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Herbal Marijuana Alternatives Investigation: K2 and Spice: A Masters Thesis

Christopher D. Rosenbaum
University of Massachusetts Medical School

HERBAL MARIJUANA ALTERNATIVES INVESTIGATION: K2 AND SPICE

A Masters Thesis Presented

By

CHRISTOPHER D. ROSENBAUM

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Novel Drugs of Abuse

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The signatures of the Master's Thesis Committee signifies
Completion and approval as to style and content of the Thesis

(_____)

Ed Boyer MD, PhD, Chair of Committee

(_____)

(Ed Boudreaux, PhD, Member of Committee

(_____)

Steve Bird MD, Member of Committee

(_____)

Chad Darling MD, Member of Committee

(_____)

Robert Goldberg PhD, Thesis Advisor

The signature of the Dean of the Graduate School of Biomedical Sciences signifies that the student has met all master's degree graduation requirements of the school.

(_____)
Anthony Carruthers, Ph.D.,
Dean of the Graduate School of Biomedical Sciences

Masters of Science, Clinical Investigation

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Dedication

I dedicate this thesis to my family: Meg, Ella, Anna, and Grace.

Acknowledgements

I would like to thank the following individuals for their contributions to this study (alphabetical order): Kavita Babu, Steve Bird, Edwin Boudreaux, Edward Boyer, Chad Darling, Robert Goldberg, Amanda Jenkins, Chris Long, Gaylord Lopez, Sean Ragone, Anthony Scalzo, and Julie Weber.

Abstract

Background

Herbal marijuana alternatives (HMA), legal plant products adulterated with synthetic cannabinoid receptor agonists, represent a growing public health concern. Only a few case reports describe HMA and synthetic cannabinoid's clinical toxicity. We describe an outbreak of HMA abuse primarily in the Midwest, the clinical presentation of HMA toxicity, and clinical and forensic testing.

Methods

During the course of ongoing surveillance for emerging drugs of abuse between November 2009 and August 2010, we retrospectively and prospectively identified a convenience sample comprising 81 cases of abuse of HMA products. Subject demographics, vital signs, lab results and urine were obtained (when available) and tested via gas chromatography mass spectrometry (GCMS) analysis. Samples of HMAs and synthetic cannabinoids were also analyzed via GCMS.

Results

HMA users were predominantly young males who inhaled HMAs. Analysis of their urine detected synthetic cannabinoid parent compound in one subject. GCMS analysis of synthetic cannabinoids established a reference library that confirmed the presence of synthetic cannabinoids in sampled HMA products.

Conclusion:

HMA products were available in head shops, gas stations, and via the Internet. We have confirmed the presence of synthetic cannabinoids in these HMA products. The

tachycardia, hypertension, agitation, anxiety, vomiting and hallucinations observed in this convenience sample are not readily explained by the presence of synthetic cannabinoids acting on CB_1 and CB_2 receptors. Further research must be done on HMA products and their abusers.

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List of symbols, Abbreviations or Nomenclature

GC-MS	Gas Chromatography-Mass Spectrometry
LC-MS/MS	Liquid Chromatography-Mass Spectrometry
HMA	Herbal Marijuana Alternative
CB	Cannabinoid Receptor

Preface

I was the primary author on each of these publications, and as such made the most significant contribution in each reference:

1. **Rosenbaum CD**, Ward JA, Boudreaux ED, Burstein S, Boyer EW. JWH-018, JWH-073, and Spice. Abstract – International congress of the European Association of Poison Centres and Clinical Toxicologists. Bordeaux, France. May 2010. *Clinical Toxicology* 2010; 48 (3) pp 307.
2. **Rosenbaum CD**, Ward JA, Boudreaux ED, Burstein S, Jenkins A, Bird SB, Boyer EW. JWH-018 & JWH-073 Synthetic Cannabinoids. Abstract – International congress of the European Association of Poison Centres and Clinical Toxicologists. Bordeaux, France. May 2010. *Clinical Toxicology* 2010; 48 (3) pp 307.
3. **Rosenbaum, Chris**. K2 is coming to you! ACEP Toxicology Section Newsletter. June 2010, vol 17, #2. www.acep.org/content.aspx?id=48712#story9
4. **Christopher D Rosenbaum**, Anthony J Scalzo, Christopher Long, Julie Weber, Amanda Jenkins, Gaylord Lopez, Sean Ragone. K2 & Spice abusers: a case series of clinical and laboratory findings. North American Congress of Clinical Toxicology (NACCT). Washington D.C. September 23, 2011 (Poster presentation).
5. **Rosenbaum C**, Carreiro S, Babu K. Here today, gone tomorrow…back again? A Review of: Herbal Marijuana Alternatives (Spice, K2); Synthetic Cathinones

(Bath Salts); Kratom, Salvia Divinorum, Methoxetamine and Piperazines. *Journal of Medical Toxicology* (Accepted for publication December 2011).

These were the two publications sited to generate Table 1.2; I was not the author of either of these publications.

1. Schneir AB, Cullen J, Ly BT. "Spice" girls: synthetic cannabinoid intoxication. *J Emerg Med.* Mar 2011;40(3):296-299.
2. Addiction EMCDDA. Understanding the 'Spice' phenomenon. Lisbon, Portugal 2009:1-34. http://www.emcdda.europa.eu

CHAPTER I: Introduction

History of HMAs

Herbal marijuana alternatives (HMAs) are legally available drugs of abuse growing in popularity across the U.S. and abroad. While cleverly labeled not for human consumption, HMAs are available on-line and in specialty shops as legal alternatives to marijuana that allegedly produce a similar clinical effect but are not detectable by traditional marijuana screening tests.

