COMMITTEE ON TOXICITY OF CHEMICALS IN FOOD, CONSUMER PRODUCTS AND THE ENVIRONMENT (COT)

Potential adverse effects associated with exposure to cannabidiol (CBD) by inhalation.

Introduction

1. A position paper on potential risk of cannabidiol (CBD) in CBD food products was published by the COT in July 2020, summarising the discussions by COT and COM[1]. A discussion paper on potential risks from use of topically applied CBD-containing cosmetic products was discussed in May 2020 (TOX/2020/23). Following from the evaluations of potential adverse effects associated with oral or dermal exposure, this current paper summarises available data relating to the pharmacokinetics following inhalation exposure and potential adverse effects of exposure to CBD by inhalation.

COT consideration of oral and dermal exposure to CBD

2. Relating to CBD as a novel food, COT discussions had indicated that although the available evidence base was not sufficient to undertake a risk assessment, some general conclusions could be drawn, notably: adverse effects on the liver (hepatic injury) at a CBD dose of ≤ 5 mg/kg bodyweight (bw)/day; inhibitory interactions with some medications at a CBD dose of ≤ 1 mg/kg bw/day (insufficient information was available to determine the overall range of medications that might be affected); somnolence effects noted at ≤ 10 mg/kg bw/day concerning which Members agreed that the British National Formulary warning regarding driving and operating machinery should be noted; reproductive toxicity was observed in laboratory animals treated with CBD as well as developmental effects in the offspring, however the mechanism for this was thought to be unclear at this time; CBD was considered not to be teratogenic; due to CBD's physiochemical properties it was considered likely to transfer into breastmilk and could therefore pose a risk to nursing infants. COM concluded that studies using pure (>98%) CBD indicated that CBD did not have genotoxic potential, although raw data were needed to finalise this conclusion.

3. The Committee discussed the pharmacokinetics parameters of CBD and noted that although CBD has low fasting bioavailability (<10%), consumption with food could increase CBD uptake by, for example, up to 5-fold if taken with a high fat meal.

4. During discussions of the potential risks from topically applied CBD cosmetic products, it was considered that dermal absorption of CBD was likely to be less than

[1] https://cot.food.gov.uk/sites/default/files/cbdpositionpaper290720.pdf

10% of oral absorption. Overall, it was agreed that there was insufficient data to draw conclusions on the toxicity and pharmacokinetic profile of dermally applied CBD to allow adequate risk assessment, but the COT noted that use of topical CBD products could lead to an increase in overall systemic exposure.

5. The Food Standards Agency (FSA) published consumer advice on CBD in food products in February 2020. Overall this advice indicated that consumers should think carefully before taking any CBD food products, that healthy adults should not take more than 1 mg/kg bw per day in total unless advised otherwise by a doctor, and that as a precaution pregnant or breastfeeding women and people taking medications should not consume CBD.

Search strategy

6. Searches of Scopus and PubMed for publications relating to CBD toxicity following inhalation were conducted on 18/06/2020. Details of searches are provided at Annex A.

Exposure

7. Inhalation exposure to CBD may occur via various methods, for example by inhaling combusted or heated cannabis plant or oil-extract products, or alternatively via exposure to aerosol produced from 'e-liquid' containing CBD using an electronic vaporisation device. Two analytical studies of products marketed as containing CBD targeted to exposure by inhalation were identified.

8. Peace et al. (2016) analysed two commercially available e-liquids that were indicated to contain CBD. The two products, Cloud 9 Hemp Easy Rider and Yellow Brick Road, were labelled as containing 3.3 mg/mL CBD, and it was noted that the vendor claimed that a hemp strain with the highest CBD potency was used in the manufacture of their products. E-liquids were analysed by high-performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS) and headspace gas chromatography with flame ionisation detector (HS-GC-FID). Aerosol produced from the e-liquids was analysed by solid phase microextraction gas chromatograph (SPME-GC/MS). The Cloud 9 and Yellow Brick Road e-liquids were determined to contain, respectively, 7.6 mg/L and 6.5 mg/L CBD, 3600 mg/L and 6600 mg/L ethanol, various flavouring compounds, and the carriers propylene glycol (PG) and glycerol (65:35 ratio). No other cannabinoids were detected. Aerosols generated from the e-liquids were reported to contain CBD, PG, glycerol and flavouring agents, but quantitative data were not available to evaluate at this time.

9. Hädener et al. (2019) investigated the cannabinoid content and theoretical lung availability of THC and/or CBD from various cannabis plant or oil-extract products, including marijuana flowers[2], mixed material (leaves and stems), butane

[2] The THC-rich part of the plant.

hash oil (BHO)[3], and flowers from a CBD-rich hemp plant (Cannabis Sativa L.) that is commercially available in Switzerland. Plant or oil material was analysed for content of THC, CBD, their respective precursor acids, Δ^9-tetrahydrocannabinolic acid A (THCA) and cannabidiolic acid (CBDA), and cannabinol (CBN). Findings are summarised in Table 1, below:

Table 1. Cannabinoid content (% w/w) in plant or oil samples (data from Hadener et al. 2019).

