Cannabis in Veterinary Medicine: A Critical Review

Trina Hazzah, DVM, Casara Andre, DVM, Gary Richter, DVM, Stephanie McGrath, DVM, MS

Author Contacts:
Trina Hazzah, DVM
Veterinary Cannabis Society
Los Angeles, CA 90066
Email: drtrinahazzah@gmail.com

Casara Andre, DVM
Veterinary Cannabis Education and Consulting
10474 West 39th Ave.
Wheat Ridge, CO 80033
Email: reception.desk@veterinarycannabis.org

Gary Richter, DVM
Veterinary Cannabis Society
Oakland, CA 94611
Email: garyrichterdvm@gmail.com

Stephanie McGrath, DVM, MS
Department of Clinical Sciences
College of Veterinary Medicine and Biomedical Sciences
Colorado State University
Fort Collins, CO 80523
Email: stephanie.mcgrath@colostate.edu

Abbreviations

AEA	Anandamide or N-arachidonoylethanolamide
AUC	Area under the curve
CB1	Cannabinoid type 1 receptor
CB2	Cannabinoid type 2 receptor
CBC	Cannabichromene
CBD	Cannabidiol
CBDA	Cannabidiolic acid
CBDV	Cannabidivarin
CBG	Cannabigerol
CBN	Cannabinol
CBR	Cannabinoid receptor
Cmax	Maximum concentration
CoA	Certificate of analysis
COX-2	Cyclooxygenase-2
CYP	Cytochrome P450
DDI	Drug-drug interaction
eCB	Endocannabinoid
ECS	Endocannabinoid system
FABP	Fatty acid–binding protein
GPCR	G protein–coupled receptor
MCT	Medium-chain triglyceride
OA	Osteoarthritis
PD	Pharmacodynamics
PK	Pharmacokinetics
PPAR	Peroxisome proliferator-activated receptor
THC	Delta-9-tetrahydrocannabinol
THCA	Tetrahydrocannabinolic acid
THCV	Tetrahydrocannabivarin
Tmax	Time to maximum concentration
TRPV	Transient receptor potential vanilloid
2-AG	2-arachidonoylglycerol

Abstract

This article is intended to provide the veterinary community with a concise, understandable, and clinically relevant review of cannabis medicine in companion animals. Included are descriptions of the structure and function of the endocannabinoid system (ECS), outlines of the pharmacologic effects of biologically active compounds produced by the cannabis plant (phytocannabinoids, terpenoids, flavonoids), potential clinical uses and toxicities, relevant legal updates, and an overview of the most relevant veterinary research.

The ECS is a complex neuromodulatory signaling system that regulates multiple systems throughout the body and is often up-regulated or down-regulated in times of disease. Many cannabis-derived molecules have been

shown to have multiple therapeutic benefits, including anti-inflammatory, analgesic, antineoplastic, anxiolytic, and anticonvulsant properties, through a variety of mechanisms. Although some of the naturally occurring compounds produced by the cannabis plant work in concert with the ECS, others have a unique pharmacology that produces important physiologic effects via ECS-independent mechanisms. Cannabis science is still in its infancy, and the research up to this point has centered around cannabidiol (CBD) or delta-9-tetrahydrocannabinol (THC). It is essential for veterinary practitioners to understand that the cannabis plant does not consist of a single therapeutic agent but rather a heterogeneous blend of a multitude of compounds.

Introduction

Today's intense interest in the benefits of cannabis is not a new phenomenon. Humans have been cultivating cannabis for more than 5000 years for a variety of uses, including, paper, fiber, clothing, and medicine. Long valued for its use in industry and ancient medicine practices, the therapeutic applications of the medicinal compounds produced by this plant have received increased attention in conventional medicine over recent years (1). The human cannabis market (both hemp and marijuana) has experienced dramatic growth, and projections estimate the industry will continue this impressive expansion, from roughly $12.581 billion in 2018 to a projected market of $36.903 billion by 2024 (2).

Animal caregivers are interested in, and are already exploring, the therapeutic potential of cannabis-derived products for their animals. As they do this, they are looking to veterinarians for guidance on the safety, efficacy, and clinical applications of these products (3). Additionally, the increase in access to cannabis products across the United States has resulted in a rise in both intentional and accidental exposure to animals in households with human cannabis consumers due to increased risk of unintended exposure and potential intoxication (4). In order to educate clients and effectively advocate for the safety of our patients, veterinarians must strive to remain informed about the potential risks and benefits of cannabis use in a clinical context. Although research into the use of cannabis for companion animals is in the early stages, safeguarding the welfare of patients necessitates an understanding of currently available research and advocating for continued scientific study.

Fundamental Terminology: Cannabis

The taxonomical debate on the classification of *Cannabis* spp. has been lengthy and complicated and is often a point of confusion for many. *Cannabis sativa* L. is defined by most expert botanists as a single species having subspecies or varieties. These varieties include indica, sativa, and ruderalis. Inbreeding has led to many hybrid cultivars. A *cultivar,* or cultivated variety, is defined as a plant that has been created or intentionally selectively bred with general characteristics maintained through cultivation. The lay community often uses terms such as *strain, breed,* and *type* to refer to different cultivars. The various cultivar varieties are phenotypically different plants; however, physical characteristics do not consistently correlate with chemical constituents. Cultivars are typically given names, sometimes based on arbitrary creativity or their appearance, smell, and physiological effects. New genotypes created through crossbreeding produce a unique spectrum of medicinal molecules (5–7). The word *chemovar,* on the other hand, is distinguished through a biochemical approach, with an emphasis placed on a specific molecular profile (ie, cannabinoids, terpenes, and other compounds within the plant). Varieties and cultivars often refer to the phenotypic expression, whereas chemovar refers to the chemical expression.

Although the colloquial terminology used by the consumer marketplace frequently requires clarification, the term *cannabis* may be used as a scientifically appropriate term for any plant within the *Cannabis* genus, regardless of subtype or variety.

Beyond its biological taxonomy, *Cannabis* spp. are divided into 2 subtypes based on the plant's intended use and legal classification. The first of these 2 recognized subtypes is known as "industrial hemp," "hemp," or "low-THC (delta-9-tetrahydrocannabinol) cannabis." The current legal landscape allows products that have been formulated from the hemp subtype to be easily obtained by animal caregivers through over-the-counter purchases, such as from retail outlets and online marketplaces. The second subtype is commonly described as "high-THC cannabis" or "marijuana." It should be noted that the term *marijuana* is a description of cannabis when used as a recreational product. From a scientific and medical perspective, the use of the term *high-THC cannabis* is preferred.

Low-THC Cannabis/Hemp: Industrial and Medical Uses

Historically, products derived from hemp varieties of cannabis were intended for industrial purposes, and plants were highly valued for their fiber and nutritional content rather than their production of medicinal compounds. As a consequence of intentional breeding for fiber and seeds, hemp plants traditionally produced low quantities of the psychoactive molecule THC as well as many other medicinal compounds. Medical products made from such hemp plants contain a limited number of cannabinoids such as THC and may include chemical contaminants such as heavy metals due to the inherent accumulative nature of the hemp plant.

In today's cannabis market, however, growers are able to produce hemp plants that are better suited to medical uses than their predecessors. These medicinal-use hemp plants have traded their lengthy fibrous stalks for robust flowering and resin production. They are phenotypically similar to non-hemp cannabis plants and are able to produce copious amounts of medicinally valuable compounds. Careful farming practices can also ensure that hemp intended for medical use is free of chemical residues and other contaminants. Medical practitioners should be aware of the distinction between medicinal-use hemp and industrial-use hemp because differences in growing practices, plant quality, and extraction techniques can affect the quality of the end product and its clinical safety as well as efficacy (8).

Regardless of the intended use or even the specific chemotype, cannabis containing less than or equal to 0.3% THC by dry weight at the time of harvest is legally classified as hemp in the United States. Although the medical community finds value in distinguishing between medicinal- and industrial-use plants, the term *industrial hemp* is frequently used by nonmedical industries as a catch-all to include all hemp plants, regardless of their intended end use.

High-THC Cannabis/Marijuana: Recreational and Medical Uses

According to US federal law, any cannabis plant containing more than 0.3% THC by dry weight at the time

of harvest is classified as marijuana. Current legal policies restrict access to products that have been produced from marijuana plants, and these products are only sold through licensed cannabis dispensaries as permitted by state-specific legislation. The term *marijuana,* as used by the US federal government, frequently denotes all non-hemp cannabis varieties as illicit drugs with no medicinal value. As described throughout this article, there are significant medical uses for both low-THC and high-THC cannabis varieties. Thus, the term *high-THC cannabis* is more medically appropriate than the term *marijuana*.

Modern-day, high-THC cannabis grown for the medical and recreational markets typically has much higher levels of THC than what is found in uncultivated plants (1). Consequently, extracts or products formulated from these plants frequently contain equally high or higher levels of THC. Although cannabis and their extracts with very high THC levels have medical application in specific circumstances, these products require particular caution by the end user because the THC molecule has a robust psychoactive effect within the nervous system.

The predominance of the veterinary cannabis market is currently contained within the hemp (low-THC) category; however, veterinary patients are frequently exposed to high-THC products (3). In the authors' clinical experience, animal caregivers who use cannabis products (medical or recreational) themselves are more likely to be interested in, or have already experimented with, the use of high-THC-derived products for their animals. In addition, with intentional administration of these products to animals, human cannabis users must also be educated in harm reduction strategies to prevent accidental animal exposure and unintended intoxication.