Beginning in 2004, European authorities recognized HMA abuse on the Internet.[1] In 2009, the European Monitoring Centre for Drugs and Drug Addiction released a report identifying the brand-named HMA *Spice* as a combination of plant material and intentionally added synthetic cannabinoid research chemicals.[2] Prior to this report, there was a paucity of data available describing the toxicity of the herbs or synthetic cannabinoids present in HMAs.[3] Initial synthetic cannabinoid pharmacokinetic and animal data did not easily translate into anticipated clinical effects in humans.[4-7]

Subsequently, German scientists working in concert with InterPol performed both forensic Gas Chromatography-Mass Spectrometry (GC-MS) and Liquid Chromatography-Mass Spectrometry (LC-MS/MS) testing on multiple HMA samples as well as self-experiments smoking the HMA products.[8] These scientists substantiated the belief that HMAs were not detectable via drug tests by demonstrating that neither the HMA products themselves, nor human urine samples obtained from HMA exposed

volunteers, demonstrated cross-reactivity with existing marijuana (i.e. Δ9-tetrahydrocannabinol (Δ9-THC)) immunoassays.[8] Researchers discovered that some (but not all) HMA samples contained JWH-018, oleamide, and CP 47,497-analogue.[8] Never tested in humans, JWH-018 and other JWH-compounds are non-naturally occurring aminoalkylindoles designed in the 1990's and studied by **J**ohn **W**. **H**uffman (an organic chemist at Clemson University) to better understand CB receptors.[9, 10] While oleamide is a naturally occurring fatty acid known to interact with endogenous cannabinoid receptors (CBs) in a manner similar to that of anandamide, CP 47,497 is cannabimimetic **c**yclohexyl**p**henol created by Pfizer in the 1970's.[11, 12,2] The location and manufacturing details of synthetic cannabinoids remains purely speculative.

For decades, both basic science researchers and pharmaceutical companies have designed hundreds of synthetic cannabinoids in the quest for better understanding of CB receptor-ligand binding relationships, and discovering a chemical compound with the therapeutic properties of Δ9-THC, without its psychotropic effects.[13] Most synthetic cannabinoids had not been tested in humans, so their potentially toxic effects were unknown before the current epidemic.

Japanese, U.S., and U.K. investigators subsequently identified the following synthetic cannabinoids in seized HMA samples: HU-210 (so named for **H**ebrew **U**niversity where it was created in the 1960's), JWH-073, JWH-250, and JWH-398 (**TABLE 1.1**).[2, 14-18] While little was known about the potential health risks associated with these synthetically

Table 1.1: Synthetic Cannabinoids Historically Found in HMAs

alpha-amyrine	JWH-007
acetyl vanillin	JWH-018
beta-sisterole	JWH-019
benzaldehyde	JWH-049
benzyl benzoate	JWH-073
benzophenone	JWH-081
carvone	JWH-175
CP47,497	JWH-199
Analogue V	JWH-200
Analogue VII	JWH-246
Analogue VIII	JWH-253
Analogue XVI	JWH-306
CP55,940	JWH-369
clorophene	JWH-398
cyclopentadiene	linoleic acid
delta-8-THC	methyl palmitate
delta-9-THC	Nabilone
delta-cadinene	nerolidol
dihydroxyacetone	oleamide
ethyl linoleate	palmitamide
ethyl vanillin	palmitic acid
eucalyptol	persicol
eugenol	phytol
2-furanmethanol	phytosterole
hexacosane	selina-6en-4ol
hydrochinone	squalene
hydroxybenzoic acid	stearamide
	tocopherol
	thymol
	WIN55212-2

Legend for Table 1.1
This is a list of synthetic cannabinoids historically found to be present in HMA samples

derived substances, the growing popularity of HMA products and the possibility of toxicity lead many European countries to ultimately ban the brand-named *Spice* product and other similar HMAs.

Coincident with the European ban of HMAs, the first cases of HMA abuse in the U.S. became recognized by the DEA.[2] Reports associated HMAs with dry mouth, quickened pulse, and agitation for up to 6 hours; fatalities were also reported.[19-21] An 18 year old man in the Midwest took his own life by shooting himself in 2010, after becoming agitated from smoking *K2*.[22] This growing public health threat in the Midwest inexplicably began as a geographic anomaly that ultimately spread to other parts of the country.[23] In the spring of 2011, the following synthetic cannabinoids fell under schedule-1 restriction by the DEA: JWH-018, JWH-073, JWH-200, CP 47-497, and CP 47-497 C8 homologue.[24] Currently, HMAs are still available as legal drugs of abuse, not containing any illegal substances.[25]

Pharmacology of Cannabinoid Receptors

Synthetic cannabinoids can be divided into the following 7 structural groups: 1) classical cannabinoids (HU-210), 2) cyclohexylphenols (CP 47,497), phenylacetylindoles (JWH-250), 4) naphthylmethylindenes, 5) naphthoylpyrrols, 6) naphthylmethylindoles, and 7) naphthoylindoles (JWH-018, JWH-073).[12, 26] Cannabinoid receptors are those that interact with cannabinoid-like drugs, including phytobiotics (Δ9-THC), synthetic cannabinoids (JWH-compounds), or endogenous cannabinoids (anandamide).[27, 28]

Despite these synthetic cannabinoids' structural analogy to Δ9-THC, these compounds are agonists at CBs.[28] The two types of CB receptors (CB_1 and CB_2) are G-protein coupled receptors located primarily in the central nervous system (e.g. basal ganglia, hippocampus, cortex and cerebellum) and peripheral tissues (e.g. immune cells, blood cells, and spleen) respectively.[27, 29-32] CB_1 receptors have been localized to presynaptic GABA neurons, and the CB_2 receptors on immune cells could affect cytokine release and cell migration.[7, 29, 33, 34] The peripheral CB_2 receptor effect is the likely root of Δ9-THC's anti-inflammatory effects.

