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Cannabidiol safety considerations: Development of a potential acceptable daily intake value and recommended upper intake limits for dietary supplement use

Rayetta G. Henderson [a,*], Melissa Vincent [b], Brianna N. Rivera [c], Marcel O. Bonn-Miller [d,e], Candace Doepker [b]

[a] ToxStrategies LLC, Wilmington, NC, USA
[b] ToxStrategies LLC, Cincinnati, OH, USA
[c] ToxStrategies LLC, Asheville, NC, USA
[d] Canopy Growth Corporation, Smiths Falls, Ontario, Canada
[e] Charlotte's Web, Inc., Louisville, CO, USA

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ABSTRACT

Consumer use of hemp-derived products continues to rise, underscoring the need to establish evidence-based safety guidance. The present study sought to develop recommendations for oral upper intake limits of cannabidiol (CBD) isolate. Sufficiently robust and reliable data for this purpose were identified from published human clinical trials and guideline-compliant toxicity studies in animal models. Based on the metrics used in this assessment, a potential Acceptable Daily Intake (ADI) value of 0.43 mg/kg-bw/d (e.g., 30 mg/d for 70-kg adult) was determined for the general population based on liver effects in human studies. This value applies to the most sensitive subpopulations, including children, over a lifetime of exposure and from all sources, including food. For dietary supplements with adequate product labeling intended for use by healthy adults only, a potential Upper Intake Limit (UL) of 70 mg/d was determined based on reproductive effects in animals. For healthy adults, except those trying to conceive, or currently pregnant or lactating, a conservative dietary supplement UL of 100 mg/d was identified based on liver effects; however, as the target population excludes individuals at risk for liver injury, an alternative dietary supplement UL of 160 mg/d for this population can also be considered.

1. Introduction

Worldwide use of hemp-derived consumer products continues to rise, despite a lack of consistent safety-related guidance or regulatory oversight. Hemp, typically defined as *Cannabis sativa* L. containing ≤0.3% delta-9-tetrahydrocannabinol (THC) on a dry-weight basis, contains more than 120 identified cannabinoids, as well as an array of terpenes and phenolic compounds (AHPA, 2022; EC, 2013; Rupasinghe et al., 2020; Walsh et al., 2021). A large number of pre-clinical and clinical safety studies have been conducted with the most common non-intoxicating cannabinoid in hemp, cannabidiol (CBD). In the United States (US) and Canada, a survey of 45,300 adults (age 16 years and older) demonstrated that 16.2–26.1% had used CBD-containing products in the previous twelve months (Goodman et al., 2022). Similarly, the Brightfield Group (2023) has estimated that 15% of Americans (49.8 million) use CBD regularly, reporting consumption levels ranging from ≤20 mg/day and ≥1000 mg/day. The amount of CBD consumption associated with consumer products varies by individual; for example, in one survey conducted in the United Kingdom (UK), participants reported using between ≤24 mg/day and ≥200 mg/day of CBD (Moltke and Hindocha, 2021). Continued consumer interest in these products has been attributed to perceived beneficial effects on conditions such as anxiety, pain, depression, and insomnia, as well as for well-being, relaxation, and stress relief (Goodman et al., 2022; Moltke and Hindocha, 2021; Fortin et al., 2021; Corroon and Phillips, 2018). The clinical evidence for potential therapeutic effects of CBD has been reviewed in recent publications, such as O'Sullivan et al. (2023). In addition to CBD isolate, many consumer products are hemp extracts containing a mixture of cannabinoids and terpenes, where CBD typically comprises a large fraction of the ingested material (e.g., 5–90% CBD). Thus, determining safe levels of CBD intake in dietary supplements, foods, and/or

* Corresponding author. 23501 Cinco Ranch Blvd, Suite B226, Katy, TX, 77494, USA.
E-mail address: rhenderson@toxstrategies.com (R.G. Henderson).

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Abbreviations

ADI	acceptable daily intake
AED	antiepileptic drug
AHPA	American Herbal Products Association
AIC	Akaike information criterion
ALP	alkaline phosphatase
ALT	alanine aminotransferase
AST	aspartate aminotransferase
BMA	Bayesian model averaging
BMD	benchmark dose
BMDL	benchmark dose lower limit
BMR	benchmark response
BMDS	benchmark dose software
CBD	cannabidiol
CDER	Center for Drug Evaluation and Research (US FDA)
CFR	US Code of Federal Regulations
7-COOH-CBD	7-carboxycannabidiol
DILI	drug-induced liver injury
EC	European Commission
EPA	US Environmental Protection Agency
FDA	US Food and Drug Administration
FSA	UK Food Safety Authority
GD	gestational day
GLP	Good Laboratory Practice
GRAS	Generally Recognized as Safe
HBGV	health-based guidance value
HDL	high-density lipoprotein
IPCS	International Programme on Chemical Safety
LDL	low-density lipoprotein
LOAEL	lowest-observable-adverse-effect level
LOEL	lowest-observable-effect level
MCT	medium-chain triglyceride
NDIN	New Dietary Ingredient Notification
NOEL	no-observable-effect level
NOAEL	no-observable-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
UL	upper intake limit
US	United States of America

beverages is critical to ensuring consumer safety.

Despite the amount of safety-related data on CBD, acceptable daily intake (ADI) values have not yet been established by any regulatory agency or authoritative body. An ADI is intended to apply to the general population, which includes all age groups (including children, typically 12 weeks of age and older), physiological states, and pregnant and lactating individuals (IPCS, 1987, 2020). The value is defined as the estimated amount of a substance, expressed on a body-weight basis, that can be ingested daily over a lifetime without appreciable health risk (EFSA, 2012a; IPCS, 2004).

While no regulatory-based guidance for consumption of CBD from foods or supplements by children has been established, recommended upper intake levels for adults using CBD-containing novel foods and/or dietary supplements have been established for CBD by the UK Food Safety Authority (FSA, 2023), Health Canada (2022), and the Australian Therapeutic Goods Administration (TGA, 2021). Conversely, the US Food and Drug Administration (FDA, 2022; 2023) and the European Food Safety Authority (EFSA, 2022a) have concluded the currently available data to be insufficient for this purpose, citing uncertainties in the dataset, such as a need for additional data on long-term exposure, and potential effects on liver and reproductive toxicity.

Despite the general absence of harmonized authoritative positions, consumer use of CBD and other hemp-derived products continues to grow, thus creating an immediate need to establish evidence-based recommendations for intake to enable continued safe use of CBD-containing products. A recent increase in relevant published literature provides a sufficient basis from which to accomplish this critical need. This new information further supplements the large body of human CBD clinical trial data that already exists (reviewed in Arnold et al., 2023; Chesney et al., 2020; Lo et al., 2023; Souza et al., 2022). In addition, new guideline-compliant genotoxicity, subchronic oral toxicity, and reproductive toxicity studies in animal models have recently been published which help to address data gaps highlighted previously by regulatory agencies (Henderson et al., 2023a, 2023b, 2023c; Tallon and Child, 2023).

The goal of the current study was to use a systematic approach to review the publicly available safety data to develop recommendations for oral consumer use of hemp-derived CBD isolate. Specifically, we attempted to derive: 1) a potential ADI for the general population and from all sources, and 2) recommended upper intake limits (ULs) for dietary supplement use by healthy adults.

2. Materials and methods

2.1. Development of candidate intake limit values

This study sought to derive multiple oral intake limit values. Available evidence from human clinical trials and studies in laboratory animals were considered as possible points of departure (PODs) for all values. Selections of PODs and candidate limit values were made based on the most appropriate data for each target population. The overall approach is presented in Fig. 1 and detailed methods are provided below.

2.1.1. Human studies

2.1.1.1. Toxicity data selection. First, available systematic reviews evaluating adverse events in human clinical trials with CBD were identified and reviewed to establish the critical endpoint from this dataset for the purposes of the current assessment. Next, a targeted literature search was conducted to identify publications reporting some quantitative analysis of the critical endpoint across relevant studies (see Fig. 1 and Supplemental Table S1 for details). The identified systematic reviews/meta-analyses were then reviewed for relevance based on the inclusion criteria. For inclusion, the systematic review/meta-analysis had to report incidence of the critical endpoint stratified by CBD dose level based on clinical trials with repeated oral dosing with CBD (e.g., CBD isolate, Epidiolex®, or in a combination drug such as Sativex®).

