CLINICAL & FORENSIC

Toxicology News

ISSN 2326-3253

June 2023

An AACC/CAP Educational Newsletter for Toxicology Laboratories

Food and Drug Administration Oversight of Laboratory-Developed Tests: The Ongoing Saga

By Shannon Bennett, MS, MBA, CM/QOE (ASQ)

Clinical laboratory tests can be divided into 2 broad categories: in vitro diagnostic (IVD) kits developed and produced by commercial manufacturers, and laboratory-developed tests (LDTs) developed directly by the performing laboratory. Under certain circumstances, an IVD may become an LDT if the performing laboratory modifies the IVD outside of the manufacturer's instructions. For example, using whole blood samples with an IVD kit labeled only for serum would be categorized as an LDT.

Current Regulation

The Clinical Laboratory Improvement Amendments (CLIA), enforced by the Centers for Medicare and Medicaid Services in the Department of Health and Human Services, governs clinical laboratories performing diagnostic testing. CLIA focuses on laboratory operations, such as the minimum educational requirements for laboratory staff, rules for staff training and competency, proficiency testing, equipment calibration and maintenance, and other operational considerations. CLIA makes a brief mention that laboratories must establish or verify analytical performance characteristics for new or modified tests, but at a very high level. Laboratories must provide documentation of verification or validation, available during a routine inspection, which generally occurs when a lab applies for CLIA certification, and then every 2 years.

In comparison, IVD manufacturers must submit their tests or reagents to the Food and Drug Administration (FDA), also under Department of Health and Human Services. IVDs are subject to medical device rules and cannot be sold or marketed before FDA review. The FDA has several submission pathways that are roughly aligned with perceived risk to patients and/or users of the medical device, designated by the FDA as class I (lowest risk) to class III (highest risk). Some tests are so simple and low risk they do not require any submission, which the FDA refers to as exempt tests. FDA also assigns the CLIA complexity for tests, which can cause confusion because the terminology sounds similar to the submission pathways. Tests are assigned a CLIA complexity of waived, moderate, or high complexity based upon a variety of factors that include the educational background of the person performing the test, training requirements, and complexity of interpretation. While an FDA-exempt test may be CLIA-waived, the 2 designations are distinct. As an example, many immunohistochemical stains are FDA-exempt, but CLIA has high complexity.

Lower-risk devices often go through the 510(k) pathway, which compares them against a similar (predicate) device already on the market. IVDs that successfully navigate the 510(k) process are called FDA-cleared. The highest-risk products utilize a significantly more rigorous Premarket Approval (PMA) process, which requires more evidence of safety and effectiveness, often including a prospective clinical trial. IVDs that successfully go through the PMA process are called FDA-approved. IVDs that fall between these 2 risk classifications can utilize the less frequently used De Novo submission pathway and are called FDA-granted upon completion of the submission.

IVDs and LDTs are both used for clinical diagnosis. So why are they regulated so differently?

Inside...

A Brief History Lesson

The FDA gets its statutory authority from the federal Food, Drug, and Cosmetic Act, and in 1976 the Medical Device Amendments to the act gave the agency authority to regulate medical devices, including IVDs. At the time, the FDA indicated they would leverage enforcement discretion and exclude LDTs from this regulation because they were viewed as low risk due to the low complexity of the tests and the limited patient population a given LDT would be used with. However, over time the number and sophistication of LDTs evolved, particularly with the advent of advanced technologies such as next-generation sequencing. This caught the attention of the FDA, which expressed concern with the increased level of potential risk for patients as LDTs became more complex.

The FDA has several options to enforce its authority. The agency may release guidance documents, which describe how they interpret applicable statutes or regulations but are not themselves legally binding. As such they are the weakest form of authority but provide detailed information on FDA expectations. Guidance documents may be released in draft form for public comment but may also be issued in a finalized state. Another option is the notice and comment rulemaking approach, where the FDA will promulgate new draft regulations, solicit input from the public, address those comments (though not necessarily accept them), and then the new regulations go into effect over a defined implementation period. This process gives the FDA a stronger footing as they are creating regulations, but the process can be very time-consuming. The final option is getting explicit authority through legislation.

In 2014, the FDA issued a draft guidance document outlining how the agency might begin regulating select high-risk LDTs. The agency outlined several perceived gaps in CLIA regarding the regulation of LDTs:

- Analytical validation is required but FDA views the limited statutory requirements as insufficient.
- Tests can be offered to patients before review by an outside party.
- Clinical validation is not mentioned; by comparison, IVDs require documented analytical *and* clinical validity.
- Laboratories are not required to report adverse events involving their LDTs.
- There are no provisions to remove an individual poorly performing test from the market.
- There are no specific requirements for the design, development, or production of an LDT or its components.

FDA proposed using the same class-based risk determination as for IVDs and other medical devices, focusing their attention on moderate and high-risk LDTs.

There was significant pushback from the clinical laboratory community, with many groups expressing concern that FDA was overreaching, that innovation would be stifled, and some even questioning if FDA had the statutory authority to regulate LDTs. FDA never finalized the draft guidance, noting concerns from stakeholders but also likely influenced by the 2016 presidential election. Changing tactics, FDA issued a discussion paper in 2017 that outlined broad concepts for regulating LDTs, with the stated intent of assisting Congress in drafting legislation to address any questions about statutory authority.

In 2017, the Diagnostic Accuracy and Innovation Act (DAIA) was introduced in Congress. Stakeholders from both the IVD and LDT communities helped develop the bill and sought to balance the concerns of FDA, clinical laboratories, and manufacturers. The bill introduced a new product type distinct from medical devices, in vitro clinical tests (IVCTs).

