U.S. FOOD & DRUG ADMINISTRATION

7-Hydroxymitragynine (7-OH):

An Assessment of the Scientific Data and Toxicological Concerns Around an Emerging Opioid Threat

7-Hydroxymitragynine (7-OH):

An Assessment of the Scientific Data and Toxicological Concerns Around an Emerging Opioid Threat

FDA Center for Drug Evaluation and Research Authors

Chad J. Reissig, PhD, Supervisory Pharmacologist, Controlled Substance Staff (CSS), CDER
Dominic Chiapperino, PhD, Director, CSS, CDER
Amy Seitz, PhD, MPH, Team Lead for Nonmedical Drug Use Team 3, Division of Epidemiology II (DEPI), Office of Pharmacovigilance and Epidemiology (OPE), Office of Surveillance and Epidemiology (OSE), CDER
Regina Lee, Pharm.D., Safety Evaluator, Division of Pharmacovigilance II, OPE, OSE, CDER
Rose Radin, PhD, Associate Director for Science, DEPI, OPE, OSE, CDER
Jana McAninch, MD, MPH, MS, Associate Director for Public Health Initiatives, OSE, CDER

Acknowledgments

Matthew Daubresse, DrPH, MHS, Epidemiologist, DEPI, OPE, OSE, CDER
Tamra Meyer, PhD, MPH, Associate Director for Nonmedical Drug Use, DEPI, OPE, OSE, CDER
Marta Sokolowska, PhD, Deputy Center Director for Substance Use and Behavioral Health, CDER

Table of Contents

EXECUTIVE SUMMARY

Recent reports indicate increased availability and marketing of 7-hydroxymitragynine (7-OH) in the U.S., raising public health concerns due to its pharmacology. This report provides an overview on the chemical, pharmacological, and epidemiological data on 7-OH. It focuses on the characterization of 7-OH-containing products in the marketplace, the evidence of increasing human exposures, and the extensive body of preclinical studies in the scientific literature that indicate the predominant mu opioid agonist pharmacology of 7-OH. These data sources indicate that 7-OH is a potent opioid that poses an emerging public health threat, especially when considering the increasing availability of enhanced or concentrated 7-OH products in the marketplace.

7-OH is a naturally occurring substance in the kratom plant (*Mitragyna speciosa*), but only a minor constituent that comprises less than 2% of the total alkaloid content in natural kratom leaves. However, 7-OH demonstrates substantially greater mu-opioid receptor potency than kratom's primary alkaloid constituent mitragynine, as well as other classical opioids such as morphine. In vitro studies reveal 7-OH exhibits high binding affinity for mu-opioid receptors (Ki = 7.2-70 nM), with functional activity as a mu agonist. Animal behavioral studies demonstrate its rewarding effects from self-administration and conditioned place preference methods, consistent with its opioid properties. Critically, 7-OH produces respiratory depression, physical dependence, and withdrawal symptoms characteristic of classical opioids, such as morphine, fentanyl, oxycodone, and hydrocodone.

Recently, there has been a concerning proliferation of concentrated 7-OH products that are sold over the counter and online. The enhanced amount of 7-OH in these products is likely synthetically derived through oxidate chemical conversion of mitragynine isolates or kratom extracts. Given the trace amounts of 7-OH that are naturally present in kratom, direct extraction of 7-OH from plant material would simply be unfeasible economically.

Surveillance data from multiple sources, including America's Poison Centers National Poison Data System (NPDS), Drug Enforcement Administration toxicology testing programs, and social media monitoring, suggest increasing human exposure to these concentrated 7-OH products. Clinical presentations include euphoria, sedation, respiratory depression, and opioid-like withdrawal syndromes, with users acknowledging its significant addiction potential.

The pharmacological profile, abuse liability, and emerging patterns of non-medical use establish 7-OH as a dangerous substance. Current regulatory gaps have enabled widespread availability of these products despite their opioid-like properties and necessitate immediate policy intervention to address this emerging threat to American public health.

INTRODUCTION

The Context for 7-OH Concerns

7-Hydroxymitragynine (7-OH) is a component of the plant kratom (*Mitragyna speciosa*), a tropical evergreen tree in the Rubiaceae family that grows in the wetlands of Southeast Asia (Brown et al., 2017). Kratom leaves contain over 50 alkaloids, with mitragynine and 7-OH being the primary psychoactive constituents (Warner et al., 2016). Its leaves, consumed as a tea or in dry leaf form, have been used for centuries in both medicinal and recreational settings, largely due the properties of its alkaloids mitragynine and 7-OH. Typically, 7-OH occurs in botanical kratom in amounts no more than ~.01-.04 percent by dry weight (Heywood et al., 2024). Medicinally, kratom has been used to treat headaches, diarrhea, insomnia, anxiety, opioid use withdrawal, and more, while in recreational use cases, it has been associated with feelings of euphoria (Hill et al., 2025). Currently, there are no FDA-approved drugs containing kratom or kratom-derived drug substances such as 7-OH for any therapeutic indications.

Kratom products have grown in popularity since the mid-2000's; however, kratom, mitragynine, and 7-OH have faced regulatory scrutiny in the United States due to concerns about their safety and potential for abuse. None of these substances are lawful when added to conventional foods, as dietary supplements, or as ingredients in any FDA-approved drug, and yet, these substances are still sold in various markets. At the state level, some jurisdictions have implemented restrictions on their sale and use. Until now, 7-OH has not been the sole target of a regulatory response but has always been addressed alongside the kratom plant and mitragynine.

FDA issued its first import alert for kratom in 2012. At the time, kratom was being marketed in various forms for human consumption despite a lack of approved drug uses or established safety as a dietary ingredient. In the years since, additional import alerts have been issued by the Agency. The Drug Enforcement Administration (DEA) and the Department of Health and Human Services (HHS) had given consideration to kratom, as well as its constituents, mitragynine and 7-OH, to determine whether these substances should be recommended for control under the Controlled Substances Act (CSA). Those actions were ultimately suspended in 2018, with the Assistant Secretary for Health at that time stating that the science was incomplete, and the available data were not adequate to support a recommendation to control these substances under the CSA.

Contemporary Outlook

Given the concerning trends with 7-OH and other kratom-related products, FDA has now determined that a more comprehensive assessment of available scientific and medical data on 7-OH is warranted. Many of the products available today, which are often associated with or advertised as kratom, no longer resemble botanical kratom. Instead, they contain "enhanced" or concentrated amounts of 7-OH and are formulated as powders, capsules, and liquid extracts designed to generate a stronger effect on users. Other products are explicitly advertised as 7-OH-containing products. One analysis of websites selling 7-OH products found that most (82.2 %) were formulated as chewable/sublingual tablets, shots, or gummies and marketed specifically as 7-OH only products (92%). The mean cost per recommended dose/serving was $3.97 (Hill et al., 2025).

As described below, research has shown that 7-OH is a potent mu-opioid receptor agonist, demonstrating pharmacological characteristics that define classical opioids like morphine and fentanyl. Based on its opioid pharmacology, there is significant potential for abuse of 7-OH. In fact, in various preclinical studies it has demonstrated greater potency than classical opioids. For example, 7-OH produces respiratory depression with more than 3-fold greater potency than morphine. Since the substance's therapeutic and psychoactive effects are mediated through the same mu-opioid receptor pathways as classical opioids, it can be considered to have opioid properties warranting similar regulatory consideration (Hill et al., 2025; Obeng et al., 2021).

In this report, FDA presents its new assessment of the available scientific data and literature on 7-OH, as well as more recent law enforcement data and the rapidly evolving trends in kratom-related products. FDA still has concerns about the safety of kratom products more broadly and the unlawful marketing of them under several regulated product categories in the Federal Food, Drug, and Cosmetic Act. However, there is a recognized need for more immediate action to address 7-OH because it is a substance with potent mu opioid agonist properties and significant abuse liability.

ANALYSIS OF DATA ON 7-HYDROXYMITRAGYNINE (7-OH)

7-OH Sources and Products vs. Kratom

The alkaloid 7-hydroxymitragynine (7-OH) is a naturally occurring substance in the kratom plant (*Mitragyna speciosa*), but only a minor constituent, described as early as 1994, when it was reported to comprise about 1.6% of the total alkaloid content of kratom leaves (Ponglux et al., 1994). This early reported value is in agreement with more recent assessments that have consistently demonstrated 7-OH as comprising less than 2% of the total alkaloid content in natural kratom as noted below.

