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Synthetic cannabinoids: general considerations

Nik De Brabanter[a*], Koen Deventer[a], Veronique Stove[b], Peter Van Eenoo[a]

[a]*Ghent University (UGent), Department of Clinical Biology, Microbiology and Immunology, Doping Control Laboratory, Technologiepark 30, B-9052 Zwijnaarde, Belgium.*
[b]*Department of Laboratory Medicine, Ghent University Hospital, De Pintelaan 185, B-9000 Gent, Belgium.*

**Corresponding author: Nik De Brabanter, Ghent University (UGent), Department of Clinical Biology, Microbiology and Immunology, Doping Control Laboratory, Technologiepark 30, B-9052 Zwijnaarde, Belgium, Technologiepark 30, B-9052 Zwijnaarde, Belgium.*
Email: Nikdebrabanter@gmail.com

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Abstract

Around 2008 synthetic cannabinoids were found to be present in; and responsible for the psychoactive effects of herbal mixtures with names like 'Spice' or 'K2'. In response to the increased popularity of these products, (inter)national organizations and governments started banning these cannabimimetics gradually. However, the lack of an uniform and international regulation makes it hard to control this issue.
For the different types of synthetic cannabinoids the scientific knowledge in terms of pharmacokinetics and pharmacodynamics is limited. This also means that little is known on the health of users, both on short and long term.
In the last years effort has been made to make detection of these products possible in different biological matrices. However, since the number of cannabimimetic compounds on the market appears to grow every month, both scientist and legislators run after a moving target.

Keywords: Synthetic cannabinoids, Spice, legislation, detection methods, cannabinoid receptor.

INTRODUCTION

Starting from 2004, a new generation of psychoactive substances appeared on the market. These products, with brand names like 'Spice' or 'K2', are sold as herbal mixtures and are available in many European countries (1). Packed as 'natural herbal incense' or 'room odorizers', these products can be traded legally in headshops and online stores (2,3). After smoking these mixtures, users reported cannabis-like effects on internet forums. These effects were first explained in 2008 by the detection of synthetic cannabinoids like JWH-018 as active ingredient (4) , although not mentioned on the package.

Throughout the years, more of these products were identified as additives in these packages of herbal material. As a response to the rising popularity of these compounds, several countries started monitoring and even banning these products (5).

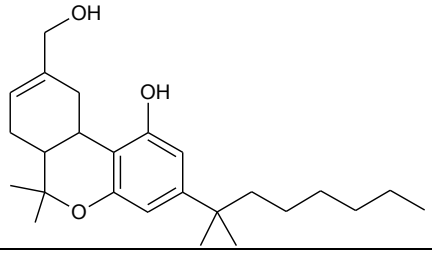
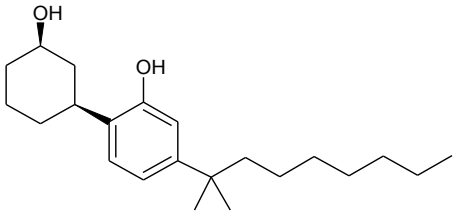
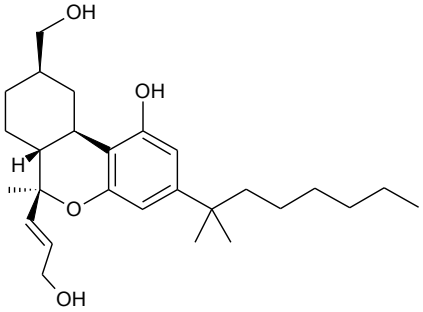
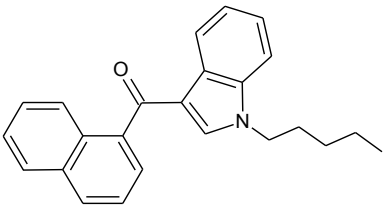
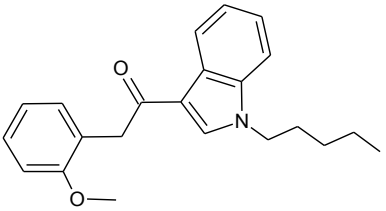
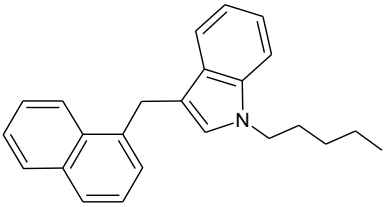
The search for compounds with THC-like properties in the human body, i.e. synthetic cannabinoid receptor agonists or briefly cannabimimetics, started in the pharmaceutical industry. In a way to separate the wanted pain-relieving effects from the unwanted psychotropic effects, several categories of products were synthesized and subjected to SAR (structure activity relationship) tests. The academic and/or pharmaceutical origin of these compounds is often reflected in the name of the product. In the best known class of JWH-compounds, these initials stands for the name of the organic chemistry professor John W. Huffman, who first synthesized these products in the 1990s. In a similar way AM (e.g. in AM-630) refers to professor Alexandros Makriyannis from Northeastern University and HU (e.g. from HU-210) to Hebrew University. Also, the pharmaceutical industry realized the potential value of these products, leading to the synthesis of the CP-family (e.g. CP-47,497) by Pfizer and the WIN-group (e.g. WIN 55,212-2) by the former Sterling Winthrop Pharmaceuticals.

