NEW DIETARY INGREDIENT NOTIFICATION FOR NPI-001, A DRIED KRATOM LEAF POWDER

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Food and Drug Administration
Department of Health and Human Services
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DATE:
21 October 2022

New Dietary Ingredient Notification for NPI-001, a Dried Kratom Leaf Powder

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SECTION A – ADMINISTRATIVE INFORMATION

A.1 Description of the New Dietary Ingredient

Johnson Foods Holding LLC (Johnson Foods) intends to act as a bulk manufacturer, distributor, and supplier of NPI-001, a new dietary ingredient (NDI). The proposed NDI, NPI-001, is a fine powder made from the dried leaves of *Mitragyna speciosa* (Korth.) Havil. (abbreviated to *M. speciosa*). *M. speciosa*, commonly referred to as "kratom," is a flowering evergreen tree belonging to the Rubiacaea (coffee) family and is native to Southeast Asia. Johnson Foods intends to market NPI-001 using either a common name (*e.g.*, kratom leaf powder), abbreviated Latin binomial name (*e.g.*, *M. speciosa* leaf powder), or the trademarked name Mitra-Leaf™, or any future trademarked names.

The term "dietary supplement" is defined in Title 21 of *United States Code* (U.S.C.) 321 (ff)[1] as, among other things:

> "*a product (other than tobacco) intended to supplement the diet that bears or contains one or more of the following dietary ingredients: (A) a vitamin; (B) a mineral; (C) an herb or other botanical; (D) an amino acid; (E) a dietary substance for use by man to supplement the diet by increasing the total dietary intake; or (F) a concentrate, metabolite, constituent, extract, or combination of any ingredient described in clause (A), (B), (C), (D), or (E).*"

NPI-001 is a dietary ingredient that qualifies as a dietary supplement that is an herb or other botanical, in accordance with 21 U.S.C. 321 (ff)(1)(C).

Additionally, Johnson Foods is not presenting evidence that the NDI NPI-001 was marketed in the United States (U.S.) before 15 October 1994, nor that NPI-001 was included in any dietary ingredient that was marketed in the U.S. before 15 October 1994[2]. Thus, under Section 413(c) (21 U.S.C. 350b), NPI-001 qualifies as an NDI. In accordance with Sections 413(a)(2) (21 U.S.C. 350b) and 413(b) (21 U.S.C. 350b)[3], Johnson Foods is providing the Secretary with information, including any citation to published articles, which is the basis on which Johnson Foods concluded that a dietary supplement containing NPI-001 will reasonably be expected to be safe, and the conditions of use under which NPI-001 will reasonably be expected to be safe.

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[1] U.S. FDA (2022a). Federal Food, Drug, and Cosmetic Act (FD&C Act): Chapter 9. Subchapter II - Definitions. 21 USC §321 - Definitions, generally [Sec. 201]. In: *U.S. Code-Title 21-Food and Drug* (Food and Drug Administration). Washington (DC): U.S. House of Representatives, Office of Law Revision Counsel. Available at: http://uscode.house.gov/browse/prelim@title21/chapter9&edition=prelim [current through Public Law 117-110 – 04/08/2022].

[2] DSHEA (1994). *Dietary Supplement Health and Education Act of 1994*. (Public Law 103-417, 103rd Congress). Washington (DC): U.S. Food and Drug Administration (U.S. FDA). Available at: https://ods.od.nih.gov/About/DSHEA_Wording.aspx.

[3] U.S. FDA (2022b). Federal Food, Drug, and Cosmetic Act (FD&C Act): Chapter 9. Subchapter IV - Food. 21 USC §350b - New dietary ingredients [Sec. 413]. In: *U.S. Code-Title 21-Food and Drug* (Food and Drug Administration). Washington (DC): U.S. House of Representatives, Office of Law Revision Counsel. Available at: http://uscode.house.gov/browse/prelim@title21/chapter9&edition=prelim [current through Public Law 117-110 – 04/08/2022].

Johnson Foods is the bulk manufacturer, distributor, and supplier of NPI-001. As such, Johnson Foods recommends the use of NPI-001 in dietary supplement products for oral consumption as a powder, capsule containing powder, or other oral dosage forms that do not physically or chemically alter the NDI or exceed the labeled conditions of use. The recommended serving size of NPI-001 is not more than 50 mg of NPI-001 per day, for intermittent daily use defined as a maximum of 15 consecutive days, and not more than 15 days per 30-day period.

A.2 Identification of Trade Secret Information

The following information and their location(s) within the dossier, as indicated in Table A.2-1, are identified as confidential trade secret and/or confidential commercial information under Title 21 of the *Code of Federal Regulations* (CFR) §20.61(d) and 190.6(e) (U.S. FDA, 2021). Johnson Foods understands that this information will be kept confidential for 90 days after the filing date of this Notice; however, Johnson Foods respectfully requests that certain information, as described in Table A.2-1 below, be kept confidential even after the 90-day period. The confidential and proprietary information is related to NPI-001's chemical composition, raw materials, manufacturing, material sources, specifications, certificates of analysis, and test methods, along with product-specific analysis.

Table A.2-1 Confidential Chapters and Sections of the New Dietary Ingredient Notification

Confidential Chapters and Sections	Explanation
Specified paragraphs of Section A.3 – Executive Summary	Confidential Commercial Information under 21 CFR §20.61(b), as specified Sections pertain to other confidential Sections described in subsequent rows of this Table.
Section B.2 to B.5 and associated Appendices (*i.e.*, Appendix A, B, C, and D) – Identity of the New Dietary Ingredient and Manufacturing Information	Trade Secret under 21 CFR §20.61(a), as Sections B.2 to B.5 and Appendix A, B, C, and D, contain the proprietary information related to the identification of NPI-001 along with the raw materials, manufacturing process, quality control steps, and a schematic diagram that should remain confidential to protect Johnson Foods' commercial valuable and proprietary data. Confidential Commercial Information under 21 CFR §20.61(b), as Sections B.2 to B.5 and Appendix A, B, C, and D contain information used in Johnson Foods' business that is customarily held in strict confidence. Disclosure of this information would allow competitors to replicate Johnson Foods' supply chain and manufacturing processes, which would, in turn, cause substantial harm to Johnson Foods' competitive situation.
Section C.2.1 to C.2.3 and associated Appendices (*i.e.*, Appendix E and F) – *in vitro* Study, *in vivo* Studies, and Clinical Study	Trade Secret under 21 CFR §20.61(a), as Section C.2 and the associated Appendices contain product-specific safety data related to Johnson Foods' NPI-001. These data are not publicly available and are therefore regarded as proprietary information. Substantial effort was required to generate the data necessary for these Sections and Appendices through research and innovation. As such, these data represent significant proprietary commercial value and should not be disclosed. Confidential Commercial Information under 21 CFR §20.61(b), as Section C.2, and the associated Appendices contain information used in Johnson Foods' business that is customarily held in strict confidence and is regarded as privileged.
Section C.4 – Comprehensive Safety Profile	Trade Secret under 21 CFR §20.61(a), as Section C.4 contains a comprehensive safety profile and narrative regarding *M. speciosa*, which required substantial effort to generate through research and

	innovation. As such, these data represent significant proprietary commercial value and should not be disclosed.

CFR = *Code of Federal Regulations*.

A.3 Executive Summary

Johnson Foods's NPI-001 is a dry powder ingredient made from *M. speciosa* tree leaves. NPI-001 is intended to be used as a dietary ingredient in a dietary supplement for oral consumption as a powder, capsule containing powder, or other oral dosage forms that do not physically or chemically alter the NDI or exceed the labeled conditions of use. The recommended serving size of NPI-001 is not more than 50 mg per day for intermittent daily use defined as a maximum of 15 consecutive days, and not more than 15 days per 30-day period.

[CONFIDENTIAL] (b) (4)

The manufacturing process (b) (4)

[END OF CONFIDENTIAL SECTION]

(b) (4)

[CONFIDENTIAL] (b) (4)

[END OF CONFIDENTIAL SECTION] Based on the risk assessment and corroborative findings from a review of published literature, Johnson Foods determined that the NDI is reasonably expected to be safe under the recommended conditions of use (50 mg/day, not more than 15 consecutive days, and not more than 15 days per 30-day period).

SECTION B – CHEMISTRY AND IDENTITY

B.1 Description of the New Dietary Ingredient Identity

Johnson Foods's NPI-001 is a dry powder ingredient made from the dried leaves of the *Mitragyna speciosa* (Korth.) Havil. plant. *M. speciosa*, commonly referred to as "kratom", is a flowering evergreen tree belonging to the Rubiacaea (coffee) family. The characteristics of the tree were described by Mr. G.D. Haviland in "*Revision of Naucleeae*," Journal of the Linnean Society, 1897 (Haviland, 1897). A complete anatomical investigation of this plant was conducted by Shellard and Lees (1965) and provides an in-depth account of the plant's macro- and microscopic features. This plant species is native to tropical and sub-tropical regions of Southeast Asia and Africa (Suhaimi *et al.*, 2016). The taxonomic classification of the botanical used to produce NPI-001 is presented in Table B.1-1, below. Complete reference texts relevant to identity are included in Appendix A.

Table B.1-1 Taxonomic Classification of the Botanical in NPI-001

Kingdom	Plantae
Phylum	Tracheophyta
Class	Magnoliopsida
Order	Gentianales
Family	Rubiaceae
Genus	*Mitragyna* Korth.
Species	*Mitragyna speciosa* (Korth.) Havil.

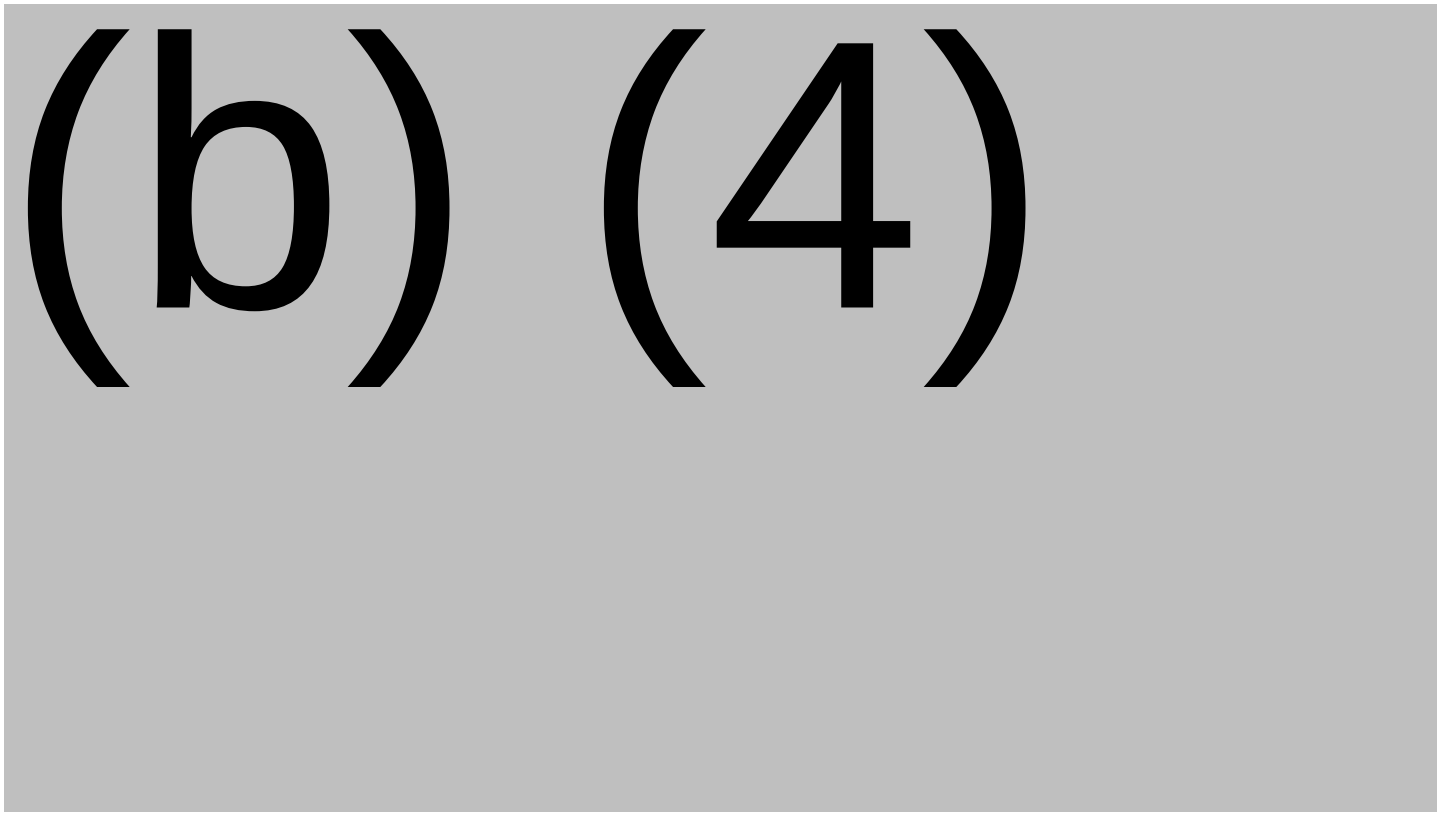
Johnson Foods manufactures NPI-001 with *M. speciosa* leaf material sourced from farms located in tropical regions, mainly in Southeast Asia. The harvested *M. speciosa* leaves are dried and milled into a powder. Johnson Foods developed appropriate specifications and process controls (Section B.3 and B.2.3, respectively) for NPI-001 that include validated methods for testing the NDI's identity and purity for conformance to the established specification. Results from batch analysis of 4 non-consecutive batches of NPI-001 demonstrate that the manufacturing process outlined in Section B.2 produces a consistent ingredient that conforms to the established specifications, as detailed in Section B.4.

B.2 Manufacturing Information [CONFIDENTIAL]

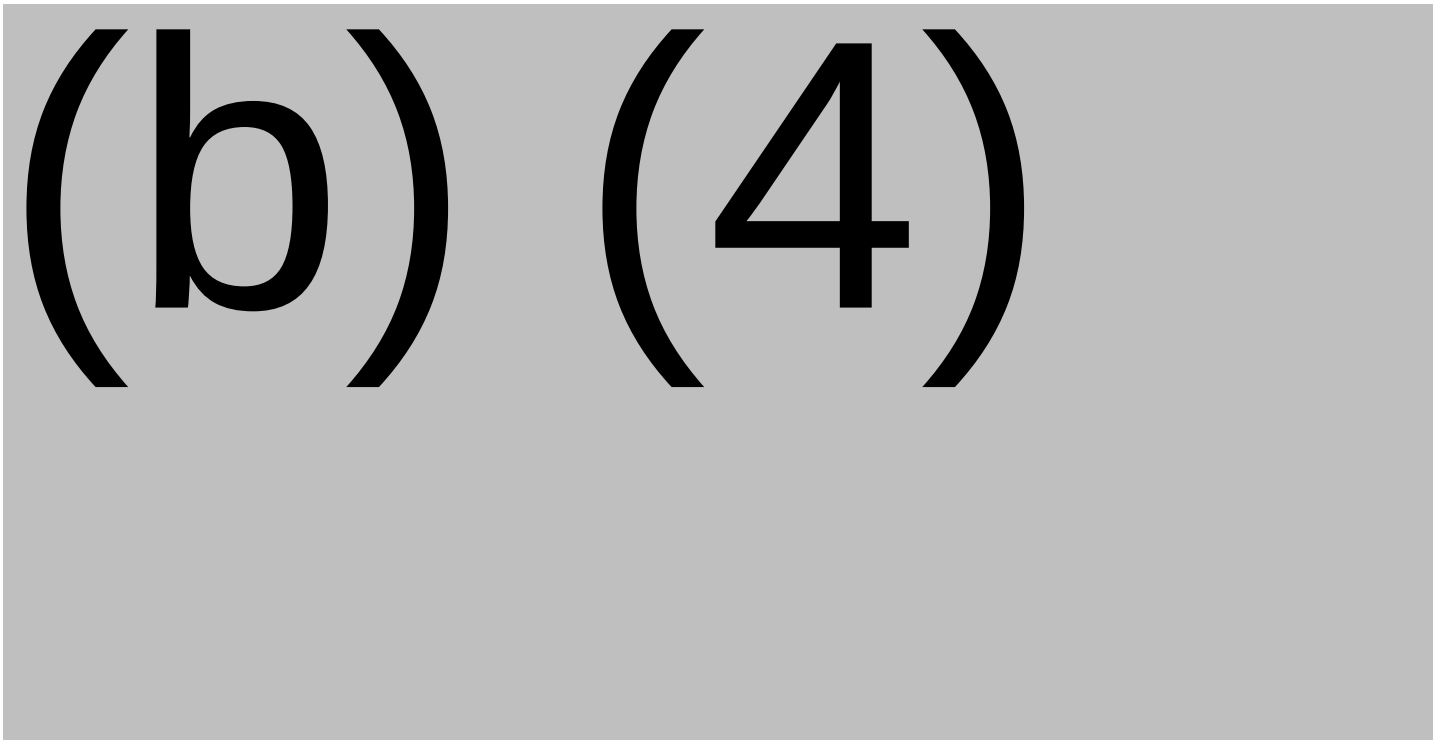
The manufacturing process (b) (4)

The manufacturing process (b) (4) , is depicted by Figure B.2-1, below.

Figure B.2-1 Schematic Overview of the NPI-001 Manufacturing Process



B.2.1 Raw Material



B.2.2 Manufacturing Process

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B.2.3 Process Controls

(b) (4)

(b) (4)

B.3 NPI-001 Specifications [CONFIDENTIAL]

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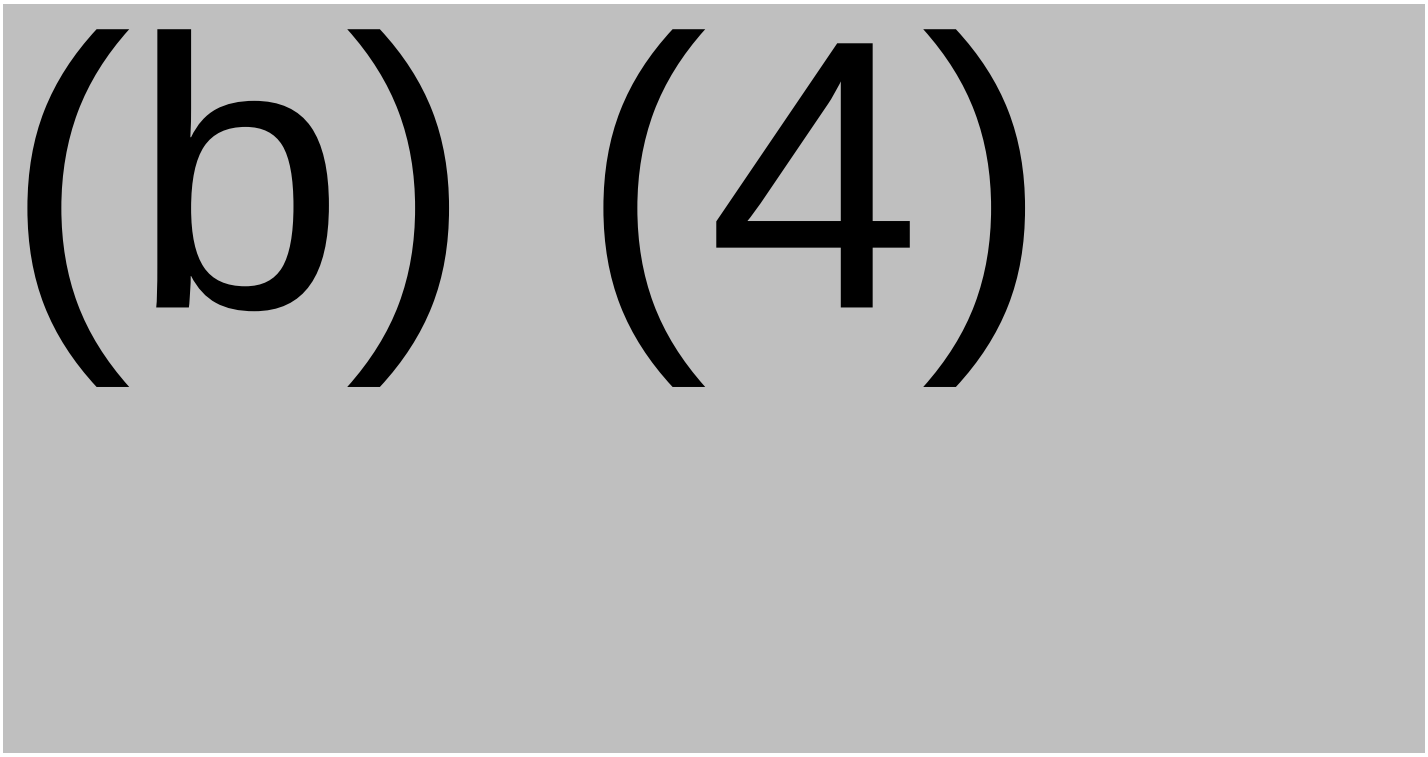
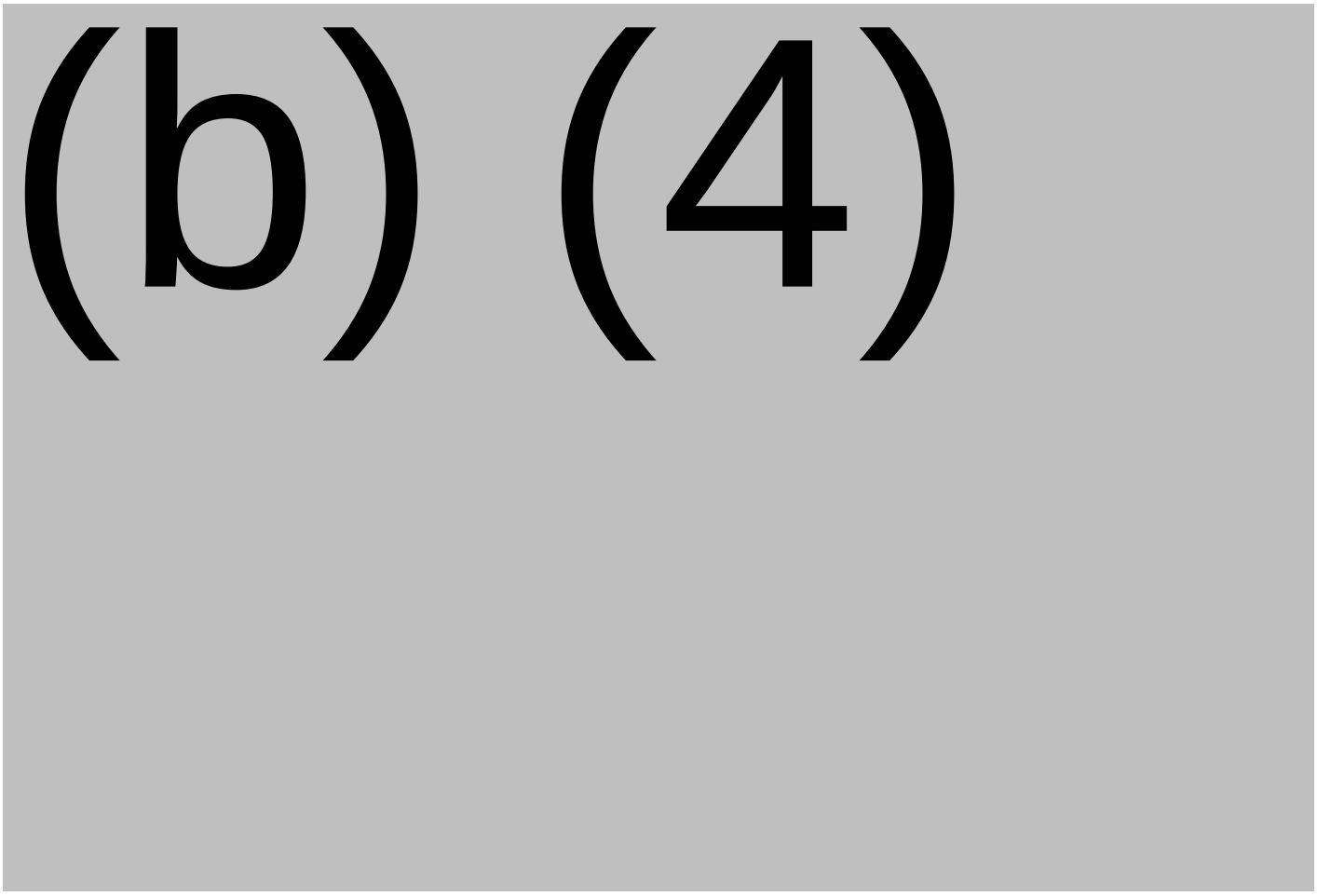
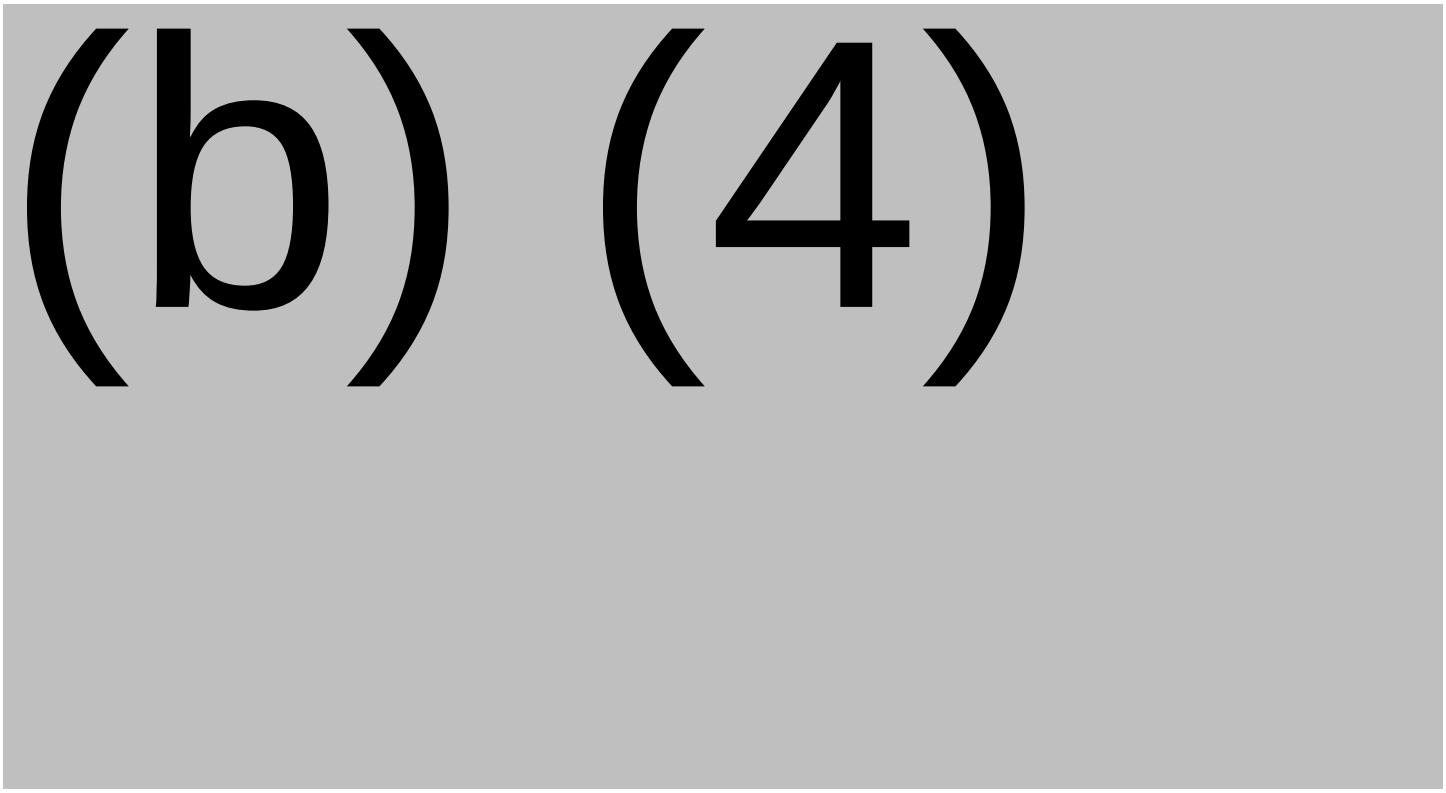
B.3.1 Methods and Specifications for Establishing Identity

(b) (4)

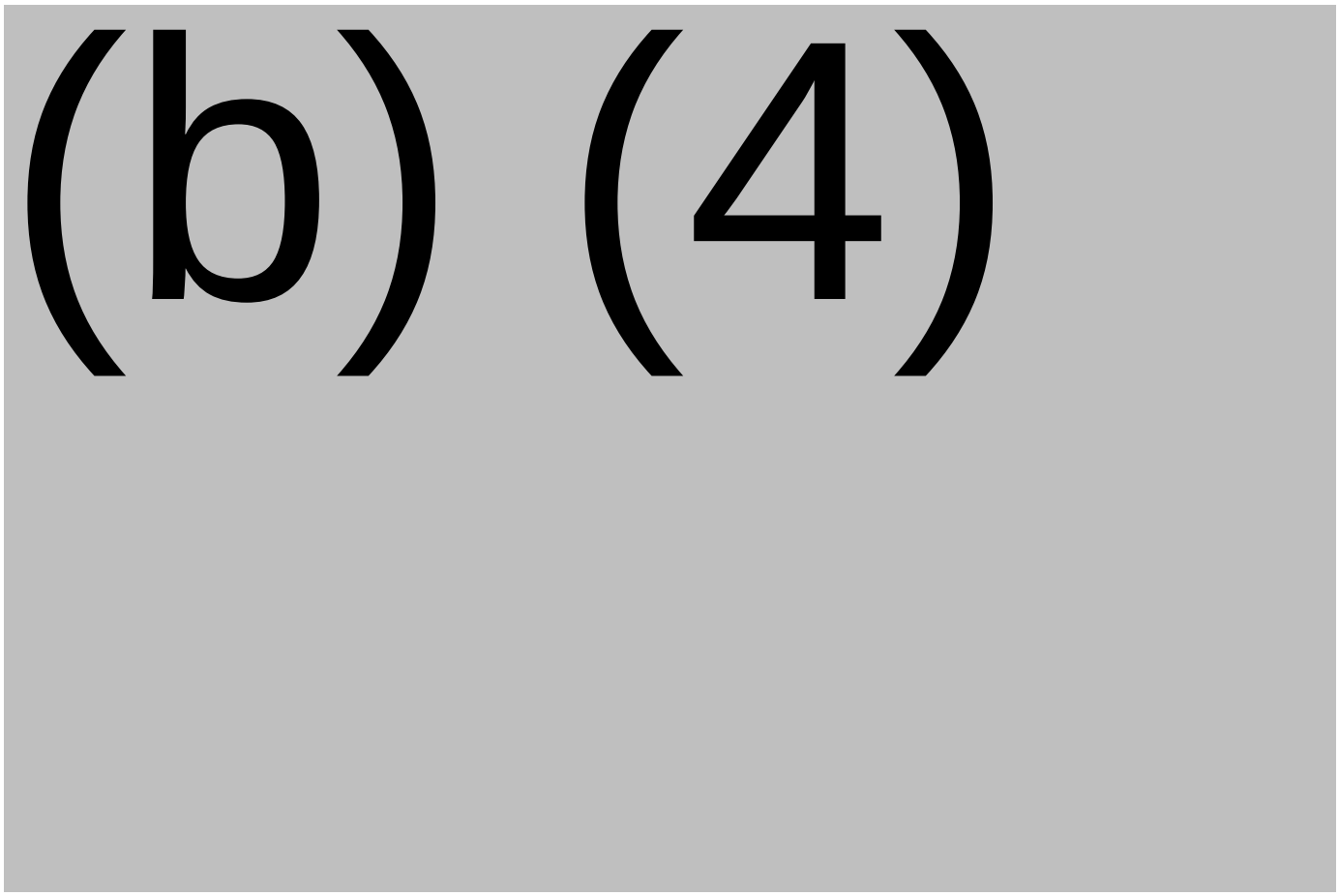


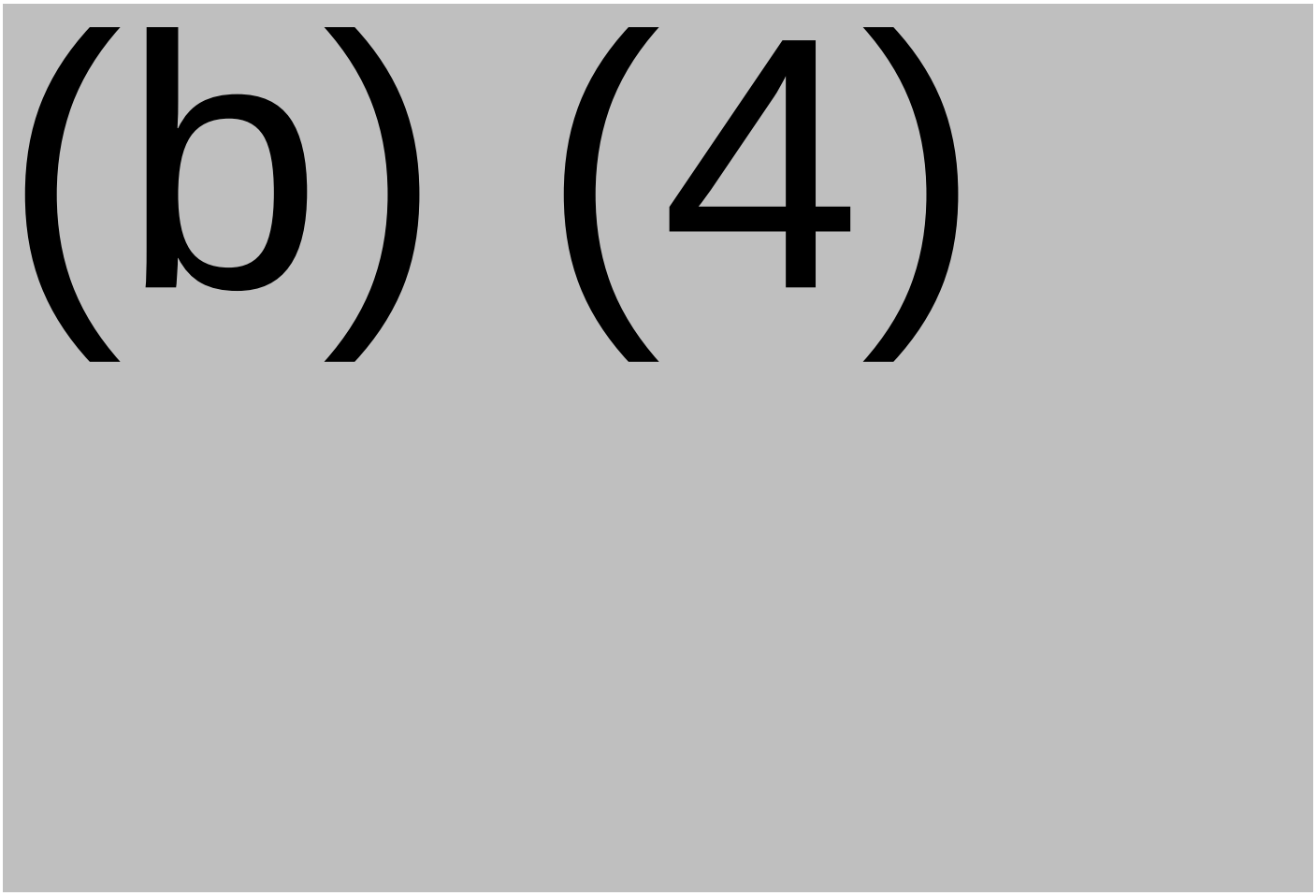
B.3.2 Methods and Specifications for Establishing Purity



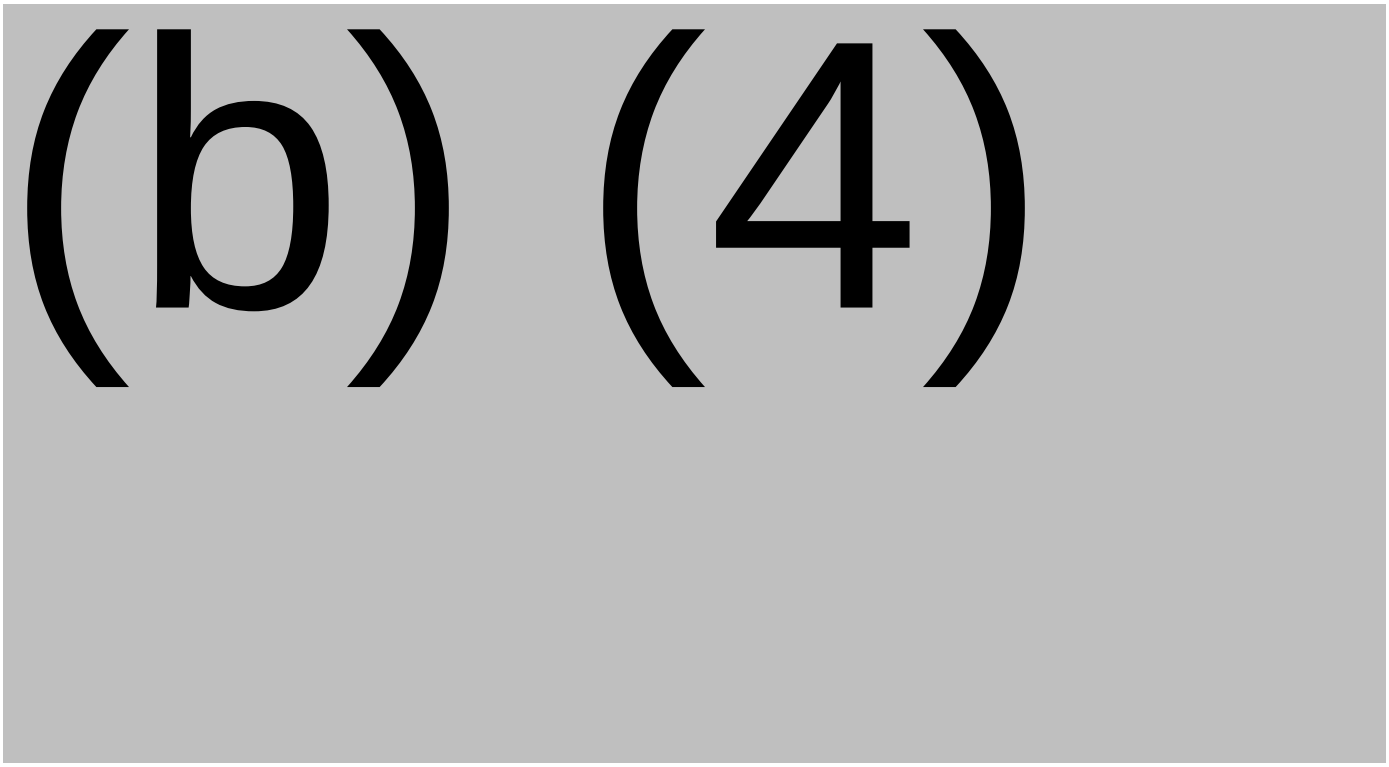


B.4 Product Analysis [CONFIDENTIAL]





B.5 Additional Product Characterization [CONFIDENTIAL]



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B.6 Intended Use Level of the New Dietary Ingredient

Johnson Foods intends for NPI-001 to be consumed intermittently with a labelled serving size of 50 mg per day and is not to be consumed more than 15 days in a row or for more than 15 days per 30-day period. NPI-001 is not intended for anyone under the age of 18 or any adult who is, or may become, pregnant or is breastfeeding.

