




Special Guest Editor

Complexity of Translating Analytics to Recent Cannabis Use and Impairment

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Abstract

While current analytical methodologies can readily identify cannabis use, definitively establishing recent use within the impairment window has proven to be far more complex, requiring a new approach. Recent studies have shown no direct relationship between impairment and Δ^9 -tetra-hydrocannabinol (Δ^9 -THC) concentrations in blood or saliva, making legal “per se” Δ^9 -THC limits scientifically unjustified. Current methods that focus on Δ^9 -THC and/or metabolite concentrations in blood, saliva, urine, or exhaled breath can lead to false-positive results for recent use due to the persistence of Δ^9 -THC well outside of the typical 3–4 h window of potential impairment following cannabis inhalation. There is also the issue of impairment due to other intoxicating substances—just because a subject exhibits signs of impairment and cannabis use is detected does not rule out the involvement of other drugs. Compounding the matter is the increasing popularity of hemp-derived cannabidiol (CBD) products following passage of the 2018 Farm Bill, which legalized industrial hemp in the United States. Many of these products contain varying levels of Δ^9 -THC, which can lead to false-positive tests for cannabis use. Furthermore, hemp-derived CBD is used to synthesize Δ^8 -THC, which possesses psychoactive properties similar to Δ^9 -THC and is surrounded by legal controversy. For accuracy, analytical methods must be able to distinguish the various THC isomers, which have identical masses and exhibit immunological cross-reactivity. A new testing approach has been developed based on exhaled breath and blood sampling that incorporates kinetic changes and the presence of key cannabinoids to detect recent cannabis use within the impairment window without the false-positive results seen with other methods. The complexity of determining recent cannabis use that may lead to impairment demands such a comprehensive method so that irresponsible users can be accurately detected without falsely accusing responsible users who may unjustly suffer harsh, life-changing consequences.

In recent years, the legalization of medicinal and recreational cannabis has been steadily expanding throughout the United States and worldwide. As of November 2023, a total of 24 US states plus the District of Columbia (D.C.) have legalized cannabis for adult recreational use, with medicinal use being approved in 37 states plus Washington, D.C. (1). The increasing acceptance of cannabis has also led to an increasing need for an accurate and objective means of detecting recent cannabis use that can potentially lead to impairment, a need that remains unmet. Throughout this review, the term impairment refers to the compromised ability to perform safety-sensitive tasks such as driving or operating heavy machinery. Current testing methodologies may lead to false-positive results for recent cannabis use associated with impairment because they focus on the main psychoactive cannabinoid found in the cannabis plant, Δ^9 -tetrahydrocannabinol (Δ^9 -THC), detectable concentrations of which can persist in common test sample matrixes such as blood (2), saliva (3–5), exhaled breath (6, 7), and urine (8, 9) well beyond the typical 3 h impairment window following inhalation (10–12) or 8 h after ingestion of cannabis edibles (3, 12). Recent research has shown, however, that there is no direct relationship between impairment and specific concentrations of Δ^9 -THC in blood or

saliva (13–21), which makes the use of legal “per se” limits for Δ^9 -THC blood concentrations, concentrations above which test subjects are considered to be legally impaired, scientifically unsupported at the present time. As used in this review, the impairment window refers to the timeframe during which individuals are potentially impaired following the use of cannabis. It has been estimated that approximately only half of cannabis users are impaired with respect to driving performance soon after smoking (22).

Compounding the issue of assessing potential impairment associated with recent cannabis use is the potential involvement of other intoxicating drugs. In a study of 921 driver fatalities in Ontario, Canada that occurred from 2016–2018, a total of 495 victims tested positive for alcohol, cannabis (Δ^9 -THC), and/or other psychoactive drugs. Of those 495, 18.2% were positive for Δ^9 -THC alone, 27.7% were positive for drugs other than Δ^9 -THC and alcohol, and 16.6% tested positive for Δ^9 -THC and at least one drug other than alcohol (23). A Norwegian study of 10 520 drivers involved in crashes from 2013–2020 found that 2133 tested positive for Δ^9 -THC above the legal limit, with 84% (1799) of those drivers also testing positive for alcohol or other drugs, including sedatives, stimulants, and opioids, above their respective legal limits

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(24). In this study, THC alone was found in only 15.7% of all THC-positive driving under the influence (DUI) drivers, which would represent only 3.2% of all DUI drivers (24), which is in agreement with a 2007 Australian study (25). In a recently published Colorado study of drugged driving prevalence, Wood reported that THC alone was found in 11.0% of all DUI drivers (26). While these studies do not prove impairment at the time of the crash, they show that it is common for Δ^9 -THC to be found in combination with other drugs, and thus it is crucial that analytical methods incorporate other potentially impairing substances when assessing suspected impairment due to recent cannabis use.

In cases of driver impairment, there is abundant evidence to show that cannabis use slightly but significantly increases crash risk (27–29), but this is also true of other psychoactive drugs (30, 31). Culpability studies focusing on severe crashes with injuries and fatalities have also shown that Δ^9 -THC significantly increases the risk of crashing (32–35). To put these risks in perspective, though, a 2016 report issued by the National Highway Traffic Safety Administration (NHTSA) found that compared to alcohol, neither Δ^9 -THC nor other psychoactive drugs were significant contributors to crash risk after controlling for alcohol use and other factors (36). This finding is supported by a recent systematic review and meta-analysis that failed to uncover any credible evidence that cannabis significantly adds to the effect of alcohol use on the risk of crashing (37).

In a more recent study, Myran et al. (38) presented evidence suggesting that cannabis legalization in Canada has led to large increases in cannabis-involved traffic injury emergency room (ER) visits compared to a time period prior to legalization, in agreement with a review showing increases in cannabis-related hospitalizations and ER visits in the United States and Canada, and an increase in traffic fatalities in some US states following legalization and commercialization of recreational cannabis (39). While there is also some data suggesting that recreational cannabis legalization is associated with a decrease in serious and fatal motor vehicle collisions, at least in the state of Washington (40), it is becoming more apparent with each passing year that the expanding legalization of recreational cannabis is leading to an overall increase in cannabis-involved crashes, a finding supported by one of the latest reviews of meta-analyses of the risks and benefits associated with cannabis use, which found convincing evidence of increased car crash risk (41).

