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Synthetic Cannabinoid Receptor Agonists: Methods of Detection and Current Knowledge on Toxicity

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Outline

I. Methods of detection

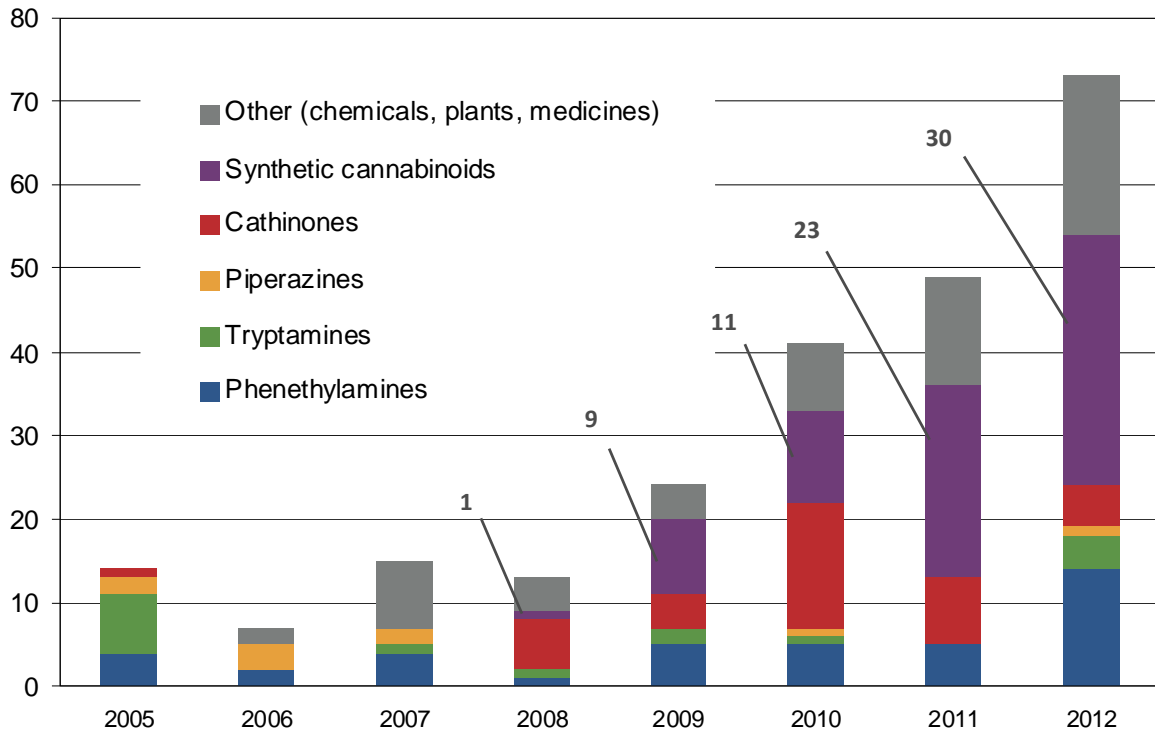
- Drug material
- Human samples (blood, oral fluid, hair, urine)
- Interpretation of analytical results

II. Toxicity

- Acute toxicity
- Long-term toxicity

Conclusions

New psychoactive substances reported through the EWS



www.emcdda.europa.eu



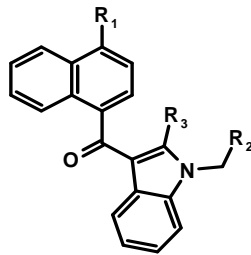
German narcotics law

- | | | | |
|------------------------|--------------------------|--------------------------|-----------------|
| • CP 47,497 | • AM-1220 | • AM-1248 | • JWH-412-5F |
| • CP 47,497-C6-homolog | • AM-1220-azepane isomer | • AM-2201 indazol isomer | • MN25 |
| • CP 47,497-C8-homolog | • AM-2201 | • AM-679 | • NNEI |
| • CP 47,497-C9-homolog | • AM-2232 | • 2NEI (APICA) | • NNEI-5F |
| • JWH-018 | • AM-2233 | • BB-22 | • PB-22 |
| • JWH-019 | • JWH-307 | • EAM-2201 | • PB-22-5F |
| • JWH-073 | • MAM-2201 | • FDU-PB-22 | • RCS-8 |
| • JWH-203 | • UR-144 | • FUB-PB-22 | • STS-135 |
| • RCS-4 ortho isomer | • XLR-11 | • JWH-020 | • THJ |
| • AM-694 | • AB-001 | • JWH-022 | • THJ-5F |
| • JWH-007 | • A 796,260 | • JWH-030 | • THJ-018 |
| • JWH-015 | • A 834,735 | • JWH-080 | • THJ-2201 |
| • JWH-081 | • AB-001-5F | • JWH-176 | • UR-144 isomer |
| • JWH-122 | • AB-005 | • JWH-180 | • XLR-11 isomer |
| • JWH-200 | • AB-FUBINACA | • JWH-182 | • XLR-12 |
| • JWH-210 | • AB-PINACA | • JWH-213 | • WIN 48,098 |
| • JWH-250 | • ADB-FUBINACA | • JWH-368 | • WIN 55,212-2 |
| • JWH-251 | • ADB-PINACA | • JWH-370 | |
| • RCS-4 | • ADB-PINACA-5F | • JWH-387 | |
| • AKB-48 (APINACA) | • ADBICA | • JWH-398 | |
| • AKB-48-5F | • ADBICA-5F | • JWH-412 | |

already banned
not listed yet

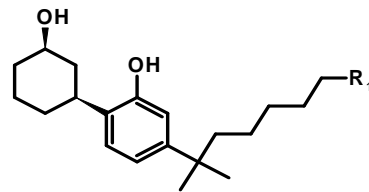
Synthetic cannabinoids: The compounds

a) Naphthoylindoles



JWH-018	JWH-073
JWH-398	JWH-200
JWH-081	JWH-015
JWH-122	JWH-210
JWH-019	JWH-007
AM-2201	JWH-020
JWH-387	AM-1220
JWH-412	MAM-2201
...	