The Endocannabinoid System

The endocannabinoid system (ECS) is a complex, widely distributed regulatory system that provides essential mechanisms for maintaining the biologic balance and feedback throughout the body. This essential neuromodulatory network has been identified in a multitude of species. From humans to birds to canines, the ECS plays an essential role in health and homeostasis (9).

The functions of the ECS have been characterized as "relax, eat, sleep, forget, and protect" (10). As both the medical and scientific communities mature in their understanding of the ECS, this system is being recognized for its importance in maintaining holistic health. The regulatory functions of the ECS are essential in the integration and modulation of complex and diverse body functions, such as appetite and digestion, energy balance, sleep patterns, immune status and inflammation, and emotional responses (10). The scientific and medical communities' deepening understanding of the ECS not only improves our understanding of the mechanisms of existing medical modalities and their impact on the ECS but also offers an interesting array of novel therapeutic targets and unique medical solutions.

The classic ECS can be conceptualized as 3 distinct components: cannabinoid receptors (CBRs), endogenous ligands of CBRs known as *endocannabinoids* (eCBs), and enzymes responsible for the activation, transportation, and breakdown of eCBs.

CBRs

CBRs are located throughout the mammalian body. Their widespread distribution and facilitation of intricate, intersystem communication provide the body with a fine-tuned biologic balancing system. The 2 CBRs currently recognized are cannabinoid type 1 (CB1) receptor and cannabinoid type 2 (CB2) receptor. CBRs belong to the class of G protein–coupled receptors (GPCRs) and have been identified as the most abundant GPCR-type receptor throughout the mammalian body (11, 12). Although these 2 receptors share a significant degree of similarity in their genetic sequence, the effects mediated throughout the body are distinct (7). As an additional layer of complexity, the CB2 receptor demonstrates different pharmacologic responses between species and different responses even within specific organ systems (13).

The CB1 receptor is distributed throughout the nervous system, including peripheral, spinal, and supraspinal sites. This receptor is strategically located with various densities based on region, cell type, and specific neurotransmitter category (14). CB1 receptors have also been identified within the cardiovascular, immune, gastrointestinal, and reproductive tissues but with far less density than within the nervous system (12). Due to its distribution, the CB1 receptor is responsible for multiple physiologic functions, such as pain modulation, movement, appetite, nausea, memory processing, and temperature regulation (11).

Through modulation of neurotransmission, CB1 receptors play a role in down-regulating the release of multiple neurotransmitters, such as glutamate and GABA, and act to maintain physiologic balance through several pathways, such as the inhibition of adenylyl cyclase, stimulation of mitogen-activated protein kinase (MAPK) cascades, modulation of ion channels, and intracellular calcium mobilization (11, 15). In addition, CB1 receptors appear to demonstrate constitutive activity, which provides a basal sympathetic tone even in the absence of an exogenous ligand (15).

The CB2 subset of CBRs are concentrated within the tissues of the immune system, with the highest density found in B lymphocytes, natural killer cells, macrophages, T lymphocytes, and the spleen (13, 16). Additionally, CB2 receptors can be found throughout peripheral organ tissues, such as the lungs, skin, bones, and gastrointestinal tract, as well as in the supportive cells of the nervous system, such as microglia and a small subset of neurons (13, 14). The numbers of CB2 receptors expressed in these tissues appear to be intimately influenced by the inflammatory process and the state of that cell's activity (14). The CB2 receptors appear to mediate the ECS's regulation of local immune and inflammatory reactions throughout the body (16). In contrast to normal, healthy tissues, the CB2 receptor may be up-regulated by 100-fold in areas of inflammation and injury (17). CB2 receptor activity modulates important regulatory functions in immune cell activation, inflammatory response, pain modulation, neuroprotection, and bone density (13).

The receptor-mediated effects of both CB1 and CB2 can be triggered by the binding of either endogenous (eCBs) or exogenous (select phytocannabinoids, terpenoids, and synthetic cannabinoids) ligands. Ligand binding at both CB1 and CB2 receptors initiates and modulates multiple cellular signaling pathways.

The wide and sometimes contradictory array of effects mediated by endogenous and exogenous cannabinoids may not be completely explained by these 2 CBRs only. This suggests the existence of a larger network of other receptors within the ECS and/or complex interactions of the ECS with non-ECS receptors and ligands (18). The intricate network of inter- and intra-ECS interactions and effects is now often referred to as the *endocannabinoidome* (19, 20).

As the study of the endocannabinoidome develops, multiple receptors and ligands outside of the classic ECS demonstrate important and complex interactions with the classical components. Although a complete review of these nonclassical or atypical receptors is beyond the scope of this review, the more well-defined atypical receptors are briefly described below.

GPR55 receptors are widely expressed throughout the body, particularly within the nervous system, and can be CB2 receptor dependent or independent to regulate immune cell migration. They may also be involved in sensory transmission, regulation of bone physiology, analgesia, energy regulation, neuroprotection, calcium modulation, and seizure regulation (18–20).

GPR18 receptors are expressed in the cerebellum, spinal cord, small intestine, immune system, lungs, and reproductive tissue. These receptors may play an important role in microglial activation in response to neuronal injury and provide neuroprotective effects (18–20).

GPR119 receptors have a more limited distribution and have been identified on pancreatic and gastrointestinal tissue and may have effects on multiple physiological processes, including energy regulation, glucose homeostasis, and appetite control (18–20).

Transient receptor potential vanilloids (TRPVs) are present within the CNS and on peripheral sensory neurons. These receptors may reduce nociception by modifying pain pathways (18–20).

Peroxisome proliferator-activated receptors (PPARs) are unique from other receptors interacting with the ECS because they are located within the cell nucleus and serve to direct or modify gene transcription (18–20).

eCBs and Enzymes

The term *cannabinoid* encompasses all chemical substances that act as ligands of the CBRs, specifically CB1 and CB2 (19). Included in this definition are the endogenous ligands of the CBRs, including the eCBs. In contrast, molecules that have activity at these CBRs but are synthesized outside of the body are known as *exogenous cannabinoids*. Examples of exogenous cannabinoids include plant-derived cannabinoids such as THC and synthetic cannabinoids.

The 2 eCBs that have been well characterized are N-arachidonoylethanolamide, referred to as *anandamide* or *AEA,* and 2-arachidonoylglycerol (2-AG). eCBs are derived from inactive precursors and are particularly generated in times of stress, disease, or injury (20). Unlike traditional neurotransmitters, such as glutamate and GABA, which are preformed and stored in intracellular vesicles until needed, eCBs are created on demand from inactive phospholipid precursors embedded in the cell membrane. Also importantly, most neurotransmitters are water-soluble and require transmembrane proteins to transport them across the cell membrane. In contrast, the eCBs (AEA and 2-AG) are uncharged lipid molecules that readily diffuse across the cellular membranes.

Due to their hydrophobic nature, eCBs cannot travel very far in aqueous mediums without the aid of a transporter. Fatty acid–binding proteins (FABPs) are responsible for the transportation of eCBs through the aqueous cytoplasm to undergo rapid deactivation. AEA is hydrolyzed by fatty acid amide hydrolase (FAAH) into arachidonic acid (AA) and ethanolamine. 2-AG is hydrolyzed by monoacylglycerol lipase (MAGL) into AA and glycerol (15, 19, 20). Additionally, cyclooxygenase-2 (COX-2) metabolizes both AEA and 2-AG (21).

Within the nervous system, eCBs mediate retrograde signaling between pre- and postsynaptic neurons. After activation from the postsynaptic neuron in response to depolarization, eCBs are released and travel in a retrograde manner across the synaptic cleft and bind to the CB1 receptors abundantly expressed on the presynaptic terminal. This transsynaptic gap communication between pre- and postsynaptic neurons provides a mechanism by which neurons are able to regulate their own stimulation or inhibition. Mediated by the control of eCB synthesis (in response to neuronal stimulation), the local action (retrograde movement across the synaptic gap), and the rapid degradation (temporal control) of ECBs, the ECS provides the nervous system with a fine-tuned regulatory mechanism (19, 22).

Apart from the classic eCBs (AEA and 2-AG), multiple other molecules are under consideration for inclusion in this group of compounds. These presumptive eCBs have low binding affinity at the primary CBRs but frequently demonstrate strong binding affinity to the atypical eCB receptors. The non-classic eCBs currently identified include N-arachidonoyl dopamine, virodhamine (a precursor to AEA), and 2-AG (noladin ether) (22).

The effects of both endogenous and exogenous cannabinoids will vary depending on the "ECS tone" of the individual patient. ECS tone is determined by the levels of endogenous cannabinoids (eCBs) and the presence and density of CBRs (23). The tone of the ECS is therefore subject to anything that changes any of these factors, such as genetic or congenital influences, or that can be acquired secondary to inflammatory conditions, increased levels of circulating catecholamines, increased sympathetic tone, and alterations in neurotransmitter production and release. eCB deficiency with resultant reduction in baseline ECS tone has been linked to a variety of chronic diseases in humans, such as fibromyalgia, migraines, and irritable bowel syndrome (23).