As mentioned previously, the ongoing pursuit of therapeutic cannabinoid discovery involves developing a CB_2 receptor-specific cannabinoid that exhibits minimal interaction with central CB_1 central receptors.[35] However, there is growing evidence to suggest that CB_2 receptors may also be present in the CNS.[4] Because synthetic cannabinoids have much greater binding affinities for CB receptors than Δ9-THC, one understands why HMA manufacturers might infer strong marijuana-like effects.[36]

Evidence for Herbal Effects

Of the herbal constituents identified in certain HMAs (**TABLE 1.2**), there is, at best, anecdotal and historical evidence to support psychotropic or marijuana-like effects.[2, 25] For instance, the Nymphaeaceae family of water lilies have been depicted on the tombs of Ramses II, Amenhotep I, and Tutankhamun and may have been used as narcotics in religious ceremonies.[3] The most well known herb is a South African plant called Wild

Table 1.2: Herbs Historically Found in HMAs

Alfalfa
Beach bean
Blue violet
Blue/Sacred lotus
Comfrey leaf
Dog rose/Rosehip
Dwarf skullcap
Gymnema Sylvestre
Honeyweed/Siberian motherwort
Horehound
Indian warrior
Lioin's ear/tail, Wild dagga
Maconha brava
Marshmallow
Neem Leaf
Nettle Leaf
Passion Flower Leaf
White and blue water lily

Legend for Table 1.2: This is a list of herbs historically found in HMAs

Dagga. While Wild Dagga is abused for its alleged euphoric effects, no pharmacologic data exists to support this alleged effect. The scientific data demonstrates anti-inflammatory, analgesic, and hypoglycemic effects in mice and rats.[37] Although makers of HMAs may also be intimating psychotropic effects via Comfrey, the pyrrolizidine alkaloids found in Comfrey plant material are also known to be hepatotoxic.[38, 39] Furthermore, there is evidence to support Rosehip use as an anti-inflammatory agent for the treatment of osteoarthritis; there is no strong mechanistic evidence to support cannabimimetic effects.[40] Neem leaf extract has demonstrated anti-inflammatory and possibly cancer-treating effects in vitro.[41]

Specific Aims

At the time of this study, *K2* was not well understood. It was our hypothesis that *K2* was a brand of HMA (renamed from *Spice* after it had been recently banned in Europe), and that it may contain synthetic cannabinoids. The primary aim of this study was to identify signs and symptoms associated with self-identified HMA abuse. Secondary aims included 1) testing HMAs to confirm the presence of synthetic cannabinoids (or other adulterants); 2) testing synthetic cannabinoids to see if they cross reacted with the drug of abuse immunoassay for marijuana; 3) testing HMA abuser's urine for presence of synthetic cannabinoids; and 4) to develop an effective means of capturing real-time data on emerging drugs of abuse (like HMAs).

CHAPTER II: Methods

Study design and setting

This was a multi-center collaborative effort involving ongoing epidemiological surveillance for emerging drugs of abuse between November 2009 and August 2010. Initially, we identified a "signal" comprising 81 cases of abuse of HMA products. In these cases, there appeared to be a shared commonality of agitation, tachycardia and hypertension. After an investigation revealed additional cases in Missouri, Kansas, Georgia and Massachusetts, and after consultation with the Centers for Disease Control (CDC), we developed case definition criteria that included patient-identified exposure to HMAs. We used that criteria to both retrospectively and prospectively identify cases of HMA exposure through the Missouri Poison Control Center, Georgia Poison Control Center, and the University of Massachusetts Medical Toxicology consultation service. Cases presented as 1) phone-calls to Poison Control Centers, 2) Emergency Department visits, or 3) in-hospital Medical Toxicology consultations. When available, data was collected regarding subject demographics, vital signs, physical exam findings, symptoms, and laboratory testing (such as qualitative urine immunoassays for typical drug of abuse like opiates, benzodiazepines, marijuana and cocaine).

Laboratory Testing

Synthetic Cannabinoid Laboratory Testing via Immunoassay

Prior to DEA scheduling of certain synthetic cannabinoids, we acquired samples of the synthetic cannabinoids JWH-015, JWH-018, JWH-073 and JWH-200 (in the form of a

white powder) via Internet vendors for $20/100mg. We spiked human urine control samples with these samples of synthetic cannabinoid powders and tested them via the Microgenics Corporation qualitative drug of abuse immunoassay to see if they generated a positive cannabinoid result.

Synthetic Cannabinoid Laboratory Testing via GC-MS

We used GC-MS to test JWH-015, JWH-018, JWH-073 and JWH-200 via a single step liquid extraction at pH 8–10 with ammonium hydroxide and dichloromethane. The extract was reconstituted with ethyl acetate. A sample was injected on Agilent 5973 GC-MS using chemstation software and an HP1 12 m capillary column. Chlorimipramine was the internal standard. Subsequently, a comprehensive list of synthetic cannabinoids was analyzed via similar GC-MS analysis and their mass spectra were entered into a GC-MS library as reference standards (against which future urine tests would be analyzed)(**TABLE 2.1**).

HMA Forensic Testing

Prior to local and federal regulations on HMA products, we acquired multiple samples of *K2* from Internet vendors and completed forensic GC-MS testing at the St. Louis forensic laboratories (**Figures 2.1-2.3**). Four milliliters of forensic samples were extracted with a 9:1, hexane: ethylacetate mixture. Deuterated internal standards were used. The first standard was an alkaline extraction and then an acid back extraction. The solvent was

