37°C for 3 h. Cultures were then washed with DME medium and covered with DME containing 10% FBS. Cell counts were performed after 20 h (approximately 1.5 normal cell cycles from the beginning of treatment) using a Bürker chamber. Additional groups of cells were treated for 20 h without S9 and for 3 h with S9, with cell counts conducted after 20 h (without S9 mix only) and 28 h (without and with S9 mix). Four cultures were also set up for determining the initial cell count. At harvest, the cells were trypsinized (Sigma-Aldrich Chemie GmbH), collected, and cell counts were determined, and the relative increase in cell counts (RICC) was calculated, which is an indicator of cytotoxicity. Precipitation was evaluated at the beginning and at the end of the treatment by the unaided eye, and pH and osmolality effects were also measured. At harvest, the aim is for the highest concentration to show a significant reduction in cell count (55 ± 5%).

The main chromosomal aberration assays were conducted for different lengths of time and in the presence and absence of S9. Concentrations of the test item used in the study were selected based on a cytotoxicity pretest which was also performed ± S9 in accordance with OECD guidance. Concentrations along with treatment and sampling times in the main test were as follows (note that 20 and 28 h are equivalent to approximately 1.5 and 2 cell cycles, respectively). For the 3 h treatment, concentrations were 2, 4, 6, and 8 µg/mL without S9 (20-h sampling time), and 7, 12, 17, and 22 µg/mL with S9 (20- and 28-h sampling times). For the 20-h treatment and 20/28-h sampling times performed without S9, the concentrations were 0.5, 1, and 2 µg/mL.

For all experimental conditions, 5×10^5 V79 cells were seeded into 92 × 17 mm dishes, using DME medium to reach the constant volume of 5 mL/plate. The culture medium of the growing cells was replaced with the test or control formulations with or without S9 mix. After exposure, the cells were washed with DME medium followed by fresh growth medium (DME + 10% FBS). Exposures were performed at 37°C, and precipitation (using the unaided eye) was determined at the beginning and the end of treatment. Additionally, pH and osmolality were measured for negative control and treatment groups.

Approximately 2.5 h prior to harvesting, cell cultures were treated with colchicine (0.2 µg/mL; Sigma-Aldrich Chemie GmbH). At harvest, cells were swollen with 0.075 M KCl (Sigma-Aldrich Chemie GmbH) hypotonic solution and washed in fixative (3:1 mixture of methanol: acetic acid; from Lach-ner Ltd and Merck KGaA, respectively). They were then dropped onto microscope slides, air-dried, and stained with 5% Giemsa (Merck KGaA) for scoring of chromosome aberration frequencies. Slides were independently coded before analysis and scored blind. Approximately 300 well-spread metaphase cells (split evenly between the duplicate slides) containing 22 ± 2 chromosomes were scored per condition. Chromatid and chromosome type aberrations (gaps, deletions, and exchanges), as well as polyploid and endoreduplicated cells, were recorded separately. Classification and nomenclature were based on established criteria. (Hamden et al., 1985; Savage 1976, 1983) Statistical analysis (with significance defined as $p < 0.05$) was performed using the chi-square test (SPSS PC + software, version 4), comparing treated group and concurrent positive control results with those of the concurrent negative control. The adequate regression analysis (Microsoft Excel software) was used to check for linear trends in the number of cells with aberrations. The test was considered positive if at least one of the test concentrations exhibited a statistically significant increase compared with the concurrent negative control; the increase was concentration-related when evaluated using an appropriate trend test, and if any of the results are outside the distribution of the laboratory historical negative control data.

2.5 | In vivo mammalian micronucleus test

The mammalian micronucleus test followed the OECD Guidelines for Testing of Chemicals No. 474 (adopted 29 July, 2016) (OECD, 2016b). The study was permitted under the Institutional Animal Care and Use Committee of Toxi-Coop Zrt.

A non-GLP preliminary solubility test was performed in which the test item was dissolved in sunflower oil (Magilab Kft) as a vehicle and stirred until homogeneity was reached. Additionally, a non-GLP preliminary toxicity test was performed to justify the main study maximum dose and to determine whether there were toxicity differences

between the sexes. Groups of two mice/sex/group were treated twice by oral gavage at doses of 500, 1000, and 2000 mg/kg bw. Animals were examined regularly for toxic signs and mortality, but no bone marrow smears were prepared.

In the main study, test item was prepared in sunflower oil at concentrations of 0, 50, 100, and 200, and was used within 15 min of preparation on the day of dosing. Cyclophosphamide (Sigma-Aldrich, Germany) dissolved in sterile water for injection (Magilab Kft, Hungary) was used as the positive control. Sunflower oil vehicle functioned as the negative control. Eight-week-old SPF Win: NMRI mice (Toxi-Coop Zrt, Budapest, Hungary) weighing 34.5–38.4 g were used as the experimental animals (two animals/sex/group for the pretest, and five males/group in the main test). Two additional males were added to the high-dose group in case any animals died at this dose. Animals were housed in groups of two per cage (pretest) and five per cage (main test), under a 12-h light/dark cycle and controlled temperature and humidity. They were fed ssniff® SR/M-Z + H diet (Experimental Animal Diets Inc., Germany) and received potable water ad libitum.

The main study dose groups were 0, 500, 1000, and 2000 mg/kg bw/day (males only). The cyclophosphamide positive control was dosed at 60 mg/kg bw/day. The test item was administered by gavage twice at 24-h intervals. The positive control was administered once intraperitoneally. All treatment volumes were 10 mL/kg bw. The animals were examined regularly for any visible reactions to treatment, and sampling was performed 24 h after the last treatment. They were sacrificed via cervical dislocation, and bone marrow was immediately obtained from femurs. Bone marrow was flushed with FBS, vortex mixed, and cells were concentrated via centrifugation. Smears of the cell pellets were made on microscope slides and dried at room temperature. Slides were then fixed with methanol (Lach-ner Ltd) and stained with Giemsa (10%) solution.

Four thousand polychromatic erythrocytes (PCEs) per animal were scored blind, and the percent of micronucleated cells (MPCEs) was calculated. Additionally, the percent of immature erythrocytes was determined for each animal by counting at least 500 erythrocytes. Statistical analysis of MCPE frequency in the positive and treatment groups with comparison to concurrent and historical controls was performed using Kruskal–Wallis nonparametric analysis of variance (ANOVA) test (SPSS PC + software version 4, SPSS, Inc.). Statistical analysis of immature erythrocytes in animals was performed using Mann–Whitney *U*-test versus control. Linear trends in mutant frequency were checked using the Microsoft Excel's adequate regression analysis. The evaluation and interpretation criteria of OECD guideline 474 were applied to the determination of clearly positive or negative results.

2.6 | 90-day repeated-dose oral toxicity study in rats

The 90-day repeated-dose toxicity study followed the OECD Guidelines for Testing of Chemicals No. 408 (adopted 25 June 2018). (OECD, 2018) The study was approved by the Institutional Animal Care and Use Committee of Toxi-Coop Zrt. and was performed according to the National Research Council Guide for Care and Use of Laboratory Animals. (National Research Council, 2011).

For this study, the test item was formulated in sunflower oil as the vehicle, at concentrations of 7.5, 12.5, and 17.5 mg/mL. Dose and vehicle selection were made on the basis of an unpublished, OECD compliant (OECD, 2008), 14-day repeated-dose range-finding study on Imperial Oil® in which no adverse effects were observed up to the highest dose (125 mg/kg bw/day) tested, along with information in the literature related to toxicologically relevant doses of hemp extracts containing various amounts of CBD (Dziwenka et al., 2020, 2021; Marx et al., 2018). Formulations in the current study were administered to SPF Han:WIST rats (Toxi-Coop Zrt., Budapest, Hungary) within 4 h of preparation until stability data of formulations were available. After that time, formulations were prepared not longer than 4 days beforehand and were stored at room temperature until use.

Analytical control of dosing formulations was performed using a validated HPLC-UV method during the study. Five samples were taken for each concentration and analyzed in replicates of three, on three different occasions throughout the study. The concentrations of Imperial Oil® in the dosing formulations ranged from 95.4% to 106% of the nominal concentrations. The test item was found to be stable in the concentration range of 5–20 mg/mL for at least 4 days at room temperature, and homogeneity was determined and found to be sufficient.

Male and female rats, ages 47–52 and 41–47 days, and weighing 221–248 g and 129–160 g, respectively, were randomly divided based on stratification by body weight into four groups of 10 rats/sex and acclimatized for 7 days. They were housed in group cages (2 animals/sex/cage) under a 12-h light–dark cycle and controlled temperature and humidity. They were given ssniff® SR/M-Z + H diet and potable water, except for overnight food deprivation prior to blood sampling. The dose groups were 0, 75, 125, and 175 mg/kg bw/day, given via gavage at a dose volume of 10 mL/kg for 90 or 91 days, for males and females, respectively.

The eyes of all animals were examined using indirect ophthalmoscopy during the acclimation period, and the procedure was repeated on all animals of the control and high-dose groups on Day 84 of the study. Animals were inspected for signs of morbidity and mortality twice per day, and clinical observations were performed cage-side once per day after treatment. Detailed clinical examinations were conducted outside the cage prior to the initial treatment, and weekly thereafter, and a functional observation battery (FOB) was performed on Day 84. Body weight measurements were taken twice during the acclimation period, prior to the first treatment, twice weekly during weeks 1–4, and weekly thereafter. Food consumption was determined weekly.

Estrous cycle was examined using vaginal smears prepared on the day of necropsy, followed by staining with 1% aqueous methylene blue solution (Acros Organics), and examination under light microscope. Animals were fasted for approximately 16 h prior to blood

sampling from the retro orbital venous plexus under Isofluran CP® anesthesia on Days 90 and 91 (males and females, respectively). The collection tubes utilized were K_3EDTA (for hematology), 9NC coagulation 3.2% (for blood coagulation), and Vacuette Z Serum Sep C/A (for clinical chemistry and thyroid hormone measurements). All except the K_3EDTA tubes were centrifuged after filling such that supernatant plasma samples could be used for evaluation.

After blood sampling, animals were exsanguinated from the abdominal aorta after verification of narcosis. Macroscopic examination of the external body, internal cavities, and organs and tissues was performed. Next, organs and tissues were removed, trimmed of adherent tissue, weighed, and preserved in 4% formaldehyde solution (Lach-Ner, Ltd.), except that the testes and epididymides were fixed in modified Davidson solution prior to storage in the formaldehyde solution for histopathologic examination. Paired organs were weighed together. Weight of pituitary and thyroid glands were determined after fixation. Fixed tissues were trimmed, processed, embedded in paraffin, sectioned with a microtome, placed on glass slides, stained with hematoxylin and eosin (both from DiaPath SPA), and examined by light microscopy. Full histological examinations were performed on the preserved organs and tissues from the control and high-dose groups. The liver, kidneys, thymus, and ovaries were examined in all dose groups based on other findings. Staining using Oil-Red-O (Renal Laboratory Ltd.) was performed on all liver samples, along with the periodic-acid Schiff reaction (Pas, Renal Laboratory Ltd.).

Statistical analysis related to body weight, food consumption, feed efficiency, hematology and blood coagulation, clinical chemistry, thyroid hormones, and organ weights was performed using SPSS PC + software version 4 (SPSS, Inc.). The heterogeneity of variance between groups was checked by Bartlett's homogeneity of variance test. When no significant heterogeneity was determined, a one-way analysis of variance (ANOVA) was performed. If a positive result was obtained, Duncan's Multiple Range test was used to assess significance of inter-group differences. If significant heterogeneity was noted, the normal distribution of data was examined using Kolmogorov–Smirnov test. In the case of non-normal distribution, the Kruskal–Wallis One-Way analysis of variance was used. If the result was positive, the Mann–Whitney *U*-test was used for inter-group comparisons. Significance was judged at probability values of $p < 0.05$ and < 0.01.

3 | RESULTS

3.1 | Bacterial reverse mutation test

In the preliminary solubility test, the test item was a clear solution in DMSO at all concentrations (1.6–50 mg/mL). When mixed with the top agar, the solution was clear at the lowest concentration (1.6 mg/mL). Suspensions were noted at higher concentrations, which were described as slightly opalescent, opalescent, and homogenous/milky at 5, 16, and 50 mg/ml, respectively.

The concentration range finding study negative control results fell within corresponding historical control ranges, and the positive control showed expected biologically relevant increases in induced revertant colonies in both bacterial test strains. No unequivocal inhibitory effect of the test item was observed, and background lawn development was not affected. No precipitate was noted but at 5000 and 1600 μg/plate, opalescent milky plates (viewed as microdrops under 40X magnification) were observed ±S9 but did not disturb scoring. There were also no test item effects noted when results were compared to the corresponding solvent and untreated control.

In the main studies, the vehicle controls demonstrated spontaneous revertant numbers compatible with the corresponding historical controls, and the concurrent positive controls showed the expected (at least threefold) increases in revertant colonies as compared to the concurrent vehicle controls in all tester strains and experimental phases, with results also compatible with historical positive control ranges. The initial mutation test showed an equivocal inhibitory effect of the test item in the TA100 strain (based on lower revertant colony numbers compared to the historical control range for the vehicle [without effects on background lawn development] at 5000 μg/plate in the absence of S9, and in the 500–5000 μg/plate range in the presence of S9), whereas in the confirmatory pre-incubation test, inhibitory/cytotoxic effects were observed in all *S. typhimurium* strains, beginning at 160, 500, and 5000 μg/plate for strains TA100, TA98, TA1537, and TA1535 in the absence of S9, and starting at 500 μg/plate for strain TA100 in the presence of S9 based on reduced background lawn development and/or reduced revertant colony numbers compared to the corresponding historical negative controls. Also opalescent milky plates (composed of microdrops at 40X magnification and consistent with a colloid chemical phenomenon) were observed at or above 1600 μg/plate in all experiments but did not disturb the scoring. Regardless of these effects, in all experimental phases there were at least five analyzable concentrations and at least three non-cytotoxic concentrations. In considering all relevant factors, the validity of the bacterial reverse mutation test was verified. No relevant increases were observed in revertant colony numbers in any of the five test strains following test item treatment with or without metabolic activation in the main studies. Summary results are presented in Table 1.

3.2 | In vitro mammalian chromosomal aberration test

The main chromosomal aberration assay test item concentrations were based on the results of a cytotoxicity pre-test. No precipitation or relevant pH or osmolality changes were observed at any concentration in the assay. Clear cytotoxicity was observed at the highest concentrations in an acceptable range per guidelines (52–54%).

In the short-term experiments with a treatment time of 3 h and sampling times of 20 h, no increases in cells with structural chromosomal aberrations were noted compared to the concurrent negative

TABLE 1 Results of the bacterial reverse mutation test.