2.1.1.2. POD development. Once the systematic review/meta-analysis to be used was selected, the paper was thoroughly reviewed to identify the most appropriate PODs based on the analyses reported by the authors (Lo et al., 2023) for developing: 1) a potential ADI, and 2) possible ULs for dietary supplement use by adults. As part of the current

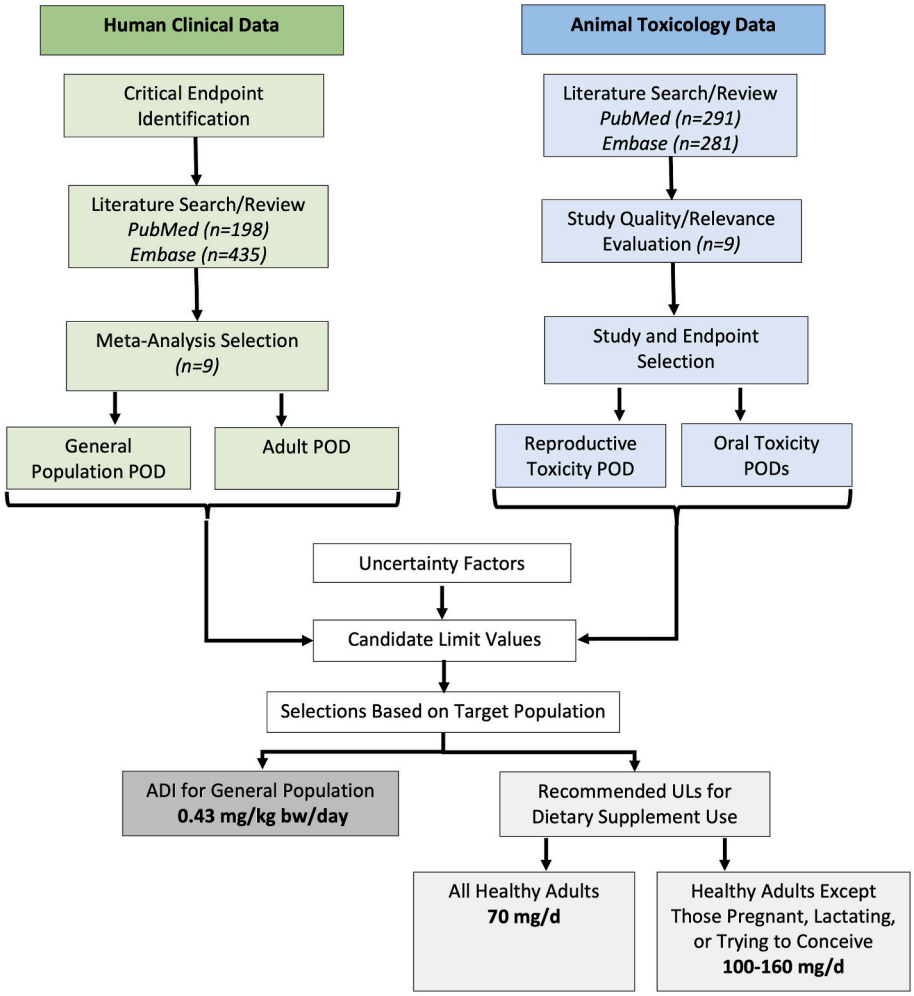

Fig. 1. Flowchart of approach used to develop potential Acceptable Daily Intake (ADI) and upper intake limit (UL) values for cannabidiol (CBD). POD, point of departure.

study, individual studies within the meta-analysis that included adults (i.e., ≥17 years) were further screened, and additional data extracted from each original study publication were reviewed to aid in the selection of a POD from the meta-analysis (Lo et al., 2023) for healthy adults.

2.1.2. *Animal studies*

2.1.2.1. Toxicity data selection. A literature search was conducted, and relevant publications were systematically identified (see Fig. 1 and Supplemental Table S2 for details). Inclusion criteria included the following minimum parameters: *in vivo* study, relevant route of exposure (oral), multiple dose levels in addition to concurrent controls, repeated dosing, mammalian species, and hemp-derived CBD isolate test material. CBD isolate was defined as ≥95% CBD and included studies with broad spectrum hemp extracts that met this definition based on minimum CBD content. Studies identified for inclusion were reviewed for relevance and quality to determine study selection for POD development. To be considered for selection, a study had to have a quality and reliability rating equivalent to a Klimisch score of K1 or K2 (Klimisch et al., 1997). Copies of full laboratory reports were made available by the respective study sponsors for publications scored as K1 and were also reviewed.

2.1.2.2. Dose-response modeling and POD development. Potential critical endpoints that meet the criteria for dose-response analyses were evaluated using benchmark dose (BMD) modeling to derive PODs for clinically relevant endpoints. Criteria included: 1) statistical or biological significance of the observed outcome, and 2) a clear dose-response relationship with responses different from controls in at least two tested doses. BMD modeling was conducted using the US Environmental Protection Agency (EPA)'s Benchmark Dose Software (BMDS, version 3.3) in accordance with US EPA BMD technical guidance (USEPA, 2012). All standard continuous models were considered.

For continuous endpoints without a clearly defined or biologically meaningful basis for determining an adverse or detrimental change in response, a standard benchmark response (BMR) of 1 SD change in mean response was used. For continuous endpoints related to reproductive or developmental toxicity, a conservative BMR of a 0.5 SD change in mean response was modeled in addition to the standard approach BMR of a 1 SD change (corresponding to an approximation of 5% and 10% extra risk). Due to the heterogeneity in variance across dose groups in the reproductive toxicity study (Henderson et al., 2023b), variance was also modeled (i.e., assumed non-constant) to improve model fit. For dichotomous endpoints, a BMR of 10% extra risk was utilized.

In accordance with US EPA guidance (USEPA, 2012), all viable models (i.e., those with adequate fit defined as $P > 0.1$) were considered for selection. Of the viable models, models with the lowest Akaike information criterion (AIC) were selected for POD derivation to ensure consistency and repeatability in model selection, consistent with US EPA guidance and practice (Haber et al., 2018; USEPA, 2012). However, the range of viable BMDLs are presented and considered for each endpoint to consider sensitivities in BMDL derivation attributable to model

dependence. Current methods propose the use of Bayesian model averaging (BMA) approaches in lieu of selecting a single model in order to better reflect the underlying uncertainties in model fitting and the subsequently predicted dose-response relationships (e.g., EFSA, 2022b). However, BMDS does not currently support BMA of continuous endpoints and EPA guidance recommends combining models only when there is not one clear best fit (US EPA, 2012).

US EPA's Categorical Regression Software (CatReg version 3.1.0.7) was used to assess dichotomous endpoints with differing levels of severity (i.e., adrenal vacuolation). A BMR of 10% Extra Risk was also applied using a Logit function and cumulative odds models with the assumption of a linear dose function.

2.1.3. Candidate intake limit value derivation

Each POD was divided by applicable uncertainty factors to derive candidate limit values for consideration in the development of a potential ADI and/or possible ULs for dietary supplement use in healthy adults. Selection of uncertainty factors considered guidance available from multiple regulatory agencies and was consistent with typical recommendations for risk assessment of chemicals in food and dietary supplements (IPCS, 2005, 2020; EFSA, 2012b; FDA, 2016; FSA, 2012). ADIs and ULs were then selected from the most sensitive and appropriate candidate values for each target population.

Equation (1). Candidate Limit Value Derivation

$$\text{Candidate Limit Value } (\text{mg} / \text{kg bw} / \text{day}) = \text{POD} \,/\, \text{UF}_{\text{inter}}\ x\text{UF}_{\text{intra}}\ x\text{UF}_{\text{extrap}} \quad (1)$$

where,

POD = point of departure (NOAEL or BMDL; mg/kg bw/day)
UF_{inter} = uncertainty factor for interspecies variation (unitless);
UF_{intra} = uncertainty factor for intraspecies variation (unitless);
UF_{extrap} = uncertainty factor for subchronic-to-chronic extrapolation (unitless).

