



Chlorpyrifos and Δ^9 Tetrahydrocannabinol exposure and effects on parameters associated with the endocannabinoid system and risk factors for obesity

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ABSTRACT

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Increased childhood and adult obesity are associated with chlorpyrifos (CPF), an organophosphate pesticide. Cannabis (Δ^9 Tetrahydrocannabinol: Δ^9 THC) use has increased globally with legalization. CPF applications on cannabis crops lacks federally regulated tolerances and may pose health risks through exposure during development and in adulthood. Both CPF and Δ^9 THC affect the endocannabinoid system (eCBS), a regulator of appetite, energy balance, and gut microbiota, which, if disrupted, increases risk for obesity and related diseases. CPF inhibits eCB metabolism and Δ^9 THC is a partial agonist/antagonist at the cannabinoid receptor (CB1R). Effects of each on obesogenic parameters were examined via literature search. Male rodents with CPF exposure showed increased body weights, dysbiosis, inflammation and oxidative stress, potentially associated with increased eCBs acting through the gut-microbiota-adipose-brain regulatory loop. Δ^9 THC generally decreased body weights via partial agonism at the CB1R, lowering levels of eCBs. Dysbiosis and/or oxidative stress associated inflammation occurred with CPF, but these parameters were not tested with Δ^9 THC. Database deficiencies included limited endpoints to compare between chemicals/age-groups, inter-study variables (dose ranges, dosing vehicle, rodent strain, treatment duration, etc.). CPF and Δ^9 THC were not tested together, but human co-chemical effects would depend on exposure ratio, subject age, exposure duration, and health status, among others. An overriding concern is that both chemicals are well-documented developmental neurotoxins in addition to their low dose effects on energy balance. A co-exposure risk assessment is warranted with increased use and lack of federal CPF regulation on cannabis.

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Introduction¹

Obesity in the United States has rapidly increased in adults to 42% (2017–2018) and in children (age 2–19) to 20% (2019; [Overweight & Obesity | CDC](#)). Childhood overweight and obesity present an increased risk for type-2 diabetes (T2D), hypertension and cardiovascular diseases in later life ([Weihrauch-Blüher and Wiegand, 2018](#)). Excessive lipid accumulation in adipose tissue has been attributed, in part, to environmental obesogenic chemicals, including pesticides ([Blanco et al., 2020](#); [Radi and Hasni, 2014](#)). One such chemical is the organophosphate insecticide, chlorpyrifos (CPF, ethyl) which primarily acts through inhibition of the serine hydrolase: acetylcholinesterase (AChE), in the peripheral and central nervous systems (CNS) ([Casida, 2017](#)). It has been extensively used in agricultural settings throughout much of the United States and internationally ([CDPR, 2018](#), [EFSA, 2014](#); [US EPA, 2020](#)). Although it generally does not bioaccumulate, it is sufficiently lipophilic to be stored in fatty tissues and appear in colostrum or milk of lactating women ([Weldon et al., 2011](#)). Agriculture workers or bystanders living near fields where CPF is used are potentially at high risk for long term effects of exposure ([CDPR, 2018](#); [US EPA, 2020](#)). Exposure in early development ([Reygnier et al., 2016](#)), during pubescence or in adulthood ([Acker and Nogueira, 2012](#); [Elsharkawy et al., 2013](#)) has resulted in conditions related to diabetes, obesity, and metabolic syndrome² in rodent models and potentially also in humans ([Velmurugan et al., 2017](#)).

The use of medicinal and recreational cannabis (main component Δ^9 Tetrahydrocannabinol: Δ^9 THC) has increased globally by 60% from 2010 to 2019, with 8.7 billion dollars in sales between 2014 and 2021 ([Marijuana Statistics 2020, Usage, Trends and Data - AmericanMarijuana](#)). While at least half of the United States has legalized cannabis, it is not federally approved (<https://www.dea.gov/controlled-substances-act>). This means that pesticide use on marijuana plants can be unregulated, lacking tolerance limits normally set by the United States Environmental Protection Agency (USEPA; <https://www.epa.gov/pesticide-tolerances>). Moreover, CPF residues have been detected in cannabis plants and products and together they potentially pose health risks ([Sandler et al., 2019](#); [Voelker and Holmes, 2015](#)). The risks depend on several variables, including use patterns (e.g., location, sex, age, race, frequency, etc.) and product (edibles, dermal/topical use, smoking dry flowers or vaping) ([Schauer et al., 2016](#); [Sexton](#)

¹ Abbreviations: AEA: anandamide; 2-AG: 2-arachidonoylglycerol CPF: chlorpyrifos; endocannabinoids (eCB); eCBS: eCB system; FAAH: fatty acid amide hydrolase; HDL: high density lipoproteins; i.p.: intraperitoneal; i.v.: intravenous; LDL/VLDL: low or very low-density lipoproteins; LOEL: lowest observed effect level; MAGL: monoacylglycerol lipase; PND: post-natal day; s.c.: subcutaneous; Δ^9 THC: Δ^9 Tetrahydrocannabinol; TC: total cholesterol; TG: triglycerides

² Metabolic syndrome is characterized by high blood pressure, insulin resistance, increased glucose, triglycerides and LDL, large waste circumference and potentially also obesity GRUNDY, S. M. 2016. Metabolic syndrome update. *Trends in Cardiovascular Medicine*, 26, 364–373.

[et al., 2016](#); [Shiplo et al., 2016](#)). In some cases, as with waxes and concentrated extracts, concentrated CPF residues can occur ([Raber et al., 2015](#)). The combined effects of CPF and chemicals in cannabis are therefore of interest but have not been evaluated.

Background and theory

CPF and Δ^9 THC have in common their effects on the endocannabinoid system (eCBS) ([Carr et al., 2020](#); [Di Marzo et al., 2011](#); [Medina-Cleghorn et al., 2014](#)). It is generally composed of (1) a G-protein-coupled receptor, cannabinoid 1 (CB1R), which is highly concentrated in the CNS, but also abundant in the peripheral tissues; (2) two principal endogenous endocannabinoids (eCB) 2-arachidonoylglycerol (2-AG) and anandamide (AEA); and (3) the serine hydrolases monoacylglycerol lipase (MAGL), and fatty acid amide hydrolase (FAAH) that metabolize 2-AG and AEA ([Di Marzo and Matias, 2005](#); [Matias and Di Marzo, 2007](#)). eCBS helps to shape neuronal connectivity in the brain during development and into adulthood ([Mato et al., 2003](#)), however, it is also involved in numerous pathways controlling appetite, including the gut-microbiota-adipose-brain regulatory loop and other parameters directly-related to energy balance ([Jo et al., 2005](#); [Quarta et al., 2010](#); [Silvestri and Di Marzo, 2013](#); [Forte et al., 2020](#)).