b) Cyclohexylphenoles

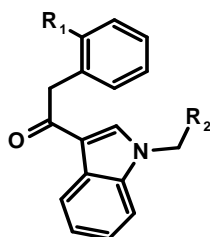


CP-47,497-C8



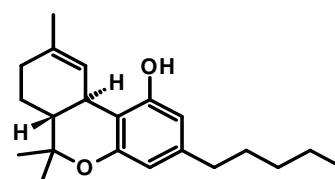
Synthetic cannabinoids: The compounds

c) Phenylacetylindoles

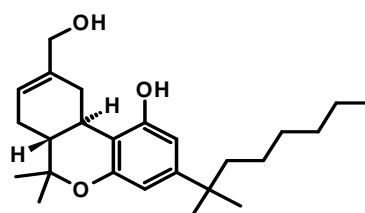


JWH-250
JWH-203
JWH-251
RCS-8
...

d) Classical cannabinoids

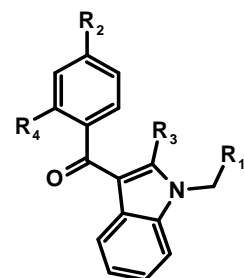


Δ 9-THC



HU-210

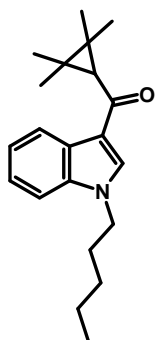
e) Benzoylindoles



AM-694
RCS-4
WIN-48,098
RCS-4-ortho-isomer
AM-2233
...

Synthetic cannabinoids: The compounds

f) Cyclopropylindoles



UR-144

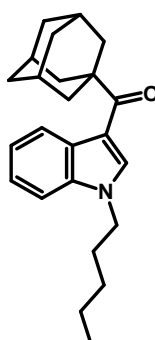
XLR-11

A-796,260

AB-005

...

g) Adamantane derived indoles/indazoles



AB-001

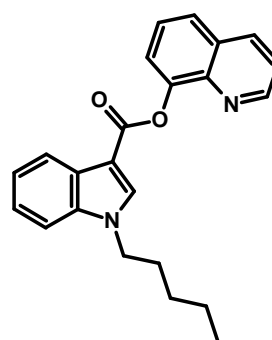
APICA (SDB-001; 2NE1)

APINACA (AKB48)

5F-APINACA (AKB48-5F)

STS-135

h) Indole quinolinylesters



PB-22

5F-PB-22

BB-22

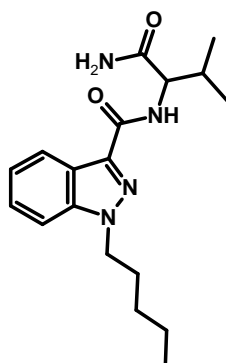
FUB-PB-22

...



Synthetic cannabinoids: The compounds

i) Indazole derivatives



AB-PINACA

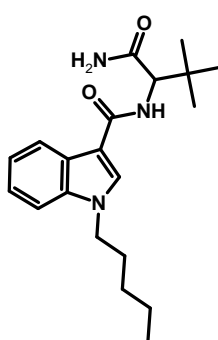
AB-FUBINACA

ADB-FUBINACA

THJ-018

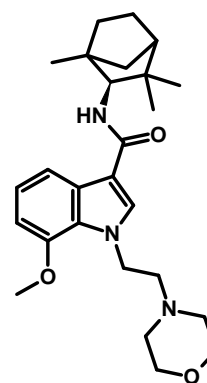
THJ-2201

j) Other structures



ADBICA

5F-ADBICA



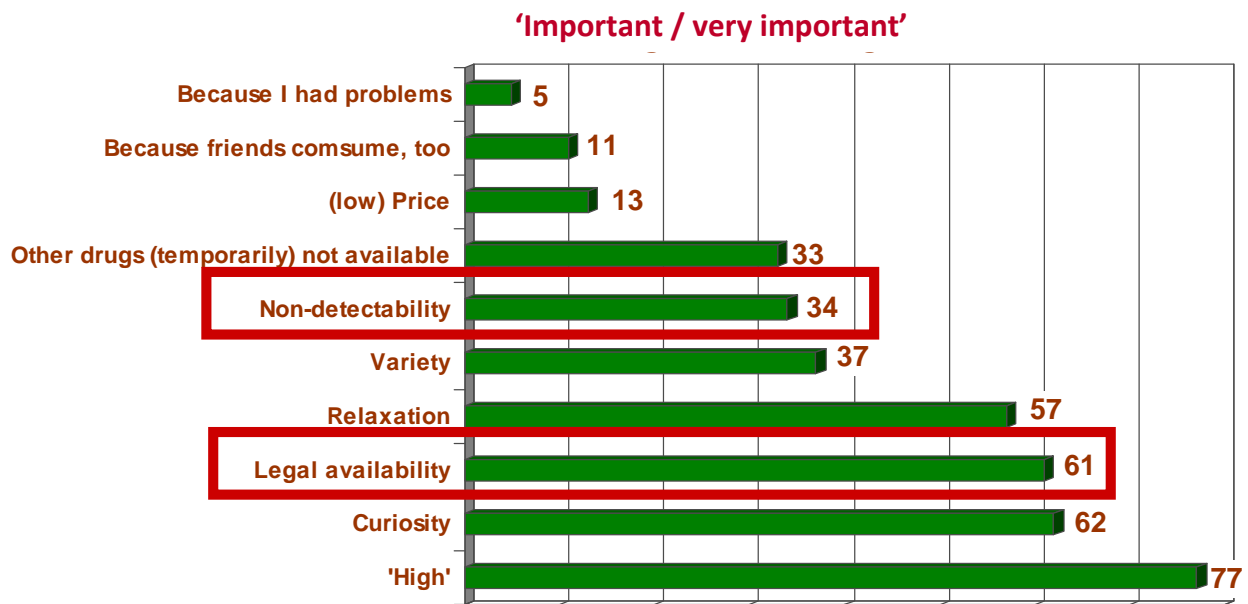
MN25

...



