MUT/2019/10

COMMITTEE ON THE MUTAGENICITY OF CHEMICALS IN FOOD CONSUMER PRODUCTS AND THE ENVIRONMENT

REVIEW OF GENOTOXICITY OF CANNABIDIOL (CBD)

Referral to COM

1. The Food Standards Agency (FSA) has asked for advice on the genotoxicity of CBD to assist in developing advice for the increasing number of risk assessments requests for CBD consumer products.

2. A Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment Committee (COT) paper (TOX/2019/32) was reviewed in July 2019 on the potential adverse effects of CBD products. The genotoxicity data was conflicting and therefore the Committee on the Mutagenicity of Chemicals in Food Consumer Products and The Environment (COM) is being asked to review the genotoxicity data.

Introduction

3. CBD has been investigated and researched in the medicinal sector for a number of years including clinical trials for treatment of epilepsy and seizures. CBD has now entered the food sector and is present in several products available for consumption. These consumable products range include beverages (beer, spirits, wine, coffee and soda style drinks), liquids (tinctures, drops, syrup, oils) chewables (gum drops) and chocolate. In addition, it is present in pet food in various formats.

4. There are various ways of manufacturing CBD which include: liquid solvents, oil extraction and supercritical carbon dioxide (CO_2) extraction. As the methodology will vary, so may the composition of the products and extracts. This should be taken into consideration in the risk assessment of the CBD products.

5. The amount of CBD present in these products varies from 2-200 mg in total. However, if used in tinctures this can vary further as the consumer controls the dosage, therefore the dosing range can be somewhat higher. In addition, foreseeable misuse may lead to lower or higher dosing than that specified (Bonn-Miller *et al*., 2017).

6. Risk assessment advice on CBD has been increasingly requested from the FSA therefore it was considered timely to obtain a view from COT.

Regulatory/Legal Status

7. The European Commission confirmed CBD's classification as a novel food in 2019 by updating the European Union (EU) Novel Food Catalogue. This means that this product was not significantly used as a food or food ingredient before 15th of May

in 1997. Therefore, before it may be placed on the market in the EU as a food or food ingredient a safety assessment under the Novel Food Regulation is required.

CBD in medicinal products

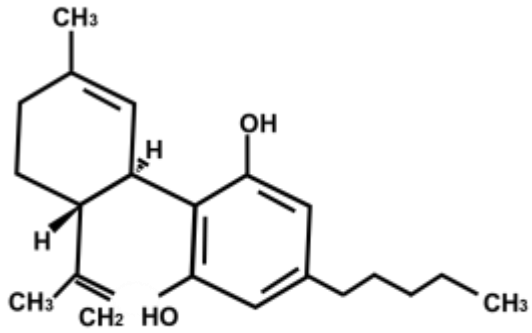
8. According to World Health Organization (WHO)[1], CBD has been demonstrated as an effective treatment of epilepsy in several clinical trials, with one pure CBD product (Epidiolex®[2]) in the United States of America (USA) approved by the U.S. Food and Drug Administration (FDA). There is also preliminary evidence that CBD may be a useful treatment for a number of other medical conditions. CBD is generally well tolerated with a good safety profile. Reported adverse effects may be as a result of drug-drug interactions between CBD and patients' existing medications.

9. In the UK, there are only a limited number of licensed medicinal products derived from or related to cannabis. One of the most common ones is Nabiximols (Sativex®[3]) which is licensed in the UK to treat MS-related muscle spasticity. Use on the NHS is limited since it is not considered cost effective by The National Institute for Health and Care Excellence (NICE[4]).

Physical and Chemical Properties

10. CBD is a type of cannabinoid found in the *Cannabis* plant which was discovered in 1940 by Roger Adams (Adams *et al*., 1940).

11. CBD is made up of 21 carbon, 30 hydrogen atoms and 2 oxygen atoms (Figure 1). CBD has a chemical formula of $C_{21}H_{30}O_2$ and a molecular weight of 314.469 g/mol. Figure 1. Structure of CBD



12. A cannabinoid is one of a class of diverse chemical compounds that acts on cannabinoid receptors known as the endocannabinoid system in cells that are considered to alter neurotransmitter release in the brain.

13. Cannabinoids can be phytocannabinoids which occur naturally in the Cannabis plant (*Cannabis Sativa*) and some other plants; and synthetic cannabinoids, manufactured artificially.

[1] https://www.who.int/medicines/access/controlled-substances/5.2_CBD.pdf
[2] https://www.epidiolex.com/
[3] https://www.mstrust.org.uk/a-z/sativex-nabiximols
[4] https://www.nice.org.uk/

Adsorption Distribution Metabolism Excretion

Absorption

Humans

14. In a randomized controlled trial, CBD oral solution (GW Pharmaceuticals Ltd., London, UK) was given at doses of 5, 10 and 20 mg/kg/d at 3, 7, and 11 days to children ages 4-10 with Dravet syndrome[5], dose proportional increases in area under the curve (AUC) plasma concentrations were produced for CBD and its metabolites (Devinsky *et al*., 2018).

15. In healthy male volunteers given 600 mg oral CBD, mean ± SD whole blood levels of CBD were 0.36 (0.64) ng/mL, 1.62 (2.98) ng/mL and 3.4 (6.42) ng/mL, respectively 1, 2 and 3 hours after administration (Martin Santos *et al*., 2012).

16. A single large oral dose of 400 mg CBD (approximated as 5.0–7.5 mg/kg) in adults was shown to yield a maximum concentration (C_{max}) of 577 nM, while a dose of 800 mg CBD (10–15 mg/kg) resulted in mean C_{max} values of up to 704 nM (Manini *et al*., 2015).

Animals

17. In animal studies, the oral bioavailability of CBD has been shown to be very low (13–19%) (Mechoulam *et al*., 2002). It undergoes extensive first pass metabolism and its metabolites are mostly excreted via the kidneys (Huestis, 2007). Plasma and brain concentrations are dose-dependent in animals, and bioavailability is increased with various lipid formulations (Zgair *et al*., 2016).

18. Interestingly, intraperitoneal (i.p.) injection of CBD corresponded to higher plasma and brain concentrations than oral administration in mice, however in rats, similar concentrations were observed for both administration routes, and brain concentrations were in fact higher following oral compared to i.p. route (Deiana *et al*., 2012).