3. Results

As discussed in the introduction, development of an ADI for any substance requires adequate available data demonstrating a lack of genotoxic effects. EFSA (2022a) and FSA (2019) previously concluded the available data on CBD to be insufficient to evaluate its potential genotoxicity; however, a more recent publication summarized the outcome of three Organisation for Economic Co-operation and Development (OECD) guideline-compliant mutagenicity and genotoxicity studies that evaluated the ability of CBD isolate to induce mutation or cause chromosomal damage (Henderson et al., 2023c). The results from this testing battery, which included an *in vivo* mammalian micronucleus test (OECD, 2016a; Test Guideline 474), an *in vitro* mammalian micronucleus test (OCED, 2016b; Test Guideline 487), and an *in vitro* bacterial review mutation test (OECD, 2020; Test Guideline 471), indicated that pure CBD isolate was nonmutagenic, nonclastogenic, and nongenotoxic under the study conditions. In addition, no increases in tumor incidence were reported in a 2-year study reviewed by CDER (2018a) conducted on a CBD botanical drug substance (up to 67% CBD); conversely, decreases in some hormonally-mediated tumors in aging animals were observed. These findings are consistent with preclinical evidence from studies investigating CBD as a potential treatment for cancer, including at multiple levels of tumor progression and via different mechanistic pathways; the data demonstrating anti-tumor effects of CBD have been summarized in review articles (O'Brien, 2022; Seltzer et al., 2020; Hinz and Ramer, 2022). Importantly, there is a lack of cancer-related adverse outcomes in clinical trials conducted with CBD for various indications (Chesney et al., 2020; dos Santos et al., 2020). In addition, CBD has been evaluated in clinical trials in cancer patients to evaluate its efficacy on treating symptoms, such as pain and nausea (O'Brien, 2022). As the available data demonstrate a lack of genotoxic and carcinogenic potential, the present study focused only on consideration of non-cancer endpoints.

Data of sufficient quality and relevance were identified and used for the purposes of this study as described in Section 3.1. Multiple PODs and candidate limit values were identified based on the most critical endpoint from human clinical trials and the most sensitive endpoints in animal models. ADI and UL values were then selected from the most sensitive candidate values based on relevance of the dataset to the target population (i.e., general population or healthy adults) and intended use (i.e., from all dietary sources and/or from dietary supplement products). An overview of the approach used in this study is presented in Fig. 1.

3.1. Data selection and PODs

3.1.1. Human data – candidate data selection

Clinical data were first reviewed to identify the critical non-cancer effect from human studies. A review of recent systematic reviews of clinical trial data from dozens of studies with CBD demonstrate that serious adverse events are primarily limited to effects on liver function tests (elevated liver enzyme levels) and upper respiratory infections (Chesney et al., 2020; dos Santos et al., 2020). Respiratory effects were deemed to occur much less frequently than liver effects and have not been reported in studies with healthy populations (Souza et al., 2022). Given that the purpose of the current study was to derive candidate values for the general population and healthy adults, respiratory effects were not considered as the critical endpoint from human studies for the approach used in the current study. Liver effects in human studies have also been flagged by regulatory agencies such as FDA (2022) as cause for concern, and by EFSA (2022a), which noted that studies in humans are needed to identify a POD for increases in liver enzymes. As such, this prevailing knowledge formed the basis for the current study focusing on incidence of elevated enzyme levels as the critical endpoint evaluated from human studies and subsequently identifying studies which reported the CBD exposure levels associated with such.

Nine meta-analyses were identified in the literature search that evaluated liver enzyme elevations related to CBD in clinical trials (Fig. 1). However, only one study met the inclusion criteria and was therefore suitable for POD development (Supplemental Table S1). The systematic review and meta-analysis by Lo et al. (2023) was the only study identified that analyzed incidence of liver enzyme elevations stratified by CBD dose. This well-designed study also encompassed the broadest dataset, widest range of population characteristics, and the most recent clinical trials. The Lo et al. (2023) meta-analysis included studies conducted with different preparations of CBD (CBD isolate and pharmaceuticals) in adults and children for up to 26 weeks, and included studies with fasted and unfasted individuals. Participants included males and females from all age groups (≥1–75 years), healthy individuals, and patients with various disease states, many of whom were on concomitant medication(s) before and/or during the trial.

The primary outcomes evaluated in Lo et al. (2023) were the proportion of elevated liver enzymes (defined as alanine aminotransferase [ALT] or aspartate aminotransferase [AST] >3x upper limit of normal [ULN] or alkaline phosphatase [ALP] >2x ULN) and drug-induced liver injury (DILI; see Lo et al. [2023] for definitions). Additional outcomes included patient characteristics, time to detection and resolution, dose, and concomitant medications. Based on analysis of 1533 participants across 28 studies, CBD doses classified as "high" by the authors of the meta-analysis (i.e., ≥1000 mg/day or ≥20 mg/kg-bw/day) and concomitant use of the antiepileptic drug valproate were each found to be significantly associated with liver enzyme elevations and DILI.

3.1.1.1. POD identification from human data for general population. The Lo et al. (2023) meta-analysis was determined to be sufficiently representative of the general population, including children. This study also

Table 1
Human clinical trials reporting incidence of elevated liver enzymes in adults following exposure to CBD (adapted from Lo et al. [2023] with additional population characteristics added).

Reference	N	Age Range (mean)	Race: White/ Black/ Other[a]	Sex (M/F)	Health Status	Co-Exposure Study	CBD Dose (mg/kg bw/ day)	Total Study Duration[b]	Participants with Elevated Liver Enzymes (DILI)	Reversal of Elevated Enzymes	Time to Resolution (days)
Studies in Adults Only											
Naftali et al. (2017)	10	18-75 (45)	NR	6/4	Crohn's disease	_	0.3[c]	8 weeks	0 (–)	_	_
Hosseini et al., 2021	6	27-34 (30)	5/0/1	3/3	Healthy	_	1.4 [c]	5 days	0 (–)	_	_
Irving et al. (2018)	29	Adult (44)	21/1/7	23/6	Ulcerative Colitis	_[f]	3.6 [c,f]	10 weeks	0 (–)	_	_
Crippa et al. (2021)	59	Adult (33)	NR	20/39	Healthy	_	4.3 [c]	4 weeks	4 (2)	with cessation	7
Consroe et al. (1991)	15	16-66 (48)	NR	8/7	Huntington's Disease	_	10	6 weeks	0 (–)	_	_
Ben-Menachem et al. (2020)	28	17-55 (30)	6/0/0	5/1	Epilepsy	VPA or Stiripentol	20	24 days	2 (2)	with cessation	20–31
Leehey et al. (2020)	13	56-75 (68)	NR	10/3[d]	Parkinson's Disease	_	20	~3 weeks	2 (2)	with cessation	14
VanLandingham et al. (2020)	16	Adult (37)	15/0/1	8/8	Epilepsy	CLB	20	31 days	2 (2)	with cessation	12–22
Morrison et al. (2019)	78	Adult (30)	62/7/8[e]	50/27	Healthy	CLB, VPA, Stiripentol	21.4 [c]	~1–2 weeks	0 (–)	_	_
Taylor et al. (2020)	30	Adult (25)	27/0/3[e]	17/13	Healthy	_	21.4 [c]	4 weeks	2 (2)	with cessation	14–28
Thai et al. (2021)	16	Adult (33)	13/1/2	6/10	Healthy	CYP1A2 caffeine probe	21.4 [c]	25 days	6 (6)	n = 5 with cessation; n = 1 unresolved	9-49 (majority 10–20)
Watkins et al. (2021)	16	Adult (29)	13/1/2	6/10	Healthy	CYP1A2 caffeine probe	21.4 [c]	25 days	6 (5)	with cessation	~10
Studies in Adults and Children											
Devinsky et al. (2016)	162	1–26	NR	80/82	Epilepsy	_	25–50	12 weeks	11 (1)	unknown	unknown
Devinsky et al. (2018)	149	3-48 (16)	NR	85	Epilepsy	_	20	14 weeks	14 (5)		unknown, within 4 months
Iannone et al. (2021)	93	3-56 (21)	NR	49/44	Epilepsy	_	18–25	26 weeks	10 (unknown)	unknown	unknown
Klotz et al. (2019)	35	Adult (32) Children (9)	NR	19/ 16[d]	Epilepsy	_	18–20	13 weeks	5 (unknown)	_	unknown
Thiele et al. (2018)	86	3-45 (15)	75/0/11	45/41	Epilepsy	_	20	14 weeks	20 (6)	n = 8 spontaneous; n = 9 with cessation or reduced dose; n = 3 reduced AED dose	unknown
Thiele et al., 2021	148	1-57 (11)	NR	86/ 62[d]	Epilepsy	_	25–50	16 weeks	28 (14)	_	unknown