	Without exogenous metabolic activation (−S9 mix)									
	Salmonella typhimurium tester strains								*Escherichia coli*	
	TA98		TA100		TA1535		TA1537		WP2*uvr*A	
Concentration (μg/plate)	Mean ± SD	MR	Mean ± SD	MR	Mean ± SD	MR	Mean ± SD	MR	Mean ± SD	MR
Initial mutation test (plate incorporation method)										
Untreated control	26.3 ± 4.2	0.9	104.0 ± 9.5	1.2	10.7 ± 3.5	1.1	6.0 ± 2.0	1.1	41.7 ± 10.3	1.3
DMSO control	30.0 ± 2.6	1.0	89.3 ± 21.7	1.0	9.7 ± 1.5	1.0	5.7 ± 1.5	1.0	33.0 ± 5.3	1.0
Water control			94.7 ± 5.7	1.0	12.7 ± 9.1	1.0			36.0 ± 9.539	1.0
5000	26.7 ± 4.0*	0.9	47.3 ± 15.3*	0.5	14.0 ± 2.6*	1.4	4.3 ± 3.1*	0.8	32.0 ± 4.6*	1.0
1600	16.7 ± 3.2*	0.6	60.0 ± 6.0*	0.7	12.3 ± 7.6*	1.3	4.0 ± 1.0*	0.7	38.0 ± 6.1*	1.2
500	20.3 ± 6.7	0.7	65.7 ± 8.6	0.7	12.7 ± 1.5	1.3	4.0 ± 2.6	0.7	40.7 ± 11.1	1.2
160	21.7 ± 3.2	0.7	55.0 ± 3.6	0.6	11.3 ± 2.1	1.2	6.3 ± 4.9	1.1	32.7 ± 1.5	1.0
50	24.7 ± 8.6	0.8	69.3 ± 15.0	0.8	14.7 ± 3.2	1.5	7.0 ± 4.0	1.2	35.0 ± 1.7	1.1
16	18.3 ± 2.5	0.6	80.3 ± 8.0	0.9	12.0 ± 3.5	1.2	6.7 ± 1.5	1.2	35.0 ± 1.7	1.1
5	25.7 ± 5.8	0.9	89.0 ± 13.1	1.0	9.7 ± 3.8	1.0	6.0 ± 1.0	1.1	30.3 ± 1.2	0.9
Positive controls	*1118.7 ± 48.4*[a]	*37.3*	648.0 ± 124.2[b]	*6.8*	*884.0 ± 72.8*[b]	*69.8*	490.7 ± 137.7[c]	*86.6*	*665.3 ± 14.0*[d]	*18.5*
Confirmatory mutation test (plate incorporation method)										
Untreated control	30.0 ± 2.00	1.41	93.0 ± 8.19	1.15	12.3 ± 3.1	0.9	7.3 ± 1.5	1.2	27.3 ± 0.6	1.0
DMSO control	21.3 ± 3.06	1.00	80.7 ± 1.15	1.00	14.0 ± 3.6	1.0	6.3 ± 2.9	1.0	26.7 ± 7.5	1.0
Water control			111.0 ± 1.7	1.0	15.3 ± 4.0	1.0			33.3 ± 7.5	1.0
5000	16.0 ± 1.00†*	0.75	31.0 ± 13.53▼*	0.38	14.3 ± 4.5†*	1.0	0.3 ± 0.6▼*	0.1	28.3 ± 4.2*	1.1
1600	11.3 ± 5.77†	0.53	25.3 ± 5.51†	0.31	9.3 ± 3.1	0.7	1.3 ± 0.6†	0.2	25.0 ± 2.6	0.9
500	12.3 ± 5.51†	0.58	23.3 ± 11.06†	0.29	12.0 ± 3.6	0.9	2.0 ± 1.0†	0.3	29.3 ± 9.3	1.1
160	15.0 ± 4.58	0.70	45.0 ± 1.00	0.56	12.7 ± 3.5	0.9	5.7 ± 4.7	0.9	27.7 ± 5.7	1.0
50	12.7 ± 2.31	0.59	62.0 ± 4.36	0.77	10.7 ± 1.2	0.8	6.0 ± 5.3	0.9	29.0 ± 2.0	1.1
16	19.3 ± 0.58	0.91	60.7 ± 4.73	0.75	11.0 ± 3.0	0.8	5.7 ± 2.1	0.9	27.7 ± 3.5	1.0
5	16.0 ± 4.58	0.75	65.3 ± 3.79	0.81	11.7 ± 1.5	0.8	6.7 ± 5.5	1.1	26.7 ± 4.2	1.0
Positive controls	*374.7 ± 29.48*[a]	*17.56*	718.7 ± 117.05[b]	*6.47*	*877.3 ± 65.2*[b]	*57.2*	*242.0 ± 40.0*[c]	*38.2*	*1000.0 ± 100.2*[d]	*30.0*
Historical control data ranges										
Untreated control	10–39		63–127		4–19		2–18		14–57	
DMSO control	10–40		59–111		3–18		2–17		15–48	
Water control	10–38		62–117		2–23		2–15		17–61	
Positive controls	*212–941*[a]		*476–1745*[b]		*482–1987*[b]		*129–2,663*[c]		*340–2,176*[d]	

TABLE 1 (Continued)

	With exogenous metabolic activation (+S9 mix)									
	S. typhimurium tester strains								*E. coli*	
	TA98		TA100		TA1535		TA1537		WP2*uvr*A	
Concentration (μg/plate)	Mean ± SD	MR	Mean ± SD	MR	Mean ± SD	MR	Mean ± SD	MR	Mean ± SD	MR
Initial mutation test (plate incorporation method)										
Untreated control	39.0 ± 5.3	1.2	106.0 ± 7.2	1.1	18.0 ± 1.0	1.3	17.7 ± 2.3	1.2	43.0 ± 6.9	1.3
DMSO control	32.7 ± 1.2	1.0	97.3 19.4	1.0	14.0 ± 1.0	1.0	15.3 ± 3.5	1.0	36.3 ± 3.8	1.0
Water control										
5000	30.0 ± 2.6*	0.9	43.7 ± 6.4*	0.4	14.3 ± 3.1*	1.0	9.0 ± 6.2*	0.6	49.3 ± 4.5*	1.0
1600	27.7 ± 1.5*	0.8	55.0 ± 2.6*	0.6	12.7 ± 0.6*	0.9	8.3 ± 4.9*	0.5	44.0 ± 8.2*	1.0
500	32.3 ± 5.9	1.0	56.7 ± 13.7	0.6	12.3 ± 4.9	0.9	13.3 ± 1.5	0.9	40.0 ± 4.4	1.2
160	31.3 ± 6.5	1.0	92.0 ± 10.8	0.9	9.7 ± 4.5	0.7	13.7 ± 3.1	0.9	49.3 ± 2.5	1.2
50	30.3 ± 4.0	0.9	100.7 ± 8.1	1.0	10.7 ± 1.5	0.8	11.3 ± 3.1	0.7	46.0 ± 5.6	1.0
16	36.7 ± 5.1	1.1	100.3 ± 11.7	1.0	16.3 ± 5.0	1.2	12.3 ± 5.0	0.8	39.3 ± 1.2	1.1
5	37.3 ± 4.9	1.1	100.0 ± 4.0	1.0	12.0 ± 2.0	0.9	14.3 ± 5.0	0.9	41.7 ± 2.5	1.1
Positive controls	*1888.0 ± 60.4*[e]	*57.8*	*942.0 ± 45.2*[e]	*9.7*	*180.7 ± 7.6*[e]	*12.9*	*213.0 ± 38.1*[e]	*13.9*	*238.0 ± 29.8*[f]	*0.9*
Confirmatory mutation test (plate incorporation method)										
Untreated control	31.3 ± 1.5	1.0	129.7 ± 2.5	1.2	11.7 ± 0.6	1.1	6.3 ± 1.5	0.8	36.3 ± 11.0	0.8
DMSO control	31.7 ± 2.9	1.0	108.0 ± 1.0	1.0	11.0 ± 4.6	1.0	8.3 ± 4.5	1.0	44.3 ± 3.5	1.0
Water control										
5000	33.0 ± 5.6*	1.0	33.3 ± 4.726*	0.31	10.67 ± 1.2*	1.0	8.0 ± 1.7*	1.0	41.0 ± 4.6*	0.9
1600	29.0 ± 11.3*	0.9	44.7 ± 3.8*	0.41	11.3 ± 4.5*	1.0	5.3 ± 4.9*	0.6	37.0 ± 3.6*	0.8
500	30.3 ± 7.4	1.0	42.0 ± 4.4	0.4	14.0 ± 2.0	1.3	8.3 ± 4.9	1.0	35.3 ± 3.2	0.8
160	35.7 ± 5.1	1.1	75.3 ± 3.5	0.7	10.3 ± 4.5	0.9	5.3 ± 0.6	0.6	41.7 ± 6.7	0.9
50	36.3 ± 2.1	1.1	96.3 ± 3.1	0.9	12.7 ± 6.4	1.2	7.7 ± 3.8	0.9	40.0 ± 6.2	0.9
16	37.3 ± 7.1	1.2	88.7 ± 13.9	0.8	15.3 ± 3.8	1.4	9.0 ± 4.6	1.1	37.7 ± 4.0	0.8
5	31.3 ± 4.7	1.0	106.0 ± 6.2	1.0	17.0 ± 5.6	1.5	8.7 ± 4.6	1.0	39.7 ± 5.0	0.9
Positive controls	*1040.0 ± 50.0*[e]	*32.8*	*813.3 ± 45.5*[e]	*7.5*	*141.3 ± 7.1*[e]	*12.8*	*101.7 ± 20.6*[e]	*12.2*	*370.0 ± 22.7*[f]	*8.3*
Historical control data ranges										
Untreated control	13–46		68–149		3–27		2–16		19–64	
DMSO control	11–42		67–130		3–23		2–18		19–65	

TABLE 1 (Continued)

	With exogenous metabolic activation (+S9 mix)									
	S. typhimurium tester strains								*E. coli*	
	TA98		TA100		TA1535		TA1537		WP2*uvr*A	
Concentration (μg/plate)	Mean ± SD	MR	Mean ± SD	MR	Mean ± SD	MR	Mean ± SD	MR	Mean ± SD	MR
Water control	13–47		71–134		5–21		2–15		22–65	
Positive controls	*555–2,740*[e]		*641–2,520*[e]		*82–233*[e]		*69–264*[e]		*142–324*[f]	

Abbreviations: DMSO, dimethyl sulfoxide; MR, mutation rate; SD, standard deviation; UP, ultrapure.

Remarks: Ultrapure water was applied as vehicle of the test item and of the positive control substances sodium azide and methyl methanesulfonate. Dimethyl sulfoxide was applied as vehicle of the positive control substances 4-nitro−1,2-phenylenediamine, 9-aminoacridine, and 2-aminoanthracene. The mutation rates obtained at each substance refer to their respective vehicles.

[a]4-Nitro−1,2-phenylenediamine (4 μg/plate).

[b]Sodium azide (2 μg/plate).

[c]9-Aminoacridine (50 μg/plate).

[d]Methyl methanesulfonate (2 μL/plate).

[e]2-Aminoanthracene (2 μg/plate).

[f]2-Aminoanthracene (50 μg/plate).

*Opalescent plates (in microscope at 40X magnification microdrops, colloid chemical phenomenon).

†Slightly reduced background lawn development.

▼Reduced background lawn development.

control or the historical range, in the absence or the presence of metabolic activation. In the remaining experiments, the cell frequency with structural chromosome aberrations were increased compared to the historical control range at the highest concentrations only (3/28 h, with S9 and with gaps; 20/20 h, without S9 and with and without gaps; and 20/28 h, without S9 and with gaps). Cell frequency with aberrations fell within historical control ranges at all other concentrations. None of the values that were increased above the historical control ranges were statistically significantly different than the concurrent negative control. The percent of aberrant cells at the dose level of 2 μg/mL was just over twofold higher than that of the vehicle control when cells were treated for 20 h (one and a half cell cycles).

There were no polyploid or endoreduplicated metaphases in either experiment under any condition. The number of aberrations found in the concurrent negative controls was in the range of the historical laboratory negative control data, whereas the concurrent positive controls caused the expected increases and were compatible with the historical positive control data. Thus, the study was considered valid. Results are summarized in Table 2.

3.3 | In vivo mammalian micronucleus test

The preliminary solubility test showed homogeneity of the test item in sunflower oil up to a concentration of 200 mg/mL. The preliminary toxicity test using two mice/sex/group showed no mortality up to the highest dose of 2000 mg/kg bw. Males and females in all three dose groups (500, 1000, and 2000 mg/kg bw) showed slight piloerection between 1 and 5 h after treatments. In addition, animals in the 2000 mg/kg bw/day dose groups showed a slight decrease in activity during the same time period. As observations were mild and transient following dose administration and no differences in findings were noted between sexes, only males were utilized in the main test, and the same doses were utilized.

In the main micronucleus test, there was no mortality, and, thus, bone marrow was not prepared from the two additional animals that had been added to the high-dose group to maintain statistical power in case of any death. There were no visible signs of reaction to treatment noted, other than the same findings of slight piloerection and decreased activity that were seen in the preliminary study. The ratio of PCEs to total erythrocytes in the 500 mg/kg bw dose group was similar to that of the concurrent and historical negative controls. However, in the 1000 and 2000 mg/kg bw groups, a statistically significant decrease in this ratio was observed compared to the concurrent and historical negative control groups, demonstrating the expected exposure of the test item to the bone marrow.

The frequencies of MPCEs for the concurrent negative and positive control mice were within acceptable ranges and compatible with the laboratory's historical control data. Concurrent positive controls showed a large, statistically significant increase in MPCEs compared to the concurrent and historical negative controls. Thus, the study was considered valid. Imperial Oil® did not induce significant increases

TABLE 2 Chromosomal abberation results.