A new draft bill, the Verifying Accurate Leading-edge IVCT Development (VALID) Act, which first began circulating in late 2018, incorporated concepts from DAIA. Like DAIA, VALID intended to provide the FDA with the explicit authority to regulate IVDs and LDTs under the same regulatory model. VALID was formally introduced on the floor of Congress in 2020, but due to the coronavirus disease (COVID) pandemic escalating during that time, no action was taken. The bill was reintroduced in 2021. In both cases, a competing bill was also introduced around the same time, the Verified Innovative Testing in American Laboratories Act. The act took the opposite approach to VALID, expressly prohibiting the FDA from regulating LDTs and instead directing Centers for Medicare and Medicaid Services to update CLIA, with the intent to address advances in technology and test complexity through the existing clinical laboratory regulations.

While the Verified Innovative Testing in American Laboratories Act had robust support from many laboratories and professional societies, only 1 Senator sponsored the bill and it struggled to gain much momentum. VALID, in contrast, had bipartisan sponsorship in both the House and Senate, making it politically more viable. VALID underwent a series of revisions based on feedback from the FDA, professional societies, patient advocacy groups, individual laboratories, and other stakeholders from its introduction in early 2021 through mid-2022. VALID was unlikely to pass as a standalone bill and would most likely be included as a rider to another piece of legislation. Fall of 2022

offered an excellent opportunity because the must-pass Medical Device User Fee Agreements reauthorization was due for a vote; the agreements provide funding for FDA regulation of medical devices and was a natural fit for inclusion of VALID.

The summer of 2022 saw a flurry of advocacy efforts from proponents and opponents of VALID. A significant number of professional societies, academic medical centers, and other stakeholders expressed grave concerns about increased FDA regulation. They highlighted issues such as increased costs due to the need for additional quality/regulatory personnel and the submissions themselves, the additional time required for FDA review, and concerns over stifling innovation and patient access as a result. Advocates such as the FDA and patient advocacy groups reiterated arguments first raised in the FDA's 2014 draft guidance, while some laboratory proponents were concerned that VALID offered a compromise position and that without it, the FDA would pursue regulating LDTs fully as medical devices.

Opponents were ultimately victorious, with the VALID Act being stripped from the user fee agreements legislation at the last moment. Another opportunity presented itself with the year-end omnibus spending bill, another must-pass piece of legislation that often serves as a catch-all bill for many additional legislative riders. Again, VALID was stripped from the omnibus at the last moment and as of early 2023, the bill's fate is an open question.

VALID Act: What Is in the Bill

The VALID Act is a lengthy 240+ pages of dense and technical legislative language—it is not an easy read. It may be helpful to understand the major components and concepts in the legislation, as it is likely that future efforts by FDA to regulate LDTs may incorporate some of them.

Grandfathering

There is not consensus on the number of LDTs currently on the market given the lack of a centralized repository for test information, but it is certainly tens or even hundreds of thousands. The 2014 FDA guidance required a review of at least some of the existing LDTs, which would have been a tremendous undertaking. The VALID Act, in contrast, grandfathers most LDTs that are on the market before 45 days of enactment. Exceptions include tests that involve home specimen collection unless it is performed with a collection device that has been reviewed by the FDA (or is exempt). Grandfathered tests would require a reporting comment indicating they have not been reviewed by the FDA. Modifications to a grandfathered test that significantly change performance claims or adversely affect performance may require FDA review and approval. Grandfathered tests are also subject to listing and adverse event reporting requirements.

Risk Classification

Submission requirements in the VALID Act are predicated on patient risk. Low-risk tests are defined as those where an undetected inaccurate result would cause minimal or immediately reversible harm to a patient. High-risk tests are defined as those where an undetected inaccurate result is reasonably likely to result in irreversible harm or death to a patient. Moderate risk tests are defined as those that are neither low or high risk, which include tests that would be considered high risk but utilize one or more mitigating measures to decrease the risk of patient harm.

Submission Content

Low-risk tests are exempt from FDA review, though laboratories would still need to document verification or validation in compliance with CLIA, list their test, and comply with adverse event reporting. The submission requirements for moderate and high-risk tests include:

- Description of the test, the intended use, methodology, and performance characteristics.
- Documented risk assessment for the test, identifying potential failure points and risk mitigation (e.g., quality control, specific process steps, etc.).
- Bibliography of applicable publications.
- Validation summary, including study description, design, limitations, and conclusions; note this includes both analytical validity as well as clinical validity.

High-risk tests must also include raw data for all validation studies and information about the laboratory's quality management system. Moderate and high-risk tests cannot be offered to patients before FDA approval.

The VALID Act includes several important exemptions from submission requirements, which do not apply to high-risk tests or tests used for screening:

- Humanitarian test exemption – tests for noncontagious conditions that affect less than 10 000 patients per year, or contagious conditions that affect less than 1 500 patients per year.
- Custom or low volume tests – tests for a unique pathology that will be used on one (custom) or <5 (low volume) patients per year.

- Manual tests – tests that are the result of direct, manual observation without the use of instruments or software.
- Public health surveillance – results are not returned to individual patients.

Modifications

Laboratories must perform a risk assessment for any test modification to determine (a) the impact on the test's performance, and (b) whether the modification will require a supplemental submission and approval before implementation. Several types of modification are exempt from submission, including modifications under a preapproved change protocol, that do not significantly change the intended use, or that do not significantly change performance claims. Changes to specimen type are explicitly mentioned as not requiring submission and approval. Note that these requirements apply regardless of whether the laboratory is modifying its own LDT or a test kit purchased from a third-party manufacturer.

Technology Certification

The concept of technology certification seeks to reduce the submission burden for laboratories, using a "center of excellence" model. A laboratory would submit information on an exemplar test that uses one or more technologies, such as flow cytometry, along with a standard validation process for that technology and information about the laboratory's quality management system. Once the technology certification is granted, the laboratory could develop new, or modify existing, tests that use the technology without the need to submit each individual test to the FDA for approval. The laboratory would still need to list the test and follow adverse event reporting requirements.

