

Δ8-THC Impact on Non-Regulated Marijuana Confirmation Testing Rates

David Kuntz, Sarah Small, Tobin Kocour, Martin Jacques, Melissa Beals, Barbara Rowland, Brett Oswald, Brittany DeWitt. Clinical Reference Laboratory (CRL), Lenexa, Kansas.

INTRODUCTION

Over the last several years, the use of Δ8-THC products has been documented as a legal alternative to Δ9-THC. The initial widespread use of Δ8-THC began around 2020 and has continued to grow even with legalization of marijuana for recreational and medical purposes. Our laboratory developed what turned into a series of LC-MS/MS methods to adequately separate Δ8-Carboxy-THC (Δ8-COOH-THC) and Δ9-Carboxy-THC (Δ9-COOH-THC); maintaining acceptable resolution between analytes required adjustments as each method was challenged with concentrations of Δ8-COOH-THC soaring into the thousands of nanograms per milliliter.

Figure A: Δ9-THC and Δ8-THC Structures

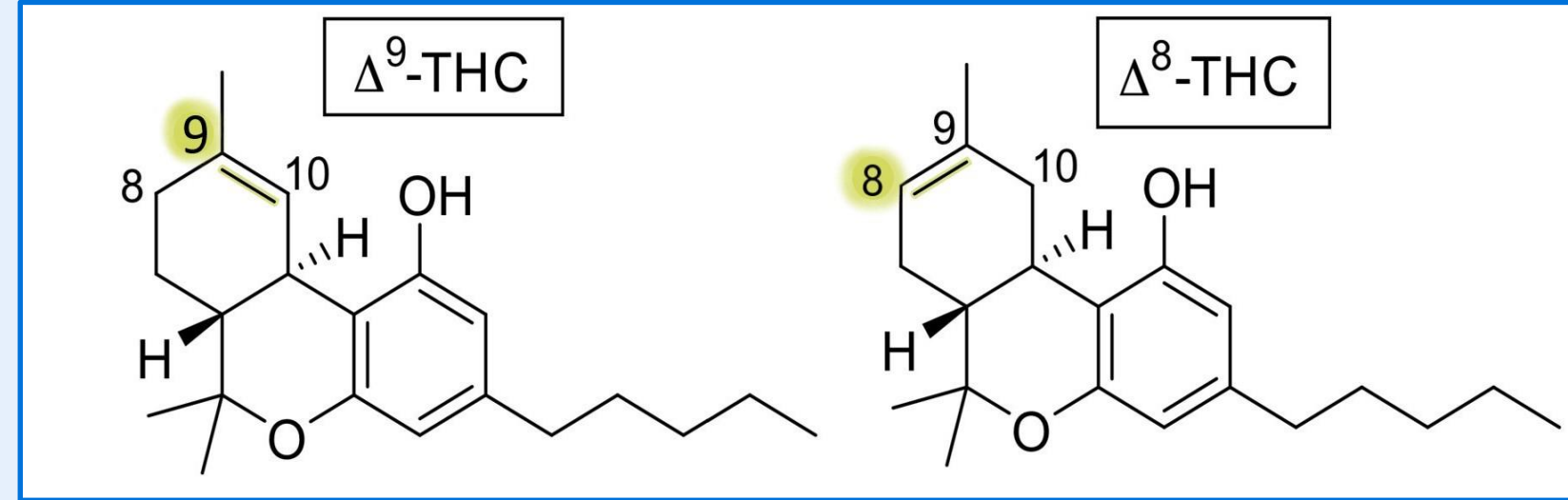
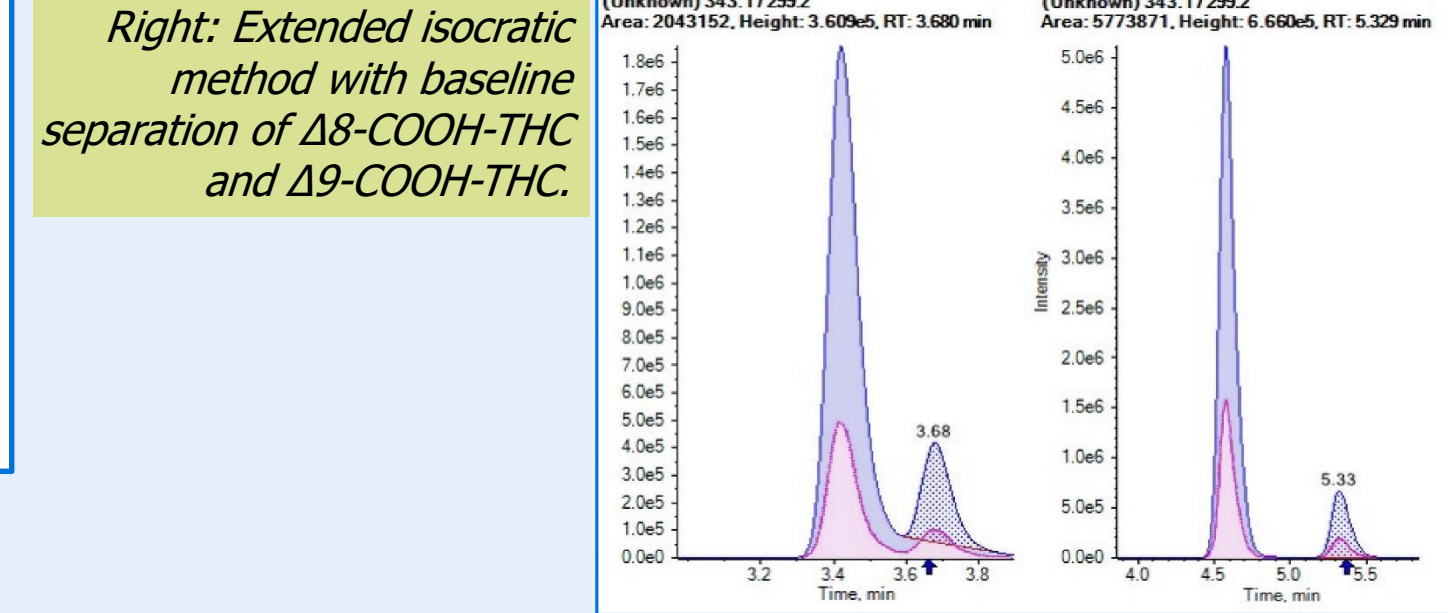


