Review of Phytocannabinoids in Low-THC Hemp Foods

February 15, 2016

Report prepared for the
NSW Ministry of Health
and the NSW Food Authority

FJ FLEMING
FOOD CONSULTING

TABLE OF CONTENTS

LIST OF TABLES, FIGURES AND APPENDICES

Tables

Figures

Appendices

EXECUTIVE SUMMARY

The Australia and New Zealand Ministerial Forum on Food Regulation (the Forum) has considered applications to approve low tetrahydrocannabinol (THC) hemp as food twice. Most recently in January 2015, the Forum agreed that these foods do not present any safety concerns, however it remained concerned about possible impacts on roadside drug testing, a lack of information on interaction with broader legislation and international treaties to which Australia is a signatory, the potential for inappropriate marketing of hemp food to send a confused message about illicit cannabis, and whether other phytocannabinoids (plant derived cannabinoids) in low-THC hemp food, such as cannabidiol (CBD), may produce therapeutic effects and require limits to be set, as previously proposed for THC.

The Food Regulation Standing Committee (FRSC) is currently overseeing work to address these information gaps. NSW Health and the NSW Food Authority are leading the work on CBD and other phytocannabinoids.

The overall objective of the research presented in this report is to determine if a level for CBD and/or other phytocannabinoids needs to be set for low-THC hemp foods, to ensure they do not provide therapeutic doses at normally consumed levels and/or as a means of distinguishing low-THC foods from therapeutic goods.

This report presents five pieces of work undertaken to address the overall objective. The work is summarised in the following table.

Project/Action	Description/deliverable	Responsibility
Review of International Regulation of Hemp as a Food	Conduct a review of published regulations and consultation with food or health jurisdictions regarding food products overseas (noting that this issue is not addressed in many jurisdictions) to determine if any jurisdictions have set limits for CBD or other cannabinoids in hemp foods.	NSW Health commissioned FJ Fleming Food Consulting to conduct this work
Nutrition and Health Claims Review	Conduct a claims review of internationally available products and sample marketing to consider the health claims being made by marketers of low-THC hemp foods in regards to other cannabinoids.	NSW Health commissioned FJ Fleming Food Consulting to conduct this work
Pharmacological actions and associated therapeutic levels of phytocannabinoids (Literature Review)	Conduct a literature review of peer review and grey literature on the cannabinoids with therapeutic potential and likely therapeutic doses.	NSW Health commissioned the Sax Institute to broker this work which was conducted by the University of Sydney
Hemp Food Product Analysis	A survey to establish levels of cannabinoids, including cannabidiol and THC, in low-THC hemp products, which could be available in the Australian and New Zealand markets.	NSW Food Authority commissioned Southern Cross University to complete this work
Dietary Assessment	Preliminary assessment to determine the maximum amount of hemp foods containing cannabidiol and other cannabinoids that could be consumed before the lowest therapeutic dose level is reached.	Food Standards Australia New Zealand (FSANZ) conducted this work.

The pharmacology and minimum therapeutic levels for THC are already well understood. The review on therapeutic potential and doses identified sufficient information to estimate a minimum adult therapeutic level for CBD of 800 mg/day (absolute dose), although further clinical trials are required to confirm this estimate. FSANZ have also advised that it may be possible to use animal data to set a limit for cannabidiolic acid (CBDA). The available information on minimum therapeutic levels for other cannabinoids is limited to preclinical studies (cellular models and animal studies) and is insufficient to estimate minimum therapeutic levels for these cannabinoids.

The predominant cannabinoids present in the 200 hemp foods from Australia, New Zealand and overseas tested were CBD, THC and their acid precursors (CBDA and Delta-8-tetrahydrocannabinolic acid (THCA)). Other cannabinoids were present at either low levels or below the limit of detection. Some hemp foods tested exceeded the THC limit proposed by FSANZ in relation to A1039.

CBD and CBDA levels in low-THC hemp foods are below those required to produce a therapeutic effect at all reasonable levels of consumption. There is no apparent need for a CBD limit to be set for low-THC hemp foods in general based on the dietary assessment. However, there are a small number of readily available CBD fortified products that may provide a therapeutic dose. Setting a CBD limit may be required to distinguish these products from foods, and to prevent them being misused by people seeking to self-medicate for serious medical conditions such as epilepsy.

There is potential for CBDA and THCA to be converted to CBD and THC respectively at temperatures above 160°C. For this reason, CBDA and THCA may need to be included in any limits proposed for CBD and THC.

No international jurisdiction which currently permits consumption of low-THC hemp foods has set a limit for any cannabinoid other than THC, so there are no international CBD limits for hemp foods. International trade rules may constrain any aspiration to set unilateral CBD limits.

Research on the pharmacology and therapeutic effects of cannabinoids is expanding rapidly and any limits proposed for cannabinoids in low-THC hemp foods may need to be reviewed as new evidence comes to light.

Claims made for hemp foods reviewed did not mention CBD or other cannabinoids, but rather related mainly to nutrition and general level claims that would be captured under Standard 1.2.7 (Nutrition, Health and Related Claims) of the Australia New Zealand Food Standards Code (the Code). However, some products marketed as nutritional supplements made high level health claims. Given these CBD fortified products may deliver a therapeutic dose of CBD, they could be regulated as therapeutic goods and product claims managed under that regime.

Any hemp foods available for sale in Australia and New Zealand would also need to comply with other relevant standards in the Code, for example, in relation to safety, claims and labelling.

Approval of low-THC hemp food products may need to be supported by education, particularly for the hemp industry, and the capacity for industry and regulators to monitor cannabinoid levels.

1. INTRODUCTION

This report brings together the research of a number of teams working on different regulatory and scientific aspects of low-THC hemp foods.

1.1 Objective & Deliverable

The **overall objective** of the work presented in this report is to determine if a level for CBD and/or other phytocannabinoids (plant derived cannabinoids) needs to be set for low-THC hemp foods:

- to ensure they do not provide therapeutic doses of phytocannabinoids at normally consumed levels; and
- as a means of distinguishing low-THC hemp foods from therapeutic goods.

The **overall deliverable** is a recommendation on how to manage CBD and other cannabinoid levels in low-THC hemp foods in Australia if this is required.

A number of individual pieces of work were undertaken to inform the objective as summarised in **Table 1** below.

A working group for this project was composed of representatives from the NSW Ministry of Health, NSW Food Authority and Food Standards Australia New Zealand (FSANZ) who had responsibility for different aspects of the project and met regularly to share and discuss key findings from their area of research.

Progress on this work was communicated to the FRSC low-THC Hemp Working Group responsible for overseeing the work on all information gaps relating to low-THC hemp food.

Table 1: Low-THC Hemp Food Projects

Project/Action	Description/deliverable	Responsibility
Review of International Regulation of Hemp as a Food	Conduct a review of published regulations and consultation with food or health jurisdictions regarding food products overseas (noting that this issue is not addressed in many jurisdictions) to determine if any jurisdictions have set limits for CBD or other cannabinoids in hemp foods.	NSW Health commissioned FJ Fleming Food Consulting to conduct this work
Nutrition and Health Claims Review	Conduct a claims review of internationally available products and sample marketing to consider the health claims being made by marketers of low-THC hemp foods in regards to other cannabinoids.	NSW Health commissioned FJ Fleming Food Consulting to conduct this work
Pharmacological actions and associated therapeutic levels of phytocannabinoids (Literature Review)	Conduct a literature review of peer review and grey literature on the cannabinoids with therapeutic potential and likely therapeutic doses.	NSW Health commissioned the Sax Institute to broker this work which was conducted by the University of Sydney
Hemp Food Product Analysis	A survey to establish levels of cannabinoids, including cannabidiol and THC, in low-THC hemp products, which could be available in the Australian and New Zealand markets.	NSW Food Authority commissioned Southern Cross University to complete this work
Dietary Assessment	Preliminary assessment to determine the maximum amount of hemp foods containing cannabidiol and other cannabinoids that could be consumed before the lowest therapeutic dose level is reached.	Food Standards Australia New Zealand (FSANZ) conducted this work.

1.2 Scope

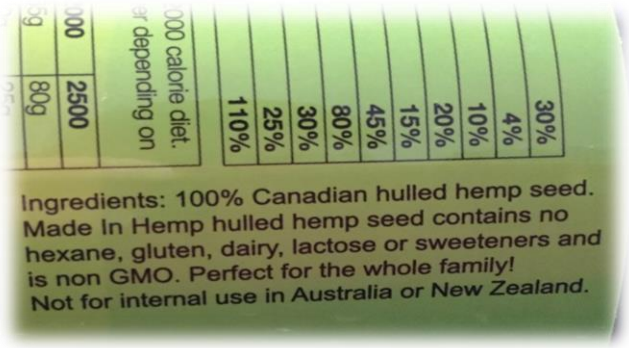
The **scope** of the project encompasses products derived from low-THC hemp that are sold or have the potential to be sold as food and food ingredients in countries where hemp is legally permitted as a food. Hemp foods marketed as dietary supplements, Traditional Chinese Medicines (TCM) and therapeutics were considered to be out of scope. However, high CBD hemp oil was considered as it is currently being promoted for therapeutic use on websites that also sell hemp food.

Hemp foods are available in a number of formats as summarised in **Table 2**. It should be noted that the proposal by FSANZ under A1039 to approve a variation to Standard 1.4.4 – Prohibited and Restricted Plants and Fungi was to permit the sale of foods derived from the seeds of low-THC hemp. In reviewing the hemp foods available for this project, products derived from parts of the plant other than the seed have been included as they are currently available for sale. Such products are generally labelled to indicate they are not intended for consumption in Australia. **Figure 1** shows some examples of these statements.

Table 2: Forms of hemp available as food and dietary supplements

PRODUCT TYPES
Hemp products
Hemp Seeds (some organic)
Toasted hemp seeds
Hulled hemp seeds (some organic)
Hemp hearts
Hemp powder
Hemp flour
Hemp protein powder (some organic)
Hemp meal
Hemp seed oil (some organic)
CBD Hemp powder
Food Products with hemp ingredients
Hemp oil infused teas; coffee and cocoa
Hemp oil infused protein bars
Hemp milks
Granola bars with hemp
CBD infused chewing gum
Dietary supplement type products
CBD Hemp Oil
Extra Strength CBD oil

Figure 1: Hemp Food labels



Phytocannabinoids

The project required CBD and/or other phytocannabinoids in hemp foods to be considered. There are some 100 terpenophenolic compounds that are known as *phytocannabinoids* (plant-derived cannabinoids) (Arnold et al, 2016).

The phytocannabinoids considered within in the scope of this work are listed below.

Table 3: Phytocannabinoids within project scope

Abbreviation	Full Name
CBD	Cannabidiol
CBDA	Cannabidiolic acid
CBDV	Cannabidivarin
THCA	Delta-8-tetrahydrocannabinolic acid
THCV	Delta-9-tetrahydrocannabivarin
THCVA	Delta-9-tetrahydrocannabivarinic acid
CBG	Cannabigerol
CBGA	Cannabigerolic acid
CBN	Cannabinol
CBC	Cannabichromene

1.3 Background

Cannabis sativa is well known as a source of the psychoactive substance, delta-9-tetrahydrocannabinol (THC). Varieties of *C. sativa* that contain no, or very low levels of THC, are commonly referred to as hemp, industrial hemp or industrial cannabis. Hemp has typically been used for industrial purposes, such as textiles, fibres, paper, building materials (fibrous parts of plant) and also as a food source (seeds). Hemp is permitted to be cultivated (under licence) in New Zealand and most Australian states and territories.

Hemp is currently not permitted to be sold as a food or food ingredient in Australia. Oil extracted from hemp seed may be sold as food in New Zealand if it complies with requirements set out in clause 26 of the New Zealand Food (Safety) Regulations 2002 (NZ FS Regs, 2002). However, a number of hemp products are available for purchase in Australia and New Zealand via retail outlets and online. Most, but not all such products are labelled to indicate they are not intended for consumption in Australia (Figure 1).

In 1998 Application A360 (Hemp as a Novel Food) to allow the use of hemp seed and hemp seed oil in food was submitted to Food Standards Australia New Zealand (FSANZ) but was rejected by the (then) Food Regulation Ministerial Council in May 2002 due to concerns about policing and the possibility that it may send a mixed message about the acceptability of cannabis.

In 2009, FSANZ received an Application seeking approval for the use of *Cannabis sativa* with low levels of THC, in both seed and seed oil, as a food (Application A1039 - Low-THC Hemp as a Food). In December 2014, the FSANZ Board approved a variation to Standard 1.4.4 to permit the sale of foods derived from the seeds of low-THC varieties of *C. sativa*. Requirements for maximum levels (MLs) of THC that may be present in hemp foods were specified and hemp seeds were only to be sold if they were non-viable. The approved variation allowed only low-THC varieties of *C. sativa* to be used as a source for food, and that only naturally-occurring THC could be present in hemp-based food (FSANZ, 2014).

The Australia and New Zealand Ministerial Forum on Food Regulation (the Forum) subsequently met in Auckland in January 2015 and resolved to reject the proposed variation to Standard 1.4.4 – Prohibited and Restricted Plants and Fungi to permit the sale of foods derived from the seeds of low-THC hemp, resulting from Application A1039.

The Forum noted FSANZ's view that foods derived from the seeds of low-THC hemp do not present any food safety concerns. However, there were several concerns, including a lack of information about interaction with broader legislation and international treaties to which Australia is a signatory, possible impacts on roadside drug testing, and the potential for inappropriate marketing of hemp food to send a confused message to consumers about the acceptability and safety of illicit cannabis.

There was also concern about a lack of information on CBD and other cannabinoid levels in hemp food. FRSC is currently overseeing work to address these information gaps. NSW Health and the NSW Food Authority are leading the work on CBD and other phytocannabinoids.

CBD is one of the major phytocannabinoids present in the hemp plant. There is no evidence of THC-like intoxication with CBD (Arnold et al, 2016). However, CBD is being investigated for its therapeutic use to treat a number of conditions, including epilepsy in children. The presence of CBD in hemp seed is primarily limited to the seed hull, with almost no CBD present in hulled seeds. However, CBD-free extraction of hemp oil is difficult as CBD is highly fat soluble and will be incorporated when seeds are pressed.

Low-THC hemp is not intended to have a therapeutic use. However, internationally, hemp food producers have recently begun fortifying pressed seed hemp oil with CBD extract to appeal to medicinal markets with therapeutic claims. High-CBD hemp oil used for therapeutic purposes is distinctly different to low-CBD hemp oil intended for use as a food. Low-CBD hemp oil is made solely from the pressed seeds of low-THC hemp plants, a process which results in only a small amount of CBD being retained in the oil. Comparatively, high-CBD hemp oil is prepared from pressed hemp seeds and fortified with concentrated extracts from other parts of the hemp plant, resulting in high-CBD content.

The therapeutic use of high-CBD hemp oil has raised concerns that patients with medical conditions such as epilepsy may be influenced by therapeutic claims on high CBD oil to self-medicate and stop taking their prescribed medications. These products are often marketed on the same websites as low-THC hemp foods, potentially blurring the food-medicine interface.

There is no evidence to suggest that CBD levels naturally present in low-THC hemp foods would have any pharmacological effects, however the levels of CBD in food products are largely unknown.

2 RESULTS

2.1 Review of International Regulation of Low-THC Hemp as Food

The **specific aim** of this work was to review published regulations and consult with food or health jurisdictions regarding hemp food products overseas, noting that this issue is not addressed in many jurisdictions.

The review has determined for the countries reviewed, there are no regulations in relation to the level of CBD or any other cannabinoids in hemp foods permitted for sale. **Table 4** summarizes the regulations investigated with respect to THC and CBD.

For the USA and Canada, where direct contact was made with representatives from their respective regulatory agencies, no advice was available as to why there has been no regulatory level considered or set for CBD or any other cannabinoids.

Further discussion with Canadian and USA regulatory authorities is recommended, potentially sharing the outcomes of the literature review, the hemp food product analysis and the dietary assessment.

A full report on this work is provided as **Appendix 1.**

Table 4: Summary of International Hemp Food Regulation

COUNTRY	THC LEVEL REGULATED	CBD LEVEL REGULATED
New Zealand	Inferred limit of 0.35% THC for hemp oil	No
Canada	Maximum level of 10 parts per million (ppm or mg/kg) for THC residues in products derived from hemp grain, such as flour and oil.	No
USA	THC limit of 10ppm (mg/kg)	No
EU	0.3% THC	No
UK	Case by case permission	
Ireland	No regulation	No
Netherlands	No set limit	No
Germany	No specific regulation but recommendations for THC limits: 5 µg/kg for non-alcoholic and alcoholic drinks; 5000 µg/kg for edible oils; 150 µg /kg for all other foods.	No
France	Not permitted	No
Greece	Not permitted	No
Spain	Not permitted	No
Austria	THC must not exceed 1-2ug/kg bw/day	No
Belgium	THC limits of oil of seed: 10 mg/kg; Seed and flour of seed: 5 mg/kg; Other foods and drinks: 0.2 mg/kg	No
Italy	Zero	No
Cyprus	No specific hemp food regulation – expectation that hemp based foods will have no detectable levels of THC	
China	No regulation	No
Hong Kong	Hemp foods not permitted	No
Singapore	Hemp foods not permitted	No

2.2 Review of Nutrition and Health Claims in Low-THC Hemp Food

The **specific objective** of this segment of work was to review internationally available hemp foods and sample marketing to consider the nutrition and health claims being made by marketers of low-THC hemp foods in regards to other cannabinoids.

The review identified a range of hemp foods. Additional products that are currently sold as dietary supplements such as (high) CBD hemp oils considered to be out of scope for this review were included here to illustrate the spectrum of claims being made at food-medicine interface.

Overall, the claims being made in relation to hemp foods can be described as nutrition content and general level type health claims.

The types of claim have been classified using the current terminology for claims in Standard 1.2.7 – Nutrition, Health and Related Claims of the Australia New Zealand Food Standards Code:

- **General level health claim** (GLHC) refers to a nutrient or substance in a food and its effect on a health function. They must not refer to a serious disease or to a biomarker of a serious disease.

  For example: calcium is good for bones and teeth.[1]

- **High level health claim** (HLHC) refers to a nutrient or substance in a food and its relationship to a serious disease or to a biomarker of a serious disease.

  For example: Diets high in calcium may reduce the risk of osteoporosis in people 65 years and over.

  For example, a biomarker health claim is: Phytosterols may reduce blood cholesterol.

- **Nutrition content claim** (NC) refers to claims about the content of certain nutrients or substances in a food, such as 'low in fat' or 'good source of calcium'.

Examples of the types of claims are shown in **Table 5** below.

Claims in relation to the hemp foods identified did not refer to or make mention of CBD or any other phytocannabinoids.

The high CBD hemp oil products also make a range of claims including HLHCs. Examples of these types of claims are shown in **Table 6** below.

The claims review identified a range of health claims in relation to available hemp foods and hemp products at the food/medicine interface. Some claims could be managed under Standard 1.2.7 and some were clearly therapeutic and not permitted or appropriate for food products.

A full report on this work is provided as **Appendix 2.**

[1] http://www.foodstandards.gov.au/consumer/labelling/nutrition/Pages/default.aspx, accessed 07.02.2016

Table 5: Hemp Food Product Claims (examples)

CLAIMS	TYPE [1]
Natural source of antioxidants, phytosterols, vitamins, minerals and fibre	NC
Great sources of omega 3 & 6. Great source of protein, folate, iron and calcium. Hemp seeds contain all 20 amino acids, including the 9 essential ones that our body cannot produce on its own. Hemp seeds are also a rich source of essential minerals, including magnesium and phosphorus for bone health, zinc to help support the immune system, and iron for oxygenating the blood.	NC; GLHC
Ideal ratio of omega 6 to omega 3 which can help the body to metabolise fat, counteract aging, increase immune system strength, lower cholesterol and help prevent cardiovascular disease. May assist with healthy skin.	NC; GLHC
The protein and fibre help to slow digestion, prevent spikes in blood sugar and help the body to sustain energy. An important aspect of hemp seed protein is a high content of arginine (97mg/g protein) and histidine (23 mg/g protein), both of which are important for growth during childhood, and of the sulfur containing amino acids methionine (20 mg/g protein) and cysteine (16 mg/g protein), which are needed for proper enzyme formation. Hemp protein also contains relatively high levels of the branched-chain amino acids that are important for the metabolism of exercising muscle. Hemp protein contains over 61mg/g of protein of Leucine – higher than cashews or chia seeds.	NC; GLHC;
Great source of protein; perfect balance of omega 3, 6 and 9; rich in vitamins and minerals; organic; Complete source of amino acids	NC
23 times mores omega 3 than olive oil improve cholesterol; 33% easily digested protein helps build muscle; high in nutrients and minerals promotes general well being	NC; GLHC
Hulled hemp seeds are a delicious source of polyunsaturated fatty acids. They contain 10 grams of omega-6 fatty acids and 3 grams of omega-3 fatty acids per serving. Hulled hemp seeds contain all 20 essential amino acids and zero trans fats.	NC
The omega 3 in hemp seed oil lowers blood pressure, reduces inflammation, decreases the risk of cardio vascular disease (CVD) and may have an effect on Alzheimer's disease. The Omega 6 helps maintain brain functions and bone health as well as stimulate hair and skin growth. Hemp Oil Australia, however, is not a cure for cancer.	NC; GLHC and HLHC
Can assist in the symptoms of high and low blood pressure, psoriasis, eczema and rheumatoid arthritis. Multiple sclerosis, schizophrenic psychosis and cancer - major preventative to these diseases. Regular intake of Pure Cold Pressed Hemp Seed Oil will help reduce the risk of arterial sclerosis and other cardiovascular diseases. The daily recommended dose is 10ml. Clinical trials showed a significant improvement in pre-menstrual stress (PMS) related symptoms over a 12-week period. The daily recommended dose is 5ml, massaged into the stomach area. Rheumatoid arthritis - effective as an anti-inflammatory.	HLHC

[1] NC = nutrition content; GLHC = general level health claim; HLHC = high level health claim

Table 6: Hemp Product Claims – High CBD oil (examples)

CLAIMS	TYPE [1]
CBD is a neuromodulator that enhances cell communication and is thought to be effective in supporting several vital bodily systems. A compound of hemp, each CBD softgel contains over 400 milligrams of hemp seed oil, an excellent source of essential fatty acids (EFAs). Deemed essential because our bodies do not naturally produce them, EFAs occur naturally in hemp in a 3:1 balance of Omega-6 over Omega-3. According to the World Health Organization, this is the perfect balance for human consumption.	NC; GLHC; HLHC
Testimonials: Dietary supplement - experience the positive effects of phytonutrients Antioxidant and anti-inflammatory properties Antiemetic (reduces nausea and prevents vomiting), anti-depressant and antipsychotic properties Natural pain killer - analgesic effect Reduce the pain in arthritis - through analgesic and anti-inflammatory properties Epilepsy - reduction in seizures Improvement in sleep; improved mental development; physical development Control of pain Thyroid cancer - reduction in tumour size Appetite suppressing properties Anti-inflammatory and anti-bacterial effects Anti-inflammatory and bone stimulant	GLHC; HLHC

[1] NC = nutrition content; GLHC = general level health claim; HLHC = high level health claim

2.3 Pharmacological actions and associated therapeutic levels of phytocannabinoids

A systematic review of the clinical and preclinical literature was conducted by the University of Sydney Lambert Initiative for Cannabinoid Therapeutics to examine the pharmacological and possible therapeutic effects of various plant-derived phytocannabinoids that are found in street cannabis and industrial hemp. Some of these phytocannabinoids may also be present from time to time in hemp seed oil and other hemp foods.

The full report on this work is provided as **Appendix 3.**

The phytocannabinoids reviewed were:

- Cannabidiol (CBD)
- Cannabidiolic acid (CBDA)
- Cannabidivarin (CBDV)
- Delta-8-tetrahydrocannabinolic acid (THCA)
- Delta-9-tetrahydrocannabivarin (THCV)
- Delta-9-tetrahydrocannabivarinic acid (THCVA)
- Cannabigerol (CBG)
- Cannabigerolic acid (CBGA)
- Cannabinol (CBN)
- Cannabichromene (CBC).

The therapeutic effects of THC are well described and FSANZ has already proposed a maximum allowable level of THC in hemp seed and oil added to food or offered for sale as food in its response to Application A1039. The review conducted by the University of Sydney therefore did not include THC.

Evidence from human trials was considered of greatest relevance. Where this was not available, evidence from in vitro and animal studies was reviewed and included.

The main research question addressed by the researchers was:

What therapeutic levels of individual cannabinoids are required to elicit the pharmacological characteristic in adults and children?

The review found that there was a paucity of good quality evidence for a therapeutic effect for many of the phytocannabinoids. In most cases, no published studies involving human administration were available, and available studies were limited to *in vitro* cellular or *in vivo* rodent preclinical studies. While a therapeutic effect in humans can be estimated by extrapolation from in vitro and animal studies to doses in humans, drawing conclusions on therapeutic oral doses in humans from animal studies has major limitations.

The majority of phytocannabinoids reviewed have no demonstrated action in humans at this stage. The only cannabinoid for which there was there was reasonably good evidence of a therapeutic effect in humans was CBD. Based on the best available evidence, an estimated lowest adult therapeutic dose for CBD was 800 mg/day. This dose estimation might be subject to change when results of Phase 3 clinical trials become available. There is the possibility of mild sedation at such doses of CBD, although the current literature is ambiguous on this point with the balance being in favour of no sedative effects. There is no evidence of THC-like intoxication with CBD.

Evidence relating to potential therapeutic effects of the remaining phytocannabinoids mostly comes from preclinical studies involving cellular models and laboratory animals. The estimated human doses calculated from animal doses may not be relevant to human consumption. There is little evidence of intoxication from oral consumption of THCA, THCV, CBDV, CBC and CBN in humans. Similarly, CBDA, THCVA, CBG and CBGA do not appear to have intoxicating properties although human studies are required to definitively rule this out.

2.4 Cannabinoids in Low-THC Hemp Products - Analysis

This work was undertaken to gather information on the levels of cannabinoids, including CBD and THC, in products which could potentially be available on the Australian market, should low-THC hemp foods be approved.

A full report on this work is provided as **Appendix 4.**

A total of 200 products were purchased between July and September 2015. Samples were purchased from health food stores around NSW, New Zealand and online (both Australian and overseas based suppliers).

The country of origin of most samples was Australia, New Zealand or Canada. There were three products categories: hemp oil (seed oil and oil capsules), hemp powder (protein powder/flour and shake powder), and hemp seed. The phytocannabinoids that were analysed in these products were:

- CBD;
- CBDA;
- THC;
- THCA; and
- total cannabinoids.

The analysis results are summarised in **Table 7**. In general, hemp oil products contained higher level of total cannabinoids compared to hemp powder and seed. The total cannabinoids for oil samples (non-capsules) ranged from 1.5 to 123 ppm, with an average of 49.4 ppm. The total cannabinoids for the four hemp oil capsule samples ranged from 40.2 to 76.5 ppm with an average of 58 ppm. All capsules contained low levels of total THC (less than 10 ppm). 77 out of 78 protein powder/flour and shake powder contained low levels of total cannabinoids, ranging from 0 to 20 ppm. Only one shake powder contained total cannabinoids of 46.3 ppm. In addition, all seed samples contained very low levels of total cannabinoids (ranging from 0 to 9 ppm).

The level of total cannabinoids varied between products. There was no obvious correlation between where the products were sourced and there was no clear correlation between the level of CBD and THC in products tested.

There were a number of products that exceeded the THC levels proposed by FSANZ in application A1039; using the total THC levels (THC + THCA) - 29 (38%) of the oil products, two (3.6%) of hemp protein powder and three (13%) of hemp shakes exceeded the levels proposed. Using just the THC levels – 11 (14%) of the oil products, two (3.6%) of hemp protein powder and three (13%) of hemp shakes exceeded the levels proposed

The proposed amendment to the Code was to permit the sale, as a food, including as an ingredient of a food, the seed and seed products from Cannabis species (spp.) with levels of delta 9-tetrahydrocannabinol (THC) as follows:

- seeds of low-THC *Cannabis sativa* – maximum of 5mg THC per kg of seeds
- oil extracted from the seed of low-THC *Cannabis sativa* – maximum of 10mg THC per kg of oil
- a beverage derived from the seed of low-THC *Cannabis sativa* – maximum of 0.2mg THC per kg of beverage
- any other substance extracted or derived from the seed of low-THC *Cannabis sativa* – maximum of 5mg THC per kg of seed or substance (FSANZ, 2014)

These results were shared with FSANZ to assist with a dietary modelling exercise to calculate the maximum amount of food that could be consumed before the lowest therapeutic level was reached and to compare this with predicted hemp food consumption amounts.

Table 7: Summary of low-THC Hemp Products Analysis Results

Product	Recommended serving size	Total CBD[2] (range – ppm)	Total CBD[2] (average – ppm)	Total THC[3] (range - ppm)	Total THC[3] (average – ppm)	Total cannabinoids[4] (range – ppm)	Total cannabinoids[4] (average – ppm)
Hemp oil (non-capsule) n= 73	15 – 30 ml	0 – 111.1	34.9	0 – 114.5 38% of samples >10ppm[5]	11.8	1.5 – 123.4	49.1
Hemp oil (capsule) n= 4	3 – 6 capsules	36.1 – 76.5	53.2	0 – 8.6	4.2	40.2 – 76.5	58
Hemp powder/flour (100% hemp) n=55	10 – 32 g	0 – 15	4.5	0 – 7.3 3.6% of samples >5ppm[6]	0.6	0 – 17	5.3
Hemp powder – shake powder (hemp protein as an ingredient) n=23	10 – 32 g	0 – 10.7	3.7	0 – 34.4 13% of samples >5ppm[6]	2.8	0 – 46.3	6.8
Hemp seed n= 45	15 – 50 g	0 – 8.7	1.4	0 – 2.8 All samples <5ppm[7]	0.3	0 – 9	1.9

[2] Total CBD = CBD + CBDA
[3] Total THC = THC+THCA
[4] Total cannabinoids mean the sum of CBD, CBDA, THC, THCA and THCV-A
[5] Maximum THC limit proposed by FSANZ for hemp oil (A1039, 2012)
[6] Maximum THC limit proposed by FSANZ for hemp powder (A1039, 2012)
[7] Maximum THC limit proposed by FSANZ for hemp seed (A1039, 2012)

2.5 Low-THC Hemp Food Dietary Assessment

This work was undertaken by FSANZ as a preliminary assessment to determine the maximum amount of hemp based food that could be consumed before the lowest therapeutic dose level for CBD and other cannabinoids was reached.

A full report on this work is provided as **Appendix 5.**

On behalf of the FRSC low-THC Hemp Working Group, NSW Health requested FSANZ undertake a preliminary dietary assessment to determine the maximum amount of hemp derived food that could be consumed by Australian and New Zealand populations without clinical effects for some selected phytocannabinoids being observed (lowest therapeutic dose). Calculations to derive the maximum levels were based on the concentrations of a range of phytocannabinoids reported in an analytical survey of hemp foods that had been commissioned by NSW Health in 2015 (Section 2.4).

FSANZ reviewed CBD, CBDA, THC and THCA in the assessment

The product groups assessed were hemp oil, hemp seeds, hemp flour, hemp protein powder and hemp-based milk substitute[8]. Hemp oil contained higher concentrations of all phytocannabinoids analysed in comparison to hemp protein powder, hemp flour and hemp seed, with hemp seed having the lowest mean concentrations.

The maximum amounts of each type of hemp food that could be consumed without clinical effect by Australian and New Zealand populations was estimated for the selected phytocannabinoids and then compared to mean and 90th percentile consumption amounts for consumers (eaters only) of similar foods, derived from national nutrition surveys (AusNNS, 1995 and NZANS, 2008). The likelihood of the estimated maximum amount of hemp food actually eaten was then determined.

FSANZ calculated the amount of hemp oil, hempseed, hemp flour, hemp protein powder and hemp-based milk substitute that could be consumed before reaching the lowest therapeutic effect dose as set out in **Table 8**.

FSANZ identified a 2016 study in rats which enabled the oral CBDA dose without adverse effects in humans to be estimated to be up to 50 mg/person (Brierley et al 2016). Some pharmacological activity was evident at estimated human doses of 5 and 50 mg/person, but not at the lowest tested dose of 0.5 mg/person. A dose of 5 mg/person, derived from this study, is considered to be a reasonably reliable estimate of the lowest oral human therapeutic dose. It was this dose of 5 mg/person rather than 0.07 mg/person (Arnold et al 2016) which was used in the dietary assessment.

[8] hemp-based milk substitute was included by FSANZ. These types of products were not included in the analysis conducted by the NSW Food Authority.

For CBDA, FSANZ concluded that:

The lowest oral human therapeutic dose of 0.07 mg/person, estimated for CBDA by Arnold et al using an extrapolation method from an IP study in rats is known to be unreliable and not an appropriate methodology for the derivation of an ML for CBDA in hemp foods.

However, given the unreliability in using animal data to estimate human therapeutic doses, it may be premature to consider setting limits for any cannabinoids for which only animal data is currently available.

Table 8: Lowest therapeutic effect doses for individual phytocannabinoids used in the dietary assessment

Phytocannabinoid	Lowest therapeutic effect dose (mg/day based on 60 kg person)	Reference	Comments
CBD	800	Arnold *et. al.* (2016) [1]	
CBDA	5	Brierley et al (2016)	FSANZ modelling (Full report – Appendix 5)
THC	0.36	FSANZ (2011)	Tolerable daily intake (TDI) for THC of 6 µg/kg bw/day, converted to a daily amount per person, using a 60 kg body weight
THCA	3.5	Arnold *et. al.* (2016)	

[1] Arnold et al is Appendix 3

When it was assumed that hemp foods contained the mean or maximum analysed concentration of CBD, CBDA and THCA, the amount of hemp foods that could be consumed without any clinical effects was greater than the predicted mean and 90th percentile consumption of these foods by consumers only.

When it was assumed that hemp foods contained the mean analysed concentration of THC, the amount of hemp foods that could be consumed without clinical effects was greater than the predicted mean and 90th percentile consumption of these foods by consumers (eaters of the food).

When it was assumed that foods contained the maximum analysed concentration of THC, the estimated amount of hemp flour, hemp-based milk substitute and hemp seed that could be consumed without clinical signs was higher the predicted mean and 90th percentile consumption of these foods by consumers (THC was not detected in any sample of hemp flour).

However, for hemp protein powder and hemp oil, the estimated amount of food that could be consumed before any clinical signs were observed was lower than the predicted mean and 90th percentile consumption of these foods by consumers; i.e. a clinical sign may occur at the mean level of consumption of hemp powder and hemp oil containing THC at the maximum reported level. FSANZ notes that using the maximum analysed concentration of THC in the calculations is not considered a realistic chronic (long-term) scenario, as it is

unlikely that hemp protein powder and hemp oil will always contain the maximum analysed concentration of THC observed in a single sample from a survey.

These findings are consistent with the FSANZ hemp food application A1039 – Low-THC Hemp as a Food (FSANZ 2011), which assessed hemp foods as safe for human consumption at the recommended maximum levels of THC content. The implementation of a maximum limit for THC in some foods would minimise the likelihood of consuming the specified foods with high THC levels (as observed in the analytical survey) and thus minimise the likelihood that consumption will lead to exceedance of the therapeutic dose for THC.

3 DISCUSSION

International Regulation of low-THC Hemp as Food

The review of international regulation of hemp foods found no regulatory limits for CBD or other cannabinoids however it is not clear in all cases whether this issue has been considered and dismissed, or not considered at all. On current information it is understood that neither the USA or Canada have considered this.

It should also be noted that while food regulators in the USA and Canada have not considered setting limits for CBD in food products, the US Food and Drug Administration (US FDA) have concluded that products that contain cannabidiol cannot be sold as dietary supplements. The USFDA have stated that:

Based on available evidence, FDA has concluded that cannabidiol products are excluded from the dietary supplement definition under section 201(ff)(3)(B)(ii) of the FD&C Act. Under that provision, if a substance (such as cannabidiol) has been authorized for investigation as a new drug for which substantial clinical investigations have been instituted and for which the existence of such investigations has been made public, then products containing that substance are outside the definition of a dietary supplement. There is an exception if the substance was "marketed as" a dietary supplement or as a conventional food before the new drug investigations were authorized; however, based on available evidence, FDA has concluded that this is not the case for cannabidiol.[9]

The US FDA have issued warning letters to companies marketing high CBD oils as unapproved drugs for the treatment of disease. The most recent letters were issued by the FDA in February 2016.[10]

This lack of international regulation for CBD and other cannabinoids in hemp foods may expose Australia and New Zealand to a risk of creating technical barriers to trade and may result in a World Trade Organisation (WTO) challenge, should regulatory limits for cannabinoids other than THC be considered. However, the preliminary assessment indicates setting limits for CBD is not likely to be necessary, as THC is the limiting component.

Nutrition and Health Claims in low-THC Hemp Foods

The review identified a range of hemp foods and additional products that are sold as dietary supplements. The claims being made in relation to hemp foods could be classified as nutrition content (NC) and general level health claims (GLHC), which are required to comply with the requirements of Standard 1.2.7 - Nutrition, Health and Related Claims of the Code. The claims focused on the nutritional profile of hemp foods and did not refer to or make mention of CBD or any other cannabinoids.

This Standard restricts the making of express or implied nutrition, health and related claims in relation to food or a property of food. Health claims need to meet certain criteria to be permitted under this Standard.

[9] http://www.fda.gov/NewsEvents/PublicHealthFocus/ucm421168.htm#dietarysuppl, accessed 14.02.2016

[10] http://www.accessdata.fda.gov/scripts/warningletters/wlSearchResult.cfm?webSearch=true&qryStr=CBD&Search=Search, accessed 14.02.2016

Under this Standard the making of therapeutic claims is strictly prohibited. This includes reference to prevention, diagnosis, cure or alleviation of a disease, disorder or condition; or to comparison of a food with a good that is represented in any way or likely to be taken to be for therapeutic use. This Standard came into effect in Australia and New Zealand on 18 January 2016. Therefore, any claims about the health benefits of hemp foods marketed in Australia and New Zealand (if they were legal) would be required to meet this Standard.

High CBD hemp oil products make a range of high level health claims (HLHC) which are currently not permitted under Standard 1.2.7. The various State and Territory Food Acts include a 'meaning' for food which includes a statement 'food does not include a therapeutic good'. Therefore, a high CBD oil may be a therapeutic good and therefore not a food.

If approval of hemp foods is progressed, manufactures and marketers of these products, both here and overseas for sale online into Australian and New Zealand would need to be aware of the requirements of Standard 1.2.7 and other regulatory measures such as the Competition and Consumer Act 2010 (CCA). Under the CCA, businesses are not permitted to make statements that are incorrect or likely to create a false impression. This applies to advertising, product packaging, and any information provided to consumers by staff, through online shopping services or messaging via social media.

The issue of dietary supplement type products will need to be addressed with respect to levels of CBD and other cannabinoids but also the claims being made. These products are out of scope for this review but their regulatory compliance will need to be considered.

Pharmacological actions and associated therapeutic levels of phytocannabinoids

The majority of phytocannabinoids reviewed have no demonstrated action in humans at this stage.

However, the researchers considered a limit could be set for CBD given that it has been demonstrated to have a therapeutic effect in humans. The lowest estimated adult therapeutic absolute dose for CBD is 800mg/day. This dose level has been used by FSANZ in their dietary assessment. The volume of food required to reach the lowest estimated therapeutic dose of CBD is summarised in Table 9.

Table 9: Amount of Hemp Foods required to achieve the therapeutic dose of CBD

Hemp Food	Estimated amount of food [1] that would need to be consumed to reach the estimated lowest therapeutic effect dose of CBD (800 mg per day) for a 60 kg person
Hemp Protein Powder	Approximately 125 – 920 kg/day
Hemp flour	Approximately 400 – 2,200 kg per day
Hemp based milk substitutes	Approximately 160 – 45,500 kg per day
Hemp oil	Approximately 35 – 100 kg per day
Hemp seed	Approximately 160 – 1,800 kg per day

[1] Lower end of range refers to an estimated amount of food at the maximum concentration of CBD analysed; the upper end of the range refers to an estimated amount of food at the mean or medium concentration of CBD analysed, whichever is the lowest concentration value.

The researchers advised that any limits set for CBD should also include CBDA given that CBDA is almost completely converted to CBD upon heating (Arnold 2016). Similarly, the researchers advised that any limits currently proposed for THC should also include THCA. FSANZ did not consider THCA in its work on A1039. However, THCA is almost completely converted to THC when it is heated and so may be heated by some consumers either intentionally or in product use, for example in the baking of biscuits or breads containing hemp flour.

The researchers advised that it was not possible to propose minimum therapeutic doses on which to base limits in hemp foods at this stage for the remaining phytocannabinoids - CBC, CBDV, CBN, CBGA, CBG, THCV and THCVA. There is limited evidence to support these compounds having intoxicating effects following oral administration and the available evidence for therapeutic potential comes from animal studies and in vitro studies. The estimated human doses calculated from animal studies may not be relevant to human consumption.

It should be noted that the therapeutic effects of cannabinoids are a new and expanding field and while at present the evidence for a therapeutic effect in humans is limited, this will need to be monitored over time as the evidence-base develops. On-going monitoring of therapeutic data and the cannabinoid content of hemp foods is likely to be required in order to identify any other cannabinoids of concern in the future.

Cannabinoids in low-THC Hemp Products - Analysis

The NSW Food Authority work provided two key pieces of information to inform this review:

1. Levels of a CBD, CBDA and total cannabinoids provided to FSANZ for the dietary assessment; and
2. Levels of THC and THCA which can be assessed against the maximum levels of THC proposed as regulatory limits in A1039.

The food product analysis based on 200 samples sourced from Australia, New Zealand and overseas found the level of total cannabinoids varied between products. There was no obvious correlation between where the products were sourced and no clear correlation between the level of CBD and THC in products tested.

There were a number of products that exceeded the THC levels proposed in application A1039; 29 (38%) of the oil products, two (3.6%) of hemp protein powder and three (13%) of hemp shakes. A1039 proposed a maximum THC limit of 10 mg/kg (ppm) in hemp oil products and 5 mg/kg in hemp flour and hemp seed. Of the 200 products analysed, 29 (38%) of the oil products, two (3.6%) hemp protein powders and three (13%) hemp shakes had levels of THC in excess of those proposed by FSANZ.

These results support the need for a THC limit to be established for hemp foods.

The results also indicate that if a new proposal for hemp foods is progressed, those who supply low-THC hemp foods will need appropriate monitoring procedures to ensure products comply with requirements.

It is important to note that high CBD oil products were excluded from the analysis because they were not considered to be food and their high CBD content would have biased the results.

Low-THC Hemp Food Dietary Assessment

The dietary assessment conducted by FSANZ is based on:

a) the hemp food product analysis results (NSW Food Authority); and

b) the relevant lowest therapeutic effect dose from either:

- Arnold *et al* (2016) (Section 2.3);
- FSANZ (2011); or
- Brierley et al (2016).

The following matrix summarises the results of the dietary assessment for each cannabinoid against each hemp food group. A tick (✓) denotes that the maximum amount of hemp food estimated to be able to be consumed before the lowest therapeutic dose level was reached was lower than the predicted consumption of that hemp based food by consumers (eaters) only.

Table 10: Low-THC hemp food cannabinoid exposure matrix

Hemp Food	CBD	CBDA	THC	THCA
Lowest estimated therapeutic effect dose (mg/day based on 60 kg person)	800	5	0.36	3.5
Hemp Protein Powder	X [1]	X	✓ [2]	X
Hemp flour	X	X	ND	ND
Hemp based milk substitutes	X	X	X	X
Hemp oil	X	X	✓	X
Hemp seed	X	X	X	X

[1] X = Estimated maximum food amount higher than predicted hemp food consumption

[2] ✓ = Estimated maximum food amount lower than predicted hemp food consumption

Of the hemp food groups assessed, THC (at the maximum analysed concentration) was the cannabinoid most likely to exceed the minimum therapeutic dose in hemp protein powder and hemp oil. The estimated amount of these foods that could be consumed without reaching a therapeutic dose was lower than the predicted mean and 90th percentile consumption of these foods; i.e. an average consumption level of hemp powder and hemp oil containing the maximum level of THC would deliver a therapeutic dose.

FSANZ notes that using the maximum analysed concentration of THC in the calculations is not considered a realistic chronic (long-term) scenario, as it is unlikely that hemp protein powder and hemp oil will always contain the maximum analysed concentration of THC observed in a single sample from a survey.

This would support setting a maximum level of THC in some hemp foods which is consistent with the recommendation of A1039.

FSANZ noted that although the 2011-12 National Nutrition and Physical Activity Survey (NNPAS) data have been published by the Australian Bureau of Statistics, the data set is not yet available for use in FSANZ's modelling system to derive food consumption amounts that include the uses of foods as ingredients in mixed foods. As a result, the assessment was conducted using the 1995 Australian National Nutrition Survey (AusNNS). If FSANZ is requested to raise a proposal for approval of low-THC hemp foods, it would be preferable to use the 2011-12 National Nutrition and Physical Activity Survey (NNPAS) data for modelling.

Other considerations

Food/Medicine interface

The issue of whether particular products are food or therapeutic goods has become more complex over recent years as health claims are made in relation to a wider range of products, including products that have traditionally been regarded as food.[11]

The Therapeutic Goods Administration (TGA) provides a tool for manufacturers to determine whether products are regulated as therapeutic goods or as a food. The "Food-Medicine Interface Guidance Tool". [12] Question 4 of the tool asks:

Is the product 'goods for which there is a standard' under the Food Standards Code?

A clear definition of hemp as food in the Code could assist in preventing products containing therapeutic levels of cannabinoids being sold as food by limiting the levels to those naturally occurring.

General Food Regulation Compliance

The Code sets out the requirements for food products with respect to composition, labelling and food safety. If low-THC foods are approved there will be other requirements that manufacturers and suppliers of hemp foods will be required to meet. There may also be requirements for amendments to the Code, for example, to set microbiological limits to ensure safety.

For example: in September 2015, hemp seed protein powder and hemp powder (marked as not for human consumption) were recalled in Australia due to salmonella contamination.

Other sections of the Code may need to be reviewed and amended to ensure that hemp foods available for sale are safe.

[11] TGA, https://www.tga.gov.au/community-qa/food-and-medicine-regulation, accessed 01.02.2016

[12] https://www.tga.gov.au/food-medicine-interface-guidance-tool-questions-explanation-and-information, accessed 01.02.2016.

Food regulation awareness

Awareness of the requirements of the Code by both local and overseas suppliers may be limited as these products are not currently required to comply.

In addition to compliance to any limits set in the future, suppliers of hemp foods would need to ensure they meet other Code requirements.

4 KEY FINDINGS

Key finding 1 – Therapeutic effects

Human data on the therapeutic effects of cannabinoids is emerging.

The review on pharmacological actions and associated therapeutic levels identified sufficient information to estimate a threshold adult therapeutic dose for CBD of 800 mg/day (absolute dose) although further clinical trials are required to confirm this estimate.

The available information on minimum therapeutic levels for the remaining phytocannabinoids CBC, CBDV, CBN, CBGA, CBG, THCV and THCVA is limited to preclinical studies (cellular models and animal studies) and is insufficient to estimate minimum therapeutic levels for these cannabinoids. However, FSANZ have advised that it may be possible to use animal data to set a limit for CBDA.

Key finding 2 - Limits

There is no apparent need for a limit to be set for cannabinoids other than THC in relation to low-THC hemp foods, as CBD and CBDA levels in these products were below those required to produce a therapeutic effect at all reasonable levels of consumption.

The FSANZ dietary assessment suggests THC is the limiting component with respect to the potential for delivery of therapeutic levels in hemp foods, but not CBD or CBDA, which is in line with the previous conclusions of A1039.

There are readily available (on line) CBD fortified products that are capable of providing a therapeutic dose and setting a CBD limit may be required to distinguish these products from food and prevent them being misused by people seeking to self-medicate for serious medical conditions such as epilepsy.

There is potential for CBDA and THCA to be converted to CBD and THC respectively at temperatures above 160°C. For this reason, CBDA and THCA may need to be included in any limits proposed for CBD and THC

If limits are to be set, they could be expressed as:

- Total phytocannabinoids defined as CBD, CBDA, THC, THCA; or
- Total CBD defined as CBD and CBDA; and
- Total THC defined as THC and THCA.

Research on the pharmacology and therapeutic effects of cannabinoids is expanding rapidly and any limits proposed for cannabinoids in low-THC hemp foods may need to be reviewed as new information comes to light.

Key finding 3 – Cannabinoids in low-THC hemp product

The predominant cannabinoids in the 200 hemp foods from Australia, New Zealand and overseas tested were CBD, THC and their acid precursors CBDA and THCA.

Other cannabinoids were present at either low levels or below the limit of detection.

Some of the hemp foods tested had THC levels in excess of the limit previously proposed by FSANZ in relation to A1039.

Key finding 4 – International regulation of CBD in low-THC hemp as food

No international jurisdiction which currently permits consumption of low-THC hemp food has set a limit for any cannabinoid other than THC, so there are no international CBD limits for foods. International trade rules may constrain any aspiration to set unilateral CBD limits.

The Forum could request FSANZ engage with key jurisdictions such as the USA and Canada to share the findings of this work and to open dialogue in relation to the potential for setting limits for CBD and/or other cannabinoids.

Key finding 6 – Nutrition and health claims

Claims made for hemp foods did not mention CBD or other cannabinoids, but rather related mainly to nutrition content and general level claims that would be captured under Standard 1.2.7 (Nutrition, Health and Related Claims) of the Code. However, some products marketed as nutritional supplements made high level health claims. Given that these products may deliver a therapeutic dose of CBD, they could be regulated as therapeutic goods and product claims managed under that regime.

Key finding 7 – Education

Approval of low-THC hemp as food may need to be supported by education, particularly for the hemp industry.

Business may need assistance to comply with any food regulatory and testing requirements for low-THC hemp foods prior to implementation of any regulation.

Key finding 8 – Food regulation

Hemp foods available for sale in Australia and New Zealand would also need to comply with other relevant standards in the Australia New Zealand Food Standards Code (the Code), for example, in relation to safety, claims and labelling.

Key finding 9 – Monitoring

Consideration will need to be given as to how to manage CBD and other cannabinoid levels in low-THC hemp foods should limits be set. Monitoring for compliance of THC /THCA and CBD/CBDA would require laboratories to have the capacity to support industry requirements in a timely and cost effective manner.

REFERENCES

Arnold JC, Allsop DJ, Lintzeris N, McGregor IS. Pharmacological actions and associated therapeutic levels of phytocannabinoids: An Evidence Check review brokered by the Sax Institute (www.saxinstitute.org.au) for the NSW Ministry of Health, 2016. [Provided as Appendix 3].

Australia New Zealand Food Standards Code (2015). Standard 1.1.1 – Structure of the Code and general provisions.

http://www.foodstandards.gov.au/code/Documents/1.1.1%20Structure%20and%20General%20Provs%20v159.pdf, accessed 14.01.2016

Australia New Zealand Food Standards Code (2015). Schedule 23 – Prohibited plants and fungi.

https://www.comlaw.gov.au/Details/F2015L00435, accessed 14.01.2016

Australian National Nutrition Survey: Food Eaten, Australia, 1995. (AusNNS, 1995).

http://www.abs.gov.au/ausstats/abs@.nsf/mf/4804.0, accessed January 2016.

Brierley DI, Samuels J, Duncan M, Whalley BJ, Williams CM (2016) Neuromotor tolerability and behavioural characterisation of cannabidiolic acid, a phytocannabinoid with therapeutic potential for anticipatory nausea. Psychopharmacology (Berl). 233(2):243-54. [Provided with Appendix 5].

FSANZ (2014). Review – Application A1039, Low-THC Hemp as a Food. [26-14].

http://www.foodstandards.gov.au/code/applications/Documents/A1039-Hemp-RevR.pdf, accessed 07.02.2016.

FSANZ. (2012). *Supporting Document 1. Safety Assessment (Approval) – Application A1039. Low-THC Hemp as a Food.* Retrieved 12 January 2016 from http://www.foodstandards.gov.au/code/applications/documents/A1039_SD1.pdf

FSANZ (2011) Final Assessment Report: Application A1039 – Low-THC hemp as a food.

http://www.foodstandards.gov.au/code/applications/documents/A1039%20Low%20THC%20hemp%20AR%20FINAL.pdf

New Zealand (NZ) Food (Safety) Regulations 2002.

http://www.legislation.govt.nz/regulation/public/2002/0396/latest/whole.html, accessed 14.01.2016

New Zealand Adult Nutrition Survey. (2008) (NZANS, 2008).

http://www.health.govt.nz/publication/2008-09-new-zealand-adult-nutrition-survey-data-tables, accessed January 2016

Acknowledgements

A document such as this is not the work of a single person or small group, it represents the collective effort and expertise of a number of organisations.

The Author wishes to acknowledge the following contributors to this review:

Food Standards Australia New Zealand

NSW Food Authority

NSW Ministry of Health

Sax Institute Knowledge Exchange Program

Southern Cross University Plant Science Laboratory

The University of Sydney Lambert Initiative for Cannabinoid Therapeutics

FJ FLEMING
FOOD CONSULTING

Fiona Fleming, Director
B.App Sc (Food Tech); MNutr Mgt; MAIFST, MAICD

FJ Fleming Food Consulting Pty Ltd
ABN:17154815531

Food Policy and Regulation | Communication and Corporate Affairs
Issues and Crisis Management | Food Safety and Quality Systems

M: 0417 461 911
T: +61 2 8091 4174
E: fiona@fjflemingfoodconsulting.com.au
S: theflemingsathome

APPENDIX 1

Review of International Regulation of Low-THC Hemp Food

Review of International Regulation of Low-THC Hemp Food

Final Report

January 2016

Report prepared for the NSW Ministry of Health

FJ FLEMING
FOOD CONSULTING

Table of Contents

1. Introduction

The Food Regulation Standing Committee (FRSC) is currently considering the regulation of low-THC hemp. NSW Health and the NSW Food Authority have been asked to assist the Committee through consideration of setting cannabidiol levels to distinguish low-THC foods from therapeutic goods if this is considered appropriate.

FJ Fleming Food Consulting (FJF FC) has been engaged by NSW Health to conduct an evidence review in relation to the international regulation of low-THC hemp foods.

The **overall objective** of this work undertaken by NSW Health and the NSW Food Authority is to determine if a level for cannabidiol (CBD) and/or other phytocannabinoids (plant derived cannabinoids) needs to be set for low THC hemp foods:

- to ensure they do not provide therapeutic doses of phytocannabinoids at normally consumed levels; and
- as a means of distinguishing low-THC foods from therapeutic goods.

The **specific aim** of this segment of the work is to conduct a review of published regulations and consultation with food or health jurisdictions regarding food products overseas (noting that this issue is not addressed in many jurisdictions)

2. Method

The starting point for the review was a document prepared by FSANZ as part of the work on Application A1039 – Low THC Hemp as a Food – Supporting Document 5 – ANZ and International Hemp Regulations which was prepared in November 2012 (FSANZ 2012). This information was based on a questionnaire sent out by FSANZ late in 2011. FSANZ prepared a summary of hemp regulations as at that point in time based on the feedback received to the questionnaire.

Regulations for the countries listed in **Table 1** were sourced and reviewed to determine if any changes had been made to the regulation of hemp foods since the FSANZ review in 2011. Regulations were sourced from the website of the regulatory agency for each country.

In the case of the USA and Canada contact was made via to request further information and clarification of regulation. The FDA was contacted directly and information provided by the Office of Food Additive Safety (Centre for Food Safety and Applied Nutrition, U.S. FDA). Contact with Canadian regulators was made via the Canadian Embassy in Canberra.

3. Results

The table below summarizes the regulations investigated and the regulation of THC and CBD.

Table 1: Summary of International Hemp Food Regulation

COUNTRY	THC LEVEL REGULATED	CBD LEVEL REGULATED
New Zealand	Inferred limit of 0.35% THC for hemp oil	No
Canada	Maximum level of 10 parts per million (ppm or mg/kg) for THC residues in products derived from hemp grain, such as flour and oil.	No
USA	THC limit of 10ppm (mg/kg)	No
EU	0.3% THC	No
UK	Case by case permission	
Ireland	No regulation	No
Netherlands	No set limit	No
Germany	No specific regulation but recommendations for THC limits: 5 µg/kg for non-alcoholic and alcoholic drinks; 5000 µg/kg for edible oils; 150 µg /kg for all other foods.	No
France	Not permitted	No
Greece	Not permitted	No
Spain	Not permitted	No
Austria	THC must not exceed 1-2ug/kg bw/day	No
Belgium	THC limits of oil of seed: 10 mg/kg; Seed and flour of seed: 5 mg/kg; Other foods and drinks: 0.2 mg/kg	No
Italy	Zero	No
Cyprus	No specific hemp food regulation – expectation that hemp based foods will have no detectable levels of THC	
China	No regulation	No
Hong Kong	Hemp foods not permitted	No
Singapore	Hemp foods not permitted	No

USA

A written (email) response was received from the US FDA as follows:

The regulation of marijuana, THC, and other physiologically active materials from the plant is quite complex in the US due to the different laws enacted at the Federal level and in the individual States. So while the States might legalize the sale and use of marijuana and derivative products, those products remain illegal in the eyes of the Federal government and may not be sold or dealt across State lines. The status of other marijuana or hemp-derived products depends on their (proposed or intended) use. If someone wanted to propose a medical use for a hemp-derived material, they would need investigational permission from FDA and they would need permission from other regulatory agencies like the Drug Enforcement Administration before they could proceed.