In recent years, the exploding popularity of hemp-derived CBD products in the United States has added yet another layer of difficulty to recent-use cannabis testing. Passage of the Agriculture Improvement Act of 2018, commonly referred to as the Farm Bill, legalized the US production of industrial hemp, which is a major source of the non-psychoactive cannabinoid CBD. At issue is the synthetic production of Δ^8 -tetrahydrocannabinol (Δ^8 -THC) through the acid-catalyzed conversion of CBD (42, 43). Δ^8 -THC is a positional isomer of Δ^9 -THC, differing only in the location of a carbon–carbon double bond (see Figure 1), and it possesses psychoactive properties similar to Δ^9 -THC (44–46), although it may be somewhat less potent (47, 48). While Δ^8 -THC occurs naturally as a degradation product of Δ^9 -THC (49, 50), it represents less than 1% of total THC (51). Products containing Δ^8 -THC in a variety of different formulations for both oral consumption, e.g., gummies, and inhalation, e.g., vaporization pens and cartridges, have become widely available throughout the United States wherever CBD and hemp products are sold (52), especially in areas where the nonmedical adult use of cannabis is still prohibited. Because they are derived from hemp, these products are perceived as being fully legal; however, Δ^8 -THC is being

synthetically produced, and the U.S. Drug Enforcement Administration (DEA) considers all tetrahydrocannabinols to be Schedule I controlled substances regardless of their Δ^9 -THC concentration if they are synthetically derived from non-cannabis materials (53), and the acid-catalyzed conversion reaction does involve some non-cannabis materials. Further clouding the issue of Δ^8 -THC legality is a May 2022 ruling from the U.S. Court of Appeals for the Ninth Circuit, which upheld Δ^8 -THC as a legal, hemp-derived product under the 2018 Farm Bill, and the fact that 18 US states, notably including California, have already banned hemp-derived Δ^8 -THC, as of September 2023 (54). The relative lack of regulation compared to traditional cannabis products containing Δ^9 -THC has led to legal and safety concerns (52, 55, 56). While certain provisions of the current Farm Bill were set to expire beginning September 30, 2023, the 5-year renewal bill, which is stalled in Congress as of this writing, is unlikely to clarify the legality of Δ^8 -THC on the federal level (57).

Another issue with the synthetic production of Δ^8 -THC is that byproducts of the reaction can include unnatural THC isomers such as Δ^7 -, Δ^{10} -, Δ^{11} -THC, and hexahydrocannabinols (HHCs; 58), the pharmacological properties of which remain largely unknown, and potentially other impurities due to poor chemistry (59). The chemical structures of selected THC isomers and analogs are shown in Figures 1 and 2, respectively. While there are no published studies on the psychoactive effects of these THC isomers in humans, Δ^{10} -THC was found to be psychoactive in pigeons, although it was far less potent compared to Δ^9 -THC (60, 61). Both Δ^7 -THC (62, 63) and Δ^{11} -THC (64, 65), also known as exo-THC, were found to be non-psychoactive in a rhesus macaque animal model. There is abundant misinformation on the internet regarding Δ^{11} -THC, where it is frequently confused with the active Δ^9 -THC metabolite 11-hydroxy- Δ^9 -THC. Regarding HHCs, they can occur as 9-methoxy-, 10-methoxy-, 9-ethoxy-, and 10-ethoxy-HHC (Figure 2A and B), depending on whether methanol or ethanol was used in the reaction (58, 66). Through hydrogenation of Δ^8 -THC and Δ^9 -THC, HHCs may also occur as the diastereomers (9R, 9S)-HHC (67, 68; Figure 2E and F), which can be found naturally in the pollen and seeds of hemp plants and were recently found in samples of hemp-derived resin (68). The psychotropic effects of HHCs are largely unknown at this time (66), but they purportedly have euphoric effects similar to Δ^9 -THC (69). One of the latest hemp-derived synthetic THC analogs to emerge on the market is Δ^8 -THC-O-acetate (Figure 2C), also known as THC-O or Δ^8 -THC acetate ester (70, 71), which has already been found in commercially available products (72) and is purportedly up to 3 times as potent as Δ^9 -THC (73). THC-O may also occur in the form of Δ^9 -THC-O-acetate (Figure 2D), which likewise possesses enhanced potency compared to Δ^9 -THC (71, 74). In a recent letter dated February 13, 2023, the DEA classifies both Δ^8 -THC-O and Δ^9 -THC-O as synthetically derived tetrahydrocannabinols that are not naturally occurring, thereby making them Schedule I controlled substances (75). The relative psychoactive potencies of the various THC isomers and analogs are shown in Table 1.

With the rapidly increasing availability of cannabis and hemp-derived products, the frequent use of other psychoactive substances in combination with cannabis, and the shortcomings of current analytical methodologies in the detection of recent cannabis use within the impairment window, it is clear that a new testing approach is needed to address the complexity of translating analytics to recent cannabis use and potential impairment. Here, we review the inadequacies of current testing methodologies for assessing potential impairment due to recent cannabis use, the lack of correlation between single measurements of Δ^9 -THC in