	S9 mix	Treatment time	Sampling time	Cytotoxicity	Number of aberrant cells (mean of duplicates/150 cells)		Percent of aberrant cells	Number of aberrations (mean of duplicates/150 cells)		Aberration subtypes (mean of duplicates/150 cells)					
										Chromosome			Chromatid		
					Gap+	Gap−	Gap−	Gap+	Gap−	Gap	Del	Exchange	Gap	Del	Exchange
Short-term experiments															
Test item															
2 μg/mL	−	3 h	20 h	1	8	3	1.667	9	3	2.5	0.5	1.0	3.5	1.0	0.0
4 μg/mL	−	3 h	20 h	17	6	2	1.333	6	2	1.0	0.5	0.0	3.0	1.5	0.0
6 μg/mL	−	3 h	20 h	33	6	3	2.000	7	4	1.0	0.0	0.5	2.0	1.0	2.0
8 μg/mL	−	3 h	20 h	54	7	4	2.333	9	5	0.0	0.0	0.5	4.0	3.0	1.5
Vehicle control	−	3 h	20 h	0	7	3	2.000	7	3	0.5	0.0	1.0	3.5	2.0	0.0
Hist. veh. control	−	3 h	20 h		4–9	2–5									
Positive control	−	3 h	20 h	54	42**	34**	22.667	56**	37**	6.0	6.0	5.0	13.0	13.0	12.5
Test item															
7 μg/mL	+	3 h	20 h	4	9	4	2.667	9	4	2.5	0.5	1.0	2.5	1.5	1.0
12 μg/mL	+	3 h	20 h	17	7	3	1.667	7	3	1.5	1.5	0.0	3.0	1.0	0.0
17 μg/mL	+	3 h	20 h	31	7	3	2.000	7	3	1.5	0.5	1.5	2.0	1.0	0.0
22 μg/mL	+	3 h	20 h	52	8	5	3.000	9	5	2.0	0.5	0.5	2.0	2.0	2.0
Vehicle control	+	3 h	20 h	0	8	4	2.333	8	4	1.5	0.5	0.5	2.5	2.5	0.0
Hist. veh. control	+	3 h	20 h		5–9	2–6									
Positive control	+	3 h	20 h	53	43**	42**	27.667	72**	48**	7.5	7.0	5.5	17.0	13.5	21.5
Test item															
7 μg/mL	+	3 h	28 h	2	7	2	1.333	7	2	0.5	1.0	0.0	4.0	1.0	0.0
12 μg/mL	+	3 h	28 h	14	7	3	1.667	7	3	2.5	0.0	0.0	1.5	1.5	1.0
17 μg/mL	+	3 h	28 h	29	7	4	2.667	7	4	1.5	1.5	1.5	1.5	0.5	0.5
22 μg/mL	+	3 h	28 h	54	10	5	3.000	12	5	3.0	1.5	0.5	4.0	2.0	0.5
Vehicle control	+	3 h	28 h	0	8	4	2.333	8	4	1.0	0.5	1.0	3.5	1.0	1.0
Hist. veh. control	+	3 h	28 h		4–9	2–5									
Positive control	+	3 h	28 h	55	45**	41**	27.333	82**	55**	9.5	5.5	8.5	17.0	14.5	26.5
Long-term experiments															
Test item															
0.5 μg/mL	−	20 h	20 h	3	6	2	1.333	6	2	0.5	0.5	0.0	3.5	0.0	1.5
1 μg/mL	−	20 h	20 h	19	6	3	1.667	7	3	1.5	0.5	0.0	2.5	2.0	0.0
2 μg/mL	−	20 h	20 h	52	11	8	5.000	17*	10*	2.5	2.0	4.0	4.5	2.5	1.0
Vehicle control	−	20 h	20 h	0	8	4	2.333	8	4	0.5	0.5	1.5	4.0	1.5	0.0

TABLE 2 (Continued)

	S9 mix	Treatment time	Sampling time	Cytotoxicity	Number of aberrant cells (mean of duplicates/150 cells)		Percent of aberrant cells	Number of aberrations (mean of duplicates/150 cells)		Aberration subtypes (mean of duplicates/150 cells)					
										Chromosome			Chromatid		
					Gap+	Gap−	Gap−	Gap+	Gap−	Gap	Del	Exchange	Gap	Del	Exchange
Hist. veh. control	–	20 h	20 h		4–9	2–5									
Positive control	–	20 h	20 h	53	42**	36**	24	79**	52**	9.5	6.5	7.5	17.5	14.5	23.5
Test item															
0.5 μg/mL	–	20 h	28 h	1	7	3	1.667	7	3	1.5	0.5	0.5	3.0	1.5	0.0
1 μg/mL	–	20 h	28 h	20	7	3	2.000	7	3	1.5	0.0	3.0	2.0	0.0	0.0
2 μg/mL	–	20 h	28 h	54	12	7	4.667	13	7	1.5	1.0	4.0	4.0	1.0	1.0
Vehicle control	–	20 h	28 h	0	7	3	2.000	7	3	2.5	0.0	0.0	1.0	3.0	0.0
Hist. veh. control	–	20 h	28 h		4–9	2–5									
Positive control	–	20 h	28 h	55	44**	40**	26.333	68**	47**	6.0	6.0	6.0	15.0	13.0	22.0

Abbreviations: –, without S9 mix; +, with S9 mix; del, deletion; gap-, excluding gaps; gap+, including gaps; Hist. veh. control, historical vehicle control (range).
Vehicle control, DMSP; positive control with S9 mix, cyclophosphamide monohydrate; positive control short-term treatment without S9 mix, ethyl methanesulfonate.
**$p < 0.01$ compared to concurrent and historical vehicle controls.

in MPCE frequency compared to the concurrent negative (vehicle) or historical controls, and the values were compatible with the historical control data for this laboratory. The results of the study are summarized in Table 3.

3.4 | 90-day repeated-dose oral toxicity study in rats

There were no mortalities in the 90-day repeated dose study in rats. Animals exhibited generally normal behavior and physical condition during clinical observations throughout the study and during the FOB on day 84. Some salivation and nuzzling of the bedding material were observed in animals after treatment. Increased salivation was noted in the control, low-, mid-, and high-dose groups in 0/10, 3/10, 7/10, and 9/10 male and 0/10, 1/10, 1/10, 7/10 female animals, respectively. Nuzzling of the bedding material was noted in 0/10, 2/10, 3/10, and 2/10 male and 0/10, 1/10, 2/10, and 7/10 female animals, respectively. Both findings ceased after a short duration in all animals.

Mean terminal body weight and overall body weight gain values were slightly decreased in the 125 and 175 mg/kg bw/day male groups (body weight was decreased by 6 and 7%, respectively, compared to control). Transient statistically significantly decreased mean body weight gains were also noted mainly in mid- and high-dose male groups during several days in the study, although values did not follow a dose-dependent trend. Female body weight and body weight gain values in treated groups did not differ from controls at the end of the study. Body weight results are shown in Tables 4 and 5. Slight transient differences in food consumption with respect to controls were noted as decreased in all male treatment groups during week seven, decreased in the female 75 mg/kg bw/day group during week seven, and increased in the female 175 mg/kg bw/day group during weeks four and five (see Table 6). Ophthalmologic examination results were normal in all animals examined and did not differ between the high-dose and controls at the end of the treatment period.

With regard to hematology measurements (see Table 7), male animals showed statistically significantly lower mean percentage of eosinophils (EOS) in the mid-dose group; lower hemoglobin concentration (HGB), lower mean hematocrit (HCT), and shorter mean prothrombin time (PT) in all three treatment groups; and lower mean corpuscular volume (MCV), mean corpuscular hemoglobin content (MCH), and mean corpuscular hemoglobin concentration (MCHC), and shorter mean activated partial thromboplastin time (APTT) in high-dose animals compared to controls. Female animals showed a statistically significantly lower mean percentage of EOS, HGB, MCV, MCH, and MCHC in mid- and high-dose animals compared to controls. All significant hematology and blood coagulation values fell well within the laboratory's historical control ranges for both males and female rats.

There were some clinical chemistry and thyroid parameters in treated groups that showed statistically significant differences

TABLE 3 Results of the in vivo mammalian micronucleus test.

Groups ($n = 5^{\dagger}$)	Sampling time (hours following final treatment)	Total number of PCEs analyzed	MPCE (per 4000 PCE)		PCE/PCE + NCE	
			Mean	± SD	Mean	± SD
Historical control range	24	620,000	3–8	—	0.46–0.57	—
Vehicle control	24	20,000	5.60	1.14	0.52	0.00
500 mg/kg bw	24	20,000	5.20	1.64	0.52	0.01
1000 mg/kg bw	24	20,000	5.60	1.14	0.48$^{\Phi}$U	0.01
2000 mg/kg bw	24	20,000	5.80	0.84	0.46$^{\Phi}$U	0.01
Positive control (60 mg/kg bw)	24	20,000	143.80*/**	6.98	0.37$^{\Phi}$U	0.02

Abbreviations: MPCE, micronucleated polychromatic erythrocytes, NCE, normochromic erythrocytes; PCE, polychromatic erythrocytes.
†Historical Control ($n = 155$).
*$p < 0.05$ compared to concurrent negative control. **p < 0.01 compared to historical control. $^{\Phi}p < 0.01$ compared to concurrent negative and historical control.
U: Mann–Whitney *U*-test versus control.

compared to control values (see Table 8). In males, these included higher mean activity of alanine amino transferase (ALT) in the mid- and high-dose groups, lower mean concentration of bilirubin (TBIL) in the low- and high-dose groups, elevated mean concentrations of urea and blood urea nitrogen (BUN) in the high-dose group, lower mean concentration of calcium (Ca^{2+}) in the low-dose group, and lower mean concentration of total protein (TPROT) in the mid-dose groups. All three male treatment groups showed slight but significantly decreased free thyroxine (FT4) values compared to controls. In females, statistically significant differences in values as compared to controls were elevated urea and BUN in the high-dose group, lower mean concentration of glucose (GLUC) in the mid-dose group, higher mean concentration of low-density lipoprotein (LDL) in all three treatment groups, higher mean concentration of cholesterol (CHOL) and high density lipoprotein (HDL) in the mid- and high-dose groups, and lower mean albumin: globulin ratio (A/G) in the high-dose group. The FT4 value was significantly decreased in high-dose females compared to controls. As with hematology and blood coagulation values, significant clinical chemistry and thyroid values fell well within the laboratory's historical control ranges for both males and females.

Individual findings were noted in a few animals during the macroscopic examination (see Table 9). In male animals, slight renal pelvic dilation was observed in all three treatment groups, although it did not appear to follow a dose-dependent pattern. Brownish-red color of the thymus in one animal of the low- and high-dose groups, and point-like hemorrhages in the thymus were noted in one animal of the high-dose group. In female animals, a hernia diaphragmatica was noted in one animal of the control group, a brownish-red mustard seed-sized hard formation in the ovary was noted in one animal of the low-dose group, a smaller than normal right ovary was seen in one animal of the mid-dose group, a pinprick-sized cyst was seen in an ovary of a single animal in the high-dose group, a brownish-red colored thymus was noted in one animal of the low-dose group, slight dilation of the renal pelvis was observed in one animal of the high-dose group, and dilation of the uterine lumen was seen in all dose groups, with highest levels noted in the control animals.

Absolute and relative organ weight differences between treated and control groups, along with percent differences, are summarized in Tables 10–12. As shown in the tables, both male and female animals had statistically significantly increased liver weights (absolute and relative to body and brain weights) in all dose groups. Values generally fell above the upper limit of the historical control range in the mid- and high-dose groups (except that male absolute liver weights were only outside of historical controls in the high-dose group, and female liver to body weight ratio was outside the historical control range in all three treatment groups). Weights of the testes and epididymides in males were significantly decreased in the high-dose group only (absolute and relative to brain weight); however, the values fell well-within historical control values. Uterine weights in females were decreased in the mid- and high-dose groups (absolute and relative to brain weights) but did not clearly follow a dose-dependent pattern and also fell well within historical control values. Ovary weights were increased compared to controls in the high-dose group only (absolute and relative to body weight) and were not outside historical controls. Adrenal gland weights were increased compared to controls in males, but not females (absolute and relative to body and brain weights), which was only significant in the mid- and high-dose groups, and again fell within historical control ranges except that the significant finding in male adrenal to body weight ratio high-dose animals fell just above the historical control range. Pituitary gland weights were decreased significantly in the high-dose group male and female dose groups (absolute and relative to brain weights in males, as well as relative to body weight in females), and in the mid-dose group in females (absolute and relative to brain weight). Laboratory historical control values have not been fully established for this parameter. Thyroid gland weights were increased in males-only in all three dose groups (absolute and relative to body and brain weights). Weights of the testes, epididymides, and uterus were decreased, and ovary weights were increased statistically significantly compared to controls, but all fit well within the historical control weight ranges.

Histopathological examinations (see Table 13) showed slight dilation of the pelvis of the kidneys in 0, 1, 2, and 1 male animals in the

TABLE 4 Body weight results.

Group mg/kg bw/day		Body weight (g) on days 0	4	7	11	14	18	21	25	28
Male										
0	Mean	235.9	259.0	269.9	286.4	302.0	320.4	330.6	342.5	350.4
($n = 10$)	SD	7.8	8.4	10.3	11.2	11.9	14.4	16.0	15.3	17.2
75	Mean	233.8	256.3	267.7	284.0	299.9	318.0	328.2	339.5	347.4
($n = 10$)	SD	4.9	7.4	8.2	8.4	10.2	11.0	12.3	12.2	12.7
	± %	−1	−1	−1	−1	−1	−1	−1	−1	−1
125	Mean	233.3	254.6	266.1	281.7	295.7	314.8	323.7	332.8	340.3
($n = 10$)	SD	4.9	7.3	8.8	9.2	10.2	11.7	13.0	14.5	14.0
	± %	−1	−2	−1	−2	−2	−2	−2	−3	−3
175	Mean	234.4	254.8	267.9	283.4	296.2	312.8	323.1	331.2	339.3
($n = 10$)	SD	5.2	6.1	5.9	7.8	8.2	11.7	13.3	13.9	15.3
	± %	−1	−2	−1	−1	−2	−2	−2	−3	−3
Test for significance		NS	NS	NS	NS	NS	NS	NS	NS	NS
Historical control range min–max (mean)		176–259 (218.9)	201–284 (241.9)	218–304 (260.4)	243–323 (278.7)	256–342 (295.7)	270–367 (311.0)	276–376 (322.7)	288–393 (336.2)	294–404 (345.9)
Female										
0	Mean	143.3	156.0	160.2	170.4	179.7	188.5	192.5	198.1	199.7
($n = 10$)	SD	7.7	7.8	7.9	8.5	9.9	10.8	12.7	13.5	12.5
75	Mean	143.5	155.4	161.3	172.8	182.8	191.5	195.9	199.2	203.4
($n = 10$)	SD	7.2	8.7	7.1	8.1	8.1	11.2	9.8	11.4	9.3
	± %	0	0	1	1	2	2	2	1	2
125	Mean	140.9	152.3	160.4	171.3	179.8	187.4	191.2	196.4	202.1
($n = 10$)	SD	4.2	6.3	7.7	8.9	8.7	7.8	8.2	10.2	9.9
	± %	−2	−2	0	1	0	−1	−1	−1	1
175	Mean	140.6	151.8	160.8	172.1	183.0	193.5	197.3	201.7	207.1
($n = 10$)	SD	7.1	7.3	6.7	7.7	9.0	10.5	10.8	10.6	11.1
	± %	−2	−3	0	1	2	3	2	2	4
Test for significance		NS	NS	NS	NS	NS	NS	NS	NS	NS
Historical control range min–max (mean)		129–178 (150.2)	142–195 (162.7)	146–202 (171.0)	160–214 (181.4)	164–217 (187.4)	172–229 (194.3)	176–228 (200.1)	180–242 (207.9)	190–247 (210.8)

TABLE 4 (Continued)