The *Cannabis sativa* Plant

With more than 750 bioactive compounds, the cannabis plant is chemically diverse with numerous constituents, including phytocannabinoids, terpenoids, flavonoids, carbohydrates, fatty acids and their esters, amides, amines, phytosterols, and phenolic compounds (24). To understand whole-plant medicine is to appreciate the phenomenon of the interactions that can occur between these molecules within biological systems. These complex molecular interactions are referred to as the "entourage effect," a term that more recently has been used to describe the therapeutic potential of the interactions between the plant's individual compounds to provide powerful medicinal benefits. This term, however, was first introduced by Professor Raphael Mechoulam referring to the ECS and how the "inactive" metabolites markedly increase the activity of the primary endogenous cannabinoids, AEA and 2-AG.

Plants within the genus *Cannabis* are considered polypharmaceuticals, full of hundreds of compounds, some of which are synergistic or additive with one another and others with an opposing effect. In some cases, the result is an increased therapeutic benefit compared to any single compound. Use of the entourage effect should be an important clinical consideration until the medical community truly understands the exact combinations and concentrations of individual compounds required to treat specific diseases. The first step in understanding this clinical application of whole-plant medicine is to understand the 3 classes of medicinal compounds found within the cannabis plant.

Phytocannabinoids

Phytocannabinoids are exclusively produced in the cannabis plant and consist of more than 150 naturally occurring compounds, some that bind to the eCB receptors with various affinities (25). Within the plant, cannabinoids are predominantly present in the form of their acidic precursors as carboxylic acids. For example, THC and cannabidiol (CBD) are present within the plant as tetrahydrocannabinolic acid (THCA) and cannabidiolic acid (CBDA), respectively. Under the influence of heat, light, and time, these acidic precursors are converted into their neutral, more stable forms by the removal of a carboxyl group and release CO_2, known as the process of *decarboxylation* (26).

The major phytocannabinoids, THC and CBD, are the most well studied and well known. Interestingly, these phytocannabinoids are chemical isomers, but their atomic arrangements differ. Despite the slight variance in molecular structure, the physiologic effects of both compounds vary greatly. For example, both CBD and THC bind to receptors in the brain and impact factors such as sleep, mood, and anxiety, but only THC produces the intoxicating effects that have been associated with high-THC plant varieties.

Although THC and CBD may be the most well-known phytocannabinoids, the cannabis plant produces many others. Below is a summary of the mechanisms of action of the most commonly studied major and minor cannabinoids.

THC: THC is a partial agonist at both CB1 and CB2 and is largely responsible for the intoxicating effects noted with cannabis use. Some potential therapeutic effects of THC include analgesia, muscle relaxation, and antiemetic and anticonvulsant properties (25). Additionally, THC has immunomodulatory and anti-inflammatory properties that are produced partly through the activation of the CB2 receptor. THC has been shown to have greater anti-inflammatory activity when compared to both aspirin and hydrocortisone (27). Other medical benefits of THC include bronchodilation, gastrointestinal support (including in inflammatory bowel disease), reduced intraocular pressure, antineoplastic effect, neuroprotective activity, and sleep support (28–34).

CBD: Unlike THC, CBD does not have any inherent intoxicating effects. However, one of the common CBD myths is that this molecule is not psychoactive. The CBD molecule is able to achieve changes in brain function and results in clinically detectable alterations in perception, mood, and consciousness, and consequently, it is frequently used as a psychoactive compound with anxiolytic and antidepressant effects. CBD has also demonstrated the ability to reduce the negative effects noted secondary to THC (ie, psychotoxicity, paranoia, and tachycardia). These modulatory effects likely occur via multiple mechanisms, such as negative allosteric modulation of eCB receptors, competitive inhibition of the cytochrome P450 enzyme complex, and competition for FABPs (35, 36). CBD, when coadministered orally with THC, inhibits cytochrome P450 and slows the metabolism of THC to its more psychoactive metabolite, 11-hydroxy-Δ^9-THC (36).

CBD influences the availability and activity of eCBs, particularly AEA, by binding to FABP and reducing the degradation of AEA by FAAH in rodent models. This allows the body to be exposed to AEA over prolonged periods of time and may explain, in part, the action of CBD in modulating the eCB tone (37).

CBD is currently considered a partial agonist at CB2 and an antagonist at CB1 with low binding affinity at the orthostatic site of the CB1 receptor (25). However, these mechanisms were observed at concentrations that are likely not physiologically relevant and are, consequently, difficult to extrapolate to clinical applications. As of 2019, the specific mechanisms of action for these biological effects remained unclear. There are at least 65 molecular targets for CBD, its activity is multimodal, and the majority of functional targets are non-CBR dependent (38).

CBD molecules work on numerous other receptor systems throughout the body, such as TRPVs; nuclear receptor PPARs; GPCRs 55, 18, and adenosine A2A receptor; and 5-HT1A (serotonin) receptors (39). Due to its promiscuous binding activity, CBD has proven to be an extremely diverse and active pharmacological compound. The CBD molecule has demonstrated multiple therapeutic applications and properties, such as analgesia, antioxidant, anti-inflammatory, antiemetic, antidiabetic, antineoplastic, anxiolytic, cardioprotective, and bone strengthening (32, 40–46). CBD has been found to be more protective against glutamate neurotoxicity than either ascorbate or α-tocopherol, indicating that it is a potent antioxidant (47). This molecule has received significant scientific interest as an anticonvulsant agent, and in 2018, for the first time, the FDA approved a cannabis-based CBD pharmaceutical (a) (48, 49).

Cannabigerol: Cannabigerol (CBG) (when in its acid form) is considered to be the parent molecule of the majority of phytocannabinoids. It is non-psychotropic and a weak partial agonist at CB2 and possibly at CB1 (50). With prolonged stimulation, it can desensitize TRPV type 1 (TRPV1), and it is also considered a potent inhibitor of transient receptor potential melastatin 8 (TRPM8), making it a potential therapeutic tool for multiple different cancers as well as other disease processes (51). It has analgesic and anti-inflammatory effects via multiple mechanisms such as activation of PPARγ, reducing proinflammatory cytokines such as interleukin-1β (IL-1β), tumor necrosis factor α (TNF-α), and interferon-γ (IFN-γ), and is a potent α-2 adrenoreceptor agonist (52, 53). CBG produces muscle relaxation through GABA reuptake inhibition at a greater level than CBD or THC (54). Finally, CBG has modest antifungal action but is a powerful antibacterial agent against methicillin-resistant *Staphylococcus aureus* (MRSA) (55). CBG is typically found in relatively low concentrations in most cannabis plants, but recent and more advanced breeding work has yielded cannabis chemovars with high CBG potencies.

Cannabichromene: Cannabichromene (CBC) is a non-psychotropic cannabinoid with inflammation modulatory properties through selective binding at the CB2 receptors and its ability to bind with transient receptor potential channels of both TRPV1 and ankyrin type-1 receptor (TRPA1). These modulatory effects were augmented when CBC and THC were coadministered (40, 56, 57). Finally, CBC has been shown to possess antibiotic and antifungal activity (58).

Cannabinol: Cannabinol (CBN) is an oxidative by-product of THC found predominantly in aged cannabis products and was the first phytocannabinoid to be isolated in its pure form. CBN has significantly weaker binding affinity to both CB1 and CB2 receptors compared to THC, and based on anecdotal reports and older publications, it produces greater sedation when combined with THC than when administered alone (59, 60). CBN has anticonvulsant properties, albeit less than either CBD or THC (61). It also shows similar therapeutic properties to other phytocannabinoids, such as anti-inflammatory (via inhibitory activity on COX and lipoxygenase [LOX]) and antibacterial activity (62). Also, because it has TRPV2 (high-threshold thermosensor) agonist activity, CBN could be a potential treatment option for burns (63).

THCA and CBDA: The minor cannabinoids THCA and CBDA are the 2 naturally occurring, thermally unstable, non-psychotropic precursors of THC and CBD, respectively. They are considered more bioavailable and have minimal to no ability to cross the blood-brain barrier. Due to the extra carboxyl group, these precursors are structurally larger in size than their neutral forms and are therefore unable to effectively bind to the CBRs. Consequently, their therapeutic value is derived from other mechanisms. Both THCA and CBDA have been shown to have anti-inflammatory, antiemetic, antineoplastic, and anticonvulsant properties (64–69). A 2008 study demonstrated that CBDA had essentially equal COX-2 inhibition in comparison to 2 different conventional NSAIDs (diclofenac and indomethacin) (70). A subsequent study showed that CBG, cannabigerol acid (CBGA), and THCA also demonstrated COX-2 inhibition (71).

Cannabidivarin and (–) Δ^9-Tetrahydrocannabivarin: Cannabidivarin (CBDV) is a propyl analogue of CBD and has been shown to have anticonvulsant activity (72). (–) Δ^9-tetrahydrocannabivarin (THCV) is a propyl analogue of THC. In contrast to THC, however, THCV is a CB1 antagonist at lower doses and a CB1 agonist at higher doses (73, 74). THCV has previously demonstrated prominent anticonvulsant properties in rodent models (75). In addition, THCV was found to produce weight loss, possess hypophagic properties, and decrease body fat and serum leptin concentrations in obese mice (76). Recently, THCV has been postulated to be beneficial for type 2 diabetes by improving glucose tolerance and increasing insulin sensitivity in mouse models (77).