Sample	THC	THCA	Total THC	CBD	CBDA	Total CBD	CBN
Marijuana flowers	2.7	16.7	17.3	1.1	not done	1.1	not done
Mixed material	0.5	0.5	0.9	not done	not done	not done	<0.1
BHO	13.1	66.5	71.4	1.4	not done	1.4	0.6
CBD-rich hemp flowers	<0.1	<0.1	<0.1	0.3	6.8	6.3[a]	<0.1

[a] corrected for loss of CO_2.

10. Samples were subsequently either completely combusted (plant material) or heated on a titanium nail (dabbing). Condensate was collected and analysed for cannabinoid content. THCA and CBDA were not detectable in the condensates, indicating that decarboxylation of the precursors during the burning/evaporation process was complete. Theoretical (maximum) lung availability was reported as the percentage of chemical in plant or oil material that was recovered in the smoke/vapour condensate: for THC, 26.7% for marijuana flowers, 12.8% for mixed material, and 75.5% for BHO; for CBD, 20% from CBD-hemp flowers. Authors conclusions mostly related to THC, in particular the high lung availability from dabbing which may explain the increased psychoactive and unwanted side effects (not detailed further) associated with this route of cannabis administration. Regarding CBD, authors concluded that lung availability of CBD from CBD-containing hemp would be similar to that of THC from THC-containing marijuana flowers. It was however noted that the methodology was based on an assumption of inhalation of 100% of the combusted material (mainstream smoke) and did not account for factors such as loss of material into the air between puffs (side-stream smoke).

Pharmacokinetics

11. COT has previously discussed the pharmacokinetic parameters of CBD following oral[4] and dermal (TOX/2020/23) exposure. It was noted that for oral exposure "although CBD has low fasting bioavailability (<10%), consumption with food could increase uptake by, for example, up to 5-fold if taken with a high fat

[3]'Hash oil' is extracted with solvents from high THC-content cannabis plants and residues are mixed with vegetable oil. The resulting product, which has a high THC concentration, is inhaled after heating on a titanium nail mounted on a water pipe, a process called 'dabbing'.
[4] https://cot.food.gov.uk/sites/default/files/cbdpositionpaper290720.pdf

meal". In addition, it was noted that "In humans, the most common adverse reactions in humans are somnolence, decreased appetite, diarrhoea, pyrexia, fatigue, and vomiting. The most frequent cause of discontinuations was transaminase elevation. Studies in animals have also shown transaminase elevation, liver injury as well as reproductive toxicity". The Committee considered that the dermal absorption of CBD "would be quite low (<10% of oral absorption) but given the lipophilic nature of CBD, repeat application of these products could result in CBD accumulating in the stratum corneum from where it might slowly diffuse into the systemic circulation". It was acknowledged that there was "insufficient information on the pharmacokinetics and toxicity of dermally applied CBD to allow an adequate risk assessment of the safety of CBD in cosmetics to be undertaken".

12. One report has been identified regarding the pharmacokinetics of inhaled CBD in humans. Ohlsson et al. (1986) evaluated single-dose kinetics of deuterium-labelled CBD in humans after smoking or intravenous (i.v.) administration. Five young male regular or infrequent marijuana smokers were followed for 72 hours after application of 20 mg $^{2}H_{2}$-CBD i.v. infusion[5] or an estimated 18.8-19.4 mg $^{2}H_{2}$-CBD by smoking[6]. Mean plasma levels of $^{2}H_{2}$-CBD measured over the time-course are indicated in Table 2, and kinetic parameters calculated for the five individual subjects are shown in Table 3, below. Systemic availability determined by comparing areas under the plasma concentration versus time curves for the two treatments was 31±13% (range, 11-45%), with a four-fold variation over the five subjects. The range of plasma clearance was calculated as 960-1560 mL/min (mean, 1240±240 mL/min). Mean volume of distribution was 2520±470 L (32.7±8.6 L/kg; range, 22.5-45.5 L/kg). Half-life was estimated as 31±4 hours after smoking and 24±6 hours after i.v. injection. Authors concluded that, similarly to THC, CBD is a high-clearance drug with a large distribution volume that is eliminated very slowly from the body. Distribution volume and plasma clearance for CBD were noted to be somewhat larger than published values for THC, while half-lives were about the same for the two compounds.

[5] 2 mg/mL in 95% ethanol solution injected over a 2-min period
[6] The participant smoked as much as possible of one marijuana placebo cigarette spiked with 20 mg $^{2}H_{2}$-CBD

Table 2. Mean plasma $^{2}H_{2}$-CBD levels measured in five subjects after administration i.v. or by smoking (data from Ohlsson et al. 1986)