Table 2.1: Synthetic Cannabinoids Entered Into GC-MS Reference Library

AM-2201	1-(5-fluoropentyl)-3-(1-naphthoyl)indole
CP-47,497	2-[(1R,3S)-3-hydroxycyclohexyl]- 5-(2-methyloctan -2-yl)phenol
HU-210	3-(1,1'-dimethylheptyl)-6aR,7,10,10aR-tetrahydro-1-hydroxy-6,6-dimethyl-6H-dibenzo[b,d]pyran-9-methanol
HU-211	3-(1,1'-dimethylheptyl)-6aR,7,10,10aR- tetrahydro-1-hydroxy-6,6-dimethyl- 6H-dibenzo[b,d]pyran-9-methanol
HU-308	4-[4-(1,1- dimethylheptyl)-2,6-dimethoxyphenyl]-6,6-dimethyl-bicyclo[3.1.1]hept-2-ene-2-methanol
HU-331	4-[4-(1,1- dimethylheptyl)-2,6-dimethoxyphenyl]-6,6-dimethyl-bicyclo[3.1.1]hept-2-ene-2-methanol
JWH-018	1-pentyl-3-(1-naphthoyl)indole
JWH-019	1-hexyl-3-(naphthalen-1-oyl)indole
JWH-073	naphthalen-1-yl-(1-butylindol-3-yl)methanone
JWH-081	4-methoxynaphthalen-1-yl-(1-pentylindol-3-yl) methanone
JWH-122	(4-methyl-1-naphthalenyl)(1-pentyl-1H-indol-3-yl)-methanone
JWH-200	(1-(2-morpholin-4-ylethyl)indol-3-yl)-naphthalen-1-ylmethanone
JWH-203	2-(2-chlorophenyl)-1-(1-pentyl-1H-indol-3-yl)-ethanone
JWH-210	(4-ethyl-1-naphthalenyl)(1-pentyl-1H-indol-3-yl)-methanone
JWH-250	1-(1-pentyl-1H-indol-3-yl)-2-(2-methoxyphenyl)-ethanone
JWH-251	2-(2-methylphenyl)-1-(1-pentyl-1H-indol-3-yl)-methanone
JWH-398	(4-chloronaphthalen-1-yl)(1-pentylindolin-3-yl)-methanone

Legend for Table 2.1: This is a list of synthetic cannabinoids analyzed in this study and entered into the GC-MS library as references

Figure 2.1

Legend 2.1: This is a package of the HMA K2 (contents inside plastic bag)


Figure 2.2
Legend 2.2: These are the contents of Figure 2.1, the HMA K2

Figure 2.3

Legend 2.3: These are the contents from a separate K2 sample than the contents seen in Figure 2.1 and 2.2. Note the white granular particulate scattered throughout this sample.

evaporated and reconstituted with the derivatizing agent BSTFA, heated for 15 minutes at 100 degrees, and then 2 microliters were injected.

Human Urine Sample Laboratory Testing

After obtaining IRB approval (or waiver) and subjects' informed consent, we obtained 22 urine samples from self-identified HMA abusers presenting to Emergency Departments for complaints directly self-attributed to HMA exposures. Each urine sample was de-identified and stored in a plastic specimen container before being sent at either room temperature or frozen on dry ice and mailed (via Federal Express ® overnight shipping) to the University of Massachusetts toxicology laboratory or the St. Louis toxicology laboratory, for independent confirmatory testing. After arriving in each laboratory, the urine sample was tested at room-air temperature (or thawed to room-air temperature when appropriate) and analyzed via GC-MS analysis for known xenobiotics and illicit drugs (including the synthetic cannabinoids).

Creation of Toxicology Database

With IRB approval, we used the University of Massachusetts information technology support structure to create a toxicology database (TOXIDARE) in order to facilitate prospective data collection efforts and further research on emerging drugs of abuse in cases presenting to the UMass Medical Toxicology Consultation service. This Internet-based database was created using REDCap © (Research Electronic Data Capture) technology, based out of Vanderbilt University.[42] Access to data entry was created for

Emergency Medicine residents during their Toxicology rotation, Medical Toxicology fellows and attending physicians.

Primary Data Analysis and Outcome Measures

Data analysis of patient information was performed via descriptive statistics including ranges, means, medians and modes calculated with Microsoft Office Excel, 2008 ©. Laboratory data analysis was performed by methods previously described in this study.

CHAPTER III: Clinical Results

Characteristics of Study Subjects

We identified a total of 81 cases of HMA abuse between November 2009 and August 2010 who presented to Poison Control Centers via phone call, EMS personnel, Emergency Departments, or Medical Toxicology services. Patients were primarily young males (the youngest subject was 12 years old) who smoked or inhaled HMA (**TABLE 3.1**). When presenting to a health care provider, subjects commonly had elevated heart rates and blood pressures. The most commonly reported symptoms were anxiety, confusion and agitation. Fifteen patients reported nausea or vomiting. There were 3 reported seizures, and an additional subject (who did not seize) called the Missouri Poison Control Center because he was concerned after his friend "seized" after smoking the same *K2* product. Nine subjects reported visual hallucinations. Reportedly within one hour of K2 exposure, one subject committed suicide with a firearm after reporting paranoia, thinking he was "in hell" according to non-medical provider accounts. Subjects most commonly required 4-24 hours of observation in the ED, with 6 subjects requiring hospital floor admission for up to 3 days. One individual required the administration of BIPAP and ultimate ICU admission for respiratory difficulty (**TABLE 3.2**).

In this surveillance study, subjects most frequently purchased HMAs from local head shops or gas stations, and the most frequently reported HMAs were *K2*, *Spice*, and *K2 Summit* (**TABLE 3.3**).

Table 3.1: Demographics, Vitals, Signs and Symptoms

Demographics	
Number of subjects	81
Male sex	
n (percentage)	64 (80%)
(number subjects with available data)	80
Age (years)	
(range)	12 to 46
mean, median, mode	22.3, 20, 18
(number subjects with available data)	71
Route of exposure	
inhalation/ingestion	78/1
(number subjects with available data)	79

Vital signs	
Temerature (Celcius)	
range	36.3 to 36.4
mean, median, mode	36.9, 36.9, 36.6
(number subjects with available data)	11
Heart rate (beats per minute)	
range	49 to 180
mean, median, mode	122, 120, 120
(number subjects with available data)	43
Mean arterial pressure (mmHg)	
range	57 to 140
mean, median, mode	99, 100, 87
(number subjects with available data)	32
Respiratory Rate (RR per minute)	
range	13 to 28
mean, median, mode	19, 18, 18
(Number subjects with available data)	12

Signs and Symptoms Number subjects/ Number available data (%)	
Anxiety Y/N	37/51 (73)
Confusion Y/N	25/44 (57)
Pupils dilated Y/N	11/28 (41)
Agitation Y/N	15/46 (33)
Pallor Y/N	6/25 (24)
Sweating Y/N	5/23 (22)
Hallucinations Y/N	9/44 (20)
Seizure Y/N	3/44 (7)
Nausea or Vomiting reported	15
Suicide	1