Products containing NPI-001 will include the following warning:

> *For adult use only. Keep out of reach of children. If you are pregnant, breastfeeding, taking medications, planning medical or surgical procedures, or have a medical condition, talk to your doctor before use. Discontinue use and inform your health care provider if any adverse reactions occur.*

SECTION C – SAFETY AND TOXICOLOGY

C.1 Safety Narrative for the New Dietary Ingredient

As described in Section A.1, Johnson Foods intends to act as a bulk supplier of NPI-001, a fine powder derived from the dried leaves of the kratom (*M. speciosa* [Korth.] Havil.) plant. Johnson Foods is not presenting evidence that NPI-001 was present in the U.S. food supply as an article used for food, nor in a dietary supplement containing NPI-001. Therefore, under 21 U.S.C. 350b, NPI-001 is defined as a new dietary ingredient.

In accordance with Section 201(ff) [21 U.S.C. 321(ff)], and as per the 2016 FDA Draft Guidance, Dietary Supplements: New Dietary Ingredient Notifications and Related Issues: Guidance for Industry (U.S. FDA, 2016, 2022a), this section provides the basis for Johnson Foods' conclusion that a dietary supplement containing the NDI is reasonably expected to be safe under the supplement's labeled conditions of use [21 U.S.C. 350b(a)(2)].

While kratom and kratom-derived products have an extensive history of use both domestically and globally, NPI-001 is an ingredient that was not previously marketed by Johnson Foods. The basis of safety of Johnson Foods's NPI-001 at the proposed daily serving size is supported by the results from a battery of product-specific nonclinical and clinical toxicity studies conducted with NPI-001 and described in Section C.2. The battery of *nonclinical* toxicological studies includes an *in vitro* bacterial reverse mutation assay, 2 *in vivo* studies of genotoxic potential (micronucleus and comet assays) conducted in rats, and a 28-day repeated-dose toxicity study in rats. Results from a single-dose and 15-day multiple-dose trial conducted with NPI-001 in humans also provides strong evidence to support the safety of NPI-001 at the intended serving size described within this Notification (*i.e.*, 50 mg/day, intermittent daily use).

A comprehensive review of published literature was also conducted to identify studies on other kratom and kratom-derived products that further substantiate that NPI-001 is reasonably expected to be safe under the labeled conditions of use. A summary of relevant studies and why they corroborate the basis of safety established for Johnson Foods' NPI-001 is included in Section C.3. This section includes a discussion on kratom pharmacology, toxicology, surveys and case studies, herb-drug interactions, and potential for abuse, dependence, and withdrawal.

The totality of the scientific evidence summarized in Section C.2 to C.3 is used to create the comprehensive safety profile of the NDI presented in Section C.4 and includes discussions regarding the NDI's computed margin of safety, Acceptable Daily Intake (ADI), and Expected Daily Intake (EDI). Together, the scientific evidence presented in this New Dietary Ingredient Notification (NDIN) supports the conclusion that the use of NPI-001 is reasonably expected to be safe under the recommended conditions of use (50 mg/day, not more than 15 consecutive days, and not more than 15 days per 30-day period).

C.2 Studies to Support Safety of NPI-001

(b) (4)

C.2.1 *In vitro* Study [CONFIDENTIAL]

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C.2.2 *In vivo* Studies [CONFIDENTIAL]

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C.2.3 Clinical Study [CONFIDENTIAL]

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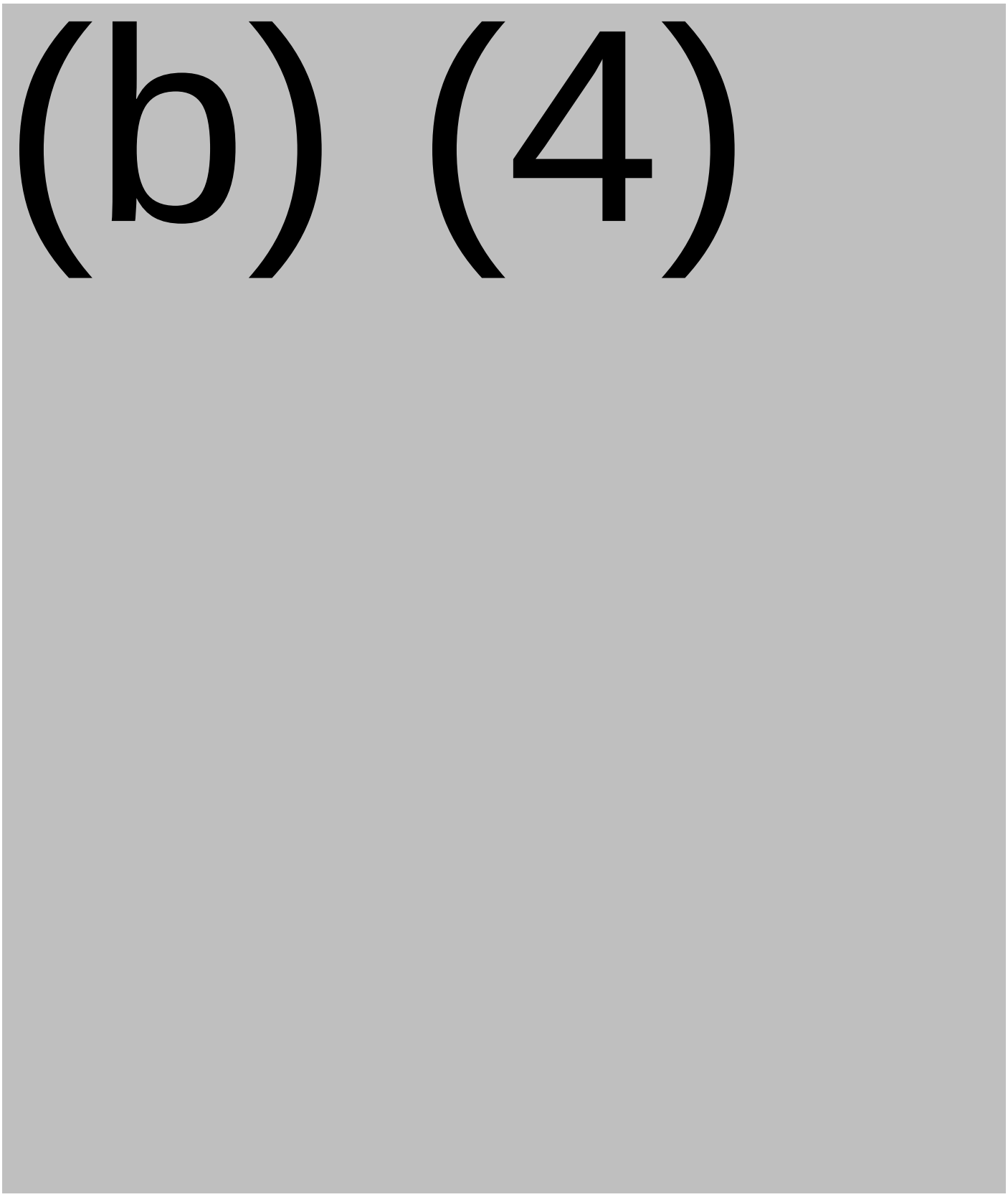
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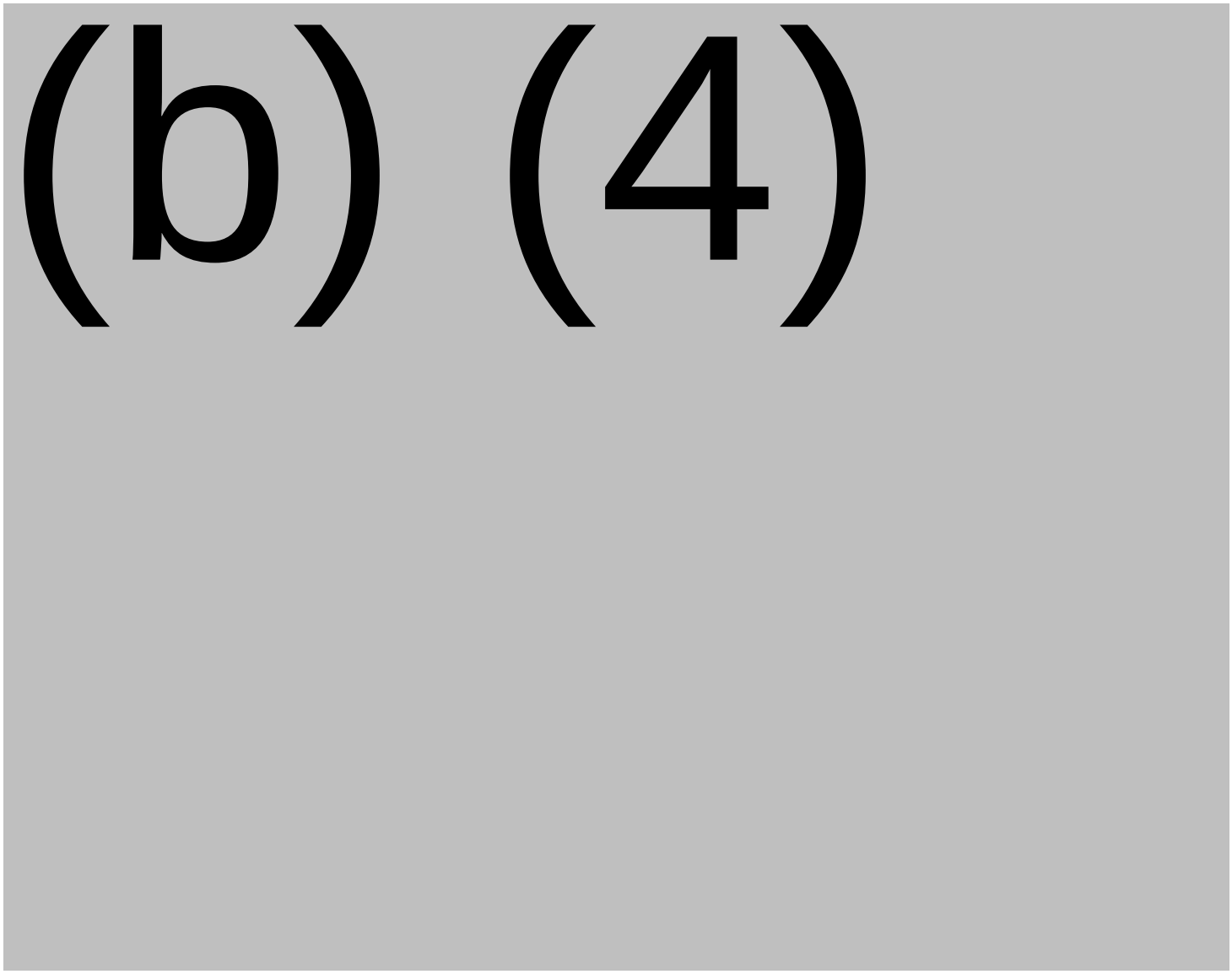
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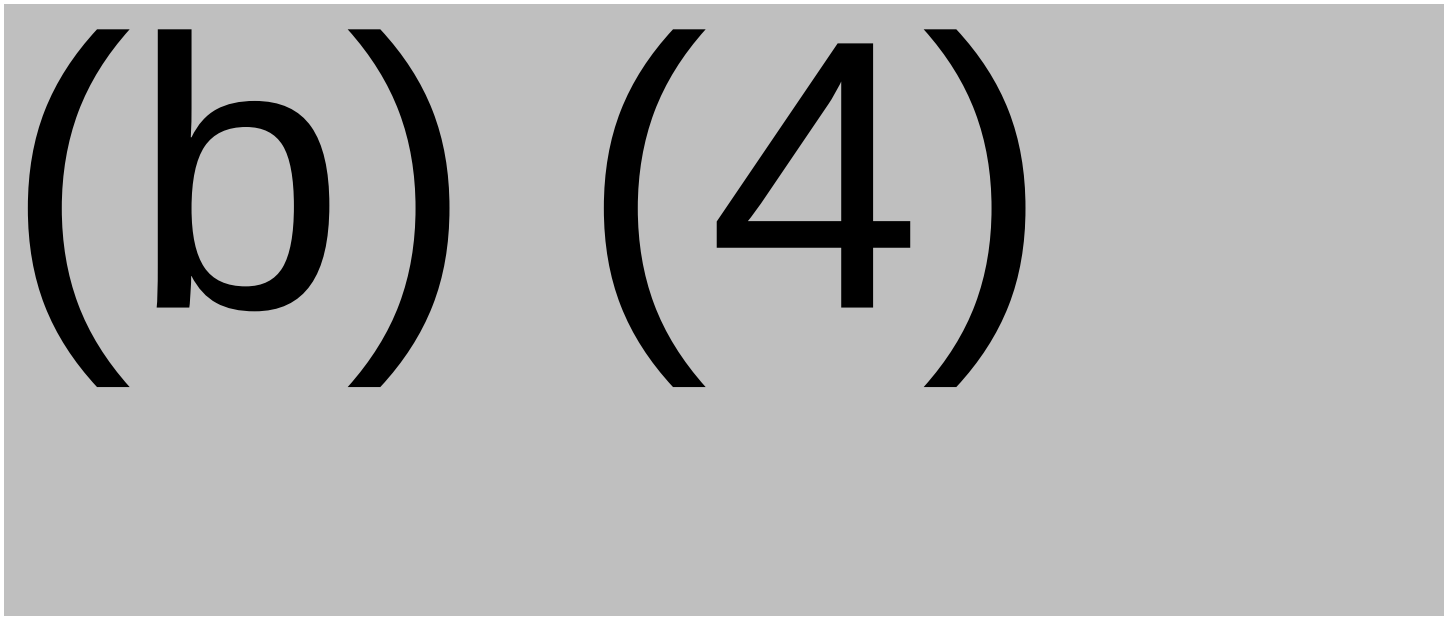
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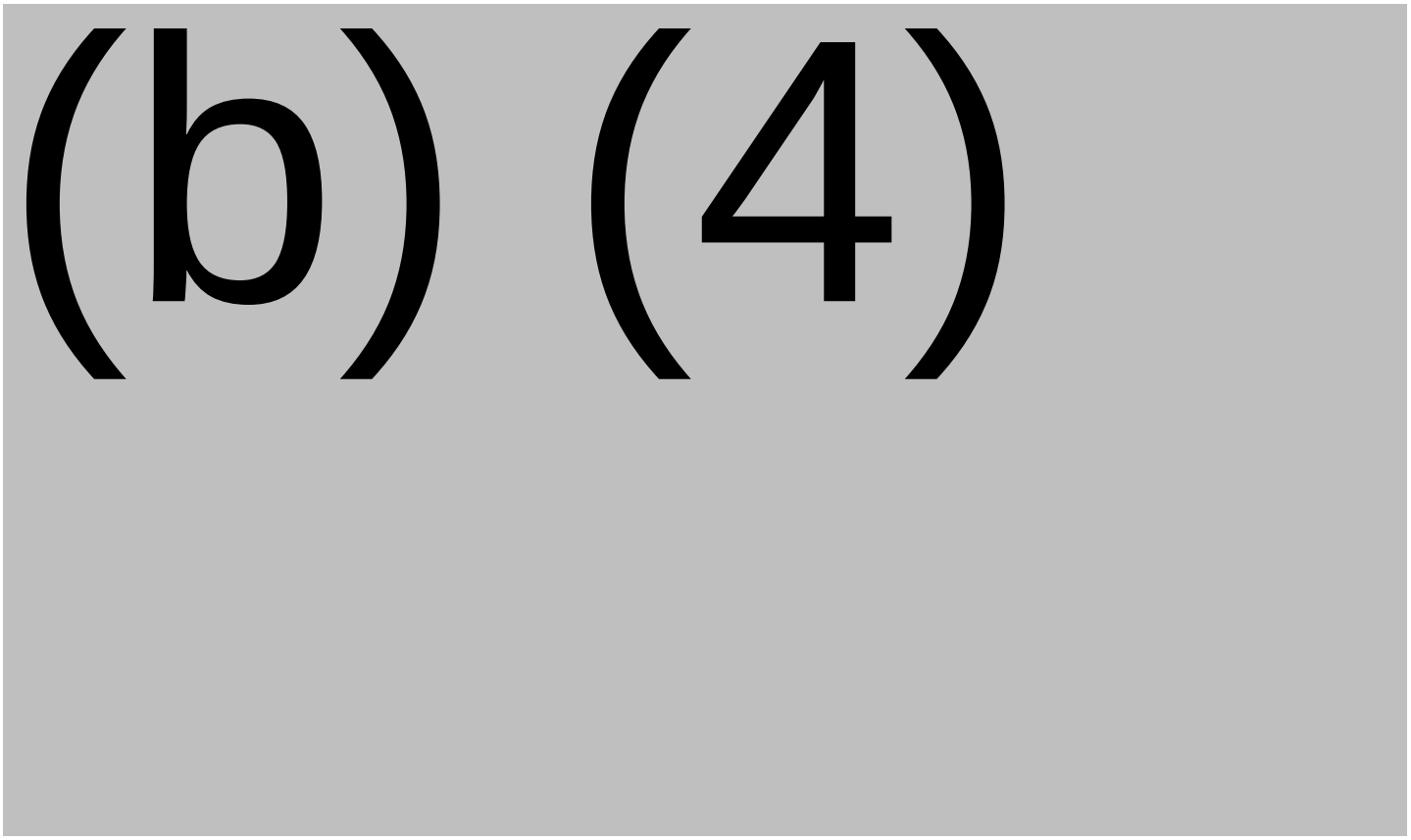
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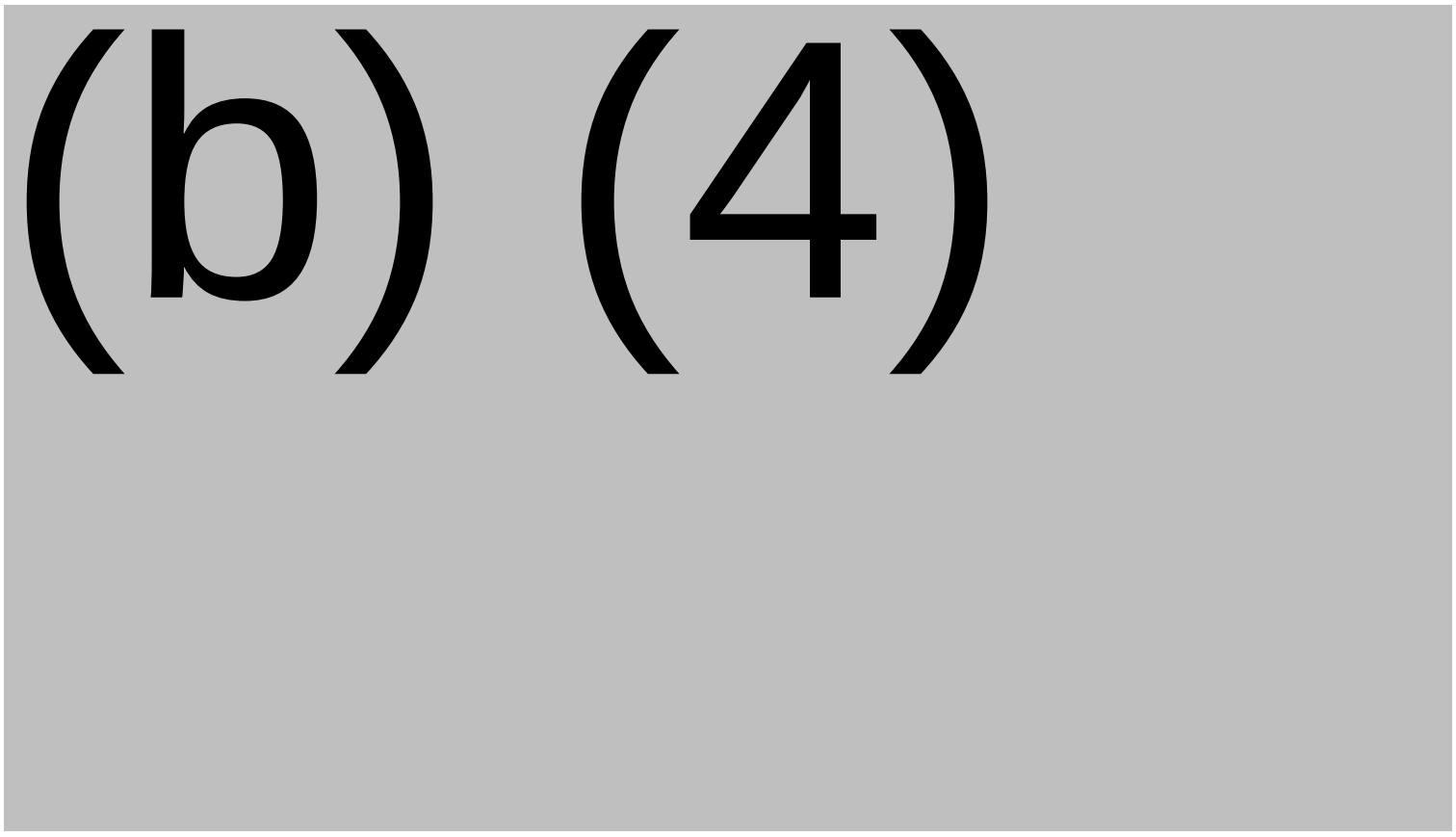
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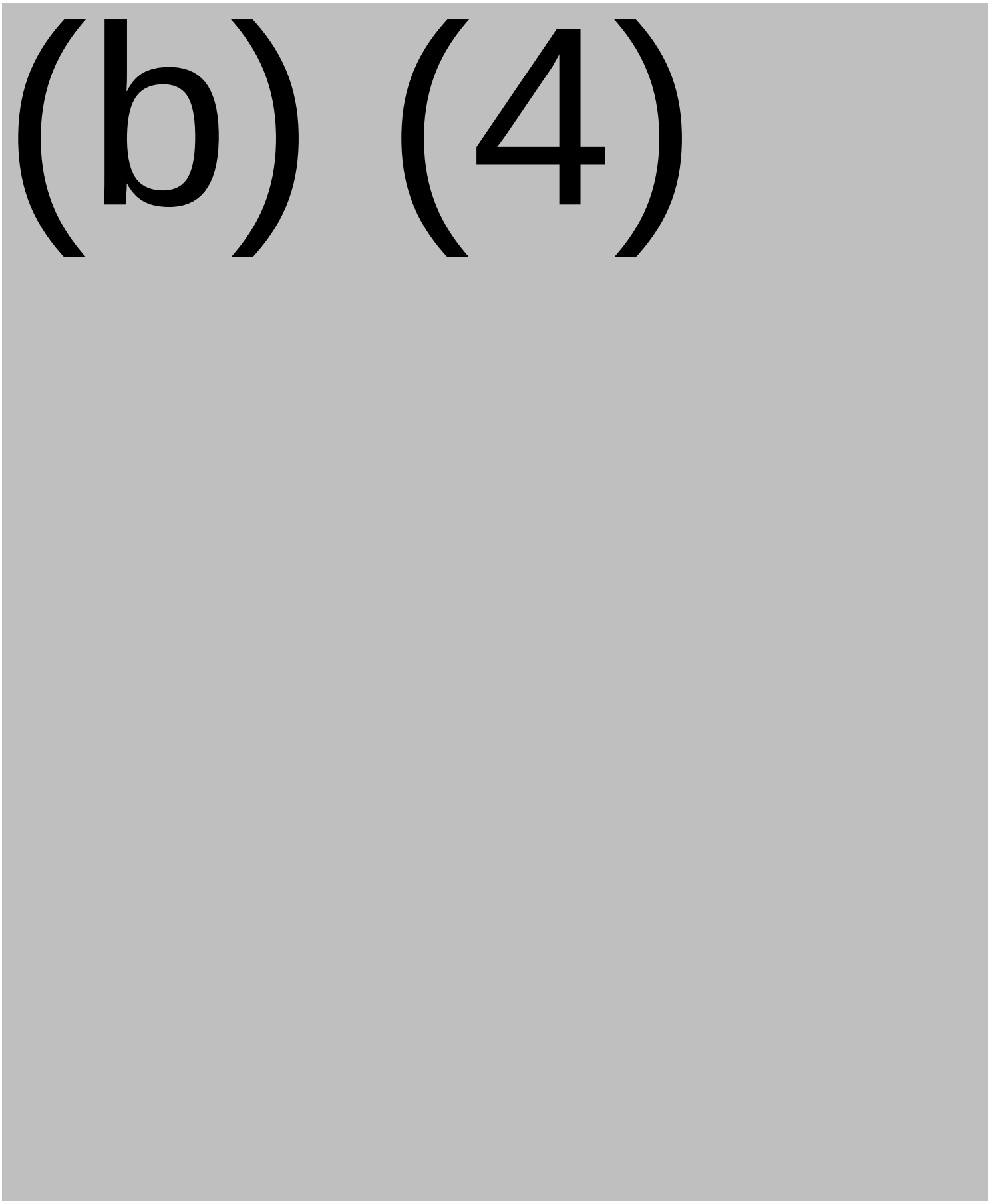
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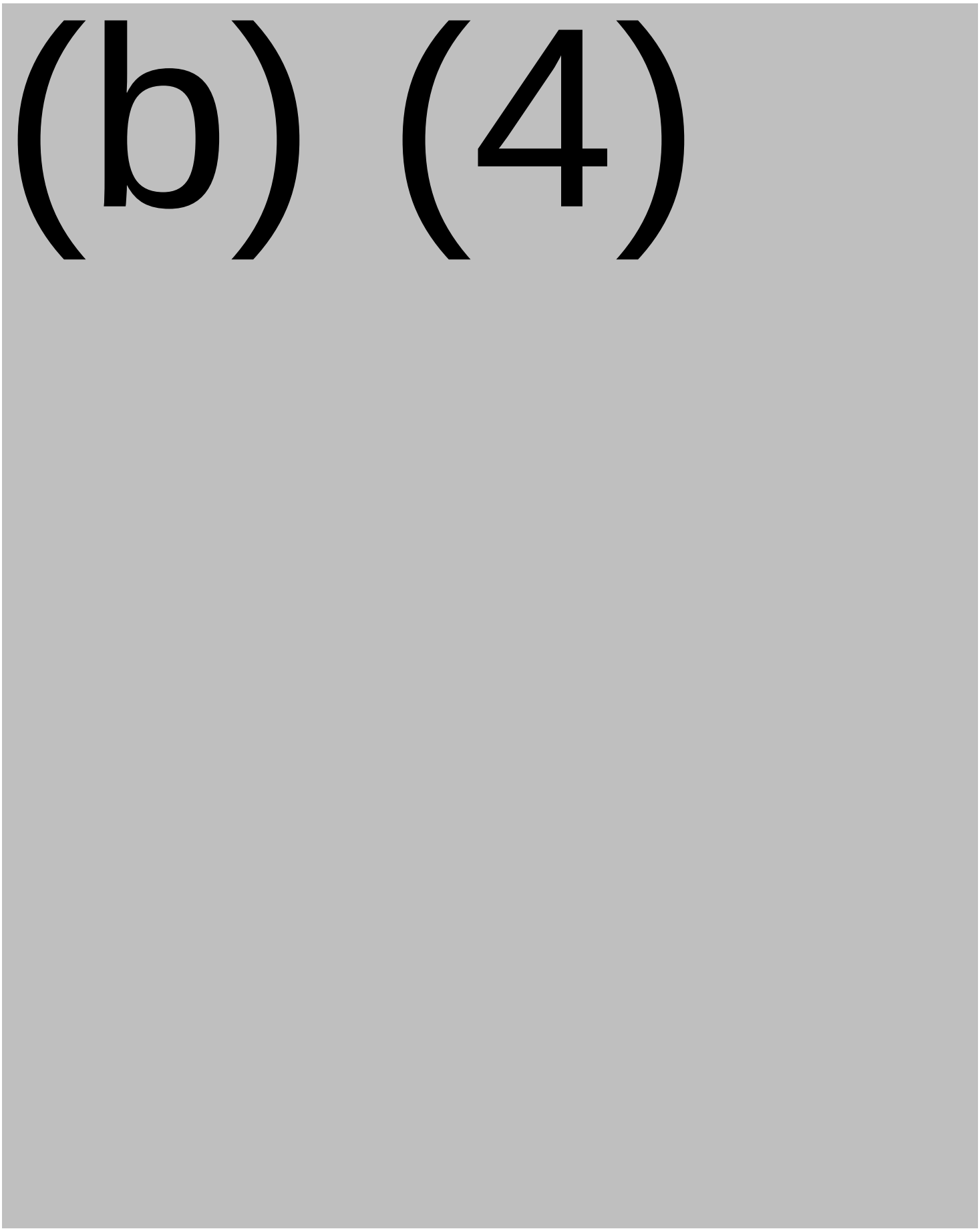
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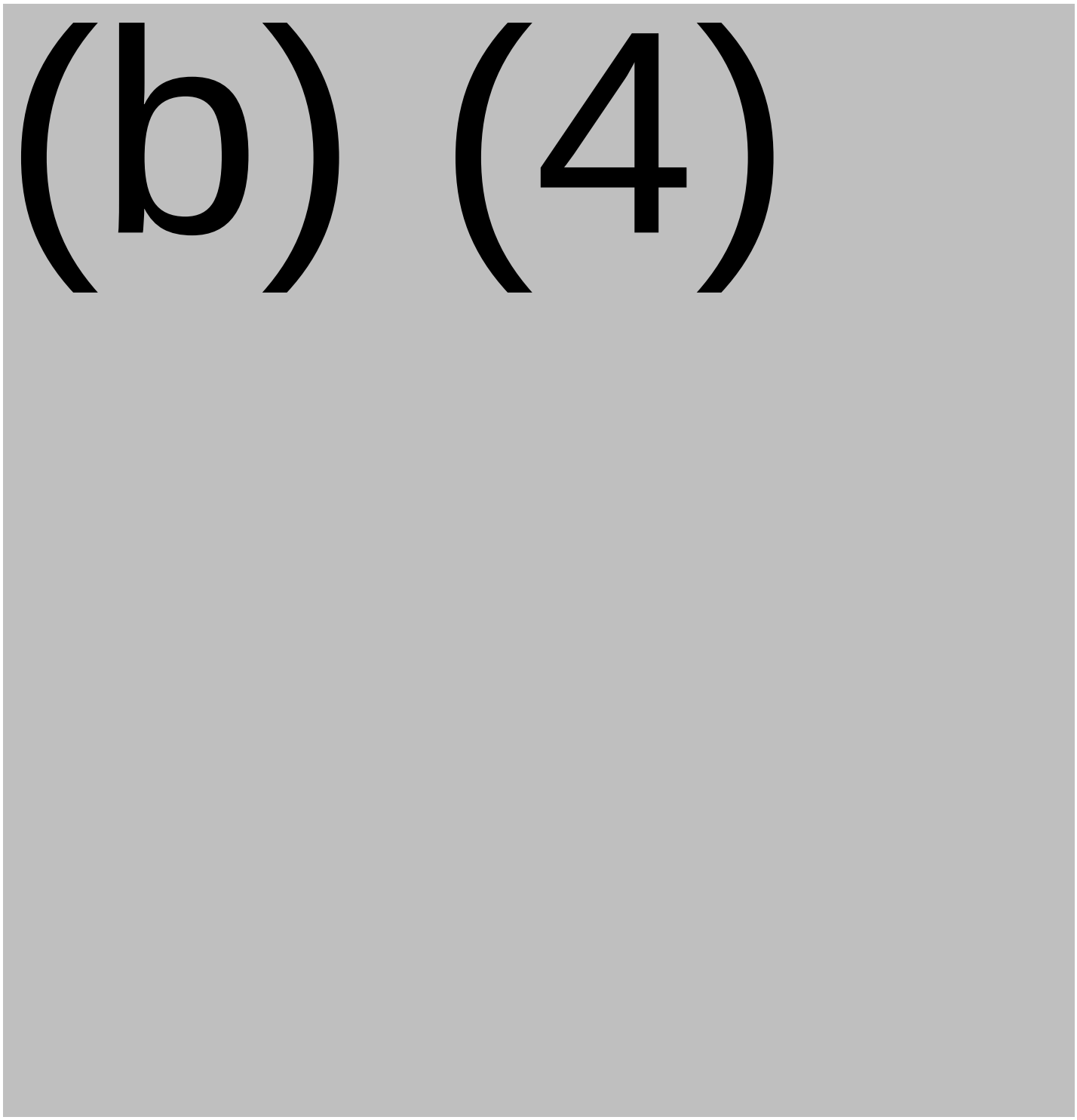
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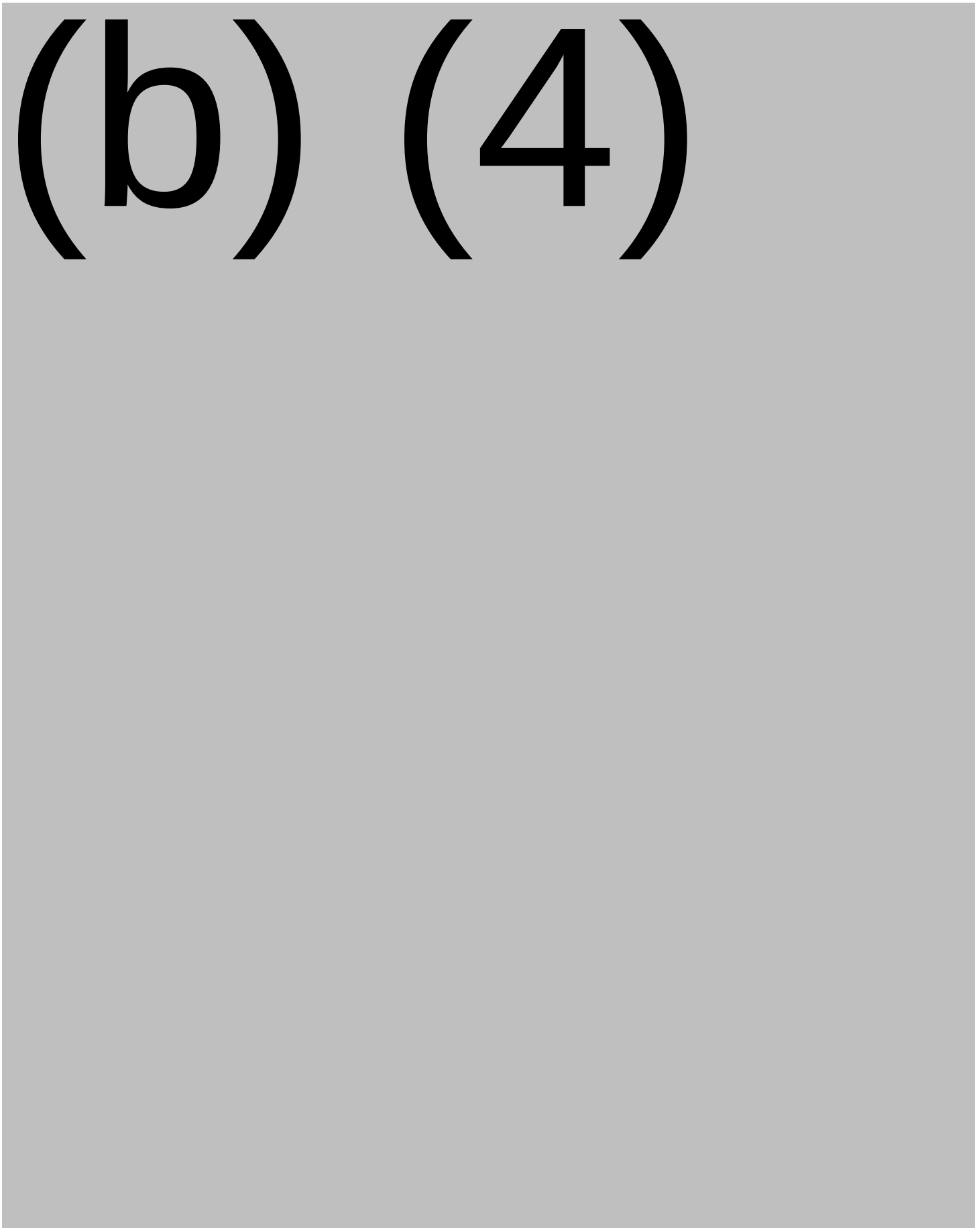
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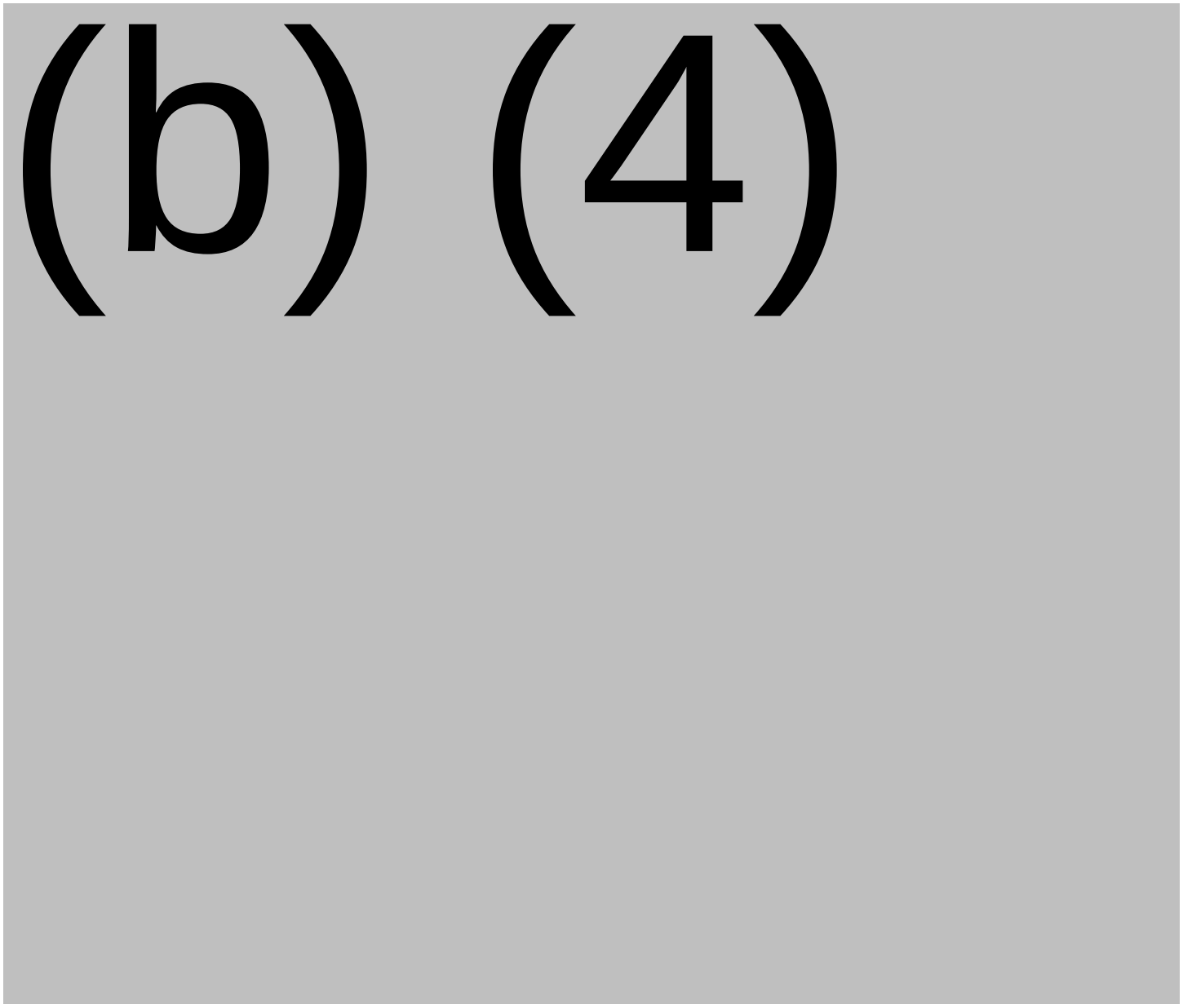
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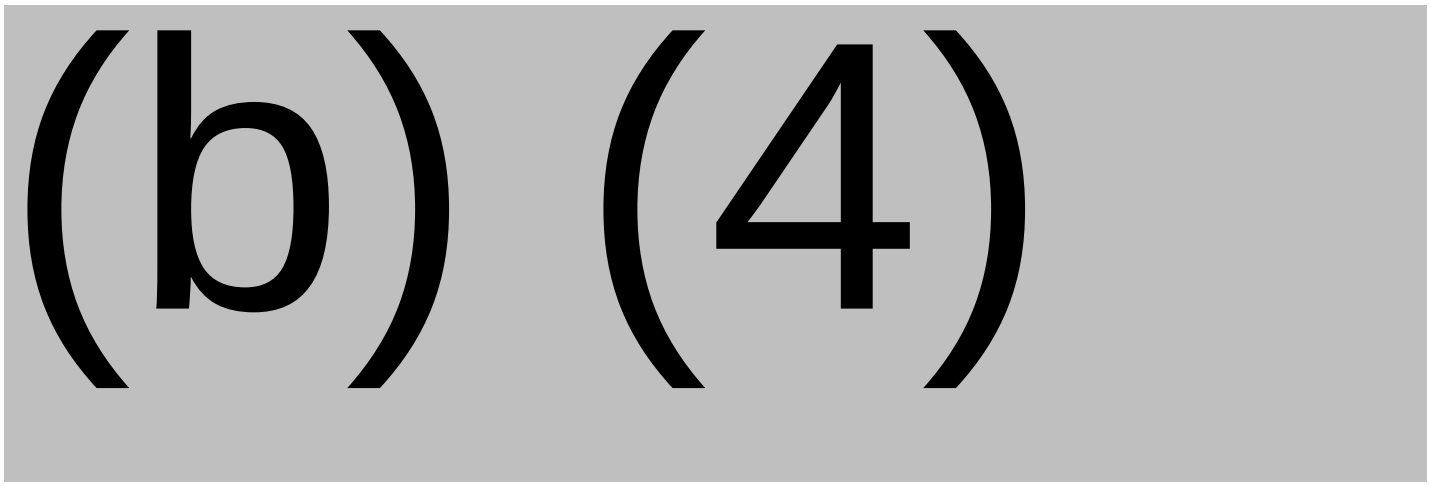
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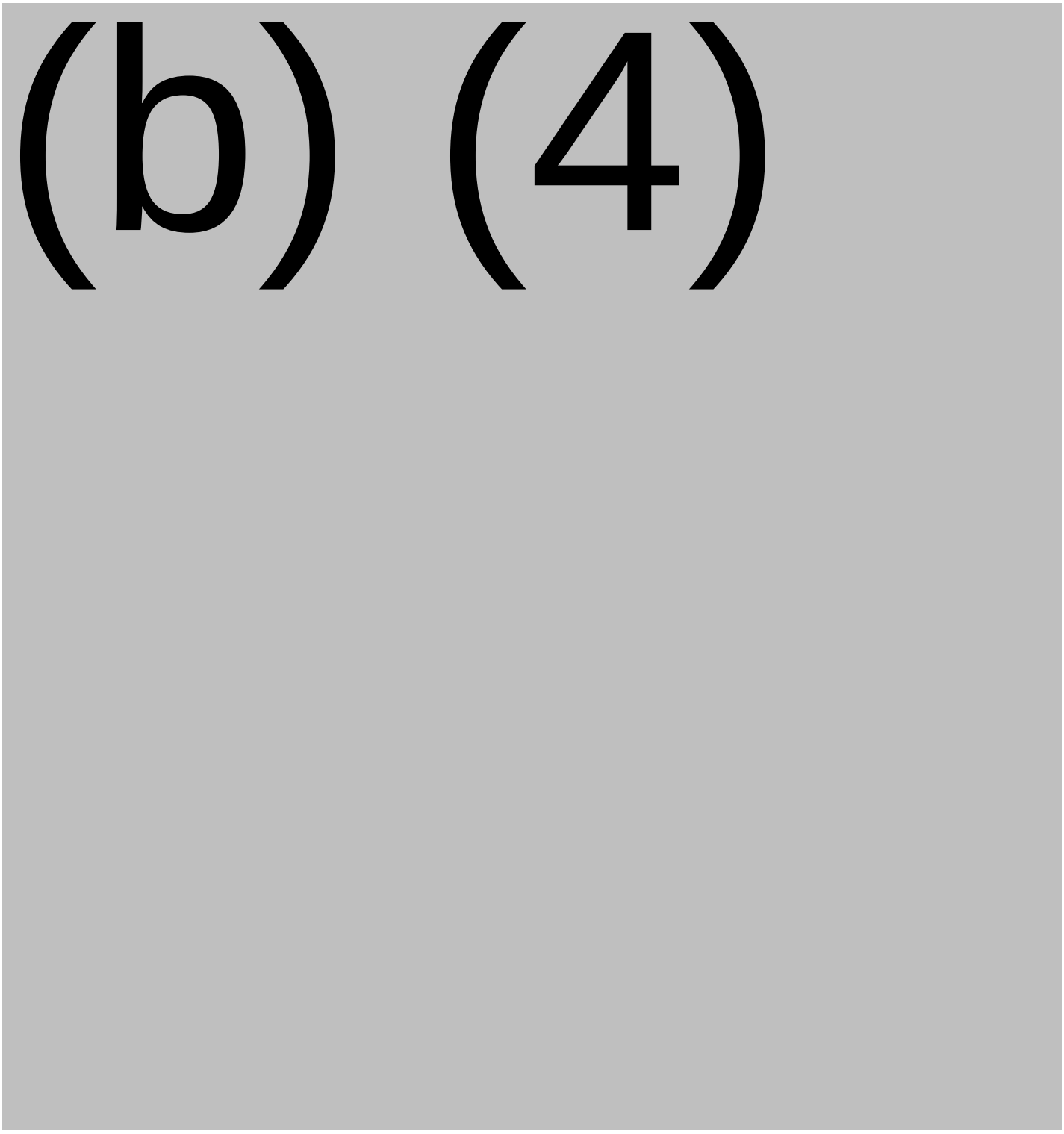
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C.3 Other Evidence of Safety