	20 mg i.v.		**19.2 ± 0.2 mg smoking**	
Time (h)	Mean ± SD (ng/mL)	Range (ng/mL)	Mean ± SD (ng/mL)	Range (ng/mL)
0.05	686 ± 239	356-972	110 ± 55	41.8-191
0.1	286 ± 81	205-383	68.7 ± 31.4	23.9-111
0.17	160 ± 50	104-224	42.9 ± 24.3	12.4-72
0.25	115 ± 24	90.8-151	32.6 ± 16.7	11.7-51.1
0.5	74.4 ± 11.7	59.2-90.2	23.7 ± 10.2	10.0-35.1
1.0	48.4 ± 10.7	37.0-61.0	10.2 ± 6.6	2.98-17.8
1.5	32.0 ± 12.4	22.2-49.8	9.88 ± 4.22	5.49-16.4
2	22.1 ± 7.0	17.3-34.2	8.78 ± 3.51	5.38-13.7
3	13.7 ± 4.9	6.98-18.6	4.03 ± 1.55	1.70-5.53
4	10.5 ± 4.4	3.42-15.5	3.09 ± 1.11	1.92-4.38
8	2.91 ± 0.85	1.77-3.83	1.04 ± 0.54	0.41-1.67
12	1.68 ± 0.54	1.21-2.36	0.56 ± 0.24	0.25-0.83
24	0.97 ± 0.24	0.56-1.16	0.31 ± 0.13	0.19-0.46
48	0.49 ± 0.15	0.24-0.64	0.17 ± 0.06	0.10-0.24
72	0.24 ± 0.20	0.08-0.46	0.13 ± 0.04	0.80-0.18

Table 3. Kinetic parameters for 2H2-CBD disposition in five subjects after application i.v. or via smoking (data from Ohlsson et al. 1986)

	20 mg i.v.				Smoking			
Subject	AUC_{0-72h} (ng/mL min x 10^{-3})	Plasma clearance (mL/min)	Distribution volume (L/kg)	$t_{1/2}$ (h)	Estimated amount (mg)	AUC_{0-72h} (ng/mL min x 10^{-3})	Estimated systemic availability (%)	$t_{1/2}$ (h)
1	12.80	1560	35.5	22	19.4	5.64	45	27
2	15.78	1270	31.5	23	19.3	4.74	30	33
3	14.92	1340	22.5	18	18.8	4.47	32	27
4	29.04	1050	45.5	33	19.0	7.05	39	35
5	20.82	960	28.6	24	19.3	2.35	11	33
Mean ±SD	16.67 ± 3.23	1240 ± 240	32.7 ± 8.6	24	19.2 ± 0.3	4.85 ± 1.72	31 ± 13	31 ± 4

				± 6				

Human data

Clinical case reports.

13. Webster (2019) reported a case of acute pneumonitis associated with vaping of CBD oil. A 54-year-old man presented after three days of symptoms of shortness of breath and blood-tinged sputum. The patient reported that he had started to vape CBD oil via an electronic vaporisation device two days prior to onset of symptoms. The publication did not provide any further information regarding the exposure. Chest X-ray y showed bilateral infiltrate most prominent in the perihilar region, and a CT scan revealed bilateral ground-glass opacities with sparing of the lung apices. Laboratory tests indicated a 'WBC' count[7] of 10.1 and a urine drug screen that was "positive for cannabinoids", however no further details were given. The patient's respiratory status worsened over several days despite treatment with antibiotics, but subsequently improved with corticosteroid (prednisone) treatment.

14. Blagev et al. (2019) published a summary of data relating to the outbreak of vaping-associated lung injury (E-cigarette, or Vaping, product use – Associated Lung Injury; 'EVALI') that was noted in the US during 2019-2020. The main focus of the report was to document clinical parameters (symptoms, treatments, prognosis) in a cohort of 60 EVALI patients. However, some details of which products had been inhaled via electronic vaping devices during the 90 days prior to presentation of EVALI were reported in the publication[8] and these data are summarised in Table 4, below. According to the report, "All patients had vaping exposure to nicotine, tetrahydrocannabinol, or both, as required by the case definition." However, it is unclear if the authors considered CBD oil to fall under the umbrella of THC in this definition, as summation of numbers of patients who used either nicotine-only, THC-only, or nicotine + THC only accounts for 57/60 of the cohort.

[7] Assumed to refer to white blood cell count, although this is not specified in the publication. Units were not stated.
[8] Data were obtained via interview of patients or their proxy.

Table 4. Product use reported by 'EVALI' patients in the study of Blagev et al. (2020) (data from Table 1 of Blagev et al. 2020).

Product vaped via electronic device during the 90 days prior to onset of EVALI	**Number of patients (%) (n_{total} = 60)**
Nicotine	40 (67%)
Nicotine only	10 (17%)
THC	47 (78%)
THC only	18 (30%)
Nicotine and THC	29 (48%)
CBD oil	4 (5%)

Clinical studies

15. A small, randomised, placebo-controlled study indicated that CBD in the form of an inhaler may aid in tobacco smoking cessation, with no adverse effects on mood (Morgan et al. 2013). A group of 24 regular smokers (≥10 cigarettes per day) who expressed an intention to quit smoking were randomised to provision for one week with an inhaler delivering either CBD (400 µg per dose, in 5% ethanol) or placebo (5% ethanol) (n=6 subjects per sex per group). Authors considered that bioavailability of CBD from use of the device was >65% (personal communication with the authors from the manufacturer). Participants were instructed to use the inhaler when they felt like smoking and to record variables including number of cigarettes smoked and inhaler use, cravings, and mood parameters. In-clinic tests were conducted on day 1 and day 7, including exhaled carbon monoxide, craving scale questionnaire, and a 16-item mood rating scale to assess key side effects one hour pre- and post-treatment. Total number of cigarettes smoked over the week of treatment was significantly reduced in the CBD group but not in the placebo group compared with pre-study levels, but this effect did not persist on subsequent follow-up. Analysis of qualitative data at days 1 and 7 indicated no main effect of treatment type on level of craving (reduced in both groups from day 1 to day 7), sedation (increased in both groups), depression (no change) or anxiety (decreased in both groups).

