Short Communication

Using the BMD Approach to Derive Acceptable Daily Intakes of Cannabidiol (CBD) and Tetrahydrocannabinol (THC) Relevant to Electronic Cigarette Liquids

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Abstract

Background: In the past 60 years, *Cannabis sativa* L. has been an object of increasing interest because of the psychotropic effects of some of its constituents. These effects mainly arise from the cannabinoid Δ^9-tetrahydrocannabinol (Δ^9-THC). *C. sativa* species also synthesize and accumulate the non-psychotropic compound cannabidiol (CBD). Due to their therapeutic potential, both cannabinoids are an object of medical research and drug development. More recently, CBD has received increasing interest as an ingredient in electronic cigarette liquids (e-liquids). This trend may have been reinforced by health and disease-related claims, often based on clinical studies, which are used to advertise CBD. CBD liquids may be based on full-spectrum hemp extracts, CBD isolates, or synthetic CBD, all of which may contain some residual levels of Δ^9-THC from either natural content (in the extracts) or from possible degradation of CBD to Δ^9-THC, which may occur during storage. There is uncertainty about safety regarding the consumption of CBD (and Δ^9-THC) in e-liquids. The aim of this publication was to present an approach for a toxicological risk assessment of CBD and Δ^9-THC relevant to e-liquids by using the benchmark dose (BMD) approach. **Materials and Methods**: Before an analysis to estimate a reference dose (RfD) for both cannabinoids, a systematic review of dose-response data was conducted. The data obtained were analyzed using the BMD approach to derive a benchmark dose lower confidence limit (BMDL). The BMDL was used as a point of departure to estimate the RfD. **Results**: No adequate human data suitable for dose-response modeling were identified. Based on animal data, the RfD values for the most sensitive endpoints were selected. For CBD, an RfD for acute exposure of 1 mg/kg body weight (bw) was estimated. For Δ^9-THC, an acute RfD was found to be 0.006 mg/kg bw. Additionally, the RfD for chronic exposure to CBD was estimated to be 4 mg/kg bw per day. The respective endpoints for CBD were a reduction in norepinephrine turnover and a reduction in uterus weight. The endpoint for Δ^9-THC was a change in blood pressure. **Conclusions**: Because of the limited availability and quality of dose-response data, it cannot be excluded that the estimated RfD values might be afflicted with considerable uncertainties. Therefore, it is recommended to conduct further research on dose-response data, preferably from human studies.

Keywords: cannabis; cannabidiol; Δ^9-tetrahydrocannabinol; CBD; Δ^9-THC; e-cigarette; e-liquid; inhalation; risk assessment; benchmark dose; toxicology

1. Introduction

During recent years, cannabinoids such as cannabidiol (CBD) and Δ^9-tetrahydrocannabidiol (Δ^9-THC) in particular have faced increasing research interest, due to several beneficial effects attributed to the compounds [1]. Cannabinoid formulations are currently used as a therapeutic approach for treating neuropathic disorders, such as severe forms of treatment-resistant epilepsy [2,3]. In 2018, the prescription anticonvulsant Epidiolex, a drug containing CBD as the pharmacologically active compound, was approved by the Food and Drug Administration (FDA) in the United States of America [4]. Since then, the drug has been used to treat the Lennox-Gastaut syndrome (LGS) and the Dravet syndrome (DS) in children [5]. In addition to the reported antiepileptic effects of CBD, anxiolytic, antipsy-

chotic, and anti-inflammatory effects have been reported in humans [2,6]. Further potentially beneficial effects have been mentioned in several controlled clinical trials, which investigated the therapeutic effects of CBD preparations administered to patients suffering from schizophrenia [2].

While Δ^9-THC directly interacts with the endocannabinoid receptors CB1 and CB2, molecular interactions of CBD occur only with low affinity at those targets [7,8]. A major benefit of the administration of CBD-based drugs is a low incidence of adverse events in patients and volunteers in randomized controlled trials (RCTs) [9].

Different modes of action (MOAs) for CBD have been elucidated. CBD interacts with multiple cellular pathways. For example, through interaction with cytochrome P450 metabolic pathways, CBD can increase effects of Δ^9-THC

[10]. Other target receptors for CBD are TRPA1, as well as TRPV1/V2 and TRPM8, which are involved in the nociceptive system.

CBD is further known to increase beneficial effects of THC observed in studies co-administering CBD and Δ^9-THC [11]. The drug Nabiximols is used in the oromucosal spray Sativex to treat spasticity and pain in multiple sclerosis and contains CBD and Δ^9-THC in equal amounts. Sativex delivers 2.7 mg Δ^9-THC and 2.5 mg CBD per application [12]. Administration of both compounds in combination allows for administering greater amounts of Δ^9-THC to patients [2].

Due to strict legislative regulations in most countries and its analgesic effects, Δ^9-THC is primarily administered as the prescription drug dronabinol during chemotherapy in cancer treatment as an antinociceptive and antiemetic agent and as an antiemetic in patients being affected by AIDS [13–15].