Food / Uptake

19. Studies have shown that plasma levels of CBD were increased when CBD was administered with food or in a fed state, or when a meal is consumed post-administration. Oral capsules with piperine[6] pronanolipospheres also increased AUC and maximum concentration (C_{max}). This is also demonstrated in animal studies; co-administration of lipids with oral CBD increased systemic availability by almost 3-fold in rats (Zgair *et al*., 2016) and a pro-nanoliposphere formulation increased oral bioavailability by about 6-fold (Cherniakov *et al*., 2017). CBD is considered a highly lipophilic molecule; therefore it has been suggested that CBD may dissolve in the fat

[5] Dravet syndrome: previously known as severe myoclonic epilepsy of infancy (SMEI), is a type of epilepsy with seizures that are often triggered by hot temperatures or fever.
[6] Piperine: along with its isomer chavicine, is the alkaloid responsible for the pungency of black pepper and long pepper.

content of food, increasing its solubility, and absorption and therefore bioavailability as demonstrated by numerous pharmacological drugs (Winter *et al*., 2013).

Distribution

20. CBD is rapidly distributed into the tissues with a high volume of distribution of ~32 L/kg. It has been suggested that, CBD may preferentially accumulate in adipose tissues due to its high lipophilicity (Fasinu *et al*., 2016, Ohlsson *et al*., 1986).

Metabolism

21. CBD is extensively metabolized by experimental animals and humans (Huestis, 2005). Metabolism of CBD is regulated by biotransformation routes usually observed for phytocannabinoids (Harvey & Mechoulam, 1990; Samara, Bialer, & Harvey, 1991), although several metabolic pathways have been described in different animal species and in humans.

22. In the Devinsky *et al* 2018 randomized controlled trial, CBD oral solution (GW Pharmaceuticals Ltd., London, UK) was given at doses of 5, 10 and 20 mg/kg/d at 3, 7, and 11 days to children ages 4-10 with Dravet syndrome. At all doses and timepoints, 7-COOH-CBD was the most abundant circulating metabolite while concentrations of 6-OH-CBD were consistently <10% those of CBD, based on $AUC_{0–t}$. For each analyte, exposure (based on $AUC_{0–t}$ at end of treatment) increased in a dose-related manner, with no major deviation from dose proportionality. Qualitative data generated for the 7-OH-CBD metabolite also showed a dose-proportional increase, with plasma exposures less than that of CBD. At end of treatment, 7-COOH-CBD levels were 13–17 times those of CBD. Total variability in $AUC_{0–t}$ for CBD was moderate to high (% coefficient of variation (%CV) 20%–121%), and was substantially higher for the metabolites (%CV 57%–1750%). There was no effect of repeated CBD administration on the 6-OHCBD:CBD ratio for $AUC_{0–t}$, but there was a marked increase in the 7-COOH-CBD:CBD ratio at end of treatment. The authors suggested a greater accumulation of this metabolite and that this was a major route of biotransformation.

23. Furthermore, CBD is subjected to multiple reactions including hydroxylation, oxidation to carboxylic acids, conjugation, epoxidation and beta-oxidation (Harvey & Mechoulam, 1990; Samara, Bialer, & Harvey, 1990a).

24. CBD is extensively metabolised in the liver. The primary route is hydroxylation to 7-OH-CBD which is then metabolised further. A study in human liver microsomes (HLMs) demonstrated that CBD was metabolized by pooled HLMs to eight monohydroxylated metabolites. Seven recombinant human CYP enzymes were identified as capable of metabolising CBD: CYP1A1, CYP1A2, CYP2C9, CYP2C19, CYP2D6, CYP3A4, and CYP3A5. The two main isoforms involved are CYP3A4 and CYP2C19 (Jiang *et al*., 2011).

25. It has been demonstrated that CBD interferes with hepatic drug metabolism of some compounds (Samara, Brown, & Harvey, 1990) by inactivating cytochrome

P450s of 3A and 2C subfamilies. Such interactions have to be considered in case of CBD co-administration with other drugs metabolized through these routes.

Excretion

26. CBD in its free state and as its glucuronide are primarily excreted in urine and has a half-life of 9 hours (Samara, Bialer, & Harvey, 1990b). The metabolites derived from 7-OH-CBD are excreted in faeces and urine (Hawksworth *et al*., 2004).
A single-dose pharmacokinetic study in beagle dogs using oral doses of 2 mg/kg/ and 8 mg/kg CBD, demonstrated that the CBD half-life of elimination median was 4.2 h (3.8–6.8 h). Median maximal concentration of CBD oil in serum were 102.3 ng/mL (60.7–132.0 ng/mL; 180 nM) and 590.8 ng/mL (389.5–904.5 ng/mL; 1.2 µM) respectively and was reached after 1.5 and 2 h. The AUC time 0-24 hours were 367 (183-437) ng-hr/mL at the 2mg/kg and 2,658 (1,753-3,048) ng-hr/mL at 8mg/kg (Wakshlag *et al*., 2018).

CBD toxicity

27. In animals, the adverse effects of CBD included developmental toxicity, embryo-fetal mortality, spermatogenesis reduction, central nervous system inhibition and neurotoxicity, organ weight alterations, hepatocellular injuries, male reproductive system alterations and hypotension (Rosenkratz *et al*., 1981; Marx *et al*., 2018; Ewing *et al*., 2019). Preliminary data sets suggest adverse reproductive effects (Carvalho *et al*., 2018).

28. In human studies, the Bergamaschi review (2011) reported no significant effect/side effects (including heart rate, blood pressure, psychological measurements, urine examinations) CBD dosing range from 5-1500 mg per person (Hollister *et al*., 1973, Crippa *et al*., 2011, Crippa *et al*., 2010, Fusar-Poli *et al*., 2009, Crippa *et al*., 2004, Zuardi *et al*., 1993, Consroe *et al*., 1979, Hallak *et al*., 2010, Mincis *et al*., 1973, Zuardi *et al*., 2006, Zuardi *et al*., 2010). From the limited data available it appears that single doses of CBD between 20-1500 mg per person do not cause side effects and are considered to be well tolerated (Bergamaschi *et al*., 2011). However, there are limited/no long term data available.