In general, it are lipid soluble, non-polar molecules, containing 20 to 26 carbon atoms (6). Based upon this chemical structure, synthetic cannabinoids can be divided into different classes (Table 1) (1).

- Classical cannabinoids: structurally related to THC from *Cannabis sativa*.
- Non-classical cannabinoids: cyclohexylphenols or 3-arylcyclohexanols
- Hybrid cannabinoids: structural combinations of both classical and non-classical cannabinoids.
- Aminoalkylindoles;
  - Naphthoylindoles
  - Phenylacetylindoles
  - Naphthylmethylindoles
  - Benzoylindoles
- Eicosanoids: endocannabinoids and synthetic analogs
- Others: diarylpyrazoles, naphthoyl-pyrroles, etc.

It should be noted that, depending on the source, the classifications can vary. In the light of an internationally uniformed approach, referring to the (abovementioned) classification of a

leading institution as the United Nations Office on Drugs and Crime (UNODC) is recommended.

Classical cannabinoids e.g. THC, HU-210, AM906, …	
Non-classical cannabinoids e.g. CP-47,497-C8, CP-55,940, HU-308, …	
Hybrid cannabinoids e.g. AM-4030	
Naphthoylindoles e.g. JWH-018, JWH-073, JWH-122, JWH-200, …	
Phenylacetylindoles e.g. JWH-250, RCS-8, JWH-203, …	
Naphthylmethylindoles e.g. JWH-175, JWH-184, JWH-185, …	

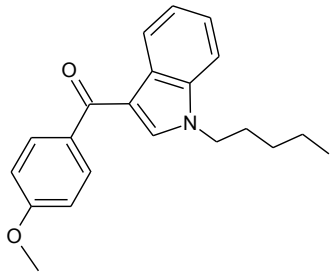
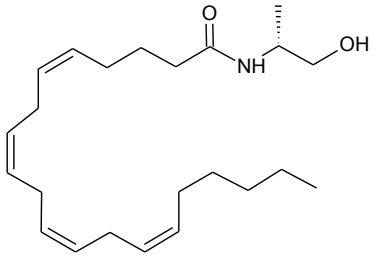
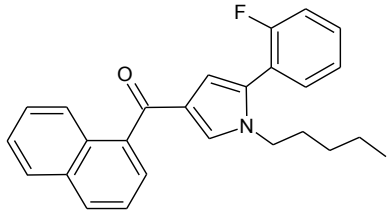
Benzoylindoles e.g. AM-630, AM-2233, RCS-4, …	
Eicosanoids e.g. anandamide, methanandamide, …	
Others e.g. JWH-307, CRA-13, …	

Table 1. Classification of synthetic cannabinoids according to the UNODC (1), with some typical examples. The underlined compound is illustrated in the second column.

ABUSE OF SYNTHETIC CANNABINOIDS

Packages of 'Spice' usually contain approximately 3g of herbal material and are often sold in head shops, gas stations or via internet shops. The price varies around €10/g, which is considered expensive compared to traditional cannabis (4). It is promised that the inhalation of the blends of psychoactive plants gives the user a similar experience as marijuana, only using legal alternatives. A survey in the US showed that 'Spice' products were primarily smoked, but also administration via vaporization, oral and rectal ingestion were reported (7).