Johnson Foods performed a comprehensive review of the published literature to identify studies on other kratom and kratom-derived products that provide additional substantiation that NPI-001 is reasonably expected to be safe under the labeled conditions of use. Kratom's history of use, prevalence, pharmacology, toxicology, case studies, retrospective studies, potential NDI-drug interactions, and potential for abuse, dependence and withdrawal were investigated. In addition, this thorough review includes research pertaining to other kratom-derived test articles, mitragynine, and 7-hydroxymitragynine.

NPI-001 is a fine powder produced from dried leaves of the *M. speciosa* plant, without use of any techniques that chemically alter the raw material. As such, it may be similar to the historically consumed ingredient. However, based on the information presented in this notification, NPI-001 has not previously been marketed, nor is it present in the U.S. food supply as an article used for food nor as an ingredient in a dietary supplement. Moreover, numerous adulterated kratom products are reportedly available on the internet that could confound published results related to the pure leaf material presented herein (Arndt *et al.*, 2011; Lydecker *et al.*, 2016; Nacca *et al.*, 2020; LeSaint *et al.*, 2022). Therefore, in the absence of compositional data that may directly relate test articles from published studies to NPI-001, the applicability of the referenced literature may be limited.

Johnson Foods conducted a review of the literature using 3 databases (TOXNET, PubMed, and Google Scholar) to identify English-language articles published as of 15 May 2022. Search terms included "kratom," "mitragynine," "7-hydroxymitragynine," "7-hydroxymitragynine," "Mitragyna," and "ketum." Studies relevant to the history of use, safety, pharmacology, mechanism of action, prevalence, and adverse effects of *M. speciosa*, mitragynine, 7-hydroxymitragynine were included in this review.

C.3.1 History of Use of *M. speciosa*

M. speciosa, as well as preparations derived from its leaves, are often referred to as "kratom," but can also be referred to as "ketum," "thom," "thang," and "biak" (Sethi *et al*., 2020). *M. speciosa* is native to the tropical southeast Asia region where it also has a rich history of use. Since NPI-001 is manufactured using *M. speciosa* leaves without the use of any techniques that chemically alter the raw material, it may be compositionally like the historically consumed ingredient. However, Johnson Foods is proposing a serving size for NPI-001 that is significantly lower than reported serving sizes of *M. speciosa* leaves as described in this section. NPI-001 is proposed for use as an NDI at a serving size up to 50 mg/day (*i.e*., 0.4 to 0.6 mg mitragynine/day) and is not to be consumed more than 15 days in a row or for more than 15 days per 30-day period, nor is it intended for anyone under the age of 18 or any adult who is pregnant or breastfeeding.

Kratom has been used in its native southeast Asia for centuries as an ingredient in cooking (*e.g*., to wrap fish, stews), or consumed through chewing, smoking, brewing into a tea, or as part of a commonly consumed tar-like extract (Adkins *et al*., 2011; Henningfield *et al*., 2019). While there were anecdotal reports of various consumption regimens to achieve desired effects, there does not appear to be any widely accepted dosing regimen. Low-dose usage of kratom, when the plant is used for its caffeine-like effects, is generally consumed at 5 g or less per occasion; however, the raw plant material is also reported to be commonly consumed at levels up to 15 g per occasion (Swogger *et al.*, 2022).

Consumption of kratom in the form of a traditional tea/juice was evaluated in a study of 58 daily long term kratom users (≥5 years kratom use history) consuming an average of 3.5 cups/glasses per day. This daily consumption of 76.3 to 114.8 mg mitragynine per day did not alter the hematological and biochemical parameters of kratom users (Singh *et al.*, 2018). Another study of 13 regular long-term kratom users (>20 years kratom use history; daily intake ≥87.5 mg mitragynine) found no significant alterations in hematological, kidney, liver, thyroid, inflammatory, or gastrointestinal analytes (Singh *et al.*, 2020a,b)

Use of Johnson Foods' NPI-001 as an NDI at a serving size up to 50 mg/day (*i.e.*, 0.4 to 0.6 mg mitragynine/day, based on product specifications proposed in Section B.3) is a proposed use serving size much lower than traditional kratom use considered to be safe. The proposed serving size of NPI-001 (*i.e.,* 50 mg/day) is expected to be safe.

C.3.2 Safety of *M. speciosa* and Mitragynine

As described above, published literature pertaining to *M. speciosa* leaf material may be relevant to the safety review of NPI-001. Since mitragynine is unique to *M. speciosa*, Johnson Foods created an identity specification for which the NDI is strictly controlled (see Section B.3). Therefore, use of Johnson Foods' NPI-001 at the intended use level described in Section B.6 (*i.e.*, 50 mg/day; 0.714 mg/kg body weight/day for a 70-kg adult) corresponds to a maximum daily intake of mitragynine of approximately 0.10 mg/kg body weight/day based on the maximum established mitragynine content in NPI-001 (see Section B.3). Therefore, corroborative safety data pertaining to mitragynine, and its metabolite 7-hydroxymitragynine, are considered in the safety evaluation of NPI-001 in addition to data pertaining to *M. speciosa* leaf material.

A summary of the genotoxicity and mutagenicity assays conducted with *M. speciosa* and mitragynine is presented in Table C.3.2.2.1-1 below, while the results of the repeated-dose studies are summarized in Table C.3.2.2.2-1. Additional discussions on pharmacology, toxicology, prevalence, retrospective, observational, and case studies; NDI-drug interactions; and abuse, dependence, and withdrawal potential are consistent with expected safety of NPI-001 that is established from the product-specific safety studies and the history of consumption in other jurisdictions. The results of these corroborative studies provide evidence to demonstrate that the proposed use of NPI-001 does not pose toxicological concern at the proposed use conditions.

C.3.2.1 Pharmacology

Mitragynine, the marker of identity for NPI-001, has a complex pharmacological profile that includes activity at multiple receptors. Although the role of each binding site in mediating *M. speciosa*'s effects is not completely understood, research has been published describing activity at several receptor sites of interest, including the Mu Opioid Receptor (MOR) system. In addition, major interspecies differences were noted in recent scientific investigations emphasizing the importance of human research findings, including those related to abuse potential and withdrawal.

Receptor-level functional characterization (*i.e.*, [35S] GTPγS binding) of mitragynine and related natural alkaloids at human μ-, κ-, and δ-opioid receptors revealed that mitragynine and 7-hydroxymitragynine are partial agonists of the human μ-opioid receptor and competitive antagonists at κ- and δ-opioid receptors (Kruegel *et al*., 2016). In mice, mitragynine from kratom extract had lower affinity and potency at the μ-opioid receptor than morphine; importantly however, it also is unable to induce comparable phosphorylation and GTPγS stimulation to morphine and other prototypical opioids.

Unlike full μ-opioid agonists morphine and fentanyl, mitragynine does not activate the β-arrestin-2 pathway implicated in many of the adverse effects of μ-opioid agonists including respiratory depression and constipation (Váradi *et al.*, 2016; Eastlack *et al.*, 2020). These data support early reports of Macko *et al.* (1972) of reduced respiratory depression from mitragynine compared to codeine.

Mitragynine's pharmacology is also different from prototypical opioids due to its binding to κ-opioid, δ-opioid, dopamine D1 and D2, α-adrenergic (α1A, α1B, α1D, α2A, α2B, and α2C), serotonin 5-HT_{2C} and 5-HT_7, and A_{2A} adenosine receptors (Matsumoto *et al.*, 1996, 2005; Boyer *et al.*, 2008; Prozialeck *et al.*, 2012; Stolt *et al.*, 2014; Obeng *et al.*, 2020). Most recently, potential mitragynine activity was also identified at the 5-HT_{1A} receptor (León *et al.*, 2021). Many recent publications (in the last 10 years) refined the consensus understanding of mitragynine pharmacology and documented that *Mitragyna* alkaloids binding at the μ-opioid receptor is distinct from that of classical opioids (Henningfield *et al.*, 2022a).

Inter-species differences were identified between mitragynine efficacy at the μ-opioid receptor. Mitragynine is a competitive antagonist at the mouse μ-opioid receptor but a weak partial agonist (median effective concentration [EC_{50}] = 339 ± 178 nM; maximal efficacy [E_{max}] = 34%) at the human μ-opioid receptor in a range of *in vitro* bioluminescence resonance energy transfer functional assays (Kruegel *et al.*, 2016). This binding affinity is much lower than what was previously reported for mitragynine (Takayama *et al.,* 2002; Matsumoto *et al.*, 2004). The inhibitory constant (K_i) of mitragynine at the μ-opioid receptor was reported to range from 161 to 230 nM (Váradi *et al.*, 2016; Maxwell *et al.*, 2021).

At the κ-opioid receptor, Obeng *et al.* (2020) reported the K_i of mitragynine as 198 ± 30 nM. In a study of synthetic and receptor signaling of the *Mitragyna* alkaloids, mitragynine was a competitive antagonist with a median inhibitory concentration (IC_{50}) of 8.5 ± 7.6 μM and a pA_2 value (an estimate of dissociation constant [K_d]) of 1.4 ± 0.40 μM, which contrasts its observed partial agonist activity at the μ-opioid receptor (Kruegel *et al.*, 2016). In the same study, mitragynine was also characterized as a low-potency antagonist at the human δ-opioid receptor.

Radioligand binding inhibition by mitragynine on different receptor systems in *in vitro* human receptor models is summarized in Table C.3.2.1-1, below, as reported by Boyer *et al.* (2008).

Based on the evidence presented, mitragynine's pharmacology is distinct from prototypical opioids. Unlike full μ-opioid agonists such as morphine and fentanyl, mitragynine does not activate the β-arrestin-2 pathway implicated in the adverse effects of μ-opioid agonists including respiratory depression and constipation.

Table C.3.2.1-1 Inhibition of Radioligand Binding by Mitragynine at Select *in vitro* Human Receptor Systems (Reproduced from Boyer *et al.*, 2008)

Receptor System	% Inhibition
Adenosine (A_{2A})	65.66
Adrenergic (α_2)	61.92
Dopamine (D_2S)	54.22
Opioid (μ)	89.52
Opioid (κ)	90.21
Opioid (δ)	7.00
Serotonin ($5HT_{2c}$)	58.77
Serotonin ($5HT_7$)	64.41

Table C.3.2.1-1 Inhibition of Radioligand Binding by Mitragynine at Select *in vitro* Human Receptor Systems (Reproduced from Boyer *et al.*, 2008)

Receptor System	% Inhibition
Dissociation constants for opioid receptor binding	
Opioid (μ)	204 ± 26 nM
Opioid (κ)	455 ± 47 nM
Opioid (δ)	2,250 ± 120 nM

C.3.2.1.1 Physicochemical Characteristics

Mitragynine's partition coefficient was 249 in heptane/phosphate buffer at pH 7.6 and its pKa was 7.06 in a study of molecular stereochemistry on the metabolism of corynantheidine-type alkaloids (Beckett and Morton, 1967). More recently, the Log P of mitragynine was reported as 1.73, based on ultraviolet absorption, with a pKa of 8.11 ± 0.11 (Ramanathan *et al.*, 2015).

In a comprehensive evaluation of the physicochemical characteristics of mitragynine and a *M. speciosa* alkaloid extract [produced using a methanol-chloroform and acid-base extraction method described by Kong *et al.* (2011)], parameters such as aqueous solubility, plasma protein binding, metabolic stability, and permeability were evaluated (Kong *et al.*, 2017a). The alkaloid extract and mitragynine were highly soluble in aqueous solution at pH 4.0 but less soluble at pH 7.4, resulting in greater absorption from the intestine than in the stomach. The metabolic stability of mitragynine in both preparations was assessed following a 30-minute incubation with rat liver microsomes. Results indicated high metabolic stability (89.8 ± 1.5% for the alkaloid extract and 84.5 ± 1.9% for mitragynine) (Kong *et al.*, 2017a). Alkaloids in the extract and mitragynine were further tested in a plasma protein binding assay conducted by incubating human plasma with 4 μg/mL alkaloid extract or 10 μM for 1 hour at 37°C. The alkaloid extract and mitragynine were moderately bound (92.2 and 85.4%, respectively) to human plasma suggesting low distribution and drug clearance (Kong *et al.*, 2017a). Similar results utilizing equilibrium dialysis found 95% plasma protein binding when 5 to 15 μM mitragynine was incubated with human plasma at 37°C for 24 hours (Manda *et al.*, 2014).

C.3.2.1.2 Metabolism Studies Conducted *in vitro*

In vitro mitragynine metabolism studies are helpful in suggesting pharmacokinetic pathways and rates of metabolism. Comprehensive summaries of studies conducted to investigate the metabolism of mitragynine and alkaloid-containing kratom extracts *in vitro* are included below.

7-Hydroxymitragynine was first demonstrated as the major metabolite of mitragynine (65.8%) following *O*-demethylation in rabbit liver microsomes (Beckett and Morton, 1967). The primary cytochrome P450 (CYP) enzyme responsible for mitragynine metabolism is the most abundant CYP3A4 enzyme, with minor contribution from CYP2D6 and CYP2C9 (Kamble *et al.*, 2019). In human liver microsomes, mitragynine was extensively metabolized, primarily to its *O*-demethylated and monooxidative metabolites, with CYP3A4 playing the predominant role in the formation of 7-hydroxymitragynine. CYP2D6 polymorphism produced metabolites of minor abundance, indicating that polymorphisms were of minimal importance.

P-glycoprotein is important for removing potentially toxic compounds from the brain; thus, knowing if mitragynine and P-glycoprotein interact is important if mitragynine is co-administered with drugs that are P-glycoprotein substrates. In an *in vitro* absorption, distribution, metabolism, and excretion (ADME) study, mitragynine inhibited P-glycoprotein activity with an EC_{50} of 18.2 μM (Manda *et al.*, 2014). Mitragynine also showed moderate permeability across MDR-MDCK monolayers (model for the blood:brain barrier [BBB]) with no significant efflux, resulting in the classification of diffusible for mitragynine. In studies of different

drug interactions with the adenosine triphosphate (ATP)-dependent P-glycoprotein efflux mechanism, mitragynine did not significantly stimulate ATPase or P-glycoprotein activity compared to the non-stimulation propranolol control (Meyer *et al*., 2015). However, later, an increase in P-glycoprotein activity by kratom extract (3-fold), the alkaloid fraction (4-fold), and 9 individual alkaloids (4- to 6-fold) was shown due to activation of the pregnane X receptor that increases expression of P-glycoprotein (Manda *et al*., 2017). Ya *et al*. (2019) cautioned that considering free mitragynine concentrations and the concentrations necessary to produce these effects, these findings may not be clinically relevant.

Mitragynine pharmacokinetics in the brain were further investigated in an *in vitro* primary porcine brain endothelial cell BBB model (Yusof *et al.*, 2019). The rates of mitragynine and 7-hydroxymitragynine passage and interaction with the efflux transporter P-glycoprotein showed that mitragynine crossed from the apical-to-basolateral (blood-to-brain) side more readily than its more polar metabolite, 7-hydroxymitragynine; however, p-glycoprotein effluxed both compounds. Mitragynine had an 18-fold higher brain tissue uptake in the brain slice assay compared to 7-hydroxymitragynine. Mitragynine accumulated inside brain parenchymal cells, while 7-hydroxymitragynine entry was restricted. Together, these data document rapid absorption of mitragynine, but not 7-hydroxymitragynine, across the BBB (Ramanathan *et al.*, 2015).

Mitragynine also showed moderate permeability across Caco-2 cells (similar cells to those at the intestinal barrier) (Manda *et al.*, 2014). In addition, transport studies across Caco-2 cell monolayers were performed to evaluate the extent of oral absorption in human intestines (Meyer *et al.*, 2015). Mitragynine was a weak inhibitor of P-glycoprotein transport in both apical to basal and basal to apical membrane transfers. Transport across Caco-2 cell monolayers is not reflective of transport across the BBB but suggests possible interactions affecting oral absorption of P-glycoprotein substrates in the intestine. The Parallel Artificial Membrane Permeability Assay (PAMPA) showed high alkaloid and mitragynine membrane permeability at pH 7.4 but low permeability at pH 4.0 due to the higher charge of the mitragynine amine group at the lower pH (Kong *et al.*, 2017a). These data indicate rapid absorption of mitragynine across intestinal membranes at a neutral pH and lower absorption in acidic environments such as the stomach (Ramanathan *et al.*, 2015).

Mitragynine was stable in simulated intestinal fluid, human liver microsomes, and S9 fractions (Manda *et al*., 2014). The wide variability observed in pharmacological studies of mitragynine was attributed to its hydrophobicity, poor water solubility (<100 µg/mL), high variability of release in simulated biological fluids, and acid degradation characteristics (Ramanathan *et al*., 2015).

In a recent *in silico* investigation utilizing quantum chemical calculations to complement experimental nonclinical findings, mitragynine primarily metabolized *via* hydrolysis of the methyl ester at position 16, O-demethylation of the 9-methoxy group, and O-demethylation of the 17-methoxy group (Limpanuparb *et al*., 2019). Further metabolism included subsequent conjugation with glucuronic acid at 1 of these 3 positions, subsequent conjugation with sulfate only at the 9-methoxy group, and combinations of these steps.

Mitragynine is primarily metabolized by CYP3A4, the most abundant CYP enzyme, with minor contributions from CYP2D6 and CYP2C9. CYP2D6 polymorphism produced metabolites of minor abundance, indicating that polymorphisms were of minimal importance. Mitragynine also crossed the BBB more readily than its metabolite 7-hydroxymitragynine but was not a P-glycoprotein substrate in the brain. Mitragynine's permeability at pH 7.4 is much higher than at pH 4.0, which indicates that primary absorption will through the intestine rather than the stomach. In an intestinal model cell assay, mitragynine was a weak P-glycoprotein inhibitor but not a substrate, consistent with its activity at brain P-glycoprotein.

C.3.2.1.3 Metabolism Studies Conducted *in vivo*

In vivo mitragynine pharmacokinetics were variable based on the model, species and gender, vehicle, whether the alkaloid was pure or part of an extract, and whether the extract was aqueous, methanolic, or an alternate preparation.

C.3.2.1.3.1 Summaries of Studies Conducted in Rodents

In a study of rodent mitragynine metabolism, 6 rats received a single oral dose of 40 mg/kg mitragynine dissolved in propylene glycol, followed by blood collection for up to 15 hours (Janchawee *et al.*, 2007). Mitragynine was rapidly absorbed with a C_{max} of 630 ± 180 ng/mL at 1.83 ± 1.25 hours, and a T_{max} with an absorption rate constant (K_a) of 1.43 ± 0.90 h^{-1} and a high volume of distribution (V_d)/oral bioavailability (F) of 89.5 ± 30.3 L/kg. The elimination rate constant (λz) was 0.07 ± 0.01 h^{-1}, with a clearance (CL)/F) of 1.60 ± 0.58 L/h. The half-lives of absorption ($t_{1/2\ ab}$) and elimination ($t_{1/2\ \lambda z}$) were 0.48 ± 0.36 and 9.43 ± 1.74 hours, respectively. The mean residence time ($MRT_{0\rightarrow\infty}$) was 14.0 ± 2.8 hours. Detection was based on ultraviolet (UV) absorption offering limited sensitivity and specificity.

Single oral doses of 50, 100, 200 and 400 mg/kg oral methanolic kratom extract provided dose-dependent protection against castor oil-induced diarrhea in rats and inhibited intestinal transit (Chittrakarn *et al.*, 2008). The antidiarrheal effect was not antagonized by naloxone indicating the effect was not solely mediated by the μ-opioid receptor. Extract treatment for 15 and 30 days did not decrease intestinal transit time indicating that adaptation had occurred. There were no effects on cholecystokinin concentrations that regulate intestinal motility and suppress food intake by inhibiting gastric emptying.

Following administration of a lower single oral dose of 20 mg/kg to 8 rats, the mean ± standard error of the mean (SEM) plasma C_{max} was 424 ± 61.8 ng/mL occurring with a mean T_{max} of 1.3 ± 0.2 hours, and a mean $t_{1/2\ \lambda z}$ of 3.9 ± 0.5 hours (de Moraes *et al.*, 2009). The mean apparent total clearance was 6.4 ± 0.4 L/hour·kg and the mean apparent V_d was 37.9 ± 0.5 L/kg based on liquid chromatography with tandem mass spectrometry (LC-MS/MS) identification and quantification of mitragynine.

Philipp et al. (2009) identified 7 phase I mitragynine metabolites, 1 sulphonate, and 4 glucuronide phase II metabolites in Wistar rat urine 24 hours after 40 mg/kg gastric intubation of mitragynine. The phase I metabolites included the hydrolysis product of the methylester in position 16, O-demethylation of the 9-methoxy and the 17-methoxy group, followed by intermediate aldehydes and oxidation to carboxylic acids or reduction to alcohols. The phase I metabolites included 9-O-desmethylmitragynine; 16-carboxymitragynine; 9-O-desmethyl-16-carboxymitragynine; 17-O-desmethyl-16,17-dihydromitragynine; 9,17-O-bisdesmethyl-16,17-dihydromitragynine; 17-carboxy-16,17-dihydromitragynine; and 9-O-desmethyl-17-carboxy-16,17-dihydromitragynine. Phase II conjugation with sulphate or glucuronic acid followed.

In another study, the effects of methanolic, aqueous, and total alkaloid extracts on glutathione S-transferase (GST) activity were studied in male Sprague-Dawley rat liver cytosol (Azizi *et al.*, 2010). At the highest 750 μg/mL doses, GST activity was inhibited 61% by the methanolic, 50% by the aqueous, and 43% by the total alkaloid extract ($p<0.001$). However, in the *in vivo* rat study, GST activity increased after exposure to all 3 extracts, but significantly (129%) after the 100 mg/kg aqueous extract compared to the control. GST induction could increase protection against the toxic effects of electrophilic chemicals or metabolites.