AED, antiepileptic drug; CLB – clobazam; DILI – drug induced liver injury; NR – not reported; VPA – valproate.
[a] Other defined as Asian, Alaska Native, American Indian, or multiple.
[b] Includes periods of titration for some studies.
[c] Calculated from reported dose in mg/d and assuming a 70-kg adult.
[d] Only males reported, calculated number of females from total N.
[e] Includes individuals who identified as multiple races.
[f] The test material was a CBD-rich botanical extract containing primarily CBD and smaller amounts of other compounds such as cannabigerol, terpenoids, flavonoids, sterols, and 3.2%–4.7% THC.

included subpopulations of patients on antiepileptic drugs (AEDs), and therefore more sensitive to the critical endpoint of liver toxicity than the general population. As such, 1000 mg/day CBD could be considered the no-observed-adverse-effect level (NOAEL) for liver effects in humans. This is further supported by similar findings in sensitivity tests using a trichotomized dose range (<300 mg/day vs. 300–999 mg/day vs. ≥1000 mg/day) reported by Lo et al. (2023). It should be noted that the lower-bound value of 300 mg/day in the trichotomized analysis was based on a single clinical trial with possible limitations (Crippa et al., 2021; as described in the next section) and is therefore likely an overly conservative departure point. Nevertheless, to ensure the selected POD for the general population from this study to be considered as a candidate value for a possible ADI is sufficiently protective, the lower end of the mid-dose from the trichotomized analysis of 300 mg/day (equivalent to 4.3 mg/kg-bw/day in adults) was carried forward for the POD (as a NOAEL) for ADI development.

3.1.1.2. POD identification from human data for healthy adult population. Studies from the Lo et al. (2023) meta-analysis that included adult participants were further assessed to determine an appropriate POD for healthy adults for possible UL development for dietary supplement use (Table 1). A single study in healthy volunteers reported 4/59 cases of elevated liver enzymes with a p value of 0.06, and for which Lo et al. (2023) identified a potential risk of bias arising from the randomization process (Crippa et al., 2021). Of the remaining 17 studies with adult participants, incidences of elevated enzyme levels were reported starting at very high clinical doses, generally starting at 20 mg/kg-bw/day (equivalent to 1400 mg/day) (Ben-Menachem et al., 2020; Leehey et al., 2020; VanLandingham et al., 2020; Morrison et al., 2019; Taylor et al., 2020; Thai et al., 2021; Watkins et al., 2021; Devinsky et al., 2016, 2018; Thiele et al., 2018, 2021). No liver effects were reported in a study of Huntington's Disease patients receiving 10 mg/kg-bw/day (equivalent to 700 mg/day) for six weeks (Consroe et al., 1991) or in studies with CBD doses below 4.3 mg/kg-bw/day (300 mg/day). Given that the target population for dietary supplements in the current study is healthy adults, the risk analysis from Lo et al. (2023) is considered sufficiently protective for liver effects when appropriate product labeling is used on packaging for dietary supplements. A POD of 1000 mg/day (equivalent to 14.3 mg/kg-bw/day for adults) was selected for UL development for dietary supplement use in healthy adults.

3.1.2. Animal studies

Nine studies from the peer review literature were identified that met the *a priori* criteria in laboratory animals (Supplemental Table S2). Four of these studies were guideline studies equivalent to a Klimisch quality and reliability score of K1 (Klimisch et al., 1997; Henderson et al., 2023a, 2023b; Tallon and Child, 2023). The remaining studies included in the database were assigned a Klimisch score of K2, i.e., reliable with restrictions (Carvalho et al., 2018a, 2018b, 2022; Rosenkrantz et al., 1981; Vaughn et al., 2021). Following consideration of all available data, two K1 studies were selected to meet the objectives of the study based on robustness of the respective study designs.

Of note, information available from data in the Epidiolex non-clinical review package available as summaries from FDA were not considered publicly available and, as such, would have been assigned a Klimisch score of K4 for the purposes of the current assessment (CDER, 2018a). Findings from studies reviewed by CDER (2018a) were generally consistent with those of the K1 studies identified and support the conclusions of the present study for CBD isolate.

3.1.2.1. Candidate data selection from animal studies – reproductive toxicity. Reproductive toxicity was identified as the most sensitive endpoint following exposure to CBD in guideline-compliant studies. The study by Henderson et al. (2023b) was selected for reproductive and developmental toxicity, as this study had the lowest NOAEL (i.e., most conservative) for this endpoint. In addition, this study included CBD exposure to both sexes starting prior to mating, continuing through weaning for females, and dosing offspring directly through postnatal day (PND) 42. This OECD Test Guideline 421 (OECD, 2016c) study was conducted according to good laboratory practice (GLP) and included extended postnatal dosing and hormone analysis (thyroid hormones and testosterone). Male and female rats were administered 0, 30, 100, or 300 mg/kg-bw/day hemp-derived CBD isolate (>99%) and a NOAEL of 100 mg/kg-bw/day was identified for female reproductive toxicity and neonatal toxicity. The most sensitive endpoint in offspring identified in the Henderson et al. (2023b) study was that of decreased pup body weight in both sexes of the highest dose group in the postnatal period. Many of the same endpoints evaluated in the Henderson et al. (2023b) study were also assessed in the prenatal toxicity study reported by Tallon and Child (2023) conducted according to OECD Test Guideline 414 (OECD, 2018a); however, this study included only prenatal dosing in females. While there were no significant changes in fetal litter weights observed in the Tallon and Child (2023) study, these findings may not be indicative a lack of postnatal body weight effects in offspring. For comparison, the body weights of male pups were not statistically different at birth but were significantly lower by PND 4 in the Henderson et al. (2023b).

Treatment-related mortality and decreased body weight in parental males and females were reported at 300 mg/kg-bw/day; severe maternal toxicity was associated with litter loss of some females in this dose group (Henderson et al., 2023b). Mean pup weights from the remaining three dams in the 300 mg/kg-bw/day group were lower than those of controls during the postnatal period. BMD modeling estimated a $BMDL_{0.5SD}$ of 118 or 121 mg/kg-bw/day for reduced male and female pup weight at PND 21, respectively (Supplemental Table S3 for full range of derived BMDLs). As the NOAEL from this study was lower than the BMDL, both values for pup bodyweight were carried forward as PODs.

Other findings in this study were considered but determined not to be suitable for POD development. Hepatocellular hypertrophy correlating with hypertrophy/hyperplasia in the thyroid gland and lower mean thyroid hormone levels were reported in parental males and females (100 and 300 mg/kg-bw/day). Hepatocellular hypertrophy without other changes in histopathology or clinical chemistry measures indicative of liver toxicity is considered adaptive and non-adverse, as described in a review by Hall et al. (2012). No concomitant increases in ALT or other markers of hepatobiliary damage were observed, and effects on triglycerides were not considered biologically significant, as they were not associated with lesions reflecting alterations in lipid metabolism in this study. The findings suggest induction of both phase 1 and phase 2 metabolic enzymes involved in thyroid hormone elimination, which is consistent with the increase in thyroid hormone clearance (i.e., reduced thyroid hormone levels) and thyroid follicular cell hypertrophy observed in this study (CDER, 2018b; Papineni et al., 2015; Noyes et al., 2019). Similar effects were reported in other studies with CBD (CDER, 2018a; Henderson et al., 2023a; Tallon and Child, 2023).

3.1.2.2. Candidate data selection from animal studies – subchronic oral toxicity. The present study also sought to identify a potential UL for dietary supplement use for individuals not pregnant/lactating or trying to conceive. As such, the most sensitive PODs from studies other than those evaluating reproductive toxicity were also considered. Two subchronic studies conducted according to OECD Test Guideline 408 (OECD, 2018b) were identified. As doses up to 140 mg/kg-bw/day were not associated with adverse effects in the study by Henderson et al. (2023a), the subchronic study reported by Tallon and Child (2023) was selected. In this study, male and female rats were dosed by oral gavage with 0, 30, 115, 230, or 460 mg/kg-bw/day of CBD. The test material was a hemp-derived broad-spectrum extract diluted in medium-chain

triglyceride (MCT) oil with a CBD content >97%.