Legend for Table 3.1
These are the demographics, signs and symptoms for study subjects

Table 3.2: Health Care Intervention

Nature of presentation Number of subjects (%) (Data available for 73 subjects)	
Emergency Department then discharged home (observation ranged 4 to 24 hours)	47 (64)
Home calls to poison center	13 (18)
Admission to hospital floor (admitted up to 3 days)	6 (8)
EMS scene evaluation only	4 (5)
Prison referral phone call	1 (1)
Admission to hospital ICU	1(1)
Suicide	1 (1)

Treatment Rendered (Number of subjects receiving)	
IV fluids	14
Benzodiazepines	14
Ondansetron	3
Bipap	1
Insulin/glucose/lasiz/kayexalate	
Albuterol/calcium gluconate	1
Hydromorphone	1
Adenosine/potassium	1

Legend for Table 3.2
This is table shows what type of interaction each subject had with the health care system, and what type of treatment they received

Table 3.3: HMA details reported by subjects

Where HMA purchased	
Head Shop	11
Gas Station	6

Types of HMAs reported (Total reported 76)	
K2 (1 case: subject unclear if K2 or Flash 2)	43
Spice	11
K2 Summit	6
K2 Pink	3
Spike	3
Spike Gold	3
Spike Diamond	2
Star Orbit	2
K2 Serenity	1
K2 Blonde	1
Blueberry Spice	1

Legend for Table 3.3
This table shows where subjects purchased their HMAs (according to self report) as well as the HMA brand names.

Laboratory Findings

Of the serum chemistry laboratory results obtained, there were 3 subjects with K^+ values < 2.8 mEquiv/L; one subject had a K^+ value of 1.7 mEquiv/L (**TABLE 3.4**). Urine immunoassay testing ordered during hospital evaluation revealed positive THC results in 5 subjects; of these, one individual admitted to smoking marijuana as well as *K2*.

Urine GC-MS Testing

Of the 22 subjects who volunteered their urine for research testing, only one subject's urine returned a positive result for a synthetic cannabinoid (JWH-018). Testing demonstrated the presence of various xenobiotics, most commonly caffeine and nicotine (**TABLE 3.5**).

Table 3.4: Laboratory Results

Lab Results	
Na (mmol/L)	
range	132 to 146
mean, median, mode	139, 139, 140
(number subjects with available data)	22
K (mmol/L)	
range	1.7 to 6.5
mean, median, mode	3.6, 3.6, 3.8
(number subjects with available data)	23
Bicarbonate (mmol/L)	
range	15 to 28
mean, median, mode	25, 25, 24
(number subjects with available data)	21
Cl (mEquiv/L)	
range	95 to 129
mean, median, mode	104, 103, 103
(number subjects with available data)	20
BUN (mg/dL)	
range	10 to 25
mean, median, mode	15, 13, 13
(number subjects with available data)	19
Cr (mg/dL)	
range	<0.5 to 2.08
mean, median, mode	0.98, 0.9, 1.1
(number subjects with available data)	20
Calcium (nonionized) (mg/dL)	
range	4 to 11
mean, median	9.2, 9.5
(number subjects with available data)	14
Serum Glucose (mg/dL)	
range	64 to 218
mean, median, mode	126, 115, 92
(number subjects with available data)	21

Lab results	
Number of subjects (%)	
(Data available for 26 subjects)	
Urine drug immunoassay	
THC	5 (19)
Urine drug immunoassay	
amphetamine	4 (15)
Urine drug immunoassay	
benzodiazepine	2 (7)
Urine drug immunoassay	
opiates/opiates	1 (4)

Legend for table 3.4
This table shows the subject lab results as well as the results of urine immunoassay testing

Table 3.5: Subject Urine GC-MS Testing Results

Xenobiotics Detected	
Caffeine	16
Nicotine/ Cotinine	8
Acetaminophen	1
Amphetamine	1
Bupropion	1
Citalopram	1
Dextromethorphan	1
Diltiazem	1
Diphenhydramine	1
Lamotrigine	1
Lidocaine	1
Methadone	1
Phenytoin	1
Venlafaxine	1

Legend for Table 3.5
This shows the xenobiotics discovered by GC-MS analysis of subject's urine, other than JWH-018

CHAPTER IV: Forensic Laboratory Results

Synthetic Cannabinoid Testing Via Urine Immunoassay

After adding samples of JWH-015, JWH-018, JWH-073 and JWH-200 to control human urine specimens, we ran the Microgenics EMIT immunoassay test that detects Δ9-THC. We found that none of these pure JWH-compounds generated a positive immunoassay result for THC.

Synthetic Cannabinoid Evaluation via GC-MS

We performed GC-MS analysis of 18 synthetic cannabinoids (**TABLE 2.1**). Mass spectra results were available for each of these compounds, and they were each entered into the GC-MS library, against which each subject's volunteered urine was tested.

Forensic Testing of HMA Sample

Through forensic GC-MS analysis of multiple acquired samples of HMAs, we confirmed the presence of the following synthetic cannabinoids: RCS4, JWH-018, JWH-73, JWH-081, and JWH-122.

Chapter V: Conclusion

Coincident with the Midwestern U.S. HMA epidemic that broke out during the Spring of 2010, we confirmed the presence of synthetic cannabinoids in HMAs. We also confirmed the presence of synthetic cannabinoids in one subject's volunteered urine sample via GC-MS analysis. Subjects with untoward symptoms resulting from self-identified HMA inhalation most commonly manifested tachycardia, hypertension, agitation, anxiety and vomiting. We identified cases of seizure and suicide associated with HMA exposure.

Synthetic cannabinoids likely present in HMAs did not trigger a positive THC result by urine immunoassay testing directed at Δ9-THC. This illustrates one of the likely reasons for HMAs' popularity, which is avoidance of drug detection.