***In vivo* studies**

16. Javadi-Paydar et al. (2018) reported that inhalation of THC and/or CBD induced hypothermia and antinociceptive effects to an equivalent level in male and female in Wistar rats. Animals (n=8 per sex) implanted with radiotelemetry transmitters were exposed to test compound in sealed vapour inhalation chambers and then moved to separate chambers for experimental recordings, made over a 240-min period. An electronic vaping system was used to produce aerosols which were passed through the inhalation chamber mixed with ambient air. The test

compounds, either Δ^9-THC (12.5, 25, 50, 100, or 200 mg/mL) or CBD (100 or 400 mg/mL), were dissolved in PG vehicle. Different exposure concentrations were achieved either by adjustment of the amount of the compound in the PG vehicle (for THC) or by modification of duration of exposure in the inhalation chamber to a fixed-dose concentration (for CBD). Measurements of test compound concentrations in the air to which rats were exposed in the exposure chambers were not reported.

17. Several experiments were conducted on the same set of rats, with a 7-day interval between each experiment-set, described in chronological order in the following narrative. Telemetry data were analysed with Analysis of Variance (ANOVA) with repeated measures factors for the Drug Inhalation Condition, the Time post-initiation of vapour and oestrous phase (where relevant). Tail-withdrawal latencies were analysed by ANOVA with repeated measures factors of Drug Treatment Condition and Water Bath temperature and between-groups factor of sex. Plasma levels of THC were analysed with between-groups ANOVA due to the design with factors for Time post-initiation of inhalation, drug Concentration in the PG and/or sex. Significant effects within group were followed with post hoc analysis using Tukey correction for all multi-level, and Sidak correction for two-level comparisons.

18. Experiment 1. Exposure to 12.5, 25, 50, or 100 mg/mL THC for 30 min indicated a dose-dependent decrease in body temperature in male and female rats. Effects were significant[9] at 25, 50, and 100 mg/mL (females) or 50 and 100 mg/mL THC (males) compared with baseline readings, and at 50 and 100 mg/mL THC (females and males) compared with vehicle-only exposure. For female rats, activity levels varied significantly across the recording period, but this was not associated with vapour inhalation dose. Male rats showed significantly less activity with 50 or 100 mg/mL THC exposure compared with vehicle-only condition. CBD was not included in this experiment.

19. Experiment 2. This test-set conducted in female rats indicated no difference in response to THC exposures at different points in the oestrous cycle. CBD was not tested in this experiment.

20. Experiment 3. Body temperature and activity level were measured in male rats exposed for 30 min to PG vehicle or 100 mg/mL CBD (in counter-balanced order), then to vehicle, 200 mg/mL THC, or 200 mg/mL THC + 100 mg/mL CBD (in counter-balanced order). CBD, THC, and THC+CBD exposures were associated with significant reduction in body temperature and activity level compared with baseline[10] and compared with vehicle-only exposure. Effects were significantly greater with THC and THC+CBD compared with CBD only, but there was no significant difference in effect level between the THC and THC+CBD conditions.

[9] Throughout the report, effects were indicated on figures as significantly different from vehicle (*) or baseline (#). However, in accompanying p values were not given.
[10] Body temperature was also reduced after vehicle-only exposure compared with baseline.

21. Experiment 4. This study assessed effects of exposure to 100 mg/mL CBD for 10, 20, or 40 min on temperature and activity rate. In female rats, the following changes were observed: decreased body temperature compared with baseline (20 min and 40 min CBD) or compared with vehicle-only exposure (40 min CBD); decreased activity rate compared with baseline and compared with vehicle-only exposure (10 min, 20 min, and 40 min CBD). In male rats, body temperature was reduced compared with baseline (vehicle exposure, 40 min CBD), compared with vehicle-only exposure (20 min and 40 min CBD), and compared with the 10 min CBD condition (20 min and 40 min CBD). Activity rate was reduced after 20 min CBD compared with vehicle-only exposure.

22. Experiment 5. This was similar to Experiment 3, but conducted in male and female rats, with test exposures of 100 mg/mL CBD, 25 mg/mL THC, or 100 mg/mL CBD + 25 mg/mL THC. In both male and female rats, body temperature and activity rate were significantly lower compared with baseline[11] and compared with vehicle-only exposure after exposure to CBD, THC, and THC+CBD. Body temperature and activity rate decreased to a greater extent with exposure to THC or THC+CBD compared with CBD only. There were also some differences (not further defined) noted between the THC compared with THC+CBD conditions.

23. Experiment 6 indicated that exposure to THC increased latency to a noxious stimulus (tail withdrawal latency using a water bath at 48, 50, and 52 ^{o}C) in both male and female rats and this was not affected by oestrous stage. CBD was not tested in this experiment.