Gut microbiota participate in gut-brain crosstalk to maintain host energy homeostasis, where increased circulating eCBs disrupt this balance ([Forte et al., 2020](#)). Most gut microbiota (~90%) are from 5 phyla consisting primarily of *Bacteroidetes* and *Firmicutes* at 90–99% but also *Actinobacteria*, *Proteobacteria* and *Verrucomicrobia* and within those phyla are a wide variety of species and strains in the intestine ([Rinninella et al., 2019](#)). Dysbiosis occurs when there is a decrease in bacterial diversity or in the ratio of healthy bacteria (*Bacteroidetes/Firmicutes*) compared to bacterial strains associated with pathogenic activity (e.g., *Enterobacteriaceae* family of *Proteobacteria*) ([Castaner et al., 2018](#); [Levy et al., 2017](#)) caused by environmental toxins like CPF. When pathogenic bacteria predominate ([Cani et al., 2014](#); [Levy et al., 2017](#)), the eCB system is activated in the gut, along with increases in CB1 receptors, while FAAH is downregulated ([Di Marzo, 2008b](#); [DiPatrizio, 2016](#); [Matias and Di Marzo, 2007](#)). Circulating lipopolysaccharides (LPS) endotoxins from G negative bacteria cause metabolic endotoxemia-induced inflammation, a “leaky gut” that increases risk for insulin resistance and obesity ([Cani et al., 2007](#); [Cani et al., 2008](#); [Di Marzo and Silvestri, 2019](#)). [Forte et al. \(2020\)](#) reviewed data showing that increased LPS and eCB are associated with increased fasting glucose and insulin, hepatic adipose, and whole-body weight gains. These risk factors are associated with obesity, T2D, gastrointestinal cancer and inflammatory bowel disease ([Forte et al., 2020](#)).

CPF and Δ^9 THC are metabolized by the following P450s: CYP1A2, 3A4, 2C9, 2C19, 2B6, 2D6 and eliminated by glucuronidation ([Qian et al., 2019](#), [Testai et al., 2010](#); [Watanabe et al., 2007](#)). With co-exposures, CYP induction could reach a tipping point for irreversible adverse effects even when the individual CPF and Δ^9 THC doses are

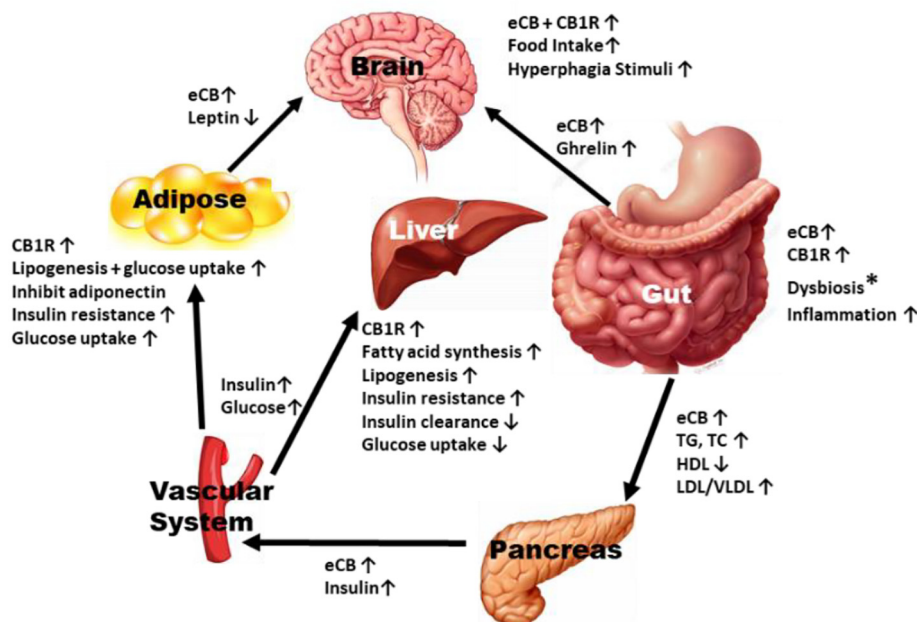


Fig. 1. This diagram is an indication of where CPF or an obesogenic diet would lead to an over-active eCBS. Δ^9 -THC intake would downregulate CB1R levels and potentially attenuate these effects.

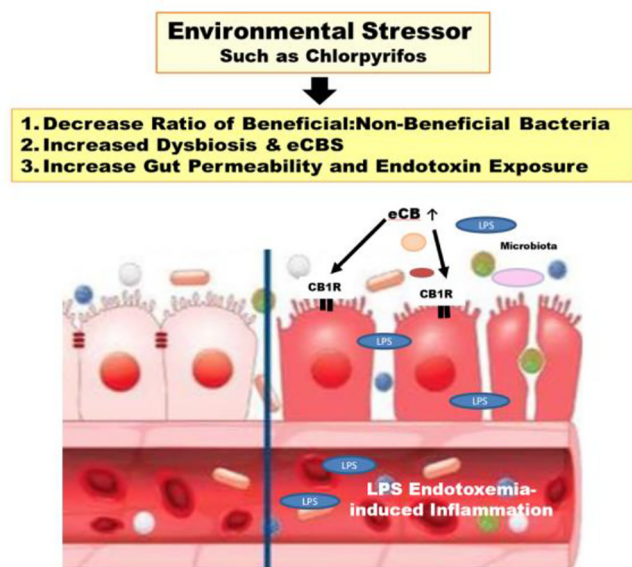


Fig. 2. Dysbiosis occurring from an environmental stressor such as CPF. Δ^9 -THC would tend to promote a healthy balance in microbiota (discussed in the text).

within regulatory limits (Saili et al., 2020; Shah et al., 2016). The tipping point also depends on an individual’s ability to handle various exposure loads based on age, genetic makeup, health status, and diet, among other influences (Hewitt et al., 2007; Bernasconi et al., 2019). These risk factors are often difficult to characterize in humans since hepatic metabolism studies are, by necessity, generally performed *in vitro* (Bernasconi et al., 2019).

While both CPF and Δ^9 -THC affect the eCBS, their modes of action (MOA) are different. After absorption into tissues and/or interacting at the CB1R, CPF inhibits FAAH and MAGL centrally and peripherally, preventing the breakdown of eCBs and allowing their buildup (Buntyn et al., 2017; Carr et al., 2020). This action has been shown to occur at doses of less than or equal to 0.5 mg/kg/day (Buntyn

et al., 2017; Carr et al., 2020; Carr et al., 2017; Leung et al., 2019). Increased eCB levels inhibit the release of excitatory or inhibitory neurotransmitters (e.g., γ -aminobutyric acid [GABA] or glutamate, norepinephrine and ACh) in the CNS and peripheral tissues (Leung et al., 2019) affecting various aspects of appetite and energy balance (Di Marzo and Matias, 2005). The effects would occur with the overabundance of and overstimulation by eCBs as shown in Fig. 1.

Over activation of the eCBS also affects the microbiome. Fig. 2 depicts the effects on an imbalance in normal microbiota (dysbiosis) resulting from an environmental stressor such as CPF. It is known that the eCBS is activated by eCB crosstalk with increased plasma levels of LPS from Gram negative gut microbiota (Muccioli et al., 2010).

Δ^9 -THC acts as a partial agonist/antagonist at the CB1R and the extent of agonist activity affecting food intake pathways generally depends on dose and treatment durations (Di Marzo, 2011). There is a bimodal dose effect in mice receiving acute intraperitoneal (i.p.) Δ^9 -THC treatment (Bellocchio et al., 2010). At 1 mg/kg Δ^9 -THC mice showed increased food intake, where at 2.5 mg/kg/day, food intake was decreased; however, at both doses there was increased glutamatergic and decreased GABAergic transmission in the CNS. Glutamatergic transmission through CB1R stimulates food intake, where CB1R action in GABAergic neurons inhibits food intake (Di Marzo, 2011). Thus, aspects of the Δ^9 -THC MOA involve selection of neuronal type in the brain, depending on exposure. Epidemiological studies showed that acute cannabis use was associated with appetite stimulation, while chronic use decreased risk of obesity, insulin resistance and diabetes mellitus (reviewed in: Farokhnia et al. (2020)). These data support cross talk between cannabis, insulin, and the pancreas.