Registration and Listing, Clinical Test Information System

All clinical laboratories developing IVCTs must register with the FDA, and newly registered laboratories are subject to inspection. All IVCTs a laboratory develops must be listed with the following information:

- Test name and FDA listing number.
- CLIA certificate number.
- Submission pathway used (e.g., approval pathway, technology certification, and specific exemption).
- Indications for use.
- Summary of analytical and clinical performance.
- Description of conformance with applicable mitigating measures or recognized standards.

FDA will develop a database, the Clinical Test Information System to house registration and listing information.

Quality Requirements

Only laboratories that meet the CLIA requirements for high-complexity testing are eligible to develop IVCTs, which may include some modifications to FDA-cleared or approved test kits. In addition, laboratories must ensure their IVCTs meet user needs through the use of design controls, and must have defined acceptance criteria for relevant activities such as reagent handling, a corrective and preventative action process, and a mechanism to document complaints and their follow-up.

Adverse Event Reporting

VALID requires laboratories to establish an adverse event documentation system. Should an IVCT cause or contribute to the death of a patient, the laboratory would need to notify the FDA within 5 calendar days after becoming aware of such an event. In addition, laboratories would need to submit quarterly reports describing inaccurate IVCT results and any absence, delay, or discontinuation of patient care as a result.

Fees

The existing medical device regulations enforced by FDA are significantly funded through user fees, which operate in 5-year cycles. The VALID Act requires the establishment of a new user fee program to partially fund IVCT regulation. The fees are set through a series of negotiations between the FDA and relevant stakeholders, often including performance goals for the FDA, for example, 90% of reviews will occur within X days of submission.

Despite its length, the VALID Act is just a framework; the FDA will need to develop significant additional regulations and guidance documents to specify all of the details that will make IVCT regulation work. The legislation spells out a 5-year transition plan, where the FDA will spend the first 2 to 3 years developing these details as well as the Clinical Test Information System, and laboratories will have the remaining time to move into compliance. Any IVCT developed and marketed during this transition time that is not grandfathered or otherwise exempt will eventually need to be submitted. While the concepts in the VALID Act have come a long way and moved in a more laboratory-friendly direction, FDA regulation of LDTs/IVCTs would be a significant change for the clinical laboratory industry.

Additional Reading

- FDA 510k pathway: https://www.fda.gov/medical-devices/premarket-submissions-selecting-and-preparing-correct-submission/premarket-notification-510k
- FDA De Novo pathway: https://www.fda.gov/medical-devices/premarket-submissions-selecting-and-preparing-correct-submission/de-novo-classification-request
- FDA PMA pathway: https://www.fda.gov/medical-devices/premarket-submissions-selecting-and-preparing-correct-submission/premarket-approval-pma
- FDA draft guidance document on LDTs: https://www.fda.gov/media/89841/download
- VALID Act (version ultimately struck from MDUFA): https://www.congress.gov/bill/117th-congress/senate-bill/4348/text
- FDA Medical Device Exemptions 510(k) database: https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfpcd/315.cfm?GMPPart=864#start
- FDA CLIA Waived Analytes database: https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfClia/analyteswaived.cfm

The author has nothing to disclose.

Shannon Bennett, MS, MBA, CM|QOE (ASQ) is the Director of Regulatory Affairs for the Department of Laboratory Medicine and Pathology at Mayo Clinic in Rochester, Minnesota. Email: bennett.shannon@mayo.edu.

Smoke Rings and Fused Rings: Making Sense out of THC Isomers and Analogs from a Structural Perspective

By Gregory C. Janis

Introduction and Background

The 2018 Agriculture Improvement Act (commonly known as the Farm Bill) reinvigorated the long-dormant industry of commercial hemp production. The bill sets rules to distinguish legal hemp production from still-illegal marijuana cultivation; those rules inadvertently created a new industry and spawned a revolution in amateur medicinal chemistry. As legal hemp production soared after the Farm Bill, so did interest and production of cannabidiol (CBD). Eventually, an entrepreneur with at least basic chemistry knowledge realized that CBD is a good starting material to create other, more active, and potentially more profitable compounds. Through a simple acid condensation reaction, CBD can easily be converted into Δ^9-tetrahydrocannabinol (Δ^9-THC). Secondary reactions can then be utilized to isomerize Δ^9-THC into Δ^8-THC. Before 2018, Δ^8-THC was known to be active but less potent than Δ^9-THC and it was known that Δ^8-THC is found naturally when Δ^9-THC degrades to the more thermodynamically favorable Δ^8-THC isomer as marijuana ages post-harvest (1). Little else was known about Δ^8-THC, and most marijuana users had no knowledge of Δ^8-THC. This lack of knowledge of Δ^8-THC even extended to producers and dispensaries in states where marijuana had been legalized for medicinal or recreational use. However, after the 2018 Farm Bill, Δ^8-THC quickly overcame its anonymity and entered the mainstream where it was touted as legal weed-lite packaged in vapes and edible products.

The evolution of CBD-derived Δ^9-THC analogs did not end with Δ^8-THC. Purportedly, an accident of chemistry involving residual fire retardants sprayed onto a hemp crop led a manufacturer to inadvertently produce Δ^{10}-THC while processing CBD. The reaction was later reproduced at scale using a catalyst of food-grade radical initiators; subsequently, Δ^{10}-THC vapes and gummies began appearing in smoke shops. Since then, alternative chemical pathways have been utilized to further move the double bond within the backbone of Δ^9-THC (see Fig. 1), and alter other aspects of the basic structure of Δ^9-THC. The chemistry required to further modify THC is more complex than creating Δ^8-THC from CBD, increasing the potential for side reactions and unanticipated byproducts. Without regulations governing the purity of hemp-derived consumer products, there is no guarantee that byproducts have been removed from the final product (2). The current legal status of hemp-derived products is discussed below. Byproducts detected in vapes include *iso*-tetrahydrocannabifuran (*iso*-THC), and many smaller compounds; little is known about the activity or potential health impact of these byproducts, especially when heated and inhaled. The structure of *iso*-THC and other compounds mentioned throughout this article can be found in Fig. 2.