Terpenoids

Terpenes and terpenoids are arguably the largest and most diverse group of phytochemicals found in nature. More than 50,000 have been identified to date, with at least half synthesized by plants (78). Although the terms are often used interchangeably, terpenes are hydrocarbons, whereas terpenoids have been modified by an extra atom (usually oxygen). These volatile, aromatic organic hydrocarbons are responsible for the aroma and taste of many plants, including cannabis. They are classified by the number of isoprene units present in their structure, with the most common being monoterpenes (2 isoprene units), sesquiterpenes (3 isoprene units), and diterpenes (4 isoprene units). Monoterpenes in particular are found in nature as a major constituent of terpenic

oils or essential oils. Similar to other strong-smelling plants, the development of terpenes in cannabis began for adaptive purposes—to attract pollinators and repel and protect injured tissues in plants from the attack of herbivores, insects, and parasites.

More than 200 terpenes have been identified from various chemovars within the *Cannabis* genus (39). Every cultivar of cannabis produces a unique terpene profile and may, consequently, have equally unique therapeutic effects. Terpene molecules have the ability to contribute their own physiologic and therapeutic effect. They may also interact synergistically with other compounds within the plant to enhance the clinical outcome (39).

Monoterpenes, such as limonene, myrcene, and pinene, usually are the predominant terpenes within most cannabis plants. Limonene is the precursor to several other monoterpenes and demonstrates anxiolytic, antidepressant, and antineoplastic effects and may be beneficial for gastrointestinal reflux. β-Myrcene, also found in hops and mango, has anti-inflammatory, analgesic, and anxiolytic properties (39). Linalool, commonly found in lavender plants, has demonstrated activity as an anti-inflammatory, local analgesic, anxiolytic, and anticonvulsant agent (39).

Due to their volatile nature, monoterpenes may be lost during processing, drying, and storage of plant material. The resulting terpene profile consequently leads to higher proportions of sesquiterpenes, especially caryophyllene. β-Caryophyllene, also found in black pepper, is the most common sesquiterpenoid found in the cannabis plant and is unique because it is the only known terpene that acts as a potent, full CB2 receptor agonist (39). Given the lack of psychoactivity of CB2 agonists, β-caryophyllene offers enormous promise as a therapeutic immunomodulatory compound. It also demonstrates anxiolytic, antioxidant, and anti-inflammatory effects mediated by both PPARγ and CB2 receptors (79). β-Caryophyllene may work in concert with specific cannabinoids to produce a profound and durable anti-inflammatory response as well as offer gastric protective properties (39).

Many factors play a role in the diversity of a plant's terpene profile, including genetics; exposure to light, heat, and humidity; and the character of the soil. Additionally, as mentioned above, the terpene profile of a cannabis product is greatly affected by production methods, some of which lead to terpene loss. Although a comprehensive discussion of terpenes and their interactions with cannabinoids is beyond the scope of this article, clinicians should recognize that some terpenes have physiologic effects on their own. The presence of terpenes in a product may affect how cannabinoids are absorbed (including increasing permeability of cell membranes such as the blood-brain barrier) and can work in a synergistic, additive, or perhaps even an opposing manner with cannabinoids.

Flavonoids

Flavonoids are a diverse group of naturally occurring polyphenolic compounds that contribute to the plant's vivid color pigmentation, which also serves to attract pollinators. Approximately 20 different types of flavonoids have been produced within the cannabis plant, and similar to terpenoids, many of these compounds have been shown to have anti-inflammatory, neuroprotective, and antitumor effects (80).

Of the numerous flavonoids that exist within the plant, the cannaflavin compounds are unique to cannabis. Cannaflavin A inhibits prostaglandin E2 (PGE_2) with 30 times the activity of aspirin (81). A recent study demonstrated an isomer of cannaflavin B decreased survival of 2 pancreatic cancer cell models as well as pancreatic cells treated with radiotherapy. Additionally, in vivo results demonstrate that cannaflavin B has therapeutic efficacy in delaying both local and metastatic tumor progression as well as increasing survival times in animals with pancreatic cancer (82).

Clinical Use

Cannabis is currently being used for a variety of disease processes in human medicine, and there are multiple publications (both in vivo and in vitro) supporting its medicinal use. Due to the legal environment surrounding access to cannabis products, only a few in vivo studies have been performed in veterinary medicine thus far. The most common and scientifically justified clinical applications of cannabis in veterinary medicine to date are analgesia for osteoarthritis (OA) and anticonvulsant activity for epilepsy (83, 84).

Intriguing applications for medical cannabis being studied in humans include its use as an antineoplastic, anxiolytic, anti-inflammatory (beyond OA), anticonvulsant,

gastrointestinal support, and a neuroprotective agent (85–90). As is common in veterinary medicine, practitioners must rely on non-veterinary-specific research when considering possible therapeutic benefits of medical modalities in their patients. The multitude of documented uses in human medicine propose a myriad of potential applications for the veterinary practitioner beyond the treatment of OA and seizures. The balance between potential benefits and risks of the "off label" use of any medicine or modality is the standard of care for veterinary professionals, and this tradition of translational medicine applies equally to cannabis medicine.

Toxicity

THC is typically considered the limiting factor when dosing cannabis products in veterinary patients. Canines in particular have a higher density of CB1 receptors in their cerebellum compared to any other species studied (91). When dogs receive excessive amounts of THC, either via accidental ingestion or overdose, they develop a unique array of clinical signs referred to as *static ataxia*. Dogs with static ataxia frequently present with a sawhorse stance, sway back and forth, and abruptly catch themselves from falling. Excessive THC exposure in dogs can also lead to urinary incontinence, severe lethargy/stuporous appearance, agitation, tachycardia or bradycardia (dose dependent), hypersalivation, and hypothermia (92).

The majority of dogs experiencing intoxication after high-THC cannabis ingestion recover completely with supportive care and monitoring. Dogs with severe clinical signs that are unable to eat or drink may require hospitalization and IV fluid support. The use of intralipid therapy to bind the highly lipophilic THC is a valid treatment option to reduce clinical signs in severe cases of toxicity (92). Anecdotally, CBD has been used to counteract the effects of THC toxicity in humans. It is postulated that CBD's negative allosteric modulation at the CB1 receptor and its potential to slow the conversion of THC to the more psychoactive metabolite, 11-hydroxy-THC, are responsible for "blunting" some of the more extreme effects of THC toxicity. No studies have been published to confirm this, however, and legal restrictions surrounding the use of CBD in a clinical setting makes using it as a THC toxicity antidote impractical.

Unlike opioid receptors, there are no CBRs in the respiratory centers of the brain. Thus, even with extreme overdoses of cannabis, there is no chance of respiratory depression. One study concluded there is no known LD_{50} for THC in dogs after doses of 3000 mg/kg of pure THC were administered. Although no fatalities directly related to THC occurred, 2 dogs in the study died from aspiration pneumonia secondary to severe sedation (93). This fatal secondary complication, although uncommon, has occurred in instances in which pets unintentionally have ingested large amounts of THC and timely medical support was not available.

CBD, by contrast, has very few side effects and a wide margin of safety in both humans and veterinary patients (94). CBD is well tolerated at much higher doses than would be typically used in a clinical setting (95). In addition, given the effects of CBD on common biological targets associated in drug metabolism (eg, cytochrome P450 family 2, subfamily C, member 19 [CYP2C19], CYP2D6, and CYP2C9) and excretion, clinicians should be aware of the potential for drug-drug interactions (DDIs) and adverse drug events (ADEs) (84). It is, however, the author's experience that both DDIs and ADEs are exceedingly rare at the doses we use in a clinical veterinary setting. Specific information regarding the safety of CBD in veterinary patients is discussed in the Relevant Veterinary Research section.

Product Terminology and Safety

As is the case with any medicine, the selection of the product and formulation is critical. This is particularly true regarding any cannabis product given the inconsistencies in terminology, labeling, product content, and quality that are prevalent throughout the cannabis industry. These inconsistencies are at least partially due to the lack of federal regulation by the FDA of CBD-based products for humans and animals.

Other than the restriction of "drug claims" by the FDA, there is little by way of oversight when it comes to terminology used by manufacturers to describe cannabis products. Much of this industry-specific jargon is unfamiliar to veterinary professionals and deserves clarification and standardization. Three of the most frequently encountered terms used to describe the molecular content of a cannabis product are *full spectrum*, *broad spectrum*, and *isolate*.

Full-spectrum products are intended to be products formulated from a cannabis extract that still contain the

various components of cannabinoids, terpenes, and flavonoids that were present in the original whole plant. Full-spectrum products theoretically contain the highest amount and diversity of compounds. Even the most minor cannabinoids and terpenes have the potential of "boosting" the effects of the major cannabinoids, CBD and THC. Many practitioners use the concept of the entourage effect when providing recommendations to patients to obtain better clinical outcomes.

Broad-spectrum products, by comparison, are cannabis products that contain some, but not all, of the components from the original whole plant, and these products are typically void of THC at measurable levels. One must consider that the manufacturing methods to remove THC from a cannabis product can potentially lead to the loss or significant reduction of other compounds, such as terpenes and minor cannabinoids.

Finally, *isolate* products are purified single compounds such as CBD or THC. They are considered more of a pharmaceutical product because they are void of all other compounds. They require higher dosages to achieve a therapeutic effect and have demonstrated an increased incidence of negative side effects (96).