Group mg/kg bw/day		Body weight (g) on days								
		35	42	49	56	63	70	77	84	89
0	Mean	371.7	385.8	398.6	412.0	427.1	437.1	448.4	455.3	463.1
($n = 10$)	SD	17.8	19.1	20.5	23.4	24.4	25.8	28.5	29.8	30.9
75	Mean	365.3	381.8	395.7	412.1	423.4	434.3	448.1	455.3	463.6
($n = 10$)	SD	15.3	16.6	17.5	20.7	21.8	22.6	25.1	28.5	29.6
	± %	−2	−1	−1	0	−1	−1	0	0	0
125	Mean	358.2	370.6	381.7	394.8	404.6	413.9	424.4	427.2	433.8
($n = 10$)	SD	17.1	18.9	20.3	22.8	24.0	24.9	26.1	28.0	28.3
	± %	−4	−4	−4	−4	−5	−5	−5	−6	−6*
175	Mean	355.4	369.7	379.2	391.6	401.5	412.0	422.0	427.3	431.9
($n = 10$)	SD	17.7	20.7	21.7	25.6	27.3	29.9	31.2	32.4	32.2
	± %	−4	−4	−5	−5	−6*	−6	−6	−6*	−7*
Test for significance		NS	NS	NS	NS	DN	NS	NS	DN	DN
Historical control range min–max (mean)		304–425 (364.7)	314–444 (380.9)	328–461 (396.8)	338–491 (411.2)	334–508 (422.1)	358–522 (431.3)	369–541 (441.7)	371–562 (449.3)	377–569 (453.2)
Female										
0	Mean	207.1	212.0	218.2	224.4	227.2	231.1	232.9	235.5	238.1
($n = 10$)	SD	12.9	14.5	13.3	15.5	14.1	15.6	16.6	18.8	18.9
75	Mean	209.4	216.0	220.6	224.9	227.4	231.4	234.5	235.3	237.6
($n = 10$)	SD	11.1	9.6	8.5	9.9	9.8	9.4	9.8	9.3	10.5
	± %	1	2	1	0	0	0	1	0	0
125	Mean	207.3	212.8	219.6	219.0	220.7	224.1	227.0	227.6	229.1
($n = 10$)	SD	10.7	9.8	12.5	12.7	13.2	11.3	11.7	13.6	11.5
	± %	0	0	1	−2	−3	−3	−3	−3	−4
175	Mean	214.2	220.1	224.0	226.1	228.1	232.3	233.1	233.3	235.0
($n = 10$)	SD	12.5	14.2	14.4	13.8	15.9	15.5	14.6	15.7	14.4
	± %	3	4	3	1	0	1	0	−1	−1
Test for significance		NS	NS	NS	NS	NS	NS	NS	NS	NS
Historical control range min–max (mean)		196–248 (218.1)	194–252 (225.6)	202–261 (232.2)	204–268 (235.9)	211–273 (241.2)	206–274 (244.4)	215–283 (247.1)	220–287 (251.0)	219–299 (256.3)

Abbreviations: DN = Duncan's multiple range test; NS = not significant; SD = standard deviation.
*$p < 0.05$, and **$p < 0.01$.

TABLE 5 Body weight gain results.

Group mg/kg bw/day		Body weight gain (g) between days													Sum.
		0–7	7–14	14–21	21–28	28–35	35–42	42–49	49–56	56–63	63–70	70–77	77–84	84–89	0–89
Male															
0	Mean	34.0	32.1	28.6	19.8	21.3	14.1	12.8	13.4	15.1	10.0	11.3	6.9	7.8	227.2
(*n* = 10)	SD	4.6	4.4	4.9	2.9	3.6	3.2	3.9	4.8	4.1	2.9	3.7	2.8	2.9	26.8
75	Mean	33.9	11.4	16.3	15.9	17.9	16.5	13.9	16.4	11.3*	10.9	13.8	7.2	8.3	229.8
(*n* = 10)	SD	4.5	2.7	2.7	3.0	3.6	4.0	3.8	3.8	3.6	2.2	4.4	4.5	2.7	25.7
125	Mean	32.8	29.6	28.0	16.6	17.9*	12.4	11.1	13.1	9.8**	9.3	10.5	2.8*	6.6	200.5*
(*n* = 10)	SD	6.0	2.9	4.0	2.7	3.7	2.3	3.0	5.2	1.6	2.9	3.2	4.0	2.8	26.1
175	Mean	33.5	28.3*	26.9	16.2*	16.1**	14.3	9.5*	12.4	9.9**	10.5	10.0	5.3	4.6*	197.5*
(*n* = 10)	SD	5.8	3.7	5.6	4.2	4.0	4.3	2.5	4.8	3.5	3.4	3.9	3.0	4.6	32.4
Test for significance		NS	DN	NS	DN	DN	NS	DN	NS	DN	NS	NS	DN	DN	DN
Historical control range min–max (mean)		21–59 (41.5)	18–61 (35.3)	10–44 (27.1)	10–35 (23.1)	10–27 (18.9)	4–25 (16.2)	3–26 (15.9)	4–30 (14.4)	−7–19 (10.9)	−1–24 (9.2)	3–20 (10.4)	−3–21 (7.6)	−5–12 (3.9)	151–321 (234.3)
Female															
0	Mean	16.9	19.5	12.8	7.2	7.4	4.9	6.2	6.2	2.8	3.9	1.8	2.6	2.6	94.8
(*n* = 10)	SD	4.0	2.6	4.8	4.5	3.2	4.1	3.9	3.7	3.6	3.6	3.4	3.3	2.4	15.0
75	Mean	17.8	21.5	13.1	7.5	6.0	6.6	4.6	4.3	2.5	4.0	3.1	0.8	2.3	94.1
(*n* = 10)	SD	2.9	3.5	3.9	3.1	3.5	2.6	3.6	2.3	2.5	3.7	2.9	4.3	3.0	8.6
125	Mean	19.5	19.4	11.4	10.9	5.2	5.5	6.8	−0.6**	1.7	3.4	2.9	0.6	1.5	88.2
(*n* = 10)	SD	5.5	3.5	3.6	6.7	4.7	3.3	5.1	4.0	3.6	4.1	2.0	4.2	4.8	13.3
175	Mean	20.2	22.2	14.3	9.8	7.1	5.9	3.9	2.1*	2.0	4.2	0.8	0.2	1.7	94.4
(*n* = 10)	SD	3.8	4.7	3.4	3.4	3.3	4.3	3.0	3.5	4.5	4.4	5.2	3.3	4.1	10.1
Test for significance		NS	NS	NS	NS	NS	NS	NS	DN	NS	NS	NS	NS	NS	NS
Historical control range min–max (mean)		6–33 (20.8)	5–29 (16.4)	1–25 (12.8)	−1–24 (10.6)	−3–23 (7.3)	−2–20 (7.6)	−2–14 (6.6)	−3–13 (3.4)	−5–16 (5.6)	−5–13 (3.3)	−4–11 (2.5)	−4–20 (3.9)	−3–14 (5.3)	62–140 (106.1)

Abbreviations: DN = Duncan's multiple range test; NS = not significant; SD = standard deviation.
*$p < 0.05$, and **$p < 0.01$.

TABLE 6 Food intake results.

			Daily mean food consumption (g/animal/day)					
Group mg/kg bw/day		Weeks Days	1 0–7	2 7–14	3 14–21	4 21–28	5 28–35	6 35–42
Male								
0	Mean		17.4	16.6	15.7	15.1	15.0	14.2
	SD		1.02	1.28	1.24	1.55	1.01	1.32
75	Mean		16.9	15.9	14.8	14.9	14.4	13.9
	SD		0.56	0.67	0.76	0.76	0.39	0.58
	± %		−3	−4	−5	−1	−4	−2
125	Mean		16.7	15.8	15.4	14.2	14.0	13.4
	SD		0.46	1.04	1.78	1.45	1.07	1.08
	± %		−4	−5	−2	−6	−7	−6
175	Mean		16.5	15.5	14.1	13.8	13.9	13.7
	SD		0.90	0.78	1.21	1.06	0.81	1.20
	± %		−5	−7	−10	−9	−7	−4
Test for significance			NS	NS	NS	NS	NS	NS
Historical control range min–max (mean)			19.6–28.1 (23.4)	203–29.3 (24.3)	20.1–29.3 (24.2)	19.1–27.3 (23.8)	19.1–27.6 (23.4)	18.7–28.1 (23.1)
Female								
0	Mean		11.4	11.3	10.5	10.2	10.3	9.9
	SD		0.52	0.69	0.91	0.59	0.75	1.11
75	Mean		11.8	11.3	10.7	10.3	10.1	10.1
	SD		0.87	0.47	0.41	0.25	0.43	0.22
	± %		4	0	2	1	−2	3
125	Mean		11.4	11.2	10.4	10.1	10.5	10.1
	SD		1.21	0.75	0.65	0.40	0.41	0.20
	± %		0	−1	−1	−1	2	2
175	Mean		11.0	11.5	11.5	11.2	11.2	10.6
	SD		0.29	0.35	1.08	1.02	0.46	1.00
	± %		−3	1	10	10*	9*	8
Test for significance			NS	NS	NS	DN	DN	NS
Historical control range min–max (mean)			13.9–19.9 (16.6)	13.6–20.1 (16.9)	13.8–19.1 (16.7)	14.1–19.7 (16.6)	13.4–19.1 (16.4)	12.8–20.9 (16.3)

TABLE 6 (Continued)

Group mg/kg bw/day		Daily mean food consumption (g/animal/day)						
		7 42–49	8 49–56	9 56–63	10 63–70	11 70–77	12 77–84	13 84–89
0	Mean	16.4	14.0	14.2	13.3	12.2	11.2	11.1
	SD	1.38	0.99	1.08	1.18	1.42	1.33	1.38
75	Mean	14.5	12.7	13.4	12.5	12.3	11.4	11.8
	SD	0.66	0.95	1.30	1.19	1.48	1.30	0.86
	± %	−12*	−10	−5	−6	1	2	6
125	Mean	13.5	12.2	12.8	11.6	11.3	11.0	11.3
	SD	1.10	1.23	1.14	0.59	0.51	0.30	0.62
	± %	−18**	−13	−10	−12	−7	−2	1
175	Mean	13.5	12.4	13.1	12.3	12.3	12.1	12.2
	SD	1.15	1.56	1.14	1.34	1.43	1.68	1.43
	± %	−18**	−12	−8	−7	1	8	9
Test for significance		DN	NS	NS	NS	NS	NS	NS
Historical control range min–max (mean)		18.7–28.9 (23.4)	18.9–28.7 (23.2)	19.4–27.4 (23.2)	18.7–27.1 (22.7)	19.0–28.3 (22.8)	17.6–27.4 (22.9)	18.8–28.0 (23.6)
Female								
0	Mean	11.4	10.1	9.8	9.5	8.8	8.7	8.9
	SD	0.83	0.98	0.78	0.70	0.70	0.63	1.03
75	Mean	10.2	8.9	9.9	9.2	8.8	8.5	8.6
	SD	0.11	0.44	0.94	0.49	0.41	0.43	0.22
	± %	−10**	−12	1	−3	1	−3	−4
125	Mean	11.0	9.0	10.5	9.6	9.3	9.4	9.5
	SD	1.43	0.89	0.85	0.67	0.86	0.65	0.76
	± %	−3	−11	7	0	6	7	6
175	Mean	11.2	9.9	10.2	10.3	9.6	9.6	10.0
	SD	1.02	1.52	0.92	1.16	0.72	1.29	1.23
	± %	−2	−2	4	8	10	10	12
Test for significance		U	NS	NS	NS	NS	NS	NS
Historical control range min–max (mean)		12.5–20.9 (16.6)	12.5–19.4 (16.4)	12.3–21.0 (16.5)	12.0–20.0 (16.1)	12.8–20.0 (16.1)	12.4–21.6 (16.3)	13.6–22.0 (17.1)

Abbreviations: DN = Duncan's multiple range test; NS = not significant; SD = standard deviation; U = Mann–Whitney *U*-test versus control.
*$p < 0.05$, and **$p < 0.01$.

TABLE 7 Hematology and blood coagulation results.

Group mg/kg bw/day		WBC ($\times 10^9$/L)	NEU (%)	LYM (%)_	MONO (%)	EOS (%)	BASO (%)	RBC ($\times 10^{12}$/L)	HGB (g/L)
Male									
0	Mean	6.24	18.21	78.42	1.81	1.24	0.08	8.98	163.70
(*n* = 10)	SD	0.82	4.71	4.92	0.71	0.37	0.04	0.36	2.95
75	Mean	5.43	26.16	70.08	2.29	1.12	0.05	8.80	157.00**
(*n* = 10)	SD	1.93	16.73	17.85	0.94	0.59	0.05	0.40	5.31
125	Mean	6.08	18.81	77.80	2.16	0.72**	0.04	8.65	153.20**
(*n* = 10)	SD	0.98	5.74	5.87	0.78	0.21	0.05	0.46	3.82
175	Mean	5.83	23.78	72.21	2.33	1.30	0.04	8.64	147.60**
(*n* = 10)	SD	1.57	11.55	12.47	0.67	1.13	0.05	0.35	4.03
Test for significance		NS	NS	NS	NS	U	NS	NS	U
Historical control range min–max (mean)		4.3–10.6 (7.2)	10.3–35.7 (18.7)	60.1–86.5 (76.9)	1.2–6.7 (2.4)	0.7–2.8 (1.5)	0.0–0.2 (0.1)	8.1–9.9 (9.1)	145–183 (163.5)
Female									
0	Mean	4.01	24.20	71.82	1.83	1.84	0.07	7.97	151.60
(*n* = 10)	SD	0.91	19.92	20.49	0.43	1.16	0.05	0.13	3.98
75	Mean	3.60	20.55	75.47	2.42	1.17	0.05	8.03	149.50
(*n* = 10)	SD	1.33	11.87	12.71	0.91	0.33	0.05	0.15	3.63
125	Mean	4.30	19.19	77.31	2.05	0.96*	0.05	8.09	143.90**
(*n* = 10)	SD	0.82	6.05	6.18	0.80	0.30	0.05	0.23	3.18
175	Mean	4.75	14.71	81.48	2.15	1.03*	0.04	8.04	143.20**
(*n* = 10)	SD	0.69	4.13	4.27	0.31	0.29	0.05	0.37	4.39
Test for significance		NS	NS	NS	NS	U	NS	NS	DN
Historical control range min–max (mean)		1.5–9.2 (4.6)	7.6–50.2 (18.1)	43.7–88.7 (77.4)	1.0–5.0 (2.2)	0.8–3.7 (1.8)	0.0–0.2 (0.1)	7.4–10.1 (8.3)	141–175 (154.8)

TABLE 7 (Continued)