Activity

Two cannabinoid receptors are well described in the literature. Type I cannabinoid (CB1) receptors are concentrated in the central nervous system and are believed to be the cannabinoid receptor subtype involved in mood, somnolence, and hunger. Type II cannabinoid (CB2) receptors are primarily associated with the immune system and are involved in regulating inflammation and neuropathic pain.

With each new structural derivation of the THC molecule, sellers make new claims about the

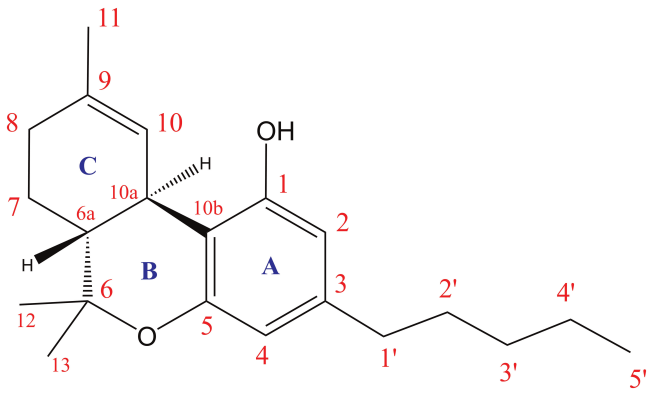


Fig. 1. Structure of Δ^9-tetrahydrocannabinol (Δ^9-THC) with carbon atoms labeled (in red) according to the dibenzopyran numbering system, labeled fused rings (in blue), and indicating *trans* stereochemistry across the 6a,10a bond of the natural phytocannabinoid.

activity of their products. Some derivatives are marketed as being Δ^9-THC-like, other derivatives are marketed as being extremely potent, others proclaim unique "highs" differing in some way from the effects of Δ^9-THC, and others are marketed with the initially surprising claim of having stimulating properties. Most claims are based upon the initial experiences of the first few people who experimented with the previously uncharacterized chemical products, and these claims may be confounded by poor product purity or incomplete structural characterization of the products. Little scientific data exists detailing the activity of most of the individual Δ^9-THC derivatives. However, some generalized inferences can be made from studies of cannabinoid receptor binding and activation, although these binding studies are typically decades old using technologies that now appear dated.

To understand the proliferation of CBD-derived THC analogs and their effects, we need to have a basic understanding of the structure–activity relationships (SARs) of phytocannabinoids with Δ^9-THC as the archetypal compound for the family. A structural representation of Δ^9-THC is shown in Fig. 1. THC is comprised of an n-hexyl alkyl chain (carbons 1' through 5'), and 3 fused rings. The alkyl side chain is bonded to an aromatic A-ring with a phenolic hydroxyl group at the carbon-1 (C-1) position. A central pyran ring comprises the B-ring, differentiating THC from the open-ring isomer CBD. Despite the well-known psychoactivity differences between THC and CBD, the pyran ring is not considered essential to cannabinoid receptor affinity or activation; for example, the B-ring is lacking in some highly potent synthetic cannabinoids such as CP 47,497. The third fused ring, or C-ring, is a cyclohexene ring with a single methyl group (carbon-11) bound to carbon-9. SAR studies reveal 3 key structural components impacting cannabinoid binding affinities to the CB1 receptor: the allylic chain, the phenolic group at C-1, and the orientation of C-9 and functional groups attached to C-9 relative to the rest of the molecule.

Alkyl Chain

The first structural feature to consider is the lipophilic nature of the aliphatic chain bound to the A ring (numbered 1' through 5' in Fig. 1), and its impact on potency. Shortening the chain length reduces the potency of the compound. As a primary example, Δ^9-tetrahydrocannabivarin (Δ^9-THCV) differs from Δ^9-THC only by having a shorter aliphatic chain length of 3 carbons. That structural change dramatically reduces the binding affinity of Δ^9-THCV to the CB1 receptor, imparting Δ^9-THCV with properties of a CB1 antagonist capable of blocking the effects of other phytocannabinoids and endocannabinoids (3). In contrast, increasing the lipophilic nature of the chain increases the potency of the cannabinoid. The compound Δ^9-tetrahydrocannabiphorol (Δ^9-THCP) (Fig. 2) possesses a linear aliphatic chain consisting of 7 carbon atoms (2 longer than THC). Allegedly, Δ^9-THCP possesses 30× the potency of Δ^9-THC as a result of the longer aliphatic chain. Both Δ^9-THCV and Δ^9-THCP occur naturally in cannabis as minor (Δ^9-THCV) and trace (Δ^9-THCP) constituents. However, CBD-derived versions of both compounds (in addition to the 6-carbon version Δ^9-tetrahydrocannabihexol (Δ^9-THCH) possessing ~10× the potency of Δ^9-THC) are sold in concentrated forms but are marketed very differently. THCH and Δ^9-THCP are sold for the intense high resulting from their significantly higher affinity for CB1 receptors. Δ^9-THCV on the other hand is being sold specifically for the pharmacological effects of a CB1 receptor antagonist. Δ^9-THCV is touted as a treatment for ADHD and as an appetite suppressant. Users report that Δ^9-THCV provides a stimulation effect free from the negative side effects associated with traditional stimulant medications. The claims surrounding Δ^9-THCV have not been approved by the FDA, but the limited research that is available is intriguing and warrants additional studies.