Six rats received a single 1.5 mg/kg intravenous (IV) mitragynine or 50 mg/kg oral mitragynine dose in 20% Tween 20 aqueous solution, and after 2 weeks the alternate dose regimen (Parthasarathy *et al.*, 2010). Blood samples were collected for 24 hours after the IV and 48 hours after the oral mitragynine doses and analyzed by HPLC with UV detection. Following oral administration, mitragynine showed an erratic and prolonged absorption as indicated by longer T_{max} (4.5 ± 3.6 hours) and a low C_{max} of 700 ± 210 ng/mL, followed by gradual elimination. Mitragynine oral absorption was incomplete as evidenced by the 33-fold lower area under the concentration-time curve (AUC) compared to the IV dose. The calculated absolute bioavailability for mitragynine was 3.0%, presumably due to its poor aqueous solubility. These data were refuted by multiple later studies documenting higher bioavailability.

Eight rats received 5 mg/kg IV mitragynine in 1% Cremophore EL in saline *via* the jugular vein (Vuppala *et al.*, 2011). Plasma was analyzed for mitragynine in samples collected for 8 hours finding a mean ± standard deviation (SD) C_{max} of 3,900 ± 700 ng/mL and a mean T_{max} of 1 minute. The mean $t_{1/2}$ was 2.6 ± 0.4 hours, the mean Vd was 8.2 ± 2.2 L/kg, and the mean CL was 1.2 ± 0.2 L/hour·kg.

Azizi *et al.* (2013) treated Sprague-Dawley rats with methanolic (50, 100, and 200 mg/kg), aqueous (50, 100, and 200 mg/kg), and a total alkaloid extract (5, 10 and 20 mg/kg) of *M. speciosa via* oral gavage to study its effects on the CYP and uridine diphosphate (UDP)-glucuronosyl transferase (UGT) drug metabolizing enzymes. Significant inductive effects of mitragynine-containing extracts on UGT and CYP450 activity were observed, potentially increasing clearance of drugs metabolized through this pathway.

Phytochemicals present in the organic kratom fraction and lyophilized kratom tea increased the observed oral bioavailability of mitragynine in rats compared to those dosed with oral mitragynine alone (Jaiswal *et al.*, 2014). The authors suggested that presence of permeability/solubility enhancers, CYP450 enzyme inhibitors, and/or gastrointestinal motility inhibitors in the tea or its organic fraction improved mitragynine's oral bioavailability.

Six rats received single 10 mg/kg IV mitragynine doses in 10% Tween 20 (Kong *et al.*, 2017a). Blood and brain microdialysate samples were collected over 30 ± 2 minute(s) up to 7.5 hours. The ratio of the unbound concentration of mitragynine in the brain and plasma (AUC_{brain}/AUC_{plasma}) was calculated. Unbound mitragynine was detected in blood and brain within 30 minutes after administration, demonstrating rapid and good BBB permeability, with a C_{max} of 1,516 ± 145.3 ng/mL and 920 ± 51.2 ng/mL in blood and brain, respectively. Mitragynine elimination was biphasic with a sharp decrease in the initial plasma concentration (distribution phase) followed by slow drug clearance (elimination phase), with a long 13-hour *beta*-phase half-life. Large Vd 9.8 L/kg in plasma and 16.9 L/kg in brain was observed. Mitragynine showed good permeability across the BBB with an AUC_{brain}/AUC_{plasma} ratio of 65.83 ± 4.54%. High BBB permeability was not predicted based on reported high plasma protein binding. Tween 20 was utilized to improve solubility in blood and might have enhanced drug permeability into the central nervous system (CNS). Mitragynine showed a two-compartmental drug elimination pattern with half-life ($t_{1/2}$) of approximately 13 hours.

The pharmacokinetics of a single 5 mg/kg IV mitragynine hydrochloride dose was compared to those of a single 20 mg/kg oral mitragynine hydrochloride dose, lyophilized kratom tea, and 20 mg/kg mitragynine in the organic fraction of lyophilized kratom tea in rats (Avery *et al.*, 2019). After IV mitragynine, there was a biexponential decrease in the concentration-time profile, showing the fast distribution of mitragynine from the systemic circulation to the peripheral compartments. The V_d for the peripheral compartment (V2) was 6.3 ± 1.4 L/kg and 1.7 ± 0.2 L/kg for the central compartment (V1) demonstrating high tissue distribution. The terminal $t_{1/2}$ was 6.0 ± 1.5 hours. Oral bioavailability of mitragynine hydrochloride, lyophilized kratom tea, and the lyophilized kratom tea organic fraction was 1.5 to 1.8-fold higher than that of mitragynine alone. After any oral dose there were two C_{max}, the first by 1.5 hours post-dose and the second about 2.8 to 3.8 hours after dosing that could be due to enterohepatic recirculation (although this was not observed

after the IV dose), delayed gastric emptying, or variability in absorption. Clearance and V_d after oral mitragynine hydrochloride were 7.1 ± 0.5 L/hour·kg and 33.8 ± 3.1 L/kg, respectively. Oral bioavailability of mitragynine was 17.0, 25.1, and 31.2% for mitragynine hydrochloride, lyophilized kratom tea, and its organic fraction, respectively.

Jagabalan *et al.* (2019) investigated the intestinal permeability of mitragynine *in situ* in rat small intestine in the absence/presence of P-glycoprotein and/or CYP3A4 inhibitors. Mitragynine demonstrated high intestinal effective permeability (P_{eff}; 1.11 x 10^{-4} cm/second) like the highly permeable drug propranolol (P_{eff} 1.27 x 10^{-4} cm/second). Addition of azithromycin (P-glycoprotein inhibitor) and ciprofloxacin (CYP3A4 inhibitor) or both had no effect on intestinal permeability of mitragynine across the rat small intestine, suggesting that mitragynine is not a P-glycoprotein substrate.

The pharmacokinetics of 11 alkaloids (mitragynine, 7-hydroxymitragynine, corynantheidine, speciogynine, speciociliatine, paynantheine, corynoxine, corynoxine-B, mitraphylline, ajmalicine, and isospeciofoline) in 4 rats were described following administration of the traditional oral lyophilized kratom tea and a commercial kratom product, OPMS liquid shot in water (Kamble *et al*., 2021). The mitragynine content in these preparations was 366 mg/kg in the kratom tea corresponding to a human equivalent dose (HED) of 5.7 mg/kg and 9.6 mg/kg for OPMS. Only mitragynine, 7-hydroxymitragynine, speciociliatine, and corynantheidine were quantifiable at 8 hours post dose, and their dose-normalized systemic exposure was higher (1.6 to 2.4-fold) following the administration of the commercial OPMS liquid. The dose-normalized C_{max} values for mitragynine were 11.1 ± 1.1 ng/mL at a T_{max} of 1.3 ± 0.3 hours, and 11.7 ± 1.6 ng/mL at a T_{max} of 3.1 ± 1.7 hours following the kratom tea and OPMS liquid oral doses, respectively. Further, the dose-normalized $AUC_{0\text{-}24}$/dose for mitragynine was 83.7 ± 6.4 and 136.1 ± 13.1 h·kg·ng/mL/mg following kratom tea and OPMS liquid oral administration, respectively. These results indicate a slower rate of absorption and increased systemic exposure of mitragynine (1.6-fold) following the OPMS liquid dose as compared to kratom tea. However, no change in the percentage ratio of AUC_{0-24} of 7-hydroxymitragynine to AUC_{0-24} mitragynine (3.4% and 3.1% in kratom tea and OPMS liquid studies, respectively) was observed, suggesting that the extent of metabolism of mitragynine to 7-hydroxymitragynine with both formulations was comparable.

The 7-hydroxymitragynine dose was negligible (<0.1 mg/kg), but C_{max} of 4.3 ± 0.8 (kratom tea) and 4.0 ± 0.6 ng/mL OPMS were observed, consistent with the metabolism of mitragynine into 7-hydroxymitragynine (Kamble *et al.*, 2021). Since the elimination phase was not achieved for mitragynine or 7-hydroxymitragynine, *i.e.*, the percentages of area under the concentration-time curve from time zero extrapolated to infinity (% $AUC_{0\text{-}inf}$) were greater than 20%, the plasma $t_{1/2}$ and $AUC_{0\text{-}inf}$ were not calculated following the OPMS liquid dose. OPMS liquid showed an extended exposure of kratom alkaloids as compared to kratom tea.

A physiologically-based pharmacokinetic (PBPK) model that predicts mitragynine concentrations in plasma, lung, brain, liver, fat, and slowly and rapidly perfused tissues after IV and oral doses was developed from 2 rat studies and the Trakulsrichai *et al.* (2015) human study to guide optimal mitragynine dosing regimens (Ya *et al.*, 2021). The developed PBPK model included biologically relevant features of breast cancer-resistant protein (BCRP) in brain, CYP3A4-mediated metabolism in the liver, and diffusion-limited transport in fat. The AUC_{brain}/AUC_{plasma} ratio of mitragynine following oral administration is only half that compared to IV. Mitragynine as a highly lipophilic compound showed higher and more persistent concentrations in fat than other tissues. The estimated AUC_{brain}/AUC_{plasma} ratio was 0.66 following IV administration indicating the role of BCRP-mediated efflux transport. After oral administration, the predicted AUC_{brain}/AUC_{plasma} ratio was approximately 0.5, slightly lower than those of the IV administration potentially due to a slow dissolution rate from the gut and the low oral bioavailability of mitragynine.

C.3.2.1.3.2 Summaries of Studies Conducted in Dogs

The safety and pharmacokinetic properties of mitragynine in female Beagle dogs were evaluated after single oral 5 mg/kg and 0.1 mg/kg IV mitragynine doses in a cross-over design of oral dose, followed by a 3-week washout period before administration of the IV dose (Maxwell *et al.*, 2020). Plasma mitragynine concentrations using non-compartmental analysis following IV mitragynine showed a large V_d of 6.3 ± 0.6 L/kg and high clearance 1.8 ± 0.4 L/h/kg. After oral mitragynine, the first plasma mitragynine C_{max} was 278 ± 47.4 ng/mL occurring rapidly within 0.5 hours and the 7-hydroxymitragynine C_{max} was 31.5 ± 3.3 ng/mL at 1.7 ± 0.6 hours. A second C_{max} (237 ± 50 ng/mL) also was observed at 1.9 ± 0.7 hours. The authors suggested that delayed gastric emptying, absorption from multiple gastrointestinal sites, and/or the poor solubility of mitragynine in water could explain the multiple C_{max} values. The metabolic ratio calculated as AUC 7-hydroxymitragynine/AUC mitragynine after 5 mg/kg mitragynine was 12.6 ± 1.6%. After IV administration, 7-hydroxymitragynine concentrations were below the limit of quantification (LOQ). The bioavailability of oral mitragynine in dogs was 69.6% or 2.5- and 4.2-fold higher than that determined in rats (Kruegel and Grundmann, 2018; Avery *et al.*, 2019).

No major adverse events occurred after either dose, although all dogs experienced mild transient sedation immediately after dosing that lasted 2 to 4 hours after the oral 5 mg/kg dose and for up to 1 hour following the 0.1 mg/kg IV dose (Maxwell *et al.*, 2020). Stress-related signs such as pacing, barking, jumping, and spinning were reduced. After oral mitragynine, 2 dogs displayed lip licking and excessive drooling, likely due to poor palatability of the oral solution. Although nausea cannot be excluded, excessive drooling resolved within 30 minutes, and all dogs ate readily when offered food 2 hours after dosing. One dog transiently panted. There were no clinically significant changes in vital signs, physical examinations, clinical laboratory tests, or hematology results after either dose. The observed mild hypoglycemia possibly was due to delayed sample processing. During IV dose selection, generalized hives and hyperthermia were noted in 1 dog at 1 mg/kg mitragynine, and they resolved after diphenhydramine injection.

The oral bioavailability for mitragynine in rodents appears to be between 17 to 32%. In canines, a single study computed oral bioavailability to be 69%—more than 2-fold greater than that of rodents. Mitragynine and alkaloid-containing extractions induced GST activity, which may increase protection from toxic electrophilic molecules or metabolites, and induced CYPs and UGT drug-metabolizing enzymes that may increase clearance of drugs metabolized through this pathway. Recent *in vivo* rodent studies indicate mitragynine is not a P-glycoprotein substrate. The metabolic ratio of mitragynine to 7-hydroxymitragynine in canines was 12.6 ± 1.6%.

C.3.2.1.4 Human Metabolism Studies

Mitragynine metabolites were identified in human urine from mitragynine users and compared to mitragynine metabolites in rat urine after controlled gastric intubation (Philipp *et al*., 2009). Mitragynine was extensively metabolized in rats and humans with some differences in phase I metabolism but greater differences in phase II metabolism. In humans, 3 sulphate and 3 glucuronide metabolites were identified, while in rats, 4 glucuronide and 1 sulphate conjugate were observed. Human urine samples (n=120) sent for identification of kratom or krypton intake (kratom and O-desmethyltramadol) showed the presence of mitragynine, 9-O-desmethyl-16-carboxymitragynine, 9-O-desmethyl-mitragynine, 16-carboxy-mitragynine, 16-carboxy-paynantheine, 9-O-desmethyl-paynantheine, 9-O-desmethyl-speciogynine, 9-O-desmethyl-speciociliatine, and 16-carboxy-speciociliatine. The three most prevalent analytes were 9-O-desmethyl-16-carboxymitragynine, 9-O-desmethyl-mitragynine, and 16-carboxy-mitragynine, which identified kratom use in 98 of 120 human urine samples.

Le *et al.* (2012) provided urine mitragynine concentrations from individuals suspected of kratom use of 1.2 to greater than 50,000 ng/mL mitragynine. All samples also contained 7-hydroxymitragynine, and small concentrations of other metabolites, but no histories of use were available.

In the only human controlled mitragynine pharmacokinetics study conducted prior to 2022, 10 male chronic kratom users drank 6.3, 10 or 11.2 mg mitragynine in a tea decoction for 7 days to normalize mitragynine exposure prior to a loading dose of 6.3 to 23 mg mitragynine on Day 8 for the pharmacokinetics study (Trakulsrichai *et al.*, 2015). Different doses were utilized to confirm linearity in pharmacokinetics. Subjects were not required to stop their usual kratom intake outside of the study. One subject's pharmacokinetics were abnormal and were presented separately. For the other 9 subjects, T_{max} was 0.83 ± 0.35 hours, terminal $t_{1/2}$ was 23.2 ± 16.1 hours, V_d/F 38.0 ± 24.3 L/kg and the apparent total clearance was 98.1 ± 51.3 L/h/kg, where F is the bioavailability of mitragynine. The oral bioavailability of mitragynine was not known in humans, so the bioavailability data from rats, 3.0 ± 1.5%, was utilized for F. Notably, more recent data from Kruegel and Grundmann suggest that the true oral bioavailability of mitragynine in rats is approximately 20 to 30% (Kruegel and Grundmann, 2018); thus, these data utilizing a 3% oral bioavailability may not be accurate. C_{max} and AUC varied with dose, ranging from 18.5 to 105 ng/mL and 62 to 670 ng/h mL, and were dose-related. WinNonLin non-compartmental modeling of the data revealed a linear two-compartment model. One subject received the 10 mg dose for 7 days and on Day 8 displayed an abnormal drug concentration profile over time, with a C_{max} of about 32 ng/mL and a consistent concentration of about 20 ng/mL throughout the 25-hour monitoring period. Urine excretion of unchanged mitragynine was as low as 0.14%, documenting the importance of hepatic mitragynine metabolism. The long, terminal elimination $t_{1/2}$ and large V_d were different than in animal studies, suggesting pharmacokinetic differences between the species.

Lee *et al.* (2018) analyzed 10 human urine samples for mitragynine and its 2 major metabolites, 16-carboxy mitragynine and 9-O-demethyl mitragynine. 7-Hydroxymitragynine and its metabolites were not evaluated. Mitragynine glucuronide-conjugated metabolites were successfully hydrolyzed while sulphate-conjugated metabolites were resistant to enzyme hydrolysis.

Due to mitragynine's lipophilicity and poor water solubility at physiological pH, mitragynine is classified as a Class II Drug according to the Biopharmaceutical Classification System (BCS) (Ya *et al.*, 2019). Dissolution is one of the major factors influencing mitragynine oral bioavailability indicating that development of an appropriate salt form of mitragynine may increase its oral bioavailability.

A tea was prepared by steeping 2 g kratom containing 39 mg mitragynine, 11.8 mg paynantheine, 10.2 mg speciociliatine, 6.4 mg speciogynine, 1.3 mg mitraciliatine, 1.2 mg isopaynantheine, and no detectable 7-hydroxymitragynine in hot water (Tanna *et al.*, 2022). The tea was consumed within 10 minutes by 6 healthy participants with a history of kratom use and willingness to abstain from kratom for several weeks. Blood was collected within the research unit for 12.25 hours, and daily up until 120 hours after dosing when participants returned to the clinic. Pooled urine was collected from 0 to 12 and 12 to 24 hours on Day 1 after dosing, followed by 24-hour pooled collections for the next 4 days. Samples were analyzed by LC-MS/MS with a LOQ of 0.23 nM. A median concentration-time profile was used rather than individual concentration-time curves to derive the pharmacokinetic data due to sparse data points and multiple peaks in individual participants during the absorption phase, presumably due to delayed gastric emptying. Eight participants were enrolled; 2 female participants were withdrawn due to nausea and vomiting. Of the 6 participants, 4 were White (1 male, 3 females), 1 was Black (male) and 1 was Multiracial (male), and their ages ranged from 26 to 40 years. Five completed the study and there were no serious adverse effects. Other than the nausea and vomiting experienced by the 2 withdrawn participants, the kratom tea was well tolerated.

Noncompartmental analysis of pharmacokinetic parameters of 7 measured alkaloid components of a well-characterized kratom product, following consumption of the product as a tea, are presented in Table C.3.2.1.4-1, below. This table was reproduced from the study conducted by Tanna *et al.* (2022). Similarly, plasma concentration-time profiles for mitragynine, mitragynine diastereomers, 7-hydroxymitragynine, paynantheine, and isopaynantheine following oral kratom administration are presented in Figure C.3.2.1.4-1—also reproduced from Tanna *et al.* (2022).

Compartmental analysis of the median concentration time profiles of all kratom alkaloids revealed that a two-compartment model with first-order input and elimination rate, elimination from the central compartment, and a lag time with a weighting factor of $1/y^2$ best described the data based on goodness-of-fit criteria. All alkaloids were quantifiable in the plasma within 15 minutes after tea consumption. Pronounced pharmacokinetic differences between alkaloids with the 3S configuration (mitragynine, speciogynine, paynantheine) and alkaloids with the 3R configuration (mitraciliatine, speciociliatine, isopaynantheine) were attributed to differences in V_d and clearance. The 7-hydroxymitragynine/mitragynine C_{max} and $AUC_{0\text{-}inf}$ ratios ranged from ~0.24 to 0.27. As 7-hydroxymitragynine was below the LOQ in the ground powder, the 7-hydroxymitragynine was produced by mitragynine metabolism. Minimal amounts of the kratom alkaloids were excreted unchanged in the urine.

The 3S alkaloids exhibited a shorter median time to maximum concentration (1 to 2 *vs.* 2.5 to 4.5 hours), lower AUC (430 to 490 vs. 794 to 5,120 nM * hour), longer terminal $t_{1/2}$ (24 to 45 vs. ~12 to 18 hours), and higher apparent V_d during the terminal phase (960 to 12,700 vs. ~46 to 130 L) compared to the 3R alkaloids. Follow-up mechanistic *in vitro* studies suggested differential hepatic/intestinal metabolism, plasma protein binding, blood to-plasma partitioning, and/or distribution coefficients to explain the pharmacokinetic differences between the 2 alkaloid types.

Table C.3.2.1.4-1 Noncompartmental-Analysis-Derived Pharmacokinetics of Kratom Alkaloids in Healthy Adult Participants (n=5 completers) Administered a Well-Characterized Kratom Product (2 g) as a Tea (reproduced from Tanna *et al.*, 2022)

Alkaloid (mg/g kratom powder)	Median (Range)			
	Plasma		Urine	
Mitragynine (19.48 ± 0.81)	$t_{1/2}$ (h)	45.3 (31.9–50.2)	A_e (nmol)	102 (78–134)
	T_{max} (h)	1 (0.75–1.5)	f_e	0.0010 (0.0008–0.0013)
	C_{max} (nM)	81.9 (50.1–177)	CL_R (L/h)	0.194 (0.129–0.291)
	$AUC_{0\text{-}120}$ (nM×h)	388 (300–1,240)		
	$AUC_{0\text{-}inf}$ (nM×h)	420 (324–1,360)		
	V_z/F (L)	12,700 (5,190–19,700)		
	CL/F (L/h)	233 (71.7–302)		
Speciogynine (3.18 ± 0.13)	$t_{1/2}$ (h)	23.5 (16.1–28.3)	A_e (nmol)	258 (210–317)
	T_{max} (h)	2 (1–3.5)	f_e	0.016 (0.013–0.020)
	C_{max} (nM)	51.4 (34.2–121)	CL_R (L/h)	0.451 (0.282–0.723)
	$AUC_{0\text{-}120}$ (nM×h)	469 (368–1,080)		
	$AUC_{0\text{-}inf}$ (nM×h)	477 (379–1,120)		
	V_z/F (L)	962 (584–1,235)		
	CL/F (L/h)	33.5 (14.3–42.1)		
Mitraciliatine (0.647 ± 0.035)	$t_{1/2}$ (h)	17.8 (11.2–24.7)	A_e (nmol)	586 (461–744)
	T_{max} (h)	4.5 (3.5–6.5)	f_e	0.18 (0.14–0.23)
	C_{max} (nM)	73.5 (34.9–98.6)	CL_R (L/h)	0.361 (0.271–0.481)
	$AUC_{0\text{-}120}$ (nM×h)	1,160 (1,030–3,460)		

Table C.3.2.1.4-1 Noncompartmental-Analysis-Derived Pharmacokinetics of Kratom Alkaloids in Healthy Adult Participants (n=5 completers) Administered a Well-Characterized Kratom Product (2 g) as a Tea (reproduced from Tanna *et al.*, 2022)

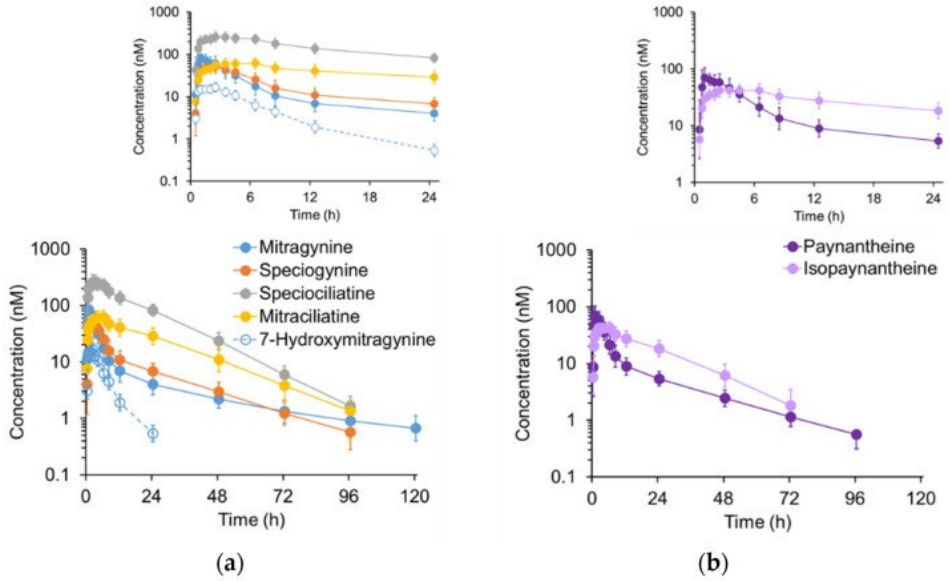
Alkaloid (mg/g kratom powder)	Median (Range)			
	Plasma		Urine	
	$AUC_{0\text{-}inf}$ (nM×h)	1,160 (1,040–3,520)		
	V_z/F (L)	46.0 (26.2–74.0)		
	CL/F (L/h)	2.78 (0.92–3.11)		
Speciociliatine (5.12 ± 0.26)	$t_{1/2}$ (h)	12.3 (10.4–21.1)	A_e (nmol)	2,350 (1,920–2,870)
	T_{max} (h)	2.5 (1–3.5)	f_e	0.091 (0.075–0.11)
	C_{max} (nM)	308 (154–380)	CL_R (L/h)	0.482 (0.327–0.709)
	$AUC_{0\text{-}120}$ (nM×h)	5,110 (3,190–7,550)		
	$AUC_{0\text{-}inf}$ (nM×h)	5,120 (3,200–7,560)		
	V_z/F (L)	130 (60.1–159)		
	CL/F (L/h)	5.01 (3.40–8.04)		
Paynantheine (5.86 ± 0.26)	$t_{1/2}$ (h)	27.0 (17.7–30.8)	A_e (nmol)	101 (81.9–124)
	T_{max} (h)	1 (0.75–2.5)	f_e	0.0034 (0.0028–0.0042)
	C_{max} (nM)	61.1 (56.4–157)	CL_R (L/h)	0.185 (0.115–0.296)
	$AUC_{0\text{-}120}$ (nM×h)	428 (383–917)		
	$AUC_{0\text{-}inf}$ (nM×h)	438 (389–956)		
	V_z/F (L)	1,940 (1,370–2,620)		
	CL/F (L/h)	67.4 (30.9–76.0)		
Isopaynantheine (0.512 ± 0.010)	$t_{1/2}$ (h)	14.4 (11.8–20.9)	A_e (nmol)	269 (226–320)
	T_{max} (h)	4.5 (2.5–6.5)	f_e	0.10 (0.087–0.12)
	C_{max} (nM)	48.8 (26.2–68.2)	CL_R (L/h)	0.262 (0.172–0.401)
	$AUC_{0\text{-}120}$ (nM×h)	784 (662–2,040)		
	$AUC_{0\text{-}inf}$ (nM×h)	794 (667–2,130)		
	V_z/F (L)	55.5 (36.6–76.0)		
	CL/F (L/h)	3.25 (1.21–3.87)		
7-Hydroxymitragynine (<LOQ[a])	$t_{1/2}$ (h)	5.67 (5.03–6.52)	A_e (nmol)[b]	179 (120–268)
	T_{max} (h)	1 (0.75–2.5)	f_e	NA
	C_{max} (nM)	16.1 (11.9–22.2)	CL_R (L/h)	2.03 (1.57–2.63)
	$AUC_{0\text{-}120}$ (nM×h)	103 (57.5–120)		
	$AUC_{0\text{-}inf}$ (nM×h)	106 (60.8–126)		
	$C_{max,m}/C_{max,p}$	0.27 (0.07–0.28)		
	$AUC_{0\text{-}inf,m}/AUC_{0\text{-}inf,p}$	0.24 (0.07–0.29)		

A_e = cumulative amount excreted unchanged in the urine from time zero to 120 h; $AUC_{0\text{-}120}$ = area under the concentration-time curve over 120 hours; AUC_{inf} = area under the concentration-time curve from time zero extrapolated to infinity; $AUC_{0\text{-}inf,m}/AUC_{0\text{-}inf,p}$ = 7-hydroxymitragynine-to-mitragynine $AUC_{0\text{-}inf}$ ratio; CL/F = oral clearance; CL_R = renal clearance; C_{max} = maximum plasma concentration; f_e = fraction of amount of kratom alkaloid measured in 2 g of K51 powder excreted unchanged in the urine; h = hour(s); LOQ = limit of quantification; NA = not applicable; $t_{1/2}$ = terminal half-life; T_{max} = time to reach C_{max}; $C_{max,m}/C_{max,p}$ = 7-hydroxymitragynine-to-mitragynine C_{max} ratio; V_z/F = apparent volume of distribution during the terminal phase.

[a] LOQ of the analytical method was not defined by study authors.

[b] Calculated based on unhydrolyzed urine data.

Figure C.3.2.1.4-1 Plasma Concentration-Time Profiles for (a) Mitragynine, Mitragynine Diastereomers, and 7-Hydroxymitragynine, and (b) Paynantheine and Isopaynantheine Following Oral Administration of Kratom (reproduced from Tanna *et al.*, 2022)



h = hour(s).
Kratom tea was prepared with 2 g of yellow Indonesian Micro Powder (K51) in 240 mL hot water (80°C), which was allowed to steep for 3 minutes. A sugar packet (4 g) was added to improve palatability. The prepared tea was cooled to 50°C before administration to the participants. Symbols and error bars denote geometric means and 90% confidence intervals, respectively. Insets show the 0- to 24-hour profiles to better visualize alkaloid concentrations during the intensive sampling period after administration of the kratom tea.

C.3.2.2 Nonclinical Toxicological Studies

C.3.2.2.1 *In vitro* Studies of Mitragynine and *M. speciosa*

The potential genotoxicity, mutagenicity, and cytotoxicity of mitragynine and extracts of *M. speciosa* were evaluated in a wide range of *in vitro* tests and the results are presented in Table C.3.2.2.1-1. The results from standard genotoxicity and mutagenicity studies (*i.e.*, bacterial reverse mutation, mouse lymphoma gene mutation, and comet assays) conducted with purified mitragynine or aqueous *M. speciosa* extract were consistently negative (Ghazali *et al.*, 2011; Saidin *et al.*, 2015; Oliveira *et al*., 2016) and consistent with experimental data presented in Section C.2. The only positive result was a genotoxicity study conducted by Oliveira *et al.* (2016). However, the samples tested were purchased online and at retail stores in Europe and lacked appropriate labeling. Additionally, while the samples were tested for mitragynine content, they were not tested for adulterants or other ingredients that may have elicited these results.

Moreover, the cytotoxicity of *M. speciosa* extracts and mitragynine was evaluated in numerous studies conducted with a wide range of cell types. Briefly, an aqueous extract of *M. speciosa* was not observed to elicit cytotoxicity at concentrations up to 500 µg/mL (Goh *et al.*, 2021). Other studies conducted with methanolic extracts of *M. speciosa* and mitragynine had IC_{50} values of 8 to 134 µg/mL and 0.3 to 240 µM, respectively (Jamil *et al.*, 2013; Goh *et al.*, 2014, 2021; Kong *et al.*, 2017b; Matsunaga *et al.*, 2017; Rusli *et al.*, 2019; Domnic *et al.*, 2021). Additionally, in a series of cell patch-clamp assays, the IC_{50} values were reported to range from 333 to 910 nM mitragynine (Lu *et al.*, 2014; Tay *et al.*, 2019).

Table C.3.2.2.1-1 Summary of *in vitro* Studies Conducted with *Mitragyna speciosa* and Mitragynine Preparations

Test Article	Assay	Test System	Concentrations (+/- metabolic activation)	Result[a]	Reference
Mitragyna speciosa aqueous extracts					
M. speciosa aqueous extract (mitragynine content NR)	Bacterial reverse mutation assay	*Salmonella* Typhimurium TA98 and TA100	3.1, 12.5, or 50 mg/mL (± S9)	Negative	Ghazali *et al.* (2011)
M. speciosa methanolic extracts					
M. speciosa leaf alkaloid extract; methanol-chloroform and acid-base extractions (40.9% mitragynine)	XTT cell proliferation assay	Human (HepG2 and WRL 68) and rat (Clone 9) hepatocytes	0.05–300 µg/mL	IC_{50} values: HepG2 = 22.9 µg/mL WRL 68 = 27.4 µg/mL Clone 9 = 37.0 µg/mL	Kong *et al.* (2017b)
M. speciosa methanolic alkaloid extract powders; (0.08–19% mitragynine)	Comet assay	Intestinal Caco-2 and neuronal SH-SY5Y cells	0, 2.5, 5, 10, 20, 30, 40, and 60 µg/mL	IC_{50} values: Caco-2 = 9–49 µg/mL; SH-SY5Y = 2–15 µg/mL; Comet assay = Positive	Oliveira *et al.* (2016)
Kratom extracts (2–7% mitragynine) (MeOH, EtOH, and EtOAc)	MTT cell viability assay	HEK293 and HeLa Chang liver cells	7.8–500 µg/mL	IC_{50} >500 µg/mL for aqueous, methanolic, and ethanolic extracts	Goh *et al.* (2021)
M. speciosa leaf methanolic extract (30% mitragynine)	Cell patch-clamp assay	HEK293-hERG1a/1b recombinant cells	0.001–10 µM	IC_{50} = 333 nM	Tay *et al.* (2019)
M. speciosa leaf methanolic extract (30% mitragynine)	Cell patch-clamp assay	hERG-HEK cardiomyocytes	0.01–100 µM	IC_{50} = 910 nM	Lu *et al.* (2014)
M. speciosa leaf methanolic and alkaloid extracts (not further characterized)	Cytotoxicity assay	Nasopharyngeal carcinoma (NPC)/ HK1 cell	Methanolic extract, up to 300 µg/mL Alkaloid extract, up to 100 µg/mL	Methanolic extract IC_{50} = 134 µg/mL Alkaloid extract IC_{50} = 32 µg/mL	Domnic *et al.* (2021)
M. speciosa methanol-chloroform extract (42% mitragynine)	Cytotoxicity assay	HepG2, HEK293, MCL-5, cHol, SH-SY5Y cells	10, 50, 100, 250, 500, 1,000 µg/mL	IC_{50} values: HepG2 = 230.8 µg/mL; MCL-5 = 410 µg/mL; cHol = 282.1 µg/mL; HEK293 = 282.1 µg/mL; SY5Y = 91.2 µg/mL	Saidin (2008)
M. speciosa methanolic leaf extract (30% mitragynine)	Cytotoxicity assay	Caco-2 cells	0.001–10 µM mitragynine	8 µg/mL	Rusli *et al.* (2019)
M. speciosa methanol-chloroform extract (42% mitragynine)	Gene mutation assay (MLA)	L5178Y	5–40 µg/mL	Negative	Saidin (2008)

Table C.3.2.2.1-1 Summary of *in vitro* Studies Conducted with *Mitragyna speciosa* and Mitragynine Preparations

Test Article	Assay	Test System	Concentrations (+/- metabolic activation)	Result[a]	Reference
Mitragynine					
Pure mitragynine (purity NR)	Comet assay	Intestinal Caco-2 and neuronal SH-SY5Y cells	2.5 and 5.0 µg/mL	Negative	Oliveira *et al.* (2016)
Mitragynine (98% purity) prepared from *M. speciosa*	Mouse lymphoma gene mutation assay	L5178 TK murine lymphoma cells	0–75 µM (± S9)	Negative	Saidin *et al.* (2015)
	Trypan blue exclusion cell viability assay	HEK293, MCL-5, and SH-SY5Y cells	NR	IC_{50} values: HEK293 = 75 µM; MCL-5 = 240 µM; SH-SY5Y = 80 µM	
Mitragynine (98% purity) extracted and isolated from Kratom leaf	AlamarBlue® cell viability assay	SK-N-SH neuroblastoma cells	3.9–1,257 µM	IC_{50} = 77.3 µM	Jamil *et al.* (2013)
Mitragynine (99% purity) extracted and isolated from Kratom leaf	MTT cell antiproliferation assay	HCT116 and K562 cancer cell lines	6.5–200 µM	IC_{50} values: HCT116 = 47.1 µM; K562 = 25.2 µM	Goh *et al.* (2014)
Mitragynine (≥99% purity)	MTT cell viability assay	HEK293 and HeLa Chang liver cells	7.8–500 µg/mL	IC_{50} values: HEK293 = 112 µM; HeLa Chang = 210 µM	Goh *et al.* (2021)
Pure mitragynine (purity NR)	XTT cell proliferation assay	Human (HepG2 and WRL 68), and rat (Clone 9) hepatocytes	0.01–120 µg/mL	IC_{50} values: HepG2 = 10.6 µg/mL; WRL 68 = 6.4 µg/mL; Clone 9 = 16.0 µg/mL	Kong *et al.* (2017b)
Pure mitragynine (purity NR)	Cytotoxicity assay	Human aortic endothelial cells	20 and 60 µM	LC_{50} values: HAE = 43.1 µg/mL; BEAS-2B = 67.2 µg/mL; SK-N-SH = 50.9 µg/mL	Matsunaga *et al.* (2017)
Pure mitragynine (purity NR)	Cytotoxicity assay	HepG2, HEK293, MCL-5, cHol, SH-SY5Y cells	0.3–75 µM	IC_{50} values: SH-SY5Y = 75 µM HEK293 = 240 µM	Saidin (2008)
Pure mitragynine (purity NR)	Mouse lymphoma gene mutation assay	L5178Y murine lymphoma cells	5–30 µg/mL	Negative	Saidin (2008)

EtOAc = ethyl acetate; EtOH = ethanol; HEK = human embryonic kidney; hERG = human ether-a-go-go-related gene; IC_{50} = median inhibitory concentration; MeOH = methanol; MTT = 3-[4,5-dimenthylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide; NR = not reported; LC_{50} = median lethal concentration; XTT = 2,3-bis(2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxanilide.