There has been an independent determination of GRAS (general recognition of safety) status for vegetable oil derived from seeds of industrial hemp, as well as for the press cake from those seeds (provided as ***Appendix 1****). Since industrial hemp has low THC content and no THC occurs in the seeds themselves, we have not found cause to object to that determination.*

Cannabidiol is a substance that is under current clinical investigation for its utility as a medicine. It does not carry GRAS status, as there is no consensus in the scientific community that this material meets the criteria for such. Because it is now the subject of clinical drug trials it is not allowed to be added to food nor may it be marketed as a dietary supplement under Federal law. It may, however, be investigated for its utility as a medicine if proper FDA procedures for new drug investigations are followed.

There is no allowable amount of THC in food in the US.[1]

Canada

A written (email) response was received from Health Canada as follows:

Canada has not considered setting levels for cannabinoids, other than tetrahydrocannabinol (THC), in foods.

Health Canada has conducted some research on the risks of cannabinoids in foods, cosmetics and nutraceuticals following the promulgation of the Industrial Hemp Regulations in 1998. However, this research is considered preliminary and has not been updated since 2001.[2]

The consultant and a representative from NSW Health also met with representatives from Health Canada in December 2015. This meeting did not provide any further information relevant to regulation of hemp as food. An additional contact for the Health Products and Food Branch section of Health Canada was provided to the consultant in late December 2015. Contact was initiated via email but no response has been received at the time of completing this report.

[1] Email response from Michael Adams, Deputy Director, Office of Food Additive Safety, Centre for Food Safety and Applied Nutrition. U.S. Food and Drug Administration. July 2015

[2] Email response from David Ingham, Trade Commissioner, High Commission of Canada, Canberra, ACT. September 2015.

4. Findings

The **specific aim** of this segment of the work is to conduct a review of published regulations and consultation with food or health jurisdictions regarding food products overseas (noting that this issue is not addressed in many jurisdictions).

The review has determined for the countries reviewed, there are no regulations in relation to the level of CBD or any other cannabinoids in hemp food products permitted for sale in the respective jurisdiction. Table 1 summarizes the regulations investigated and the regulation of THC and CBD.

For the USA and Canada where direct contact was made with representatives from the respective regulatory agencies, no advice was available as to why there has been no regulatory level considered or set for CBD or any other cannabinoids.

Further discussion with Canadian and US regulatory authorities is recommended, potentially sharing the outcomes of the literature review, the hemp food product analysis and the dietary assessment.

References

FSANZ. (2011). *Supporting Document 6. International Hemp Regulations – Application A1039. Low THC Hemp as a Food*.

http://www.foodstandards.gov.au/code/applications/documents/A1039_AR_%20SD6.pdf, accessed 11.02.2016

Appendix 1

Notice of GRAS Exemption Claims – Hempseed Oil (November, 1999)

http://www.fda.gov/downloads/Food/IngredientsPackagingLabeling/GRAS/NoticeInventory/UCM265977, accessed 11.02.2016

APPENDIX 2

Review of Nutrition and Health Claims in Low-THC Hemp Food

Review of Nutrition and Health Claims in Low-THC Hemp Food

Final Report

December 2015

Report prepared for the NSW Ministry of Health

FJ FLEMING
FOOD CONSULTING

Table of Contents

1. Introduction

The Food Regulation Standing Committee (FRSC) is currently considering the regulation of low-THC hemp. NSW Health and the NSW Food Authority have been asked to assist the Committee through consideration of setting cannabidiol levels to distinguish low-THC foods from therapeutic goods if this is considered appropriate.

FJ Fleming Food Consulting (FJF FC) has been engaged by NSW Health to conduct an evidence review in relation to the regulation of low-THC hemp foods.

The **overall objective** of this work undertaken by NSW Health and the NSW Food Authority is to determine if a level for cannabidiol (CBD) and/or other phytocannabinoids (plant derived cannabinoids) needs to be set for low THC hemp foods:

- to ensure they do not provide therapeutic doses of phytocannabinoids at normally consumed levels; and
- as a means of distinguishing low-THC foods from therapeutic goods.

The **specific objective** of this segment of work is to conduct a claims review of internationally available products and sample marketing to consider the health claims being made by marketers of low-THC hemp foods in regards to other cannabinoids.

2. Method

The search strategy used to identify health claims currently being made in relation to commercially available hemp food products is summarised in Table 1 below.

Products identified in the search are available for purchase in Australia and overseas markets.

Table 1: Search Parameters

PARAMETER	DETAIL	COMMENTS
Scope of Search	Hemp food products available for sale: • direct to consumers via the internet; • via retail outlets.	
Methods used	• Google Alerts using search terms described; • Review of company websites for marketing materials; • Review of products collected by NSW Food Authority for testing	
Key sources of information	Company websites; Product packaging; Product marketing materials; testimonials; FDA website; materials provided by NSW Health	As this exercise was not a scientific literature review, databases such as PubMed were not included in the scope of the search.
Search terms	Hemp; hemp foods; hemp food products; hemp benefits; hemp health; cannabinoid; cannabidiol; delta 9-tetrahydrocannabinol; hemp CBD oil; hemp seed oil; cannabis	
Search engine	Google alerts were set up using the search terms described	
Search dates	Google alerts were set up in June and monitored daily until August 18th. The alerts will continue to be monitored until the end of September.	
Search technique	Snowballing – the products and companies identified through the google alerts were subsequently investigated further via their websites and associated links	For example: searches were extended from a company's website to other marketing channels and from company market and financial information to their products.
Inclusion criteria	Products marketed as foods and which would comply with the Australia New Zealand Food Standards Code were included. Products containing low levels of THC.	While hemp food products are currently not approved to be sold in Australia
Exclusion criteria	Products marketed as medicines were not included. Products containing THC at > 0.3% were not included	

3. Hemp Food Claims

The following tables provide a summary of the products and the types of claims currently being made in advertising and marketing of hemp food products in Australia and internationally.

The types of claim have been classified using the current terminology for claims in Standard 1.2.7 – Nutrition, Health and Related Claims of the Australia New Zealand Food Standards Code.

General level health claim (GLHC) refer to a nutrient or substance in a food and its effect on a health function. They must not refer to a serious disease or to a biomarker of a serious disease.

An example is: calcium is good for bones and teeth.[1]

High level health claim (HLHC) refer to a nutrient or substance in a food and its relationship to a serious disease or to a biomarker of a serious disease. For example: Diets high in calcium may reduce the risk of osteoporosis in people 65 years and over.

Nutrition content claim (NC) are claims about the content of certain nutrients or substances in a food, such as 'low in fat' or 'good source of calcium'. The claims are about –

(a) the presence or absence of –

- (i) a biologically active substance; or
- (ii) dietary fibre; or
- (iii) energy; or
- (iv) minerals; or
- (v) potassium; or
- (vi) protein; or
- (vii) carbohydrate; or
- (viii) fat; or
- (ix) the components of any one of protein, carbohydrate or fat; or
- (x) salt; or
- (xi) sodium; or
- (xii) vitamins; or

(b) glycaemic index or glycaemic load;

that does not refer to the presence or absence of alcohol, and is not a health claim.

[1] http://www.foodstandards.gov.au/consumer/labelling/nutrition/Pages/default.aspx, accessed 07.02.2016

Table 2: Forms of hemp available as food and dietary supplements

PRODUCT TYPES
Hemp products
Hemp Seeds (some organic)
Toasted hemp seeds
Hulled hemp seeds (some organic)
Hemp hearts
Hemp powder
Hemp flour
Hemp protein powder (some organic)
Hemp meal
Hemp seed oil (some organic)
CBD Hemp powder
Food Products with hemp ingredients
Hemp oil infused teas; coffee and cocoa
Hemp oil infused protein bars
Hemp milks
Granola bars with hemp
CBD infused chewing gum
Dietary supplement type products
CBD Hemp Oil
Extra Strength CBD oil

The searches conducted for the purpose of this review were limited to food products however some high CBD hemp oil products were included as they were also available for sale via similar channels to the hemp food products. These products were generally marketed with droppers or syringes to deliver a defined quantity of oil.

Table 3: Hemp Food Product Claims (examples)

The claims listed below are examples of the types of each type of claim – a full listing of claims is provided as **Appendix 1.**

CLAIMS	TYPE [1]
Natural source of antioxidants, phytosterols, vitamins, minerals and fibre	NC
Great sources of omega 3 & 6. Great source of protein. Folate, Iron, Calcium. Hemp seeds contain all 20 amino acids, including the 9 essential ones that our bodies cannot produce on its own. Hemp seeds are also a rich source of essential minerals, including magnesium and phosphorus for bone health, zinc to help support the immune system, and iron for oxygenating the blood.	NC; GLHC
Ideal ratio of omega 6 to omega 3 which can help the body to metabolise fat, counteract aging, increase immune system strength, lower cholesterol and help prevent cardiovascular disease. May assist with healthy skin	NC; GLHC
The Protein and fibre help to slow digestion, prevent spikes in blood sugar and help the body to sustain energy. An important aspect of hemp seed protein is a high content of arginine (97mg/g protein) and histidine (23 mg/g protein), both of which are important for growth during childhood, and of the sulfur containing amino acids Methionine (20 mg/g protein) and cysteine (16 mg/g protein), which are needed for proper enzyme formation. Hemp protein also contains relatively high levels of the branched-chain amino acids that are important for the metabolism of exercising muscle. Hemp protein contains over 61mg/g of protein of Leucine – higher than cashews or chia seeds.	NC; GLHC;
Great source of protein; perfect balance of omega3, 6 and 9; rich in vitamins and minerals; organic; Complete source of amino acids	NC
23 times mores omega 3 than olive oil improve cholesterol; 33% easily digested protein helps build muscle; high in nutrients and minerals promotes general well being	NC; GLHC
Hulled hemp seeds are a delicious source of polyunsaturated fatty acids. They contain 10 grams of omega-6 fatty acids and 3 grams of omega-3 fatty acids per serving. Hulled hemp seeds contain all 20 essential amino acids and zero trans fats.	NC
The omega 3 in hemp seed oil lowers blood pressure, reduces inflammation, decreases the risk of CVD and may have an effect on Alzheimer's disease. The Omega 6 helps maintain brain functions and bone health as well as stimulate hair and skin growth. Hemp Oil Australia, however, is not a cure for cancer.	NC; GLHC and HLHC
Can assist in the symptoms of high and low blood pressure, psoriasis, eczema and rheumatoid arthritis. Multiple sclerosis, schizophrenic psychosis and cancer - major preventative to these diseases. Regular intake of Pure Cold Pressed Hemp Seed Oil will help reduce the risk of arterial sclerosis and other cardiovascular diseases. The daily recommended dose is 10ml. Clinical trials showed a significant improvement in PMS related symptoms over a 12-week period. The daily recommended dose is 5ml, massaged into the stomach area. Rheumatoid arthritis - effective as an anti-inflammatory.	HLHC

[1] NC = nutrition content; GLHC = general level health claim; HLHC = high level health claim

Table 4: Hemp Product Claims – High CBD oil (examples)

CLAIMS	TYPE [1]
CBD is a neuromodulator that enhances cell communication and is thought to be effective in supporting several vital bodily systems. A compound of hemp, each CBD softgel contains over 400 milligrams of hemp seed oil, an excellent source of essential fatty acids (EFAs). Deemed essential because our bodies do not naturally produce them, EFAs occur naturally in hemp in a 3:1 balance of Omega-6 over Omega-3. According to the World Health Organization, this is the perfect balance for human consumption.	NC; GLHC; HLHC
Testimonials: ○ Dietary supplement - experience the positive effects of phytonutrients ○ Antioxidant and anti-inflammatory properties ○ Antiemetic (reduces nausea and prevents vomiting), anti-depressant and antipsychotic properties ○ Natural pain killer - analgesic effect ○ Reduce the pain in arthritis - through analgesic and anti-inflammatory properties ○ Epilepsy - reduction in seizures ○ Improvement in sleep; improved mental development; physical development ○ Control of pain ○ Thyroid cancer - reduction in tumour size ○ appetite suppressing properties ○ anti-inflammatory and anti-bacterial effects; useful in preventing certain forms of cancer ○ anti-inflammatory and bone stimulant	GLHC; HLHC

[1] NC = nutrition content; GLHC = general level health claim; HLHC = high level health claim

4. Findings

The objective of this segment of work is to conduct a claims review of internationally available products and sample marketing to consider the health claims being made by marketers of low-THC hemp foods in regards to other cannabinoids.

The review identified a range of hemp food products and additional products that are sold as dietary supplements. The claims being made in relation to hemp food products could be classified as nutrition content (NC) and general level health claims (GLHC) which are required to comply with the requirements of Standard 1.2.7 - Nutrition, Health and Related Claims. The claims focused on the nutritional profile of hemp food products and did not refer to or make mention of CBD or any other cannabinoids.

This Standard restricts the making of express or implied nutrition, health and related claims in relation to food or a property of food. Health claims need to meet certain criteria to be permitted under this Standard.

Under this Standard the making of therapeutic claims is strictly prohibited. This includes reference to prevention, diagnosis, cure or alleviation of a disease, disorder or condition; or to comparison of a food with a good that is represented in any way or likely to be taken to be for therapeutic use. This Standard came into effect in Australia and New Zealand on 18 January 2016. Therefore, any claims about the health benefits of hemp foods marketed in ANZ (if they were legal) would be required meet this Standard.

The high CBD hemp oil products also make a range of high level health claims (HLHC) which are currently not permitted under Standard 1.2.7. The various Food Acts include a 'meaning' for food which includes a statement 'food does not include a therapeutic good'. Therefore, a high CBD oil may be a therapeutic good and therefore not a food.

If approval of hemp food products is progressed, ANZ manufactures and marketers of these products, both here and overseas (for sale online into ANZ) would need to be aware of the requirements of Standard 1.2.7 and other regulatory measures such as the Competition and Consumer Act 2010 (CCA). Under the CCA, businesses are not allowed to make statements that are incorrect or likely to create a false impression. This applies to advertising, product packaging, and any information provided to consumers by staff, through online shopping services or messaging via social media.

The issue of "dietary supplement" type products will need to be addressed with respect to both levels of CBD and other cannabinoids but also the claims being made. These products are out of scope for this review but their regulatory compliance will need to be considered.

Appendix 1

Claims Review – Full Listing of Claims

NSW Health - Hemp Foods Project

CLAIMS REVIEW - APPENDIX 1

Objective: *Review of internationally available products and sample marketing to consider the health claims being made by marketers of low -THC hemp foods in regards to other cannabinoids.*

Search Terms: *hemp; hemp foods; hemp food products; hemp benefits; hemp health; cannabinoid; cannabidiol; delta 9-tetrahydrocannabinol; hemp CBD oil; hemp seed oil; cannabis;*

PRODUCT NAME/BRAND	PRODUCT DESCRIPTION	COUNTRY	DISTRIBUTION	CLAIM	CLAIM TYPE	Amount of THC; CBD or other Cannabinoid	POSITIONED AS MEDICINE OR FOOD	FORMAT (Pack, website, advertising)	COMPANY	COMMENTS	LINKS
			1=direct to consumer from overseas via the internet; 2=via an Australian distributor (retailer sale); 3= Aust distributor website; 4= Aust distributor website that links to overseas site				F= food; M= medicine; DS=dietary supplement				
Hemp Foods Australia	Organic Hulled Hemp seed (various pack sizes)	Made in Aust but exported for food use	1; 2; 3	2000mg of Omega 3 per spoonful; 6400mg of complete protein. Natural source of antioxidants, phytosterols, vitamins, minerals and fibre. 100% Australian certified organic	Nutrition content claims			Pack and website	Hemp Foods Australia		http://www.hempfoods.com.au/
Hemp Foods Australia	Whole Hemp seed (various pack sizes)	Made in Aust but exported for food use		100% Australian certified organic. Great sourced of omega 3 & 6. Great source of protein. Folate, Iron, Calcium. Hemp seeds contain all 20 amino acids, including the 9 essential ones that our bodies cannot produce on its own. Hemp seeds are also a rich source of essential minerals, including magnesium and phosphorus for bone health, zinc to help support the immune system, and iron for oxygenating the blood.	Nutrition content claims and GLHC			Pack and website	Hemp Foods Australia		
Hemp Foods Australia	Organic Hemp oil (various pack sizes). Cold-pressed hemp seed oil	Made in Aust but exported for food use		ideal ratio of omega 6 to omega 3 which can help the body to metabilise fat, counteract aging, increase immune system strength, lower cholesterol and help prevent cardiovascular disease. May assist with healthy skin	General level and high level health claims			Website	Hemp Foods Australia		
Hemp Foods Australia		Made in Aust but exported for food use		The omega 3 in hemp seed oil lowers blood pressure, reduces inflammation, decreases the risk of CVD and may have an effect on Alzheimer's disease. The Omega 6 helps maintain brain fuctions and bone health as well as stimulat hair and skin growth. Hemp Oil Australia, however, is not a cure for cancer.	General level and high level health claims			Website	Hemp Foods Australia		
Hemp Foods Australia	Organic Hemp Protein powder (various pack sizes)	Made in Aust but exported for food use		The Protein and fibre help to slow digestion, prevent spikes in blood sugar and help the body to sustain energy. An important aspect of hemp seed protein is a high content of arginine (97mg/g protein) and histidine (23 mg/g protein), both of which are important for growth during childhood, and of the sulfur containing amino acids Methionine (20 mg/g protein) and cysteine (16 mg/g protein), which are needed for proper enzyme formation. Hemp protein also contains relatively high levels of the branched-chain amino acids that are important for the metabolism of exercising muscle. Hemp protein contains over 61mg/g of protein of Leucine – higher than cashews or chia seeds.	Nutrition content claims and GLHC			Website	Hemp Foods Australia		
Hemp Foods Australia	Overall - Hemp Nutrition	Made in Aust but exported for food use		Highest amounts of PUFAs; highest quantity and quality of protein; GLA believed to be important for preventing inflammation; CLA; gut cleansing fibre; minerals; vitam E; B vitamins and the only known plant food source on Vitamin D3 - the bone building sunshine vitamin	Nutrition content claims and GLHC			Website	Hemp Foods Australia		http://www.hempfoods.com.au/hemp-nutrition/#.VbQwx_mqpBc
SANI HEMP Australia	Hemp Seed oil - cold pressed, organic	Distributed by Hemp Health Products Australia. Product of the UK - packaging in Australa		Can assist in the symptoms of high and low blood pressure, psoriasis, eczema and rheumatoid arthritis. Multiple sclerosis, schizophrenic phychosos and cancer - major preventative to these diseases. Regular intake of Pure Cold Pressed Hemp Seed Oil will help reduce the risk of arterial sclerosis and other cardiovascular diseases. The daily recommended dose is 10ml. Clinical trials showed a significant improvement in PMS related symptoms over a 12 week period. The daily recommended dose is 5ml, massaged into the stomach area. Rheumatiod arthritis - effective as an anti inflammatory.	Nutrition content claims; GLHC and HLHC	Our product undergoes testing for THC content when imported and is considered like many Hemp Seed Oils to contain a very low content of THC. The amount of THC is below the Australian government standards and is imported and checked through Australian Customs for sale in Australia but is approved for use by customers		Website & brochure	SANI HEMP AUSTRALIA		http://www.organicsonabudget.com.au/sani-hemp-australia-hemp-seed-oil-200ml/; http://www.hemphealth.com.au/history_of_hemp/why_hemp_seed_oil
Eco Farms	Organic hemp seeds	Australia	2								
Made in Hemp	Organic Hemp seed oil	Australian owned (Long Jetty NSW). Product of Canada	2	Great source of protein; perfect balance of omega3, 6 and 9; rich in vitamins and minerals; organic; Complete source of amino acids	Nutrition content claims			Website and product label	Made in Hemp		http://www.madeinhemp.com.au/273/Hemp-Seed-Products/
Made in Hemp	50% Organic Hemp Protein powder	Australian owned (Long Jetty NSW). Product of Canada		Great source of protein; perfect balance of omega3, 6 and 9; rich in vitamins and minerals; organic; Complete source of amino acids	Nutrition content claims			Website and product label			
Made in Hemp	Organic Hulled Hemp Seed	Australian owned (Long Jetty NSW). Product of Canada		Great source of protein; perfect balance of omega3, 6 and 9; rich in vitamins and minerals; organic; Complete source of amino acids	Nutrition content claims			Website and product label			
								Website and product label			
Forest Superfoods	Hulled Hemp seeds	Canada		23 times mores omega 3 than olive oil improve cholesterol; 33% easily digested protein helps build muscle; high in nutrients and minerals promotes general well being	Nutrition content claims and GLHC			Website and product label			http://forestsuperfoods.com.au/hemp-protein-superfood-buy-online-organic.html
Forest Superfoods	Organic Hemp Protein powder	Canada		One of the most digestible proteins on the planet	Nutrition content claims and GLHC			Website and product label			
Forest Superfoods	Organic Hemp Seed Oil	Canada						Website and product label			
EM Superfoods	Raw hemp seeds hulled certified organic	Grown in Inner Mongolia	Online								www.em-superfoods.com.au
Vitality Trading Co	Tasmanian Hemp Meal	Tasmania	2, 3???								https://www.vitaminking.com.au/Hemp-Protein-Meal-1kg-by-Vitality-Trading.html
Every bit organic	Hemp seed oil	Australia	3 ??								
ViPova (Lexaria)	Hemp oil infused black tea; Earl Grey tea; Herbal Cherry Balc tea; Organic Green tea; De-caf English breakfast; Herbal Chai tea; The hemp oil is infused within dried evaporated non fat milk	Canada		targeted to contain ~10mg of CBD for just $3.00 per serving					LEXARIA	We actually have a patent-pending method by which we infuse our organically sourced high purity hemp oil INSIDE the molecules of other ingredients. We work with lipids because the human endocannabinoid system is itself lipid based.	www.vipora.com
Lexaria Energy	Hemp oil infused protein bar	Canada							LEXARIA	Not yet available on the market (11.08.2015)	http://www.lexariaenergy.com/
Hemp Health Inc	CBD Rich hemp Oil. Nutritional supplement	Manufactured in the USA		Notes that they cannot make claims due to FDA regulations		15.9 - 25% CDB; THC < 1%					https://hemphealthinc.com/about-our-cbd/

Hemp Health Inc	CBD Powder. Dietary supplement	Manufactured in the USA				0% THC; 100% CBD			https://hemphealthinc.com/shop/pharma-cbd-powder/pharma-cbd-powder/
Hemp Health Inc	Hemp CBD dietary supplement capsules	Manufactured in the USA				Hemp oil (stalk and stem) = 130mg/capsule; CBD = 25mg/capsule			
Hemp Health Inc	CBD Oral Spray	Manufactured in the USA				Hemp oil (stalk and stem) = 8mg/pump; CBD = 1mg/pump			
Love Hemp	CBD Oil							CBD Oils UK	http://www.cbdoilsuk.com/
Daily Greens Hemp Milks	Complete-protein milk-alternatives made with organic hemp, A combination of hemp seeds, greens and unique ingredients such as matcha green tea and blue-green algae	USA		They are a complete protein, contain the perfect proportion of Omegas 3 + 6, and are high in iron and calcium. No mention of cannabinoids	Content claim		Website	Daily Greens	http://drinkdailygreens.com/hemp-milks/
Lariese	Raw hulled hemp seeds (Organic)	Produced and packaged in NZ	2 & 3	Raw hemp is the most complete source of nutrition on earth		NZ THC free hemp seeds			http://lariese.com.au/product-category/hemp-products/
Lariese	Raw cold pressed hemp seed oil (organic)	Produced and packaged in NZ	2 & 3	Essential fatty acids in this oil is fundamental in restoring health and the immune function. These properties could assist with problems associated with heart, liver, kidney, bowel, gallbladder, arthritis, immunity, auto-immune disorder, hormone balance, reproduction, PMS, menopause, allergies, metabolism, nerve and brain function, attention span, muscle recovery, physical performance, lactic acid reduction in joints, eczema and skin health. It is anti-bacterial, anti-funagal, anit-microbial and anti yeast.	Nutrition content; GLHC and HLHC				https://lariese.com/product/certified-organic-edible-hemp-seed-oil/
Lariese	Raw Hemp protein (organic)	Produced and packaged in NZ			Nutrition content and GLHC	THC free			https://lariese.com/product/hemp-protein-powder-certified-food-grade/
Lariese	Raw hemp flour	Produced and packaged in NZ		Gluten Free	Nutrition content				https://lariese.com/product/hemp-flour-certified-food-grade/
Lariese biona	Hemp seed oil	UK	2						
Pot-o-Coffee	CBD infused single serve coffee, tea and cocoa. Pot-O-Coffee product line consists of two variants for cannabis infusion; one infused with cannabis plant-extracted tetrahydrocannabinol (THC) oil, while the other is infused with non-psychotropic cannabidiol (CBD) derived from agricultural based Hemp Oil.	USA		Many CBD users claim to receive medical relief without experiencing the "high" effect that is typically associated with cannabis use.		10mg per CBD infused product	News article		http://www.benzinga.com/pressreleases/15/07/p5698939/pot-o-coffee-heats-up-the-cannabis-market-by-introducing-marijuana-infu; http://www.potocoffee.coffee/#about
CannazALL	Hemp CBD oils	USA						Hemp Life Today	http://www.hemplifetoday.com/lp/
Dietary Supplements	CBD Tincture			Provide links on their webstie to google.com search for a range of "ailments" - anxiety; inflammation; chronic pain; spasms; sleep disorders; rheumatiod arthritis; diabetes; alcoholism; MS; schizophrenia; PTSD; epilepsy; neurological disorders; Parkinsons and much more!		1 dropper = 10mg CBD; recommended use is 25-50mg/day up to 100mg for more aggressive CBD theraphy	Website and Google.com		
	Extra strength CBD oil	USA				300mg CBD per syringe			
Life Series	Hemp Protein Powder	USA		Hemp seed protein powder is an easily digestible, vegan protein that contains all 20 essential amino acids as well as omegas 6 and 3 fatty acids.	Nutriiton content		Website	Life Series Products	http://lifeseriesproducts.com/product/hemp-protein-powder-16-oz/
Life Series	Hulled hemp seeds	USA		Hulled hemp seeds are a delicious source of polyunsaturated fatty acids. They contain 10 grams of omega-6 fatty acids and 3 grams of omega-3 fatty acids per serving. Hulled hemp seeds contain all 20 essential amino acids and zero trans fats.	Nutriiton content	Hemp oil - 490mg; CBD - 10mg	Website		
Life Series	Hemp flour	USA		Hemp seed flour is a great source of natural plant-based protein, fiber and omegas 6 and 3 fatty acids. It is full of B vitamins including thiamine, vitamin B6 and folate, and contains all 20 essential amino acids.	Nutriiton content		Website		
Life Series	Toasted Hemp seeds	USA		Toasted hemp seeds offer a deliciously high source of fiber and are loaded with digestible, vegan protein containing a balance of all 20 essential amino acids. Our toasted hemp seeds are packed with omegas 6 and 3 essential fatty acids, B vitamins, vitamin E and folic acid.	Nutriiton content		Website		
Life Series	CBD Softgels - daily supplement	USA		CBD is a neuromodulator that enhances cell communication and is thought to be effective in supporting several vital bodily systems. A compound of hemp, each CBD softgel contains over 400 milligrams of hemp seed oil, an excellent source of essential fatty acids (EFAs). Deemed essential because our bodies do not naturally produce them, EFAs occur naturally in hemp in a 3:1 balance of Omega-6 over Omega-3. According to the World Health Organization, this is the perfect balance for human consumption.	GLHC and nutrition content	Hemp oil - 490mg; CBD - 10mg & 20mg CBD & 60mg CBD	Website		
Cannaka	a line of botanically complete hemp oil products with added terpenes. Cannaka products are 100% natural, containing cannabinoids and terpenes derived from industrial hemp and other botanicals			Cannaka products promote a healthy inflammatory response and support the central nervous system by supporting the endocannabinoid system.				Cannaka	
Nutiva	Organic Hemp Protein Hi Fibre	USA	1						http://www.mynaturalmarket.com/contact_us.html
	Hemp oil			Ideal 3:1 omega 6 to omega 3 ratio. Can help the body to metabolize fat					
Navitas Naturals	Hemp seed oil Organic Raw Hemp Protein Powder	Canada	1	One of the best vegetarian sources of protein and is highly absorbable. Additionally the seeds contain all the essential amino cids making hemp a complete protein. Hemp seeds possess an ideally balanced ration of O3 and O6 acids. Excellent source of Mg, Iron and dietary fibre.					
Jarrow Formulas	Hemp protein								
Now Foods	Organic Toasted hemp seeds	USA		The seeds of the hemp plant have valuable naturally occuring essential fatty acids, included omega 3 and omega 6 and their easily digestible protein content has amino acids in a perfect ration for healthy nutrition					
Hippie Butter	Hemp seed oil Hemp seed protein powder	Canada							
Earth Circle Organics	hemp protein powder	Canada		Provides your body with the complete plant protein					

Bob's Red Mill	Hulled Hemp seed Hemp protein powder	USA	2	Good source of protein and contains all of the essential amino acids						
Health and Happiness	Hemp oil	Australia								
Eternal Delight	Chai Hemp protein									
Eternal Delight	Lush wellness sports powder	NZ		Balanced energy - increased; Stamina - Healthy radiant skin; Mental clarity - Healthy liver; Stronger immune system; improved digestion wellness; Health cholesterol levels; healthy blood sugar; cardiovascular wellness						eternaldelight.co.nz
Eternal Delight	Hemp powder - muscle pro cappuccino			Helps to keep your waistline trim by forming a gel in your stomach that can curb your calorie intake by making you feel fuller for longer. Improve endurance strength; energy; mental focus; coordination and performance; muscle tissue recovery; conversion of fat cells to lean muscle: delav of muscular fatieue						
Melbourne Hemp	Hemp seed oil		2							
	Hemp protein		2							
G.R.E.E.N. HEMP AUSTRALIA	Hemp seed oil									
Harveys	Hemp seed oil	Canada								
Manitoba Harvest	Raw shelled hemp seeds Hemp oil	Canada	2							
Kind and Strong	Granola bar - honey smoked BBQ - almond protein bar	USA							contains hemp seeds - level not declared	
	Granola bar - hickory smoked - almond protein bar	USA							contains hemp seeds - level not declared	
	Granola bar - Thai sweet chilli - almond protein bar	USA							contains hemp seeds - level not declared	
Narcotic Nutrition	Granola bar - Roaster jalapeno - almond protein bar	USA							contains hemp seeds - level not declared	
Absolute organics	Granola bar - honey mustard - almond protein bar	USA							contains hemp seeds - level not declared	
Just Hemp Foods										http://www.justhempfoods.com/
Elixinol ™	CBD Hemp Oil (contains 18%CBD). Dietary supplement.	USA		Dietary supplement - experience the positive effects of phytonutrients		10ml = 1,800mg CBD. THC <0.3%		Website	Elixinol	Marketed as a dietary supplement	http://elixinolcbd.com/pages/cbd-hemp-oil
Elixinol ™	Available in all US States (no permit or prescription required)			Antioxidant and anti-inflammatory properties						http://elixinolcbd.com/blogs/buyers-guide?page=2
Elixinol ™				Antiemetic (reduces nausea and prevents vominting), anti-depressant and antipsychotic properties	High level and General level health claims - not approved in ANZ					
Elixinol ™				Natural pain killer - analgesic effect						
Elixinol ™				Reduce the pain in arthritis - through analgesic and anti inflamm properties						
Elixinol ™	Elixinol Testimonials			Epilepsy - reduction in seizures						
Elixinol ™				Improvement in sleep; improved mental development; physical development						
Elixinol ™				Control of pain						
Elixinol ™				Thyroid cancer - reduction in tumor size						
Elixinol ™										
Elixinol ™	THVC			appetite suppresing propeties						
Elixinol ™	CBG			anti inflammatory and anti-bacterial effects; useful in preventing certain forms of cancer						
Elixinol ™	CBC			anti inflammatory and bone stimulant						
UltraCBD	Hemp Oil Supplements - 200mg cannbinoid extract			may assist in promoting overall wellness and easing anxiety caused by everyday stress.						http://ultracbd.com/
Loft Teas	Natural remedy teas infused with CBD	Produced in the US, CBD from Denmark and the Netherlands	Online	Website talks about high amounts of CBD in relation to epilepsy, muscle spasms, aniety, inflammation and pain but not directly related to this product - described as a wellness supplement - aimed at opening up the cannabis market to women		10mg CBD/serve	Food			http://www.lofttea.com/#landing

APPENDIX 3

Pharmacological actions and associated therapeutic levels of phytocannabinoids:

an Evidence Check review

saxinstitute

Evidence Check

Pharmacological actions and associated therapeutic levels of phytocannabinoids

An **Evidence Check** rapid review brokered by the Sax Institute for the NSW Ministry of Health. January 2016.

An **Evidence Check** rapid review brokered by the Sax Institute for the NSW Ministry of Health.
January 2016.

This report was prepared by:

Jonathon C. Arnold, David J. Allsop, Nicholas Lintzeris, Iain S. McGregor

January 2016

Enquiries regarding this report may be directed to the:

Head
Knowledge Exchange Program
Sax Institute
www.saxinstitute.org.au
knowledge.exchange@saxinstitute.org.au
Phone: +61 2 91889500

Suggested Citation:

Arnold JC, Allsop DJ, Lintzeris N, McGregor IS. Pharmacological actions and associated therapeutic levels of phytocannabinoids: an Evidence Check review brokered by the Sax Institute (www.saxinstitute.org.au) for the NSW Ministry of Health, 2016.

Pharmacological actions and associated therapeutic levels of phytocannabinoids

An **Evidence Check** rapid review brokered by the Sax Institute for The NSW Ministry of Health. January 2016.

This report was prepared by Jonathon C. Arnold, David J. Allsop, Nicholas Lintzeris, Iain S. McGregor

LAMBERT INITIATIVE
FOR CANNABINOID THERAPEUTICS

THE UNIVERSITY OF SYDNEY

Contents

1 Executive summary

Pharmacological actions and associated therapeutic levels of phytocannabinoids

A systematic review of the clinical and preclinical literature was conducted to examine the pharmacological and possible therapeutic effects of various plant-derived cannabinoids (phytocannabinoids) that are found in street cannabis and industrial hemp. Some of these phytocannabinoids may also be present from time to time in hemp seed oil and related products.

The phytocannabinoids of interest were:

- Cannabidiol (CBD)
- Cannabidiolic acid (CBDA)
- Cannabidivarin (CBDV)
- Delta-8-tetrahydrocannabinolic acid (THCA)
- Delta-9-tetrahydrocannabivarin (THCV)
- Delta-9-tetrahydrocannabivarinic acid (THCVA)
- Cannabigerol (CBG)
- Cannabigerolic acid (CBGA)
- Cannabinol (CBN)
- Cannabichromene (CBC).

The specific research questions posed to be addressed were:

1. What are the pharmacological characteristics of individual cannabinoids; and
2. What therapeutic levels of individual cannabinoids are required to elicit the pharmacological characteristic in adults and children?

The therapeutic effects of delta-9-tetrahydrocannabinol (THC) are well described and Food Standards Australia New Zealand (FSANZ) has already proposed a maximum allowable level of THC in hemp seed and oil added to food or offered for sale as food in their proposal in response to Application A1039.[i] The review therefore did not include THC.

Evidence from human trials was considered of greatest relevance. Where this was not available evidence from in vitro and animal studies was reviewed and included.

[i] A1039 proposes a variation to Standards in the Australia New Zealand Food Standards Code that would allow:

- Cannabis sativa seeds to be added to food or offered for sale as food if the seeds contain not more than 5 mg/kg delta 9-tetrahydrocannabinol, each seed is a non-viable seed and each seed is a hulled seed.
- All or any of the following seed products may be added to food or offered for sale as food: oil extracted from Cannabis sativa seeds if the oil contains not more than 10 mg/kg delta 9-tetrahydrocannabinol; a beverage derived from Cannabis sativa seeds if the beverage contains not more than 0.2 mg/kg/ delta 9- tetrahydrocannabinol and any other substance extracted or derived from Cannabis sativa seeds if the substance contains not more than 5 mg/kg delta 9-tetrahydrocannabinol.

Introduction

Cannabis sativa has been used for millennia for its myriad therapeutic effects. Following a few decades of prohibition, recent times have seen resurgent interest in the therapeutic potential of cannabis as well as the legalisation of medicinal cannabis in various countries.

This new interest has coincided with a dramatic increase in our knowledge of the cannabis plant, which is now known to contain more than 100 terpenophenolic compounds that are known as 'phytocannabinoids' (plant-derived cannabinoids) in addition to several hundred other compounds including monoterpenoids, sesquiterpenoids and flavonoids.

It is generally accepted that the characteristic intoxicating effects of cannabis are almost exclusively due to the action of the phytocannabinoid 'delta-9-tetrahydrocannabinol' (THC). THC acts at cannabinoid CB1 receptors (CB1Rs) in the brain to produce intoxicating effects that are reversible by CB1R antagonist drugs. Administration of pure THC to humans or non-human primates produces intoxication that is very similar to cannabis itself.

Only a few phytocannabinoids other than THC have been administered in purified form to humans. These include 'cannabidiol' (CBD), 'tetrahydrocannabivarin' (THCV), 'cannabichromene' (CBC), 'cannabidivarin' (CBDV), 'tetrahydrocannabinolic acid' (THCA) and 'cannabinol' (CBN). For CBD, a reasonable number of human studies exist, but for THCV, CBC, CBDV and CBN there are very few studies and they are generally of poor quality. Overall, it appears that none of these five phytocannabinoids appear to have THC-like intoxicating properties, although there is some evidence of mild intoxication with CBN and THCV following intravenous dosing, and there may be some mild sedation or somnolence with high oral doses of CBD, although the data are inconclusive on this point.

With the other phytocannabinoids, where human data are scarce or non-existent, intoxicating properties (or lack thereof) can only be inferred from either (1) studies of CB1R binding in vitro, in which CB1R binding can predict THC-like intoxicating effects, and (2) studies of THC-like effects in laboratory animals, which when carefully conducted can be broadly predictive of 'cannabimimetic' (cannabis-like) intoxicating effects in humans.

Phytocannabinoid content of cannabis plant material and other hemp preparations

The flowering heads of typical street cannabis in Australia contain a mean of 15% THC which is mostly in the form of non-psychoactive 'delta-9-tetrahydrocannabinolic acid' (THCA) (approximately 13.5%) with only 1.5% actual THC content. Heating the plant material to 160˚C or greater causes the conversion of plant based THCA to THC, a process known as 'decarboxylation'. This explains why cannabis is typically smoked, baked or vaporised by recreational users to obtain intoxicating effects. The levels of other phytocannabinoids in the flowering heads of Australian street cannabis is very low, typically <0.2%, suggesting that very large amounts of cannabis would need to be consumed to obtain any relevant pharmacological actions from these compounds.

Fibre hemp varieties of cannabis display an entirely different cannabinoid profile, with very low levels of THC (<0.5%) and THCA (<0.5%) but correspondingly high levels of CBD (approximately 3.5%) and its acidic form 'cannabidiolic acid' (CBDA) (approximately 10.5%). There is no evidence of intoxicating effects of CBDA. There is some evidence of mildly sedating and anxiolytic effects of CBD, but no evidence of THC-like intoxication. Other phytocannabinoids such as 'cannabidivarin' (CBDV) and 'cannabigerol' (CBG) may be present in hemp varieties at levels of 0.5% or less.

Hemp seed oils prepared by the cold pressing of cannabis seeds are acclaimed for their high levels of omega-6 and omega-3 polyunsatured fatty acids, and also for containing a diverse array of essential amino acids. Cannabinoids are apparently not synthesised within the hemp seed. However traces of cannabinoid contamination may result from the pressing of hemp seed oil probably due to residual contamination from the hull.

Comprehensive reports of the cannabinoid content of hemp seed oils are not readily available. It has generally been assumed that if made from the seeds of low THC hemp fibre varieties then only miniscule, trace levels of CBD, CBDA and sometimes THC will be present, typically at a range of 1–10 parts per million (ppm). However, one very recent analysis from Croatia[1] that included a diverse array of hempseed products, found CBD present at a range of 3–250 ppm, THC at a range of 4–243 ppm and CBN at a range of 2–8 ppm. More data are clearly needed relating to possible cannabinoid content of local Australian hemp seed products.

Diverse pharmacological targets of phytocannabinoids

In considering the possible 'therapeutic' actions of phytocannabinoids it is important to consider their pharmacological effects at multiple targets in the brain and body other than the CB1R. These include:

a) **The cannabinoid CB2 receptor:** The cannabinoid CB2 receptor (CB2R) is located largely in the immune system and this is an important therapeutic target related to neuropathic pain and inflammation. The CB2R is also an emerging target for novel therapeutics in the addictions field
b) **The endocannabinoid system:** The brain and body contain at least 2 endogenous cannabinoids called 'anandamide' and 2-AG. Some phytocannabinoids can modulate levels of these endocannabinoids by acting on endocannabinoid synthetic enzymes (NAPE-PLD and DAGL) or degradative enzymes (FAAH and DAGL). Such actions could theoretically produce a range of THC-like effects, although research on this topic is still at an elementary stage
c) **Transient Receptor Potential (TRP) channels:** There are more than 28 different TRP channel types in the human brain and body and these have a diverse range of functions relating to pain, inflammation, temperature sensing, taste and visceral function. Endocannabinoids, and some phytocannabinoids, act at TRP channels and this may underlie some therapeutic actions, particularly analgesic and anti-inflammatory effects
d) **Miscellaneous other receptors:** There is evidence for an action of some phytocannabinoids on a range of other receptors including serotonin (e.g. 5-HT_{1A}), noradrenaline (e.g. α2) and peroxisome-proliferator activated receptors (e.g. PPAR- α).

Pharmacological actions and possible therapeutic effects of individual phytocannabinoids

The relevant pharmacological and therapeutic actions of various phytocannabinoids, excluding THC, are summarised as follows. However it should be noted that the majority of phytocannabinoids reviewed have no demonstrated action in humans at this stage. We therefore estimated the lowest therapeutic dose (mg) in humans by extrapolating from the lowest dose effective in treating the disease in an in vivo animal model. While this dose may be instructive to the reader's consideration of what future limits might be, we do not endorse setting limits for cannabinoids in hemp food products based on therapeutic doses that were estimated from animal studies at this point in time. Future human studies would be required for this given major limitations in the translatability of animal research.

CBC (Cannabichromene)

CBC is a relatively common phytocannabinoid that is produced from CBG in the cannabis plant. There is little evidence for THC-like effects of CBC at CB1Rs, although there is evidence of sedation and catalepsy in laboratory animals at very high intravenous doses. CBC has a range of intriguing in vitro and in vivo actions in preclinical models that imply therapeutic efficacy as an anti-inflammatory agent and also perhaps as a mild sedative, analgesic and antibiotic. However, further confirmation in human studies is required.

CBC has no demonstrated therapeutic actions in humans. We therefore estimated the lowest therapeutic dose (mg) in humans by extrapolating from the lowest dose effective in treating disease in an in vivo animal model. The lowest therapeutic dose we found in the scientific literature was 1 mg/kg administered intraperitoneally (IP) which was effective in reducing inflammation of the colon (colitis) in mice.[2] As IP phytocannabinoid doses achieve on average 7 times higher tissue concentrations than oral doses in rodents, [3] the oral dose needed to achieve equivalent plasma levels might be as high as 7 mg/kg in mice. Applying calculations relating to interspecies comparison to this dose (footnote[ii]), the estimated therapeutic oral dose for CBC in a 60 kg human to produce anti-inflammatory effects is calculated at around 35 mg. Given the major limitations of animal to human dose extrapolation, this estimated dose should not be used to set a limit of CBC in hemp food products. To produce sedative, hypothermic or analgesic effects 20–80 fold higher doses are required, namely 0.7–3.0 g. It is very implausible that such dose would be achieved with even excessive consumption of any current hemp seed or hemp oil preparations.

CBDA (Cannabidiolic acid)

CBDA has never been administered to a human as a pure substance. However, the preclinical evidence described above suggests that CBDA is unlikely to have intoxicating effects in humans. CBDA only binds to CB1Rs at relatively high concentrations and does not activate the receptor. It does not appear to produce THC-like cannabimimetic effects in laboratory animals.

CBDA has various pharmacological actions that might be clinically relevant based on the effective concentrations and doses used in cell systems and animal studies respectively. Some effects have been shown at very low concentrations, including antiemetic effects and the potentiation of 5-HT_{1A} receptor activity.

The lowest CBDA dose we identified in the literature to be effective in an animal model of disease was 0.001 mg/kg (IP). This dose inhibited lithium-induced conditioned gaping (an animal model of anticipatory nausea) in rats.[4] As IP phytocannabinoid doses achieve on average 7 times higher tissue concentrations than oral doses in rodents,[3] the oral dose needed to achieve equivalent plasma levels might be 0.007 mg/kg in rats. Applying interspecies calculations to this dose, the estimated therapeutic oral dose of CBDA for this anticipatory nausea therapeutic effect in a 60 kg human would be approximately 0.07 mg[2]. However, we do not endorse setting limits for cannabinoids in hemp food products based on therapeutic doses that were estimated from animal studies.

CBDA is the precursor to CBD in the cannabis plant and is converted to CBD by heat. Given this fact, it is likely prudent to set a similar limit with CBDA as to that set for CBD, as heating CBDA produces CBD.

[ii]Please refer to the Introduction to the Review section under *"Caveat 3: Difficulties in extrapolation from preclinical concentrations/doses to therapeutic levels in humans"* in the full report for an explanation of the FDA-recommended animal to human dose calculation and its significant limitations in being able to predict a human therapeutic dose. There is high likelihood that potential therapeutic effects in *in vitro* and *in vivo* animal models may not translate to humans. If they do translate, there is no certainty that the human oral doses will reflect animal doses**. We do not endorse setting limits for cannabinoids in hemp food products based on therapeutic doses that were estimated from animal studies.**

CBD (Cannabidiol)

CBD has numerous pharmacological actions and is already showing strong clinical promise in the treatment of epilepsy, anxiety and psychosis. Complications may arise with pharmacokinetic interactions when CBD is combined with other medications such as opioids or anticonvulsants.

CBD appears to lack significant intoxicating effects in animals or humans, although some studies indicate the possibility of mild sedative effects of CBD at oral doses ranging from 200–800 mg.[5-7] However, the majority of studies in which evidence was presented suggest no sedative effects, even with oral doses as high as 1280 mg.

Across the many human studies performed with CBD, the lowest oral dose at which a therapeutically relevant effect was observed was 800 mg administered daily, which reduced psychotic symptoms in schizophrenia patients.[8] We used this study to obtain our lowest therapeutic dose (800 mg) on the basis of it being in a clinical population (i.e. not in healthy humans), being of robust experimental design and that it reported a clearly defined therapeutic effect. Please note though that this study is a Phase 2 RCT and thus cannot provide sufficient evidence for CBD to be formally approved as a therapeutic agent. Larger scale Phase 3 RCT trials are necessary for this, some of which are being conducted now with results forthcoming in 2016. We therefore conclude that a human oral dose of 800 mg of CBD constitutes a threshold therapeutic dose based on the best available evidence. This dose estimation might be subject to change when results of Phase 3 trials become available.

CBDV (Cannabivarin)

CBDV is the propyl homologue of CBD and has very low affinity for human CB1Rs or CB2Rs. It has powerful effects on various TRP channels, perhaps the most potent TRP effects of any of the phytocannabinoids. CBDV is clearly of major therapeutic interest as an anticonvulsant, given its current investigation in human clinical trials. Although comparatively little work has been done with CBDV, there is nothing to suggest CB1 affinity or intoxicating effects. Evidence for inflammatory and antiemetic actions are preliminary and reflect individual preclinical studies. Anticonvulsant effects may reflect actions of CBDV at TRPV1 channels.

The lowest CBDV dose we identified in the literature to be effective in an animal model of disease was 50 mg/kg IP which inhibited tonic convulsions induced by audiogenic seizures in rats.[9] As IP doses of CBDV achieve 1.6 times higher brain concentrations (the site of anticonvulsant drug action) than oral doses in rats,[3] the oral dose needed to achieve equivalent plasma levels would likely be around 80 mg/kg. Applying interspecies conversion calculations to this dose, the estimated human oral therapeutic dose in a 60 kg human would be approximately 774 mg.[2]

CBGA (Cannabigerolic acid)

CBGA is the precursor to both CBDA and THCA in the cannabis plant and is found at low levels in street cannabis. CBGA has never been administered to humans as a pure substance. It is unlikely to have intoxicating effects in humans given its lack of affinity at CB1Rs. However, appropriate preclinical and human studies would be required to rule this out definitively. CBGA has various pharmacological actions that may or may not be clinically relevant given the relatively high concentrations and doses needed to affect cell systems. In the absence of clinical evidence it is difficult to determine what constitutes a therapeutic dose of CBGA. Extrapolation from animals to humans is also not possible, as CBGA has never been tested in an in vivo animal study.

CBG (Cannabigerol)

Cannabigerol (CBG) is formed by non-enzymatic decarboxylation from CBGA.CBG has never been administered to a human as a pure substance. It binds to the CB1Rs only at relatively high concentrations and does not activate the receptor. It does not produce THC-like cannabimimetic effects in animals. It is therefore unlikely to have intoxicating effects in humans based on preclinical evidence.

CBG has various pharmacological actions that might be clinically relevant based on the effective concentrations and doses used in cell systems and animal studies respectively. Some effects have been shown at nanomolar concentrations such as α_2-adrenoceptor agonism and CB1R antagonism.

The lowest CBG dose we identified in the literature to be effective in an animal model of disease was 3 mg/kg IP which inhibited the size of colon tumors in a mouse xenograft model.[10] As IP phytocannabinoid doses achieve 60.9 times higher plasma concentrations than oral doses in mice,[3] the oral dose needed to achieve equivalent plasma levels might be as high as 183 mg. Applying interspecies conversion calculations to this dose, the estimated therapeutic oral dose in a 60 kg human would be approximately 892 mg[2].

CBN (Cannabinol)

CBN was the first cannabinoid to be isolated from the cannabis plant (in 1895) and many studies have examined its pharmacological activity. CBN may have mild psychoactivity when large quantities of the drug are administered intravenously to humans. This presumably reflects lower efficacy of CBN at CB1Rs than THC, despite its higher affinity. In the absence of good quality clinical studies, it is difficult to determine a therapeutic dose of CBN.

The lowest CBN dose we identified in the literature to be effective in an animal model of disease was 5 mg/kg which delayed the onset of symptoms in a mouse model of ALS when administered subcutaneously.[11] A subcutaneous phytocannabinoid dose would achieve on average 7 times higher tissue concentrations than oral doses in rodents,[3] the oral dose needed to achieve equivalent plasma levels would be around 35 mg/kg. Applying interspecies conversion calculations to this dose, the estimated therapeutic oral dose in a 60 kg human for this indication would be 171 mg[2].

In a recent analysis of hempseed oils, CBN was present in some preparations at a concentration approaching 10 mg/kg.[1] This would suggest that consuming 5 kg (5.5 L) of such hemp seed oil would be necessary to obtain a non-psychoactive dose of 50 mg CBN.

THCA (Delta-9-tetrahydrocannabinolic acid)

THCA is the chemical precursor of THC in the cannabis plant. THCA is formed from cannabigerolic acid (CBGA) by the enzyme THCA synthase. Heating cannabis plant material to around 160˚C causes the decarboxylation of THCA to THC by a non-enzymatic reaction. Overall the weight of evidence suggests that THCA is unlikely to have intoxicating effects in humans. This includes a single study in which THCA was administered to human participants.

The lowest THCA dose we identified in the literature to be effective in an animal model of disease was 0.05 mg/kg IP which had anti-nausea effects in rats.[12] As IP phytocannabinoid doses achieve on average 7 times higher tissue concentrations than oral doses in rodents,[3] the oral dose needed to achieve equivalent plasma levels would be around 0.35 mg/kg. Applying interspecies conversion calculations to this dose, the estimated therapeutic oral dose in a 60 kg human for this indication would be 3.5 mg[2].

It should be noted however that THCA is readily converted to THC by heating plant material. At 100˚ Celsius it is reported that 80% of THCA is converted to THC.[13] THCA is quite stable in the short term (24 hours) even at high temperatures such as 50˚C, although there is a relatively small but significant conversion to THC at room temperature (e.g. a 2% THC content can increase to 5.6% THC across a year of measurement). [13]

Thus THCA content should be measured in hemp food products and maintained at low levels given that it could be readily converted to THC via heating by knowledgeable consumers. Similar concentrations of THCA in seed, oil, and beverages might be adopted to that proposed for THC in FSANZ Application A1039.

THCVA (Delta-9-tetrahydrocannabivarinic acid)

THCVA is formed in the cannabis plant from cannabigerovarinic acid (CBGVA). There is extremely limited evidence available with which to form an opinion about the psychopharmacological, therapeutic and intoxicating actions of THCVA. THCVA has cytotoxic effects in prostate cancer cells at medium micromolar concentrations. Without more evidence it is impossible to estimate a human therapeutic dose.

THCV (Delta-9-tetrahydrocannabivarin)

THCV is the propyl homologue of THC differing only in its slightly shortened alkyl side chain. The literature on THCV is very limited, with only a handful of human studies of very limited quality. It is generally thought that THCV has antagonist effects at the CB1R which oppose the intoxicating effects of THC. This forms the basis of current interest in the appetite-suppressant effects of THCV as a treatment for obesity and metabolic disorders.

Nonetheless, there is an absence of good quality clinical evidence to determine a therapeutic dose of THCV. When administered to humans at a dose of 7 mg intravenously there were some mild to moderate subjective effects in a small number of participants that were approximately 25% the potency of THC. A more recent brain imaging study administering 10 mg oral THCV did not assess any therapeutic outcomes, although it did report no significant differences between the THCV dose and placebo on scores for mood, energy and affect.

The lowest THCV dose we identified in the literature to be effective in an animal model of disease was 0.25 mg/kg IP which reduced seizure severity in a rat model of epilepsy.[14] As IP doses of THCV achieve 5.4 times higher brain concentrations (the site of anticonvulsant drug action) than oral doses in rats,[3] the oral dose required would be around high 1.35 mg/kg. Applying the FDA calculation to this dose, the estimated therapeutic oral dose in a 60 kg human is 13 mg[2]. We list this dose as our estimated lowest therapeutic dose owing to the human studies listed not quantifying any therapeutic effect, although it is reassuring that the animal calculation closely matches the maximum dose recorded in humans (10 mg oral), which yielded no noticeable intoxication or mood effects.

Conclusions

This review demonstrates a paucity of good quality evidence for many of the phytocannabinoids. For most there are no published studies involving human administration, and therapeutic plasma levels in humans are undefined. Available studies are limited to in vitro cellular or in vivo rodent preclinical studies and some qualified inferences can be made from such data regarding possible therapeutic levels in humans. Such information provides some clues regarding effective therapeutic doses in humans, but extrapolation from these preclinical in vitro concentrations and in vivo rodent doses to humans can only be made with caution. Drawing conclusions on therapeutic oral doses in humans from animal studies has major limitations. What appears to be a potent therapeutic action in an animal may not translate to humans, or even if it does, the therapeutic doses achieved in humans may be radically different to those observed in animal studies.

There is reasonably good evidence of the therapeutic effects of CBD in humans at 800 mg (absolute dose). There is the possibility of mild sedation at such doses of CBD, although the current literature is ambiguous on this point with the balance being in favour of no sedative effects. There is no evidence of THC-like intoxication with CBD.

Evidence relating to potential therapeutic effects of the remaining phytocannabinoids mostly comes from preclinical studies involving cellular models and laboratory animals. Some evidence is available relating to effects arising from the consumption of THCA, THCV, CBDV, CBC and CBN in humans. In general, there is little evidence of intoxication with these phytocannabinoids following an oral route of administration. However, CBN and THCV may be mildly intoxicating following intravenous administration of relatively high doses. Only limited preclinical evidence is available for CBDA, THCVA, CBG and CBGA. None of these phytocannabinoids appear to have intoxicating properties although human studies are required to definitively rule this out.

The specific conclusions for setting limits in hemp-derived products are:

1. **A limit could be set for CBD** given that it has therapeutic effects in humans (lowest human therapeutic absolute dose is 800 mg)
2. **The same limit might also be set for CBDA** (i.e. 800 mg) given it is almost completely converted to CBD upon heating
3. **A limit could be set for THCA** identical to that already set by FSANZ for THC. THCA is almost completely converted to THC when it is heated and so might be heated by some consumers seeking intoxication
4. **At this stage limits may not be required for the remaining phytocannabinoids CBC, CBDV, CBN, CBGA, CBG, THCV and THCVA**. No strong evidence supports these compounds having intoxicating effects following oral administration. The evidence for therapeutic potential comes only from animal studies and so the estimated human doses calculated from animal studies may not be relevant to human consumption.

2 Introduction to the review

Cannabis sativa has been used for millennia for its myriad therapeutic effects. Following a few decades of prohibition, recent times have seen resurgent interest in the therapeutic potential of cannabis as well as the legalisation of medicinal cannabis in various countries.

This new interest has coincided with a dramatic increase in our knowledge of the cannabis plant, which is now known to contain more than 100 terpenophenolic compounds that are known as 'phytocannabinoids' (plant-derived cannabinoids).

