

# Free and Glucuronide Whole Blood Cannabinoids' Pharmacokinetics after Controlled Smoked, Vaporized, and Oral Cannabis Administration in Frequent and Occasional Cannabis Users: Identification of Recent Cannabis Intake

Matthew N. Newmeyer,<sup>1,2</sup> Madeleine J. Swortwood,<sup>1</sup> Allan J. Barnes,<sup>1</sup> Osama A. Abulseoud,<sup>1</sup> Karl B. Scheidweiler,<sup>1\*</sup> and Marilyn A. Huestis<sup>1,3</sup>

**BACKGROUND:** There is increasing interest in markers of recent cannabis use because following frequent cannabis intake,  $\Delta^9$ -tetrahydrocannabinol (THC) may be detected in blood for up to 30 days. The minor cannabinoids cannabidiol, cannabinol (CBN), and THC-glucuronide were previously detected for  $\leq 2.1$  h in frequent and occasional smokers' blood after cannabis smoking. Cannabigerol (CBG),  $\Delta^9$ -tetrahydrocannabivarin (THCV), and 11-nor-9-carboxy-THCV might also be recent use markers, but their blood pharmacokinetics have not been investigated. Additionally, while smoking is the most common administration route, vaporization and edibles are frequently used.

**METHODS:** We characterized blood pharmacokinetics of THC, its phase I and phase II glucuronide metabolites, and minor cannabinoids in occasional and frequent cannabis smokers for 54 (occasional) and 72 (frequent) hours after controlled smoked, vaporized, and oral cannabis administration.

**RESULTS:** Few differences were observed between smoked and vaporized blood cannabinoid pharmacokinetics, while significantly greater 11-nor-9-carboxy-THC (THCCOOH) and THCCOOH-glucuronide concentrations occurred following oral cannabis. CBG and CBN were frequently identified after inhalation routes with short detection windows, but not detected following oral dosing. Implementation of a combined THC  $\geq 5$   $\mu\text{g/L}$  plus THCCOOH/11-hydroxy-THC ratio  $< 20$  cutoff produced detection windows  $< 8$  h after all routes for frequent smokers; no occasional smoker was

positive 1.5 h or 12 h following inhaled or oral cannabis, respectively.

**CONCLUSIONS:** Vaporization and smoking provide comparable cannabinoid delivery. CBG and CBN are recent-use cannabis markers after cannabis inhalation, but their absence does not exclude recent use. Multiple, complementary criteria should be implemented in conjunction with impairment observations to improve interpretation of cannabinoid tests.

Clinicaltrials.gov Identifier: NCT02177513

© 2016 American Association for Clinical Chemistry

Cannabis continues to be the most commonly abused drug worldwide (1) and the most frequently identified drug in cases submitted to state and local laboratories in the US (2).  $\Delta^9$ -Tetrahydrocannabinol (THC)<sup>4</sup>—the primary psychoactive constituent—showed the largest increase in US weekend nighttime driving prevalence from 8.6% in 2007 to 12.6% in 2013–2014 (3), furthering public health and safety concerns.

THC metabolism produces the active metabolite 11-hydroxy-THC (11-OH-THC) (4, 5) followed by oxidation to 11-nor-9-carboxy-THC (THCCOOH). Peak THC concentrations occur during smoking, with peak 11-OH-THC concentrations occurring close to smoking, while THCCOOH concentrations peak  $< 1$ –4 h later (6). THC, 11-OH-THC, and THCCOOH were detected in some chronic frequent cannabis smokers' blood up to 30, 3, and  $> 33$  days during sustained, mon-

<sup>1</sup> Chemistry and Drug Metabolism Section, IRP, National Institute on Drug Abuse, National Institutes of Health, Baltimore, MD; <sup>2</sup> Program in Toxicology, University of Maryland, Baltimore, Baltimore, MD; <sup>3</sup> University of Maryland School of Medicine, Baltimore, MD.

\* Address correspondence to this author at: Chemistry and Drug Metabolism, Clinical Pharmacology and Therapeutics Research Branch, NIDA-IRP, NIH, Biomedical Research Center, 251 Bayview Blvd., Baltimore, MD 21224. Fax 410-550-2971; e-mail kscheidweiler@intra.nida.nih.gov.

Received July 4, 2016; accepted September 6, 2016.

Previously published online at DOI: 10.1373/clinchem.2016.263475

© 2016 American Association for Clinical Chemistry

<sup>4</sup> Nonstandard abbreviations: THC,  $\Delta^9$ -tetrahydrocannabinol; 11-OH-THC, 11-hydroxy-THC; THCCOOH, 11-nor-9-carboxy-THC; CBD, cannabidiol; CBN, cannabinol; CBG, cannabigerol; THCV,  $\Delta^9$ -tetrahydrocannabivarin; THCVCOOH, 11-nor-9-carboxy-THCV; DPX, disposable pipette extraction;  $C_{\text{max}}$ , maximum concentration;  $t_{\text{max}}$ , time to  $C_{\text{max}}$ ;  $t_{\text{last}}$ , last detection time;  $\text{AUC}_{0 \rightarrow 54\text{h}}$ , AUC up to 54 h (occasional smokers);  $\text{AUC}_{0 \rightarrow 72\text{h}}$ , AUC up to 72 h (frequent smokers); LOQ, limit of quantification.

itored abstinence (7). THC's possible prolonged detection in frequent smokers' blood creates difficulties in result interpretation, including driving under the influence of drugs cases, since it may be detected beyond the window of acute impairment (8). Minor cannabinoids were evaluated as recent use markers (9, 10); THC-glucuronide, cannabidiol (CBD), and cannabinol (CBN) were detected in frequent smokers' blood up to 0.6, 0.5, and 2.1 h, respectively, after smoking a 6.8% THC, 0.25% CBD, and 0.21% CBN cigarette (10).

Other minor cannabinoids such as cannabigerol (CBG),  $\Delta^9$ -tetrahydrocannabivarin (THCV), and its metabolite 11-nor-9-carboxy-THCV (THCVCOOH) may also serve as recent use markers; however, their blood pharmacokinetics have not been characterized, with previous identification only in cannabis smokers' urine (11–13).

While smoking is the most common cannabis administration route, vaporization and consumption of cannabis edibles are common. A survey showed that 29.8% of US adults aged  $\geq 18$  years ever consumed cannabis via “edibles or drinks” and 9.9% used a “vaporizer or other electronic device,” respectively (14), indicating the importance of characterizing multiple cannabis administration routes.

An evaluation of the Volcano<sup>®</sup> Medic vaporization device for cannabis administration demonstrated THC delivery comparable to that from smoking (15). Following use of the device by occasional-to-moderate cannabis smokers, blood pharmacokinetics of multiple cannabinoids (16) were similar to previously published data after smoking (9, 10). Cannabinoid pharmacokinetic data following various oral administrations are available (17–23); administered daily doses range from 0.39 (18) up to 90 (24) and 120 mg (21) THC. Direct comparisons of cannabinoid whole blood pharmacokinetics, including minor cannabinoids, following vaporized or oral doses to those after smoked cannabis were never conducted.

The present study evaluated blood pharmacokinetics of THC, its phase I and phase II glucuronide metabolites, and minor cannabinoids in frequent and occasional smokers after smoked, vaporized, and oral cannabis administration. Participants received every cannabis dose to permit direct comparisons between administration routes. Minor cannabinoids and recommended and novel cutoffs were evaluated as indicators of recent cannabis intake.

## Materials and Methods

### PARTICIPANTS

Healthy adults between ages 18 and 50 years were recruited for this National Institute on Drug Abuse Intramural Research Program Institutional Review Board–, Federal Drug Administration–, and Drug Enforcement

Administration–approved study. Individuals received a comprehensive medical and psychological evaluation. Inclusion criteria included a mean self-reported cannabis intake frequency  $\geq 2\times$  per month but  $\leq 3\times$  per week (occasional smokers) or  $\geq 5\times$  per week (frequent smokers) over the previous 3 months and a positive urine cannabinoid screen (frequent smokers). Exclusion criteria included systolic blood pressure  $>140$  mmHg, diastolic blood pressure  $>90$  mmHg, or heart rate  $>100$  bpm; clinically significant electrocardiogram abnormality; inability to discontinue use of contraindicated medication; physical dependence on any drug other than cannabis, caffeine, or nicotine; medicinal cannabis use; medical condition or history of neurological illness; history of clinically significant cannabis adverse event; donation of  $>450$  mL blood within 8 weeks of study dosing; pregnant or nursing women; interest or participation in a drug abuse treatment program within 90 days of study dosing; and any history of food allergy or sensitivity to gluten, dairy, egg, soy, and/or chocolate. Individuals provided written, informed consent before being admitted to the study.

### STUDY DESIGN

The study was randomized, double-blind, and placebo controlled with a crossover and double-dummy design. Participants entered the secure research unit approximately 19 h before dosing to preclude acute intoxication. Cannabis cigarettes were obtained through the National Institute on Drug Abuse. Active cigarettes [0.734 (0.05) g] contained 6.9% (0.95%) (approximately 50.6 mg) THC, 0.20% (0.01%) (approximately 1.5 mg) CBD, and 0.44% (0.08%) (approximately 3.3 mg) CBN. Placebo cigarettes [0.713 (0.05) g] contained 0.001% (0.000%) THC, no detectable CBD, and 0.004% (0.000%) CBN. Throughout 4 dosing sessions, participants were administered one active or placebo cannabis-containing brownie followed by one active or placebo cigarette or one active or placebo vaporized ground cannabis dose (210 °C, Volcano Medic, Storz & Bickel). No more than one active dose was administered per session and the oral dose was followed by either smoking or vaporization in 2 sessions each. Participants consumed the oral dose ad libitum for 10 min followed by 10 min to consume the smoked or vaporized dose ad libitum. Frequent smokers remained on the unit up to 72 h postdose and were required to leave the unit for  $\geq 72$  h before next session admission to minimize acute withdrawal symptoms. Occasional smokers remained on the unit up to 54 h postdose but were allowed to remain on the unit for multiple sessions; they were not dosed more frequently than their self-reported intake frequency.

Oral cannabis doses were prepared with Duncan Hines<sup>®</sup> Double Fudge brownie mix according to the manufacturer's instructions for cake-like brownies. Indi-

vidual, equal portions of the wet batter were poured into a muffin container. The contents of either an active or placebo cigarette were ground, baked for 30 min at 121 °C in aluminum foil, and mixed into one individual portion. Following baking and cooling, individual doses were stored frozen, but allowed to thaw refrigerated overnight before dosing.