29. In cells, CBD has been shown to be a potent inhibitor of hepatic drug metabolism and it has been shown that CBD interacts with drug metabolizing enzymes *i.e.* the cytochrome p450 family (Bih *et al*., 2015, Jones *et al*., 1972, Stout, 2014)

Genotoxicity of CBD

In vitro genotoxicity studies

Bacteria

30. In Marx *et al*., 2018 study, the mutagenic potential of the CBD (~96%- from CV Sciences, Inc. (San Diego, CA) supplied the test article, a proprietary supercritical CO_2 extract of the aerial parts of hemp) was evaluated in a bacterial reverse mutation test using *Salmonella typhimurium* (TA98, TA100, TA1535, and TA1537) and *Escherichia coli* WP2uvrA (Moltox, Inc., Boone, NC) in the presence and absence of activated rat liver S9 (Moltox, Inc., Boone, NC). Concentrations were 5, 16, 50, 160, 500, 1600, and 5000 µg/plate. No substantial increases in revertant colony numbers were observed in any of the five tester strains following treatment with the test article in the presence or absence of metabolic activation (S9) at any concentration level. Sporadic increases in revertant colony numbers compared to vehicle control were observed in both experiments, reflecting the biological variability of the applied test system; however, there was no tendency of dose related increases and mutation rates remained within the historical control data range. Full results shown in Tables 1 and 2.

31. The following strain specific positive controls, for the experiments without metabolic activation, were used to demonstrate the effectiveness of the test: 4-Nitro-1,2-phenylenediamine (NPD) (4 µg/plate) was used for TA98, sodium azide (SAZ) (2 µg/plate) for TA100 and TA1535, 9-aminoacridine (9-AA) (50 µg/plate) for TA1537, and methyl methane sulfonate (MMS) (2 µg/plate) for WP2. The positive control for experiments with metabolic activation was 2-aminoanthracene (2-AA) (2 µg/plate and 50 µg/plate for all *S. typhimurium* strains and the *E. coli* WP2uvrA strain, respectively). Two negative (vehicle) control groups were utilized because of the different solubility of the test article and positive control items. DMSO served as the vehicle control for the test article, NPD, 9-AA, and 2-AA and ultrapure water (ASTM type 1, prepared by Direct-Q5 system, Millipore) for SAZ and MMS. Colony numbers were determined by manual counting, from which mean values, standard deviations, and mutation rates were calculated. A result was considered positive if a dose related increase in revertant colonies occurred and/or a reproducible biologically relevant positive response for at least one dose group occurred in at least one strain with or without metabolic activation. A result was considered biologically relevant if the increase was twice that of negative controls for strain TA100 or if the increase was three times that of negative controls for all other strains.

Table 1: Summary table of the results of the initial mutation test (taken from Marx *et al* 2018).

Initial mutation test (plate incorporation test)*

Concentrations (µg/plate)	*Salmonella typhimurium* tester strains																*Escherichia coli*			
	TA 98				TA 100				TA 1535				TA 1537				WP2uvrA			
	-S9		+S9		-S9		+S9		-S9		+S9		-S9		+S9		-S9		+S9	
Mean values of revertants per plate and mutation rate (MR)	Mean	MR	Mean	MR	Mean	MR	Mean	MR	Mean	MR	Mean	MR	Mean	MR	Mean	MR	Mean	MR	Mean	MR
Untreated control	13.7	0.89	19.3	1.09	84.0	1.02	105.0	1.12	8.7	1.18	11.7	1.05	12.7	0.93	11.5	0.89	15.5	0.95	21.7	1.16
DMSO control	15.3	1.00	17.7	1.00	82.0	1.00	93.7	1.00	7.3	1.00	11.3	1.00	13.7	1.00	12.7	1.00	14.0	1.00	18.7	1.00
Ultrapure Water control	–	–	–	–	83.3	1.00	–	–	8.3	1.00	–	–	–	–	–	–	14.0	1.00	–	–
5000	11.0	0.72	14.0	0.79	54.0	0.66	78.0	0.83	3.7	0.50	4.7	0.41	4.7	0.34	2.0	0.16	13.0	0.93	18.0	0.96
1600	13.3	0.87	18.0	1.02	56.0	0.68	70.3	0.75	9.3	1.27	8.0	0.71	12.7	0.93	9.7	0.76	10.7	0.76	18.0	0.96
500	12.3	0.80	22.0	1.25	60.7	0.74	86.3	0.92	8.7	1.18	10.0	0.88	10.0	0.73	12.0	0.95	11.3	0.81	19.3	1.04
160	11.0	0.72	23.0	1.30	89.3	1.09	92.3	0.99	8.0	1.09	11.3	1.00	11.7	0.85	12.7	1.00	14.3	1.02	19.7	1.05
50	15.3	1.00	24.0	1.36	98.3	1.20	87.0	0.93	7.3	1.00	9.7	0.85	12.0	0.88	13.7	1.08	11.0	0.79	16.7	0.89
16	15.3	1.00	20.0	1.13	86.0	1.05	91.7	0.98	9.0	1.23	9.0	0.79	12.0	0.88	12.7	1.00	14.0	1.00	17.7	0.95
5	13.3	0.87	17.0	0.96	84.3	1.03	101.0	1.08	9.0	1.23	12.0	1.06	13.3	0.98	12.3	0.97	14.0	1.00	18.3	0.98
NPD (4)	208.0	13.57	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
SAZ (2)	–	–	–	–	1008.0	12.10	–	–	1066.7	128.00	–	–	–	–	–	–	–	–	–	–
9AA (50)	–	–	–	–	–	–	–	–	–	–	–	–	574.0	42.00	–	–	–	–	–	–
MMS (2 µL)	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	422.7	30.19	–	–
2AA (2)	–	–	1469.3	83.17	–	–	2506.7	26.76	–	–	105.0	9.26	–	–	116.7	10.79	–	–	–	–
2AA (50)	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	209.0	11.20

*DMSO was used as the vehicle for the test article and positive control substances: NPD, 9AA, and 2AA. Ultrapure water was used as the vehicle for SAZ and MMS. The mutation rate of the test item and the untreated control is given referring to the DMSO.

Table 2: Summary table of the results of the confirmatory mutation test (taken from Marx *et al.,* 2018).