The best-known phytocannabinoid is 'delta-9-tetrahydrocannabinol' (THC), which was first reported by Raphael Mechoulam in 1964. THC is the main psychoactive (or intoxicating) ingredient of cannabis, and produces its characteristic intoxicating effects primarily through an action at brain 'CB1 cannabinoid receptors' (CB1Rs).

THC is available in Australia in purified capsule form as an S8 medication (Dronabinol) for the relief of chemotherapy-induced nausea and vomiting. It is also available as an oromucosal spray (in combination with the non-psychoactive phytocannabinoid 'cannabidiol' (CBD) in the S8 medication nabiximols (Sativex). Nabiximols is currently approved in more than 25 countries, primarily for the relief of spasticity in multiple sclerosis.

Phytocannabinoids other than THC and CBD are generally not well characterised for their pharmacological effects. Few have been administered in purified form to humans under controlled conditions. Nonetheless, it is inferred from receptor binding characteristics, and various preclinical animal studies, that these phytocannabinoids lack the distinctive intoxicating and psychoactive effects of THC. Indeed, as early as 1970, Raphael Mechoulam had separated hashish into various purified cannabinoids (including CBG, CBC and CBN), reporting that only a THC-containing extract produced intoxicating like behavioural effects in monkeys (these included akinesia, apathy, reddening of the eyes, drowsiness and tameness).[15]

However, the non-intoxicating phytocannabinoids can have other pharmacological effects that may imbue them with therapeutic potential in disease states such as cancer, inflammation, pain and epilepsy. These include subtle actions on the endocannabinoid system of the brain and body via interactions with specific enzymes, as well as a range of actions at diverse receptor targets such as transient receptor potential (TRP) channels and serotonin (5-HT) receptors. These are outlined in detail in the current review.

In cannabis plants, the phytocannabinoids that tend to dominate are either THC and its acid precursor THCA (in Australian street cannabis these represent on average 15% of weight of flowering heads), or CBD and its acid precursor CBDA (which in industrial hemp can represent around 15% of weight of flowering heads). The other phytocannabinoids such as CBG, CBC, CBN and THCV are typically present in much lower quantities (0–0.5%), meaning that their pharmacological effects will tend to be of lesser significance in plant material, extracts or oils.

The phytocannabinoids of interest in the current review are shown in Table 1 alongside their typical concentration in (a) Australian street cannabis, and (b) industrial hemp.

Table 1: Phytocannabinoids of interest and their concentration in street cannabis and industrial hemp

	Abbreviation	Name	Australian Street Cannabis (%)[1]	Industrial Hemp (%)[2]
1.	THC	Delta-9-tetrahydrocannabinol	1.45	0.52
2.	THCA	Delta-9-tetrahydrocannabinolic acid	12.95	0.42
3.	THCV	Delta-9-tetrahydrocannabivarin	0	0
4.	THCVA	Delta-9-tetrahydrocannabivarinic acid	-	-
5.	CBD	Cannabidiol	0	3.56
6.	CBDA	Cannabidiolic acid	0.04	10.62
7.	CBDV	Cannabidivarin	-	0.35
8.	CBG	Cannabigerol	0.08	0.22
9.	CBGA	Cannabigerolic acid	0.13	0
10.	CBN	Cannabinol	0.03	0
11.	CBC	Cannabichromene	0.03	0.43

[1] Mean content of phytocannabinoids in police seized cannabis in NSW Australia as reported by Swift et al. (2013).

[2] Mean content of an industrial hemp cultivar grown by Australian company 'Ecofibre' in Kentucky (USA) and analysed by Bill Arnold at the University of Colarado (personal communication).

CB1 and CB2 cannabinoid receptors

As noted above, the distinctive intoxicating effects of THC are largely, and perhaps exclusively, due to the action of THC as a partial agonist at CB1 cannabinoid receptors (CB1Rs) in the brain.

When given to rats and mice, THC causes four distinctive behavioural and physiological changes that are commonly known as the 'tetrad'. These are (1) lowered body temperature (hypothermia), (2) analgesia, (3) an inhibition of locomotor activity (sedation), and (4) a characteristic waxy immobility known as 'catalepsy'. The CB1R dependence of these effects is shown by the ability of CB1R antagonist drugs to reverse these tetrad effects,[16] and the absence of such effects in mice genetically engineered to lack the CB1R.[17]

Additionally, a wide range of novel 'synthetic cannabinoid' drugs, such as those found in recreational products like 'Spice' and 'Kronik', are invariably found to be CB1R agonists, and have potent THC-like (sometimes called 'cannabimimetic') subjective effects in humans, and characteristic behavioural and physiological effects in the tetrad test battery in rodents.[18]

In 1992, a second cannabinoid receptor was reported, 'the CB2 receptor' (CB2R).[19] The CB2R is mostly found in the periphery in immune organs such as the spleen and thymus, and in white blood cells and macrophages. Although the CB2R is found in the brain, this often reflects induced expression on activated microglia following brain damage or neuroinflammation, with only sparse expression on neurons.[20] Although THC is an agonist at CB2Rs as well as CB1Rs, stimulation of the CB2R not lead to intoxication. A range of selective CB2R agonists have been developed and are under Phase 2 and Phase 3 human clinical trials in the treatment of pain and inflammation.[21]

In considering the pharmacological actions of drugs acting at CB1Rs and CB2Rs it is important to consider both 'affinity' and 'efficacy'.

Affinity refers to the extent to which a drug binds to a receptor, and is usually expressed as a drug concentration (nanomolar > micromolar > millimolar). A drug with high affinity at a receptor requires very low concentrations to competitively bind to that receptor, and this is expressed by pharmacologists in terms of it having 'nanomolar' or 'micromolar affinity'.

Efficacy refers to the extent to which a drug causes a functional effect in a cell: so a drug can bind to a receptor with nanomolar affinity while causing minimal biochemical changes within that cell (e.g. the firing of a neuron). This is referred to as 'low efficacy'. Antagonist drugs tend to have high affinity and no efficacy (they occupy the receptor without causing a functional effect) while agonists have high affinity and high efficacy.

THC is generally found to have nanomolar affinity but only moderate efficacy at both CB1Rs and CB2Rs and consequently is considered to be a 'partial agonist' at these receptors.

The endocannabinoid system

The human brain and body contain two major endocannabinoid substances that bind to CB1Rs and CB2Rs under natural conditions and in doing so influence a diverse range of physiological processes. These two ligands are the fatty acid amide substances '2-arachidonyl glycerol' (2-AG) and 'arachidonylethanolamide' (anandamide). When administered in high doses to laboratory animals both anandamide and 2-AG produce THC-like behavioural and physiological effects that are CB1R mediated.[22, 23]

Most phytocannabinoids are neither CB1R nor CB2R agonists, but some have modulatory effects on endogenous levels of 2-AG and anandamide and these might conceivably lead to indirect cannabis-like ('cannabimimetic') effects. For example, anandamide is broken down by the enzyme 'fatty acid amide hydrolase' (**FAAH**) and drugs that inhibit FAAH can increase endocannabinoid levels, sometimes causing subtle THC-like effects.[24] Similarly 2-AG is broken down by monoacylglycerol lipase (**MAGL**) such that inhibiting this enzyme causes 2-AG levels to rise and may also cause cannabimimetic effects.[25, 26]

Other important endocannabinoid related enzymes are diacylglycerol-alpha (**DAGLα**) and diacylglycerol-beta (**DAGLß**) that play key roles in the synthesis of 2-AG. The main enzyme involved in anandamide synthesis is N-acylphosphatidylethanolamine-phospholipase D (**NAPE-PLD**). Interfering with the synthetic enyzmes (DAGLα, DAGLß and NAPE-PLD) can reduce levels of endocannabinoids.

Thus, to better understand the pharmacological actions of phytocannabinoids, it is important to consider that some of them have indirect actions on the endocannabinoid system via FAAH, MAGL, DAGL and NAPE-PLD.

Transient Receptor Potential (TRP) Ion Channels

There is accumulating evidence that both endocannabinoids and phytocannabinoids exert important therapeutically relevant pharmacological actions upon targets other than CB1Rs, CB2Rs, and the endocannabinoid system.

An important set of targets are the transient receptor potential (TRP) ion channels, a total of 28 different types of which have been characterized in the brain and body.[27-29] These channels have an important and diverse range of functions, including sensing noxious and thermal stimuli, involvement in inflammatory, gustatory and gastrointestinal function, and many other physiological processes.

Some of the therapeutic effects of phytocannabinoids outlined in this review are thought to involve actions at these TRP channels, although evidence is still at a formative stage. Of particular importance is the ability

of endocannabinoids and phytocannabinoids to activate and desensitize some of these TRP channels. This action may be critical to their analgesic and anti-inflammatory therapeutic effects.

Accordingly, these actions are reported for each phytocannabinoid in the current review. The major TRP ion channels of interest are:

- **TRPA1.** This receptor plays an important role in pain caused by mechanical stress and perhaps noxious cold. The TRPA1 receptor is also sensitive to pungent stimuli such as mustard oil, wasabi, wintergreen and cinnamon. TRPA1 plays an important role in inflammatory processes
- **TRPV1.** This receptor plays a key role in sensing noxious heat and acidity. It is responsible for the burning sensation of chili peppers (capsaicin). Activation of TRPV1 leads to a painful, burning sensation and a compensatory reduction in body temperature. Overexpression and overactivation of TRPV1 is observed in various painful conditions
- **TRPV2.** This receptor is a homologue of TRPV1 and is similarly sensitive to high temperatures (> 52˚C)
- **TRPV3.** This receptor is activated by pleasant warm temperatures, but not by noxious heat. TRPV3 has been recently implicated in pruritic dermatitis and skin inflammation and is also widely expressed in the gastrointestinal tract
- **TRPV4.** This receptor plays a variety of roles in growth and development and in vascular and osmotic regulation. It is sensitive to a wide range of osmotic, mechanical and chemical cues
- **TRPM8.** This receptor is the primary sensor of cold in humans and has sensitivity to substances such as menthol, eucolyptol and geraniol. The activation of TRPM8 sends a cool and soothing sensation that alleviates pain.

Investigating the intoxicating effects of phytocannabinoids

As noted above, very few studies have administered individual phytocannabinoids to humans under controlled laboratory or clinical conditions.

Indeed, the current review could only find studies of acceptable quality that involved human administration of cannabidiol (CBD) and tetrahydrocannabivarin (THCV). In addition there were a few early human studies involving administration of cannabinol (CBN) or cannabichromene (CBC) and a single unpublished German study involving administration of terahydrocannabinolic acid (THCA). There were no published studies apparent in relation to, THCVA, CBG, CBGA, CBDA and CBDV. In cases where no human studies are available the possible intoxicating (or lack of) effects can be inferred from two principle sources. These are:

- **Tetrad effects in laboratory animals.** As noted above, when THC is administered to rats or mice it produces characteristic physiological and behavioural changes sometimes known as the 'tetrad'. These four key indicators are hypothermia, analgesia, sedation, and catalepsy. An absence of such effects at high doses of a phytocannabinoid strongly implies a lack of THC-like CB1R agonist or intoxicating effects
- **CB1 receptor affinity and efficacy.** Further evidence that a compound either possesses or lacks THC-like effects comes from the standard pharmacological method of 'competitive radioligand binding assays'. This tests whether a compound competes for CB1R binding in vitro against a known ligand for the CB1R such as THC, CP 55,940 or WIN 55,212-2, which is usually tagged with a radioactive label. If a substance fails to compete with THC at CB1Rs in this assay then it will be very unlikely to have THC-like psychoactive effects.

In interpreting the pharmacological actions of individual phytocannabinoids, it is also important to bear in mind several caveats that are described in detail below. These include:

1. possible additive and subtractive interactions between phytocannabinoids when present in a mixture;
2. the transformation of acid phytocannabinoids by heating, when cannabis plant material or extracts are smoked or vaporized; and
3. hazards in extrapolating from preclinical research outcomes in laboratory animals to humans.

Caveat 1: Interactions between phytocannabinoids

Cannabis plant material and cannabis extracts contain numerous different phytocannabinoids and there is emerging evidence of pharmacologically relevant interactions between these individual components, including supra-additive (synergistic) interactions, inhibitory interactions and a variety of other 'entourage effects'.[30, 31] Such effects are the subject of much speculation, particularly in considering whether therapeutic effects are best obtained with purified individual cannabinoids, or broad-spectrum 'artisanal' plant material and extracts.

For example, both CBD and CBG diminished the proliferation of human leukemia cells when applied individually to these cells.[32] However, the combination of a lower concentrations of each of these cannabinoids promoted a significantly greater anti-proliferative effect. Supra-additive effects were also observed in this study between CBD and CBDA, and CBGV and CBGA.

Alternatively, CBG may inhibit some of the therapeutic actions of CBD. For example, CBG was found to prevent the antiemetic actions on CBD in rats.[33] In this instance, a cannabinoid extract may then be sub-optimal as an antiemetic agent.

Perhaps the greatest attention in the literature has been focused on the interaction between THC and CBD. In humans there is evidence that consumption of cannabis containing CBD leads to fewer psychosis-inducing and memory-impairing effects than cannabis containing only high THC with no CBD. A number of recent studies support this contention.[30, 34-36]

Caveat 2: The effects of heat

It is also important to realise that heating cannabis plant material causes the conversion of acid phytocannabinoids to non-acid varieties. Thus, as can be seen in Table 1, street cannabis contains relatively low amounts of THC (mean = 1.45%) but large amounts of the non-psychoactive phytocannabinoid THCA (mean = 12.95%). Heating cannabis plant material above 160˚C causes the rapid conversion (decarboxylation) of THCA into THC. This is generally why cannabis plant material is smoked, vaporized or baked by recreational users in order to achieve psychoactive effects. If cannabis plant material is ingested without heating (e.g. 'juicing') then very different cannabinoid levels and physiological effects are achieved.

Similarly, heat causes the conversion of CBDA into CBD, and CBGA into CBG. Thus, it is important to consider not only the cannabinoid content of a given preparation, but the potential transformational effects of heating on phytocannabinoid profile.

Caveat 3: Extrapolation from preclinical concentrations/doses to therapeutic levels in humans

As noted above, for many of the phytocannabinoids considered in this review there are no published human studies involving their administration, and so therapeutic plasma levels in humans are undefined. For most of these phytocannabinoids, however, there are published studies involving in vitro cellular or in vivo rodent preclinical studies and some qualified inferences can be made from such data regarding possible therapeutic levels in humans. For in vitro (cellular) experiments, molar doses of phytocannabinoids are

usually presented where some therapeutic effect is observed, while with in vivo studies in laboratory animals doses are usually presented in terms of mg/kg.

So for example, it might be the case that a phytocannabinoid prevents the proliferation of cancer cells when applied to these cells at a 10 micromolar concentration, or it may cause analgesic effects in a mouse when injected intraperitoneally (IP) at a dose of 80 mg/kg. As outlined below, such information provides some clues regarding effective therapeutic doses in humans, but extrapolation from these preclinical in vitro concentrations and in vivo rodent doses to humans should only be made with caution.

Interpreting molar concentrations from in vitro studies

When interpreting concentrations from in vitro cellular studies, the lower the concentration at which a given effect occurs, the more likely that it will be clinically relevant in humans. In general, nanomolar (nM) to low micromolar (μM) concentrations are those that would be attained from the administration of a pure substance to a human. When a cellular effect requires much higher micromolar or millimolar concentrations of a cannabinoid, it is unlikely to be therapeutically relevant.

Accounting for differences between systemic and oral dosing in animal studies

Most preclinical studies involving laboratory animals administer phytocannabinoids intraperitoneally (IP), while the primary route of administration for hemp-derived products involves oral administration. It thus becomes important to consider how therapeutic effects obtained with IP dosing in rodents can be related to these same effects when a phytocannabinoid is given via oral dosing to humans. Oral administration of phytocannabinoids leads to 'first-pass metabolism' whereby the drugs are substantially metabolised by the liver prior to reaching the brain or other tissues. Oral administration of phytocannabinoids also leads to more variable and generally poorer absorption into the blood stream than IP injection. As a result, the oral route usually requires much higher doses to achieve equivalent tissue concentrations than IP injection.

A recent study directly compared the tissue concentrations achieved for the phytocannabinoids CBD, CBG, THCV and CBDV following oral and IP dosing in mice.[3] On average, IP dosing achieved 7 fold higher phytocannabinoid tissue concentrations than oral dosing. Therefore, in the current review, when considering possible therapeutic concentrations of uncharacterized phytocannabinoids, we have generally estimated the effective oral dose from animal studies by multiplying the effective IP dose by 7, except in a few cases nin which more specific and detailed information was available.

Extrapolating from rodent doses to a human therapeutic dose

An additional correction is necessary when extrapolating from doses in laboratory animal species (such as rats and mice) into humans. This 'interspecies scaling' factor primarily relates to differences in body surface area between species. The USA Food and Drug Administration (FDA) provides information on how to calculate the approximate human equivalent dose (HED) for doses given to different laboratory animals. This involves dividing the dose by 12.3 (mice), 6.2 (rat), 4.6 (guinea pig) or 3.1 (rabbit) to yield the human mg/kg dose.

So, for example, a 100 mg/kg dose in a rat would represent approximately (100/6.2) 16 mg/kg dose in a human. To then calculate the total human dose in mg, we would multiply this mg/kg dose by 60 (since the average weight of an adult in 60 kg).

So in the case above, the human equivalent dose (HED) for a 100 mg/kg dose in a rat would equal 16 mg/kg x 60 = approximately 1 gram. However, if the drug had been given to a rat using an IP route of administration then a multiplication factor would be applied (x 7) to account for oral administration, meaning that the human equivalent oral dose would become 7 grams.

3 Methodology: search strategy

To conduct the current review, the specified phytocannabinoids listed in Table 1 (above) were systematically reviewed according to their pharmacological and possible therapeutic effects with regard to the following therapeutic indication terms: Anti-inflammatory; Antibiotic; Anticonvulsant: Antifungal; Analgesic; Anxiolytic; Antipsychotic; Antioxidant; Antispasmodic; Antiemetic; Anti-ischemic; Anticancer; Antidiarrhoeal; Antibacterial; Antidepressant; Anti-Psoriasis/skin disorders; Anti-tussive, Anti-glaucoma; Antileishmanial; Euphoriant; Metabolic; Sedative.

Inclusion criteria

All included evidence sources had to be available in English language.

Literature search

1. The review included peer reviewed publications in academic databases including: Cochrane, PubMed and Google Scholar
2. Grey literature was included in the search (e.g. Government reports, policy statements and issue papers, conference proceedings, theses and dissertations, research reports, newsletters and bulletins, fact sheets). Grey literature searches used: Google, Google Scholar.

Search terms

Searches were conducted for the terms listed in publication titles, abstracts, keywords, and database subject headings. The search was conducted for the period up to December 2015. Terms within columns were combined usin**g** the Boolean operator 'OR', and the resulting strings will be combined using the Boolean operator 'AND'.

The titles and abstracts of all records obtained from database searches were examined, and full texts of all potentially relevant publications retrieved and examined to determine whether they hold relevant information. Additional relevant studies were identified from the reference lists of those studies meeting the inclusion criteria.

Quality assessment

Preclinical animal and cellular studies were labelled as such. The methodological quality of studies involving human cannabinoid administration meeting the inclusion criteria was assessed using the NHMRC criteria for levels of evidence as outlined below:

- **Level I:** Evidence obtained from a systematic review of all relevant randomised controlled trials
- **Level II:** Evidence obtained from at least one properly designed randomised controlled trial
- **Level III-1:** Evidence obtained from well-designed pseudo-randomised controlled trials (alternate allocation or some other method)
- **Level III-2:** Evidence obtained from comparative studies with concurrent controls and allocation not randomised (cohort studies), case control studies, or interrupted time series with a control group

- **Level III-3:** Evidence obtained from comparative studies with historical control, two or more single-arm studies, or interrupted time series without a parallel control group
- **Level IV:** Evidence obtained from case series, either post-test or pre-test and post-test.

Data extraction

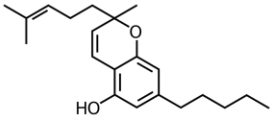
Where available, the following information was extracted from publications and tabulated prior to descriptive review:

1. Pharmacological characteristic (i.e. anti-inflammatory, anti-cancer etc.)
2. Therapeutic levels per pharmacological characteristic (dose of specified cannabinoid delivered in study)
3. Best level of evidence per pharmacological characteristic (i.e. in vitro assay, animal, human)
4. Relevance of source of evidence (i.e. detailed brief description of the major study design elements)
5. References (all papers reviewed are cited in the relevant table in the appendix for each cannabinoid reviewed)
6. Key research question addressed (primary aim or hypothesis of study).

Format of results

A descriptive review is given for each cannabinoid and relevant therapeutic action in the main section of the report, and the tabulated version of data extracted are provided in the Tables presented in the Appendix.

4 Individual cannabinoid summaries



CANNABICHROMENE (CBC)

Introduction

Cannabichromene (CBC) is a relatively common phytocannabinoid found in both street cannabis and industrial hemp. It was first isolated from hashish and described by Gaoni and Mechoulam in 1966.

CBC is produced in the cannabis plant from the precursor cannabigerol (CBG) via the action of the enzyme CBC synthase. The original report of Gaoni and Mechoulam (1966) said that *"when administered to a dog, CBC caused sedation and ataxia"*. However, a subsequent study failed to replicate this effect[37] Subsequent research has suggested that CBC is generally non-intoxicating and non-psychoactive.

Levels of CBC in Australian street cannabis were reported to be very low (0.06%) by Swift et al. (2013) while USA and UK studies have shown slightly higher levels of around 0.2–0.35%.[38, 39] In hashish and hash oil, levels of CBC are generally higher, at around 0.7% and 0.9%, respectively.[39]

Relevant pharmacological actions

CB1 and CB2 receptor affinity

CBC has relatively low affinity for the human CB1R (Ki = 713 μM) and CB2R (Ki = 256 μM).[40] Very low affinity for mouse CB1Rs was confirmed by Booker et al. (2009).[41] This implies a low likelihood of CBC producing intoxicating THC-like effects in humans.

Effects on the endocannabinoid system

CBC does not appear to affect either FAAH or MAGL, suggesting an absence of modulatory effects on endocannabinoid levels. However, CBC inhibits the cellular uptake of anandamide.[42]

Effects on TRP channels

CBC is a very potent agonist at TRPA1 channels (EC50 = 0.06 μM)[42, 43] and this has been linked to analgesic actions.[44] CBC has only weak actions at TRPM8 and TRPV1 channels.

Effects on other targets

There appear to be no relevant studies of CBC actions at other receptors.

Evidence for intoxicating and other behavioural effects

Evidence for intoxicating effects in humans and non-human primates

An early review by Turner et al. (1980)[45] refers to a publication by Isbell et al. (1967) where consumption of CBC was found to have no intoxicating effects (the original paper could not be sourced at this time). CBC did not have intoxicating effects in the Rhesus monkey when given at a low dose intravenously with CBG, CBN and cannabicyclol (CBL).[15]

Tetrad effects in rodents

Davis and Hatoum[46] showed that CBC (up to 75 mg/kg) caused a modest decrease in locomotor activity while Hatoum et al.[47] demonstrated hypothermic effects. More recently, El-Alfy et al.[48] found that CBC caused significant decreases in locomotor activity and body temperature at 80 mg/kg but did not cause any catalepsy. CBC was recently found to exert antinociceptive effects in the tail flick test via TRPA1 receptors.[44] In a comprehensive assessment, DeLong et al.[49] found that the highest dose of CBC tested (100 mg/kg, IV) produced all four tetrad effects in mice (catalepsy, hypothermia, reduced locomotor activity and analgesia). However, these effects were not reversed by a CB1R antagonist suggesting that CBC was working to produce such effects through a different receptor.

Therapeutic potential

Appendix Table 3 presents details of therapeutic potential for CBC.

Anti-inflammatory effects

CBC (1 mg/kg) displayed strong anti-inflammatory effects in a murine model of colitis. This was associated with a TRPA1 mediated reduction in nitric oxide production in peritoneal macrophages.[2] CBC also reduced inflammation following injection of lipopolysaccharide or carrageenan into the paws of mice[49, 50] or croton oil topically onto the ears of mice.[51] In this latter study the anti-inflammatory effects of CBC were less than indomethacin, a standard anti-inflammatory medication.

Analgesic effects

CBC (up to 75 mg/kg) had mild analgesic effects in mice and also potentiated the analgesic effects of THC.[46] Only a trend towards analgesic effects with CBC were seen in the tail flick test in one study with mice[48] but more convincing effects were obtained in another study when a higher intravenous dose of 100 mg/kg was used.[49] No antinociceptive effects of CBC were evident however in an acetic acid model of visceral pain in rats.[41]

Anticonvulsant effects

CBC (up to 75 mg/kg) had modest anticonvulsant effects in mice in the electroshock model.[46]

Antidepressant-like effects

CBC (20–80 mg/kg) had antidepressant-like effects in the mouse forced swim and tail suspension tests.[48]

Sedative effects

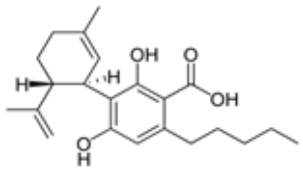
CBC was able to prolong the sleep time induced by the barbiturate hexobarbital in mice,[47] while (as noted above) several groups have shown reduction in locomotor activity in rodents with CBC, consistent with a sedative like effect.

Conclusions

There is little evidence for THC-like effects of CBC at CB1Rs, although there is evidence of sedation and catalepsy in laboratory animals at very high intravenous doses. CBC has a range of intriguing in vitro and in vivo actions in preclinical models that imply therapeutic efficacy as an anti-inflammatory agent and also perhaps as a mild sedative, analgesic and antibiotic. However, further confirmation in human studies is required.

The doses required for pharmacological effects are generally quite high with the exception of the effect on colitis in mice which required only 1 mg/kg IP.[2] As IP phytocannabinoid doses achieve on average 7 times higher tissue concentrations than oral doses in rodents [3], the oral dose needed to achieve equivalent plasma levels might be as high as 7 mg/kg in humans. Applying the FDA calculation to this dose, the estimated

therapeutic oral dose in a 60 kg human for CBC to produce anti-inflammatory effects would be 35 mg.[iii] To produce sedative, hypothermic or analgesic effects 20–80 fold higher doses are required, namely 0.7–3.0 grams. It is very implausible that such dose would be achieved with even excessive consumption of any current hemp seed or hemp oil preparations.



CANNABIDIOLIC ACID (CBDA)

Introduction

CBDA is the chemical precursor to CBD in the cannabis plant. CBDA is formed from cannabigerolic acid (CBGA) by the enzyme 'CBDA synthase'. It is then decarboxylated to CBD by a non-enzymatic reaction catalysed by heating. CBDA is found in Australian street cannabis at very low levels with only an average of 0.14% of the weight of cannabis. However industrial hemp may have much higher levels of CBDA (> 10%) as shown in Table 1 above.[52, 53] Very little research has been conducted on the pharmacological properties of CBDA, and it has not been administered to humans under controlled conditions, which limits the strength of our conclusions when evaluating its pharmacological activity and therapeutic actions.

Relevant pharmacological actions

CB1 and CB2 receptor affinity

CBDA binds to murine CB1Rs with low affinity (e.g. THC binds at low nM whereas CBDA bind at low µM).[54] CBDA does not activate the CB1R and thus appears to be a weak CB1R antagonist.[54] To the best of our knowledge effects of CBDA on CB2Rs have not been studied.

Effects on the endocannabinoid system

CBDA may reduce levels of endocannabinoids by inhibiting the 2-AG synthesizing enzyme DAGLα at concentrations around 20 µM.[42] CBDA also inhibits N-acylethanolamine acid amide hydrolase (NAAA) at 20 µM,[42] an enzyme that degrades N-palmitoylethanolamine (PEA). PEA is a lipid mediator that activates the PPAR-α receptor which plays an important role in energy balance and is vital for ketogenesis. CBDA does not inhibit the function of the anandamide degradative enzyme FAAH, the 2-AG degradative enzyme MAGL, and does not affect anandamide reuptake.[42]

Effects on TRP channels

CBDA also modulates various TRP channels at low to medium µM concentrations (1–20 µM) (TRPA1 agonist, TRPM8 antagonist, and weak TRPV1 and TRPV4 agonist).[42] It was ineffective in modulating TRPV2 and TRPV3 channels.

---

[3] Please refer back to Introduction to the Review section above under *"Caveat 3: Difficulties in extrapolation from preclinical concentrations/doses to therapeutic levels in humans"* for an explanation of the FDA-recommended animal to human dose calculation and its significant limitations in being able to predict a human therapeutic dose. There is high likelihood that potential therapeutic effects in in vitro and in vivo animal models may not translate. If they do translate, there is no certainty that the human oral doses will reflect animal doses. **We do not endorse setting limits for cannabinoids in hemp food products based on therapeutic doses that were estimated from animal studies.**

Effects on neurotransmitter receptors

CBDA influences other receptor targets as shown in various in vitro studies. However the functional and therapeutic significance of all these interactions remains to be demonstrated. CBDA potently potentiates the activity of the 5-HT_{1A} receptor (0.1–100 nM) which is a relevant target for emesis, depression, anxiety and pain.[54] CBDA blocks the GPR55 receptor at 1–10 μM. GPR55 is often viewed of as the third cannabinoid receptor.[55]

Evidence for intoxicating and other behavioural effects

Evidence for intoxicating effects in humans

CBDA to the best of our knowledge has never been administered to humans as a pure compound. A proper controlled psychopharmacological study is required to specifically confirm whether CBDA possesses or lacks psychoactive effects.

Tetrad effects in rodents

CBDA did not suppress locomotor activity in rats at doses up to 1 mg/kg,[4] although higher doses should be tested to further confirm an absence of cannabimimetic effects. Overall, it would seem unlikely that CBDA is psychoactive.

Therapeutic potential

Several preclinical studies have evaluated the therapeutic potential of CBDA (see Appendix Table 4) but there are no relevant human studies.

Anti-inflammatory effects

CBDA inhibits cyclooxygenase enzymes,[56] the targets of NSAIDs and aspirin. However there are conflicting findings pertaining to its potency and likely clinical relevance in this regard.[56, 57]

Antiemetic effects

Preclinical research shows CBDA has potent antiemetic actions. In shrews (*Suncus murinus*) CBDA potently inhibited vomiting induced by lithium chloride, the chemotherapeutic agent cisplatin and by motion at 0.1–0.5 mg/kg.[54] These effects appeared to be mediated by 5-HT_{1A} receptors rather than CB1Rs. In rats CBDA reduced lithium-induced conditioned gaping (a model of anticipatory nausea) at doses as low as 0.001 mg/kg.[4] Moreover, subthreshold doses of both CBDA and THCA interacted to significantly reduce lithium-induced conditioned gaping. A subthreshold dose of CBDA was also shown to potentiate the actions of the established antiemetics metoclopramide and ondansetron in this model.[58, 59]

Anticancer effects

CBDA inhibits human breast cancer cell migration in vitro and reduces the expression of genes involved in metastasis at doses as low as 5 μM.[60, 61] Interestingly, CBDA did not inhibit the proliferation of breast cancer cells, unlike the structurally similar phytocannabinoid CBD. However, the opposite was observed for migration whereby CBDA was effective and CBD was not. CBDA also inhibited human glioma cells and rat thyroid cancer cells in vitro at around 10–20 μM.[62]

Antibacterial effects

Drug resistance is a major issue in the treatment of bacterial infections and various phytocannabinoids are effective in inhibiting the growth of drug-resistant bacterial strains. CBDA has potent antibacterial activity at low μM concentrations against various drug-resistant strains of *Staphylococcus aureus*.[63]

Antidiarrhoeal effects

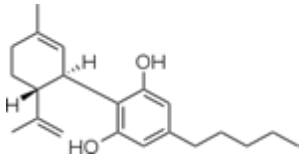
CBDA may have potential as an antidiarrhoeal agent: it inhibited contraction of shrew intestine at 1–30 μM concentrations.[64] These effects were not mediated by CB1 or CB2 receptors.

Conclusions

CBDA has never been administered to a human as a pure substance. However, the preclinical evidence described above suggests that CBDA is unlikely to have intoxicating effects in humans. CBDA only binds to CB1Rs at relatively high concentrations and does not activate the receptor. It does not appear to produce THC-like cannabimimetic effects in laboratory animals.

CBDA has various pharmacological actions that might be clinically relevant based on the effective concentrations and doses used in cell systems and animal studies respectively. Some effects have been shown at very low concentrations, including antiemetic effects and the potentiation of 5-HT_{1A} receptor activity.

The lowest CBDA dose we identified in the literature to be effective in an animal model of disease was 0.001 mg/kg (IP). This dose inhibited lithium-induced conditioned gaping (an animal model of anticipatory nausea) in rats.[4] As IP phytocannabinoid doses achieve on average 7 times higher tissue concentrations than oral doses in rodents,[3] the oral dose needed to achieve equivalent plasma levels might be 0.007 mg/kg. Applying the FDA calculation to this dose, the estimated therapeutic oral dose of CBDA for this anticipatory nausea therapeutic effect in a 60 kg human is approximately 0.07 mg.[iv]



CANNABIDIOL (CBD)

Introduction

Cannabidiol (CBD) is a phytocannabinoid that is present at a wide variety of concentrations across different cannabis strains.[38] In Australian street cannabis, levels of CBD were low to non-existent.[52] As noted in Table 1 above, CBD is more prevalent in industrial hemp cultivars (which have very low THC) and in hashish (which tends to have similar CBD and THC levels).[39] CBD is produced in the cannabis plant from the carboxylic acid precursor (CBDA) through a decarboxylation process involving heating. As with THCA, CBDA is produced from the common precursor CBGA by the enzyme 'cannabidiolic acid synthase'.

CBD is one of the best-studied cannabinoids in humans, a result of it exhibiting broad-spectrum therapeutic potential.[65-67]

Relevant pharmacological actions

CB1 and CB2 receptor affinity

CBD has low micromolar affinity for the CB1R and CB2R. Despite this low affinity, CBD has displayed a capacity in some studies to antagonise effects of CB1R and CB2R agonists at nanomolar concentrations.[68, 69] This may reflect negative allosteric modulation by CBD at the CB1R and CB2R.

[iv] Please refer back to Introduction to the Review section above under *"Caveat 3: Difficulties in extrapolation from preclinical concentrations/doses to therapeutic levels in humans"* for an explanation of the FDA-recommended animal to human dose calculation and its significant limitations in being able to predict a human therapeutic dose. There is high likelihood that potential therapeutic effects in in vitro and in vivo animal models may not translate. If they do translate, there is no certainty that the human oral doses will reflect animal doses. **We do not endorse setting limits for cannabinoids in hemp food products based on therapeutic doses that were estimated from animal studies.**

Effects on the endocannabinoid system

CBD can increase levels of endocannabinoids via inhibition of the anandamide degradative enzyme FAAH.[8] CBD can also inhibit the enzymes which break down PEA and 2-AG.[42] CBD also has been shown to inhibit AEA cellular uptake (with an IC50 around 25 μM).[42, 70] CBD does not appear to have any effect on MAGL and DAGL.[42]

Effects on TRP channels

CBD activates and desensitises TRPV1 channels in vitro, which may be relevant to its effects on epileptiform activity.[71] CBD also activates TRPA1, TRPV1–3, TRPV4 and blocks TRPM8 channels.[69, 72] The functional significance of these actions for pharmacological activity requires further investigation.[42]

Effects on other receptor systems

CBD is a neurochemically promiscuous compound and affects numerous other targets including enhancing 5-HT_{1A} receptor activation and PPAR-gamma receptors.[72, 73] There is some suggestive evidence of CBD being an allosteric modulator at mu and delta opioid receptors, D2 dopamine receptors and GABA-A receptors.[74] CBD also exerts a bidirectional effect on intracellular calcium levels, depending on the excitability of cells, by targeting mitochondria.[75]. CBD also antagonizes 5-HT3ARs, enhances alpha-3GlyR, and inhibits the Cav3 ion channel as well as adenosine reuptake.[72, 76]

Evidence for intoxicating and other behavioural effects

Evidence for intoxicating effects in humans

CBD is well tolerated across a wide range of doses in humans.[77-79] Acute CBD administration in humans by oral, inhalation, or intravenous routes does not induce any significant intoxicating and/or toxic effects.[80] CBD has been administered to humans in doses as high as 1500 mg (orally)[81] or 30 mg (intravenously) with no reported adverse events. CBD is reported to have been safe and well tolerated with both acute and chronic administration to healthy subjects.[82, 83] Chronic administration of CBD for 30 days to healthy volunteers, at daily oral doses ranging from 10–400 mg, did not induce any significant alteration in neurological, psychiatric or clinical measures.[84]

Limited safety data exist for long-term administration of pure CBD in humans, although there have been many patient-years of exposure to nabiximols (Sativex) (which contains both CBD and THC) which is an approved medication in more than 25 different countries. CBD is currently in Phase 3 clinical trials in the USA (Epidiolex) for the treatment of intractable pediatric epilepsy, and a variety of other CBD-rich plant extracts are being used worldwide for the same indication.

Tetrad effects in rodents

CBD does not induce classic tetrad effects of hypolocomotion, analgesia, catalepsy and hypothermia in mice, although it did produce mild hypothermia at higher doses.[48, 85]

Other considerations

The main area of caution with the use of CBD in humans relates to its inhibitory effects on several cytochrome P450 isoenzymes, including CYP1A2, CYP2B6, CYP2C9, CYP2D6, and CYP3A4. This is especially important in the management of chronic pain and epilepsy, since conventionally used analgesics (opioids and non-opioids) and anticonvulsants are sometimes metabolised via these pathways (e.g. CYP2D6 and CYP3A4).[86] There is also a theoretical risk of immunosuppression from CBD as it suppresses interleukin 8 and 10 production and induces lymphocyte apoptosis in vitro, but this has not been demonstrated clinically.[87, 88]

Therapeutic potential

CBD is a major focus of current medicinal cannabinoid research. There are at least 59 currently active clinical trials of CBD listed on clinicaltrials.gov for indications including schizophrenia, inflammatory bowel disease, pediatric epilepsy, addiction, chronic pain, cancer, Huntington's Disease, Multiple Sclerosis, diabetes, nausea and vomiting, bipolar disorder and ADHD. Of all the cannabinoids reviewed here CBD has the largest number of level II RCTs in clinical and healthy populations and a robust preclinical literature (Appendix Table 5).

Anxiolytic effects

CBD exhibits anxiolytic effects in humans. A double blind RCT involving 24 social anxiety patients demonstrated reductions in anxiety with 600 mg CBD (orally) administered prior to delivering an anxiety-provoking public speech.[89] A smaller study with 10 social anxiety patients undergoing SPECT imaging showed that CBD reduced anxiety-related brain activity.[90]

Antipsychotic effects

A phase II double blind RCT administered 800 mg CBD/day versus an amisulpride (standard antipsychotic medication) active control to 42 hospitalised schizophrenic patients and found therapeutically relevant improvement in PANSS scores in both groups indicating therapeutic equivalence of CBD. CBD exhibited far fewer side effects (e.g. extrapyramidal symptoms) than amisulpride.[8]

Sedative effects

Of the 20 papers identified where CBD had been given to humans, 8 showed no signs of sedation, and 5 studies reported some form of sedation, somnolence or extended sleep duration. Sedation was either not measured or not discussed in the remaining 7 human studies.

Looking in more detail at the 5 papers reporting some form of sedation, one administered pure CBD at a range of doses between 10 and 600 mg to a small number of healthy volunteers across a number of poorly designed phase I experiments,[91] with a minority of subjects self reporting somnolence (in 3 of 5 experiments). The same paper also reports a clinical trial of CBD (40, 80 or 160 mg) as a hypnotic medication compared to nitrazepam (5 mg) or placebo using a within-subjects design over 5 weeks, in 15 participants (family members of the investigator) self-reporting poor sleep. The study found that 160 mg CBD resulted in several (but not all) measures on a self-made sleep quality questionnaire being significantly improved, including longer sleep duration than placebo or 5 mg nitrazepam.[91] However sleep duration might not equate with sedation per se.

Another recent paper, this time with no control group, reported somnolence in 25% of treatment resistant epileptic children[92] although the lack of control group and the use of concomitant anti-epileptic drugs, including clobazam which is sleep-inducing, renders this finding unusable in drawing firm conclusions on the sedation issue.

An experimental model of impaired perception during psychotic states was tested with 200 mg CBD in 9 healthy male volunteers and reported sedative effects in the text but with no details of how sedation was measured, and no data.[7]

A small study of the effects of 300 (n=7) and 600 (n=4) mg CBD on plasma prolactin in healthy human volunteers recorded a sedative effect on a self evaluation scale.[6]

Finally 400 mg oral CBD was given to 10 healthy male volunteers in a SPECT imaging study of the effects of CBD on cerebral blood flow, reporting increases in mental sedation measured using the Visual Analogue Mood Scale.[5]

Anticonvulsant effects

CBD displays anticonvulsant effects across different preclinical epilepsy models.[93-96] In the maximal electroshock model of epilepsy in mice CBD (120 mg/kg IP) exhibited therapeutically relevant anticonvulsant effects.[97, 98] Similarly, sezures were suppressed at 100 mg/kg (IP) CBD in a rat PTZ epilepsy model[96] and at 1, 10, and 100 mg/kg (IP) in a rat pilocarpine induced seizure model.[95] In that same study a rat penicillin seizure model demonstrated improvements in mortality due to seizures, and fewer tonic-clonic seizures with >/= 10 mg/kg (IP) CBD.[96] In mice PTZ and MES models of epileptic activity seizures were suppressed at 200 ng CBD (intracerebroventricular; ICV) and at 20–200 ng CBD (ICV) respectively.[94] CBD at 50 mg/kg (IP) also suppressed seizures in a chronic administration model of PTZ induced seizures where rats were administered PTZ daily for 28 days.[93]

In humans, a 2014 Cochrane review on CBD for epilepsy identified four RCTs (with a total of 48 patients) being administered 200–300 mg CBD per day for 1–3 months.[99] The effects on seizure frequency were mixed and the review deemed the studies were of insufficient quality to draw conclusions about the efficacy of CBD for epilepsy at this time. A proprietary oral formulation of CBD (Epidiolex) is currently in testing in the USA for the treatment of pediatric epilepsy with ongoing blinded placebo controlled trials in progress. One report from an open label uncontrolled dose ranging study did find that daily doses of CBD titrated up to an average of 22.9 mg/kg resulted in a median reduction in the weekly rate of convulsive seizures of 34.6% across multiple drug-resistant epilepsy syndromes and seizure types in treatment resistant epileptic children.[92]

Analgesic effects

Daily oral treatment with CBD (2.5–20 mg/kg) reduced hyperalgesia to thermal and mechanical stimuli in rat models of neuropathic pain (sciatic nerve constriction) and a rat inflammatory pain model (complete Freund's adjuvant intraplantar injections).[100] CBD (3 nmol) reduced the ongoing activity of ON and OFF neurons and induced antinociceptive responses in the tail flick-test measured by extracellular electrical activity of ON and OFF neurons of the rostral ventromedial medulla in anaesthetised rats.[44] CBD (2.5–10 mg/kg IP also prevented chemotherapy induced mechanical sensitivity in mice, an effect that was reversed by a 5-HT_{1A} antagonist, but not by CB1R or CB2R antagonists.[101]. In humans, a double blind RCT in 24 patients with a neurological pain condition demonstrated therapeutically relevant pain relief at an average of 24 mg CBD per day delivered as a sublingual spray.[102] However the study is confounded by the use of a 'CBD rich plant extract' leaving room for uncertainty as to the presence of other cannabinoids beside CBD.

Anti-inflammatory effects

There is preclinical evidence for anti-inflammatory effects of CBD in the 1–30 mg/kg range. Preclinical studies also suggest efficacy of CBD in inflammatory bowel disease.[103-105]

Antioxidant

CBD in the 2–4 µM range was an effective antioxidant in rat cortical neuron cultures exposed to toxic levels of glutamate.[106] CBD in the 5–10 mg/kg range decreased cellular markers of oxidative stress in several mouse models including a chemically-induced oxidative stress[107] and an alcohol-induced stenosis model.[108]

Anti-ischaemic effects

There is substantial and convincing preclinical work showing anti-ischaemic effects of CBD, particularly with neonatal animals and the hypoxic ischaemia encephalopathy (HIE) models where CBD is effective in the 0.1–5 mg/kg range. This work has progressed with considerable success in neonatal piglets.[109, 110] Human clinical trials in this area are imminent.

Anticancer effects

There is abundant preclinical in vitro work examining the antiproliferative properties of CBD in a range of cancers, including Kaposi sarcoma,[111] breast cancer,[112] lung cancer,[113] bladder cancer,[114] glioblastoma,[115-117] leukemia,[118] and colon cancer.[119] In cellular models, CBD induces cell death in the low micromolar range (0.25–2 μM), although higher doses are required for some cancers.

Antispasmodic

CBD has been extensively trialed in humans in combination with THC (in the form of Nabiximols) for spasticity in Multiple Sclerosis (MS). However, only a single study has trialed CBD alone (700 mg/day oral for 6 weeks) as an antispasmodic, but no reductions in chorea severity were observed. Mouse models of MS have demonstrated significant efficacy of CBD as an antispasmodic in the 5 mg/kg range with CBD ameliorating signs of autoimmune encephalomyelitis[120] and motor deficits in the chronic phase of the disease.

Antiemetic

CBD at 20 mg/kg suppressed nicotine-, lithium chloride (LiCl)- and Cisplatin-induced vomiting in the asian house shrew *S. murinus*, as well as lithium chloride-induced conditioned gaping in rats. However CBD was ineffective at a higher dose (40 mg/kg).[121] Similar dose response profiles have been observed previously in the house musk shrew,[122], with 5 mg/kg inhibiting vomiting but 40 mg/kg CBD inducing vomiting.[123]

Antidepressant

CBD had significant antidepressant-like effects at 30 mg/kg (IP) in the forced swim and tail suspension tests in mice (standard preclinical models of antidepressant effects). CBD was ineffective at doses of 3, 10 and 100 mg/kg.[124] Significant antidepressant-like effects were obtained in other study with CBD at 200 mg/kg IP.[48]

Anti-psoriasis/skin disorders

In vitro assays have started to explore the effects of CBD on skin cell growth and proliferation, with therapeutically relevant effects found in the 0.5–10 μM range. CBD inhibited the growth of cultured human sebocytes and human skin organ culture in the 1–10 μM range, suggesting a possible acne treatment.[125]

Conclusions

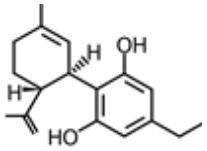
CBD has numerous pharmacological actions and is already showing strong clinical promise in the treatment of epilepsy, anxiety and psychosis. Complications may arise with pharmacokinetic interactions when CBD is combined with other medications such as opioids or anticonvulsants.

CBD appears to lack significant intoxicating effects in animals or humans. While some studies suggest mild sedative effects at doses ranging from 200–800 mg,[5-7, 92] the majority of studies do not, and a lack of sedative effects has been reported even at doses as high as 1280 mg. Further studies are required to verify the nature, reliability and severity of the putative sedative effects obtained with CBD.

Across all of the human studies performed with CBD, the lowest dose at which a therapeutically relevant effect was observed in a double blind RCT with an adequate control group (amisulpride) in a clinical population was 800 mg/day (oral) in the control of symptoms of schizophrenia.[8] Please note though that this study is a Phase 2 RCT and thus cannot provide sufficient evidence for CBD to be formally approved as a therapeutic agent. Larger scale Phase 3 RCT trials are necessary for this, some of which are being conducted now with results forthcoming in 2016. We therefore conclude that a human oral dose of 800 mg of CBD constitutes a threshold therapeutic dose based on the best available evidence. This dose estimation might be subject to change when results of Phase 3 trials become available. The therapeutic dose of 800 mg would be reached easily with oral dosing of a high CBD concentration product such as Elixinol, which contains 180 mg/ml CBD.

While there are other reports of CBD administration to humans achieving a therapeutic response at lower doses in clinical populations, all of those studies had methodological flaws leading to ambiguity in interpretation and a chance of bias (i.e. no control group and variable dose uncertainty,[92] uncertain timing of outcome measurement,[84] use of 'CBD rich' plant extract of unknown purity,[102] or a very small sample size and subjective outcomes).[90] Additionally it should be noted that it is our opinion that across the full range of human studies administering CBD (including healthy human subjects) there has been inadequate assessment of dose response for any particular indication, and that it is imperitive that this work be carried out.

CANNABIDIVARIN (CBDV)



Introduction

Cannabidivarin (CBDV) is the propyl homologue of cannabidiol (CBD), differing only in the length of its alkyl side chain (CBD has a pentyl side chain while CBDV has a shorter propyl slide chain). CBDV was first discovered in hashish by Vollner and colleagues in 1969.[126]

CBDV is generally present in quite low levels in cannabis plant material. However, CBDV was not examined in the analysis of Australian street cannabis by Swift et al.,[52] the analysis of UK cannabis by Potter et al.[38] or the analysis of USA cannabis/hashish by Mehmedic et al.[39]

Recent therapeutic interest in CBDV has centred around its anticonvulsant effects that have been widely established in animal models. GW Pharmaceuticals currently have a Phase 2A clinical trial of CBDV (known as GWP42006) underway in the USA examining its safety and efficacy for the treatment of focal seizures in adult humans as well as its pharmacokinetic profile. GW Pharmaceuticals have also developed a CBDV enriched botanical extract known as CBDV-BDS (botanical drug substance). Despite this, there are no available published studies of CBDV effects in humans.

Relevant pharmacological actions

CB1 and CB2 receptor affinity

CBDV has very low affinity for human CB1R (Ki=14,711 μM) and CB2R (Ki=574 μM).[40] Similarly, Hill et al. (2013) showed very low affinity for CBDV at human CB1Rs.[127] This indicates a very low likelihood of CBDV producing THC-like intoxicating effects in humans.

Effects on the endocannabinoid system

CBDV strongly inhibits DAGLα, which may cause reduced synthesis of the endocannabinoid 2-AG. CBDV does not appear to affect either FAAH or MAGL, suggesting no likely effect on endocannabinoid enzymatic breakdown. CBDV inhibits the cellular uptake of anandamide.[42]

Effects on TRP channels

CBDV is an agonist at TRPA1 channels (EC50 = 0.42 μM), TRPV1 channels (EC50 = 3.6 μM)[42] and TRPV4 channels (EC50 = 0.9 μM).[128] It also has antagonist effects at TRPM8 channels (IC50 = 0.9 μM). Following initial stimulation, CBDV elicits rapid desensitization of TRPV1, TRPV2 and TRPA1 channels.[71]

Effects on other targets

There appear to be no relevant studies of CBDV actions at other receptors.

Evidence for intoxicating and other behavioural effects

Evidence for intoxicating effects in humans

To our knowledge there are no published studies of CBDV effects in humans, although the ongoing Phase 2 clinical trials involving CBDV implies that the compound lacks obvious intoxicating effects.

Tetrad effects in rodents

To our knowledge there are no reports of CBDV effects on the rodent tetrad battery. CBDV (50–200 mg/kg) had no effects in the beam test of motor co-ordination and the grip strength test of muscle relaxation in rodents.[9]

Therapeutic potential

Appendix Table 6 presents details of therapeutic effects relevant to CBDV.

Anti-inflammatory effects

CBDV reduced the inflammation caused by topical administration of croton oil onto the ears of mice, albeit to a lesser extent than indomethacin.[51]

Anticonvulsant effects

CBDV (50–200 mg/kg)had significant anticonvulsant effects on the electroshock (100 mg/kg), audiogenic (50 mg/kg) and pentylenetetrazole-induced seizure models in rodents (100 mg/kg).[9] CBDV also reduced the duration of epileptiform-like burst firing in hippocampal neurons.[71] The cannabis extract that is enriched in CBDV (CBDV-BDS) also had powerful anticonvulsant effects in rodents.[127]

Antiemetic effects

CBDV at a dose of 200 mg/kg reduced gaping in rats to a saccharin solution that had been paired with the emetic agent lithium chloride.[129] This implies an anti-nausea effect of CBDV.

Conclusions

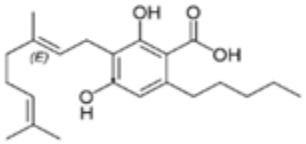
CBDV is clearly of major therapeutic interest as an anticonvulsant, given its current investigation in human clinical trials. Although there has been comparatively little work done with CBDV, there is nothing in its profile to suggest CB1 affinity or intoxicating effects. Evidence for inflammatory and antiemetic actions are preliminary and reflect single preclinical studies. Anticonvulsant effects may reflect actions of CBDV at TRPV1 channels.

The lowest CBDV dose we identified in the literature to be effective in an animal model of disease was 50 mg/kg IP which inhibited tonic convulsions induced by audiogenic seizures in rats.[9] As IP doses of CBDV achieve 1.6 times higher brain concentrations (the site of anticonvulsant drug action) than oral doses in rats,[3] the oral dose needed to achieve equivalent plasma levels might be as high as 80 mg/kg. Applying the FDA calculation to this dose, the estimated human oral therapeutic dose in a 60 kg human is 774 mg.[v]

---

[v] Please refer back to Introduction to the Review section above under *"Caveat 3: Difficulties in extrapolation from preclinical concentrations/doses to therapeutic levels in humans"* for an explanation of the FDA-recommended animal to human dose calculation and its significant limitations in being able to predict a human therapeutic dose. There is high likelihood that potential therapeutic effects in in vitro and in vivo animal models may not translate. If they do translate, there is no certainty that the human oral doses will reflect animal doses. **We do not endorse setting limits for cannabinoids in hemp food products based on therapeutic doses that were estimated from animal studies.**

CANNABIGEROLIC ACID (CBGA)



Introduction

Cannabigerolic acid (CBGA) is the precursor to both THCA and CBDA in the cannabis plant. It is found in street cannabis at very low levels: on average 0.28% of the total weight of cannabis.[52] CBGA comes in A and B forms however most scientific papers do not specify which they are using. Very little research has been conducted on the pharmacological properties of CBGA which seriously limits the strength of the conclusions that can be reached regarding its pharmacological activity and therapeutic potential.

Relevant pharmacological actions

CB1 and CB2 receptor affinity

CBGA does not appear to have affinity at CB1Rs: however the actual data supporting this conclusion were not provided.[130] To the best of our knowledge CBGA actions on CB2Rs have not been studied.

Effects on the endocannabinoid system

CBGA may reduce levels of the endocannabinoid 2-AG by inhibiting the 2-AG synthesizing enzyme DAGLα, but this occurs at relatively high concentrations of CBGA (30 μM).[42] CBGA did not inhibit the function of the anandamide degradative enzyme FAAH, the 2-AG degradative enzyme MAGL, or affect anandamide reuptake.[42]

Effects on TRP channels

CBGA affects various protein targets in vitro: however the functional and therapeutic significance of these effects remains to be demonstrated. CBGA modulates various TRP channels at low to medium μM concentrations (1–10 μM) (TRPA1 agonist, TRPM8 antagonist).[42] It also blocks TRPV1 (20 μM), TRPV3 (13 μM) and TRPV4 (30 μM) at higher concentrations that may not be clinically relevant.[42, 128]

Evidence for intoxicating and other behavioural effects

Evidence for intoxicating effects in humans

CBGA to the best of our knowledge has never been administered to humans as a pure compound. A proper controlled psychopharmacological study is required to specifically confirm whether CBGA has psychoactive effects.

Tetrad effects in rodents

CBGA has not been tested in the cannabinoid tetrad in rodents.

Therapeutic potential

Several preclinical studies that have evaluated the therapeutic potential of CBGA (see Appendix Table 7).

Anti-inflammatory effects

CBGA inhibits cyclooxygenase enzymes in vitro,[56] but only at relatively high concentrations that are unlikely to be clinically relevant.

Anticancer effects

CBGA inhibited human leukaemia cell proliferation, albeit at relatively high concentrations.[32]

Antibacterial effects

CBGA has potent antibacterial activity at low μM concentrations against various drug-resistant strains of *Staphylococcus aureus*.[63]

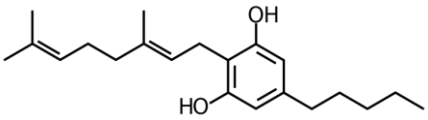
Anti-leishmanial effects

CBGA has antileishmanial properties, killing this parasitic protozoan at μM concentrations.[131]

Conclusions

CBGA has never been administered to humans as a pure substance. It is unlikely to have intoxicating effects in humans given its lack of affinity at CB1Rs. However studies testing in the rodent tetrad and in humans are needed to rule this out definitively. It has various pharmacological actions that may or may not be clinically relevant given the relatively high effective concentrations and doses needed to affect cell systems. In the absence of clinical evidence it is difficult to determine what constitutes a therapeutic dose of CBGA. Extrapolation from animals to humans is also not possible, as CBGA has never been tested in an in vivo animal study.

CANNABIGEROL (CBG)



Introduction

Cannabigerol (CBG) is formed by non-enzymatic decarboxylation from CBGA. It is found in Australian street cannabis at low levels with on average 0.93% of the weight of cannabis, although some plants had up to 15% CBG.[41]

Relevant pharmacological actions

CB1 and CB2 receptor affinity

CBG binds to human CB1Rs with relatively low affinity (e.g. THC binds at low nM whereas CBG bind at high nM; it also binds CB2Rs).[40] CBG does not appear to be effective in mobilizing G-protein that is necessary to activate CB1Rs, and thus behaves as a CB1R and CB2R antagonist in the submicromolar range.[132]

Effects on the endocannabinoid system

CBG does not inhibit the function of the anandamide degradative enzyme FAAH but inhibited anandamide uptake at 11 μM.[42, 128] CBG inhibits the 2-AG degradative enzyme MAGL at high μM concentrations that are unlikely to be clinically relevant.[42]

Effects on TRP channels

CBG modulates various TRP channels at the submicromolar range. It activates TRPA1 at 700 nM and antagonises TRPM8 at 160 nM.[42] It also activates TRPV1-4 in the 1–10 μM range.