Little is known on the exact composition and the properties of the used plants and in many cases the ingredients listed on the package do not cover the content either (8,9). The manufacturers of these blends make users believe the effects are caused by the mix of plant material used. However, research on the botanical material showed that most of the plant species do not have psychoactive properties and are therefore only used to dilute the added cannabimimetics (9). Moreover, the producers try to present their products as natural and safe in order to circumvent the marijuana policy of governments. The UNODC concluded that producers respond very fast to changes in legislations by making small modifications to the new products launched (1).

The success of this 'legal-highs' business is reflected in the increasing

number of web shops selling these products online. In 2009, the European Monitoring Centre for Drugs and Drugs addiction (EMCDDA) found 115 online shops offering psychoactive substances in Europe, in 48% of these 'Spice' products were offered. In a recent report mention is made of an increase to 314 online shops in 2011 and 690 in January 2012 (10). Moreover, an investigation via Google® performed by the Belgian Monitoring Centre for Drugs and Drug Addiction (BMCDDA), showed that all new products reported in 2010 were already offered for sale online even before their existence was picked up by the Belgium Early Warning System for Drugs (BEWSD) (11). Also the list of products with names like 'Spice gold', 'Yucatan Fire' and 'Lava Red' continued to rise (8). Furthermore, recent investigation in Poland demonstrated that many herbal blends contained more than one psychoactive ingredient (12).

A survey of the member states of the European Union by the EMCDDA showed that in 2009, 'Spice' products were identified in 21 out of the 30 countries. At that time no products containing synthetic cannabinoids were found in Belgium. In 2011 however, Belgian laboratories reported 11 synthetic cannabinoids to the European Early Warning System (EWS) and for the first time a complete laboratory capable of producing and packing synthetic cannabinoids was dismantled in Belgium. Similar facilities were reported in Ireland and the Netherlands and are the link between the producers – mainly located in China and India – and the customers in Europe. Since these substances can be produced cheaply, it is clear that these businesses are very lucrative (10). During the production process, the synthesized synthetic cannabinoids are distributed over the dried plant material. This is usually done by homogenization with cannabinoids in the crystalline form or by spraying the products dissolved in an organic solvent. However, recently also the starting materials are being sold as 'research chemicals' via online shops or traders (13).

The rate at which a specific product is spreading, is also noteworthy. In Europe, JWH-018 was first reported by Austria in December 2008. Only in the first year after this, eight more neighboring countries confirmed this finding, followed by ten more in the next months. Similar developments were reported for other compounds like JWH-073 (13 countries), CP47, 497 (10 countries), JWH-122 (14 countries), JWH-081 (10 countries) and AM-2201 (11 countries) (11). In 2011, already 23 new synthetic cannabimimetics were reported through the European Early Warning System (EWS), in 2012, another 30 followed. With a current total of 84 compounds (May 2013), the synthetic cannabinoid receptor agonists are, despite their recent introduction, already the largest drug family monitored by the EMCDDA (14).

An internet search learned that mostly young people - especially men - aged between 25 and 40 are using Spice-like products. The reasons are various; ranging from previous cannabis users looking for a substitute over people in search of legal drugs to experimental users

seeking sensation (8). In 2008, German authorities found a strong increase in the interest for these products after a period of biased media attention in which their use as legal cannabis substitutes was announced. Once the presence of synthetic compounds was demonstrated and some of these products were banned, the opposite trend was observed, leaving only the users looking for a cannabis substitute to avoid positive testing (1).

Even in sport drug testing, the first cannabimimetics were reported in the statistics of the World Anti-Doping Agency (WADA). However, with only 3 positive cases in 2011, the number remains small compared to the traditional THC abuse in sport competitions (n = 442 in 2011) (15).

LEGISLATION

Since synthetic cannabinoids are currently not controlled under the UN Drug control conventions, the legal status of these compounds depends on the drug laws of individual countries (1).

In Europe, the first actions by governments were taken in 2009. The first discovered compounds JWH-018, HU-210 and CP47,479 and its homologues were included in national drug laws in Austria, Germany, France, Luxembourg, Sweden, Estonia, Poland, Hungary and the United Kingdom. A recent report on the evolution of the situation in Poland over the last few years, showed that both the compounds and the way of trading changed in response to the successive actions of the Polish government (12). As mentioned earlier, it is sufficient to make a small change in the drug structure to stay one step ahead of the legislator. Therefore, the UK and Ireland started using generic definitions to include products which will appear in the future (8). Recently, other European countries also adopted this strategy in substitution for the earlier used approach of individual listing of already identified synthetic cannabinoids (10).