To date, compounds where the basic structure of THC has been modified at the alkyl sidechain are not major players in the market of CBD-derived phytocannabinoids analogs compared with the dominant class of C-ring modified THC analogs (discussed later). Perhaps the lower prevalence of marketed alkyl derivatives is due to the increased complexity of the synthetic chemistry required to produce these compounds from a starting material of CBD.

C-1 Phenol

The second structural feature relevant to the activity of phytocannabinoids and their derivatives is the phenolic hydroxyl group at position 1 of the A-

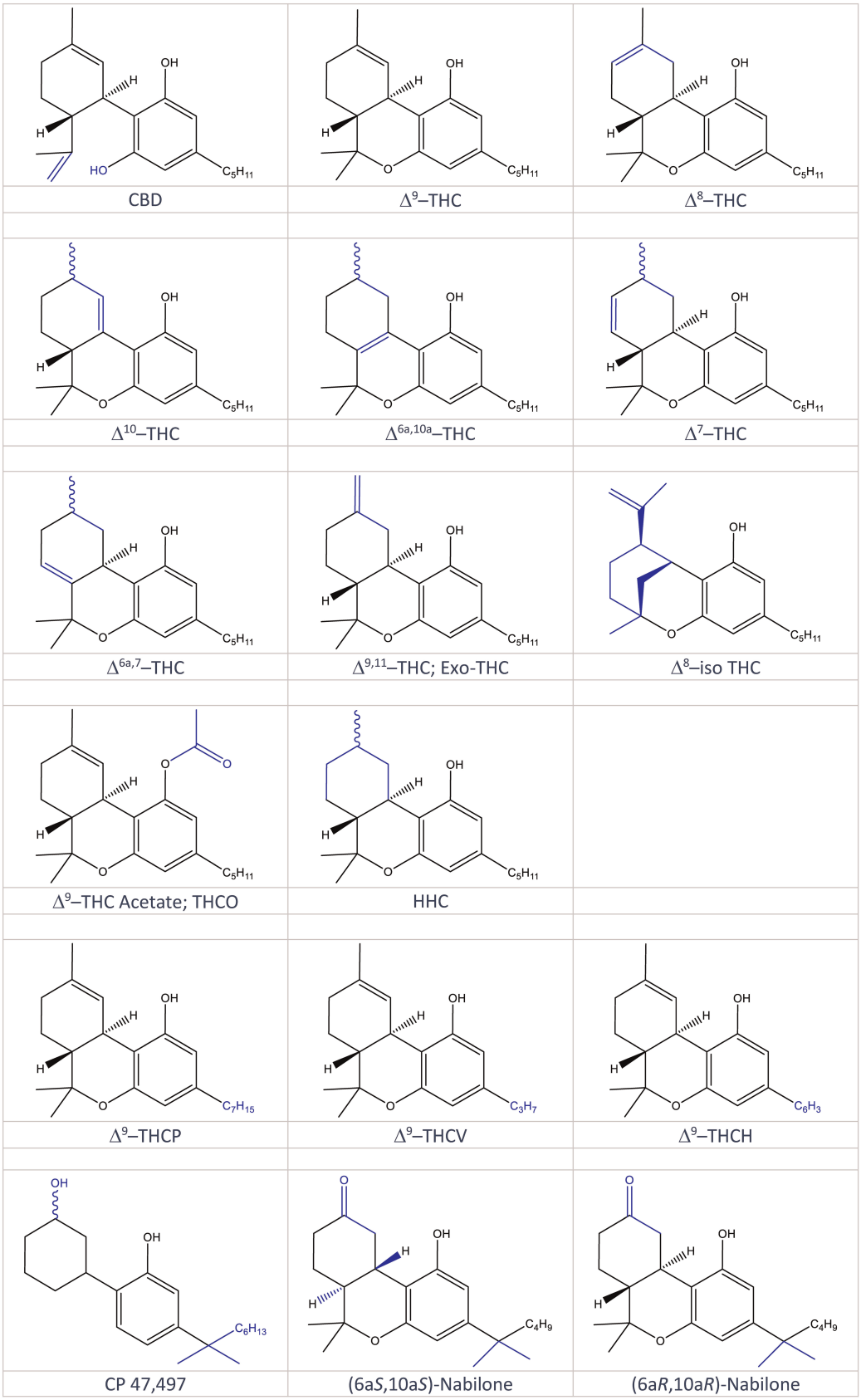
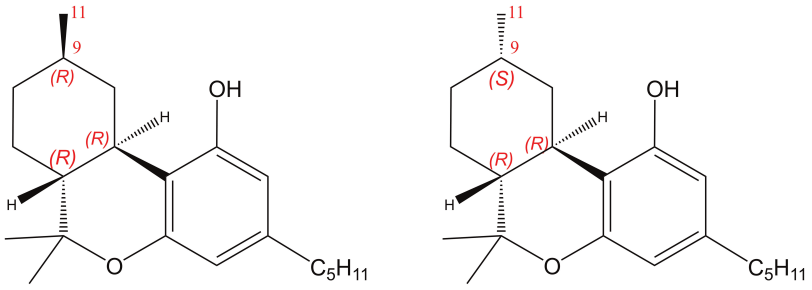


Fig. 2. Representative structures of phytocannabinoids and derivatives; differences from Δ^9-tetrahydrocannabinol (Δ^9-THC) are highlighted in blue.



(9R)-HHC (9S)-HHC

Fig. 3. Structure of hexahydrocannabinol (HHC) illustrating the two stereoisomers at C-9 and the implications on the position of C-11. Shown on the left is the active (9*R*)-HHC; on the right is the inactive (9S)-HHC isomer.

ring. Alterations to the phenolic hydroxyl group can result in dramatic differences in selectivity, and phenolic modifications have been shown to independently alter a compound's binding affinity to CB1 versus CB2 receptors (4). For the CBD-derived THC derivatives frequently encountered today, acetate conjugates are the primary example of this structural modification. The chemistry required to acetylate the phenolic hydroxyl is relatively simple, allowing these derivatives to be easily produced. Derivatives of both Δ^8- and Δ^9-THC acetylated at the C-1 position are widely available in many areas and sold as vapes and gummies labeled as THCO, THCO-Ac, or THCO acetate.