Tallon and Child (2023) identified a NOAEL of 230 mg/kg-bw/day CBD for female rats based on a combination of effects, concluding that these findings may suggest dysfunction in mineralocorticoid production of the adrenal glands. The following effects were reported in females of the 460 mg/kg-bw/day group as supporting this NOAEL determination by the study authors: moderate/marked vacuolation of the adrenal gland zona glomerulosa, decreased serum electrolyte levels (sodium and chloride), increased urine volume, and decreased urine specific gravity. However, all noted clinical chemistry and urinalysis parameters were within their respective historical control values for the laboratory and were recovered following a 35-day recovery period (Product Safety Labs, 2022). Findings of adrenal vacuolation were also fully resolved after the recovery period; in addition, there was no evidence of cellular degeneration or necrosis within the affected regions. Vacuolization has been noted to be increased by chemicals that interfere with steroid synthesis and may represent the accumulation of cholesterol and other steroid precursors (Brändli-Baiocco et al., 2018). As described below, observed changes in this study related to cholesterol, low-density lipoprotein (LDL), and high-density lipoprotein (HDL), were considered non-adverse. Based on the totality of this information, it is plausible that the observed effects as described by Tallon and Child (2023) are not truly adverse but rather represent an adaptable response. However, despite being reversible, the large magnitude of change in cholesterol levels in females of the 460 mg/kg-bw/day could be concluded to be adverse and was, therefore, considered as a possible precursor for aldosterone production in the zona glomerulosa, one possible pathway related to mineralocorticoid production leading to the other observed effects (Ghafar, 2019; Rosol et al., 2001).

To present the most conservative approach to developing candidate limit values for CBD, adrenal vacuolation in females and the endpoints theorized to be associated with this finding (i.e., changes in serum cholesterol and electrolyte levels) were carried forward as PODs from this study. For serum cholesterol level, a $BMDL_{1SD}$ of 155 mg/kg-bw/day was derived based on the changes in serum cholesterol in female rats (see Supplemental Table S4 for full range of derived BMDLs). For comparison, effects that are potentially secondary to increases in serum cholesterol in this hypothetical pathway, such as vacuolation of the adrenal glomerulosa and changes in electrolytes (Cl and Na), were also considered (Supplemental Table S4). The selected $BMDL_{1SD}$ for chloride response in female rats was 310 mg/kg-bw/day; of note, all BMD estimates derived from models of the chloride responses in female rats were estimated to be higher than the maximum tested dose. BMDL estimates derived from models of the sodium responses in female rats showed model dependence; however, the best fit models estimated a $BMDL_{1SD}$ of 226 mg/kg-bw-day. Vacuolation of the adrenal glomerulosa occurred only in females and primarily in the high-dose group; however, the location of vacuolation was not quantitatively reported. Therefore, total incidence of adrenal vacuolation by dose level in females was carried forward for modeling purposes. To account for differing levels of severity in the adrenal gland vacuolation, the dose-response was modeled using CatReg; moderate or marked vacuolation was considered adverse as discussed in Section 3.1.2.2. Estimated BMD(L)s of 1350 (1280) mg/kg-bw/day and 910 (610) mg/kg-bw/day were calculated for the moderate and marked vacuolation, respectively. A BMD(L) of 550 (470) mg/kg-bw/day was estimated for mild vacuolation. Each of these estimated BMD(L)s was higher than the reported no-observable-effect-level (NOEL) of 230 mg/kg bw/day; therefore, the NOEL was also carried as the POD for adrenal vacuolation. This sensitivity analysis confirms that derivation of a POD based on increases in cholesterol in female animals is protective of possible downstream effects, including adrenal vacuolation and changes in serum electrolyte levels (Fig. 2). However, as all clinical chemistry parameters remained within the laboratory's historical control levels (Product Safety Labs, 2022) and all parameters were fully reversed following a recovery period, the POD derived from changes in serum cholesterol is an overly conservative estimate and likely overprotective.

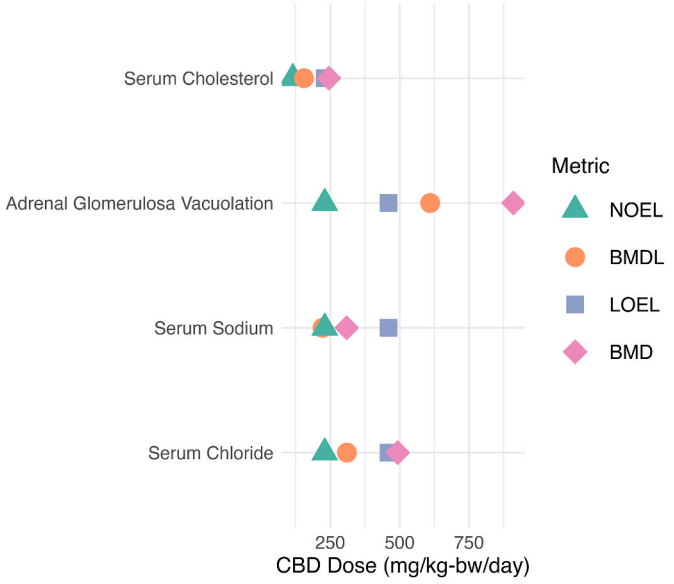

Fig. 2. Most sensitive endpoints selected for modeling from the selected 90-day oral toxicity study in female rats (Tallon and Child, 2023). Effects on serum cholesterol levels are shown relative to other potential downstream effects, including adrenal glomerulosa vacuolation and changes in serum electrolytes. BMD, benchmark dose; BMDL, lower limit; LOEL, low-observable-effect level; NOEL, no-observable-effect level.

Other findings in this study were considered but determined not to be suitable for POD development. Adrenal vacuolation in males was considered to be non-adverse, as it was fully reversible following the recovery period, was limited to the zona fasciculata, and was not associated with observed effects on serum cholesterol or electrolyte levels or urine parameters. Such lesions are proposed to represent the accumulation of cholesterol and other steroid precursors, which provides one possible explanation for these effects following exposure to CBD and which has been reported previously in laboratory rats administered cannabinoids (Dziwenka et al., 2020). In addition, adrenal cortical vacuolization is considered a background lesion in laboratory rats, and non-specific cytotoxic effects in the cortex have been reported following high doses of xenobiotics (Laast et al., 2014; Rosol et al., 2001). Observed hepatocellular hypertrophy, thyroid hypertrophy/hyperplasia, and decreased mean thyroxine (T4) levels (but not triiodothyronine [T3] or thyroid stimulating hormone [TSH]) are considered non-adverse, as described above for Henderson et al. (2023b). Finally, observed increases in HDL and LDL, and cholesterol levels, all in female animals, were not associated with lesions that reflect alterations in lipid metabolism in the liver; in addition, all levels were within the laboratory's historical control values (Product Safety Labs, 2022). All other observed changes, such as histopathological lesions observed in some organs, were reversible, unrelated to clinical chemistry changes, and/or otherwise determined not to be adverse.

3.2. Development of candidate limit values

To develop candidate intake limit values for consideration as a potential ADI for the general population and possible ULs for use in dietary supplements, candidate POD values derived for critical effects in human and animal studies conducted with CBD were divided by the following uncertainty factors, where applicable. A summary of the PODs and resulting candidate intake limit values is presented in Table 2.

The approach used was based on expert judgement and guidance available from authoritative bodies (IPCS, 2005, 2020; EFSA, 2012b; FDA, 2016; FSA, 2012). All animal-based PODs were divided by the

Table 2
Point of departure (POD) and array of candidate intake limit values from human and animal studies.