Confirmatory mutation test (preincubation test)*

Concentrations (µg/plate)	*Salmonella typhimurium* tester strains																*Escherichia coli*			
	TA 98				TA 100				TA 1535				TA 1537				WP2uvrA			
	-S9		+S9		-S9		+S9		-S9		+S9		-S9		+S9		-S9		+S9	
Mean values of revertants per plate and mutation rate (MR)	Mean	MR	Mean	MR	Mean	MR	Mean	MR	Mean	MR	Mean	MR	Mean	MR	Mean	MR	Mean	MR	Mean	MR
Untreated control	14.7	0.98	22.7	1.33	102.0	1.02	126.3	1.10	8.7	0.93	8.0	0.83	9.3	1.27	9.0	0.84	13.3	0.77	19.3	1.16
DMSO control	15.3	1.00	17.0	1.00	100.0	1.00	114.3	1.00	9.3	1.00	9.7	1.00	7.3	1.00	10.7	1.00	17.3	1.00	16.7	1.00
Ultrapure water control	–	–	–	–	86.7	1.00	–	–	13.0	1.00	–	–	–	–	–	–	17.7	1.00	–	–
5000	5.0	0.33	16.7	0.98	44.3	0.44	61.3	0.54	0.3	0.04	9.0	0.93	0.7	0.09	4.7	0.44	12.7	0.73	16.3	0.98
1600	9.0	0.59	23.3	1.37	46.7	0.47	71.3	0.62	2.7	0.29	8.3	0.86	2.0	0.27	8.7	0.81	16.3	0.94	17.7	1.06
500	8.3	0.54	18.0	1.06	45.7	0.46	69.7	0.61	6.0	0.64	9.3	0.97	0.7	0.09	8.3	0.78	14.3	0.83	15.3	0.92
160	12.3	0.80	26.7	1.57	80.3	0.80	91.0	0.80	8.3	0.89	10.0	1.03	4.3	0.59	9.3	0.88	10.7	0.62	20.3	1.22
50	12.3	0.80	26.3	1.55	94.7	0.95	100.7	0.88	10.0	1.07	10.0	1.03	7.7	1.05	10.3	0.97	16.0	0.92	18.0	1.08
16	14.0	0.91	29.0	1.71	94.7	0.95	101.0	0.88	9.3	1.00	8.7	0.90	7.7	1.05	11.7	1.09	12.3	0.71	19.3	1.16
5	14.3	0.93	23.0	1.35	94.7	0.95	115.7	1.01	10.3	1.11	13.0	1.34	7.3	1.00	11.3	1.06	16.0	0.92	18.3	1.10
NPD (4)	310.0	20.22	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
SAZ (2)	–	–	–	–	1389.3	16.03	–	–	3104.0	238.77	–	–	–	–	–	–	–	–	–	–
9AA (50)	–	–	–	–	–	–	–	–	–	–	–	–	586.0	79.91	–	–	–	–	–	–
MMS (2 µL)	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	898.7	50.87	–	–
2AA (2)	–	–	989.3	58.20	–	–	1752.0	15.32	–	–	113.3	11.72	–	–	133.7	12.53	–	–	–	–
2AA (50)	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	181.3	10.88

∗DMSO was applied as vehicle of the test article and positive control substances: NPD, 9AA, and 2AA; and the ultrapure water was applied as vehicle for the SAZ and MMS. The mutation rate of the test item and the untreated control is given referring to the DMSO.

Mammalian cells

32. An *in vitro* mammalian chromosomal aberration test was performed to determine whether CBD (~96%- from CV Sciences, Inc. (San Diego, CA) supplied the test article, a proprietary supercritical CO_2 extract of the aerial parts of hemp) 10, 20, 30, 50, 60, 70 and 90 µg/mL could induce structural chromosomal aberrations in cultured V79 Chinese hamster lung cells (Marx *et al*., 2018). In Experiment A, V79 cultures (5×10^5 cells/group) were exposed to the negative control or each test article concentration for a three-hour period with (50, 70, and 90 µg/mL) and without (10, 20, and 30 µg/mL) metabolic activation. Groups of cells were also exposed to the respective positive controls (ethyl ethanesulfonate and cyclophosphamide). Following the exposure period, the cells were washed with Dulbecco's Modified Eagle's Medium containing 5% fetal bovine serum, and growth medium was added. Sampling was made 20 hours following the start of treatment. All individual test article and negative and positive control experiments were carried out in duplicate, and the Relative Increase in Cell Counts was calculated. Experiment B was conducted as described for Experiment A except that the exposure period without metabolic activation was 20 hours (while exposure with metabolic activation remained 3 hours), and sampling was made after 20 hours for groups treated without metabolic activation and after 28 hours (to cover the potential for mitotic delay) for groups treated both with and without metabolic activation. The test article concentrations were 50, 70, and 90 µg/mL with S9 metabolic activation and 1.25, 2.5, and 5 µg/mL without activation. The authors stated that CBD was considered as nonclastogenic if there were no statistically significant increases in the number of metaphases with aberrations in dose groups compared to the negative control and/or if the number of metaphases with aberrations was within the range of the laboratory's historical control data.

33. In the negative control group, the percentage of cells with structural aberrations was equal to or less than 5%, confirming the suitability of the V79 cell line used. The concurrent positive controls caused the expected biologically relevant increases of cells with structural chromosome aberrations as compared to current solvent and historical controls. CBD did not induce an increase in the number of cells with aberrations or rates of polyploidy or end reduplicated metaphases at concentrations ranging from 10 to 90 µg/mL. There were no statistically significant differences between treatment and the solvent control groups, and no dose-response relationships were noted. Full results in Table 3.

Table 3. Summary of chromosomal aberration test results (Table taken from Marx *et al*., 2018).

Concentration	S9 mix	Treatment	Harvest	Mean aberrant cells (per 200 cells)		Number of aberrations	
µg/mL		Time (h)	Time(h)	Incl. gaps	Excl. gaps	Incl. gaps	Excl. gaps
Experiment A [1]							
Test Article							
10	–	3	20	7	4	7	4
20	–	3	20	6	3	8	3
30	–	3	20	7	4	8	4
Vehicle control	–	3	20	6	3	6	3
Positive control	–	3	20	60**	48**	100**	63**
Hist. veh. control[4]	–	3	20	0.35–11.99	0.00–7.30	n/a	n/a
Test Article							
50	+	3	20	8	5	9	5
70	+	3	20	7	3	7	3
90	+	3	20	9	5	10	5
Vehicle control	+	3	20	7	4	7	3
Positive control	+	3	20	69**	58**	130**	85**
Hist. veh. control[4]	+	3	20	0.00–15.19	0.53–5.47	n/a	n/a
Experiment B [2]							
Test Article							
1.25	–	20	20	6	2	6	2
2.5	–	20	20	6	3	6	3
5	–	20	20	8	3	8	3
Vehicle control	–	20	20	6	3	7	3
Positive control	–	20	20	55**	48**	112**	60**
Hist. veh. control[4]	–	20	20	2.58–10.42	1.39–4.94	n/a	n/a
Experiment B [3]							
Test Article							
1.25	–	20	28	8	4	8	4
2.5	–	20	28	7	4	8	4
5	–	20	28	8	4	8	4
Vehicle control	–	20	28	6	3	6	3
Positive control	–	20	28	67**	62**	146**	84**
Hist. veh. control[4]	–	20	28	2.70–11.30	3.00–3.00	n/a	n/a
Test Article							
50	+	3	28	9	4	10	5
70	+	3	28	9	4	10	4
90	+	3	28	7	3	8	4
Vehicle control	+	3	28	7	4	7	4
Positive control	+	3	28	63**	54**	140**	105**
Hist. veh. control[4]	+	3	28	4.73–8.27	0.00–6.97	n/a	n/a

