STAKEHOLDER PANEL ON ALKALOIDS OF KRATOM

Background & Fitness for Purpose

(Mitragynine & 7-OH Mitragynine)

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Kratom Roadmap

- Background
- Significance
- Regulatory Guidance
- General Analytical Needs
- Existing Methods and Techniques
- Challenges
- Fitness for Purpose Statement
Background (Agency History)

- 2006 (street drug language in labeling)
- 2011 – Develop tox regulatory strategy for “low hanging fruit” using NDI adulteration charge
  - no safety data or safety concern based on MOA
  - “Legal High” Ingredients
  - Centrally Active Ingredients
  - Analysis of importation
- 2012 – Dietary Supplement (54) Import Bulletin written for Kratom
- Thousands of kilos of kratom detained/refused
- “Operation Log Jam” – not a massive nationwide crackdown on kratom

Background (Synonyms)

(*Mitragyna Speciosa Korth*)

- Biak-biak
- Katawn
- Krton
- Krypton
- Kakuan
- Mambog
- Madat
- Maeng Da
- Thom
- Ithang
- Thang
- Ketum
- Kedemba
- Red Vein/White Vein
- Nauclea
- Gratom, Cratom, Krathom
Background

- Botanical (fits within 201(ff))
- Leaves are traditionally chewed and ingested
- Trees grow 12-30 ft, leaves are 4-7 inches long
- Kratom contains over 25 different alkaloids (mitragynine and 7-hydroxymitragynine)
- Different geographical varieties - vary in their alkaloid composition and potency
  - Malaysia and Borneo – have more red vein
  - Sumatra and Bali – have more white vein
  - Thailand has Maeng Da variety (potent red vein)

Background

- Each leaf – 0.5% alkaloids
- 20 leaves – 17 mg mitrag
- White Vein/Red Vein
- White – euphoric, energetic & stimulating
- Red – more sedating
- Whether stimulating or sedating - depends mostly on the alkaloid dose ingested
- Alkaloid amounts can vary markedly within the white or red vein leaf varieties at different harvest times and due to variations in climate
Background

- Country or region where leaves were picked (i.e. Thai, Indo, Maeng Da, Sumatran, Malaysian, Bali)
  - “Bumblebee” Origin - Vietnam
  - Maeng Da (premium) – Thailand
  - Rifat Red Vein - Indonesia

- Country on product name tells you the variety
  - Sumatra, Bali – more white vein
  - Malasia, Borneo – have more red vein

- Grade of Mitragyna speciosa (i.e. Premium, Super, Ultra, extract, concentrated, Pimp Grade)

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New way to take my Kratom...

I parachute it. Take a piece of toilet paper (thinnest you can get) and peel the layers off each other so that you have a very thin piece of toilet paper you can see through. Add 1 tsp on the paper, put on back of tongue and guzzle water. Wahhh LAHH no nasty taste and hits you quickly...

Some of you may say “no way” or “not in a million years”... if you won’t trip or anything, there are mild visuals at higher doses (i did 4 grams of the 15x extract once and just saw some traces and vivid looking colors, pulsing computer screen etc nothin big. And FYI 6 grams is 4 times an average dose, lol. I suggest parachuting the entire container (assuming its a 3 gram one like all the kratom i’ve seen) and down it with something thick and strong tasting like some kind of juice or gatorade since the shits bitter, but i always parachute. it tastes like ass when mixed with water. oh and to get the best absorption and best buzz, follow with a shot or two which potentiate the effects. under 1.5 or so grams its more of a stimulant though.
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Background (Matrix)

- Whole Leaf
- Crushed Leaf
- Powder
- Capsules, Tablets
- Extracts
- Tinctures
- Relaxation DS “Drinks”
- Shots
- Mists
- Teas
- Resins & Brownies

Background (Claims)

- Euphoria, Stress Relief at low doses
- Pain relief and opiate withdrawal at higher doses
- Depression
- Muscle aches and pains
- Flu-like symptoms
- Combat nausea and diarrhea
- Insomnia
- Restless leg syndrome

I buy salvia every few weeks online and today they sent me a sample of Kratom Powder. Has anyone tried drinking this? What am i getting my self in to? I have done some research but i would rather hear from you guys.

Re: Kratom becoming illegal in several US states

As a Virginia resident and avid supporter and user of Kratom this is horrible.