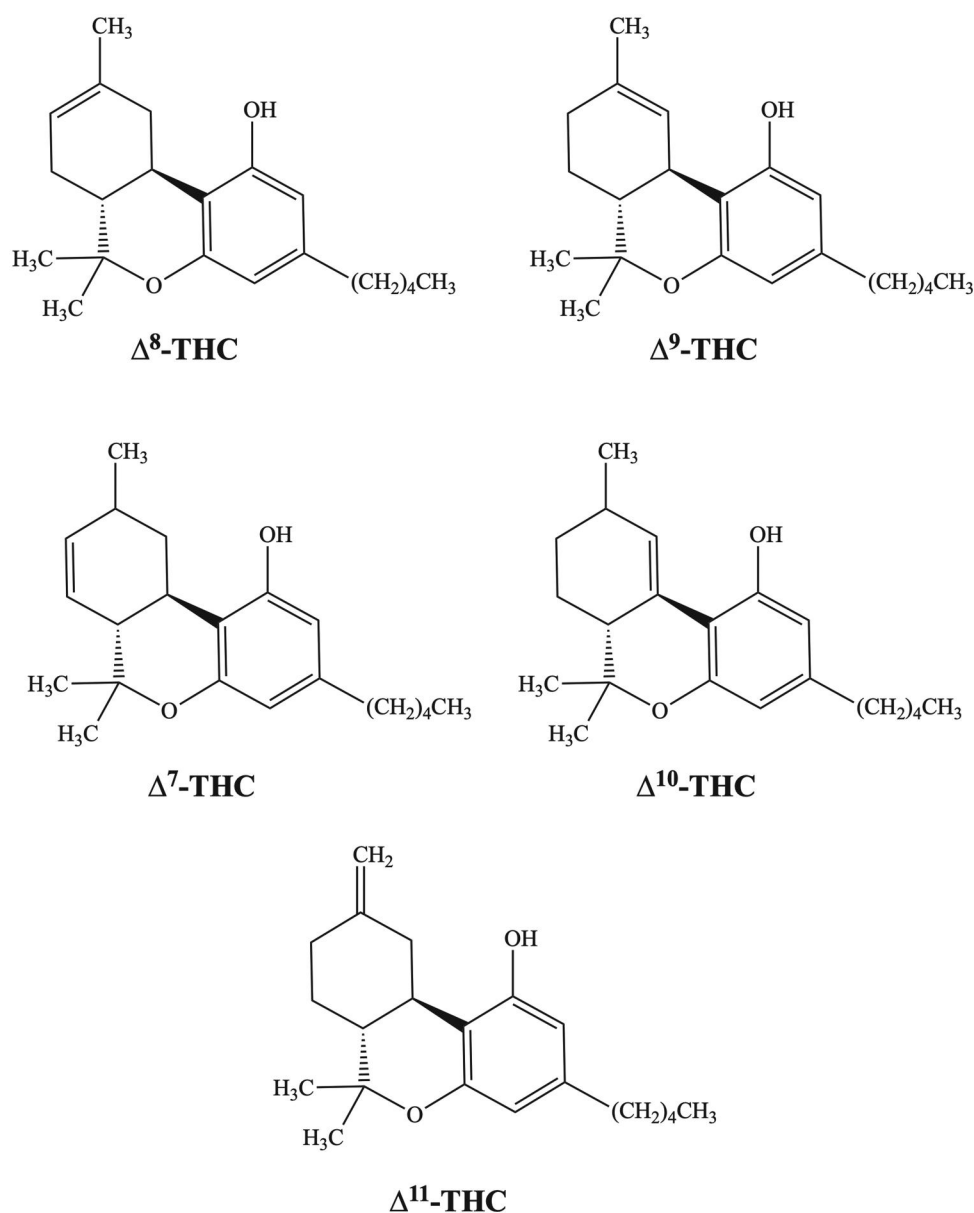


Figure 1. Chemical structures of THC isomers.

biological matrixes and impairment, the impairment potential of Δ^8 -THC, other THC isomers and analogs, and the need for their incorporation into standard testing methods, and the potential involvement of other psychoactive drugs and how they can complicate the assessment of impairment due to recent cannabis use. We then close with a discussion of a newly developed comprehensive testing approach based on exhaled breath and blood sampling that incorporates kinetic changes and the presence of key cannabinoids to detect recent cannabis use within the impairment window, particularly within the first hour after smoking, when the likelihood of impairment is greatest (11, 77), without the false-positive results seen with other methods.

The Inadequacies of Current Testing Methods for Assessing Cannabis Impairment

Testing methodologies currently being employed for detecting cannabis use include urine, blood, oral fluid (saliva), and hair

analysis. As an alternative test matrix, exhaled breath is being explored for its potential to better identify recent cannabis use within the impairment window. Each of these testing methods, and the reasons why they are inadequate for accurately detecting recent cannabis use within the window of potential impairment, are discussed below. For an excellent review of current cannabinoid analytical methodologies and how they are being employed, refer to Karschner et al. (78). It is worth noting that impairment can be affected by the development of tolerance (discussed later), use history, and route of administration such that the impairment window can be shorter, longer, or in some cases nonexistent (22).

Urine Testing

Urine testing is a common, cost-effective means of determining prior cannabis use, and it has traditionally been employed, for example, in routine workplace drug screening and drug use monitoring in parolees. However, in frequent cannabis users, metabolites of Δ^9 -THC such as 11-nor-9-carboxy- Δ^9 -THC (Δ^9 -