[1]Positive controls: (–S9): ethyl methanesulfonate (1.0 µL/mL); (+S9): cyclophosphamide (5.0 µg/mL).
[2]Positive control: (–S9): ethyl methanesulfonate (0.4 µL/mL).
[3]Positive controls: (–S9) ethyl methanesulfonate (0.4 µL/mL); (+S9): cyclophosphamide (5.0 µg/mL).
[4]Numbers reported are the 95% confidence interval.
**p < 0.01 to the concurrent vehicle control and to the historical vehicle control.
n/a, not available; veh., vehicle; Hist., historical; Incl., including; Excl., excluding

34. In another study which was performed by single cell electrophoresis assay (Comet assay) with Caco-2 cells demonstrated that 24 hour exposure to CBD at 10 µM 3144.6 µg/L) alone did not significantly affect DNA damage after (Aviello *et al*., 2011). DNA damage (% DNA tail) was quantified using at least 75 cells per gel were scored and each sample was evaluated in triplicate (*n*=3 independent experiments).

35. The results of the Russo *et al* (2019) study demonstrated that CBD caused formation of comets (which reflect single and double strand breaks and apurinic sites), oxidation of DNA bases and induction of micronuclei (MN) in human cells-human liver cell line (HepG2) and buccal-derived cells (TR146). The HepG2 cells were exposed to the CBD (CBD, CAS 13956-29-1, purity 99.95%) was obtained from LGC Standards GmbH (Germany) for 3 hours and 24 hours (3 hours: dose range 0.66, 2.0, 6.0, 18.0 and 54 µM (207.5, 415.1, 1886.8 and 16981. 1 µg/L), 24 hours: dose range 0.22, 0.66, 2.0, 6.0 and 18 µM (69.2, 207.5, 628.9 and 5660.4 µg/L)). TR146 cells were treated with the cannabinoids for 3 hours (dose range 2.00, 6.0, 18.0 and 54.0 µM). In all experiments, solvent controls (methanol) and positive controls (H_2O_2, 50 µM) were included. CBD caused DNA damage in both cell types (HepG2 and TR146). In the liver-derived cells *i.e.* HepG2, significant induction of damage was seen with both compounds at concentrations ≥ 6.0 µM after 3 hours. The results are summarized in Table 4. When the cells were treated for 24 hours, clear damage was observed with the lower concentrations (≥2.0 µM). In the TR146 cells, positive findings were obtained under identical conditions, *i.e.*, induction of comets was detected at concentrations ≥ 6.0 µM after 3 h. The authors stated that CBD was more active than its propyl analogue cannabidivarin (CBDV) in both cell lines, when the cells were exposed for 3 hours, the extent of DNA damage which was seen with the former compound under identical conditions was approximately threefold higher. To find out if the compounds were converted to mutagenic metabolites by liver enzymes, an additional experimental series was realized, in which S9 mix (which contains active phase I enzymes) was added to the incubation during the treatment of TR146 cells with CBD. Addition of the enzyme homogenate caused induction of DNA damage in TR146 cells, but no such effect was seen when the liver enzymes were inactivated by heating. To investigate if CBD causes oxidative damage of DNA bases, experiments were conducted with lesion-specific enzymes (Formamidopyrimidine DNA glycosylase (FPG) and endonuclease III (ENDO III)). This showed that CBD caused oxidation of purines and pyrimidines. Even with the lowest levels (0.66 µM), significant induction of comet formation was observed. Furthermore, to find out if treatment of human liver-derived cells leads to formation of MNi, which reflect structural and numerical chromosomal aberrations, cytome MN experiments were conducted with HepG2 cells. CBD caused induction of MNi at low concentrations (≥0.22 µM). Additionally, a significant increase of other nuclear anomalies: Nuclear buds (Nbuds) and nucleoplasmatic bridges (NPBs), as well as induction of cell death (necrosis and apoptosis) was observed after treatment.

Table 4: Impact of CBD on MN formation and on the rates of various nuclear aberrations in HepG2 cells (taken from Russo *et al* 2019).

Compounds	Concentrations (μM)	CPBI Mean ± SD	CT %	BN-MN[a] Mean (‰) ± SD	MNi[b] Mean (‰) ± SD	Nbuds Mean (‰) ± SD	NPBs Mean (‰) ± SD	Necrosis Mean (‰) ± SD	Apoptosis Mean (‰) ± SD
Neg. Ctrl	0	2.04 ± 0.03	–	5.25 ± 0.35	5.75 ± 0.35	4.75 ± 0.35	3.50 ± 0.71	6.25 ± 0.35	3.00 ± 0.71
CBD	0.07	2.00 ± 0.08	3.92	6.50 ± 1.41	6.50 ± 1.41	16.00 ± 2.12*	5.25 ± 0.35	16.25 ± 1.77*	13.50 ± 0.71*
	0.22	1.93 ± 0.04	10.60	21.00 ± 1.41*	31.00 ± 2.12*	25.50 ± 2.83*	8.50 ± 1.41*	21.00 ± 0.70*	25.25 ± 3.18*
	0.66	1.83 ± 0.04	20.22	31.25 ± 2.47*	46.25 ± 3.89*	37.25 ± 1.06*	10.00 ± 1.41*	30.75 ± 1.77*	29.00 ± 1.41*
	2.00	1.72 ± 0.01	30.76	39.25 ± 3.89*	53.25 ± 2.47*	43.00 ± 2.83*	14.00 ± 0.71*	33.50 ± 2.12*	37.25 ± 1.77*
SC[c]		1.80 ± 0.00	23.05	5.00 ± 1.41	6.25 ± 0.35	5.50 ± 1.41	3.25 ± 1.06	6.75 ± 1.06	3.00 ± 0.71

CBPI cytokinesis-block proliferation indices, *CT* cytostasis (%), HepG2 cells were treated with different concentrations of the test compounds for 3 h. Numbers represent results (means±SD) obtained in two independent experiments, and in each experiment, two cultures were made per experimental point. Four slides were prepared and 2000 cells were evaluated. All statistical calculations are based on comparisons between results which were obtained with cells which had been treated with the test compounds and results which were obtained with corresponding solvent controls.