Kratom is a major player in why I’ve been heroin free for close to 6 months now. Yes, I’ve slipped up with Suboxone but 90% of the time I keep the PAINS away with just a few grams of Kratom. For people like me this just sends me back to the local black market which caused my problems in the first place.

Virginia, don’t ban Kratom! ughh
Background (Public Health Interest)

- Kratom – banned in Thailand - Kratom Act 2486
- 2nd most abused substance in Thailand today
- Not scheduled by DEA but on their list of Drugs and Chemicals of concern
- botanical marketed as a “legal high”, “Not for Human Consumption”, “Incense”, and “Ethnobotanical” that are coming through US ports coded as dietary supplements and dietary ingredients
- ingested products with centrally active alkaloids
- products with a clear CNS antinociceptive profile
- sold in head shops (they are the new K2, Spice, and Bath Salts)

Refusals

- Detained and refused 35,000 (low estimate) kilos kratom in 30 months
- IB (2012, 2013) and IA (2/2014)
New warning about Kratom use

Local recovery clinic sees increase in addicts

Updated: Sunday, 12 Aug 2012, 5:00 PM CDT
Published: Saturday, 11 Aug 2012, 11:15 PM CDT

AUSTIN (KXAN) - A local drug treatment facility has seen a sharp surge in people checking in for addiction to a substance called Kratom.

"It's a legal opiate- it's a legal heroin," said Angela Vickrey, Director of Admissions for the Austin Recovery Center, where they have treated seven patients for Kratom addiction in the last 90 days. "It basically has the same effect that opiates do- opiates being heroin and pain medication in large quantities- in low dose- it's a stimulant."
Background (Kratom Alkaloid Breakdown)

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Alkaloid %</th>
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<tbody>
<tr>
<td>Mitragynine</td>
<td>66</td>
<td>Isomitraphylline</td>
<td>&lt;1</td>
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<tr>
<td>Paynantheine</td>
<td>8.6 - 9</td>
<td>Mitraphylline</td>
<td>&lt;1</td>
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<tr>
<td>Speciogynine</td>
<td>6.6 - 7</td>
<td>Mitraciliatine</td>
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<td>7-Hydroxymitragynine</td>
<td>2</td>
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<td>Speciociliatine</td>
<td>0.8 - 1</td>
<td>Ajmalicine (Raubasine)</td>
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<td>Mitragynine oxindole B</td>
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<td>Akuammigine</td>
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<td>Ciliaphylline</td>
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<td>9-Hydroxcorynantheidine</td>
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<td>Corynantheidine (δ-yohimbine)</td>
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<td>Mitaversine</td>
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<td>Corynoxeine</td>
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<td>Isorhynchophylline</td>
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Background (Structures)

- Mitragynine
  - C₂₃H₃₀N₂O₄
  - MW 398.495

- Paynantheine
  - C₂₃H₂₈N₂O₄
  - MW 396.479

- Ajmalicine (δ-yohimbine or raubisine)
  - C₂₁H₂₄N₂O₃
  - MW 352.43

- 7-Hydroxymitragynine
  - C₂₃H₃₀N₂O₅
  - MW 414.49
Significance (Public Health Interest)

- Leaves of *Mitragyna speciosa* are used to suppress pain and mitigate opioid withdrawal
- Known substitute for opium (banned in Thailand)
- Number 2 abused plant in Thailand and Malaysia
- Dietary ingredients should not be addictive
- Readily available psychoactive plant
- Most detained/refused entry by FDA over the past 3 years – largest hauls are $200k+
- Import Alert (2/2014), previously Import Bulletin
• Methoxyl group at C9 – supposedly responsible for analgesic activity of mitragynine/7-hydroxymitragynine
• Corynantheidine (9-demethoxymitragynine)
• Chromophore + wavelength shift

• Alkaloid substitution for kratom minus alkaloids marketed for relaxation?
• Yohimbe and Rauwolfia alkaloids (corynantheidine, corynanthine, yohimbine)

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**Significance (Public Health Interest)**

• IA 66-41 (import alert as a drug) – in place for many years
• Why did the problem get worse despite an FDA IA?
  – The IA 66-41 = covered plant material and product imports coded as a drug or drug ingredient entry (66) or for street drug language on labeling
  – Overseas suppliers - switched to code it as 54
  – The new IA 54-14 covered finished supplements and raw botanical material without making drug claims
Significance (Public Health Interest)

- It is the dietary ingredient of 2014 and beyond
  - 3-14-2014 voluntary recall of kratom capsules, powder, maeng da leaf powder and extracts by Smiley National Inc. (Class II Recall)
  - Who will be caught in the dragnet next?
  - Open ended seizures?