Venous blood was collected through an indwelling peripheral catheter into grey-top potassium oxalate (8 mg)/sodium fluoride (10 mg) Vacutainer<sup>®</sup> tubes (BD) on admission (−19 h); before initiation of smoking/vaporization (−1.5 h); at smoking/vaporization initiation ( $t = 0.00$  h), then every 2 min for 12 min ( $t = 0.20$  h); at 0.25, 0.50, 1.5, 2.5, 3.5, 5.0, 8.0, 10, 12, 14, 20, 26, 32, 38, 44, and 50 h after smoking/vaporization initiation for all participants; at 54 h for occasional smokers only; and at 56, 62, 68, and 72 h for frequent smokers only. Blood was aliquoted into 3.6 mL Nunc<sup>®</sup> cryotubes (Thomas Scientific) and stored at −20 °C until analysis.

#### WHOLE BLOOD ANALYSIS

Whole blood cannabinoids were quantified via a previously published LC-MS/MS method (25). Briefly, 0.2 mL whole blood was precipitated via acetonitrile, and cannabinoids were extracted with disposable pipette extraction (DPX) WAX-S tips (DPX Labs); a diluted aliquot of the organic phase was injected onto a 5500 QTRAP (SCIEX). Linear ranges were 0.5–100 µg/L for THC and THCCOOH; 0.5–50 µg/L for 11-OH-THC, CBD, CBN, and THC-glucuronide; 1–100 µg/L for CBG, THCV, and THCVCOOH; and 5–500 µg/L for THCCOOH-glucuronide. Interassay imprecisions were ≤8.5% CV and recovered concentrations were 88.9%–115% of target concentration.

#### DATA ANALYSIS

Differences in demographic data between groups were evaluated with  $t$ -tests. Noncompartmental pharmacokinetic analyses were performed with Phoenix<sup>®</sup> WinNonlin<sup>®</sup> 6.4 for Windows (Pharsight Software). Analyses of maximum concentration ( $C_{\max}$ ), baseline-adjusted  $C_{\max}$  (baseline concentrations subtracted from postdose  $C_{\max}$ ), time to  $C_{\max}$  ( $t_{\max}$ ), last detection time ( $t_{\text{last}}$ ), and AUC up to 54 h or 72 h ( $\text{AUC}_{0 \rightarrow 54\text{h}}$ , occasional smokers;  $\text{AUC}_{0 \rightarrow 72\text{h}}$ , frequent smokers) differences between administrations were performed with SPSS<sup>®</sup> Statistics 20 for Windows (IBM). For analytes that were detected after all administrations, differences were evaluated by repeated-measures ANOVA with data from frequent and occasional smokers analyzed separately. The Greenhouse–Geisser correction was used for sphericity violations. If a significant overall dose effect was observed, planned Helmert contrasts were performed; contrast 1 compares oral dosing to the combined inhaled doses, contrast 2 compares smoking and vaporization. For analytes only de-

tected after smoking and vaporization, paired-sample  $t$ -tests were conducted. Group differences were evaluated with separate repeated-measures ANOVA, with group included as a between-subject factor; if a significant dose–group interaction was observed group differences after each administration were evaluated with  $t$ -tests. Detection times and rates were determined at recommended and novel cutoffs. Missing data were excluded from statistical evaluations. Significance was attributed to a 2-tailed  $P < 0.05$ .

## Results

#### PARTICIPANTS

Table 1 summarizes 11 frequent and 9 occasional cannabis smokers' demographic information (ages 19–46 years, 75% male, 75% African American). Participant K was originally recruited as an occasional cannabis smoker, but reclassified as a frequent smoker because baseline and postdose THC and metabolite concentrations were consistent with published frequent smoker data (9, 10). Participant H reported last use approximately 10 days before session 1 admission, despite self-reporting smoking 5× per week during screening; the participant reported smoking within 0.6–18.7 h of admission to subsequent sessions. Occasional smokers began smoking at a significantly older age ( $P = 0.011$ ), smoked on a significantly fewer number of days out of the previous 14 ( $P < 0.001$ ), and smoked significantly less per smoking occasion ( $P = 0.021$ ).

#### PHARMACOKINETIC EVALUATION

Overall 1632 blood samples (940 frequent, 692 occasional) were collected. Because participant K was originally recruited as an occasional smoker, no specimens were collected from 56 to 72 h. Fig. 1 depicts concentration-time curves and Table 2 summarizes THC, 11-OH-THC, THCCOOH, THCVCOOH, and THCCOOH-glucuronide pharmacokinetic results. Time to  $C_{\max}$  for these analytes occurred significantly later after oral dosing than after inhaled doses in both groups (Table 2, Helmert contrast 1). THC  $C_{\max}$  was significantly lower after oral compared to inhaled doses within each group (frequent,  $P = 0.001$ ; occasional,  $P = 0.010$ ). Conversely, both groups' observed (frequent,  $P = 0.005$ ; occasional,  $P < 0.001$ ) and frequent smokers' baseline-adjusted THCCOOH  $C_{\max}$  ( $P = 0.025$ ) and frequent smokers' baseline-adjusted ( $P = 0.02$ ) and occasional smokers' observed ( $P = 0.004$ ) THCCOOH-glucuronide  $C_{\max}$  after oral dosing were significantly greater than after inhaled doses. 11-OH-THC  $C_{\max}$  ( $P = 0.016$ ) and  $t_{\text{last}}$  ( $P < 0.001$ ) after oral dosing was significantly greater and later in occasional smokers only. Significant differences between smoking and vaporization (Table 2, Helmert contrast 2) were only observed in fre-

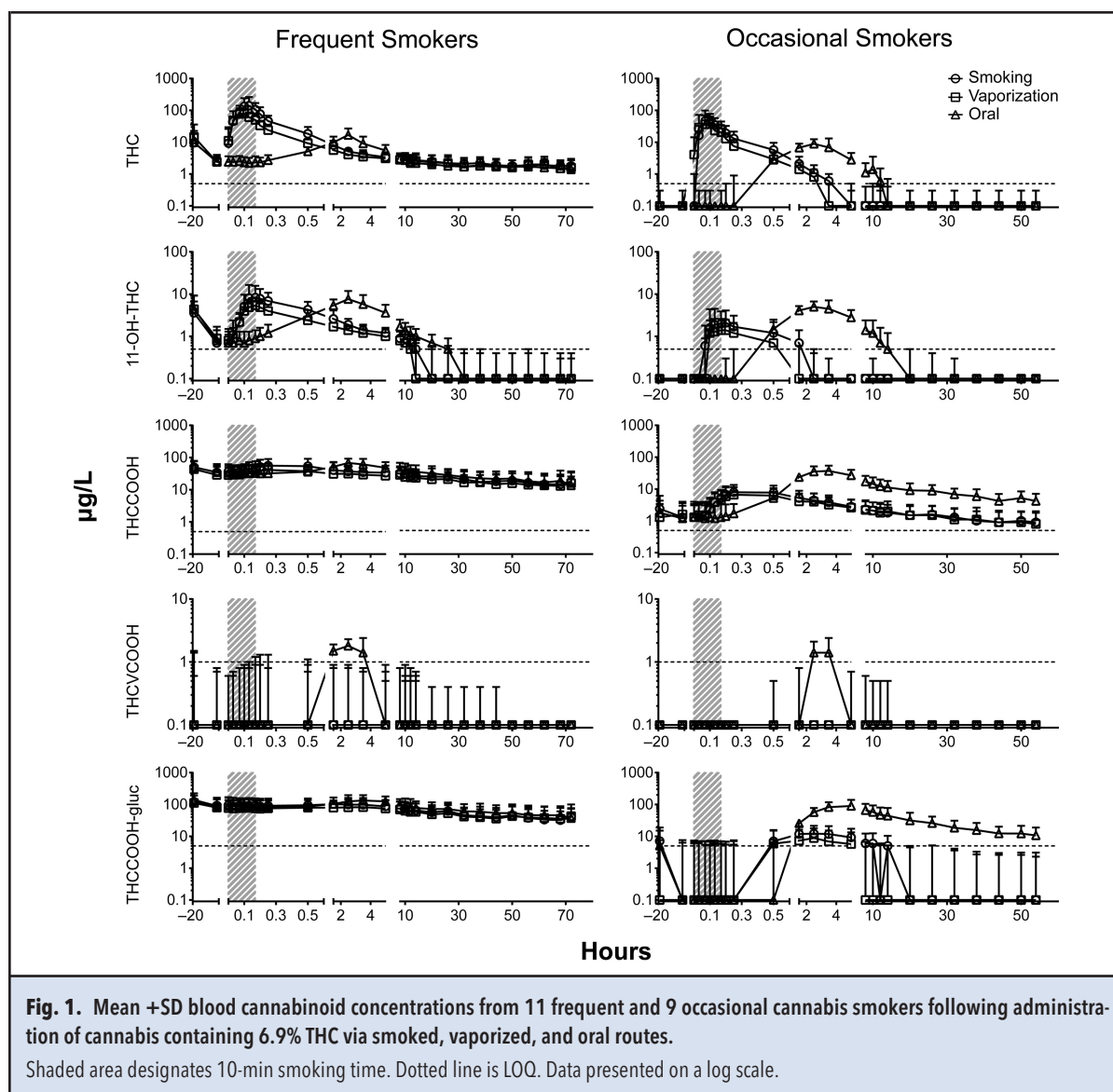
**Table 1.** Demographic data and cannabis smoking histories for 11 frequent and 9 occasional smokers.