24. Experiment 7 was similar to previous experiments (3, 5) except that test exposures were 400 mg/mL CBD, 100 mg/mL THC, or 400 mg/mL CBD + 100 mg/mL THC. Findings for body temperature were similar to those in experiment 5, except no significant difference was noted between THC and CBD conditions for female rats. In females, activity rate was described as significantly different[12] for the corresponding time-point compared with vehicle-only exposure for CBD and for THC+CBD; significantly different between CBD and THC+CBD, and significantly different between THC and THC+CBD. In males, activity rate was significantly increased across all exposure conditions at 30 min after start of exposure compared with all other time-points and was significantly lower than baseline after 150-240 min.

25. Experiment 8 evaluated plasma kinetics of THC. Plasma concentrations were increased at 35 and 60 min after start of exposure, with higher plasma THC levels at the highest (200 mg/mL) compared with two lower-dose (25, 100 mg/mL) test concentrations. The highest plasma concentrations were determined at the first evaluation time-point (35 min after start of exposure). There was no sex difference. Plasma kinetics of CBD were not evaluated.

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[11] Body temperature was also reduced compared with baseline after vehicle-only exposure.

[12] The narrative does not indicate whether the difference was an increase or decrease; from the figure provided, the direction of change appears to vary at different time-points, but the data in the graphs are somewhat difficult to interpret.

26. Authors concluded that evaluation of effects of inhalation exposure to Δ^9-THC, CBD, and their combination, on hypothermia, hypoactivity, and antinociception showed similar effects in male and female Wistar rats. Regarding CBD, exposure reduced body temperature in male and female rats in a dose-dependent manner. Combined exposure to THC+CBD led to additive effects on hypothermia at a 1:4 THC:CBD ratio, but this was not seen when the exposure ratio was 1:2. The authors noted that prior studies using intraperitoneal (i.p.) application indicated that CBD may oppose effects of THC on temperature responses in rodents, and that CBD applied alone by i.p. or intravenous (i.v.) injection had no effect on thermoregulation, which they suggested my indicate possible differences depending on route of administration.

27. A follow-up study, focussing on CBD, was reported by Javadi-Paydar et al. (2019). The study was conducted using Sprague-Dawley and Wistar rats implanted with radiotelemetry transmitters (n=6-8 per sex per group). Animals had been used in previous studies evaluating effects of exposures to other compounds including THC, nicotine, and MDMA. Exposures to aerosols produced via an electronic vaporiser were effected in an inhalation chamber, and temperature and activity responses evaluated in separate recording chambers, as described in Javadi-Paydar et al. (2018) (see paragraphs 16-26 above). Test compounds for inhalation were CBD (100, 200, or 200 mg/mL), Δ^9-THC (25, 50, or 100 mg/mL), and nicotine bitartrate (30 mg/mL), in a vehicle of PG. For i.p. injection, CBD was dissolved in a 1:1:8 mix of ethanol:cremulphor:saline. Plasma levels of CBD were analysed with ANOVA with repeated measures factors of Drug Condition and Time post-initiation of vapor or Time post-injection. A between-subjects factor was included for sex. Temperature and activity rate (counts per minute) data were collected via the radiotelemetry system on a 5 minute schedule and analysed in 30 minute averages (the time point refers to the ending time, i.e. 60 = average of 35-60 minute samples). Telemetry data were analysed with Analysis of Variance (ANOVA) with repeated measures factors for the Drug Condition and the Time post-initiation of vapor or Time post-injection. Comparison with the pre-treatment baselines and the vehicle conditions. The tail withdrawal latencies were analysed with 'within-subject' factors for pre/post inhalation and for inhalation condition. Any significant effects within group were followed with post hoc analysis using Tukey correction for all multi-level, and Sidak correction for any two-level, comparisons. The following set of experiments was conducted.

28. Experiment 1 evaluated plasma kinetics of CBD on exposure via inhalation or i.p. injection. Male and female Wistar rats were exposed to CBD either by inhalation (100 or 400 mg/mL for 30 min) or by i.p injection (10 or 30 mg/kg bw). Blood samples were drawn at 35 and 120 min after the start of exposure. CBD exposure produced concentration-dependent effects on plasma CBD. Three-way statistical analysis showed a significant difference attributable to sex (higher in females than males) [$F(1, 14) = 7.34$; $p<0.05$], sampling time (higher at 35 min than 120 min) [$F(1, 14) = 171.6$; $p<0.0001$], CBD concentration [$F(1, 14) = 35.82$; $p<0.0001$], and interaction of sex with time [$F(1, 14) = 4.62$; $p<0.05$]. Post-hoc tests confirmed a significant

concentration-related difference in plasma CBD immediately after the inhalation session and a significant reduction 120 min after the start of inhalation. For i.p. injection, three-way analysis indicated significant effects on plasma CBD of time after injection [$F(1,14) = 19.76$; $p<0.001$], dose [$F(1, 14) = 20.96$; $p<0.001$], and interaction of dose with time [$F(1, 14) = 6.74$; $p<0.05$]. Post-hoc tests confirmed significant differences between doses at 35 min and a difference between time points for the 30 mg/kg bw dose.

