MUT/2020/01

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

REVIEW OF GENOTOXICITY OF CANNABIDIOL (CBD) UPDATE

Background and 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 assessment 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[1]) on the potential adverse effects of CBD products was reviewed in July 2019. The genotoxicity data was conflicting and therefore the Committee on the Mutagenicity of Chemicals in Food Consumer Products and The Environment (COM) was asked to review the genotoxicity data.

3. At their meeting in October 2019, the COM concluded that the *in vitro* and *in vivo* genotoxicity studies were inadequate and therefore a conclusion on the genotoxic potential of CBD could not be reached.

4. With the cooperation of GW Pharmaceuticals, the manufacturers of Epidiolex[2®] the medicinal form of CBD, the COT Secretariat were able to examine and discuss recent clinical and non-clinical data on the medicinal form of CBD which has now become available online.

5. In January 2020, the COT were given an update on CBD on the medicinal data that is publicly available online (TOX/2020/02)[3]. This included some new genotoxicity data. The COT therefore referred the new genotoxicity data to be reviewed by the COM.

6. This paper provides details on the newly available genotoxicity studies on the medicinal form of CBD.

Epidiolex® Data

7. Epidiolex® is a prescription medicine[4] that is used to treat seizures associated with Lennox-Gastaut syndrome (LGS) [5]or Dravet syndrome[6] in patients 2 years of age and older. Symptoms include multiple types of seizure (fits), abnormal electrical

[1] https://cot.food.gov.uk/sites/default/files/tox2019-32.pdf
[2] https://www.epidiolex.com/
[3] https://cot.food.gov.uk/sites/default/files/tox202002cbd.pdf
[4] https://www.accessdata.fda.gov/drugsatfda_docs/label/2018/210365lbl.pdf
[5] Lennox–Gastaut syndrome (LGS): a complex, rare, and severe childhood-onset epilepsy.
[6] 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.

activity in the brain, learning disability and behavioural problems. These conditions are rare, and Epidiolex was designated an 'orphan medicine[7]'.

8. The information presented is scientific data from the Epidiolex® medicine approval package. Some of the data that is publicly available data is in a redacted form. Only the publicly available information has been used in this paper.

Product Formulation

9. The medicinal substance is produced from an extract of *Cannabis sativa L*. plants (*i.e.* botanical substance CBD > 98% purity). The medicinal product is a 100 mg/mL, non-sterile, non-preserved, non-aqueous oral solution of CBD dissolved in sesame oil, flavouring agent strawberry flavour, sucralose and dehydrated alcohol. The medicinal product is packaged in a 105 mL amber glass bottle. It contains no ingredients made from a gluten-containing grain (wheat, barley, or rye)[8].

Ethanol in the formulation

10. Each ml of Epidiolex® contains 79 mg of ethanol. The maximum recommended single dose of Epidiolex® (10 mg/kg) will increase the concentration of ethanol in the body by about 13 mg/l. For an adult weighing 70 kg, this is equivalent to 14 millilitres (ml) of beer, or 6 ml of wine per dose.

Genotoxicity data from Epidiolex

Taken from the European Medical Agency (EMA) Assessment report[9]

11. The below is the EMAs review of genotoxicity data including conclusions.

12. CBD, purified CBD and CBD as botanical drug substance[10] (BDS) were evaluated in a range of *in vitro* and *in vivo* standard genotoxicity assays. Only studies performed with purified CBD and Cannabidiol Oral Solution (CBS-OS) are summarised in Table 1.

Table 1

Type of test/study ID/GLP	Test system	Concentration range/ Metabolising system/dose	Results Positive/negative/equivocal
Gene mutations in bacteria (GWOR0910/GLP)	Salmonella strains TA98, TA100, TA1535, TA1537, and TA102	1.6 – 320 µg purified CBD	Negative
Chromosomal aberrations *in vivo* (GWOR0903/GLP)	Rat, micronuclei in bone marrow	125, 250, 500 mg/kg per os[11] (p.o.) CBD-OS	Negative
DNA damage *in vivo* (GWTX1510/GLP)	Rat Alkaline COMET Assay	125, 250, 500 mg/kg p.o. purified CBD	Negative

[7] Orphan medicine: a medicine used in rare diseases.
[8] https://www.accessdata.fda.gov/drugsatfda_docs/label/2018/210365lbl.pdf
[9] https://www.ema.europa.eu/en/documents/assessment-report/epidyolex-epar-public-assessment-report_en.pdf
[10] A botanical drug product is intended for use in the diagnosis, cure, mitigation, treatment or prevention of disease in humans. A botanical drug product consists of vegetable materials, which may include plant materials, algae, macroscopic fungi, or combinations thereof.
[11] Per os is sometimes used as an abbreviation for medication to be taken orally.