| Participant        | Sex | Age, years | Race and ethnicity | BMI, kg/m <sup>2</sup> | Age at first use <sup>a</sup> | Lifetime years smoked <sup>a</sup> | Cannabis intake frequency <sup>a</sup> | Time between last use and admission <sup>b</sup> | Number of days used in last 14 <sup>b</sup> | Mean joint equivalents per smoking occasion <sup>b</sup> |
|--------------------|-----|------------|--------------------|------------------------|-------------------------------|------------------------------------|----------------------------------------|--------------------------------------------------|---------------------------------------------|----------------------------------------------------------|
| Frequent smokers   |     |            |                    |                        |                               |                                    |                                        |                                                  |                                             |                                                          |
| A                  | M   | 21         | AA <sup>c</sup>    | 26.5                   | 16                            | 5                                  | Daily                                  | 17.2 h                                           | 14                                          | 5                                                        |
| B                  | M   | 22         | AA                 | 31.0                   | 15                            | 7                                  | Daily                                  | 19.3 h                                           | 10                                          | 4                                                        |
| C                  | M   | 19         | AA                 | 19.8                   | 13                            | 6                                  | Daily                                  | 18.7 h                                           | 14                                          | 4                                                        |
| D                  | F   | 23         | AA                 | 31.9                   | 13                            | 10                                 | Daily                                  | 7.9 h                                            | 14                                          | 10                                                       |
| E                  | M   | 38         | AA                 | 32.2                   | 12                            | 26                                 | Daily                                  | 2.4 h                                            | 14                                          | 15                                                       |
| F                  | F   | 29         | AA                 | 31.0                   | 11                            | 18                                 | Daily                                  | 1.9 h                                            | 14                                          | 20                                                       |
| G                  | M   | 38         | AA                 | 22.0                   | 16                            | 22                                 | Daily                                  | 2.1 h                                            | 14                                          | 7                                                        |
| H                  | M   | 34         | AA                 | 23.0                   | 14                            | 20                                 | 5×/Week                                | 239.7 h <sup>d</sup>                             | 2 <sup>d</sup>                              | 2 <sup>d</sup>                                           |
| I                  | M   | 21         | AA                 | 25.0                   | 11                            | 10                                 | Daily                                  | 0.7 h                                            | 14                                          | 5                                                        |
| J                  | M   | 25         | AA                 | 19.0                   | 13                            | 12                                 | 5×/Week                                | 5.8 h                                            | 14                                          | 2                                                        |
| K                  | M   | 31         | AA                 | 16.8                   | 15                            | 16                                 | 2–3×/Week <sup>e</sup>                 | 5.1 h <sup>e</sup>                               | 4 <sup>e</sup>                              | 2.5 <sup>e</sup>                                         |
| Mean               |     | 27.4       |                    | 25.3                   | 13.5 <sup>f</sup>             | 13.9                               |                                        | 8.4 h                                            | 13.6 <sup>f</sup>                           | 8.0 <sup>f</sup>                                         |
| SD                 |     | 6.9        |                    | 5.6                    | 1.8                           | 6.9                                |                                        | 7.8 h                                            | 1.3                                         | 6.0                                                      |
| Median             |     | 25.3       |                    | 25.0                   | 13.0                          | 12.3                               |                                        | 5.8 h                                            | 14.0                                        | 5.0                                                      |
| Occasional smokers |     |            |                    |                        |                               |                                    |                                        |                                                  |                                             |                                                          |
| L                  | M   | 24         | AA                 | 36.3                   | 17                            | 7                                  | 2×/Month                               | 1.4 days                                         | 3                                           | 2                                                        |
| M                  | M   | 21         | AA                 | 23.0                   | 13                            | 8                                  | 2×/Week                                | 0.7 days                                         | 4                                           | 2                                                        |
| N                  | M   | 25         | W                  | 24.2                   | 21                            | 4                                  | 2×/Week                                | 13.0 days                                        | 1                                           | 3                                                        |
| O                  | M   | 40         | W                  | 28.3                   | 18                            | 22                                 | 2×/Week                                | 30.7 days                                        | 0                                           | 2                                                        |
| P                  | F   | 46         | AA                 | 31.0                   | 26                            | 20                                 | 2×/Week                                | 0.4 days                                         | 4                                           | 4                                                        |
| Q                  | M   | 33         | AA                 | 30.7                   | 16                            | 17                                 | 2×/Month                               | 22.8 days                                        | 0                                           | 3                                                        |
| R                  | F   | 22         | W                  | 22.0                   | 16                            | 6                                  | 2×/Week                                | 1.7 days                                         | 4                                           | 1                                                        |
| S                  | F   | 22         | W                  | 23.0                   | 14                            | 8                                  | 2×/Week                                | 1.1 days                                         | 10                                          | 2                                                        |
| T                  | M   | 31         | W                  | 21.7                   | 22                            | 9                                  | 1–2×/Week                              | 1.8 days                                         | 2                                           | 2                                                        |
| Mean               |     | 29.4       |                    | 26.7                   | 18.1 <sup>f</sup>             | 11.3                               |                                        | 8.2 days                                         | 3.1 <sup>f</sup>                            | 2.3 <sup>f</sup>                                         |
| SD                 |     | 8.6        |                    | 5.1                    | 4.2                           | 6.3                                |                                        | 11.4 days                                        | 3.1                                         | 0.9                                                      |
| Median             |     | 24.9       |                    | 24.2                   | 17.0                          | 8.5                                |                                        | 1.7 days                                         | 3.0                                         | 2.0                                                      |
| P value            |     |            |                    |                        | 0.011                         |                                    |                                        |                                                  | <0.001                                      | 0.021                                                    |

<sup>a</sup> Data collected during screening.  
<sup>b</sup> Data collected on admission to Session 1.  
<sup>c</sup> AA, African American; W, white.  
<sup>d</sup> Self-reported data on admission inconsistent with data received at screening. Data excluded from statistics.  
<sup>e</sup> Self-reported data inconsistent with biological sample concentrations. Data excluded from statistics.  
<sup>f</sup> Significant difference between groups.

quent smokers; observed ( $P = 0.041$ ) and baseline-adjusted ( $P = 0.039$ ) THC  $C_{\max}$  and baseline-adjusted THCCOOH  $C_{\max}$  ( $P = 0.014$ ) were significantly greater after smoking. Baseline THCCOOH concentrations were 10.0–78.1  $\mu\text{g/L}$  before smoking sessions and 1.1–85.5  $\mu\text{g/L}$  before vaporization sessions; baseline THC concentrations were 0.7–5.3  $\mu\text{g/L}$  and 0.6–4.9  $\mu\text{g/L}$ , respectively.

At frequent smokers' final collection time (72 h postdose), 100%, 90.9%, and 100% of specimens were THC positive after smoked, vaporized, and oral cannabis; concentrations were 0.5–4.3, 0.7–3.7, and 0.8–2.5  $\mu\text{g/L}$ , respectively. In contrast, 100%, 100%, and 88.9% of occasional smokers' specimens were negative at the final collection (54 h postdose) after smoked, vaporized, and oral cannabis, respectively; one participant's blood



THC concentration 54 h after oral dosing was  $0.6 \mu\text{g/L}$ . Frequent smokers' observed and baseline-adjusted THC  $C_{\text{max}}$  were significantly greater than occasional smokers' after smoking (observed,  $P = 0.027$ ; adjusted,  $P = 0.030$ ) and vaporization (observed,  $P = 0.035$ ; adjusted,  $P = 0.048$ ) only.

All frequent smokers' specimens were  $\geq$ THCCOOH limit of quantification (LOQ,  $0.5 \mu\text{g/L}$ ) and 100%, 90.9%, and 100% were  $\geq$ THCCOOH-glucuronide LOQ ( $5.0 \mu\text{g/L}$ ) 72 h after smoked, vaporized, and oral cannabis, respectively. For occasional smokers, 66.7%, 55.6%, and 100% of specimens were THCCOOH positive at 54 h. THCCOOH-glucuronide was  $>$ LOQ in 77.8%, 66.7%, and 100% of occasional smokers after smoked, vaporized, and oral doses, respectively; among those, THCCOOH-

glucuronide was detected at 54 h in 28.6%, 16.7% and 77.8% of participants. THCCOOH  $C_{\text{max}}$  was significantly higher in frequent as compared to occasional smokers regardless of administration route ( $P < 0.001$ ). In contrast, frequent smokers' THCCOOH-glucuronide  $C_{\text{max}}$  was significantly greater following smoking ( $P = 0.001$ ) and vaporization ( $P = 0.003$ ) only, but occasional smokers' baseline-adjusted  $C_{\text{max}}$  following oral dosing was significantly greater ( $P = 0.019$ ) than that of frequent smokers.

For 11-OH-THC, 90.9%, 81.8%, and 90.9% of frequent smokers were negative 72 h after smoked, vaporized and oral doses, respectively;  $t_{\text{last}}$  varied among those participants (5.0–56, 2.5–38, and 5.0–50 h, respectively). Occasional smokers' 11-OH-THC  $t_{\text{last}}$  [mean (range)] after smoking [1.7 (0.25–3.5) h] and va-

**Table 2.** Summary of observed and baseline-adjusted  $C_{\max}$ ,  $t_{\max}$ ,  $t_{\text{last}}$ , and  $AUC_{0 \rightarrow 72 \text{ h}}$  (frequent smokers) or  $AUC_{0 \rightarrow 54 \text{ h}}$  (occasional smokers) after smoked, vaporized, and oral cannabis doses in 11 frequent and 9 occasional smokers.<sup>a</sup>