Figure B: Left: Sample chromatogram from standard Δ9-COOH-THC analysis exhibiting Δ8-COOH-THC interference; Right: Extended isocratic method with baseline separation of Δ8-COOH-THC and Δ9-COOH-THC.



OBJECTIVE

The main goals of this study were to determine the prevalence of Δ8-COOH-THC in non-regulated workplace drug testing specimens, and reveal the impact of Δ8-COOH-THC on the confirmation rate for samples that screened positive by immunoassay.

METHODS

In April of 2023, a total of 1,455 urine specimens were initially screened by immunoassay as part of the donor's drug testing panel and then confirmed by LC-MS/MS. The confirmation assay was validated in accordance with National Laboratory Certification Program guidelines for federal drug testing, including interference studies involving 11-Hydroxy-THC, Cannabinol, Cannabidiol, and 126 other drugs. (See Tables 1 and 2 for UHPLC-MS/MS parameters other analytical method information, and Figure C for chromatography acceptance criteria.) The laboratory collected Δ8-COOH-THC and Δ9-COOH-THC ions for all specimens with quantitative results for both. The data were correlated with the reason for test, state of collection, and the ultimate reporting result for marijuana and other associated drugs (such as cocaine, methamphetamine, benzodiazepines, etc.). Results for Δ8-COOH-THC were only reported to the client when requested as part of the drug testing panel.

