The Implications of the Production of Synthetic Cannabinoids Receptor Agonists as they Relate to Forensic Toxicology

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Implications of SCRA Production in Forensic Toxicology

Novel psychoactive substances [NPS] are drugs that mimic existing drugs, are newly developed or discovered in existing products, and have psychoactive effects in humans. These are further developed into six primary "effect" groups including opioids, stimulants, sedatives/hypnotics, classic hallucinogens, dissociatives, and synthetic cannabinoid receptor agonists (United Nations Office on Drugs and Crime [UNODC], 2022a). This paper will explore the history, distribution, and development of synthetic cannabinoid receptor agonists, as well as their impact on the medicolegal and criminal justice communities within the context of forensic toxicology.

Background

Cannabis is a botanic product, the oldest known usage of which is dated back to 5,000 years ago, found in what is now known as Romania (Bridgeman et al., 2017). The earliest documented evidence of cannabis used in a medicinal capacity is dated around 400 CE, when the psychoactive component Δ^6-tetrahydrocannabinol (Δ^6-THC) was found in ashes at an ancient Jewish shrine (Bridgeman et al., 2017; Fox, 2020). The marijuana plant was also used medicinally in the United States, the first description of this found in the United States Pharmacopeia in 1850 but was dropped from the Pharmacopeia in 1942 (Bridgeman et al., 2017).

Cannabis use and sale was first federally restricted in 1937 with the advent of the Marihuana Tax Act, and legal penalties for possession were increased in 1951 with the enactment of the Boggs Control Act and increased again in 1956 with the passage of the Narcotics Control Act. Federal prohibition occurred with the Controlled Substances Act of 1970. The aforementioned legislative actions also created limitations on academic research of the drug (Bridgeman et al., 2017). These restrictions may have also contributed to the market for the

production of synthetic cannabinoids. California was the first state to legalize medicinal cannabis use in 1996, and since that time, 44 other states have decriminalized some use of cannabis, either recreationally, medicinally, or some combination of the two (Bridgeman et al., 2017).

Synthetic cannabinoids receptor agonists, or simply, synthetic cannabinoids, were first detected in 2008 as an additive to herbal smoking blends. These blends were produced and sold on the Internet and in specialized shops, also known as "head shops" (UNODC, 2022a). Prior to 2008, "herbal highs" were available from these venues, but had minor psychoactive effects on the consumer until 2004, when the chemical composition changed to include synthetic cannabinoids, the active psychoactive compound (Laboratory and Scientific Section, UNODC, 2020).

In 1988, a synthetic analogue of THC, also termed a "classical cannabinoid," HU-210, was first created in Israel, containing a potency of over 100 times that of THC, and can still be found in synthetic cannabinoids globally. Simultaneously, Pfizer pharmaceutical company developed another group of synthetic cannabinoids, termed non-classical, and were developed as a potential analgesic. This class includes cyclohexylphenols or "CP compounds." Classical and non-classical cannabinoids are structurally similar, although other dissimilar varieties unrelated to THC are available at the same venues, including aminoalkylindoles, phenylacetylindoles, and benzoylindoles, the first of which is the most prevalent on the current market in the form of JWH-018 (UNODC, 2022b). Other chemical classifications include hybrid cannabinoids (e.g. $C_{27}H_{42}O_4$), aminoalkylindoles (e.g. $C_{26}H_{26}N_2O$), aminoalkylindazoles (e.g. $C_{22}H_{25}NO$), eicosanoids (e.g. $C_{26}H_{39}NO_2$), and others (e.g. $C_{26}H_{24}O_2$) (Laboratory and Scientific Section, UNODC, 2020).

The most recent synthetic cannabinoids demonstrate an even broader structural diversity, presumably to avoid the legislative measures taken to control these substances. These synthetic substances are generally added to herbal blends by soaking or spraying but can also be found in crystal form and are sprinkled in the blend, producing an inhomogeneous mixture. The pure form of synthetic cannabinoids is found in the form of white to grey or yellow to brown fine, lipophilic, crystalline powders. These compounds are soluble in non-polar to medium polarity solvents including methanol, acetonitrile, and acetone, although they demonstrate low solubility in water. The progression of the chemical changes in synthetic cannabinoids may include changes to the tail, such as length of the alkyl chain or a halide substitution; changes to the linked group, such as adamantyl or methoxy phenyl; changes to the link, such as esters and amides; or changes to the core, such as indazole or pyrazole (Laboratory and Scientific Section, UNODC, p.2, 2020).