In the last decade, the use of electronic cigarettes (e-cigarettes) has experienced a significant increase in many countries as an attractive alternative to smoking. E-cigarettes or vaporizers are deemed a reduced risk alternative to conventional cigarettes and have found a wide application as a substitute delivery system. The devices operate by heating so-called e-liquids until vaporization. The aerosol is inhaled by the consumer. E-liquids contain dissolved aroma compounds in an organic matrix such as glycerol and propylene glycol [16]. Recent cases of e-cigarette associated lung injury (EVALI) raised concerns about the safety of e-cigarettes. In 2020, 2807 cases of EVALI have been reported, resulting in the deaths of 68 consumers in the United States [17]. In other countries e-cigarette associated cases of hospitalization and even deaths have been reported [18]. Some mechanisms leading to EVALI have been investigated in animal models, such as oxidative stress and inflammatory reactions in lung epithelia resulting in a damaging of alveolar tissues and cells [19], which is assumed to be the result of the accumulation of vitamin E acetate in alveolar tissue.

The beneficial effect of CBD concerning bone health is subject to current research. CBD preparations were found to reduce bone loss and promote bone healing in rats [20,21]. However, it needs to be elucidated, whether exposure to CBD via inhalation of CBD liquids has the same effect on humans. This is relevant concerning the discussed osteotoxic effect of nicotine and flavoring agents in e-liquids on bone integrity [22].

E-liquid preparations containing the non-psychotropic CBD are commercially available. In the European Union (EU), e-liquids containing CBD are not necessarily legal for sale because CBD may be a compound not in compliance with Art. 7 No. 6a of directive 2014/40/EU, which prohibits the placing on the market of tobacco products, which contain additives that create the impression that a tobacco product has a health benefit [23]. Due to narcotic laws in most countries, e-liquids are also not allowed to exceed certain thresholds of Δ^9-THC, which vary between member states of the European Union and the United Kingdom [24]. Despite regulatory difficulties, a wide variety of commercially available e-liquids containing CBD is available on the market in different concentrations and the popularity of e-cigarettes has offered cannabis users new ways of consuming cannabis and its preparations [25].

This study aimed to conduct a benchmark dose modeling for CBD and Δ^9-THC in order to find thresholds for an acceptable level of the 2 compounds in e-liquids.

2. Materials and Methods

2.1 Literature Research

The first step in this study was to obtain suitable data from the scientific literature. Only *in vivo* data from studies in different animal models and human data were considered appropriate. The accepted animal models were mice, rats, and rhesus monkeys. The data acquisition was carried out using the Google Scholar search engine, the PubMed database of the National Library of Medicine, and the Cochrane Library, as well as additional online resources such as databases provided by publishers, including Nature and Science.

The major search terms and keywords used are listed below. To complement the data acquisition, combinations and variations of search terms and keywords were used as well. Slashes separating keywords in the list below indicate, that the separated keywords were used individually or in combination with each other.

Search terms and keywords:
• toxicity of CBD/Δ^9-THC
• acute/chronic toxicity of CBD/Δ^9-THC
• acute/chronic toxicity of CBD/Δ^9-THC
• CBD/Δ^9-THC inhalation in rats/mice/monkeys/humans
• vaporized CBD/Δ^9-THC in rats/mice/monkeys/humans
• oral CBD/Δ^9-THC in rats/mice/monkeys/humans
• intravenous (i.v.) CBD/Δ^9-THC in rats/mice/monkeys/humans
• bioavailability of oral and inhaled CBD/Δ^9-THC in rats/mice/monkeys/humans
• randomized controlled trials for CBD/Δ^9-THC administration in humans
• combinations of the search terms and keywords mentioned above with author names

A further research strategy, which has been found useful, was to directly search for publications found in references of studies and reviews.

To be included, the literature data had to meet several criteria. A study considered for inclusion in this research had to have administered at least 3 different doses and a control group receiving vehicle. Dose spacing was not considered relevant. Furthermore, applied doses had to be administered in mg/kg of body weight. Studies reported to have administered only fixed doses of CBD or

Δ^9-THC without considering the respective subject's body weight were excluded, as precise information about dosage was preferred. The number of animals per dose group had to be declared. Studies reporting concomitant treatment with other compounds were not included.

The reported results were expected to be presented as a mean effect with a standard deviation. Alternatively, the standard error of a dose group could be converted into a standard deviation by multiplying the value of the error with the square root of the number of animals in the respective dose group.

The test substances that were used had to be as pure as possible. The results of formulations with combinations of CBD with Δ^9-THC were considered unreliable. Studies that investigated the effects of mixtures such as hashish or marihuana preparations (smoked cannabis) were excluded. Studies in humans that received concomitant medication were also not included.

Data, which were only available plotted in diagrams, were included if data points for mean effect values and standard deviations were distinguishable from other plotted values. For numerical retrieval of plotted data, a dedicated plugin for ImageJ image processing software Version 1.8.0 (National Institutes of Health, Bethesda, MD, USA) was applied [26].

2.2 Benchmark Dose Modeling

In toxicological risk assessment, the benchmark dose (BMD) approach is a more advanced statistical method than the established No Observed Adverse Effect Level (NOAEL) approach [27]. The BMD approach was first introduced by Crump [28] as an alternative method to NOAEL.