Instrument Parameters

Table 1: UHPLC-MS/MS Parameters

UHPLC System	Shimadzu Nexera	LC-30AD Pumps SIL-30AC Autosampler
Injection Volume	20 µL	
Analytical Column	Phenomenex Kinetex® 2.6µm C18 100Å, 150 x 2.1mm (00F4462-AN)	
Guard Column	Phenomenex SecurityGuard™ ULTRA Cartridges, UHPLC C18 2.1mm (AUJ-8782)	
Column Temp.	50°C	
Mobile Phase	A: 2mM (NH ₄)HCO ₃ in H ₂ O w/0.02% Acetic Acid B: Acetonitrile C: Methanol	
Flow Rate	0.600 mL/min	
Run Time	5.5 minutes	
Mass Spectrometer	Sciex Triple Quad 6500, QTRAP 6500	
Ionization	Source Type: Electrospray Ionization (ESI) Negative	
Source Temp.	650°C	
Data Analysis	MultiQuant by Sciex	

Table 2: Analyte Transitions and Elution Order

Analyte	Internal Standard	Precursor Ion	Product Ion Quantifier	Product Ion Qualifier	Elution Order
Δ9-COOH-THC	Δ9-COOH-THC-D9	343.1	299.2	245.1	Peak 4
Δ9-COOH-THC-D9		352.1	308.2	254.1	Peak 3
Δ8-COOH-THC	Δ8-COOH-THC-D6	343.1	299.2	245.1	Peak 2
Δ8-COOH-THC-D6		349.1	305.2	251.1	Peak 1

Figure C: Acceptance Criteria for Chromatography

Relative Retention Time (RRT)	±2% of expected RRT of the analyte/internal standard pair established by the batch calibrator	
Internal Standard (IS) Response	Total IS peak area = ≥10% of calibrator IS peak area	
Symmetry / Peak Shape	Gaussian peaks; asymmetry at 10% of peak height = <3.0 for IS and quant. peaks	
Resolution	Adjacent peaks ≥90% resolved (± 10% valley/peak height ratio)	
Ion Ratios (Qualifiers)	Ratio of abundance of quantitative to qualifier ion = ±20% of target ratio established by batch calibrator	

RESULTS / DISCUSSION

All specimen data used was de-identified and detached from client affiliation for this study. Original results for Δ9-COOH-THC were reported to the client in accordance with their drug testing policy. Results for Δ8-COOH-THC were only reported in two instances. In past years, the Δ9-COOH-THC confirmation rate was nearly 100% based on screening positivity rates; however, the confirmation rate for one week in April 2023 fell to 83.6%. Specimens containing only Δ8-COOH-THC accounted for 10.3% of the "nonconfirming" samples. The remaining 6% included three samples with high levels of Cannabidiol (CBD), one specimen positive for Δ9-THC with no trace of the Δ9-COOH-THC metabolite, and seven samples that were negative for both metabolites, which may have contained other drugs known to cause false-positive immunoassay results (e.g. Protonix, Efavirenz) or alternative cannabinoids.