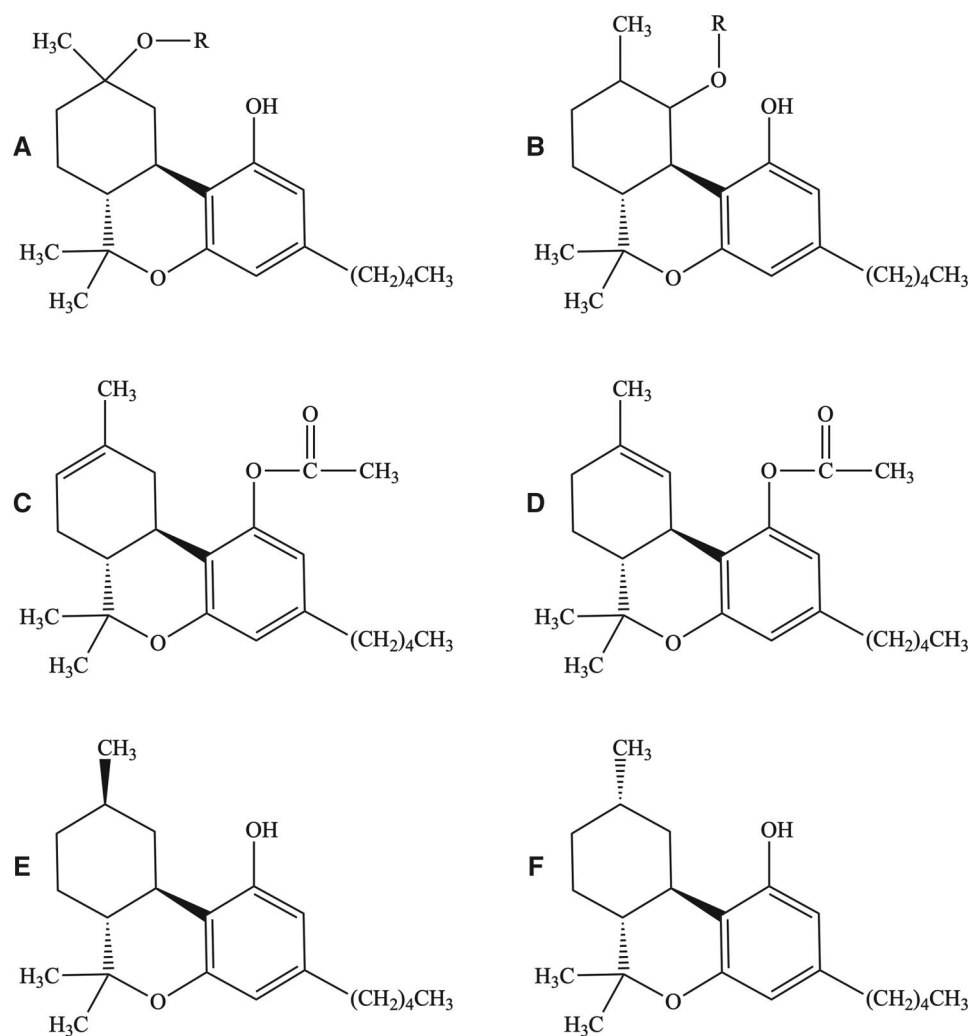


Figure 2. Chemical structures of THC analogs. (A) 9-methoxy- (R= CH₃) and 9-ethoxy-hexahydrocannabinol (HHC) (R= CH₂CH₃); (B) 10-methoxy- (R= CH₃) and 10-ethoxy-HHC (R= CH₂CH₃); (C) Δ⁸-THC-O-acetate; (D) Δ⁹-THC-O-acetate; (E) 9R-HHC; (F) 9S-HHC.

Table 1. Relative psychoactive potencies of THC isomers and analogs

Compound	Relative potency	References
Δ ⁹ -THC	+++	(76)
Δ ⁸ -THC	++	(44, 47, 48)
Δ ⁷ -THC	-	(62, 63, 65)
Δ ¹⁰ -THC	+/-	(60, 61)
Δ ¹¹ -THC	-	(64, 65)
9R, 9S-HHC	++	(64, 65, 69)
9- and 10-methoxy-HHC	Unknown	(66)
9- and 10-ethoxy-HHC	Unknown	(66)
Δ ⁸ -THC-O-acetate	+++++	(73)

THC-COOH) may be detectable in urine for several weeks after most recent use, and Δ⁹-THC itself has been detected in urine up to 24 days following most recent use (8, 9). Passive cannabis exposure from secondhand smoke also has the potential to produce a positive urine test for Δ⁹-THC-COOH (79), which could become problematic in certain settings, e.g., workplace drug testing. Furthermore, the consumption of products containing hemp-derived Δ⁸-THC, which have been increasing in popularity, especially in the United States as previously discussed, may lead to a false-positive urine screen for cannabis use due to common analytical methods confusing Δ⁸-THC-COOH with Δ⁹-THC-COOH (80)

as a result of their identical masses. While different approaches to utilizing urinary cannabinoid data have been studied in regard to determining recent use (8, 81), none of these methods is capable of determining cannabis use within the impairment window, and thus urine testing is not useful in this context.

Blood Testing

In suspected cases of driving under the influence (DUI) of cannabis, blood testing has been the gold standard. There are, however, a number of reasons why blood testing, at least as it is currently being employed, is inadequate for establishing recent cannabis use within the impairment window. In law enforcement settings, blood samples are typically not collected until approximately 1.5 to 4 h following a traffic stop or collision (82, 83), a time when plasma Δ⁹-THC concentrations may have already fallen to very low or undetectable levels. Furthermore, current testing approaches utilize one blood draw, which will at best provide a single measure of Δ⁹-THC and metabolite concentrations. This is problematic because recent research has shown that there is no meaningful correlation between impairment and specific Δ⁹-THC blood levels (13–21; more on this below). This has become a real issue in US states and other countries that have arbitrarily established legal “per se” Δ⁹-THC blood concentration limits for the determination of cannabis impairment. It has also been shown that

Δ^9 -THC can be detected in the blood of chronic users after 30 days of abstinence (2), and that exercise can increase the blood level of Δ^9 -THC due to release from fat stores (84). As is the case with urine testing, the potential of a positive blood test result for Δ^9 -THC and/or Δ^9 -THC-COOH exists following passive exposure to cannabis smoke (78, 85, 86). Using a blood Δ^9 -THC cutoff level of ≥ 5 ng/mL in combination with a concentration ratio of Δ^9 -THC-COOH to 11-hydroxy- Δ^9 -THC of < 20 has been suggested as a means of detecting recent cannabis use (87); however, while this approach can detect recent use within 8–12 h depending on route of consumption and usage history, it cannot be employed as a reliable means of identifying recent use within the impairment window following inhaled or oral consumption.