Population/ Species	Endpoint	POD Type	POD (mg/kg-bw/day)	Composite UF (unitless)	Candidate Limit Values (mg/kg-bw/day)	Use in ADI and UL Development Based on Population and Intended Use
Studies in Humans						
All participants	**Elevated liver enzymes/ DILI**	**NOAEL**	**4.3**	**10**[a]	**0.43**	**Selected for ADI for general population**
Adults	**Elevated liver enzymes/ DILI**	**NOAEL**	**14.2**	**10**[a]	**1.42**	**Selected as conservative UL for dietary supplement use by all healthy adults, except those pregnant/lactating or trying to conceive**
Studies in Animals						
Rat (male/ female)	Pup bodyweight	$BMDL_{0.5SD}$	120	100[b]	1.20	*Not selected*
Rat (male/ female)	**Pup bodyweight**	**NOAEL**	**100**	**100**[b]	**1.00**	**Selected as UL for dietary supplement use by all healthy adults**
Rat (female)	Cholesterol level	$BMDL_{1SD}$	155	100[b]	1.55	*Not selected*
Rat (female)	**Vacuolation of adrenal glomerulosa**	**NOEL**	**230**	**100**[b]	**2.30**	**Selected as alternative UL for dietary supplement use by all healthy adults, except those pregnant/lactating or trying to conceive**
Rat (female)	Serum sodium level	$BMDL_{1SD}$	222	100[b]	2.22	*Not selected*
Rat (female)	Serum chloride level	$BMDL_{1SD}$	310	100[b]	3.10	*Not selected*

Grey shading denotes endpoints considered as PODs based on one possible pathway leading to the observed effects in 300 mg/kg-bw/d females, which may be considered adaptive. See Fig. 2 for overview of findings based on these endpoints. Healthy adults in this study are defined as those without medical conditions or currently taking any medications. BMDL – benchmark dose lower confidence limit; DILI – drug induced liver injury; NOEL – no-observable-effect level; NOAEL – no-observable-adverse-effect level; POD – point of departure; UF – uncertainty factor.

[a] $UF_{intra} = 3$; $UF_{extrap} = 3$.

[b] $UF_{inter} = 10$; $UF_{intra} = 3$; $UF_{extrap} = 3$.

standard default factor of 10-fold to account for interspecies variation in toxicokinetics and toxicodynamics (UF_{inter}). Available data from human studies at very high doses of CBD show consistent clinical effects (toxicodynamics) across a broad range population characteristics, including sensitive subpopulations for effects on liver function. In addition, extensive toxicokinetic data from these studies and others also demonstrate that steady state is reached in approximately 3–4 weeks or less (CDER, 2018b; Crippa et al., 2021; Schultz et al., 2022; Taylor et al., 2018, 2020; Thai et al., 2021; Wheless et al., 2019), with a variation of about 3.5% in CBD concentration (Schultz et al., 2022). The observed variation between human subjects may reflect a prolonged half-life due to differences in metabolism, distribution, and/or accumulation in fatty tissues due to the lipophilic nature of CBD (Hosseini et al., 2021; Millar et al., 2018). The study by Shultz et al. (2022) provides key information on potential interindividual variability based on characterization using a three-compartment model, which accounted for the multi-compartmental pharmacokinetics of CBD, including distribution in fatty tissues. Despite the available data, a conservative three-fold uncertainty factor for intraspecies variation (UF_{intra}) was selected based on expert judgment and applied to PODs from human and animal data. Finally, as discussed above, steady state following repeated exposure is reached within ~4 weeks for CBD and only slightly longer for the major circulating metabolite in humans (CDER, 2018c; Taylor et al., 2018). Therefore, studies in adults and children ranging from 5 days to 26 weeks (Table 1) were considered to provide relevant information for assessing longer term exposure to CBD. However, as there may still be uncertainties associated with the dataset, all PODs were divided three-fold to account for extrapolation from subchronic to chronic (UF_{intra}) based on expert judgement. Of note, while WHO does not include a default uncertainty factor for extrapolation from subchronic to chronic exposure, EFSA (2012b) and FSA (2012) recommend a factor of two (2), and FDA (2016) guidance for New Dietary Ingredient Notifications (NDINs) recommends a 10-fold factor when human data are not available.

3.2.1. *Acceptable daily intake for the general population*

For deriving an ADI based on the data reviewed and methods employed in this study, candidate PODs were considered for human liver effects as determined for the general population (all study participants) and for effects on offspring body weight, as the most sensitive endpoint in laboratory animals (Table 2). Taking the lowest candidate value based on all participants in the Lo et al. (2023) meta-analysis, 0.43 mg/kg-bw/day (30 mg/day in adults) was selected for the proposed ADI.

3.2.2. *Recommended upper intake limits for dietary supplement use by healthy adults*

For the purpose of this study, "healthy adults" excludes those treated for or diagnosed with any medical condition, or currently taking any medications. The candidate value of 1.42 mg/kg-bw/day based on adults only from the Lo et al. (2023) meta-analysis was considered for this population (Table 2). However, reproductive toxicity observed rats (Henderson et al., 2023b) was more sensitive than the liver effects from human studies. The candidate $BMDLs_{0.5SD}$ for this endpoint conservatively assumes that the changes in pup weight at PND 21 are attributable to reproductive effects and not, at least in part, secondary to severe maternal toxicity. Additional viable BMD models estimated more conservative $BMDL_{0.5SD}$ values, some of which were derived at doses lower than the NOAEL of 100 mg/kg-bw/day (range of 66–122 mg/kg-bw/day, see Supplemental Table S3). However, these alternative models were not selected based on AIC, residuals, and visual fit. To consider the uncertainty in BMDL derivation, a range of candidate values is presented for reproductive toxicity based on the selected $BMDL_{0.5SD}$ of 120 mg/kg-bw/day and the study NOAEL of 100 mg/kg-bw/day (Table 2). Based on the most sensitive of the candidate values, 70 mg/d was selected as the potential UL for dietary supplement

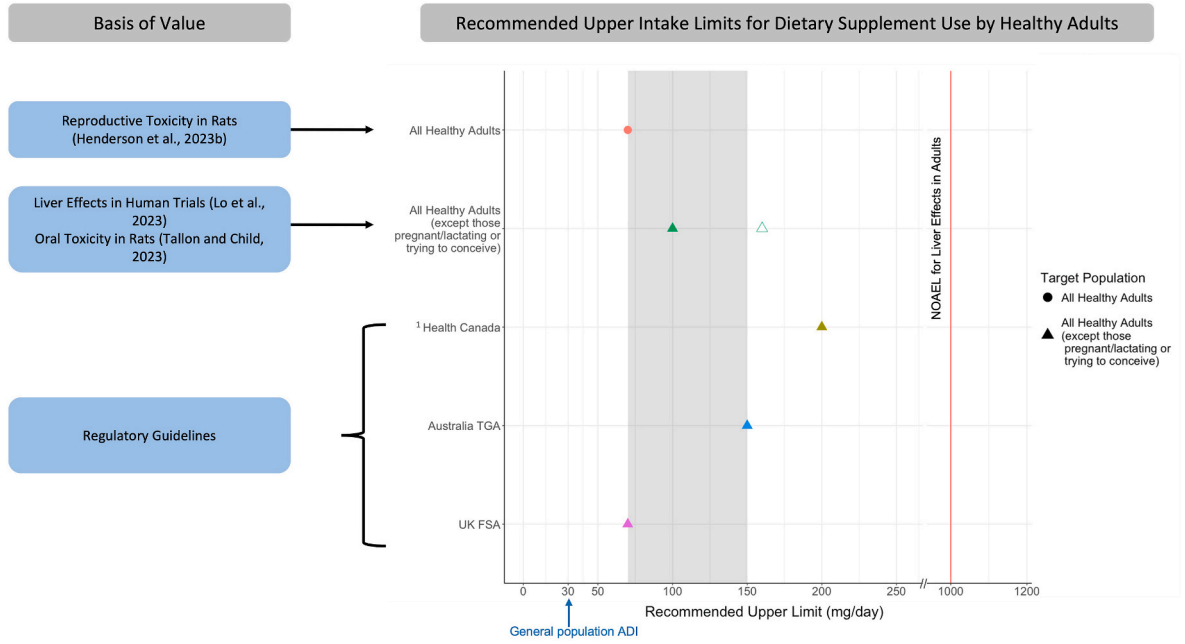

Fig. 3. Recommended upper intake limits (ULs) for dietary supplement use of CBD by healthy adults based on a default body weight of 70-kg. The potential values derived in this study are presented relative to those recommended by regulatory agencies for use in supplements, including food use for the UK FSA (Health Canada, 2022[1]; TGA, 2021; FSA, 2023). All limits are intended for use with warnings (e.g., labeling) for individuals taking medications or otherwise susceptible to liver effects. Grey shading denotes regulatory guidelines without associated limitations for duration of use. The red vertical line represents the NOAEL determined in this study and based on Lo et al. (2023) for elevated liver enzymes in adults from human clinical trials. As described in the text, the true NOAEL value for liver effects could be higher. Target population type is represented by symbol shape: square is for healthy adults except those trying to conceive, or those currently or planning to become pregnant or lactate; triangle is for all healthy adults. The open triangle represents a potentially higher UL based on animal data. The potential ADI for general population use developed in this study is presented for context. ADI, acceptable daily intake; NOAEL, no-observable-adverse-effect level; TGA, Therapeutic Goods Administration; UK FSA, United Kingdom Food Safety Authority.

use by all healthy adults (Table 2 and Fig. 3).