7-OH has the chemical structure shown in Figure 1. Its IUPAC name is methyl (E)-2-[(2S,3S,7aS,12bS)-3-ethyl-7a-hydroxy-8-methoxy-2,3,4,6,7,12b-hexahydro-1H-indolo[2,3-a]quinolizin-2-yl]-3-methoxyprop-2-enoate, and it has the molecular formular $C_{23}H_{30}N_2O_5$, with a molecular weight of 414.40 amu.

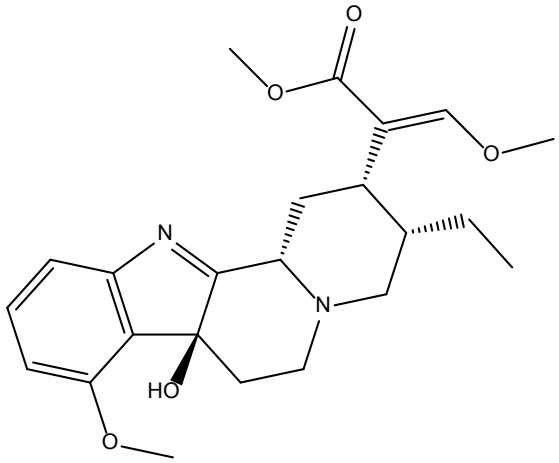

Figure 1. 7-Hydroxymitragynine Chemical Structure

Although details are not well-known, 7-OH is present in some products in amounts far exceeding its natural levels in the kratom plant. The 7-OH in these products is likely derived from the kratom plant. These 7-OH-enhanced products likely involve additional chemical synthetic steps by the producers of these products, converting the more abundant plant alkaloid mitragynine into 7-OH via chemical oxidation.

Data are available regarding 7-OH as a percentage of the total alkaloid content in kratom, and also as a percentage of dried botanical kratom leaf material and other kratom-derived products in the U.S. marketplace. One recent review reports 7-OH as comprising 2% of the total alkaloid content in kratom (Hossain et al., 2023) and this result can be extended to samples of kratom grown in the U.S. (Leon et al., 2009). In another analysis of 13 commercial products purported to contain kratom, the 7-OH content by weight ranged from 0.01-0.04% (Kikura-Hanajiri et al., 2009) a finding in agreement with others that have reported 7-OH to account for less than 0.05% by weight, substantially lower than reported mitragynine amounts (Kruegel et al., 2019). A more

recent study used ecological momentary assessment to evaluate the motivations and patterns of use of adult U.S. kratom consumers (Smith, Panlilio, Feldman, et al., 2024; Smith, Panlilio, Sharma, et al., 2024). As part of the study, subjects provided samples for quantitative testing of their own kratom products that they obtained and were self-administering. Across the 341 samples, the 7-OH content (expressed as a percentage by weight/weight or weight/volume, as indicated) ranged from below the limit of quantitation (< 0.005%) to a maximum of 0.21% with a mean of 0.01% (Sharma et al., 2025). These data suggest 7-OH is present in botanical kratom (i.e., leaf) at relatively low or trace amounts and may be a postharvest oxidative derivative of mitragynine (Karunakaran et al., 2024).

Common forms of kratom sold online include powders, capsules, resin extracts, crushed leaves, and tablets, although loose powder and prepared capsules have been reported to be the most frequently used formulations (Garcia-Romeu et al., 2020; Smith, Panlilio, et al., 2024). While kratom use characteristics are complicated by the diversity of products in the marketplace, survey studies have reported on consumption patterns. Garcia-Romeu collected data from regular kratom users and found that most users reported using 1-3g (49%) or 4-6g (33.4%) of botanical kratom per consumption (Garcia-Romeu et al., 2020). In other survey studies, the self-reported average consumption of kratom powder was 4-5 g per serving with serving sizes ranging between 2.6- 7.5 g (Rogers et al., 2024; Smith et al., 2022). When quantifying the amount of mitragynine consumed through the use of kratom, individuals self-reported consuming an average of 31.3 mg of mitragynine/serving and a range of 78.3 – 134.6 mg of mitragynine per day (Sharma et al., 2025).

Mitragynine, as the most abundant alkaloid in kratom, accounts for about 66% of the *total alkaloid content* of kratom and less than 2% of dried leaf content *by weight*, although there are reports of regional and seasonal variability in the tree's alkaloid composition (Arndt et al., 2011; Leon et al., 2009; Sengnon et al., 2023). For example, Chear and colleagues collected fresh kratom leaves from different locations in Peninsular Malaysia and determined their alkaloid profiles. The mitragynine concentration ranged from 9.38 to 18.85 mg/g or 0.38% to 1.89% of dried leaf weight while the 7-OH concentration ranged from 0.05 to 0.15 mg/g or 0.005% to 0.015% (Chear et al., 2021).

Despite the low amounts of 7-OH in botanical kratom, there are reports of its more-enhanced presence in commercial kratom-related products (Grundmann et al., 2024), although some products have been identified in reports from nearly a decade ago. For example, Lydecker and colleagues tested eight commercially available kratom products for their alkaloid content(s). In seven of the eight products tested, they found levels of 7-OH to be 109-509% higher than expected, based on naturally occurring levels of 7-OH reported in the kratom plant (Lydecker et al., 2016). More recently, the Tampa Bay Times purchased twenty kratom-derived products from local stores. One of those products consisted of pressed pills and contained 15 mg/pill of 7-OH, an amount far greater than observed in any botanical kratom preparation to date (Ogozalek, 2023). In addition to the verified amounts of 7-OH in the products obtained by Lydecker et al. and the Tampa Bay Times, other products *labeled* and/or *purported* to have high levels of 7-OH appear to be readily available for purchase online.

In summary, the low amounts of 7-OH in natural botanical kratom products is well-established as a percentage of alkaloid content, as a percentage of dried kratom leaf material, and in products representing other dosage forms made from natural kratom and consistent with its natural

composition. However, there are also a concerning and increasing number of products being sold that have unexpectedly and unnaturally high levels of 7-OH. This poses a threat to public health that is more clearly understood based on the pharmacological properties and effects of 7-OH, discussed in the preclinical data section below, and also in the limited information available on known patterns of human use and resulting harms discussed below. These sections will present and discuss the evidence in the available data that establishes the mu opioid agonist pharmacology associated with 7-OH in particular.

Patterns of 7-OH Use, Human Exposures, and Law Enforcement Data

There are several sources of information to characterize the current patterns of 7-OH use and the resulting harms to individuals who knowingly or unknowingly are exposed to 7-OH at significant doses from 7-OH-enhanced products, as described in the subsections below.

National Drug Early Warning System (NDEWS)

The National Drug Early Warning System (NDEWS) provides real-time surveillance from sentinel sites across U.S. to detect early signals of potential drug epidemics using novel (e.g., street reporting, web monitoring) and traditional data sources (e.g., OD deaths, treatment admissions).

NDEWS analyzed Reddit posts mentioning 7-OH during January to September 2024 and found that posts increased over this time. These posts are broad and can vary in content but have included warnings from Reddit users about respiratory depression, potency, dependence and long-lasting withdrawal (NDEWS, 2024).

Social Media

A variety of social media outlets were assessed for mentions and/or discussions of 7-OH. Websites included:

- erowid.org - a member-supported organization providing access to information about psychoactive plants, chemicals, and related issues;
- bluelight.org - an international message board that educates the public about responsible drug use by promoting free discussion, advocating harm reduction, and attempting to eliminate misinformation;
- reddit.com - online forum that functions as a vast collection of user-driven communities, known as sub-Reddits, each centered around specific topics.