29. Experiment 2 evaluated effects of inhalation exposure to CBD on body temperature in male Wistar rats. Animals were exposed by inhalation to PG vehicle or 100 mg/mL PG for 30 min (counter-balanced order) then 3 weeks later to PG vehicle or 400 mg/mL CBD (counter-balanced). Body temperature was significantly reduced after inhalation of both CBD doses compared with baseline and compared with PG exposure. Statistical analysis using ANOVA showed significant effects of time after initiation of exposure [$F(7, 49) = 12.92$; $p<0.0001$], vape condition [$F(3, 21) = 16.49$; $p<0.0001$], and interaction of factors [$F(21, 147) = 15.01$; $p<0.0001$] on body temperature. Post-hoc tests confirmed that temperature was lower than baseline and vehicle-condition at 60-120 min (100 mg/mL CBD) or 60-180 min (400 mg/mL CBD). Data relating to effects of exposures on activity rates were not clearly presented: Figure 3 of the publication appears to show that activity rates were reduced after all treatments (vehicle or CBD), and the narrative notes that post-hoc testing indicated a significantly lower activity rate at 60 min associated with 400 mg/mL CBD compared with all other conditions. In a follow-on study, rats pre-treated by i.p. injection with varying concentrations of the 5-HT_{1A} antagonist, WAY-100635, were subsequently exposed by inhalation to 100 mg/mL CBD or 100 mg/mL THC, in counter-balanced order. ANOVA showed significant effects of dosing condition [$F(5, 35) = 7.54$; $p<0.0001$] and time after initiation of exposure on (reduction in) body temperature [$F(7, 49) = 9.02$; $p<0.0001$], however there were no differences between saline or WAY-100635 pre-treatment groups[13].

30. Experiment 3 investigated effects of inhalation exposure to CBD, with or without co-exposure to nicotine, on body temperature in male Sprague-Dawley rats. Experimental sets were conducted at a minimum 3-4 day intervals. Animals were exposed by inhalation to PG vehicle, 30 mg/mL nicotine, 400 mg/mL CBD, or a combination of the three, in counter-balanced order. Experiments were subsequently repeated using 100 mg/mL CBD. Exposure to vehicle or nicotine did not alter body temperature at any time point. CBD treatment induced hypothermia and this effect was enhanced by nicotine. ANOVA indicated significant effects of time [$F(7, 42) = 131.8$; $p<0.0001$)], vape condition [$F(7, 42) = 19.39$; $p<0.0001$)], and interaction of factors [$F(49, 294) = 7.52$; $p<0.0001$)]. Post-hoc tests confirmed a significant difference between temperatures observed after 100 mg/mL or 400 mg/mL CBD, both in the absence (between 60-210 min) or presence (60-150 min) of nicotine. There was also a significant difference between 400 mg/mL CBD compared with 400

[13] Effects of WAY-100635 to act as a 5-HT_{1A} antagonist were confirmed by positive-control studies in which pre-treatment with WAY-100635 attenuated response to a known 5-HT_{1A} agonist, 8-OH-DPAT.

mg/mL CBD + nicotine (at 120 min). Analysis of activity rate indicated significant effects of time, vape condition, and interaction of factors. Post-hoc tests confirmed that activity rate at 60 min was significantly higher after nicotine exposure compared with all other conditions. Finally, rats pre-treated with varying doses of WAY-100635 were exposed by inhalation to 100 mg/mL CBD. In these experiments, pre-treatment with WAY-100635 significantly attenuated CBD-induced hypothermia, as compared with saline pre-treatment.

31. Experiment 4 evaluated whether inhalation exposure to CBD would modify the antinociceptive effects (tail withdrawal latency) of THC inhalation in male Wistar rats. Rats were exposed in a first experiment for 30 min to 400 mg/mL CBD or 100 mg/mL THC, then in the next experiment to 25 mg/mL or 50 mg/mL THC, and in a final experiment to 50 mg/mL THC (alone) or 50 mg/mL THC + 200 mg/mL CBD. Tail withdrawal latency was not affected by exposure to 400 mg/mL CBD. Latency was significantly slowed after exposure to 50 mg/mL or 100 mg/mL THC [Pre/Post: $F(1, 5) = 15.98$; $P<0.05$; Vapor Condition: $F(1, 5) = 5.39$; $P=0.068$; Interaction: $F(1, 5) = 7.79$; $P<0.05$]. The final experiment showed no significant effect of co-exposure to CBD on reduction in tail-withdrawal latency induced by 50 mg/mL THC [Pre/Post: $F(1, 5) = 33.09$; $P<0.005$; Vapor Condition: $F(1, 5) = 5.52$; $P=0.066$; Interaction: $F(1, 5) = 4.77$; $P=0.081$].

32. Authors concluded that: vapour-inhalation of CBD produces concentration-related plasma CBD levels in Wistar rats that are within the range achieved by i.p. injection of 10 or 30 mg/kg bw; CBD inhalation induces dose-related hypothermia in Sprague-Dawley rats and this is partially attenuated by 5-HT_{1A} receptor blockade; nicotine inhalation enhances the effect of CBD; and CBD (alone) did not affect nociception, nor did CBD co-treatment attenuate THC-associated nociception.