Regulatory Guidance

- Slot kratom appropriately (comfrey, ephedra)
- Kratom could fit under 201(ff) as an herb or other botanical (alkaloids with opioid activity are the issue)
- Kratom (mitragynine or 7-hydroxymitragynine) = adulterated based on 402(f)(1)(B)
- Kratom (mitragynine and 7-hydroxymitragynine removed) = allowable? (all other alkaloids left - cause relaxation)
- At what level would they be acceptable?
- Zero tolerance? Agency has to make that case
Regulatory Guidance

➢ Kratom – burden is on the Agency
   • Labeling as kratom or one of its synonyms alone does not imply adulteration
   • FDA must confirm presence of the alkaloids with quantitative level

➢ Remember:
   • Comfrey minus pyrrolizidine alkaloids
   • Ephedra minus alkaloids
   • Rauwolfia minus reserpine
   • RYR minus lovastatin

Regulatory Guidance (Import Alert)

• IA 54-15 DETENTION/REFUSAL
  ➢ Label (kratom, Mitragyna speciosa, mitragynine extract, biak-biak, cratom, gratom, ithang, kakuam, katawn, kedemba, ketum, krathom, krton, mambog, madat, Maeng da leaf, nauclea, Nauclea speciosa, or thang)

  ➢ Quantitative level for mitragynine and 7-hydroxymitragynine
General Analytical Needs

- Mitragynine and 7-hydroxymitragynine are exclusive to *speciosa*
  - the only 2 alkaloids likely of interest to the Agency (safety)
  - Physiology/pharm is lagging behind on the other alkaloids (binding data)
- Currently only 1 FDA lab performs all analyses for kratom
- Need - a quantitative method for these 2 alkaloids
- NPA submitted FOIA for FDA's quantitative method
- FDA has not used a screening method
- Matrix can vary – finished forms (tablets, capsules), liquid extracts, concentrated extracts, dry whole leaf, powder (detentions have mostly involved raw botanical form and liquids)

Existing Methods - General

Three independent chromatographic methods coupled with 2 detection systems
1) GC with mass spectrometry
2) Supercritical fluid chromatography with diode array detection
3) High-performance liquid chromatography with mass spectrometry and diode array detection
Techniques Used for Detection of Mitragynine and Other Indole and Oxindole Alkaloids in Kratom

Chromatographic Methods
- HPLC (most common)
- GC
- SFC

Detection Systems for Indole Alkaloids
- Diode Arrays
- Mass-specific
  - Quadrupole
  - Linear ion trap
  - Triple quadrupole MS

Existing Methods

LC-MS with quadrupole mass spec (scan mode)
- Analytes:
  - Mitragynine
  - 7-hydroxymitragynine
  - other indole alkaloids
- Direct quantitation
- gradient elution with MeOH and water (46 min analysis time)
- Matrix
  - raw plant material
  - commercial products of kratom

Example:
Existing Methods

Linear Ion Trap MS

✓ Analytes:
  • Mitragynine, Paynantheine
✓ HPLC for metabolic studies, Matrix (urine)
✓ Ion Trap – MS for quantitation and detailed structural analysis of metabolites in 20-30 min

Examples:

Existing Methods

Triple Quadrupole MS

✓ Analyte (mitragynine) in biological samples
✓ Internal standard (Ajmalicine)
✓ Extraction with methanol/water and C18 column in 30 min
✓ ESI/MS/MS
✓ Detection limit of 0.02 ng/mL
✓ LOQ 100 ng/mL

Example:
Existing Methods

Triple Quadrupole MS

✓ Analyte (mitragynine) in biological sample
✓ Internal standard (amitriptyline), liquid-liquid one step extraction (MTBE), separated on Acquity UPLC column, isocratic elution
✓ LOQ 1 ng/mL (range 1 – 5000 ng/mL)

Example:

✓ mitragynine and amitriptyline extracted with hexane-isoamyl alcohol, resolved on Lichrospher RP-SelectB column (9.8 and 12.9 min respectively)
✓ LOQ 0.2 ng/mL (range 0.2-1000 ng/mL)

Example:

Existing Methods

Triple Quadrupole MS

✓ Analyte (7-hydroxymitragynine) in biological sample (rat plasma)
✓ Internal standard (tryptoline)
✓ isocratic elution, separation on Acquity UPLC(TM) BEH C18 column (2.5 min run time)
✓ LOQ 10 ng/mL (range 10-4000 ng/mL)

Examples:
Existing Methods

GC – MS Method

✓ Analyte (mitragynine)
✓ no quantitation or validation attempted
✓ HP-5 capillary column at high temp (200 °C)
✓ mitragynine eluted as symmetrical peak in < 17 min
✓ identification: comparison of experimental mass spectra with library

Examples:

Existing Methods

GC – MS Method

✓ Analytes (metabolites of mitragynine, paynantheine, speciogynine, speciociliatine)
✓ Matrix (urine)
✓ enzymatic cleavage of conjugates, solid-phase extraction, and trimethylsilylation
✓ LOQ: 100 ng/mL
✓ GC-MS analysis of underivatized mitragynine and other indole alkaloids requires high temp.
✓ little control available for optimization of resolution of the analytes

Example:
Existing Methods

SFC Method

- historically used for analysis of indole alkaloids in Madagascar periwinkle
- faster, more economical, environmental consideration (better than HPLC)
- Analytes (mitragynine, 7-hydroxymitragynine, others)
- Matrix (leaves of *M. speciosa*)
- Head-to-head comparison (SFC-DAD, UHPLC-MS-DAD, GC-MS)
- First SFC method for analysis of mitragynine in plant material
- elution (7 min) with 15% methanol as eluent
- mitragynine – resolved from speciogynine and speciociliatine

Example:

Challenges

- GC Methods
  - not commonly applied for *M. speciosa*, particularly in the wide variety of matrices encountered
  - High temperatures required to elute the alkaloids – restricts parameter adjustment for resolution of any alkaloid mixture
  - inadequate resolution of mitragynine and speciociliatine
  - Reliance on mass-specific detection – EI, ESI, and ESI MS/MS spectra of mitragynine, speciogynine, and speciociliatine are nearly identical
  - Interference of speciociliatine – would probably not be detected by either DAD or MS detectors
  - Methods should account for interference
  - derivatization may be necessary to overcome poor chrom. res.
Challenges

- **LC Methods**
  - Classical LC with bonded stationary phases and aqueous/organic eluents coupled to mass detection
  - Viable for *M. speciosa* alkaloids in plant material and various fluids (Kikura-Hanajiri, 2009)
  - Resolution of diastereoisomers is OK (eluent pH dependent order)
  - MS/MS increases sensitivity and selectivity
  - Problems with mass specific detection (as stated previously)
  - Pure analytical standards (labor intensive isolation from natural products)

- **SFC (plant material)**
  - SFC with liquid carbon dioxide modified with organic liquid as eluent
  - Unexplored, effective
  - Advantages of mass-specific detection and MS/MS are applicable to SFC and HPLC
  - Requires less organic liquids than HPLC
  - Successfully applied for separation of enantiomers and diastereoisomers, resolves diastereoisomer resolution in *M. speciosa*
  - Faster, better resolution, separations orthogonal to HPLC
Fitness for Purpose Statements

• Screening Method?
• Identification/Confirmation Method

“Screening Method”

The method must be able to screen known and unknown (unexpected) analytes of concern in various dietary supplement matrices.

- No unknown analytes
- Only mitragynine and 7-hydroxymitragynine
- Not seeing economic adulteration
- What does it buy you? Added cost, no bang
- FDA does not use screening here
Fitness for Purpose Statement

“AOAC Kratom Method”

The method must be able to identify known indole alkaloids, which adulterate the product, in a broad range of matrices, including dry and liquid finished products, concentrated extracts, and dry leaf material. The method should be able to quantitate the analytes for which appropriate standards are available and account for interfering compounds.

Questions for SPDS Discussion

“Identification/Confirmation Method”

- Arming decision making – (combination of alkaloids)
- Limit of quantitation?
- Range?
- Acceptable level(s) – Agency concern – below
- List of targeted analytes? Just 2 for now?
- Resolution of diastereoisomers?
- Handle wide variety of matrices?
- Cost of the analysis
QUESTIONS??

Thanks
NPA CEO and Executive Director Dr. Daniel Fabricant
Darryl Sullivan
Dawn Frazier