It is important to note that all considerations of these social media sources are, at best, anecdotal in considering the risks and abuse potential associated with 7-OH products. However, it is clear that there is fairly widespread understanding of the availability of products specifically targeting high levels of the substance 7-OH, distinct from kratom products generally. In analyzing these social media posts, some relevant themes have been identified and include mention of the following: euphoria and an opioid-like “buzz”/high as motivation for consuming 7-OH; availability of “candy-like” formulations which users acknowledge as having a risk of overconsumption to their own detriment; perceptions of therapeutic value of 7-OH in self-treating pain and anxiety; concerns over loss of access to these products if they were to be banned; acknowledgement that use of these products could lead to overdose and serious

outcomes including death; and acknowledgement that use could lead to addiction and has caused users to experience withdrawal symptomology much like that produced by other commonly abused opioids.

Drug Enforcement Administration Toxicology Testing Program (DEA TOX)

The Drug Enforcement Administration Toxicology Testing program (DEA TOX) conducts analyses of voluntarily submitted leftover or previously collected biological samples from drug overdose victims to identify novel psychoactive substances (NPS) and other drugs of abuse in subjects with fatal and nonfatal overdose. The DEA TOX database was queried for reports of mitragynine, 7-OH, or mitragynine pseudoindoxyl from 2019-2025. A total of 103 cases, some fatal and some non-fatal, were identified in this selected sample; this database does not include all overdose cases, and the number of samples voluntarily submitted for analysis may vary year to year based on unknown factors.

It is notable that the utility of the DEA TOX data is limited because it generally cannot be discerned whether deaths are related to mitragynine, 7-OH, or mitragynine pseudoindoxyl, or some combination thereof. In addition, although 7-OH and mitragynine pseudoindoxyl are not typically found in appreciable amounts in fresh kratom leaves (Hill et al., 2025), both are metabolites of mitragynine, complicating forensic assessments of causality (Kamble et al., 2020). These are significant limitations in making inferences from these data; however, the number of fatal overdose cases in which one or more of these substances were detected for 2023 to 2025 are approximately three-fold higher than for the years 2019 through 2022, coinciding with the more recent entry of more-concerning kratom-related products in the marketplace, such as 7-OH.

Human Exposures in Pharmacokinetic Studies

Pharmacokinetic (PK) data for 7-OH are sparse, as to our knowledge, no clinical studies have been performed using isolated or purified 7-OH. Nonetheless, there are 7-OH PK data derived from a small number of studies using botanical kratom. Most available clinical PK data for 7-OH are variable, which may be for several reasons such as genetic differences in kratom plants, different formulations (e.g., teas, capsules, etc.), and methods of analysis. Much of the data is also from non-controlled studies making it difficult to interpret the results. Huestis and colleagues conducted a randomized, between-subject, double-blind, placebo-controlled dose escalation study of 500-4000 mg encapsulated dried kratom leaf powder corresponding to mitragynine doses of 6.65-53.2 mg. Twelve subjects enrolled in the study (n=12). Blood plasma levels of mitragynine and 7-OH were assessed after a single dose, and then again after 15 days of continuous dosing. According to the study authors, peak plasma levels of 7-OH (i.e., C_{max}values) and exposure (i.e., area under the curve, (AUC)) were lower than mitragynine but increased in a dose proportional manner and ranged from 3.6 to 22.7 ng/mL while the time to peak plasma levels (i.e.,T_{max} values) ranged from 1.2 – 1.8 h. The half-life of 7-OH increased with increasing dose and ranged from a mean of 1.7 to 4.7 hours. During the multiple dose phase of the study, 7-OH steady state was reached in about 7 days (Huestis et al., 2024).

In another study examining the PK properties of 7-OH, sixteen healthy subjects (n=16) received kratom tea containing 23.6 mg of mitragynine. Subjects were administered tea in two sessions: once with tea alone, and in a second session following pretreatment with itraconazole, a

CYP3A4 inhibitor. The 7-OH C_{max} was 12.81±3.39 ng/mL which occurred 1.7 h after administration (T_{max}). In the second session after pretreatment with itraconazole (200 mg), the C_{max} decreased 56% with a concomitant 43% decrease in AUC. These data describe the PK of 7-OH and demonstrate that the metabolism of mitragynine to 7-OH is heavily dependent on CYP3A4 (Mongar et al., 2024).

Tanna et. al., assessed the PK of a single orally administered dose of kratom (2 g), in the form of a tea, to healthy adult subjects (n = 5 completers). According to the authors, there were only trace amounts of 7-OH (< LOQ) in the starting product, therefore, the assumption was made that 7-OH was generated from the metabolism of mitragynine *in vivo.* The authors identified a PK difference between enantiomers of kratom alkaloids in either the 3S or 3R configuration. 7-OH has a 3S configuration which, according to the authors, leads to a shorter T_{max}, lower exposure (AUC), longer terminal half-life, and a higher volume of distribution during the terminal phase compared to the 3R alkaloids. Measured 7-OH in plasma samples demonstrated that 7-OH had a C_{max} = 16.1 nM, T_{max} = 1h, half-life = 5.67h, and an AUC0-120h = 103nM x h.(Tanna et al., 2022).

Epidemiological Data Sources

Limitations with the Epidemiological Data Sources

Because 7-OH appears to be a novel, emerging public health threat, the ability of public health surveillance systems to monitor 7-OH specific risks may be limited. For example, large national surveys such as the National Survey on Drug Use and Health include questions about use of kratom, but not 7-OH. Additionally, there may be a lack of awareness among consumers of kratom-related products that they are obtaining 7-OH enhanced products, and thus use of 7-OH would likely be underreported in data collected using self-report. Many forensic laboratories test for mitragynine as a marker of kratom use. In these cases, 7-OH overdose cases and fatalities may incorrectly be classified as kratom and/or mitragynine-related (Smith, Boyer, et al., 2024). Furthermore, toxicology reports documenting presence of 7-OH are difficult to interpret, because 7-OH is a known metabolite of mitragynine in humans. All of these issues complicate the real-world assessment of risks associated with use of 7-OH containing products as distinct from risks associated with kratom and other mitragynine-containing products.

FDA's Adverse Event Reporting System

Although FDA's Adverse Event Reporting System (FAERS) has documented cases reporting adverse events (13 cases, including 2 deaths) suspected to involve 7-OH, ambiguity about the contributory role of 7-OH from uncharacterized products or concomitant medications and underlying disease limits interpretation. Therefore, we do not include further analysis of these FAERS cases here.

America's Poison Centers, National Poison Data System

National Poison Data System (NPDS) receives near real-time data from the nation's poison centers (PC), providing information and assistance to callers on exposures to prescription drugs, over-the-counter medications, unapproved products, and other substances. PC healthcare professionals systematically follow up on exposure cases to document medical and clinical effects. Quality control measures are used to ensure data accuracy and completeness. Notably, 7-OH specific NPDS codes were only recently added (Feb-May 2025), and therefore the NPDS reporting period is limited to 2/1/2025-4/30/2025. As shown below, there were a total of 53 exposure cases involving 7-OH during this time period, the majority of which involved abuse-related reasons for use (i.e., "intentional abuse"). Most single-substance 7-OH exposure cases resulted in minor or moderate clinical outcomes, with several documented has having major clinical outcomes.

Table 1. National Poison Data System Closed Human Exposure Cases*, 2/1/2025-4/30/2025

	Number of exposure cases**	Number of abuse cases**	Single substance exposure cases	Single substance abuse cases
Total cases involving 7-OH	53	24	37	16
Reason				
Adverse drug reaction	4		2	
Intentional- abuse	24		16	
Intentional- misuse	4		3	
Intentional - Suspected suicide	2		0	
Other – Withdrawal	8		6	
Unintentional – general	4		4	
Unintentional- misuse	1		1	
Unintentional therapeutic error	4		3	
Unknown reason	2		2	
Related clinical outcomes				
Minor			6	3
Moderate			13	6
Major			3	1
Not followed, minimal clinical effects possible			5	3
Unable to follow, judged as potentially toxic exposure			1	0
Age				
<18 years	6	1	5	0
≥ 18 years	46	23	32	16
Unknown age	1	0	0	0

*Excludes cases classified as 'confirmed non-exposure'
**Cases may involve other substances, besides 7-OH
Related clinical outcomes include cases with clinical effects deemed "related" to exposure based on timing, severity, and assessment of clinical effects by Poison Center Specialists. Definitions available from America's Poison Centers: NPDS Full Report 2023. Page 235.