Effects on neurotransmitter receptors

CBG is a highly potent α_2-adrenoceptor agonist, in mouse brain and mouse vas deferens, activating this receptor at 0.2 nM and 73 nM respectively.[132] It also antagonizes 5-HT_{1A} receptors at 1μM.[132] CBG also inhibits the synaptic uptake of noradrenaline, 5-HT and GABA at 50–67 μM, however these concentrations are very high and unlikely to be clinically relevant.[133]

Eicosanoid enzymes

CBG stimulated phospholipase A2 at 10 μM,[134] an enzyme that converts phospholipids or diacylglycerol into arachidonic acid, a precursor to the production of endocannabinoids and also eicosanoids such as prostaglandin, leukotrienes and thromboxanes. It also inhibited lipoxygenase in the 1–10 μM range, an enzyme involved in the production of leukotrienes from arachidonic acid.[135]

Evidence for intoxicating and other behavioural effects

Evidence for intoxicating effects in humans

CBG to the best of our knowledge has never been administered to humans as a pure compound. A proper controlled psychopharmacological study is required to specifically confirm whether CBG lacks psychoactive effects.

Tetrad effects in rodents

CBG did not exhibit THC-like activity in mice, rats, gerbils and non-human primates, consistent with it lacking psychoactivity.[15, 136] Moreover, CBG was without effect up to 80 mg/kg in the mouse tetrad test of cannabimimetic activity (locomotor suppression, catalepsy, hypothermia and analgesia).[48] Another classic effect of THC is promoting conjunctival erythema, an effect that was not observed with CBG administration in cats.[137] Taken together it would seem highly unlikely that CBG has pronounced intoxicating or psychoactive effects.

Therapeutic potential

Several preclinical studies have evaluated the therapeutic potential of CBG (see Appendix Table 8) but there are no relevant studies in humans.

Anti-inflammatory effects

There is preclinical evidence for anti-inflammatory properties of CBG. CBG has beneficial actions in a mouse model of colitis, where it reduced the concentrations of various inflammatory markers such as cytokines and interleukin-1β (IL-1β).[138] The anti-inflammatory effects of CBG were also observed in peritoneal macrophages at low μM concentrations. CBG inhibited cyclooxygenase 2, however at a concentration that is not likely to be clinically relevant.[56]

Antioxidant effects

There is in vitro evidence that CBG is an antioxidant in colon cancer cells.[138] CBG had a subtle, low efficacy, prooxidant effect as evidenced by a reduction in the levels of glutathione, an antioxidant, in mouse primary cultured dopamine brain cells. However, this did not translate into any loss of viability in the cells, questioning the overall physiological significance of this observation.[40]

Antibacterial effects

CBG inhibited the growth of bacterial strains that are resistant to drug treatment, with potent antibacterial activity at low μM concentrations against various drug-resistant strains of *Staphylococcus aureus* [63] and also *Mycobacterium intracellular* in vitro.[131]

Anti-psoriasis/skin disorders

The endocannabinoid system regulates skin physiology and all major components of the endocannabinoid system are found in human epidermis, the outmost layer of the skin.[139, 140] CBG potently inhibited the proliferation and differentiation of keratinocytes in vitro, a major cellular component of the epidermis. Keratinocytes contain keratin, a fibrous structural protein that provides strength and flexibility to the skin.[139, 140] These results suggest that topical applications of cannabinoid products for skin disorders such as psoriasis might be justified, but this would require further preclinical and clinical examination.

Anticancer effects

CBG reduces the proliferation of various cancer cells in vitro including human leukaemia, breast, prostate, glioma and neuroblastoma cells at μM concentrations.[10, 32, 40, 62, 141] One of the more promising applications is colorectal cancer, where CBG reduced the proliferation of cancer cells in vitro, but also reduced the size of

human colorectal cancer tumours grafted onto mice in vivo [10]. These studies have yet to be translated into the clinic.

Huntington's Disease

Repeated exposure to CBG reduced the motor dysfunction and neuronal cell loss observed in toxin-induced and genetic mouse models of Huntington's disease. These beneficial neuroprotective effects were associated with reductions in markers of neuroinflammation and oxidative stress.[142].

Bladder dysfunction

CBG potently inhibited chemical-induced contractions of the mouse and human bladder in organ bath preparations at 10 nM.[143] The therapeutic significance of this finding remains to be determined.

Antiglaucoma

CBG potently inhibited intraocular pressure in cats, suggesting utility as an antiglaucoma agent.[137]

Conclusions

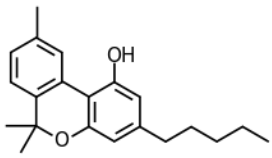
CBG has never been administered to a human as a pure substance. It binds to the CB1Rs only at relatively high concentrations and does not activate the receptor. It does not produce THC-like cannabimimetic effects in animals. It is therefore unlikely to have intoxicating effects in humans based on preclinical evidence.

CBG has various pharmacological actions that might be clinically relevant based on the effective concentrations and doses used in cell systems and animal studies respectively. Some effects have been shown at nanomolar concentrations such as α_2-adrenoceptor agonism and CB1R antagonism.

The lowest CBG dose we identified in the literature to be effective in an animal model of disease was 3 mg/kg IP which inhibited the size of colon tumors in a mouse xenograft model.[10] As IP phytocannabinoid doses achieve 60.9 times higher plasma concentrations than oral doses in mice,[3] the oral dose needed to achieve equivalent plasma levels might be as high as 183 mg. Applying the FDA calculation to this dose, the estimated therapeutic oral dose in a 60 kg human would be 892 mg.[vi]

[vi] Please refer back to Introduction to the Review section above under *"Caveat 3: Difficulties in extrapolation from preclinical concentrations/doses to therapeutic levels in humans"* for an explanation of the FDA-recommended animal to human dose calculation and its significant limitations in being able to predict a human therapeutic dose. There is high likelihood that potential therapeutic effects in in vitro and in vivo animal models may not translate. If they do translate, there is no certainty that the human oral doses will reflect animal doses. **We do not endorse setting limits for cannabinoids in hemp food products based on therapeutic doses that were estimated from animal studies.**

CANNABINOL (CBN)



Introduction

Cannabinol (CBN) was the first cannabinoid isolated from the cannabis plant in 1895, and as a result of this long history there are many studies that have examined its pharmacological activity.[144] CBN is mostly formed through the oxidation of THC, and is found in trace amounts in street cannabis in Australia constituting on average 0.09% of the plant.[52] If cannabis plant material is stored longer without being consumed, particularly at room temperature, so CBN levels increase.

Relevant pharmacological actions

CB1 and CB2 receptor affinity

CBN binds CB1 and CB2 receptors at high nanomolar concentrations, significantly higher than THC which binds at the low nanomolar range.[145] Like THC, CBN is a partial agonist at the CB1 receptor.[146]

Effects on the endocannabinoid system

CBN does not appear to modulate endocannabinoid enzymes such as DAGLα, MAGL and FAAH. Nor does it influence anandamide uptake.[42]

Effects on TRP channels

CBN is a TRPA1 agonist and a TRPM8 antagonist at nanomolar concentrations.[42] It also activates TRPV1, TRPV2, TRPV3 and TRPV4 at between 6–20 μM, but with low efficacy.[42, 128]

Evidence for intoxicating and other behavioural effects

Evidence for intoxicating effects in humans

At least four studies have administered CBN to humans. One study found that 600 mg CBN given orally every day for 20 days induced no chromosomal abnormalities in 27 healthy subjects.[147] In another study[148] infrequent cannabis users were given access to intravenous CBN. They were told to self-administer the drug until they felt they had reached a sufficient level of 'high'. The amount of THC delivered to achieve intoxication was much less than for CBN. However CBN was still self-administered and to a greater extent than the non-psychoactive CBD which produced no noticeable effect. The participants reported that they felt CBN effects (at high doses) were mild but enjoyable, but much less intense than smoking cannabis. In another human study, 25 mg oral THC increased heart rate, decreased estimates of the passage of time, and increased feelings of being drugged, drunk, dizzy and drowsy. No such effects were observed with 50 mg oral CBN.[149] In a final study, oral CBN was administered at a maximum of 1200 mg to human cannabis smokers and failed to induce dose-related physiological effects, such as bronchodilation.[150]

Tetrad effects in rodents

CBN promotes modest locomotor suppression in mice at high doses (40–80 mg/kg).[48] However, no effects were observed on catalepsy, body temperature or antinociception. CBN produced conjunctival erythemia (red eyes) in cats, a common effect of THC administration.[137] CBN substituted for THC in the drug discrimination paradigm in rats and monkeys, suggesting CBN and THC have similar subjective qualities in laboratory animals.[151]

Therapeutic potential

Appendix Table 9 presents details of therapeutic effects relevant to CBN.

Immunosuppressant effects

Immunosuppression may be beneficial in autoimmune conditions, where an overactive immune system causes pathology. In immortalized astroglial cells, CBN inhibited the levels of nitrites released in response to the immune challenges lipopolysaccharide (LPS) and interferon-γ (IFNγ) when administered in nanomolar concentrations.[152] This effect was mediated by CB1Rs. Similar CBN inhibition of IFNγ-induced nitrite concentrations were seen in macrophages, albeit at lower potency (10 μM).[153]

Analgesic effects

CBN had antinociceptive effects at 50 mg/kg in the acetic acid model of visceral pain in mice. This was mediated by CB1 receptors.[41] THC was far more potent with equivalent effects at a 1 mg/kg dose. CBN had similar antinociceptive potency in the hot-plate test in mice.[154]

Anticonvulsant effects

CBN at a very high dose of 250 mg/kg showed anticonvulsant activity in the maximum electroshock test in mice.[155]

Antidiarrhoeal effects

CBN inhibited the enhanced gastrointestinal motility caused by the administration of the intestinal inflammatory agent croton oil in mice, albeit with much less potency that the synthetic CB1R agonist WIN 55,212-2. This was mediated by CB1 receptors and croton oil increased CB1 receptor expression in the intestine.[156] CBN at 11–20 mg/kg also decreased small intestine and colonic propulsion.[157, 158]

Anti-psoriasis/skin disorders

CBN potently inhibited the proliferation and differentiation of keratinocytes in vitro, a major cellular component of the epidermis.[140] These results suggest that topical applications of cannabinoid products for skin disorders such as psoriasis might be justified, but would require further preclinical and clinical examination.

Anticancer effects

CBN reduces the proliferation of human leukaemia and neuroblastoma cells at μM concentrations.[40]

Appetite stimulant effects

CBN stimulated feeding behavior in rats at 26 mg/kg and this effect was mediated by CB1 receptors.[159]

Antibacterial effects

CBN has potent antibacterial activity at low μM concentrations against drug-resistant strains of *Staphylococcus aureus*.[63]

Amyotrophic lateral sclerosis (ALS)

CBN at 5 mg/kg daily for 12 days delayed the onset of symptoms in a mouse model of ALS which involves mutations in the superoxide dismutase 1 gene (SOD1).[11] SOD1 is an enzyme that detoxifies reactive oxygen species.

Neuroprotective effects

CBN at 4 μM inhibited apoptosis and NF-κβ induced by the anticancer drug camptothecin and TNFα in primary mice cortical cells cultures.[160]

Antioxidant effects

CBN's chemical structure resembles the antioxidant vitamin E. Submicromolar concentrations of CBN inhibited cell death promoted by serum starvation via an antioxidant mechanism of action.[161] CBN also showed an antioxidant profile like CBD and THC using cyclic voltammetry with a electron donating profile similar to that of the known antioxidant, butylhydroxy-toluene (BHT).[106]

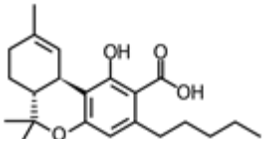
Conclusions

CBN may have mild psychoactivity when large quantities of the drug are administered intravenously to humans. This presumably reflects lower efficacy of CBN at CB1Rs than THC, despite its higher affinity. In the absence of adequate clinical evidence it is difficult to determine a therapeutic dose of CBN.

In a recent analysis of cannabinol in hempseed oils CBN was present at a maximum concentration approaching 10 mg/kg.[1] This would mean that consuming 5 kg (5.5 L) of hemp seed oil would be required to reach a non-psychoactive CBN dose of 50 mg.

The lowest CBN dose we identified in the literature to be effective in an animal model of disease was 5 mg/kg administered subcutaneously which delayed the onset of symptoms in a mouse model of ALS.[11] As systemic phytocannabinoid doses achieve on average 7 times higher tissue concentrations than oral doses in rodents,[3] the oral dose needed to achieve equivalent plasma levels might be as high as 35 mg/kg. Applying the FDA calculation to this dose, the estimated therapeutic oral dose in a 60 kg human for this indication would be 171 mg.[vii]

TETRAHYDROCANNABINOLIC ACID (THCA)



Introduction

THCA is the chemical precursor of THC in the cannabis plant. THCA is formed from cannabigerolic acid (CBGA) by the enzyme THCA synthase. Heating cannabis plant material to around 160˚C causes the decarboxylation of THCA to THC by a non-enzymatic reaction.

THCA is the most abundant cannabinoid found in police seized Australian street cannabis, representing on average almost 13% of the weight of cannabis flowering heads (up to 40% in some samples).[52]

THCA is generally considered to be non-psychoactive. However, very little research has been conducted on the pharmacological properties of THCA and this limits the strength of our conclusions when evaluating its pharmacological activity and toxicity.

[vii] Please refer back to Introduction to the Review section above under *"Caveat 3: Difficulties in extrapolation from preclinical concentrations/doses to therapeutic levels in humans"* for an explanation of the FDA-recommended animal to human dose calculation and its significant limitations in being able to predict a human therapeutic dose. There is high likelihood that potential therapeutic effects in in vitro and in vivo animal models may not translate. If they do translate, there is no certainty that the human oral doses will reflect animal doses. **We do not endorse setting limits for cannabinoids in hemp food products based on therapeutic doses that were estimated from animal studies.**

Relevant pharmacological actions

CB1 and CB2 receptor affinity

A single study suggests that THCA binds to human CB1 receptors with similar affinity to THC.[40] However, this requires further verification and, in any case, there is no evidence that it activates the receptor (low 'efficacy') in a way that would be necessary to achieve intoxication. The same report suggests that THCA also binds the human CB2 receptor at low nM concentrations,[40] but this requires further independent verification.

Effects on the endocannabinoid system

THCA inhibits the 2-AG synthesizing enzyme DAGLα and the degradative enzyme MAGL [42] at > 10 μM. THCA does not inhibit the function of the anandamide degradative enzyme FAAH.[42]

Effects on TRP channels

THCA blocks TRPM8 channels and activates TRPA1 at submicromolar concentrations.[42] The functional significance of these actions for pharmacological activity is unclear at present.

Evidence for intoxicating and other behavioural effects

Evidence for intoxicating effects in humans

Anecdotal reports suggest that 'juicing' cannabis, that is, the oral consumption of liquefied raw cannabis plant material, which is very rich in THCA, does not produce intoxication. This is despite probable gram quantities of THCA being administered in juiced products due to the high concentration of THCA in plant material.[162] A proper controlled psychopharmacological study is required to specifically confirm whether THCA lacks psychoactive effects.

Other human studies

Only one study could be located that administered THCA to humans (10 mg oral and 5 mg intravenous). While the study has been cited by others as evidence of no psychoactive effects of THCA, the subjective effects of THCA were not explicitly assessed.[163] The primary focus of the study was to determine THCA pharmacokinetics. The highest serum level achieved following an oral dose of 10 mg THCA was 600 ng/ml (1.6 μM concentration) and with the intravenous 5 mg dose the level was 1100 ng/ml (3 μM concentration).[164] It is worth noting that THCA is not converted into THC in humans or rats in vivo.[12, 13]

Tetrad effects in rodents

THCA did not suppress locomotor activity or produce hypothermia in rats, two typical actions of the psychoactive cannabinoid THC.[12] However, studies assessing higher doses may be required to completely rule out cannabimimetic actions.

Therapeutic potential

Several preclinical studies that have evaluated the therapeutic potential of THCA (see Appendix Table 10), but there are no relevant studies in humans.

Antiemetic effects

The most impressive potential therapeutic effect of THCA emerging from preclinical studies is an antiemetic action. In shrews (*Suncus murinus*) THCA potently inhibited vomiting induced by lithium chloride at 0.05 mg/kg.[12] In rats THCA also reduced lithium-induced conditioned gaping (a model of anticipatory nausea) at plasma levels of around 16 ng/ml or 0.043 μM.[12] This suggests that the effective plasma level could be attained from consuming medium μg to low mg quantities in humans when reflecting upon the data of Wohlfarth et al. (2012) where low μM levels were attained in the plasma following a small oral dose of 10 mg.

The antiemetic effects of THCA appear mediated by cannabinoid CB1 receptors, as a cannabinoid CB1 receptor antagonist reversed the ability of THCA to reduce emesis in rodents.[12]. Thus THCA might activate the receptor directly or increase levels of endocannabinoids like anandamide and 2-AG.

Anti-inflammatory effects

There is also preclinical evidence for anti-inflammatory properties of THCA, perhaps via inhibition of cyclooxygenase enzymes.[56] These enzymes are the drug targets of various non-steroidal anti-inflammatory drugs (NSAIDs) and aspirin. However THCA has relatively low potency at this target and this mechanism is unlikely to be clinically relevant. THCA also inhibits the release of inflammatory cytokines such as TNF-α from macrophages.[162]

Antioxidant effects

There is in vitro evidence that THCA may also be a weak antioxidant.[165]

Conclusions

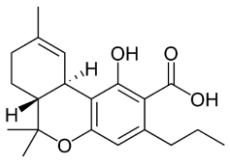
Overall the weight of evidence suggests that THCA is unlikely to have intoxicating effects in humans.

However, it is problematic that THCA is readily converted to THC by heating plant material e.g. at 100 degrees or more 80% of THCA is converted to THC.[13] THCA is quite stable in the short term (24 hours) even at high temperatures such as 50˚C, although there is a relatively small but significant conversion to THC at room temperature across a year of observation (e.g. a 2% THC content can increase to 5.6% THC).[13]

Thus THCA content should be measured in hemp food products and maintained at low levels given that it could be readily converted to THC via heating by knowledgeable consumers. Similar concentrations of THCA in seed, oil, and beverages might be adopted to that proposed for THC in FSANZ Application A1039.

The lowest THCA dose we identified in the literature to be effective in an animal model of disease was 0.05 mg/kg IP which had anti-nausea effects in rats.[12] As IP phytocannabinoid doses achieve on average 7 times higher tissue concentrations than oral doses in rodents,[3] the oral dose needed to achieve equivalent plasma levels might be as high as 0.35 mg/kg. Applying the FDA calculation to this dose, the estimated therapeutic oral dose in a 60 kg human for this indication would be 3.5 mg.[viii]

TETRAHYDROCANNABIVARINIC ACID (THCVA)



Introduction

THCVA is formed in the cannabis plant from cannabigerovarinic acid (CBGVA). Very little research has been conducted on the pharmacological properties of THCVA and this limits the strength of our conclusions when evaluating its pharmacological activity and toxicity.

[viii] Please refer back to Introduction to the Review section above under *"Caveat 3: Difficulties in extrapolation from preclinical concentrations/doses to therapeutic levels in humans"* for an explanation of the FDA-recommended animal to human dose calculation and its significant limitations in being able to predict a human therapeutic dose. There is high likelihood that potential therapeutic effects in in vitro and in vivo animal models may not translate. If they do translate, there is no certainty that the human oral doses will reflect animal doses. **We do not endorse setting limits for cannabinoids in hemp food products based on therapeutic doses that were estimated from animal studies.**

Relevant pharmacological actions

CB1 and CB2 receptor affinity

To the best of our knowledge no study has examined the binding of THCVA to cannabinoid receptors.

Effects on the endocannabinoid system

THCVA does not appear to modulate endocannabinoid enzymes such as DAGLα, MAGL and FAAH, and does not affect anandamide uptake.[42]

Effects on TRP channels

THCVA modulates various TRP channels at low to medium micromolar concentrations. It antagonises TRPM8 channels at 1 μM and activates TRPA1 at 16 μM.[42] It also activates TRPV1, TRPV3 and TRPV4 at relatively high micromolar concentrations and with low efficacy (26 μM, 48 μM and 4 μM respectively).[42, 128]

Evidence for intoxicating and other behavioural effects

Evidence for intoxicating effects in humans

THCVA has never been administered to humans as a pure compound.

Tetrad effects in rodents

THCVA has not been examined in the tetrad test battery.

Therapeutic potential

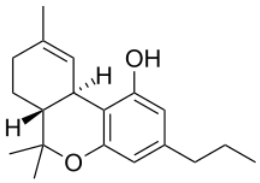
Note that there is no summary table in the Appendix for this phytocannabinoid owing to an overall lack of research activity.

Anticancer effects

One GW Pharmaceuticals patent ('Phytocannabinoids in the treatment of cancer' US 20130059018 A1) reported that THCVA induces apoptosis in hormone-sensitive and hormone-insensitive prostate cancer cells in culture at 25 μM.

Conclusions

There is extremely limited evidence available to form an opinion about the psychopharmacological, therapeutic and intoxicating actions of THCVA. THCVA has cytotoxic effects in prostate cancer cells at medium micromolar concentrations. Without more evidence it is impossible to estimate a human therapeutic dose.



TETRAHYDROCANNABIVARIN (THCV)

Introduction

THCV is the propyl homologue of THC differing only in its slightly shortened alkyl side chain. It was discovered in the 1960s.[166]

THCV occurs naturally at low levels in the cannabis plant with variation across different strains. THCV was detectable in 36% of 206 illicit cannabis seizures analysed in NSW,[52] while cannabis "cigarettes" supplied for human research by the US National Institute of Drug Abuse (NIDA) contained an average of 0.12% (0.96 mg) THCV.[167] Furthermore, GW Pharmaceuticals flagship product nabiximols (Sativex) also contains THCV (~1% of the total cannabinoid content).

THCV appears to be the only phytocannabinoid discovered to date that acts as an antagonist at CB1Rs. It is a 'neutral' antagonist making it a potentially safer alternative to the 'inverse' CB_1 agonist SR141716

(rimonabant),[168] which was marketed in Europe as a treatment for metabolic disorders and obesity until serious neuropsychiatric side effects became apparent.[169, 170]

Relevant pharmacological actions

CB1 and CB2 receptor affinity

THCV binds to the CB1R with similar affinity to THC,[41] but unlike THC it has low efficacy and therefore acts as an antagonist.[171] THCV displaces the highly potent synthetic cannabinoid compound CP55940 from human CB1 and CB2 receptors.[69] While, THCV exhibits neutral antagonist properties in mice at doses less than 3 mg/kg, it may act as a partial agonist of the CB1Rs and CB2Rs when doses exceed 10 mg/kg.[69]

Other in vitro evidence come from experiments with murine cerebellar slices indicates that THCV can block activation of neuronal CB1 receptors.[172] In this study THCV prevented the inhibition of GABA release caused by the CB1R agonist WIN 55212 and mimicked the effects of the CB1 receptor antagonist/inverse agonist AM251 in increasing GABA release.

THCV (68.4 nM) also activated CB2 receptors in vitro,[173] exhibiting high affinity for the CB2 receptor, signaling as a partial agonist.[30]

Effects on the endocannabinoid system

THCV can block CB1-mediated effects of endogenously released endocannabinoids when administered in vivo. THCV did not appreciably inhibit DAGL, MAGL, FAAH or NAAA.[42, 128]

Effects on TRP channels

THCV stimulates TRPV3 channels with high efficacy (50–70% of the effect of ionomycin) and potency (EC50~3.7 μm).[128] THCV stimulated TRPV4 channels with moderate to high efficacy (30–60% of the effect of ionomycin) and potency.[42, 128] TRPV1 was also stimulated and desensitised by THCV, and THCV-BDS was the most potent cannabinoid trialed at TRPA1 and at TRPM8 and was a potent activator of TRPV2.[42, 128]

Evidence for intoxicating and other behavioural effects

Evidence for intoxicating effects in humans

Two human studies have reported administration of THCV. Pure THCV was first administered to 6 humans in 1974 at a dose of 7 mg intravenously, and was generally well tolerated. One subject experienced no subjective effect while the remaining subjects experienced mild to moderate effects similar to THC but at approximately 25% the potency.[174] In a later study 10 mg THCV was administered to 20 healthy humans [175] and reportedly enhanced activation of key reward and aversion related areas of the brain, a profile that was distinct from the prototypical CB1R antagonist rimonabant.[176] No euphoria, sedation, or any changes in mood or affect were found in this study.[175]

Tetrad effects in rodents

Early pharmacological experiments with THCV indicated that it induced signs of catalepsy in the mouse ring test [166] but with a potency in mouse and human four or five times weaker than THC. THCV also produces antinociception in the tail flick test.[177] This is consistent with the notion that THCV activates CB1Rs weakly at higher doses.

Therapeutic potential

Refer to Appendix Table 11.

Metabolic

Owing to its 'neutral' antagonist properties at CB_1 receptors there is interest in the possibility that THCV might modify food and appetite-related phenomena. THCV, like the CB1R antagonist AM251, reduced the

food intake and body weight of non-fasted and fasted mice when administered singly[178] and reduced the food intake and body weight of mice rendered obese with a junk food diet when administered repeatedly over 30–45 days.[179]

Anxiolytic effects

THCV did not have anxiogenic properties, but nor was it demonstrated to be anxiolytic at 2.5mg/kg (IV) in a rat model of anxiety.[180]

Antipsychotic effects

THCV had antipsychotic-like effects in a rat phencyclidine model of psychosis.[181]

Anticonvulsant effects

THCV (20 μM) significantly reduced seizure-like activity in rat brain slices in vitro. THCV (0.25 mg/kg) significantly reduced seizure incidence in the PTZ model in rats when tested in vivo.[14]

Analgesic effects

THCV administered alone (50 mg/kg) did not have antinociceptive effects, but prevented the antinociceptive effects of THC in an acetic acid stretching model of rodent visceral pain.[41] However THCV (5 mg/kg) exhibited analgesic effects when tested in an inflammatory pain model, most likely due to CB2R activation.[182]

Anti-inflammatory effects

THCV (0.3 or 1 mg/kg IP) decreased signs of inflammation in a rat paw inflammatory pain model using intraplantar injection of carrageenan or formalin, and these effects were blocked with the use of CB1 and CB2 receptor antagonists.[183]

Antioxidant

THCV provided signs of neuroprotection in the form of an attenuation of the loss of tyrosine hydroxylase-positive neurons in rats lesioned with 6-hydroxydopamine and in mice lesioned with lipopolysaccharide (LPS).[182]

Anti-ischaemic effects

THCV activated CB2 receptors in vitro, and decreased tissue injury and inflammation in vivo, associated with ischaemia-reperfusion injury, partly via CB2 receptor activation.[173]

Conclusions

The literature on THCV, particularly in humans, is clearly very limited. However, THCV shows some exciting promise as a treatment for metabolic disorders. In the absence of sound clinical evidence it is impossible to determine a therapeutic dose of THCV.

The lowest THCV dose we identified in the literature to be effective in an animal model of disease was 0.25 mg/kg IP which reduced seizure severity in a rat model of epilepsy.[14] As IP doses of THCV achieve 5.4 times higher brain concentrations (the site of anticonvulsant drug action) than oral doses in rats,[3] the oral dose required could be as high 1.35 mg/kg. Applying the FDA calculation to this dose, the estimated therapeutic oral dose in a 60 kg human is 13 mg.[ix]

[ix] Please refer back to Introduction to the Review section above under *"Caveat 3: Difficulties in extrapolation from preclinical concentrations/doses to therapeutic levels in humans"* for an explanation of the FDA-recommended animal to human dose calculation and its significant limitations in being able to predict a human therapeutic dose. There is high likelihood that potential therapeutic effects in in vitro and in vivo animal models may not translate. If they do translate, there is no certainty that the human oral doses will reflect animal doses. **We do not endorse setting limits for cannabinoids in hemp food products based on therapeutic doses that were estimated from animal studies.**

5 General conclusions and summary of therapeutic levels

Table 2 (below) summarises the information from the preceding sections to give our estimate of the lowest effective oral human therapeutic dose for each of the phytocannabinoids of interest, as well as the potential of each phytocannabinoid to produce THC-like intoxicating effects. Note that most of the therapeutic effects listed are potentially beneficial to human consumers, so these limits should not be necessarily seen as providing a level at which hemp-derived products should become restricted or scheduled. Note, also, the previously described caveats involved in extrapolating from studies with laboratory animals into the human situation, and in inferring oral human doses from rat or mouse systemic doses.

Obviously, proposed limits of phytocannabinoid levels will become better contextualised once more information on actual concentrations of the phytocannabinoids in hemp seed, hemp seed oil and hemp food products can be provided. The data being collected by our colleagues at Southern Cross University may be important to allow us to determine the potential hazards or therapeutic benefits accruing from consumption of specific hemp-derived products produced in, or imported into, Australia. In the absence of such data it is difficult at present to formulate an opinion on the feasibility of setting a total cannabinoid limit in such products.

There is reasonably good evidence of the therapeutic effects of CBD in humans at 800 mg (absolute dose). There is the possibility of mild sedation at such doses of CBD, although the current literature is ambiguous on this point with the balance of studies suggesting no sedative effects. There is no evidence of THC-like intoxication with CBD.

Evidence relating to potential therapeutic effects of the remaining phytocannabinoids mostly comes from preclinical studies involving cellular models and laboratory animals. Some evidence is available relating to effects arising from the consumption of THCA, THCV, CBDV, CBC and CBN in humans. In general there is little evidence of intoxication with these phytocannabinoids using an oral route of administration. However, CBN and THCV may be mildly intoxicating at relatively high intravenous doses. Only limited preclinical evidence is available for CBDA, THCVA, CBG and CBGA. On the basis of such evidence, none of these phytocannabinoids appear to have intoxicating properties.

The specific conclusions for setting limits in hemp-derived products are (also see Table 2 below):

1. **A limit could be set for CBD** given that it has therapeutic effects in humans (lowest human therapeutic absolute dose is 800 mg)
2. **The same limit might also be set for CBDA** (i.e. 800 mg) given it is almost completely converted to CBD upon heating
3. **A limit could be set for THCA** identical to that already set by FSANZ for THC. THCA is almost completely converted to THC when it is heated and so might be heated by some consumers seeking intoxication
4. **At this stage limits may not be required for the remaining phytocannabinoids CBC, CBDV, CBN, CBGA, CBG, THCV and THCVA**. No strong evidence supports these compounds having intoxicating effects following oral administration. The evidence for therapeutic potential comes only from animal studies and so the estimated human doses calculated from animal studies may not be relevant to human consumption.

Table 2: Lowest human therapeutic doses of the phytocannabinoids and potential intoxicating effects

Note: Except for CBD, all phytocannabinoid "therapeutic doses" have been estimated from animal studies. We do not endorse setting limits of cannabinoids in hemp food products based on therapeutic doses that are estimated from animal studies.

	Phyto-cannabinoid	Set limit? (dose)	Estimated lowest therapeutic oral dose in 60 kg human*	Species from which therapeutic dose was calculated	Indication for therapeutic dose	Ref	Potential to induce intoxication
1	CBC	No, insufficient data	35 mg	Mice	Colitis	2	No
2	CBDA	Possibly** (800 mg)	0.07 mg	Rat	Anti-nausea	4	No
3	CBD	Possibly (800 mg)	800 mg (absolute dose)	Human	Schizophrenia	8	No#
4	CBDV	No, insufficient data	774 mg	Rat	Seizures	9	No
5	CBGA	No, insufficient data	No data		-	-	Unknown
6	CBG	No, insufficient data	892 mg	Mice	Colon cancer	10	No
7	CBN	No, insufficient data	171 mg	Mice	Amyotrophic Lateral Sclerosis	11	Possible at high i.v. dose
8	THCA	Yes## Use FSANZ THC limit	3.5 mg	Rat	Nausea	12	No
9	THCVA	No, insufficient data	No data	-	-	-	Unknown
10	THCV	No, insufficient data	13 mg	Rat	Seizures	14	Possible at high i.v. dose

* Calculated using FDA method www.fda.gov/downloads/Drugs/.../Guidances/UCM078932.pdf

** CBDA is converted to CBD with heating

Weak evidence for sedative effects in humans – most likely not present but requires further testing

Note issues of THCA conversion to THC

6 References

1. Petrovic M, Debeljak Z, Kezic N, Dzidara P. Relationship between cannabinoids content and composition of fatty acids in hempseed oils. Food Chemistry. 2015;170:218-25.

2. Romano B, Borrelli F, Fasolino I, Capasso R, Piscitelli F, Cascio M, et al. The cannabinoid TRPA1 agonist cannabichromene inhibits nitric oxide production in macrophages and ameliorates murine colitis. British Journal of Pharmacology. 2013;169(1):213-29.

3. Deiana S, Watanabe A, Yamasaki Y, Amada N, Arthur M, Fleming S, et al. Plasma and brain pharmacokinetic profile of cannabidiol (CBD), cannabidivarine (CBDV), Delta(9)-tetrahydrocannabivarin (THCV) and cannabigerol (CBG) in rats and mice following oral and intraperitoneal administration and CBD action on obsessive-compulsive behaviour. Psychopharmacology (Berl). 2012;219(3):859-73.

4. Rock EM, Limebeer CL, Navaratnam R, Sticht MA, Bonner N, Engeland K, et al. A comparison of cannabidiolic acid with other treatments for anticipatory nausea using a rat model of contextually elicited conditioned gaping. Psychopharmacology (Berl). 2014;231(16):3207-15.

5. Crippa JA, Zuardi AW, Garrido GE, Wichert-Ana L, Guarnieri R, Ferrari L, et al. Effects of cannabidiol (CBD) on regional cerebral blood flow. Neuropsychopharmacology. 2004;29(2):417-26.

6. Zuardi AW, Guimaraes FS, Moreira AC. Effect of cannabidiol on plasma prolactin, growth hormone and cortisol in human volunteers. Braz J Med Biol Res. 1993;26(2):213-7.

7. Leweke FM, Schneider U, Radwan M, Schmidt E, Emrich HM. Different effects of nabilone and cannabidiol on binocular depth inversion in Man. Pharmacol Biochem Behav. 2000;66(1):175-81.

8. Leweke FM, Piomelli D, Pahlisch F, Muhl D, Gerth CW, Hoyer C, et al. Cannabidiol enhances anandamide signaling and alleviates psychotic symptoms of schizophrenia. Transl Psychiatry. 2012;2:e94.

9. Hill AJ, Mercier MS, Hill TD, Glyn SE, Jones NA, Yamasaki Y, et al. Cannabidivarin is anticonvulsant in mouse and rat. British Journal of Pharmacology. 2012;167(8):1629-42.

10. Borrelli F, Pagano E, Romano B, Panzera S, Maiello F, Coppola D, et al. Colon carcinogenesis is inhibited by the TRPM8 antagonist cannabigerol, a Cannabis-derived non-psychotropic cannabinoid. Carcinogenesis. 2014;35(12):2787-97.

11. Weydt P, Hong S, Witting A, Moller T, Stella N, Kliot M. Cannabinol delays symptom onset in SOD1 (G93A) transgenic mice without affecting survival. Amyotroph Lateral Scler Other Motor Neuron Disord. 2005;6(3):182-4.

12. Rock EM, Kopstick RL, Limebeer CL, Parker LA. Tetrahydrocannabinolic acid reduces nausea-induced conditioned gaping in rats and vomiting in Suncus murinus. British Journal of Pharmacology. 2013;170(3):641-8.

13. Taschwer M, Schmid MG. Determination of the relative percentage distribution of THCA and Delta-THC in herbal cannabis seized in Austria - Impact of different storage temperatures on stability. Forensic Sci Int. 2015;254:167-71.

14. Hill AJ, Weston SE, Jones NA, Smith I, Bevan SA, Williamson EM, et al. Delta(9)-Tetrahydrocannabivarin suppresses in vitro epileptiform and in vivo seizure activity in adult rats. Epilepsia. 2010;51(8):1522-32.

15. Mechoulam R, Shani A, Edery H, Grunfeld Y. Chemical basis of hashish activity. Science. 1970;169(3945):611-2.

16. Compton DR, Aceto MD, Lowe J, Martin BR. In vivo characterization of a specific cannabinoid receptor antagonist (SR141716A): inhibition of delta 9-tetrahydrocannabinol-induced responses and apparent agonist activity. J Pharmacol Exp Ther. 1996;277(2):586-94.

17. Zimmer A, Zimmer AM, Hohmann AG, Herkenham M, Bonner TI. Increased mortality, hypoactivity, and hypoalgesia in cannabinoid CB1 receptor knockout mice. Proc Natl Acad Sci U S A. 1999;96(10):5780-5.

18. Banister SD, Moir M, Stuart J, Kevin RC, Wood KE, Longworth M, et al. Pharmacology of Indole and Indazole Synthetic Cannabinoid Designer Drugs AB-FUBINACA, ADB-FUBINACA, AB-PINACA, ADB-PINACA, 5F-AB-PINACA, 5F-ADB-PINACA, ADBICA, and 5F-ADBICA. ACS Chem Neurosci. 2015;6(9):1546-59.

19. Munro S, Thomas KL, Abu-Shaar M. Molecular characterization of a peripheral receptor for cannabinoids. Nature. 1993;365(6441):61-5.

20. Malfitano AM, Basu S, Maresz K, Bifulco M, Dittel BN. What we know and do not know about the cannabinoid receptor 2 (CB2). Semin Immunol. 2014;26(5):369-79.

21. Nevalainen T. Recent development of CB2 selective and peripheral CB1/CB2 cannabinoid receptor ligands. Curr Med Chem. 2014;21(2):187-203.

22. Burston JJ, Sim-Selley LJ, Harloe JP, Mahadevan A, Razdan RK, Selley DE, et al. N-arachidonyl maleimide potentiates the pharmacological and biochemical effects of the endocannabinoid 2-arachidonylglycerol through inhibition of monoacylglycerol lipase. J Pharmacol Exp Ther. 2008;327(2):546-53.

23. Long JZ, Nomura DK, Vann RE, Walentiny DM, Booker L, Jin X, et al. Dual blockade of FAAH and MAGL identifies behavioral processes regulated by endocannabinoid crosstalk in vivo. Proc Natl Acad Sci U S A. 2009;106(48):20270-5.

24. Sokolic L, Long LE, Hunt GE, Arnold JC, McGregor IS. Disruptive effects of the prototypical cannabinoid Delta(9)-tetrahydrocannabinol and the fatty acid amide inhibitor URB-597 on go/no-go auditory discrimination performance and olfactory reversal learning in rats. Behav Pharmacol. 2011;22(3):191-202.

25. Walentiny DM, Vann RE, Wiley JL. Phenotypic assessment of THC discriminative stimulus properties in fatty acid amide hydrolase knockout and wildtype mice. Neuropharmacology. 2015;93:237-42.

26. Wise LE, Long KA, Abdullah RA, Long JZ, Cravatt BF, Lichtman AH. Dual fatty acid amide hydrolase and monoacylglycerol lipase blockade produces THC-like Morris water maze deficits in mice. ACS Chem Neurosci. 2012;3(5):369-78.

27. Caterina MJ. TRP channel cannabinoid receptors in skin sensation, homeostasis, and inflammation. ACS Chem Neurosci. 2014;5(11):1107-16.

28. Janero DR, Makriyannis A. Terpenes and lipids of the endocannabinoid and transient-receptor-potential-channel biosignaling systems. ACS Chem Neurosci. 2014;5(11):1097-106.

29. Premkumar LS. Transient receptor potential channels as targets for phytochemicals. ACS Chem Neurosci. 2014;5(11):1117-30.

30. McPartland JM, Duncan M, Di Marzo V, Pertwee RG. Are cannabidiol and Delta(9) - tetrahydrocannabivarin negative modulators of the endocannabinoid system? A systematic review. British Journal of Pharmacology. 2015;172(3):737-53.

31. Russo EB. Taming THC: potential cannabis synergy and phytocannabinoid-terpenoid entourage effects. British Journal of Pharmacology. 2011;163(7):1344-64.

32. Scott KA, Shah S, Dalgleish AG, Liu WM. Enhancing the activity of cannabidiol and other cannabinoids in vitro through modifications to drug combinations and treatment schedules. Anticancer Res. 2013;33(10):4373-80.

33. Rock EM, Goodwin JM, Limebeer CL, Breuer A, Pertwee RG, Mechoulam R, et al. Interaction between non-psychotropic cannabinoids in marihuana: effect of cannabigerol (CBG) on the anti-nausea or anti-emetic effects of cannabidiol (CBD) in rats and shrews. Psychopharmacology (Berl). 2011;215(3):505-12.

34. Englund A, Morrison PD, Nottage J, Hague D, Kane F, Bonaccorso S, et al. Cannabidiol inhibits THC-elicited paranoid symptoms and hippocampal-dependent memory impairment. J Psychopharmacology. 2013;27(1):19-27.

35. Morgan CJ, Freeman TP, Schafer GL, Curran HV. Cannabidiol attenuates the appetitive effects of Delta 9-tetrahydrocannabinol in humans smoking their chosen cannabis. Neuropsychopharmacology. 2010;35(9):1879-85.

36. Morgan CJ, Schafer G, Freeman TP, Curran HV. Impact of cannabidiol on the acute memory and psychotomimetic effects of smoked cannabis: naturalistic study: naturalistic study [corrected]. Br J Psychiatry. 2010;197(4):285-90.

37. Gaoni Y, Mechoulam R. The isolation and structure of delta-1-tetrahydrocannabinol and other neutral cannabinoids from hashish. J Am Chem Soc. 1971;93(1):217-24.

38. Potter DJ, Clark P, Brown MB. Potency of delta 9-THC and other cannabinoids in cannabis in England in 2005: implications for psychoactivity and pharmacology. J Forensic Sci. 2008;53(1):90-4.

39. Mehmedic Z, Chandra S, Slade D, Denham H, Foster S, Patel AS, et al. Potency trends of Delta9-THC and other cannabinoids in confiscated cannabis preparations from 1993 to 2008. J Forensic Sci. 2010;55(5):1209-17.

40. Rosenthaler S, Pohn B, Kolmanz C, Huu CN, Krewenka C, Huber A, et al. Differences in receptor binding affinity of several phytocannabinoids do not explain their effects on neural cell cultures. Neurotoxicol Teratol. 2014;46:49-56.

41. Booker L, Naidu PS, Razdan RK, Mahadevan A, Lichtman AH. Evaluation of prevalent phytocannabinoids in the acetic acid model of visceral nociception. Drug Alcohol Depend. 2009;105(1-2):42-7.

42. De Petrocellis L, Ligresti A, Moriello AS, Allara M, Bisogno T, Petrosino S, et al. Effects of cannabinoids and cannabinoid-enriched Cannabis extracts on TRP channels and endocannabinoid metabolic enzymes. British Journal of Pharmacology. 2011;163(7):1479-94.

43. De Petrocellis L, Vellani V, Schiano-Moriello A, Marini P, Magherini PC, Orlando P, et al. Plant-derived cannabinoids modulate the activity of transient receptor potential channels of ankyrin type-1 and melastatin type-8. J Pharmacol Exp Ther. 2008;325(3):1007-15.

44. Maione S, Piscitelli F, Gatta L, Vita D, De Petrocellis L, Palazzo E, et al. Non-psychoactive cannabinoids modulate the descending pathway of antinociception in anaesthetized rats through several mechanisms of action. British Journal of Pharmacology. 2011;162(3):584-96.

45. Turner CE, Elsohly MA, Boeren EG. Constituents of Cannabis sativa L. XVII. A review of the natural constituents. J Nat Prod. 1980;43(2):169-234.

46. Davis WM, Hatoum NS. Neurobehavioral actions of cannabichromene and interactions with delta 9-tetrahydrocannabinol. Gen Pharmacol. 1983;14(2):247-52.

47. Hatoum NS, Davis WM, Elsohly MA, Turner CE. Cannabichromene and delta 9-tetrahydrocannabinol: interactions relative to lethality, hypothermia and hexobarbital hypnosis. Gen Pharmacol. 1981;12(5):357-62.

48. El-Alfy AT, Ivey K, Robinson K, Ahmed S, Radwan M, Slade D, et al. Antidepressant-like effect of delta9-tetrahydrocannabinol and other cannabinoids isolated from Cannabis sativa L. Pharmacol Biochem Behav. 2010;95(4):434-42.

49. DeLong GT, Wolf CE, Poklis A, Lichtman AH. Pharmacological evaluation of the natural constituent of Cannabis sativa, cannabichromene and its modulation by Delta(9)-tetrahydrocannabinol. Drug Alcohol Depend. 2010;112(1-2):126-33.

50. Turner CE, Elsohly MA. Biological activity of cannabichromene, its homologs and isomers. J Clin Pharmacol. 1981;21(8-9 Suppl):283S-91S.

51. Tubaro A, Giangaspero A, Sosa S, Negri R, Grassi G, Casano S, et al. Comparative topical anti-inflammatory activity of cannabinoids and cannabivarins. Fitoterapia. 2010;81(7):816-9.

52. Swift W, Wong A, Li KM, Arnold JC, McGregor IS. Analysis of cannabis seizures in NSW, Australia: cannabis potency and cannabinoid profile. PLoS One. 2013;8(7):e70052.

53. Weiblen GD, Wenger JP, Craft KJ, ElSohly MA, Mehmedic Z, Treiber EL, et al. Gene duplication and divergence affecting drug content in Cannabis sativa. New Phytol. 2015.

54. Bolognini D, Rock EM, Cluny NL, Cascio MG, Limebeer CL, Duncan M, et al. Cannabidiolic acid prevents vomiting in Suncus murinus and nausea-induced behaviour in rats by enhancing 5-HT1A receptor activation. British Journal of Pharmacology. 2013;168(6):1456-70.

55. Anavi-Goffer S, Baillie G, Irving AJ, Gertsch J, Greig IR, Pertwee RG, et al. Modulation of L-alpha-lysophosphatidylinositol/GPR55 mitogen-activated protein kinase (MAPK) signaling by cannabinoids. J Biol Chem. 2012;287(1):91-104.

56. Ruhaak LR, Felth J, Karlsson PC, Rafter JJ, Verpoorte R, Bohlin L. Evaluation of the cyclooxygenase inhibiting effects of six major cannabinoids isolated from Cannabis sativa. Biol Pharm Bull. 2011;34(5):774-8.

57. Takeda S, Misawa K, Yamamoto I, Watanabe K. Cannabidiolic acid as a selective cyclooxygenase-2 inhibitory component in cannabis. Drug Metab Dispos. 2008;36(9):1917-21.

58. Rock EM, Parker LA. Suppression of lithium chloride-induced conditioned gaping (a model of nausea-induced behaviour) in rats (using the taste reactivity test) with metoclopramide is enhanced by cannabidiolic acid. Pharmacol Biochem Behav. 2013;111:84-9.

59. Rock EM, Parker LA. Effect of low doses of cannabidiolic acid and ondansetron on LiCl-induced conditioned gaping (a model of nausea-induced behaviour) in rats. British Journal of Pharmacology. 2013;169(3):685-92.

60. Takeda S, Okajima S, Miyoshi H, Yoshida K, Okamoto Y, Okada T, et al. Cannabidiolic acid, a major cannabinoid in fiber-type cannabis, is an inhibitor of MDA-MB-231 breast cancer cell migration. Toxicol Lett. 2012;214(3):314-9.

61. Takeda S, Okazaki H, Ikeda E, Abe S, Yoshioka Y, Watanabe K, et al. Down-regulation of cyclooxygenase-2 (COX-2) by cannabidiolic acid in human breast cancer cells. J Toxicol Sci. 2014;39(5):711-6.

62. Ligresti A, Moriello AS, Starowicz K, Matias I, Pisanti S, De Petrocellis L, et al. Antitumor activity of plant cannabinoids with emphasis on the effect of cannabidiol on human breast carcinoma. J Pharmacol Exp Ther. 2006;318(3):1375-87.

63. Appendino G, Gibbons S, Giana A, Pagani A, Grassi G, Stavri M, et al. Antibacterial cannabinoids from Cannabis sativa: a structure-activity study. J Nat Prod. 2008;71(8):1427-30.

64. Cluny NL, Naylor RJ, Whittle BA, Javid FA. The effects of cannabidiolic acid and cannabidiol on contractility of the gastrointestinal tract of Suncus murinus. Arch Pharm Res. 2011;34(9):1509-17.

65. Campos AC, Moreira FA, Gomes FV, Del Bel EA, Guimaraes FS. Multiple mechanisms involved in the large-spectrum therapeutic potential of cannabidiol in psychiatric disorders. Philosophical Transactions of the Royal Society of London Series B: Biological Sciences. 2012;367(1607):3364-78.

66. Zuardi AW. Cannabidiol: from an inactive cannabinoid to a drug with wide spectrum of action. Rev Bras Psiquiatr. 2008;30(3):271-80.

67. Izzo AA, Borrelli F, Capasso R, Di Marzo V, Mechoulam R. Non-psychotropic plant cannabinoids: new therapeutic opportunities from an ancient herb. Trends Pharmacol Sci. 2009;30(10):515-27.

68. Taura F, Sirikantaramas S, Shoyama Y, Yoshikai K, Shoyama Y, Morimoto S. Cannabidiolic-acid synthase, the chemotype-determining enzyme in the fiber-type Cannabis sativa. FEBS letters. 2007;581(16):2929-34.

69. Pertwee RG. The diverse CB1 and CB2 receptor pharmacology of three plant cannabinoids: delta9-tetrahydrocannabinol, cannabidiol and delta9-tetrahydrocannabivarin. British Journal of Pharmacology. 2008;153(2):199-215.

70. Bisogno T, Hanus L, De Petrocellis L, Tchilibon S, Ponde DE, Brandi I, et al. Molecular targets for cannabidiol and its synthetic analogues: effect on vanilloid VR1 receptors and on the cellular uptake and enzymatic hydrolysis of anandamide. Br J Pharmacol. 2001;134(4):845-52.

71. Iannotti FA, Hill CL, Leo A, Alhusaini A, Soubrane C, Mazzarella E, et al. Nonpsychotropic plant cannabinoids, cannabidivarin (CBDV) and cannabidiol (CBD), activate and desensitize transient receptor potential vanilloid 1 (TRPV1) channels in vitro: potential for the treatment of neuronal hyperexcitability. ACS Chem Neurosci. 2014;5(11):1131-41.

72. Cascio M, Pertwee R. Known pharmacological actions of nine nonpsychotropic phytocannabinoids. In: Pertwee RG, editor. Handbook of Cannabis. Oxford, United Kingdom: Oxford University Press; 2014.

73. Russo EB, Burnett A, Hall B, Parker KK. Agonistic properties of cannabidiol at 5-HT1a receptors. Neurochem Res. 2005;30(8):1037-43.

74. Kathmann M, Flau K, Redmer A, Trankle C, Schlicker E. Cannabidiol is an allosteric modulator at mu- and delta-opioid receptors. Naunyn Schmiedebergs Arch Pharmacol. 2006;372(5):354-61.

75. Ryan D, Drysdale AJ, Lafourcade C, Pertwee RG, Platt B. Cannabidiol targets mitochondria to regulate intracellular Ca2+ levels. J Neurosci. 2009;29(7):2053-63.

76. Pertwee R, Cascio M. Known pharmacological actions of delta-9-tetrahydrocannabinol and of four other chemical constituents of cannabis that activate cannabinoid receptors. In: Pertwee RG, editor. Handbook of cannabis. Oxford, UK: Oxford University Press; 2014.

77. Mechoulam R, Peters M, Murillo-Rodriguez E, Hanus LO. Cannabidiol–recent advances. Chem Biodivers. 2007;4(8):1678-92.

78. Petitet F, Jeantaud B, Reibaud M, Imperato A, Dubroeucq MC. Complex pharmacology of natural cannabinoids: evidence for partial agonist activity of delta9-tetrahydrocannabinol and antagonist activity of cannabidiol on rat brain cannabinoid receptors. Life Sciences. 1998;63(1):PL1-6.

79. Zuardi AW, Shirakawa I, Finkelfarb E, Karniol IG. Action of cannabidiol on the anxiety and other effects produced by delta 9-THC in normal subjects. Psychopharmacology. 1982;76(3):245-50.

80. Zuardi A, Guimarães F. Cannabidiol as an anxiolytic and antipsychotic. Cannabis in Medical Practice. 133-141. Jefferson, NC, USA: McFarland & Company, Inc; 1997.

81. Zuardi AW, Crippa JA, Hallak JE, Moreira FA, Guimaraes FS. Cannabidiol, a Cannabis sativa constituent, as an antipsychotic drug. Brazilian Journal of Medical & Biological Research. 2006;39(4):421-9.

82. Crippa JA, Zuardi AW, Hallak JE. [Therapeutical use of the cannabinoids in psychiatry]. Rev Bras Psiquiatr. 2010;32 Suppl 1:S56-66.

83. Crippa JA, Zuardi AW, Martin-Santos R, Bhattacharyya S, Atakan Z, McGuire P, et al. Cannabis and anxiety: a critical review of the evidence. Hum Psychopharmacol. 2009;24(7):515-23.

84. Cunha JM, Carlini EA, Pereira AE, Ramos OL, Pimentel C, Gagliardi R, et al. Chronic administration of cannabidiol to healthy volunteers and epileptic patients. Pharmacology. 1980;21(3):175-85.

85. Long LE, Chesworth R, Huang XF, McGregor IS, Arnold JC, Karl T. A behavioural comparison of acute and chronic Delta9-tetrahydrocannabinol and cannabidiol in C57BL/6JArc mice. Int J Neuropsychopharmacol. 2010;13(7):861-76.

86. Yamaori S, Kushihara M, Yamamoto I, Watanabe K. Characterization of major phytocannabinoids, cannabidiol and cannabinol, as isoform-selective and potent inhibitors of human CYP1 enzymes. Biochem Pharmacol. 2010;79(11):1691-8.

87. Srivastava MD, Srivastava BI, Brouhard B. Delta9 tetrahydrocannabinol and cannabidiol alter cytokine production by human immune cells. Immunopharmacology. 1998;40(3):179-85.

88. Wu HY, Chu RM, Wang CC, Lee CY, Lin SH, Jan TR. Cannabidiol-induced apoptosis in primary lymphocytes is associated with oxidative stress-dependent activation of caspase-8. Toxicol Appl Pharmacol. 2008;226(3):260-70.

89. Bergamaschi MM, Queiroz RH, Chagas MH, de Oliveira DC, De Martinis BS, Kapczinski F, et al. Cannabidiol reduces the anxiety induced by simulated public speaking in treatment-naive social phobia patients. Neuropsychopharmacology. 2011;36(6):1219-26.

90. Crippa JA, Derenusson GN, Ferrari TB, Wichert-Ana L, Duran FL, Martin-Santos R, et al. Neural basis of anxiolytic effects of cannabidiol (CBD) in generalized social anxiety disorder: a preliminary report. Journal of Psychopharmacology. 2011;25(1):121-30.

91. Carlini EA, Cunha JM. Hypnotic and antiepileptic effects of cannabidiol. 21. 1981;J. Clin. Pharmacol.(21):417S-27S.

92. Devinsky O, Marsh E, Friedman D, Thiele E, Laux L, Sullivan J, et al. Cannabidiol in patients with treatment-resistant epilepsy: an open-label interventional trial. The Lancet Neurology. 2015.

93. Mao K, You C, Lei D, Zhang H. High dosage of cannabidiol (CBD) alleviates pentylenetetrazole-induced epilepsy in rats by exerting an anticonvulsive effect. Int J Clin Exp Med. 2015;8(6):8820-7.

94. Shirazi-zand Z, Ahmad-Molaei L, Motamedi F, Naderi N. The role of potassium BK channels in anticonvulsant effect of cannabidiol in pentylenetetrazole and maximal electroshock models of seizure in mice. Epilepsy Behav. 2013;28(1):1-7.

95. Jones NA, Glyn SE, Akiyama S, Hill TD, Hill AJ, Weston SE, et al. Cannabidiol exerts anti-convulsant effects in animal models of temporal lobe and partial seizures. Seizure. 2012;21(5):344-52.

96. Jones NA, Hill AJ, Smith I, Bevan SA, Williams CM, Whalley BJ, et al. Cannabidiol displays antiepileptiform and antiseizure properties in vitro and in vivo. J Pharmacol Exp Ther. 2010;332(2):569-77.

97. Karler R, Turkanis SA. Cannabis and epilepsy. Adv Biosci. 1978;22-23:619-41.

98. Consroe P, Benedito MA, Leite JR, Carlini EA, Mechoulam R. Effects of cannabidiol on behavioral seizures caused by convulsant drugs or current in mice. Eur J Pharmacol. 1982;83(3-4):293-8.

99. Gloss D, Vickrey B. Cannabinoids for epilepsy. The Cochrane database of systematic reviews. 2014;3:CD009270.

100. Costa B, Trovato AE, Comelli F, Giagnoni G, Colleoni M. The non-psychoactive cannabis constituent cannabidiol is an orally effective therapeutic agent in rat chronic inflammatory and neuropathic pain. Eur J Pharmacol. 2007;556(1-3):75-83.