BN–MNi binucleated cells with micronuclei, *MNi* micronuclei, *Nbuds* nuclear buds, *NPBs* nucleoplasmatic bridges, Neg. Ctrl cells cultivated in medium, SC solvent control, Pos. Ctrl cyclophosphamide (500 μg/ml)
*Significant differences from solvent control values (Dunnett test, p≤0.05)
[a] Number of binucleated cells with MN
[b] Total number of MN from binucleated cells
[c] Methanol was used as solvent control [0.06% (v/v) in experiments with CBD

In vivo genotoxicity studies

36. Marx *et al*., 2018 performed an *in vivo* mouse micronucleus test. Specific pathogen-free mice were utilized for the study. Humaqua (sterile water, TEVA Pharmaceutical Works Private Ltd., Co.) was used as the negative control and as the vehicle for administration of the positive control (cyclophosphamide (Sigma-Aldrich, Germany)). Sunflower oil was also used as a negative control, as well as the solvent for the test article. A single dose of CBD (~96%- from CV Sciences, Inc. (San Diego, CA) supplied the test article, a proprietary supercritical CO_2 extract of the aerial parts of hemp) was administered by gavage to two male and female mice at a concentration of 2000 mg/kg body weight (bw), and the animals were observed at regular intervals for signs of toxicity and mortality. On the basis of the results of the preliminary toxicity test, single oral gavage doses of 500 (n = 5), 1000 (n = 5), and 2000 (n = 10) mg/kg bw were chosen for the main study. Male Crl:NMRI BR mice were randomly divided into five groups: a negative control (n = 10), positive control (n = 5), and the three test groups. The positive control, cyclophosphamide 60 mg/kg bw, was given intraperitoneal injection. Two extra animals were included in the high-dose group in order to maintain statistical power in case any animals died before the scheduled sacrifices. No significant differences were observed in frequency of micronucleated polychromatic erythrocytes (MPCEs) between the three dose groups compared to the negative control, and all results were within the laboratory's historical control range. Compared to the negative control group, the numbers of polychromatic erythrocytes (PCEs) at 24 and 48 hours sampling times in the 500 and 1000 mg/kg bw groups were similar. In the 2000 mg/kg bw dose group, the number of PCEs was slightly decreased compared to the negative control group at the 24 and 48 hours sampling time points. The effect was not biologically significant but demonstrated exposure of the bone marrow to the test article. A large, statistically

significant increase in MPCE frequency was observed in the positive control group compared to negative control. The authors stated that the cyclophosphamide-treated mice had MPCE counts that were slightly higher (61.40/2000 PCE) than historical controls (54.03/2000 PCE) but this deviation did not influence the quality or integrity of the study. Full results in Table 5.

Table 5. Summary table of the results for the mouse micronucleus test (taken from Marx *et al*., 2018).

Groups (mg/kg bw)	Sampling time (hour)	Total number of PCEs analyzed	MPCE (per 4000 PCE)		PCE/NCE	
			Mean	±SD	Mean	±SD
Negative control 0	24	20000	4.40	1.14	1.13	0.04
500	24	20000	4.60	0.55	1.09	0.12
1000	24	20000	4.60	1.14	1.06	0.08
2000	24	20000	5.20	0.84	0.91	0.03
Positive control 60	24	20000	122.80**	6.02	0.46	0.10
Negative control 0	48	20000	4.40	0.89	1.12	0.12
2000	48	20000	5.20	1.30	0.94	0.10

Positive control: cyclophosphamide.
Negative control: 1% aqueous methylcellulose.
**: $p < 0.01$ to the concurrent negative control and to the historical control.

37. In contrast, another *in vivo* study by Zimmerman and Raj (1980) reported that CBD exerts a positive mutagenic effect in hybrid mice. In the subacute treatment group, hybrid male mice (C57BL X C3H)F_1 were injected intraperitoneally for 5 consecutive days with CBD (99% dry-dissolved in DMSO) at 10mg/kg. This demonstrated that CBD statistically caused a greater incidence of micronuclei (5%-average of 5 mice) than the DMSO controls. In the acute treatment hybrid male mice (C57BL X C3H)F_1 were injected intraperitoneally with CBD (99% dry-dissolved in DMSO- 1, 5, 10 and 25 kg/kg-means of 4 animals in each treatment group) for 24 hours. This showed CBD dosage dependent increased number of micronuclei (6-9%). Using the mice from the subacute treatment group *i.e.* hybrid male mice (C57BL X C3H)F_1 were injected intraperitoneally for 5 consecutive days with CBD (99% dry-dissolved in DMSO) at 10mg/kg, chromosomal analysis from bone marrow cells was undertaken. CBD induced nuclear aberrations in the mice. The average value of aberrations was 14.3%.

Summary/Conclusions

38. *In vitro* studies using bacteria demonstrated no mutagenic effects post exposure to CBD (Marx *et al*., 2018). Furthermore, comet assay with Caco-2 cells demonstrated that 24 hours exposure to CBD at 10 µM alone did not significantly affect DNA damage Aviello *et al*., 2011 or induce structural chromosomal aberrations in cultured V79 Chinese hamster lung cells (10 to 90 µg/mL) (Marx *et al*., 2018).

39. In contrast, DNA damage was observed in V79 cells. CBD caused formation of comets, oxidation of DNA bases and induction of micronuclei (MN) in human cells-human liver cell line (HepG2) and buccal-derived cells (TR146). At 24 hours, clear damage was observed with the lower concentrations (≥2.0 µM). In the TR146 cells, positive findings were obtained under identical conditions, *i.e*., induction of comets was detected at concentrations ≥ 6.0 µM after 3 hours (Russo *et al* (2019).

40. A recent *in vivo* study using the micronucleus test demonstrated no significant differences were observed in frequency of micronucleated polychromatic erythrocytes (MPCEs) in mice at single oral gavage doses of 500, 1000 and 2000 mg/kg bw (Marx *et al,.* 2018).

41. On the other hand, an early study in the 1980s demonstrated that CBD induced nuclear aberrations in mice (i.p) at 10mg/kg. The average value of aberrations was 14.3% Zimmerman and Raj (1980).

QUESTIONS FOR THE COM

The COM are asked to comment on the genotoxicity studies provided.

i) Has an appropriate range of studies been conducted to come to a conclusion on the genotoxic potential of CBD?
   a) *In vivo*?
   b) *In vitro*?

ii) Will different products require a case by case basis based on method of extraction?

iii) Any future research recommended?

iv) Any other comments you may have?