Note: This analysis used the case listing data in NPDS to identify and characterize cases documented as involving 7-OH. As of July 2025, an in-depth review NPDS case narrative data was ongoing; this further review may yield different numbers from those presented here.

Summary of Epidemiological Data and 7-OH Concerns

Available surveillance data indicate that abuse of 7-OH is occurring and is associated with serious harms; however, as noted previously, it is difficult to quantify the public health burden because surveillance systems do not provide estimates for the prevalence of 7-OH use and are only beginning to track the specific involvement of 7-OH enhanced products in exposure cases and overdoses. The current epidemiologic data on 7-OH exposures often lack sufficient detail to distinguish with confidence involvement of botanical kratom products from 7-OH enhanced products.

Preclinical Data Characterizing 7-OH Pharmacology

Although there are limited data from human studies to characterize effects of 7-OH in humans, as noted above, there is a large body of in vitro and animal studies that provide extensive evidence of 7-OH as a potent mu opioid agonist, as described in below subsections.

In Vitro Data

Receptor Binding Studies

7-OH has been shown to have affinity and activity at mu opioid receptors. In a study using human embryonic kidney (HEK) cells with cloned, human opioid receptors, 7-OH demonstrated high affinity for the mu opioid receptor (Ki = 47 nM) relative to kappa (Ki = 188 nM) and delta opioid receptors (Ki = 219 nM) (Kruegel et al., 2016). In a second study using HEK 293 cells expressing human mu and other opioid receptors, 7-OH demonstrated high affinity for mu opioid receptors (Ki = 16 ± 1 nM) and its affinity was greater than mitragynine (Ki = 238 ± 28 nM) and lower than morphine (Ki = 1.50 ± 0.04 nM) (Todd et al., 2020). Using an in vitro radioligand binding assay with CHO cells expressing murine-derived opioid receptors, 7-OH demonstrated relatively high affinity for mu-opioid receptors (Ki = 37 ± 4 nM), relative to mitragynine (Ki = 230 ± 47 nM), although its affinity was lower than morphine (Ki = 4.6 ± 1.8 nM) (Varadi et al., 2016). Other studies conducted using whole brain homogenates of guinea pig brain tissue have also demonstrated that 7-OH has high affinity at mu opioid receptors (Ki = 8.0 nM) relative to kappa (Ki = 6.7 nM) and delta opioid receptors (Ki = 6.8 nM) (Matsumoto et al., 2004). Obeng and colleagues evaluated the binding affinity of 7-OH using human recombinant HEK 293 cells expressing mu opioid receptors. Their results are in agreement with the data presented above where the authors found that 7-OH binds with high affinity (Ki = 7.2 nM) to mu opioid receptors relative to delta (Ki = 236 nM) and kappa (Ki = 74.1 nM) receptor subtypes (Obeng et al., 2020). A number of additional binding studies are in keeping with the data described above, demonstrating the affinity of 7-OH for mu opioid receptors across a variety of binding assays (Chakraborty et al., 2021; Matsumoto et al., 2008; Obeng et al., 2021; Takayama et al., 2002).

The results of the receptor binding studies with 7-OH are in keeping with *in silico* receptor binding models that suggest 7-OH has high affinity for the mu opioid receptor. The *in silico* modeling results were subsequently confirmed with a radioligand binding assay where 7-OH demonstrated high affinity for cloned, human mu opioid receptors (K_i = 70 nM). (Ellis et al.,

2020). Collectively, the available receptor binding data demonstrate the affinity and binding of 7-OH to mu opioid receptors.

Functional Studies

Many of the studies referenced above performed additional assessments of 7-OH to determine its functional activity after binding (i.e., agonist or antagonist effects). These studies have consistently demonstrated that 7-OH produces mu-opioid agonist effects. For example, Kruegel and colleagues examined the functional activity of 7-OH and mitragynine in HEK cells expressing opioid receptors using a bioluminescence resonance energy transfer (BRET) assay. Both mitragynine and 7-OH functioned as partial agonists, producing Emax values of 34% and 47% respectively and EC_{50} values of 339 ± 178 nM and 34.5 ± 4.5 nM (Kruegel et al., 2016). Activation of the mu opioid receptor pathway was also investigated using forskolin-stimulated cyclic adenosine monophosphate (cAMP) accumulation in Chinese Hamster Ovary (CHO) cells expressing mu opioid receptors. In this assay, 7-OH produced a maximal activation (Emax) of 85.9%, a value similar to that produced by the positive control comparators DAMGO (86.2%) and morphine (86.9%). These data suggest 7-OH acts a full mu opioid agonist (Todd et al., 2020). Similarly, Matsumoto and colleagues concluded that 7-OH was "found to have an opioid agonist property on μ- and/or κ-opioid receptors" based on its ability to inhibit contraction of isolated guinea pig ileum. In this assay, 7-OH displayed approximately 13-fold greater potency than morphine and 46-fold greater potency than mitragynine. The inhibition was reversed by naloxone, suggesting the effects are mediated via mu opioid receptors (Matsumoto et al., 2004). Other functional assays produced results that are aligned with Matsumoto and colleagues. For example, using a cAMP mobilization assay as a measure of functional effects, 7-OH acted as a full agonist with an EC_{50} of 7.6 nM, and was more potent than mitragynine (EC_{50} 307.5 nM) (Obeng et al., 2020). Likewise, when evaluating the agonist activity of 7-OH in an electrically stimulated guinea pig ileum, 7-OH acted as a full agonist and was more potent than morphine (Takayama et al., 2002). Finally, using a [^{35}S] GTPγS functional assay, 7-OH produced an Emax of 77% with an EC_{50} of 53.4 nM, further demonstrating its agonist effects (Varadi et al., 2016).

<u>Animal Data on Behavioral and Physiological Effects</u>

Conditioned Place Preference

Conditioned place preference (CPP) is a commonly utilized animal model to study the rewarding effects of drugs. In this paradigm, an animal is conditioned to associate a particular environment with a drug treatment, and an alternative environment with a non-drug condition. After repeated sessions, the animal is then observed under non-drug conditions to determine which environment the animal prefers. CPP is established if the animal spends more time in the drug-paired compartment vs. the vehicle-paired compartment (Mombelli, 2022; Prus et al., 2009). Many drugs of abuse produce CPP, though notably, it is not a direct measure of reinforcing effects.

Using the CPP paradigm, several studies have demonstrated the ability of 7-OH to produce rewarding effects and that it does so more potently than morphine. Gutridge and colleagues employed C57BL/6 mice and demonstrated the development of CPP after 3 mg/kg 7-OH. CPP was observed after both doses although 7-OH required more sessions (4 sessions) whereas morphine (6 mg/kg) was able to establish CPP in two sessions (Gutridge et al., 2020). Similarly,

other studies have demonstrated the ability of 7-OH (2 mg/kg) to produce CPP, and that it does so with greater potency than morphine (Matsumoto et al., 2008).

Drug Discrimination

Drug discrimination is an experimental method in which animals identify whether a test drug produces interoceptive effects similar to those produced by a drug to which the animals are trained to differentiate from placebo, and which has known pharmacological properties. If the known drug is one with abuse potential, drug discrimination methods can be used to predict if a test drug will have abuse potential in humans (Balster & Bigelow, 2003; Solinas et al., 2006).

For abuse assessment purposes, an animal is trained to press one bar when it receives a known drug of abuse (the training drug) and another bar when it receives placebo. A challenge session with the test drug determines which of the two bars the animal presses more often as an indicator of whether the test drug is more like the known drug of abuse or more like placebo. A test drug is said to have "full generalization" to the training drug when the test drug produces bar pressing >80% on the bar associated with the training drug (Ator & Griffiths, 2003; Swedberg, 2016; Walker, 2018; Young, 2009). A test drug that generalizes to a known drug of abuse will likely be abused by humans (Balster and Bigelow, 2003).

Male Sprague Dawley rats were trained to discriminate morphine (5.0 mg/kg i.p.) from saline using a 30 min pretreatment time and FR10 schedule of reinforcement. After successful training, substitution tests with 7-OH (0.3, 1.0 and 3.0 mg/kg) were performed. The highest dose of 7-OH (3.0 mg/kg) produced complete substitution for the morphine stimulus cue. Moreover, pretreatment with naloxone significantly reversed the 7-OH substitution and resulted in saline-like responding. Notably, in this study, 7-OH was more potent than morphine (Harun et al., 2015).