101. Ward SJ, McAllister SD, Kawamura R, Murase R, Neelakantan H, Walker EA. Cannabidiol inhibits paclitaxel-induced neuropathic pain through 5-HT(1A) receptors without diminishing nervous system function or chemotherapy efficacy. British Journal of Pharmacology. 2014;171(3):636-45.

102. Wade DT, Robson P, House H, Makela P, Aram J. A preliminary controlled study to determine whether whole-plant cannabis extracts can improve intractable neurogenic symptoms. Clin Rehabil. 2003;17(1):21-9.

103. Borrelli F, Aviello G, Romano B, Orlando P, Capasso R, Maiello F, et al. Cannabidiol, a safe and non-psychotropic ingredient of the marijuana plant Cannabis sativa, is protective in a murine model of colitis. J Mol Med (Berl). 2009;87(11):1111-21.

104. Schicho R, Storr M. Topical and systemic cannabidiol improves trinitrobenzene sulfonic acid colitis in mice. Pharmacology. 2012;89(3-4):149-55.

105. De Filippis D, Esposito G, Cirillo C, Cipriano M, De Winter BY, Scuderi C, et al. Cannabidiol reduces intestinal inflammation through the control of neuroimmune axis. PLoS One. 2011;6(12):e28159.

106. Hampson AJ, Grimaldi M, Axelrod J, Wink D. Cannabidiol and (-)Delta9-tetrahydrocannabinol are neuroprotective antioxidants. Proc Natl Acad Sci U S A. 1998;95(14):8268-73.

107. Hao E, Mukhopadhyay P, Cao Z, Erdelyi K, Holovac E, Liaudet L, et al. Cannabidiol Protects against Doxorubicin-Induced Cardiomyopathy by Modulating Mitochondrial Function and Biogenesis. Mol Med. 2015;21:38-45.

108. Yang L, Rozenfeld R, Wu D, Devi LA, Zhang Z, Cederbaum A. Cannabidiol protects liver from binge alcohol-induced steatosis by mechanisms including inhibition of oxidative stress and increase in autophagy. Free Radic Biol Med. 2014;68:260-7.

109. Alvarez FJ, Lafuente H, Rey-Santano MC, Mielgo VE, Gastiasoro E, Rueda M, et al. Neuroprotective effects of the nonpsychoactive cannabinoid cannabidiol in hypoxic-ischemic newborn piglets. Pediatr Res. 2008;64(6):653-8.

110. Pazos MR, Mohammed N, Lafuente H, Santos M, Martinez-Pinilla E, Moreno E, et al. Mechanisms of cannabidiol neuroprotection in hypoxic-ischemic newborn pigs: role of 5HT(1A) and CB2 receptors. Neuropharmacology. 2013;71:282-91.

111. Maor Y, Yu J, Kuzontkoski PM, Dezube BJ, Zhang X, Groopman JE. Cannabidiol inhibits growth and induces programmed cell death in kaposi sarcoma-associated herpesvirus-infected endothelium. Genes Cancer. 2012;3(7-8):512-20.

112. McAllister SD, Christian RT, Horowitz MP, Garcia A, Desprez PY. Cannabidiol as a novel inhibitor of Id-1 gene expression in aggressive breast cancer cells. Mol Cancer Ther. 2007;6(11):2921-7.

113. Ramer R, Rohde A, Merkord J, Rohde H, Hinz B. Decrease of plasminogen activator inhibitor-1 may contribute to the anti-invasive action of cannabidiol on human lung cancer cells. Pharm Res. 2010;27(10):2162-74.

114. Yamada T, Ueda T, Shibata Y, Ikegami Y, Saito M, Ishida Y, et al. TRPV2 activation induces apoptotic cell death in human T24 bladder cancer cells: a potential therapeutic target for bladder cancer. Urology. 2010;76(2):509 e1-7.

115. Torres S, Lorente M, Rodriguez-Fornes F, Hernandez-Tiedra S, Salazar M, Garcia-Taboada E, et al. A combined preclinical therapy of cannabinoids and temozolomide against glioma. Mol Cancer Ther. 2011;10(1):90-103.

116. Marcu JP, Christian RT, Lau D, Zielinski AJ, Horowitz MP, Lee J, et al. Cannabidiol enhances the inhibitory effects of delta9-tetrahydrocannabinol on human glioblastoma cell proliferation and survival. Mol Cancer Ther. 2010;9(1):180-9.

117. Solinas M, Massi P, Cinquina V, Valenti M, Bolognini D, Gariboldi M, et al. Cannabidiol, a non-psychoactive cannabinoid compound, inhibits proliferation and invasion in U87-MG and T98G glioma cells through a multitarget effect. PLoS One. 2013;8(10):e76918.

118. McKallip RJ, Jia W, Schlomer J, Warren JW, Nagarkatti PS, Nagarkatti M. Cannabidiol-induced apoptosis in human leukemia cells: A novel role of cannabidiol in the regulation of p22phox and Nox4 expression. Mol Pharmacol. 2006;70(3):897-908.

119. Aviello G, Romano B, Borrelli F, Capasso R, Gallo L, Piscitelli F, et al. Chemopreventive effect of the non-psychotropic phytocannabinoid cannabidiol on experimental colon cancer. J Mol Med (Berl). 2012;90(8):925-34.

120. Kozela E, Lev N, Kaushansky N, Eilam R, Rimmerman N, Levy R, et al. Cannabidiol inhibits pathogenic T cells, decreases spinal microglial activation and ameliorates multiple sclerosis-like disease in C57BL/6 mice. British Journal of Pharmacology. 2011;163(7):1507-19.

121. Rock EM, Bolognini D, Limebeer CL, Cascio MG, Anavi-Goffer S, Fletcher PJ, et al. Cannabidiol, a non-psychotropic component of cannabis, attenuates vomiting and nausea-like behaviour via indirect agonism of 5-HT(1A) somatodendritic autoreceptors in the dorsal raphe nucleus. British Journal of Pharmacology. 2012;165(8):2620-34.

122. Parker LA, Kwiatkowska M, Burton P, Mechoulam R. Effect of cannabinoids on lithium-induced vomiting in the Suncus murinus (house musk shrew). Psychopharmacology (Berl). 2004;171(2):156-61.

123. Kwiatkowska M, Parker LA, Burton P, Mechoulam R. A comparative analysis of the potential of cannabinoids and ondansetron to suppress cisplatin-induced emesis in the Suncus murinus (house musk shrew). Psychopharmacology (Berl). 2004;174(2):254-9.

124. Zanelati TV, Biojone C, Moreira FA, Guimaraes FS, Joca SR. Antidepressant-like effects of cannabidiol in mice: possible involvement of 5-HT1A receptors. British Journal of Pharmacology. 2010;159(1):122-8.

125. Olah A, Toth BI, Borbiro I, Sugawara K, Szollosi AG, Czifra G, et al. Cannabidiol exerts sebostatic and antiinflammatory effects on human sebocytes. J Clin Invest. 2014;124(9):3713-24.

126. Vollner L, Bieniek D, Korte F. [Hashish. XX. Cannabidivarin, a new hashish constituent]. Tetrahedron Lett. 1969(3):145-7.

127. Hill TD, Cascio MG, Romano B, Duncan M, Pertwee RG, Williams CM, et al. Cannabidivarin-rich cannabis extracts are anticonvulsant in mouse and rat via a CB1 receptor-independent mechanism. British Journal of Pharmacology. 2013;170(3):679-92.

128. De Petrocellis L, Orlando P, Moriello AS, Aviello G, Stott C, Izzo AA, et al. Cannabinoid actions at TRPV channels: effects on TRPV3 and TRPV4 and their potential relevance to gastrointestinal inflammation. Acta Physiol (Oxf). 2012;204(2):255-66.

129. Rock EM, Sticht MA, Duncan M, Stott C, Parker LA. Evaluation of the potential of the phytocannabinoids, cannabidivarin (CBDV) and Delta(9) -tetrahydrocannabivarin (THCV), to produce CB1 receptor inverse agonism symptoms of nausea in rats. British Journal of Pharmacology. 2013;170(3):671-8.

130. Ahmed SA, Ross SA, Slade D, Radwan MM, Zulfiqar F, Matsumoto RR, et al. Cannabinoid ester constituents from high-potency Cannabis sativa. J Nat Prod. 2008;71(4):536-42.

131. Radwan MM, Ross SA, Slade D, Ahmed SA, Zulfiqar F, Elsohly MA. Isolation and characterization of new Cannabis constituents from a high potency variety. Planta Medica. 2008;74(3):267-72.

132. Cascio MG, Gauson LA, Stevenson LA, Ross RA, Pertwee RG. Evidence that the plant cannabinoid cannabigerol is a highly potent alpha2-adrenoceptor agonist and moderately potent 5HT1A receptor antagonist. British Journal of Pharmacology. 2010;159(1):129-41.

133. Banerjee SP, Snyder SH, Mechoulam R. Cannabinoids: influence on neurotransmitter uptake in rat brain synaptosomes. J Pharmacol Exp Ther. 1975;194(1):74-81.

134. Evans AT, Formukong E, Evans FJ. Activation of phospholipase A2 by cannabinoids. Lack of correlation with CNS effects. FEBS letters. 1987;211(2):119-22.

135. Evans FJ. Cannabinoids: the separation of central from peripheral effects on a structural basis. Planta Medica. 1991;57(7):S60-7.

136. Grunfeld Y, Edery H. Psychopharmacological activity of the active constituents of hashish and some related cannabinoids. Psychopharmacologia. 1969;14(3):200-10.

137. Colasanti BK, Craig CR, Allara RD. Intraocular pressure, ocular toxicity and neurotoxicity after administration of cannabinol or cannabigerol. Exp Eye Res. 1984;39(3):251-9.

138. Borrelli F, Fasolino I, Romano B, Capasso R, Maiello F, Coppola D, et al. Beneficial effect of the non-psychotropic plant cannabinoid cannabigerol on experimental inflammatory bowel disease. Biochem Pharmacol. 2013;85(9):1306-16.

139. Pucci M, Rapino C, Di Francesco A, Dainese E, D'Addario C, Maccarrone M. Epigenetic control of skin differentiation genes by phytocannabinoids. British Journal of Pharmacology. 2013;170(3):581-91.

140. Wilkinson JD, Williamson EM. Cannabinoids inhibit human keratinocyte proliferation through a non-CB1/CB2 mechanism and have a potential therapeutic value in the treatment of psoriasis. J Dermatol Sci. 2007;45(2):87-92.

141. Han DS, Jung KH, Jung WY, Oh IK, Kang KU, Baek SH. Synthesis and cytotoxic effects of deoxy-tomentellin. Arch Pharm Res. 2000;23(2):121-7.

142. Valdeolivas S, Navarrete C, Cantarero I, Bellido ML, Munoz E, Sagredo O. Neuroprotective properties of cannabigerol in Huntington's disease: studies in R6/2 mice and 3-nitropropionate-lesioned mice. Neurotherapeutics. 2015;12(1):185-99.

143. Pagano E, Montanaro V, Di Girolamo A, Pistone A, Altieri V, Zjawiony JK, et al. Effect of Non-psychotropic Plant-derived Cannabinoids on Bladder Contractility: Focus on Cannabigerol. Nat Prod Commun. 2015;10(6):1009-12.

144. Robson P. Therapeutic aspects of cannabis and cannabinoids. Br J Psychiatry. 2001;178:107-15.

145. McPartland JM, Glass M, Pertwee RG. Meta-analysis of cannabinoid ligand binding affinity and receptor distribution: interspecies differences. British Journal of Pharmacology. 2007;152(5):583-93.

146. Pertwee RG, Howlett AC, Abood ME, Alexander SP, Di Marzo V, Elphick MR, et al. International Union of Basic and Clinical Pharmacology. LXXIX. Cannabinoid receptors and their ligands: beyond CB(1) and CB(2). Pharmacological Reviews. 2010;62(4):588-631.

147. Matsuyama SS, Fu TK. In vivo cytogenetic effects of cannabinoids. J Clin Psychopharmacol. 1981;1(3):135-40.

148. Perez-Reyes M, Timmons MC, Davis KH, Wall EM. A comparison of the pharmacological activity in man of intravenously administered delta9-tetrahydrocannabinol, cannabinol, and cannabidiol. Experientia. 1973;29(11):1368-9.

149. Karniol IG, Shirakawa I, Takahashi RN, Knobel E, Musty RE. Effects of delta9-tetrahydrocannabinol and cannabinol in man. Pharmacology. 1975;13(6):502-12.

150. Gong H, Jr., Tashkin DP, Simmons MS, Calvarese B, Shapiro BJ. Acute and subacute bronchial effects of oral cannabinoids. Clin Pharmacol Ther. 1984;35(1):26-32.

151. Jarbe TU, Hiltunen AJ. Cannabimimetic activity of cannabinol in rats and pigeons. Neuropharmacology. 1987;26(2-3):219-28.

152. Esposito G, Izzo AA, Di Rosa M, Iuvone T. Selective cannabinoid CB1 receptor-mediated inhibition of inducible nitric oxide synthase protein expression in C6 rat glioma cells. J Neurochem. 2001;78(4):835-41.

153. Coffey RG, Yamamoto Y, Snella E, Pross S. Tetrahydrocannabinol inhibition of macrophage nitric oxide production. Biochem Pharmacol. 1996;52(5):743-51.

154. Chesher GB, Dahl CJ, Everingham M, Jackson DM, Marchant-Williams H, Starmer GA. The effect of cannabinoids on intestinal motility and their antinociceptive effect in mice. British Journal of Pharmacology. 1973;49(4):588-94.

155. Karler R, Cely W, Turkanis SA. The anticonvulsant activity of cannabidiol and cannabinol. Life Sciences. 1973;13(11):1527-31.

156. Izzo AA, Fezza F, Capasso R, Bisogno T, Pinto L, Iuvone T, et al. Cannabinoid CB1-receptor mediated regulation of gastrointestinal motility in mice in a model of intestinal inflammation. British Journal of Pharmacology. 2001;134(3):563-70.

157. Pinto L, Izzo AA, Cascio MG, Bisogno T, Hospodar-Scott K, Brown DR, et al. Endocannabinoids as physiological regulators of colonic propulsion in mice. Gastroenterology. 2002;123(1):227-34.

158. Shook JE, Burks TF. Psychoactive cannabinoids reduce gastrointestinal propulsion and motility in rodents. J Pharmacol Exp Ther. 1989;249(2):444-9.

159. Farrimond JA, Whalley BJ, Williams CM. Cannabinol and cannabidiol exert opposing effects on rat feeding patterns. Psychopharmacology (Berl). 2012;223(1):117-29.

160. Juttler E, Potrovita I, Tarabin V, Prinz S, Dong-Si T, Fink G, et al. The cannabinoid dexanabinol is an inhibitor of the nuclear factor-kappa B (NF-kappa B). Neuropharmacology. 2004;47(4):580-92.

161. Chen Y, Buck J. Cannabinoids protect cells from oxidative cell death: a receptor-independent mechanism. J Pharmacol Exp Ther. 2000;293(3):807-12.

162. Verhoeckx KC, Korthout HA, van Meeteren-Kreikamp AP, Ehlert KA, Wang M, van der Greef J, et al. Unheated Cannabis sativa extracts and its major compound THC-acid have potential immuno-modulating properties not mediated by CB1 and CB2 receptor coupled pathways. Int Immunopharmacol. 2006;6(4):656-65.

163. Wohlfarth A. Pharmakokinetik und Metabolismus von Δ9-Tetrahydrocannabinolsäure A im Menschen: Freiburg University; 2012.

164. Wohlfarth A, Roth N, Auwarter V. LC-MS/MS analysis of Delta9-tetrahydrocannabinolic acid A in serum after protein precipitation using an in-house synthesized deuterated internal standard. J Mass Spectrom. 2012;47(6):778-85.

165. Moldzio R, Pacher T, Krewenka C, Kranner B, Novak J, Duvigneau JC, et al. Effects of cannabinoids Delta(9)-tetrahydrocannabinol, Delta(9)-tetrahydrocannabinolic acid and cannabidiol in MPP+ affected murine mesencephalic cultures. Phytomedicine. 2012;19(8-9):819-24.

166. Gill EW, Paton WD, Pertwee RG. Preliminary experiments on the chemistry and pharmacology of cannabis. Nature. 1970;228(5267):134-6.

167. ElSohly MA, deWit H, Wachtel SR, Feng S, Murphy TP. Delta9-tetrahydrocannabivarin as a marker for the ingestion of marijuana versus Marinol: results of a clinical study. Journal of Analytical Toxicology. 2001;25(7):565-71.

168. Meye FJ, Trezza V, Vanderschuren LJ, Ramakers GM, Adan RA. Neutral antagonism at the cannabinoid 1 receptor: a safer treatment for obesity. Mol Psychiatry. 2013;18(12):1294-301.

169. Christensen R, Kristensen PK, Bartels EM, Bliddal H, Astrup A. Efficacy and safety of the weight-loss drug rimonabant: a meta-analysis of randomised trials. Lancet. 2007;370(9600):1706-13.

170. Doggrell SA. Is rimonabant efficacious and safe in the treatment of obesity? Expert Opin Pharmacother. 2008;9(15):2727-31.

171. Thomas A, Stevenson LA, Wease KN, Price MR, Baillie G, Ross RA, et al. Evidence that the plant cannabinoid Delta9-tetrahydrocannabivarin is a cannabinoid CB1 and CB2 receptor antagonist. British Journal of Pharmacology. 2005;146(7):917-26.

172. Ma YL, Weston SE, Whalley BJ, Stephens GJ. The phytocannabinoid Delta(9)-tetrahydrocannabivarin modulates inhibitory neurotransmission in the cerebellum. British Journal of Pharmacology. 2008;154(1):204-15.

173. Batkai S, Mukhopadhyay P, Horvath B, Rajesh M, Gao RY, Mahadevan A, et al. Delta8-Tetrahydrocannabivarin prevents hepatic ischaemia/reperfusion injury by decreasing oxidative stress and inflammatory responses through cannabinoid CB2 receptors. British Journal of Pharmacology. 2012;165(8):2450-61.

174. Hollister LE. Structure-activity relationships in man of cannabis constituents, and homologs and metabolites of delta9-tetrahydrocannabinol. Pharmacology. 1974;11(1):3-11.

175. Tudge L, Williams C, Cowen PJ, McCabe C. Neural Effects of Cannabinoid CB1 Neutral Antagonist Tetrahydrocannabivarin on Food Reward and Aversion in Healthy Volunteers. Int J Neuropsychopharmacol. 2014.

176. Horder J, Harmer CJ, Cowen PJ, McCabe C. Reduced neural response to reward following 7 days treatment with the cannabinoid CB1 antagonist rimonabant in healthy volunteers. Int J Neuropsychopharmacol. 2010;13(8):1103-13.

177. Pertwee RG. GPR55: a new member of the cannabinoid receptor clan? British Journal of Pharmacology. 2007;152(7):984-6.

178. Riedel G, Fadda P, McKillop-Smith S, Pertwee RG, Platt B, Robinson L. Synthetic and plant-derived cannabinoid receptor antagonists show hypophagic properties in fasted and non-fasted mice. British Journal of Pharmacology. 2009;156(7):1154-66.

179. Wargent ET, Zaibi MS, Silvestri C, Hislop DC, Stocker CJ, Stott CG, et al. The cannabinoid Delta(9)-tetrahydrocannabivarin (THCV) ameliorates insulin sensitivity in two mouse models of obesity. Nutr Diabetes. 2013;3:e68.

180. O'Brien LD, Wills KL, Segsworth B, Dashney B, Rock EM, Limebeer CL, et al. Effect of chronic exposure to rimonabant and phytocannabinoids on anxiety-like behavior and saccharin palatability. Pharmacol Biochem Behav. 2013;103(3):597-602.

181. Cascio MG, Zamberletti E, Marini P, Parolaro D, Pertwee RG. The phytocannabinoid, Delta(9)-tetrahydrocannabivarin, can act through 5-HT(1)A receptors to produce antipsychotic effects. British Journal of Pharmacology. 2015;172(5):1305-18.

182. Garcia C, Palomo-Garo C, Garcia-Arencibia M, Ramos J, Pertwee R, Fernandez-Ruiz J. Symptom-relieving and neuroprotective effects of the phytocannabinoid Delta(9)-THCV in animal models of Parkinson's disease. British Journal of Pharmacology. 2011;163(7):1495-506.

183. Bolognini D, Costa B, Maione S, Comelli F, Marini P, Di Marzo V, et al. The plant cannabinoid Delta9-tetrahydrocannabivarin can decrease signs of inflammation and inflammatory pain in mice. British Journal of Pharmacology. 2010;160(3):677-87.

184. Makwana R, Venkatasamy R, Spina D, Page C. The effect of phytocannabinoids on airway hyper-responsiveness, airway inflammation, and cough. J Pharmacol Exp Ther. 2015;353(1):169-80.

185. Costa B, Colleoni M, Conti S, Parolaro D, Franke C, Trovato A, et al. Oral anti-inflammatory activity of cannabidiol, a non-psychoactive constituent of cannabis, in acute carrageenan-induced inflammation in the rat paw. Naunyn-Schmiedebergs Archiv fur Experimentelle Pathologie und Pharmakologie. 2004;369(3):294-9.

186. Carrier EJ, Auchampach JA, Hillard CJ. Inhibition of an equilibrative nucleoside transporter by cannabidiol: a mechanism of cannabinoid immunosuppression. Proc Natl Acad Sci USA. 2006;103(20):7895-900.

187. Ribeiro A, Ferraz-de-Paula V, Pinheiro ML, Vitoretti LB, Mariano-Souza DP, Quinteiro-Filho WM, et al. Cannabidiol, a non-psychotropic plant-derived cannabinoid, decreases inflammation in a murine model of acute lung injury: role for the adenosine A(2A) receptor. Eur J Pharmacol. 2012;678(1-3):78-85.

188. Sacerdote P, Martucci C, Vaccani A, Bariselli F, Panerai AE, Colombo A, et al. The nonpsychoactive component of marijuana cannabidiol modulates chemotaxis and IL-10 and IL-12 production of murine macrophages both in vivo and in vitro. J Neuroimmunol. 2005;159(1-2):97-105.

189. McPartland JM. Pathogenicity of Phomopsis ganjae on Cannabis sativa and the fungistatic effect of cannabinoids produced by the host. Mycopathologia. 1984;149-153.

190. Van Klingeren B, Ten Ham M. Antibacterial activity of delta9-tetrahydrocannabinol and cannabidiol. Antonie Van Leeuwenhoek. 1976;42(1-2):9-12.

191. Zuardi AW, Cosme RA, Graeff FG, Guimaraes FS. Effects of ipsapirone and cannabidiol on human experimental anxiety. J Psychopharmacol. 1993;7(1 Suppl):82-8.

192. Zuardi AW, Morais S, Guimaraes F, al e. Anti-psychotic effect of cannabidiol. Journal of Clinical Psychiatry. 1995;56(485-486).

193. Zuardi AW, Hallak JE, Dursun SM, Morais SL, Sanches RF, Musty RE, et al. Cannabidiol monotherapy for treatment-resistant schizophrenia. J Psychopharmacol. 2006;20(5):683-6.

194. Hallak JE, Machado-de-Sousa JP, Crippa JA, Sanches RF, Trzesniak C, Chaves C, et al. Performance of schizophrenic patients in the Stroop Color Word Test and electrodermal responsiveness after acute administration of cannabidiol (CBD). Rev Bras Psiquiatr. 2010;32(1):56-61.

195. Consroe P, Laguna J, Allender J, Snider S, Stern L, Sandyk R, et al. Controlled clinical trial of cannabidiol in Huntington's disease. Pharmacol Biochem Behav. 1991;40(3):701-8.

196. Mecha M, Feliu A, Inigo PM, Mestre L, Carrillo-Salinas FJ, Guaza C. Cannabidiol provides long-lasting protection against the deleterious effects of inflammation in a viral model of multiple sclerosis: a role for A2A receptors. Neurobiol Dis. 2013;59:141-50.

197. Castillo A, MR T, J F-R. The neuroprotective effect of cannabidiol in an in vitro model of newborn hypoxic-ischemic brain damage in mice is mediated by CB(2) and adenosine receptors. Neurobiol Dis. 2010;37:434–40.

198. Pazos MR, Cinquina V, Gomez A, Layunta R, Santos M, Fernandez-Ruiz J, et al. Cannabidiol administration after hypoxia-ischemia to newborn rats reduces long-term brain injury and restores neurobehavioral function. Neuropharmacology. 2012;63(5):776-83.

199. Fouad AA, Al-Mulhim AS, Jresat I. Cannabidiol treatment ameliorates ischemia/reperfusion renal injury in rats. Life Sciences. 2012;91(7-8):284-92.

200. Mechoulam R, Carlini EA. Toward drugs derived from cannabis. Naturwissenschaften. 1978;65(4):174-9.

201. Ames FR, Cridland S. Anticonvulsant effect of cannabidiol. S Afr Med J. 1986;69(1):14.

202. Trembly B, Sherman M. Double-blind clinical study of cannabidiol as a secondary anticonvulsant. Marijuana '90 International Conference on Cannabis and Cannabinoids; July 8–11; Kolympari, Crete1990.

203. Colasanti BK, Lindamood C, 3rd, Craig CR. Effects of marihuana cannabinoids on seizure activity in cobalt-epileptic rats. Pharmacol Biochem Behav. 1982;16(4):573-8.

204. Turkanis SA, Smiley KA, Borys HK, Olsen DM, Karler R. An electrophysiological analysis of the anticonvulsant action of cannabidiol on limbic seizures in conscious rats. Epilepsia. 1979;20(4):351-63.

205. Fusar-Poli P, Crippa JA, Bhattacharyya S, Borgwardt SJ, Allen P, Martin-Santos R, et al. Distinct effects of {delta}9-tetrahydrocannabinol and cannabidiol on neural activation during emotional processing. Arch Gen Psychiatry. 2009;66(1):95-105.

206. Hill AJ, Jones NA, Smith I, Hill CL, Williams CM, Stephens GJ, et al. Voltage-gated sodium (NaV) channel blockade by plant cannabinoids does not confer anticonvulsant effects per se. Neurosci Lett. 2014;566:269-74.

207. Farrimond JA, Whalley BJ, Williams CM. Non-Delta(9)tetrahydrocannabinol phytocannabinoids stimulate feeding in rats. Behav Pharmacol. 2012;23(1):113-7.

208. Holland ML, Panetta JA, Hoskins JM, Bebawy M, Roufogalis BD, Allen JD, et al. The effects of cannabinoids on P-glycoprotein transport and expression in multidrug resistant cells. Biochem Pharmacol. 2006;71(8):1146-54.

209. Silvestri C, Paris D, Martella A, Melck D, Guadagnino I, Cawthorne M, et al. Two non-psychoactive cannabinoids reduce intracellular lipid levels and inhibit hepatosteatosis. J Hepatol. 2015;62(6):1382-90.

210. Dennis I, Whalley BJ, Stephens GJ. Effects of Delta9-tetrahydrocannabivarin on [35S]GTPgammaS binding in mouse brain cerebellum and piriform cortex membranes. British Journal of Pharmacology. 2008;154(6):1349-58.

7 Appendices: Cannabinoid evidence tables

Table 3: Summary table for CBC

Pharmacological characteristic	Effective concentration/dose	Level of evidence	Source of evidence	Reference	Key findings
Anti-inflammatory	10–100 mg/kg (IV)	Preclinical	Mouse: LPS-induced paw edema	[49]	*CBC reduces paw edema*
	120–480 mg/kg (IP)	Preclinical	Rat: Carrageenan-induced paw edema	[50]	*CBC reduces paw edema*
	1 mg/kg	Preclinical	Mouse: Model of colitis	[2]	*CBC prevents inflammation of the colon*
	0.3–1.0 µmol/cm^2	Preclinical	Mouse: Topical croton oil induced ear edema	[51]	*CBC prevents inflammation*
Antifungal	0.39–25 µg/ml	Preclinical	In vitro: Agar well diffusion assay	[50]	*Antifungal effects of CBC*
Anticonvulsant	25–75 mg/kg (IP)	Preclinical	Mouse: Maximal Electroshock method	[46]	*Anticonvulsant effects of CBC*
Antibiotic	0.39–25 µg/ml	Preclinical	In vitro: Agar well diffusion assay	[50]	*Antibacterial effects of CBC*
Analgesic	30–100 mg/kg (IV)	Preclinical	Mouse: Tail-flick assay	[49]	*Analgesic effects of CBC in mice*
	3–6 nmol to PAG	Preclinical	Rat: Tail-flick assay	[44]	*Actions of CBC in PAG in analgesia*

Sedative	100 mg/kg (IV)	Preclinical	Mouse: Tetrad	[49]	*CBC produces tetrad effects*
	75 mg/kg (IP)	Preclinical	Mouse: Locomotor activity test	[46]	*CBC reduces spontaneous activity*
	50 mg/kg (IP)	Preclinical	Mouse: Body temperature	[47]	*CBC causes hypothermia*

Table 4: Summary table for CBDA

Pharmacological characteristic	Effective concentration/dose	Level of evidence	Source of evidence	Reference	Key findings
Anti-inflammatory	470 μM (IC_{50})	Preclinical	In vitro: COX assay	[56]	*CBDA inhibits COX*
	20 μM COX1, 2.2 μM COX2 (IC_{50}).	Preclinical	In vitro: COX assay	[57]	*CBDA inhibits COX*
Antibiotic	5.6 μM (MIC)	Preclinical	In vitro: Antibacterial assays	[63]	*CBD inhibits drug-resistant bacteria*
Antiemetic	Effective as low as 0.001 mg/kg (IP)	Preclinical	Rat and shrew: Gaping toward LiCl–paired taste and vomiting	[4] [58] [59] [54]	*CBDA has antiemetic effects in rodents*
Anticancer	5–25 μM	Preclinical	In vitro: Human breast cancer cells	[61] [60]	*CBDA inhibits breast cancer migration*
	20–30 μM	Preclinical	In vitro: Human leukaemia cells (ALL and AML)	[32]	*CBDA inhibits leukaemia cell proliferation*
	13–>25 μM	Preclinical	In vitro: Human and rat cancer cells	[62]	*CBDA inhibits tumour growth*
Antidiarrhoeal	1–30 μM	Preclinical	In vitro: Shrew intestine organ bath	[64]	*CBDA inhibits intestinal contraction*
Anxiolytic	Up to 1 mg/kg	Preclinical	Rat: Fear conditioning	[4]	*CBDA doesn't affect conditioned fear*

Antitussive	Tested up to 20 mM	Preclinical	Guinea pig: Citric acid-induced coughs	[184]	*CBDA doesn't inhibit cough*
Sedative	Up to 1 mg/kg	Preclinical	Mice: Locomotor activity	[4]	*CBDA doesn't affect locomotor activity*

Table 5: Summary table for CBD

Pharmacological characteristic	Effective concentration/dose	Level of evidence	Source of evidence	Reference	Key findings
Anti-inflammatory	Oral: 5–40 mg/kg	Preclinical	Rats: Carrageenan-induced inflammation in the rat paw	[185]	*CBD reduced edema and hyperalgesia*
	1 mg/kg, IP	Preclinical	Mice: Lipopolysaccharide-induced inflammation	[186]	*CBD suppressed serum TNF production induced by lipopolysaccharide*
	20mg/kg IP	Preclinical	Mice: Inflammatory acute lung injury model	[187]	*CBD decreases biological markers of inflammation*
	30 mg kg(-1) either oral and IP	Preclinical	Mice and cells: Production of interleukin (IL)-12 and IL-10	[188]	*CBD increased IL-12 and IL-10 with anti-inflammatory effects*
	1–10 mg/kg i.v	Preclinical	Mice and cells: Colitis induced in mice	[103]	*CBD reduced colon injury in mice and reactive oxygen species production and lipid peroxidation*
	10 mg/kg IP and 20 mg/kg Intra-rectal and 20 mg/kg oral	Preclinical	Mice: Application of CBD for colonic inflammation in mice	[104]	*CBD improved colonic inflammation. Oral CBD did not.*
	10mg/kg IP	Preclinical	Mouse and human cellular: Ulcerative colitis intestinal biopsies	[105]	*CBD downregulated biomarkers of enteric glia-mediated neuroinflammation*
Antifungal	Petroleum ether extract chromatograph	Preclinical	Fungus: Germination in petri dish	[189]	*CBD inhibited P. ganjae conidia germination and hyphal growth*

Antibiotic	0.5–2 µg/mL	Preclinical	Bacterial cultures: *Staphylococcus aureus*	63	*CBD inhibited Staphylococcus aureus growth*
	1–5 µg/mL	Preclinical	Bacterial cultures: Staphylococci and streptococci	190	*CBD inhibited growth in broth cultures.*
Analgesic	24 mg/day sublingual spray	NHMRC LII	Human clinical: RCT in 24 adult patients with neurological symptoms (e.g. MS, Spinal cord injury, brachius plexus damage)	102	*CBD reduced pain significantly* *No sedation*
	Oral 2.5–20 mg/kg (neuropathic) and 20 mg/kg (inflammatory)	Preclinical	Rats: Neuropathic (sciatic nerve constriction) and inflammatory pain (adjuvant intraplantar injection)	100	*CBD reduced hyperalgesia to thermal and mechanical stimuli.*
	3 nmol intra-vl-PAG microinjection	Preclinical	Rats and cellular: Extracellular electrical activity of ON/OFF neurons of the rostral ventromedial medulla and tail flick	44	*CBD reduced the ongoing activity of ON and OFF neurons and induced antinociception in the tail flick-test*
	2.5–10 mg/kg IP	Preclinical	Mice: Chemotherapy-induced neuropathic pain	101	*CBD prevented chemotherapy induced mechanical sensitivity*
Anxiolytic	Oral 1 mg/kg	NHMRC LII Note: healthy humans	Human: RCT in 8 healthy human adults	79	*CBD caused a reduction in THC induced anxiety;* *No sedation*
	Oral 300 mg	NHMRC LII	Human: Public speaking task in 10 healthy humans adults	191	*CBD reduced anxiety after the test* *No sedation*
	Oral 400 mg	NHMRC LII	Human: SPECT imaging RCT of 10 healthy male adult volunteers	5	*CBD decreased subjective anxiety and increased mental sedation*
	Oral 400 mg	NHMRC LII	Human: SPECT RCT in10 adult	90	*CBD reduced subjective anxiety –*

			patients with Social Anxiety Disorder		*reductions of brain activity in the limbic regions observed.* *No sedation*
	Oral 600 mg	NHMRC LII	Human: RCT simulated public speaking test of anxiety using adult healthy controls and treatment naive Social Anxiety Disorder patients	[89]	*CBD reduced anxiety, cognitive impairment and discomfort in giving speech* *No sedation*
Antipsychotic	Oral 1500 mg	NHMRC LIV	Human: *Single person case study in an adult psychotic patient*	[192]	*CBD reduced psychotic symptoms* *Unknown effect on sedation*
	Oral 1280 mg	NHMRC LIV	Human: case study (n=3 schizophrenic adults) over 30 days	[193]	*CBD reduced Brief Psychiatric Rating Scale scores* *No sedation*
	Oral 300mg and 600 mg	NHMRC LII	Human: RCT with Schizophrenic adult patients	[194]	*Low dose CBD) improved stroop colour word test but not high dose* *Unknown effect on sedation*
	Oral 800 mg/day	NHMRC LII	Human: RCT CBD vs amisulpride in 42 schizophrenic adult patients	[8]	*CBD improved PANSS scores (as did amisulpride) but with fewer side effects* *Unknown effect on sedation*
Antioxidant	10 mg/kg IP	Preclinical	Mice: DOX induced oxidative stress	[107]	*CBD improved DOX-induced oxidative/nitrative stress and cell death*
	5 mg/kg IP	Preclinical	Mice: ethanol gavage model of oxidative stress in liver	[108]	*CBD protects liver from alcohol-generated oxidative stress-induced steatosis*

	2–4 µM	Preclinical	In vitro: Rat cortical neuron cultures exposed to glutamate	[106]	*CBD prevented hydroperoxide-induced oxidative damage*
Antispasmodic	Oral CBD 700 mg/day for 6 weeks	NHMRC LII	Human: 15 neuroleptic-free adult patients with Huntington's Disease	[195]	*No improvement in chorea severity* *No sedation*
	5mg/kg IP	Preclinical	Mice: Microglial activation in a mouse model (autoimmune encephalomyelitis) of MS	[120]	*CBD ameliorates signs of autoimmune encephalomyelitis*
	5mg/kg IP	Preclinical	Mice: Murine encephalomyelitis virus-induced demyelinating disease	[196]	*CBD ameliorates motor deficits with reduced microglial activation and pro-inflammatory cytokine production*
Antiemetic	20 mg/kg SC	Preclinical	Shrew/Rats: antiemesis (shrews) and antinausea (rats) In vitro: 5-HT1A receptors	[121]	*CBD suppressed nicotine, LiCl and cisplatin induced vomiting and conditioned gaping in rats*
	2.5 mg/kg IP	Preclinical	Shrew: Lithium chloride (LiCl) induced vomiting model	[122]	*CBD lower doses produced suppression and higher doses producing enhancement of Li-induced vomiting*
	5 mg/kg IP	Preclinical	Shrew: Cisplatin induced vomiting test with CBD vs ondansetron	[123]	*CBD suppressed vomiting at 5 mg/kg but potentiated it at 40 mg/kg*
Antiischaemic	100 µM	Preclinical	In vitro: Experimentally induced hypoxic ischaema in brain slices of mice	[197]	*CBD reduced acute and apoptotic HI brain damage*
	1 mg/kg IV	Preclinical	Rats: Experimentally induced hypoxic ischaema	[198]	*CBD reduced brain excitotoxicity, oxidative stress and inflammation seven days after HI*

	1mg/kg IV	Preclinical	Pigs: Experimentally induced hypoxic ischaema	[110]	*CBD reduced viable neuron damage, improved EEG, reduced excitotoxicity, oxidative stress and inflammation (brain IL-1 levels)*
	0.1mg/kg -PV	Preclinical	Pigs: Experimentally induced hypoxic ischaema	[109]	*CBD improved brain tissue oxygenation during the first 3H after Hypoxic Ischaema, and partial EEG recovery*
	5 mg/kg, IV	Preclinical	Rats: Induced ischaema	[199]	*CBD ameliorated ischaemia/reperfusion-induced kidney damage*
Anticonvulsant	Oral or gastric tube 22.9 mg/kg (patient body weights not reported)	NHMRC LIV	Human open label single arm (no control) in 162 treatment resistant epileptics aged between 1 and 30 yrs old (mean age 10 yrs)	[92]	*CBD reduced seizure frequency by 34.6% across all epilepsy and seizure types* *Somnolence was reported in 25% of cases but effects of CBD confounded by concommittant AEDs*
	Oral 200–300mg/day	NHMRC LII	Human RCT: Chronic administration of CBD for 3–18 weeks to adult patients with temporal lobe epilepsy	[84]	*CBD improved seizure control* *Unknown effect on sedation*
	Oral 200mg/day	NHMRC LII	Human RCT: Non blinded with a placebo arm administered CBD for 3 months (age of participants not reported)	[200]	*2 of 4 receiving CBD were seizure free, 1 had partial improvement, 1 no change* *Unknown effect on sedation*

	-	NHMRC LII	Human RCT in treatment resistant epilepsy (letter to editor only, does not state age of participants)	[201]	*No difference in seizure frequency (Oral CBD 200–300 mg/day for 4 weeks)* *Unknown effect on sedation*
	-	NHMRC LII	Human RCT in adults with treatment resistant epilepsy	[202]	*No change in seizure frequency (Oral CBD 300 mg/day for 6 months with cross over)* *Unknown effect on sedation*
	120 mg/kg	Preclinical	Mice: Maximal electroshock model of epilepsy	[97]	*Significant anticonvulsant effects of CBD*
	-	Preclinical	Rats: Cobalt induced focal seizures	[203]	*Not therapeutically relevant at 60mg/kg on cobalt induced focal seizure*
	94.9–481.7 mg/kg IP	Preclinical	Mice: Transcorneal (electroshock) current or convulsant drugs in mice	[98]	*CBD significantly reduced seizures resulting from: MES, PIC, INH, PTZ and BIC* *Strychnine convulsions not reduced*
	0.3–3 mg/kg CBD IP	Preclinical	Rats: Electrically kindled seizures	[204]	*Significant antiepileptiform activtity*
	30 mg/kg IP	Preclinical	Mice: Forced swimming test	[124]	*CBD reduced immobility; 5-HT(1A) receptor blockade removed antidepressant effects* *CBD (3, 10, 100 mg/kg) did not reduce immobility*

Antidepressant	200mg/kg IP	Preclinical	Mice: Automated mouse forced swim (FST) and tail suspension (TST) tests	[48]	*Significant antidepressant effect*
	0.25 to 1.0 µM	Preclinical	In vitro: Kaposi sarcoma	[111]	*CBD inhibits growth and induces programmed cell death*
Anticancer	1.0–1.9 µmol/L	Preclinical	In vitro: Breast cancer	[112]	*CBD was able to inhibit Id-1 expression at the mRNA and protein level inhibiting metastatic breast cancer spread*
	0.01 µM or 0.1 µM	Preclinical	In vitro: Human lung cancer cell lines	[113]	*CBD caused 29% and 63% inhibition of A549 cell invasion*
	3 µmol and 30 µmol	Preclinical	In vitro: Urothelial carcinoma cells – viability assays	[114]	*The viability of T24 cells decreased with increasing concentrations of CBD*
	7.5 mg/kg/day	Preclinical	In vitro: Glioblastoma multiforme cell culture and xenografts into mice	[115]	*CBD reduces cell growth in TMZ resistant cells*
	0.4–1.4 µM	Preclinical	In vitro: Glioblastoma multiforme cell culture	[116]	*CBD enhanced the inhibitory properties of Δ9-THC on growth and survival*
	12.5 or 25 mg/kg	Preclinical	In vitro: Leukemia cell line viability	[118]	*CBD induced apoptosis*
	1 and 5 mg/kg in mice 0.01–10 µM In cell lines	Preclinical	Animal and In vitro: Chemopreventive effect of CBD on colon cancer in mice	[119]	*CBD reduced ACF, polyps and tumours in mice. In cell lines, CBD protected DNA from oxidative damage*
	1–12 µM	Preclinical	In vitro: Glioma cell line proliferation studies	[117]	*CBD inhibited U87-MG and T98G cell proliferation and invasiveness*

Sedative	Oral 160mg	NHMRC LII	Human: Blinded RCT in 15 adult Insomniac volunteers	[91]	*CBD increased sleep duration at 160 mg but not at 40 or 80 mg*
	Oral or gastric tube 22.9 mg/kg (patient body weights not reported)	NHMRC LIV	Human open label single arm (no control) in 162 treatment resistant epileptics aged between 1 and 30 yrs old (mean age 10 yrs)	[92]	*Somnolence was reported in 25% of cases but effects of CBD confounded by concommittant AEDs and lack of control group*
	Oral 300 and 600 mg	NHMRC LII	Human: RCT in Healthy adult humans	[6]	*Self report sedation at 300 and 600 mg CBD*
	Oral 200mg	NHMRC LII	Human: RCT schizophrenia model in healthy adult male volunteers	[7]	*CBD caused sedation*
	Oral 400 mg	NHMRC LII	Human: SPECT imaging RCT of 10 healthy male adult volunteers	[5]	*CBD decreased subjective anxiety and increased mental sedation on a VAS*
	-	NHMRC LII	Human: fMRI study of CBD and THC effects on emotion in adults.	[205]	*600 mg oral CBD DID NOT cause sedation*
	-	NHMRC LII	Human: RCT of 8 healthy human adults, double blind CBD vs to diazepam and placebo	[79]	*NOT sedating at 1 mg/kg oral*
Anti-psoriasis/skin disorders	1–10 µM	Preclinical	In vitro: Acne model cultured human sebocytes and human skin organ culture	[125, 139]	*Suppressed the proliferation of acne vulgaris*
	0.5 µM	Preclinical	In vitro: Skin cell growth and maturation	[139, 205]	*Repressed cell differentiation*

Table 6: Summary table for CBDV

Pharmacological characteristic	Effective concentration/dose	Level of evidence	Source of evidence	Reference	Key findings
Anti-inflammatory	0.3–1.0 µmol/cm^2	Preclinical	Mouse: Topical croton oil induced ear edema	[51]	*CBDV prevents inflammation*
Antiemetic	200 mg/kg (IP)	Preclinical	Rat: Gaping towards a LiCL-paired taste	[129]	*CBDV has anti-nausea effects*
Anticonvulsant	50–200 mg/kg (IP)	Preclinical	Rat: Variety of seizure models	[127]	*CBDV has anticonvulsant effects*
	87–422 mg/kg (CBDV-BDS, IP)	Preclinical	Rat: Variety of seizure models	[9]	*CBDV-BDS has anticonvulsant effects*
	10 µM	Preclinical	In vitro: Burst firing in hippocampal slices	[71]	*CBDV prevents epileptiform activity*

Table 7: Summary table for CBGA

Pharmacological characteristic	Effective concentration/dose	Level of evidence	Source of evidence	Reference	Key findings
Anti-inflammatory	460 µM COX1 and 200 µM COX2	Preclinical	In vitro: COX assay	[56]	*CBGA weakly inhibits COX1 and COX2*
Anti-leishmanial	33 µM IC_{50}	Preclinical	In vitro: *Leishmania Donovani*	[131]	*CBGA kills parasitic protozoa*
Antibiotic	5.5–11 µM MIC	Preclinical	In vitro: Antibacterial assay	[63]	*CBGA inhibits drug-resistant bacteria*
Anticancer	30–40 µM	Preclinical	In vitro: Leukaemia cells (ALL and AML)	[32]	*CBGA inhibits proliferation of leukaemia cells*

Table 8: Summary table for CBG

Pharmacological characteristic	Effective concentration/dose	Level of evidence	Source of evidence	Reference	Key findings
Anti-inflammatory	270 μM	Preclinical	In vitro: COX assay	[56]	*CBG weakly inhibits COX*
	1–10 μM 1, 5 and 30 mg/kg	Preclinical	In vitro: Peritoneal macrophages Mice: Chemical-induced colitis	[138]	*CBG reduces colitis*
Antioxidant	1–10 μM	Preclinical	In vitro: Reactive oxygen species in colon cells	[138]	*CBG has antioxidant effects*
	0.1–1 μM	Preclinical	In vitro: Mouse cultured dopamine neurons	[40]	*CBG doesn't affect neuronal cell viability, but has low efficacy prooxidant effects*
Antibiotic	47 μM IC_{50}	Preclinical	In vitro: Mycobacteria assay	[131]	*CBG kills mycobacterium intracellular*
	3–6 μM MIC	Preclinical	In vitro: Antibacterial assay	[63]	*CBG has antibacterial properties*
Antipsoriasis/skin disorders	0.5 μM	Preclinical	In vitro: Skin cells	[139]	*CBG inhibits skin cell growth and maturation*
	2.3 μM	Preclinical	In vitro: Skin cells	[140]	*CBG inhibits skin cell growth and maturation*

Anticancer	10–30 µM 3–10 mg/kg	Preclinical	In vitro: Human colorectal cancer cells; Mouse: xenograft model	[10]	*CBG kills colorectal cancer cells*
	10–15 µM	Preclinical	In vitro: Human leukaemia cells (ALL and AML)	[32]	*CBG inhibits proliferation of leukaemia cells*
	8–20 µM	Preclinical	In vitro Human and rat cancer cells	[62]	*CBG inhibits tumour growth*
	0.1–1 µM	Preclinical	In vitro: Neuroblastoma cells	[40]	*CBG modestly inhibits cell proliferation*
	45 µM	Preclinical	In vitro: KB cells	[141]	*CBG inhibits proliferation of oral carcinoma cells*
Huntington's disease (HD)	10 mg/kg	Preclinical	Mouse model of HD	[142]	*CBG displays neuroprotective, anti-inflammatory and antioxidant effects*
Bladder function	10 nM	Preclinical	In vitro: Mouse and human bladder organ bath	[143]	*CBG inhibits mouse and human bladder contractility*
Antiglaucoma	0.48 mg/day	Preclinical	Cat: IOP measurement	[137]	*CBG lowers intraocular pressure*
Anticonvulsant	Up to 200 mg/kg	Preclinical	Rat: PTZ-chemical-induced seizures	[206]	*CBG was not an anticonvulsant*
Antitussive	Tested up to 20 mM	Preclinical	Guinea pig: Citric acid-induced coughs	[184]	*CBG was ineffective in treating cough*

Appetite stimulant	No effect up to 17.6 mg/kg	Preclinical	Rat: Feeding behaviour	[207]	*CBG did not stimulate feeding*
Sedative	Up to 80 mg/kg	Preclinical	Mice: Tetrad	[48]	*CBG did not have tetrad effects*

Table 9: Summary table for CBN

Pharmacological characteristic	Effective concentration/dose	Level of evidence	Source of evidence	Reference	Key findings
Respiratory/bronchial dilator	-	NHMRC LII	Human: RCT compared to THC and CBD with a diazepam control.	[150]	*CBN 1200 mg oral was not a bronchodilator*
Safety – cytogenetic abnormalities	-	NHMRC LII	Human trial: Cytogenetic effects in a prospective double blind RCT study	[147]	*600 mg CBN oral daily for 20 days led to no chromosomal abnormalities.*
Immunosuppressant	700 nM	Preclinical	In vitro: Rat C6 glioma cells	[152]	*CBN inhibits LPS and IFNγ-induced nitrite release*
	10 µM	Preclinical	In vitro: Mice macrophages	[153]	*CBN inhibits IFNγ-induced nitrite release*
Neuroprotective	4 µM	Preclinical	In vitro: Mouse cortical neurons	[160]	*CBN inhibits anticancer drug and TNFα-induced apoptosis*
Anticonvulsant	250 mg/kg IP	Preclinical	Mice: Maximum electroshock test	[155]	*CBN has anticonvulsant effects*
Antibiotic	1 µg/ml (MIC)	Preclinical	In vitro: Antibacterial assay	[63]	*CBN inhibits bacterial growth*
Analgesic	50 mg/kg	Preclinical	Mice: Acetic acid-induced abdominal stretching	[41]	*CBN inhibits nociception*
	32 mg/kg	Preclinical	Mice: Hot-plate test	[154]	*CBN inhibits nociception*

Pharmacological characteristic	Effective concentration/dose	Level of evidence	Source of evidence	Reference	Key findings
Amyotrophic lateral sclerosis	5 mg/kg/day s.c. for 12 days	Preclinical	Mice: SOD transgenics	[11]	*CBN delays symptom onset in mouse model of ALS*
Antioxidant	nM concentrations	Preclinical	In vitro: Leukaemia and 3T3 cells	[161]	*CBN inhibits serum starved cell death*
	Not defined	Preclinical	Cyclic voltammetry	[106]	*CBN has antioxidant properties*
Anticancer	36 µM	Preclinical	In vitro: Human leukaemia cells (ALL)	[10, 208]	*CBN reverses multidrug resistance*
	0.1–10 µM	Preclinical	In vitro: Neuroblastoma cells	[40]	*CBN modestly reduced cell viability*
Antipsoriasis/skin disorders	2.1 µM	Preclinical	In vitro: Skin cells	[140]	*CBN inhibits proliferation of skin cells*
Appetite stimulant	26 mg/kg p.o.	Preclinical	Rat: Feeding behaviour	[207]	*CBN stimulated feeding*
Antidiarrhoeal	1.36 µmol/mouse	Preclinical	Mice: Croton oil inflammatory model	[156]	*CBN inhibits intestinal motility*
	11.2 mg/kg IC_{50}	Preclinical	Mice: colonic propulsion	[157]	*CBN inhibits colonic propulsion?*
	12–20 mg/kg	Preclinical	Rat: GIT motility	[158]	*CBN inhibits gastric emptying and small intestine motility*
Antiglaucoma	0.48 mg/day	Preclinical	Cat: IOP	[137]	*CBN lowers Intraocular pressure*

Pharmacological characteristic	Effective concentration/dose	Level of evidence	Source of evidence	Reference	Key findings
Euphoriant	CBN (200 µg/kg) IV	NHMRC LII	Human trial	[148]	*CBN was capable of producing a THC like high at high doses*
	Oral CBN 50 mg	NHMRC LII	Human trial	[149]	*CBN had no effect on any physiological or subjective measures of intoxication*

Table 10: Summary table for THCA

Pharmacological characteristic	Effective Concentration/Dose	Level of evidence	Source of evidence	References	Key findings
Anti-inflammatory	630–1700 µM	Preclinical	In vitro: COX assay	[56]	*THCA weakly inhibits COX*
	40–160 µM	Preclinical	In vitro: Macrophages	[162]	*THCA inhibits LPS-induced TNFα release*
Antioxidant	10 µM	Preclinical	In vitro: MPP toxicity in rat dopamine neurons	[165]	*CBN is a free radical scavenger and modestly reverses damage in in vitro model of PD*
Antiemetic	0.05 mg/kg IP at 0.043 µM in plasma	Preclinical	Rat and shrew: LiCl-induced gaping and vomiting	[12]	*THCA has antiemetic actions*

Table 11: Summary table for THCV

Pharmacological characteristic	Effective concentration/dose	Level of evidence	Source of evidence	Reference	Key findings
Metabolic	-	NHMRCLII	Human: fMRI RCT	[175]	*10 mg THCV increases neural responding to rewarding and aversive stimuli but not subjective response*
	3 mg/kg	Preclinical	Mice: Assessment of feeding behavior	[178]	*3 mg/kg reduced the food intake and body weight*
	0.1, 0.5, 2.5 and 12.5mg/kg	Preclinical	Mice: Insulin sensitivity in dietary induced (DIO) and genetically obese mice	[179]	*THCV dose-dependently reduced glucose intolerance*
	5 µM in zebrafish 12.5 mg/kg in obese mice	Preclinical	Animal and In vitro: Models of non alcoholic fatty liver disease	[209]	*THCV increased yolk lipid mobilization (zebrafish) and inhibited the development of hepatosteatosis (obese mice) respectively*
Anti-inflammatory	0.3 or 1 mg/kg-1 IP (Inflammation) 5 mg/kg IP (Pain)	Preclinical	Rat: Injection of carrageenan or formalin in a rat paw pain/analgesia model	[183]	*THCV can activate CB2 receptors in vitro and decrease signs of inflammation and inflammatory Pain.*
Analgesic	-	Preclinical	Mice: Acetic acid stretching test, a visceral pain model	[41]	*THCV (50 mg/kg) IP did not produce antinociceptive effects but blocked the antinociceptive effects of THC.*
Anxiolytic (Anxiogenic)	-	Preclinical	Rat: Light/Dark immersion model of anxiety	[180]	*THCV (2.5 mg/kg IV) was not anxiolytic (or anxiogenic)*

Antipsychotic	100 nM – 5-HT1A receptor activation 2 mg/kg – rat psychosis	Preclinical	Rat and in vitro: Rat brainstem and human 5-HT1A binding assays and rat phencyclidine model of psychosis	[181]	*THCV enhanced 5-HT1A receptor activation.* *THCV exhibited significant antipsychotic effects*
Antioxidant	2 mg/kg IP	Preclinical	Mice and rats: Neuroprotection in lesioned mice and rats	[182]	*THCV attenuated lesion related neuronal loss by antioxidant activity (or CB2 activation)*
Antiischaemic	68.4 nM (in vitro) 3 or 10 mg/kg IP (in mice)	Preclinical	Mice and in vitro: experimental ischaemia and In vitro CB2 binding assays	[173]	*D8-THCV activated CB2 receptors, and decreased tissue injury and inflammation in vivo*
Anticonvulsant	10–20 µM (in vitro) 0.25 mg/kg IP (in rats)	Preclinical	Rats and In vitro: Brain slices of epileptiform activity and a generalised PTZ seizure model	[14]	*THCV significantly reduced seizure activity in rat brain slice* *THCV significantly reduced seizure incidence in PTZ model in rats*
	1 and 5 µM	Preclinical	In vitro: Testing inhibitory effects on neurotransmission in mouse membranes	[210]	*1 and 5 µM THCV caused a non CB receptor depression in basal [35S]GTPgS binding*
	5–58 µM	Preclinical	In vitro: Inhibitory neurotransmission at interneurone-Purkinje cells (PC)	[172]	*THCV induced decreases in spike firing suggest a mechanism of PC inhibition*
Euphoriant	7 mg IV	NHMRC LII	Human: Exploratory administration to healthy humans to compare to THC effects	[174]	*THCV produced mild to moderate effects similar to THC but at ~25% strength*

APPENDIX 4

Cannabinoids in Low-THC Hemp Products - Analysis

Cannabinoids in Low THC Hemp Products

8 February 2016

Executive Summary

Application A1039 proposed to amend Standard 1.4.4 – Prohibited and Restricted Plants and Fungi of the Australia New Zealand Food Standards Code (the Code) to permit the sale of foods derived from the seeds of low delta-9-tetrahydrocannabinol (THC) hemp. The Application was rejected due to a lack of information on some areas such as law enforcement, road drug testing, levels of cannabidiol (CBD) and other cannabinoids, and marketing concerns. A number of projects were proposed to address the information gaps.

This project was undertaken to gather information on the levels of cannabinoids, including cannabidiol (CBD) and THC, in products which could potentially be available on the Australian market should low-THC hemp food products be approved.

A total of 200 products were purchased between July and September 2015. Samples were purchased from health food stores around NSW, New Zealand and online (both Australian and overseas based).

The country of origin for most samples was Australia, New Zealand or Canada. There were three products categories: hemp oil (seed oil and oil capsules), hemp powder (protein powder/flour and shake powder), and hemp seed. Products were tested to determine the level of major cannabinoids (CBD, CBD-A, THC, THC-A) and total cannabinoids.