The benchmark dose (BMD) is defined by the United States Environmental Protection Agency (EPA) as the exposure level that corresponds to a percentage increase (usually 5 or 10%) in the probability of an adverse event (response) compared to a control scenario with no exposure [27]. This change in response to an exposure is called a benchmark response (BMR). Therefore, a BMR of 5% would be defined as a 5% increase in the number of subjects with an incidence of an adverse event. Benchmark models provide not only a BMD but also a confidence interval, which contains the value of the respective BMD. The limits of the confidence intervals obtained are called the benchmark dose lower limit (BMDL) and the benchmark dose upper limit (BMDU). In toxicological risk assessment the BMDL is used for estimating a reference value for consumer safety.

The BMD and its respective confidence interval, with the BMDL being the Point of Departure (POD), are calculated by fitting multiple statistical models. The benchmark dose software (BMDS), which has been developed by the EPA [29], performs automated fitting of selected models to dose-response data retrieved from toxicological studies.

The output delivered by the software contains the results of the models, namely, the BMD, the BMDL and the BMDU, with a recommendation for the most suitable model. The most suitable model is determined by comparing the Akaike information criteria generated in the output.

Before a BMD analysis could be carried out by using the BMDS, settings needed to be adjusted in the main workspace window. The data type obtained from publications included in this assessment was found to be continuous. For the benchmark response, the default setting for continuous data of one standard deviation (1SD) was selected.

2.3 Dose-Conversion and Route-to-Route Extrapolation for Inhalation Exposure from Animal BMDLs

To obtain RfD values for inhalation in humans, a concept has been designed for converting animal BMDLs into RfD values. This was an important step because most BMDLs considered reliable were obtained from analyzing dose-response data from animal studies in other routes besides inhalation (i.e., oral or i.v.). The conversion is carried out in several steps.

Initially, route-specific RfDs in humans have been calculated from animal BMDL for the same route by using an uncertainty factor (UF) of 10 to account for interspecies variability and an additional UF of 10 to account for intraspecies variability.

$$RfD_{human,route} = \frac{BMDL_{animal,route}}{UF_{interspecies} * UF_{intraspecies}}$$

This equation yields an RfD for exposure in humans by the same administration route as used in the animal model. The next step calculates a theoretical RfD for the same dose-dependent response after an intravenous (i.v.) administration in humans ($RfD_{human,i.v.}$). By definition, the intravenous administration of a chemical compound results in systemic bioavailability (F) of the substance of 100%, which means, that 100% of the injected compound is available in a subject's blood circuit [30]. This means that the concentration value of the $RfD_{human,i.v.}$ can equal the plasma concentration of a substance that is injected intravenously. The $RfD_{human,i.v.}$ can be calculated by dividing the previously determined $RfD_{human,route}$ by the route-specific bioavailability in humans designated here as $F_{human,route}$ for the same route the test substance was administered in the animal model:

$$RfD_{human,i.v.} = \frac{RfD_{animal,route}}{F_{human,route}}$$

This step is necessary because it allows one to calculate the final estimated RfD for inhalation in humans ($RfD_{human,inhal.}$). The $RfD_{human,inhal.}$ can be determined by dividing the $RfD_{human,i.v.}$ by the bioavailability for a sub-
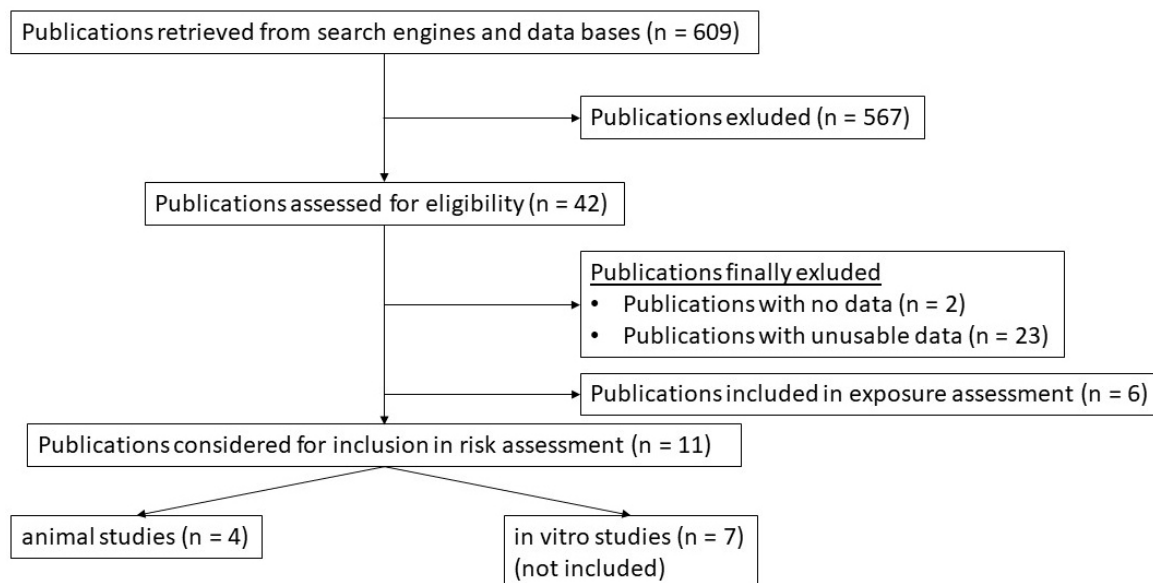
Fig. 1. Flow-chart diagram of all studies reviewed for this risk assessment. Publications were finally included after published dose-response data were considered suitable for a risk assessment using the BMD approach.