While it remains unknown how the combined exposures might disrupt the eCBS, in theory, exposure to both CPF and Δ^9 -THC could have opposing effects on energy balance. Δ^9 -THC intake would downregulate CB1R levels and potentially attenuate the effects of eCBS overactivation by CPF (Di Marzo et al., 2004; Forte et al., 2020; Horvath et al., 2012). The current study presents a qualitative assessment of CPF and Δ^9 -THC effects on parameters influencing energy balance associated with the eCBS, along with discussion of potential consequences from co exposures.

Table 1
Measured Parameters Associated with Risk Factors for Obesity from Treatment with CPF or Δ⁹THC in Male Rodents.

Reference	Species/ Strain	Treatment Regimen Duration & Dose (mg/kg/d)	Day Tested	LOEL	Body Weight	Food Intake	Adiposity	Glucose	Insulin/IR	TG	TC	Inflammatio n	Oxid. Stress	Microbiota
CPF Treatment During Gestation with Offspring Assessment														
1	Wistar Dam	Gavage (rapeseed oil) GD 1-PND 60	PND 60	1.0*										
2	Wistar Dam	Gavage (rapeseed oil) GD 1-21	PND 1, 21, 60	1.0*	W									
3	Wistar Dam	Gavage (rapeseed oil) GD 1-PND 21	PND 60	1.0	W			F	F, S					
4	Long-Evans Dam	Gavage (corn oil) GD 7-PND 21	PND 22, 95-101	2.5	W									
CPF Treatment During the Neonatal / Perinatal Time Frame														
5	Sprague-Dawley Pup	s.c. (DMSO) PND 1-4	PND 120	1.0*	W			F	F, S					
6	Wistar Pup	Gavage (corn oil) PND 10-15	PND 16, 21, 35	1.0*	W									
7	Wistar Pup	Gavage (corn oil) PND 10-15	PND ~195	1.0*	W									
CPF Treatment at Weaning														
8	Wistar	Gavage (Tween20/saline) PND 21-196	Weekly PND 21-196	0.3*	W				S			@		
9	CD-1 C57Bl/6	Gavage (corn oil) PND 21-84	Day 84	5.0	W			F	IR, S			#		↑LPS
	CD-1 (ICR)				W			F	IR, S			#		↑LPS
CPF Treatment During Adolescence														
10	Wistar	Gavage (olive oil) 14-28 days	Days 14 or 28	0.2										
11	Wistar	Gavage (corn oil) 90 days	Day 90	3.0	G									
12	Wistar	Gavage (soya oil) 112 days	Day 112	4.8*								#		
13	Wistar	Gavage (soya oil) 120 days	Day 120	4.8*								#		
CPF Treatment During Adulthood														
14	Wistar	Gavage (ethanol) 32 d	Day 32	0.01										
8	Wistar	Gavage (Tween20/saline) 175 d	Weekly PND 56-196	0.3*	W				IR			@		
15	Wistar	Gavage (Tween20/saline) 63 d	Day 63	0.3	W			F	F, S					
16	Wistar	Gavage (corn oil) 91 d	Day 91	1.3				F					X	
17	Wistar	Gavage (corn oil) 90 d	Days 3 or 14	2.5										
18	Wistar	Gavage (olive oil) 45 d	Day 45	5.0*									X	
19	Long-Evans	s.c. (DMSO/saline) 120 d	Days 60, 90, 120	5.0*	W									
20	KE Mice	Gavage (corn oil) 30 d	Day 30	1.0*	W							#		↑LPS
21	C57Bl/6	Gavage (corn oil) 84 d	Day 84	1.0	W							\$		
22	C57Bl/N6	Diet (Diet) 56 d	Day 56	2.0*	W			F	IR, S					
Δ⁹THC Treatment During Gestation with Offspring Assessment														
23	Wistar Dam	i.p. (saline/cremaphor) GD 6-22	PND 21, 245	3.0*	W			F	F, S					
Δ⁹THC Treatment During the Neonatal/Perinatal Time Frame														
24	Ivanovas Pup	i.p. (propylene glycol/saline) PND 16-87	PND 87	4.0*	G									
25	Wistar Pup	s.c. (Tween80/saline) PND 4-14	PND 14	5.0*	G									
Δ⁹THC Treatment at Weaning														
26	Long-Evans	i.p. (cremaphor/saline) PND 21-35	PND 35	5.0*	G									
	Wistar				G									
27	Sprague-Dawley	Gavage (sesame oil) PND 22-40	PND 40	5.0	W									
Δ⁹THC Treatment During Adolescence														
28	Sprague-Dawley	i.p. (methanol) 28 days	Day 63-84	1.5	W			F	F, S					
29	Fischer 344	Gavage (corn oil) 91 days	Day 91	5.0	W									
30	Long-Evans	i.p. (saline) PND 35-75	PND 75	5.6*	W									
31	ddY Mice	i.p. (saline) PND 35-42	PND 43	4.0	W									
Δ⁹THC Treatment During Adulthood														
32	Sprague-Dawley	i.p. (saline) 7 d	Day 7	3.0*										

Abbreviations: F: fasted; G: body weight gain measured; i.p.: intraperitoneal; IR: insulin resistance measured; LOEL: lowest observed effect level; PND: post-natal day; s.c. subcutaneous; S: serum insulin levels measured; TC: total cholesterol; TG: triglyceride; W: body weight measured

References: 1. Joly Condette et al. (2014); 2. Joly et al. (2013); 3. Reygnier et al. (2016); 4. Lassiter and Brimijoin (2008); 5. Slotkin et al. (2005); 6. Perez-Fernandez et al. (2020a); 7. Perez-Fernandez et al. (2020b); 8. Li et al. (2019); 9. Liang et al. (2019); 10. Łukaszewicz-Hussain (2011); 11. Akhtar et al. (2009); 12. Uchendu et al. (2017); 13. Uchendu et al. (2018); 14. Kopjar et al. (2018); 15. Fang et al. (2018); 16. Wang et al. (2009); 17. Alvarez et al. (2008); 18. Roshanravan et al. (2020); 19. Meggs and Brewer (2007); 20. Zhao et al. (2016); 21. Zhang et al. (2021); 22. Peris-Sampedro et al. (2015b); 23. Gillies et al. (2020); 24. Gupta and Elbracht (1983); 25. O’Shea and Mallet (2005); 26. Keeley et al. (2015); 27. Dow-Edwards and Zhao (2008); 28. Beydogan et al. (2019); 29. NTP (1998); 30. Weed et al. (2016); 31. Rahminiwati and Nishimura (1999); 32. Coskun and Bolkent (2014).

Symbols: “*” – Indicates only dose used in the study; # - Inflammation in liver; @ - Serum levels of proinflammatory cytokines; \$ - Intestinal epithelium; X - Oxidative stress was based on lipid peroxidation associated with membrane fluidity; Green, yellow, and red indicate increase, equivocal or decreased compared to controls, respectively.

Methods

Data sources and search strategy

A literature search was conducted by methods similar to the Patient, Intervention, Comparison, Outcome (PICO) of evidence-based practice (Eriksen and Frandsen, 2018; Santos et al., 2007). It was not a conventional Systematic Review requiring two or more persons to review an abstract (PICO vs. Systematic Review described in: Eriksen and Frandsen (2018)) but it was designed to obtain data involving search terms that most closely focused on the subject.