Stepping beyond issues of terminology, the lack of regulatory oversight and potential inconsistencies from one product description to the next has led to a "buyer beware" environment. It is critical for both animal caregivers and veterinarians to understand how to evaluate cannabis companies and products for transparency, safety, and quality. As an agricultural product, cannabis extracts may potentially be contaminated with pesticides, fungicides, herbicides, heavy metals, bacteria pathogens, mycotoxins, and potential residual solvents from the extraction process (depending on the specific method used) (97). The FDA is taking steps to improve the regulatory pathway for lawful marketing of cannabis-derived products for humans and animals.

Due diligence in assessing the safety and potential efficacy of a cannabis product must include the evaluation of a Certificate of Analysis (CoA). A CoA is a laboratory evaluation of a cannabis product providing an objective measurement of its contents. Ideally, a CoA should come from a third-party laboratory that meets International Organization for Standardization/International Electrotechnical Commission (ISO/IEC) 17025, displaying levels of each cannabinoid and terpene as well as levels of any contaminants or potential pathogens present. As a natural product containing multiple phytochemicals, no plant or plant extract contains exactly the same spectrum of phytochemicals. Each individual extraction varies in content. Therefore, it is critical to evaluate a CoA that is specific to the current batch or lot being produced. By evaluating the CoA, veterinarians and animal caregivers are better able to predict the physiologic action of the medicine (via cannabinoid and terpene profiles) and screen for product safety by ensuring that harmful contaminants are undetectable.

Dosing

Considerations for dosing cannabis in the veterinary patient are multifactorial and, to an extent, somewhat unique compared to other drugs and botanicals. Most pharmaceuticals follow a familiar pharmacokinetics (PK) pattern by demonstrating a linear dosing curve and thus displaying a direct linear relationship between increasing dose and increased efficacy until a maximum level of efficacy is reached. Dosing above this point of maximal effect may lead to an increase in negative side effects with little to no increase in therapeutic value.

The clinical impression of many cannabis practitioners, however, is that cannabis follows a biphasic dose response curve. Similar to pharmaceuticals, products with a biphasic dose response demonstrate a direct relationship between increased dose and efficacy until reaching a point of maximal efficacy. Increasing the dose beyond the level of maximal effect will, however, lead to decreased clinical efficacy as well as increased negative side effects. When using products that create a biphasic curve response, there is a "sweet spot" for dosing that provides the optimal clinical effect. Dosing above or below this level leads to suboptimal results (98).

There are, however, data to suggest the biphasic phenomenon is not consistent throughout all cannabis preparations. In one study comparing a CBD isolate to a broad-spectrum CBD-dominant extract, the isolate displayed a biphasic curve and the broad-spectrum formula showed a linear dose response. Additionally, the formulation used in the study was predominantly CBD (17.9%), with much smaller amounts of THC (1.1%), CBC (1.1%), and CBG (0.2%) as well as trace amounts of CBN and CBDV. This is an excellent illustration of both the entourage effect and how very

small quantities of cannabinoids can drastically affect the PK and, presumably, the pharmacodynamics (PD) of medical cannabis formulations (99).

In addition to biphasic dosing considerations, the specific milligrams of cannabinoids (ie, CBD, THC), cannabinoid ratio, and terpene content must be considered. The ratio of cannabinoids in a medical preparation is reported to have an important impact on its effects. For example, "CBD-dominant" formulations with relatively little or no THC have been shown to have efficacy in treating seizures, mild pain, and anxiety (83, 84, 100). Formulations with an even ratio of THC and CBD may have greater effects for moderate to severe pain, gastrointestinal conditions, and certain cancers, and "THC-dominant" products with relatively little CBD are often used for severe pain, such as cancer pain and pain from spinal or neurogenic origins (101, 102).

Broadly speaking, full-spectrum formulations are preferred by most clinical cannabis experts due to the anticipated increase in therapeutic benefit from the entourage effect. As mentioned previously, the milligram amount of THC in a dose of medicine is always the limiting factor. Because of the possibility of THC sensitivity in cannabis-naive patients and the effects of the biphasic dosing curve, it is ideal to follow a "start low and go slow" dosing regimen—specifically, beginning with very low doses and incrementally increasing the dose every 5 to 7 days based on the patient's response and tolerance to the product. This accomplishes the dual goals of minimizing the chance of negative effects related to THC and allowing both the animal caregiver and veterinarian to identify and evaluate clinical effects and determine the optimal dose for each individual patient.

Most cannabis products are dosed orally at an interval of every 12 hours; however, depending on the severity of the condition treated (ie, seizures, anxiety, pain), the patient's tolerance, and the molecular profile of the product, dosing may be implemented every 6 to 8 hours. Based on a previous publication that evaluated PK of CBD, it was demonstrated that the median $T_{½}$ of elimination of CBD specifically in canines was approximately 4 hours at both 2 mg/kg and 8 mg/kg doses when administered a full-spectrum hemp-derived CBD oil (with equal amounts of CBD and CBDA and minor amounts of other compounds) (83).

With regard to THC, negative side effects, such as severe sedation and static ataxia, are never acceptable when cannabis is used medically in animals. Due to concerns of dysphoria, some practitioners start initially with a minimal nighttime dose and increase to BID dosing as tolerance occurs and the therapeutic window widens. Depending on the condition being treated, these initial small doses of THC may be subtherapeutic and, consequently, the dose may need to be titrated up to find the clinically effective dose for that specific patient's condition. Using the "start low and go slow" method greatly optimizes the chances of finding the patient's dosing "sweet spot" while also minimizing the risk of THC-induced adverse effects. Preclinical research demonstrates that tolerance may occur through persistent agonism of the CB1 receptors secondary to prolonged exposure to high doses of THC. Down-regulation of the ECS is thought to occur through receptor desensitization initially and then may be followed by membrane receptor internalization (98).

The margin of safety for CBD is so broad, there is little concern for negative side effects with the exception of idiosyncratic responses. Although the current research evaluating seizures and OA suggests doses of 2 mg/kg BID of a CBD-dominant product, many veterinarians and animal caregivers have found efficacy at much lower doses. In addition, due to the high cost of CBD on a per milligram basis, it may be advisable to begin dosing at approximately 0.3 mg/kg of CBD BID and incrementally increase the dose over time until efficacy is achieved (103).

The future of cannabis medicine will provide transformational opportunities in integrative and personalized medicine practices. Every patient (human or animal) has a unique ECS whose state depends on multiple factors, including genetics, chronicity of disease, age, concomitant medications, comorbidities, and environmental influences such as stress. Because each patient has an individualized response to cannabis medicine, the future use of genetic testing may be a helpful tool for clinicians to identify the exact molecular profile best suited for each patient.

Relevant Veterinary Research

To date, there is a dearth of peer-reviewed veterinary research describing the PK, safety profile, DDIs, and effectiveness and dosing of cannabis in companion animals. However, as the legal landscape changes and the stigma is gradually lifted, the CBD molecule from the cannabis

plant is rapidly becoming more favorably regarded by, and accessible to, the general public. Fortunately, regulations governing research are also changing, such that the gap between science and the everyday use of cannabis is slowly closing.

PK and Safety Studies

Although previous reports have questioned the bioavailability of CBD in dogs, there have been several more recent studies that have evaluated PK/PD in greater detail (**Table 1**) (104).

CBD PK analysis of a CBD-dominant hemp product administered via 3 delivery methods (oral CBD-infused oil, oral CBD-infused capsules, and CBD-infused transdermal cream applied to the pinnae) was performed using 2 single doses (approximately 5 mg/kg and 10 mg/kg) given to healthy research dogs, randomly divided into groups of 5 dogs (105). Overall, the CBD-infused oil delivered orally offered the highest maximum concentration (Cmax), longest $T_{1/2}$, and greatest systemic exposure (represented by the area under the curve [AUC]) (**Table 1**). The dogs were subsequently administered 5 mg/kg or 10 mg/kg BID of the oral CBD-infused oil, the oral CBD-infused capsules, or the CBD-infused transdermal cream applied to the pinnae (same dosing groups as the single-dose phase of the study) for a 6-week period, during which time they were monitored for adverse effects (**Table 2**) (94). Significant adverse effects included diarrhea, which occurred in all dogs, and elevated serum alkaline phosphatase (ALP), which occurred in the dogs receiving the oral forms of the CBD-infused product only. Although diarrhea was reported as a potential adverse effect, without a control group it is impossible to rule out unrelated causes, such as housing relocation, stress, and diet changes. Overall, the study showed that oral and transdermal CBD were measurable in the plasma and well tolerated.

A PK study using 4 healthy research dogs was included as the first arm of an OA efficacy study, in which a full-spectrum hemp-derived CBD oil (with equal amounts of CBD and CBDA and minor amounts of other compounds) was administered orally at single doses of 2 mg/kg and 8 mg/kg of CBD/CBDA combination (1 mg/kg and 4 mg/kg of CBD) specifically, with a 2-week washout period between each experiment (**Table 1**) (83). A similar PK study was performed using oral soft chews (50%/50% mix of CBD/CBDA) dosed at 2 mg/kg CBD/CBDA combination (1 mg/kg of CBD) BID to healthy research dogs (**Table 1**) (106). The dogs were then orally dosed with 2 mg/kg CBD/CBDA-infused soft chews BID for 84 days to assess adverse events. Loose stool and vomiting were rare reported adverse events, at an occurrence of 3.3% and 0.45%, respectively. There were no CBC or serum biochemistry changes outside the reported reference range for any dog during the study period, further supporting the apparent short-term safety profile of oral CBD.