For dietary supplement products targeted for healthy adults except those trying to conceive (males and females) and those currently pregnant and/or lactating, additional labeling is recommended. For this population, data from the reproductive toxicity study were not relevant. Therefore, the value of 1.42 mg/kg-bw/day based on liver enzyme effects in adults only from Lo et al. (2023) was the most sensitive and is recommended as a conservative UL. However, given that the majority of individuals at risk for adverse live effects (e.g., those taking medications or with medical conditions) are not included in the target population, candidate PODs based on the potential pathway involving cholesterol levels, vacuolation of the adrenal glomerulosa, and serum electrolyte levels from the Tallon and Child (2023) study were also considered (Table 2). These data are presented together in Fig. 2. Given the lack of adverse effects, cholesterol levels alone were determined not to be appropriate as the basis of a UL. Therefore, the value of 160 mg/d based on the NOAEL as identified Tallon and Child (2023) was identified as a possible alternative UL, where there is confidence that the target population is not at risk for liver effects and/or is monitored by their physician (Tallon and Child, 2023).

4. Discussion

Sufficient data from human and animal studies were available to characterize endpoints associated with oral exposure to CBD, including liver effects in human clinical trials and reproductive and developmental toxicity in animal models. Possible oral intake limit values for three target consumer populations were developed based on an array of PODs derived from studies determined to be of the highest quality and relevance. It is anticipated that the recommended values described herein should be informative for risk assessors and regulators interested in characterizing human health hazards and determining recommendations for consumer intake of CBD.

Quality data from human studies is typically prioritized over animal data for risk assessment purposes, especially when the resulting value is more conservative than that derived from animal studies. The proposed ADI for CBD of 0.43 mg/kg-bw/day developed in this study is based on data from 28 human clinical trials, ~18 of which included sensitive subpopulations for the critical effect of elevated liver enzymes (Lo et al., 2023). By definition, an ADI is intended to apply to all individuals in the general population, including all age groups, physiological states, and pregnant and lactating individuals (IPCS, 1987, 2020). In adults, this equates to 30 mg/day of CBD ingested over a lifetime without appreciable health risk. It should be noted that applying the default uncertainty factors of 10x each for inter- and intraspecies variation, where relevant, as utilized by WHO (IPCS, 2005), or considering a margin of exposure of 100-fold relative to animal data is used in Generally Recognized as Safe (GRAS) in the US (US CFR Section 170.22) would have resulted in the same selected ADI value.

The potential ADI value derived in the present study differs from the health-based guidance value (HBGV) derived by Lachenmeier et al. (2023). In this publication, the authors sought to provide recommendations for risk management in the European Union until such time as the novel foods process is finalized. The final HBGV determined by Lachenmeier et al. (2023) of 0.14 mg/kg-bw/day (10 mg/day for a 70-kg adult) was based on the elevated liver enzymes observed in the single study by Crippa et al. (2021), where the dose of 300 mg/day was considered a NOAEL and divided by a total UF of 30 (3-fold for LOAEL to NOAEL extrapolation and 10-fold for intraspecies variation). In the present study, 300 mg/day as NOAEL was used as a POD for the ADI calculation based on the Lo et al. (2023) meta-analysis; however, the composite UF of 10 (3-fold for intraspecies variation and 3-fold for extrapolation to chronic) resulted in a calculated ADI of 0.43 mg/kg-bw/day. Also of note, a recent review published by Nyland and

Moyer (2022) concluded the available data are insufficient to establish a safe level of CBD consumption from food. While the authors stated that their review was "not intended to identify a specific safe dose for food", the study sought to "reveal enough data to inform dose quantities". For comparison to the present study, Nyland and Moyer (2022) considered only four human clinical trials and one rodent study with CBD, plus one feline study with a CBD mixture (50% CBD).

Dietary supplements can and should be marketed with specific labeling for intake; as opposed to food, this allows for consideration of special populations. The recommended ULs for dietary supplement use in the current study are intended for healthy adults, except those treated for or diagnosed with any medical condition, or currently taking any medications. A possible dietary supplement UL of 70 mg/day for CBD was developed for all healthy adults based on reproductive effects observed in an animal model. For products including additional labeling to exclude use by individuals trying to conceive (males and females) and those currently pregnant and/or lactating, a conservative UL of 100 mg/day for CBD is proposed based on liver effects in adults from human studies. However, an alternative UL of 160 mg/day could be considered, given that the target population should exclude those at risk for liver injury with proper labeling of dietary supplement products to include warnings for individuals taking medications or with medical conditions. All proposed ULs fall below or within the same range as those recommended by three regulatory agencies for CBD and are far below reported levels of adverse clinical liver effects in adults (Fig. 3). A recommended maximum daily intake of 70 mg/day for healthy non-pregnant, non-lactating adults from food and supplement sources has been maintained by UK FSA since February 2020 (FSA, 2023). In Australia, over-the-counter CBD-containing products are approved in adults up to a maximum of 150 mg/day, when provided by a pharmacist (without a prescription) (TGA, 2021). In addition, Health Canada (2022) has concluded oral doses from 20 to 200 mg/day to be safe and tolerable in healthy adults for short-term use only (a maximum of 30 days). In some cases, the reviews conducted to support these determinations included selection of critical effect levels from laboratory studies, and the application of uncertainty factors to calculate possible reference doses. However, it does not appear that these calculations were ultimately used to identify each agency's respective recommended daily maximum intake level. Rather, these recommendations appear to have been based on an overall assessment that considered effects observed at exposures in human studies, with contextual support from studies in animal models.

Overall, findings from the key study used to derive PODs for liver effects in human trials with CBD are consistent with those from other reviews (Arnold et al., 2023; Chesney et al., 2020; dos Santos et al., 2020; Souza et al., 2022). Similar effects on liver enzymes are not generally seen in laboratory studies with rodents or felines (CDER, 2018a; Kulpa et al., 2021). These differences may bring into question the relevance of animal data to human health risk assessment. While CBD metabolism involves the same pathways between humans and laboratory animal models, the major circulating compound after CBD ingestion in humans is the 7-COOH-CBD metabolite, whereas CBD is the most prominent circulating compound in rats (CDER, 2018a; CDER, 2018c; Deabold et al., 2019; Harvey et al., 1991). One possible explanation for the differences in effects on liver enzymes between species may be due to the differences in primary circulating metabolites. For example, available *in vitro* data suggest that 7-COOH-CBD may act directly on hepatic mitochondria to cause elevations in serum ALT (CDER, 2018b). In addition, a potential interaction between valproate and CBD at the mitochondria has been suggested as a possible mechanism for this effect (CDER, 2018b). This is an important observation given that the Lo et al. (2023) meta-analysis identified concomitant use of valproate as the only other risk factor for elevated enzyme levels (121/159 cases). Also of importance, elevated liver enzymes have not been observed in observational studies where consumers ingested typical dietary supplement levels of CBD (mean CBD isolate 50.3–63.6 mg/day) for 30-≥60 days (Kaufmann et al., 2021, 2022).

Liver enzyme effects in humans may be due to higher circulating levels of 7-COOH-CBD and therefore represent a mechanism different than that observed on the liver in rat studies. The observed hepatocellular hypertrophy in guideline studies with rats has been concluded to be adaptive, reversible, and non-adverse (CDER, 2018a; Henderson et al., 2023a, 2023b; Tallon and Child, 2023). In addition, it should also be noted that the liver effects in these studies may have been exacerbated by interactions of the vehicle oil and the CBD at the target liver sites. However, as can be the case with adaptive effects not associated with toxicologically significant findings, it was considered whether such effects may have become adverse at very high doses in females of the Tallon and Child (2023) study (Hall et al., 2012). While each of these parameters was fully reversible following a recovery period, these findings were conservatively used as candidate PODs for the current study. Of note, the lack of effects in male rats at this same dose is likely attributed to sex differences, where the bioavailability in females has been shown to be higher (MacNair et al., 2023; Moazen-Zadeh et al., 2023).