- **Problem Identification:** A concern was the potential for exposure to CPF and Δ^9 -THC through use of cannabis products.
- **Specific Question:** Is there a potential for disruption of the eCBS by either chemical and is there evidence that they affect energy balance or contribute to risk factors for obesity?
- **Search Strategy:** The search strategy sought to curate through electronic sources peer-reviewed journal articles, books, and reports containing the toxicological data available to explore the subject. The open access Abstract Sifter tool was used to query PubMed, facilitate compilation of results and sort, and select pertinent articles (Baker et al., 2017). Searches were also performed with Google Scholar, Science Direct and Research Gate, as needed to obtain articles related to the primary subjects. PubMed Medical Subject Headings (MeSH: <https://meshb.nlm.nih.gov/search>) were queried, along with other index terms for CPF and Δ^9 -THC in relation to risk factors for obesity or imbalance in energy utilization (Supplemental Table 1). Δ^9 -THC was chosen as the cannabis component of concern, to focus on the ingredient with the likelihood of a dominant exposure. Among the search terms were: “adipose”, “body weight,” body weight gain OR body weight change,” “cholesterol,” “food intake OR food consumption,” “ghrelin,” “glucose,” “HDL OR high-density lipoprotein,” “high fat,” “insulin,” “insulin resistance,” “LDL OR low-density lipoprotein,” “leptin,” “gut microbiome OR microbiota (gut microorganisms),” “obese OR obesity,” “oxidative stress,” “triglycerides,” “VLDL OR very low-density lipoprotein”, “environmental obesogen,” and others (Supplemental Table 1). Literature was also obtained from citations listed in screened studies. Review articles were a source of additional articles.

Data inclusion or exclusion

Study selection based on route of exposure

Oral, intravenous (i.v.), intraperitoneal (i.p.) or subcutaneous (s.c.) and inhalation are commonly used and well-characterized routes given that absorption is assumed to be 100% for CPF (Griffin et al., 1999; Testai et al., 2010, US EPA, 2011; CDP, 2018) and Δ^9 -THC (Wiley et al., 2021; Nguyen et al., 2016; Nguyen et al., 2020a; Nguyen et al., 2020b; Taffe et al., 2020), where dermal studies are less sensitive due to the skin as a barrier to absorption. Dermal studies for CPF were excluded because absorption is low (~1–3%) and anticipated toxicological effects would not exceed those observed by other routes (US EPA, 2011; Nolan et al., 1984). The Health Effects Test Guideline 21-day dermal rat study showed no effects at the highest CPF dose (10 mg/kg/day) (US EPA, 2011). Another CPF dermal study in mice treated only the tail skin for 2 weeks and showed a slight decrease in plasma ChE at 101 mg/kg/day (only dose) (Krishnan et al., 2012). CPF inhalation studies were also excluded because four Health Effects Test Guideline 90-day inhalation studies showed no systemic or cholinesterase effects at the highest attainable vapor concentration (Corley et al., 1986; US EPA, 2011; US EPA, 2014) and other repeated dose inhalation studies performed with technical grade CPF were not

found in the open literature. While both dermal and inhalation exposures to agricultural workers and inhalation exposure by spray drift to bystanders are a concern, the most sensitive point of departure for CPF risk assessment was based on developmental neurotoxicity after **oral** treatment in animal studies (~0.1 mg/kg/day) (CDPR, 2018). That value was used to calculate human risk by all routes.

There were 13 inhalation studies performed with technical grade Δ^9 -THC in the open literature, but they were excluded for the following reasons: 1) they were acute (single administration), 2) the doses used in the repeat intervals were above the LOEL (~5.0 mg/kg/day) and into the range of overt toxicity (10–20 mg/kg/day), 3) a graded dose regimen was used, or 4) only female animals were examined. Δ^9 -THC lacked dermal toxicity characterization (CompTox Chemicals Dashboard (epa.gov) in the open literature. While inhalation and dermal exposures are expected in users of cannabis products, the Δ^9 -THC risk assessment (EFSA, 2015) determined that neurotoxicity from **oral** administration to human subjects provided the most sensitive point of departure (~2.5 mg/kg/day) as a basis for calculating human risk by all routes.

Data Inclusion

- Studies performed in rat and mouse models throughout all life stages were selected initially to determine whether age at exposure affected risk (Fig. 3; Supplemental Table 2).
- Studies including healthy male and female rodents (rats and mice) on a standard diet.
- Repeated dose studies were selected to capture effects from long-term, low-dose exposure scenarios which is a likely representation of cannabis use patterns, with potential CPF co-exposure.
- Study acceptance was not limited by treatment vehicle (e.g., DMSO, corn oil, etc.).
- Year of publication and/or geographical locations were not restricted, but the articles needed to be in English or English translations (Fig. 3).

Data exclusion

- Acute studies were excluded because the major concern is chronic exposure leading to irreversible effects, where there is greater potential for reversal and repair with acute exposure.

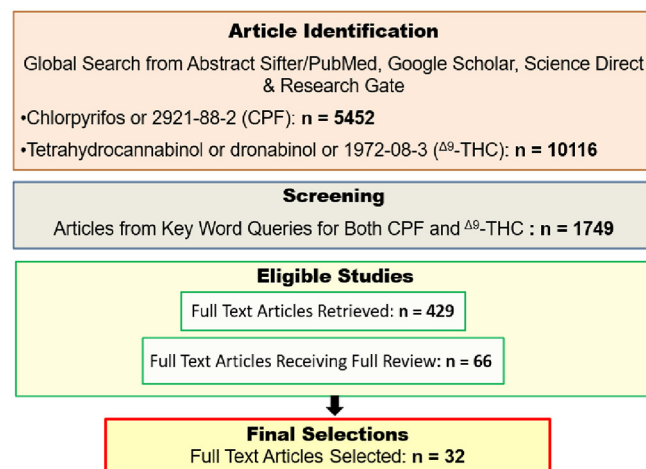


Fig. 3. Sequence of steps involved in the review to filter for potential CPF and Δ^9 -THC effects on the eCB system associated with energy imbalance or obesity.

- Use of diabetic, obese, or genetically modified rodent strains were excluded because the purpose was to investigate effects of CPF and Δ^9 -THC on healthy animals on a standard, non-obesogenic diet.
- Studies were excluded where the animal strain and age at treatment were not described.
- If exposure was to “cannabis” (cannabinoid mixtures, cannabinoids other than Δ^9 -THC or synthetic cannabinoids) or CPF as a formulated product or if the chemical characterization was not described, the study was excluded because cannabinoids other than Δ^9 -THC or inerts added to CPF formulations could stimulate effects unrelated to the active ingredients under investigation.
- Studies using dose levels known to induce overt toxicity were obtained in the initial search but were excluded because the exposures would not be representative of what would generally occur in humans (e.g., CPF > 5.0 mg/kg/day or Δ^9 -THC 10–20 mg/kg/day) (Ambali et al., 2011; ElMazouly et al., 2011; NTP, 1998).
- If the full text article was unavailable after corresponding with authors through email or Research Gate, then it was excluded.

References were selected and their “pdf” copies were sent to Endnote20 (October 23, 2020, release). Each study was reviewed and the specifics (e.g., chemical exposure, vehicle, sex, animal age at treatment, strain, dosing schedule, doses used, testing dates post-treatment and endpoints) were compiled (Fig. 3; Supplemental Table 2).

Review of studies

The selected studies were reviewed for effects related to treatment by CPF or Δ^9 -THC associated with increases, decreases or no effect on body weight/body weight gain, food intake, adipose, free-fatty acids (FFA), triglycerides (TG), total cholesterol (TC), low or very low-density lipoproteins (LDL/VLDL), high density lipoproteins (HDL), glucose, insulin, insulin resistance, ghrelin, leptin, inflammation, oxidative stress, or microbiota. Initial reviews included both male and female rats and mice and these data are in Supplemental Table 2. Ultimately studies in male rodents were selected for analysis because most of the available results were in males.