Group mg/kg bw/day		HCT (L/L)	MCV (fL)	MCH (pg)	MCHC (g/L)	PLT ($\times 10^9$/L)	RET (%)	PT (sec)	APTT (sec)
Male									
0	Mean	0.47	52.90	18.26	345.20	750.00	2.08	10.28	11.48
(*n* = 10)	SD	0.01	1.68	0.83	6.96	87.36	0.40	0.19	1.02
75	Mean	0.46*	52.12	17.88	342.80	784.70	2.05	10.04**	11.19
(*n* = 10)	SD	0.02	0.87	0.55	7.07	45.58	0.26	0.14	0.81
125	Mean	0.45**	51.96	17.76	341.70	744.90	1.98	10.11*	11.08
(*n* = 10)	SD	0.01	2.14	1.09	7.62	78.92	0.23	0.13	0.63
175	Mean	0.44**	50.98*	17.12**	335.80**	740.20	2.01	10.05**	10.42**
(*n* = 10)	SD	0.01	1.45	0.57	4.49	66.29	0.20	0.21	0.70
Test for significance		DN	DN	DN	DN	NS	NS	DN	DN
Historical control range min–max (mean)		0.4–0.5 (0.5)	49.9–60.2 (52.5)	16.3–20.7 (17.9)	315–361 (341)	339–1,062 (817.5)	1.2–2.3 (1.8)	10.0–11.2 (10.5)	10.2–15.6 (13.1)
Female									
0	Mean	0.43	53.65	19.03	354.80	868.80	2.62	9.86	11.94
(*n* = 10)	SD	0.01	1.03	0.42	8.47	157.29	0.61	0.24	1.34
75	Mean	0.43	53.21	18.64	350.40	847.90	2.11	9.99	12.77
(*n* = 10)	SD	0.01	1.05	0.44	3.50	67.60	0.42	0.16	1.02
125	Mean	0.42	51.92**	17.78**	342.70**	825.40	2.17	9.78	12.66
(*n* = 10)	SD	0.01	1.21	0.44	3.89	123.68	0.59	0.19	1.19
175	Mean	0.42	52.04**	17.83**	342.60**	808.10	2.38	9.84	11.93
(*n* = 10)	SD	0.01	1.43	0.65	6.06	114.75	0.42	0.15	0.81
Test for significance		NS	DN	DN	U	NS	NS	NS	NS
Historical control range min–max (mean)		0.4–0.5 (0.5)	50.1–57.7 (54.5)	17.2–20.1 (18.6)	319–365 (341.1)	450–1,127 (812.9)	1.2–3.6 (2.2)	9.5–10.5 (9.9)	11.1–16.1 (13.5)

Abbreviations: BASO = DN = Duncan's multiple range test; EOS = eosinophil; HCT = hematocrit; HGB = hemoglobin; LYM = lymphocyte; MCH = mean corpuscular hemoglobin; MCHC = mean corpuscular hemoglobin concentration; MCV = mean corpuscular volume; MONO = monocyte; NEU = neutrophil; NS = not significant; PLT = platelet; RBC = red blood cell; RET = reticulocytes; U = Mann–Whitney *U*-test versus control; WBC = white blood cell.

*$p < 0.05$, and **$p < 0.01$.

TABLE 8 Clinical chemistry and thyroid results.

Group Mg/kg bw/day		ALT (U/L)	AST (U/L)	ALP (U/L)	TBIL (µmol/L)	CREA (µmol/L)	UREA (mmol/L)	BUN (mg/dL)	GLUC (mmol/L)	CHOL (mmol/L)	HDL (mmol/L)	LDL (mmol/L)
Male												
0	Mean	51.30	95.70	134.10	2.85	29.50	3.64	10.19	6.78	1.86	1.34	0.25
(*n* = 10)	SD	8.34	18.18	38.72	0.51	3.95	0.48	1.33	0.43	0.23	0.21	0.05
75	Mean	52.00	99.00	138.90	2.26**	29.10	3.62	10.14	6.66	1.77	1.19	0.24
(*n* = 10)	SD	8.71	27.42	38.22	0.42	2.88	0.42	1.16	0.26	0.25	0.18	0.07
125	Mean	66.90**	88.90	158.40	2.53	28.90	4.07	11.40	6.73	1.84	1.19	0.27
(*n* = 10)	SD	13.44	6.52	59.17	0.34	2.33	0.40	1.11	0.49	0.21	0.14	0.08
175	Mean	66.00**	98.10	173.80	2.39*	31.20	4.45**	12.46**	7.00	1.82	1.29	0.19
(*n* = 10)	SD	12.30	20.14	48.30	0.43	3.71	0.83	2.32	0.48	0.16	0.12	0.07
Test for significance		DN	NS	NS	DN	NS	DN	DN	NS	NS	NS	NS
Historical control range min–max (mean)		26.0–70.0 (42.5)	65.0–131.0 (87.0)	62.0–209.0 (98.8)	0.4–2.5 (1.3)	20.0–35.0 (27.3)	3.3–8.9 (6.6)	NE	4.7–9.2 (6.2)	1.4–3.1 (2.3)	NE	NE
Female												
0	Mean	44.20	85.30	76.00	2.35	29.00	4.53	12.68	6.46	1.92	1.67	0.13
(*n* = 10)	SD	9.45	20.56	47.79	0.29	3.56	0.98	2.73	0.60	0.49	0.45	0.04
75	Mean	40.90	69.40	56.40	2.41	28.80	4.46	12.49	6.22	2.23	1.94	0.18*
(*n* = 10)	SD	8.39	11.28	18.17	0.24	2.25	0.59	1.65	0.61	0.32	0.26	0.04
125	Mean	49.50	92.30	77.90	2.57	28.00	4.25	11.90	5.71**	2.49**	2.15**	0.19*
(*n* = 10)	SD	7.74	30.92	40.07	0.36	4.37	0.63	1.77	0.57	0.48	0.41	0.05
175	Mean	52.50	83.50	54.60	2.34	30.20	5.50*	15.40*	6.00	2.68**	2.25**	0.24**
(*n* = 10)	SD	9.48	21.89	15.43	0.29	4.57	1.25	3.49	0.49	0.42	0.37	0.06
Test for significance		NS	NS	NS	NS	NS	DN	DN	DN	DN	DN	DN
Historical control range min–max (mean)		28.0–133.0 (54.6)	66.0–249.0 (104.2)	22.0–162.0 (45.2)	0.5–3.6 (1.7)	24.0–40.0 (30.9)	3.8–9.5 (6.3)	NE	4.0–7.3 (5.5)	1.1–2.8 (2.0)	NE	NE

TABLE 8 (Continued)

Group Mg/kg bw/day		Pi (mmol/L)	Ca^{++} (mmol/L)	Na^{+} (mmol/L)	K^{+} (mmol/L)	Cl^{-} (mmol/L)	ALB (g/L]	TPROT (g/L]	A/G	FT3 (ng/dL)	FT4 (ng/dL)	TSH (ng/mL)
0	Mean	2.15	2.76	144.81	4.65	102.34	46.30	66.00	2.39	0.32	2.08	6.87
(*n* = 10)	SD	0.12	0.08	2.07	0.39	1.49	1.73	2.04	0.33	0.03	0.21	1.43
75	Mean	2.02	2.66**	144.98	4.50	101.66	44.65	64.71	2.24	0.32	1.85*	8.19
(*n* = 10)	SD	0.12	0.06	0.74	0.19	0.92	2.03	3.42	0.24	0.04	0.23	1.97
125	Mean	2.09	2.71	145.03	4.58	102.40	44.68	63.33*	2.40	0.31	1.58**	7.58
(*n* = 10)	SD	0.18	0.06	0.94	0.19	1.06	1.37	1.65	0.16	0.05	0.27	4.00
175	Mean	2.05	2.74	144.44	4.46	102.44	46.37	67.20	2.25	0.31	1.56**	7.68
(*n* = 10)	SD	0.17	0.06	1.21	0.41	1.23	2.23	3.23	0.22	0.05	0.17	2.74
Test for significance		NS	DN	NS	NS	NS	NS	DN	NS	NS	DN	NS
Historical control range min–max (mean)		1.5–2.3 (1.9)	2.4–2.9 (2.9)	141.2–148.4 (144.1)	4.1–5.2 (4.5)	96.8–103.2 (99.4)	40.1–47.3 (43.8)	59.9–70.1 (64.9)	1.5–2.6 (2.1)	0.192–0.498 (0.280)	1.28–2.94 (2.19)	4.37–26.65 (10.03)
Female												
0	Mean	1.73	2.69	142.63	4.09	101.42	53.27	70.02	3.23	0.36	1.75	7.89
(*n* = 10)	SD	0.26	0.06	0.95	0.33	1.35	2.60	3.58	0.49	0.05	0.22	4.55
75	Mean	1.75	2.70	141.89	3.89	100.77	53.38	70.46	3.18	0.33	1.66	7.48
(*n* = 10)	SD	0.13	0.07	1.12	0.30	0.80	2.89	4.03	0.41	0.05	0.18	3.05
125	Mean	1.83	2.70	142.59	4.14	101.21	54.47	72.37	3.06	0.35	1.60	7.71
(*n* = 10)	SD	0.15	0.07	1.15	0.35	1.26	3.38	4.71	0.28	0.04	0.26	2.99
175	Mean	1.77	2.72	142.77	4.13	102.26	52.77	71.92	2.76*	0.33	1.48*	7.63
(*n* = 10)	SD	0.21	0.06	1.97	0.25	2.18	2.40	2.55	0.29	0.05	0.24	4.29
Test for significance		NS	NS	NS	NS	NS	NS	NS	DN	NS	DN	NS
Historical control range min–max (mean)		0.8–2.1 (1.5)	2.4–2.9 (2.6)	140.9–146.5 (143.7)	3.1–4.6 (4.1)	97.6–105.0 (100.6)	43.8–57.6 (49.6)	56.5–78.9 (68.4)	1.7–3.7 (2.7)	0.235–0.482 (0.320)	0.87–2.40 (1.54)	2.46–25.99 (6.85)

Notes: A/G, albumin to globulin ratio; ALB, albumin; ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BUN, blood urea nitrogen; Ca^{++}, calcium; CHOL, cholesterol; Cl^{-}, chloride; CREA, creatinine; DN, Duncan's multiple range test; FT3, free three-iodothyronine; FT4, free tetra-iodothyronine; GLUC, glucose; HDL, high-density lipoprotein; K^{+}, potassium; LDL, low-density lipoprotein; Na^{+}, sodium; NE, laboratory historical control data not yet established—new parameter in accordance with OECD 408 (25 June 2008); NS, Not Significant; Pi, inorganic phosphorous; SD, standard deviation; SS, statistically significant compared to control; TBIL, total bilirubin; TPROT, total protein.

*$p < 0.05$, and **$p < 0.01$.

TABLE 9 Necropsy findings.

Organs	Observations	Frequency of observations[a] 0 mg/kg bw/day	75 mg/kg bw/day	125 mg/kg bw/day	175 mg/kg bw/day
Male					
Kidneys	No macroscopic findings	10/10	8/10	8/10	7/10
	Pelvis, dilation, slight	0/10	**1/10**	**2/10**	**1/10**
Thymus	Brownish-reddish colored	0/10	**1/10**	0/10	**1/10**
	Point-like hemorrhages	0/10	0/10	0/10	**1/10**
Female					
Uterus	No macroscopic findings	4/10	7/10	8/10	7/10
	Dilation	**6/10**	**2/10**	**1/10**	**2/10**
Diaphragm	Hernia – including pea-sized liver	**1/10**	0/10	0/10	0/10
Ovaries	Brownish-reddish hard formation	0/10	**1/10**	0/10	0/10
	Smaller than normal	0/10	0/10	**1/10**	0/10
	Cyst	0/10	0/10	0/10	**1/10**
Kidneys	Pelvis, dilation, slight	0/10	0/10	0/10	**1/10**
Thymus	Brownish-reddish colored	0/10	**1/10**	0/10	0/10

[a]Number of animals with observations/number of animals examined.

control, low-, mid-, and high-dose groups, respectively. This finding was also noted in one female animal in the high-dose group. Some male animals also showed minimal to moderate centrilobular hepatocellular vacuolation, including in 4, 6, 6, and 10 animals of the same respective groups as stated above. In the control male animals, the vacuolation was rated as minimal in all four animals. In low-dose animals, three were scored as minimal, two as mild, and one as moderate. In mid-dose animals, one was scored as minimal, four as mild, and one as moderate. In high-dose animals, three were scored minimal, two as mild, and five as moderate. Female rats did not display this finding in any dose group. Oil-Red-O and Pas staining of male livers was performed because of the positive histopathological findings in controls and treated animals. Oil-Red-O revealed no positive lipid findings in any animal, whereas Pas positive material (glycogen) was noted in all male livers in the control and treatment groups. One male and female rat in the low-dose groups and two male animals of the high-dose group had noted congestion of the thymus gland. One female high-dose group animal also had a noted ovarian cyst. Dilation of the uterus was noted in 6/10 animals of the control group and 2/10 animals in the high-dose group.

4 | DISCUSSION

A *C. sativa* ethanolic extract containing approximately 85% CBD (Imperial Oil®) was the test item in the current studies. The extract was assessed for in vitro and in vivo genotoxicity as well as for any toxicity from repeated dosing in rats over a period of 90 days. The bacterial reverse mutation test, which has the ability to detect point mutations induced by test items causing base changes or frameshift mutations in the genome of specific amino-acid requiring bacterial strains, showed no induction of gene mutations under the conditions of the study. In the in vitro chromosomal aberration study (which assesses the potential of a test item to produce breaks in one or both DNA strands that can result in chromosomal fragments), a 3-h treatment with the test item in both the absence and presence of metabolic activation did not induce structural chromosomal aberrations in V79 cells up to the cytotoxic concentrations after a 20-h sampling time. However, a 3-h treatment time with a 28-h sampling time, and 20-h treatments with 20- and 28-h sampling times using Imperial Oil® resulted in increased number of cells containing structural chromosome aberrations at the highest concentrations, which were not statistically significant compared to the concurrent negative control. The absolute number of aberrations were also increased in the same experiments, although the values were only statistically significant compared to controls at the 20 h and not the 28-h sampling time point. It should be noted that the cell is the experimental unit of this study per OECD 473 guidance; thus, it is the number/% of cells with aberrations and not the absolute number of aberrations that should be evaluated.

The mouse micronucleus study, which is performed to determine if a test item causes genotoxic effects in vivo via damage to chromosomes or the mitotic apparatus of erythroblasts, revealed no statistically or biologically significant increases in MPCE frequency compared to negative or laboratory historical controls. This was true up to the dose of 2000 mg/kg bw (the highest dose tested in the study). The extract was considered non-genotoxic under the conditions of the in vivo test.

Other genotoxicity studies of hemp extracts containing CBD (as well as no significant THC) found in the literature have not

TABLE 10 Organ weights.