A recent study explored the PK of a cannabis oil that contained both THC and CBD in fasting and fed dogs (107). A total of 6 healthy Labrador Retrievers were used for the study and administered the product at a dose of 1.5 mg/kg THC and 0.0375 mg/kg CBD after being both fasted and fed (**Table 1**). No detectable plasma CBD was found at any time point. It is interesting to note that the fasted dogs had a greater AUC in this study. Given the highly lipophilic nature of THC, they would have been expected to have greater bioavailability in a fed state. The authors hypothesized that perhaps the lipophilicity of the olive oil base maximized the bioavailability in the fasted state, whereas in the fed state the meal may have sequestered the phytocannabinoids, slowing the absorption phase. This is in contrast to previous studies in humans and rodents, in which cannabinoids were absorbed faster and achieved higher Cmax and AUCs (108). Based on this study, when cannabis is given along with a high-fat meal, it is sufficient to activate intestinal lymphatic transport and lead to increased systemic exposure of cannabinoids. Finally, a recent study evaluating 8 dogs that received 2 mg/kg of a full-spectrum CBD-dominant oil demonstrated that feeding enhanced absorption as did feeding soft chews compared to giving the hemp-derived CBD oil without feeding (b). The PK processes are dynamic, change over time, and may be affected by the frequency and magnitude of drug exposure. Also, dose, route of administration, vehicle (tincture, capsule, edible), and physiological factors, such as absorption and rates of metabolism and excretion, can influence drug concentrations in circulation.

Another study investigated the PK of a CBD pharmaceutical (c) when administered using single or multiple administrations to healthy dogs via sublingual delivery (**Table 1**) (109). After the single-dose administration and testing, the dogs were subsequently treated with

Table 1. Pharmacokinetics Summarized				
	Dog	**Dog**	**Dog**	**Dog**
Author	Samara et al. 1988[104]	Bartner et al. 2018[105]	Gamble et al. 2018[83]	Lebkowska-Wieruszewska et al. 2019[107]
Cohort info	6 research dogs	30 research dogs	4 research Beagles	6 healthy Labrador Retrievers
Product format	IV: in 70% ethanol PO: gelatin capsule	Hemp seed oil	Olive oil	Olive oil
Dose (mg/kg)	IV: 45 and 90 mg total dose CBD PO: 180 mg total dose CBD	75 and 150 mg total dose CBD	2 CBD/CBDA 8 CBD/CBDA	1.5 THC 0.0375 CBD
Route	IV and PO	PO	PO	PO
Fasted or fed	Not reported	Fed	Fasted	Fasted and fed
AUC (ng/mL/hour)	IV: 45 = 2706 ± 519; 90 = 6095 ± 1741	135.6 ± 46.3; 297.6 ± 112.8	367 (183–437); 2658 (1753–3048)	69.94 (20.26–95.43); 24.00 (7.84–58.65)
Tmax (hours)	Not reported	Not reported	1.5 (1.0–2.0); 2.0 (1.0–2.0)	1.25 (0.5–4) (fasted); 5 (0.75–8) (fed)
Cmax (ng/mL)	Not reported	625.3 ± 164.3; 845.5 ± 262.2	102.3 (60.7–132.0); 590.8 (389.5–904.5)	24.34 (9.2–77.1); 7.10 (3.6–11.4)
$T_{1/2}$ (hours)	Not reported	199.7 ± 55.9; 127.5 ± 32.2	4.2; 4.2	1.74 (0.80–3.5) (fasted); 1.86 (0.86–3.01) (fed)
MRT (hours)	IV: 45 = 7.0 ± 3.5; 90 = 7.5 ± 2.7	217 ± 46; 298 ± 43	5.6 (4.2–9.1); 5.6 (5.1–7.0)	3.98 (1.49–7.07) (fasted); 5.47 (1.08–7.34) (fed)
Bioavailability	13%–19%	Not reported	Not reported	Fed: 48.22% Fasted: not reported
Comment(s)	Plasma levels of the PO CBD were undetected in 3 dogs and barely detectable in the other 3 dogs	None	Full-spectrum hemp-derived product (with equal amounts of CBD and CBDA and minor amounts of other compounds) The dose was actually 1 mg/kg and 4 mg/kg of each compound	CBD was not detected in plasma at any point

	Dog	Cat	Dog	Dog
Author	Deabold et al. 2019[106]	Deabold et al. 2019[106]	Fernández et al. 2020[109]	Boothe et al. 2019[b]
Cohort info	8 research Beagles	8 research DSH cats	6 research Beagles	8 research Beagles
Product format	Soft chew	Fish oil	Oromucosal spray	Oil and soft chew
Dose (mg/kg)	2 CBD/CBDA BID	2 CBD/CBDA BID	8.1 THC 7.5 CBD	2 (for both oil and soft chew) of CBD
Route	PO	PO	PO	PO
Fasted or fed	Fasted	Fasted	Fasted	Fasted and fed
AUC (ng/mL/hour)	1297 ± 210 (SEM)	164 ± 29 (SEM)	THC: 94.9 CBD: 60.4	Oil (fasted) = 763 Oil (fed) = 1419 Soft chew (fed) = 1140
Tmax (hours)	1.4 ± 0.2 (SEM)	2.0 ± 0.6 (SEM)	2 (single dose) 1 (14 days)	Oil (fasted) = 74.0 Oil (fed) = 2.0 Soft chew (fed) = 4.0
Cmax (ng/mL)	301 ± 63 (SEM)	43 ± 9 (SEM)	THC: 18.5 CBD: 10.5	Oil (fasted) = 130 Oil (fed) = 304 Soft chew (fed) = 301
$T_{1/2}$ (hours)	1.0 ± 0.2 (SEM)	1.5 ± 0.2 (SEM)	Not reported	Oil (fasted) = 5.5 Oil (fed) = 6.2 Soft chew (fed) = 5.6
MRT (hours)	1.4 ± 0.3 (SEM)	3.5 ± 1.4 (SEM)	Not reported	Oil (fasted) = 10 Oil (fed) = 7.1 Soft chew (fed) = 7.7
Bioavailability	Not reported	Not reported	Not reported	Not reported
Comment(s)	50%/50% mix of CBD/CBDA The dose was actually 1 mg/kg of each compound BID	50%/50% mix of CBD/CBDA The dose was actually 1 mg/kg of each compound BID	Other than Tmax, all other parameters were stable from the single to 14-day dosing	None

*The fraction of administered drug that reaches systemic circulation (drug administered/amount absorbed).

Abbreviations: DSH, domestic short-haired; MRT, mean residence time.

Table 2. Safety and Tolerability Studies		
	Dog	**Dog**
Author	McGrath et al. 2018[94]	Vaughn et al. 2020[95]
Study design	Study dogs were randomly assigned to receive CBD in the form of microencapsulated oil beads (capsule), CBD-infused oil, or CBD-infused transdermal cream Safety and tolerability study	Randomized, placebo-controlled, blinded, parallel study Safety and tolerability of escalating doses
Study duration	6 weeks	Up to 10 escalating doses of the oils were planned, with at least 3 days separating doses
Cohort info	30 research dogs	20 healthy Beagles
Product format	All forms had hemp seed oil as base	Sunflower oil and MCT oil
Dose (mg/kg)	Randomized to receive a dose of 5 BID or 10 BID	Dose range CBD-dominant oil: CBD: 1.7–64.7 (18.3–640.5 mg) THC: 0.06–2.4 (0.7–24.2 mg) THC-dominant oil: THC: 1.9–52.4 (24.9–597.6 mg) CBD/THC oil: CBD: 1.4–13.4 (17.6–140.8 mg) THC: 0.97–9.2 (12.1–96.6 mg)
Route	PO	Oral gavage
Fasted or fed	Fed	Fasted
Testing parameters	Assessment of health and blood samples (CBC/chemistry/bile acids) and urinalysis performed	Assessment via body temperature, respiration rate, and heart rate Blood samples (CBC/chemistry) performed

the same dose daily for 14 days. They reported a similar THC/CBD PK profile but with a time to maximum concentration (Tmax) of 1 hour. The authors noted that the plasma levels in the multiple-dose condition were higher than after the single dose, suggesting that the phytocannabinoids may accumulate in the fat tissues and other organs and undergo a progressive and slow release when plasma levels are low.

A recent study investigated the safety of escalating oral doses of CBD, THC, and a combination of both compounds in healthy research dogs. It showed that, at the doses used in this study, CBD was better tolerated than THC in that particular cohort of dogs (95). The dogs were assigned to one of 5 treatment groups (n = 4/group): CBD-dominant oil, THC-dominant oil, CBD/THC oil (1.5:1), sunflower oil placebo, and medium-chain triglyceride (MCT) oil placebo. The CBD-dominant oil was administered up to approximately 62 mg/kg, the THC-dominant oil up to approximately 49 mg/kg, and the CBD/THC oil up to approximately 12 mg/kg CBD/8 mg/kg THC (**Table 2**). The sunflower and MCT oil were given orally up to volumes of 45 mL and 35 mL, respectively. Adverse events were observed in all dogs, most of which were considered mild (diarrhea, vomiting, lethargy, ataxia), with the fewest being in the CBD-dominant oil group. There were no moderate or severe adverse events seen in the dogs receiving the CBD-dominant oil. In the dogs receiving THC-dominant oil or CBD/THC oil combination, moderate to severe side effects were noted, including ataxia, hypothermia, and lethargy. All of these adverse effects are not surprising with THC, especially considering that the experimental design did not account for tolerance that occurs when the dose of THC is appropriately escalated in a clinical

setting. This requires a consistent but gradual dose titration method (not *q* 3-day dosing), which can significantly lower or eliminate toxicity seen with THC. The primary serum biochemical change was an increase in liver enzymes in some of the dogs: 1 dog in the CBD-dominant oil group and 1 dog in the CBD/THC oil group experienced an increase in ALP of 2.9- and 3.6-fold baseline, respectively, which approached or exceeded the upper limit of the reference range, when given at the highest dose. No other significant abnormalities were noted. This study offers the first controlled scientific comparison of the safety and tolerability of oil containing primarily CBD to oil containing higher amounts of THC in dogs, concluding that the CBD-dominant oil was better tolerated.