Results

Search results

The data in Table 1 present an overall summary of studies. Not all parameters were measured at each life-stage or for each chemical, which limited direct comparisons of effects between chemicals. General age at treatment, treatment regimen, age at testing, dose(s) used, and effect in male rats and mice were reported. Female data were presented in Supplemental Table 2, along with complete descriptions of protocols for studies selected for this work. Some parameters were not added to Table 1 (FFA, leptin, ghrelin, HDL, LDL and VLDL) because they were measured in few studies and the results did not add to overall data interpretation (data for these parameters included in Supplemental Table 2).

Data summary

Overall conclusions were based on qualitative observations because most of the studies were not performed according to Health Effects Test Guidelines (US EPA, 1998) but were designed to examine a specific effect (i.e., body weight, etc.; Table 1). As such, they may have used only one dose which precluded determination of a LOEL or Benchmark Dose due to lack of a dose–response. Δ^9 -THC studies were mainly designed to test for neurotoxicity, but body weights and sometimes other parameters related to energy balance were available. Variables among studies included rat strains (6), mouse strains (5), doses admin-

istered, method of administration (i.e., gavage, diet, intravenous (i.v.), intraperitoneal (i.p.), subcutaneous (s.c.)), vehicle (i.e., olive oil, sesame oil, corn oil, soya oil, rapeseed oil, DMSO, methanol, Tween80, Tween20, propylene glycol, saline, cremaphor, ethanol), treatment durations (~4–175 days), age at treatment and testing, dietary composition, and test laboratory. Of the 10 measured parameters, or categories listed in Table 1, most were examined only with CPF. In most cases, fasting serum glucose was measured but it was not always stated in the methods section of the respective studies. In some cases, both serum insulin and insulin resistance were tested as designated in Table 1. Studies were initially organized by life-stage, but the overall results did not support or highlight differences. While the sequence of the studies in Table 1 is by treatment age, it is for convenience but does not indicate specific age-related effects.

Body weight effects

CPF (Table 1): Body weights were increased at doses as low as 0.3 mg/kg/day and up to 5.0 mg/kg/day in rats and mice treated during gestation- to weaning, at weaning and in adulthood (Table 1). There were no effects on body weights from neonatal/perinatal or adolescent treatment. In some cases, body weight increases were accompanied by increased adiposity but not necessarily by increased food intake.

In utero CPF exposure at 1.0 mg/kg/day would be from metabolism in treated dams for the entire gestation period and include placental transfer of the major metabolite CPF-oxon (Mattsson et al., 2000). Pup body weights could be affected from eCBS overactivity based the knowledge of placental CPF-oxon transfer and evidence of eCBS central and peripheral disruptions in juvenile rats at 0.5 mg/kg/day (Fig. 2) (Buntyn et al., 2017; Carr et al., 2020).

Body weight was not a sensitive endpoint in Sprague-Dawley and Wistar male pups treated neo/perinatally (PND 1–4 or PND 10–15) at 1.0 mg/kg/day CPF, possibly due to immature metabolic capacity (Perez-Fernandez et al., 2020a; Perez-Fernandez et al., 2020b; Slotkin et al., 2005) (Table 1). Neonatal/perinatal pediatric CPF-metabolizing nuclear receptor (e.g., pregnane-x-receptor: PXR) and CYP enzyme (CYP1A2, 3A4, 2C9, 2C19, 2B6) induction in Phase 1 metabolism may be 0–60% of adult values depending upon the CYP subfamily, inter- and intraindividual variability, assay test methods and available specimens (Allegaert and van den Anker, 2019; Sadler et al., 2016; Vyhldal et al., 2006). Time of peak AChE inhibition for CPF administered by gavage (corn oil vehicle) at 3.0 mg/kg/day in brain of PND 11 pups vs. adult Sprague-Dawley females was 8 and 6 h, respectively, indicating that CPF metabolism was slower in younger animals (Marty et al., 2012). Hence, the eCBS may not have been disrupted to the extent that body weight effects occurred due to lack of metabolic activation of CPF to CPF-oxon. It is also possible that the longer treatment at other life stages had effects simply based on the greater exposure duration.

CPF at 0.3 mg/kg/day showed increased body weights in weanling rats (0.3 mg/kg/day; Tween20/saline vehicle; Table 1) but not in rats at 3.0 mg/kg/day during adolescence (corn oil vehicle; Table 1). The higher absorptive properties of Tween20/saline vs. corn oil vehicles when CPF was administered by gavage may explain the difference (Marty et al. 2007; Smith et al. 2009). It may also be that metabolic capability of rats during adolescence is increased, compared to weaning, such that the tipping point for toxicity was not reached. Activation to detoxification ratios for CPF depend on age, as well as many other interindividual and intraindividual ability to handle various exposure loads (Eaton et al., 2008; Ginsberg et al., 2004; Ginsberg et al., 2009; Ginsberg et al., 2017). Interpretation of these data was challenging because the Li et al. (2019) study used only 0.3 mg/kg/day CPF and measured absolute body weights, where Akhtar et al. (2009) treated at 3.0, 6.0 and 9.0 mg/kg/day and measured body weight gain.

Adult Wistar rats in two different studies showed either no effect or an increase in body weights at 0.3 mg/kg/day CPF with the same vehi-

cle and chronic treatment of 63-, or 196-days (Fang et al., 2018; Li et al., 2019). While there is not a clear understanding of why the difference occurred, it is possible that 0.3 mg/kg/day CPF is a threshold for body weight effects in Wistar rats. The differences in the controls, study methods or times of assessment may have determined a result above or below the threshold.

Adult KM³ and C57BL/6 mice treated with CPF at 1.0 mg/kg/day by gavage (corn oil) for 30 or 85 days, respectively did not have effects on body weight (Zhang et al., 2021; Zhao et al., 2016). Adult C57BL/6 mice treated at a higher dose had increased body weight and food intake at 2.0 mg/kg/day when treated 56 days with CPF in diet (Peris-Sampedro et al., 2015a). The vehicle used the above studies (corn oil vs. diet) may have affected CPF absorption. While 2.0 mg/kg/day CPF is above the dose initiating effects on the eCBS, it may be on the border of where body weight effects might occur in mice.

Δ^9 -THC (Table 1): One of the most consistent findings from Δ^9 -THC treatment was body weight decrease in rodents (Table 1). Since Δ^9 -THC acts through the eCBS, it is assumed that the body weight decrements occurred by agonism/antagonism at the CB1R. The threshold for Δ^9 -THC effect on body weight, regardless of age, vehicle, or treatment duration, appeared to be 3.0 mg/kg/day.

Body weights, pancreatic weights and glucose in plasma were decreased in Wistar male offspring of dams treated i.p. GD 6–22 at 3.0 mg/kg/day Δ^9 -THC (Gillies et al., 2020). PND 1 pup body weight decreases could have occurred through partial agonist activity of Δ^9 -THC at the placental CB1 receptor in the dams, while not affecting maternal food intake or body weights. Glucose, transported through the placenta to the fetus by glucose transporter proteins (e.g., GLUT1) (Acosta et al., 2015), is decreased by 35% in the placenta after Δ^9 -THC exposure during gestation, resulting in a decreased fetal body weight (Natale et al., 2020). In human subjects, developmental delays associated with low birth weight and cannabis use during pregnancy are known to occur (reviewed in: Fried and Smith (2001)). Hence, while protecting against potential risks for obesity, Δ^9 -THC negatively affects neuronal pathways during critical hormonal and neurodevelopmental changes (Berghuis et al., 2007; BERGHUIS, 2005; Fride, 2004; Fride, 2008). Moreover, low birth weight and body weight decreases potentially due to Δ^9 -THC partial agonist activity at the CB1R, can *increase* risk for diseases related to T2 diabetes, and hypertension in later life (Schieve et al., 2016; Alexander et al., 2014).