| Analyte and parameter                      | n  | Smoking                | Vaporization           | Oral                   | Overall        |                  | Oral vs inhaled<br>(contrast 1) |                  | Smoking vs<br>vaporization<br>(contrast 2) |              |
|--------------------------------------------|----|------------------------|------------------------|------------------------|----------------|------------------|---------------------------------|------------------|--------------------------------------------|--------------|
|                                            |    |                        |                        |                        | F              | P                | F                               | P                | F                                          | P            |
| THC frequent smokers                       |    |                        |                        |                        |                |                  |                                 |                  |                                            |              |
| C <sub>max</sub> µg/L                      | 11 | 153, 117 (52.8-471)    | 87.1, 88.0 (24.7-170)  | 17.7, 15.6 (4.7-34.8)  | 12.48          | <b>0.004</b>     | 20.35                           | <b>0.001</b>     | 5.52                                       | <b>0.041</b> |
| Baseline-adjusted C <sub>max</sub> µg/L    | 11 | 151, 114 (51.6-467)    | 84.7, 83.1 (23.5-169)  | 15.3, 14.3 (1.4-32.4)  | 12.56          | <b>0.004</b>     | 20.40                           | <b>0.001</b>     | 5.61                                       | <b>0.039</b> |
| t <sub>max</sub> h                         | 11 | 0.12, 0.13 (0.00-0.17) | 0.09, 0.10 (0.03-0.17) | 2.5, 2.5 (1.5-3.5)     | 309.25         | <b>&lt;0.001</b> | 313.12                          | <b>&lt;0.001</b> | 2.10                                       | 0.174        |
| t <sub>last</sub> h <sup>b</sup>           | 9  | >72, >72               | 65, >72 (5.0->72)      | >72, >72               | - <sup>c</sup> | -                | -                               | -                | -                                          | -            |
| AUC <sub>0→72 hr</sub> µg·h/L <sup>b</sup> | 9  | 200, 163 (94.3-377)    | 174, 136 (32.9-324)    | 167, 166 (104-244)     | 0.96           | 0.404            | -                               | -                | -                                          | -            |
| THC occasional smokers                     |    |                        |                        |                        |                |                  |                                 |                  |                                            |              |
| C <sub>max</sub> µg/L                      | 9  | 51.6, 44.4 (1.3-174)   | 47.8, 34.8 (5.2-137)   | 10.3, 10.1 (3.6-22.5)  | 4.02           | <b>0.039</b>     | 11.37                           | <b>0.010</b>     | 0.04                                       | 0.840        |
| t <sub>max</sub> h                         | 9  | 0.11, 0.10 (0.07-0.17) | 0.11, 0.10 (0.03-0.17) | 2.3, 2.5 (1.5-3.5)     | 92.50          | <b>&lt;0.001</b> | 93.44                           | <b>&lt;0.001</b> | 0.18                                       | 0.681        |
| t <sub>last</sub> h                        | 9  | 10.1, 3.5 (0.20-44)    | 3.6, 2.5 (0.25-12)     | 14.9, 12.0 (5.0->54)   | 2.45           | 0.118            | -                               | -                | -                                          | -            |
| AUC <sub>0→54 hr</sub> µg·h/L              | 9  | 20.4, 18.0 (0.1-43.9)  | 11.7, 9.9 (0.9-22.3)   | 43.4, 37.5 (11.9-114)  | 7.26           | <b>0.006</b>     | 7.71                            | <b>0.024</b>     | 4.08                                       | 0.078        |
| 11-OH-THC frequent smokers                 |    |                        |                        |                        |                |                  |                                 |                  |                                            |              |
| C <sub>max</sub> µg/L                      | 11 | 9.8, 7.2 (1.9-30.9)    | 5.7, 6.2 (1.6-10.7)    | 8.1, 7.5 (2.2-14.3)    | 1.83           | 0.186            | -                               | -                | -                                          | -            |
| Baseline-adjusted C <sub>max</sub> µg/L    | 11 | 9.0, 6.5 (1.9-30.2)    | 4.8, 4.2 (1.6-9.8)     | 7.3, 6.2 (0.9-13.7)    | 2.06           | 0.153            | -                               | -                | -                                          | -            |
| t <sub>max</sub> h                         | 11 | 0.21, 0.20 (0.10-0.50) | 0.19, 0.17 (0.10-0.50) | 2.3, 2.5 (1.5-3.5)     | 84.98          | <b>&lt;0.001</b> | 87.06                           | <b>&lt;0.001</b> | 0.28                                       | 0.606        |
| t <sub>last</sub> h <sup>d</sup>           | 10 | 30.1, 20.0 (5.0->72)   | 25.6, 11.0 (2.5->72)   | 31.5, 29.0 (5.0->72)   | 0.69           | 0.513            | -                               | -                | -                                          | -            |
| AUC <sub>0→72 hr</sub> µg·h/L <sup>d</sup> | 10 | 31.0, 20.6 (6.7-75.3)  | 27.3, 13.5 (4.1-80.1)  | 51.9, 54.3 (11.7-81.5) | 4.75           | <b>0.022</b>     | 5.71                            | <b>0.041</b>     | 0.48                                       | 0.505        |
| 11-OH-THC occasional smokers               |    |                        |                        |                        |                |                  |                                 |                  |                                            |              |
| C <sub>max</sub> µg/L                      | 8  | 2.8, 1.9 (0.5-8.7)     | 2.0, 1.6 (0.7-3.5)     | 5.5, 5.1 (2.4-11.0)    | 6.28           | <b>0.011</b>     | 9.93                            | <b>0.016</b>     | 0.712                                      | 0.427        |
| t <sub>max</sub> h                         | 8  | 0.22, 0.19 (0.10-0.50) | 0.15, 0.15 (0.10-0.20) | 2.4, 2.5 (1.5-3.5)     | 97.68          | <b>&lt;0.001</b> | 99.78                           | <b>&lt;0.001</b> | 3.79                                       | 0.093        |
| t <sub>last</sub> h                        | 8  | 1.7, 1.5 (0.25-3.5)    | 1.5, 1.0 (0.25-3.5)    | 14, 11 (5.0-32)        | 17.35          | <b>0.004</b>     | 17.71                           | <b>0.004</b>     | 0.26                                       | 0.628        |
| AUC <sub>0→54 hr</sub> µg·h/L              | 8  | 2.6, 2.3 (0.1-7.9)     | 1.8, 1.2 (0.2-4.3)     | 32.7, 24.1 (11.4-81.6) | 15.27          | <b>0.006</b>     | 15.40                           | <b>0.006</b>     | 0.89                                       | 0.378        |

Continued on page 1585

Continued on page 1585

**Table 2. Summary of observed and baseline-adjusted  $C_{max}$ ,  $t_{max}$ ,  $t_{last}$  and  $AUC_{0-72\text{ h}}$  (frequent smokers) or  $AUC_{0-54\text{ h}}$  (occasional smokers) after smoked, vaporized, and oral cannabis doses in 11 frequent and 9 occasional smokers.<sup>a</sup> (Continued from page 1584)**

| Analyte and parameter                                | n  | Smoking                | Vaporization           | Oral                    | Overall        |                  | Oral vs inhaled<br>(contrast 1) |                  | Smoking vs<br>vaporization<br>(contrast 2) |              |  |
|------------------------------------------------------|----|------------------------|------------------------|-------------------------|----------------|------------------|---------------------------------|------------------|--------------------------------------------|--------------|--|
|                                                      |    |                        |                        |                         | F              | P                | F                               | P                | F                                          | P            |  |
| THCCOOH frequent smokers                             |    |                        |                        |                         |                |                  |                                 |                  |                                            |              |  |
| C <sub>max</sub> µg/L                                | 11 | 58.3, 50.7 (15.6-137)  | 42.0, 41.4 (13.5-98.0) | 71.5, 71.8 (33.5-119)   | 6.57           | <b>0.006</b>     | 12.76                           | <b>0.005</b>     | 3.12                                       | 0.108        |  |
| Baseline-adjusted C <sub>max</sub> µg/L              | 11 | 23.5, 20.0 (5.7-64.9)  | 13.0, 12.5 (4.1-31.3)  | 36.4, 35.3 (4.3-99.4)   | 7.24           | <b>0.004</b>     | 6.92                            | <b>0.025</b>     | 8.92                                       | <b>0.014</b> |  |
| t <sub>max</sub> h                                   | 11 | 0.28, 0.25 (0.00-0.50) | 0.25, 0.25 (0.13-0.50) | 2.7, 2.5 (2.5-3.5)      | 404.71         | <b>&lt;0.001</b> | 482.80                          | <b>&lt;0.001</b> | 0.40                                       | 0.539        |  |
| t <sub>last</sub> h <sup>b</sup>                     | 9  | >72, >72               | >72, >72               | >72, >72                | - <sup>c</sup> | -                | -                               | -                | -                                          | -            |  |
| AUC <sub>0→72</sub> hr µg·h/L <sup>b</sup>           | 9  | 1302, 1135 (426-3702)  | 1289, 857 (162-4138)   | 1604, 1048 (747-3990)   | 1.44           | 0.266            | -                               | -                | -                                          | -            |  |
| THCCOOH occasional smokers                           |    |                        |                        |                         |                |                  |                                 |                  |                                            |              |  |
| C <sub>max</sub> µg/L                                | 9  | 8.4, 7.4 (0.7-17.5)    | 7.2, 5.3 (1.4-15.9)    | 39.8, 37.8 (12.5-70.4)  | 33.26          | <b>&lt;0.001</b> | 34.50                           | <b>&lt;0.001</b> | 0.91                                       | 0.368        |  |
| t <sub>max</sub> h                                   | 9  | 0.31, 0.25 (0.10-0.50) | 0.33, 0.25 (0.20-0.50) | 2.9, 3.5 (1.5-3.5)      | 97.14          | <b>&lt;0.001</b> | 99.25                           | <b>&lt;0.001</b> | 0.14                                       | 0.720        |  |
| t <sub>last</sub> h                                  | 9  | 46, >54 (12->54)       | 40, >54 (8.0->54)      | >54, >54                | - <sup>c</sup> | -                | -                               | -                | -                                          | -            |  |
| AUC <sub>0→54</sub> hr µg·h/L                        | 9  | 93.4, 77.0 (9.0-215)   | 87.9, 46.9 (12.4-277)  | 554, 570 (141-918)      | 42.85          | <b>&lt;0.001</b> | 44.83                           | <b>&lt;0.001</b> | 0.10                                       | 0.755        |  |
| THCVCOOH frequent smokers                            |    |                        |                        |                         |                |                  |                                 |                  |                                            |              |  |
| C <sub>max</sub> µg/L                                | 4  | 2.4, 2.4 (1.8-3.1)     | 1.7, 1.8 (1.2-2.1)     | 2.1, 2.0 (1.1-3.4)      | 0.94           | 0.442            | -                               | -                | -                                          | -            |  |
| t <sub>max</sub> h                                   | 4  | 0.22, 0.23 (0.17-0.25) | 0.52, 0.21 (0.17-1.5)  | 3.0, 3.0 (2.5-3.5)      | 29.08          | <b>0.001</b>     | 41.91                           | <b>0.007</b>     | -                                          | -            |  |
| t <sub>last</sub> h                                  | 4  | 16, 9.0 (0.5-44)       | 7.2, 7.3 (0.25-14)     | 13, 12 (2.5-26)         | 0.37           | 0.707            | -                               | -                | -                                          | -            |  |
| AUC <sub>0→72</sub> hr µg·h/L                        | 4  | 20.9, 12.2 (1.3-57.8)  | 9.8, 7.8 (0.26-23.1)   | 20.4, 19.5 (2.1-40.6)   | 0.38           | 0.696            | -                               | -                | -                                          | -            |  |
| THCVCOOH occasional smokers                          |    |                        |                        |                         |                |                  |                                 |                  |                                            |              |  |
| C <sub>max</sub> µg/L                                | 8  | -                      | -                      | 1.9, 1.9 (1.1-2.7)      | -              | -                | -                               | -                | -                                          | -            |  |
| t <sub>max</sub> h                                   | 8  | -                      | -                      | 2.6, 2.5 (1.5-3.5)      | -              | -                | -                               | -                | -                                          | -            |  |
| t <sub>last</sub> h                                  | 8  | -                      | -                      | 6.2, 5.0 (2.5-14)       | -              | -                | -                               | -                | -                                          | -            |  |
| AUC <sub>0→54</sub> hr µg·h/L                        | 8  | -                      | -                      | 9.1, 8.0 (1.1-21.1)     | -              | -                | -                               | -                | -                                          | -            |  |
| THCCOOH-glucuronide frequent smokers                 |    |                        |                        |                         |                |                  |                                 |                  |                                            |              |  |
| C <sub>max</sub> µg/L                                | 11 | 113, 106 (49.9-248)    | 89.0, 66.8 (18.1-225)  | 139, 113 (84.9-304)     | 3.12           | 0.066            | -                               | -                | -                                          | -            |  |
| Baseline-adjusted C <sub>max</sub> µg/L <sup>e</sup> | 9  | 25.8, 14.1 (5.0-70.7)  | 10.9, 10.6 (0.8-23.8)  | 53.0, 57.1 (10.3-75.7)  | 12.59          | <b>0.001</b>     | 19.19                           | <b>0.002</b>     | 3.62                                       | 0.094        |  |
| t <sub>max</sub> h                                   | 11 | 1.1, 0.5 (0.0-3.5)     | 1.8, 1.5 (0.03-3.5)    | 3.4, 3.5 (1.5-5.0)      | 10.49          | <b>0.004</b>     | 46.03                           | <b>&lt;0.001</b> | 1.03                                       | 0.335        |  |
| t <sub>last</sub> h <sup>b</sup>                     | 9  | >72, >72               | 67, >72 (26->72)       | >72, >72                | - <sup>c</sup> | -                | -                               | -                | -                                          | -            |  |
| AUC <sub>0→72</sub> hr µg·h/L <sup>b</sup>           | 9  | 3733, 2283 (1473-9740) | 3693, 2090 (276-12038) | 4554, 3079 (2165-11345) | 0.95           | 0.407            | -                               | -                | -                                          | -            |  |
| Continued on page 1586                               |    |                        |                        |                         |                |                  |                                 |                  |                                            |              |  |