The branding of the packages tends to be of professional quality and are sold under names such as “Spice Gold,” “Bliss,” and “Black Mamba.” These are generally smoked but may also be ingested, and typically contain 0.5-3 grams of herbal blend dosed with one or more synthetic cannabinoids (UNODC, 2022b). In the early years, usage was restricted to a small community of experimental users, but in 2008 and with the aid of the Internet, achieved popularity in European countries as “legal alternatives” to cannabis or “research chemicals.” Since that time, many new synthetic cannabinoids have been detected in the market in variable amounts, quantified in milligrams to kilograms (Laboratory and Scientific Section, UNODC, p.1, 2020).

Discussion

Pharmacodynamics and Pharmacokinetics

Due to the chemical structure of these synthetic cannabinoids, they can bind to the cannabinoid receptors found in the body (Bridgeman et al., p. 181, 2017). These cannabinoid receptors, CB_1 and CB_2, are found in human cells in the nervous system, internal organs, connective tissues, glands, and immune system, which are all part of the endocannabinoid (eCB) system (Bridgeman et al., p. 181, 2017; Laboratory and Scientific Section, UNODC, p.5, 2020). Generally, CB_1 receptor is found in the brain and nervous system, while CB_2 is found in the spleen and is believed to mitigate some immunological processes in vivo (Laboratory and Scientific Section, UNODC, p.5, 2020).

The eCB system functions in homeostasis, generally characterized as "eat, sleep, relax, forget, and protect" (Bridgeman et al., p. 180, 2017). eCBs work both antagonistically and symbiotically in the body, functioning in both the pathology of some disorders, while serving in a prophylactic manner against other diseases and conditions (Bridgeman et al., 2017). A deficiency in naturally occurring eCBs has been linked to the pathogenesis of depression, schizophrenia, Huntington's and Parkinson's disease, anorexia, and infantile failure to thrive (Bridgeman et al., 2017).

Naturally occurring cannabinoids, apart from endocannabinoids, are restricted to the general chemical metabolites of cannabis, Δ^9-tetrahydrocannabinol, the only psychoactive compound of cannabis, and cannabidiol. However, the broader chemical composition of synthetic cannabinoids grants more potential affinity for the binding sites of either of CB_1 and CB_2, or both (Laboratory and Scientific Section, UNODC, p.5, 2020). The pharmacological

mechanism in synthetic cannabinoids that produces psychoactive effects is the activation of the CB_1 receptor (UNODC, 2022a).

Although most herbal blends do not contain cannabis, synthetic cannabinoids produce similar psychotropic effects including euphoria, enhanced sensory perception, and antinociception, the latter of which induces an analgesic benefit by triggering a pain-relieving response in the body. Synthetic blends may also share the negative effects of cannabis including tachycardia or tachyarrhythmia, psychological disorders, and potential carcinogenic effects, although substance-specific effects are difficult to ascertain, as synthetic cannabis is often laced with several different chemicals in different concentrations (UNODC, 2022a; Ameri, 1999). For this reason, data on human toxicity is limited although a study by Ludger et al. (2012) demonstrated increasing suicide rates with preceding use of synthetic cannabinoids (UNODC, 2022a).

Analytical Methodology and Instrumentation

Due to the large and varied number of chemical compounds in synthetic cannabinoids, presumptive testing may not be ideal, although microscopy may be able to demonstrate the presence of crystals in the sample. A more accurate, inexpensive, and rapid approach may be to utilize thin layer chromatography (TLC), which may also reduce the opportunity for contamination due to the single-use plates and products. Analysis of constantly evolving, uncontrolled substances is complex, and must involve identification of the chemical structure of the analyte (qualification), followed by quantification of the chemical components. Considerations for analysis include sampling techniques, homogeneity of the sample, extraction of the substance from any source material (e.g., herbal blend, paper), sensitivity of the

methodology used, as well as selection of appropriate reference material (Laboratory and Scientific Section, UNODC, pp.47-48, 2020).

Gas chromatography-mass spectrometry (GC-MS) is recognized as the gold standard for qualitative analysis for substances of this nature, although thin layer chromatography (TLC), ion mass spectrometry (IMS), or liquid-chromatography-mass spectrometry (LC-MS) could also be used depending on the available resources to the analyst. GC-MS offers precise chromatographic resolution and the separation for identification of active ingredients by their electron impact-mass spectra (EI-MS). Limitations for this methodology may include difficulty in analysis of similar isomers. For this reason, a secondary analysis of the substance using gas chromatography-infra-red detection (GC-IRD) or nuclear magnetic resonance (NMR) spectroscopy to accurately identify similar isomers may be necessary (Laboratory and Scientific Section, UNODC, pp.49-50, 2020).