In a second study, the discriminative stimulus effects of 7-OH were examined in separate groups of rats trained to discriminate either morphine (3.2 mg/kg i.p., 15 min pretreatment) or mitragynine (32 mg/kg i.p., 30 min pretreatment) from saline. After successful acquisition of discrimination training 7-OH was administered in substitution tests. 7-OH was administered i.p., with a 15 min pretreatment time in a dose range of 0.1-17.8 mg/kg. In the morphine-trained rats, 7-OH produced complete substitution at doses above 0.56 mg/kg, with the 1.0 mg/kg dose producing 100% drug-lever-appropriate responding and a resultant ED_{50} of 0.28 mg/kg. Notably, the dose-response curve was shifted to the left, demonstrating an increased potency of 7-OH relative to morphine. In addition, pretreatment with 0.032 mg/kg naltrexone shifted the dose-response curve to the right suggesting substitution was mediated via mu-opioid receptors (Obeng et al., 2021). Taken together, the drug discrimination data demonstrate the ability of 7-OH to substitute and mimic the stimulus effects of morphine, and that 7-OH is more potent in doing so. These data are a strong indication that 7-OH produces subjective effects in humans that are similar to opioids, along with an associated abuse potential.

Self-Administration

Self-administration is a method that assesses whether a drug produces reinforcing effects that increase the likelihood of behavioral responses in order to obtain additional drug (i.e., whether an animal will press a lever for a drug injection). Drugs that are self-administered by animals are

likely to produce rewarding effects in humans, which is indicative of abuse potential. Generally, a good correlation exists between those drugs that are self-administered by animals and those that are abused by humans (Balster & Bigelow, 2003; Brady et al., 1987; Johanson & Schuster, 1981; Panlilio & Goldberg, 2007). It is notable that self-administration is a behavior that is produced by drugs that have been placed into every schedule of the CSA. Additionally, rates of self-administration for a particular drug will go up or down if the available drug dose or the work requirement (bar pressing for drug) is altered. Positive results from a self-administration study provide an abuse potential signal, suggesting that a drug has rewarding properties, but not necessarily that it produces more rewarding effects than another drug in humans.

7-OH produces reinforcing effects and is self-administered by rodents. In the study, rodents were trained to self-administer morphine (100 µg/infusion) and faded to 50 µg/infusion once stable responding was achieved. Thereafter, extinction sessions were performed to confirm acquisition of the self-administration training prior to substitution tests. Substitution tests were performed with 7-OH doses of 2.5, 5, 10 and 20 µg/infusion. In the substitution tests, 7-OH produced an inverted U-shaped curve and the number of infusions for 5 and 10 µg/infusion of 7-OH were significantly greater than vehicle, demonstrating the reinforcing effects of 7-OH (Hemby et al., 2019).

The self-administration of 7-OH was blocked by both a mu opioid antagonist (naloxonazine) and a delta opioid antagonist (naltrindole), suggesting its reinforcing effects are mediated via opioid receptors. In addition, peak morphine self-administration occurred at 50 µg/infusion while peak 7-OH infusions occurred at 5 µg/infusion, demonstrating a substantially increased potency of 7-OH relative to morphine.

There are some pharmacokinetic (PK) data available from animal studies involving the administration of isolated, i.e., single entity, 7-OH. Following a single oral dose (1 mg/kg 7-OH) to beagle dogs, absorption was rapid, with a peak plasma concentration (i.e., Cmax) of 56 ± 1.6 ng/mL 15 minutes post-dose. The elimination half-life was slower, producing a mean of 3.6 ± 0.5 h. No AEs were observed, and no abnormal laboratory findings were reported (Maxwell et al., 2021). In adult male and female mice, the PK parameters of 7-OH were investigated after a single oral dose of 50 mg/kg 7-OH. The tissue distribution of 7-OH was observed in descending order: liver > kidney > spleen > lung > brain. Plasme C_{max} values were 0.6 and 09 µg/mL in males and females with a T max value of 0.5 hr. Area under the curve (AUC) values over 48 hours (AUC_{0-48} hr* µg/mL) were 1.4 and 2.9 in male and female mice (Berthold et al., 2022).

Antinociceptive Effects

The antinociceptive effects of 7-OH were investigated in mice using the tail flick and hot plate tests. These tests are commonly used to examine pain and analgesic effects in rodents (D'Amour & Smith, 1941). In these tests, rodents are subject to a heat stimulus and timed for the duration it takes to move their tail (i.e., tail flick) or produce a response such as jumping, licking, or shaking of limbs (i.e., hot plate).

In the tail flick test, subcutaneous administration of 7-OH (2.5 – 10 mg/kg) produced both time and dose-related antinociceptive effects. Notably, the dose-effect curve for 7-OH was shifted to the left, indicating a greater potency than the positive control comparator, morphine. Similar results were observed in the hot plate test, and when morphine and 7-OH were administered

orally. Naloxone (2 mg/kg s.c.) inhibited the effects of 7-OH and morphine in both tests (Matsumoto et al., 2004; Matsumoto et al., 2008). Concurrent results were observed by Obeng and colleagues using the hot plate test. In their study, 7-OH (0.0032 – 3.2 mg/kg, i.v.) produced maximum antinociceptive effects and was more potent morphine but less potent than fentanyl when administered intravenously. Likewise, naltrexone (0.1 mg/kg) reversed the antinociceptive effects of 7-OH suggesting the antinociception was mediated via mu opioid receptors (Obeng et al., 2020).

Respiratory Depression

A major risk of opioid exposure and cause of opioid-induced death is respiratory depression (Baldo & Rose, 2022; Bateman et al., 2023). To examine the respiratory effects of 7-OH in rodents, whole body plethysmography was used in freely moving, awake rats. Both morphine (10 and 32 mg/kg, i.v.) and 7-OH (1, 3.2, and 10 mg/kg, i.v.) induced significant respiratory depression as assessed by minute volume, tidal volume, and breathing frequency. The mu-opioid agonist naloxone (1.0 mg/kg i.v.) reversed these effects, a finding consistent with the mu opioid effects of 7-OH (Zuarth Gonzalez et al., 2025). These data highlight a potential risk factor of 7-OH exposure and suggest 7-OH may expose individuals to similar risks as classic opioids, including respiratory depression.

Physical Dependence and Withdrawal

It is well-established that chronic administration of opioids leads to the development of tolerance and physical dependence that may culminate into a withdrawal syndrome. In parallel with some of the hot plate tests described above, the ability of 7-OH to produce physical dependence and withdrawal was examined. Mice were treated with subcutaneous 7-OH (10 mg/kg b.i.d.) or morphine (10 mg/kg b.i.d.) for five days. Tolerance was assessed as a reduction of analgesia in the hot plate test. After five days of treatment, both morphine and 7-OH showed a decreased analgesic response on the hot plate test, demonstrating the development of tolerance. In addition, cross-tolerance was also observed between morphine and 7-OH suggesting a similar mechanism of action between the drugs. Finally, after five days of escalating doses of 7-OH and morphine (8-45 mg/kg b.i.d.) the development of withdrawal was assessed with a 3 mg/kg s.c., dose of naloxone injected two hours after 7-OH administration. Both morphine and 7-OH treatment produced signs of withdrawal such as jumping, rearing, urination, ptosis, forepaw tremor, and diarrhea (Matsumoto et al., 2005).

Summary of Preclinical Data

From the studies described above, 7-OH has high affinity for mu opioid receptors and functional activity as an agonist at these receptors. Consistent with this pharmacological activity, 7-OH is self-administered by animals, substitutes for morphine in drug discrimination studies, produces antinociception, and physical dependence leading to withdrawal when administered to rodents. Moreover, 7-OH has consistently demonstrated an increased potency relative to morphine in preclinical rodent studies. These observations suggest 7-OH has pharmacological properties representative of a full mu opioid agonist and an associated high potential for abuse.