Chapter VI: Strengths and Limitations

Strengths

The present study had several strengths, which included prospective data collection on a novel emerging drug of abuse. At the time of the study, there were only isolated case reports of HMA abuse and associated clinical symptoms. The multi-center and multidisciplinary effort involving Poison Control Specialists, physicians and laboratory experts in forensic analysis maximized the number of cases and allowed for confirmatory testing.

Limitations

Missing Data

There were several limitations that must be kept in mind in interpreting the present results. There was a significant amount of missing data in this investigation. Data collection took place contemporaneously with the occurrence of the HMA epidemic, primarily located in the Midwest. Because of the need to collect as much information as possible, a data collection template was provided to Poison Control Center specialists in Missouri, Georgia, Kansas and toxicologists at the University of Massachusetts. This multistate collaborative effort was needed in order to maximize the number of cases. Despite extensive educational efforts and requests to collect relevant subject details, it was remarkably difficult to collect information. Many subject encounters took place over the phone, and subjects were not willing to participate in a lengthy data collection interview. When subjects presented to a health care facility, Poison Control Center

specialists again depended on the ability and willingness of the often-busy practicing physician or nurses in the Emergency Department.

Vital sign and laboratory information was often collected only one time during a subjects hospital stay. Depending on when vital signs, physical exams and laboratory testing were completed in relation to the time of exposure to HMAs, the results might have been considerably different.

Selection Bias

Because we did not know what the clinical effects of HMAs were prior to undertaking this study, it was not possible to develop a tailored data collection tool for confirmatory vital signs, laboratory findings, physical exam findings, or physical/psychiatric complaints. Initially, we noted a common thread of agitation and sympathomimetic vital signs, so those details were part of our data collection instrument. We did not initially note the presence of nausea or vomiting, therefore neither descriptor was a specific field on the data collection form. Thus, the 15 cases of spontaneously reported nausea and vomiting likely under-represent the true incidence of vomiting in our study subjects.

Inability to confirm subjects took HMAs

Few subjects volunteered samples of their HMAs to health care personnel or law enforcement for confirmatory testing and analysis. Therefore, it was not possible to validate our case-definition of self-identified HMA exposure with confirmatory lab

testing. Some data may, therefore, not represent HMA effects, but rather those of another drug of abuse.

GC-MS urinalysis confirmed only 1 case of synthetic cannabinoid exposure

First, it is possible that the subjects in this study were not exposed to synthetic cannabinoids. Also, parent compounds of synthetic cannabinoids may not be stable or have a sufficient $t_{1/2}$ to allow for detection hours after HMA exposure. Too much time may have elapsed between 1) HMA exposure and urine sample collection, or 2) urine collection and toxicology testing. It was not possible to determine how much time elapsed between exposure to HMAs and urine collection. Synthetic cannabinoid parent compounds may also not be stable for transfer at freezing temperatures. It was also possible (as has been suggested by other authors) that better synthetic cannabinoid detection in urine would come via testing for metabolites.[43]

HMAs changing contents

Although we tested for the most likely and available synthetic compounds, as identified by existing evidence at the time, it is possible that the subjects in this study were exposed to HMAs containing synthetic cannabinoids not accounted for by the 18 synthetic cannabinoids in our GC-MS library.

Chapter VII: Discussion

Subsequent to the completion of this study, researchers have been able to detect synthetic cannabinoid metabolites in human urine via GC-MS testing.[43, 44] If we conducted a similar study again with HMAs and HMA abusers, we would direct our urine testing at previously identified and possibly novel metabolites of synthetic cannabinoids known to contaminate HMAs. However, due to the DEA listing many synthetic cannabinoids as schedule 1 substances, the ability to conduct such research has been significantly impaired.

It is difficult to explain the seemingly sympathomimetic vital sign and physical exam abnormalities that appeared in the subjects in our surveillance study. Delta-9-THC may be associated with psychosis, but it has been associated with both bradycardia and tachycardia.[45-47] Moreover, the synthetic cannabinoid dronabinol is an FDA approved pharmacotherapy for often protracted vomiting secondary to chemotherapy.[48] Therefore, the vomiting seen in our subject population would not be a symptom predicted to result from a synthetic cannabinoid interacting with CB receptors similarly to Δ9-THC.

We considered the possibility of stimulants (and not synthetic cannabinoids) causing the symptoms seen in our subjects. However, our GC-MS analysis would have likely been able to detect typical stimulants (like amphetamines and other phenylalanine-based xenobiotics), and it only did so in 2 of our subjects. It is important to note that mephedrone was quite popular in Europe, and *Bath Salts* were beginning their rapid rise

to popularity around the time of the HMA epidemic. Mephedrone is 4-methylmethcathinone, which is a synthetic stimulant derived from cathinone – a substance found in the plant Khat.[49] *Bath Salts* ultimately proved to contain sympathomimetics (e.g. MDPV, amphetamines and cathinone-derivatives), which were confirmed to be present in seized samples of *Bath Salts* by law-enforcement.[50] While unable to detect MDPV or certain cathinones at the time of the present study, our GC-MS library was able to detect amphetamines and mephedrone. We did not detect mephedrone. Researchers have subsequently described urinalysis testing that would easily allow for stimulants like mephedrone and MDPV that we would utilize for further observational studies on emerging drugs of abuse.[51]

Synthetic cannabinoids may cause sympathomimetic symptoms by reverse signaling disinhibition, via GABA inhibition (thus leaving unopposed excitatory glutamate). The hallucinations seen by one of our subjects may be explained by synthetic cannabinoid's association with serotonergic activity.[52] Seizures may manifest as cataplexy, a phenomenon explained by Martin et al who demonstrated synthetic cannabinoid activity on dopaminergic D_1 and D_2 receptors.[53] There are other published case reports of seizures associated with synthetic cannabinoid exposure.[54]

The REDCap © database we created designed to address many of the data-collection difficulties experienced in our study. Prospective data collection for emerging drugs of abuse (with respect to vital signs, physical exam findings, symptoms and laboratory

findings) is difficult due to the nature of drug abuse and how patients present to the health care system. Much of the access to drug-abusing patients comes via phone calls to Poison Control Centers. Poison Control specialist's jobs are to provide appropriate medical advice and direct patients to hospital care when appropriate. Rigorous data collection for research purposes, as a goal, is not often compatible or possible in this environment. Furthermore, the Poison Control Center database does not easily lend itself to research-quality data collection. It is my hope than the creation of the UMass REDCap © database will allow for more meaningful prospective data collection for emerging drugs of abuse (like HMAs), in order to contribute to the medical literature and Emergency Medicine community in a more scientifically rigorous fashion.