The results are summarised in the Table 1. In general, oil products contained higher level of total cannabinoids compared to hemp powder and seed. The total cannabinoids for oil samples (non-capsules) ranged from 1.5 to 123 ppm, with an average of 49.4 ppm. The total cannabinoids for the four hemp oil capsule samples ranged from 40.2 to 76.5 ppm with an average of 58 ppm. All capsules contained low level of total THC (less than 10 ppm). 77 out of 78 protein powder/flour and shake powder contained low level of total cannabinoids, ranging from 0 to 20 ppm. Only one shake powder contained total cannabinoids of 46.3 ppm. In addition, all seed samples contained very low level of total cannabinoids (ranged from 0 to 9 ppm).

The level of total cannabinoids varied between products. There was no obvious correlation between where the products were purchased from and there was no clear correlation between the level of CBD and THC in products tested.

Lastly, there were a number of products that exceeded the THC levels proposed in the application A1039[1]; using the total THC levels - 29 (38%) of the oil products, two (3.6%) of hemp protein powder and three (13%) of hemp shakes exceeded the levels proposed[2].

The results of analysis of these products have been shared with Food Standards Australia New Zealand (FSANZ) to assist with a dietary assessment exercise to inform the overall project objective to determine if a level for cannabidiol (CBD) and/or other phytocannabinoids need to be set for low-THC hemp foods to ensure they do not provide therapeutic levels at normally consumed levels to distinguish low-THC foods from therapeutic goods.

[1] A1039 proposed a maximum THC limit of 10 mg/kg (ppm) in hemp oil products and 5 mg/kg in hemp flour and hemp seed (FSANZ, 2012)

[2] Using just the THC levels – 11 (14%) of the oil products, two (3.6%) of hemp protein powder and three (13%) of hemp shakes exceeded the levels proposed

Table 1: Summary of Analysis Results

Product	Recommended serving size	Total CBD[3] (range – ppm)	Total CBD[3] (average – ppm)	Total THC[4] (range - ppm)	Total THC[4] (average – ppm)	Total cannabinoids[5] (range – ppm)	Total cannabinoids[5] (average – ppm)
Hemp oil (non-capsule) n= 73	15 – 30 ml	0 – 111.1	34.9	0 – 114.5 38% of samples >10ppm[6]	11.8	1.5 – 123.4	49.1
Hemp oil (capsule) n= 4	3 – 6 capsules	36.1 – 76.5	53.2	0 – 8.6	4.2	40.2 – 76.5	58
Hemp powder/flour (100% hemp) n=55	10 – 32 g	0 – 15	4.5	0 – 7.3 3.6% of samples >5ppm[7]	0.6	0 – 17	5.3
Hemp powder – shake powder (hemp protein as an ingredient) n=23	10 – 32 g	0 – 10.7	3.7	0 – 34.4 13% of samples >5ppm[7]	2.8	0 – 46.3	6.8
Hemp seed n= 45	15 – 50 g	0 – 8.7	1.4	0 – 2.8 All samples <5ppm[8]	0.3	0 – 9	1.9

[3] Total CBD = CBD + CBD-A
[4] Total THC = THC+THC-A
[5] Total cannabinoids mean the sum of CBD, CBD-A, THC, THC-A and THCV-A
[6] Maximum THC limit proposed by FSANZ for hemp oil (A1039, 2012)
[7] Maximum THC limit proposed by FSANZ for hemp powder (A1039, 2012)
[8] Maximum THC limit proposed by FSANZ for hemp seed (A1039, 2012)

Background

Application A1039 proposed to amend Standard 1.4.4 – Prohibited and Restricted Plants and Fungi of the Australia New Zealand Food Standards Code (the Code) to permit the sale of foods derived from the seeds of low delta-9-tetrahydrocannabinol (THC) hemp. The Ministerial Forum for Food Regulation (FoFR) rejected this application on 30 January 2015 due a lack of information on the issues outlined below and requested the Food Regulation Standing Committee (FRSC) to address these gaps.

The application was rejected due to concerns about information gaps relating to:

1. Law enforcement;
2. roadside drug testing;
3. levels of cannabidiol and other cannabinoids; and
4. marketing concerns.

This project provides information to address item (3) above.

Aim

The aim of this project was to gather information on the levels of cannabinoids, including cannabidiol (CBD) and tetrahydrocannabinol (THC), in products which could potentially be available on the Australian market should low-THC food products be approved.

Materials and Methods

Samples

A total of 200 samples were purchased between July and September 2015. There were three categories of product:

1. Hemp oil
   - seed oil; and
   - oil capsules
2. Hemp powder
   - protein powder/flour (100% hemp as the ingredient); and
   - shake powder (hemp as an ingredient)
3. Hemp seed (hulled)

Table 2. The number of samples for each product category

Category	Sub category	Number of samples
Hemp oil	Seed oil	73
	Capsules	4
Hemp powder	Protein powder / flour	55
	Shake powder	23
Hemp seed	Hulled	45
Total		**200**

Samples were purchased from health food stores around NSW, New Zealand and online (both Australian and overseas based).

The country of origin of the products was also noted. Most samples were from Australia, New Zealand or Canada (**Table 3**).

Table 3. The number of samples according to the country of origin

Country of origin	Hemp oil	Hemp powder	Hemp seed
Australia	23	22	9
New Zealand	13	28	5
Canada	28	21	13
Other countries (e.g. China, UK, Mongolia, USA)	5	4	14
Not stated on the labels	8	3	4

Prior to analysis, samples were kept under temperature control (similar to the condition that the products were sold) and in the original packaging. All products were tested before their use-by-date.

Method of analysis

Analysis was conducted by the Southern Cross Plant Science Laboratory at the Southern Cross University. Southern Cross Plant Science has expertise in the area of industrial hemp research and analysis.

The testing was done using LC-MS HPLC to determine the level of major cannabinoids (CBD, CBD-A (cannabidiolic acid), THC, THC-A (tetrahydrocannabinolic acid)) and total cannabinoids. Where cannabinoids not included in the list were present, they have been calculated as CBD. THCV-A (tetrahydrocannabivarin acid) has also been reported because some samples contained appreciable amounts of this.

Table 4. Limit of detection (LOD), limit of quantification (LOQ) and uncertainty of measurement for each analyte

Analyte	LOD (ppm)	LOQ (ppm)	Uncertainty of measurement +/- (%)
CBD	0.05	0.5	4.61
CBD-A	0.05	0.5	1.83
THC	0.5	1	8.10
THC-A	0.2	0.5	3.86

Each sample was tested in triplicate (A, B, C) with each sub-sample tested in duplicate. So each sample resulted in six readings for each analyte. In this report, the average value of the six readings is reported as the result for each sample. All results are expressed in ppm (mg/kg).

In this report:

- total CBD means the sum of CBD and CBD-A;
- total THC means the sum of THC and THC-A;
- total cannabinoids mean the sum of CBD, CBD-A, THC, THC-A and THCV-A.

Results and Discussion

The results are summarised in the **Appendix 1.** The frequency distribution for total CBD, total THC and total cannabinoids for each type of product is presented in **Appendix 2.**

Hemp oil

There were two distinct types of hemp oil products:

- Hemp seed oil – when stated on the labels, most of the products claimed that the oil was obtained using a cold-press method. Cold pressing refers to oils obtained through pressing seeds without using any heat or solvent. Oil samples were packaged in glass or plastic bottles. Samples purchased from retail shops were generally displayed under refrigeration but all samples purchased online were delivered at ambient temperatures. About three quarters of oil products had labels that stated refrigeration is needed at all times or after opening.
- Hemp oil capsules – products were sold in a plastic container at room temperature. They were sold as an essential fatty acid (omega 3 & 6) supplements.

A total of 73 hemp oil and four hemp oil capsules were tested.

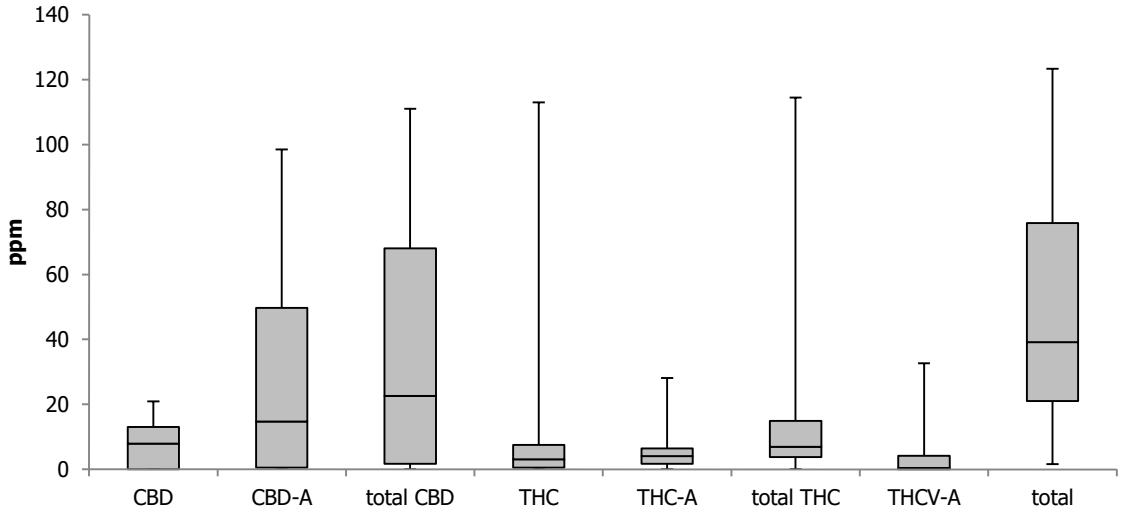
The total cannabinoids for oil samples (non-capsules) ranged from 1.5 to 123 ppm, with an average of 49.1 ppm **(Figure 1)**. Seven samples were found to contain total cannabinoids of greater than 100 ppm. The top two highest products were:

- A Canadian product purchased online from a US website. It contained total cannabinoids of 123 ppm which comprised of total CBD at the level of 111 ppm and 12 ppm total THC. Four other oil samples from the same company (all different batches, purchased in Australia and overseas) were tested and all had variable levels of total cannabinoids with the level of total CBD ranging from 21 to 111 ppm (average 44 ppm) and the total THC level ranging from 3.7 to 8.5 ppm.
- An Australian product and contained total cannabinoids of 123 ppm (total CBD level of 8 ppm and THC of 115 ppm). The product was purchased online and labelled as carrier oil.

The total cannabinoids for the four hemp oil capsule samples ranged from 40.2 to 76.5 ppm with an average of 58 ppm. All products contained low level of total THC (less than 10 ppm).

A1039 proposed a Maximum THC limit of 10 mg/kg (ppm) in hemp oil products (FSANZ, 2012). Using that limit as a guide, 29 (38%) and 11 (14%) of products would be classified as non-compliant based on the levels for total THC and THC only, respectively.

Figure 1. Box plots for hemp oil samples



Hemp powder

Two sub-categories of hemp powder were sampled:

- Hemp protein powder or flour – products with 100% hemp as the ingredient. The labels stated the products could be mixed with water, juices, or milk to create shakes, smoothies or power drinks. They could also be added to baked goods, pancakes or oatmeal to boost protein content.
- Hemp shake powder – products with hemp protein as an ingredient. Other common ingredients were vegetable proteins, spices and flavouring. The proportion of hemp protein in these products was not stated on the labels.

A total of 55 hemp protein powder/flour samples were tested. Total cannabinoids levels were low ranging from 0 to 17 ppm, with an average of 5.3 ppm **(Figure 2)**. The highest level of total cannabinoids was detected in a Canadian product purchased from a USA website. This product also had the highest level of total THC at 7.3 ppm.

A further 23 hemp shake powder products were also tested. The total cannabinoids levels were low for 22 of these, ranging from 0 to 20 ppm with an average of 5 ppm **(Figure 3)**. However, one product had a total cannabinoids concentration of 46.3 ppm with total CBD of 10.7 ppm and total THC of 34.4 ppm. This was a New Zealand product purchased directly from the manufacturer's website. Another batch of the same product contained a total cannabinoids level of 5.7 ppm, with no THC.

A1039 proposed a Maximum THC Limit of 5 mg/kg (ppm) for hemp powder products (FSANZ, 2012). Using that limit as a guide, two (3.6%) powder/flour samples and three (13%) shake samples would be considered non-compliant.

Figure 2. Box plots for hemp protein powder/flour samples

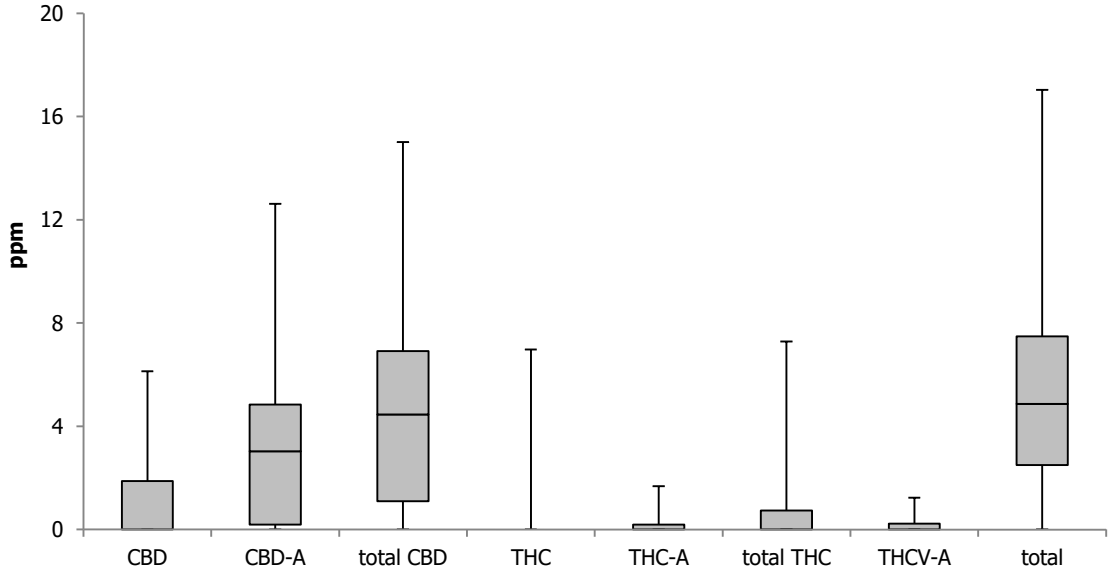
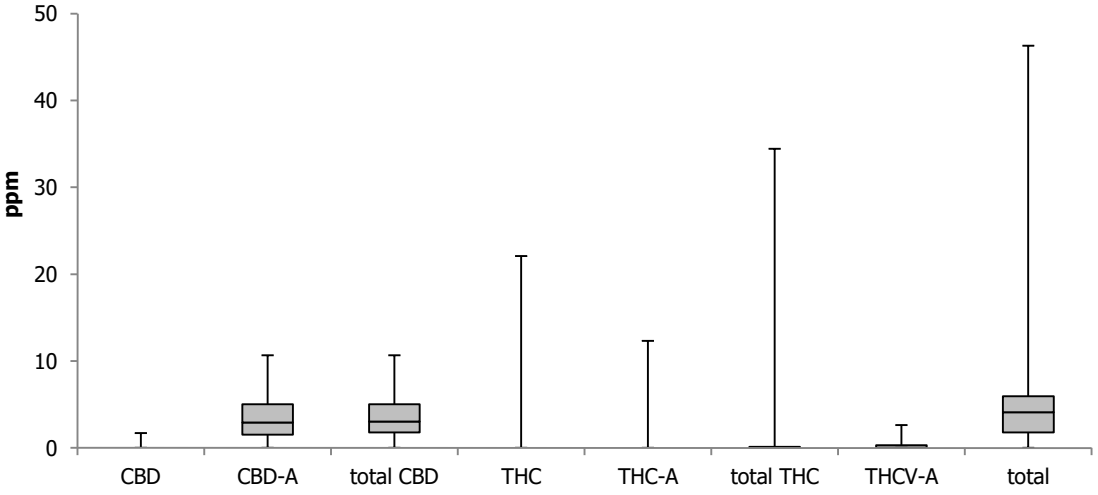


Figure 3. Box plots for hemp shake powder samples

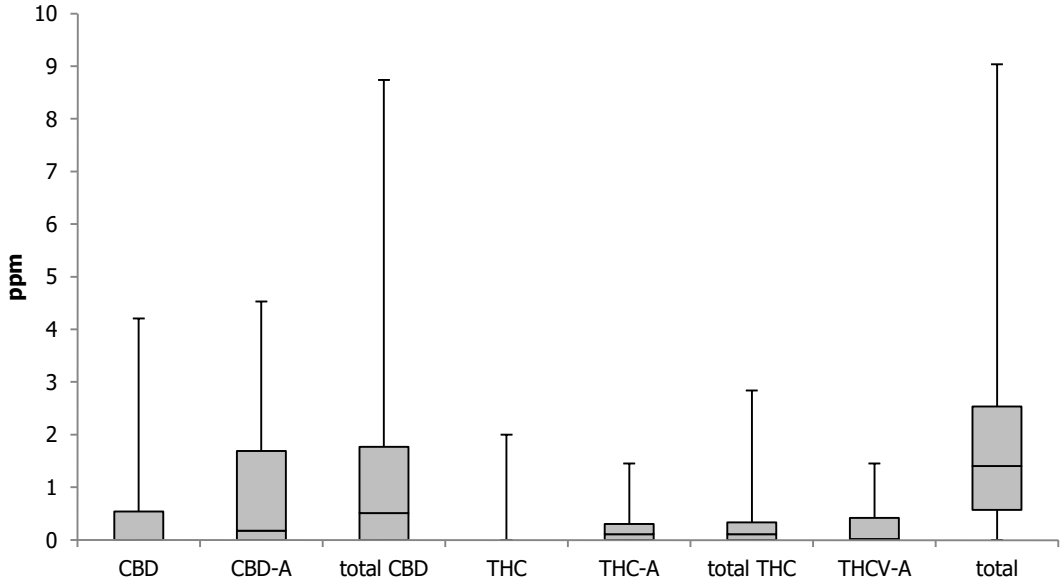


Hemp seed

A total of 45 samples of hulled hemp seed were tested. Total cannabinoids levels were very low, ranging from 0 to 9 ppm with an average of 1.9 ppm **(Figure 4)**. The highest level of total cannabinoids was detected in a Canadian product purchased in Australia. The cannabinoids in this product were mostly CBD at the level of 8.7 ppm.

A1039 proposed a Maximum THC Limit of 5 mg/kg (ppm) in hemp seed products (FSANZ, 2012). Using that limit as a guide, all of the samples would be considered compliant.

Figure 4. Box plots for hemp seed samples



Batch variation

When possible, a number of samples of the same product were purchased to observe variation between different batches and within a batch. Most samples from the same batch had similar concentration of total cannabinoids. No significant variation was observed between batches either, except for one product (oil B) **(Table 5)**.

Table 5. Batch variability observations

Product	No of samples	Findings
Oil A	8	Products came from 7 batches. The total cannabinoids levels ranged from 15 to 22.5 ppm, with an average of 19.9 ppm. The two products that came from the same batch had total cannabinoids levels of 19.6 and 21.1 ppm.
Oil B	7	Products came from 3 batches. The total cannabinoids levels ranged from 35.9 to 79.9 ppm, with an average of 52.7 ppm. Batch 1 (3 samples) total cannabinoids = 35.9, 37.4 and 38 ppm Batch 2 (3 samples) total cannabinoids = 55.9, 58.6 and 63 ppm Batch 3 (1 sample) total cannabinoids = 80 ppm
Protein powder A	11	Products came from 9 batches. The total cannabinoids levels ranged from 0 to 5.5 ppm, with an average of 2 ppm. The two products that came from the same batch had total cannabinoid levels of 2.2 and 2.5 ppm.
Protein powder B	6	Products came from 2 batches. The total cannabinoids levels ranged from 2.5 to 8 ppm, with an average of 5.3 ppm. Batch 1 (2 samples) – total cannabinoids = 2.8 and 2.6 ppm Batch 2 (4 samples) – total cannabinoids = 8, 8, 4.2, 6.2 ppm
Seed A	9	All products came from different batches. The total cannabinoids ranged from 0 to 2.9 ppm, with an average of 0.5 ppm.
Seed B	5	All products came from different batches. The total cannabinoids levels ranged from 0.5 to 3.5 ppm, with an average of 1.5 ppm.

Point of purchase variation

Samples were purchased in Australia, either at retail or online, New Zealand, and through overseas websites. To simplify the analysis, only the mean value of the total CBD, total THC and total cannabinoids are presented in **Table 7**.

Oil products purchased from NZ and international websites had an average total cannabinoids level of 64 ppm, whereas oil products purchased from Australia (both from stores and online) had an average of 44 ppm. For all other product groups, there was no significant difference in average total cannabinoids level based on point of purchase.

Table 6 outlines the number of samples that would exceed the THC limits proposed in the A1039 based on the point of purchase.

Table 6. Number of samples exceeding the proposed maximum limit for total THC based on point of purchase

Place of purchase	Hemp oil	Hemp oil capsule	Hemp protein powder/flour	Hemp shake	Hemp seed
Australia (store)	11/25 (44%)	0/1	0/19	-	0/24
Australia (online)	14/30 (47%)	-	1/21 (5%)	1/5 (20%)	0/13
New Zealand	1/10 (10%)	0/3	0/7	2/16 (13%)	-
Overseas	3/8 (38%)	-	1/8 (13%)	0/2	0/8

Table 7. Average value of total CBD, total THC and total cannabinoid for products based on the point of purchase

	oil			oil capsule			protein powder			shake			seed		
	total CBD	total THC	total cannabinoids	total CBD	total THC	total cannabinoids	total CBD	total THC	total cannabinoids	total CBD	total THC	total cannabinoids	total CBD	total THC	total cannabinoids
Australia (store)	31.7	9.6	43	44.8	5.3	50.7	4	0.3	4.4				1	0.2	1.4
Australia (online)	28.2	14.2	45.3				4.4	0.6	5.1	4.2	2.4	6.9	2.2	0.3	2.8
NZ	56.8	6.6	63.7	56	3.8	60.5	6.8	0.1	7.1	3.7	3.3	7.3			
overseas	42.7	16.7	64.2				4	1.9	6.4	2	0.2	2.2	1	0.6	2

Summary of Findings

- In general, oil products contained higher level of total cannabinoids compared to hemp powder and seed.
- The level of CBD and THC in products varied between products. There was no obvious correlation between where the products were purchased.
- There was no clear correlation between the level of CBD and THC in products tested.
- There were a number of products that exceeded the THC levels proposed in the application (A1039); using the total THC levels, 29 (38%) of the oil products, two (3.6%) of hemp protein powder and three (13%) of hemp shakes.
- Setting limits means that industry will need to test their products before releasing them to the market. Regulatory bodies may also need to do random testing of products for compliance.

References

Elixinol. (n.d*). What are the CBD oil benefits?* Retrieved 12 January 2016 from https://elixinol.com/education/cbd-is-good-for-me/

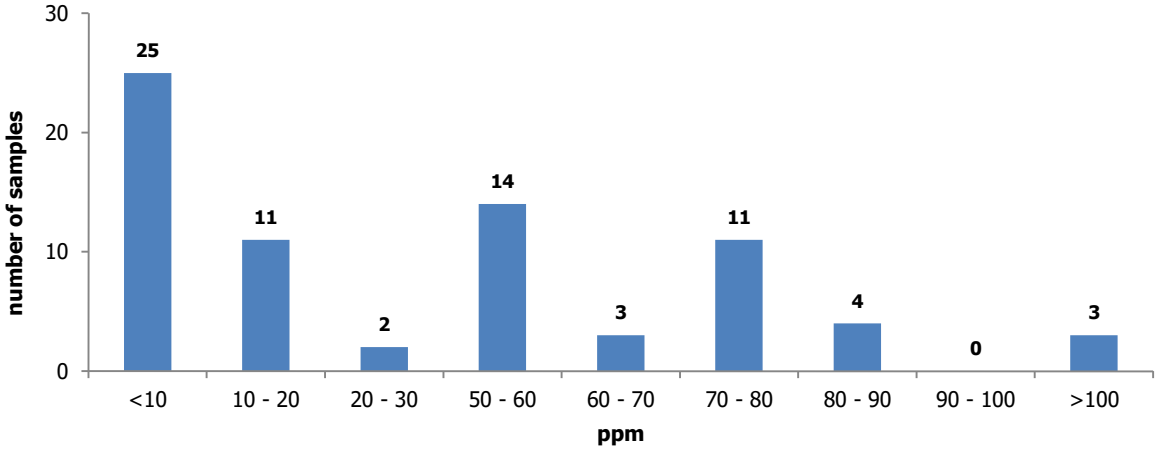
FSANZ. (2012). *Supporting Document 1. Safety Assessment (Approval) – Application A1039. Low THC Hemp as a Food.* Retrieved 12 January 2016 from http://www.foodstandards.gov.au/code/applications/documents/A1039_SD1.pdf

Appendix 1. Average, standard deviation, minimum and maximum value of total CBD, total THC and total cannabinoids per product type

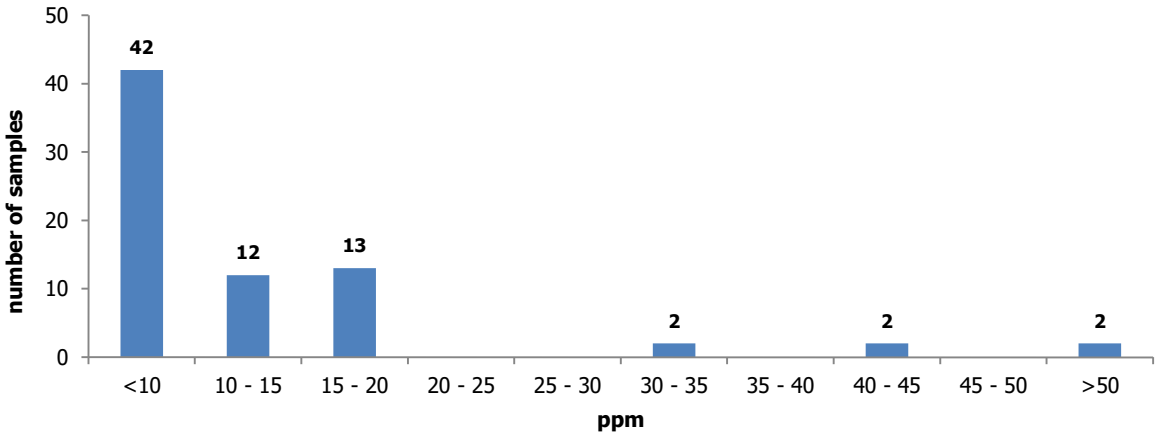
Type of product	Sub-category	No of samples	total CBD				total THC				total cannabinoids			
			average	sd	min	max	average	sd	min	max	average	sd	min	max
Oil	Hemp oil	73	34.9	33.1	0	111.1	11.8	16.3	0	114.5	49.1	31	1.5	123.4
	Hemp oil capsule	4	53.2	17.4	36.1	76.5	4.2	3.7	0	8.6	58	15.9	40.2	76.5
Powder	Protein powder / flour	55	4.5	3.7	0	15	0.6	1.4	0	7.3	5.3	3.9	0	17
	shakes	23	3.7	2.8	0	10.7	2.8	7.9	0	34.4	6.8	9.9	0	46.3
Seed		45	1.4	1.9	0	8.7	0.3	0.5	0	2.8	1.9	1.9	0	9

Appendix 2. Frequency distribution for total CBD, total THC and total cannabinoids for each type of products

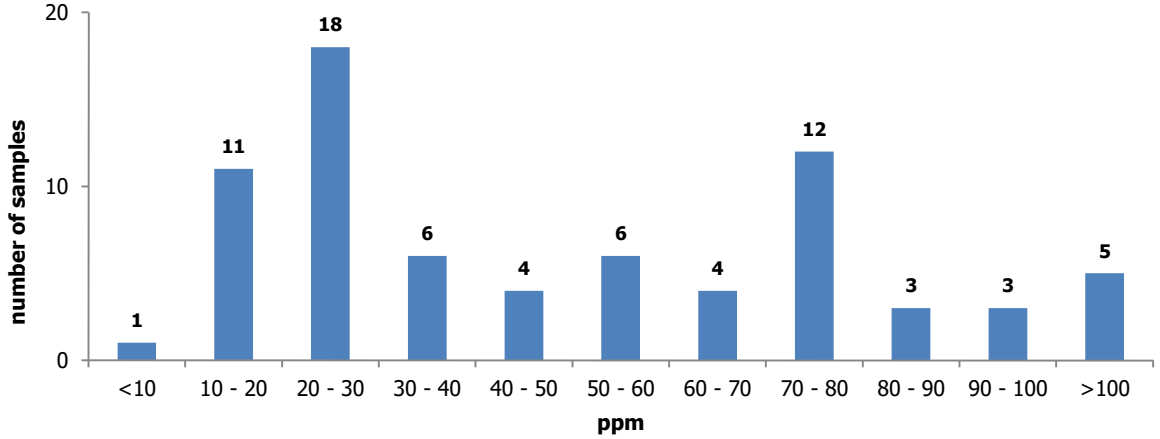
Frequency distribution for total CBD in hemp oil samples (n = 75)



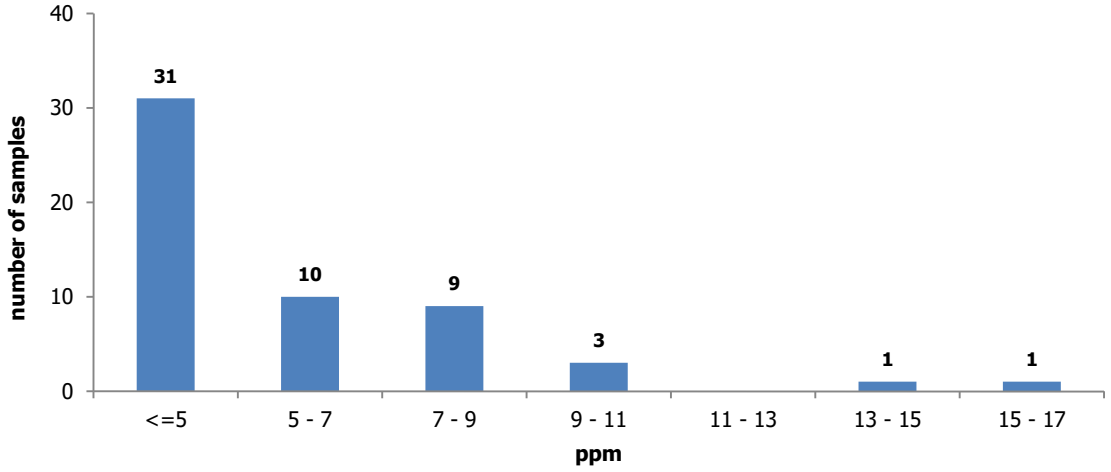
Frequency distribution for total THC in hemp oil samples (n = 75)



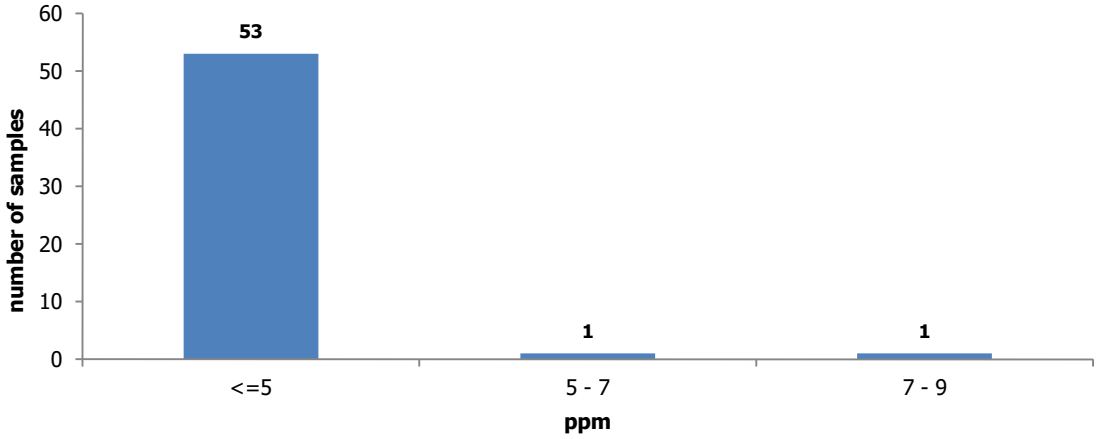
Frequency distribution for total cannabinoids in hemp oil samples (n = 75)



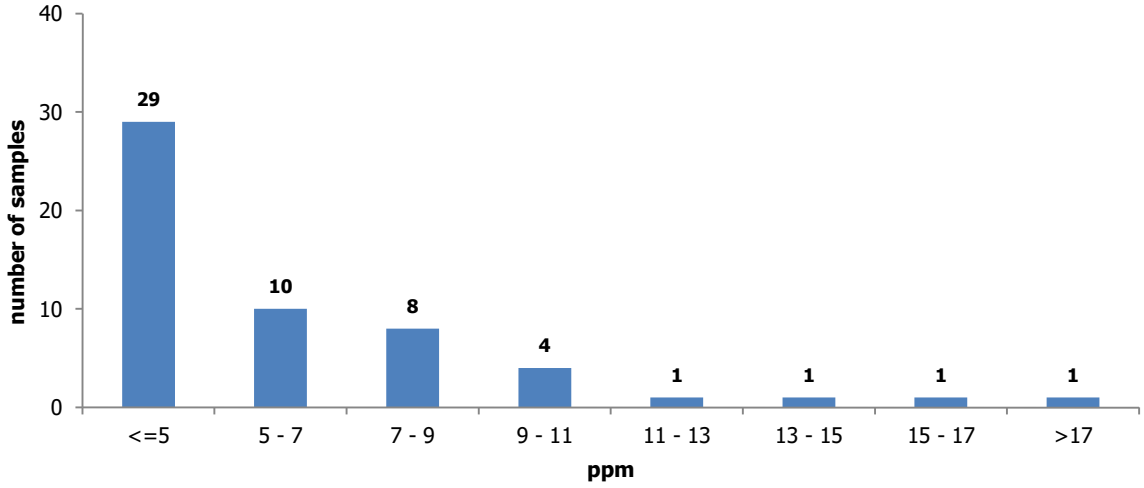
Frequency distribution for total CBD in hemp powder/flour samples (n = 55)



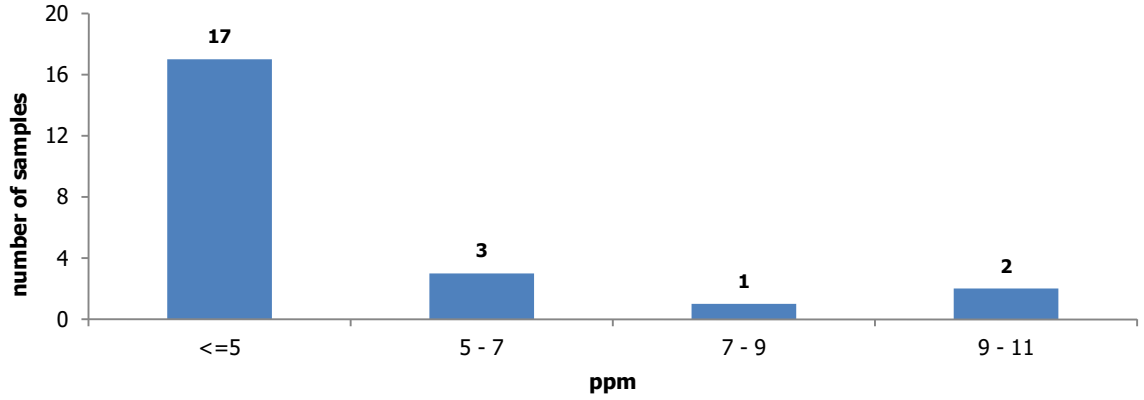
Frequency distribution for total THC in hemp powder/flour samples (n = 55)



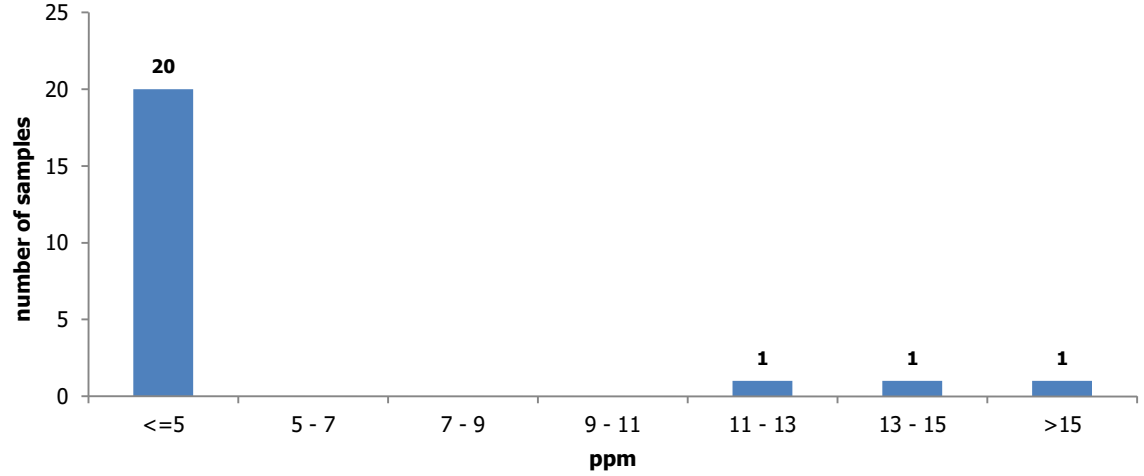
Frequency distribution for total cannabinoids in hemp powder/flour samples (n = 55)



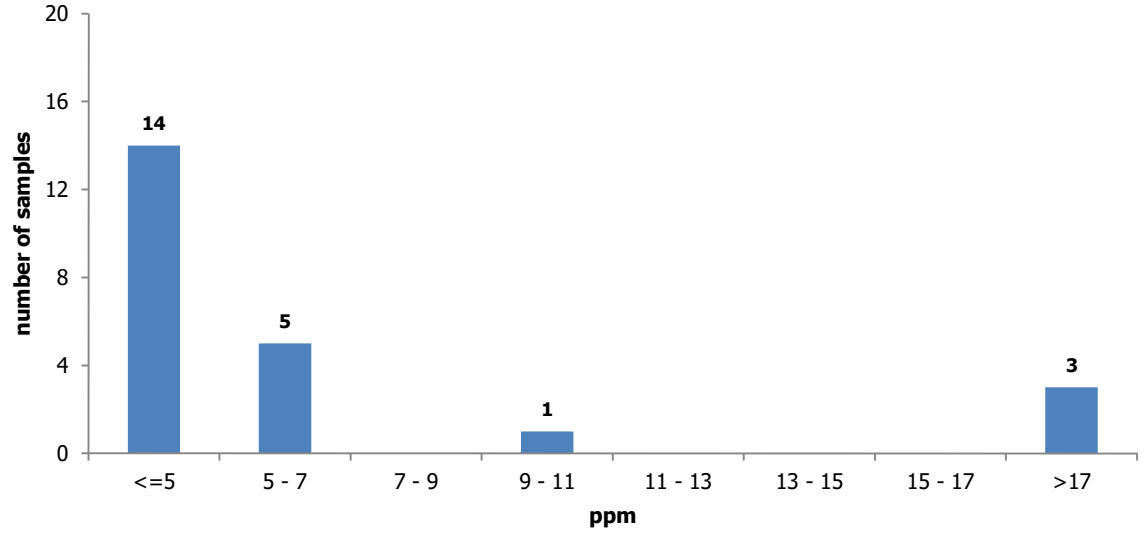
Frequency distribution for total CBD in hemp shake samples (n = 23)



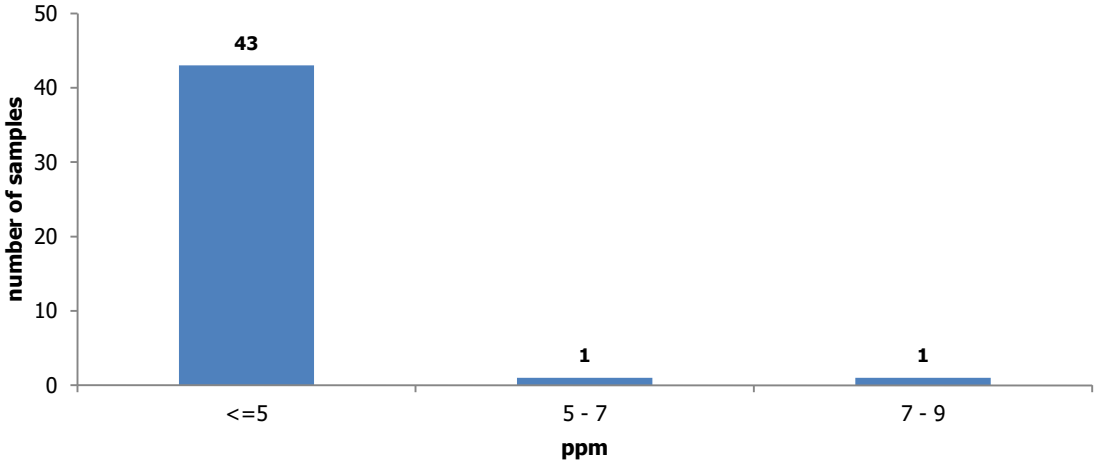
Frequency distribution for total THC in hemp shake samples (n = 23)



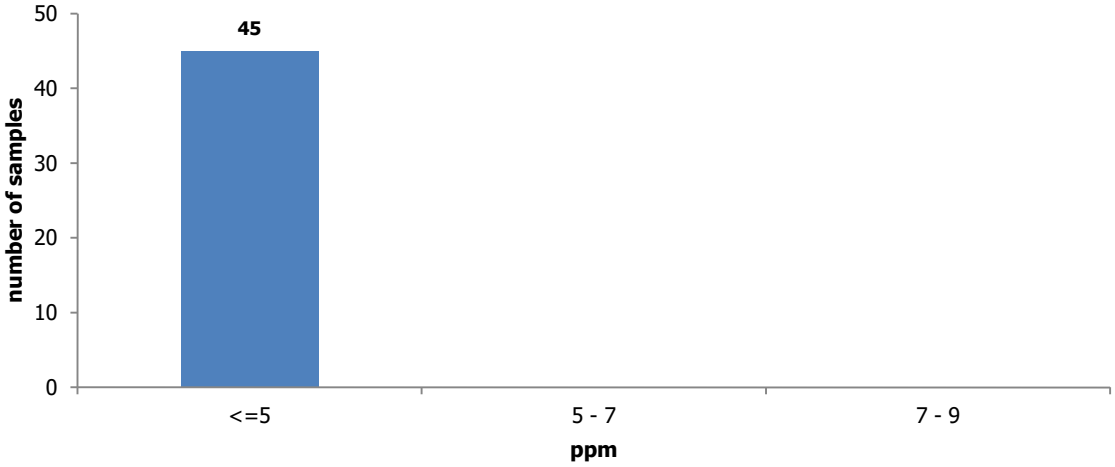
Frequency distribution for total cannabinoids in hemp shake samples (n = 23)



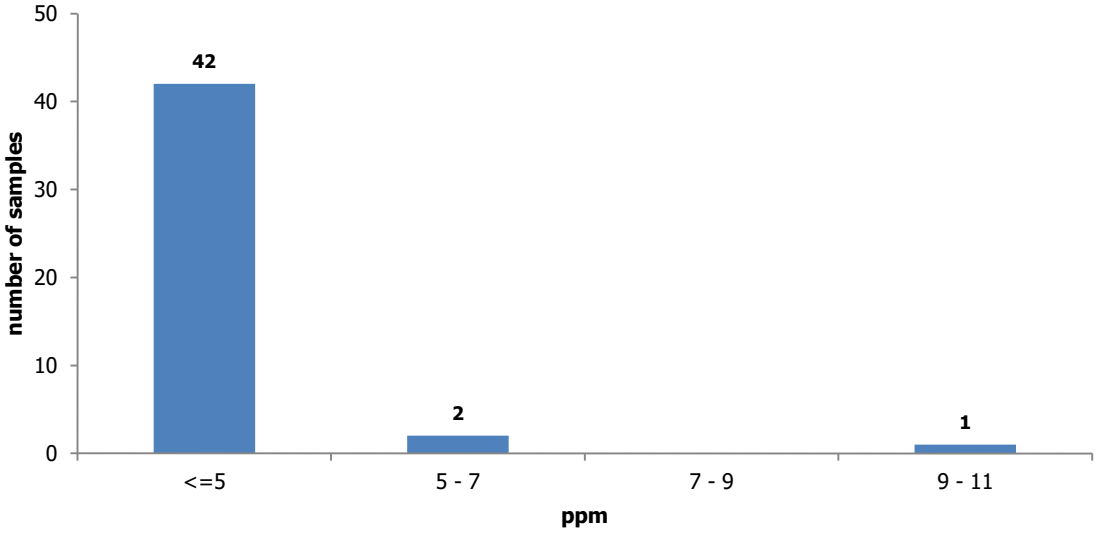
Frequency distribution for total CBD in hemp seed samples (n = 45)



Frequency distribution for total THC in hemp seed samples (n = 45)



Frequency distribution for total cannabinoids in hemp seed samples (n = 45)



APPENDIX 5

FSANZ

Low-THC Hemp Food - Dietary Assessment

FOOD STANDARDS
Australia New Zealand
Te Mana Kounga Kai – Ahitereiria me Aotearoa

Low THC Hemp Project

Draft estimation of the amount of food that can be consumed before reaching the lowest therapeutic dose for a range of phytocannabinoids in hemp foods, for consumers of hemp foods only

---

Table of contents

Executive summary

On behalf of the FRSC Low THC Hemp Working Group, NSW Health requested that Food Standards Australia New Zealand (FSANZ) undertake a preliminary dietary assessment to determine the maximum amount of hemp derived food that could be consumed by Australian and New Zealand populations without clinical effects for some selected phytocannabinoids being observed (lowest therapeutic dose). Calculations to derive the maximum levels were based on the concentrations of a range of phytocannabinoids, including cannabidiol (CBD) and tetrahydrocannabinol (THC), reported in an analytical survey of hemp foods that had been commissioned by NSW Health in 2015.

Hemp oil contained higher concentrations of all phytocannabinoids analysed in comparison to hemp protein powder, hemp flour and hemp seed, with hemp seed having the lowest mean concentrations.

The maximum amounts of each type of hemp food that could be consumed without clinical effect by Australian and New Zealand populations was estimated for selected phytocannabinoids and then compared to mean and 90th percentile consumption amounts for consumers (eaters only) of similar foods, derived from national nutrition surveys. The likelihood of the estimated maximum amount of hemp food actually eaten was then determined.

When it was assumed that hemp foods contained the mean or maximum analysed concentration of CBD, CBD-A and THC-A, the amount of hemp foods that could be consumed without any clinical effects was greater than the predicted mean and 90th percentile consumption of these foods by consumers only.

When it was assumed that hemp foods contained the mean analysed concentration of THC, the amount of hemp foods that could be consumed without clinical effects was greater than the predicted mean and 90th percentile consumption of these foods by consumers (eaters of the food).

When it was assumed that foods contained the maximum analysed concentration of THC, the estimated amount of hemp flour, hemp-based milk substitute and hemp seed that could be consumed without clinical signs was higher the predicted mean and 90th percentile consumption of these foods by consumers (THC was not detected in any sample of hemp flour). However, for hemp protein powder and hemp oil the estimated amount of food that could be consumed before any clinical signs were observed was lower than the predicted mean and 90th percentile consumption of these foods by consumers; i.e. a clinical sign may occur at the mean level of consumption of hemp powder and hemp oil consumption containing THC at the maximum concentration level. FSANZ notes that using the maximum analysed concentration of THC in the calculations is not considered a realistic chronic (long-term) scenario as it is unlikely that hemp protein powder and hemp oil always contains the maximum analysed concentration of THC observed in a single sample from a survey.

These findings are consistent with the FSANZ hemp food application A1039 – Low THC Hemp as a Food (FSANZ 2011), which assessed hemp foods as safe for human consumption at the recommended maximum levels of THC content. The implementation of a maximum limit for THC in some foods would minimise the likelihood of consuming the specified foods with high THC levels (as observed in the analytical survey) and thus minimise the likelihood that consumption leads to exceedances of the therapeutic dose for THC.

1. Introduction

1.1 Background

Food Standards Australia New Zealand (FSANZ) was requested to undertake preliminary calculations by NSW Health on behalf of the FRSC Low THC Hemp Working Group to determine the maximum amount of food derived from hemp that could be consumed by Australian and New Zealand populations without clinical effects for selected phytocannabinoids being observed (lowest therapeutic effect levels), including situations where the food contained the chemical of interest at the maximum concentration analysed.

NSW Health commissioned an analytical survey of hemp foods to ascertain the concentrations of a range of phytocannabinoids – including cannabidiol (CBD) and tetrahydrocannabinol (THC) – in low hemp products. A total of 200 samples of hemp foods, incorporating hemp protein powder, hemp flour, hemp seed and hemp oil were analysed for phytocannabinoids by the Southern Cross Plant Science Laboratory at the Southern Cross University. These results were provided to FSANZ in December 2015 by NSW Health for the purposes of undertaking the assessment.

At the request of NSW Health, the Lambert Initiative for Cannabinoid Therapeutics, Faculty of Science and Faculty of Medicine at the University of Sydney conducted a literature review to determine the pharmacological actions and associated therapeutic levels of phytocannabinoids (Arnold *et al* 2016). FSANZ was also asked to review the draft report by Arnold *et al* (2016), in particular the section on cannabidiolic acid (CBD-A).

FSANZ calculated the amount of hemp oil, hemp-based milk substitute, hemp seeds, hemp flour and hemp protein powder that could be consumed before reaching the lowest therapeutic effect dose using: a) the analytical survey results; and b) the relevant lowest therapeutic effect dose from either Arnold *et al* (2016), FSANZ (2011) or Brierley et al (2016). These calculations were undertaken for hemp food consumers only. See Section 1.3 below for further details about the lowest therapeutic effect dose used for individual phytocannabinoids.

1.2 Concentrations of phytocannabinoids in low THC hemp foods

The phytocannabinoids that were analysed in hemp oil, hemp powder and hemp seed were:

- Cannabidiol (CBD)
- Cannabidiolic Acid (CBD-A)
- Tetrahydrocannabinol (THC)
- Tetrahydrocannabinolic acid (THC-A); and
- Total cannabinoids.

A summary of the mean, median and maximum analytical results for each of the hemp foods listed above can be found in Table 7 of Appendix 1. FSANZ further divided the hemp powder category into hemp protein powder and hemp flour. Hemp oil contained higher concentrations of all phytocannabinoids analysed in comparison to hemp protein powder, hemp flour and hemp seed (see Figure 1). Hemp seed contained the lowest mean concentrations.

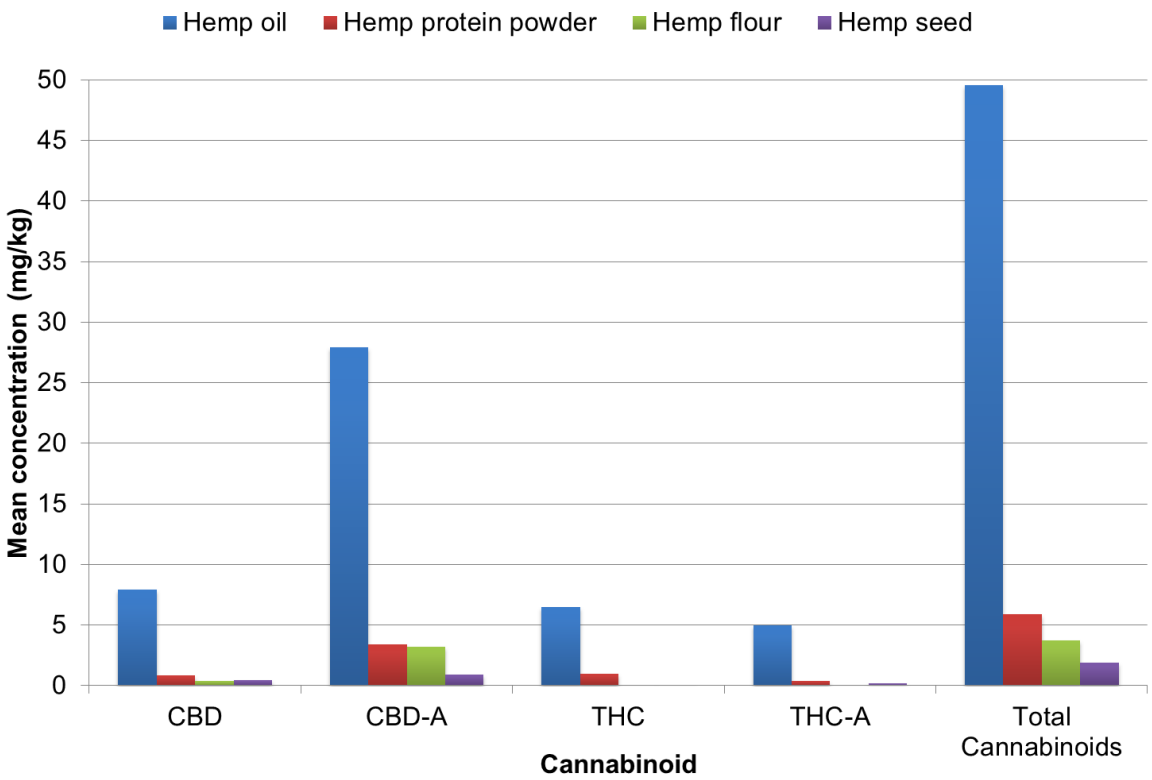


Figure 1: Mean concentrations of phytocannabinoids in hemp foods

1.3 Lowest therapeutic effect doses for phytocannabinoids

The lowest therapeutic effect doses used in the dietary exposure assessment are listed in Table 1 below.

Table 1: Lowest therapeutic effect doses for individual phytocannabinoids

Phytocannabinoid	Lowest therapeutic effect dose (mg/day based on 60 kg person)	Reference	Comments
CBD	800	Arnold *et. al.* (2016)	
CBD-A	5	Brierley et al (2016)	See Attachment 1
THC	0.36	FSANZ (2011)	Tolerable daily intake (TDI) for THC of 6 µg/kg bw/day, converted to a daily amount per person, using a 60 kg body weight
THC-A	3.5	Arnold *et. al.* (2016)	

2. Estimation of the amount of hemp food that can be consumed before reaching the lowest therapeutic effect dose for various phytocannabinoids

2.1 Methodology

The amount of food that could be consumed before reaching the lowest therapeutic dose for each phytocannabinoid was estimated using (1) the mean, median and maximum phytocannabinoid concentrations in the analysed hemp foods (oil, powder and seed); and (2) the lowest therapeutic effect levels for each phytocannabinoid, using Equation 1 below.

$$\text{Amount of food that can be consumed before reaching lowest therapeutic effect dose} = \frac{\text{Concentration of phytocannabinoid in the food}}{\text{Lowest therapeutic effect dose}}$$

Equation 1: Calculation of the amount of hemp food that can be consumed before reaching the lowest therapeutic effect dose for various phytocannabinoids

The results from these calculations were compared against the predicted mean and P90 food consumption of hemp foods (for hemp food consumers only), using appropriate substitute foods as a proxy to derive consumption amounts from national nutrition survey data for Australians aged 2 years and above and for New Zealanders aged 15 years and above. The process for selecting the proxy foods is detailed in Section 2.2 below. In this assessment, FSANZ used the most up-to-date available detailed food consumption data from the national nutrition surveys as outlined below.

- 1995 Australian National Nutrition Survey (AusNNS) is a one day 24-hour recall survey of 13,858 respondents aged 2 years and above (with 10% of respondents undertaking a second 24-hour recall on a non-consecutive day). Results in this assessment are for Day 1 only. A respondent is counted as a consumer if the food was consumed on Day 1 only.
- 2008-09 New Zealand Adult Nutrition Survey (2008 NZANS) is a one day 24-hour recall survey of 4,721 respondents aged 15 years and above (with 25% of respondents undertaking a second 24-hour recall on a non-consecutive day). The results in this assessment are for Day 1 only. A respondent is counted as a consumer if the food was consumed on Day 1 only

Although the 2011-12 National Nutrition and Physical Activity Survey (NNPAS) data have been published by the Australian Bureau of Statistics, the data set is not yet available for use in FSANZ's modelling system to derive food consumption amounts that include the uses of foods as ingredients in mixed foods. However, from past assessments FSANZ has observed that the Australian and New Zealand food supplies are similar, as are food consumption patterns across the two countries. Hence it is predicted that the results for Australians are likely to be similar to those for the New Zealand population based on the more up-to-date 2008-09 NZANS data.