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 prominent metabolite (CDER, 2018a; Vaughn et al., 2020). Mixed results related to elevated liver enzyme levels have been observed in studies with canines using mixtures containing CBD and/or where CBD was co-administered with another compound, as well as at least one study using a CBD isolate (Mejia et al., 2021; Gamble et al., 2018; McGrath et al., 2018; Vaughn et al., 2020; Doran et al., 2021; Klatzkow et al., 2023). Upon review of the available data in canines, Ukai et al. (2023) concluded the following: "Three- and 6-month safety studies of chronic administration of CBD-rich product at 2 and 4 mg/kg/d showed safe administration with no alterations in [clinical blood chemistry] CBC and occasional rises in serum ALP as the primary observation in some dogs … This elevation suggests potential differences in hepatic cannabinoid metabolism and potential upregulation of cytochrome p450 metabolism … Clinically, rises in ALP concurrently with CBD use is not accompanied by rises in other liver-associated parameters such as [gamma-glutamyl transferase] GGT and bilirubin. Therefore, an ALP rise of this nature is often innocuous …"

The effects seen in the studies with rats were considered relative to observed effects on liver and/or thyroid parameters in studies with canines as well as monkeys and determined not to be toxicologically significant for the purposes of our study. In addition, the data available and included in this assessment from clinical trials provides the most relevant information to inform on potential liver effects in humans. In monkeys, relative but not absolute liver weights increased in males exposed orally to 30–100 mg/kg bw/day CBD for 90 days (Rosenkrantz et al., 1981). Liver weights were reported to be mostly within the normal range ("slightly elevated") after the recovery period. In addition, thyroid gland weights were reduced in male monkeys in this same study. However, there were no related histopathological or clinical chemistry changes observed in these animals. In a separate study in monkeys that also looked at thyroid hormone levels, T4 levels increased with CBD exposure for 90 days (Esber et al., 1979). This finding contrasts with results of the Henderson et al. (2023b) and Tallon and Child (2023) studies, which reported decreases in circulating thyroid hormone levels in some rats following CBD exposure. Nevertheless, findings from these studies may warrant further consideration when conducting an assessment on CBD.

Decisions made in the present study in the selection of toxicity data, critical endpoints (PODs), and uncertainty factors were based on regulatory precedence and application of expert judgment. As is the case with any assessment of this type, various uncertainties are inherent. For example, an important consideration is that not all dosing regimens (and subsequent POD development) can be directly compared between the studies reviewed as part of the current assessment. While all studies included used CBD isolate as the test material, a small number of the

human trials also administered other medications (e.g., AEDs, caffeine), which may have affected bioavailability of CBD in these studies. In addition, given that CBD is highly lipophilic, gastrointestinal absorption has been shown to be greater when administered with food or oils. The Lo et al. (2023) meta-analysis included some human studies with non-fasted individuals but also included studies with fasted subjects. Importantly, the toxicology studies selected as the basis for POD development in the current study administered CBD in edible oils (i.e., olive oil, MCT) to nonfasted animals. While these protocols for dosing were designed to increase the bioavailability of CBD to more adequately assess potential adverse effects, some uncertainty remains when comparing the results across all studies reviewed in the current assessment for the purposes of developing upper intake values such as an ADI.

Of note, the present study did not directly address some endpoints of concern raised by global regulatory agencies associated with CBD exposure, such as drug interactions. CBD is metabolized by CYP450, specifically the following isoenzymes: CYP3A4, CYP2C19, CYP2D6 and additionally by CYP1A1, CYP1A2, and CYP2C9 (Balachandran et al., 2021; Brown and Winterstein, 2019). CBD is transformed into both active (e.g., 7-OH-CBD, 7-COOH-CBD) and inactive metabolites following metabolism (Taylor et al., 2018). These metabolites are then further metabolized to inactive compounds and eliminated by the liver. Due to CBD being metabolized by a common pathway for metabolism of other drugs, it is important for future study to continue to understand the potential impact of CBD on other drug response and adverse effects. For example, if CBD is given with a CYP3A4 inducer, you will see a significant reduction in peak plasma concentration of CBD. If given with a CYP3A4 inhibitor, you can see a significant increase in peak plasma concentration of CBD (Balachandran et al., 2021; Brown and Winterstein, 2019). CBD inhibits CYP2C19 enzymes and therefore drugs metabolized by CYP2C19 have the potential to have blood levels elevated. In addition, fatigue and somnolence are reported side effects of CBD and could be compounded if co-administered with a central nervous system-active medication (pharmacologic drug interaction; Balachandran et al., 2021).

Many botanically-derived food and supplement ingredients are known to interact with CYP450s, thus having the potential for dug-botanical interactions (Sprouse and van Breemen, 2016). While the potential for such interactions exists with CBD consumption, the risk of such should be considered relative to the intended exposure and concomitant medication use. The drug interaction potential with oral CBD (Epidiolex) has been described following administration of doses above 750 mg twice daily or 20 mg/kg bw/day (Ben-Menachem et al., 2020; Morrison et al., 2019; VanLandingham et al., 2020). As discussed in Lo et al. (2023) and presented in Table 1, oral CBD can cause dose-related elevation of liver enzymes that generally resolve with discontinuation, dose reduction, and/or spontaneously. The intended population for dietary supplements in the present study is defined as healthy adults, and product labeling to exclude individuals taking medications may preclude concerns for potential drug interactions. For the general population, derivation of the ADI in the present study using standard risk assessment is considered to be protective of potential drug interactions, as the POD was based on markers of liver toxicity in human trials that included sensitive subpopulations, including those taking AEDs. Nevertheless, those taking any medications, including individuals taking oral contraceptives (i.e., ethinyl estradiol), should take potential interactions into consideration. While some clinical trials have included participants taking concomitant hormonal birth control (e.g., Taylor et al., 2020; Thai et al., 2021; Watkins et al., 2021), additional research is needed in this area. One clinical trial investigating the potential interaction between CBD and oral contraceptives has been completed; however, study results were not available at time of this publication (Ramanadhan, 2023).

The outcomes of the current assessment are applicable to hemp-derived CBD isolates and broad-spectrum extracts similar to the test articles in the reviewed studies, i.e., ≥95% CBD, and may include small fractions of other cannabinoids and/or terpenes. Given consumer interest in hemp extract-based products, application of the current study to broad-spectrum extracts with lower CBD content should also be considered. For example, the ULs developed in this study may be appropriately applied to extracts with >90% CBD content, especially if the toxicological profile of other constituents are included in the overall safety assessment and can minimize uncertainty associated with the non-CBD fraction. In addition, data from other safety studies or human trials can be used to provide additional corroborative information to support safe use of these products.

This state-of-the-science assessment meets an urgent need to provide guidance to regulators and other entities seeking to provide recommendations for consumer use based on the currently available data. The hazard assessment, dose-response modeling, and development of limit values in the present study were based on the most robust dataset available in the public literature and the methods employed by the authors. This assessment can be refined as additional data become available, in particular, human clinical trials with lower doses of CBD and pre-clinical study data on the potential developmental neurotoxicity of CBD. The recommendations herein represent possible ADI and UL values based solely on the authors' assessments and do not reflect any regulatory guidelines. Different conclusions may be reached by regulators or other risk assessors that have access to additional high-quality data, such as those used to support the Epidiolex submission or other proprietary data, as well as on the approaches used.

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CRediT authorship contribution statement

Rayetta G. Henderson: Conceptualization, Methodology, Investigation, Writing – original draft, Writing – review & editing, Supervision, Project administration. **Melissa Vincent:** Methodology, Formal analysis, Writing – original draft, Writing – review & editing. **Brianna N. Rivera:** Visualization, Writing – review & editing. **Marcel O. Bonn-Miller:** Resources, Funding acquisition, Review. **Candace Doepker:** Methodology, 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 and Charlotte's Web, Inc. Author MOB-M was a previous employee of Canopy Growth Corporation, receiving stock options during employment, and is currently (during the conduct and drafting of this study) an employee of Charlotte's Web, receiving stock options during employment. MOB-M also served on the Board of Directors for AusCann Group Holdings Limited and currently serves on the Board of Directors for DeFloria, LLC. To limit any real or perceived bias, neither MOB-M nor related funding bodies were involved in the selection of studies, analysis of data, or determination of derived limits reported herein. ToxStrategies, a private consulting firm providing services on toxicology and risk assessment issues, received funds for conducting this work. Authors RH, MV, BR, and CD were employees of ToxStrategies during the conduct and drafting of this study; no personal fees were received.

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.yrtph.2023.105482.

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