Users of acetylated THC describe a more intense and psychedelic experience compared with THC; THCO is often stated as being 3× the potency of THC. If that assessment is accurate and not overly impacted by the myriad of uncontrolled factors confounding user reports, the reason for a different user experience is not totally clear. Minimal pharmacological data on the acetylated phenol derivatives exist. The presence of the acetate group could alter CB1 receptor activation; early SAR studies indicated the acetate derivatives retained activity while other derivatives at this position were inactive. Additionally proposed is the idea that the acetate group improves the absorption and bioavailability of the active compound. In this hypothesis, THCO is a pro-drug being activated when converted to THC within the body. In this hypothesis, THC acetate could extend the half-life of THC by protecting the molecule from first-pass metabolism. While this hypothesis is plausible when comparing the effects of edible forms of THCO acetate versus THC, the hypothesis is less logical when pondering inhaled drugs (smoked or vaped) as first-pass metabolism is not applicable to this route of administration.

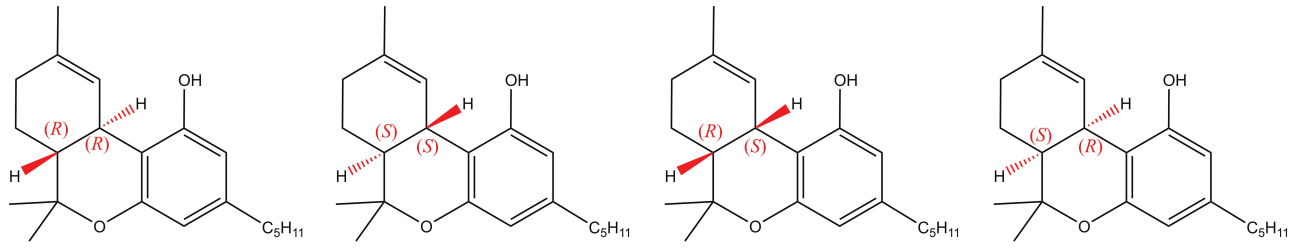
C-Ring and Carbon 11

The third structural feature involved in the SAR of Δ^9-THC is the position of carbon-11 off of the C-ring. Plant biosynthesis fuses the B and C rings of THC across carbons 6A and 10A in a *trans* configuration. This orientation and the existence of a double bond between C9 and C10 restricts the structural conformations of the C-ring with a half-chair conformation the most energetically favorable. In this conformation, C-11 of Δ^9-THC and Δ^8-THC are roughly planar to the B-ring (5).

Movement of the double bond from the 9 position to the 8 position alters electronic spatial interactions between the phenolic hydroxyl and the allylic proton of C-10 and alters the conformation of the C-ring. Thus, the relative orientation of C-11 is slightly altered in Δ^8-THC; this minor shift in the position of C-11 may explain the lower potency of Δ^8-THC compared with Δ^9-THC.

The orientation of C-11 can move more dramatically if the double bond in the C-ring does not involve C-9, or the double bound is removed altogether (as is the case for hexahydrocannabinol [HHC]). These structures are saturated at C-9 that creates an additional chiral center at C-9; meaning, C-11 can be in either an *R* or *S* configuration (see Fig. 3). According to the SAR studies, equatorial conformations are expected to retain activity, while axial conformations have significantly decreased activity. If your organic chemistry is rusty, axial and equatorial conformations can be, respectively, oversimplified to roughly perpendicular and roughly parallel to the general plane of the ring.

The existence of C-9 stereoisomers present in Δ^9-THC analogs other than Δ^8-THC may help explain the conflicting information concerning the activity of analogs where C-11 may be in an R or S conformation. Due to restrictions the double bond places on the C-ring conformation, moving or removing the double bond can change the lowest energy conformation of the C-ring away from the half-chair conformation seen with Δ^8-THC and Δ^9-THC. Changing the shape of the C-ring can alter the relative position of C-9 stereoisomers. Meaning, axial and equatorial conformations of C-11 may not neatly align with R and S configurations. However, it is clear that statements concerning the activity of C-9–saturated analogs are imprecise if those claims



- trans (6aR,10aR)- 9-THC Natural Confirmation

+ trans (6aS,10aS)- 9-THC

cis (6aR,10aS)- 9-THC

cis (6aS,10aR)- 9-THC

Fig. 4. Structure of the 4 Δ^9-tetrahydrocannabinol (Δ^9-THC) isomers across the 6a,10a bond fusing the B and C-rings.

do not specify the stereochemistry of C-9. This fallacy appears to have impacted some early investigations into the potency of C-9–saturated THC analogs and also impacts the claims made by users and manufacturers of HHC, Δ^{10}-THC, and other C-9–saturated derivatives. Only studies using isometrically pure analogs will be able to answer these questions. Current synthetic pathways used to create consumer C-9–saturated analogs do not appear capable of producing pure isomers at C-9, but a stereochemical preference may be present in some synthetic pathways.

While the stereochemistry of C-11 relative to C-9 greatly alters potency, CB1 receptor activity does not require a methyl group attached to C-9. The methyl group can be absent or other functional groups can take the place of C-11 and the compounds retain activity. For example, the synthetic CB receptor agonist nabilone replaces C-11 with a ketone functionality attached to C-9. Additionally, the 11-hydroxy metabolites of Δ^9-THC are known to be active. For now, there does not appear to be a significant presence of CBD-derived consumer products where the C-11 methyl group has been replaced with another functional group.