In vitro studies

33. Muthumalage and Rahman (2019) investigated effects of exposure to varying concentrations of CBD in liquid or aerosol on generation of reactive oxygen species (ROS) and inflammatory mediators in lung epithelial cells (BEAS-2B and NHBE), macrophages (U937), and lung fibroblast cells (HFL-1). Potential anti-inflammatory effects of CBD and/or dexamethasone were studied using monocytes and epithelial cells. CBD treatment showed differential effects on IL-8 and MCP-1, and acelluar and cellular ROS levels. CBD treatment significantly attenuated LPS-induced NF-kB activity, IL-8, and MCP-1 release from macrophages. Cytokine array evaluation indicated a differential cytokine response from CBD, with inflammatory mediators either induced (IL-8, serpin E1, CXCL1, IL-6, MIF, IFNγ, MCP-1, RANTES, TNFα) or reduced (MCP-1/CCL2, CCL5, eotaxin, and IL-2). CBD and dexamethasone treatments individually both reduced LPS-induced IL-8 levels, but in combination were antagonistic, via MCPIP. Authors concluded that CBD differentially regulated basal proinflammatory response and attenuated both LPS-induced cytokine release and NF-kB activity in monocytes, similar to dexamethasone. Thus, CBD has a differential inflammatory response and acts as an anti-inflammatory agent in

proinflammatory conditions, but acts as an antagonist with steroids, overriding the anti-inflammatory potential of steroids when used in combination.

34. Leigh and Goniewicz (2020) studied the effects of exposure to aerosols produced from e-liquids containing combinations of PG, nicotine, CBD, and flavouring chemicals on cell viability, metabolic activity, and production of inflammatory mediators in cultured H292 human bronchial epithelial cells. Primary e-liquid constituents were purchased commercially as follows: 'Easy Rider', labelled as 1.7 mg/mL CBD, and containing unspecified flavouring compounds with a 'fruity' smell[14]; 'pure' CBD liquid, labelled as containing 33.3 mg/mL CBD; nicotine (≥99% purity); PG (≥99% purity). Commercial CBD-containing products were listed as industrial hemp derived and GC/MS analysis confirmed the absence of THC. Test liquids were then mixed in the laboratory as follows: PG-only (PG); PG + 1.7 mg/mL CBD (PG+CBD); PG + 1.7 mg/mL nicotine (PG+NIC); PG + 1.7 mg/mL CBD + 1.7 mg/mL nicotine (PG+CBD+NIC); Easy-Rider + 1.7 mg/mL nicotine (EasyRider[CBD+flavour]+NIC). Aerosols were produced using an eGO ENDS device, and control exposures were run using ambient air. Cells were exposed to air or aerosol in an air liquid interface setup.

35. Cellular metabolic activity, measured by neutral red uptake, was significantly decreased as follows: all test products compared with air control; PG-NIC and EasyRider[CBD+flavour]+NIC exposures compared with PG; PG+CBD compared with PG+NIC; PG+CBD and EasyRider[CBD+flavour]+NIC compared with PG+NIC+CBD.

36. Cell viability, measured by trypan blue assay, was significantly decreased in all CBD exposure conditions, as follows: PG+CBD, PG+CBD+NIC, EasyRider[CBD+flavour]+NIC compared with air control and compared with PG.

37. Levels of the cytokines/chemokines, IL-1β, IL-6, IL-10, CXCL1, CXCL2, CXCL10, measured by ELISA, were mostly increased by exposures to nicotine and/or CBD-containing aerosols. In general, the highest levels were observed with CBD-containing exposures, in particular where the exposure contained both CBD and nicotine. For four pro-inflammatory cytokines/chemokines (IL-1β, IL-6, CXCL1, CXCL10), highest levels were observed under the EasyRider[CBD+flavour]+NIC exposure condition.

38. Authors concluded that exposure to nicotine and CBD aerosols (separately) induces cytotoxic and inflammatory effects, with CBD producing stronger responses than nicotine. It was also concluded that co-exposure to nicotine and CBD produces additive cytotoxic and proinflammatory effects. Observation that levels of the anti-inflammatory IL-10 were substantially increased by exposure to aerosols containing PG+CBD or PG+CBD+NIC led the authors to hypothesise that CBD-containing

[14] GC/MS analysis indicated the presence of CBD, 2,3-butanediol, acetoin, acetone alcohol, benzaldehyde, and PG in the Easy-Rider e-liquid.

aerosol may be cytotoxic by a mechanism of necrosis, however this was not tested experimentally.

Summary/conclusions

39. The evidence base relating to potential adverse effects of inhalation exposure to CBD was small, including two studies on the presence of CBD in plant extracts or e-liquids, one study on pharmacokinetics of CBD in humans after smoking, one clinical case report, one report of products used by EVALI patients, a small-scale clinical study of a CBD inhaler to aid smoking cessation, a set of two experimental studies in rats exposed to CBD in inhalation chambers, and two studies that investigated effects of CBD exposure on lung cells *in vitro*.