Oral Fluid

Compared to urine and blood testing, oral fluid offers a convenient, non-invasive means of assessing drug use, including cannabis. A number of different drugs of abuse and their metabolites, e.g., Δ^9 -THC, can be detected in oral fluid (88). Oral fluid testing devices are already being employed for impaired driving investigations in countries such as Canada, Australia, Norway, and the United States, where multiple states have legalized the collection of oral fluid for use in DUI testing (89). Similar to blood, detectable concentrations of Δ^9 -THC in oral fluid can persist well beyond the window of impairment (3–5). In frequent cannabis users, Δ^9 -THC was detected in oral fluid for an average of 61 h after smoking, with some subjects still exhibiting meaningful Δ^9 -THC concentrations at 72 h post-smoking (5). The oral cavity is also subject to contamination from environmental smoke (79, 90), which could result in a false-positive test result, as is the case with urine and blood testing. In field testing of oral fluid performed by a popular commercial testing device, false-positive and false-negative rates of 14.5 and 13.4%, respectively, were encountered for cannabis use (91). A similar rate of false-positive test results was reported for another competing oral fluid testing device in a clinical study evaluating oral fluid cannabinoid pharmacokinetics following consumption of cannabis edibles (92). One of the latest oral fluid testing devices in development claims to offer rapid, on-site quantification of Δ^9 -THC (93). While oral fluid screening may be useful in a workplace setting or for routine drug use monitoring in parolees, for example, it cannot be used to reliably assess recent use within the impairment window.

Hair Analysis

Of the available testing methods, hair analysis is probably the least informative with respect to assessing recent cannabis use associated with impairment. While hair collection is non-invasive and can be directly observed, thereby limiting the possibility of sample adulteration, the cannabinoid detection window in hair is the longest of any of the test matrixes currently being used. Evidence of cannabis use, typically confirmed by detection of Δ^9 -THC-COOH and/or 11-OH- Δ^9 -THC to rule out passive exposure (94, 95), can be found in hair up to 90 days or longer since last use depending on the length of the hair strands examined (96–98). Such a broad detection window can be very useful in applications such as forensic analyses and workplace drug testing, but hair analysis cannot provide any useful information regarding recent cannabis use within the impairment window. Perhaps the biggest drawback to hair testing for recent use is the fact that it takes approximately 2 weeks for newly formed hair to emerge above the scalp (99), and thus hair analysis can, at best, detect cannabis use only as recently as 2 weeks ago.

Exhaled Breath

Exhaled breath has emerged as a very promising alternative test matrix to blood and oral fluid for establishing recent cannabis use within the impairment window. While it has been known for 40 years that Δ^9 -THC can be detected in exhaled breath (100), only relatively recently has this matrix been explored as a potential means of establishing recent cannabis use within the impairment window (101, 102). Exhaled breath testing for recent use is predicated on a short period of detection for Δ^9 -THC within the impairment window. A study by Himes et al. suggested that Δ^9 -THC is generally detectable in breath for only about 2 h after smoking even in chronic users (101), which is shorter than the impairment window following cannabis inhalation, but more recent studies have shown that Δ^9 -THC remains detectable in breath up to several days since last use (6, 7). This is a major finding because no meaningful correlation has yet been established between impairment and Δ^9 -THC levels in any matrix tested to date (13–21). Because the leading technologies for breath-based testing for recent cannabis use, such as described by Lynch et al. (6) and others (103), rely solely on the detection of Δ^9 -THC, there is a real potential for false-positive test results due to the presence of Δ^9 -THC in breath outside of the impairment window following cannabis use through inhalation (smoking or vaporization), not to mention the fact that the detection of Δ^9 -THC in breath alone is uninformative with respect to impairment. Finally, as there are currently no published data showing detection of Δ^9 -THC, its metabolites, or any other cannabinoids in exhaled breath following the consumption of cannabis edibles, breath testing can detect only the inhaled use of cannabis at the present time.

As discussed below, a new testing approach that incorporates a combination of exhaled breath and blood testing has been developed (104) that can detect the recent inhaled use of cannabis within the impairment window without the false-positive results seen with other methods.

Single Measurements of Δ^9 -THC in Blood, Oral Fluid, and Breath Do Not Correlate With Impairment

As pointed out above, previous studies have failed to demonstrate a clear relationship between impairment and specific concentrations of Δ^9 -THC in blood or in oral fluid (13–21). To determine whether this is also true in exhaled breath, pre- and post-smoking blood and breath levels of Δ^9 -THC and other cannabinoids and their relationship to impairment were examined in a clinical study, the results of which were recently published (19).

In agreement with previous studies, the results of this study showed that a majority of a group of 30 test subjects had pre-smoking Δ^9 -THC blood concentrations that exceeded the legal per se limits currently in place in five US states (Illinois, Montana, Ohio, Nevada, and Washington), which range from 2–5 ng/mL (105), in the absence of impairment (19). The results also showed that the post-smoking duration of impairment appeared to be inversely related to baseline blood Δ^9 -THC concentrations, and that the subjects with the shortest duration of impairment tended to have the lowest incidence of horizontal gaze nystagmus (HGN), which is a potential indicator of psychomotor impairment, 3 h post-smoking (19). In this study there were a total of nine subjects who were tested on at least three different occasions. Baseline pre-smoking blood sample analysis

pattern of other cannabinoids observed in breath [cannabinol (CBN), THCV, CBC, cannabigerol (CBG), and CBD] within the first hour post-vaporization in this study was likewise consistent with what was observed in these subjects after they had smoked Δ^9 -THC cannabis (104). Interestingly, CBC and THCV were seen only within the first hour post-vaporization of the Δ^8 -THC product, which suggests that these cannabinoids may be indicators of recent use and, potentially, impairment as observed in our previous study (104), although it should be noted that no impairment window has yet been established for Δ^8 -THC. While the product vaporized by the subjects in this study was labeled as hemp-derived Δ^8 -THC, it was not surprising to see these other cannabinoids in breath and blood because all of them are commonly found in hemp (115, 116).

Other than Δ^8 -THC, there are a number of other unnatural THC isomers, including Δ^7 -THC, Δ^{10} -THC, Δ^{11} -THC, and HHCs that can be produced as byproducts of the acid-catalyzed conversion of hemp-derived CBD into Δ^8 -THC, as previously discussed. Not including the HHCs, all of the THC isomers, along with the natural cannabinoids CBD and CBC, share the same monoisotopic parent mass as well as fragment masses, which makes chromatographic separation critical in liquid chromatography–tandem mass spectrometry (LC–MS/MS)-based methods. Standard analytical methods typically include only Δ^9 -THC and can easily misidentify the other isomers as Δ^9 -THC due to co-migration on the LC column, which is common. Importantly, some of these THC isomers, e.g., 9-ethoxy-HHC and Δ^9 -THC, and a previously unknown cannabinoid (iso-tetrahydrocannabifuran), have already been identified in hemp-derived Δ^8 -THC products (117). Another issue with the various THC isomers and their metabolites is immunologic cross-reactivity in common immunoassay procedures. Using a cannabinoid ELISA kit with samples of whole blood, it was recently shown that Δ^{10} -THC, Δ^{11} -THC, THC-O, and carboxy- Δ^8 -THC exhibited varying degrees of cross-reactivity (118).