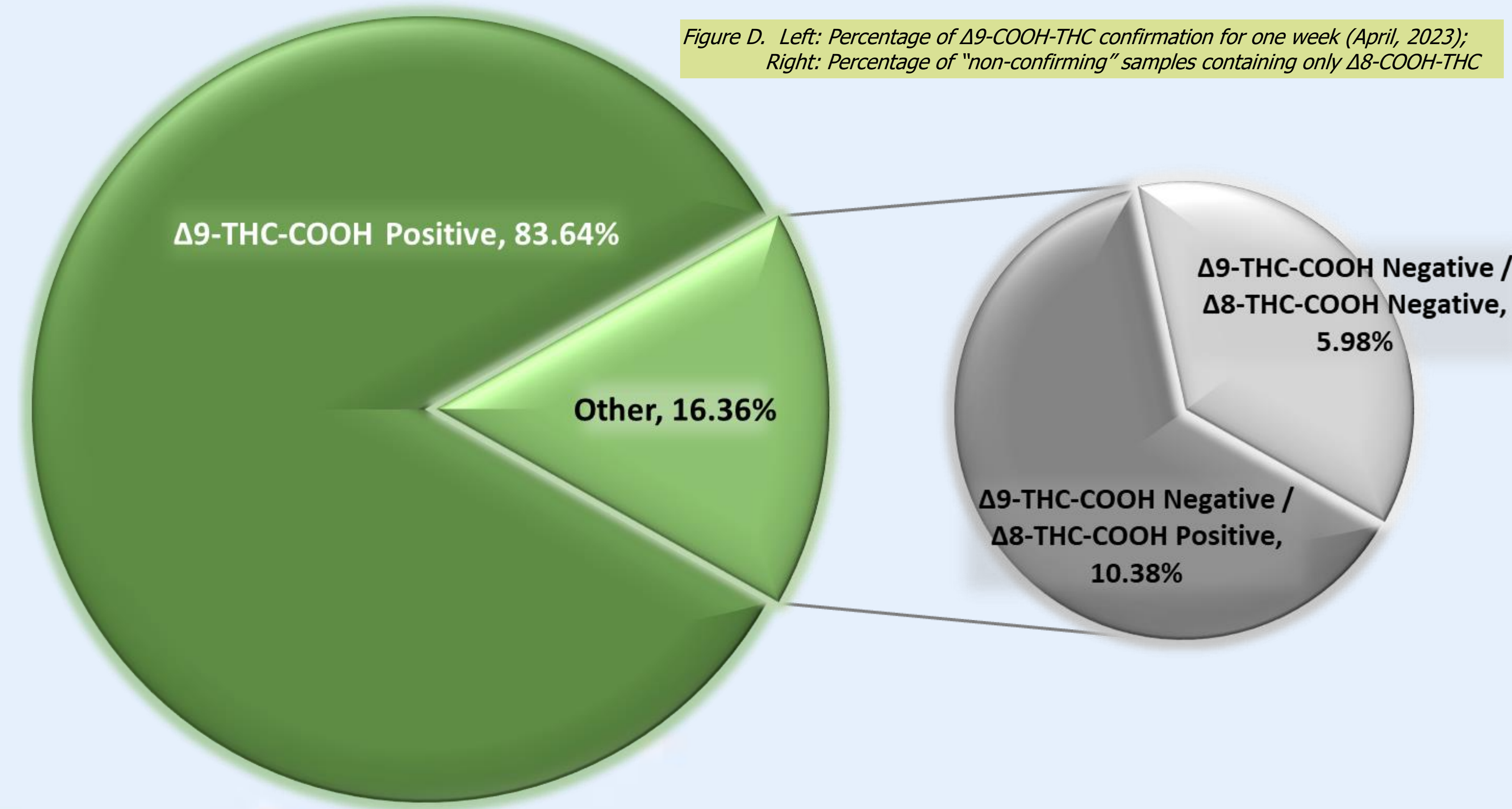