Body weight gains were decreased in male Ivanovas (Sprague-Dawley) and Wistar pups treated peri/postnatally by i.p. (PND 16–87) with Δ^9 -THC at 4.0 mg/kg/day (Gupta and Elbracht, 1983) or s.c. (PND 4–14) at 5.0 mg/kg/day (O'Shea and Mallet, 2005). Previous reports have shown that Δ^9 -THC > 2.5 mg/kg/day in rats, results in decreased body weights (Bellocchio et al., 2010). However, as with low birth weights, a decrease in body weight during early development can be an adverse health effect. CB1R activation is essential in initiation of milk suckling in neonatal mice by facilitating innervation and activation of tongue muscles (Fride et al., 2005; Fride et al., 2009). Partial Δ^9 -THC agonism/antagonism (dose-dependent) at the CB1R will affect neurotransmission and suckling behavior in neonates, ultimately affecting body weight and inability to thrive. Δ^9 -THC exposure during the perinatal/preweaning period has been shown to have lasting negative effects on neurodevelopmental behaviors (Campolongo et al., 2011; Mohammed et al., 2018; Newsom and Kelly, 2008).

Long-Evans and Wistar males treated i.p. at weaning/peri-adolescence (PND 21–35)⁴ with 5.0 mg/kg/day Δ^9 -THC showed

decreased body weight gains after 2 weeks of treatment (Keeley et al., 2015). Sprague-Dawley rats were treated PND 22–40 at 5.0 mg/kg/day but there were no effects on body weight (Dow-Edwards and Zhao, 2008). It is notable that body weight effects were different in two studies at the same dose with treatment for approximately the same duration. However, the rat strains differed, along with routes of administration and vehicle, among other variables. The i.p. injection exposure route and vehicle (cremaphor/saline) facilitated absorption, with greater tissue availability as compared to exposure by gavage (Iwaniec and Turner, 2013). For example, Dow-Edwards and Zhao (2008) attributed the lack of significant behavioral effects at 5.0 mg/kg/day to slow absorption of Δ^9 -THC administered orally compared to i.p. injection.

At 1.5 mg/kg/day Δ^9 -THC dose, Sprague-Dawley rats treated for 28 days by i.p. in adolescence showed slight but not significant decreases in body weight (Beydogan et al., 2019). Decreased body weights in adolescent male Fischer 344 and Long-Evans rats were seen at higher doses (5.0 mg/kg/day: gavage; 5.6 mg/kg/day: i.p.) (NTP, 1998; Weed et al., 2016). The decreased body weights in Long-Evans rats were not accompanied by effects on food intake (Weed et al., 2016), however, human subjects showed decreased Body Mass Index with chronic cannabis use along with increased caloric intake (Alshaarawy and Anthony, 2019; Smit and Crespo, 2001).

Effects on the developing brain with *peri*-adolescent/adolescent exposure may outweigh the protective effects on energy balance. Human exposure to Δ^9 -THC in childhood/*peri*-adolescence would more likely be second hand (e.g., second-hand smoke/vaping), rather than direct. Yet even these exposures could adversely affect brain development since areas of the cerebellum and amygdala continue to develop into early adulthood (Clancy et al., 2001; Rice and Barone, 2000). Cannabis use during adolescence and early adult ages results in cognitive and behavioral deficits (Camchong et al., 2017; Kasten et al., 2017; Nguyen et al., 2020b; Rubino et al., 2009). Further, current increases in cannabis use trends among pre-teens and adolescents (ages > 12 years) have shown that flavored vaping is perceived as beneficial compared to smoking (Knapp et al., 2019). Pilin et al. (2021) have stated this age is a time of experimentation with cannabis; often influenced by parental or peer behaviors.

Glucose and Insulin/Insulin resistance

CPF (Table 1): Overall, it appeared that insulin and insulin resistance were more sensitive targets than glucose, potentially related to dose and treatment duration. At higher CPF doses, insulin effects could have inhibited breakdown of eCBS, resulting in overstimulation in peripheral organs (Di Marzo, 2008a; Buntyn et al., 2017). eCBS modulate insulin-regulated glucose uptake *in vitro*, and the affected glucose and insulin levels may be indicators of insulin resistance (Bellocchio et al., 2008; Gasperi et al., 2007; Motaghedi and McGraw, 2008). The lack of effects on glucose or insulin levels in Sprague-Dawley pups treated at 1.0 mg/kg/day PND 1–4 (s.c. DMSO) may be due to the short exposure duration at a dose where the rats could metabolize and eliminate CPF (Slotkin et al., 2005).

Decreased glucose and (fasted) serum insulin occurred in adult Wistar rats treated 63 days with CPF at 0.3 mg/kg/day (Fang et al., 2018). At a low CPF dose (i.e., 0.3 mg/kg/day), the insulin decrease should coincide with a normal decrease in glucose associated with eCBS affecting insulin-regulated glucose uptake (Bellocchio et al., 2008; Gasperi et al., 2007; Motaghedi and McGraw, 2008).

Δ^9 -THC: It was concerning that after gestational treatment, pups showed decreases in glucose with Δ^9 -THC at 3.0 mg/kg/day, because an imbalance in glucose during brain development can have detrimental effects (Chugani, 1998). It was also notable because the regulatory No-observed-effect-level in rodents for Δ^9 -THC is 5.0 mg/kg/day (National Institute of Technology and Evaluation, Tokyo, Japan: [Search Results - NITE-CHRIP \(NITE Chemical Risk Information Platform\)](#) based on NTP (1998)) and 2.5 mg/kg/day in humans (EFSA,

³ Chinese Kun Ming (KM) mouse strain most closely genetically related to BALB/C and C57BL/6 strains ZHANG, X., ZHU, Z., HUANG, Z., TAN, P. & MA, R. Z. 2007. Microsatellite Genotyping for Four Expected Inbred Mouse Strains from KM Mice. *Journal of Genetics and Genomics*, 34, 214–222.

⁴ Rat adolescence is initiated approximately PND 28–42; SPEAR, L. P. 2000. The adolescent brain and age-related behavioral manifestations. *Neuroscience & Biobehavioral Reviews*, 24, 417–463, *ibid.*, *ibid.*

2015, EFSA, 2020). Therefore, fetuses exposed to supposedly “safe” levels of Δ^9 -THC, may end up with low birth weight and developmental deficits.

Δ^9 -THC had no effects on insulin at 1.5 or 3.0 mg/kg/day across life stages (Beydogan et al., 2019; Coskun and Bolkent, 2014; Gillies et al., 2020).

Triglycerides and total cholesterol

TG was a more sensitive endpoint than TC for CPF but there did not appear to be a dose, strain or treatment-related association (Table 1). TC was a sensitive endpoint for Δ^9 -THC, with an increase at lower doses (1.5 and 3.0 mg/kg/day). The response for TG did not appear to be related to dose or dosing regimen when measured in adolescence and adulthood, however, TC was increased at both doses. The greater effects on TC at 1.5 mg/kg/day Δ^9 -THC may be due to the methanol vehicle administered i.p., since methanol, due to its greater lipophilicity, would facilitate Δ^9 -THC absorption to a greater extent than saline (PubChem (nih.gov); CompTox Chemicals Dashboard | Home (epa.gov)). Saline, with lower absorptive properties for lipophilic compounds may have resulted in fewer effects even though the higher dose (3.0 mg/kg/day Δ^9 -THC, also administered i.p., in the same strain of rat, showed no effects.