Coinciding with the emergence of hemp-derived Δ^8 -THC products in 2019 was a marked increase in cases of electronic vaping-associated lung injury (EVALI), which was eventually found to be strongly associated with vitamin E (α -tocopherol) acetate (VEA; 119) used as a diluent in cannabis vaping oils; however, the involvement of other additives and adulterants cannot be ruled out (120). The long, 16-carbon aliphatic tail of VEA is thought to be responsible for altering the viscosity of the alveolar surfactant layer (58), and when vaporized upon heating, VEA undergoes thermal decomposition to form ketene, which is a potent lung toxicant (121, 122). THC-O has also been shown to form ketene during vaporization (71, 74), and the aliphatic tails of Δ^8 -THC and other THC isomers, though shorter than VEA, may still be long enough to disrupt the surfactant layer in the lung alveoli (58). While there is a lack of published toxicological data on Δ^8 -THC and other THC isomers that are produced during the acid-catalyzed conversion of CBD, it is indeed very interesting that cases of EVALI began to sharply increase with the introduction of hemp-derived Δ^8 -THC products. In a subset of 300 cannabis-based vaping liquid products analyzed during the EVALI epidemic investigation, 10% of these products were found to contain major quantities of Δ^8 -THC, Δ^{10} -THC, and Δ^{11} -THC (123).

The Necessity of Testing for Other Psychoactive Drugs When Assessing Recent Cannabis Use and Impairment

Cannabis is frequently used in combination with alcohol and/or other psychoactive drugs, which can greatly complicate an

assessment of impairment due to recent cannabis use. In order to rule out the potential involvement of other intoxicating substances in cases of suspected cannabis DUI, for example, analytical methods need to incorporate commonly used psychoactive drugs such as opiates, methamphetamine, benzodiazepines, barbiturates, and synthetic cannabinoids, in addition to Δ^9 -THC, its metabolites and various isomers, and other phytocannabinoids. If a subject is exhibiting signs of impairment after being involved in a workplace accident, for example, and cannabis use is detected, this does not rule out the possibility of other drugs being involved. Should the subject be found to be outside of the cannabis impairment window using the comprehensive testing approach presented below, and test positive for other psychoactive substance(s), the observed impairment is unlikely to be due to cannabis, especially if alcohol is involved, as discussed below.

Synthetic cannabinoids are a diverse class of molecules that are designed as potent agonists of the endocannabinoid receptors CB₁ and CB₂. Compared to the phytocannabinoid Δ^9 -THC, which is a partial agonist, most synthetic cannabinoids are full agonists of the endocannabinoid receptors, leading to more intense psychoactive effects as well as undesired side effects (124). Recreational use of these compounds began in the mid-1990s (124), and they have continued to proliferate ever since. Over 200 synthetic cannabinoids from at least six different chemical classes have so far been identified, some of which are similar to the natural phytocannabinoids and some of which are similar to the natural endocannabinoids anandamide and 2-arachidonoylglycerol (124). These compounds are typically applied to plant-based materials and sold under catchy trade names such as Spice, K2, AK-47, Mr Happy, Scooby Snax, Kush, and Kronik. Smoking is by far the most common route of administration, but synthetic cannabinoids may also be taken orally as tablets, powders, and herbal infusions (124).

In a systematic review and meta-analysis on the combined effects of cannabis and alcohol on driving performance, Simmons et al. found that, with the exception of lateral lane position variability, which is known to be significantly affected by cannabis, the combination of cannabis and alcohol did not significantly affect driving performance measures, including crash risk, hazard reaction time, and speed variability, compared to alcohol alone (125). This finding is in agreement with an earlier NHTSA report stating that neither Δ^9 -THC nor other psychoactive drugs were significant contributors to crash risk after controlling for alcohol use and other factors (36), as well as more recent publications examining the crash risks associated with the use of cannabis and alcohol alone and in combination (37, 126). However, these findings should not be interpreted to mean that cannabis use does not significantly affect crash risk, but rather that the risk posed by cannabis (27–29) does not significantly add to the risk already posed by alcohol consumption.

Comprehensive Breath Test for Confirming Recent Use of Inhaled Cannabis Within the Impairment Window

The complex nature of cannabinoid pharmacokinetics and pharmacodynamics calls for a new approach for effectively determining recent use of cannabis within the impairment window, which cannot be established using currently available testing methods. To address this critical unmet need for the benefit of law enforcement, employers, and others, a comprehensive test was developed based on pharmacological changes in Δ^9 -THC and other cannabinoids that occur with time in exhaled breath and blood, a

test that improves the accuracy of breath-based testing by incorporating a confirmatory blood test to prevent false-positive results (104). It was hypothesized that a two-sample testing strategy (collecting two samples separated by a known time interval) could be used to detect cannabinoids in breath during their distribution phases, which occur only during the first few hours after smoking. Liquid chromatography–high-resolution mass spectrometry (LC–HRMS) bioanalytical methods for the quantification of Δ^9 -THC and other cannabinoids in exhaled breath and whole blood (127) were developed and validated for this purpose. A confirmatory blood test could be used to compare relative levels of cannabinoids in breath. Higher relative levels of cannabinoids in breath compared to blood would be indicative of very recent use because it is not possible for higher relative levels of cannabinoids to redistribute back into the breath from the blood. Coupled with physical and subject self-assessments of impairment, it was believed that the results of the test could also be tied to recent use within the impairment window after smoking, which is the most common route of cannabis administration (128). Summarized below is the clinical development of a comprehensive breath and blood-based test and its application in determining recent cannabis use and impairment after smoking.