In Belgium the first legislative actions were taken in 2011, by adding the first seven compounds to the list of prohibited psychotropic substances. In 2013 six more cannabimimetics were listed (Table 2). Unfortunately, to this day, no generic definitions have come into force.

In the United States, the Synthetic Drug Abuse Prevention Act of 2012 placed cannabimimetic agents in Schedule I, making manufacturing, distributing, or possessing these products illegal. Besides this federal law, several states and even individual cities have taken additional measures to control ‘Spice’ abuse (16).

Since, similar to classical cannabis, the synthetic analogues are predominantly smoked, it is not inconceivable that passive inhalation of the smoke can result in positive testing. Once this was observed for cannabis (17), threshold concentrations were installed to distinguish active from passive use (18). However, up to now, this possibility has not been studied for the cannabimimetics currently flooding the market.

In general legislation, both within and outside Europe, is too diverse and therefore not efficient to tackle international issues as ‘legal highs’.

KB 2011-09-26/16, art. 1,011 Effective date: 23/10/2011	KB 2013-03-20/08, art. 1,012 Effective date: 22/04/2013
JWH-018	AM-694
JWH-073	AM-2233
JWH-250	WIN 48,098
JWH-398	JWH-307
CP-47,497	A-796,260
HU-210	XLR-11 (5F-UR144)
JWH-210	

Table 2. Synthetic cannabinoids present on the list of prohibited psychotropic substances in Belgium.

PHARMACOKINETICS

Contrary to the classical THC for which the pharmacokinetics have been investigated (19,20), no such data are available for the synthetic analogues.

As described in several reports on the experiences of 'Spice' users, the effect is quickly noticeable after smoking a few grams of herbal material (2,6,16). These observations are supported by a recent study on the quantification of JWH-018 in blood after smoking the incense 'Smoke' (21); the maximum concentrations were found 5 min post-smoking. This shows that after inhalation, the absorption via the lungs and the distribution over organs like the brain takes place in a few minutes (1). It was found that the measured maximum blood concentrations of JWH-018 were already decimated after 3h and the parent compound was detectable until 48h after administration (21).

Investigation of the metabolisation of cannabimimetics is not as straightforward as for pharmaceutical approved agents. Since there are little or no pharmacological data available for these compounds, human administration in order to perform excretion studies is ethically questionable. Therefore, most studies use models to reveal the metabolic pathways in the human body. In one of the most common approaches human liver microsomes are used to investigate the metabolisation *in vitro* (22–25), in the search for a more complete model with higher complexity also *in vivo* mice experiments are performed (26). In some cases, human urine samples are available from caught users (27–29) or conducted self-experiments (30).

In general, these compounds are excessively metabolized in the human body. In all metabolic studies on compounds of the aminoalkylindole family described up to now a similar series of modifications were found: single or multiple hydroxylations, carboxylation, dehydrogenation, dealkylation and dihydrodiol formation. From the data obtained using *in vivo* models or positive urine samples, is was found that these metabolites are mainly excreted as

glucuronide and/or sulphate conjugates in urine (26). Mainly the monohydroxylated (31) and carboxylated (28) metabolites are found in the highest quantities in urine.

PHARMACODYNAMICS

Although the effects of *Cannabis sativa* and derivates are known for centuries, it was only in the last twenty years that the interactions in the human body were revealed (6,32).

Today, two cannabinoid receptors are described in the human body. Both CB1 and CB2 are G-protein coupled receptors with an important function in intercellular signaling. The CB1 receptor is distributed in the brain and the central nervous system, mainly expressed presynaptically, and decreases the release of neurotransmitters like dopamine (33). Activation of the CB1 receptor is responsible for the psychotropic effects assigned to cannabis use.

CB2 receptors are located in immune cells and interfere in the regulation of the inflammatory process (19). Therefore in the medical field, research has focused on receptor agonists selective for this CB2 receptor aiming for the therapeutic effects and hereby avoiding the psychotropic effects induced by the interaction with the CB1 receptor.