Group mg/kg bw/day		Organ weight (g) Body weight	Brain	Liver	Kidneys	Heart	Thymus	Spleen	Testes	Epididymides	eminal vesiclesprostate	Adrenals	Pituitary	Thyroids
Male														
0	Mean	456.6	2.15	10.72	2.23	1.09	0.39	0.62	3.67	1.79	2.77	0.062	0.012	0.018
($n = 10$)	SD	30.71	0.08	1.09	0.27	0.09	0.06	0.06	0.33	0.23	0.37	0.009	0.003	0.002
75	Mean	456.3	2.12	13.68	2.32	1.05	0.41	0.57	3.64	1.74	2.86	0.069	0.011	0.021
($n = 10$)	SD	28.01	0.07	0.90	0.16	0.10	0.10	0.05	0.23	0.14	0.37	0.012	0.002	0.002
	± %	0	−1	28**	4	−4	5	−8	−1	−3	3	11	−9	19**
125	Mean	425.4	2.14	14.31	2.22	1.04	0.36	0.58	3.63	1.67	2.63	0.076	0.010	0.022
($n = 10$)	SD	28.36	0.09	1.40	0.12	0.10	0.05	0.05	0.22	0.13	0.27	0.010	0.002	0.002
	± %	−7*	0	33**	−1	−5	−8	−6	−1	−7	−5	21*	−13	27**
175	Mean	423.7	2.15	15.33	2.16	1.06	0.35	0.58	3.39	1.56	2.48	0.091	0.009	0.020
($n = 10$)	SD	31.28	0.06	1.74	0.21	0.08	0.07	0.09	0.22	0.12	0.34	0.013	0.002	0.003
	± %	−7*	0	43**	−3	−2	−11	−5	−8*	−13**	−11	46**	−21*	13*
Test for significance		DN	NS	DN	NS	NS	NS	NS	DN	DN	NS	DN	DN	DN
Historical control range min–max (mean)		363–548 (439.3)	2.00–2.35 (2.16)	8.20–14.6 (10.86)	1.95–3.19 (2.38)	0.93–1.37 (1.08)	0.25–0.59 (0.40)	0.51–0.93 (0.69)	2.99–4.43 (3.60)	1.20–1.91 (1.50)	1.45–3.25 (2.38)	0.041–0.091 (0.066)	NE	NE

Group mg/kg bw/day		Body weight	Brain	Liver	Kidneys	Heart	Thymus	Spleen	Uterus	Ovaries	—	Adrenals	Pituitary	Thyroids
Female														
0	Mean	235.7	1.95	6.78	1.42	0.78	0.31	0.44	0.94	0.082	—	0.077	0.014	0.016
($n = 10$)	SD	18.88	0.06	0.65	0.11	0.06	0.04	0.06	0.16	0.014	—	0.009	0.003	0.004
75	Mean	234.5	1.98	7.89	1.36	0.74	0.29	0.40	0.89	0.082	—	0.078	0.014	0.017
($n = 10$)	SD	11.21	0.06	0.47	0.07	0.03	0.04	0.05	0.15	0.018	—	0.011	0.002	0.003
	± %	−1	1	16**	−5	−4	−4	−9	−5	−1		1	−1	6
125	Mean	224.7	1.95	9.01	1.47	0.74	0.30	0.42	0.78	0.092	—	0.085	0.012	0.016
($n = 10$)	SD	11.43	0.07	0.49	0.10	0.05	0.04	0.06	0.12	0.021	—	0.011	0.001	0.002
	± %	−5	0	33**	3	−4	−2	−5	−17*	12		10	−14*	3
175	Mean	231.4	1.96	10.20	1.46	0.76	0.29	0.41	0.79	0.102	—	0.086	0.012	0.016
($n = 10$)	SD	15.32	0.08	0.62	0.15	0.08	0.06	0.07	0.12	0.016	—	0.012	0.001	0.004
	± %	−2	0	50**	3	−1	−7	−9	−16*	24*		12	−15*	3
Test for significance		NS	NS	DN	NS	NS	NS	NS	DN	DN	—	NS	DN	NS
Historical control range min–max (mean)		208–297 (243.8)	1.83–2.17 (1.99)	5.18–8.53 (6.30)	1.36–2.34 (1.52)	0.63–0.85 (0.71)	0.18–0.47 (0.32)	0.32–0.56 (0.46)	0.42–1.11 (0.71)	0.07–0.14 (0.10)	—	0.063–0.104 (0.081)	NE	NE

Abbreviations: DN = Duncan's multiple range test; NE = not yet established; NS = not significant.
*$p < 0.05$, and **$p < 0.01$.

TABLE 11 Organ weights relative to body weight.

Group mg/kg bw/day		Organ weight relative to body weight (%)											
		Brain	Liver	Kidneys	Heart	Thymus	Spleen	Testes	Epididymides	Seminal Vesicles prostate	Adrenals	Pituitary	Thyroids
Male													
0	Mean	0.471	2.347	0.488	0.238	0.085	0.136	0.804	0.392	0.606	0.0137	0.0025	0.0038
($n = 10$)	SD	0.022	0.148	0.043	0.014	0.014	0.016	0.061	0.042	0.068	0.0018	0.0007	0.0004
75	Mean	0.467	2.998	0.508	0.229	0.089	0.124	0.800	0.382	0.629	0.0151	0.0023	0.0046
($n = 10$)	SD	0.029	0.074	0.034	0.015	0.018	0.009	0.059	0.040	0.094	0.0022	0.0003	0.0005
	± %	−1	28**	4	−4	5	−9	−1	−3	4	11	−9	19**
125	Mean	0.506	3.363	0.522	0.244	0.084	0.137	0.853	0.393	0.619	0.0178	0.0024	0.0052
($n = 10$)	SD	0.038	0.203	0.027	0.024	0.011	0.015	0.035	0.030	0.058	0.0020	0.0005	0.0005
	± %	7*	43**	7	3	−1	1	6	0	2	30**	−7	36**
175	Mean	0.509	3.612	0.509	0.251	0.082	0.138	0.805	0.370	0.586	0.0214	0.0021	0.0047
($n = 10$)	SD	0.034	0.210	0.036	0.010	0.019	0.016	0.088	0.026	0.077	0.0019	0.0003	0.0006
	± %	8*	54**	4	5	−3	1	0	−6	−3	57**	−15	22**
Test for significance		DN	U	NS	NS	NS	NS	NS	NS	NS	DN	NS	DN
Historical control range min–max (mean)		0.403–0.606 (0.496)	2.055–3.156 (2.470)	0.452–0.634 (0.542)	0.211–0.284 (0.247)	0.063–0.129 (0.090)	0.119–0.194 (0.157)	0.642–0.963 (0.823)	0.279–0.424 (0.343)	0.360–0.716 (0.530)	0.009–0.020 (0.015)	NE	NE

Group mg/kg bw/day		Brain	Liver	Kidneys	Heart	Thymus	Spleen	Uterus	Ovaries	—	Adrenals	Pituitary	Thyroids
Female													
0	Mean	0.832	2.876	0.606	0.329	0.129	0.188	0.401	0.0351	—	0.0330	0.0059	0.0066
($n = 10$)	SD	0.062	0.168	0.045	0.016	0.014	0.015	0.070	0.0062	—	0.0051	0.0010	0.0013
75	Mean	0.845	3.369	0.580	0.317	0.125	0.172	0.382	0.0349	—	0.0332	0.0059	0.0070
($n = 10$)	SD	0.046	0.195	0.039	0.016	0.015	0.015	0.070	0.0082	—	0.0041	0.0007	0.0010
	± %	2	17**	−4	−4	−3	−9	−5	−1		1	0	7
125	Mean	0.870	4.019	0.656	0.331	0.134	0.188	0.348	0.0409	—	0.0378	0.0053	0.0071
($n = 10$)	SD	0.055	0.305	0.062	0.025	0.015	0.031	0.061	0.0090	—	0.0047	0.0005	0.0009
	± %	5	40**	8*	0	3	0	−13	16		15	−10	9
175	Mean	0.848	4.412	0.631	0.331	0.123	0.174	0.342	0.0444	—	0.0374	0.0051	0.0069
($n = 10$)	SD	0.067	0.223	0.029	0.038	0.022	0.022	0.056	0.0076	—	0.0055	0.0006	0.0016
	± %	2	53**	4	1	−5	−7	−15	26*		13	−13*	5
Test for significance		NS	DN	DN	NS	NS	NS	NS	DN	—	NS	DN	NS
Historical control range min–max (mean)		0.731–1.000 (0.818)	2.183–3.189 (2.580)	0.508–0.951 (0.626)	0.236–0.333 (2.36)	0.078–0.169 (0.130)	0.139–0.227 (0.187)	0.161–0.465 (0.295)	0.029–0.054 (0.040)	—	0.025–0.045 (0.033)	NE	NE

Abbreviations: DN = Duncan's multiple range test; NE = not yet established; NS = not significant; U = Mann–Whitney *U*-test versus control.

*$p < 0.05$, and **$p < 0.01$.

TABLE 12 Organ weight relative to brain weight.

		Organ weight and body weight relative to brain weight (%)											
Group mg/kg bw/day		Body weight	Liver	Kidneys	Heart	Thymus	Spleen	Testes	Epididymides	Seminal vesicles prostate	Adrenals	Pituitary	Thyroids
Male													
0	Mean	21,268.3	499.41	103.95	50.66	18.01	28.80	170.97	83.37	128.81	2.91	0.54	0.81
($n = 10$)	SD	1007.36	43.39	12.29	3.79	3.04	3.18	15.21	10.19	15.40	0.42	0.14	0.09
75	Mean	21,503.1	644.74	109.03	49.30	19.23	26.68	171.51	81.86	134.67	3.25	0.49	0.98
($n = 10$)	SD	1317.57	44.77	6.35	4.05	4.73	2.68	10.44	7.05	17.37	0.53	0.07	0.09
	± %	1	29**	5	−3	7	−7	0	−2	5	12	−8	20**
125	Mean	19,871.8	668.98	103.52	48.43	16.70	27.13	169.43	78.05	122.87	3.54	0.47	1.04
($n = 10$)	SD	1526.57	72.72	7.17	4.43	2.66	1.99	12.80	7.07	13.71	0.47	0.08	0.10
	± %	−7*	34**	0	−4	−7	−6	−1	−6	−5	21*	−13	27**
175	Mean	19,734.6	714.06	100.36	49.40	16.07	27.17	157.94	72.82	115.07	4.24	0.42	0.92
($n = 10$)	SD	1340.52	77.45	8.03	3.22	3.08	3.88	9.20	4.88	13.16	0.56	0.06	0.11
	± %	−7*	43**	−3	−2	−11	−6	−8*	−13**	−11	46**	−21*	13*
Test for significance		DN	DN	NS	NS	NS	NS	DN	DN	NS	DN	DN	DN
Historical control range min–max (mean)		16,500–24,796 (20,326)	375.45–660.63 (501.92)	88.64–144.34 (109.91)	42.73–61.99 (50.06)	11.31–26.29 (18.34)	22.47–41.15 (31.91)	146.64–196.02 (166.35)	55.16–85.65 (69.42)	65.47–154.76 (109.75)	1.95–4.27 (3.07)	NE	NE

Group mg/kg bw/day		Body weight	Liver	Kidneys	Heart	Thymus	Spleen	Uterus	Ovaries	—	Adrenals	Pituitary	Thyroids
Female													
0	Mean	12,073.9	346.85	72.92	39.68	15.63	22.74	48.20	4.23	—	3.95	0.71	0.79
($n = 10$)	SD	863.04	27.69	4.42	2.60	2.17	2.66	7.97	0.73	—	0.48	0.11	0.17
75	Mean	11,862.0	399.19	68.72	37.54	14.85	20.43	45.13	4.13	—	3.95	0.70	0.83
($n = 10$)	SD	607.12	24.03	4.06	1.60	1.86	2.27	7.80	0.93	—	0.58	0.10	0.14
	± %	−2	15**	−6	−5	−5	−10	−6	−2		0	−2	6
125	Mean	11,529.9	462.55	75.25	38.02	15.40	21.66	40.07	4.74	—	4.34	0.61	0.82
($n = 10$)	SD	701.41	33.70	3.49	1.72	1.99	3.38	6.91	1.15	—	0.54	0.05	0.09
	± %	−5	33**	3	−4	−1	−5	−17*	12		10	−14*	4
175	Mean	11,852.9	521.67	74.86	39.12	14.60	20.70	40.28	5.24	—	4.42	0.60	0.81
($n = 10$)	SD	884.51	26.70	7.72	4.22	3.07	3.40	5.86	0.84	—	0.57	0.07	0.19
	± %	−2	50**	3	−1	−7	−9	−16*	24*		12	−15*	3
Test for significance		NS	DN	NS	NS	NS	NS	DN	DN	—	NS	DN	NS
Historical control range Min–max (mean)		10,000–13,686 (12,274)	263.82–408.74 (316.83)	66.67–125.81 (76.71)	30.29–44.09 (35.72)	8.96–21.86 (15.91)	17.11–28.87 (22.95)	20.85–51.63 (35.94)	3.49–7.00 (4.89)	—	2.99–5.56 (4.06)	NE	NE

Abbreviations: DN = Duncan's multiple range test; NE = not yet established; NS = not significant.
*$p < 0.05$, and **$p < 0.01$.

TABLE 13 Histopathology.

Organs[a]	Observations	Incidence of observations per group[b] 0 mg/kg bw/day	75 mg/kg bw/day	125 mg/kg bw/day	175 mg/kg bw/day
Male					
Kidneys	Pelvis, dilation, slight	0/10	1/1	2/2	1/10
Liver	Vacuolation of hepatocytes[c]	4/10	6/10	6/10	10/10
	Oil-red-o staining	0/10	0/10	0/10	0/10
	Pas positive material	10/10	10/10	10/10	10/10
Thymus	Congestion	0/10	1/1	–	2/10
Female					
Kidneys	Pelvis, dilation, slight	0/10	–	–	1/10
Liver	Focal fibrosis in Glisson's capsule	1/10	–	–	0/10
Ovaries	Cyst	0/10	0/1	0/1	1/10
	Atrophy of hair follicles	1/10	–	–	0/10
Skin	Subacute dermatitis	1/10	–	–	0/10
Thymus	Congestion	0/10	1/1	–	0/10
Uterus	Dilation	6/10	–	–	2/10

[a]Organs examined with no lesions in 10/10 animals in control and high-dose groups, or in mid-dose animals histopathologically studied because of a gross lesion are not shown: adrenals glands, aorta, bone marrow, brain, cecum, colon, duodenum, eyes + optic nerve, epididymides, esophagus, harderian glands, heart, ilium, jejunum, lachrymal glands, lungs, mammary gland, mesenteric lymph nodes, quadriceps muscle, pancreas, pituitary, prostate, rectum, salivary glands, sciatic nerve, seminal vesicle, spinal cord, spleen, stomach, submandibular lymph nodes, thyroid + parathyroid, testes, trachea, urinary bladder, and vagina.
[b]Number of animals with observation/number of animals examined.
[c]Severity scoring: control, minimal; 75 mg/kg, minimal-mild; 125 mg/kg, mild; 175 mg/kg, mild–moderate.
– means no data (not examined).

suggested genotoxic effects. Marx et al. (2018) performed the same three genotoxicity tests on a 25% CBD hemp extract. The extract did not induce an increase in cells with chromosomal aberrations, including at concentrations up to 5 μg/mL (1.25 μg/mL CBD) without metabolic activation for 20 h of treatment and sampling times of 20 and 28 h. This is compared to the current study in which non-statistically significant but biologically relevant increase in cells with aberration was noted under the same study conditions at a concentration of 2 μg/mL (1.7 μg/mL CBD). Additionally, no genotoxicity findings occurred in bacterial reverse mutation or mouse micronucleus tests in the Marx et al. assessment. Dziwenka et al. (2021) performed a bacterial reverse mutation test and a mouse micronucleus test on their 25% CBD hemp extract, both of which showed no indication of genotoxicity. A chromosomal aberration study was not published on that particular test item. There is also some evidence in the literature of the chemoprotective effects of pure CBD (Aviello et al., 2012). Overall, the fact that the current in vitro chromosomal aberration study showed a positive result after the longer treatment period suggests potential clastogenicity, but the fact that this was only seen without metabolic activation and was not statistically significantly different than the concurrent negative control, no mutagenicity was noted in the bacterial reverse mutation test or genotoxicity in the biological in vivo system (mouse micronucleus assay), and previous studies on hemp extracts containing CBD did not show positive findings, suggesting that overall genotoxic concern for Imperial Oil® is low.