Online-survey: Motives for consumption (%)

(,Legal highs')



Goethe-Universität
Frankfurt am Main Institut für Sozialpädagogik und Erwachsenenbildung



UNIVERSITÄTS
KLINIKUM REIBURG

Methods of detection



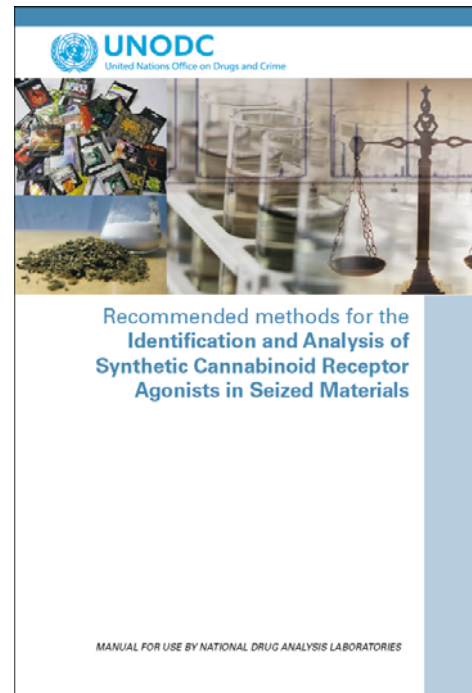
UNIVERSITÄTS
KLINIKUM REIBURG

Methods of detection

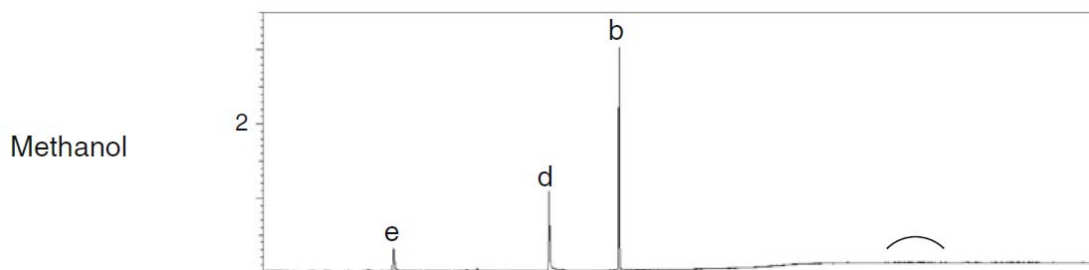
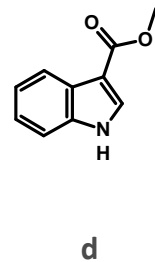
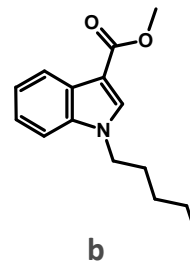
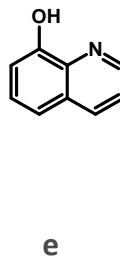
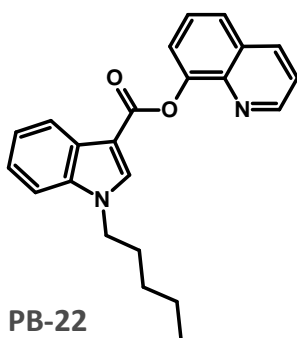
Drug material:

- Fast detection, on site
(IMS or IR, no color tests available!)
- TLC(-DESI-MS), HPLC-DAD, LC-MS/MS, GC-FID, HRMS
- Gold standard: GC-MS

Problem: Quick identification of new compounds (NMR)

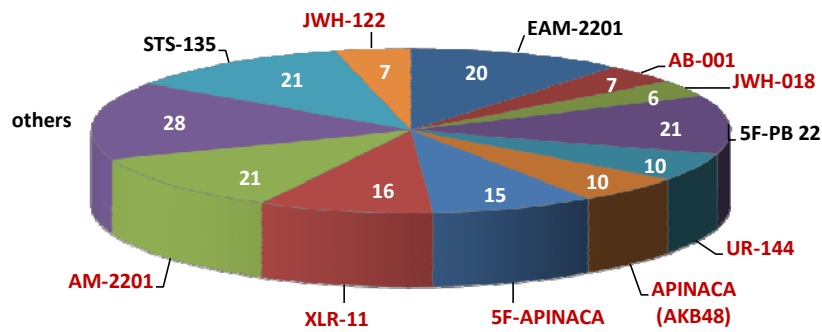


Thermal degradation of carboxylates



Synthetic Cannabinoids - Monitoring

85% positiv herbal blends



NEW:

- 5F-APINACA (june 2013)
- 5F-PB-22 (june 2013)
- STS-135 (august 2013)
- THJ-018 (january 2014)
- THJ-2201 (january 2014)

since march 2013:

160 herbal blends,
24 of them without any synthetic
cannabinoid

Quantification of SCs in drug material

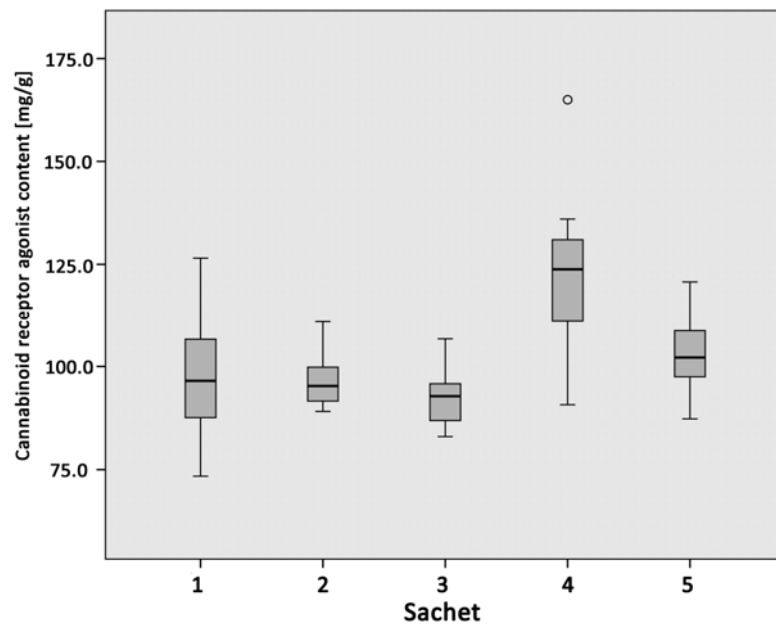
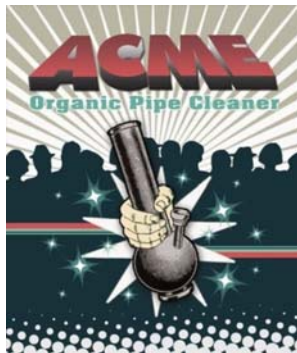
HPLC-DAD:

Analysis of 313 sachets (31 brands)

„Complete“ analysis of 34 sachets (21 brands)



Inhomogeneities in the ‚herbal mixture‘ ACME



Product weight: 2 g
 Aliquots: 200 mg
 SC: JWH-210
 K_i CB₁: 0.46 ± 0.03 nM



Replacement of the active ingredient

Product	Mean SC content [mg/g]	Synthetic cannabinoid	K_i CB ₁ [nM]
Blaze	160.7	JWH-307	7.7 ± 1.8
	152.8	JWH-210	0.46 ± 0.03
Peace	59.4	JWH-210	0.46 ± 0.03
	38.9	XLR-11	24
Vegas Titanium	49.2	JWH-210	0.46 ± 0.03
	45.9	XLR-11	24

The added amount of synthetic cannabinoids often stays the same despite significant differences in the binding affinity of the ‚newly‘ added synthetic cannabinoid

→ Intoxications likely to occur



Methods of detection

Drug material:

Fast detection, on site (IMS or IR, no color tests available!)

TLC(-DESI-MS), HPLC-DAD, LC-MS/MS, GC-FID, HRMS

Gold standard: GC-MS

Problem: Quick identification of new compounds (NMR)

Human samples:

Analytical techniques: LC-MS/MS, GC-MS/MS, HR-MS, IA(?)

Blood, oral fluid, hair: Unchanged compounds

Urine: Metabolites

Problems: Sensitivity, reference standards, metabolism

Interpretation of analytical results?



Covered analytes

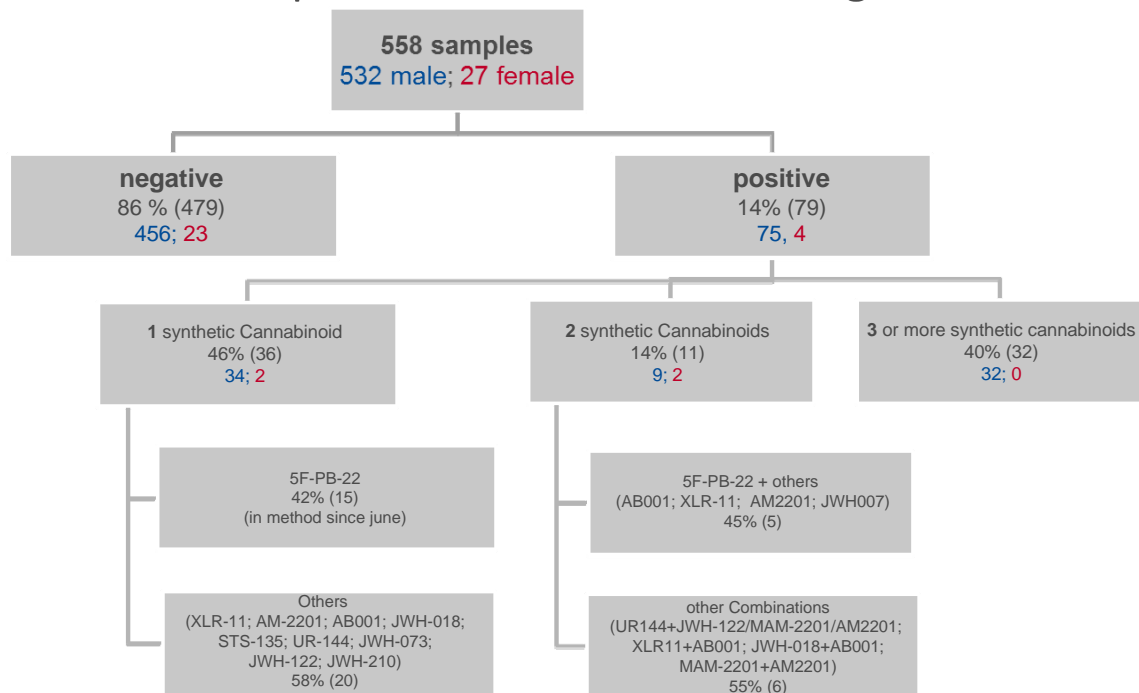
Blood, (oral fluid), hair:

75 compounds

(most quantitative, LLOQ 0.1 ng/mL in serum)



synthetic cannabinoids samples since march 2013 –august 2013



19

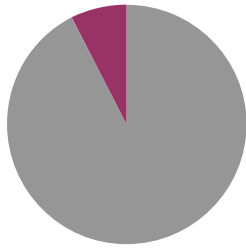
Sensitivity!