Next to endocannabinoids, plant derived and other exogenous cannabinoids act as agonists of both receptors with varying affinity. Classical cannabinoids like THC have comparable affinity for both receptors, about 40 nM, without a major selectivity for a particular receptor (34). As shown in Table 3, this is different for synthetic cannabinoids. The affinity of the most prevalent cannabimimetic compounds is significantly higher, especially towards the CB1 receptor. With this in mind, it can be expected that compounds with lower potency (i.e. lower affinity for the CB1 receptor) than classical THC will not be used in 'Spice'-like products. Nevertheless JWH-015 was recently detected in a herbal blend in Latvia (1).

When using data on receptor affinities, one should be careful when using exact numbers. Indeed, depending on the used experimental set-up, variation in the values is possible (34). In short, the receptor affinity (K_i) is determined as the ability of the given compound to displace a potent radio labeled cannabinoid (usually tritiated CP-55,940 or tritiated WIN-55,512-2) from their binding sites (35). For a potent cannabinoid, low concentrations will be sufficient to achieve this. Since this concentration (IC50) is proportional to the receptor affinity K_i, the more potent the cannabinoid, the lower K_i (Table 3).

HEALTH RISKS

Although there are case reports describing the effects experienced immediately after the use of 'Spice' (2,3,16), little or no information is available on the long term effects or the consequences of regular use.

In 2009, Zimmermann et al. reported on a patient who showed symptoms of a physical withdrawal syndrome after using 'Spice Gold' on a

Compound	Ki - CB1 (nM)	Ki - CB2 (nM)
HU-210	0.06 (34)	0.52 (34)
JWH-122	0.7 (36)	1.2 (36)
JWH-073	8.9 (35,37)	27 (35), 38 (37)
JWH-018	9 (35)	2.9 (35)
CP47,497	9.54 (38)	
JWH-250	11(39)	33(39)
Δ9-THC	39.5 (34)	40 (34)
JWH-015	383 (34), 164(35)	13.8 (34,35)

Table 3. Receptro affinities for both cannabinoid receptors for some common cannabimimetics.

daily basis for about 8 months. While the patient initially used only 1g of product every day, the decreasing effect experienced made him increase the dose up to 3g daily. Both physical (sweating, tremor, insomnia, nausea, etc.) as psychological (depression, desperation, desire for 'Spice Gold') effects were observed the first days of treatment in hospital (40). In another paper, psychosis was diagnosed in ten patients after smoking herbal blends containing synthetic cannabinoids, which lasted months after the final use (41).

Recently, compound specific data related to harm assessment have been included in the European Database on New Drugs (EDND). For two compounds, chronic physical damage after use is mentioned. It is related to learning difficulties and cognitive ailment for HU-210 and JWH-018, respectively. Moreover, for both compounds physical dependence (withdrawal symptoms) was reported, together with psychological dependence for JWH-018 and JWH-122 (11).

Predicting the possible effects of a particular herbal blend, is almost impossible. It was shown that the content of these packages varies significantly and is often not in accordance with the indications on the package. Toxicological data on the used plant material are not available, and then again, mostly the indications on the packages with regards to the herbal material are not reliable (9). Concerning the added synthetic cannabinoids, it was shown that concentrations can vary (5) and that some blends may contain two or more active compounds (42). Although research showed that the used chemicals are of high purity (43), the presence of impurities with unknown toxicity cannot be ruled out. In that way, it is not possible to estimate the impact when smoking a few grams of a given mixture. In general, the observed effects are very diverse and highly dependent on the type of herbal blend or synthetic cannabinoid(s) used. Most described psychoactive effects are: alterations in mood (from euphoria to anxiety) (5), hallucinations, agitated behavior and hyperreflexia (16). Medical investigation showed symptoms like increased pulse rates (6) and blood

pressure, flushed skin, dilated pupils and nausea (16). It was reported that the major psychotropic and physical effects disappear after 6h to 8h (5,6).

Together with the fact that these cannabimimetics have stronger affinities for the cannabinoid receptors compared to THC, it is not unlikely that overdosing would lead to life-threatening intoxications. This is confirmed in case studies (44) and reflected in the increasing statistics of Poison Control Centers in the US: in 2011 there were reports of over 4000 synthetic marijuana exposures in a period of 8 months, which is an increase of 52% compared to 2010 (45).