The 90-day repeated-dose study was performed in rats to assess the potential health hazards that likely to occur from repeated exposure to the test item and provide information on any major toxic effects or target organs as well as a NOAEL. The study length covers post-weaning maturation and growth of the rats well into adulthood. No mortality occurred throughout the study. Increased salivation and nuzzling up of bedding materials was noted after treatments, which was also seen in the Marx et al. study of a 25% CBD supercritical CO_2 hemp extract (Marx et al., 2018). These findings are considered likely reactions to the test item but are transient and not toxicologically concerning.

The modest but significant decreased body weight in the 125 and 175 mg/kg bw/day male animals compared to controls at the end of the study may also be because of test item consumption by the animals, although the values were considered slight at ≤10% decreased (often the point at which toxicological concern is considered) compared to controls (van Berlo et al., 2022). Specifically, the relative decreases were −6 and −7% in the mid- and high-dose male groups (which are equivalent to approximately 106 and 150 mg/kg bw/day of CBD), respectively. There was no clear correlation between slight transient differences in food consumption between groups and body weight results. Note that significantly decreased body weight compared to controls was also noted in the Marx et al. (2018) study in mid- and high-dose males (equivalent to approximately 94 and 187 mg/kg bw/day of CBD, respectively), although in that study the

values were greater than or equal to 10% difference compared to controls (Marx et al., 2018). Dziwenka et al. (2021) also noted decreased body weight in the highest-dose males and females compared to controls (equivalent to approximately 82 mg/kg bw/day CBD), and the difference was approximately 10% in males but less in females (Dziwenka et al., 2021).

There were various slightly statistically significant differences noted in hematological values compared to controls in both male and female treated animals. Values that showed statistically significant and dose-dependent patterns were decreased HGB and MCHC in males and females, and decreased HCT, MCV, MCH, and APTT in males-only. Similar hematology findings were noted in the Marx et al. study including decreases in MCH and MCHC in males and females and decreases in HGB and HCT in males only, lending credence to the consideration that they are test item-related effects (Marx et al., 2018). However, in both studies, the changes were slight and remained within historical control values and, thus, are not considered to be clinically or toxicologically relevant at the doses studied.

Various clinical chemistry and thyroid parameter differences were also noted in the current study. The only values that appeared to follow a dose-dependent pattern were cholesterol value increases in females only (CHOL, HDL, and LDL), FT4 decreases (in males and potentially females), and potentially UREA and BUN increases in both males and females. FT4 was statistically significantly decreased in all male groups dose dependently, while the dose-related pattern in females became statistically significant at the high dose only. Nonetheless, these changes were not accompanied by correlating findings in TSH (which remained well within historical control data ranges) or statistically significant differences in FT3 (the active hormone). The changes in UREA and BUN appeared potentially dose related in males (but not females) beginning at the mid dose; however, statistical significance compared to controls only occurred in only high-dose groups of both sexes. Because all differences in the above parameters were slight in magnitude and fell within historical control ranges of the laboratory, and were not associated with relevant organ findings, they were not considered of toxicological concern. None of these findings were identified in the Dziwenka et al. (2021) study, and the only similarity described in the Marx et al. (2018) study was an increase in CHOL in the high-dose females (Dziwenka et al., 2021; Marx et al., 2018).

Liver weight elevations in both male and female treated animals compared to controls (absolute and relative) were dose-dependent and were outside of historical control values. Liver weight increases in males only were accompanied by hepatocellular vacuolation (in the absence of substantial changes in liver enzyme activity levels and adverse liver histopathological changes such as necrosis) and were without correlating findings in females. Such changes are commonly considered non-adverse adaptive effects in studies of various xenobiotics that utilize/induce CYP450 enzymes for metabolism (Baldrick et al., 2020; Keller et al., 2012; Pandiri et al., 2017; Ramaiah et al., 2017). Indeed, metabolism of CBD in both rats and humans is mainly via CYP450 activity, especially CYP2C19 and CYP3A4, although other isozymes are also involved (Foster et al., 2019; Jiang et al., 2011; Lucas et al., 2018). Although there was a slight statistically significant increase in ALT (well under onefold relative to control) noted in male animals of the mid- and high-dose groups compared to controls, there was no clear dose-dependent pattern (the high-dose value was slightly decreased compared to the mid-dose level, but still statistically elevated compared to the control), and the levels remained within the historical control range and overall were considered non-toxicologically relevant. Male animals (including those of the control group) also showed an increase in vacuolation of hepatocyte cells. Oil-Red-O staining results suggested that the vacuolation was not related to lipids, whereas Pas staining revealed glycogen accumulation (in all control and treated animals). Necrosis and/or inflammatory changes were not present. Nonlipid cytoplasmic vacuolation is thought to reflect an adaptive response connected to energy disturbance, likely because of increased metabolic demand related to the xenobiotic rather than a toxicological effect; this type of adaptive vacuolation is generally because of glycogen accumulation, as is noted in the current findings (Nayak et al., 1996; Suttie et al., 2018).

Other organ weight differences compared to controls included an increase in adrenal weight which was statistically significant in males, but the increased trend in weight noted in females was not significant. In both males and females, the adrenal weight differences appeared dose dependent. No histopathological changes occurred in the adrenal glands, and the weight increases were considered part of an adaptive response, which is not an uncommon finding in animal studies (Everds et al., 2013). Adrenal weights were also increased in the Marx et al. study higher dose groups (Marx et al., 2018). Increased thyroid weight in male groups given the test item did not show a dose-dependent pattern, and the finding was not seen in female animals, nor were there any related histopathological findings in the males. Because of this, the thyroid weight finding was not considered related to any toxicological effect. The decreased testes, epidiymides and uterine weights, and increased ovary weights were statistically significant compared to controls, but all fit well within the historical control weight ranges, and no gross or histopathological lesions were noted upon examination of these organs. The decreased testes, epidiymides, and uterine weights were also not different when looked at relative to body weight, and thus could be an artifact of the slight overall decrease in body weight. The findings were not considered toxicologically relevant. Slight decreases in epididymides and ovary weights in the Marx et al. study were also noted after test article administration, and also fell within/marginal to the historical control range (Marx et al., 2018).

The few remaining gross and histopathological observations were considered individual lesions in animals that were unrelated to the test item. Dilation of the renal pelvis, ovarian cysts, and dilation of uterine horns without other histopathological lesions, such as degeneration, inflammation, or fibrosis, are common background findings in untreated laboratory rats without toxicological significance. As the various observations were of very low incidence, were not dose dependent, did not correlate with any other findings, and/or are commonly seen in this species of animal, they were not considered of toxicological relevance.

5 | CONCLUSIONS

In the current safety assessment, an ethanolic extract of *C. sativa* containing ~85% CBD showed an overall low concern for genotoxicity based on the results of in vitro and in vivo assays, even with the non-statistically significant high-dose results that exceeded the historical control range under some test conditions in the chromosomal aberration assay. A 90-day repeated dose study in rats showed mild findings considered related to the test item and indicative of an adaptive response of the organism. Based on the overall observations, the NOAEL for male and female Han:WIST rats was considered to be 175 mg/kg bw/day. As there were several findings in the study that were considered non-adverse, the No Observed Effect Level (NOEL) was determined to be <75 mg/kg bw/day. These results add to the totality of safety evidence on hemp and CBD extracts for human oral consumption.

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CONFLICTS OF INTEREST STATEMENT

The authors have no conflict of interest to report.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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EXHIBIT F

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JOHNS HOPKINS
MEDICINE
DEPARTMENT OF PSYCHIATRY AND BEHAVIORAL SCIENCES

05/01/2023

Mr. Jared Stanley
Charlotte's Web

Hi Jared,

I'm writing this letter to reiterate what we have discussed previously by phone. As you know, I am currently running a research study that is evaluating the effects of oral doses of cannabidiol (CBD) in medium-chain triglyceride (MCT) oil (100mg/mL) alone and in combination with multiple doses of delta-9-tetrahydrocannabinol (THC). The THC doses we are evaluating are 0.5mg, 1mg, 2mg, 2.8mg, and 3.7mg/mL. Study participants are healthy adults who have not used any cannabis product for at least one month. During the study, they are assigned to one of the above dose conditions, and are given a single dose in the laboratory where we assess subjective drug effects, cognitive performance, heart rate, blood pressure, and drug pharmacokinetics. They then take the same dose of the drug at home twice daily for 14 days, returning for additional assessments on Day 2, 7, and 14.

To date, 38 participants have completed the study. Ten participants have received each of the 100mg CBD, 100mg CBD + 3.7mg THC, and 100mg CBD + 2.8mg THC dose conditions, and a total of 8 participants have received the 100mg CBD + 0.5mg THC, 100mg CBD + 1mg THC, or 100mg CBD + 2mg THC dose conditions.

Evaluation of the study data show that none of the CBD + THC dose conditions differ from the CBD only dose condition on any measure of cognitive performance impairment. These include measures of psychomotor ability, working memory, divided attention, and higher order cognitive functioning. Subjective drug effects have generally been small in magnitude, and aggregate data show no differences between CBD + THC dose conditions and the CBD only dose condition. Adverse events have been mostly very mild, related to gastrointestinal discomfort, and have all spontaneously resolved.

If I can answer any questions or be of additional help, please let me know.

With kind regards,

Ryan Vandrey, PhD

The Pharmacokinetic and Pharmacodynamic Effects of Oral Cannabidiol (CBD) Under Acute and Chronic Exposure Conditions

David Wolinsky[1], C. Austin Zamarripa[1], Tory Spindle[1], Edward J. Cone[1], Ruth E. Winecker[2], Ron R. Flegal[3], David Kuntz[4], Ryan Vandrey[1]

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BACKGROUND

- Hemp (cannabis with <0.3% THC) was removed as a controlled substance with the passage of the 2018 Agriculture Improvement Act (or "Farm Bill").
- High CBD/low THC products have become widely available for consumers as a result, yet little research on the behavioral pharmacology or toxicology of these products has been conducted.
- Prior study in our lab showed acute exposure to low THC (0.39%) cannabis resulted in positive urine drug tests for a subset of participants.
- Current project extends this research to oral products at or slightly above the "hemp" THC threshold for a 100mg CBD dose after acute and twice daily exposure for 2 weeks.
- Results will help inform consumers about occupational and legal risks of hemp use, as well as inform guidelines related to drug testing methodology and interpretation.

METHODS

Participants (N=16) are healthy adults who have used cannabis or hemp/CBD at least once and lack a dependence on cannabis or other substances.
Goal will be recruitment of 60 subjects—20 for each of the three study arms, each with a different study drug.

Study Drugs:

1) 1 mL Cannabis oil containing 100 mg CBD/3.7 mg THC (0.39% THC)
2) 1 mL Cannabis oil containing 100 mg CBD/2.8 mg THC (0.3% THC)
3) 1 mL Cannabis oil containing 100 mg CBD/0 mg THC (0.0% THC)

CBD oil prepared through mixture of purified, hemp-derived CBD and purified THC mixed in medium-chain triglyceride oil.

Procedures
Participants will undergo three outpatient phases:

-Phase 1: Initial drug administration session where pharmacokinetics and pharmacodynamics are assessed before and after first acute dose.

-Phase 2: Use of study drug twice daily for 14 days, with laboratory assessments on Days 2, 7 and 14.

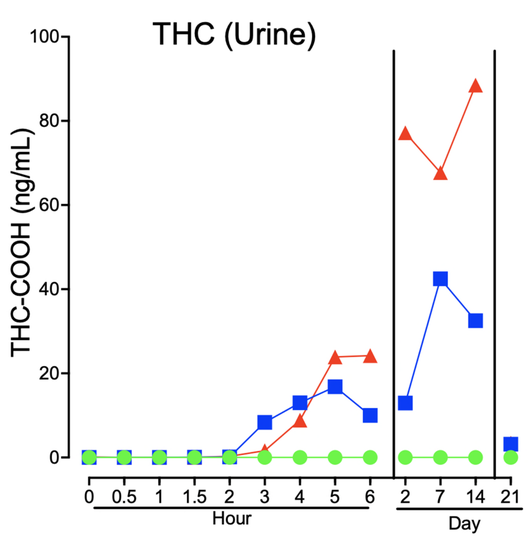
-Phase 3: 1-week washout period followed by laboratory assessments on Day 21

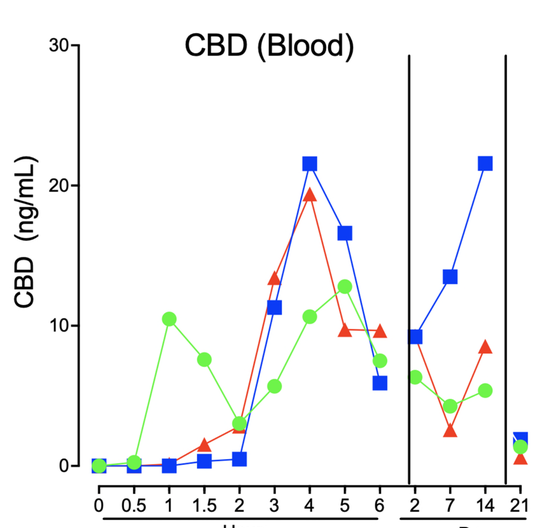
Assessments
- Qualitative and quantitative cannabinoids in oral fluid (OF) and urine
- Vital signs (HR and BP)
- Drug Effect Questionnaire (DEQ; 100mm VAS)
- Divided Attention Task (DAT)
- Digit Symbol Substitution Task (DSST)
- Paced Serial Addition Task (PASAT)