The stereochemistry of the fusing of the B and C-rings is another interesting feature of the SARs of cannabinoids. The plant exclusively fuses these 2 rings with both C-6A and C-10A in an *R* configuration. As a result, the bond between 6A and 10A is in a *trans* configuration (see Fig. 4). The *cis* configuration removes CB receptor activity, yet the opposite *trans* configuration (6a*S*, 10a*S*) seen in nabilone maintains activity. Additionally, the derivative $\Delta^{6a,10a}$–THC is without stereochemistry at these 2 locations, yet the 9*R* isomer of $\Delta^{6a,10a}$–THC is believed to maintain activity. These alternate configurations of Δ^9-THC at the 6a,10a bond have not been fully explored.

Legal Status of CBD-Derived THC Analogs

Since the inception of the Controlled Substance Act in 1970, marijuana and all phytocannabinoids have been classified as Schedule I drugs with a high potential for abuse and no legitimate use. Passage of the 2018 Farm Bill legalized naturally occurring phytocannabinoids except Δ^9-THC, and that federal protection extends to Δ^8-THC. The US Drug Enforcement Agency (DEA) affirmed this stance as it pertains to Δ^8-THC in a letter dated September 15, 2021 (6). However, that document does not provide any clarity to the legal status of other CBD-derived THC isomers where natural origins within hemp remain disputable, such as Δ^7-THC, HHC, etc. Similarly, the DEA has not released guidance addressing Δ^9-THCV or Δ^9-THCP. However, these compounds are clearly naturally occurring phytocannabinoids, and appear to fall under the protection of the 2018 Farm Bill. However, the protection provided by the Farm Bill to natural phytocannabinoids could quickly change as the Farm Bill must be renewed every 5 years, which occurs in 2023. The renewal could provide Congress the opportunity to alter or clarify the rules of the Farm Bill as they apply to phytocannabinoids.

Until earlier this year, there was no clear guidance on the legal status of the acetate derivatives of Δ^9-THC or Δ^8-THC. If Δ^9-THCO acetate is a prodrug biologically converted to a Schedule I compound (Δ^9-THC), the acetate derivatives appear to run afoul of DEA scheduling rules stating a prodrug of a scheduled compound is also scheduled. Additionally, neither compound naturally occurs within cannabis; as such, the acetate derivatives are not protected by the language of the Farm Bill. Using that rationale, the DEA released its position stating acetate esters of both Δ^8-THC and Δ^9-THC are Schedule I controlled substances in a letter dated February 13, 2023 (7).

Impact of CBD-Derived THC Analogs on Analytical Toxicology

From an analytic perspective, CBD-derived cannabinoids are a newly realized complexity to toxicology. Only a few years ago, the term "THC" unambiguously indicated Δ^9-THC. Now the THC

isomers commercially produced from CBD seem to endlessly proliferate; when including stereoisomers around the C-9 position there are 11 isomers where only the C-ring is modified; if you factor in potential isomers involving the stereochemistry of carbons 6a and 10a the isomer count jumps to 30. Many of these share similar or matching fragmentation patterns via both electron impact ionization (for gas chromatography–mass spectrometry analysis) and collision-induced fragmentation (used in most liquid chromatography–mass spectrometry applications). Chromatographic separation of the isomers adds significant complexity and time to analysis. For better or for worse, the *R* and *S* isomers of the C-9–saturated compounds easily resolve from each other via high-performance liquid chromatography; meaning each isomer must be accounted for in the analysis. Even when intending to measure 1 or 2 cannabinoids in an assay, the others must be evaluated as potential sources of assay interference.

When analyzing cannabinoid metabolites in biological samples we enter into an unknown world. Human metabolism studies for cannabinoids other than Δ^9-THC and CBD do not exist. In some cases it appears safe to assume the metabolites will be similar to those of Δ^9-THC; that is, a C-11 hydroxy metabolite, a C-11 carboxy metabolite, and subsequently a phase II glucuronide conjugate, 11-carboxy Δ^9-THC-glucuronide exist. Such is the case for Δ^8-THC. However, parallel metabolism may not be a safe assumption for other CDB-derived THC analogs. From a chemical perspective, saturating C-9 removes the energetic driving force to oxidize C-11. However, enzymatic processes within the cytochrome P450 system may overcome the energetic disadvantage and generate familiar-looking metabolites. If so, the other THC isomers may result in 11-hydroxy, 11-carboxy, and glucuronide conjugated 11-carboxy metabolites. Alternatively, these compounds may preferentially skip Phase I metabolism, and the parent compounds could undergo glucuronidation at the C-1 hydroxyl position. Alternatively, the changes in the molecular structures could direct metabolic processes to target other regions of the molecules, such as oxidizing the alkyl chain or sites within the C-ring. This variable includes the possibility that the metabolic fate of THC analogs may differ based on the confirmation of different stereoisomers; in other words, (9*R*) Δ^{10}-THC may result in different metabolites than the (9*S*) isomer of Δ^{10}-THC. As human metabolism studies are unlikely to occur with most of these derivatives, we will be able to elucidate the human metabolism of these compounds only through careful examination of case studies. Until that time, analytical toxicology is handicapped by an ever-expanding number of compounds of wildly varying CB receptor activity and with unknown metabolic fates.

References

1. Hanuš LO, Meyer SM, Muñoz E, Taglialatela-Scafati O, Appendino G. Phytocannabinoids: a unified critical inventory. Nat Prod Rep 2016;33:1357–92.
2. Meehan-Atrash J, Rahman I. Novel Δ8-tetrahydrocannabinol vaporizers contain unlabeled adulterants, unintended byproducts of chemical synthesis, and heavy metals. Chem Res Toxicol 2022;3573–76.
3. Pertwee RG. The diverse CB1 and CB2 receptor pharmacology of three plant cannabinoids: delta9-tetrahydrocannabinol, cannabidiol and delta9-tetrahydrocannabivarin. Br J Pharmacol 2008;153:199–215.
4. Prandi C, Blangetti M, Namdar D, Koltai H. Structure-activity relationship of cannabis derived compounds for the treatment of neuronal activity-related diseases. Molecules 2018;23:1526.
5. Reggio PH, Greer KV, Cox SM. The importance of the orientation of the C9 substituent to cannabinoid activity. J Med Chem 1989;32:1630–35.
6. Boos TL. Drug Enforcement Administration. https://albop.com/oodoardu/2021/10/ALBOP-synthetic-delta8-THC-21-7520-signed.pdf (Accessed June 2023).
7. Boos TL. Drug Enforcement Administration. https://www.documentcloud.org/documents/23608864-dea-thco-response-to-kight (Accessed June 2023).