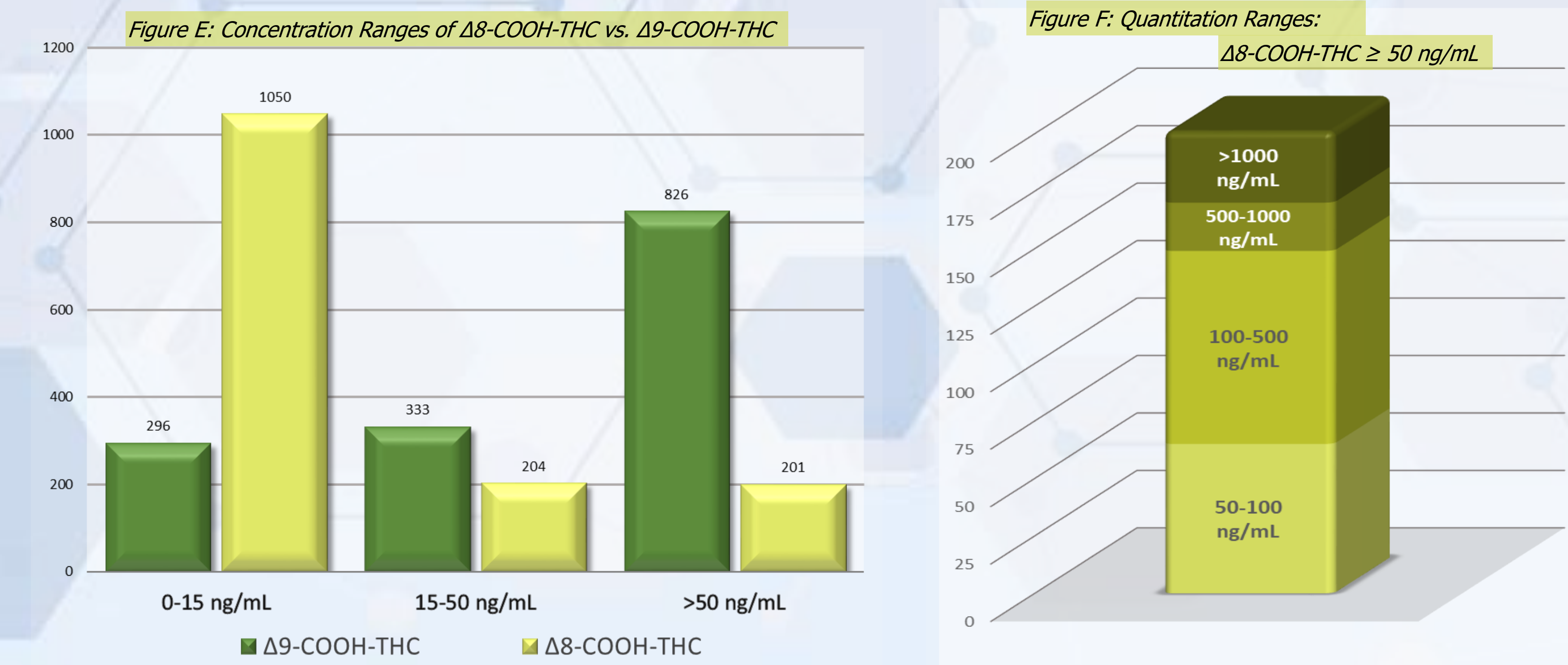