Table 1. Bioavailability of CBD and Δ^9-THC in humans after oral administration and inhalation.

Test substance	Administration route	Bioavailability	Reference
CBD	oral	19%	Mechoulam *et al.* [6]
	inhalation	31%	Lucas *et al.* [31]
Δ^9-THC	oral	20%	Grotenhermen *et al.* [1]
	inhalation	37%	Lunn *et al.* [32]

stance after inhalation in humans:

$$RfD_{human,inhalation} = \frac{RfD_{human,i.v.}}{F_{human,inhalation}}$$

This approach allows for the estimation of an RfD for inhalation in humans from BMDLs derived from animal models for different administration routes and can also be used to convert applied doses, documented in the literature, to doses for inhalation, which would lead to the same observed adverse effect. This facilitated a comparison of the estimated RfDs with the RfDs in the literature. To perform this approach for extrapolation, the values of the respective bioavailability for each route need to be known. The respective bioavailability values for human exposure to CBD and Δ^9-THC were retrieved from literature and can be found in Table 1 (Ref. [1,6,31,32]). The table shows the values of the respective bioavailability obtained from the references. When a range between 2 values was presented, the highest value was selected.

3. Results

Before conducting this risk assessment, a literature review was performed to obtain usable dose-response data (Fig. 1). Data that met the inclusion criteria were included

for a BMD analysis using the benchmark dose software (BMDS) provided by the EPA [29]. A total of 609 publications were screened for usable dose-response data, of which 567 publications were excluded. Of the remaining 42 studies, 2 studies did not contain dose-response data and 23 studies had no usable dose-response data. Six studies were included for exposure assessment and have been used to develop the dose conversion model, which has been introduced before. Among 11 studies that were considered to be used for a risk assessment using BMD modeling, 4 animal studies contained sufficient dose-response data for a BMD analysis.

This article presents the lowest BMD and BMDL values obtained by conducting BMD analyses with dose-response data from animal studies that administered CBD or Δ^9-THC via different administration routes. The results obtained from the BMD analysis of the included dose-response data are presented in Table 2 (Ref. [33–35]). The lowest BMDLs for long-term inhalation of CBD in humans was found to be approximately 26 mg/kg bw (*p*-value: 0.7). The BMDL was derived from an oral-study by Marx *et al.* [34], who administered CBD preparations in rats for 90 consecutive days. The observed endpoint was a change in the weight of the uterus. A single acute BMDL of 4.3 mg/kg bw was derived from a study by Steger *et al.* [33] (*p*-value:

Table 2. Results of the BMD modeling of data obtained from animal studies in rats.

Author	Model	Study type	Compound	Route of administration	Doses [mg/kg bw]	Endpoint	BMD	BMDL	p-value [a]
Marx *et al.* [34] *[b]	rats	90-day study; daily administration	CBD	Oral	0 (Control), 100, 360, 720	Uterus weight	79	26	0.7
Steger *et al.* [33]	rats	acute	CBD	Single oral dose	0 (Control), 0.1, 1, 10	Norepinephrine turnover	6.8	4.3	0.06
Siqueira *et al.* [35] *[b]	rats	acute	Δ^9-THC	Intravenous	0 (Control), 1, 2, 5, 10	Change in mean arterial blood pressure	3.05	0.22	0.4

[a] The *p*-values provide information about accuracy of predicted BMD models. A *p*-value greater than 0.1 indicates that the model fits the data (*p*-value 1.0 = perfect fit).

[b] Asterisks (*) indicate that the authors reported some statistically significant responses among their published dose-response data, which result from the administration of the corresponding doses.

0.06). The respective endpoint was found to be a change in norepinephrine (NE) turnover. This BMDL was derived from a study by Steger *et al.* [33] who administered oral CBD ranging from concentrations of 0 mg/kg bw (Control) to 10 mg/kg bw to rats. A BMDL of acute exposure to Δ^9-THC has been derived from Siqueira *et al.* [35] who injected solutions of Δ^9-THC in mice with a dose range of 0 mg/kg bw to 10 mg/kg bw. The observed adverse effect was a change in mean arterial blood pressure at a BMDL of 0.22 mg/kg bw (*p*-value: 0.4).

4. Discussion

4.1 Dose-Response Modeling of CBD and Δ^9-THC

During research for retrieving published data for deriving an RfD, different toxicological endpoints regarding physiological and psychological parameters were considered. However, not all parameters are considered equally relevant. To achieve an appropriate evaluation of the safety of CBD and Δ^9-THC, toxicological endpoints were prioritized. Endpoints of higher clinical importance were considered changes in blood pressure, heart rate, blood parameters, cell titers in blood specimens, endocrinological parameters, as well as changes in organ weights, as these findings might indicate long-term health effects for consumers. The endpoints of lesser significance are supposedly changes in nociception, body temperature, and food intake. Another prioritization refers to the experimental model and administration route of the compounds. Per definition, dose-response data retrieved from human studies (e.g., RCTs) is considered to be more reliable than data from animal models. Furthermore, data from inhalation and i.v. application of both compounds are preferred over RfD values retrieved from studies using oral administration.