2.2 Assumptions

The following assumptions were made in preparing the data for the estimation of the amount of hemp food that can be consumed before reaching the lowest therapeutic effect dose for various phytocannabinoids:

- the hemp food sample categories (hemp oil, hemp powder / flour and hemp seed) are as provided by the NSW Food Authority;
- mean analytical phytocannabinoid concentrations were derived by averaging the value for each replicate sample (i.e. 6 replicates averaged), then averaging these results for each sample type;
- median analytical phytocannabinoid concentrations were derived by averaging the value for each replicate sample (i.e. 6 replicates averaged), then taking the P50 of these results for each sample type;
- maximum analytical phytocannabinoid concentrations were derived by finding the maximum value for replicate samples for each sample type;
- an analytical result of zero is a 'not detected' result;
- for the purposes of estimating hemp flour consumption, it was assumed that hemp flour could be used in a similar manner to flour from all grains (e.g. wheat, barley, rice, corn, rye, oat) and to corn and wheat starches. However, due to the technical limitations of substituting wheat flour with hemp flour, it was assumed that only 25% of grains and corn and wheat starches could be replaced by hemp flour;
- for the purposes of estimating hemp protein powder consumption, it was assumed that hemp protein powder could be used in a similar way to dairy and soy based protein powders;
- for the purposes of estimating hemp oil consumption, it was assumed that hemp oil could be used in a similar way to canola, cottonseed, olive, rice bran, safflower, soybean and sunflower oils;
- for the purposes of estimating hemp seed consumption, it was assumed that hemp seeds could be used in a similar way to linseed, poppy seed, sesame seed, sunflower, chia and mustard seeds;
- in ANZFA's (2002) previous assessment of hemp foods (Application A360), the ML for THC for hemp-based non-dairy milk was set at 4% of that for hemp seeds (i.e. 0.2 mg/kg for hemp-based non-dairy milk; 5 mg/kg for hemp seed). Hemp based non-dairy milk is made from soaking hemp seeds in liquid. Therefore, it was assumed that hemp-based milk substitute is made from 4% hemp seeds;
- for the purposes of estimating hemp-based milk substitute consumption, it was assumed that hemp-based milk substitute could be used in a similar way to legume-based, cereal-based or nut- or seed-based milk alternatives;
- since the consumption of legume-, cereal-, nut- and seed-based milk substitutes is low or absent in the 1995 Australian National Nutrition Survey (1995 AusNNS), it was assumed that milk substitutes account for approximately 3% of the dairy milk volume consumed (excluding yoghurts, cheese, butter, cream, ice cream etc.). In ANZFA's (2002) previous assessment of hemp foods (Application A360), it was estimated that approximately 3% of milk consumers drank soy beverages in the 1995 AusNNS, therefore a 3% adjustment factor was given to mammalian milk consumption;
- for the purposes of calculating the amount of hemp food that can be eaten before a therapeutic effect may be seen, it was assumed that only one type of hemp food is consumed at a time; and
- the lowest therapeutic dose level is as per Table 1.

2.3 Results

2.3.1 Hemp protein powder

The consumption of hemp protein powder was estimated from the 1995 AusNNS and 2008 NZANS by assuming that hemp protein powder is used in a similar way to protein powders derived from dairy or soy. The predicted mean and P90 consumption of hemp protein powder for Australians aged 2 years and above and for New Zealanders aged 15 years and above is listed in Table 2 below. Predicted mean consumption of hemp protein powder is 40 – 50 grams per day, with P90 consumption estimated at 96 – 104 grams per day.

Table 2: Predicted mean and P90 consumption of hemp protein powder

Country	Population group	Nutrition Survey	Average body weight (kg)	Predicted consumption of hemp protein powder, for consumers only (grams per day)	
				Mean	P90
Australia	2 years and above	1995 AusNNS	66.7	40	96
New Zealand	15 years and above	2008 NZANS	78.6	50	104

The amount of hemp protein powder that would need to be consumed before reaching the lowest therapeutic effect dose for the phytocannabinoids analysed was estimated for each phytocannabinoid. These results are discussed below and are summarised in Appendix 1.

2.3.1.1 CBD

For all analytical concentrations of CBD in hemp protein powder, the estimated amount of food that would need to be consumed to reach the lowest therapeutic effect dose (800 mg/ day) for a 60 kg person is large (approximately 126.9 – 919.5 kg / day) and is far in excess of the predicted mean and P90 consumption of hemp protein powder for Australians aged 2 years and above and for New Zealanders aged 15 years and above (see Table 2).

At the estimated P90 consumption of hemp protein powder containing the mean, median or maximum concentration of CBD, Australians and New Zealanders would not reach the lowest therapeutic effect dose for CBD.

2.3.1.2 CBD-A

The amount of hemp protein powder that can be consumed before the lowest therapeutic dose threshold is reached for CBD-A (5 mg/day for a 60 kg person) was estimated to be between 357 grams / day (based on the maximum CBD-A concentration in hemp protein powder) and 1,654 grams / day (at the median CBD-A concentration in hemp protein powder).

At the P90 consumption of hemp protein powder (see Table 2) containing the mean, median or maximum concentration of CBD-A, Australians and New Zealanders would not reach the lowest therapeutic effect dose for CBD-A.

2.3.1.3 THC

The amount of hemp protein powder that can be consumed before the lowest therapeutic dose threshold is reached for THC (0.36 mg/day for a 60 kg person) was estimated to be between 13 grams / day (based on the maximum THC concentration in hemp protein powder) and 360 grams / day (at the mean THC concentration in hemp protein powder).

Using the mean THC concentration in hemp protein powder, the amount of hemp protein powder that can be consumed before reaching the lowest therapeutic effect dose is higher than the predicted P90 consumption of hemp protein powder (see Table 2) for Australians aged 2 years and above and New Zealanders aged 15 years and above. Consequently, Australians and New Zealanders would be unlikely to achieve a therapeutic dose of THC, even at the P90 of consumption of hemp protein powder.

If it is assumed that hemp protein powder always contains THC at the maximum analysed concentration, Australians and New Zealanders would be likely to achieve a therapeutic dose of THC, even at the mean consumption of hemp protein powder (see Table 2). However, it is not considered a realistic chronic (long-term) scenario as it is unlikely that hemp protein powder always contains the maximum analysed concentration observed in a single sample in the survey.

2.3.1.4 THC-A

The amount of hemp protein powder that can be consumed before the lowest therapeutic dose threshold is reached for THC-A (3.5 mg/day) was estimated to be between 269 grams / day (based on the maximum THC-A concentration in hemp protein powder and 9,722 grams / day for a 60 kg person (at the mean THC-A concentration in hemp protein powder). These amounts of hemp protein powder are far in excess of the predicted P90 consumption of hemp protein powder for Australians aged 2 years and above (see Table 2).

At the P90 consumption of hemp protein powder (see Table 2) containing the mean, median or maximum concentration of THC-A, Australians and New Zealanders would not reach the lowest therapeutic effect dose for THC-A.

2.3.1.5 Conclusion

The quantity of hemp protein powder that would need to be consumed to reach the lowest therapeutic dose for CBD, CBD-A and THC-A is much higher than the predicted P90 consumption of hemp protein powder by Australians aged 2 years and above and New Zealanders aged 15 years and above.

Australians and New Zealanders are unlikely to achieve a therapeutic dose of THC, even at the P90 of consumption of hemp protein powder, when THC is present at the mean concentration in hemp protein powder. However, Australians and New Zealanders may achieve a therapeutic dose of THC, at the mean consumption of hemp protein powder when THC is present at the maximum concentration in hemp protein powder (see Figure 2). However, it is not considered a realistic chronic (long-term) scenario as it is unlikely that hemp protein powder always contains the maximum analysed concentration observed in a single sample in the survey.

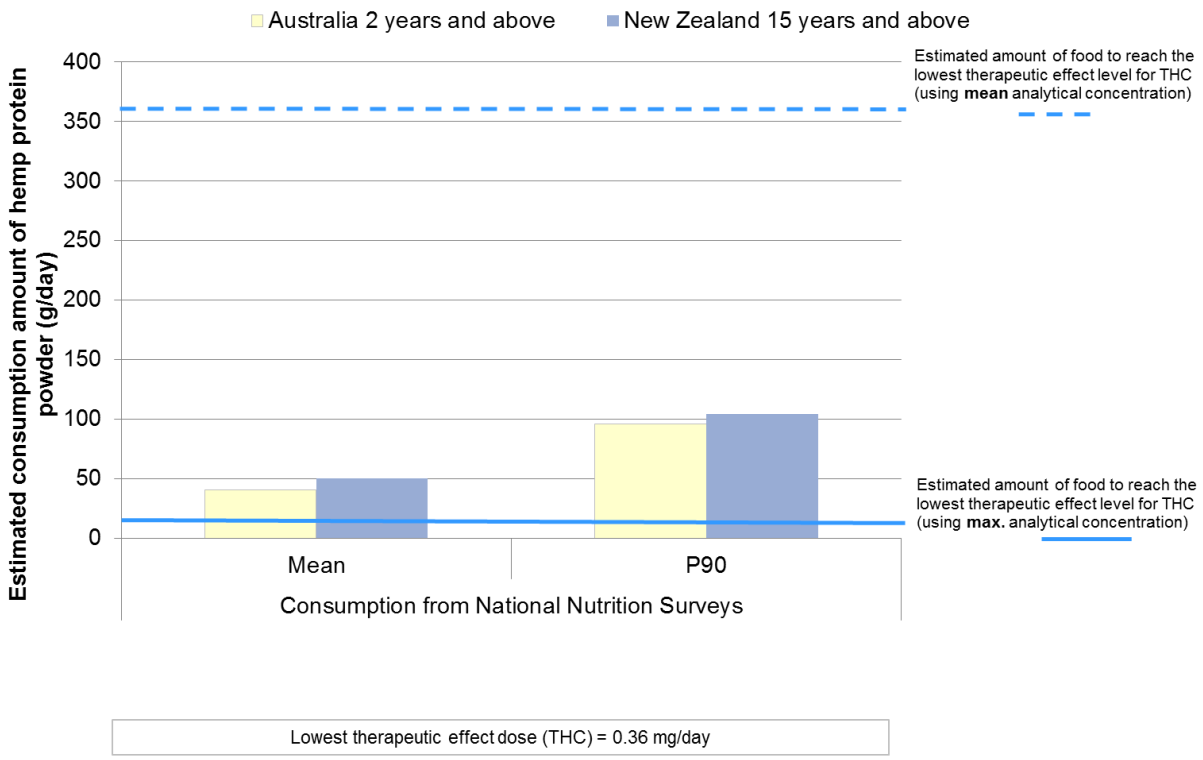


Figure 2: Comparison between mean and P90 consumption of foods from National Nutrition Surveys and the estimated amount of hemp powder / flour that could be consumed before reaching lowest therapeutic dose for THC

2.3.2 Hemp flour

The consumption of hemp flour was estimated from the 1995 AusNNS and 2008 NZANS by assuming that 25% of all flour from all grains (e.g. wheat, barley, rice, corn, rye, oat) and corn and wheat starches are substituted with hemp flour. The predicted mean and P90 consumption of hemp flour for Australians aged 2 years and above and for New Zealanders aged 15 years and above is listed in Table 2 above. Predicted mean consumption of hemp flour is 25 – 26 grams per day, with P90 consumption estimated at 44 – 49 grams per day.

Table 3: Predicted mean and P90 consumption of hemp flour

Country	Population group	Nutrition Survey	Average body weight (kg)	Predicted consumption of hemp flour, for consumers only (grams per day)	
				Mean	P90
Australia	2 years and above	1995 AusNNS	66.7	25	44
New Zealand	15 years and above	2008 NZANS	78.6	26	49

The amount of hemp flour that would need to be consumed before reaching the lowest therapeutic effect dose for the phytocannabinoids analysed was estimated for each phytocannabinoid. These results are discussed below and are summarised in Table 9 in Appendix 1.

2.3.2.1 CBD

For all analytical concentrations of CBD in hemp flour, the estimated amount of food that would need to be consumed to reach the lowest therapeutic effect dose (800 mg/day) for a 60 kg person is large (approximately 400 – 2,222 kg / day) and is far in excess of the predicted mean and P90 consumption of hemp flour for Australians aged 2 years and above and for New Zealanders aged 15 years and above (see Table 2).

At the estimated P90 consumption of hemp flour containing the mean, median or maximum concentration of CBD, Australians and New Zealanders would not reach the lowest therapeutic effect dose for CBD.

2.3.2.2 CBD-A

The amount of hemp flour that can be consumed before the lowest therapeutic dose threshold is reached for CBD-A (5 mg/day for a 60 kg person) was estimated to be between 704 grams / day (based on the maximum CBD-A concentration in hemp flour) and 2,689 grams / day (at the median CBD-A concentration in hemp flour).

At the P90 consumption of hemp flour (see Table 2) containing the mean, median or maximum concentration of CBD-A, Australians and New Zealanders would not reach the lowest therapeutic effect dose for CBD-A.

2.3.2.3 THC

THC was not detected in hemp flour. Therefore, no quantity has been set for the amount of hemp flour that can be consumed before the lowest therapeutic dose threshold is reached for THC.

2.3.2.4 THC-A

THC-A was not detected in hemp flour. Therefore, no quantity has been set for the amount of hemp flour that can be consumed before the lowest therapeutic dose threshold is reached for THC-A.

2.3.2.5 Conclusion

The quantity of hemp flour that would need to be consumed to reach the lowest therapeutic dose for CBD, CBD-A, THC and THC-A is much higher than the predicted P90 consumption of hemp flour by Australians aged 2 years and above and New Zealanders aged 15 years and above, even when it is assumed that hemp flour always contains the maximum analysed concentrations of phytocannabinoids.

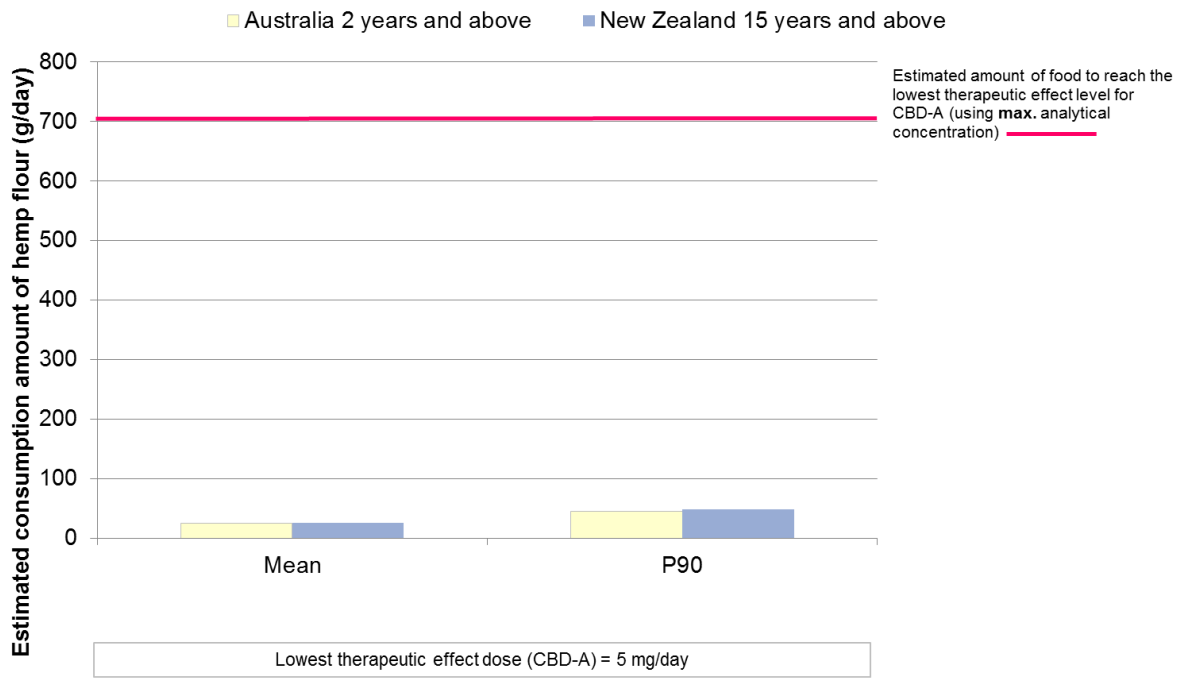


Figure 3: Comparison between mean and P90 consumption of foods from National Nutrition Surveys and the estimated amount of hemp flour that could be consumed before reaching lowest therapeutic dose for CBD-A

2.3.3 Hemp-based milk substitutes

The consumption of hemp-based milk substitute was predicted from the 1995 AusNNS by assuming that approximately 3% of the dairy milk volume consumed (excluding yoghurts, cheese, butter, cream, ice cream etc.) is substituted with hemp-based milk substitute. For the 2008 NZANS it was assumed that hemp-based milk substitute could be substituted for legume-based, cereal-based or nut- or seed-based milk substitutes. The predicted mean and P90 consumption of hemp-based milk substitute for Australians aged 2 years and above and for New Zealanders aged 15 years and above is listed in Table 4 below. Predicted mean consumption of hemp-based milk substitute is 228 – 299 grams per day, with P90 consumption at 510 – 649 grams per day.

Table 4: Predicted mean and P90 consumption of hemp –based milk substitute

Country	Population group	Nutrition Survey	Average body weight (kg)	Predicted consumption of hemp-based milk substitute, for consumers only (grams per day)	
				Mean	P90
Australia	2 years and above	1995 AusNNS	66.7	299	649
New Zealand	15 years and above	2008 NZANS	78.6	228	510

Table 10 in Appendix 1 details the estimated amount of hemp-based milk substitute that would need to be consumed before reaching the lowest therapeutic effect dose for the phytocannabinoids analysed.

2.3.3.1 CBD

For all analytical concentrations of CBD in hemp-based milk substitute, the estimated amount of food that would need to be consumed to reach the lowest therapeutic effect dose (800 mg/day) is large (4,053– 45,528 kg / day) and is far in excess of the estimated mean and P90 consumption of hemp-based milk substitute (see Table 4) for Australians aged 2 years and above and for New Zealanders aged 15 years and above.

2.3.3.2 CBD-A

It was estimated that 25.9 – 726.3kg of hemp-based milk substitute could be consumed per day, depending on the analytical concentration used (mean, median or maximum concentration), before reaching the lowest therapeutic effect dose. At the P90 consumption of hemp-based milk substitute (see Table 4) containing the mean, median or maximum concentration of CBD-A, Australians and New Zealanders would not reach the lowest therapeutic effect dose for CBD-A.

2.3.3.3 THC

The amount of hemp-based milk substitute that can be consumed before the lowest therapeutic dose threshold is reached for THC (0.36 mg/day) was estimated to be between 4.0 kg / day (based on the maximum THC concentration in hemp seeds, extrapolated to a concentration for hemp-based milk substitutes) and 122.3kg / day for a 60 kg person (based on the mean THC concentration in hemp seeds, extrapolated to a concentration for hemp-based milk substitutes).

At the P90 consumption of hemp-based milk substitute (see Table 4) containing the mean, median or maximum concentration of THC, Australians and New Zealanders would not reach the lowest therapeutic effect dose for THC.

2.3.3.4 THC-A

The amount of hemp-based milk substitutes that can be consumed before the lowest therapeutic dose threshold is reached for THC-A (3.5 mg / day) was estimated to be between 52.5kg / day (based on the maximum THC-A concentration in hemp-based milk substitutes) and 817.5kg / day for a 60 kg person (at the mean THC-A concentration in hemp-based milk substitutes). These amounts of hemp-based milk substitutes are far in excess of the predicted P90 consumption of hemp-based milk substitutes (see Table 4) for Australians aged 2 years and above and New Zealanders aged 15 years and above.

2.3.3.5 Conclusion

The quantity of hemp-based milk substitutes that would need to be consumed to reach the lowest therapeutic dose for CBD, CBD-A, THC and THC-A is much higher than the estimated P90 consumption of hemp-based milk substitutes by Australians aged 2 years and above and New Zealanders aged 15 years and above. Therefore, Australians and New Zealanders are unlikely to achieve a therapeutic dose of CBD, CBD-A, THC or THC-A, due to the consumption of hemp-based milk substitutes, even at the P90 of consumption (see Figure 4).

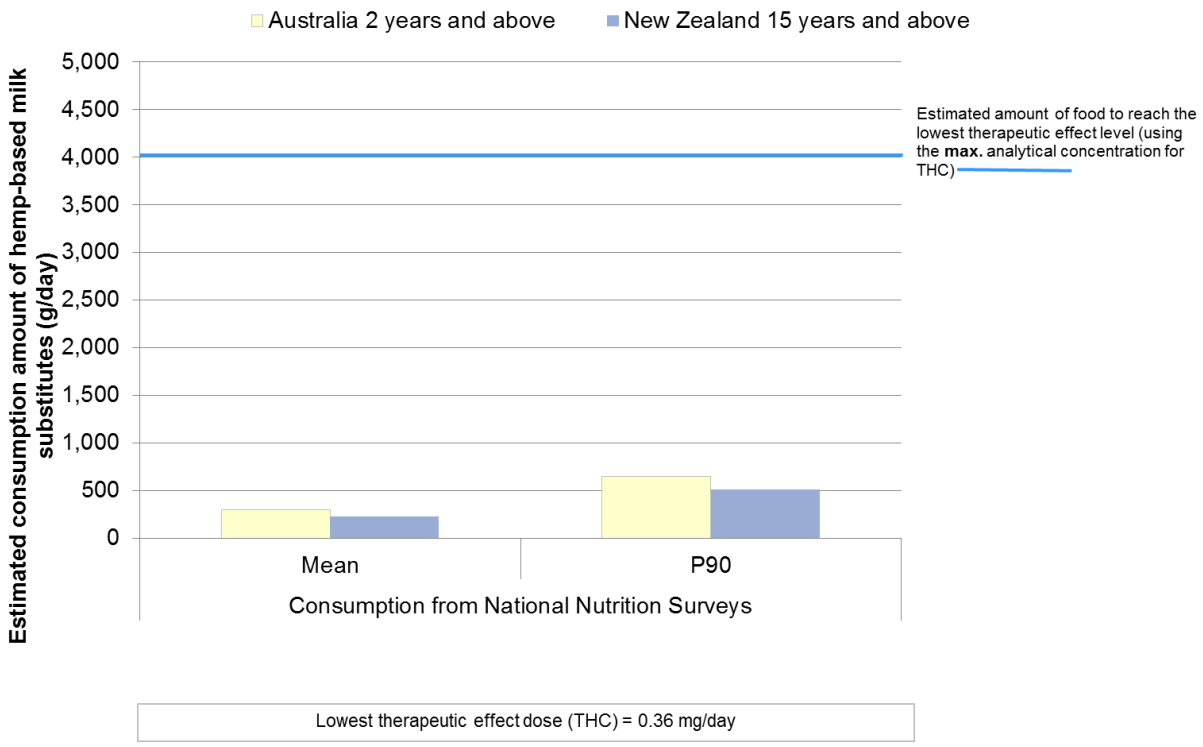


Figure 4: Comparison between mean and P90 consumption of foods from National Nutrition Surveys and the estimated maximum amount of hemp-based milk substitute that could be consumed before reaching lowest therapeutic dose for THC

2.3.4 Hemp oil

The consumption of hemp oil was estimated from the 1995 AusNNS and 2008 NZANS by assuming that hemp oil could be substituted for canola, cottonseed, olive, rice bran, safflower, soybean and sunflower oils. The predicted mean and P90 consumption of hemp oil for Australians aged 2 years and above and for New Zealanders aged 15 years and above is listed in Table 5 below. Predicted mean consumption of hemp oil is 7.5 – 8.3 grams per day, with P90 consumption estimated at 16 – 18 grams per day.

Table 5: Predicted mean and P90 consumption of hemp oil

Country	Population group	Nutrition Survey	Average body weight (kg)	Predicted consumption of hemp oil for consumers only (grams per day)	
				Mean	P90
Australia	2 years and above	1995 AusNNS	66.7	8.3	18
New Zealand	15 years and above	2008 NZANS	78.6	7.5	16

Table 11 in Appendix 1 details the estimated amount of hemp oil that would need to be consumed before reaching the lowest therapeutic effect dose for the phytocannabinoids analysed.

2.3.4.1 CBD

For all analytical concentrations of CBD in hemp oil, the estimated amount of food that would need to be consumed to reach the lowest therapeutic effect dose (800 mg/day) is large (approximately 34.8 – 100.9 kg/ day) and is far in excess of the predicted mean and P90 consumption of hemp oil (see Table 5) for Australians aged 2 years and above and for New Zealanders aged 15 years and above.

2.3.4.2 CBD-A

It was estimated that approximately 50 – 244 grams of hemp oil could be consumed per day, depending on the analytical concentration used (mean, median or maximum concentration). At the P90 consumption of hemp oil (see Table 5) containing the mean or maximum concentration of CBD-A, Australians and New Zealanders would not reach the lowest therapeutic effect dose for CBD-A.

2.3.4.3 THC

The amount of hemp oil that can be consumed before the lowest therapeutic dose threshold is reached for THC (0.36 mg/day) was estimated to be between 2.8 grams / day (based on the maximum THC concentration in hemp oil) and 118 grams / day for a 60 kg person (at the median THC concentration in hemp oil).

If THC is present always present in hemp oil at the mean or median analytical concentration, Australians and New Zealanders would not reach the lowest therapeutic effect dose for THC at the P90 level of consumption (see Table 4).

If THC is present always present in hemp oil at the maximum analytical concentration, Australians and New Zealanders would reach the lowest therapeutic effect dose for THC at the mean and P90 levels of consumption (see Table 6). However, it is not considered a realistic chronic (long-term) scenario as it is unlikely that hemp oil always contains the maximum analysed concentration observed in a single sample in the survey.

2.3.4.4 THC-A

The amount of hemp oil that can be consumed before the lowest therapeutic dose threshold is reached for THC-A (3.5 mg/day) was estimated to be between 115 grams / day (based on the maximum THC-A concentration in hemp oil) and 879 grams/ day for a 60 kg person (at the median THC-A concentration in hemp oil). These amounts of hemp oil are far in excess of the predicted P90 consumption of hemp oil (see Table 6) for Australians aged 2 years and above and New Zealanders aged 15 years and above.

2.3.4.5 Conclusion

The quantity of hemp oil that would need to be consumed to reach the lowest therapeutic dose for CBD, CBD-A and THC-A is much higher than the predicted P90 consumption of hemp oil by Australians aged 2 years and above and New Zealanders aged 15 years and above.

Australians and New Zealanders are unlikely to achieve a therapeutic dose of THC, even at the P90 of consumption of hemp oil, when THC is present at the mean or median concentration in hemp oil. However, Australians and New Zealanders would be likely to achieve a therapeutic dose of THC, at the mean consumption of hemp oil when THC is present at the maximum concentration in hemp oil (see Figure 5). However, this is considered an unlikely chronic (long-term) scenario, as it is unlikely that hemp oil always

contains the maximum analysed concentration observed in a single sample in the survey.

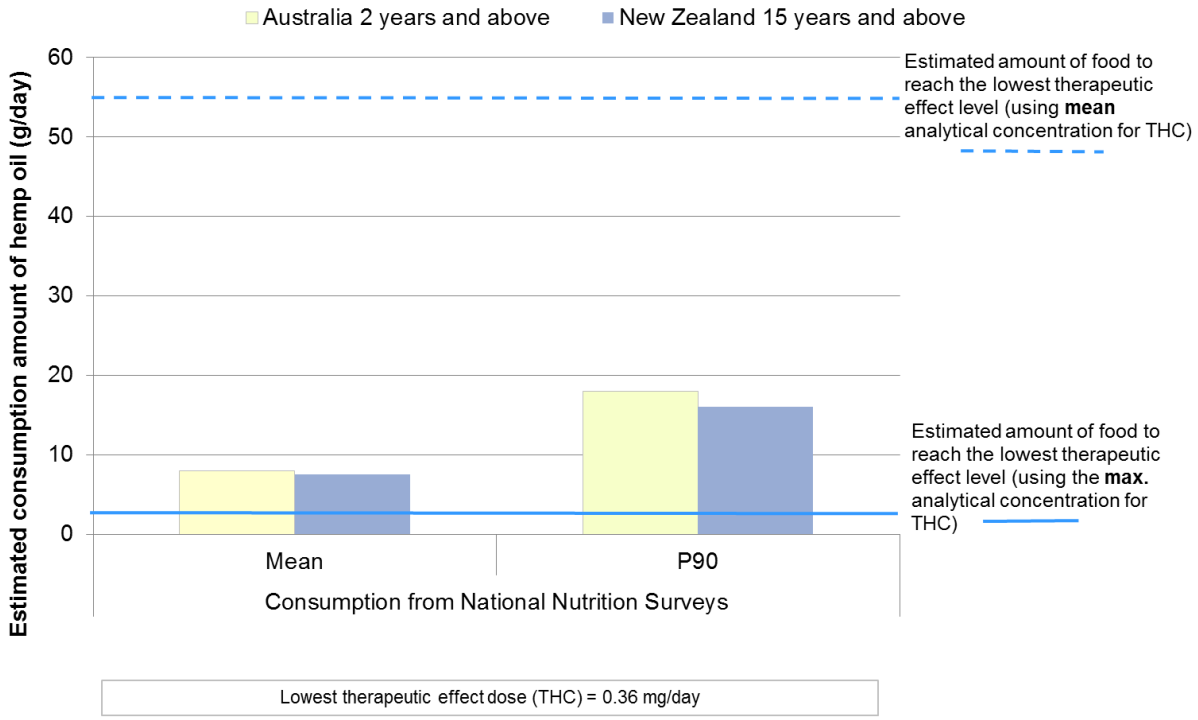


Figure 5: Comparison between mean and P90 consumption of foods from National Nutrition Surveys and the estimated maximum amount of hemp oil that could be consumed before reaching lowest therapeutic dose for THC

2.3.5 Hemp seed

The consumption of hemp seed was estimated from the 1995 AusNNS and 2008 NZANS by assuming that hemp seeds could be substituted for linseed, poppy seed, sesame seed, sunflower, chia and mustard seeds. The predicted mean and P90 consumption of hemp seed for Australians aged 2 years and above and for New Zealanders aged 15 years and above is listed in Table 6 below. Predicted mean consumption of hemp seed is 2.6 – 3.3 grams per day, with P90 consumption estimated at 6.3 – 8.8 grams per day.

Table 6: Predicted mean and P90 consumption of hemp seed

Country	Population group	Nutrition Survey	Average body weight (kg)	Predicted consumption of hemp seed, for consumers only (grams per day)	
				Mean	P90
Australia	2 years and above	1995 AusNNS	66.7	2.6	6.3
New Zealand	15 years and above	2008 NZANS	78.6	3.3	8.8

Table 12 in Appendix 1 details the estimated amount of hemp seeds that would need to be consumed before reaching the lowest therapeutic effect dose for the phytocannabinoids analysed.

2.3.5.1 CBD

For all analytical concentrations of CBD in hemp seeds, the estimated amount of food that would need to be consumed to reach the lowest therapeutic effect dose (800 mg/day) is large (162.1 – 1,821.1 kg / day) and is far in excess of the predicted mean and P90 consumption of hemp seeds (see Table 6) for Australians aged 2 years and above and for New Zealanders aged 15 years and above.

2.3.5.2 CBD-A

It was estimated that 1– 29 kilograms of hemp seed could be consumed per day before reaching the lowest therapeutic effect dose (5 mg/day for a 60 kg person), depending on the analytical concentration used (mean, median or maximum concentration). At the P90 consumption of hemp seeds (see Table 6) containing the mean or maximum concentration of THC, Australians and New Zealanders wouldn't reach the lowest therapeutic effect dose for CBD-A.

2.3.5.3 THC

The amount of hemp seeds that can be consumed before the lowest therapeutic dose threshold is reached for THC (0.36 mg/day) was estimated to be between 161 grams / day (based on the maximum THC concentration in hemp seeds) and 4,893 grams / day for a 60 kg person (at the mean THC concentration in hemp seeds). These amounts of hemp seeds are higher than the estimated P90 consumption of hemp seeds (see Table 6) for Australians aged 2 years and above and New Zealanders aged 15 years and above.

2.3.5.4 THC-A

The amount of hemp seeds that can be consumed before the lowest therapeutic dose threshold is reached for THC-A (3.5 mg/day) was estimated to be between 2.1kg /day (based on the maximum THC concentration in hemp seeds) and 32.7 kg/ day for a 60 kg person (at the median THC concentration in hemp seeds). These amounts of hemp seeds are far in excess of the predicted P90 consumption of hemp seeds (see Table 6) for Australians aged 2 years and above and New Zealanders aged 15 years and above.

2.3.5.5 Conclusion

The quantity of hemp seeds that would need to be consumed to reach the lowest therapeutic dose for CBD, CBD-A, THC or THC-A is much higher than the predicted P90 consumption of hemp seeds by Australians aged 2 years and above and New Zealanders aged 15 years and above (see Figure 6).

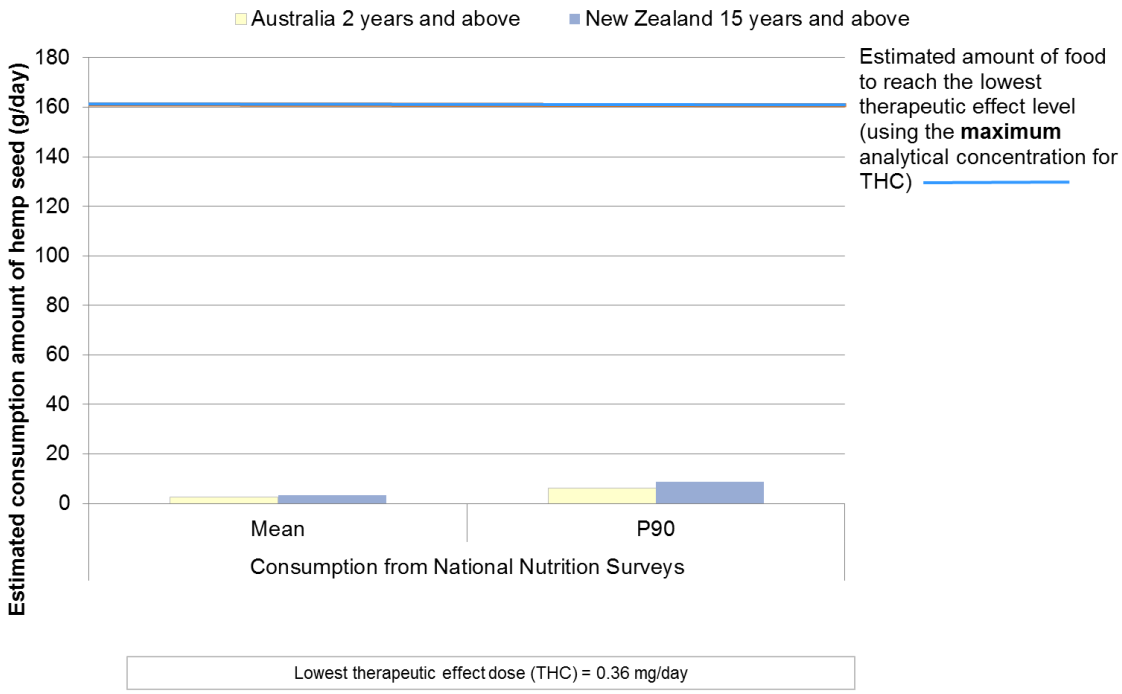


Figure 6: Comparison between mean and P90 consumption of foods from National Nutrition Surveys and the estimated maximum amount of food that could be consumed before reaching lowest therapeutic dose for one or more of the cannabinoids analysed, for hemp seeds

3. Conclusions

Hemp oil contained higher concentrations of all phytocannabinoids analysed in comparison to hemp protein powder, hemp flour and hemp seed, with hemp seed having the lowest mean concentrations.

The maximum amounts of each type of hemp food that could be consumed without clinical effect by Australian and New Zealand populations for selected phytocannabinoids was estimated and then compared to mean and 90th percentile consumption amounts for consumers (eaters only) of similar foods; the latter derived from national nutrition surveys. The likelihood of the estimated maximum amount of hemp food actually eaten was then determined.

When it was assumed that hemp foods contained the mean or maximum analysed concentration of CBD, CBD-A and THC-A, the amount of hemp foods that could be consumed without any clinical effects was greater than the predicted mean and 90th percentile consumption of these foods by consumers only.

When it was assumed that hemp foods contained the mean analysed concentration of THC, the amount of hemp foods that could be consumed without clinical effects was greater than the predicted mean and 90th percentile consumption of these foods by consumers (eaters of the food).

When it was assumed that foods contained the maximum analysed concentration of THC, the estimated amount of hemp flour, hemp-based milk substitute and hemp seed that could be consumed without clinical signs was higher the predicted mean and 90th percentile consumption of these foods by consumers (THC was not detected in any sample of hemp

flour). However, for hemp protein powder and hemp oil the estimated amount of food that could be consumed before any clinical signs were observed was lower than the predicted mean and 90th percentile consumption of these foods by consumers; i.e. a clinical sign may occur at the mean level of consumption of hemp powder and hemp oil consumption containing THC at the maximum concentration level. FSANZ notes that using the maximum analysed concentration of THC in the calculations is not considered a realistic chronic (long-term) scenario as it is unlikely that hemp protein powder and hemp oil always contains the maximum analysed concentration of THC observed in a single sample from a survey.

These findings are consistent with the FSANZ hemp food application A1039 – Low THC Hemp as a Food (FSANZ 2011) which assessed hemp foods as safe for human consumption at the recommended maximum levels of THC content. The implementation of a maximum limit for THC in some foods would minimise the likelihood of consuming the specified foods with high THC levels (as observed in the analytical survey) and thus minimise the likelihood that consumption leads to exceedances of the therapeutic dose for THC.

4. References

ANZFA (2002) Final Assessment Report: Application A360 – Use of industrial hemp as a novel food.
http://www.foodstandards.gov.au/code/applications/documents/A360_Final%20AR.pdf

Arnold JC, Allsop DJ, Lintzeris N, McGregor IS (2015) Pharmacological actions and associated therapeutic levels of phytocannabinoids. Lambert Initiative for Cannabinoid Therapeutics, Faculty of Science and Faculty of Medicine University of Sydney. Unpublished review.

Brierley DI, Samuels J, Duncan M, Whalley BJ, Williams CM (2016) Neuromotor tolerability and behavioural characterisation of cannabidiolic acid, a phytocannabinoid with therapeutic potential for anticipatory nausea. Psychopharmacology (Berl). 233(2):243-54.

FSANZ (2011) Final Assessment Report: Application A1039 – Low THC hemp as a food.
http://www.foodstandards.gov.au/code/applications/documents/A1039%20Low%20THC%20hemp%20AR%20FINAL.pdf

Appendix 1: Detailed analytical and dietary exposure assessment results

Table 7: Mean, median and maximum analytical concentrations of various phytocannabinoids in hemp foods

Hemp Food	Analytical Concentration (mg/kg)Ψ														
	CBD			CBD-A			THC			THC-A			Total Cannabinoids		
	Mean	*Median*	*Max.*	*Mean*	*Median*	*Max.*	*Mean*	*Median*	*Max.*	*Mean*	*Median*	*Max.*	*Mean*	*Median*	*Max.*
Hemp oil	7.9	8.9	23	28	20	99	6.5	3.0	126	5	4.0	30	50	41	137
Hemp flour	0.36	nd	2.0	3.2	1.9	7.1	nd	nd	nd	nd	nd	nd	3.7	3.2	8.2
Hemp protein powder	0.87	nd	6.3	3.4	3.0	14	1.0	nd	26	0.36	nd	13	5.9	4.5	48
Hemp seed	0.44	nd	4.9	0.93	0.17	4.8	0.074	nd	2.2	0.21	0.11	1.7	1.9	1.4	10

Ψ – all analytical concentrations have been rounded to the nearest whole number or 2 significant figures

Table 8: Estimated maximum amount of hemp protein powder that can be consumed before a therapeutic effect level may be observed, for the various cannabinoids analysed

Phytocannabinoid	Lowest Therapeutic Dose[#] (mg per day)	Maximum amount of hemp protein powder that can be consumed before lowest therapeutic dose is reached (grams per day)		
		Mean analytical conc.	Median analytical conc.	Maximum analytical conc.
CBD	800	919,540	Not determined (analytical result is 'nd')	126,984
CBD-A	5	1,470	**1,654**	357
THC	0.36	**360**	Not determined (analytical result is 'nd')	**13**
THC-A	3.5	9,722	Not determined (analytical result is 'nd')	269

# for a 60 kg person
nd = analytical method did not detect the presence of the substance in the food
Bold text indicates the lowest amount of food that can be consumed before the lowest therapeutic dose is reached for any one of the phytocannabinoids

Table 9: Estimated maximum amount of hemp flour that can be consumed before a therapeutic effect level may be observed, for the various cannabinoids analysed

Phytocannabinoid	Lowest Therapeutic Dose[#] (mg per day)	Maximum amount of hemp flour that can be consumed before lowest therapeutic dose is reached (grams per day)		
		Mean analytical conc.	Median analytical conc.	Maximum analytical conc.
CBD	800	2,222,222	Not determined (analytical result is 'nd')	400,000
CBD-A	5	**1,562**	**2,689**	**704**
THC	0.36	Limit not set (analytical result is 'nd')	Not determined (analytical result is 'nd')	Limit not set (analytical result is 'nd')
THC-A	3.5	Limit not set (analytical result is 'nd')	Not determined (analytical result is 'nd')	Limit not set (analytical result is 'nd')

# for a 60 kg person
nd = analytical method did not detect the presence of the substance in the food
Bold text indicates the lowest amount of food that can be consumed before the lowest therapeutic dose is reached for any one of the phytocannabinoids

Table 10: Estimated maximum amount of hemp-based milk substitute that can be consumed before a therapeutic effect level may be observed, for the various cannabinoids analysed

Phytocannabinoid	Lowest Therapeutic Dose# (mg per day)	Maximum amount of hemp-based milk substitute that can be consumed before lowest therapeutic dose is reached (grams per day)		
		Mean analytical conc.	Median analytical conc.	Maximum analytical conc.
CBD	800	45,528,927	Not determined (analytical result is 'nd')	4,053,752
CBD-A	5	134,257	**726,341**	25,978
THC	0.36	**122,335**	Not determined (analytical result is 'nd')	**4,033**
THC-A	3.5	415,700	817,569	52,535

# for a 60 kg person
nd = analytical method did not detect the presence of the substance in the food
Bold text indicates the lowest amount of food that can be consumed before the lowest therapeutic dose is reached for any one of the phytocannabinoids

Table 11: Estimated maximum amount of hemp oil that can be consumed before a therapeutic effect level may be observed, for the various cannabinoids analysed

Phytocannabinoid	Lowest Therapeutic Dose# (mg per day)	Maximum amount of hemp oil that can be consumed before lowest therapeutic dose is reached (grams per day)		
		Mean analytical conc.	Median analytical conc.	Maximum analytical conc.
CBD	800	100,954	90,378	34,833
CBD-A	5	179	244	50
THC	0.36	**55**	**118**	**2.8**
THC-A	3.5	701	879	115

for a 60 kg person
Bold text indicates the lowest amount of food that can be consumed before the lowest therapeutic dose is reached for any one of the phytocannabinoids

Table 12: Estimated maximum amount of hemp seed that can be consumed before a therapeutic effect level may be observed, for the various cannabinoids analysed

Phytocannabinoid	Lowest Therapeutic Dose[#] (mg per day)	Maximum amount of hemp seed that can be consumed before lowest therapeutic dose is reached (grams per day)		
		Mean analytical conc.	Median analytical conc.	Maximum analytical conc.
CBD	800	1,821,157	Not determined (analytical result is 'nd')	162,150
CBD-A	5	5,370	**29,053**	1,039
THC	0.36	**4,893**	Not determined (analytical result is 'nd')	**161**
THC-A	3.5	16,628	32,702	2,101

# for a 60 kg person
nd = analytical method did not detect the presence of the substance in the food
Bold text indicates the lowest amount of food that can be consumed before the lowest therapeutic dose is reached for any one of the phytocannabinoids

Attachment 1: Pharmacological activity of cannabinoids other than THC in hemp foods

Executive Summary

The Australia and New Zealand Ministerial Forum on Food Regulation (The Forum) is currently considering information gaps in relation to low-THC[1] hemp as a food. Part of this work is concerned with the levels of cannabinoids other than THC in hemp foods, and whether it is appropriate to set maximum levels (MLs) for these cannabinoids because of their potential for pharmacological activity.

NSW Health provided FSANZ with an unpublished draft review on the pharmacology of cannabinoids other than THC. FSANZ was asked to review the draft report by Arnold *et al* (2015), in particular the section on cannabidiolic acid (CBD-A).

The review provided estimates of the lowest oral dose that may have a therapeutic effect in humans for 8 cannabinoids. An unexpectedly low effective oral dose for cannabidiolic acid (CBDA) was derived from a study using intraperitoneal (IP) administration. In the absence of an appropriate oral dosing study a more robust estimate of an appropriate oral dose for CBDA in humans would be to consider a structure activity response relationship. However, an oral administration study published after the Arnold et al review was completed, has enabled an appropriate effective oral dose estimate for CBDA in humans to be calculated as being 5 mg/person.

Background

The Australia and New Zealand Ministerial Forum on Food Regulation (The Forum) is currently considering information gaps in relation to low-THC hemp as a food[2]. Part of this work is concerned with the levels of cannabinoids other than THC in hemp foods, and whether it is appropriate to set MLs for these cannabinoids because of their potential for pharmacological activity.

Unpublished review on cannabinoid pharmacology

NSW Health provided FSANZ with an unpublished review on cannabinoids that was authored by researchers from the Lambert Initiative for Cannabinoid Therapeutics, University of Sydney (Arnold et al 2015). The review includes estimates of the lowest oral human therapeutic doses (LOHTD) of 8 cannabinoids. Excluding cannabidiol (CBD), for which an LOHTD of 800 mg/day was derived from a human study, the estimated LOHTDs were derived from studies in mice and rats. The estimated LOHTDs ranged from 0.07 to 892 mg/person.

An LOHTD of 0.07 mg/person was derived for cannabidiolic acid (CBDA) from a study in which single doses of the substance were administered by intraperitoneal (IP) injection into rats (Rock et al 2014). Arnold et al stated in their review that no human study on CBDA could be located. In addition, no animal studies on CBDA by the oral route were cited.

---

[1] Δ^9-tetrahydrocannabinol

[2] Australia and New Zealand Ministerial Forum on Food Regulation. FINAL COMMUNIQUÉ. 20 November 2015. http://www.health.gov.au/internet/main/publishing.nsf/Content/foodsecretariat-communiqu%C3%A9s-15_20Nov

Arnold et al multiplied the lowest IP "effect" dose (0.001 mg/kg bw) by a factor of 7 to derive an oral dose in rats of 0.007 mg/kg bw, stating that "IP phytocannabinoid doses achieve on average 7-times higher tissue concentrations than oral doses in rodents". A human equivalent dose of 0.07 mg/person (of bodyweight 60 kg) was derived from the estimated oral rat dose of 0.007 mg/kg bw using scaling based on body surface area (US FDA 2005).

The above factor of 7 to convert an IP dose to an equivalent oral dose was derived from studies on four other cannabinoids, not from CBDA, and the variability observed among these cannabinoids was large (Deiana et al 2012). The bioavailability of CBDA by IP or oral routes is not known and cannot be predicted with sufficient accuracy to reliably estimate an oral dose that would be equivalent to an IP dose.

Oral study on CBDA administered to rats

A study published after the Arnold et al review investigated the tolerability of CBDA administered orally to rats at single doses of 0.05, 0.5 or 5 mg/kg bw (Brierley et al 2016). These dose levels correspond to extrapolated human doses of 0.5, 5, or 50 mg for a 60 kg person based on body surface area scaling (US FDA 2005). No adverse effects were observed at any dose. CBDA had no adverse effects on performance in neuromotor tolerability tests and did not affect feeding behaviours. CBDA was negative in two of three tests designed to assess anxiety-like behaviour. In the third anxiety test, statistically significant reductions in a measure of anxiety were observed at doses of 0.5 and 5 mg/kg bw.

Assuming that an oral dose of 0.5 mg/kg bw in rats is an approximate lowest effect dose, the resulting LOHTD (5 mg/person) is approximately 70-times greater than the LOHTD of 0.07 mg/person derived by Arnold et al from an IP rat study.

Conclusion

The lowest oral human therapeutic dose of 0.07 mg/person, estimated for CBDA by Arnold et al using an extrapolation method from an IP study in rats is known to be unreliable and not an appropriate methodology for the derivation of an ML for CBDA in hemp foods. In the absence of an appropriate oral dosing study a more robust estimate could be obtained if a structure activity relationship were to be considered. However, fortuitously a rat study was published during the time at which FSANZ was undertaking a peer review of the Arnold et al review. This 2016 study in rats enabled the oral CBDA dose without adverse effects in humans to be estimated to be up to 50 mg/person (Brierley et al 2016). Some pharmacological activity was evident at estimated human doses of 5 and 50 mg/person, but not at the lowest tested dose of 0.5 mg/person. A dose of 5 mg/person, derived from this study, is considered to be a reasonably reliable estimate of the lowest oral human therapeutic dose.

References

Arnold JC, Allsop DJ, Lintzeris N, McGregor IS (2016) Pharmacological actions and associated therapeutic levels of phytocannabinoids. Lambert Initiative for Cannabinoid Therapeutics, Faculty of Science and Faculty of Medicine University of Sydney. Unpublished review.

Brierley DI, Samuels J, Duncan M, Whalley BJ, Williams CM (2016) Neuromotor tolerability and behavioural characterisation of cannabidiolic acid, a phytocannabinoid with therapeutic potential for anticipatory nausea. Psychopharmacology (Berl). 233(2):243-54.

Deiana S, Watanabe A, Yamasaki Y, Amada N, Arthur M, Fleming S, Woodcock H, Dorward P, Pigliacampo B, Close S, Platt B, Riedel G (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 (Berl). 219(3):859-73.

Rock EM, Limebeer CL, Navaratnam R, Sticht MA, Bonner N, Engeland K, Downey R, Morris H, Jackson M, Parker LA (2014) A comparison of cannabidiolic acid with other treatments for anticipatory nausea using a rat model of contextually elicited conditioned gaping. Psychopharmacology (Berl). 231(16):3207-15.

US FDA (2005) Guidance for Industry: Estimating the Maximum Safe Starting Dose in Initial Clinical Trials for Therapeutics in Adult Healthy Volunteers. http://www.fda.gov/downloads/Drugs/.../Guidances/UCM078932.pdf

---

Rock et al (2014): additional study details

The rats in the Rock et al study had been conditioned by IP injection of lithium chloride to exhibit a nausea response associated with a specific cage environment (anticipatory nausea: AN[3]). The number of mouth "gaping" responses over a 5 min period was used as a measure of AN. Gaping was defined as "large amplitude openings of the mouth with simultaneous retractions of the corners of the mouth exposing incisors".

Groups of AN-conditioned male rats (6–8/group) received CBDA by IP injection at single doses of 0.0001, 0.001, 0.01, 0.1 or 1 mg/kg bw. Compared to vehicle control, CBDA suppressed the gaping response at doses of 0.001–0.1 mg/kg bw, but not at the high dose of 1 mg/kg bw. No adverse effects associated with CBDA were reported at any dose.

[3] Patients undergoing chemotherapy can experience AN prior to a round of treatment. Rock et al (2014) state that currently there is no effective treatment for AN.

Psychopharmacology (2016) 233:243–254
DOI 10.1007/s00213-015-4100-1

ORIGINAL INVESTIGATION

Neuromotor tolerability and behavioural characterisation of cannabidiolic acid, a phytocannabinoid with therapeutic potential for anticipatory nausea

Daniel I Brierley[1,2] · **James Samuels**[1] · **Marnie Duncan**[3] · **Benjamin J Whalley**[2] · **Claire M Williams**[1]

Received: 19 March 2015 / Accepted: 27 September 2015 / Published online: 6 October 2015

Abstract

Rationale Anticipatory nausea (AN) is a poorly controlled side effect experienced by chemotherapy patients. Currently, pharmacotherapy is restricted to benzodiazepine anxiolytics, which have limited efficacy, have significant sedative effects and induce dependency. The non-psychoactive phytocannabinoid, cannabidiolic acid (CBDA), has shown considerable efficacy in pre-clinical AN models, however determination of its neuromotor tolerability profile is crucial to justify clinical investigation. Provisional evidence for appetite-stimulating properties also requires detailed investigation.

Objectives This study aims to assess the tolerability of CBDA in locomotor activity, motor coordination and muscular strength tests, and additionally for ability to modulate feeding behaviours.

Methods Male Lister Hooded rats administered CBDA (0.05–5 mg/kg; p.o.) were assessed in habituated open field (for locomotor activity), static beam and grip strength tests. A further study investigated whether these CBDA doses modulated normal feeding behaviour. Finally, evidence of anxiolytic-like effects in the habituated open field prompted testing of 5 mg/kg CBDA for anxiolytic-like activity in unhabituated open field, light/dark box and novelty-suppressed feeding (NSF) tests.

Results CBDA had no adverse effects upon performance in any neuromotor tolerability test, however anxiolytic-like behaviour was observed in the habituated open field. Normal feeding behaviours were unaffected by any dose. CBDA (5 mg/kg) abolished the increased feeding latency in the NSF test induced by the 5-HT_{1A}R antagonist, WAY-100,635, indicative of anxiolytic-like effects, but had no effect on anxiety-like behaviour in the novel open field or light/dark box.

Conclusions CBDA is very well tolerated and devoid of the sedative side effect profile of benzodiazepines, justifying its clinical investigation as a novel AN treatment.

Keywords Cannabidiolic acid · Cannabinoid · WAY-100,635 · Anticipatory nausea · Chemotherapy · Tolerability · Appetite · Anxiety · Open field · Novelty-suppressed feeding

Abbreviations

CBDA	Cannabidiolic acid
CBD	Cannabidiol
pCB	Phytocannabinoid
CINV	Chemotherapy-induced nausea and vomiting
AN	Anticipatory nausea
CDP	Chlordiazepoxide
NK1	Neurokinin 1
5-HT	5-Hydroxytryptamine
5-HT_{1A}R	5-Hydroxytryptamine receptor type 1A

Introduction

Chemotherapy treatment commonly causes distressing and debilitating side effects in cancer patients, including acute and delayed vomiting (Martin 1996); acute, delayed and

✉ Claire M Williams
claire.williams@reading.ac.uk

[1] School of Psychology and Clinical Language Sciences, University of Reading, Reading, UK

[2] School of Chemistry, Food and Nutritional Sciences, and Pharmacy, University of Reading, Reading, UK

[3] GW Pharmaceuticals Ltd, Cambridge, UK

anticipatory nausea (Rock et al. 2014b); reduced food intake and bodyweight (Hainsworth and Hesketh 1992); and fatigue (Ahlberg et al. 2003). These chemotherapy-induced nausea and vomiting (CINV) symptoms are highly distressing for patients, adversely affecting quality of life to the point where some will delay and even consider refusing future cycles of chemotherapy treatment (Janelsins et al. 2013). It is estimated that, without prophylaxis, CINV would be experienced by up to 80 % of patients, with prevalence and severity varying according to the individual chemotherapy regimen employed (dos Santos et al. 2012). Many commonly prescribed chemotherapy drugs are classified as highly emetogenic within typical dose ranges, including cisplatin, cyclophosphamide (>1500 mg/m^2) and carmustine, all of which lead to CINV in >90 % of patients without effective prophylaxis (Hesketh 2008; Roila et al. 2010). Cisplatin, the most extensively studied highly emetogenic chemotherapy drug, elicits a biphasic CINV response, comprising an acute phase (within 24 h) and delayed phase (24–120 h), each with distinct pathogeneses and sensitivities to anti-emetic treatments (Martin 1996).

The effective control of the acute phase of CINV is achieved in approximately half of patients undergoing highly emetic chemotherapy using 5-HT$_3$ receptor antagonists (e.g. ondansetron) in combination with a glucocorticoid (e.g. dexamethasone). However, the delayed phase of CINV remains poorly controlled by this combination of drugs (Hickok et al. 2003). More recently, it has been shown that adjunctive use of neurokinin 1 (NK1) receptor antagonists (e.g. apripitant) with conventional anti-emetic treatment regimens can significantly reduce the incidence of delayed vomiting (Navari et al. 1999; Campos et al. 2001; Hesketh et al. 2003). Indeed, the combination therapy of NK1 receptor antagonist, 5HT$_3$R antagonist and dexamethasone is now strongly recommended for CINV prophylaxis in highly emetogenic regimes as it provides complete control of vomiting in both the acute and delayed phases of CINV in 60–70 % of patients (Kris et al. 2006; Roila et al. 2010). Despite these advances, the control of delayed nausea, and the consequences of incomplete control of acute and/or delayed vomiting, remains problematic and requires new anti-emetic strategies (Hesketh 2008; Janelsins et al. 2013).

Incomplete or ineffective control of nausea can cause increased anxiety, depression and the development of anticipatory nausea in patients (Rock et al. 2014b). Anticipatory nausea (AN) manifests as nausea (sometimes accompanied by vomiting) *prior* to administration of chemotherapy, and occurs in up to 20 % of patients before any one chemotherapy cycle and in up to 30 % of patients by the fourth cycle (Roscoe et al. 2011). AN is widely considered to be a form of Pavlovian classical conditioning, in which the cues of the clinical environment become associated with the nausea and vomiting induced by chemotherapy (Nesse et al. 1980; Matteson et al. 2002) and, as such, is not controlled by standard antiemetic treatments (Janelsins et al. 2013; Kamen et al. 2014). Furthermore, once AN has developed, patients also report more severe acute nausea following subsequent cycles of chemotherapy (Bovbjerg 2006).