[a] Results are reported in this table as either "negative" or "positive" as they pertain to mutagenicity or genotoxicity unless stated otherwise.

C.3.2.2.2 *In vivo* Studies of Mitragynine and *M. speciosa*

Numerous studies conducted to evaluate the toxicity of mitragynine or *M. speciosa* in animals are summarized in Table C.3.2.2.2-1. Briefly, the median lethal dose (LD_{50}) for oral *M. speciosa* extracts and isolated mitragynine in mice range from 173 to 4,900 mg/kg body weight and 477 to 548 mg/kg body weight, respectively. The test articles utilized in these tests were often poorly characterized and none of the referenced literature used a *M. speciosa* dried leaf suspension. Additionally, the use of extraction solvents other than water or aqueous ethanol to prepare a test article is considered a chemical alteration of the raw material. While Johnson Foods used a methanolic extract for an *in vitro* study of NPI-001 due to study feasibility issues, *in vivo* studies conducted using a dried leaf suspension are most relevant to the safety evaluation of NPI-001.

In a study of acute toxicity, Kamal *et al*. (2012) administered a single dose (175, 500, and 2,000 mg/kg body weight) of aqueous *M. speciosa* extract (not further characterized) to male and female Sprague-Dawley rats and observed a decrease in the mean corpuscular hemoglobin concentration in females, along with non-dose dependent findings in male hematological parameters. In a 10-day repeated-dose study conducted with an aqueous *M. speciosa* decoction (not further characterized), an increase in GST activity was observed following consumption of 0.175 mg/kg body weight/day (Guenther *et al.*, 2019 [abstract only]). In a separate study conducted in rats, aqueous *M. speciosa* extract (1% mitragynine) elicited an increase in GST activity in rats administered 100 mg/kg body weight/day over a 14-day repeated-dose test (Azizi *et al.*, 2010), though methanolic and total alkaloid extract did not have this effect. In this study, a NOAEL of 50 mg/kg body weight/day can be concluded for the *M. speciosa* aqueous extract test article.

In both the Kamal *et al.* (2012) and Guenther *et al.*, (2019) investigations, compositional data pertaining to the test articles were not provided, and thus the relevance to NPI-001 is unclear.

Respiration was measured in awake, freely moving CD-1 mice after oral morphine, mitragynine, 7-hydroxymitragynine, and the CYP3A inhibitor ketoconazole using whole body plethysmography (Hill *et al.*, 2022) Three mg/kg oral mitragynine did not produce significant changes in respiration, while 10, 30, and 90 mg/kg mitragynine induced significant prolonged respiratory depression that was not further increased at the higher doses. Oral administration of calculated equi-effective doses of mitragynine (5.5 mg/kg), 7-hydroxymitragynine (1.9 mg/kg), and morphine (3.8 mg/kg) induced significant respiratory depression. Pre-treatment with 50 mg/kg oral ketoconazole for 30 minutes significantly reduced mitragynine (5.5 mg/kg)-induced respiratory depression. Pre-treatment with the same dose and timing of ketoconazole did not affect 7-hydroxymitragynine respiratory depression. A ceiling effect was documented for the respiratory depressant effects of mitragynine as doses higher than 10 mg/kg produced the same level of effect, while 7-hydroxymitragynine induced a dose-dependent effect on mouse respiration. Inhibition of CYP3A metabolism reduced mitragynine-induced respiratory depression without impacting the effects of 7-hydroxymitragynine. The limited rate of conversion of mitragynine into its active metabolite produced a ceiling effect of the mitragynine-induced respiratory depression in mice. These data suggest that such "metabolic saturation" at high doses may underlie an improved safety profile of mitragynine; however, it is unclear if this effect in mice translates to human pharmacokinetics.

In a similarly designed study, Henningfield *et al.* (2022b), respiratory effects of oral mitragynine and oral oxycodone in rats were compared using a study design recommended by FDA scientists for evaluating the respiratory effects of opioids (Xu *et al.*, 2020). Blood gases, physiological and behavioral observations, and mitragynine pharmacokinetics were assessed for 12 hours after 20, 40, 80, 240 and 400 mg/kg oral mitragynine isolate and 6.75, 60 and 150 mg/kg oral oxycodone hydrochloride. Oxycodone administration produced significant dose-related respiratory depressant effects and pronounced sedation with 1 death

each at 60 and 150 mg/kg. Mitragynine did not yield significant dose-related respiratory depressant or life-threatening effects. Sedative-like effects, milder than produced by oxycodone, were evident at the highest mitragynine dose. Maximum oxycodone and mitragynine plasma concentrations were dose-related. Consistent with mitragynine's pharmacology that includes partial μ-opioid receptor agonism with little recruitment of the respiratory depressant activating β-arrestin pathway, mitragynine produced no evidence of respiratory depression at doses many times higher than known to be taken by humans.

Table C.3.2.2.2-1 Summary of the Toxicology Studies Conducted *in vivo* with *Mitragyna speciosa* and Mitragynine Preparations

Test Article	Species	Route and Dose (mg/kg bw/day)	Duration	Significant Findings	Reference
Mitragyna speciosa					
M. speciosa alkaloid extracts (solvent not specified)					
M. speciosa alkaloid extract (not further characterized)	Mouse (M; Swiss albino)	Oral; dose for each successive animal was adjusted up or down depending on the previous outcome	Single dose	LD_{50} = 173 mg/kg bw	Reanmongkol *et al.* (2007)
M. speciosa alkaloid extract (22% mitragynine; solvent not specified)	Mouse (M; Swiss albino)	Oral; 20, 50, 160, 320, and 400 mg/kg bw	Single dose	ED_{50} = 194 mg/kg for antinociceptive effects	Sabetghadam *et al.* (2013a)
		Oral; 175 and 2,000 mg/kg bw	Single dose	No toxic effects following administration of 175 mg/kg bw; however, all mice died at the 2,000 mg/kg dose level LD_{50} = 591 mg/kg bw	
M. speciosa aqueous extracts					
Aqueous *M. speciosa* extract (1% mitragynine)	Rat (M; Sprague-Dawley)	Oral (gavage); 50, 100, and 200 mg/kg bw/day	14 days	Increase in GST activity in rats administered 100 mg/kg bw/day	Azizi *et al.* (2010)
Aqueous *M. speciosa* decoction (tea; not further characterized)	Mouse (C57BL/6)	Oral (diet); 0.175 mg/kg bw/day	10 days	Increase in liver size observed following consumption of kratom tea	Guenther *et al.* (2019) [abstract only]
Aqueous *M. speciosa* extract (not further characterized)	Rat (Sprague-Dawley)	Oral (gavage); 175, 500, and 2,000 mg/kg	Single dose	Decrease in mean corpuscular hemoglobin concentration for females in the 500 and 2,000 mg/kg bw groups Decrease in albumin, calcium, and cholesterol for males in the 175 mg/kg bw group LD_{50} >2,000 mg/kg bw	Kamal *et al.* (2012)

Table C.3.2.2.2-1 Summary of the Toxicology Studies Conducted *in vivo* with *Mitragyna speciosa* and Mitragynine Preparations

Test Article	Species	Route and Dose (mg/kg bw/day)	Duration	Significant Findings	Reference
***M. speciosa* methanolic extracts**					
Methanolic *M. speciosa* extract (1.6% mitragynine)	Rat (M; Sprague-Dawley)	Oral (gavage); 50, 100, and 200 mg/kg bw/day	14 days	No evidence of toxicity present at any dose level Increase in GST activity in rats administered 100 mg/kg bw/day	Azizi *et al.* (2013)
Methanolic *M. speciosa* extract (not further characterized)	Mouse (M; Swiss albino)	Oral; dose for each successive animal was adjusted up or down depending on the previous outcome	Single dose	LD_{50} = 4,900 mg/kg bw	Reanmongkol *et al.* (2007)
Methanolic *M. speciosa* extract (not further characterized)	Rat (F; Sprague-Dawley)	Oral; 1,000 mg/kg bw (acute) or 500 mg/kg bw/day (subacute)	Single dose (acute) or 28 (subacute) days	Severe sinusoidal congestion with enlarged hepatocytes and numerous vacuolation observed in subacute group Increase in vacuolation of uterine tissue cell lining in subacute group	Sakaran *et al.* (2014)
Methanolic *M. speciosa* extract (1.6% mitragynine)	Rat (M; Sprague-Dawley)	Oral (gavage); 100, 500, and 1,000 mg/kg	14 days	No evidence of toxicity present at any dose level	Harizal *et al.* (2010)
Methanolic *M. speciosa* extract (not further characterized)	Rat (M; Sprague-Dawley)	Oral (gavage); 100, 200, and 500 mg/kg	28 days	Decrease in AST activity in rats administered 500 mg/kg bw/day	Ilmie *et al.* (2015)
Mitragynine (Purity Reported)					
Mitragynine (99.5% purity)	Mouse (M; Swiss Webster)	Oral; 62.5 to 500 mg/kg	NR	LD_{50} = 548 mg/kg	Smith *et al.* (2019)
Mitragynine (98% purity)	Rat (M; Wistar)	Oral (gavage); 60 mg/kg bw	Single dose	No observed adverse events	Janchawee *et al.* (2007)
Mitragynine (94.5% purity)	Rat (Sprague-Dawley)	Oral; 1, 10, or 100 mg/kg bw/day	28 days	Decreased bw in females in 100 mg/kg bw/day group Increased ALT and AST activity, and increased liver weight in rats from 100 mg/kg bw/day group	Sabetghadam *et al.* (2013b)
Mitragynine (>99% purity)	Rat (Sprague-Dawley)	Oral; 20, 40, 80, 240 and 400 mg/kg	Single dose	No significant respiratory depression based on blood gas analysis	Henningfield *et al.* (2022b)

Table C.3.2.2.2-1 Summary of the Toxicology Studies Conducted *in vivo* with *Mitragyna speciosa* and Mitragynine Preparations

Test Article	Species	Route and Dose (mg/kg bw/day)	Duration	Significant Findings	Reference
Mitragynine (≥98% purity)	Dog (F; Beagle)	Oral; 5 mg/kg	Single dose	No observed adverse events	Maxwell *et al.* (2020)
Mitragynine (Purity NR)					
Mitragynine (purity NR)	Mouse (M; Swiss albino)	Oral; 175 and 1,300 mg/kg bw	Single dose	No toxic effects following administration of 175 mg/kg bw; however, all mice died at the 1,300 mg/kg dose level LD_{50} = 477 mg/kg bw	Sabetghadam *et al.* (2013a)
Mitragynine (purity NR)	Rat (M; Sprague-Dawley)	Oral; 525 and 807 mg/kg	Single dose	No mortality	Macko *et al.* (1972)
Mitragynine (purity NR)	Rat (Sprague-Dawley)	Oral; 8 mg/kg bw/day	5 days	Slight diarrhea observed in 3 of 12 animals; no other effects observed	
Mitragynine (purity NR)	Rat (Charles River)	Oral (gavage); 5 or 50 mg/kg bw/day	5 days/week for 6 weeks	No observed adverse events	
Mitragynine (purity NR)	Mouse (CD-1)	Oral; 3, 10, 30, and 90 mg/kg	Single dose	Respiratory depression with ceiling effect at doses ≥10 mg/kg	Hill *et al.* (2022)

Table C.3.2.2.2-1 Summary of the Toxicology Studies Conducted *in vivo* with *Mitragyna speciosa* and Mitragynine Preparations

Test Article	Species	Route and Dose (mg/kg bw/day)	Duration	Significant Findings	Reference
Mitragynine (purity NR)	Dog (Beagle)	Oral; 16.1 mg/kg bw/day for 5 days, followed by 2 days of 32.2 mg/kg bw/day	7 days	No observed adverse events	Macko *et al.* (1972)
Mitragynine (purity NR)	Dog (Beagle)	Oral; 8 to 80 mg/kg	NR	No observed adverse events	
Mitragynine (purity NR)	Dog (Beagle)	Oral; Group I: Control Group II: 5 mg/kg bw/day Group III: 20 mg/kg bw/day for 3 weeks, 40 mg/kg bw/day for next 4 weeks, and 40 mg/kg bw/day following 6-week washout period	Group II: 6 days/week for 8 weeks Group III: 50 days, followed by 42 days of washout, and another 10 days of exposure	Clinical findings (*i.e.*, leukopenia, granulocytopcnia, lymphocytosis, monocytosis, and atypical and immature lymphocytes) emerged following administration of 40 mg/kg bw/day, which reversed during washout and re-emerged when dosing resumed Moderately severe granulocytic hyperplasia observed in high-dose males Lymph nodes hyperplasia and diffuse increased sinusoidal cellularity in the livers of high-dose dogs	

ALT = alanine aminotransferase; AST = aspartate aminotransferase; bw = body weight; ED_{50} = median effective dose; F = female; GST = glutathione S-transferase; LD_{50} = median lethal dose; M= male; NR = not reported.

C.3.2.3 Surveys, Retrospective Studies, Observational Studies, and Case Studies of Human Exposure

Recent surveys of kratom use in humans indicate current significant use of kratom in the U.S. (see Section C.3.2.3.1). Despite this, few longitudinal studies exist, and most human exposure data are reported as case reports or retrospective and observational studies. Johnson Foods conducted a thorough search of the available scientific literature to better understand the prevalence of *M. speciosa* use, product form (*i.e.*, dry leaf powder, leaf extract, liquid suspension, *etc.*), and serving sizes, and to identify any severe or common effects in humans and on various organ systems that may have bearing on the safety assessment of NPI-001. Due to the nature of case reporting, the evidence presented is anecdotal and often without proper context including test article characterization, description, pattern of use, or other confounding variables, thus limiting direct applicability to the safety assessment of NPI-001.

C.3.2.3.1 Prevalence and Survey Data

Despite significant global use of kratom or kratom-derived products, the historical presence and use of kratom in the U.S. is not well documented. Anecdotal reports indicate that the Hmong, and other peoples of Southeast Asia, brought the plant with them as they migrated to the U.S. in the latter 20th Century (Adkins *et al.*, 2011; Henningfield *et al.*, 2019). Various national and sub-national efforts were made to collect data on prevalence of use of drugs and other substances; however, kratom is only included as a measure in the National Survey on Drug Use and Health (NSDUH – DHHS-SAMHSA, 2020). Other commonly used measures, such as the Monitoring the Future (MTF) survey, the National Forensic Laboratory Information Service (NFLIS), and the Treatment Episodes Data Set (TEDS), either i) do not collect data on kratom use, or ii) do not contain reports of kratom use as measured numbers are below the threshold for inclusion in reports. In addition to nationally representative surveys to measure kratom use, there are surveys of self-described kratom and self-reported adverse events.

In the U.S., estimates of the prevalence of kratom use vary widely across surveys depending on the measures (*e.g.*, "past 30 day," "past year," or "lifetime") and survey methodology. Thus, estimates range from approximately 2 million past year users to more than 16 million. These findings and differences across surveys were summarized by Henningfield *et al.* (2022a), and their summary table comparing the results across surveys are presented below in Table C.3.2.3.1-1.

Table C.3.2.3.1-1 Estimate Number of Kratom Consumers in the U.S. from Published Surveys and Estimates from Kratom Importers and Retailers (adapted from Henningfield *et al.*, 2022a)

Sources	Methods	Prevalence
NSDUH 2019 data reported in 2020 (DHHS-SAMHSA, 2020)	U.S. federal survey by SAMHSA (n=67,625) Nationally representative sample with face-to-face interviews % estimates of U.S. population aged ≥12 years (≥18 years presented in this slide)	Lifetime: 0.5% Past year: 0.7% Past month: 0.3% Past year, adult users Estimate: 1,790,000
Schimmel *et al.* (2021)	U.S. survey by RADARS System panel of paid responders (n=59,714) Nonprobability sample with online self-administration % estimates of U.S. population aged ≥18 years	Lifetime: 1.3% Past year: 0.8% Past year, adult users Estimate: 2,040,000
Covvey *et al.* (2020)	U.S. survey through Qualtrics Panels (n=1,842) Nonprobability sample with online self-administration % estimates of U.S. population aged 18–59 years	Lifetime: 6.1% Past year: 4.1% Past month: 3.5% Past year, adult users Estimate: 10,500,000

Table C.3.2.3.1-1 Estimate Number of Kratom Consumers in the U.S. from Published Surveys and Estimates from Kratom Importers and Retailers (adapted from Henningfield *et al.*, 2022a)

Sources	Methods	Prevalence
American Kratom Association (AKA) – AKA, 2019[a]	Estimate of the number of U.S. kratom consumers based on Southeast Asian kratom export volume in consultation with kratom vendors and retailers	15,600,244 estimated U.S. kratom consumers
Botanical Education Alliance and AKA – BEA & AKA, 2016[b]	U.S. surveys of kratom vendors and retailers 2,014–2,016	3–5 million kratom consumers

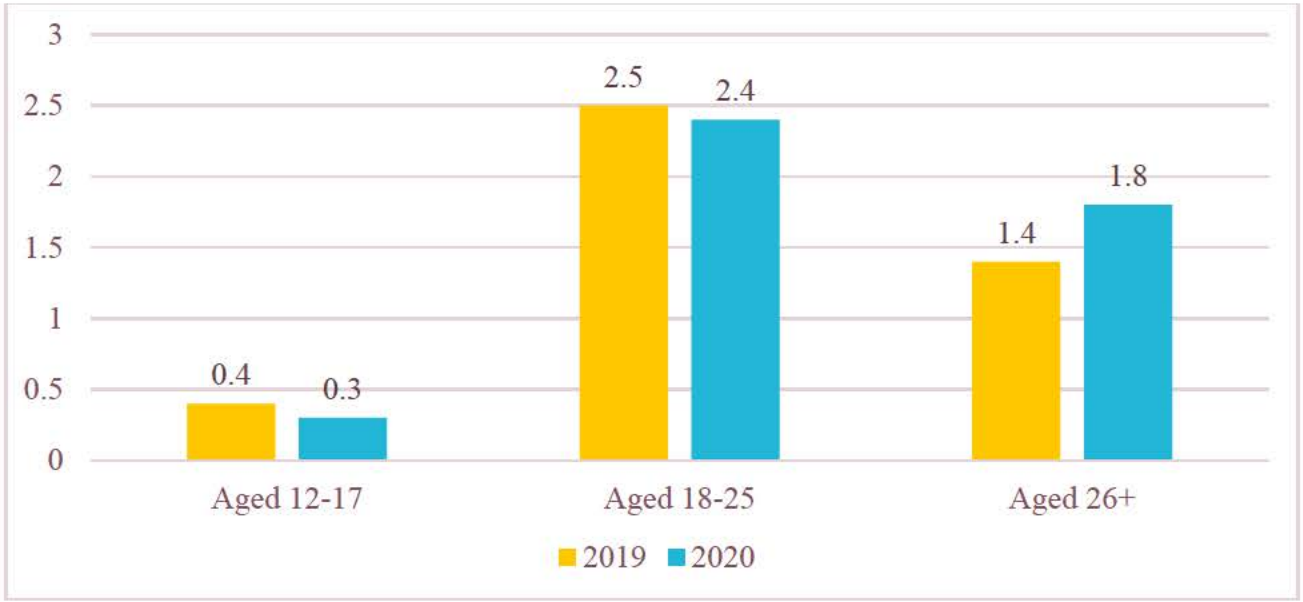
AKA = American Kratom Association; NSDUH = National Survey on Drug Use and Health; RADARS = Researched Abuse, Diversion and Addiction-Related Surveillance; SAMHSA = Substance Abuse and Mental Health Services Administration; U.S. = United States.
[a] https://web.archive.org/web/20191019101051/https://www.americankratom.org/images/Kratom_Population_2019.pdf.
[b] https://www.prnewswire.com/news-releases/groups-dea-ban-of-natural-herb-kratom-could-cause-billions-in-industry-losses-harm-more-than-three-million-americans-300336610.html.

C.3.2.3.1.1 Nationally Representative Prevalence Data in the U.S.

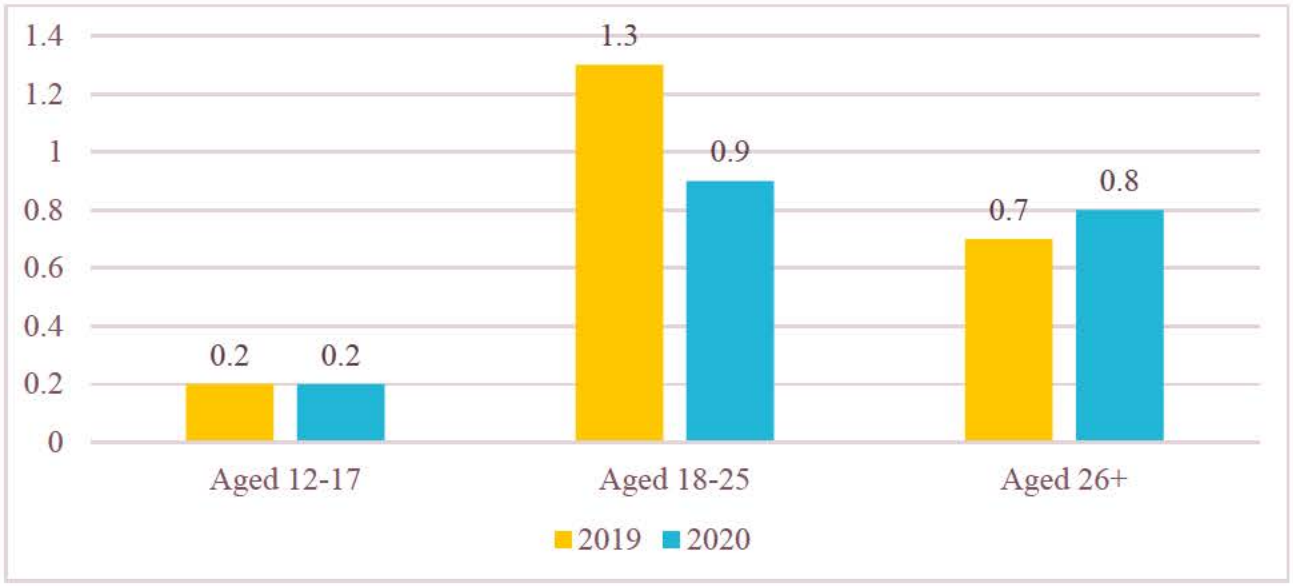
The NSDUH is a national survey that collects and presents information on health and substance use by youth aged 12 to 17 and adults aged 18 or older (DHHS-SAMHSA, 2020). While NSDUH is generally considered a reliable estimate of substance use prevalence, it is not necessarily suited to track use of emerging products (Palamar *et al.*, 2015). Beginning in 2019, NSDUH began surveying its respondents on kratom use. In 2019, approximately 1.4% of respondents aged 12 and older reported lifetime kratom use, increasing to 1.8% in 2020 (SAMHSA, 2020, 2021). Figure C.3.2.3.1-1 provides the prevalence of lifetime, past year, and past month kratom use by age group in 2019 and 2020.

Figure C.3.2.3.1.-1 Lifetime (a), Past Year (b), and Past Month (c) Kratom Use in the U.S. by Age Group in 2019 & 2020

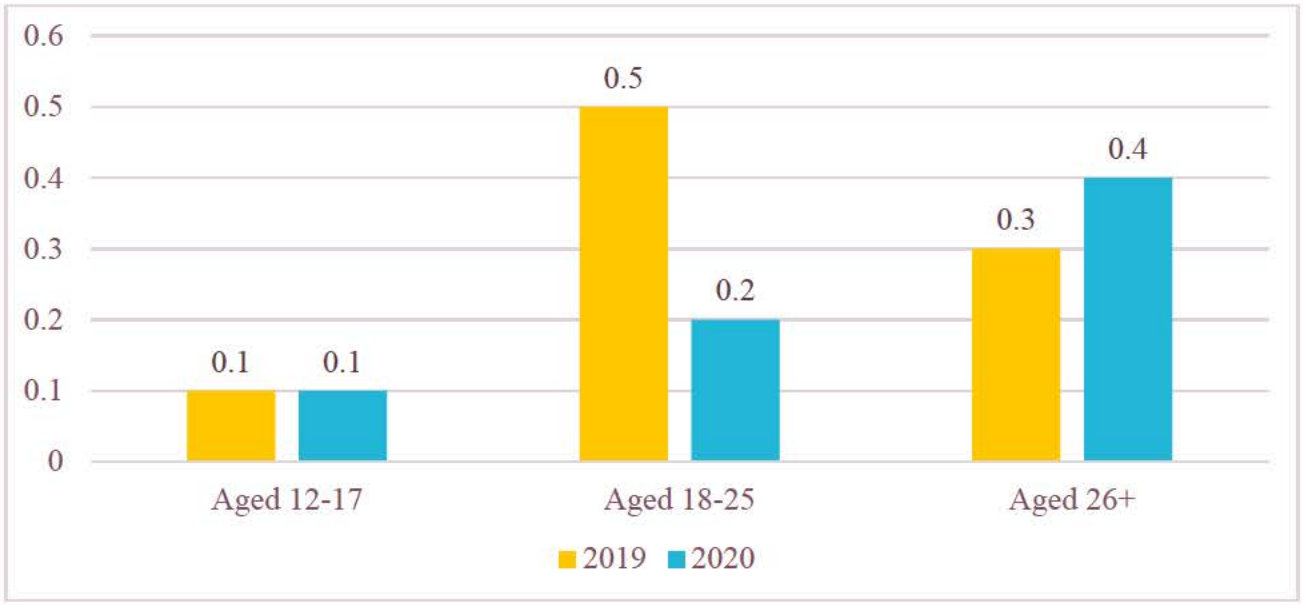
A) Lifetime



b) Past Year



c) Past Month



[1] Source: Substance Abuse and Mental Health Services Administration (SAMHSA)'s public online data analysis system (PDAS) Survey: National Survey on Drug Use and Health, 2019, https://pdas.samhsa.gov/#/survey/NSDUH-2019-DS0001/crosstab/?column=CATAGE&results_received=true&row=KRATFLG&run_chisq=false&weight=ANALWT_C .
[2] Source: Substance Abuse and Mental Health Services Administration (SAMHSA)'s public online data analysis system (PDAS) Survey: National Survey on Drug Use and Health, 2020, https://pdas.samhsa.gov/#/survey/NSDUH-2020-DS0001/crosstab/?column=CATAGE&results_received=true&row=KRATOMFLAG&run_chisq=false&weight=ANALWTQ1Q4_C .

In a review of the 2019 NSDUH data, Xu *et al.* (2021) identified the prevalence of lifetime kratom use to be approximately 1.5% in U.S. individuals over the age of 12. Among those who used kratom, approximately 50.9% used the plant more than 1 year before the survey, 28.4% used it within a year of the survey, and 20.7% used it within the previous month. Most lifetime kratom users were males between the ages 18 and 34.

Moreover, in an internet survey of a nationally representative sample of U.S. adults aged 18 to 59 (n=1,842), it was reported that 6.1% used kratom at least once in their life (Covvey *et al*., 2020). Most respondents were between the ages of 25 and 44 years old, male, and employed. In 2020, respondents to the Q3 2018 – Q1 2019 Researched Abuse, Diversion and Addiction-Related Surveillance Non-Medical Use of Prescription Drugs Program were examined, and among 59,714 respondents aged 18 years or older, 1.3% reported lifetime use of kratom, representing approximately 3.4 million adults in the U.S. (Schimmel *et al.*, 2021). Similarly, to estimate the total number of kratom users in the U.S., the American Kratom Association surveyed export data from several Indonesian commercial export associations (documenting an average of 1,950 metric tons of kratom per month to the U.S.) and compared these data with approximate use levels. Based on an estimated daily 4 to 6 g/day per consumer, the monthly kratom U.S. import numbers yield a potential range of 10.8 to 16.3 million regular kratom consumers (Henningfield *et al.*, 2019). Recent surveys of kratom use in humans indicate that there is currently a substantial use of kratom worldwide (see Section C.3.1).

In summary, various surveys attempting to quantify the prevalence of kratom use in the U.S. reported that approximately 1 to 6% of U.S. adults (approximately 2.5 to 15 million U.S. adults) have consumed an *M. speciosa* product in their lifetime.

C.3.2.3.1.2 Surveys of Self-Described *M. speciosa* Users in the U.S.

In a survey of 8,049 kratom users in the U.S., consumption of ≤8 g of raw plant matter per occasion was most common (>95% of users) (Grundmann, 2017). Mean concentrations of mitragynine and 7-hydroxymitragynine were reported as 1.8% and 0.02%, respectively, in kratom leaf (Kikura-Hanajiri *et al.*, 2009) and from 1.7 to 2.0% for mitragynine or 0.03% for 7-hydroxymitragynine in kratom leaf powder (Kikura-Hanajiri *et al*., 2009; Lydecker *et al.*, 2016). Mitragynine and 7-hydroxymitragynine concentrations also were much greater in kratom resin than in dried leaves. Based on these data, maximum expected human exposure to mitragynine and 7-hydroxymitragynine from kratom use (leaves or powder) is 180 and 3.4 mg/day (*i.e.*, 2.5 and 0.05 mg/kg body weight for a 70-kg individual), respectively (Kruegel and Grundmann, 2018). In the anonymous cross-sectional online survey of 8,049 current kratom users, 20.9% of respondents described dose-dependent adverse effects (primarily gastrointestinal) following high (≥5 g doses) and frequent (≥22 doses/week) kratom intake (Grundmann, 2017). Adverse effects were more frequent when daily kratom doses exceeded 8 g, but the need for outpatient treatment or hospitalization had a low incidence of 0.65%.

A serving size of 5 grams or greater and a serving frequency of 3 times per day or more may be associated with adverse events, but these events generally appear to be mild and self-manageable without intervention from a medical provider in most cases. Such a serving size and frequency significantly exceeds Johnson Foods' proposed conditions of use for NPI-001.

C.3.2.3.2 Hepatotoxicity

Chronic use of kratom is associated with rare instances of acute liver injury in small numbers of susceptible individuals with idiosyncratic reactions (Pantano *et al.*, 2016). It is difficult to correlate kratom consumption and hepatic injuries since they frequently involve multiple concomitant drugs or exposure to contaminants in herbal products. The assessment of causality should be performed using the Roussel Uclaf Causality Assessment Method (RUCAM) that is specific for the liver and validated for hepatotoxicity; however, this methodology only recently was applied to suspected cases of kratom-induced hepatotoxicity (Schimmel and Dart, 2020). In a total of 26 case reports and abstracts and 27 internet user forum posts, the median latency to symptom onset was 20.6 days, with the most common signs and symptoms abdominal discomfort,

jaundice, pruritis, dark urine, chills, and light-colored stool. The median age was 31.5 years (range 19 to 70), 65% of patients were male, and a wide variety of kratom formulations were involved, including leaf powder, tea, and other unknown variations. Human liver biopsies were also reviewed with histology indicating predominantly cholestatic but also biochemical changes, although it was not clear which subgroups of users were considered most at risk. The authors cautioned that the case studies were frequently low-quality human evidence, often lacking documentation required to elucidate a causal relationship between kratom use and the observed hepatotoxicity.

Patients presenting to emergency departments for kratom-related hepatotoxicity sometimes report dark-colored urine, light-colored stools, profound weakness, weight loss, nausea, vomiting, fever, yellow skin, scleral icterus, pruritus, and/or night sweats (Alsarraf *et al*., 2019). In these cases, kratom use is typically reported to be 14 to 21 g/day, a level of consumption that far exceeds those proposed for Johnson Foods' NPI-001 (*i.e.*, 50 mg/day).

A retrospective, single poison control center study on hepatotoxicity examined calls from healthcare facilities related to kratom exposure from 2002 to 2016 (Cumpston *et al*., 2018). Out of 12 patients exposed to kratom or kratom-related products, only 1 had elevated transaminases and bilirubin after first presenting with nausea, abdominal pain, and jaundice. The patient had underlying nonalcoholic steatohepatitis, had discontinued lupus medications 1 month prior, and initiated 3 times a day kratom use. Transaminase concentrations improved after N-acetylcysteine treatment for 21 hours.

From 2004 to 2018, 8 of 404 cases associated with herbal and dietary supplement cases of liver injury involved kratom based on reports to the Drug-Induced Liver Injury Network (Navarro *et al*., 2019). Kratom products were used for a median of 22 days (range 15 to 49) in those 8 cases before onset of injury, and a causal association was reported in 7; however, all cases involved concomitant ethanol use. There were reports of jaundice (5 individuals), itching (6 individuals), abdominal pains (5 individuals), and fever (3 individuals); no rashes were reported. Six of 8 individuals were hospitalized, no treatments were described, and all individuals eventually recovered.

In a more recent review of kratom's potential to cause liver injury, which included epidemiologic, animal, and mechanistic aspects, 132 kratom cases reported to the FDA Center for Food Safety and Applied Nutrition Adverse Event Reporting System from 2004 to 2008 were reviewed (Schimmel and Dart, 2020). Of these, 15 were described as liver toxicity cases but details were unknown and causality could not be estimated. In the FDA Adverse Event Reporting System (FAERS)[4] from 2008 through March 2019, there were 408 reports under mitragynine/herbals with 15 listed as possible liver injury. No clear deaths from kratom or mitragynine liver injury were identified and not a single case in the FAERS database was identified without sufficient exclusion of alternate etiologies or underlying alcoholic cirrhosis. Hepatic coagulopathy was not described; although 1 case in the FAERS database reported severe coagulopathy, no conclusions could be drawn due to poor documentation. Additionally, hepatic encephalopathy grade I was described in a single case report and 2 cases in the FAERS database reported elevated serum ammonia with no documentation of encephalopathy. Latency to onset of liver injury was unclear.

Kratom-induced liver injury appears rare and was primarily associated with idiosyncratic reactions without relation to a specific serving size, serving type, or duration of use. Based on the large number of self-

[4] U.S. FDA (2021). *FDA Adverse Event Reporting System (FAERS) Public Dashboard*. Silver Spring (MD): U.S. Food and Drug Administration (U.S. FDA), Center for Food Safety & Applied Nutrition (CFSAN). Available at: https://www.fda.gov/drugs/questions-and-answers-fdas-adverse-event-reporting-system-faers/fda-adverse-event-reporting-system-faers-public-dashboard [content current as of: 10/22/2021].

described kratom users and the small number of kratom-related liver injury cases reported, kratom-induced liver injury is not common, but onsets rapidly (within 21 days) when it does occur.