This study might present one of the first approaches for a risk assessment for CBD and Δ^9-THC in e-liquids. From 11 publications included in this risk assessment, 4 studies provided dose-response data, which were considered to provide usable BMDL values. Depending on the respective toxicological endpoint, the RfD values obtained

clearly differ for both CBD and Δ^9-THC.

This finding indicates different susceptibilities of tissues and organs to inhaled CBD and Δ^9-THC. Comparison with the literature referred to in this publication supports this observation, as tissue or organ-specific dose-depending responses have been reported. The results for CBD suggest that blood parameters, hormone levels and enzyme titers are in general less susceptible to CBD than changes in physiological parameters [34].

Uterus weight in animal models was altered by a relatively low dose of CBD (RfD 4 mg/kg bw). This endpoint may be relevant, as hormones and therefore hormone producing glands, tissues and organs have an essential function in the development of an organism, especially during prenatal development and adolescence.

From Steger *et al.* [33], an RfD for Δ^9-THC of approximately 1 mg/kg bw for a reduction in plasma FSH has been derived. Lower levels in plasma FSH induced by surpassing a critical dose of Δ^9-THC might be responsible for a reported reduction in fertility in both males and females. This finding might be a cause for a reduction in human fertility most likely due a reduction in reproductive hormones when Δ^9-THC is consumed extensively over a long period. However, Δ^9-THC induced effects on sex hormones have been reported to be reversible [36]. One reported mechanism, which could be responsible for the observed reduction in plasma FSH, is caused by Δ^9-THC acutely inhibiting the release of gonadotropin-releasing hormone (GnRH) [37,38] in the hypothalamus. GnRH stimulates the production of FSH and LH. The release of FSH and LH is responsible for the secretion of testosterone in males and estradiol and progesterone in females. This effect is probably mediated by CB1 receptors, which have been found in the hypothalamus and gonad cells [37,39,40].

Another effect observed after Δ^9-THC exposure is an increase in (mean) blood pressure. According to the findings in this study, a change in mean blood pressure can be observable after surpassing an estimated RfD of approximately 0.006 mg/kg bw [35]. For a consumer with a body weight of 70 kg, this would be equivalent to a total intake

of 0.42 mg Δ^9-THC per administration session. In contrast to this finding, Johnson *et al.* [41] reported a significant increase in systolic and diastolic blood pressure in human subjects who inhaled more than 10 mg Δ^9-THC in marijuana cigarettes. Solowij *et al.* [42] reported a significant increase in blood pressure in human subjects who inhaled 8 mg of Δ^9-THC by vaporization, which would equal an intake of approximately 0.1 mg/kg bw assuming a body weight of 70 kg. In a study by Friedman *et al.* [43], the authors have found, that a total of approximately 0.25 mg/kg (about 0.1 mg/kg bw) of Δ^9-THC administered intravenously to dogs had no significant effect on blood pressure. Converting this dose into an inhaled dose for humans by considering an uncertainty factor of 100 and bioavailability for inhalation of 37% would yield an estimated inhalation dose of approximately 0.007 mg/kg bw of Δ^9-THC in humans. Note that these exemplary values are not actual RfD values, which were derived from points of departure but only serve as a comparison for the estimated RfD.

Data used for estimating an RfD for inhalation of 0.04 mg/kg bw Δ^9-THC in rhesus monkeys [44] was unfortunately not based on statistically significant dose-response data but was still included in the discussion because a change in thymus weight may result in altered endocrinological parameters, which could lead to serious adverse events.

4.2 Comparison with Previous Assessments of CBD and Δ^9-THC in the Literature

In 2015, the EFSA postulated an oral ARfD for Δ^9-THC of 0.001 mg/kg bw using an UF of 30 with an increase in pulse rate as one of the respective endpoints [45]. Converting this dose for a 70 kg person into an (A)RfD for inhalation considering an oral bioavailability of Δ^9-THC of 20% [1] and bioavailability for inhalation of 37% [32] by route-to-route extrapolation would deliver an acceptable dose of 0.135 μg/kg bw or 0.95 μg/d for a 70 kg person, respectively.

A published review of risk assessments [46] derived a LO(A)EL of 2.8 mg/d for cannabis inhalation from a study by Ramaekers *et al.* [47]. After conducting a dose conversion, the theoretical NOAEL would yield an RfD for the inhalation of cannabis of a total of 0.028 mg/d. In comparison, the RfD for inhalation of pure Δ^9-THC estimated from Siqueira *et al.* [35] for a 70 kg person would be 0.42 mg/d according to the results of the BMD modelling presented in Table 2, which has been obtained after a dose conversion.