A total of 10 pharmacologic parameters were found to be associated with recent cannabis use based on the two-point breath sampling strategy. These included the presence of CBN, CBC, CBG, Δ^9 -THCV, and cannabigerolic acid (CBGA), and short half-lives (<60 min) for Δ^9 -THC, CBN, CBC, CBG, and Δ^9 -THCV (104). Interestingly, CBC and Δ^9 -THCV were detected in breath only during the peak impairment window (the first hour post-smoking), making these cannabinoids key indicators of recent cannabis use through inhalation. All subjects from whom breath samples were collected self-reported peak impairment within the first 20 min post-smoking, which coincided with the shortest cannabinoid half-lives and peak incidence of HGN (104). An 11th parameter to emerge was the breath/blood ratio of Δ^9 -THC, which proved to be a key confirmatory indicator of recent use. This ratio was computed by dividing the Δ^9 -THC peak area ratio to the internal standard in breath by the corresponding peak area ratio in blood. All of these ratios were <2 when measured pre-smoking, while all values were >2 when assessed immediately after smoking (104). Compared to the average pre-smoking ratio, average breath/blood Δ^9 -THC ratios measured immediately after smoking up to 180 min post-smoking remained significantly greater (104). To test positive based on the two-point breath and one-point blood test, a subject must exhibit a breath/blood Δ^9 -THC ratio ≥ 2 , in addition to a short Δ^9 -THC half-life and one or more other indicator of recent use in breath.

In this study, all 44 subjects (100%) from whom both blood and breath samples were collected tested positive for recent use during the peak impairment window after smoking using the two-point breath and one-point blood test. Pre-smoking, 0% (0/34) of subjects tested positive for recent use, indicating no false-positive test results (104). A positive test result using the two-point breath and one-point blood test indicates that a subject has used cannabis recently through inhalation, i.e., smoking or vaping, and that they are within the 3 h impairment window. Interestingly, approximately 68% of these subjects had detectable levels of Δ^9 -THC in their breath at baseline prior to smoking (104), in agreement with recent reports (6, 7). This finding reinforces the fact that the mere presence of Δ^9 -THC in breath does not conclusively demonstrate recent use within the impairment window, which could prove to be a major shortcoming of the commercial cannabis breathalyzers currently in development. This

comprehensive test, which incorporates a breath/blood Δ^9 -THC ratio and the presence and half-lives of key cannabinoids in breath, definitively established recent cannabis use within the impairment window (104).

A potential limitation of this testing approach is the possibility for false-negative results. Evidence of recent cannabis use in breath declines rapidly beyond the first hour after smoking, so it is possible that breath samples collected during the second or third hour after cannabis inhalation may no longer exhibit sufficient evidence of recent use, even though the subject may still be impaired. A false-negative result obviously favors the test subject, while a false-positive result could have potentially serious consequences, including wrongful termination of employment and prosecution. With this test, it is possible to observe short Δ^9 -THC half-lives in breath and breath/blood Δ^9 -THC ratios ≥ 2 when concentrations of Δ^9 -THC are very low, at or near assay LOQs. In order to avoid false-positive test results due to assay variation at low levels, it is important to ensure that concentrations fall within the validated range of the assay. To resolve borderline breath/blood Δ^9 -THC ratios, i.e., ratios ranging from 1 to 3, additional evidence of recent use within the impairment window is needed.

Because evidence of recent cannabis use in breath dissipates rapidly, it is critical that breath samples are collected as soon as possible following a workplace incident, for example, or once a need for testing arises, in order to prevent the loss of breath evidence. On-site breath collection using a device such as the one employed in this study (104) can easily be performed without specialized training. Likewise, the blood sample can be collected on site using one of the commercially available capillary blood draw devices designed to be used without specialized training. For detecting the recent inhaled use of cannabis, a two-point breath test is the most practical application. In the event that a test subject is positive for only a short Δ^9 -THC half-life in breath, the matching blood sample can optionally be used to confirm recent use if desired. A matching blood sample would always be collected to allow testing for other drugs that can induce impairment, including prescription drugs, and it can be additionally analyzed for cannabinoid content if needed.

Discussion

The complexity of using analytics to determine recent cannabis use and potential impairment demands a comprehensive testing approach that includes all known isomers of THC, synthetic cannabinoids, and commonly abused psychoactive drugs, both illegal and prescription, in order to ensure that suspected cases of cannabis-induced impairment are actually due to cannabis use and that test subjects used recently enough to be impaired, i.e., within the impairment window. Currently established modes of testing based on single measurements of Δ^9 -THC and/or its metabolites in urine, blood, oral fluid, and hair have proven to be inadequate for this purpose, basically leading to “best guess” assumptions regarding cannabis use and potential impairment. As discussed at length in this review, recent studies have firmly established that the use of per se legal limits for Δ^9 -THC in blood or oral fluid in various US states and abroad is simply not supported by science due to cannabis-use history and the development of tolerance, but for those wrongly accused the consequences can be very real, potentially leading to loss of employment, financial ruin, even incarceration. By itself, standardized field sobriety testing, originally developed for the detection of alcohol intoxication, has proven to be inadequate with respect

to cannabis impairment (129, 130). A recently published clinical trial found that 19% of cannabis smokers (23/121) tested non-impaired 70 min after smoking, while over 49% of placebo smokers (31/63) were incorrectly deemed impaired (131). Combining oral fluid toxicology and field sobriety testing was found to eliminate false-positive test results in placebo smokers, but proved to be of no value in improving the rate of false negatives in the cannabis smokers (21). Identifying changes in oculomotor control unique to cannabis may improve the accuracy of field sobriety testing, but this approach remains unproven (132).