CONCLUSIONS

The data described in this report indicate that 7-OH has a significant potential for abuse and associated harms. Conclusively, 7-OH has high affinity and agonist activity at mu opioid receptors. Consistent with this pharmacological mechanism of action, 7-OH demonstrates rewarding effects in that it is self-administered by animals and also produces conditioned place preference, two well-established animal behavioral models measuring rewarding effects as a predictor of abuse potential in humans. In animal drug discrimination studies, 7-OH substitutes for morphine with full generalization. 7-OH is also demonstrated to produce antinociception consistent with opioid pharmacology, and to produce physical dependence when administered to rodents, as evidenced by a classic set of withdrawal signs associated with opioid withdrawal upon discontinuation of opioid administration. Moreover, 7-OH in all above models has consistently demonstrated an increased potency relative to morphine.

Due to the fact that 7-OH is both a metabolite of mitragynine and naturally present in low amounts in botanical kratom, using toxicology results to identify 7-OH as a primary or sole contributor in human exposures is challenging. There is also a need for improved clinical awareness and population surveillance to better characterize patterns of 7-OH use, the products that people are obtaining, and individual treatment needs following 7-OH exposure. Additionally, questions on 7-OH are not generally included in national surveys, and other data sources that rely on self-reported use of 7-OH likely underestimate the number of 7-OH exposure cases, as individuals may be unaware of the distinction from kratom products. Nonetheless, since specific codes were added earlier this year to document 7-OH exposure cases, U.S. poison centers have identified multiple single-substance cases of 7-OH exposure resulting in serious adverse clinical outcomes. Also, although anecdotal, social media and online forums indicate growing awareness and use of 7-OH, and many testimonials of the negative opioid-mediated effects users have experienced, including 7-OH dependence, associated withdrawal syndrome, and addiction.

In the current marketplace in the U.S., 7-OH is increasingly being marketed over-the-counter and online, in concentrated forms or sufficient doses to cause harms to those individuals engaging, knowingly or unknowingly, in use of 7-OH. Based on demonstrated pharmacology, repeated or prolonged use of 7-OH would lead to tolerance, physical dependence, and potentially to opioid addiction— typical of mu opioid agonist drugs of abuse. This public health threat is troubling and requires immediate and impactful policies to educate consumers and take regulatory action that limits access to 7-OH containing products.

REFERENCES

Ator, N. A., & Griffiths, R. R. (2003). Principles of drug abuse liability assessment in laboratory animals. *Drug Alcohol Depend*, *70*(3 Suppl), S55-72. https://doi.org/10.1016/s0376-8716(03)00099-1

Baldo, B. A., & Rose, M. A. (2022). Mechanisms of opioid-induced respiratory depression. *Arch Toxicol*, *96*(8), 2247-2260. https://doi.org/10.1007/s00204-022-03300-7

Balster, R. L., & Bigelow, G. E. (2003). Guidelines and methodological reviews concerning drug abuse liability assessment. *Drug Alcohol Depend*, *70*(3 Suppl), S13-40. https://doi.org/10.1016/s0376-8716(03)00097-8

Bateman, J. T., Saunders, S. E., & Levitt, E. S. (2023). Understanding and countering opioid-induced respiratory depression. *Br J Pharmacol*, *180*(7), 813-828. https://doi.org/10.1111/bph.15580

Berthold, E. C., Kamble, S. H., Raju, K. S., Kuntz, M. A., Senetra, A. S., Mottinelli, M., Leon, F., Restrepo, L. F., Patel, A., Ho, N. P., Hiranita, T., Sharma, A., McMahon, L. R., & McCurdy, C. R. (2022). The Lack of Contribution of 7-Hydroxymitragynine to the Antinociceptive Effects of Mitragynine in Mice: A Pharmacokinetic and Pharmacodynamic Study. *Drug Metab Dispos*, *50*(2), 158-167. https://doi.org/10.1124/dmd.121.000640

Brady, J. V., Griffiths, R. R., Hienz, R. D., Ator, N. A., Lukas, S. E., & Lamb, R. J. (1987). Assessing Drugs for Abuse Liability and Dependence Potential in Laboratory Primates. In M. A. Bozarth (Ed.), *Methods of Assessing the Reinforcing Properties of Abused Drugs* (pp. 45-85). Springer New York. https://doi.org/10.1007/978-1-4612-4812-5_3

Chakraborty, S., Uprety, R., Slocum, S. T., Irie, T., Le Rouzic, V., Li, X., Wilson, L. L., Scouller, B., Alder, A. F., Kruegel, A. C., Ansonoff, M., Varadi, A., Eans, S. O., Hunkele, A., Allaoa, A., Kalra, S., Xu, J., Pan, Y. X., Pintar, J.,…Majumdar, S. (2021). Oxidative Metabolism as a Modulator of Kratom's Biological Actions. *J Med Chem*, *64*(22), 16553-16572. https://doi.org/10.1021/acs.jmedchem.1c01111

Chear, N. J., Leon, F., Sharma, A., Kanumuri, S. R. R., Zwolinski, G., Abboud, K. A., Singh, D., Restrepo, L. F., Patel, A., Hiranita, T., Ramanathan, S., Hampson, A. J., McMahon, L. R., & McCurdy, C. R. (2021). Exploring the Chemistry of Alkaloids from Malaysian Mitragyna speciosa (Kratom) and the Role of Oxindoles on Human Opioid Receptors. *J Nat Prod*, *84*(4), 1034-1043. https://doi.org/10.1021/acs.jnatprod.0c01055

Cinosi, E., Martinotti, G., Simonato, P., Singh, D., Demetrovics, Z., Roman-Urrestarazu, A., Bersani, F. S., Vicknasingam, B., Piazzon, G., Li, J. H., Yu, W. J., Kapitany-Foveny, M., Farkas, J., Di Giannantonio, M., & Corazza, O. (2015). Following "the Roots" of Kratom (Mitragyna speciosa): The Evolution of an Enhancer from a Traditional Use to Increase Work and Productivity in Southeast Asia to a Recreational Psychoactive Drug in Western Countries. *Biomed Res Int*, *2015*, 968786. https://doi.org/10.1155/2015/968786

D'Amour, F. E., & Smith, D. L. (1941). A METHOD FOR DETERMINING LOSS OF PAIN SENSATION. *The Journal of Pharmacology and Experimental Therapeutics*, *72*(1), 74-79. https://doi.org/10.1016/S0022-3565(25)03823-6

Ellis, C. R., Racz, R., Kruhlak, N. L., Kim, M. T., Zakharov, A. V., Southall, N., Hawkins, E. G., Burkhart, K., Strauss, D. G., & Stavitskaya, L. (2020). Evaluating kratom alkaloids using PHASE. *PLoS One*, *15*(3), e0229646. https://doi.org/10.1371/journal.pone.0229646

Garcia-Romeu, A., Cox, D. J., Smith, K. E., Dunn, K. E., & Griffiths, R. R. (2020). Kratom (Mitragyna speciosa): User demographics, use patterns, and implications for the opioid