				Highest concentration [ng/mL]*	Median concentration [ng/mL]
Table 6. Results of the analysis of 227 serum samples tested				11	0.28
*Serum concentrations exceeding the highest calibrator were				11	0.40
Analyte	Positive samples (n)	Overall positives [%]	Abundance in samples		
JWH-018	23	2.8	10.1	7.1	0.85
JWH-019	14	1.7	6.1	6.0	0.35
JWH-073	6	0.7	2.6		
JWH-081	6	0.7	2.6	230	0.52
JWH-122	144	17.3	63.4		
JWH-200	1	0.1	0.4	-	-
JWH-203	5	0.6	2.2		
JWH-210	182	21.8	80.2	6.0	0.69
JWH-307	8	1.0	3.5		
AM-2201	65	7.8	28.6	46	0.77
RCS-4	3	0.4	1.3	53	0.49
				9.5	0.31
				-	-



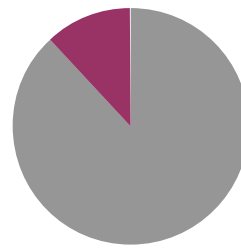
184 hair samples were analysed...

- 87 hair samples from 2010



Positive rate: 8 %

- 97 hair samples from 2011



Positive rate: 13 %

Positive rates of hair samples are very similar to positive rates of serum and urine samples...

BUT...



Synthetic cannabinoids in hair –
contribution of contamination by
side-stream smoke or handling of
drug material



Covered analytes

Blood, (oral fluid), hair:

75 compounds

(most quantitative, LLOQ 0.1 ng/mL in serum)

Urine:

Metabolites of 32 compounds

(mainly qualitative, LOD ~0.005 - 0.020 ng/mL)

AB-001, AB-FUBINACA, AB-PINACA, AKB-48, AKB48-5F, AM-694, AM-2201, APICA, BB-22, EAM-2201, JWH-007, JWH-018, JWH-019, JWH-073, JWH-081, JWH-122, JWH-200, JWH-203, JWH-210, JWH-250, JWH-307, JWH-398, MAM-2201, PB-22, PB-22-5F, RCS-4, STS-135, THJ-018, THJ-2201, UR-144, UR-144 Isomer, XLR-11

Hutter et al. 2012 Journal of Mass Spectrometry



How to identify metabolites

Freiburg approach, human samples:

- Urine samples from patients (paired with positive blood samples, best $n > 5$ per compound)
- Recording of mass spectra of anticipated metabolites (EPI)
- Confirmation using High Resolution Mass Spectrometry
- Identification of the most abundant metabolites (best $n > 3$)
- Development of an LC-MS/MS method for target metabolites

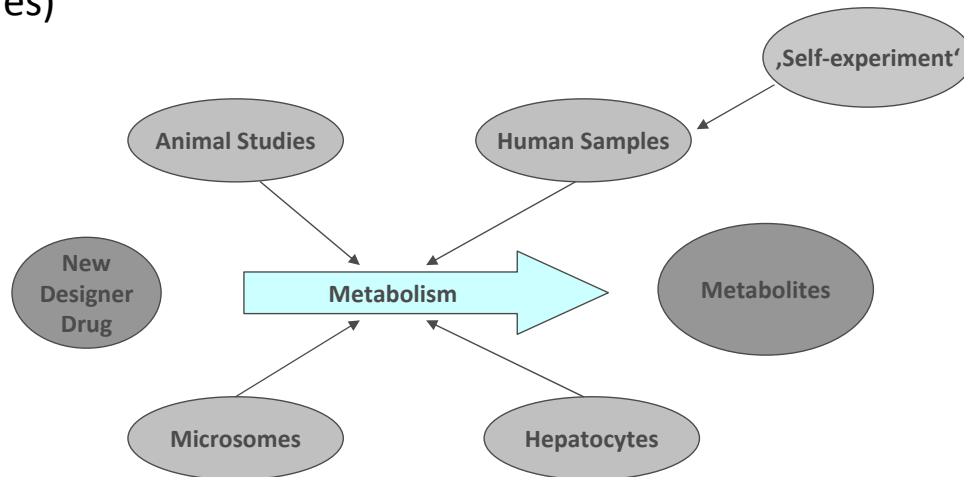
**Complex metabolism,
monohydroxylated compounds are the main targets**



How to identify metabolites

Problem:

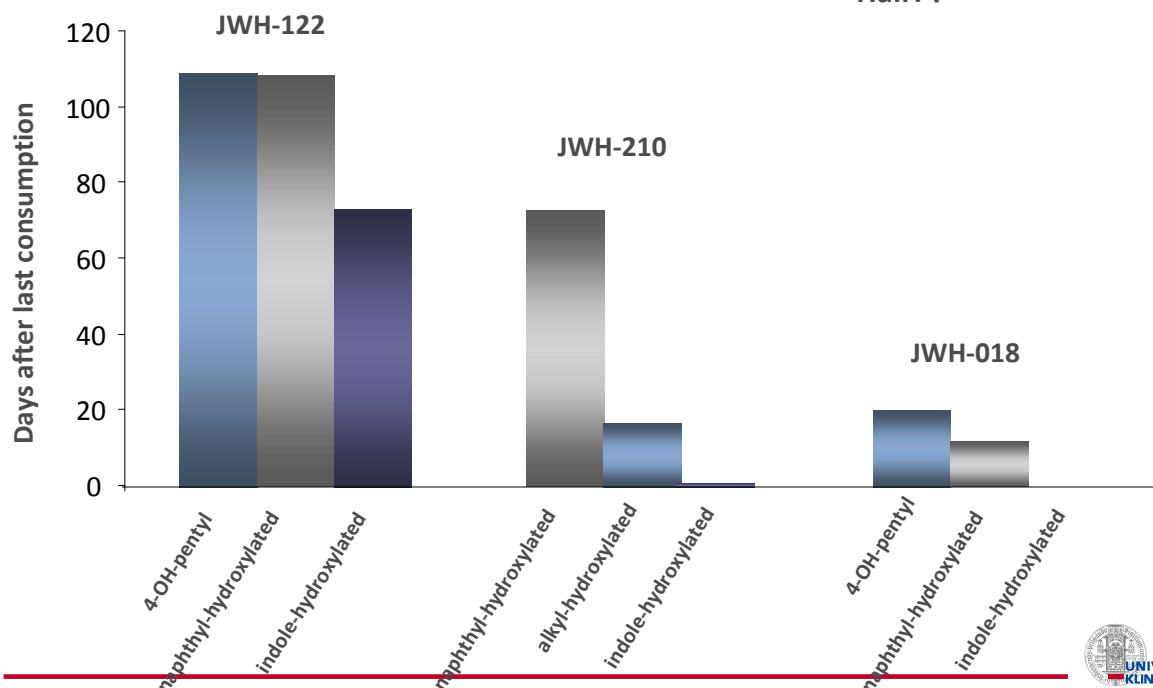
- > Not enough paired samples for rather 'exotic' compounds
- > 'Mixed' consume (more than one compound in blood samples)



Interpretation of results –window of detection

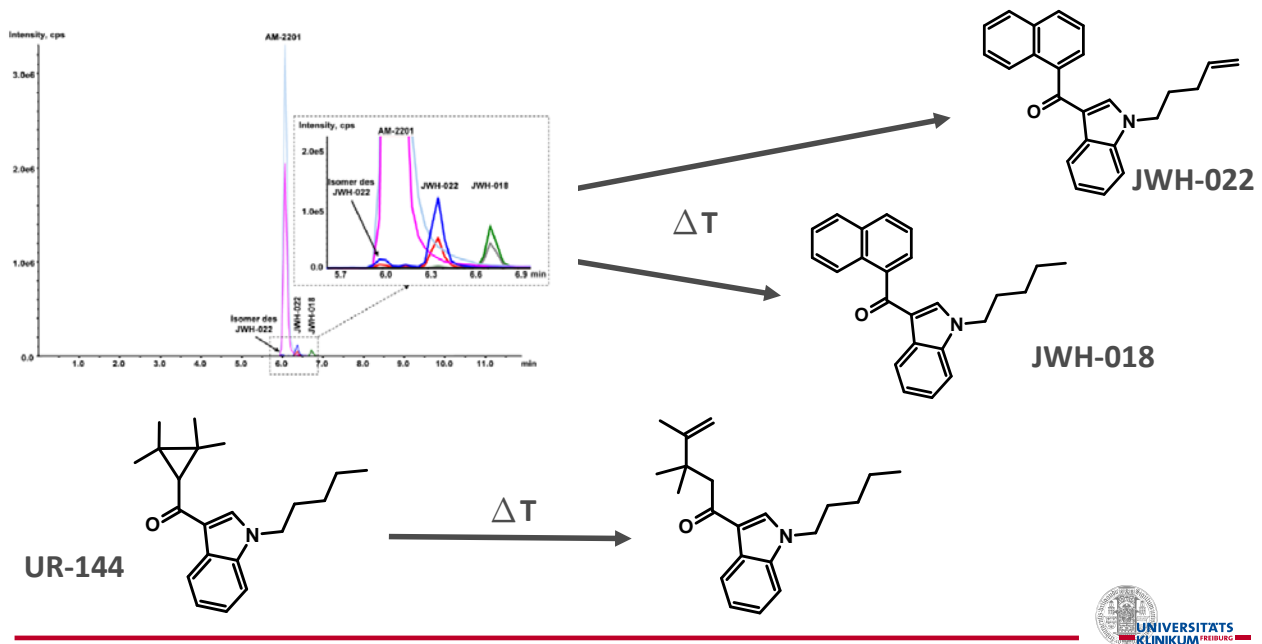
- Long terminal elimination half-lives

Blood: Similar range
Hair: ?