13. The genotoxic potential of CBD has been evaluated in a standard test battery of *in vitro* and *in vivo* assays according to ICH S2(R1) -Genotoxicity testing and data interpretation for pharmaceuticals intended for human use[12]. All tests concluded CBD to be negative for genotoxic potential.

14. A genotoxicity assessment of 7-COOH-CBD[13] using non-Good Laboratory Practice (GLP) test material in an Ames Test (GWTX18016) was provided. Results from this study showed that 7-COOH-CBD did not induce mutation in 5 *Salmonella typhimurium* strains (TA98, TA100, TA102, TA1535 and TA1537) under the conditions selected for this study. However, test item output from the scaled-up manufacture will produce appropriately characterised material to conduct genotoxicity GLP studies planned with both 7-OH-CBD and 7-COOH-CBD. GLP genotoxicity studies are awaited via post-authorization measure commitment.

Taken from the Center for Drug Evaluation and Research (CDER) Food and Drug Administration (FDA) Report[14]

15. The below is the CDER/FDA's report review of genotoxicity data including conclusions.

In vitro

- Reverse mutation in five histidine-requiring strains of *Salmonella typhimurium* (Study No. GWOR0910, report dated 6/18/09, GLP)

16. Pure CBD (Batch # 30301) was tested in the Ames assay using tester Salmonella strains TA98, TA100, TA1535, TA1537, and TA102 in the presence and absence of Aroclor 1254-induced rat liver S9 using the plate incorporation method at concentrations up to 5000 ug/plate. No positive mutagenic responses were observed with any of the tester strains in either the presence or absence of S9 activation. Under the conditions of the study, pure CBD was negative in the Ames assay.

In vivo

- Induction of micronuclei in the bone marrow of treated rats (Study No. GWOR0903, report dated 2/09/10, GLP).

17. Pure CBD (Batch # 30301) was evaluated for its potential to increase the incidence of micronucleated polychromatic erythrocytes (MNPCEs) in rat bone marrow cells. Male Sprague Dawley rats (6/grp) received 2 oral gavage (20 mL/kg) doses of 0 (sesame oil), 125, 250, or 500 mg/kg/day. The positive control group was dosed once with cyclophosphamide (CPA 20 mg/kg) on the second day of dosing. Bone marrow smears were prepared from sacrificed animals approximately 24 hours following the final administration on Day 3. In addition to the micronucleus animals, two groups of satellite animals were dosed with vehicle and 500 mg/kg/day Pure CBD for confirmation of exposure (but not toxicokinetic (TK)).

[12] https://www.ema.europa.eu/en/ich-s2-r1-genotoxicity-testing-data-interpretation-pharmaceuticals-intended-human-use
[13] 7-COOH-CBD : CBD metabolite
[14] https://www.accessdata.fda.gov/drugsatfda_docs/nda/2018/210365Orig1s000PharmR.pdf

18. Clinical signs (lethargy, ataxia, piloerection, anogenital soiling, and unkempt appearance) generally first became apparent on Day 3. CBD-treated rats exhibited group mean MNPCE frequencies similar to those for the vehicle control group and which also fell within the laboratory's historical vehicle control range. A small increase (NS) was observed at the low dose, but was attributable to a single animal (number 556) that exhibited an elevated number of MNPCEs (11 MNPCE/2000 PCE analyzed), exceeding historical control values. However, since all other CBD treated animals in this group (and all others) demonstrated MNPCE frequencies consistent with concurrent and historical vehicle controls, this isolated increase appeared to be and was considered in the report to be spurious. Negative (vehicle) and positive controls performed as expected. Thus, it can be concluded that under the conditions of the study Pure CBD was negative in the rat bone marrow micronucleus assay.