40. A kinetic study conducted in humans found CBD to be a high-clearance drug with a large distribution volume, eliminated slowly from the body. Kinetic parameters were noted to be similar to those of THC in the published literature. A clinical case report described acute pneumonitis associated with vaping CBD, which was resolved by corticosteroid treatment. Data on EVALI patients indicated that a small number of those who reported product use may have used CBD rather than THC-containing products, but this is not very clear from the published report. The small-scale clinical study indicated that *ad lib* use of a CBD inhaler for one week showed some efficacy in reducing levels of cigarette smoking, without producing any negative effects on mood. Experimental studies *in vivo* indicated that inhalation of CBD induced a dose-dependent effect of hypothermia. Effects were partially attenuated by 5-HT_{1A} receptor blockade and were enhanced by co-exposure to nicotine. CBD exposure alone had no effect on nociception and co-exposure to CBD did not attenuate antinociceptive effects of THC. Studies *in vitro* indicated varying effects of exposure to CBD in aerosols to alter levels of inflammatory mediators, with one study suggesting that co-exposure to CBD may antagonise to the anti-inflammatory effects of the steroid, dexamethasone.

Questions for the Committee

41. Members are asked to consider the information in this paper, and in particular:

i. Does the pharmacokinetic profile of inhaled CBD pose a safety concern or raise any further questions regarding its use in products targeted for inhalation exposure?

ii. Is there adequate information on the pharmacokinetics and toxicity of inhaled CBD to generate an adequate risk assessment regarding the safety of its use in products targeted for inhalation exposure?

iii. Do the Committee have any comments on the potential for drug interactions arising from inhaled CBD exposure and how this relates to its use in products targeted for inhalation exposure?

iv. Considering the toxicity and PK profile of inhaled CBD and the levels of CBD determined in various products, does its use pose a potential safety concern?

v. Do the Committee, given the information presented, consider there to be a risk arising from the cumulative exposure arising from the use of multiple CBD-containing products including but not limited to products targeted for inhalation exposure?

NCET at WRc/IEH-C under contract supporting the PHE COT Secretariat
November 2020

Abbreviations

BHO	Butane hash oil
CBD	Cannabidiol
CBDA	Cannabidiolic acid
CBN	Cannabinol
i.p.	intra-peritoneal
i.v.	intra-venous
PG	Propylene glycol
THC	Δ^9-tetrahyrocannabinol
THCA	Δ^9-tetrahydrocannabinolic acid A

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COMMITTEE ON TOXICITY OF CHEMICALS IN FOOD, CONSUMER PRODUCTS AND THE ENVIRONMENT (COT)

Inhalation toxicology of cannabidiol (CBD).

Details of Literature search carried out by NCET at WRc/IEH-C

Searches were carried out on 18/06/2020 to identify literature on potential adverse effects of CBD following inhalation, as follows.

Scopus

(TITLE-ABS-KEY (cannabidiol OR cbd) AND TITLE-ABS-KEY (inhal* OR vapo*r OR aerosol)) AND NOT (TITLE-ABS-KEY (beryllium)) AND (LIMIT-TO (LANGUAGE , "English")) AND (EXCLUDE (SUBJAREA , "MATE") OR EXCLUDE (SUBJAREA , "ENGI") OR EXCLUDE (SUBJAREA , "PHYS") OR EXCLUDE (SUBJAREA , "CHEM") OR EXCLUDE (SUBJAREA , "ENER") OR EXCLUDE (SUBJAREA , "CENG") OR EXCLUDE (SUBJAREA , "MATH") OR EXCLUDE (SUBJAREA , "AGRI") OR EXCLUDE (SUBJAREA , "EART") OR EXCLUDE (SUBJAREA , "SOCI") OR EXCLUDE (SUBJAREA , "ARTS") OR EXCLUDE (SUBJAREA , "BUSI")) AND (EXCLUDE (SUBJAREA , "MEDI"))

PubMed

(TITLE-ABS-KEY (cannabidiol OR cbd) AND TITLE-ABS-KEY (inhal* OR vapo*r OR aerosol)) AND NOT (TITLE-ABS-KEY (beryllium)) AND (LIMIT-TO (LANGUAGE , "English")) AND (EXCLUDE (SUBJAREA , "MATE") OR EXCLUDE (SUBJAREA , "ENGI") OR EXCLUDE (SUBJAREA , "PHYS") OR EXCLUDE (SUBJAREA , "CHEM") OR EXCLUDE (SUBJAREA , "ENER") OR EXCLUDE (SUBJAREA , "CENG") OR EXCLUDE (SUBJAREA , "MATH") OR EXCLUDE (SUBJAREA , "AGRI") OR EXCLUDE (SUBJAREA , "EART") OR EXCLUDE (SUBJAREA , "SOCI") OR EXCLUDE (SUBJAREA , "ARTS") OR EXCLUDE (SUBJAREA , "BUSI")) AND (EXCLUDE (SUBJAREA , "MEDI"))

A total of 119 citations were identified by combination of the two searches. Text and reference lists of citations identified were also inspected for further publications of relevance. A brief updated search of PubMed was conducted on 12/09/2020.