The author discloses salary/consultant fee from Labcorp and stocks/bonds in Labcorp.

Gregory C. Janis, MS, is Associate Vice President of Research and Development for Labcorp and leads a multi-site development group focused on toxicology testing. E-mail: janisg@labcorp.com.

Mass Spectrometry & Advances in the Clinical Lab: Conference Recap

By Bridgit O. Crews, PhD, DABCC (CC, TC)

The annual conference for Mass Spectrometry & Advances in the Clinical Lab (MSACL) took place in the picturesque coastal town of Monterey, CA, from April 2–7. This conference brings together experts and professionals from the field of mass spectrometry and clinical laboratory medicine to discuss the latest clinical advancements and current analytical challenges. The conference kicked off on Monday with a series of short courses on specific topics ranging from R applications and dashboards to LC-MS/MS techniques and sample preparation. These courses provide participants with practical information and tips and are generally relevant to both research and clinical practice.

On Tuesday, the short courses continued, offering participants an opportunity to enhance their learning in their respective areas of interest. The official start of the conference was on Tuesday evening, with the presentation of the Michael S. Bereman Award and the MSACL Distinguished Contribution Award, in recognition of achievements made in the field of clinical mass spectrometry. A welcome reception followed, providing an opportunity to network and interact with fellow attendees.

Wednesday began with industry-sponsored workshops providing an opportunity to gain insight into the focus areas of interest for different companies. These events included brief presentations on current instruments and applications by both vendor scientists and end users.

The plenary sessions on Wednesday focused on research applications with a strong emphasis on ion mobility mass spectrometry. Ion mobility mass spectrometry is a rapidly evolving field that offers new possibilities for analyzing complex samples with high efficiency and accuracy. The plenary sessions featured renowned speakers who presented their latest research findings and discussed the future prospects of ion mobility mass spectrometry as well as current challenges.

Scientific sessions began on Wednesday and included a dedicated track on Toxicology/Therapeutic Drug Monitoring (TDM). These sessions covered a wide range of topics, such as the evaluation of analytical techniques for drug checking, therapeutic drug monitoring of venlafaxine and its metabolites, and optimizing TDM for patients with adenine phosphoribosyl transferase deficiency, a rare inborn error of purine metabolism producing elevated levels of insoluble 2,8-dihydroxyadenine (DHA) and resulting in kidney stone formation and crystal-induced kidney damage. Treatment includes allopurinol or febuxostat, but some patients do not respond. The speaker presented an LC-MS/MS method to monitor both drugs and DHA.

In the afternoon, a session in the Toxicology/TDM track focused on tackling tough issues in toxicology LC-MS/MS method development, including the selection of what drugs to include, whether or not to include hydrolysis and appropriate measuring ranges. This session sparked lively discussions among participants sharing insights on method development challenges in toxicology-focused LC-MS/MS. A separate afternoon session addressed clinical LC-MS/MS user training and the need for more focused training and certification options for individuals involved in this high-complexity area of the lab.

All sessions continued on Thursday, and of particular interest to toxicologists on this day was a practical training track. This track included several extended sessions, starting with pediatric toxicology testing strategies: A case-based discussion. The following sessions delved into practical considerations for sample preparation selection, evaluating the trade-offs between sensitivity, cost, and turnaround time. The final session focused on the interpretation of urine drug testing results. Parallel tracks included presentations on the quantitation of therapeutic monoclonal antibodies.

Throughout the 3-day conference, there were many toxicology-related posters spread among vendor exhibits with multiple opportunities to meet new people, exchange information, and discuss clinical and analytical challenges. MSACL will return to Monterey, CA, on March 17–24, 2024. This meeting offers educational grants and financial assistance for conference registration fees. These include young investigator grants, but also trainee grants and lab director grants for individuals that may have little background in mass spectrometry but are interested in learning how to apply this technology in their own laboratory (1).

Reference

1. MSACL. https://www.msacl.org (Accessed April 19, 2023).

The author has nothing to disclose.

Bridgit O. Crews, PhD, is an associate professor at Washington University in St. Louis and chair of the CFTN *Editorial Advisory Board. E-mail: bridgit@wustl.edu.*

Clinical & Forensic Toxicology News provides practical and timely information on the clinical, forensic, technical, and regulatory issues faced by toxicology laboratories. Each issue includes articles authored by experts.

Clinical & Forensic Toxicology News is an educational service of the Forensic Urine Drug Testing (FUDT) Accreditation Program. Cosponsored by the American Association for Clinical Chemistry and the College of American Pathologists, the program includes three components: FUDT accreditation, the FUDT proficiency testing survey, and this newsletter. The accreditation program is the responsibility of CAP. The surveys are sponsored jointly by AACC and CAP. The digital newsletter is published quarterly by AACC, 900 Seventh St., N.W., Suite 400, Washington, DC 20001, (800) 892-1400 or (202) 857-0717. Email: CFTNews@aacc.org.

Clinical & Forensic Toxicology News does not accept advertising and is supported solely by its readers. The 2023 annual subscription price is $92, $65 for AACC members. To subscribe, email custserv@aacc.org.

Opinions expressed are those of the authors and do not represent the position of AACC or CAP.