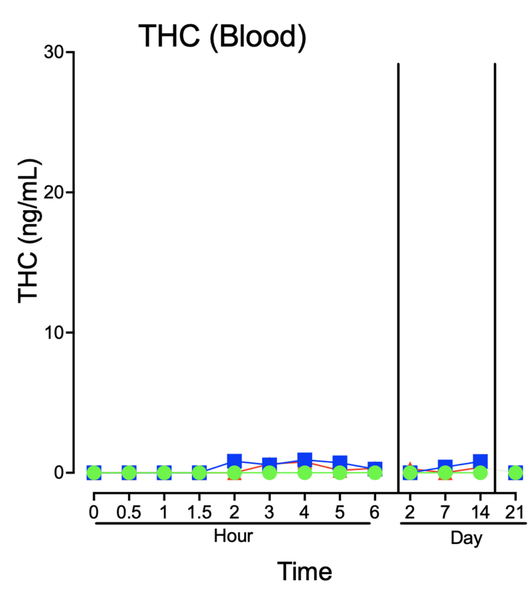
STUDY TIMELINE

	Day 1 Hour 0*	Day 1 Hour 0.5	Day 1 Hour 1	Day 1 Hour 1.5	Day 1 Hour 2	Day 1 Hour 3	Day 1 Hour 4	Day 1 Hour 5	Day 1 Hour 6	Day 2**	Day 7**	Day 14**	Day 21
Drug Taken	X									X	X	X	
Vitals	X	X	X	X	X	X	X	X	X	X	X	X	X
Blood/Urine /Oral Fluid Sampling	X	X	X	X	X	X	X	X	X	X	X	X	X
Hair Sample (Optional)	X												X
DEQ	X	X	X	X	X	X	X	X	X	X	X	X	X
DAT	X	X	X	X	X	X	X	X	X	X	X	X	X
DSST	X	X	X	X	X	X	X	X	X	X	X	X	X
PASAT	X	X	X	X	X	X	X	X	X	X	X	X	X

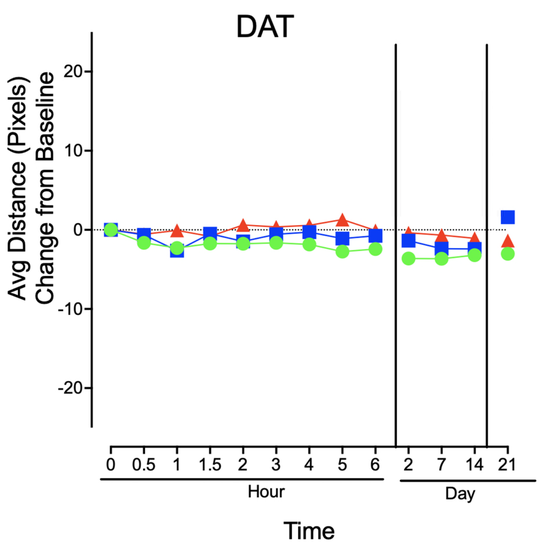
BIOMARKERS

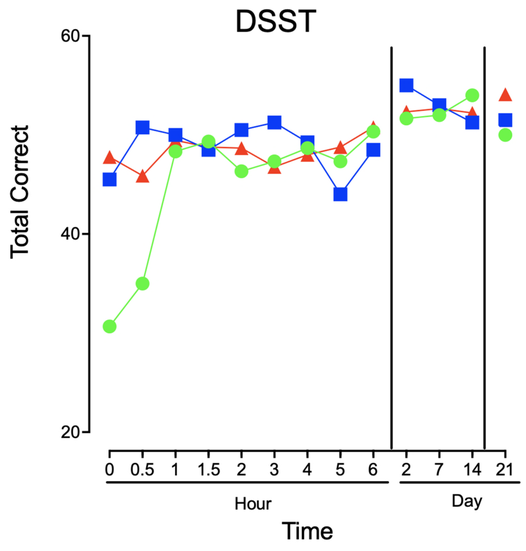


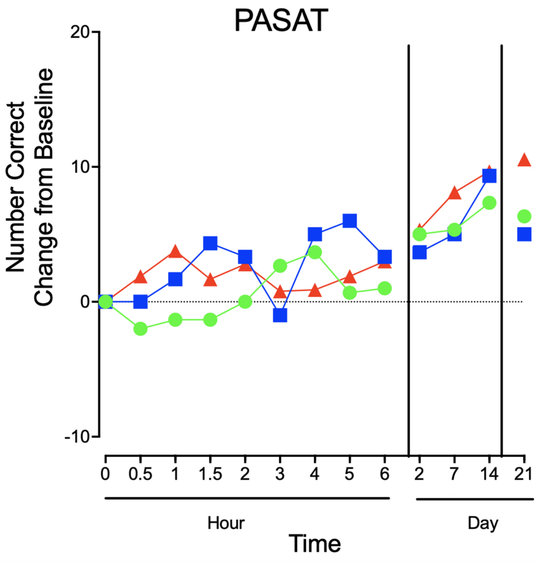




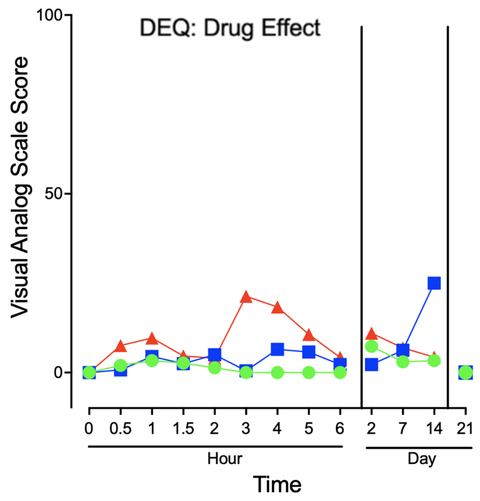
COGNITIVE PERFORMANCE

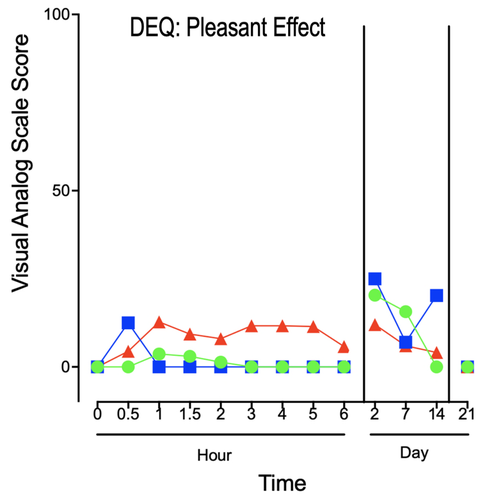






SUBJECTIVE DRUG EFFECTS





SUMMARY OF PRELIMINARY DATA

- All 4 participants receiving 3.7mg THC + 100mg CBD screened and confirmed positive on urine drug tests using federal mandatory guidelines.
- 2 of 3 participants receiving 2.8mg THC + 100mg CBD screened and confirmed positive on urine drug tests using federal mandatory guidelines.
- No positive urine drug tests in the 100mg CBD (no THC) dose condition.
- 2.8mg and 3.7mg THC associated with mild subjective drug effects, but no impact on subjective cognitive ability.
- Blood THC concentrations were generally low, none detected in CBD only dose condition. One sample tested > 2ng/mL, a cutoff sometimes used for roadside DUI evaluations.
- One participant (3.7mg THC dose) dropped out of the study early due to subjective drug effects that he felt would have impaired ability to drive (not included in data shown here).
- No impact of any dose condition on cognitive performance for other study participants.

ACKNOWLEDGMENTS

Special thanks to research staff at the Johns Hopkins BPRU and to Canopy Growth Corporation and THC Pharm GmBH for material support

DISCLOSURES

Study funded by the Substance Abuse and Mental Health Services Administration (SAMHSA) and NIDA training grant T32-DA07962. Dr. Spindle has served as a consultant for Canopy Health Innovations Inc. Dr. Vandrey has served as a consultant or received honoraria from Canopy Health Innovations Inc., MyMD Pharmaceuticals, Mira1a Pharmaceuticals, Jazz Pharmaceuticals, WebMD, Syqe Medical Ltd., and Radicle Science Inc.

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EXHIBIT G

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Investigating the Simultaneous Effects of Cannabidiol and Caffeine

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Introduction

- Approximately 85% of adults in the US consume at least one caffeinated product daily (Mitchell et al., 2014).
- Caffeine is widely consumed but there is little information regarding potential interaction effects with other substances.
- Like caffeine, cannabis is used at elevated rates, and is the most consumed illicit substance worldwide (Ferré et al., 2013; Temple et al., 2017; WHO, 2022).
- Cannabidiol (CBD) products, including those combined with caffeine, are widely available across the U.S. and are commonly used among young adults (Moltke & Hindocha, 2021).
- Despite widespread use of both caffeine and CBD, limited studies (two animal studies and three human trials; Nazarrio et al., 2015; Owolabi et al., 2017; Thai et al., 2021; Zamarripa et al., 2023; Bansal et al., 2023) have examined their simultaneous administration.
  - CBD increased caffeine C_{max} by 15% (Thai et al., 2021).
  - High doses of CBD (i.e., 640mg), but not THC, increased concentrations of caffeine in blood plasma in healthy adults (Zamarripa et al., 2023; Bansal et al., 2023).
- Given the dearth of research on this topic, the availability of commercially combined caffeine and CBD products in the U.S., and the widespread consumption of both substances, it is critical to characterize the potential effects of simultaneous use.

Public Significance

- The current study is the first to examine the potential behavioral self-reported simultaneous and/or interactions between CBD and caffeine in humans.

Participants & Materials

Participants:

- N = 54; 64.8% female; M_{age} = 20.24, M_{BMI} = 23.11
- Daily caffeine users ($M_{mg/day}$ = 418, $SD_{mg/day}$ = 231, $range_{mg/day}$ = 202 to 1257) who did not consume cannabinoids.

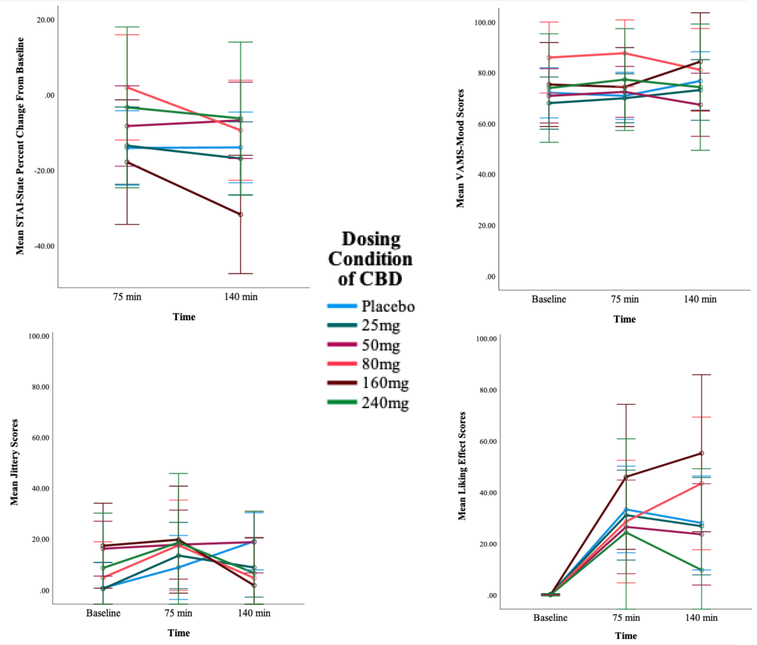
Measures:

- Caffeine Consumption Questionnaire-Revised (CCQ-R; Irons et al., 2014), Visual Analogue Mood Scale (VAMS; mood item; 0-100), State-Trait Anxiety Inventory-State (STAI-S; Spielberger et al., 1983; 20-80), Drug Effect Questionnaire (DEQ; 0-100)

Study Drugs:

- Two 100mg capsules of caffeine
- CBD isolate soft-gels suspended in MCT oil

Results



Outcomes Summary

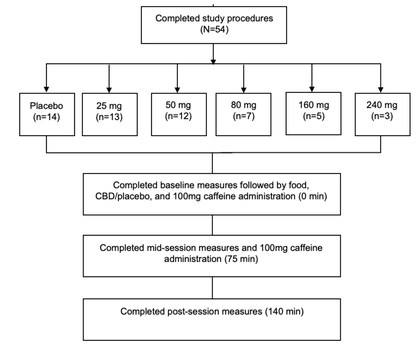
Outcome		F	p	η_p^2	1-β
STAI-State	Interaction effect	1.57	0.19	0.14	0.50
	Main effect of time	5.33	0.025	0.10	0.62
	Main effect of dose	1.37	0.25	0.13	0.44
VAMS-Mood	Interaction effect	0.99	0.46	0.10	0.49
	Main effect of time	0.27	0.73	0.01	0.09
	Main effect of dose	0.88	0.50	0.08	0.29
Jittery	Interaction effect	0.87	0.57	0.08	0.43
	Main effect of time	1.71	0.19	0.03	0.35
	Main effect of dose	0.86	0.52	0.08	0.28
Liking of Drug Effect	Interaction effect	0.63	0.79	0.06	0.31
	Main effect of time	23.41	<.001	0.33	1.00
	Main effect of dose	0.81	0.55	0.08	0.27

Note. A series of two-way, mixed ANOVAs were conducted. For STAI analyses, percent change was used. Change scores were not used for other analyses because of a plausible value of 0. When the assumption of Box's M Test of Equality of Covariance was violated, Wilks' Lambda corrections were made. When the assumption of sphericity was violated, Greenhouse-Geisser adjustments were made.

- All outcomes yielded small effect sizes and low power (see presenter for DEQ outcomes not noted on poster).
- No interaction or dose effects emerged for above analyses.
- State anxiety (STAI-S) decreased across time, regardless of dose.
- Liking of drug effect (DEQ) increased across time, regardless of dose.
- 160mg of CBD yielded generally lower state anxiety (24 and 37% reductions) and jitteriness (DEQ) and higher mood (VAMS-mood) and liking of drug effects (DEQ) compared to other doses (observed variability was less than or equal to that of other doses across outcomes).

Procedure

- Prior to study session, participants received study materials.
- Sessions occurred via Zoom (beginning no later than 10:30am).
- Participants completed baseline measures.
- Ingested their assigned CBD dose and 100mg caffeine.
- Ingested a snack with 20 mg of fat provided by the researchers (e.g., peanut butter, cheese whisps).
- At 75 minutes, participants ingested the remaining 100 mg of caffeine and completed measures again.
- At 140 minutes, participants completed measures again.
- Participants were compensated with $30 or two bottles of CBD.



Discussion

The current study provides preliminary evidence that acute simultaneous ingestion of CBD, relative to placebo, and 200mg caffeine does not impact self-reported drug effects in a sample of healthy young adults who consumer caffeine regularly.

Limitations and Future Directions:

- The current study yielded small effects sizes and limited power.
- All participants consumed caffeine daily and were healthy young adults who did not consume cannabinoids.
- Percent change from baseline calculations were not possible for scores ranging from 0-100 (i.e., VAMS, DEQ).
- Future work should examine CBD/caffeine effects among varied populations (including caffeine-naïve, clinical samples, & others).
- Caffeine and CBD both exhibit intra-subject variability in their pharmacokinetic and pharmacodynamic outcomes (Hammani and Alvi, 2017; Zamarripa et al., 2022).
- Future work should investigate simultaneous/interaction effects using within-subject comparisons to reduce individual variability influences.