It is shown above that the RfD for Δ^9-THC estimated from a BMDL was found to be above the dose values retrieved from literature and adjusted to (A)RfD values for inhalation for comparison [45,47]. It is advisable to prefer the oral (A)RfD of 0.001 mg/kg bw from the EFSA study because EFSA provided an already determined ARfD, which only needs to be adjusted by conducting a dose conversion for a 70 kg person into an inhalation dose and extrapolated

by using the same method applied for the BMDLs. Furthermore, EFSA is the only agency that mentioned an increase in heart rate as one of the observed endpoints.

In conclusion, the RfD of 0.006 mg/kg bw, which has been derived from dose-response data published by Siqueira *et al.* [35], was found to be less conservative than the (A)RfD derived from EFSA, which might be due to the respective BMDL (if converted to an oral BMDL) resulting from a BMD analysis with a 5% confidence interval.

It is recommended to further investigate whether a $BMDL_{10}$ would deliver a more appropriate RfD, when more dose-response data are available.

The BMDL and RfD values estimated for hematological endpoints and endpoints regarding clinical chemistry observations were found to be on average much higher than the acute and chronic BMDL and RfD values found for more susceptible endpoints. This observation indicates that the respective organs and systems were less susceptible to CBD. Although endocrinologically active organs were found to be more susceptible to CBD, considerable deviations in estimated BMDL and RfD values for endocrinologically active organs were observed during this study. Endpoints that indicate high tolerability of CBD were assumed to be unreliable and were excluded from the further evaluation because adverse effects have already been observed at significantly lower doses of CBD in the literature [34]. Devinsky *et al.* [3] reported elevated levels of aspartate (AST) and alanine aminotransferase (ALT) in more than 30 seizure patients who received an oral dose of 10 or 20 mg/kg bw of CBD per day in a 14-day trial [3]. For comparison with the RfD values obtained from the BMD analysis of dose-response data obtained from Marx *et al.* [34] for the respective endpoints, the oral dose of 10 mg/kg bw of CBD could be converted into an inhalation dose of approximately 1.7 mg/kg bw. In comparison an RfD of 107 mg/kg bw for AST-levels was derived from Marx *et al.* [34] by using the BMD approach. Other adverse events reported in this study were diarrhea, vomiting, mild upper respiratory tract infection, and pyrexia. CBD concentrations in the study by Devinsky *et al.* [3] were far below the corresponding RfD values estimated by Marx *et al.* [34]. This discrepancy might be due to the use of different test subjects. As Devinsky investigated administration in humans [3], it should be assumed that humans have a higher sensitivity to CBD than rats. It appears in this case that the used uncertainty factor of 100 did not account for this difference in sensitivity.

For CBD, some limited NOAEL values have been published. The study by Marx *et al.* [34], which was also included in this risk assessment, derived an oral NOAEL in hemp extract of 100 mg/kg bw in male rats, which can be converted into an RfD for inhalation of 17 mg/kg bw in humans, which corresponds to a NOAEL for CBD of approximately 1 mg/kg bw after dose conversion. In 2019, the European Medicines Agency (EMA) [48] published a

NOAEL for i.v. application of CBD in beagle dogs of 15 mg/kg bw/d for hepatological endpoints in a 14-day study, which can be converted via dose conversion to an RfD of approximately 0.5 mg/kg bw/d. In comparison, the lowest RfD estimated from the study in rats by Marx *et al.* [34] was 2 mg/kg bw for a statistically significant change in liver weight. The BMD model might therefore estimate a larger BMDL to be a more appropriate POD than NOAEL values obtained from literature. To account for consumer safety, it is recommended to prefer the more conservative NOAEL published by EMA [48] or the NOAEL from Marx *et al.* [34] as a more reliable POD over the BMDL derived in this study.

Unfortunately not enough adequate dose response data could be retrieved from online sources in ordert to conduct BMD modelling for further toxicological endpoints like local (airway) effects or cognitive and psychological effects. Most animal studies retrieved for this BMD analysis only reported acute systemic effects. A change in uterus weight in rats [34] was the only long-term effect reported in this study, as this was found to be the lowest RfD for a relevant endpoint.

It should be emphasized that this comparison between extrapolated RfDs from BMDL values and published PODs or dose-response data in humans and animals can only represent an attempt to elucidate, whether BMD modelling of the data available provided RfD values, which may be comparable to literature data. Therefore, RfDs obtained in this study should not be considered as alternative doses to already established threshold doses of CBD or Δ^9-THC. In order to obtain reliable threshold doses, BMD modelling using more appropriate dose-response data is recommended.

4.3 Initial Risk Assessment of E-Liquids

To suggest an initial approach for a risk assessment of CBD applicable to e-liquids, a theoretical consumption scenario will be considered. E-liquids contain different amounts of CBD in a glycerol/propylene glycol matrix. During our analyses, e-liquids with the highest concentrations of CBD typically contained about 100 g/L CBD in a 10 ml container. The consumer safety of these products should be assessed in a hypothetical consumption scenario with the following considerations:

The pulmonary system will be exposed to the entire amount of CBD contained in the e-liquid, and the hypothetical consumer will be exposed to an entire 10 mL of CBD-liquid. Exposure to 10 ml of e-liquid per day was also assumed in this case, considering the worst-case exposure to vaping e-liquids ranging between 5 and 25 mL/day.