At present, treatment options for AN remain limited, with clinical recommendations focussed on prophylaxis against the initial manifestation of AN through adequate control of acute nausea and vomiting (Basch et al. 2012). In patients who develop AN due to a failure of adequate control, recommendations are limited to behavioural interventions such as systemic desensitisation and progressive muscle relaxation (Figueroa-Moseley et al. 2007) or the use of non-specific benzodiazepine anxiolytic drugs (Kamen et al. 2014). While behavioural interventions, in particular systemic desensitisation, are considered the most promising option currently available, a systematic review has highlighted the limited evidence for their efficacy (Lotfi-Jam et al. 2008). Furthermore, a lack of suitably trained personnel in treatment settings has been identified as an ongoing difficulty for the implementation of such interventions (Roscoe et al. 2011). The use of benzodiazepine anxiolytics is supported by two small clinical trials. Razavi et al. (1993) investigated the use of alprazolam as an adjunct to psychological support to prevent AN in 57 women undergoing adjuvant chemotherapy for breast cancer. They found a significant reduction in AN rate at second assessment (0 vs 18 %), concluding alprazolam treatment delays the occurrence of AN. In a larger trial where lorazepam was administered with anti-emetic therapy (metoclopramide, clemastine and dexamethasone) in 180 patients receiving high-dose cisplatin, Malik et al. (1995) reported a significantly higher complete response to AN in patients receiving lorazepam (52 vs 35 %); however, these patients also experienced significantly higher occurrences of sedation (92 vs 37 %) and amnesia (32 vs 1 %). In addition to the debilitating side effects and dependency induced by benzodiazepine treatment, their efficacy against AN is also reduced during multiple chemotherapy cycles (Roila et al. 2010). Thus, there remains an unmet clinical need for convenient, effective and well-tolerated pharmacotherapies for AN.

Recently, a number of pre-clinical studies have identified the non-psychoactive phytocannabinoid, cannabidiolic acid (CBDA), as a potential novel pharmacotherapy for the treatment of AN (Bolognini et al. 2013; Rock and Parker 2013a; Rock and Parker 2013b; Rock et al. 2014a). Parker and colleagues assessed the ability of CBDA and a number of other phytocannabinoids to prevent cisplatin- or lithium chloride (LiCl)-induced vomiting (a model of acute vomiting) in house musk shrews and in rats to prevent LiCl-induced gaping (a model of acute nausea) or context-induced gaping (a conditioned model of AN). The potential for these drugs to enhance saccharin palatability was also assessed in the latter model (see Rock et al. 2014b for review of animal models). In studies using CBDA, low doses (0.01–0.5 mg/kg; i.p.) attenuated acute vomiting in shrews, and both acute and anticipatory nausea in rats, with the latter effect blocked by the 5-HT$_{1A}$R

antagonist WAY-100,635 (Bolognini et al. 2013). The same study reported an enhancement of saccharin palatability, as measured by unconditioned hedonic reactions. Further studies demonstrated that subthreshold doses of CBDA (0.1–0.5 μg/kg) potentiated the suppression of acute nausea by the anti-emetics ondansetron or metoclopramide (Rock and Parker 2013a; Rock and Parker 2013b). When CBDA was compared to the anti-emetic ondansetron or the benzodiazepine anxiolytic chlordiazepoxide (CDP) in the rat model of AN, both CBDA and CDP showed considerable efficacy, while ondansetron was ineffective (Rock et al. 2014a). Interestingly, in this study, rats were tested in an activity chamber for 15 min immediately following the AN trial, which demonstrated the expected benzodiazepine-induced suppression of locomotor activity in rats treated with CDP, but not in those with CBDA. These studies demonstrate that, in rodent models, CBDA is a highly potent treatment for both AN and acute nausea and vomiting. They also provide limited data to suggest CBDA may stimulate aspects of feeding under non-pathological conditions and lack the sedative effects of benzodiazepine anxiolytics.

To justify clinical investigation of CBDA as a novel AN treatment, it is crucial that the neuromotor tolerability profile is investigated in detail, to determine whether or not it elicits the side effects which have compromised the utility of benzodiazepines for this indication. Rock et al. (2014a) have shown that CBDA does not suppress spontaneous locomotor activity at doses ≤1 mg/kg; however, this represents the sum total of published tolerability data for CBDA. To provide a more complete assessment of CBDA tolerability, the first part of our study administered CBDA to rats across a greater dose range (0.05–5 mg/kg), after which they completed a battery of tests for effects on locomotor activity, balance, fine motor control and muscular strength. The previously reported observation that CBDA enhanced unconditioned saccharin palatability raises the intriguing possibility that CBDA may directly stimulate feeding behaviour, and thus may have additional therapeutic potential for the attenuation of chemotherapy-induced anorexia and/or cancer cachexia. In the second part of our study, we directly assessed the potential hyperphagic actions of CBDA using a well-established pre-feed paradigm for investigation of hyperphagic activity, which we have previously demonstrated for a number of other phytocannabinoids (Williams et al. 1998; Williams and Kirkham 2002; Farrimond et al. 2012a; Farrimond et al. 2012b).

Although the primary aims of this study were to determine the neuromotor tolerability and feeding behaviour profiles of CBDA, an additional follow-up experiment was also conducted to assess the anxiolytic effects of CBDA. During our battery of locomotor tasks, there was the suggestion of putative anxiolytic-like effects seen in the habituated open field test. As a final experiment therefore, using three tests of anxiety-like behaviour, CBDA was assessed alone and in combination with WAY-100,635, a 5-HT_{1A}R silent antagonist, as this receptor has previously been shown to block the effects of CBDA in models of acute and anticipatory nausea (Bolognini et al. 2013).

Methods

Drugs

CBDA (GW Pharmaceuticals, UK) was dissolved directly into sesame oil (by sonication at room temperature) to a maximal working concentration of 5 mg/ml. Working solutions of 0.5 and 0.05 mg/ml were prepared by serial dilution in sesame seed oil. WAY-100,635 (Sigma-Aldrich, UK) was dissolved directly into sterile 0.9 % saline vehicle (by vortex at room temperature), with a working concentration of 0.1 mg/ml prepared from frozen aliquots of 1 mg/ml stock solution. All drugs were prepared freshly each test day and protected from light until administration.

CBDA or sesame seed oil vehicle were administered per orem (p.o.) via a syringe placed into the cheek pouch at 1 ml/kg dosing volume, while WAY-100,635 or saline vehicle were administered intraperitoneally (i.p.) at an injection volume of 1 ml/kg.

Animals

Young adult male Lister Hooded rats (Harlan, UK), weighing 200–225 g on delivery, were housed in pairs in temperature- and humidity-controlled rooms with reversed light cycles (dim red light 12:00–24:00), with standard laboratory chow and water available ad libitum. A total of 60 rats were used in these experiments. All experiments were performed at the University of Reading in accordance with the principles of laboratory animal care, UK Home Office regulations [Animals (Scientific Procedures) Act 1986] and the ARRIVE guidelines for reporting experiments involving animals (Kilkenny et al. 2010; McGrath et al. 2010).

Experimental designs

Experiments 1 and 2 (neuromotor tolerability and acute feeding tests) were conducted using a within-subjects design, with all experimental units (individual animals) receiving 0.05, 0.5 and 5.0 mg/kg CBDA and vehicle according to a pseudo-random, counterbalanced, Latin square protocol. All animals received doses separated by a minimum 48 h washout period. On test days, animals were administered CBDA or vehicle 60 min prior to commencement of testing, consistent with our previously published studies of oral cannabinoid administration (Williams et al. 1998).

Experiment 3 (anxiety-like behavioural tests) was conducted using a between-subjects 2×2 design. Animals received

either WAY-100,635 (0.1 mg/kg) or saline and either CBDA (5 mg/kg) or sesame seed oil vehicle to yield four groups: saline/vehicle, saline/CBDA, WAY/vehicle and WAY/CBDA. WAY-100,635 or saline were administered 15 min prior to CBDA or vehicle (as per Parker and colleagues' protocol), with a further 60 min allowed for drug assimilation prior to commencement of testing. Animals were randomly allocated to the four treatment groups, and then further divided into five equally distributed blocks for daily testing, such that two animals from each group were tested on each day of the week, then again 1 week later. During the first week, animals completed the open field and light/dark box tests consecutively in a single session, followed by the novelty-suppressed feeding test 7 days later. The test order of groups was counterbalanced across the five test days each week.

Experiment 1 procedure (neuromotor tolerability)

Prior to testing, animals (n=12) were subjected to a 5-day habituation process, consisting of daily handling, vehicle drug administration and habituation to open field and static beam test procedures. On test days, all procedures were conducted during the first half of the dark period (12:00–18:00) in the same room as the animals were housed. All test equipment was cleaned with 70 % ethanol and allowed to dry completely between animals. All tasks were presented in the following order with animals having a 5 min rest period in their home cage between tasks.

Open field This consists of a 1.1×1.1×0.4 m black acrylic-lined box, delineated into 25 equal squares to form a 3×3 central sector and a single square wide peripheral sector. The open field was illuminated by dim red light (~10 lx). Animals were placed in the corner of the open field and left for 5 min with behaviour video recorded for offline coding using Observer XT software (Noldus, Netherlands). Locomotor activity was quantified based on the number of times animals crossed the lines on the open field floor, with time spent in the central area of the field and latency to first entry used to quantify anxiety-like behaviour (i.e. degree of thigmotaxis). It should be noted that the habituation animals received for this test is necessary for within-subjects assessment of drug-induced changes of locomotor activity; however as a consequence, the aversive/novel nature of the environment is attenuated. As such, a novel (i.e. unhabituated) open field test, as conducted in experiment 3, is more typically used when investigation of anxiety-like behaviour is the primary purpose of the test.

Static beam The apparatus consisted of a 3.2 cm diameter cylindrical beam, 1 m long and 0.5 m above floor level, with a bright light positioned at the start and an enclosed goal box at the end. Animals were placed at the start of the beam and allowed a maximum of 5 min to successfully traverse its length to reach the goal box. Animals were then given a 2 min rest period in home cages prior to repeating the test. Tests were video recorded for offline coding using Observer XT software (Noldus, Netherlands). In the static beam test, performance generated four outcome measures, based on successful completion or length of beam traversed prior to falling (pass rate and distance travelled), number of times paws were fully extended past the beam (foot slips) and time taken to traverse the middle 50 cm of beam (speed).

Forelimb grip strength Animals completed two repeats of the forelimb grip strength test, separated by a 30 s rest period. Animals were placed with forelimbs gripping a trapeze bar connected to a digital force gauge (FH50, Sauter GmbH, Germany), then uniformly pulled by the tail base away from bar along the horizontal plane until grip was released and peak force recorded.

Analysis All behavioural coding was conducted by an experimenter blinded to treatment allocation. For static beam and forelimb grip strength outcome measures, where animals were subjected to two tests during the battery, data represent the mean of the two technical repeats, with the exception of pass rate on static beam in which a score of 0–2 was allocated based on number of successfully completed tests. All continuous data were analysed using SPSS 18 (IBM, UK) by one-way repeated measures ANOVA (ordinal pass rate data were analysed by Friedman's ANOVA), with degrees of freedom and p values corrected where assumptions of sphericity were violated (using Greenhouse-Geisser correction). When significant overall dose effects were observed, planned comparisons of all dose groups vs vehicle group were conducted to reveal any significant pairwise comparisons. Results were considered significant if $p<0.05$.

Experiment 2 procedure (acute feeding)

Acute feeding experiments were conducted in pre-satiated animals according to a well-established paradigm for the detection of hyperphagia following administration of cannabinoids (Williams et al. 1998). Animals were habituated to handling (10 days), vehicle dosing and the pre-feed procedure (7 days), and the testing apparatus (5 days) prior to commencement of testing. The pre-feed procedure was conducted at the onset of the dark period, when animals (n=8) were transferred to individual cages containing 30.5±0.5 g of highly palatable wet-mash food. The wet-mash comprised 1 part Rat and Mouse Expanded Ground Diet (SDS, Witham, UK) and 1.25 parts tap water. Animals were allowed 2 h to consume the wet-mash, following which they were returned to their home cages and quantity of wet-mash consumed was measured. Animals were habituated to this pre-feed procedure until a stable

consumption level was reached, as indicated by a non-significant main effect of test day by one-way ANOVA across four consecutive habituation days ($F_{3, 28}$=0.653, p=0.588).

On test days, the pre-feed procedure was again conducted, immediately after which animals were administered CBDA or vehicle and replaced in home cages for 1 h for drug assimilation, during which time food was unavailable. Animals were then placed into feeder cages for 2 h, during which time food consumption and locomotor activity were recorded on automated food intake and infrared photobeam activity systems (TSE Systems, Germany and Ugo Basile, Italy respectively) and behaviour was video recorded. Animals were then returned to home cages at the end of the experiment, with food available ad libitum until the following test procedure ≥48 h later. Quantity of food consumed was confirmed manually by weighing the remaining chow pellets in food hoppers and any crumbs in spillage trays below the cages, and subtracting these from the initial weight of chow in the hopper. The automated food intake system provided data output on the time, duration and size of each feeding bout, which were confirmed from video recordings as genuine feeding episodes as opposed to exploratory interactions with food hoppers. Feeding bouts were combined into 'meals', defined as feeding bouts consuming ≥0.5 g and separated by ≥900 s, criteria previously shown to more accurately reflect the natural process of food consumption (Williams and Kirkham 2002; Farrimond et al. 2012b).

Analysis Data were analysed to provide measures of appetitive and consummatory behaviours, using the parameters of latency to first meal (appetitive) and meal sizes and durations (consummatory) in addition to total intake amounts. Ambulatory locomotor activity was quantified over the test duration using the number of infrared beam breaks. All continuous data were analysed using SPSS 18 (IBM, UK) by one-way repeated measures ANOVA, with degrees of freedom and p values corrected where assumptions of sphericity were violated (using Greenhouse-Geisser correction). When significant overall dose effects were observed, planned comparisons of all dose groups vs vehicle group were conducted to reveal any significant pairwise comparisons. Results were considered significant if $p<0.05$.

Experiment 3 procedure (unconditioned anxiety test battery)

Animals (n=40) were habituated to home environment and handling for 10 days prior to testing, and additionally to p.o. vehicle dosing and transfer to individual holding cages on the last 2 days of habituation. One day prior to the start of testing, all animals were tested for baseline levels of spontaneous locomotor activity, in which ambulatory activity was measured in an infrared photobeam activity cage (Ugo Basile, Italy) for 5 min. These data confirmed that randomisation to treatment group (as detailed above) had been successful, due to non-significant effects of treatment group on baseline activity ($F_{3, 36}$=1.342, p=0.276) or bodyweight ($F_{3, 36}$=0.4829, p=0.695).

All testing was completed during the first half of the dark period (12:00–18:00) in the same room as the animals were normally housed. On test days, animals were administered drugs at 30 min intervals from the onset of the dark period, such that all animals commenced testing 60 min after receiving CBDA or vehicle. Following drug administration, animals were placed in individual holding cages for the drug assimilation and inter-test rest periods. During testing in week 1, animals completed the open field test followed by the light/dark box test, separated by a 5-min rest period. During testing in week 2 (novelty-suppressed feeding), animals were food deprived in their home cages for 16–18 h prior to testing (dependent on test order).

Open field The open field test was conducted exactly as described for experiment 1; however, animals had not previously been habituated to the procedure/apparatus so the field represented a novel environment. Test data were analysed as described for experiment 1.

Light/dark box The apparatus consisted of an enclosed, black acrylic chamber (40×40×20 cm) connected via a small entrance hole to an open, white acrylic chamber of the same dimensions. The light sector was illuminated by a 60 W white lamp such that light levels were ~500 lx, in contrast to ~5 lx in the dark sector. Animals were placed into the light chamber facing the entrance hole and behaviour was video recorded for 5 min. Animals were then returned to home cages and equipment was cleaned with 70 % ethanol and allowed to dry completely. Movement between the sectors was recorded via an overhead digital video camera for subsequent offline coding using The Observer XT software (Noldus, The Netherlands), blinded to treatment group, with the number of entries and duration spent within the light sector quantified.

Novelty-suppressed feeding This task was conducted in a 1.1×1.1×0.4 m white-walled arena with a sawdust-covered floor. The field was illuminated by bright white light (~450 lx) and 10 standard chow pellets were placed on a large circular piece of filter paper in the centre. Animals were placed in the corner facing the centre and allowed a maximum of 10 min to begin feeding. Latency to onset of feeding (defined as pellet held in both paws and animals sat on haunches while eating) was timed manually and subsequently confirmed from the digital video recording of the test. As soon as an animal began feeding, it was removed from the open field and placed in an individual holding cage containing a weighed quantity of standard laboratory chow. It was allowed to feed ad libitum for 30 min, after which the quantity of food consumed was

recorded and the animal was returned to its home cage. The test thus generated outcome measures of latency to feeding onset and post-test food intake.

Analysis For all outcome measures, data were analysed by two-way independent ANOVA (CBDA × WAY). Where significant interactions were observed, follow-up analysis by one-way independent ANOVA and Tukey's post-hoc comparisons were conducted. To avoid attrition bias resulting from missing data points due to technical errors in data capture (two animals in light/dark box and two in novelty-suppressed feeding), data were analysed on an intention-to-treat basis, with missing data replaced by simple imputation methods (group means). Analysis on a per protocol basis with all animals with missing data excluded did not alter the experiment's conclusions. Results were considered significant at $p<0.05$.

Results

Experiment 1: neuromotor tolerability tests

To determine the viability of CBDA as a potential clinical candidate for the treatment of AN without the sedative effects typical of benzodiazapines, we first assessed its neuromotor tolerability profile using a battery of tests designed to reveal any effects on locomotor activity, balance and fine motor control and muscular strength. In addition to assessing locomotor activity, the habituated open field can provide an indication of any putative anxiolytic or anxiogenic activity.

Open field test

CBDA had no effect on locomotor activity at any dose when assessed in the open field test (Fig. 1a), with no significant overall effect of dose observed for the number of lines crossed ($F_{3,\ 33}=0.405$, $p=0.750$). However, a significant attenuation of anxiety-like behaviour was apparent, with total time spent in the central sector (Fig. 1b) increased with increasing CBDA dose ($F_{3,\ 33}=8.40$, $p<0.0005$). Planned comparisons revealed a significantly increased time spent in the central sector by both 0.5 mg/kg ($p=0.005$) and 5.0 mg/kg ($p<0.0005$) groups compared to vehicle-treated animals. In contrast, CBDA treatment had no effect upon latency to first entry into the central sector, a further measure of anxiety-like behaviour ($F_{3,\ 33}=0.769$, $p=0.52$).

Static beam test

CBDA had no effect at any dose on any measure of balance or motor coordination as assessed in the static beam test (Table 1). Neither balance, as assessed by pass rate ($F_{r\ 3}=3.522$, $p=0.318$), nor distance travelled ($F_{3,\ 33}=0.673$, $p=0.574$) was affected by CBDA treatment. Fine motor coordination was similarly unaffected, with CBDA treatment having no effect at any dose upon the number of foot slips made ($F_{3,\ 33}=0.605$, $p=0.617$) or time to cross the beam ($F_{3,\ 33}=1.105$, $p=0.361$).

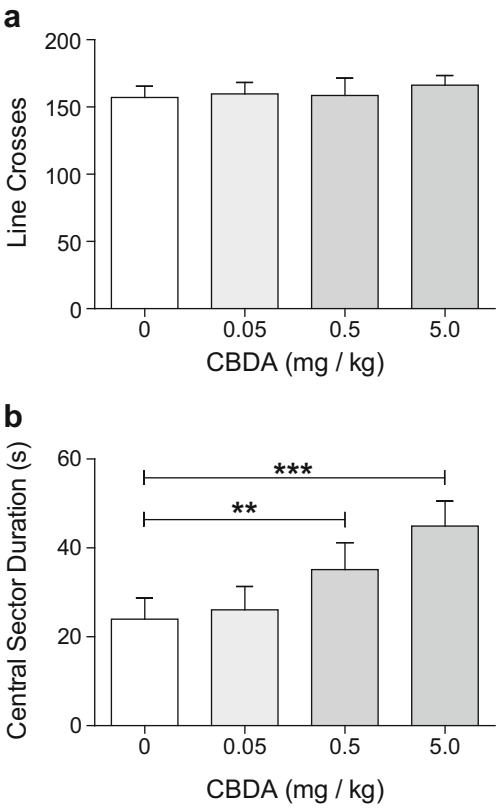


Fig. 1 Effects of CBDA treatment on behavioural parameters in habituated open field test, conducted as part of the neuromotor tolerability test battery (experiment 1). Ambulatory locomotor activity (**a**) as measured by number of line crosses was unaffected by any dose, however anxiolytic-like effects, as measured by increased time spent in central sector (**b**), were observed following 0.5 and 5.0 mg/kg CBDA treatment. Data presented as means±SEM and analysed by one-way repeated measures ANOVA and planned comparisons (all groups vs vehicle), all groups $n=12$, $^{**}p<0.01$, $^{***}p<0.001$

Grip strength test

The forelimb grip strength test (Table 1) for muscular strength and functional neurotoxicity revealed no significant overall dose effect of CBDA ($F_{1.5,\ 16.2}=1.109$, $p=0.335$).

The results from experiment 1 demonstrate that CBDA, at doses up to 5 mg/kg, is well tolerated and exerts no deleterious effects on locomotor activity, balance, fine motor control or muscular strength. Furthermore, the dose-dependent increase in central sector duration suggests that CBDA may possess anxiolytic-like properties. These findings support its viability as a novel treatment of anticipatory nausea, without the neuromotor side effects typical of the benzodiazepine anxiolytics currently in clinical use. In light of this favourable

Table 1 Performance parameters in static beam and grip strength tests, conducted as part of the neuromotor tolerability test battery (experiment 1)

CBDA (mg/kg)	0	0.05	0.5	5.0
Static beam test				
Pass rate (%)	100	95.8	95.8	100
Distance Travelled (m)	1.00 (±0.00)	0.98 (±0.02)	0.98 (±0.03)	1.00 (±0.00)
Footslips (per m)	1.17 (±0.26)	1.14 (±0.29)	0.73 (±0.28)	1.04 (±0.14)
Speed (m/s)	0.174 (±0.019)	0.151 (±0.022)	0.180 (±0.017)	0.133 (±0.020)
Grip strength test				
Grip strength (kgf)	0.787 (±0.057)	0.792 (±0.038)	0.935 (±0.114)	0.854 (±0.047)

Data presented as means±SEM, all groups $n=12$

tolerability profile, the ability of CBDA to stimulate feeding behaviours was investigated using the same dose range employed in experiment 1.

Experiment 2: test of hyperphagia in pre-satiated rats

To determine whether previously reported increases in saccharin palatability following CBDA administration were indicative of hyperphagic properties, we investigated the effects of CBDA on feeding behaviour in pre-satiated rats. As shown in Fig. 2a, CBDA dose exerted no significant overall effect on total food intake during the 2 h test period ($F_{1.4, 9.5}=0.336$, $p=0.641$). There was also no significant overall effect of CBDA dose on ambulatory locomotor activity (Fig. 2b) within the feeding chambers ($F_{3, 21}=0.309$, $p=0.819$), further validating our findings in the habituated open field test.

A more granular analysis of meal pattern microstructure parameters (Fig. 3) revealed no significant overall effect of CBDA dose on latency to meals 1 or 2 ($F_{3, 21}=0.348$, $p=0.791$ and $F_{3, 21}=0.546$, $p=0.656$ respectively), size of meals (meal 1: $F_{3, 21}=0.709$, $p=0.557$; meal 2: $F_{3, 21}=0.541$, $p=0.659$) or duration of meals (meal 1: $F_{1.5, 10.4}=0.832$, $p=0.429$; meal 2: $F_{3, 21}=0.821$, $p=0.399$). That the latency to first feeding episode was approximately 90 min into the test session for all groups demonstrates that the pre-satiation procedure was effective, and further corroborates the lack of CBDA effect on total food intake.

The results of experiment 2 demonstrate that CBDA, in the dose range tested, did not modulate any aspect of feeding behaviour in pre-satiated rats. Based on the results from experiments 1 and 2, a further study was conducted to assess whether the putative anxiolytic-like effect of CBDA could be validated in the novel open field and light/dark box tests. Additionally, CBDA was assessed in the novelty-suppressed feeding test, to investigate whether motivation to eat could be increased under anxiogenic-like conditions which typically suppress feeding behaviour. The 5-HT_{1A}R activation-dependent mechanism previously reported for CBDA in the AN model (Bolognini et al. 2013) led us to further investigate whether any anxiolytic-like effects were sensitive to 5-HT_{1A}R antagonist challenge in these tests.

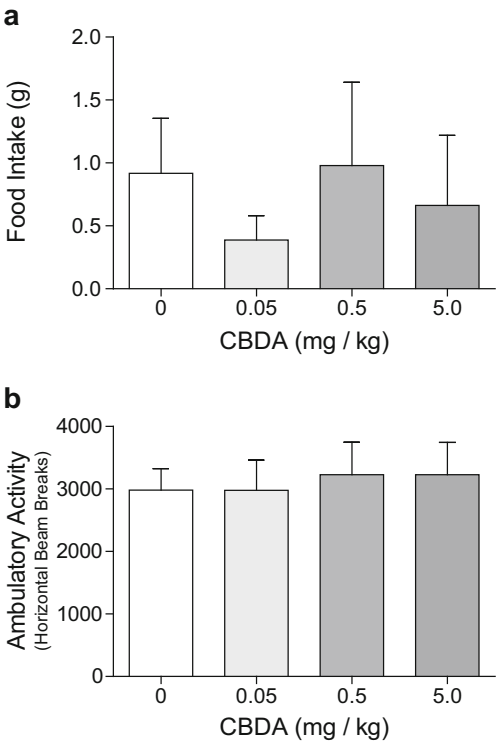


Fig. 2 Food intake and ambulatory locomotor activity during 2 h feeding test in pre-satiated rats (experiment 2). CBDA had no effect on total chow consumed (**a**) or total locomotor activity (**b**) at any dose. Data presented as mean±SEM and analysed by one-way repeated measures ANOVA, all groups $n=8$

Experiment 3: anxiety-like behaviour tests

Open field test

The total time spent in the central sector of the open field did not show significant main effects of either CBDA ($F_{1, 36}=0.177$, $p=0.676$) or WAY-100,635 administration ($F_{1, 36}=0.156$, $p=0.695$), nor was any interaction observed ($F_{1, 36}=0.042$, $p=0.838$). Locomotor activity within the open field, as measured by the number of line crosses, again did not show significant main effects of either CBDA ($F_{1, 36}=2.908$, $p=0.097$) or WAY-100,635 administration ($F_{1, 36}=0.613$, $p=0.439$), nor was any interaction observed ($F_{1, 36}=0.391$, $p=0.536$). However, a significant interaction was observed between the effects of WAY-100,635 and CBDA ($F_{1, 36}=5.270$, $p=0.028$) on latency to first entry into the central sector. Further analysis of this interaction using one-way ANOVA did not show a significant overall effect of treatment group

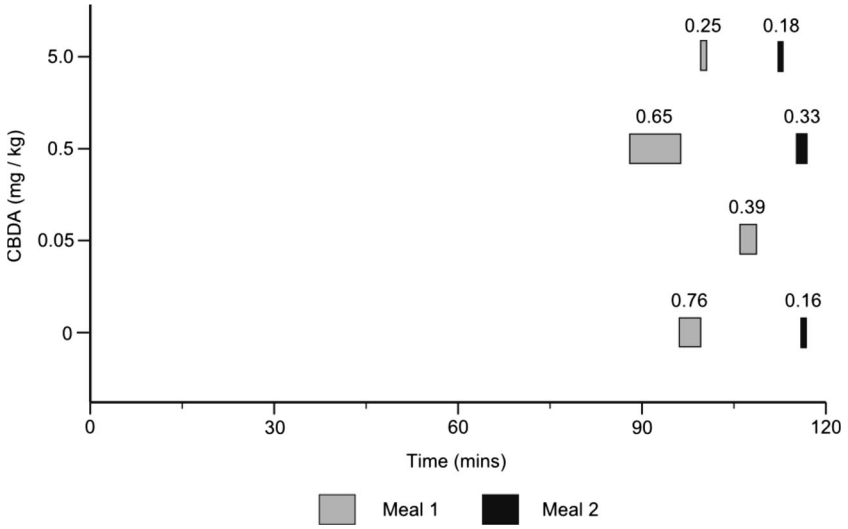


Fig. 3 Graphic summary of meal pattern microstructure parameters from experiment 2. Left edge of boxes positioned along *x*-axis according to meal latencies, box widths scaled to meal durations and meal sizes (in g) given above. CBDA had no effect on any of these measures at any dose. Note that no animals consumed a second meal in the 0.05 mg/kg group, hence this box is omitted from the figure, and group mean meal sizes below the 0.5-g meal criteria reflect that a number of animals consumed only ≤1 meal. Data presented as means and analysed by one-way repeated measures ANOVA, all groups $n=8$

($F_{3, 36}=1.879$, $p=0.151$) or any significant pairwise comparisons, indicating a lack of meaningful drug effect.

Light/dark box test

The number of entries into the light sector of the box did not show significant main effects of CBDA ($F_{1, 36}=1.677$, $p=0.204$) or WAY-100,635 ($F_{1, 36}=0.995$, $p=0.325$), nor was any interaction observed ($F_{1, 36}=0.379$, $p=0.542$). The total time spent in the light sector of the box did not show significant main effects of CBDA ($F_{1, 36}=1.096$, $p=0.302$) or WAY-100,635 ($F_{1, 36}=0.237$, $p=0.629$), nor was any interaction observed ($F_{1, 36}=0.501$, $p=0.484$).

Novelty-suppressed feeding test

A significant interaction was observed between the effects of CBDA and WAY-100,635 ($F_{1, 36}=7.551$, $p=0.009$) on latency to onset of feeding (Fig. 4a). Follow-up analysis revealed a significant overall effect of treatment group ($F_{3, 36}=10.619$, $p<0.0005$), due to an increased latency to feed in animals treated with WAY-100,635 alone vs vehicle control animals ($p=0.017$) or those treated with CBDA alone ($p<0.0005$). This increased latency was completely abolished in animals treated with both CBDA and WAY-100,635 ($p<0.0005$). Post-test food intake in home cages (Fig. 4b) did not show significant main effects of either CBDA ($F_{1, 36}=1.266$, $p=0.268$) or WAY-100,635 administration ($F_{1, 36}=2.056$, $p=0.160$), nor was any interaction observed ($F_{1, 36}=1.811$, $p=0.187$). The lack of effect of either drug on post-test food intake indicates that their effect on latency to feed was due to modulation of anxiety-like behaviour alone, and not confounded by effects on appetite.

Discussion

Our results suggest CBDA is well-tolerated, since it failed to produce any neuromotor side effects at any dose tested. In the same dose range, CBDA also had no modulatory effect on feeding behaviour in healthy, pre-satiated rats. However, CBDA did abolish the potentiated suppression of feeding behaviour in the NSF test induced by the 5-HT_{1A}R antagonist WAY-100,635. Thus, CBDA does not appear to increase appetite per se, but may selectively stimulate feeding under putatively anxiogenic conditions which suppress feeding behaviour, possibly via 5-HT_{1A}R-mediated mechanisms.

The battery of neuromotor tolerability tests used in this study has previously been utilised to assess other phytocannabinoids against drugs with known clinical neuromotor side effects (Hill et al. 2012; Hill et al. 2013). The benzodiazepine class of drugs, which are used clinically to attenuate AN, cause significant sedative side effects, decrease activity in the OFT (reviewed in Prut and Belzung 2003) and impair performance in the static beam (Stanley et al. 2005) and forelimb grip strength assays (Meyer et al. 1979; Ferguson and Paule 1996). Thus, these tests have predictive validity for assessment of the neuromotor tolerability profile of novel compounds for AN treatment. In our experiments, CBDA did not affect activity in the open field or cause any detrimental effects on any performance measure in either static beam or grip strength tests at any dose tested, the range of which was comparable to that used in previous studies in

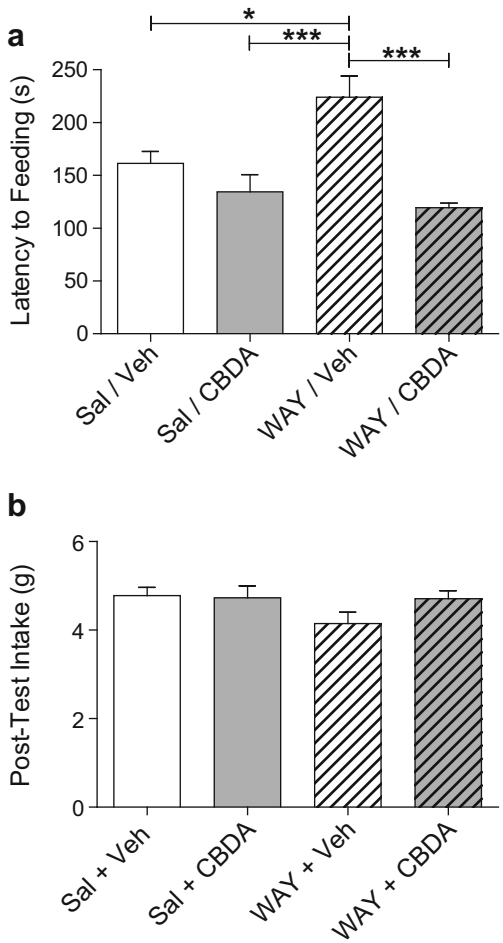


Fig. 4 Effects of CBDA (5 mg/kg) and the 5-HT_{1A}R antagonist WAY-100,635 (0.1 mg/kg) in the novelty-suppressed feeding test, conducted in a modified open field as part of the anxiety-like behaviour test battery (experiment 3). Treatment with WAY-100,635 alone elicited an anxiogenic-like effect by increasing latency to feeding onset, which was abolished by co-treatment with CBDA (**a**). Home cage food intake in the 30 min following the test was unaffected by either drug (**b**). Data presented as means±SEM and analysed by two-way ANOVA, followed by one-way ANOVA and Tukey's post-hoc comparisons, all groups n= 10, *p<0.05, ***p<0.001

models of acute and anticipatory nausea. The lack of effect on locomotor activity in the OFT is consistent with a previously published report that CBDA (0.0001-1 mg/kg; i.p.) did not affect distance travelled in a 15-min activity chamber test (Rock et al. 2014a), although doses of the benzodiazepine CDP which suppressed AN (5–10 mg/kg) exerted a sedative effect in this test. The observation that ambulatory locomotor activity during the duration of the feeding test (experiment 2) was also unaffected by any dose of CBDA further confirms the lack of sedative effect, even over an extended test period (2 h) -considerably longer than that typically used for activity tests (Curzon et al. 2009). The present study extended the investigation of potential sedative effects to include measures of motor coordination, using the static (walking) beam assay, which can more sensitively predict clinical sedative effects than the more commonly used rotarod test (Stanley et al. 2005; Hill et al. 2012). CBDA had no effect at any dose on performance measures of balance or fine motor control in this test. The final component of the tolerability test battery, the forelimb grip strength test, demonstrated that CBDA administration did not result in drug-induced muscle relaxation at any dose. These results validate and considerably extend the preliminary evidence for the lack of sedative effects of CBDA, supporting its potential as a novel treatment for AN unlikely to have the compromised clinical utility of benzodiazepines (Malik et al. 1995; Rock et al. 2014b).

A previously published study of the effects of CBDA in the AN model reported increased unconditioned hedonic reactions to saccharin (i.e. increased palatability), which the authors speculated could indicate an appetite-enhancing effect (Bolognini et al. 2013). Such an effect could have an additional clinical utility by attenuating the comorbid anorectic effects of chemotherapy treatment (Hainsworth and Hesketh 1992) and/or cancer anorexia-cachexia syndrome (Stephens and Fearon 2008).

To investigate whether this suggestion of an appetite-enhancing effect could first be validated in healthy rats under more naturalistic feeding conditions (than intraoral cannula-delivered saccharin responses), experiment 2 was conducted using a well-established test of hyperphagia. The acute feeding test in pre-satiated rats has been utilised in many previous studies in our lab to sensitively determine hyperphagic actions of pharmacological compounds, providing detailed information on both food intake and the microstructure of meal patterns (Williams et al. 1998; Williams and Kirkham 2002; Farrimond et al. 2010a; Farrimond et al. 2010b; Farrimond et al. 2012a). In vehicle-dosed rats, feeding behaviour during the test period is minimal, typically comprising one to two small meals with a total consumption of ≤1 g, occurring after ~90 min (Farrimond et al. 2012b). Consistent with this typical baseline level of consumption, no significant effect was seen on total food intake following administration of any dose of CBDA, with rats consuming 0.4–1 g over 2 h. The latency to consumption of the first meal, a measure of appetitive feeding behaviour (motivation to eat), was similarly unaffected by CBDA treatment, and neither were consummatory behaviour measures of meal size or duration. These data indicate that, at oral doses of 0.05–5 mg/kg, CBDA does not modulate total food intake or any aspects of meal microstructure. This is in contrast to the reported effect on saccharin palatability, however it should be noted that the previously reported effect was only seen at 0.01 mg/kg, but not at 0.1–5 mg/kg, and furthermore the behavioural model and route of administration were also different (Bolognini et al. 2013). It therefore remains possible that CBDA may have appetite-stimulating effects only at very low doses, or selectively for hedonic foods over regular chow, however the present data does not support any effects on feeding behaviour at doses ≥0.05 mg/kg. However,

in light of the effects seen in the NSF test presented here, it may be the case that (at least at higher doses) CBDA selectively stimulates feeding under putatively anxiogenic conditions, which is more consistent with the positive effects seen in the AN model, and may be more clinically useful. As such, further investigation of CBDA actions on feeding in models of chemotherapy- or anxiety-induced anorexia is warranted.

An interesting observation made during the neuromotor tolerability study was the dose-dependent increase in the time rats spent in the central sector of the habituated open field. This test was primarily designed as a test of sedative/stimulant effects, and hence rats were habituated to the open field to achieve stable baseline activity prior to CBDA administration. However, the lack of locomotor activity modulation in this test (as measured by line crosses) suggests this observation may still be indicative of an anxiolytic-like effect. Cannabidiol (CBD), produced by spontaneous decarboxylation of CBDA (Cluny et al. 2011), has well-documented anxiolytic-like effects in both animals and humans (reviewed by de Schier et al. 2012) which appears to be primarily facilitated by 5-HT_{1A}R-mediated neurotransmission (Campos et al. 2012). However, to the best of our knowledge, only a single study of the anxiolytic-like effects of CBDA has been published to date. In this study, CBDA (0.001–1 mg/kg, i.p.) was assessed for the ability to attenuate conditioned freezing to a shock-paired tone; however, expression of conditioned freezing was not modified by any dose (Rock et al. 2014a). The suggestion of an anxiolytic-like effect of CBDA in the habituated open field test, and the paucity of published data in anxiety-like behavioural models for this cannabinoid, prompted us to further investigate the effects of CBDA in three typical models of unconditioned anxiety-like behaviour. As the greatest effect in the habituated open field was seen following administration of 5 mg/kg CBDA, and previous reports implicated indirect 5-HT_{1A}R activation in AN models (Bolognini et al. 2013; Rock et al. 2014a), we investigated this dose with and without pre-treatment with the selective 5-HT_{1A}R antagonist WAY-100,635, at the same dose used by Parker and colleagues as a behaviourally silent antagonist in their AN studies. In the novel (unhabituated) open field test, the more aversive nature of the environment was apparent from both the reduced central sector duration in control rats (16 vs 24 s in the habituated OFT) and number of line crosses (96 vs 157 in the habituated OFT). However, in this test, CBDA had no effect on central sector duration or number of line crosses, suggesting that in this more aversive environment CBDA did not have significant anxiolytic-like effects, and thus CBDA has limited, if any, efficacy within this test. Consistent with the results from the novel open field, in the light/dark box test, which is another test based on the conflict between rats' exploratory drive and fear of bright or exposed areas (Bourin and Hascoët 2003), CBDA also had no effect on either number of entries or duration spent in the light sector, which would be indicative of an anxiolytic-like effect. In both tests, administration of 0.1 mg/kg WAY-100,635, alone or in combination with CBDA, also had no effect on any measure of anxiety-like behaviour or general locomotor activity. This indicates that this dose, which was behaviourally silent in previous AN studies, was also appropriate as a silent antagonist challenge in the open field and light/dark box tests, and that no interaction occurred with CBDA relevant to behavioural outcomes in these tests.

A third test of anxiety-like behaviour was conducted using the novelty-suppressed feeding (or hyponeophagia) test, which differs from the open field and light/dark box tests in that the conflict arises between the innate aversion to bright unfamiliar spaces and the desire to feed (following a period of food deprivation) rather than to explore a novel environment (Britton and Britton 1981; Dulawa and Hen 2005). The NSF test is sensitive to numerous drugs with known anxiolytic activity, including the 5-HT_{1A}R agonist 8-OH-DPAT (Rex et al. 1998; Zhang et al. 2010), and also demonstrates the anxiogenic-like activity of the 5-HT_{1A}R antagonist NAN-190 (Zhang et al. 2010) and increased anxiety-like behaviour in 5-HT_{1A}R knockout mice (Gross et al. 2000). In the present study, treatment with CBDA alone did not affect the latency to feed, however WAY-100,635 treatment alone significantly increased latency, indicative of an anxiogenic-like effect. That administration of WAY-100,635 alone had an anxiogenic-like effect in this test was unexpected, given that the dose of this compound was chosen as a behaviourally silent antagonist challenge, which had no effect in either previous AN studies (Bolognini et al. 2013) or in the novel open field or light/dark box tests in the present study. While this increased latency is consistent with the work of Zhang et al. (2010) using the 5-HT_{1A}R antagonist NAN-190, it should also be noted that a 0.3-mg/kg dose of WAY-100,635 given to mice in the NSF test was behaviourally silent (Duvvuri et al. 2009), and that in other tests of anxiety-like behaviour this compound can be anxiogenic or even anxiolytic dependent on dose and test type (Sánchez 1996; Griebel et al. 1999; Griebel et al. 2000). Interestingly, in rats which were administered both CBDA and WAY-100,635 in the present NSF test, this anxiogenic-like effect of WAY-100,635 was completely abolished. The results from the post-test home cage intake test did not show significant effects of either drug or their combination, ruling out confounding effects on appetite, consistent with results from the acute feeding study. Similarly, the lack of effect of either drug on the number of line crosses in the novel open field test rules out possible confounding effects of locomotor activity modulation. It thus appears that this dose of WAY-100,635, while behaviourally silent in the open field and light/dark box tests, elicits an anxiogenic-like response in the NSF test, and that this response is antagonised by CBDA, despite this cannabinoid having no anxiolytic-like effect when administered alone. Such pharmacological

effects, while seemingly robust in terms of the data obtained, are less than straightforward to interpret based on the present experiments alone.

Previous studies demonstrated that while the ability of CBDA to attenuate nausea is abolished by pretreatment with WAY-100,635, in vitro binding experiments suggested this is via an indirect enhancement of 5-HT_{1A}R activation rather than direct activation (Bolognini et al. 2013). The results from experiment 3 thus provides some further support for the notion that CBDA has limited efficacy as a typical 5-HT_{1A}R agonist anxiolytic, but under certain anxiogenic conditions does possess anxiolytic-like activity, presumably via indirect modulation of 5-HT_{1A}R-mediated neurotransmission. While beyond the scope of the present study, it may be valuable to further characterise the locus and mechanism of this activity.

The present report provides vital further data in support of CBDA as a novel treatment for anticipatory nausea, which is unlikely to elicit the compromising sedative effects of the benzodiazepine anxiolytics currently in clinical use. CBDA appears to have some anxiolytic-like activity, specific to models of feeding suppression or nausea involving alterations in 5-HT_{1A}R-dependent neurotransmission. CBDA did not modulate feeding behaviour in healthy rats, however these and previous data suggest beneficial effects on feeding may occur under pathological anxiogenic conditions, further investigation of which is warranted. While such investigations may provide evidence of further therapeutic potential for such conditions, the tolerability data presented here strongly supports clinical investigation of CBDA as a non-sedative alternative to benzodiazepine anxiolytics for the treatment of AN.

Acknowledgments This research was supported by grants to CMW and BJW by GW Pharmaceuticals and Otsuka Pharmaceuticals, and in part by the University of Reading Research Endowment Trust Fund to DIB.

Compliance with ethical standards

Conflict of interest The work reported was funded in part by grants to BJW and CMW from GW Pharmaceuticals and Otsuka Pharmaceuticals. The original study concept was discussed with the sponsor (GW Pharmaceuticals) although all subsequent study design, data collection, analysis and interpretation were conducted independently by the authors. The report was approved by the sponsor company prior to submission, and the authors retain full control of all primary data.

References

Ahlberg K, Ekman T, Gaston-Johansson F, Mock V (2003) Assessment and management of cancer-related fatigue in adults. Lancet 362: 640–650. doi:10.1016/S0140-6736(03)14186-4

Basch E, Prestrud AA, Hesketh PJ et al (2012) Antiemetic use in oncology: updated guideline recommendations from ASCO. Am Soc Clin Oncol Educ Book 532:532–540. doi:10.14694/EdBook_AM.2012.32.532

Bolognini D, Rock EM, Cluny NL et al (2013) Cannabidiolic acid prevents vomiting in *Suncus murinus* and nausea-induced behaviour in rats by enhancing 5-HT1A receptor activation. Br J Pharmacol 168: 1456–1470. doi:10.1111/bph.12043

Bourin M, Hascoët M (2003) The mouse light/dark box test. Eur J Pharmacol 463:55–65. doi:10.1016/S0014-2999(03)01274-3

Bovbjerg DH (2006) The continuing problem of post chemotherapy nausea and vomiting: contributions of classical conditioning. Auton Neurosci 129:92–98. doi:10.1016/j.autneu.2006.07.016

Britton DR, Britton KT (1981) A sensitive open field measure of anxiolytic drug activity. Pharmacol Biochem Behav 15:577–582. doi:10.1016/0091-3057(81)90212-4

Campos AC, Moreira FA, Gomes FV et al (2012) Multiple mechanisms involved in the large-spectrum therapeutic potential of cannabidiol in psychiatric disorders. Philos Trans R Soc B Biol Sci 367:3364–3378. doi:10.1098/rstb.2011.0389

Campos D, Pereira JR, Reinhardt RR et al (2001) Prevention of cisplatin-induced emesis by the oral neurokinin-1 antagonist, MK-869, in combination with granisetron and dexamethasone or with dexamethasone alone. J Clin Oncol 19:1759–1767

Cluny NL, Naylor RJ, Whittle BA, Javid FA (2011) The effects of cannabidiolic acid and cannabidiol on contractility of the gastrointestinal tract of *Suncus murinus*. Arch Pharm Res 34:1509–1517. doi:10.1007/s12272-011-0913-6

Curzon P, Zhang M, Radek R, Fox G (2009) The behavioral assessment of sensorimotor processes in the mouse: acoustic startle, sensory gating, locomotor activity, rotarod, and beam walking. Methods Behav. Anal, Neurosci

Dos Santos LV, Souza FH, Brunetto AT et al (2012) Neurokinin-1 receptor antagonists for chemotherapy-induced nausea and vomiting: a systematic review. J Natl Cancer Inst 104:1280–1292. doi:10.1093/jnci/djs335

Dulawa SC, Hen R (2005) Recent advances in animal models of chronic antidepressant effects: the novelty-induced hypophagia test. Neurosci Biobehav Rev 29:771–783. doi:10.1016/j.neubiorev.2005.03.017

Duvvuri V, Risbrough VB, Kaye WH, Geyer MA (2009) 5-HT1A receptor activation is necessary for 5-MeODMT-dependent potentiation of feeding inhibition. Pharmacol Biochem Behav 93:349–353. doi:10.1016/j.pbb.2009.05.014

Farrimond JA, Hill AJ, Whalley BJ, Williams CM (2010a) Cannabis constituents modulate δ9-tetrahydrocannabinol-induced hyperphagia in rats. Psychopharmacology (Berl) 210:97–106. doi:10.1007/s00213-010-1821-z

Farrimond JA, Whalley BJ, Williams CM (2012a) Non-Δ^9tetrahydrocannabinol phytocannabinoids stimulate feeding in rats. Behav Pharmacol 23:113–117. doi:10.1097/FBP.0b013e32834ed832

Farrimond JA, Whalley BJ, Williams CM (2012b) Cannabinol and cannabidiol exert opposing effects on rat feeding patterns. Psychopharmacology (Berl) 223:117–129. doi:10.1007/s00213-012-2697-x

Farrimond JA, Whalley BJ, Williams CM (2010b) A low-Δ9tetrahydrocannabinol cannabis extract induces hyperphagia in rats. Behav Pharmacol. doi:10.1097/FBP.0b013e328340a062

Ferguson SA, Paule MG (1996) Effects of chlorpromazine and diazepam on time estimation behavior and motivation in rats. Pharmacol Biochem Behav 53:115–122. doi:10.1016/0091-3057(95)02002-0

Figueroa-Moseley C, Jean-Pierre P, Roscoe JA et al (2007) Behavioral interventions in treating anticipatory nausea and vomiting. J Natl Compr Canc Netw 5:44–50

Griebel G, Rodgers RJ, Perrault G, Sanger DJ (1999) Behavioural profiles in the mouse defence test battery suggest anxiolytic potential of 5-HT 1A receptor antagonists. Psychopharmacology (Berl) 144: 121–130. doi:10.1007/s002130050984

Griebel G, Rodgers RJ, Perrault G, Sanger DJ (2000) The effects of compounds varying in selectivity as 5-HT1A receptor antagonists in three rat models of anxiety. Neuropharmacology 39:1848–1857. doi:10.1016/S0028-3908(00)00074-5

Gross C, Santarelli L, Brunner D et al (2000) Altered fear circuits in 5-HT(1A) receptor KO mice. Biol Psychiatry 48:1157–1163. doi:10.1016/S0006-3223(00)01041-6

Hainsworth JD, Hesketh PJ (1992) Single-dose ondansetron for the prevention of cisplatin-induced emesis: efficacy results. Semin Oncol 19:14–19

Hesketh PJ (2008) Chemotherapy-induced nausea and vomiting. N Engl J Med 358:2482–2494. doi:10.1056/NEJMra0706547

Hesketh PJ, Grunberg SM, Gralla RJ et al (2003) The oral neurokinin-1 antagonist aprepitant for the prevention of chemotherapy-induced nausea and vomiting: a multinational, randomized, double-blind, placebo-controlled trial in patients receiving high-dose cisplatin—the Aprepitant Protocol 052 Study Gr. J Clin Oncol 21:4112–4119. doi:10.1200/JCO.2003.01.095

Hickok JT, Roscoe JA, Morrow GR et al (2003) Nausea and emesis remain significant problems of chemotherapy despite prophylaxis with 5-hydroxytryptamine-3 antiemetics: a University of Rochester James P. Wilmot Cancer Center Community Clinical Oncology Program Study of 360 cancer patients treated in treated in the community. Cancer 97:2880–2886. doi:10.1002/cncr.11408

Hill AJ, Mercier MS, Hill TDM et al (2012) Cannabidivarin is anticonvulsant in mouse and rat. Br J Pharmacol 167:1629–1642. doi:10.1111/j.1476-5381.2012.02207.x

Hill TDM, Cascio M-G, Romano B et al (2013) Cannabidivarin-rich cannabis extracts are anticonvulsant in mouse and rat via a CB1 receptor-independent mechanism. Br J Pharmacol 170:679–692. doi:10.1111/bph.12321

Janelsins MC, Tejani MA, Kamen C et al (2013) Current pharmacotherapy for chemotherapy-induced nausea and vomiting in cancer patients. Expert Opin Pharmacother 14:757–766. doi:10.1517/14656566.2013.776541

Kamen C, Tejani MA, Chandwani K et al (2014) Anticipatory nausea and vomiting due to chemotherapy. Eur J Pharmacol 722:172–179. doi:10.1016/j.ejphar.2013.09.071

Kilkenny C, Browne W, Cuthill IC et al (2010) Animal research: reporting in vivo experiments: the ARRIVE guidelines. Br J Pharmacol 160:1577–1579. doi:10.1111/j.1476-5381.2010.00872.x

Kris MG, Hesketh PJ, Somerfield MR et al (2006) American Society of Clinical Oncology guideline for antiemetics in oncology: update 2006. J Clin Oncol 24:2932–2947. doi:10.1200/JCO.2006.06.9591

Lotfi-Jam K, Carey M, Jefford M et al (2008) Nonpharmacologic strategies for managing common chemotherapy adverse effects: a systematic review. J Clin Oncol 26:5618–5629. doi:10.1200/JCO.2007.15.9053

Malik IA, Khan WA, Qazilbash M et al (1995) Clinical efficacy of lorazepam in prophylaxis of anticipatory, acute, and delayed nausea and vomiting induced by high doses of cisplatin. A prospective randomized trial. Am J Clin Oncol 18:170–175

Martin M (1996) The severity and pattern of emesis following different cytotoxic agents. Oncology 53(Suppl 1):26–31. doi:10.1159/000227637

Matteson S, Roscoe J, Hickok J, Morrow GR (2002) The role of behavioral conditioning in the development of nausea. Am J Obstet Gynecol 186:S239–S243. doi:10.1067/mob.2002.122597

McGrath JC, Drummond GB, McLachlan EM et al (2010) Guidelines for reporting experiments involving animals: the ARRIVE guidelines. Br J Pharmacol 160:1573–1576. doi:10.1111/j.1476-5381.2010.00873.x

Meyer OA, Tilson HA, Byrd WC, Riley MT (1979) A method for the routine assessment of fore- and hindlimb grip strength of rats and mice. Neurobehav Toxicol 1:233–236

Navari RM, Reinhardt RR, Gralla RJ et al (1999) Reduction of cisplatin-induced emesis by a selective neurokinin-1-receptor antagonist. L-754,030 Antiemetic Trials Group. N Engl J Med 340:190–195. doi:10.1056/NEJM199901213400304

Nesse RM, Carli T, Curtis GC, Kleinman PD (1980) Pretreatment nausea in cancer chemotherapy: a conditioned response? Psychosom Med 42:33–36

Prut L, Belzung C (2003) The open field as a paradigm to measure the effects of drugs on anxiety-like behaviors: a review. Eur J Pharmacol 463:3–33. doi:10.1016/S0014-2999(03)01272-X

Razavi D, Delvaux N, Farvacques C et al (1993) Prevention of adjustment disorders and anticipatory nausea secondary to adjuvant chemotherapy: a double-blind, placebo-controlled study assessing the usefulness of alprazolam. J Clin Oncol 11:1384–1390

Rex A, Voigt JP, Voits M, Fink H (1998) Pharmacological evaluation of a modified open-field test sensitive to anxiolytic drugs. Pharmacol Biochem Behav 59:677–683. doi:10.1016/S0091-3057(97)00461-9

Rock E, Parker L (2013a) Effect of low doses of cannabidiolic acid and ondansetron on LiCl-induced conditioned gaping (a model of nausea-induced behaviour) in rats. Br J Pharmacol. doi:10.1111/bph.12162

Rock EM, Limebeer CL, Navaratnam R et al (2014a) A comparison of cannabidiolic acid with other treatments for anticipatory nausea using a rat model of contextually elicited conditioned gaping. Psychopharmacology (Berl). doi:10.1007/s00213-014-3498-1

Rock EM, Limebeer CL, Parker LA (2014b) Anticipatory nausea in animal models: a review of potential novel therapeutic treatments. Exp Brain Res. doi:10.1007/s00221-014-3942-9

Rock EM, Parker LA (2013b) Suppression of lithium chloride-induced conditioned gaping (a model of nausea-induced behaviour) in rats (using the taste reactivity test) with metoclopramide is enhanced by cannabidiolic acid. Pharmacol Biochem Behav 111:84–89. doi:10.1016/j.pbb.2013.08.012

Roila F, Herrstedt J, Aapro M et al (2010) Guideline update for MASCC and ESMO in the prevention of chemotherapy- and radiotherapy-induced nausea and vomiting: results of the Perugia consensus conference. Ann Oncol 21(Suppl 5):v232–v243. doi:10.1093/annonc/mdq194

Roscoe JA, Morrow GR, Aapro MS et al (2011) Anticipatory nausea and vomiting. Support Care Cancer 19:1533–1538. doi:10.1007/s00520-010-0980-0

Sánchez C (1996) 5-HT(1A) receptors play an important role in modulation of behavior of rats in a two-compartment black and white box. Behav Pharmacol 7:788–797

de Schier ARM, Ribeiro NP, Silva AC et al (2012) Cannabidiol, a *Cannabis sativa* constituent, as an anxiolytic drug. Rev Bras Psiquiatr 34(Suppl 1):S104–S110. doi:10.1590/S1516-44462012000500008

Stanley JL, Lincoln RJ, Brown TA et al (2005) The mouse beam walking assay offers improved sensitivity over the mouse rotarod in determining motor coordination deficits induced by benzodiazepines. J Psychopharmacol 19:221–227. doi:10.1177/0269881105051524

Stephens N, Fearon K (2008) Anorexia, cachexia and nutrition. Medicine (Baltimore) 36:78–81. doi:10.1016/j.mpmed.2007.11.004

Williams CM, Kirkham TC (2002) Observational analysis of feeding induced by Delta9-THC and anandamide. Physiol Behav 76:241–250. doi:10.1016/S0031-9384(02)00725-4

Williams CM, Rogers PJ, Kirkham TC (1998) Hyperphagia in pre-fed rats following oral delta9-THC. Physiol Behav 65:343–346. doi:10.1016/S0031-9384(98)00170-X

Zhang J, Huang X-Y, Ye M-L et al (2010) Neuronal nitric oxide synthase alteration accounts for the role of 5-HT1A receptor in modulating anxiety-related behaviors. J Neurosci 30:2433–2441. doi:10.1523/JNEUROSCI.5880-09.2010