Bibliography

1. Dresen S, Ferreiros N, Putz M, Westphal F, Zimmermann R, Auwarter V. Monitoring of herbal mixtures potentially containing synthetic cannabinoids as psychoactive compounds. *J Mass Spectrom.* Oct 2010;45(10):1186-1194.

2. Addiction EMCfDaD. Understanding the 'Spice' phenomenon. Lisbon, Portugal2009:1-34.

3. Emboden WA. Transcultural use of narcotic water lilies in ancient Egyptian and Maya drug ritual. *J Ethnopharmacol.* Jan 1981;3(1):39-83.

4. Morgan NH, Stanford IM, Woodhall GL. Functional CB2 type cannabinoid receptors at CNS synapses. *Neuropharmacology.* Sep 2009;57(4):356-368.

5. Montecucco F, Burger F, Mach F, Steffens S. CB2 cannabinoid receptor agonist JWH-015 modulates human monocyte migration through defined intracellular signaling pathways. *Am J Physiol Heart Circ Physiol.* Mar 2008;294(3):H1145-1155.

6. Lombard C, Nagarkatti M, Nagarkatti P. CB2 cannabinoid receptor agonist, JWH-015, triggers apoptosis in immune cells: potential role for CB2-selective ligands as immunosuppressive agents. *Clin Immunol.* Mar 2007;122(3):259-270.

7. Ameri A, Wilhelm A, Simmet T. Effects of the endogeneous cannabinoid, anandamide, on neuronal activity in rat hippocampal slices. *Br J Pharmacol.* Apr 1999;126(8):1831-1839.

8. Auwarter V, Dresen S, Weinmann W, Muller M, Putz M, Ferreiros N. 'Spice' and other herbal blends: harmless incense or cannabinoid designer drugs? *J Mass Spectrom.* May 2009;44(5):832-837.

9. Atwood BK, Huffman J, Straiker A, Mackie K. JWH018, a common constituent of 'Spice' herbal blends, is a potent and efficacious cannabinoid CB receptor agonist. *Br J Pharmacol.* Jun 2010;160(3):585-593.

10. Huffman JW. CB2 receptor ligands. *Mini Rev Med Chem.* Jul 2005;5(7):641-649.

11. Leggett JD, Aspley S, Beckett SR, D'Antona AM, Kendall DA. Oleamide is a selective endogenous agonist of rat and human CB1 cannabinoid receptors. *Br J Pharmacol.* Jan 2004;141(2):253-262.

12. Thakur GA, Nikas SP, Makriyannis A. CB1 cannabinoid receptor ligands. *Mini Rev Med Chem.* Jul 2005;5(7):631-640.

13. Pavlopoulos S, Thakur GA, Nikas SP, Makriyannis A. Cannabinoid receptors as therapeutic targets. *Curr Pharm Des.* 2006;12(14):1751-1769.

14. Microgram. JWH-073 (purported "Spice" ingredient) in Virginia2009.

15. Uchiyama N, Kikura-Hanajiri R, Ogata J, Goda Y. Chemical analysis of synthetic cannabinoids as designer drugs in herbal products. *Forensic Sci Int.* May 20 2010;198(1-3):31-38.

16. Zuba D, Byrska B, Maciow M. Comparison of "herbal highs" composition. *Anal Bioanal Chem.* Apr 2011;400(1):119-126.

17. Vardakou I, Pistos C, Spiliopoulou C. Spice drugs as a new trend: mode of action, identification and legislation. *Toxicol Lett.* Sep 1 2010;197(3):157-162.

18. Hudson S, Ramsey J, King L, et al. Use of high-resolution accurate mass spectrometry to detect reported and previously unreported cannabinomimetics in "herbal high" products. *J Anal Toxicol.* Jun 2010;34(5):252-260.

19. http://www.wfaa.com/news/health/North-Texas-teens-death-linked-to-K2-use-100386899.html.

20. http://abelpharmboy.wordpress.com/2010/08/14/k2-spice-associated-with-death-of-young-indiana-mother-of-two/.

21. Auwarter V DS, Weinmann MW, Putz M, Ferreiros N. 'Spice' and other herbal blends: harmless incense or cannabinoid designer drugs? *Journal of Mass Spectrometry.* 2008;44.

22. http://www.pitch.com/plog/archives/2010/06/22/iowa-teen-david-rozga-smokes-k2-freaks-out-and-commits-suicide.

23. Vearrier D, Osterhoudt KC. A teenager with agitation: higher than she should have climbed. *Pediatr Emerg Care.* Jun 2010;26(6):462-465.

24. http://www.justice.gov/dea/programs/forensicsci/microgram/mg2011/mg0111.pdf

25. Schneir AB, Cullen J, Ly BT. "Spice" girls: synthetic cannabinoid intoxication. *J Emerg Med.* Mar 2011;40(3):296-299.

26. Dowling G, Regan L. A method for CP 47, 497 a synthetic non-traditional cannabinoid in human urine using liquid chromatography tandem mass spectrometry. *J Chromatogr B Analyt Technol Biomed Life Sci.* Feb 1 2011;879(3-4):253-259.

27. Howlett AC, Barth F, Bonner TI, et al. International Union of Pharmacology. XXVII. Classification of cannabinoid receptors. *Pharmacol Rev.* Jun 2002;54(2):161-202.