DETECTION OF SYNTHETIC CANNABINOIDS ABUSE

In the past, several screening procedures – both via immunological and chromatographic techniques – have been developed to screen for the use of products from *Cannabis sativa* in different matrices (18,46–49). The abuse of the growing group of cannabimimetics, synthesized over the last years, however, cannot be detected with these existing methods.

For the identification of spiked substances in the herbal material in particular, a more or less standard strategy is used. The herbal material is extracted and subsequently analyzed by means of a chromatographic technique mostly combined with mass spectrometric detection (50). Next, the outcome is compared with databases containing the already known synthetic cannabinoids (51). If it turns out to be an unknown compound, the structure is elucidated by using high resolution mass spectrometry (HRMS) or NMR (Nuclear Magnetic Resonance) technology (52–54).

When it comes to detection in the human body, different approaches can be used depending on the type of biological sample available. In serum or whole blood, both the unchanged target compound and its metabolites are present and can be extracted and analyzed by means of liquid chromatography (LC) (55,56). The methods developed up till now mostly target the parent compounds, since this eliminates the need for the time-consuming search for metabolites and allows the quick update of the method after the release of a new compound in the future (57,58). For oral fluid testing, detection of this parent compound is possible, even via direct injection on the LC system (59). However, it should be noted that detection in the latter matrix is limited to a few hours after consumption (60).

If urine is the matrix of choice, knowledge on the metabolisation is essential, since no unchanged parent compound is found to be excreted. To detect these metabolites, an enzymatic hydrolysis is usually performed, followed by an extraction and analysis by means of liquid chromatography coupled to mass spectrometry (LC-MS) (61). For JWH-018 it was found that monohydroxylated (31) and carboxy metabolites (28,62) are excreted in the highest concentrations, which makes these the metabolites of choice to implement in routine screening methods. Similar results are found for other indole-based cannabimimetics

(26,29,63,64). Based upon these findings, chromatographic methods are developed and validated to screen for synthetic cannabinoid metabolites in urine (65,66). Also commercial tests for synthetic cannabinoids became available, promising a detection window of 72 h after a single use. Peer-reviewed data on detection times - although rare - indicate similar ranges (30,67). No information is available on the accumulation in the body for chronic users. However, in those cases detection in urine would be possible up to 3 weeks (67).

Immunochemical-based detection methods have the advantage of being cheaper and faster than the chromatographic procedures referred to above, but the development and implementation was long in coming. Indeed, developing such immunoassays is a challenging task given the great structural variety between the compounds of the cannabimimetics family. Only recently, the first screening method, using enzyme linked immunosorbent assays (ELISA) for the detection of metabolites of the naphthoylindole group in urine, has been described in literature (68). Gradually, also commercial kits for high-throughput screening of synthetic cannabinoids have become available (69,70). For any positive outcome however, a confirmatory analysis by means of the more selective chromatographic techniques remains essential.

It should be noted that correct identification of these products remains a difficult task since the availability of reference material is lagging behind on the rapid release of new products on the market (6). The latter makes it also difficult to keep screening methods up to date, since the existing methods are not able to detect non-target (i.e. currently unknown) compounds. To close this gap, an open screening approach whereby the method is capable of detecting a class of cannabinoids in a non-targeted way could be a solution.

PERSPECTIVES - CONCLUSIONS

Despite the increasing number of actions taken by governments and other (inter)national institutions, the 'Spice' issue is still expanding. The list of synthetic cannabinoids detected continues to grow and the statistics on hospitalizations due to the use of these herbal blends are following the same trend. Although effective interventions of the authorities are necessary to tackle these problems, strict legislation also has a downside. The total ban on these products takes away the opportunity to investigate the therapeutic properties. Taking into account the successful use of plant derived cannabis in medicine, there is demand to provide the possibility to do research that leads towards the medicinal use of these synthetic analogues (71).

It is clear that further research in this field is necessary. When it comes to pharmacodynamics, so far only the properties of the parent compound are investigated. However, recent data show that also the formed metabolites remain active in the human body by binding to both cannabinoid receptors (72).

For routine testing, methods should

be developed to improve detection in different biological matrices. Given the rapidly growing number of products appearing on the market, an open-screening approach could be a big step forward. When routine screening becomes more common, there will be a need for uniform regulations taking also into account the problem of passive inhalation, as known for THC smoke.

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