When considering bioavailability after inhalation of 31% [31], a total of approximately 31 mg CBD should reach the blood circuit of consumers. With an assumed average body weight of 70 kg, a human would inhale approximately 1.43 mg/kg bw/d CBD.

The amount of CBD in the respective e-liquid would

exceed the lowest estimated RfD for inhalation of approximately 1 mg/kg bw/d of CBD by a factor of two. This approach refers to an RfD for inhalation derived from a study in Sprague-Dawley rats [33]. The lowest RfD for inhalation derived from a study in humans was found to be approximately 87 mg/kg bw/d. This dose would allow a safe inhalation of the mentioned CBD-liquid. It should be emphasized, however, that endocrinological endpoints appear more susceptible to lower doses of both CBD and Δ^9-THC, than physiological parameters. The lowest RfD derived from the 90-day trial in rats by Marx *et al.* [34] was estimated to be 5 mg/kg bw/d. Among animal studies, this RfD may be considered the most suitable dose because a 90-day study is a more realistic approach to assess the potential long-term health risks of a consumer product, which is most likely to be consumed daily. It is therefore questionable, whether the lower RfD of 1 mg/kg bw/d for CBD derived from the rat model in Steger *et al.* [33] should be preferred over an alternative RfD obtained from another study, especially because the dose-response data in the study has not been statistically significant. It should be further noted that dose-response data considered reliable enough was rare in animal and human studies. Furthermore, strict precautions were taken when estimating the respective RfDs for inhalation by considering the highest value for bioavailability after inhalation [31]. It is also rather unrealistic to assume that 30% of all CBD contained in the e-liquid is fully absorbed because a significant proportion of vaporized e-liquid is usually exhaled, before pulmonary absorption can be completed, as was assumed in this scenario. These considerations could have led to an overestimation of the toxicity of CBD inhaled in humans. Therefore using this preliminary approach, an e-liquid containing 100 mg of CBD per milliliter could still be consumed with only a very low risk of adverse effects, especially when using an RfD of approximately 5 mg/kg bw/d derived from Marx *et al.* [34].

4.4 Limitations of the Assessment

A major challenge in conducting this risk assessment was the limited availability of sufficient usable toxicological data on inhaled CBD and Δ^9-THC to derive a BMDL for both compounds. Therefore, data from studies on animal models was required, which used various routes of administration and different formulations containing the compounds. This makes considerations regarding species and administration route dependent metabolism of both compounds relevant, as metabolites of CBD and Δ^9-THC can have observable effects on animals and humans. Because most of the results, which were considered to be reliable, were obtained from studies in rats, this section focuses mainly on the differences in drug metabolism of Δ^9-THC and CBD between rats and humans.

The metabolism of CBD and Δ^9-THC differs from humans to animals and is route dependent. After oral administration, both compounds face extensive metabolism

resulting in significantly reduced bioavailability, as chemical compounds face more enzymatic reactions and different reactive environments such as saliva and gastric juices. Oral bioavailability of Δ^9-THC is limited and ranges between 4 and 20% due to extensive metabolism in the hepatic first pass effect [1,49]. Due to its lipophilicity, Δ^9-THC is quickly distributed and accumulated in fat tissues, as well as in the brain and muscle, further reducing the plasma concentration of the compounds after the administration [50–53]. Δ^9-THC is largely metabolized in the hepatic first-pass effect by CYP 450-mediated microsomal hydroxylation and oxidation. One of the main metabolites of Δ^9-THC formed by CYP2C9 is 11-OH-THC [1,31,54]. Measured levels of this compound are higher after oral ingestion compared to inhalation [55,56]. THC-COOH is the predominant glucuronide conjugate and is assumed to be an inactive metabolite. For 11-OH-THC, psychotropic effects comparable to those of Δ^9-THC have been reported [57]. However, since BMDLs for psychotropic effects were not included, this finding was considered not relevant for estimated RfD values in this study.

Furthermore, it should be considered that Δ^9-THC can be redistributed back into the blood circuit from the tissue in which it has accumulated [58–60]. This is particularly relevant for heavy regular cannabis consumers, to whom a greater bioaccumulation in adipose tissue can be expected.

Regarding the knowledge about the toxicology of Δ^9-THC metabolites, it has been reported, that 11-OH-THC has similar or even greater psychotropic effects compared to Δ^9-THC [61]. 11-COOH-THC, in contrast, has no reported psychological effects, but presumable analgesic and anti-inflammatory effects could not be excluded [62].

Regarding Δ^9-THC, oral bioavailability and bioavailability after inhalation of cannabis is limited mainly due to the lipophilic properties of the compounds. Harvey *et al.* [63] found that CBD undergoes similar metabolic reactions in humans, rats and dogs, namely, carboxylation to carbonic acids, epoxidation, hydroxylation at multiple sites, oxidation, and beta-oxidation as well as conjugation [63]. However, differences between humans and rats regarding CBD metabolism have been observed. CBD metabolism in rats appears not to favor conjugation reactions of CBD, unlike in human subjects. The major metabolites in rats are acid metabolites of CBD originating from beta-oxidation. C-6 and C-7 hydroxylation was found to be more frequent in rat urine, whereas in humans, hydroxylated 7-COOH-CBD derivatives were more common [64]. The CBD metabolites 7-OH-CBD and 7-COOH-CBD were mentioned to have detectable antinociceptive and anti-inflammatory activities in animals and patients [65].