Finally, a single-dose PK evaluation followed by a 12-week safety assessment in cats, in which 8 healthy research cats were administered a CBD-infused fish oil (50%/50% mix of CBD/CBDA) orally BID, was recently published (**Table 1**) (106). The cats were subsequently administered 2 mg/kg of the CBD/CBDA mixed oil orally BID for 84 days. The CBC and serum chemistry values remained within the reference range for 7 of the 8 cats at all time points; 1 cat had a persistently elevated ALT (above the upper limit of the reference range) throughout the study period. The main adverse events reported were excessive licking and head shaking. Cats appear to absorb or eliminate CBD differently than dogs. Overall, the absorption kinetics showed maximum serum concentrations are approximately one-fifth of what was observed in the dogs, with a longer retention time and $T_{1/2}$. It should be noted that dogs received their CBD-dominant oil in a soft chew form and cats received it in fish oil. The delivery method and form can certainly affect absorption and bioavailability, and perhaps the cats may have lost some of the product with the excessive head shaking, salivation, or vomiting.

The PK/PD studies cited above demonstrated substantial variation in the study design as well as some of the PK parameters. There are many potential reasons that could account for the differences in bioavailability and metabolism in patients dosed with cannabinoids, such as the product formats used, whether they were given fasted or with food (type of food variable as well; high fat vs. low fat), and individualized patients' ECS, age, and body condition. In addition, it is imperative to understand that PK results do not always correlate with clinical outcome, which emphasizes the importance and necessity of further clinical research.

Epilepsy

In 2018, the FDA approved the first plant-derived cannabinoid medicine (a) for the treatment of seizures associated with 2 severe pediatric forms of epilepsy, Lennox-Gastaut syndrome and Dravet syndrome. The clearly demonstrated anticonvulsant properties of CBD subsequently prompted research in veterinary medicine. Although there are various theoretical and proposed mechanisms involved in CBD's anticonvulsant effects, the true mechanism of action is still unknown. Current research has been primarily focused on CBD reducing neuronal excitability through its effects on modulation of intracellular Ca^{2+} (specifically its ability to antagonize GPR55 and desensitize TRPV1) and inhibition of adenosine transport (110).

A randomized, blinded, controlled clinical trial in client-owned dogs with naturally occurring intractable idiopathic epilepsy compared the effect of adjunctive administration of a CBD-dominant hemp oil with conventional treatment alone on seizure frequency (84). A total of 16 dogs completed the study; 9 dogs received a CBD-dominant oil in addition to their standard anticonvulsant therapy, and 7 dogs received placebo in addition to their standard anticonvulsant therapy for a total of 12 weeks. Standard anticonvulsant therapy consisted of phenobarbital, potassium bromide, levetiracetam, and zonisamide, either alone or in combination. Of the 9 dogs, 8 dogs in the CBD-dominant oil group experienced a reduction in mean monthly seizure frequency as compared with 3 of 7 dogs in the placebo group. Further, there was a significant reduction in mean seizure frequency in the CBD-dominant oil group as compared with the placebo group. However, using the definition of a responder as a dog that undergoes at least a 50% reduction in seizure frequency, there was no difference in the number of responders between the group receiving the CBD-dominant oil and the placebo group. Interestingly, a negative correlation was found between seizure frequency and CBD plasma concentrations. The study concluded that although the data were promising, additional research is necessary to determine the overall usefulness of CBD as an anticonvulsant in dogs with idiopathic epilepsy (**Table 3**). Of note, in humans and possibly other animals, CBD is metabolized by the CYP

system in the liver and inhibits several isoenzymes, leading to the speculation that CBD could affect the metabolism of certain anticonvulsant therapies. However, the above study revealed no significant change in serum phenobarbital concentration in dogs following CBD treatment.

OA and Pain

The first study evaluating the clinical effect of CBD on OA pain in dogs was a randomized, blinded, crossover study (83). A total of 16 client-owned dogs with clinically and radiographically confirmed OA completed the study. Dogs were randomly assigned to receive either 2 mg/kg of a full-spectrum hemp-derived CBD oil (with equal amounts of CBD and CBDA and minor amounts of other compounds) orally or a placebo every 12 hours for 4 weeks with a 2-week washout period between treatments. The primary outcome parameters were veterinary assessment of pain, lameness, and weight-bearing and owner assessments using the Canine Brief Pain Inventory (CPBI) and Hudson activity scores. CBPI and Hudson scores significantly decreased, as compared to baseline, specifically showing a decrease in pain and increase in activity, with CBD treatment at weeks 2 and 4 as compared to the placebo arm. The veterinarian-assessed pain scores decreased 2 and 4 weeks after the initiation of treatment with the CBD-dominant oil as compared to baseline. No changes were perceived in the lameness and weight-bearing scores in either group at any time point. Of note, veterinary pain scores decreased from baseline in dogs treated with NSAIDs. The study concluded that the CBD-dominant oil helped increase comfort and activity in dogs; however, larger, long-term studies are needed (**Table 3**).

A second randomized, blinded, placebo-controlled OA study in client-owned dogs with naturally occurring OA was recently published (111). Dogs were included in the study if they had been diagnosed with OA by a veterinarian and had owner-assessed pain, a detectable lameness, and painful joint(s) on palpation. All medications were discontinued 2 weeks prior to study enrollment. During the study period, dogs were randomly assigned to receive either placebo, 20 mg/day (0.5 mg/kg) naked CBD, 50 mg/day (1.2 mg/kg) naked CBD, or 20 mg/day liposomally encapsulated CBD. All products were CBD isolates. Dogs were evaluated by a veterinarian at baseline and day 30 for mobility as assessed by walking, running, and standing from a sitting and laying position on a 5-point scale. Owner evaluations were made at weeks 4 and 6 using the Helsinki Chronic Pain Index (HCPI), a validated 11-item assessment tool. Results of this study revealed that owner assessment did not change with placebo or 20 mg/day naked CBD. However, with administration of 50 mg/day naked CBD and 20 mg/day liposomal CBD, significant reductions in pain were noted. A 17-fold increase in bioavailable circulating CBD following oral administration of the liposomal formulation as compared to the naked isolate was found. Veterinary assessment was similar, observing improvements in all assessment categories only in the dogs receiving 50 mg/day naked CBD and 20 mg/day liposomal CBD (**Table 3**).

Finally, an unblinded pilot study assessing the effect of escalating doses of a hemp-derived CBD-dominant oil was conducted on a total of 32 dogs with pain associated with OA. An informal gait and pain assessment were determined by the veterinarian at the start of the study. Each dog was administered 0.25 mg/kg CBD oil orally every 24 hours for 3 days and then every 12 hours thereafter. Pain assessments by a veterinarian and owner assessments were evaluated every 2 weeks for the 90-day study period. The CBD dose was escalated by 0.5 to 0.75 mg/kg/dose until an acceptable pain level was reached. Of the 32 dogs that completed the study, 2 dogs were considered nonresponders by both the owner and the veterinarian. Of the 30 dogs that benefited from CBD oil, the dose required to reach the pain level goal was 0.3 to 4.12 mg/kg every 12 hours. Of the 23 dogs that were taking gabapentin at the time of enrollment, 10 dogs were able to discontinue the gabapentin after the addition of the CBD oil to their pain management protocols, and 11 dogs were able to reduce their gabapentin dose. Although the vast majority of dogs in this study appeared to benefit from CBD, the lack of a placebo group and objective outcome parameters used to assess lameness could have had a significant effect on the perceived positive outcome (**Table 3**) (103).

Importantly, all of these studies showed positive outcomes of CBD treatment based on owner and veterinary assessment in a small population of dogs with naturally occurring OA of single or multiple joints. The objective parameters, such as accelerometry, gait analysis, clinical metrology instruments, or inflammatory biomarkers in synovial fluid, were not used in any of these studies. Therefore, larger, standardized, objective studies are warranted because chronic pain associated with OA is a common and devastating disease in canine patients.