Continued on page 1586

**Table 2. Summary of observed and baseline-adjusted  $C_{\max}$ ,  $t_{\max}$ ,  $t_{\text{last}}$  and  $AUC_{0 \rightarrow 72 \text{ h}}$  (frequent smokers) or  $AUC_{0 \rightarrow 54 \text{ h}}$  (occasional smokers) after smoked, vaporized, and oral cannabis doses in 11 frequent and 9 occasional smokers.<sup>a</sup> (Continued from page 1585)**

| Analyte and parameter                  | n | Smoking                | Vaporization          | Oral                   | Overall        |                  | Oral vs inhaled |              | Smoking vs vaporization |       |
|----------------------------------------|---|------------------------|-----------------------|------------------------|----------------|------------------|-----------------|--------------|-------------------------|-------|
|                                        |   |                        |                       |                        | F              | P                | F               | P            | F                       | P     |
| THCCOOH-glucuronide occasional smokers |   |                        |                       |                        |                |                  |                 |              |                         |       |
| C <sub>max</sub> , µg/L                | 5 | 19.4, 21.4 (11.8-25.0) | 15.1, 16.1 (5.3-23.7) | 124, 124 (70.9-178)    | 36.40          | <b>0.004</b>     | 36.82           | <b>0.004</b> | 3.53                    | 0.134 |
| t <sub>max</sub> <sup>a</sup> , h      | 5 | 2.1, 1.5 (1.5-3.5)     | 1.9, 2.5 (0.5-2.5)    | 4.7, 5.0 (3.5-5.0)     | 29.88          | <b>&lt;0.001</b> | 52.07           | <b>0.002</b> | 0.29                    | 0.621 |
| t <sub>last</sub> <sup>b</sup> , h     | 5 | 36, 32 (14->54)        | 25, 32 (2.5->54)      | >54, >54               | - <sup>c</sup> | -                | -               | -            | -                       | -     |
| AUC <sub>0-54 h</sub> , µg·h/L         | 5 | 365, 350 (139-631)     | 259, 279 (10.5-605)   | 2266, 2024 (1748-3578) | 40.14          | <b>0.003</b>     | 41.78           | <b>0.003</b> | 2.14                    | 0.217 |

<sup>a</sup> Data are presented as mean, median (range). Repeated-measures analysis of variance F-statistic and P value for overall dose effect and planned Helmet contrasts are reported (contrast 1 evaluated the difference between the variance from oral dosing and the combined variance from smoked and vaporized dosings, contrast2 evaluated the difference in variances from smoked and vaporized dosing). Only participants whose blood was positive for analyses after all cannabis administrations were included in analyses. Bolded P values designate significance.

<sup>b</sup> n = 9 because the 72-h collection for participant D was missed owing to catheter blockage and participant K (originally recruited as an occasional smoker, therefore blood was only collected up to 54 h postdose) was still positive at the final collection.

<sup>c</sup> Statistical evaluation could not be performed because all participants were still positive after at least 1 cannabis administration.

<sup>d</sup> n = 10 because the 72-h collection for participant D was missed owing to catheter blockage (data from participant K is included because the participant was negative at the final collection).

<sup>e</sup> n = 9 because 2 participants' postdose concentrations did not exceed baseline concentrations after all cannabis administrations.

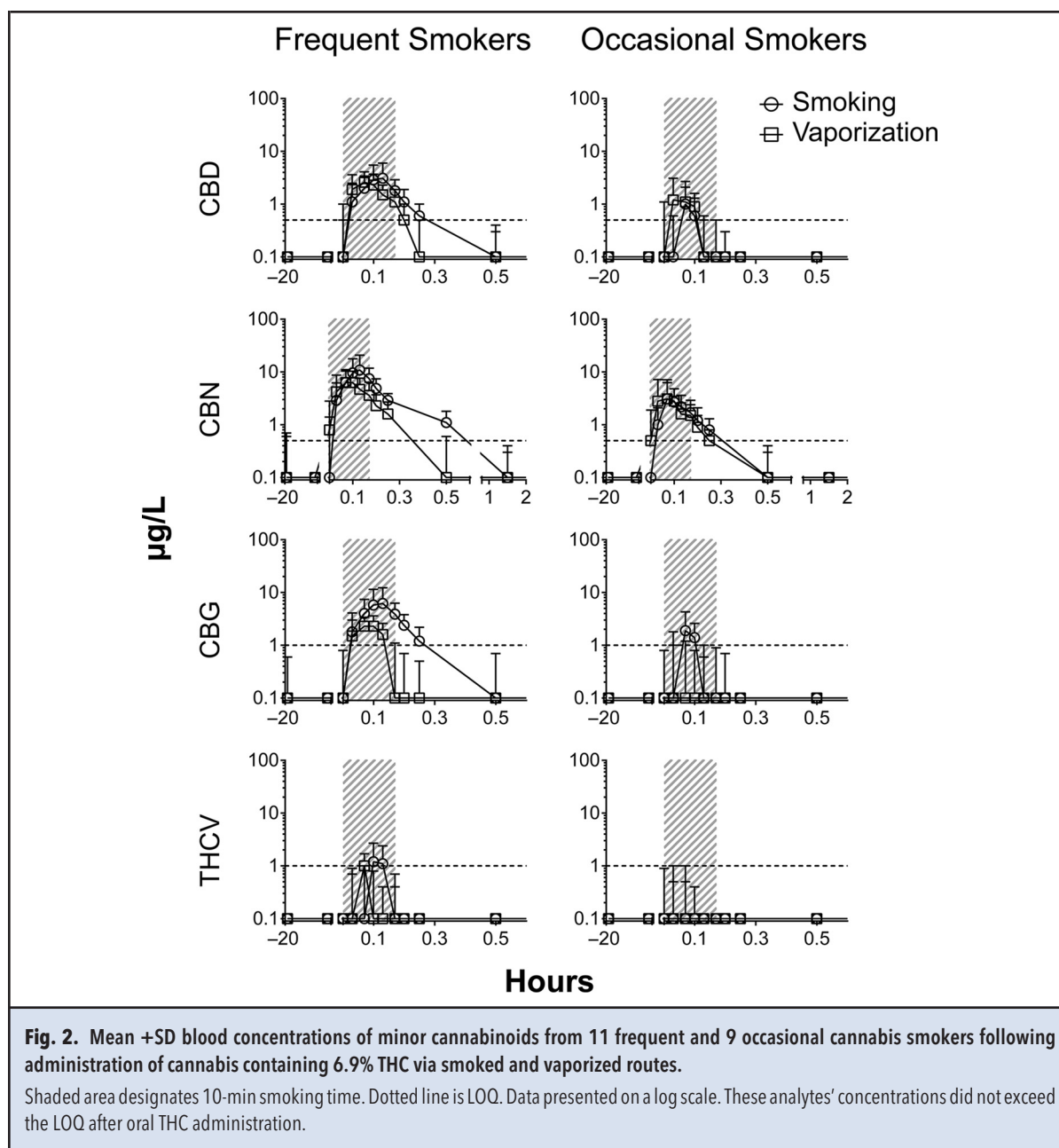
<sup>a</sup> Data are presented as mean, median (range). Repeated-measures analysis of variance F-statistic and P-value for overall dose effect and planned Helmert contrasts are reported (contrast 1 evaluated the difference between the variance from oral dosing and the combined variance from smoked and vaporized dosings, contrast 2 evaluated the difference in variances from smoked and vaporized dosing). Only participants whose blood was positive for analytes after all cannabis administrations were included in analyses. Bolded P values designate significance.

<sup>b</sup> n = 9 because the 72-h collection for participant D was missed owing to catheter blockage and participant K (originally recruited as an occasional smoker, therefore blood was only collected up to 54 h postdose) was still positive at the final collection.

<sup>c</sup> Statistical evaluation could not be performed because all participants were still positive after at least 1 cannabis administration.

<sup>d</sup> n = 10 because the 72-h collection for participant D was missed owing to catheter blockage (data from participant K is included because the participant was negative at the final collection).

<sup>e</sup> n = 9 because 2 participants' postdose concentrations did not exceed baseline concentrations after all cannabis administrations.



porization [1.5 (0.25–3.5) h] were significantly shorter than after oral dosing [14 (5.0–32) h,  $P = 0.004$ ]. Frequent smokers' observed ( $P = 0.003$ ) and baseline-adjusted 11-OH-THC  $C_{max}$  ( $P = 0.015$ ) were significantly greater and  $t_{last}$  significantly later ( $P = 0.008$ ) than occasional smokers', regardless of dose.

Frequent smokers' THCVCOOH detection rates were 45.5%, 63.6%, and 100% after smoked, vaporized, and oral cannabis, respectively, while THCVCOOH was detected in occasional smokers only after oral dosing (88.9%). Observed concentrations were relatively low

( $\leq 3.4 \mu\text{g/L}$ ) but remained  $> 1.0 \mu\text{g/L}$  for prolonged times (maximum  $t_{last}$  were 14–44 h). THCVCOOH was detected on admission to  $\geq 1$  session in 63.6% of frequent smokers (1.1–4.6  $\mu\text{g/L}$ ); 27.3% were THCVCOOH positive at baseline (1.2–1.9  $\mu\text{g/L}$ ).