epidemic. *Drug Alcohol Depend*, *208*, 107849. https://doi.org/10.1016/j.drugalcdep.2020.107849
Grundmann, O., Garcia-Romeu, A., McCurdy, C. R., Sharma, A., Smith, K. E., Swogger, M. T., & Weiss, S. T. (2024). Not all kratom is equal: The important distinction between native leaf and extract products. *Addiction*, *119*(1), 202-203. https://doi.org/10.1111/add.16366
Gutridge, A. M., Robins, M. T., Cassell, R. J., Uprety, R., Mores, K. L., Ko, M. J., Pasternak, G. W., Majumdar, S., & van Rijn, R. M. (2020). G protein-biased kratom-alkaloids and synthetic carfentanil-amide opioids as potential treatments for alcohol use disorder. *Br J Pharmacol*, *177*(7), 1497-1513. https://doi.org/10.1111/bph.14913
Harun, N., Hassan, Z., Navaratnam, V., Mansor, S. M., & Shoaib, M. (2015). Discriminative stimulus properties of mitragynine (kratom) in rats. *Psychopharmacology (Berl)*, *232*(13), 2227-2238. https://doi.org/10.1007/s00213-015-3866-5
Hemby, S. E., McIntosh, S., Leon, F., Cutler, S. J., & McCurdy, C. R. (2019). Abuse liability and therapeutic potential of the Mitragyna speciosa (kratom) alkaloids mitragynine and 7-hydroxymitragynine. *Addict Biol*, *24*(5), 874-885. https://doi.org/10.1111/adb.12639
Hill, K., Boyer, E. W., Grundmann, O., & Smith, K. E. (2025). De facto opioids: Characterization of novel 7-hydroxymitragynine and mitragynine pseudoindoxyl product marketing. *Drug Alcohol Depend*, *272*, 112701. https://doi.org/10.1016/j.drugalcdep.2025.112701
Hossain, R., Sultana, A., Nuinoon, M., Noonong, K., Tangpong, J., Hossain, K. H., & Rahman, M. A. (2023). A Critical Review of the Neuropharmacological Effects of Kratom: An Insight from the Functional Array of Identified Natural Compounds. *Molecules*, *28*(21). https://doi.org/10.3390/molecules28217372
Huestis, M. A., Brett, M. A., Bothmer, J., & Atallah, R. (2024). Human Mitragynine and 7-Hydroxymitragynine Pharmacokinetics after Single and Multiple Daily Doses of Oral Encapsulated Dried Kratom Leaf Powder. *Molecules*, *29*(5). https://doi.org/10.3390/molecules29050984
Johanson, C. E., & Schuster, C. R. (1981). Animal models of drug self-administration. *Advances in Substance Abuse*, *2*, 219-297.
Kamble, S. H., Leon, F., King, T. I., Berthold, E. C., Lopera-Londono, C., Siva Rama Raju, K., Hampson, A. J., Sharma, A., Avery, B. A., McMahon, L. R., & McCurdy, C. R. (2020). Metabolism of a Kratom Alkaloid Metabolite in Human Plasma Increases Its Opioid Potency and Efficacy. *ACS Pharmacol Transl Sci*, *3*(6), 1063-1068. https://doi.org/10.1021/acsptsci.0c00075
Karunakaran, T., Vicknasingam, B., & Chawarski, M. C. (2024). Phytochemical analysis of water and ethanol liquid extracts prepared using freshly harvested leaves of Mitragyna speciosa (Korth.). *Nat Prod Res*, 1-8. https://doi.org/10.1080/14786419.2024.2362428
Kikura-Hanajiri, R., Kawamura, M., Maruyama, T., Kitajima, M., Takayama, H., & Goda, Y. (2009). Simultaneous analysis of mitragynine, 7-hydroxymitragynine, and other alkaloids in the psychotropic plant “kratom” (Mitragyna speciosa) by LC-ESI-MS. *Forensic Toxicology*, *27*(2), 67-74. https://doi.org/10.1007/s11419-009-0070-5
Kruegel, A. C., Gassaway, M. M., Kapoor, A., Varadi, A., Majumdar, S., Filizola, M., Javitch, J. A., & Sames, D. (2016). Synthetic and Receptor Signaling Explorations of the Mitragyna Alkaloids: Mitragynine as an Atypical Molecular Framework for Opioid Receptor Modulators. *J Am Chem Soc*, *138*(21), 6754-6764. https://doi.org/10.1021/jacs.6b00360
Leon, F., Habib, E., Adkins, J. E., Furr, E. B., McCurdy, C. R., & Cutler, S. J. (2009). Phytochemical characterization of the leaves of Mitragyna speciosa grown in U.S.A. *Nat Prod Commun*, *4*(7), 907-910. https://www.ncbi.nlm.nih.gov/pubmed/19731590

Lydecker, A. G., Sharma, A., McCurdy, C. R., Avery, B. A., Babu, K. M., & Boyer, E. W. (2016). Suspected Adulteration of Commercial Kratom Products with 7-Hydroxymitragynine. *J Med Toxicol*, *12*(4), 341-349. https://doi.org/10.1007/s13181-016-0588-y

Matsumoto, K., Horie, S., Ishikawa, H., Takayama, H., Aimi, N., Ponglux, D., & Watanabe, K. (2004). Antinociceptive effect of 7-hydroxymitragynine in mice: Discovery of an orally active opioid analgesic from the Thai medicinal herb Mitragyna speciosa. *Life Sci*, *74*(17), 2143-2155. https://doi.org/10.1016/j.lfs.2003.09.054

Matsumoto, K., Horie, S., Takayama, H., Ishikawa, H., Aimi, N., Ponglux, D., Murayama, T., & Watanabe, K. (2005). Antinociception, tolerance and withdrawal symptoms induced by 7-hydroxymitragynine, an alkaloid from the Thai medicinal herb Mitragyna speciosa. *Life Sci*, *78*(1), 2-7. https://doi.org/10.1016/j.lfs.2004.10.086

Matsumoto, K., Takayama, H., Narita, M., Nakamura, A., Suzuki, M., Suzuki, T., Murayama, T., Wongseripipatana, S., Misawa, K., Kitajima, M., Tashima, K., & Horie, S. (2008). MGM-9 [(E)-methyl 2-(3-ethyl-7a,12a-(epoxyethanoxy)-9-fluoro-1,2,3,4,6,7,12,12b-octahydro-8-methoxyindolo[2,3-a]quinolizin-2-yl)-3-methoxyacrylate], a derivative of the indole alkaloid mitragynine: a novel dual-acting mu- and kappa-opioid agonist with potent antinociceptive and weak rewarding effects in mice. *Neuropharmacology*, *55*(2), 154-165. https://doi.org/10.1016/j.neuropharm.2008.05.003

Maxwell, E. A., King, T. I., Kamble, S. H., Raju, K. S. R., Berthold, E. C., Leon, F., Hampson, A., McMahon, L. R., McCurdy, C. R., & Sharma, A. (2021). Oral Pharmacokinetics in Beagle Dogs of the Mitragynine Metabolite, 7-Hydroxymitragynine. *Eur J Drug Metab Pharmacokinet*, *46*(3), 459-463. https://doi.org/10.1007/s13318-021-00684-2

McCurdy, C. R., Sharma, A., Smith, K. E., Veltri, C. A., Weiss, S. T., White, C. M., & Grundmann, O. (2024). An update on the clinical pharmacology of kratom: uses, abuse potential, and future considerations. *Expert Rev Clin Pharmacol*, *17*(2), 131-142. https://doi.org/10.1080/17512433.2024.2305798

Mombelli, E. (2022). Animal Models of Drug Addiction. In S. Della Sala (Ed.), *Encyclopedia of Behavioral Neuroscience, 2nd edition (Second Edition)* (pp. 674-681). Elsevier. https://doi.org/https://doi.org/10.1016/B978-0-12-819641-0.00118-3

Mongar, P., Jaisi, A., Inkviya, T., Wungsintaweekul, J., & Wiwattanawongsa, K. (2024). Effects of Itraconazole on Pharmacokinetics of Mitragynine and 7-Hydroxymitragynine in Healthy Volunteers. *ACS Pharmacol Transl Sci*, *7*(3), 823-833. https://doi.org/10.1021/acsptsci.3c00335

NDEWS. (2024). National Drug Early Warning System Weekly Briefing. (195). https://ndews.org/newsletter/weekly-briefing-issue-195/

Obeng, S., Kamble, S. H., Reeves, M. E., Restrepo, L. F., Patel, A., Behnke, M., Chear, N. J., Ramanathan, S., Sharma, A., Leon, F., Hiranita, T., Avery, B. A., McMahon, L. R., & McCurdy, C. R. (2020). Investigation of the Adrenergic and Opioid Binding Affinities, Metabolic Stability, Plasma Protein Binding Properties, and Functional Effects of Selected Indole-Based Kratom Alkaloids. *J Med Chem*, *63*(1), 433-439. https://doi.org/10.1021/acs.jmedchem.9b01465

Obeng, S., Wilkerson, J. L., Leon, F., Reeves, M. E., Restrepo, L. F., Gamez-Jimenez, L. R., Patel, A., Pennington, A. E., Taylor, V. A., Ho, N. P., Braun, T., Fortner, J. D., Crowley, M. L., Williamson, M. R., Pallares, V. L. C., Mottinelli, M., Lopera-Londono, C., McCurdy, C. R., McMahon, L. R., & Hiranita, T. (2021). Pharmacological Comparison of Mitragynine and 7-Hydroxymitragynine: In Vitro Affinity and Efficacy for mu-Opioid Receptor and Opioid-Like Behavioral Effects in Rats. *J Pharmacol Exp Ther*, *376*(3), 410-427. https://doi.org/10.1124/jpet.120.000189