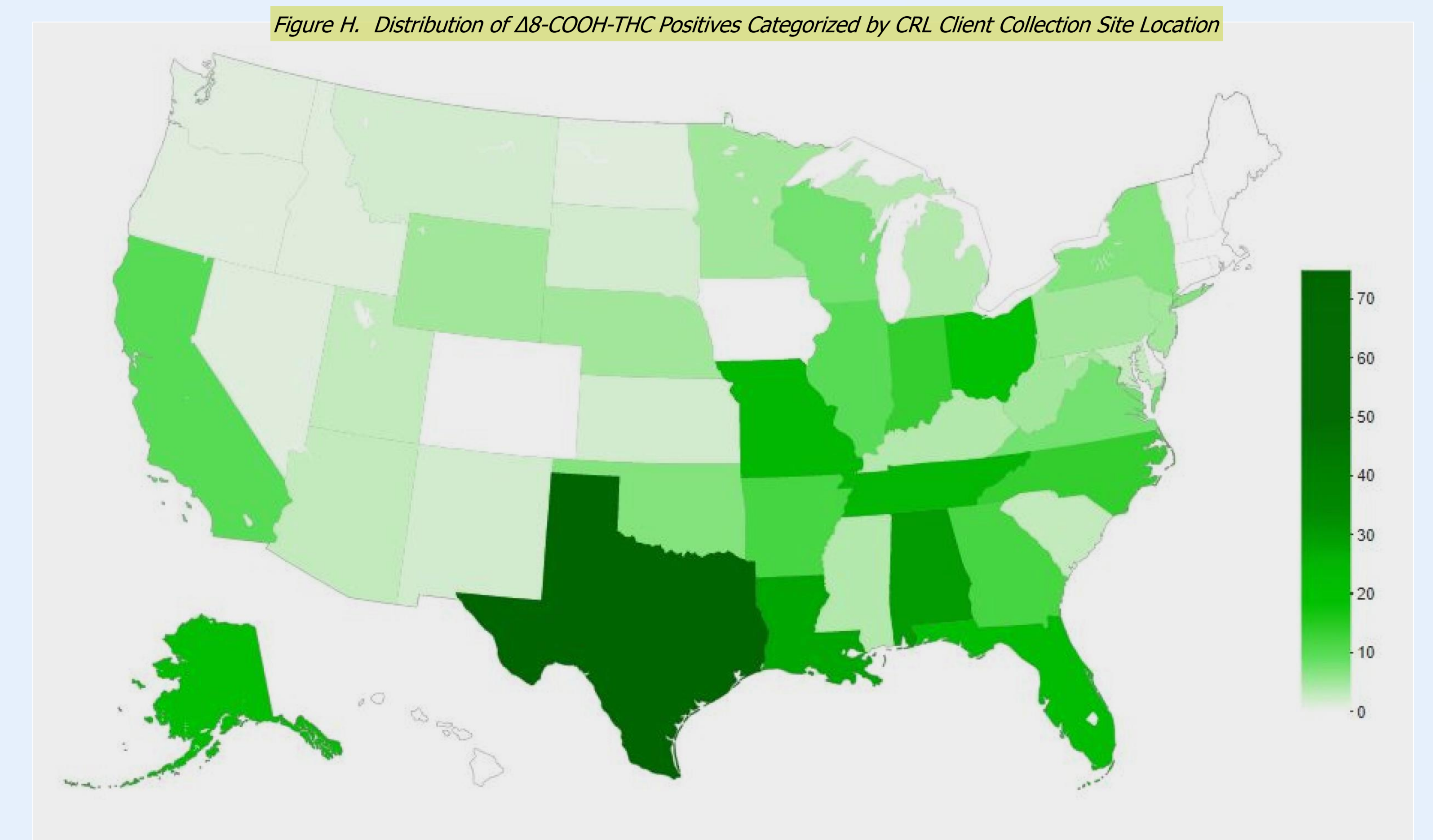
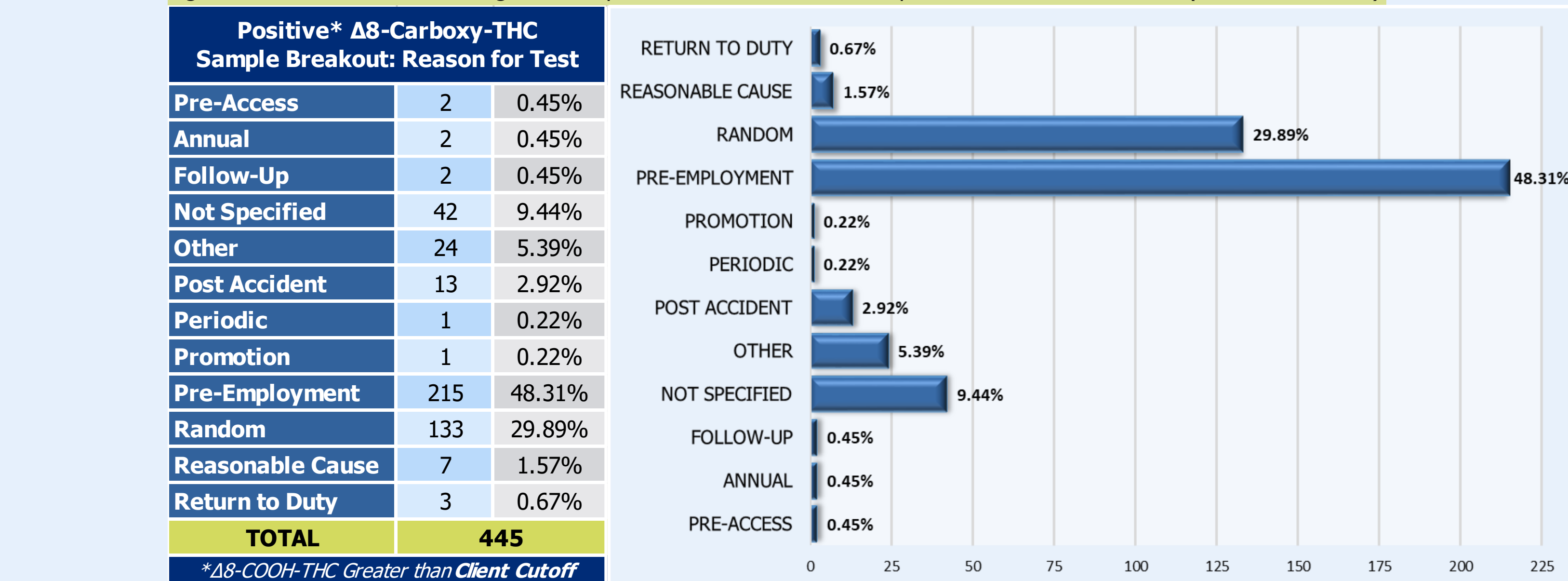
Figure D: Left: Percentage of Δ9-COOH-THC confirmation for one week (April, 2023); Right: Percentage of "non-confirming" samples containing only Δ8-COOH-THC