Fig. 2 shows concentration-time curves and Table 3 summarizes CBD, CBN, CBG, and THCV pharmacokinetic results, present only after smoked and vaporized cannabis. Frequent smokers' CBD, CBN, CBG, and THCV detection rates were 100%, 100%, 100%, and 54.5%, respectively, after smoking and 90.9%, 100%,

**Table 3.** Summary of observed  $C_{\max}$ ,  $t_{\max}$ ,  $t_{\text{last}}$ , and  $AUC_{0 \rightarrow 72 \text{ h}}$  (frequent smokers) or  $AUC_{0 \rightarrow 54 \text{ h}}$  (occasional smokers) after smoked and vaporized cannabis administrations in 11 frequent and 9 occasional smokers for minor cannabinoids.<sup>a</sup>

| Analyte and parameter                                               | n | Smoking                | Vaporization           | t     | P            |
|---------------------------------------------------------------------|---|------------------------|------------------------|-------|--------------|
| CBD frequent smokers 10                                             |   |                        |                        |       |              |
| $C_{\max}$ , $\mu\text{g/L}$                                        |   | 3.6, 2.9 (0.6–10.9)    | 3.0, 2.8 (1.1–6.0)     | 0.78  | 0.454        |
| $t_{\max}$ , h                                                      |   | 0.11, 0.12 (0.03–0.17) | 0.08, 0.07 (0.03–0.13) | 2.07  | 0.068        |
| $t_{\text{last}}$ , h                                               |   | 0.28, 0.25 (0.13–0.50) | 0.23, 0.23 (0.13–0.50) | 0.74  | 0.480        |
| $AUC_{0 \rightarrow 72 \text{ h}}$ , $\mu\text{g} \cdot \text{h/L}$ |   | 0.6, 0.5 (0.0–1.7)     | 0.5, 0.4 (0.2–1.0)     | 0.85  | 0.418        |
| CBD occasional smokers 5                                            |   |                        |                        |       |              |
| $C_{\max}$ , $\mu\text{g/L}$                                        |   | 1.8, 0.9 (0.6–5.4)     | 1.5, 1.5 (0.7–2.2)     | 0.39  | 0.719        |
| $t_{\max}$ , h                                                      |   | 0.09, 0.07 (0.07–0.13) | 0.09, 0.10 (0.07–0.13) | −0.34 | 0.749        |
| $t_{\text{last}}$ , h                                               |   | 0.15, 0.17 (0.10–0.20) | 0.16, 0.17 (0.10–0.20) | −0.24 | 0.821        |
| $AUC_{0 \rightarrow 54 \text{ h}}$ , $\mu\text{g} \cdot \text{h/L}$ |   | 0.1, 0.1 (0.0–0.4)     | 0.2, 0.1 (0.1–0.3)     | −0.25 | 0.813        |
| CBN frequent smokers 11                                             |   |                        |                        |       |              |
| $C_{\max}$ , $\mu\text{g/L}$                                        |   | 11.6, 9.1 (3.1–37.9)   | 7.1, 6.4 (0.8–16.1)    | 2.16  | 0.056        |
| $t_{\max}$ , h                                                      |   | 0.12, 0.13 (0.03–0.17) | 0.09, 0.07 (0.07–0.13) | 2.47  | <b>0.033</b> |
| $t_{\text{last}}$ , h                                               |   | 0.8, 0.5 (0.25–1.5)    | 0.5, 0.25 (0.25–1.5)   | 1.22  | 0.250        |
| $AUC_{0 \rightarrow 72 \text{ h}}$ , $\mu\text{g} \cdot \text{h/L}$ |   | 2.7, 2.0 (0.5–7.1)     | 1.5, 1.0 (0.2–4.0)     | 2.50  | <b>0.031</b> |
| CBN occasional smokers 8                                            |   |                        |                        |       |              |
| $C_{\max}$ , $\mu\text{g/L}$                                        |   | 4.0, 3.1 (0.7–13.5)    | 4.6, 4.1 (0.7–14.1)    | −0.28 | 0.786        |
| $t_{\max}$ , h                                                      |   | 0.11, 0.10 (0.07–0.17) | 0.09, 0.09 (0.03–0.17) | 0.76  | 0.472        |
| $t_{\text{last}}$ , h                                               |   | 0.36, 0.38 (0.13–0.50) | 0.27, 0.25 (0.17–0.50) | 1.09  | 0.311        |
| $AUC_{0 \rightarrow 54 \text{ h}}$ , $\mu\text{g} \cdot \text{h/L}$ |   | 0.8, 0.7 (0.1–1.8)     | 0.6, 0.6 (0.0–1.4)     | 0.49  | 0.639        |
| CBG frequent smokers 10                                             |   |                        |                        |       |              |
| $C_{\max}$ , $\mu\text{g/L}$                                        |   | 6.9, 5.1 (1.8–22.7)    | 3.0, 2.9 (1.5–5.0)     | 2.32  | <b>0.046</b> |
| $t_{\max}$ , h                                                      |   | 0.12, 0.13 (0.03–0.17) | 0.08, 0.09 (0.03–0.13) | 2.15  | 0.060        |
| $t_{\text{last}}$ , h                                               |   | 0.27, 0.25 (0.13–0.50) | 0.16, 0.17 (0.10–0.25) | 2.65  | <b>0.026</b> |
| $AUC_{0 \rightarrow 72 \text{ h}}$ , $\mu\text{g} \cdot \text{h/L}$ |   | 1.2, 0.8 (0.2–3.7)     | 0.4, 0.3 (0.2–0.7)     | 2.51  | <b>0.033</b> |
| CBG occasional smokers 5                                            |   |                        |                        |       |              |
| $C_{\max}$ , $\mu\text{g/L}$                                        |   | 3.0, 2.0 (1.4–8.1)     | 2.2, 1.7 (1.0–5.1)     | 0.54  | 0.621        |
| $t_{\max}$ , h                                                      |   | 0.10, 0.07 (0.07–0.17) | 0.08, 0.07 (0.03–0.13) | 0.73  | 0.508        |
| $t_{\text{last}}$ , h                                               |   | 0.15, 0.17 (0.10–0.20) | 0.11, 0.10 (0.07–0.13) | 1.80  | 0.147        |
| $AUC_{0 \rightarrow 54 \text{ h}}$ , $\mu\text{g} \cdot \text{h/L}$ |   | 0.3, 0.2 (0.1–0.7)     | 0.1, 0.1 (0.0–0.3)     | 1.18  | 0.304        |
| THCV frequent smokers 5                                             |   |                        |                        |       |              |
| $C_{\max}$ , $\mu\text{g/L}$                                        |   | 2.5, 2.1 (1.5–4.9)     | 1.7, 1.7 (1.4–1.9)     | 1.41  | 0.232        |
| $t_{\max}$ , h                                                      |   | 0.11, 0.10 (0.10–0.13) | 0.06, 0.07 (0.03–0.10) | 3.94  | <b>0.017</b> |
| $t_{\text{last}}$ , h                                               |   | 0.16, 0.17 (0.13–0.17) | 0.11, 0.10 (0.07–0.11) | 2.23  | 0.090        |
| $AUC_{0 \rightarrow 72 \text{ h}}$ , $\mu\text{g} \cdot \text{h/L}$ |   | 0.3, 0.2 (0.1–0.4)     | 0.2, 0.2 (0.1–0.2)     | 1.66  | 0.172        |
| THCV occasional smokers 1                                           |   |                        |                        |       |              |
| $C_{\max}$ , $\mu\text{g/L}$                                        |   | 2.8                    | 1.2                    | –     | –            |
| $t_{\max}$ , h                                                      |   | 0.07                   | 0.07                   | –     | –            |
| $t_{\text{last}}$ , h                                               |   | 0.07                   | 0.07                   | –     | –            |
| $AUC_{0 \rightarrow 54 \text{ h}}$ , $\mu\text{g} \cdot \text{h/L}$ |   | 0.1                    | 0.0                    | –     | –            |

<sup>a</sup> Data are presented as mean, median (range). Paired-samples, t-statistics, and P values are reported. Only participants whose blood was positive for analytes after both cannabis administrations were included in analyses. Bolded P values designate significance; these analytes' concentrations did not exceed limit of quantification after oral cannabis administration.

90.9%, and 72.7%, respectively, after vaporization. Occasional smokers' CBD, CBN, CBG, and THCV detection rates were 66.7%, 88.9%, 77.8%, and 11.1%, respectively, after smoking and 77.8%, 100%, 66.7%, 33.3%, respectively, after vaporization. Frequent smokers' CBD ( $P = 0.016$ ), CBN ( $P = 0.032$ ), and CBG ( $P = 0.015$ )  $t_{\text{last}}$  were significantly later than occasional smokers' regardless of inhalation method.

THC-glucuronide detection rates after smoked, vaporized, and oral cannabis were 44.4%, 0.0%, and 55.5%, respectively, for frequent smokers, and 0.0%, 0.0%, and 11.1%, respectively, for occasional smokers (THC-glucuronide data not shown). Among frequent users positive after smoking, THC-glucuronide was detected at 0.50 h (0.5–1.1  $\mu\text{g/L}$ ,  $n = 4/9$ ), and once each at 0.25 h (0.5  $\mu\text{g/L}$ ) and on admission (0.8  $\mu\text{g/L}$ ). After oral cannabis, frequent smokers' THC-glucuronide  $C_{\text{max}}$  (0.6–1.2  $\mu\text{g/L}$ ,  $n = 5/9$ ) occurred 1.5–3.5 h postdose. The first positive samples were observed 1.5–2.5 h after oral dosing and  $t_{\text{last}}$  occurred 2.5–5 h postdose. One occasional smoker's blood was THC-glucuronide positive 3.5 h after oral dosing (0.7  $\mu\text{g/L}$ ).

Recommended and novel cutoffs were evaluated for identifying recent cannabis use (Fig. 3). At 72 h following all doses, frequent smokers' detection rates with THC  $\geq 1$  and 2- $\mu\text{g/L}$  cutoffs were 70%–80% and 11%–30%, respectively; with a THC  $\geq 5$ - $\mu\text{g/L}$  cutoff, there were no positive results 26, 20, and 12 h after smoking, vaporization, and oral dosing, respectively. Occasional smokers' detection times were shorter with THC  $\geq 1$ –5- $\mu\text{g/L}$  cutoffs following smoking and vaporization since no blood sample was positive at 1.5–5 and 1.5–3.5 h, respectively; none were positive 20, 14, and 12 h after oral dosing with THC  $\geq 1$ , 2, or 5  $\mu\text{g/L}$ , respectively. With a novel cutoff THC/11-OH-THC ratio  $> 1$  plus THCCOOH/11-OH-THC ratio  $< 15$ , no frequent smoker was positive 20, 5, and 14 h after smoking, vaporization, and oral dosing, respectively, while no occasional smoker was positive 5, 3.5, and 14 h. Finally, with a combined THC  $\geq 5$   $\mu\text{g/L}$  plus THCCOOH/11-OH-THC  $< 20$ , no frequent smoker was positive 8 h after any administration; no occasional smoker was positive 1.5 h after smoking or vaporization or 12 h after oral cannabis.