C.3.2.3.3 Renal Toxicity

Kidney function of 88 regular kratom users with an average of 11 years of use and 83 healthy controls was studied through the evaluation of the users' urinary protein profiles (Jasim *et al*., 2022). Their urinary creatinine concentrations were in the normal range but their urinary protein and protein:creatinine ratio were higher than controls, suggesting possible early-stage kidney injury. The kratom products consumed, typical doses consumed and other drug and substance use were not characterized, though the authors did report 27% of kratom users tested positive for illicit substances. In addition, the authors reported a substantially longer duration of use and larger serving size than the currently proposed serving size and frequency of NPI-001.

C.3.2.3.4 Cardiovascular Toxicity

In a study of cardiovascular kratom toxicity, no significant differences were identified in the prevalence of abnormal ECGs of 100 regular kratom users (28%) and 100 control individuals (32%), except for significantly higher odds of sinus tachycardia (odds ratio = 8.61; 95% confidence interval = 1.06 to 70.17, p=0.035) in the kratom users (Leong Bin Abdullah and Singh, 2021a). The odds of observing borderline QT corrected for heart rate (QTc) intervals were significantly higher for kratom users compared to control subjects, regardless of age of first use, duration of use, daily quantity consumed, and the length of time that elapsed between last kratom use and ECG assessment. However, there were no differences in the odds of having prolonged QTc intervals between kratom users and controls. The estimated average daily intake of mitragynine consumed by these kratom users was 434 mg. Prolonged QTc intervals were identified in another study of 9 regular kratom users without history of polysubstance use; however, they did not show any risk of cardiovascular impairment or other signs of toxicity (Leong Bin Abdullah and Singh, 2021b).

The presented data show no significant difference in prolonged QTc, and an increase in odds of sinus tachycardia at an average mitragynine intake of 434 mg. Besides the increased risk of sinus tachycardia at doses substantially higher than the proposed intake of NPI-001 (0.7 mg mitragynine/day), the research did not show any risk of cardiovascular impairment or other signs of toxicity.

C.3.2.3.5 Neurological Toxicity

There were no serious adverse effects following ingestion of 6.3 to 11.2 mg oral mitragynine in a tea decoction for 7 days and an additional 6.3 to 23 mg oral mitragynine in tea to 10 male chronic kratom users (Trakulsrichai *et al*., 2015). All subjects developed tongue numbness after drinking the tea and had increased blood pressure and heart rate. Interestingly, the onset was delayed to 8 hours after drinking the tea and occurred later than the mitragynine T_{max}.

Cognitive function in 70 regular kratom users and 25 controls was evaluated using the Cambridge Neuropsychological Test Automated Battery (Singh *et al.*, 2019). Relative to controls, those who consumed large amounts of kratom tea—>more than 3 glasses daily or 72.5 to 74.9 mg mitragynine—had impaired performance on the Paired Associates Learning task, reflecting deficits in visual episodic memory and new learning. Overall, the 2 groups were comparable on other neuropsychological domains, with >3 glasses daily of kratom tea not impairing motor, memory, attention, or executive function of regular kratom users.

Overall, there is no indication that kratom or its constituents exhibit neurological toxicity even at serving sizes that greatly exceed conditions of use described for NPI-001.

C.3.2.3.6 Endocrine Toxicity

Long-term kratom tea/juice consumption (≥4 glasses/day), providing a daily mitragynine dose of 76.2 to 115 mg/day did not impair testosterone, follicle-stimulating hormone (FSH), and luteinizing hormone (LH), or hematological and biochemical parameters in 19 regular kratom users (Singh *et al*., 2018). There is no indication that kratom or its constituents exhibit endocrine toxicity.

C.3.2.3.7 Potential Deaths Associated with Mitragynine and *M. speciosa*

In a 2019 Centers for Disease Control and Prevention (CDC) *Morbidity and Mortality Weekly Report*, multiple drugs were detected in nearly all 152 unintentional overdose deaths involving kratom between July 2016 and December 2017 (Olsen *et al.*, 2019). Fentanyl or its analogs were the most common drugs detected in kratom-associated overdoses and were described as cause of death in 65.1% of these overdoses, followed by heroin (32.9%), benzodiazepines (22.4%), prescription opioids (19.7%), and cocaine (18.4%). Only 0.56% of the 27,338 U.S. overdose deaths during that period were listed as kratom-involved.

A review of death certificates in Colorado from 1999 to 2017 for any mention of kratom or mitragynine identified 15 cases where either were mentioned (Gershman *et al*., 2019). Eleven of these deaths identified 2 to 8 additional drugs in the decedent's biological samples, with 8 cases involving opioids. Further investigation of police reports and additional comprehensive toxicology testing of the 4 cases listed as mitragynine only showed that 14 of 15 deaths "clearly involved multiple drugs," with mitragynine concentrations varying widely, from 16 to 4,800 ng/mL. No blood was available for the last case to identify any other drugs that might be present. The authors concluded that careful examination of deaths apparently due only to kratom must include comprehensive toxicology screening.

In the United Kingdom, 156 cases were identified by searching mortality registers for cases involving fatal overdoses or deaths associated with kratom or mitragynine (Corkery *et al.*, 2019). Ninety-five percent had a drug abuse history and professed use for self-medication, recreation, relaxation, bodybuilding, and avoiding positive drug tests. In only 6 of all reported cases with available postmortem toxicology data (n=129) was mitragynine the sole substance identified. Mitragynine concentrations were available for 71 cases (45.8%) but unavailable/not given for 84 cases (54.2%). There was a lack of detailed reports providing mitragynine and 7-hydroxymitragynine concentrations in poisoning intoxications and fatalities, and almost no data on mitragynine or kratom doses. In some cases, 7-hydroxymitragynine blood concentrations suggest artificial adulteration of kratom products with this analyte. The mean mitragynine concentration in cases where mitragynine was the only detected substance was 2,128 ng/mL (range 16 to 16,000).

Twenty death investigation cases from 2017 and 2018 had mitragynine detected during toxicology testing of central and/or peripheral blood, liver, gastric contents, brain, and vitreous humor (Mata and Andera, 2020). The median age of the deceased was 26 years with a range of 21 to 60 years, and 25% were female (Mata and Andera, 2020), similar to the 23.7% identified in the Olsen *et al.* (2019) data. The non-specific finding of heavy lungs was observed in many of the cases, but the most common other drugs detected in 15 of 20 cases were narcotic analgesics that also produce heavy lungs. The other drugs found were CNS depressants in 13 cases, alcohol and/or benzodiazepines in 7 cases, prescription drugs in 11 cases, cannabis in 2 cases, cocaine in 1 case, and methamphetamine in 1 case. In no case was mitragynine the only drug identified. Mitragynine postmortem central blood concentrations by LC-MS/MS were 10 to 4,310 ng/mL, with a 123 ng/mL median and a 626 ng/mL mean. In 13 cases where peripheral and central blood were analyzed, mitragynine concentrations were similar to central blood (24.6 to 3,420 ng/mL), with a mean of 903 ng/mL and a median of 226 ng/mL. Postmortem redistribution ratios were determined for the 13 cases in which central and peripheral blood were evaluated (range 0.37 to 1.26, with 1 outlier removed) with a mean ratio of 0.75 and a median of 0.79. These data do not suggest significant postmortem

redistribution of mitragynine. Of the 20 identified cases of overdose, the mode of death was deemed suicide in 2 cases, natural death by severe coronary artery disease in 1 case, and accidental overdose in 17 cases. Mitragynine and other drugs were listed in 11 of the 17 overdoses, with most having confounding health conditions and most indicating additional other drug use. Three cases with the highest mitragynine concentrations (1,250 to 4,310 ng/mL central blood) only described mitragynine as the cause of death even though other drugs were also detected. These cases were the first in which mitragynine was reported by the medical examiner in Orange County, California as sole cause of death.

In another study, 35 kratom-associated deaths in Northern Nevada between 2015 and 2020 were examined and 27 cases with concentrations ranging from 8.7 to 1,800 ng/mL were identified (Schmitt *et al*., 2021). Of these cases, 8 had other non-mitragynine causes of death, with concentrations ranging from 110 to 980 ng/mL. In 1 case, the sole intoxicant listed as cause of death was mitragynine with a blood concentration of 950 ng/mL; however, aripiprazole, an atypical antipsychotic, was present at 310 ng/mL, and phenibut, a CNS depressant prescribed to treat anxiety and insomnia, was found at the scene, but no method was available for analysis in the laboratory. Thus, this case also could involve multiple-drug toxicity. There was no statistically significant difference in blood concentrations between cases where mitragynine was not listed as a cause of death (mean, 315 ± 297.2 ng/mL) and cases in which mitragynine contributed to death (mean, 269.4 ± 382.5 ng/mL; $P<0.201$). Opioids were also detected in 81.5% (n=22) of the cases; benzodiazepines and/or alcohol in 33.3% (n=9); amphetamine/methamphetamine in 25.9% (n=7); and nitrous oxide in 7.4% (n=2).

In addition, a review of the literature containing all published mitragynine or kratom deaths identified 127 reports; 117 with combined drug toxicity cause of deaths, and 10 that were listed as kratom or mitragynine only (Schmitt *et al*., 2021). Mitragynine blood concentrations in the combined drug toxicity fatalities ranged from 10 to 4,800 ng/mL. Only 3 of 10 fatalities identifying only kratom as cause of death had quantified blood mitragynine results of 260, 1,400, and 1,900 ng/mL. The highest blood concentration, 1,900 ng/mL was in the blood of a 33-year-old male with a 21-year history of drug abuse. Toxic mitragynine concentrations are not established in humans and are complicated by the potential effects of additional co-intoxicants, variations in individual metabolism, and tolerance development from chronic use of mitragynine or co-ingested opioids.

A retrospective study conducted by Jittasopa and Srisont (2021) examined autopsy reports from Ramathibodi Hospital in Thailand from January 2015 to December 2019 that included blood samples positive for mitragynine. Of 2,160 autopsy cases, 24 were positive for mitragynine, with amphetamine, antihistamines, and ethanol as the most common concomitant drugs detected. The most observed pathological findings were pulmonary edema (7 cases) and coronary atherosclerosis (6 cases). One case involved a 43-year-old man whose pathological findings showed chronic asthma with a high concentration of mitragynine in the blood (3.6 mg/L), although no other substances were detected.

Overall, kratom-involved deaths make up a small percentage of total accidental overdose deaths, and even fewer cases involved kratom alone. Most cases were identified either by case report or blood analysis to include some combination of prescription drugs, illicit drugs, or other naturally occurring substances that are known to have dangerous side effects and patterns of use, including accidental overdose death. Based on the large number of self-described kratom users and the small number of deaths associated solely with kratom, the risk for kratom-related overdose death appears low. Further, deaths associated solely with kratom often lacked complete toxicological testing to identify concomitant illicit substance use.

C.3.2.3.8 Case Studies

In addition to reviewing observational and retrospective studies concerning kratom-related injury and death, Johnson Foods identified case reports of adverse events from kratom use in the published literature. Due to the nature of case reporting, the evidence presented is anecdotal and often without proper context. Many of these case reports involve users with pre-existing conditions and concomitant use of multiple drugs or substances. Additionally, most case reports lacked a satisfactory description of the kratom product consumed, the dose and duration of use, and analytical testing of the suspected product for confirmation of its identity and for the presence of contaminants. Lastly, many case reports had incomplete toxicological testing for potential concomitant substances. A summary of adverse case reports involving kratom use is provided in Table C.3.2.3.8-1.

Table C.3.2.3.8-1 Summary of *M. speciosa* Adverse Case Reports

Age and Gender	Dose, Duration, and Kratom Type	Existing Conditions	Symptoms/ Outcome	Required Hospitalization? (Y/N)	Concomitant Substances	Reference
Hepatotoxicity						
40, F	UNK dose and type; 1 time/week; 1 mo	Prediabetes, GERD, cluster headaches	Abdominal pain, elevated LFT, hepatic injury/ Symptoms resolved (10 weeks)	N	Nettle leaf supplements, Oral contraceptives	Aldyab *et al.* (2019)
70, M	UNK dose; 2 times/day; 4 days	Hypertension, osteoarthritis	Jaundice, elevated LFT nausea, fatigue, profound weakness, weight loss/ Discharged, symptoms resolved	N	Oxycodone	Antony and Lee (2019)
27, M	UNK dose and type; 2 weeks	N/A	Epigastric abdominal pain, fevers, chills, nausea, elevated LFT/ Discharged	N	Opiates, THC	Botejue *et al.* (2021)
36, F	UNK dose and type; "several years"	Hepatic steatosis, ventral hernia repair, OUD, choledocholithiasis	Jaundice, lethargy, poor appetite, vomiting, diarrhea, scleral icterus, moderate ascites, pitting edema, elevated LFT/ Transferred to liver transplant center	Y	N/A	
58, M	UNK dose; 1 tablespoon; 1 yr; Experience Alternatives Inc., "pure maeng da kratom leaf"	Schizoaffective disorder	Jaundice, liver injury/ Discharged	Y	Quetiapine, sertraline	Dorman *et al.* (2015)
52, M	1.5 g/day; 2 mo; Crushed leaves	Shoulder strain	Yellow discoloration of eyes, skin, elevated LFT/ Discharged, symptoms resolved (4-week follow-up)	N	Acetaminophen (800 mg)	Fernandes *et al.* (2019)
37, F	3 g over 3 days; Powdered kratom	Depression, obesity	Nausea, decreased appetite, fatigue, jaundice, elevated LFT/ Discharged, symptoms resolved at 6-day follow-up)	Y	Venlafaxine	Gandhi *et al.* (2020)

Table C.3.2.3.8-1 Summary of *M. speciosa* Adverse Case Reports

Age and Gender	Dose, Duration, and Kratom Type	Existing Conditions	Symptoms/ Outcome	Required Hospitalization? (Y/N)	Concomitant Substances	Reference
21, M	12 capsules; 10 g in 2 days before exposure; 2 weeks	N/A	Vomiting, fatigue, abdominal pain, brown urine, elevated LFT/ Discharged	Y	THC	Griffiths *et al.* (2018)
38, M	3 large teas; "long" duration	Stimulant use disorder, OUD, AUD, PTSD	Elevated LFT, liver injury/ Discharged, continued kratom use and admitted to the hospital several months later with elevated LFT	Y	Chlordiazepoxide, buprenorphine/ naloxone	Jensen *et al.* (2021)
25, M	UNK dose; 4–6 teaspoons; 2 weeks; Powdered kratom (Thai Pimp, Malaysian Green)	N/A	Abdominal pain, brown discoloration of urine, jaundice, pruritus, elevated LFT/ Discharged, symptoms resolved at 47-day follow-up	Y	N/A	Kapp *et al.* (2011)
37, F	UNK dose; 1 yr; 3 capsules	Hypertension, ADHD, chronic back pain	Nausea, abdominal pain, vomiting, diarrhea, elevated LFT, acute liver injury, acute kidney injury, pancolitis/ Liver transplant, discharged, dialysis	Y	N/A	Khan *et al.* (2021)
31, M	UNK dose; 2 weeks; tea	N/A	Tea-colored urine, malaise, fatigue, fever, elevated LFT/ Discharged	Y	N/A	Mousa *et al.* (2018)
47, M	UNK dose; 3 weeks; capsules	Obesity, hypertension, prediabetes, anxiety, major depressive disorder, hypertriglyceridemia	Dark urine, pruritis, subjective fevers, reduced appetite, fatigue, elevated LFT/ Discharged, symptoms resolved	N	Valsartan, metoprolol tartrate, escitalopram, clonazepam, fexofenadine, acetaminophen	Osbourne *et al.* (2019)
36, M	UNK dose; "few weeks"; Powdered kratom	N/A	Loss of consciousness, elevated LFT/ Discharged, symptoms resolved (Week 2 discharge)	Y	N/A	Palasamudram *et al.* (2019)

Table C.3.2.3.8-1 Summary of *M. speciosa* Adverse Case Reports

Age and Gender	Dose, Duration, and Kratom Type	Existing Conditions	Symptoms/ Outcome	Required Hospitalization? (Y/N)	Concomitant Substances	Reference
28, M	UNK dose and type; 1 week	AUD, OUD, GERD	Loss of consciousness, respiratory failure, acute renal failure, severe metabolic acidosis, rhabdomyolysis, shock liver/ Discharged with hemodialysis catheter, discharged to SUD program	Y	N/A	Patel *et al.* (2021)
38, M	UNK dose, duration, and type	N/A	Dark urine, light-colored stools, fever, chills, chest pain, shortness of breath, elevated LFT/ Discharged	Y	Tylenol (5 doses)	Riverso *et al.* (2018)
45, F	UNK dose; 6–10+ pills/day; 2 mo	Crohn's disease, breast cancer status post chemotherapy, radiation, bilateral mastectomy with reconstruction, and chronic pain	Loss of consciousness, liver injury, renal injury, rhabdomyolysis, elevated LFT/ Hemodialysis, discharged	Y	N/A	Sangani *et al.* (2021)
32, M	60 tablets over 1 week; powder, tablet	Hypertension, anxiety, low back pain	Nausea, fatigue, joint pains, night sweats, pale stools, urine, yellow looking skin, elevated LFT/ Discharged, symptoms resolving	Y	Acetaminophen	Tayabali *et al.* (2018)
31, M	UNK dose and duration; "smoked kratom"	OUD, AUD, SUD	Loss of consciousness, rhabdomyolysis, acute renal injury, hyperkalemia, hypocalcemia, liver injury, necrotic leg muscle/ Discharged, fasciotomy, skin graft	Y	Venlafaxine, caffeine	Tobarran *et al.* (2022)
18, M	UNK dose, duration, and type	SUD, depression, anxiety	severe nausea, vomiting, weakness, dizziness, elevated LFT, possible sepsis/ Discharged, symptoms resolved	Y	Buspirone, duloxetine, quetiapine, gabapentin, propranolol	Zuberi *et al.* (2019)
Seizures						
43, M	4 teas/day; >3.5 yr	Chronic pain	Tonic-clonic seizure/ Discharged	N	Modafinil	Boyer *et al.* (2008)
49, F	UNK dose and type; daily over 1 mo	Tonic-clonic epilepsy	Tonic-clonic seizure/ Discharged, symptoms resolved at 4-month follow-up	N	Lacosamide, eslicarbazepine	Burke *et al.* (2021)

Table C.3.2.3.8-1 Summary of *M. speciosa* Adverse Case Reports

Age and Gender	Dose, Duration, and Kratom Type	Existing Conditions	Symptoms/ Outcome	Required Hospitalization? (Y/N)	Concomitant Substances	Reference
37, F	UNK dose and type; intermittently over <2 yr	Tonic-clonic epilepsy	Tonic-clonic seizure/ Discharged, symptoms resolved at 1-year follow-up	N	Levetiracetam, THC, benzodiazepines	Burke *et al.* (2021)
24, M	UNK dose and type; 1 mo	OUD	Tonic-clonic seizure/ Discharged, symptoms resolved at 1-year follow-up	N	N/A	Burke *et al.* (2021)
24, M	600 mg/day; UNK duration and type	Asperger syndrome, depression, SUD	Loss of consciousness, hypothermic, seizure, acute rhabdomyolysis/ Discharged, withdrawal, symptoms resolved after 45 days treatment	Y	N/A	Diep *et al.* (2018)
64, M	UNK dose and duration; Kratom-Datura tea	Colostomy repair, chronic pain, depression, alcohol use, tobacco use	Loss of consciousness, seizure, tachycardia/ Discharged	Y	Datura	Nelsen *et al.* (2010)
19, M	2–8 g/day; >2 yr; pills	Anxiety, ADHD	Seizures/ Symptoms resolved, lost to follow-up	Y	Lisdexamfetamine dimesylate, alprazolam, alcohol, marijuana	Tatum *et al.* (2018)
Deaths						
26, M	UNK dose, duration, and type	N/A	Cardiorespiratory arrest/ Death	Y	Codeine ("standard dose")	Aggarwal *et al.* (2018)
22, M	UNK dose and duration; Powdered kratom ("Red Vein")	Psychosis, anxiety, SUD	Death (possible loss of consciousness leading to fall from window)	N	Unknown tablet, benzodiazepines, etizolam, fluoxetine	Domingo *et al.* (2017)
20, M	UNK dose and duration; Powdered kratom	Autism	Death	N	N/A	Domingo *et al.* (2017)

Table C.3.2.3.8-1 Summary of *M. speciosa* Adverse Case Reports

Age and Gender	Dose, Duration, and Kratom Type	Existing Conditions	Symptoms/ Outcome	Required Hospitalization? (Y/N)	Concomitant Substances	Reference
20, M	UNK dose, duration, and type	N/A	Death	N	39 separate supplements, prescription, and nonprescription medications found at the scene; morphine, promethazine, propylhexedrine, acetaminophen measured in blood samples	Holler *et al.* (2011)
27, M	UNK dose, duration, and type	Asperger syndrome, bipolar disorder, SUD	Death	N	Valproic acid, quetiapine	Hughes (2019)
Middle age/ gender UNK	UNK dose and duration; Powdered kratom	SUD, psychiatric disease	Overdose/ Death	N	Zopiclone, citalopram, lamotrigine	Karinen *et al.* (2014)
33, M	UNK dose, duration, and type	OUD, SUD, depression, anxiety	Death	N	THC, caffeine, cotinine, naloxone	Matson and Schenk (2019)
24, M	UNK dose, duration, and type	AUD, depression, multiple suicide attempts	Death	N	Ethanol, diphenhydramin, mirtazapine, venlafaxine	McIntyre *et al.* (2015)
17, M	UNK dose and duration; 1 bottle; Liquid kratom	OUD, chronic back pain, depression, suicide attempts	Death	N	Dextromethorphan, diphenhydramin, temazepam, 7-amino-clonazepam	Neerman *et al.* (2013)

Table C.3.2.3.8-1 Summary of *M. speciosa* Adverse Case Reports

Age and Gender	Dose, Duration, and Kratom Type	Existing Conditions	Symptoms/ Outcome	Required Hospitalization? (Y/N)	Concomitant Substances	Reference
33, M	UNK dose, duration, and type	Hypertension, obesity, anxiety	Loss of consciousness, vomiting, respiratory distress, asystole/ Death	Y	Prescribed alprazolam, sertraline, metroprolol, and pantoprazole; Found by person: U-47700, acetylcodeine, acetaminophen, 6-monoacetylmorphine, cocaine, heroin, caffeine, papaverine, meconin, noscapine, etizolam, threo-4-fluoromethylphenidate, acetylpsilocin	Walsh *et al.* (2019)
56, F	UNK dose and duration; Powdered kratom	COPD, asthma	Death	N	Oxycodone, lorazepam	Wang and Walker (2018)
Nephrotoxicity						
28, M	UNK dose and type; 1 week	AUD, OUD, GERD	Loss of consciousness, respiratory failure, acute renal failure, severe metabolic acidosis, rhabdomyolysis, shock liver/ Discharged with hemodialysis catheter, discharged to SUD program	Y	N/A	Patel *et al.* (2021)
45, F	UNK dose; 6–10+ pills/day; 2 mo	Crohn's disease, breast cancer status post chemotherapy, radiation, bilateral mastectomy with reconstruction, and chronic pain	Loss of consciousness, liver injury, renal injury, rhabdomyolysis, elevated LFT/ Hemodialysis, discharged	Y	N/A	Sangani *et al.* (2021)
31, M	UNK dose and duration; "smoked kratom"	OUD, AUD, SUD	Loss of consciousness, rhabdomyolysis, acute renal injury, hyperkalemia, hypocalcemia, liver injury, necrotic leg muscle/ Discharged, fasciotomy, skin graft	Y	Venlafaxine, caffeine	Tobarran *et al.* (2022)

Table C.3.2.3.8-1 Summary of *M. speciosa* Adverse Case Reports

Age and Gender	Dose, Duration, and Kratom Type	Existing Conditions	Symptoms/ Outcome	Required Hospitalization? (Y/N)	Concomitant Substances	Reference
Cardiovascular Toxicity						
35, M	UNK dose, duration, and type	OUD, cocaine use	Overdose; Tests showed low pCO2, low HCO3, base excess, and elevated white blood cell count, creatinine, potassium, and anion gap/ Discharged on Day 4	Y	N/A	Hall and Hall (2021)
30-35, M	UNK dose, duration, and type	N/A	Loss of consciousness, bradypnea, hypercapnia, bradycardia, arterial hypotension/ Mechanical ventilation, Akrinor™, 0.4 mg naloxone without effect, UNK outcome	Y	Cyclopropylfentanyl, cyclopropylnorfentanyl, acetylfentanyl, 4-ANPP, U-47700, caffeine	Müller *et al.* (2019)
15, F	45 capsules (500 mg) (22,500 mg total); UNK duration	Depression	Attempted suicide, dry mouth, dizziness, restlessness, palpitations, nausea, vomiting, tachycardia, hypokalemia, elevated lactic acid/ Transferred to inpatient pediatric psychiatric unit	Y	N/A	Wong and Mun (2020)
Neurological Toxicity						
22, M	UNK dose, duration, and type	SUD	Headache, edema in occipital lobe, frontal lobe, and brainstem, occipital lobe hemorrhage/ Discharged	Y	Dextroamphetamine (6 tablets), fluoxetine, quetiapine	Castillo *et al.* (2017)
43, M	6–8 pills/day; pills occasionally (UNK duration), 1 bottle liquid kratom; liquid kratom once	PTSD, TBI, Chronic adrenal insufficiency, SUD	Visual and auditory hallucinations, delusions of grandeur/ Discharged	Y	Cymbalta, Adderall, testosterone	Cutlip *et al.* (2021)
39, F	UNK dose and type; "a few months"	N/A	Lightheadedness, palpitations, weakness, catatonia, paralysis, tachycardia/ Discharged	N	N/A	Matos-Casano and Nanduri (2021)

Table C.3.2.3.8-1 Summary of *M. speciosa* Adverse Case Reports

Age and Gender	Dose, Duration, and Kratom Type	Existing Conditions	Symptoms/ Outcome	Required Hospitalization? (Y/N)	Concomitant Substances	Reference
54, M	2–3 teaspoons; 1 yr; Powdered kratom (Kratom Crazy, Vivazen Botanicals Maeng Da Kratom)	Hepatitis C, AUD, OUD	Headache, incomprehensible speech, intraparenchymal hemorrhage/ Discharged, referred to SUD treatment	Y	N/A	Nacca *et al.* (2020)
37, F	3–4 g/day; UNK duration and type	Chronic pain, SUD, ADHD	Impaired driving/ UNK outcome	N	Adderall, citalopram	Wright (2018)
18, M	UNK dose, duration, and type	SUD, depression, anxiety	Severe nausea, vomiting, weakness, dizziness, elevated LFT, tremors, dizziness, lightheadedness, and near syncope, possible sepsis/ Discharged, symptoms resolved	Y	Buspirone, duloxietine, quetiapine, gabapentin, propranolol	Zuberi *et al.* (2019)
Other						
65, M	1 tsp; 4 times/day; Powdered kratom	MDD, GAD, Parkinson's Disease	N/A; Patient drank 2 oz. of Termidor, a liquid insecticide, in a previous suicide attempt. Patient reported sertraline and buspirone failed to help his anxiety and difficulty sleeping, while kratom improved his anxiety.	N/A	Sertraline, buspirone, carbidopa/levodopa	Grossman *et al.* (2020)
42, M	UNK dose, duration, and type	N/A	Poor energy, low libido/ Discharged, symptoms resolved (2-month follow-up)	N	N/A	LaBryer *et al.* (2018)
38, F	UNK dose, duration, and type	Depression, SUD	Altered mental status, decreased respiratory rate, bradypnea/ Discharged	Y	N/A	Overbeek *et al.* (2019)
53, M	2 tsp/day; UNK dose, duration, and type	Bipolar disorder, OUD	N/A; Takes kratom to help avoid opioid use relapse/ Screen tests in urine negative for opiates and other illicit drugs	N	Benztropine, carbamazepine, eszopiclone, olanzapine	Sethi *et al.* (2018)
62, F	2 tsp; single occasion; Powdered kratom	COPD, asthma	Vomiting, nausea, abdominal cramping/ Discharged	N	N/A	Singh *et al.* (2020b)

Table C.3.2.3.8-1 Summary of *M. speciosa* Adverse Case Reports

Age and Gender	Dose, Duration, and Kratom Type	Existing Conditions	Symptoms/ Outcome	Required Hospitalization? (Y/N)	Concomitant Substances	Reference

4-ANPP = 4-anilino-N-phenethyl-piperidine; ADHD = attention-deficit/hyperactivity disorder; AUD = alcohol use disorder; COPD = chronic obstructive pulmonary disease; F = female; GAD = generalized anxiety disorder; GERD = gastroesophageal reflux disease; LFT = liver function test; M = male; MDD = major depressive disorder; mo = month(s); N = no; N/A = not applicable; OUD = opioid use disorder; PTSD = post-traumatic stress disorder; SUD = substance use disorder; TBI = traumatic brain injury; THC = tetrahydrocannabinol; tsp = teaspoon; UNK = unknown; Y = yes; yr = year(s).

C.3.2.4 Interactions between NPI-001 and Other Substances

As discussed in Section C.3.2.1, mitragynine has a complex pharmacological profile including agonism and antagonism at multiple receptors and interactions with drug-metabolizing enzymes and drug-efflux systems.

C.3.2.4.1 *In vitro* Studies of Interactions Between Mitragynine, *M. speciosa*, and Other Substances

P-glycoprotein is important for effluxing potentially toxic compounds out of the brain to prevent toxicity. Mitragynine's interaction with P-glycoprotein was investigated to determine the possibility of a drug interaction if mitragynine was coadministered with drugs that are P-glycoprotein substrates. Mitragynine inhibited P-glycoprotein activity with an EC_{50} of 18.2 ± 3.6 µM (Manda *et al.*, 2014). Mitragynine showed moderate permeability across MDR-MDCK monolayers (model for the BBB) with no significant efflux, resulting in a classification of mitragynine as diffusible. In studies of different drug interactions with the ATP-dependent P-glycoprotein efflux mechanism, mitragynine did not significantly stimulate ATPase or P-glycoprotein activity compared to the non-stimulation propranolol control (Meyer et al., 2015). However, later, an increase in P-glycoprotein activity by kratom extract (3-fold), the alkaloid fraction (4-fold), and 9 individual alkaloids (4- to 6-fold) was shown due to activation of the pregnane X receptor that increases expression of P-glycoprotein (Manda *et al.*, 2017). Ya *et al.* (2019) cautioned that considering free mitragynine concentrations and the concentrations necessary to produce these effects, these findings may not be clinically relevant.

Kratom alkaloid extract effects on human recombinant CYP450 enzyme activities showed varied and inconsistent results across species and study designs. In addition, it is difficult to speculate whether these concentrations are achieved *in vivo*. The most potent inhibitory effect of a kratom alkaloid extract on CYP3A4 and CYP2D6 had an IC_{50} of 0.78 µg/mL and 0.64 µg/mL, respectively (Kong *et al.*, 2011). In addition, moderate inhibition was observed for CYP1A2, with an IC_{50} of 39 µg/mL. The IC_{50} of CYP2C19 could not be determined because inhibition was <50%. Alkaloid extract inhibition was competitive for CYP2D6, but non-competitive for CYP3A4, CYP1A2, and CYP2C19. Mitragynine noncompetitively inhibited CYP2C9 and CYP2D6 activities with 61.5 and 12.9 µM Ki, respectively, in a high-throughput luminescence assay (Hanapi *et al.*, 2013). The Ki for mitragynine inhibition of CYP3A4 in this system was much higher at 380 µM and was competitive.

Inconsistencies were also reported for the induction of enzyme activity that was investigated primarily with kratom alkaloid extract. Mitragynine induced mRNA and protein expression of CYP1A2, with an approximate 70% induction at the highest 25 µM concentration compared to the positive control (Lim *et al.*, 2013). Mitragynine was a weak CYP3A4 inducer at the transcription level, suggesting it was unlikely for mitragynine to have any significant clinical effects on CYP3A4 activity. Manda *et al.* (2017) reported induction of CYP1A2 and no induction of CYP3A4 by mitragynine. Todd *et al.* (2020) found alkaloid extract and mitragynine to have a concentration-dependent inhibition of CYP2C9, CYP2D6, and CYP3A4, with stronger effects on CYP2D6 (~1 µM mitragynine) compared to CYP2C9 and CYP3A (~10 to 100 µM mitragynine). The alkaloid extract inhibition was greater than that of mitragynine.

Haron and Ismail (2014) reported an IC_{50} of greater than 100 µM for mitragynine on phase II metabolism in rat and human liver microsomes and recombinant human UGT1A1 and UGT2B7 isoforms. Mitragynine's high IC_{50} suggests no significant inhibition of phase II metabolism. The *in vitro* inhibitory effects of mitragynine on UGT2B7, the primary UGT isoform for metabolizing opioids and non-steroidal anti-inflammatory drugs, was investigated in rat and human liver microsomes (Abdullah and Ismail, 2018). The IC_{50} of mitragynine inhibition of zidovudine glucuronidation in human and rat liver microsomes was 8.1

± 4.5 µM and 51.2 ± 6.0 µM, respectively, suggesting differences between human and rat microsomal models.

Efflux transporters such as the human breast cancer resistance protein (hBCRP) can significantly influence absorption, distribution, and excretion of drugs, influencing a drug's bioavailability and drug–drug interactions. Thirteen drugs of abuse classes were tested for their *in vitro* affinity to hBCRP; 7 compounds showed statistically significant hBCRP ATPase stimulation, with mitragynine one of the strongest stimulators (Wagmann *et al.*, 2018). Five drugs of abuse showed statistically significant hBCRP ATPase inhibition. All the drugs were shown previously to be stimulators of the P-glycoprotein ATPase and/or P-glycoprotein inhibitors (Meyer *et al.*, 2015). To predict clinical relevance of the hBCRP inhibition based on IC_{50} values, expected plasma concentrations should be considered and only limited information concerning mitragynine plasma concentrations is available as case reports (Wagmann *et al.*, 2018). Interpretation is difficult due to single cases, multiple drug intake, or incomplete toxicological investigations. Based on limited data, expected mitragynine plasma concentrations are lower than the determined $IC_{50,}$ indicating that a clinical effect is unlikely.

The potential for a mitragynine interaction with P-glycoprotein transport activity, mRNA, and protein gene expression in Caco-2 cells using molecular docking, bidirectional assay, reverse transcription quantitative real-time polymerase chain reaction, Western blot analysis, and immunocytochemistry techniques was evaluated (Rusli *et al.*, 2019). Mitragynine interacted at the nucleotide binding domain site of P-gp but not at the substrate binding site. Mitragynine inhibited P-glycoprotein transport *in vitro* by inhibiting mRNA and protein expression of P-gp in the Caco-2 cells. Based on these *in vitro* data, mitragynine is likely a P-gp inhibitor weaker than quinidine but not a substrate, suggesting that concurrent administration of mitragynine-containing kratom products and other P-gp substrate drugs could lead to an adverse drug-drug interaction.

Mitragynine concentrations from 0.25 to 250 µM induced N-demethylase and inhibited GST (IC_{50} = 11.8 to 24.4 µM) (Ya et al., 2019).