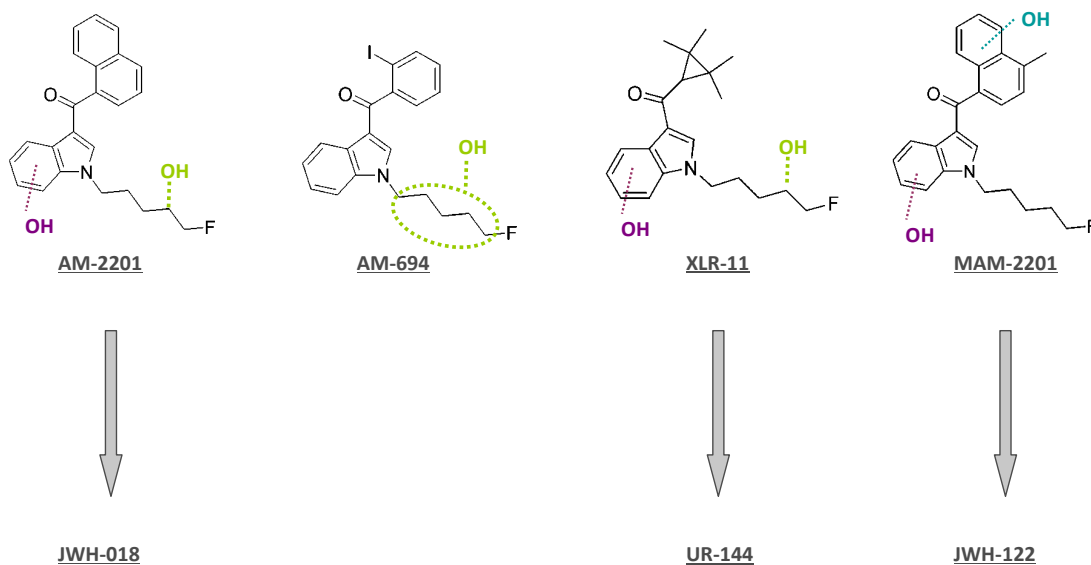


Interpretation of results

- Long terminal elimination half-lives
- Detection of artefacts, e.g. AM-2201, UR-144



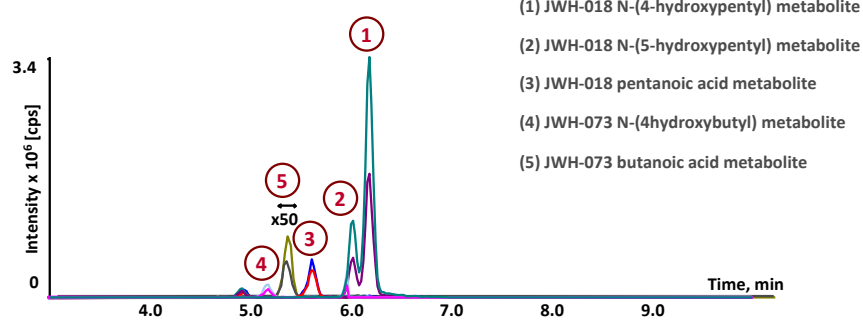
Particularities in metabolism



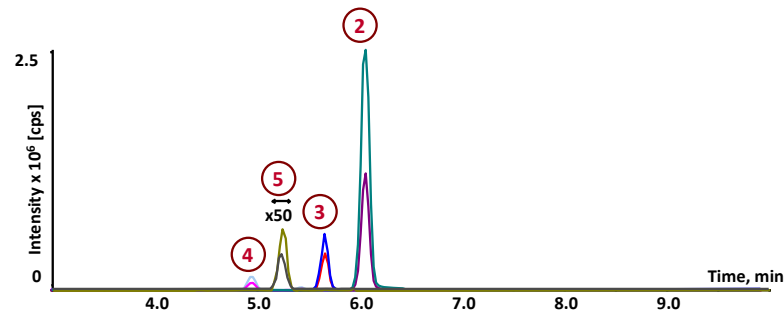
In all samples investigated
metabolites of the defluorinated analogs were detected

Metabolic formation?

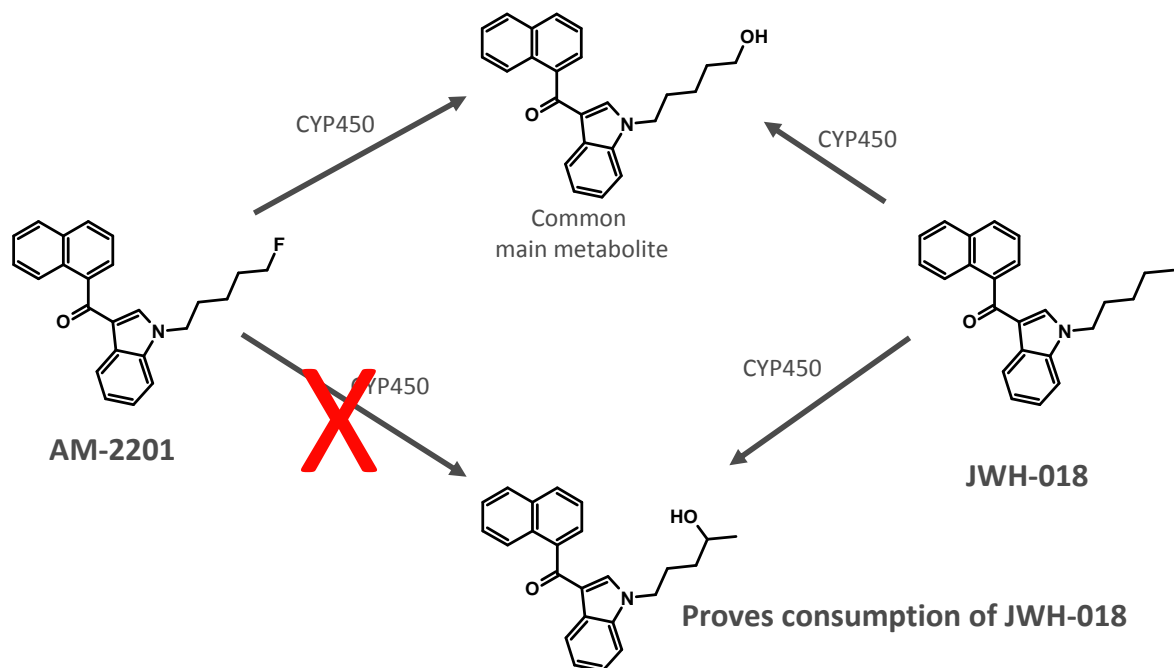
JWH-018



Oral uptake
of AM-2201



Consumption of AM-2201 or JWH-018?