28. Pertwee RG, Howlett AC, Abood ME, et al. International Union of Basic and Clinical Pharmacology. LXXIX. Cannabinoid receptors and their ligands: beyond CB and CB. *Pharmacol Rev.* Dec 2010;62(4):588-631.

29. Marriott KS, Huffman JW. Recent advances in the development of selective ligands for the cannabinoid CB(2) receptor. *Curr Top Med Chem.* 2008;8(3):187-204.

30. Di Marzo V, Bifulco M, De Petrocellis L. The endocannabinoid system and its therapeutic exploitation. *Nat Rev Drug Discov.* Sep 2004;3(9):771-784.

31. Chin CN, Murphy JW, Huffman JW, Kendall DA. The third transmembrane helix of the cannabinoid receptor plays a role in the selectivity of

aminoalkylindoles for CB2, peripheral cannabinoid receptor. *J Pharmacol Exp Ther.* Nov 1999;291(2):837-844.

32. Brown SM, Wager-Miller J, Mackie K. Cloning and molecular characterization of the rat CB2 cannabinoid receptor. *Biochim Biophys Acta.* Jul 19 2002;1576(3):255-264.

33. Verdurand M, Dalton VS, Zavitsanou K. GABA(A) receptor density is altered by cannabinoid treatment in the hippocampus of adult but not adolescent rats. *Brain Res.* Sep 10 2010;1351:238-245.

34. Napchan U, Buse DC, Loder EW. The use of marijuana or synthetic cannabinoids for the treatment of headache. *Headache.* Mar 2011;51(3):502-505.

35. Poso A, Huffman JW. Targeting the cannabinoid CB2 receptor: modelling and structural determinants of CB2 selective ligands. *Br J Pharmacol.* Jan 2008;153(2):335-346.

36. Grigoryev A, Savchuk S, Melnik A, et al. Chromatography-mass spectrometry studies on the metabolism of synthetic cannabinoids JWH-018 and JWH-073, psychoactive components of smoking mixtures. *J Chromatogr B Analyt Technol Biomed Life Sci.* May 1 2011;879(15-16):1126-1136.

37. Ojewole JA. Antinociceptive, antiinflammatory and antidiabetic effects of Leonotis leonurus (L.) R. BR. [Lamiaceae] leaf aqueous extract in mice and rats. *Methods Find Exp Clin Pharmacol.* May 2005;27(4):257-264.

38. Rode D. Comfrey toxicity revisited. *Trends Pharmacol Sci.* Nov 2002;23(11):497-499.

39. Yeong ML, Clark SP, Waring JM, Wilson RD, Wakefield SJ. The effects of comfrey derived pyrrolizidine alkaloids on rat liver. *Pathology.* Jan 1991;23(1):35-38.

40. Chrubasik C, Duke RK, Chrubasik S. The evidence for clinical efficacy of rose hip and seed: a systematic review. *Phytother Res.* Jan 2006;20(1):1-3.

41. Schumacher M, Cerella C, Reuter S, Dicato M, Diederich M. Anti-inflammatory, pro-apoptotic, and anti-proliferative effects of a methanolic neem (Azadirachta indica) leaf extract are mediated via modulation of the nuclear factor-kappaB pathway. *Genes Nutr.* May 2011;6(2):149-160.

42. Harris PA, Taylor R, Thielke R, Payne J, Gonzalez N, Conde JG. Research electronic data capture (REDCap)--a metadata-driven methodology and workflow process for providing translational research informatics support. *J Biomed Inform.* Apr 2009;42(2):377-381.

43. Sobolevsky T, Prasolov I, Rodchenkov G. Detection of JWH-018 metabolites in smoking mixture post-administration urine. *Forensic Sci Int.* Jul 15 2010;200(1-3):141-147.

44. Moran CL, Le VH, Chimalakonda KC, et al. Quantitative measurement of JWH-018 and JWH-073 metabolites excreted in human urine. *Anal Chem.* Jun 1 2011;83(11):4228-4236.

45. D'Souza DC, Perry E, MacDougall L, et al. The psychotomimetic effects of intravenous delta-9-tetrahydrocannabinol in healthy individuals: implications for psychosis. *Neuropsychopharmacology.* Aug 2004;29(8):1558-1572.

46. Vollmer RR, Cavero I, Ertel RJ, Solomon TA, Buckley JP. Role of the central autonomic nervous system in the hypotension and bradycardia induced by (-)-delta 9-trans-tetrahydrocannabinol. *J Pharm Pharmacol.* Mar 1974;26(3):186-192.

47. Young AC, Schwarz E, Medina G, et al. Cardiotoxicity associated with the synthetic cannabinoid, K9, with laboratory confirmation. *Am J Emerg Med.* Jul 28 2011.

48. Gonzalez-Rosales F, Walsh D. Intractable nausea and vomiting due to gastrointestinal mucosal metastases relieved by tetrahydrocannabinol (dronabinol). *J Pain Symptom Manage.* Nov 1997;14(5):311-314.

49. Morris K. UK places generic ban on mephedrone drug family. *Lancet.* Apr 17 2010;375(9723):1333-1334.

50. Durham M. Ivory wave: the next mephedrone? *Emerg Med J.* Dec 2011;28(12):1059-1060.

51. Bell C, George C, Kicman AT, Traynor A. Development of a rapid LC-MS/MS method for direct urinalysis of designer drugs. *Drug Test Anal.* Jul-Aug 2011;3(7-8):496-504.

52. Arevalo C, de Miguel R, Hernandez-Tristan R. Cannabinoid effects on anxiety-related behaviours and hypothalamic neurotransmitters. *Pharmacol Biochem Behav.* Sep 2001;70(1):123-131.

53. Martin AB, Fernandez-Espejo E, Ferrer B, et al. Expression and function of CB1 receptor in the rat striatum: localization and effects on D1 and D2 dopamine receptor-mediated motor behaviors. *Neuropsychopharmacology.* Jun 2008;33(7):1667-1679.

54. Schneir AB, Baumbacher T. Convulsions Associated with the Use of a Synthetic Cannabinoid Product. *J Med Toxicol.* Dec 13 2011.