Secretariat

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References

Aviello, G., Romano, B., Borrelli, F., Capasso, R., Gallo, L., Piscitelli, F., Di Marzo, V. and Izzo, A.A., 2012. Chemopreventive effect of the non-psychotropic phytocannabinoid cannabidiol on experimental colon cancer. *Journal of molecular medicine*, 90(8), pp.925-934.

Bergamaschi, M., Helena Costa Queiroz, R., Waldo Zuardi, A. and Crippa, A.S., 2011. Safety and side effects of cannabidiol, a Cannabis sativa constituent. *Current drug safety*, 6(4), pp.237-249.

Bih, C.I., Chen, T., Nunn, A.V., Bazelot, M., Dallas, M. and Whalley, B.J., 2015. Molecular targets of cannabidiol in neurological disorders. *Neurotherapeutics*, 12(4), pp.699-730.

Carvalho, R.K., Santos, M.L., Souza, M.R., Rocha, T.L., Guimarães, F.S., Anselmo-Franci, J.A. and Mazaro-Costa, R., 2018. Chronic exposure to cannabidiol induces reproductive toxicity in male Swiss mice. *Journal of Applied Toxicology*, 38(9), pp.1215-1223

Carvalho, R.K., Souza, M.R., Santos, M.L., Guimarães, F.S., Pobbe, R.L.H., Andersen, M.L. and Mazaro-Costa, R., 2018. Chronic cannabidiol exposure promotes functional impairment in sexual behaviour and fertility of male mice. *Reproductive Toxicology*, 81, pp.34-40.

Cherniakov, I., Izgelov, D., Barasch, D., Davidson, E., Domb, A.J. and Hoffman, A., 2017. Piperine-pro-nanolipospheres as a novel oral delivery system of cannabinoids: Pharmacokinetic evaluation in healthy volunteers in comparison to buccal spray administration. *Journal of Controlled Release*, 266, pp.1-7.

Consroe, P., Carlini, E.A., Zwicker, A.P. and Lacerda, L.A., 1979. Interaction of cannabidiol and alcohol in humans. *Psychopharmacology*, 66(1), pp.45-50.

Crippa, J.A.S., Derenusson, G.N., Ferrari, T.B., Wichert-Ana, L., Duran, F.L., Martin-Santos, R., Simões, M.V., Bhattacharyya, S., Fusar-Poli, P., Atakan, Z. and Filho, A.S., 2011. Neural basis of anxiolytic effects of cannabidiol (CBD) in generalized social anxiety disorder: a preliminary report. *Journal of Psychopharmacology*, 25(1), pp.121-130.

Crippa, J.A.S., Zuardi, A.W. and Hallak, J.E., 2010. Therapeutical use of the cannabinoids in psychiatry. *Brazilian Journal of Psychiatry*, 32, pp.556-566.

Crippa, J.A., Zuardi, A.W., Garrido, G.E., Wichert-Ana, L., Guarnieri, R., Ferrari, L., Azevedo-Marques, P.M., Hallak, J.E.C. and McGuire, P.K., 2004. Effects of cannabidiol (CBD) on regional cerebral blood flow. *Neuropsychopharmacology*, 29(2), p.417.

Deiana, S., Watanabe, A., Yamasaki, Y., Amada, N., Arthur, M., Fleming, S., Woodcock, H., Dorward, P., Pigliacampo, B., Close, S. and Platt, B., 2012. Plasma

and brain pharmacokinetic profile of cannabidiol (CBD), cannabidivarine (CBDV), Δ 9-tetrahydrocannabivarin (THCV) and cannabigerol (CBG) in rats and mice following oral and intraperitoneal administration and CBD action on obsessive–compulsive behaviour. *Psychopharmacology*, 219(3), pp.859-873.

Devinsky, O., Patel, A.D., Thiele, E.A., Wong, M.H., Appleton, R., Harden, C.L., Greenwood, S., Morrison, G., Sommerville, K. and GWPCARE1 Part A Study Group, 2018. Randomized, dose-ranging safety trial of cannabidiol in Dravet syndrome. *Neurology*, 90(14), pp.e1204-e1211.

Fusar-Poli, P., Crippa, J.A., Bhattacharyya, S., Borgwardt, S.J., Allen, P., Martin-Santos, R., Seal, M., Surguladze, S.A., O'Carrol, C., Atakan, Z. and Zuardi, A.W., 2009. Distinct effects of Δ9-tetrahydrocannabinol and cannabidiol on neural activation during emotional processing. *Archives of general psychiatry*, 66(1), pp.95-105.

Ewing, L.E., Skinner, C.M., Quick, C.M., Kennon-McGill, S., McGill, M.R., Walker, L.A., ElSohly, M.A., Gurley, B.J. and Koturbash, I., 2019. Hepatotoxicity of a Cannabidiol-Rich Cannabis Extract in the Mouse Model. *Molecules*, 24(9), p.1694.

Fasinu, P.S., Phillips, S., ElSohly, M.A. and Walker, L.A., 2016. Current status and prospects for cannabidiol preparations as new therapeutic agents. Pharmacotherapy: *The Journal of Human Pharmacology and Drug Therapy*, 36(7), pp.781-796

Hallak, J.E., Machado-de-Sousa, J.P., Crippa, J.A.S., Sanches, R.F., Trzesniak, C., Chaves, C., Bernardo, S.A., Regalo, S.C. and Zuardi, A.W., 2010. Performance of schizophrenic patients in the Stroop Color Word Test and electrodermal responsiveness after acute administration of cannabidiol (CBD). *Brazilian Journal of Psychiatry*, 32(1), pp.56-61.

Harvey, D.J. and Mechoulam, R., 1990. Metabolites of cannabidiol identified in human urine. *Xenobiotica*, 20(3), pp.303-320.

Hawksworth, G. and McArdle, K., 2004. Metabolism and pharmacokinetics of cannabinoids. *The Medicinal Uses of Cannabis and Cannabinoids* (Guy GW, Whittle BA, Robson PJ, eds.). Pharmaceutical Press: London, pp.205-228.

Hollister, L.E., 1973. Cannabidiol and cannabinol in man. *Experientia*, 29(7), pp.825-826.

Huestis, M.A., 2007. Human cannabinoid pharmacokinetics. *Chemistry & biodiversity*, 4(8), pp.1770-1804.

Jiang, R., Yamaori, S., Takeda, S., Yamamoto, I. and Watanabe, K., 2011. Identification of cytochrome P450 enzymes responsible for metabolism of cannabidiol by human liver microsomes. *Life sciences*, 89(5-6), pp.165-170.