Tanna *et al.* (2021) reevaluated kratom and CYP-mediated drug interaction risk using mitragynine as the marker constituent. Inhibition of a CYP in a concentration-dependent manner and by at least 50% at the highest tested concentration indicated mitragynine-mediated inhibition. A leftward shift of ≥1.5-fold in IC_{50} indicated potential time-dependent inhibition. An IC_{50} value <20 mM indicated potential clinical relevance of CYP inhibition, determined relative to the highest mitragynine concentration quantified from autopsy blood samples. Mitragynine was tested against CYP2C9 (diclofenac 4'-hydroxylation), CYP2D6 (dextromethorphan O-demethylation), and CYP3A (midazolam 19-hydroxylation) activities in human liver microsomes and CYP3A activity in human intestinal microsomes. Mitragynine showed a time-dependent inhibition of hepatic and intestinal CYP3A activity that resulted in an estimated 5.7-fold increase in exposure to the probe drug substrate midazolam. Extracts at 2 mg/mL the lowest concentration tested inhibited CYP2D6, CYP2C9, and CYP3A by 44 to 64%, 24 to 29%, and 15 to 23%, respectively. Mitragynine at 1 mM inhibited these enzymes by 57%, 21%, and 26%, respectively. Kratom extracts and mitragynine inhibited CYP3A activity in human intestinal microsomes in a concentration-dependent manner, with greater inhibition than hepatic liver microsomes at higher concentrations; at the lowest tested concentration, CYP3A activity in human intestinal microsomes was inhibited by 24% to 25% and ∼9% in hepatic liver microsomes. Methanolic kratom extracts strongly inhibited CYP2D6 and modestly inhibited CYP3A and CYP2C9 in both types of microsomes in a concentration-dependent manner, with greater effects on intestinal microsomes. Mitragynine exhibited similar trends with liver microsomes, showing the most potent inhibition against CYP2D6, followed by CYP3A and CYP2C9. The effects of mitragynine on CYP3A were similar in these microsomes. However, these data may not predict clinical relevance. Using a systematic tiered approach that considered reversible and

time-dependent inhibition of liver and intestine as sites of potential drug interactions, mitragynine was identified as a potent reversible inhibitor of CYP2D6 and a time-dependent inhibitor of CYP3A.

C.3.2.4.2 *In vivo* Studies of Interactions Between Mitragynine, *M. speciosa*, and Other Substances

Permethrin, an insecticide in mosquito coils, is hydrolyzed by carboxylesterase (Srichana *et al*., 2015). Kratom is sometimes abused in Southeast Asia by drinking a "4 x 100" cocktail (containing boiled leaf extract, cola beverages, and codeine- or diphenhydramine-containing cough syrup) that can include pyrethroid insecticides. The effects of mitragynine and an alkaloid kratom extract on *trans*-pyrethroid pharmacokinetics were investigated in rats to determine if there was a mitragynine-pyrethroid interaction. Rats received single and multiple dose pretreatment with mitragynine and the alkaloid extract prior to receiving a single oral 460 mg/kg pyrethroid dose. The pyrethroid elimination rate constant decreased, elimination half-life increased, and the metabolic ratio of pyrethroid and its metabolite phenoxybenzylalcohol decreased after treatment with mitragynine or the extract. These data suggested that pyrethroid-mitragynine and pyrethroid-alkaloid extract interactions that occur in these kratom cocktails may increase the risk of pyrethroid toxicity due to inhibition of its metabolism and elimination.

Azizi *et al.* (2013) treated Sprague-Dawley rats with methanolic (50, 100, and 200 mg/kg), aqueous (50, 100, and 200 mg/kg), and a total alkaloid extract (5, 10, and 20 mg/kg) of *M. speciosa via* oral gavage to study its effects on the CYPs and UDP-UGT drug metabolizing enzymes. Significant inductive effects of mitragynine extracts on UGT and CYP450 activity were observed, potentially increasing clearance of drugs metabolized through this pathway.

Azizi *et al.* (2010) studied the effects of methanolic, aqueous and total alkaloid extracts on GST activity in male Sprague-Dawley rat liver cytosol. At the highest 750 µg/mL doses, GST activity was inhibited 61% by the methanolic, 50% by the aqueous, and 43% by the total alkaloid extract ($p<0.001$). However, in the *in vivo* rat study, GST activity increased after exposure to all three extracts, but significantly (129%) after the 100 mg/kg aqueous extract compared to the control. GST induction could increase protection against the toxic effects of electrophilic chemicals or metabolites.

The intestinal permeability of mitragynine was also investigated *in situ* in rat small intestine in the absence/presence of P-glycoprotein and/or CYP3A4 inhibitors (Jagabalan *et al*., 2019). Mitragynine demonstrated high intestinal P_{eff} (1.11×10^{-4} cm/second)—similar to the highly permeable drug propranolol (P_{eff} 1.27×10^{-4} cm/second). Addition of azithromycin (P-glycoprotein inhibitor) and ciprofloxacin (CYP3A4 inhibitor) or both had no effect on intestinal permeability of mitragynine across the rat small intestine, suggesting that mitragynine is not a P-glycoprotein substrate. Neither is mitragynine a substrate of CYP3A4 metabolism in the small intestine of the rat.

C.3.2.4.3 Reviews of Interactions Between Mitragynine, *M. speciosa*, and Other Substances

According to a review by Ulbricht *et al.* (2013), a methanolic extract of kratom inhibited CYP isozymes 2C9, 2D6, 1A2, and 3A4, with the most potent blockade seen with CYP2D6. These data suggest that a drug-drug interaction may occur if used by patients with neurologic disorders or those using alcohol, sedatives, benzodiazepines, opioids, or opium-containing products, or stimulant substances, such as caffeine, caffeine-containing products, cocaine, yohimbine, or related compounds. Also, the co-administration with monoamine oxidase inhibitors (MAOIs) is not advised. They also concluded the concomitant use of MAOIs, ayahuasca (*Banisteriopsis caapi*), Syrian rue (*Peganum harmala*), or passionflower (*Passiflora incarnata*) with *M. speciosa* may potentially cause serious reactions. Moreover, yohimbe (*Pausinystalia yohimbe*) combined with *M. speciosa* may cause overstimulation and increased blood pressure, as also occurs with the concomitant use of caffeine.

The effects of mitragynine and kratom alkaloids on phase 1 drug metabolizing enzymes could be important for drug interactions (Kruegel and Grundmann, 2018). A CYP-mediated kratom-drug interaction is possible if CYP2D6 substrates are co-ingested, and if mitragynine concentrations in the liver or intestine reach the estimated IC_{50}. The potential exists for kratom to precipitate unfavorable interactions with other drugs metabolized through these enzymes. These observations should be confirmed *in vivo* and efforts should be made to identify the specific alkaloids responsible for the observed bioactivity. Likewise, there exists the possibility for the gross behavioral effects of kratom to differ from the pure alkaloids based not only on polypharmacology, but also on metabolic interactions between the mixed alkaloids.

C.3.2.4.4 Summary of Potential NDI-Drug Interactions

The impact of mitragynine and kratom extracts on common drug metabolizing enzymes and transport proteins varied across studies. Mitragynine inhibited CYP2D6 and P-glycoprotein *in vitro*; however, all studies described inhibition as weak. In addition, mitragynine and alkaloid-containing extractions were also observed to induce GST, which may increase protection from toxic electrophilic molecules or metabolites. Lastly, induction of aminopyrine N-demethylase and UDP-UGT activities may increase clearance of drugs such as methadone, buprenorphine, or ketamine. In some cases, results observed *in vitro* do not translate to *in vivo* models. This was the case for other herbal products such as green tea extract (Teschke et al., 2014). NDI-drug interactions may be possible, but the prevalence of reports is low compared to the prevalence of kratom use.

C.3.2.5 Abuse Potential of M. speciosa and Mitragynine and Potential to Produce Withdrawal

As stated in Section C.3.2.1, several studies showed mitragynine activity at the μ-opioid receptor, as well as κ-opioid, δ-opioid, and dopamine D1 receptors. While there are no published studies of the abuse potential of *M. speciosa* or any of its alkaloids conducted in humans, there were several nonclinical studies and case studies or retrospective observational studies relevant to abuse potential sponsored by independent academic and government-funded laboratories and published in peer-reviewed journals. None of these studies were conducted with NPI-001; however, most of the studies evaluated the most abundant alkaloid in NPI-001, mitragynine. Interspecies differences between humans and other mammals (in particular mice) in metabolism of mitragynine may also affect results.

C.3.2.5.1 Nonclinical

This notification includes 2 categories of abuse potential related studies addressing the most abundant alkaloid in NPI-001, namely, mitragynine. The studies were conducted and published in peer-reviewed scientific journals independently and without funding or input by the NDIN sponsor. The categories of studies were described in the FDA's 2017 Guidance for Industry – Assessment of the Abuse Potential of Drugs (U.S. FDA, 2017). The first category to be summarized evaluated the rewarding effects in the intravenous self-administration, conditioned place preference, and intracranial self-stimulation models, as well as comparison of the discriminative effects of mitragynine with drugs of abuse. The second category of abuse potential related studies addressed the physical dependence and withdrawal potential employing several approaches and outcome measures.

C.3.2.5.1.1 Studies Evaluating Potential Rewarding and Opioid-like Effects of Mitragynine

The rewarding effects were evaluated in the animal abuse potential model widely considered the most predictive of human abuse potential, namely, the intravenous drug self-administration model. In 2 independent studies, Yue *et al.* (2018) and Hemby *et al.* (2019) compared mitragynine to heroin and morphine, respectively. The animals robustly self-administered heroin and morphine but not mitragynine at

any of a broad range of doses. These findings were concluded by the investigators to be indicative of low relative abuse potential of mitragynine.

Both foregoing intravenous self-administration studies also included an evaluation of the potential utility of mitragynine as a treatment for opioid use disorder by evaluating the effects of pretreatment on subsequent rates of intravenous opioid self-administration. Mitragynine produced dose-dependent reductions in heroin (Yue *et al.*, 2018) and morphine self-administration (Hemby *et al.*, 2019). These findings are consistent with the reports of people with opioid use disorder that mitragynine consumption helps to relieve their opioid withdrawal and cravings (see also Grundmann, 2017; Coe *et al.*, 2019; Garcia-Romeu *et al.*, 2020; NIDA, 2021 [kratom fact website]; Henningfield *et al.*, 2022a; Harun *et al.,* 2022 [Mini Review in Neuroscience Letters]).

Drug discrimination studies found that while mitragynine can substitute for morphine in morphine-dependent rats, generalization is significantly lower than for prototypical opioid agonists, or morphine itself (Hassan *et al.*, 2020; Hiranata *et al.*, 2020; Obeng *et al.*, 2021). In another drug discrimination study, Reeve *et al.* (2020) compared mitragynine generalization to several substances in addition to morphine because mitragynine is only a partial opioid agonist and has *alpha* adrenergic and other effects. Reeve *et al.* (2020) found that mitragynine generalized most strongly to lofexidine and phenylephrine, which are not scheduled drugs of abuse (Henningfield *et al.,* 2022a). Phenylephrine is included in some over-the-counter cold medicines and lofexidine is the first FDA-approved nonopioid medicine for the treatment of opioid withdrawal.

Effects of mitragynine in the conditioned place preference (CPP) model are mixed and overall do not indicate strong rewarding effects (Henningfield *et al.*, 2022a). The ability of mitragynine to induce CPP reinstatement in rats that had previously extinguished a morphine- or mitragynine-induced CPP was evaluated (Japarin *et al.*, 2021). Following a CPP acquisition induced by either mitragynine (10 and 30 mg/kg, intraperitoneally [i.p.]) or morphine (10 mg/kg, i.p.), rats were subjected to repeated CPP extinction sessions. A low-dose priming injection of mitragynine or morphine produced a reinstatement of the previously extinguished CPP. In the second experiment of this study, a priming injection of morphine (1, 3, and 10 mg/kg, i.p.) dose-dependently reinstated a mitragynine-induced CPP. Likewise, a priming injection of mitragynine (3, 10, and 30 mg/kg, i.p.) was able to dose-dependently reinstate a morphine-induced CPP. These results suggest some level of rewarding effects; however, another CPP study involving lyophilized (freeze-dried) kratom tea which contained mitragynine, did not find CPP or rewarding effects (Wilson *et al.*, 2020). A third study by Yusoff *et al.* (2018) found that 2.5 and 5 mg/kg baclofen, a *gamma*-aminobutyric acid (GABA)-B receptor agonist, blocked expression of mitragynine-induced CPP, suggesting that mitragynine's CPP effects might be modulated by the GABA-B receptor.

Mitragynine's ability to affect brain reward thresholds was assessed in an intracranial self-stimulation (ICSS) procedure (Behnood-Rod *et al.*, 2020). Mitragynine at up to 56 mg/kg increased brain reward thresholds, showing that it is not rewarding in this procedure, and the investigators concluded that these data did not indicate abuse potential.

C.3.2.5.1.2 Studies of Potential Physical Dependence and Withdrawal Effects of Mitragynine

Harun *et al.* (2020) administered escalating doses of mitragynine (15, 20, 25, 35, and 45 mg/kg i.p.), twice daily, in rats, for 14 consecutive days. The production of physical dependence was demonstrated by the finding that naloxone administration (2 mg/kg i.p.) precipitated somatic signs of withdrawal in mitragynine-treated and morphine-treated rats; however, the global withdrawal scores were weaker in the mitragynine-treated animals than the morphine-treated animals. Withdrawal signs did not include all of those typical of

opioid withdrawal (*e.g.*, "jumping," "teeth chattering," and "grooming" were not precipitated by naloxone administration in the mitragynine-treated animals).

Furthermore, the anxiogenic withdrawal response typical during opioid withdrawal did not occur in the mitragynine-treated animals. Administration of naloxone (2 mg/kg i.p.) was evaluated in a maze study designed to examine whether the mitragynine withdrawal included the sign of anxiety behavior indicated by the percentage of center time and number of center entries. This measure of anxiety behavior was not precipitated by naloxone administration in mitragynine-treated animals, but was precipitated by naloxone administration in the morphine-treated animals, suggesting an additional qualitative difference in the withdrawal syndromes elicited in the 2 groups. These differences are in addition to the somatic differences described in the preceding paragraph. These data suggest that mitragynine withdrawal is qualitatively distinct and less severe for mitragynine as compared to morphine.

Harun *et al.* (2020) used another measure to assess physical dependence and withdrawal: whether discontinuation of mitragynine administration leads to a disruption in food-maintained operant behavior, a withdrawal sign associated with physical dependence on morphine. In this study, discontinuation of mitragynine administration did not produce response rate disruptions as it did in morphine-treated animals. Naloxone administration did, however, precipitate response rate disruptions indicating withdrawal in both mitragynine- and morphine-treated rats. This withdrawal effect was weaker and shorter lived following mitragynine as compared to morphine administration. These data indicate that, by this measure, withdrawal appears overall weaker and not readily apparent when mitragynine administration ends.

In Wilson *et al.* (2020), the effects of oral lyophilized kratom tea were evaluated in C57BL/6J and opioid receptor knockout mice. Oral kratom tea consumption produced dose-dependent antinociception at use levels ≥1 g/kg—an effect that was absent in mice lacking the MOR and reduced in mice lacking the *kappa*-opioid receptor. These kratom tea doses did not alter coordinated locomotion or induce conditioned place preference, and only briefly reduced respiration. Naloxone administration precipitated withdrawal signs in morphine-treated mice confirming that they were physically dependent to morphine. In contrast, naloxone administration did not precipitate withdrawal signs in the lyophilized kratom tea-treated animals, suggesting that they had not developed physical dependence. Administration of lyophilized kratom tea to the morphine-treated animals reduced naloxone precipitated withdrawal signs consistent with findings from other animal studies that mitragynine administration can prevent or reduce morphine withdrawal, for example, Hassan *et al.* (2020), and Yue *et al.* (2022).

Johari *et al.* (2021) found that while there do appear to be signs that mitragynine can produce physical dependence in rats, withdrawal signs are weak compared to those experiencing morphine withdrawal. Additionally, withdrawal from mitragynine was not associated with anxiogenic-like behavioral effects.

C.3.2.5.1.3 Summary

In multiple nonclinical studies assessing mitragynine's abuse potential in comparison with prototypical opioids like morphine and heroin, mitragynine consistently demonstrated weaker rewarding effects and failed to robustly support self-administration. In drug discrimination studies, mitragynine generalized most strongly to lofexidine and phenylephrine and not the prototypical opioid morphine. Finally, in conditioned place preference and intercranial self-stimulation studies, mitragynine showed low risk of abuse potential. Studies assessing physical dependence and withdrawal effects of mitragynine found that withdrawal is qualitatively distinct and less severe for mitragynine as compared to morphine. Taken together, the findings confirm that the abuse potential of mitragynine is low.

C.3.2.5.2 Case Studies

In addition to reviewing experimental evidence of abuse potential, dependence, and withdrawal, Johnson Foods identified and reviewed case studies of reported kratom-induced dependence, withdrawal, or neonatal abstinence syndrome (NAS). In these case reports, there are no reported cases of patients consuming a kratom product consistent with conditions of use described for NPI-001. In almost all cases, a myriad of pre-existing conditions and co-use of prescription or illicit drugs was also reported. Table 3.2.5.2-1 describes each referenced case report.

Table C.3.2.5.2-1 Physical Dependence, Withdrawal, and/or Neonatal Abstinence Syndrome Case Studies Associated with Use of *M. speciosa* and/or Mitragynine

Age and Gender	Dose, Duration, Kratom Type	Existing Conditions	Symptoms/ Outcome	Required Hospitalization? (Y/N)	Concomitant Substances	Reference
Dependence						
35, M	30 g/day; 3 yr; Powdered kratom (smoothies, shakes)	Anxiety	Kratom dependence/ No reported relapses in 16 months	N	Sertraline, bupropion, nicotine lozenges, alcohol, caffeine	Agapoff and Kilaru (2019)
29, M	90 g/day; >3 mo; UNK type	Bipolar disorder, PTSD, ADHD, chronic back pain	Suicidal ideation, negative mood, anhedonia, amotivation, poor concentration, fatigue, social isolation, depressed mood, kratom dependence/ Discharged, continued kratom use	Y	Aripiprazole, fluoxetine, propranolol, hydroxyzine	Anand and Hosangar (2022)
47, M	1–2 "heaping" teaspoons 3–4 times/day; <1 yr; Powdered kratom	Anxiety, depression, chronic back pain	Kratom dependence/ Discharged, symptoms resolved after buprenorphine/naloxone treatment	N	Alprazolam, escitalopram	Bowe and Kerr (2020)
36, M	120 capsules (90 g)/day; 11 yr; UNK type	OUD, AUD, tobacco use	Dizziness, high blood pressure, tachycardia, elevated temperature, sinus tachycardia, prolonged QTc, Kratom dependence/ No reported relapses in 10 months	N	Venlafaxine, quetiapine	Brogdon *et al.* (2022)
60, F	0.25 oz every 4 h/day; UNK duration and type	AUD, chronic pain, fibromyalgia, osteoarthritis	Kratom dependence, withdrawal/ No reported relapses in 9 months	N	Tramadol, pregabalin, duloxetine, oxycodone-acetaminophen	Buresh (2018)
47, M	UNK dose and type; 1 yr	Chronic low back pain, anxiety, depression, OUD	Kratom dependence/ No reported relapses in 7 months	N	Oxycodone-acetaminophen, codeine/morphine	Buresh (2018)
37, F	UNK dose; >2 yr; Capsules, extract ("Bali")	Chronic pain	Kratom dependence/ No relapses in over 1 year	N	N/A	Galbis-Reig (2016)
36, M	UNK dose; 8–10 times/day for 1.5 yr; Powdered kratom	OUD, back pain, asthma, depression, anxiety	Tramadol use, detoxify, kratom dependence/ No relapses in over 1 year	N	Snuff tobacco, gabapentin, albuterol inhaler, escitalopram	Kalin *et al.* (2020)
34, F	10 capsules/day (30 g); <2 yr; UNK type	AUD, OUD, anxiety, anemia, tonsillectomy, pregnancy, tobacco use	Kratom dependence, anxiety/ Continued MAT, tapering with goal of discontinuing	N	Sertraline	Kalin *et al.* (2020)

Table C.3.2.5.2-1 Physical Dependence, Withdrawal, and/or Neonatal Abstinence Syndrome Case Studies Associated with Use of *M. speciosa* and/or Mitragynine

Age and Gender	Dose, Duration, Kratom Type	Existing Conditions	Symptoms/ Outcome	Required Hospitalization? (Y/N)	Concomitant Substances	Reference
52, F	1 tbsp, 4–6 times/day (4–6 tbsp total); 9 mo; Powdered kratom	MDD, chronic pain	Depression, anxiety, suicidal ideation, pain, kratom dependence, sporadic jerks of limbs and neck/ Discharged, no relapse in 18 months	Y	Sertraline, trazodone, gabapentin, clonazepam	Khazaeli *et al.* (2018)
55, F	5–10 g/day; "many years"; UNK type	Chronic back pain, anxiety, depression	Withdrawal, symptoms emerged while hospitalized for an elective operation/ Discharged, symptoms resolved	Y	Acetaminophen/ oxycodone, escitalopram, cyclobenzaprine, bupropion, omeprazole, alcohol	Kucharik *et al.* (2019)
30, M	50 g/day; ~5 years; Powdered kratom	Severe anxiety	Suicide attempt, kratom dependence/ Transferred to psychiatric hospital, later developed addiction to cough syrup	Y	Escitalopram, bupropion, clonazepam, zolpidem	Lai and Wu (2021)
54, M	20 x 50 mg capsules (1,000 mg total)/day; UNK duration	OUD, AUD	Kratom dependence/ Continued MAT, tapering with goal of discontinuing	N	Illicit opioids, benzodiazepines, zolpidem, tramadol	Lei *et al.* (2021)
59, F	30 g/day; UNK duration and type	Knee pain, OUD, SUD, tobacco use	Kratom dependence/ Continued MAT, test at 6 months positive for mitragynine and 7-hydroxymitragynine, patient denied use	N	Oxycodone	Lei *et al.* (2021)
29, F	18–20 g, 3 times/day (54–60 g total); 2 yr; UNK type	OUD, Chronic low back pain, anxiety	Kratom dependence/ No reported relapses in 4 weeks	Y	N/A	MacKay and Abrahams (2018)
44, M	40 g/day; 3 yr; Powdered kratom	AUD, SUD, depression	Kratom dependence, withdrawal/ Discharged	Y	N/A	McWhirter and Morris (2010)
26, M	30 g/day; <2 yr; Powdered kratom	Chronic pain, depression	Kratom dependence, withdrawal/ Pain management switch from Tilidin to Ibuprofen and Metamizol Natrium	Y	Hydromorphone (possible kratom adulteration)	Müller *et al.* (2020)
63, M	2–3 tsp/day (est. 12 g total); 7 yr; Powdered kratom	MDD, GAD, suicidal ideation	Anxiety, depression, ruling out kratom addiction/ Discharged, continued kratom use	Y	Lorazepam, nicotine	Müller *et al.* (2021)

Table C.3.2.5.2-1 Physical Dependence, Withdrawal, and/or Neonatal Abstinence Syndrome Case Studies Associated with Use of *M. speciosa* and/or Mitragynine

Age and Gender	Dose, Duration, Kratom Type	Existing Conditions	Symptoms/ Outcome	Required Hospitalization? (Y/N)	Concomitant Substances	Reference
37, F	UNK dose, 3–4 times/day; <1 yr; Kratom tea	Restless leg syndrome, recurrent urinary tract infections, asthma, inactive genital herpes, inadequately controlled anxious depression	Kratom dependence/ Gave birth to child with NAS, entered rapid detox program, no reported relapses after 7 days	Y	Acetaminophen-methocarbamol, diphenhydramin, valacyclovir, ranitidine, loratadine, salbutamol, citalopram	Murthy and Clark (2019)
27, M	45 g/day; 2 weeks; UNK type	Back injury	Kratom dependence, anxiety, intrusive obsessive (including violent) thoughts/ Discharged, entered SUD treatment	Y	N/A	Sablaban and Gautam (2021)
20, M	30 g/day; 2 yr; UNK type	ADHD	Kratom dependence/ Continued MAT, tapering with goal of discontinuing	N	Dextramphetamine	Schmuhl *et al.* (2020)
44, M	UNK dose, 6 dropper squeezer every 6 h; 4 mo; Tincture	AUD	Kratom dependence, withdrawal, weight gain, lethargy, myxedematous face, hypothyroidism/ Discharged, symptoms resolved	Y	N/A	Sheleg and Collins (2011)
32, F	UNK dose and type; daily; 7 mo	Pregnancy	Kratom dependence/ Continued MAT, neonate showed no evidence of neonatal abstinence syndrome	N	N/A	Smid *et al.* (2018)
28, F	UNK dose; 4 mo; smoked kratom	SUD, suicide attempts, bipolar disorder	Kratom dependence, withdrawal/ Continued MAT, gave birth to child with NAS	Y	Escitalopram, lamotrigine, quetiapine, nicotine	Smid *et al.* (2018)
44, M	UNK dose and duration; Liquid "infusions"	MDD, SUD, AUD	Kratom withdrawal, elevated anxiety, tachycardia, profuse sweating, psychomotor agitation, insomnia, dysphoric mood, and emotional lability/ Discharged, stable after 9 months	N	Benzodiazepines	Vento *et al.* (2021)
36, M	20 g/day; UNK duration and type	AUD, anxiety, depression	Kratom dependence, withdrawal, anxiety after self-tapering kratom from 20 g/day to 4 g/day/ Continued MAT, tapering with goal of discontinuing	N	N/A	Weiss and Douglas (2021)

Table C.3.2.5.2-1 Physical Dependence, Withdrawal, and/or Neonatal Abstinence Syndrome Case Studies Associated with Use of *M. speciosa* and/or Mitragynine

Age and Gender	Dose, Duration, Kratom Type	Existing Conditions	Symptoms/ Outcome	Required Hospitalization? (Y/N)	Concomitant Substances	Reference
37, M	7–14 g/day; UNK duration and type	Anxiety, depression, OUD	Kratom dependence/ Continued kratom use, continued MAT, abstinent from other opioids/heroin	N	N/A	Weiss and Douglas (2021)
42, F	"Variable" dose; UNK dose, UNK dose, duration and type	Chronic pain, anxiety, depression	Kratom dependence/ Continued MAT	N	N/A	Weiss and Douglas (2021)
Withdrawal						
35, M	30 g/day; 3 yr; Powdered kratom (smoothies, shakes)	Anxiety	Kratom dependence; withdrawal symptoms: restlessness, irritability, muscle spasms, headache, gastrointestinal upset, rhinorrhea, lacrimation, sweating, joint aches/ No reported relapses in 16 months, withdrawal symptoms resolved with buprenorphine/naloxone treatment	N	Sertraline, bupropion, nicotine lozenges, alcohol, caffeine	Agapoff and Kilaru (2019)
29, M	90 g/day; >3 mo; UNK type	Bipolar disorder, PTSD, ADHD, chronic back pain	Suicidal ideation, negative mood, anhedonia, amotivation, poor concentration, fatigue, social isolation, depressed mood, kratom dependence; withdrawal symptoms: Insomnia, anorexia, diarrhea, restlessness, worsening mood, worsening anxiety, thoughts of self-harm/ Discharged, withdrawal symptoms resolved, continued kratom use	Y	Aripiprazole, fluoxetine, propranolol, hydroxyzine	Anand and Hosangar (2022)

Table C.3.2.5.2-1 Physical Dependence, Withdrawal, and/or Neonatal Abstinence Syndrome Case Studies Associated with Use of *M. speciosa* and/or Mitragynine

Age and Gender	Dose, Duration, Kratom Type	Existing Conditions	Symptoms/ Outcome	Required Hospitalization? (Y/N)	Concomitant Substances	Reference
47, M	1–2 "heaping" teaspoons, 3–4 times/day; <1 yr; Powdered kratom	Anxiety, depression, chronic back pain	Kratom dependence; withdrawal symptoms: abdominal cramping, sweating, psychomotor agitation, rhinorrhea, mood disturbances, increased anxiety and depression, and worsening back, knee, generalized body pain/ Discharged, withdrawal symptoms resolved 2 weeks after buprenorphine/naloxone treatment	N	Alprazolam, escitalopram	Bowe and Kerr (2020)
43, M	4 drinks/day; >3.5 yr; tea	Chronic pain	Tonic-clonic seizure; withdrawal symptoms: rhinorrhea, insomnia, poor concentration, constricted affect, myalgia for 10 days/ Discharged	N	Modafinil	Boyer *et al.* (2008)
36, M	120 capsules (90 g)/day; 11 yr; capsules	OUD, AUD, tobacco use	Dizziness, high blood pressure, tachycardia, elevated temperature, sinus tachycardia, prolonged QTc, kratom dependence; withdrawal symptoms: restlessness, nausea, cravings/ No reported relapses in 10 months, withdrawal symptoms resolved	N	Venlafaxine, quetiapine	Brogdon *et al.* (2022)
60, F	0.25 oz every 4 h/day; UNK duration and type	AUD, chronic pain, fibromyalgia, osteoarthritis	Kratom dependence; withdrawal symptoms: rhinorrhea, irritability/ No reported relapses in 9 months, withdrawal symptoms resolved with buprenorphine/naloxone treatment	N	Tramadol, pregabalin, duloxetine, oxycodone-acetaminophen	Buresh (2018)
47, M	UNK dose and type; 1 yr	Chronic low back pain, anxiety, depression, OUD	Kratom dependence; withdrawal symptoms: anxiety, edginess, leg shaking/ No reported relapses in 7 months, withdrawal symptoms resolved with buprenorphine/naloxone treatment	N	Oxycodone-acetaminophen, codeine/ morphine	Buresh (2018)

Table C.3.2.5.2-1 Physical Dependence, Withdrawal, and/or Neonatal Abstinence Syndrome Case Studies Associated with Use of *M. speciosa* and/or Mitragynine

Age and Gender	Dose, Duration, Kratom Type	Existing Conditions	Symptoms/ Outcome	Required Hospitalization? (Y/N)	Concomitant Substances	Reference
24, M	600 mg/day; UNK duration and type	Asperger syndrome, depression, SUD	Loss of consciousness, hypothermic, seizure, acute rhabdomyolysis; withdrawal symptoms: cravings/ Discharged, withdrawal symptoms resolved after 45 days treatment with mechanical ventilation, antibiotics, lorazepam, haloperidol, hydromorphone, sertraline, gabapentin, trazodone, zolpidem, buprenorphine, hydroxyzine, buprenorphine/naloxone	Y	N/A	Diep *et al.* (2018)
37, F	UNK dose; >2 yr; Capsules, extract ("Bali")	Chronic pain	Kratom dependence; withdrawal symptoms: blurred vision due to pupillary dilation, diaphoresis, gastrointestinal distress, anxiety, fever, bone and joint pains, increased lacrimation or rhinorrhea, tremors, and yawning/ No relapses in over 1 year, withdrawal symptoms resolved with clonidine, venlafaxine, pregabalin, naltrexone, and buprenorphine/naloxone treatment	N	N/A	Galbis-Reig (2016)
38, M	3 large teas in most recent occasion, frequent user of kratom teas	Stimulant use disorder, OUD, AUD, PTSD	Elevated LFT, liver injury; withdrawal symptoms: nausea, vomiting, abdominal pain, muscle spasms, uncontrollable limb jerking, yawning, dehydration, vomiting, diarrhea for 72 h/ Discharged, continued kratom use	Y	Chlordiazepoxide, buprenorphine/ naloxone	Jensen *et al.* (2021)
36, M	UNK dose, 8–10 times/day; 1.5 yr; Powdered kratom	OUD, back pain, asthma, depression, anxiety	Tramadol use, detoxify, kratom dependence; withdrawal symptoms: nausea, stomach cramps, diarrhea, severe anxiety, restlessness, sweats, mood swings/ No relapses in over 1 yr, withdrawal symptoms resolved with buprenorphine or naloxone treatment	N	Snuff tobacco, gabapentin, albuterol inhaler, escitalopram	Kalin *et al.* (2020)

Table C.3.2.5.2-1 Physical Dependence, Withdrawal, and/or Neonatal Abstinence Syndrome Case Studies Associated with Use of *M. speciosa* and/or Mitragynine

Age and Gender	Dose, Duration, Kratom Type	Existing Conditions	Symptoms/ Outcome	Required Hospitalization? (Y/N)	Concomitant Substances	Reference
34, F	10 capsules/day (30 g); <2 yr; capsules	AUD, OUD, anxiety, anemia, tonsillectomy, pregnancy, tobacco use	Kratom dependence, anxiety; withdrawal symptoms: irritability, stomach cramps, nausea, sweating, heart palpitations, and insomnia/ Continued MAT, tapering with goal of discontinuing, withdrawal symptoms resolved with buprenorphine, buprenorphine/naloxone	N	Sertraline	Kalin *et al.* (2020)
25, M	4–6 teaspoons; UNK duration and type	N/A	Abdominal pain, brown discoloration of urine, jaundice, pruritus, elevated LFT; withdrawal symptoms: fever, chills, insomnia, restlessness for 1 week/ Discharged, symptoms resolved (47 days follow-up)	Y	N/A	Kapp *et al.* (2011)
52, F	1 tbsp, 4–6 times/day (4–6 tbsp total); 9 mo; Powdered kratom	MDD, chronic pain	Depression, anxiety, suicidal ideation, pain, kratom dependence, sporadic jerks of limbs and neck; withdrawal symptoms: rhinorrhea, diarrhea, upset stomach, anxiety, restless legs, increased pain/ Discharged, no relapse in 18 mo, withdrawal symptoms resolved with lorazepam, clonazepam, baclofen, trazodone, gabapentin, buprenorphine/naloxone treatment	Y	Sertraline, trazodone, gabapentin, clonazepam	Khazaeli *et al.* (2018)
55, F	5–10 g/day; "many years"; UNK type	Chronic back pain, anxiety, depression	Withdrawal, symptoms emerged while hospitalized for an elective operation; withdrawal symptoms: nausea, vomiting, confusion, agitation, auditory and visual hallucinations, febrile, tachycardia, hypotensive for 12 days/ Discharged, symptoms resolved	Y	Acetaminophen/ oxycodone, escitalopram, cyclobenzaprine, bupropion, omeprazole, alcohol	Kucharik *et al.* (2019)

Table C.3.2.5.2-1 Physical Dependence, Withdrawal, and/or Neonatal Abstinence Syndrome Case Studies Associated with Use of *M. speciosa* and/or Mitragynine

Age and Gender	Dose, Duration, Kratom Type	Existing Conditions	Symptoms/ Outcome	Required Hospitalization? (Y/N)	Concomitant Substances	Reference
30, M	50 g/day; ~5 years; Powdered kratom	Severe anxiety	Suicide attempt, kratom dependence; withdrawal symptoms: anxiety, myalgia, diarrhea, yawning/ Transferred to psychiatric hospital, later developed addiction to cough syrup, resolution of withdrawal symptoms UNK	Y	Escitalopram, bupropion, clonazepam, zolpidem	Lai and Wu (2021)
62, M	1–3 tbsp (concentration UNK); 1 yr; liquid kratom	OUD, cannabis dependence, tobacco use disorder, chronic pain	Vomiting; withdrawal symptoms: chills, body aches, restlessness, decreased energy and concentration, gastrointestinal discomfort/ Kratom confirmed negative at 66 days, withdrawal symptoms resolved with buprenorphine/naloxone treatment	N	Marijuana	Lei *et al.* (2021)
54, M	Twenty 50 mg capsules (1,000 mg total)/day, UNK duration; capsules	OUD, AUD	Kratom dependence; withdrawal symptoms: muscle aches, pain, headaches, nausea, stomach cramps/ Continued MAT, tapering with goal of discontinuing, withdrawal symptoms resolved with buprenorphine/naloxone treatment	N	Illicit opioids, benzodiazepines, zolpidem, tramadol	Lei *et al.* (2021)
59, F	30 g/day; UNK duration and type	Knee pain, OUD, SUD, tobacco use	Kratom dependence; withdrawal symptoms: myalgia, paresthesia, nausea, cravings for kratom/ Continued MAT, withdrawal symptoms resolved with buprenorphine/naloxone treatment, test at 6 months positive for mitragynine and 7-hydroxymitragynine, patient denied use	N	Oxycodone	Lei *et al.* (2021)
29, F	18–20 g, 3 times/day (54–60 g total); 2 yr; UNK type	OUD, Chronic low back pain, anxiety	Kratom dependence; withdrawal symptoms: diaphoresis, rhinorrhea, myalgia, anxiety, nausea, diarrhea, piloerection/ No reported relapses in 4 weeks, withdrawal symptoms resolved with morphine treatment	Y	N/A	MacKay and Abrahams (2018)