See also MAM-2201 – JWH-122, EAM-2201 – JWH-210 or UR-144 – XLR-11



Immunochemical assay

Arnston et al. **2013**, J Anal Toxicol



Toxicity



Toxicity: Potency and efficacy

Potency: Low dose – strong effects

e.g. HU-210 (competitive binding assays, animal models)



Efficacy: Strong maximum effects

partial / full agonism ($[^{35}S]$ GTPyS, neurotransmission, animal models)

→ Many synthetic cannabinoids show higher potency and higher efficacy than *Cannabis*

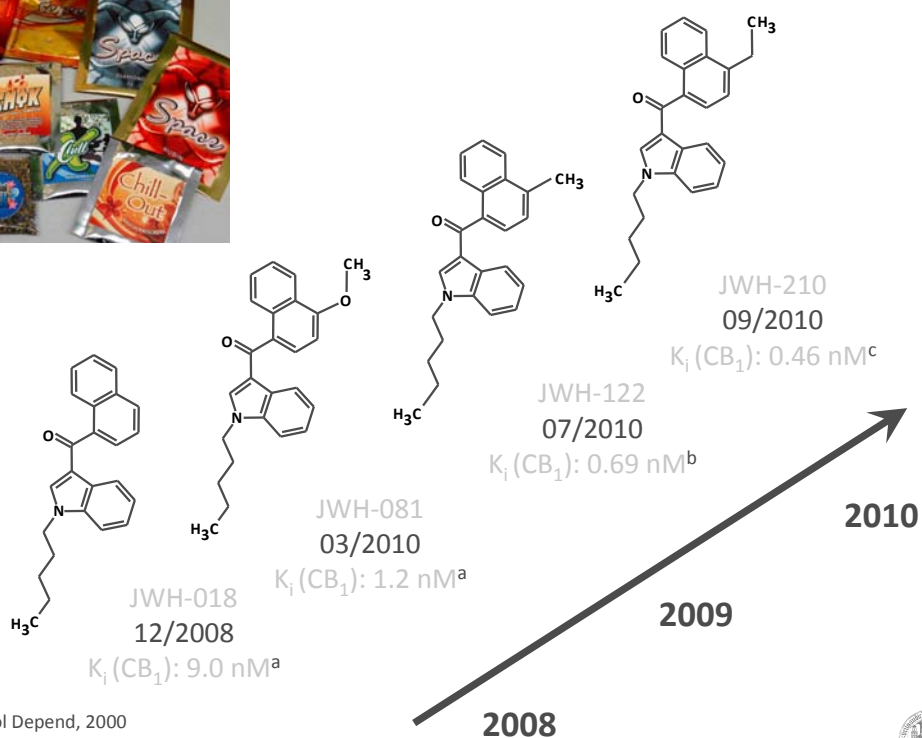
Wiley et al. 2011 Alc Drug Depend

Atwood et al. 2010 BJP (JWH-018)

Brents et al. 2011/2012 PLoS ONE/Biochem Pharm (Metabolites)



Receptor affinities



^aAung et al. Drug Alcohol Depend, 2000

^bHuffman et al. Bioorg Med Chem, 2003

^cHuffman et al. Curr Med Chem, 2005



Acute toxicity (clinical cases)

Most symptoms similar to cannabis intoxication

- Tachycardia
- Reddened eyes
- Anxiousness
- Mild sedation
- Hallucinations, acute psychosis
- Memory deficits

Symptoms not typically seen after cannabis intoxication

- Seizures
- Hypokalemia
- Hypertension
- Nausea/vomiting
- Coma
- Agitation, *violent behavior*

Schneir 2012 J Med Tox

Rosenbaum et al. 2012 J Med Chem

Forrester et al. 2011 J Add Dis

Hermanns-Clausen et al. 2012 Addiction



Fatalities...

Death cases published in the literature or under investigation

Clinical cases with resuscitation reported

Number of undetected cases?

Violent behavior under the influence



Long term toxicity

Genotoxic potential

Dose dependent cytotoxicity of CP-47,497-C8
(NG108-15 cell line: Tomiyama 2011)

AAI's: Dose dependent induction of DNA migration
(TR-146 and HepG2 cell lines: MUV, SCGE experiments: Koller et al. 2013)

Other problems:

- Risk of (persistent) psychosis
- Tolerance / addiction
- Alteration of the immune function via CB₂ receptors?



Conclusions

Methods of detection

- Regular update of methods (specialized labs)
- Urine screening remains challenging (metabolism)
- Careful interpretation of results (redistribution, wash-out)

Toxicity

- SC's show relatively high acute toxicity (cp. *Cannabis*)
- Genotoxic potential of some compounds



Acknowledgement

The EU-Commission (DG Justice), JUST/2011/DPIP/AG/3597



The German Federal Ministry of Health



The City of Frankfurt a. M., Drugs Department



Project partners:

- **Goethe University Frankfurt, Germany, Centre of Drug Research**
 - **Medical University Vienna, Austria, Institute of Cancer Research**
 - **University of Helsinki, Finland, Department of Forensic Medicine**
 - **Federal Criminal Police Office (BKA), Germany**
 - **Basis e.V. Frankfurt, Germany**
 - **University of Bern, Switzerland, Institute of Forensic Medicine**
 - **National Institute of Health and Welfare, Helsinki, Finland**
-