Inflammation, oxidative stress and microbiota

Across all life-stages, at CPF doses of 0.01 to 5.0 mg/kg/day, inflammation, oxidative stress, and microbiota changes occurred. Microbiota changes after CPF treatment at 0.3- to 5.0 mg/kg/day showed bacterial count patterns seen in obese humans or those with T2D (Karlsson et al., 2013; Qin et al., 2012). Dysbiosis leads to an increase in eCB activity, gut permeability and LPS release that would lead to an inflammatory response (Fig. 2) (Cani et al., 2016; Forte et al., 2020; Levy et al., 2017). The imbalance in beneficial to opportunistic (Gram negative) bacteria may account for the increases in LPS measured in mice (C57BL/6 and ICR) when treated PND 21–84 (Liang et al., 2019) and C57BL/6 mice treated as adults (Zhang et al., 2021) (Table 1). Both mouse strains in Liang et al. (2019) also had increased inflammation in liver, epididymal fat, ilium, and colon associated with microbiota changes (Table 1). Wistar rats treated PND 21–196 at the low dose of 0.3 mg/kg/day showed evidence of inflammation (proinflammatory cytokines in serum: MCP-1 and TNF α), however there were no effects on microbiota (Li et al., 2019). In the same study, adult rats treated PND 56–196 had increased inflammation and dysbiosis. Between the age of weaning and adulthood P450 enzymes that activate CPF and the eCBS are still developing (Berghuis et al., 2007; Fride, 2008; Tanaka, 1998; Upreti and Wahlstrom, 2016; Watanabe et al., 1993). CPF in weanlings, while absorbed through the gut, may primarily affect the brain eCBS because the P450s are not fully developed locally in the gut/liver. Inhibition of eCB breakdown in brain would affect the appetite center and potentially lead to the weight increase seen in adulthood after prolonged CPF treatment (Di Marzo, 2011) (Table 1). On the other hand, in adulthood, at low doses, CPF may be metabolized in the gut where eCBS are synthesized locally along with activation of CB1R (Di Marzo et al., 2004). The direct effects of CPF on the eCBS in the gut may lead to the inflammation and microbiota changes reported in Li et al. (2019). Further supporting the potential of a local eCBS disruption was the lack of body weight increases in treated adults indicating that CPF may not have reached the brain in sufficient levels to affect appetite.

KM mice treated with CPF at 1.0 mg/kg/day for 30 days had changes in the microbiota, accompanied by increased LPS in serum and liver inflammation (Zhao et al., 2016). The presence of proinflammatory cytokines in addition to dysbiosis, support the proposed pathway acting through disruptions in the eCBS (Cani et al., 2016; Forte et al., 2020; Levy et al., 2017).

The CB1R is involved with pro-oxidative stress, pro-inflammatory responses and cardiovascular disease (Rajesh et al., 2012). Oxidative stress likely contributed to inflammation, and both are linked to atherosclerotic diseases (Matthews and Ross, 2015). For example, oxidative stress (MDA increase; SOD and CAT decrease) was reported in conjunction with inflammation in liver after CPF treatment at 4.8 mg/kg/day (gavage, soya oil) in adolescent Wistar rats for 112–120 days (Uchendu et al., 2017; Uchendu et al., 2018). Notably, oxidative stress was observed in Wistar males even at 0.01 mg/kg/day CPF which is also below the point of departure determined by some regulatory agencies (Kopjar et al., 2018; CDPR, 2018). RBC SOD, plasma catalase and ferric reducing ability of plasma assay (FRAP: measures “antioxidant power”) were increased.

Wistar rats exposed to CPF at 5.0 mg/kg/day (corn oil vehicle) for 3 or 14 days showed increased MDA in aorta, liver, plasma, and kidney and after 3 days at 2.5 mg/kg/day, SOD was increased in plasma (Alvarez et al., 2008). Another study with Wistar males gavaged with CPF at 5.0 mg/kg/day (olive oil vehicle) showed increased oxidative stress on hematological parameters after 45 days of treatment (Roshanravan et al., 2020). However, Wistar males treated for 91 days at 1.3 mg/kg/day CPF (corn oil vehicle) showed no effects on oxidative stress possibly because this parameter was measured by lipid peroxidation in membranes and associated membrane fluidity, rather than on oxidative enzymes (e.g., SOD CAT), as was done in the other studies (Wang et al., 2009). On the other hand, the faster absorption of CPF at 1.3 mg/kg/day by the DMSO/Tween20/saline vehicle may have led to more rapid metabolism and elimination, since this dose was unlikely to overwhelm the system as might occur at higher doses (2.5 and 5.0 mg/kg/day) with corn oil or olive oil vehicles (Marty et al., 2007; Smith et al., 2009). The increased oxidative stress seen at 0.01 mg/kg/day CPF could also have been enhanced by the ethanol vehicle there is a reported increase in oxidative stress with ethanol alone compared to the negative control (Kopjar et al., 2018).

While there were no studies with Δ^9 -THC that measured effects on microbiota or oxidative stress, acting as a partial agonist/antagonist, Δ^9 -THC would decrease circulating eCBs through downregulation of CB1R to potentially counteract microbiota imbalance (Ceccarini et al., 2015; Villares, 2007). Mehrpouya-Bahrami et al. (2017) have shown that inhibition of the CB1 receptor, as would occur from Δ^9 -THC exposure, can attenuate inflammation and microbiota imbalance observed in obesity. Inflammation was measured in adult Sprague-Dawley rats at 3.0 mg/kg/day Δ^9 -THC and there were no effects (Coskun and Bolkent, 2014) but it would not be expected to occur at a dose below the NOEL/LOEL value of 5.0 mg/kg/day (NTP 1998).

Summary of observations

Predicted and observed effects on parameters related to dysbiosis and risk factors for T2D, obesity and related disorders were based on literature reviews and reports. CPF studies focused on obesogenic effects resulting from treatment and Δ^9 -THC studies focused on alleviating the effects of obesity or an obesogenic diet through Δ^9 -THC. These, among other factors limited data interpretation and the variability within and between age groups for CPF and Δ^9 -THC made direct comparisons of effects across studies challenging.

Examples of variability included:

- 11 different rodent species/strains
- 5 routes of administration
- A variety of ages at treatment
- Duration of treatment ranged from 4 to 175 days
- Variable endpoints tested across studies using different technique
- Use of a single dose rather than a range of doses and a limited dose selection with the presumed intention of getting an effect rather than determining a point of departure.

- Treatment vehicles (~13)
- Inter-test laboratory variability
- Lack of parameters directly testing eCBS
- No studies with both chemicals tested concurrently

Fig. 4 proposes a mode-of-action summary of the presumptive pathways for CPF and Δ^9 -THC leading to body weight effects, the most consistent parameter seen for both chemicals. The pathways are based on the known and/or related effects of each chemical on the eCBS and the observations in Table 1. Both chemical pathways include the neurodevelopmental effects associated with low birth weights and/or effects on the eCBS. Due to the variability listed above, and the fact that the two chemicals were not tested concurrently, conclusions were qualitative.