Ogozalek, S. (2023, December 9). The Tampa Bay Times tested 20 kratom products. Here's what we found. *Tampa Bay Times*. https://www.tampabay.com/investigations/2023/12/09/tampa-bay-times-tested-20-kratom-products-heres-what-we-found/

Panlilio, L. V., & Goldberg, S. R. (2007). Self-administration of drugs in animals and humans as a model and an investigative tool. *Addiction*, *102*(12), 1863-1870. https://doi.org/10.1111/j.1360-0443.2007.02011.x

Ponglux, D., Wongseripipatana, S., Takayama, H., Kikuchi, M., Kurihara, M., Kitajima, M., Aimi, N., & Sakai, S. (1994). A New Indole Alkaloid, 7 alpha-Hydroxy-7H-mitragynine, from Mitragyna speciosa in Thailand. *Planta Med*, *60*(6), 580-581. https://doi.org/10.1055/s-2006-959578

Prus, A. J., James, J. R., & Rosecrans, J. A. (2009). Conditioned Place Preference. In J. J. Buccafusco (Ed.), *Methods of Behavior Analysis in Neuroscience* (2nd ed.). https://www.ncbi.nlm.nih.gov/pubmed/21204336

Rogers, J. M., Weiss, S. T., Epstein, D. H., Grundmann, O., Hill, K., & Smith, K. E. (2024). Kratom addiction per DSM-5 SUD criteria, and kratom physical dependence: Insights from dosing amount versus frequency. *Drug Alcohol Depend*, *260*, 111329. https://doi.org/10.1016/j.drugalcdep.2024.111329

Sengnon, N., Vonghirundecha, P., Chaichan, W., Juengwatanatrakul, T., Onthong, J., Kitprasong, P., Sriwiriyajan, S., Chittrakarn, S., Limsuwanchote, S., & Wungsintaweekul, J. (2023). Seasonal and Geographic Variation in Alkaloid Content of Kratom (Mitragyna speciosa (Korth.) Havil.) from Thailand. *Plants (Basel)*, *12*(4). https://doi.org/10.3390/plants12040949

Sharma, A., Smith, K. E., Kuntz, M. A., Berthold, E. C., Elashkar, O. I., Guadagnoli, N., Kanumuri, S. R. R., Mukhopadhyay, S., Panlilio, L. V., Epstein, D. H., & McCurdy, C. R. (2025). Chemical Analysis and Alkaloid Intake for Kratom Products Available in the United States. *Drug Test Anal*. https://doi.org/10.1002/dta.3906

Singh, D., Narayanan, S., & Vicknasingam, B. (2016). Traditional and non-traditional uses of Mitragynine (Kratom): A survey of the literature. *Brain Res Bull*, *126*(Pt 1), 41-46. https://doi.org/10.1016/j.brainresbull.2016.05.004

Singh, D., Narayanan, S., Vicknasingam, B., Corazza, O., Santacroce, R., & Roman-Urrestarazu, A. (2017). Changing trends in the use of kratom (Mitragyna speciosa) in Southeast Asia. *Hum Psychopharmacol*, *32*(3). https://doi.org/10.1002/hup.2582

Smith, K. E., Boyer, E. W., Grundmann, O., McCurdy, C. R., & Sharma, A. (2024). The rise of novel, semi-synthetic 7-hydroxymitragnine products. *Addiction*. https://doi.org/10.1111/add.16728

Smith, K. E., Panlilio, L. V., Sharma, A., McCurdy, C. R., Feldman, J. D., Mukhopadhyay, S., Kanumuri, S. R. R., Kuntz, M. A., Hill, K., & Epstein, D. H. (2024). Time course of kratom effects via ecological momentary assessment, by product type, dose amount, and assayed alkaloid content. *Drug Alcohol Depend*, *264*, 112460. https://doi.org/10.1016/j.drugalcdep.2024.112460

Smith, K. E., Rogers, J. M., Dunn, K. E., Grundmann, O., McCurdy, C. R., Schriefer, D., & Epstein, D. H. (2022). Searching for a Signal: Self-Reported Kratom Dose-Effect Relationships Among a Sample of US Adults With Regular Kratom Use Histories. *Front Pharmacol*, *13*, 765917. https://doi.org/10.3389/fphar.2022.765917

Solinas, M., Panlilio, L. V., Justinova, Z., Yasar, S., & Goldberg, S. R. (2006). Using drug-discrimination techniques to study the abuse-related effects of psychoactive drugs in rats. *Nat Protoc*, *1*(3), 1194-1206. https://doi.org/10.1038/nprot.2006.167

Swedberg, M. D. (2016). Drug discrimination: A versatile tool for characterization of CNS safety pharmacology and potential for drug abuse. *J Pharmacol Toxicol Methods*, *81*, 295-305. https://doi.org/10.1016/j.vascn.2016.05.011

Takayama, H., Ishikawa, H., Kurihara, M., Kitajima, M., Aimi, N., Ponglux, D., Koyama, F., Matsumoto, K., Moriyama, T., Yamamoto, L. T., Watanabe, K., Murayama, T., & Horie, S. (2002). Studies on the synthesis and opioid agonistic activities of mitragynine-related indole alkaloids: discovery of opioid agonists structurally different from other opioid ligands. *J Med Chem*, *45*(9), 1949-1956. https://doi.org/10.1021/jm010576e

Tanna, R. S., Nguyen, J. T., Hadi, D. L., Manwill, P. K., Flores-Bocanegra, L., Layton, M. E., White, J. R., Cech, N. B., Oberlies, N. H., Rettie, A. E., Thummel, K. E., & Paine, M. F. (2022). Clinical Pharmacokinetic Assessment of Kratom (Mitragyna speciosa), a Botanical Product with Opioid-like Effects, in Healthy Adult Participants. *Pharmaceutics*, *14*(3). https://doi.org/10.3390/pharmaceutics14030620

Todd, D. A., Kellogg, J. J., Wallace, E. D., Khin, M., Flores-Bocanegra, L., Tanna, R. S., McIntosh, S., Raja, H. A., Graf, T. N., Hemby, S. E., Paine, M. F., Oberlies, N. H., & Cech, N. B. (2020). Chemical composition and biological effects of kratom (Mitragyna speciosa): In vitro studies with implications for efficacy and drug interactions. *Sci Rep*, *10*(1), 19158. https://doi.org/10.1038/s41598-020-76119-w

Varadi, A., Marrone, G. F., Palmer, T. C., Narayan, A., Szabo, M. R., Le Rouzic, V., Grinnell, S. G., Subrath, J. J., Warner, E., Kalra, S., Hunkele, A., Pagirsky, J., Eans, S. O., Medina, J. M., Xu, J., Pan, Y. X., Borics, A., Pasternak, G. W., McLaughlin, J. P., & Majumdar, S. (2016). Mitragynine/Corynantheidine Pseudoindoxyls As Opioid Analgesics with Mu Agonism and Delta Antagonism, Which Do Not Recruit beta-Arrestin-2. *J Med Chem*, *59*(18), 8381-8397. https://doi.org/10.1021/acs.jmedchem.6b00748

Walker, E. A. (2018). A Prospective Evaluation of Drug Discrimination in Pharmacology. *Curr Top Behav Neurosci*, *39*, 319-328. https://doi.org/10.1007/7854_2018_59

Young, R. (2009). Drug Discrimination. In J. J. Buccafusco (Ed.), *Methods of Behavior Analysis in Neuroscience* (2nd ed.). https://www.ncbi.nlm.nih.gov/pubmed/21204332

Zuarth Gonzalez, J. D., Ragsdale, A. K., Mukhopadhyay, S., McCurdy, C. R., McMahon, L. R., Obeng, S., & Wilkerson, J. L. (2025). Mitragynine and 7-Hydroxymitragynine: Bidirectional Effects on Breathing in Rats. *bioRxiv*. https://doi.org/10.1101/2025.05.16.654392