Sampling techniques such as a Bayesian model which uses probability to measure uncertainty within the model, including uncertainty in both input and output, may be the most useful technique for selecting a homogenous sample for testing, although the use of this method is more accurate with smaller samples. For larger quantities of a heterogenous nature, a hypergeometric approach may be preferrable (Laboratory and Scientific Section, UNODC, p. 50, 2020).

Both qualitative and quantitative analysis will require extraction using medium-polar or non-polar solvents which are both readily available in most drug testing laboratories. Homogenization of multiple aliquots of the sample is imperative for accurate and precise analysis of the chemical components in the sample, as well as reproducibility in testing (Laboratory and Scientific Section, UNODC, p. 50, 2020).

Quality Control and Quality Assurance

Validation of any methodology using standards of known values and at different levels should be employed. Validation data including accuracy and linearity should be kept for the purpose of substantiating any results using the methodology. Calibration verification should be completed at regular intervals and after any major maintenance on the instrument. A procedure for the testing methodology should also be made available for all testing personnel. Selection of appropriate control materials and standards is a laboratory imperative, specifically when working with seized materials.

The Scientific Working Group for the Analysis of Seized Drugs [SWGDRUG] set requirements for at least two techniques of the analysis of seized drug material to ensure both the structural data and selectivity of chemical characteristics to ensure reliability in results (SWGDRUG, p. 16, 2019). The organization also provides instruction for creating an analytical scheme utilizing increasing levels of selectivity through general or class information, chemical or physical characteristics, and most sensitive, selectivity through structural information (SWGDRUG, p. 14, 2019).

Due to the variability in chemical composition of synthetic cannabinoids, it would not be unexpected to find an unknown compound in a seized sample. For this reason, a reference sample could be synthesized from the deduction of the chemical components in the sample. For known compounds, control materials may not be readily available and may have to be ordered or created. For this reason, a standard database for new and undiscovered compounds should be created. (Laboratory and Scientific Section, UNODC, p. 57, 2020).

Contamination of the specimen prior to acquisition may be a serious issue due to the method of mass production of synthetic cannabis, which can involve inoculation of the substance

in large cement mixers and lack of standardization requirements by producers. To alleviate this liquid chromatography-tandem mass spectrometry (LC-MS/MS) may be employed (Laboratory and Scientific Section, UNODC, p. 77, 2020).

Implications for Criminal Justice

In order to provide a scientifically supported conclusion relevant to the adversarial system, laboratories must be sure to utilize the most reliable and repeatable analytical methodology. Selection of instrumentation for testing of synthetic cannabis can be costly and the time invested in proper validation of the methodology may mean longer wait times and lengthy backlogs. However, of greater concern is the ambiguous legislation provided for the control of unknown substances. A possible solution would be to invest in peer-reviewed studies to decrease the likelihood of finding completely unknown substances when testing.

Another consideration may be the establishment of standards specifically for the detection and analysis of NPSs. Expert witnesses should have some advanced background in chemistry to be able to provide unbiased and substantial testimony during criminal proceedings, which would require further research on NPSs. Finally, a presumptive testing kit for officers in the field should be developed to assist in initial testing of the suspect and to eliminate guesswork from laboratory testing.

Potential Impact on Forensic Toxicology

One of the biggest challenges facing the field of forensics is the lack of standardization of analysis, testing, and sampling procedures in laboratories. SWGDRUG recommendations should be followed to ensure that results from any testing laboratory are consistent and reliable. The complexity of the chemical components in synthetic cannabis may require more expensive equipment and higher-level education and training of the analysts. Government grants for the

study and analysis of NPSs should be applied for by these laboratories to ease costs. The difficulty in specifying psychoactive metabolites that bind with CB_1 receptors, the forensically relevant metabolite of synthetic cannabis, is furthered by the speed at which new compounds are created and distributed. This could be lessened by employing “out of the box” techniques of investigation such as utilizing the dark web market to identify new substances as they emerge or testing existing products available at specialty stores. Finally, a database should be established to share findings of new and untested synthetic cannabinoids.

Conclusion

The emergence of synthetic cannabinoids is a complex issue for forensic laboratories, with potential for serious implications for the judicial system. Consumers rarely know the danger in intaking substances that can be mass produced and distributed without regular monitoring and regulation by the Food and Drug Administration [FDA]. Prior to FDA clearance or approval, more research should be done on levels of human toxicity, chemical components in these substances, and long-term effects with usage. Government-sourced funding should be allocated for further studies on these substances to alleviate the complexity of this issue. One final possible solution is the decriminalization or legalization of cannabis throughout the states, as it has been shown to reduce cases of NPS toxicity by nearly 60% in states where marijuana is legal (LaMotte, 2022).

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