Jones, G. and Pertwee, R.G., 1972. A metabolic interaction in vivo between cannabidiol and Δ1-tetrahydrocannabinol. *British journal of pharmacology*, 45(2), pp.375-377.

Martin-Santos, R., a Crippa, J., Batalla, A., Bhattacharyya, S., Atakan, Z., Borgwardt, S., Allen, P., Seal, M., Langohr, K., Farre, M. and Zuardi, A.W., 2012. Acute effects of a single, oral dose of d9-tetrahydrocannabinol (THC) and cannabidiol (CBD) administration in healthy volunteers. *Current pharmaceutical design*, 18(32), pp.4966-4979.

Marx, T.K., Reddeman, R., Clewell, A.E., Endres, J.R., Béres, E., Vértesi, A., Glávits, R., Hirka, G. and Szakonyiné, I.P., 2018. An Assessment of the Genotoxicity and Subchronic Toxicity of a Supercritical Fluid Extract of the Aerial Parts of Hemp. *Journal of toxicology*, 2018.

Manini AF, Yiannoulos G, Bergamaschi MM, Hernandez S, Olmedo R, Barnes AJ, Winkel G, Sinha R, Jutras-Aswad D, Huestis MA, Hurd YL. Safety and pharmacokinetics of oral cannabidiol when administered concomitantly with intravenous fentanyl in humans. *Journal of addiction medicine*. 2015 May;9(3):204.

Mechoulam, R., Parker, L.A. and Gallily, R., 2002. Cannabidiol: an overview of some pharmacological aspects. *The Journal of Clinical Pharmacology*, 42(S1), pp.11S-19S.

Mincis, M., Pfeferman, A., Guimarães, R.X., Ramos, O.L., Zukerman, E., Karniol, I.G. and Carlini, E.A., 1973. Chronic administration of cannabidiol in man. Pilot study. AMB: *revista da Associacao Medica Brasileira*, 19(5), pp.185-190.

Ohlsson, A., Lindgren, J.E., Andersson, S., Agurell, S., Gillespie, H. and Hollister, L.E., 1986. Single-dose kinetics of deuterium-labelled cannabidiol in man after smoking and intravenous administration. *Biomedical & environmental mass spectrometry*, 13(2), pp.77-83.

Rosenkrantz, H., Fleischman, R.W. and Grant, R.J., 1981. Toxicity of short-term administration of cannabinoids to rhesus monkeys. *Toxicology and applied pharmacology*, 58(1), pp.118-131.

Russo, C., Ferk, F., Mišík, M., Ropek, N., Nersesyan, A., Mejri, D., Holzmann, K., Lavorgna, M., Isidori, M. and Knasmüller, S., 2019. Low doses of widely consumed cannabinoids (cannabidiol and cannabidivarin) cause DNA damage and chromosomal aberrations in human-derived cells. *Archives of toxicology*, 93(1), pp.179-188.

Samara, E., Bialer, M. and Harvey, D.J., 1990. Identification of glucose conjugates as major urinary metabolites of cannabidiol in the dog. *Xenobiotica*, 20(2), pp.177-183.

Samara, E., Bialer, M. and Harvey, D.J., 1990. Pharmacokinetics of urinary metabolites of cannabidiol in the dog. *Biopharmaceutics & drug disposition*, 11(9), pp.785-795

Stout, S.M. and Cimino, N.M., 2014. Exogenous cannabinoids as substrates, inhibitors, and inducers of human drug metabolizing enzymes: a systematic review. *Drug metabolism reviews*, 46(1), pp.86-95.

Wakshlag, J.J., Frye, C.F., Gamble, L.J., Boesch, J., Schwark, W.S., Brown, H., Wolfe, L., Mann, S. and Berthelsen, E.S., 2018. Pharmacokinetics, safety, and clinical efficacy of cannabidiol treatment in osteoarthritic dogs. *Frontiers in veterinary science*, 5, p.165.

Winter, H., Ginsberg, A., Egizi, E., Erondu, N., Whitney, K., Pauli, E. and Everitt, D., 2013. Effect of a high-calorie, high-fat meal on the bioavailability and pharmacokinetics of PA-824 in healthy adult subjects. *Antimicrobial agents and chemotherapy*, 57(11), pp.5516-5520.

Zgair, A., Wong, J.C., Lee, J.B., Mistry, J., Sivak, O., Wasan, K.M., Hennig, I.M., Barrett, D.A., Constantinescu, C.S., Fischer, P.M. and Gershkovich, P., 2016. Dietary fats and pharmaceutical lipid excipients increase systemic exposure to orally administered cannabis and cannabis-based medicines. *American journal of translational research*, 8(8), p.3448.

Zimmerman, A.M. and Raj, Y., 1980. Influence of cannabinoids on somatic cells *in vivo. Pharmacology*, 21(4), pp.277-287.

Zuardi, A.W., Crippa, J.A.D.S., Hallak, J.E.C., Moreira, F.A. and Guimarães, F.S., 2006. Cannabidiol, a Cannabis sativa constituent, as an antipsychotic drug. *Brazilian journal of medical and biological research*, 39(4), pp.421-429.

Zuardi, A.W., Cosme, R.A., Graeff, F.G. and Guimarães, F.S., 1993. Effects of ipsapirone and cannabidiol on human experimental anxiety. *Journal of psychopharmacology*, 7, pp.82-88.

Zuardi, A.W., Crippa, J.A.D.S., Dursun, S.M., Morais, S.L., Vilela, J.A.A., Sanches, R.F. and Hallak, J.E.C., 2010. Cannabidiol was ineffective for manic episode of bipolar affective disorder. *Journal of Psychopharmacology*, 24(1), pp.135-137.

Abbreviations

2-AA	2-aminoanthracene
AUC	area under the curve
CBD	cannabidiol
CBDV	cannabidivarin
COM	Mutagenicity of Chemicals in Food Consumer Products and The Environment
COT	Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment Committee
C_{max}	maximum concentration
EU	European Union
ENDO III	endonuclease III
FDA	U.S. Food and Drug Administration
FPG	Formamidopyrimidine DNA glycosylase
FSA	Food Standards Agency
HLM	human liver microsome
MPCEs	micronucleated polychromatic erythrocytes
MMS	methyl methane sulfonate
Nbuds	Nuclear buds
NPD	4-Nitro-1,2-phenylenediamine
PCEs	polychromatic erythrocytes
SAZ	sodium azide
USA	United States of America
WHO	World Health Organization