Table 3. Clinical Trial Findings Summarized				
	Dog	**Dog**	**Dog**	**Dog**
Author	Gamble et al. 2018[83]	Kogan et al. 2020[103]	Verrico et al. 2020[111]	McGrath et al. 2019[84]
Study design	Randomized, placebo-controlled, veterinarian, and owner-blinded, crossover study	Unblinded pilot clinical trial	Randomized, blinded, placebo-controlled clinical trial	Randomized, blinded, controlled clinical trial
Study duration	4-week trial with a 2-week washout period prior to crossover	90-day trial	4-week trial	12-week trial
Disease studied	OA	OA	OA	Epilepsy
Cohort info	16 client-owned dogs	32 client-owned dogs	20 client-owned dogs	26 client-owned dogs
Product format	Olive oil	Hemp seed oil	Fractionated coconut oil (for naked) and sunflower lecithin (for liposomal)	Hemp seed oil
Dose (mg/kg)	2 CBD/CBDA BID	0.3–4.12 CBD BID	Naked CBD: 0.5 (20 mg) 1.2 (50 mg) Liposomal CBD: 0.5 (20 mg)	2.5 CBD BID
Route	PO	PO	PO	PO
Fasted or fed	Fed	Fed	Not reported	Fed
Testing parameters	CBPI, Hudson activity scores, veterinary lameness and pain scores, and owner assessment	Informal gait and pain assessment determined by the veterinarian and owner	Mobility and pain assessment by veterinarians and HCPI by owners	Blood samples (including serum phenobarbital and bromide levels) Owners kept daily seizure log and filled out behavior questionnaire (C-BARQ)
Formulation type	Full-spectrum hemp-derived product (with equal amounts of CBD and CBDA and minor amounts of other compounds)	Full-spectrum hemp-derived CBD product	All CBD used was in an isolate form	Full-spectrum hemp-derived CBD product
Comment(s)	The dose was actually 1 mg/kg of each compound	Most responders were between 1.2 and 2 mg/kg BID	None	None

Abbreviation: C-BARQ, Canine Behavioral Assessment & Research Questionnaire.

Cancer

When evaluating the current literature, there is a scarcity of in vivo data evaluating the antineoplastic effect of cannabis. The majority of evidence that currently exists is through in vitro and preclinical tumor induction or xenograft models. Results from preclinical studies suggest cannabinoids elicit antitumor effects at several levels, including inhibition of tumor proliferation, inducing cell death via apoptosis and autophagy, antiangiogenic activity, inhibition of invasion and metastasis, inhibition of epithelial-to-mesenchymal transition, and eliciting an antitumor immune response (32).

Despite cannabinoids demonstrating antitumor activity in multiple cell lines, rodent cancer models, and scant human clinical trials, there are still not enough data to confirm which specific chemovars, doses, ratios, or even extraction methods are required to provide an effective and durable clinical response. It is, however, the authors' clinical experience that epithelial tumors tend to respond best to cannabinoid therapy.

Cannabinoids may play an integral role in treating cancer patients in both a definitive and palliative setting. Incorporating cannabis products can help palliatively by

reducing adverse effects seen with conventional therapies, such as chemotherapy-induced nausea, vomiting, inappetence, and neuropathy. Cannabinoids can also work synergistically with conventional therapies in an effort to increase the antitumor therapeutic potential (32). Due to the immunosuppressive effects of cannabis, clinicians should avoid giving any cannabinoid-containing product to a patient that is receiving antitumor immunotherapy. There is evidence to suggest that simultaneous exposure to both modalities can negatively affect response rates (112). Further investigation of this interaction via prospective clinical studies is needed.

Current canine cell culture studies have gleaned a preliminary view into CBD's potency as an apoptotic agent and its use with standard chemotherapies, including doxorubicin and vincristine. The first pertinent finding was that CBD could induce cell death at concentrations between 1 and 5 ug/mL in 48-hour growth and proliferative assays, whereas CBDA could not induce apoptosis, suggesting that there were targets for CBD that a simple carboxylic acid moiety on CBD could hinder. Of the 5 cell lines studied (3 osteosarcoma, 1 mammary carcinoma, and 1 lymphoma), the effects of CBD were rather universal across all cell lines. Doxorubicin and CBD treatment across all cell lines appeared to show a minor additive effect when both are at higher concentrations, although when both are at lower concentrations there may be minimal antagonism. When treating cells with CBD and vincristine, there was a clear synergistic effect noted in all cell lines (d).

Similar studies need to be performed on other compounds within the cannabis plant, such as other cannabinoids, terpenes, and flavonoids alone, and along with conventional modalities (chemotherapy, targeted therapy, and radiation therapy) to appropriately assess the antineoplastic potential in canine and feline cancer cell types. Moreover, findings in vitro or a preclinical setting are not necessarily always correlated with in vivo findings, and therefore moving forward with veterinary-specific clinical trials will help elucidate the clinically relevant antitumor effects of cannabis-derived compounds.

Legal Environment

Although various countries differ in the specifics of their cannabis regulatory policies, the majority of the worldwide regulatory framework is centered on a determination of THC levels in the plant at the time of harvest. Prior to 2018 and the Agriculture Improvement Act (2018 Farm Bill), all cannabis plants (hemp and marijuana) were included on the Schedule 1 list of controlled substances of the federal Drug Enforcement Agency (DEA) (113).

With the passing of the 2018 Farm Bill, industrial hemp and its derivatives, including CBD, became legal to grow and transport in the United States. There is, however, no FDA classification or recognition of supplements, nutraceuticals, or medical food in veterinary medicine. Until the FDA finishes its review and provides a regulatory code, it is not technically legal to sell products derived from hemp. Hemp-derived cannabis products (similar to many common veterinary supplements) are not recognized by the FDA as a food or a drug and, consequently, cannot be administered, dispensed, recommended, or prescribed with the intent to prevent, mitigate, treat, or cure a condition. Despite this, there is little to no regulation and enforcement when it comes to hemp-derived CBD. This de facto legal status has led to the enormous market of CBD products available for animals. Although the FDA has the right to confiscate and stop sales of any CBD product, they generally only do so when a company makes therapeutic claims, which are only permitted for approved drugs.

The majority of states within the United States have obtained full legalization to either or both medical and recreational access to high-THC cannabis. Additionally, the passing of the 2018 Farm Bill and subsequent descheduling of industrial hemp has made products formulated from hemp plants widely and readily available. Despite the federal descheduling within the United States, individual states continue to have more restrictive policies on hemp-derived products than the federal regulatory bodies. Additionally, the FDA continues to have regulatory authority over all cannabis or cannabis-derived compounds, regardless of the source (114). The only cannabinoid-containing products (natural and synthetic) that have received FDA approval for human use are a cannabis-based CBD product (a), dronabinol, and nabilone. At the time of this publication, there are no CBD-containing products approved for use in animals.

In contrast to industrial hemp, high-THC cannabis (marijuana) and all of its constituents, including CBD derived from it, are still listed on the DEA's Schedule 1 of controlled substances. Given the Schedule 1 status of THC

and with the exception of the 3 FDA-approved products mentioned previously, neither physicians nor veterinarians can "prescribe" cannabis. A majority of states, however, have passed medical and/or recreational cannabis laws allowing for the purchase of high-THC cannabis. Within states that have enacted medical marijuana policies, human medical providers may provide a medical "recommendation" for their patients in accordance with state-specific requirements and case criteria.

A medical recommendation from a state-certified physician allows the state to issue authorization (a medical cannabis card) for that patient to access Schedule 1–listed cannabis through a medical dispensary. States that have enacted recreational (adult-use) cannabis allow any individual older than 21 years of age to access cannabis through recreational dispensaries despite its Schedule 1 status. Currently, medical cannabis laws, and thus medical "recommendations," apply to the use of cannabis products in human patients only and do not authorize veterinarians to recommend (ie, initiate the process for a medical card), dispense, furnish, administer, or prescribe high-THC cannabis products.

Several states are beginning to address this unintentional gap in the law. Over the past 4 years, California veterinarians, pet owners, and stakeholders in the medical cannabis industry have advocated for a veterinarian's right to discuss as well as recommend cannabis for their patients, and in 2019, a first-of-its-kind legislation was passed. The most recent California-based bill advocated to provide similar legal parameters to veterinarians as their physician counterparts regarding the legal pathway for cannabis recommendation. Further efforts are ongoing, which will ensure patient safety by limiting access to medical cannabis products for animals to pet owners with a valid veterinary recommendation.

Because of the current legal status of cannabis, paradigms around the safety and use of cannabis products in veterinary patients must, for the time being, be primarily based on information extrapolated from the scant veterinary literature, human research, and anecdotal clinical reports. Until federal and state guidelines become well defined for veterinarians, it is the authors' guidance for veterinarians to exercise caution, understand the legal status of cannabis, and consult their local veterinary medical board prior to distributing any such products from their clinic.

Conclusion

Cannabis science and research are still in the early stages. The last decade has produced a wealth of published information in support of the wide variety of potential therapeutic benefits of this plant. The majority of these studies have been in vitro or are preclinical studies in rodent models. In addition, most of this research has focused solely on the 2 major phytocannabinoids, THC and CBD. With a multitude of bioactive compounds within these plants and significant variation in the phytocannabinoid, terpenoid, and flavonoid compositions of the products available, there is considerably more research to be performed.

Understanding the biological activity of all of these compounds, including the minor components, is essential to understanding and developing medical applications for these molecules. Importantly, the evaluation of PK, PD, and the safety of each of these compounds allows practitioners to develop a more complete understanding of clinical applications, dosage, side effects (short- and long-term), and potential DDIs.

As the legal landscape becomes less restrictive, veterinary practitioners should remain aware as new research becomes available, remain active in protecting their patients from harm, and provide guidance and education to clients on our current understanding of exactly how this complex plant can be used in our veterinary population.

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Endnotes

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