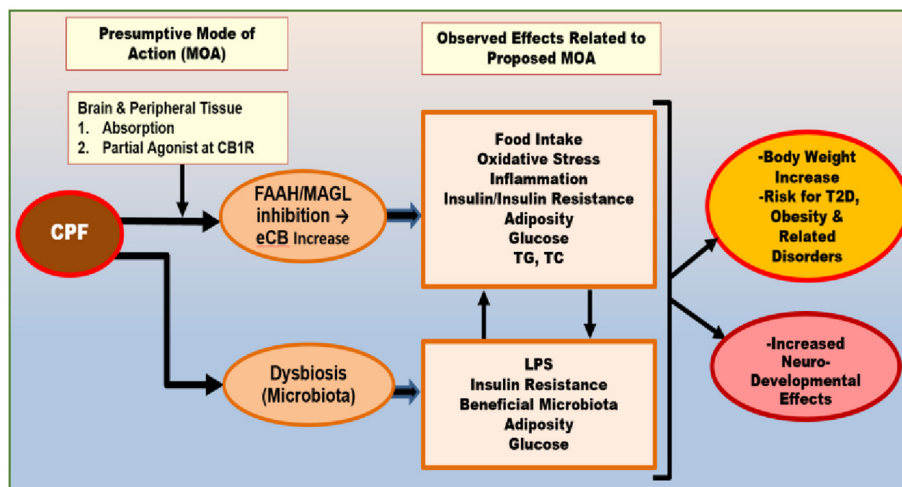
Discussion

The collective data indicated that increases in body weights/weight gains with CPF and decreases with Δ^9 -THC generally occurred across most age groups, independent of other variables. Organophosphates like CPF are implicated in the epidemic of obesity in the United States (Blanco et al., 2020; Meggs and Brewer, 2010; Radi and Hasni, 2014). CPF influence on many aspects of energy balance provided support for that association. Body weight effects could be attributed to CPF direct inhibition of FAAH and MAGL, leading to build up of AEA and 2-AG in the brain (Carr et al., 2020, Carr et al., 2017), over stimulation of the

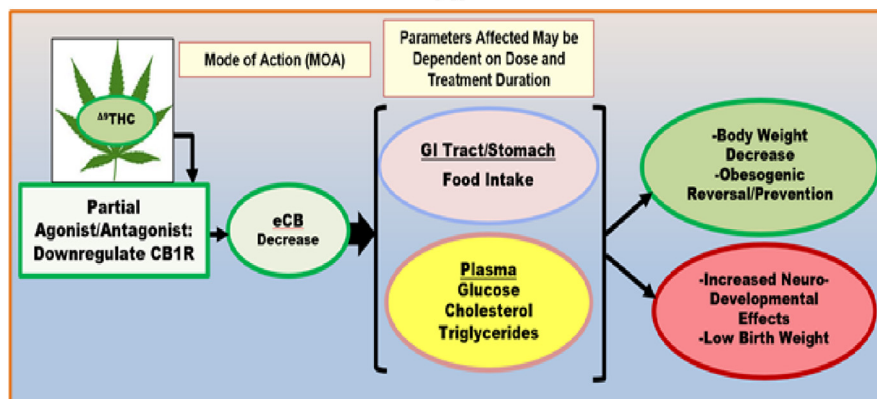
hypothalamus and nucleus accumbens resulting in increased hunger and motivation to eat (Di Marzo and Matias, 2005). Increased peripheral eCBS affecting a multitude of pathways, especially at low CPF doses, were likely associated with dysbiosis and inflammation, (Silvestri and Di Marzo, 2013) related to many diseases (e.g., T2D, metabolic syndrome, hypertension) (Borrelli and Izzo, 2009; Després et al., 2006).

Oxidative stress was a sensitive endpoint for CPF treatment in adolescence and adulthood at the lowest doses. It depleted antioxidants in tissues (i.e., SOD, CAT) and with depletion, increased inflammation occurred (Alvarez et al., 2008). Oxidative stress and/or dysbiosis, when tested together with CPF were accompanied by biomarkers for inflammation which can be precursors of obesity (Furukawa et al., 2017).

Chronic Δ^9 -THC treatment resulted in CB1R downregulation in male Wistar rat brain after treatment i.p. with 6.4 mg/kg/day (Tween80 vehicle) for 7 days (De Fonseca et al., 1994). It was also seen in human subjects by brain imaging (Hirvonen et al., 2012). Decreases in CB1R due to agonism/antagonism by Δ^9 -THC exposure was shown to increase the levels of beneficial microbiota that could alleviate inflammation associated with LPS, other endotoxins and oxidative stress observed in obesity and related diseases (Horváth et al., 2012; Cani et al., 2016; Di Marzo and Silvestri, 2019; DiPatrizio, 2016). While studies showing Δ^9 -THC effects on microbiota in non-obese rodents on a standard diet were not available for the current study, Δ^9 -THC exposure demonstrated health-protective effects



A.



B.

Fig. 4. Proposed modes of action for CPF or Δ^9 -THC and the observations associated with those pathways in rodents (Abbreviations: eCB: endocannabinoid; LPS: lipopolysaccharide; TC: total cholesterol; TG: triglyceride) A) CPF interactions with eCBS and microbiota leading to CPF effects on body weight and accompanying neurodevelopmental effect; B) Δ^9 -THC effects on the eCBS leading to decreases in body weight while inducing adverse effects on neurodevelopment.

from a high fat diet, where subjects experienced T2D and/or obesity (Cluny et al., 2015; Sharkey and Wiley, 2016; Muccioli et al., 2010; Assa-Glazer et al., 2020). Dietary induced obese (DIO) male C57Bl/6N mice treated i.p. with Δ^9 -THC at 2.0 mg/kg/day for 6 weeks had decreased weight gain, adipose mass, and food intake, compared to DIO controls (Cluny et al., 2015). These effects were accompanied by increased beneficial gut bacteria (*Akkermansia muciniphila*), known to control fat storage and adipose metabolism associated with weight loss (Everard et al. 2013).

Conclusions

While there is generally control over exposure to Δ^9 -THC, except in the form of second-hand smoke or during development *in utero*, exposures to CPF are usually unknown because residues can concentrate in cannabis products (Raber et al., 2015). There are currently several data gaps of concern associated with organophosphates affecting the eCBS which need further study, including: 1) the obesogenic effects of organophosphates like CPF on critical systems like the gut-microbiota-adipose-brain regulatory loop and appetite; 2) effects of pesticides on the eCBS during all developmental stages; and 3) MOA for co-exposures of cannabis and CPF on energy balance, obesity, and associated diseases. A useful approach could provide a standardized battery of *in vivo* and *in vitro* tests to determine a tipping point for adverse health effects or a physiologically based pharmacokinetic model to characterize co-exposures to CPF and Δ^9 -THC. Data suggest that the concern about CPF residues on cannabis might be alleviated because the MOAs for CPF and Δ^9 -THC could counteract each other and many health protective advantages of cannabis have been reported. However, the answers to these concerns are complex and would depend on working through the numerous variables mentioned above.

Even though cannabis could counter act adverse effects from CPF related to energy balance, it cannot be overlooked that both chemicals are neurodevelopmental toxicants (Leung et al., 2019; Silva, 2020; Fride et al., 2009; Fried and Smith, 2001). This is a research area that deserves further study. It is hopeful that these concerns will be addressed, and regulations will be considered.

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Author statement

I, Marilyn H. Silva, Ph.D., DABT am the sole author of this work. I initiated the concept, curated the data, analyzed the data, and was not funded for the work. I am currently retired and now I can work on projects that are of interest to me and hopefully to the scientific community as well. I performed the scientific investigations, used open access methodologies, and wrote the paper etc. I have no co-authors.

CRediT authorship contribution statement

Marilyn H. Silva: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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