The number of ways by which a subject can receive a false-positive test result for recent cannabis use are numerous under current testing methodologies. Typically, only single samples of urine, blood, oral fluid, or breath are collected, and as we have established, single measurements of Δ^9 -THC and/or its metabolites do not correlate with impairment (19). There are problems with the test methods themselves; for example, in urine cannabinoid screens, immunological cross-reactivity between THC isomers and metabolites is an issue (118). Other psychoactive substances are often used in combination with cannabis and may be the causative agent responsible for any observed impairment. Detectable concentrations of Δ^9 -THC and/or its metabolites can persist well beyond the impairment window following either inhaled or oral cannabis use. While lingering concentrations of Δ^9 -THC for days or longer beyond impairment is a well-known issue for blood and urine testing, this could be particularly problematic for breath testing devices used in field-screening or pre-access testing applications where residual Δ^9 -THC could lead to false-positive test results. The chronic use of hemp-derived CBD products, which can legally contain up to 0.3% Δ^9 -THC but often contain far higher amounts (133), could potentially lead to a positive test result for cannabis use due to the bioaccumulation of Δ^9 -THC to detectable levels. Biotransformation of CBD to Δ^9 -THC under acidic conditions may also be possible (134, 135), although unlikely (136). There is also the issue with products containing hemp-derived Δ^8 -THC, which are currently shrouded in legal controversy. The acid-catalyzed conversion of CBD to Δ^8 -THC can produce numerous THC isomers including Δ^9 -, Δ^7 -, Δ^{10} -, and Δ^{11} -THC as well as HHCs, any or all which can be mistaken for Δ^9 -THC in commonly used analytical methods that were not developed and validated for the detection of these compounds. There is also the THC analog THC-O (both Δ^8 and Δ^9 isoforms), which is synthesized from Δ^8 -THC and may possess a potency up to 3 times that of Δ^9 -THC (73).

A new test for recent cannabis use, based on two-point breath sampling with or without a one-point confirmatory blood test, has been developed that can accurately detect whether a subject has used cannabis through inhalation within the window of potential impairment, regardless of the potency of the cannabis chemovar smoked, with no false-positive results (104). The test is based on multiple parameters, including cannabinoid half-lives, which confirm distribution phase kinetics, the presence of key cannabinoids that are observed only after smoking, and a blood test to determine the breath/blood Δ^9 -THC ratio, which confirms whether the test subject was within the impairment window post-smoking. It is our belief, based on our research, that this new test provides an answer to the unmet need of determining recent cannabis use, which in turn protects the public from a safety standpoint by detecting inappropriate use, e.g., DUI, while at the same time protecting responsible cannabis users such as medicinal users from wrongful termination and prosecution. An important limitation is that while this test can identify subjects who were within the impairment window, it cannot prove actual

impairment, which would require behavioral evidence. Its value is the ability to distinguish individuals who were most likely to have been impaired due to the recent use of cannabis from those who were unlikely to have been impaired due to cannabis use. A further limitation of this approach is that it would likely be impractical to implement as part of a wide-scale roadside drug-testing scheme. Rather, it is better suited as part of a workplace drug-testing program. Now that it has become clear that legalization of recreational cannabis has resulted in a significant increase in crash risk (27–29), it is reasonable to presume that other types of incidents, e.g., workplace accidents, may also increase, and thus it is more important than ever to implement an effective means of detecting irresponsible cannabis use.

While the focus of this test has been on detection of recent inhaled cannabis use, it should be emphasized that the two-point breath and one-point blood test is not limited to just cannabis. The same testing strategy we employed for cannabis may also prove to be useful for detecting recent use of other impairing drugs such as methamphetamine, phencyclidine, and cocaine that can be administered through vaporization. Exhaled breath testing has already been proven useful for detecting multiple drug types (137, 138), and this test allows the simultaneous testing for cannabis as well as other potentially impairing drugs in both breath and blood. Practical applications include sports medicine, enforcement of workplace drug policy, and law enforcement.

Although smoking remains the most common route of administration for cannabis, other modes of delivery such as gummies, tinctures, topicals, beverages, baked goods, and dabbing are growing in popularity as the legalization of recreational cannabis continues to expand (128). With respect to cannabis edibles, further study is needed to detect recent use and impairment following oral consumption of cannabis products. Hypothetically, a similar strategy utilizing two blood samples could be deployed for detecting recent use of orally administered cannabis as well as other orally administered impairing drugs. It is well known that cannabinoid pharmacokinetics differ depending on the route of administration. Because Δ^9 -THC metabolites cannot be detected in breath, the blood may contain critical information pertaining to recent use, including concentrations of glucuronide metabolites and changes in the ratios of the Δ^9 -THC metabolites such as 11-hydroxy- Δ^9 -THC and 11-nor-9-carboxy- Δ^9 -THC to Δ^9 -THC and to each other, as previously reported (87, 127).

Conclusions

Using analytics to determine recent cannabis use and potential impairment has proven to be a complex problem due to the pharmacology of Δ^9 -THC, subject use history, and the development of tolerance to the impairing effects of Δ^9 -THC. Because of these factors, the traditional approach of analyzing single samples of various matrixes for Δ^9 -THC and/or its metabolites and the use of field sobriety testing have been found to be inadequate for this purpose. Single measurements of Δ^9 -THC and/or its metabolites cannot be correlated with impairment. Compounding the issue is the use of other psychoactive drugs in combination with cannabis, and the plethora of THC isomers and analogs that have emerged following legalization of industrial hemp in the United States, particularly THC-O, which is far more potent than Δ^9 -THC and is now being treated as a Schedule I controlled substance. A new comprehensive testing approach that involves multiple matrixes, that determines the presence of and pharmacologic changes in key compounds over time between samples,

that can distinguish between the various THC isomers and analogs, and that simultaneously tests for other common psychoactive substances is needed to accurately detect recent cannabis use within the impairment window without the false-positive results seen with other methods. The complexity of assessing potential cannabis impairment demands such a method so that irresponsible users can be accurately detected without falsely accusing responsible users who may unjustly suffer harsh, life-changing consequences such as lost jobs, lost livelihoods, and incarceration.

CRedit Author Statement

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Conflicts of Interest

All authors are the inventors of the recent cannabis-use testing methodology (US patents 17/048,737 and 18/265,541 pending) described in the present work, and all authors are founders of RCU Labs, Inc. GTW is additionally an employee of RCU Labs, Inc.

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