## Discussion

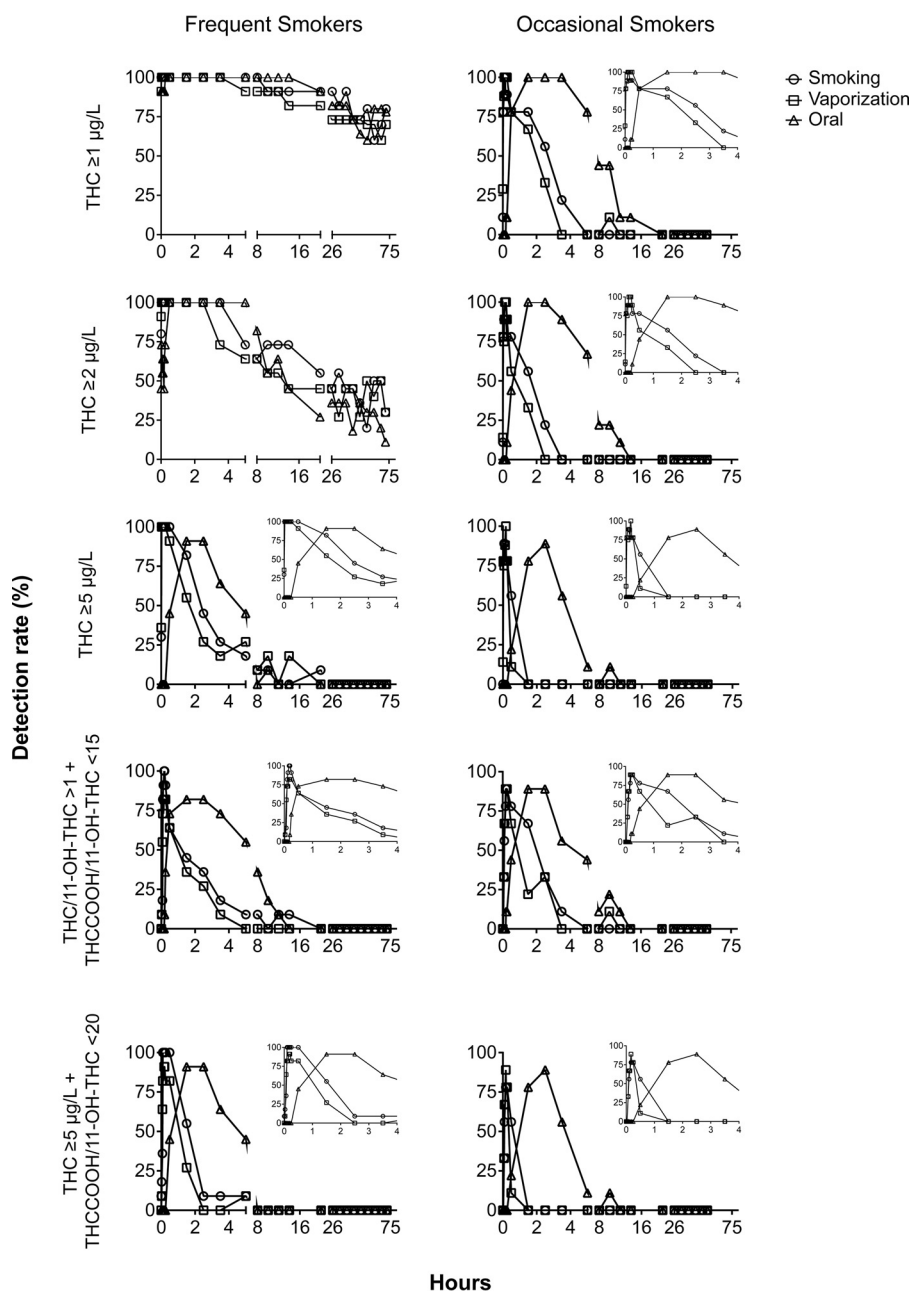
The main strengths of the study were that blood cannabinoid pharmacokinetics following three cannabis administrations were evaluated within the same participants; the cannabinoid panel was expanded to include CBG, THCV, and THCVCOOH, improving result interpretation; and inclusion of frequent and occasional smokers provided the opportunity to characterize group pharmacokinetic differences. Limitations include the small study population, due to multiple drug administra-

tion sessions and residence on a closed research unit to prevent cannabis self-administration. Additionally, the study population was made up entirely of African American and white participants, which may limit the amount of metabolic variability observed and generalizability of the results. As medical and recreational cannabis access expands, products with cannabinoid potencies greater than the single potency investigated here may be available. Finally, e-cigarettes are an increasingly popular administration route; although the pharmacokinetic profile following e-cigarette use is expected to be similar to that following inhalation, solvent effects, rapid and direct heating from the battery, and direct delivery without a balloon may affect cannabinoid delivery, requiring future investigation.

Blood THC concentrations observed 0.25–0.50 h after smoking initiation here were similar to those previously observed (9, 10). The occasional smokers' THC  $C_{\text{max}}$  after vaporizing 800 mg cannabis (6.9% THC) was more similar to those after controlled vaporized 500 mg low (2.9% THC) than to high (6.7% THC) dose cannabis (without alcohol) in occasional-to-moderate cannabis smokers (16). Differences in blood THC concentrations between studies may be from differences in extent of cannabis vaporized or self-titration. Most observed group differences are consistent with previously observed differences following smoked cannabis (10). Occasional smokers' baseline-adjusted THCCOOH-glucuronide  $C_{\text{max}}$  was significantly greater than frequent smokers' ( $P = 0.019$ ) after oral dosing only, due to frequent smokers' high baseline concentrations with small concentration increases following dosing.

Differences between frequent smokers' inhaled cannabis THC  $C_{\text{max}}$  (observed and baseline-adjusted) and baseline-adjusted THCCOOH  $C_{\text{max}}$  were unexpected based on previously published data showing comparable THC delivery following smoked and vaporized cannabis (15, 26). The conclusion by Hazekamp et al. (26) was based on vaporizing an ethanolic THC solution, with 53% of the loaded dose delivered compared to 29% from 200-mg crude flower tops. Abrams et al. (15) administered 450 mg cannabis via smoking or vaporization and compared plasma THC  $\text{AUC}_{0 \rightarrow 6\text{h}}$ ; participants used a standardized smoking procedure and 2–3 balloon inflations were required to vaporize the dose. In the present study approximately 800-mg ground bulk cannabis was vaporized once, possibly affecting vaporization efficiency and the total available cannabinoid dose. Participants inhaled doses ad libitum, resulting in variable cannabinoid doses; however, ad libitum dosing may better reflect naturalistic conditions. No differences in inhaled cannabis doses were observed in occasional smokers, likely due to similar dose titration.

Hypothesized differences between oral and inhaled cannabis (lower THC  $C_{\text{max}}$  and later  $t_{\text{max}}$  and  $t_{\text{last}}$ ) were



**Fig. 3.** Detection rates for 11 frequent and 9 occasional cannabis smokers utilizing 5 different cutoffs following administration of cannabis containing 6.9% THC via smoked, vaporized, and oral routes.

observed but some exceptions were noted; no significant difference in occasional smokers'  $\text{THC } t_{\text{last}}$  was observed. Participant P had extended THC detection after smoked (26 h) and oral (> 54 h) administrations, and participant S had extended THC detection after smoking (44 h). Frequent smokers' 11-OH-THC  $C_{\text{max}}$  was not greatest after oral cannabis, in contrast to occasional smokers,

likely due to their increased smoking efficiency. Finally, observed frequent smoker THCCOOH-glucuronide  $C_{\text{max}}$  were not significantly different between routes because of high baseline concentrations (16.5–281  $\mu\text{g/L}$  across all sessions); significance was observed after accounting for baseline concentrations (Table 2, Helmert contrast 1;  $P = 0.002$ ).

We also monitored the minor cannabinoids CBD, CBN, CBG, THCV, and THCVCOOH in blood after smoked, vaporized, and oral cannabis administrations. Currently, THCV, THCVCOOH, and CBG human data after cannabis use are available in urine only (11–13, 27). Significant differences for CBN ( $P = 0.033$ ) and THCV ( $P = 0.017$ )  $t_{\max}$  between inhaled doses in frequent smokers were observed; however, all  $t_{\max}$  occurred before the end of inhalation. Frequent smokers' significantly greater CBG  $C_{\max}$  ( $P = 0.046$ ) and later  $t_{\text{last}}$  ( $P = 0.026$ ) after smoking compared to vaporization was possibly due to inefficient CBG volatilization during vaporization. CBD and CBN  $t_{\text{last}}$  after smoking and vaporization here are similar to those observed in previous smoked (9, 10) and vaporized (16) administration studies; percent positive participants were greater in the present study due to the lower LOQ (0.5 vs 1.0  $\mu\text{g/L}$ ).

THCV is not present in synthetic dronabinol preparations (27). THCVCOOH was detected in urine after participants smoked cannabis containing THCV but not after dronabinol (28). In the present study, 63.6% of frequent smokers were THCVCOOH positive on admission, slightly >50% previously observed (13). THCV had the lowest detection rate among frequent and occasional smokers with only 5 and 1 participants, respectively, positive after both smoking and vaporization. THCV was detected in slightly more participants after vaporization, possibly indicating stability at lower vaporization temperatures. This hypothesis is supported by the THCVCOOH results: it was detected in 69.6% of frequent smokers after vaporization and 45.5% after smoking.

Based on our data, 3 groups of recent use markers were identified. THCVCOOH is not a useful marker due to its prolonged detection time after all administrations. THCV and THC-glucuronide are useful if found; detection rates were <72.7% and <45.5% overall, respectively. Thus, failure to detect these analytes does not preclude recent cannabis intake. Finally, CBG and CBN had the highest detectability and short detection windows, but were not observed after oral dosing. The value of CBD as a recent use marker cannot be determined currently without investigations of high-potency CBD cannabis, a newly available medicinal cannabis product. These groupings are based on free cannabinoid concentrations since blood was not hydrolyzed before analysis.

11-OH-THC, CBD, CBN, and THC-glucuronide were suggested as potential recent use markers in blood (7, 9, 10), identifying intake within approximately 2 h after smoking if detected. Here, frequent smokers' detection times were >72 h with THC cutoffs  $\geq 1\text{--}2\text{ }\mu\text{g/L}$ , complicating interpretation. If the THC cutoff was increased to  $\geq 5\text{ }\mu\text{g/L}$ , frequent smokers' detection times were reduced to 12–26 h, although no occasional smoker was positive 1.5 h after smoking or vaporization, demonstrating a highly limited detection window at this cutoff.