Table C.3.2.5.2-1 Physical Dependence, Withdrawal, and/or Neonatal Abstinence Syndrome Case Studies Associated with Use of *M. speciosa* and/or Mitragynine

Age and Gender	Dose, Duration, Kratom Type	Existing Conditions	Symptoms/ Outcome	Required Hospitalization? (Y/N)	Concomitant Substances	Reference
44, M	40 g/day; 3 yr; Powdered kratom	AUD, SUD, depression	Kratom dependence; withdrawal symptoms: cravings, anxiety, dread, restlessness, sweating, itch, chills, nausea, aches and pains, irritability, tremors/ Discharged, withdrawal symptoms resolved with dihydrocodeine, lofexidine, chlorpromazine treatment	Y	N/A	McWhirter and Morris (2010)
26, M	30 g/day; <2 yr; Powdered kratom	Chronic pain, depression	Kratom dependence; withdrawal symptoms: cravings, loss of analgesia/ Pain management switch from Tilidin to Ibuprofen and Metamizol-Natrium, withdrawal symptoms resolved with detoxification treatment	Y	Hydromorphone (possible kratom adulteration)	Müller *et al.* (2020)
27, M	45 g/day; 2 weeks; UNK type	Back injury	Kratom dependence, anxiety, intrusive obsessive (including violent) thoughts; withdrawal symptoms: anxiety, intrusive obsessive thoughts, rhinorrhea, lacrimation/ Discharged, entered SUD treatment, withdrawal symptoms resolved with lorazepam treatment	Y	N/A	Sablaban and Gautam (2021)
20, M	30 g/day; 2 yr; UNK type	ADHD	Kratom dependence; withdrawal symptoms: nausea, heart palpitations, irritability/ Continued MAT, tapering with goal of discontinuing, withdrawal symptoms resolved with buprenorphine/naloxone treatment	N	Dextramphetamine	Schmuhl *et al.* (2020)
44, M	UNK dose, 6 dropper squeezer every 6 h; 4 mo; Tincture	AUD	Kratom dependence, withdrawal, weight gain, lethargy, myxedematous face, hypothyroidism; withdrawal symptoms: "opiate-type withdrawal" (cramping, abdominal pain, sweating, diarrhea) for 3 days/ Discharged, symptoms resolved	Y	N/A	Sheleg and Collins (2011)

Table C.3.2.5.2-1 Physical Dependence, Withdrawal, and/or Neonatal Abstinence Syndrome Case Studies Associated with Use of *M. speciosa* and/or Mitragynine

Age and Gender	Dose, Duration, Kratom Type	Existing Conditions	Symptoms/ Outcome	Required Hospitalization? (Y/N)	Concomitant Substances	Reference
32, F	UNK dose and type; daily; 7 mo	Pregnancy	Kratom dependence; withdrawal symptoms: depression/ Continued MAT, neonate showed no evidence of neonatal abstinence syndrome, withdrawal symptoms resolved with buprenorphine treatment	N	N/A	Smid *et al.* (2018)
44, M	UNK dose and duration; Liquid "infusions"	MDD, SUD, AUD	Kratom withdrawal, elevated anxiety, tachycardia, profuse sweating, psychomotor agitation, insomnia, dysphoric mood, and emotional lability; withdrawal symptoms: restlessness, anxiety, agitation, insomnia, dysphoria, crying spells, self-harm ideation, cravings, avolition/ Discharged, stable after 9 months, withdrawal symptoms resolved with pregabalin, tapering off prescribed sertraline, bupropion, trazodone, tramadol, clomipramine treatment	N	Benzodiazepines	Vento *et al.* (2021)
36, M	20 g/day; UNK duration and type	AUD, anxiety, depression	Kratom dependence, anxiety after self-tapering kratom from 20 g/day to 4 g/day; withdrawal symptoms: anxiety/ Continued MAT, tapering with goal of discontinuing, withdrawal symptom resolved with buprenorphine/ naloxone treatment	N	N/A	Weiss and Douglas (2021)
Neonatal Abstinence Syndrome						
29, F	1–3 tablets (5 g) (total 5–15 g/day); UNK duration; tablets	Chronic low back pain, fibromyalgia, anxiety	N/A / Gave birth to child with NAS	N	Gabapentin, clonazepam, prenatal vitamins	Davidson *et al.* (2019)
Infant, F	See above entry for birth mother	N/A	NAS; reduced oral intake, jitteriness, hypertonia, sneezing, excessive crying, tachypnea, hyperthermia, excessive suck, poor feeding associated with spit up/ Discharged, symptoms resolved	Y	N/A	

Table C.3.2.5.2-1 Physical Dependence, Withdrawal, and/or Neonatal Abstinence Syndrome Case Studies Associated with Use of *M. speciosa* and/or Mitragynine

Age and Gender	Dose, Duration, Kratom Type	Existing Conditions	Symptoms/ Outcome	Required Hospitalization? (Y/N)	Concomitant Substances	Reference
UNK, F	UNK dose and duration; Kratom tea	OUD	N/A / Gave birth to child with NAS	N	N/A	Eldridge *et al.* (2018)
Infant, M	See above entry for birth mother	N/A	NAS; sneezing, jitteriness, excessive suck, facial excoriations, resting tremors, high-pitched cry, hypertonia, and irritability for 8 days/ Spontaneous improvement of symptoms, discharged	Y	N/A	
29, F	18–20 g, 3 times/day (54–60 g total); 2 yr; UNK type	OUD, chronic low back pain, anxiety	Kratom dependence/ No reported relapses in 4 weeks	Y	N/A	MacKay and Abrahams (2018)
Infant, F	See above entry for birth mother	N/A	NAS; feeding intolerance, jitteriness, irritability, emesis/ Discharged, symptoms resolved with morphine treatment	Y	N/A	
37, F	UNK dose, 3–4 times/day; <1 yr; Kratom tea	Restless leg syndrome, recurrent urinary tract infections, asthma, inactive genital herpes, inadequately controlled anxious depression	Kratom dependence/ Gave birth to child with NAS, entered rapid detox program, no reported relapses after 7 days	Y	Acetaminophen-methocarbamol, diphenhydramin, valacyclovir, ranitidine, loratadine, salbutamol, citalopram	Murthy and Clark (2019)
Infant, F	See above entry for birth mother	N/A	NAS; jitteriness, hypertonia, excessive sucking, irritability, sleeplessness, prolonged crying for 2 months/ Discharged, symptoms resolved	Y	N/A	
28, F	UNK dose; 4 mo; smoked kratom	SUD, suicide attempts, bipolar disorder	Kratom dependence, withdrawal/ Continued MAT, gave birth to child with NAS	Y	Escitalopram, lamotrigine, quetiapine, nicotine	Smid *et al.* (2018)
Infant, F	See above entry for birth mother	N/A	NAS; no specific symptoms listed/ Discharged, symptoms resolved	Y	N/A	

ADHD = attention-deficit/hyperactivity disorder; AUD = alcohol use disorder; F = female; GAD = generalized anxiety disorder; h = hour(s); LFT = liver function test; M = male; MAT = medication-assisted treatment; MDD = major depressive disorder; mo = month(s); N = no; N/A = not applicable; NAS = neonatal abstinence syndrome; OUD = opioid use disorder; PTSD = post-traumatic stress disorder; QTc = QT corrected for heart rate; SUD = substance use disorder; tbsp = tablespoon; tsp = teaspoon; UNK = unknown; Y = yes; yr = year(s).

C.3.2.5.2.1 Dependence and Withdrawal

Twenty-eight cases of kratom dependence and withdrawal were identified in literature and summarized in the table above (Table C.3.2.5.2-1). In all cases patients had pre-existing conditions, some of which included anxiety, bipolar disorder, depression, and alcohol, opioid, and/or substance use disorders. Descriptions of the kratom consumed were limited to 14 cases and included powered kratom, kratom tea, kratom tincture, liquid infusion, or capsules. One case involved a 28-year-old female who smoked kratom. Concomitant drugs and other substances were taken in 18 of the 28 cases, with 1 reporting possible kratom adulteration with hydromorphone. The duration of kratom intake in 21 of 28 cases ranged from 2 weeks to 7 years, with 9 reporting intake over multiple years. Sixteen cases reported high daily kratom doses of 5 to 90 g. Most cases required medical treatment, including buprenorphine/naloxone treatment, and 12 cases required inpatient hospitalization. Twelve cases reported symptoms that resolved or had no relapses, and in the other cases patients were discharged or continued medical treatment with no mention on the resolution of their symptoms. Most cases reported that withdrawal symptoms resolved with treatment, many times with buprenorphine/naloxone naltrexone, lorazepam, and/or haloperidol.

C.3.2.5.2.2 Neonatal Abstinence Syndrome

Five NAS cases were reported in infants of mothers who took kratom (Table C.3.2.5.2-1, above). Infants with NAS showed non-substance-specific symptoms of withdrawal. In some cases, the mothers had pre-existing conditions that included opioid or substance use disorders. Descriptions of the kratom product taken by the mother were reported in 4 cases including kratom tea, kratom tablets, and smoked kratom. The duration of kratom intake in 3 cases ranged from 4 months to 2 years and the kratom dose in 2 mothers was 5 to 15 g/day (duration unknown) and 54 to 60 g/day for 2 years. Infants with NAS required inpatient hospitalization and medical treatment including morphine. Duration of withdrawal symptoms reported in 3 cases ranged from 48 hours to 2 months and in 2 cases occurred during the hospital stay after birth.

C.3.2.5.3 Summary of Data and Relevance to NPI-001

Case studies of physical dependence, withdrawal, and neonatal abstinence syndrome associated with *M. speciosa* or mitragynine use are rare and involve high kratom doses and a long duration of use unlike those recommended for NPI-001. Many of these cases involved individuals with extensive substance use histories for medical and nonmedical purposes. The relevance of these data is limited and does not reflect the safety of NPI-001 when used as recommended.

C.3.3 Safety of 7-Hydroxymitragynine

7-Hydroxymitragynine safety data should also be considered, as this alkaloid is a metabolite of mitragynine—the most abundant alkaloid in NPI-001. NPI-001 is specified to contain less than 0.1% 7-hydroxymitragynine on a dry weight basis. Typically, less than 2% of the alkaloid content of the *M. speciosa* leaf is identified to be 7-hydroxymitragynine in other leaf products, but as with mitragynine, the content in a leaf can vary depending on the geographical region of growth and seasons of harvest (Hassan *et al*., 2013). In other mass spectrometry investigations of *M. speciosa* alkaloid content, no 7-hydroxymitragynine (Zhang *et al*., 2020) or only trace quantities (Kruegel *et al*., 2016) were identified in extractions of raw kratom plant material.

C.3.3.1 Pharmacology

7-Hydroxymitragynine is a partial agonist of the human μ-opioid receptor *in vitro*, with an EC_{50} of 34.5 nM and an E_{max} of 47%, ∼10-fold more potent than mitragynine (EC_{50} = 339 nM, E_{max} = 34%) (Kruegel *et al.*, 2016; Hemby et al., 2019). Obeng *et al.* (2020) determined a lower EC_{50} of 7.6 nM compared to the much less potent mitragynine EC_{50} of 307 nM. 7-Hydroxymitragynine also had antagonistic effects at the κ-opioid receptor with a binding constant of 115 nM. 7-Hydroxymitragynine is a G-protein biased μ-opioid receptor partial agonist in rodents, meaning that it does not act through the *beta*-arrestin pathway that is responsible for respiratory depression in prototypical opioids (Kruegel *et al.*, 2016; Váradi *et al.*, 2016). No mortality was observed in mice following oral administration of 6.25 to 50 mg/kg 7-hydroxymitragynine (Smith *et al.*, 2019).

7-Hydroxymitragynine degraded up to 27% in the low pH of simulated gastric fluid, with only 6% degradation in simulated intestinal fluid at a pH around 7 (Manda *et al.*, 2014). 7-Hydroxymitragynine had high plasma protein binding (>90%) determined by equilibrium dialysis and was metabolized by human liver microsomes with a half-life of 24 minutes. 7-Hydroxymitragynine had moderate permeability across Caco-2 (intestinal) and MDR-MDCK (BBB) monolayers with no significant efflux. 7-Hydroxymitragynine inhibited P-glycoprotein with an EC_{50} of 32.4 ± 1.9 μM.

Mitragynine is converted *in vitro* in mouse and human liver preparations to 7-hydroxymitragynine by CYP3A4, but to a greater extent in the human preparation, indicating that interspecies differences are likely important (Kruegel *et al.*, 2019). Earlier research showed that despite mitragynine being only moderately bioavailable by the oral route, it was more potent as an analgesic when administered by the oral and i.p. routes than the subcutaneous route in rats and mice (Macko *et al.*, 1972; Sabetghadam *et al.*, 2013a,b; Kruegel and Grundmann, 2018), indicating the involvement of an active metabolite produced *via* first pass metabolism. These data conflict with those of Manda *et al.* (2014) that reported mitragynine as stable when mixed with microsomes. 7-Hydroxymitragynine was much more stable in the presence of human or mouse liver microsomes than the parent mitragynine (Kruegel *et al.*, 2019).

Haron and Ismail (2014) evaluated the inhibitory potentials of mitragynine and 7-hydroxymitragynine on 4-methylumbelliferone (4-MU) glucuronidation in rat and human liver microsomes and recombinant human UGT1A1 and UGT2B7 isoforms. For human UGT1A1 isoform, 7-hydroxymitragynine strongly inhibited 4-MU glucuronidation with an IC_{50} of 7.13 ± 1.16 μM, as well as for human UGT2B7, with an IC_{50} of 26.4 ± 1.31. These data suggest that 7-hydroxymitragynine may lead to interactions if co-administered with drugs that are UGT2B7 and UGT1A1 substrates.

Mice (129S1) were treated with 10 mg/kg subcutaneous mitragynine and plasma and brain samples collected at 15 and 60 minutes after dosing analyzed by LC-MS/MS for mitragynine and 7-hydroxymitragynine (Kruegel et al*.,* 2019). Mitragynine and 7-hydroxymitragynine were detected at both time points in plasma and brain, confirming that 7-hydroxymitragynine is formed as a metabolite of mitragynine *in vivo* and that it enters the brain. Mitragynine's brain penetration was high with approximately equivalent brain and blood concentrations, while 7-hydroxymitragynine brain penetration was more modest (∼1:5 brain/plasma), although this estimation did not account for tissue binding and does not necessarily reflect free 7-hydroxymitragynine concentrations. There clearly were differences in the *in vitro* production of 7-hydroxymitragynine from mitragynine and *in vivo* production in mice where the mitragynine/7-hydroxymitragynine ratio in plasma was ∼15:1 or more. Inter-species differences in the affinity of mitragynine at the μ-opioid receptor also may mean that mitragynine and 7-hydroxymitragynine contribute to the observed effects of mitragynine.

When equianalgesic doses of 140 mg/kg subcutaneous mitragynine and 0.7 mg/kg subcutaneous 7-hydroxymitragynine were administered and analgesic activity confirmed at 15 minutes in the tail-flick assay, there was no significant difference in tail-flick latency between the 2 groups and the concentrations of 7-hydroxymitragynine between the groups were not significantly different (Kruegel et al*.,* 2019). The authors concluded that the brain concentrations of 7-hydroxymitragynine were sufficient to explain most or all of the opioid-receptor-mediated analgesic activity of mitragynine, highlighting the importance of route of administration for determining the activity of mitragynine.

C.3.3.2 Pharmacokinetics of 7-Hydroxymitragynine

C.3.3.2.1 *In vitro* 7-Hydroxymitragynine Pharmacokinetics Studies

In a recent *in silico* investigation utilizing quantum chemical calculations to complement experimental nonclinical findings, 7-hydroxymitragynine metabolism was similar to that of mitragynine (Limpanuparb *et al*., 2019). 7-Hydroxymitragynine's main metabolic pathways included hydrolysis of the only methyl ester at position 16, O-demethylation of the 9-methoxy group, O-demethylation of the 17-methoxy group, subsequent conjugation with glucuronic acid at 1 of these three positions, subsequent conjugation with sulfate only at the 9-methoxy group, and combinations of these steps. However, the metabolism of 7-hydroxymitragynine also had changes in reactivity in comparison to mitragynine, with position 17 more reactive towards demethylation and conjugation with glucuronic acid, and position 9 less reactive towards conjugation with glucuronic acid.

C.3.3.2.2 *In vivo* 7-Hydroxymitragynine Pharmacokinetics Studies

C.3.3.2.2.1 Rats

Vuppala *et al.* (2013) evaluated 7-hydroxymitragynine pharmacokinetics in 6 rats over 8 hours following 4 mg/kg IV 7-hydroxymitragynine (0.2 mL over 30 seconds in the jugular vein). Mean C_{max} was 3,000 ± 300 ng/mL at a 1- to 2-minute T_{max} and a 22.9- ± 3.6-minute terminal $t_{1/2}$. The V_d was 1,596 ± 586 mL/kg and clearance was 44.2 ± 14.8 mL/minute·kg. These data indicate that 7-hydroxymitragynine is more rapidly eliminated and has a lower V_d than mitragynine, consistent with its low stability in liver microsomes.

The pharmacokinetics of 11 alkaloids (mitragynine, 7-hydroxymitragynine, corynantheidine, speciogynine, speciociliatine, paynantheine, corynoxine, corynoxine-B, mitraphylline, ajmalicine, and isospeciofoline) in rats were described following administration of traditional oral lyophilized kratom tea and a commercial kratom product, OPMS liquid shot in water (Kamble *et al*., 2021). A dose of 366 mg/kg kratom tea delivered a HED of 5.73 mg/kg mitragynine and <0.01 mg/kg 7-hydroxymitragynine to rats, while 0.8 mL/kg OPMS delivered a HED of 9.6 mg/kg mitragynine and <0.01 mg/kg 7-hydroxymitragynine to the animals. Only mitragynine, 7-hydroxymitragynine, speciociliatine, and corynantheidine were quantifiable at 8 hours post-dose, and their dose-normalized systemic exposure was higher (1.6- to 2.4-fold) following the administration of the commercial OPMS liquid. The dose-normalized mitragynine C_{max} values were 11.1 ± 1.1 ng/mL and 11.7 ± 1.6 ng/mL following kratom tea and OPMS liquid oral doses, respectively. The T_{max} for the tea occurred earlier at 1.3 ± 0.3 hours compared to the OPMS T_{max} at 3.1 ± 1.7 hours. In addition, the dose-normalized $AUC_{0–24}$ for mitragynine was significantly lower after the kratom tea (83.7 ± 6.4 h·kg·ng/mL/mg) than after OPMS (136.1 ± 13.1 h·kg·ng/mL/mg). These results indicated a slower rate of absorption and increased systemic exposure of mitragynine (1.6-fold) following the OPMS liquid dose as compared to kratom tea.

Although the 7-hydroxymitragynine dose was negligible (<0.1 mg/kg), C_{max} of 4.3 ± 0.8 (kratom tea) and 4.0 ± 0.6 ng/mL OPMS were achieved, consistent with mitragynine metabolism into 7-hydroxymitragynine (Kamble et al., 2021). However, there was no change in the percentage ratio of AUC_{0-24} of 7-hydroxymitragynine to AUC_{0-24} mitragynine (3.4 ± 0.9% and 3.1 ± 0.5% after kratom tea and OPMS, respectively), suggesting the extent of metabolism of mitragynine to 7-hydroxymitragynine was comparable. Since the elimination phase was not achieved for mitragynine or 7-hydroxymitragynine in the 24-hour monitoring period for OPMS, the percentage of extrapolated AUC_{0-inf} was greater than 20% and the plasma $t_{1/2}$ and AUC_{0-inf} could not be accurately calculated.

Hotplate antinociception, pharmacokinetics, and tissue distribution of mitragynine and 7-hydroxymitragynine were investigated at equianalgesic oral doses in male and female C57BL/6 mice to determine the extent to which 7-hydroxymitragynine metabolized from mitragynine accounts for the antinociceptive effects of mitragynine, and to investigate sex differences (Berthold *et al.*, 2022). Equianalgesic doses of mitragynine (165 mg/kg) and 7-hydroxymitragynine (50 mg/kg) in mice were selected based on the effective dose to reach 50% maximum possible latency in the hotplate test. The opioid receptor antagonist naltrexone was utilized to determine if the mechanism of action involved the opioid receptor. The pharmacokinetic-pharmacodynamic relationship between mitragynine and 7-hydroxymitragynine as a mitragynine metabolite and after independent administration was determined, and a population pharmacokinetic/pharmacodynamic model was developed.

When administered alone, 7-hydroxymitragynine was 2.8-fold more potent than mitragynine to produce antinociception (Berthold *et al.*, 2022). At equivalent effective doses of mitragynine and 7-hydroxymitragynine, there was an 11-fold lower difference in the maximum brain concentration of 7-hydroxymitragynine achieved as a metabolite of mitragynine *versus* oral 7-hydroxymitragynine dosing (see Table C.3.3.2.2-1). The brain concentration of 7-hydroxymitragynine observed 4 hours post-dose remained 1.5-fold higher than the maximum concentration of 7-hydroxymitragynine achieved as a metabolite of mitragynine and produced an analgesic effect of <10%. The 7-hydroxymitragynine brain C_{max} as a mitragynine metabolite was 0.6 to 0.7 mg/g with an overall exposure of 1.7 to 2.2 hours * mg/g. The 7-hydroxymitragynine metabolite showed adequate BBB penetration with a brain-to-plasma ratio of 0.6 in females and 1.5 in males. These results provide strong evidence that 7-hydroxymitragynine has a negligible role in the antinociceptive effects of mitragynine in mice, refuting the data reported by Kruegel *et al.* (2019). Species differences in metabolism, brain penetration, and analyte free fraction must be considered when translating results to higher-order species.

The noncompartmental analysis of brain and plasma mitragynine and 7-hydroxymitragynine concentrations after 165 mg/kg oral mitragynine showed mitragynine was distributed in the following order: liver > kidney > lung > spleen > brain, with 7-hydroxymitragynine distributed in a similar manner but with much less 7-hydroxymitragynine in the lung, in decreasing order of liver > kidney > spleen > lung > brain. The lung-to-plasma exposure ratios for mitragynine and 7-hydroxymitragynine were 15.9 and 1.5, respectively.

Table C.3.3.2.2-1 C_{max} of Mitragynine and 7-Hydroxymitragynine in Plasma and Brain Following a Single Oral Dose of 165 mg/kg Mitragynine in Mice (Berthold *et al.*, 2022)

Parameter	Mitragynine		7-Hydroxymitragynine	
	Male	Female	Male	Female
Cmax				
Plasma (µg/mL)	12.5	6.8	0.6	0.9
Brain (µg/g wet tissue)	35.5	11.5	0.6	0.7

C_{max} = maximum plasma concentration.

C.3.3.2.2.2 Dogs

CYP3A enzymes are highly expressed in the liver and intestine, with CYP3A-mediated metabolic conversion of mitragynine to 7-hydroxymitragynine in intestinal enterocytes leading to a higher 7-hydroxymitragynine exposure after oral *versus* IV mitragynine administration (Maxwell *et al.*, 2020). These authors dosed 1 mg/kg oral mitragynine to female Beagle dogs and observed a 7-hydroxymitragynine to mitragynine AUC of 12.6%. After a single 1 mg/kg oral 7-hydroxymitragynine dose to female Beagle dogs, 7-hydroxymitragynine absorption was rapid in the 12-hour fasted animals, with a 56.4 ± 1.6 ng/mL C_{max} occurring 0.14 ± 0.01 hours post-dose (Maxwell *et al*., 2021). Whereas oral administration of mitragynine to Beagle dogs resulted in multiple plasma peaks, oral 7-hydroxymitragynine dosing produced a single peak with orderly elimination. 7-hydroxymitragynine was measurable above the LOQ of 1 ng/mL for 12 hours.

7-Hydroxymitragynine elimination was mono-exponential after oral dosing, with a mean elimination half-life of 3.6 ± 0.5 hours based on non-compartmental analysis of plasma concentration-time data. In contrast, following oral mitragynine, distribution was multi-exponential (Maxwell *et al.*, 2020), perhaps due to 7-hydroxymitragynine's higher polar surface area and lower log P compared to mitragynine, limiting its distribution to peripheral compartments. 7-Hydroxymitragynine clearance and V_d were 4.4 ± 0.6 L/hour·kg and 23.8 ± 6.7 L/kg, respectively (Maxwell *et al.*, 2021). 7-Hydroxymitragynine's elimination half-life was longer after an oral dose of mitragynine than after 7-hydroxymitragynine oral dosing, possibly due to the formation-limited pharmacokinetics of 7-hydroxymitragynine after mitragynine dosing (Maxwell *et al.*, 2020). The exposure of 7-hydroxymitragynine after mitragynine dosing was 23.1% based on metabolism of mitragynine to 7-hydroxymitragynine in Beagle dogs. Manda *et al.* (2014) previously reported that 45% of 7-hydroxymitragynine converted to mitragynine in *in vitro* human liver microsome experiments, but importantly, no conversion of 7-hydroxymitragynine to mitragynine was observed in *in vivo* experiments in Beagle dogs.

The 1 mg/kg oral 7-hydroxymitragynine dose was well tolerated in dogs with no observed adverse events or significant changes to clinical laboratory tests (Maxwell *et al.*, 2021). Dogs have well-understood physiology and similarities to humans for pharmacokinetic testing (Tibbitts, 2003). Gender-related variations in 7-hydroxymitragynine pharmacokinetics are not anticipated because of identical CYP3A/CYP3A12 expression in male and female dogs (Martinez *et al.*, 2019).

C.3.3.3 Studies of 7-Hydroxymitragynine Abuse Potential and/or Ability to Elicit Withdrawal Symptoms

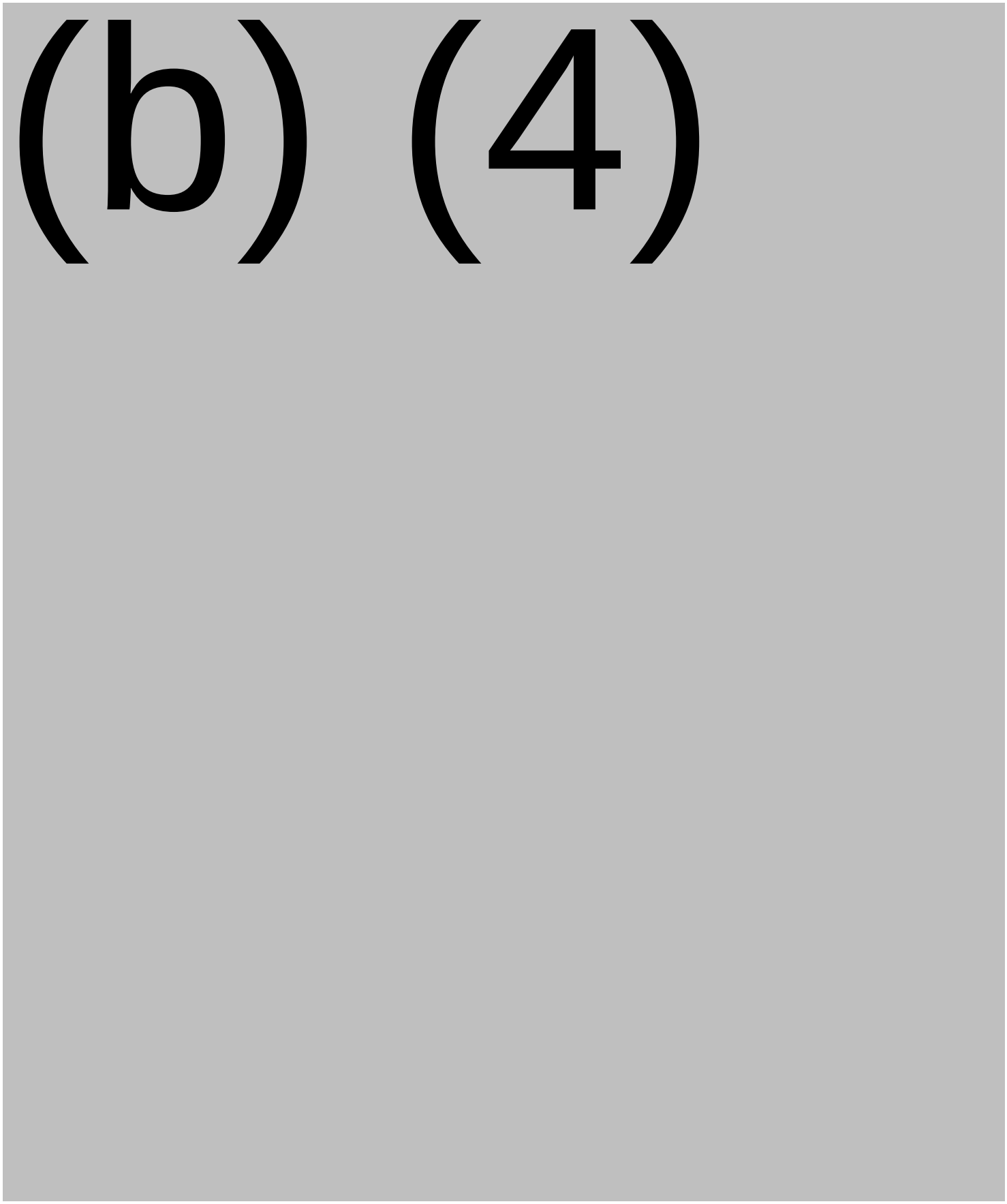
Hemby *et al*. (2019) examined the ability of mitragynine and 7-hydroxymitragynine to substitute for morphine in 344 Fischer rats. 7-Hydroxymitragynine substituted for morphine with intake dependent on the dose available [$F(4,32) = 5.3$, $p=0.0009$]. The number of infusions obtained for 5 and 10 µg/infusion were significantly greater than vehicle ($P<0.05$), confirming that these doses of 7-hydroxymitragynine functioned as reinforcing stimuli. Additionally, the authors observed that 7-hydroxymitragynine engendered and maintained IV self-administration in a dose-dependent manner.

7-Hydroxymitragynine's ability to affect brain reward thresholds was assessed in an ICSS procedure (Behnood-Rod *et al*., 2020). Low doses of 7-hydroxymitragynine did not affect the brain reward thresholds, and a high dose increased the brain reward thresholds. These data suggest that 7-hydroxymitragynine is not rewarding in this model and does not have abuse potential.

In a study by Obeng *et al*. (2021), separate groups of rats were trained to discriminate between either morphine (3.2 mg/kg) or mitragynine (32 mg/kg). Once trained, rats were tested with discriminative stimulus together with antinociception. In this study, mitragynine was observed to produce a maximum of 72.3% morphine-lever responding rate, and morphine produced a maximum of 65.4% mitragynine-lever responding rate. Other μ-opioid agonists produced high percentages of drug-lever responding in the morphine and mitragynine discrimination assays: 7-hydroxymitragynine (99.7% and 98.1%, respectively), fentanyl (99.7% and 80.1%, respectively), buprenorphine (99.8% and 79.4%, respectively), and nalbuphine (99.4% and 98.3%, respectively). In the morphine and mitragynine discrimination assays, the *kappa* agonist U69593 produced maximums of 72.3% and 22.3%, respectively, and the *delta* agonist SNC 80 produced maximums of 34.3% and 23.0%, respectively. These data indicate that 7-hydroxymitragynine fully generalized to morphine in rats, though it is unclear if this applies to humans given the lower exposure and more rapid rate of clearance of 7-hydroxymitragynine.

C.4 Comprehensive Safety Profile of the Dietary Ingredient [CONFIDENTIAL]

(b) (4)


(b) (4)

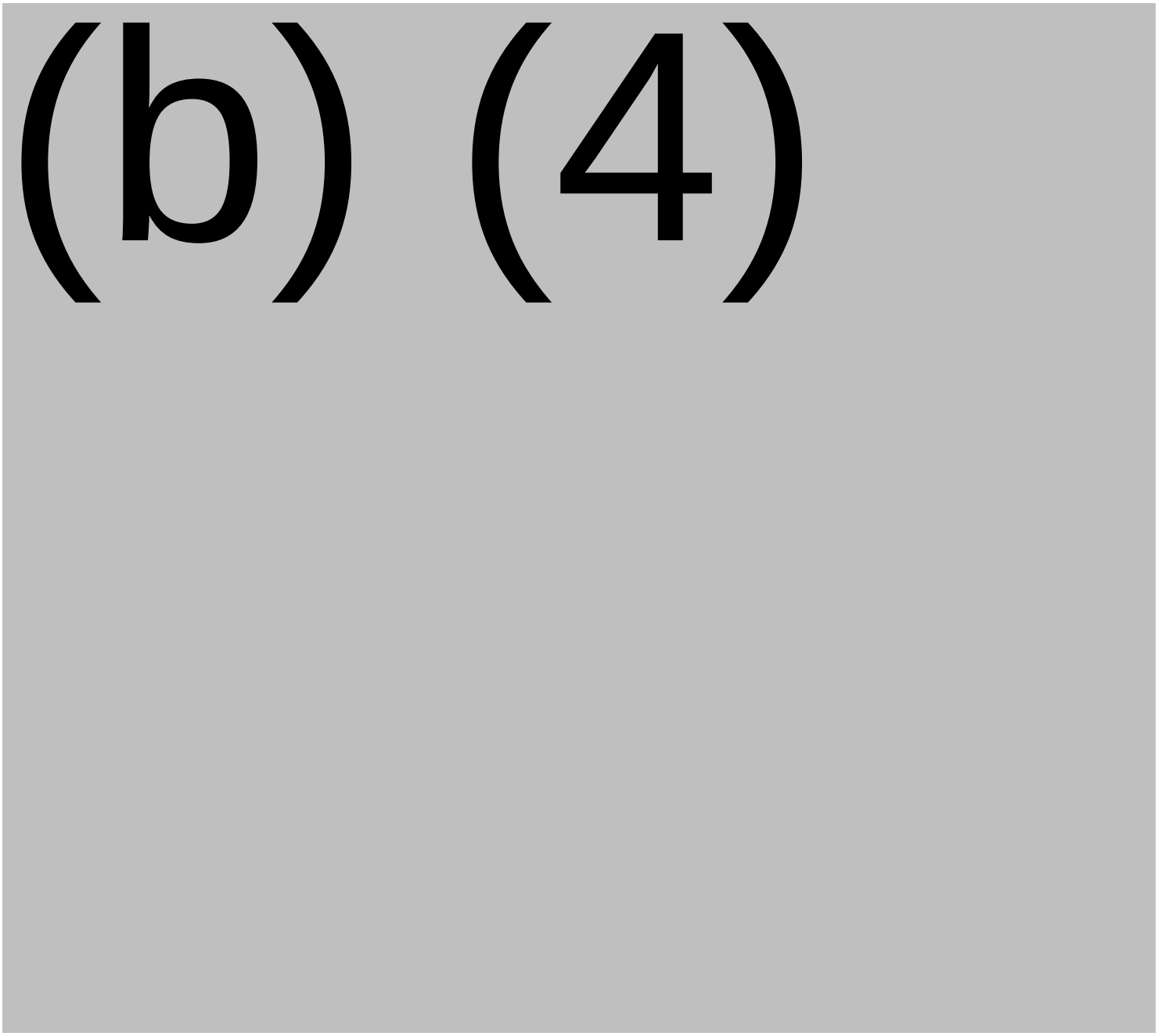

(b) (4)

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Table of CFR Sections Referenced (Title 21—Food and Drugs)

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20—Public information	20.61	Trade secrets and commercial or financial information which is privileged or confidential
58—Good laboratory practice for nonclinical laboratory studies	58	[full section]
101—Food labeling	101.9	Nutrition labeling of food
190—Dietary supplements	190.6	Requirement for premarket notification

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