CBD has been reported to be an antagonist of the type 1 vanilloid receptor in protein binding studies. Both CBD and 7-OH-CBD inhibit fatty acid amid hydrolase in rats [66]. CBD and 6-alpha/beta-OH-CBD and other hydroxy-

lated metabolites are capable of inhibiting microsomal CYP 2C and 3A in *in vivo* mouse model studies [54]. Kraemer *et al.* [67] discovered that CBD can be metabolized to its decarbonylated form in humans. However, toxicological data on decarbonylated CBD have not been found.

In conclusion, the regarded metabolites may not be of toxicological relevance for the results obtained in this risk assessment, but it should be assumed that metabolism might affect the concentration of CBD and Δ^9-THC respectively.

Note that the human metabolism of CBD and Δ^9-THC can differ between individuals and depend on a person's health status. For example, the metabolism of xenobiotics can be increased or compromised by diseases, particularly in diseases that affect the liver and gastrointestinal tract. Plasma concentrations of xenobiotics and their metabolites can vary from those in healthy individuals and might increase the risk of adverse effects, when administered. Other factors influencing metabolic rate are age and sex. Consumption of foods containing active enzymes, which may interact with xenobiotics (such as fruit juice), can also alter the bioavailability of compounds and metabolites [68].

Furthermore, it should not be excluded that other factors, which cannot be easily assessed, may impact the toxicology of CBD and Δ^9-THC, such as consumer age, gender, health status as well as consumption habits of products containing cannabis or cannabinoid preparations.

5. Conclusions

This study presents a risk assessment for inhalation of Δ^9-THC and CBD from e-liquids using the BMD approach. From a limited amount of dose-response data considered suitable for BMD modeling, several BMD values have been derived and used as points of departure for estimating RfDs. The obtained RfDs were compared with dose-response data, which have been adjusted to RfDs to facilitate comparison.

Generally, the RfDs obtained from BMD modeling were found to be higher than doses from the literature converted for comparison. To account for additional consumer safety, it is not advised to consider the RfDs from this study as reliable due to uncertainties arising from the insufficient amount and quality of dose-response data, which have been included in BMD modeling. Note that the BMD approach benefits from dose-response data, which cover a broad dose range. This would allow risk assessors to conduct a retrospective meta-analysis, which combines dose-response data from individual studies. As BMD modeling does not require a large number of test subjects per dose group, this would allow to combine dose-response data, especially from individual studies in human test subjects, to efficiently use limited dose-response data for a risk assessment. Considering that inhalation of vaporized Δ^9-THC and CBD is discussed as a comfortable and practical approach to the treatment of neurological disorders, it is advised to include dose-response data from standardized clin-

ical trials, which have administered vaporized drugs to patients and volunteers.

Further useful dose-response data could be provided by a subsequent study to Marx *et al.* [34] in rats, which uses additional or intermediate dose groups with fewer animals per dose group. The results should then be evaluated using BMD modeling.

Abbreviations

CBD, Cannabidiol; Δ^9-THC, Δ^9-Tetrahydrocannabidiol; 7-OH-CBD, 7-hydroxy cannabidiol; CYP 450, Cytochrome P 450 isoenzymes; 11-OH-THC, 11-hydroxy tetrahydrocannabinol; THC-COOH or 11-COOH-THC, Carboxy-tetrahydrocannabinol or 11-nor-9-Carboxy-THC; FSH, Follicle-stimulating hormone; LH, Luteinizing hormone; TRPA1, Transient receptor potential channels, anakyrin-protein family; TRPM8, Transient receptor potential cation channel subfamily M member 8TRPV1/V2, Transient receptor potential channels, vanilloid subtype 1/subtype 2; POD, Point of departure; (A)RfD, (Acute) reference dose; BMD, Benchmark dose; BMDL, Benchmark dose lower limit; BMDU, Benchmark dose upper limit; BMR, Benchmark response; BMDS, Benchmark dose software; MOA, Mode of action; DS, Dravet syndrome; LGS, Lennox-Gastaut syndrome; i.v., Intravenous; NOAEL, No observed adverse effect level; LO(A)EL, Lowest observed (adverse) effect level; UF, Uncertainty factor; F, Bioavailability; RCT, randomized controlled trial; EFSA, European food safety authority; US EPA, United States environmental protection agency; FDA, Food and drug administration.

Author Contributions

Conceptualization—DWL; methodology—DWL; software—AS; validation—PH; formal analysis—PH; investigation—PH, AS; resources—SGW; data curation—PH, DWL; writing original draft preparation—PH, AS; writing review and editing—DWL, ER, SGW; visualization—PH, AS; supervision—SGW, DWL, ER; project administration—DWL. All authors have read and agreed to the published version of the manuscript.

Ethics Approval and Consent to Participate

Not applicable.

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Conflict of Interest

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