Overall, 31.2% of the confirmed samples had Δ8-COOH-THC concentrations greater than the cutoff of 15 ng/mL, with the highest levels exceeding 19,000 ng/mL. There were 445 total samples that had Δ8-COOH-THC levels greater than their respective client cutoffs; of these samples, 45.8% had Δ8-COOH-THC concentrations between 15-50 ng/mL, 14.6% were between 50-100 ng/mL, 18.9% were between 100-500 ng/mL, 4.7% were between 500-1000 ng/mL, and 7.0% of Δ8-COOH-THC positives had concentrations greater than 1,000 ng/mL. See Figure E below for concentration ranges of Δ8-COOH-THC compared to those of Δ9-COOH-THC for the 1,455 sample study selection; see Figure F for analysis of Δ8-COOH-THC quantitation ranges for samples with concentrations of 50 ng/mL and greater.

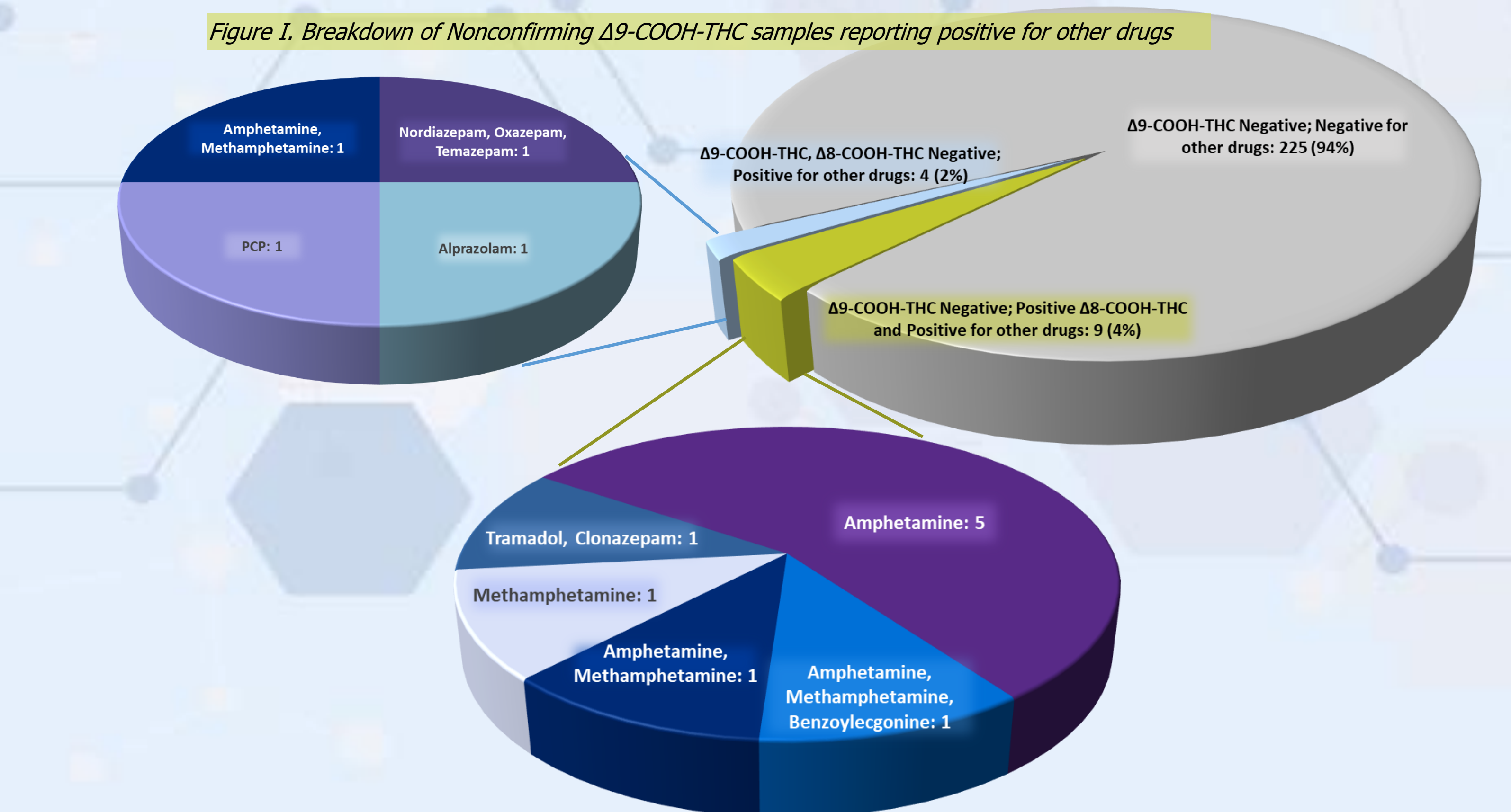


When evaluating by reason for test, collections for Pre-Employment had positive Δ8-COOH-THC greater than the reporting cutoff in 48.3% of the samples. Random urine collections were positive for Δ8-COOH-THC at a rate of 29.8%. Refer to Figure G for full breakdown of reasons for test for samples positive for Δ8-COOH-THC.

Figure G: Reasons for urine drug test for specimens with the observed presence of Δ8-COOH-THC (one week of data)



For the one-week total of 1,455 samples screening positive for Δ9-COOH-THC, 238 samples did not confirm Δ9-COOH-THC positive greater than client cutoff. Of those nonconfirming samples, 4% were positive for Δ8-COOH-THC and at least one other drug. In addition to the Δ8-COOH-THC positive results, 5 samples also tested positive for Amphetamine, and one each tested positive for the following: Alprazolam; PCP; Amphetamine and Methamphetamine; and Nordiazepam, Oxazepam, and Temazepam. The remaining 2% of Δ9-COOH-THC nonconfirming samples were also negative for Δ8-COOH-THC, but had the following positive results: Tramadol and Clonazepam; Methamphetamine; Amphetamine and Methamphetamine; and Amphetamine, Methamphetamine, and Benzoyllecgonine. See Figure I.



CONCLUSION

The data obtained in this April 2023 study are similar to a previous study in 2022; however, the amount of Δ8-Carboxy-THC appears to be increasing, as multiple samples had concentrations in the thousands of ng/mL. Due to similar psychological effects of Δ8-THC compared to Δ9-THC, impairment is substantial, a threat to public safety, and should be addressed in public and corporate policy. CRL is currently collecting ions for Δ8-COOH-THC during the analysis of all samples being confirmed for Δ9-COOH-THC. Not only does this data provide information for further studies and investigations, but results are potentially reportable based on client request.

DISCLOSURE
No relevant financial or nonfinancial relationships to disclose.