With a THC/11-OH-THC >1 plus THCCOOH/11-OH-THC <15 cutoff all participants were negative within 24 h. This cutoff has value in drug monitoring or treatment compliance programs because it can discriminate intake during the previous day from intake several days earlier, and is applicable to both smoking groups. A combined THC  $\geq 5\text{ }\mu\text{g/L}$  plus THCCOOH/11-OH-THC <20 cutoff produced detection windows <8 h for frequent smokers regardless of route, which has applications in driving under the influence of drugs settings. This cutoff was less effective for occasional smokers because all samples were negative by 1.5 h following smoking and vaporization. A THC  $\geq 1\text{ }\mu\text{g/L}$  cutoff could be useful for occasional smokers (participants were negative by 3.5–5 h after smoking and vaporization but 14 h after oral dosing); however, dose and smoking history are not typically known. Differences between frequent and occasional smokers' cannabinoid pharmacokinetics suggest that no one criterion studied to date is capable of identifying cannabis use within a single timeframe for smokers of all frequencies and after all administration routes. Therefore, blood cannabinoid results should be interpreted with multiple, complimentary criteria—presence of minor cannabinoids, THC concentrations, and analyte ratios—in conjunction with any impairment observations.

In summary, we quantified THC, its phase I and glucuronidated phase II metabolites, and minor cannabinoids in blood after smoked, vaporized, and oral cannabis to frequent and occasional smokers. Minimal differences between smoked and vaporized cannabis administrations were observed, supporting previous conclusions that vaporization provides comparable cannabinoid delivery. CBG and CBN are recent-use cannabis markers after inhalation; however, they were not detected following oral cannabis administration. Analyte concentration ratios are a complementary method for identifying recent cannabis use. Additional studies administering high-potency THC and CBD cannabis via inhaled or other edible preparations are needed.

**Author Contributions:** All authors confirmed they have contributed to the intellectual content of this paper and have met the following 3 requirements: (a) significant contributions to the conception and design, acquisition of data, or analysis and interpretation of data; (b) drafting or revising the article for intellectual content; and (c) final approval of the published article.

**Authors' Disclosures or Potential Conflicts of Interest:** Upon manuscript submission, all authors completed the author disclosure form. Disclosures and/or potential conflicts of interest:

**Employment or Leadership:** None declared.

**Consultant or Advisory Role:** None declared.

**Stock Ownership:** None declared.

**Honoraria:** None declared.

**Research Funding:** M.N. Newmeyer, The Intramural Research Program, National Institute on Drug Abuse, NIH, to institution; M.

Huestis, The Intramural Research Program, National Institute on Drug Abuse, NIH, to institution.

**Expert Testimony:** None declared.

**Patents:** None declared.

**Role of Sponsor:** The funding organizations played no role in the design of study, choice of enrolled patients, review and interpretation of data, and final approval of manuscript.

**Acknowledgments:** The authors thank Dr. Sandrine Pirard for her contribution to study design and the contributions of the clinical staffs of the Intramural Research Program, National Institute on Drug Abuse, and the Clinical Research Unit, Johns Hopkins Bayview Medical Center. This study was registered on [clinicaltrials.gov](https://clinicaltrials.gov) (NCT02177513). This research was supported by the Intramural Research Program of the National Institute on Drug Abuse, National Institutes of Health. M.N. Newmeyer acknowledges the Graduate Partnership Program, NIH.

## References

1. United Nations Office on Drugs and Crime. World drug report 2015. United Nations publication, Sales No. E.15.XI.6.
2. U.S. Drug Enforcement Administration, Office of Diversion Control. National Forensic Laboratory Information System: year 2014 annual report. Springfield (VA): US Drug Enforcement Administration; 2015.
3. Berning A, Compton R, Wochinger K. Results of the 2013–2014 national roadside survey of alcohol and drug use by drivers. Washington (DC): National Highway Traffic Safety Administration; 2015. Report No. DOT HS 812 118. Traffic Safety Facts Research Note.
4. Perez-Reyes M, Timmons MC, Lipton MA, Davis KH, Wall ME. Intravenous injection in man of  $\Delta^9$ -tetrahydrocannabinol and 11-OH- $\Delta^9$ -tetrahydrocannabinol. *Science* 1972;177:633–5.
5. Lemberger L, Martz R, Rodda B, Forney R, Rowe H. Comparative pharmacology of  $\Delta^9$ -tetrahydrocannabinol and its metabolite, 11-OH- $\Delta^9$ -tetrahydrocannabinol. *J Clin Invest* 1973;52:2411–7.
6. Huestis MA, Henningfield JE, Cone EJ. Blood cannabinoids. I. Absorption of THC and formation of 11-OH-THC and THCCOOH during and after smoking marijuana. *J Anal Toxicol* 1992;16:276–82.
7. Bergamaschi MM, Karschner EL, Goodwin RS, Scheidweiler KB, Hirvonen J, Queiroz RH, Huestis MA. Impact of prolonged cannabinoid excretion in chronic daily cannabis smokers' blood on per se drugged driving laws. *Clin Chem* 2013;59:519–26.
8. Ramaekers JG, Moeller MR, van Ruitenbeek P, Theunissen EL, Schneider E, Kauert G. Cognition and motor control as a function of  $\Delta^9$ -THC concentration in serum and oral fluid: Limits of impairment. *Drug Alcohol Depend* 2006;85:114–22.
9. Schwabe DM, Karschner EL, Gorelick DA, Huestis MA. Identification of recent cannabis use: whole-blood and plasma free and glucuronidated cannabinoid pharmacokinetics following controlled smoked cannabis administration. *Clin Chem* 2011;57:1406–14.
10. Desrosiers NA, Himes SK, Scheidweiler KB, Concheiro-Guisan M, Gorelick DA, Huestis MA. Phase I and II cannabinoid disposition in blood and plasma of occasional and frequent smokers following controlled smoked cannabis. *Clin Chem* 2014;60:631–43.
11. ElSohly MA, Feng S, Murphy TP, Warrington AW, Ross S, Nimrod A, et al. Identification and quantitation of 11-nor- $\Delta^9$ -tetrahydrocannabinol-9-carboxylic acid, a major metabolite of  $\Delta^9$ -tetrahydrocannabinol. *J Anal Toxicol* 2001;25:476–80.
12. Hidvegi E, Somogyi GP. Detection of cannabigerol and its presumptive metabolite in human urine after cannabis consumption. *Pharmazie* 2010;65:408–11.
13. Levin FR, Mariani JJ, Brooks DJ, Xie S, Murray KA.  $\Delta^9$ -Tetrahydrocannabinol testing may not have the sensitivity to detect marijuana use among individuals ingesting dronabinol. *Drug Alcohol Depend* 2010;106:65–8.
14. Schauer GL, King BA, Bunnell RE, Promoff G, McAfee TA. Toking, vaping, and eating for health or fun: marijuana use patterns in adults, U.S., 2014. *Am J Prev Med* 2016;50:1–8.
15. Abrams DI, Vizoso HP, Shade SB, Jay C, Kelly ME, Benowitz NL. Vaporization as a smokeless cannabis delivery system: a pilot study. *Clin Pharmacol Ther* 2007;82:572–8.
16. Hartman RL, Brown TL, Milavetz G, Spurgin A, Gorelick DA, Gaffney G, Huestis MA. Controlled cannabis vaporizer administration: blood and plasma cannabinoids with and without alcohol. *Clin Chem* 2015;61:850–69.
17. Hunt CA, Jones RT. Tolerance and disposition of tetrahydrocannabinol in man. *J Pharmacol Exp Ther* 1980;215:35–44.
18. Goodwin RS, Gustafson RA, Barnes A, Nebro W, Moolchan ET, Huestis MA.  $\Delta^9$ -tetrahydrocannabinol, 11-hydroxy- $\Delta^9$ -tetrahydrocannabinol and 11-nor-9-carboxy- $\Delta^9$ -tetrahydrocannabinol in human plasma after controlled oral administration of cannabinoids. *Ther Drug Monit* 2006;28:545–51.
19. Wall ME, Perez-Reyes M. The metabolism of delta-9-tetrahydrocannabinol and related cannabinoids in man. *J Clin Pharm* 1981;21:1785–895.
20. Law B, Mason PA, Moffat AC, Gleadle RI, King LJ. Forensic aspects of the metabolism and excretion of cannabinoids following oral ingestion of cannabis resin. *J Pharm Pharmacol* 1984;36:289–94.
21. Schwilke EW, Schwabe DM, Karschner EL, Lowe RH, Darwin WD, Kelly DL, et al.  $\Delta^9$ -tetrahydrocannabinol (THC), 11-hydroxy-THC, and 11-nor-9-carboxy-THC plasma pharmacokinetics during and after continuous high-dose oral THC. *Clin Chem* 2009;55:2180–9.
22. Karschner EL, Darwin WD, Goodwin RS, Wright S, Huestis MA. Plasma cannabinoid pharmacokinetics following controlled oral  $\Delta^9$ -tetrahydrocannabinol and oromucosal cannabis extract administration. *Clin Chem* 2011;57:66–75.
23. Cone EJ, Johnson RE, Paul BD, Mell LD, Mitchell J. Marijuana-laced brownies: Behavioral effects, physiologic effects, and urinalysis in humans following ingestion. *J Anal Toxicol* 1988;12:169–75.
24. Lile JA, Kelly TH, Charnigo RJ, Stinchcomb AL, Hays LR. Pharmacokinetic and pharmacodynamic profile of supratherapeutic oral doses of  $\Delta^9$ -THC in cannabis users. *J Clin Pharmacol* 2013;53:680–90.
25. Scheidweiler KB, Newmeyer MN, Barnes AJ, Huestis MA. Quantification of cannabinoids and their free and glucuronide metabolites in whole blood by disposable pipette extraction and liquid chromatography-tandem mass spectrometry. *J Chromatogr A* 2016;1453:34–42.
26. Hazekamp A, Ruhaak R, Zuurman L, van Gerven J, Verpoorte R. Evaluation of a vaporizing device (Volcano®) for the pulmonary administration of tetrahydrocannabinol. *J Pharm Sci* 2006;95:1308–17.
27. ElSohly MA, Feng S, Murphy TP, Ross SA, Nimrod A, Mehmedic Z, Fortner N.  $\Delta^9$ -tetrahydrocannabinol ( $\Delta^9$ -THCV) as a marker for the ingestion of cannabis versus Marinol. *J Anal Toxicol* 1999;23:222–4.
28. ElSohly MA, deWit H, Wachtel SR, Feng S, Murphy TP.  $\Delta^9$ -Tetrahydrocannabinol as a marker for the ingestion of marijuana versus Marinol: Results of a clinical study. *J Anal Toxicol* 2001;25:565–71.