- Rat Alkaline Comet Assay (Study No. GWTX1510, report dated 8/26/15, GLP).

19. Male Sprague Dawley rats (6/group) received single oral (gavage) doses of 0 (sesame oil), 125, 250, or 500 mg/kg/day CBD-OS. Liver samples were obtained at 24 hours after the first dose. No clinical signs of toxicity were observed at any dose. There were no microscopic pathology findings attributed to CBD-OS. There was no dose-related increase in % hedgehogs in liver cells.

20. There was an increase in group mean % tail intensity at the high dose (0.78 compared to vehicle control mean of 0.29); however, the increase was due to a single animal (#19) having an elevated % tail intensity. None of the increases were found to be statistically significant and all animals fell well within the ranges determined from the laboratory historical control data. It can be concluded that CBD-OS was negative in the rat Comet assay.

21. Purified CBD and CBD BDS were negative in the Ames test at concentrations up to 5000 µg/plate, with or without metabolic activation (S-9). In *in vivo* micronucleus assays, Purified CBD and CBD BDS did not induce micronuclei (MN) in the polychromatic erythrocytes (PCE) of the bone marrow of rats at oral (gavage) doses up to 500 or 350 mg/kg/day, respectively. CBD-OS did not induce DNA damage in the liver of rats at oral (gavage) doses of up to 500 mg/kg/day in the alkaline comet assay.

Additional information

22. Qualification of impurities - Analysis of CBD revealed 4 impurities at levels greater than the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) qualification thresholds, and the proposed limits for these impurities are ~ higher than the actual levels in the nonclinical batches. Each impurity was therefore qualified in a battery of studies including in vitro and in vivo genotoxicity tests,13 or 26- week oral (gavage) toxicity studies in the rat, and rat embryofetal development studies was in CBD toxicity studies. All other studies were performed on purified materials.

23. Aside from the bioanalytical issues, the clear species differences in CBD metabolism raise serious questions about the adequacy of the nonclinical evaluation. The failure of the sponsor to adequately evaluate the toxicity of 7-COOH-CBD was noted as a serious deficiency in the 74-day letter (dated 12/20/17). In response, the sponsor proposed to conduct additional studies with the synthesized metabolite, including genotoxicity testing (Derek for 7-COOH-CBD was negative, but this is obviously not an adequate assessment for a major metabolite). However, the sponsor's arguments for the adequacy of the toxicity studies are not supported by the available information. They state: "While GW accepts that human exposure levels are around 10 fold greater than animal exposure, there has been no safety signals in the clinical trials related to 7-COOH-CBD." But the clinical safety data obviously would not be adequate to rule out long-term effects due to the metabolite. They erroneously referenced the CH M3 (R2) Non-clinical safety studies for the conduct of human clinical trials for pharmaceuticals - M3(R2) ICH guideline[15] Q&A ("characterization of metabolite toxicity would generally be considered adequate when animal exposure is at least 50% the exposure seen in humans") and the Safety Testing of Drug Metabolites Guidance, which states that "If at least one animal test species forms this drug metabolite at adequate exposure levels (approximately equal to or greater than human exposure), as determined during toxicology testing of the parent drug, it can be assumed that the metabolite's contribution to the overall toxicity assessment has been established."

Conclusion

24. The genotoxic potential of CBD has been evaluated in a standard test battery of *in vitro* and *in vivo* assays for the medicinal approval package of Epidiolex.

25. According to the EMA Assessment report and CDER/FDA's report, all tests concluded CBD to be negative for genotoxic potential.

Questions for the COM

26. 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) Any other comments you may have?

Secretariat

February 2020

[15] https://www.ema.europa.eu/en/ich-m3-r2-non-clinical-safety-studies-conduct-human-clinical-trials-pharmaceuticals

Abbreviations

BDS	Botanical Drug Substance
CDER	Center for Drug Evaluation and Research
CBD	Cannabidiol
CBD-OS	Cannabidiol oral solution
CPA	cyclophosphamide
FDA	Food and Drug Administration
EMA	European Medical Agency
GLP	Good Laboratory Practice
MN	micronuclei
MNPCEs	micronucleated polychromatic erythrocytes
PCE	polychromatic erythrocytes