

1 **Marijuana Use by Breastfeeding Mothers and Cannabinoid Concentrations in Breast Milk**

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34
35 **Abbreviations:**

36 Δ 9-THC: Delta-9-Tetrahydrocannabinol

37 CBD: Cannabidiol

38 11-OH-THC: 11-Hydroxy-Delta-9-Tetrahydrocannabinol

39 Δ 9-carboxy-THC: Delta-9-Carboxy-Tetrahydrocannabinol

40 CBN: Cannabinol

41 LC-MS ESI: Liquid chromatography mass spectrometry electrospray ionization

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1 **Table of Contents Summary:** This study quantified levels of cannabinoids Δ 9-THC, 11-OH-
2 THC, CBD, and CBN in 54 milk samples provided by breastfeeding mothers who reported
3 recent marijuana use.
4
5 **What's Known on This Subject:** Previous data quantifying the transfer of Δ 9-THC and other
6 cannabinoids into human breast milk following maternal marijuana use are limited to several
7 case reports.
8
9 **What This Study Adds:** In 50 women reporting marijuana use while breastfeeding, Δ 9-THC
10 was measurable in 63% of milk samples, up to 6 days after last use; 11-OH-THC and CBD were
11 measurable in 9% of milk samples, and CBN was undetectable in all samples.
12

1 **Contributors' Statement Page**

2 Mrs. Bertrand designed the data collection instruments, coordinated and supervised data
3 collection and drafted the initial manuscript, and reviewed and revised the manuscript.

4
5 Dr. Chambers conceptualized and designed the study and critically reviewed and revised the
6 manuscript.

7
8 Drs. Best and Hanan carried out the assay development, sample analysis, and reviewed and
9 revised the manuscript.

10
11 Mr. Honerkamp-Smith carried out the study analyses and reviewed and revised the manuscript.

12
13 All authors approved the final manuscript as submitted and agree to be accountable for all
14 aspects of the work.

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1 **Abstract**

2 **Background and Objectives**

3 Marijuana is the most commonly used recreational drug among breastfeeding women. With
4 legalization of marijuana in several U.S. states and a 1990 study that documented psychomotor
5 deficits in infants breastfed by mothers using marijuana, there is a need for information on
6 potential exposure to the breastfed infant. The objective of this study was to quantify
7 cannabinoids in human milk following maternal marijuana use.

8 **Methods**

9 Between 2014 - 2017, 50 breastfeeding women who reported marijuana use provided 54
10 breastmilk samples to a research repository, Mommy's Milk. Concentrations of Δ 9-THC, 11-
11 OH-THC, CBD, and CBN were measured using LC-MS ESI.

12 **Results**

13 Δ 9-THC was detectable in 34 (63%) of the 54 samples up to approximately six days after last
14 reported use; the median concentration of Δ 9-THC was 9.47 ng/mL (range 1.01, 323.00). Five
15 samples had detectable levels of 11-OH-THC (range 1.33, 12.80 ng/mL) or CBD (range 1.32,
16 8.56 ng/mL). The sample with the highest concentration of CBD (8.56 ng/mL) did not have
17 measurable Δ 9-THC. CBN was not detected in any samples. Number of hours since last use was
18 a significant predictor of log Δ 9-THC concentrations (-0.03, 95% Confidence Interval [CI] -0.04,
19 -0.01, $p=0.005$). Adjusted for time since last use, the number of daily uses and time from sample
20 collection to analysis were also significant predictors of log Δ 9-THC concentrations (0.51 95%
21 CI 0.03, 0.99, $p=0.039$; 0.08, 95% CI 0.00, 0.15, $p=0.038$, respectively).

22 **Conclusions**

23 Δ 9-THC was measurable in a majority of breastmilk samples up to approximately six days after
24 maternal marijuana use.

1 **Introduction**

2 Marijuana is the most commonly used recreational drug among breastfeeding women.^{1,2}
3 However, potential infant exposure to marijuana through breastfeeding is poorly understood.
4 This question is of critical importance as human milk is the normative standard for infant feeding
5 and nutrition.³ The World Health Organization recommends exclusive breastfeeding up to six
6 months of age.⁴ Being breastfed early in life has been associated with a reduction in subsequent
7 obesity and improved performance on intelligence tests. In mothers, breastfeeding has been
8 associated with lower risks for subsequent breast and uterine cancer and Type II diabetes.^{5,6}

9 However, there is a paucity of data on the effects of maternal marijuana use among
10 infants potentially exposed through breastmilk. Case reports have documented the presence of
11 the primary psychoactive ingredient in marijuana, delta-9-tetrahydrocannabinol (Δ 9-THC), in
12 human milk.⁷ In one report, the level of Δ 9-THC measured in a milk sample provided by a
13 mother who smoked marijuana once daily was 105 ng/mL. The concentration in a sample from a
14 second mother who smoked marijuana seven times per day was 340 ng/mL. In an additional set
15 of paired milk and maternal plasma samples obtained from one of these mothers, the Δ 9-THC
16 concentration measured in the milk sample (60.3 ng/mL) was eight times higher than the
17 maternal plasma concentration of 7.2 ng/mL, suggesting that Δ 9-THC accumulated in
18 breastmilk.⁸ Furthermore, fecal samples from that mother's infant had higher concentrations of
19 other metabolites (11-hydroxy-delta-9-teretrahydrocannabinol [11-OH-THC] and Δ 9-carboxy-
20 THC) than the mother's breastmilk, suggesting the infant absorbed and metabolized the Δ 9-THC
21 following breastmilk ingestion. In another case report, one human milk sample obtained from a
22 woman with a history of drug abuse was found to have detectable levels of both Δ 9-THC (86
23 ng/mL) and 11-OH-THC (5 ng/mL).⁹ A third published study analyzed 109 randomly collected

1 milk samples with no accompanying information on maternal marijuana use; Δ^9 -THC was
2 detected in two samples and cannabidiol (CBD) in one.¹⁰

3 There are very limited data on the potential neurobehavioral effects of infant exposure to
4 cannabis through breastmilk. Astley et al (1990) reported psychomotor deficits in 55 12-month
5 old infants breastfed by mothers using cannabis compared to 81 unexposed.¹¹ In contrast, Tennes
6 et al (1985) reported no differences in motor and mental development in 27 12-month old infants
7 whose mothers used marijuana while breastfeeding compared to 35 unexposed.¹²

8 Lacking definitive data on the risk or safety of infant exposure to cannabis through
9 breastmilk, the American Academy of Pediatrics and the American Congress of Obstetricians
10 and Gynecologists advise that marijuana use should be discouraged while breastfeeding.^{13,14}
11 However, with current legalization of marijuana for recreational use in 9 U.S. states, there is an
12 urgent need for characterization of cannabinoid distribution in human milk and the
13 corresponding potential exposure of breastfeeding infants and toddlers. The purpose of this study
14 was to measure cannabinoid concentrations in breast milk in relation to dose and time since last
15 maternal marijuana use.

16

17 **Patients and Methods**

18 *Study Design*

19 In 2014, the University of California, San Diego, established Mommy's Milk, the Human
20 Milk Biorepository (HMB) for research. Volunteers residing anywhere in the U.S. and Canada
21 have been recruited into HMB through a variety of sources including social media. After
22 providing written informed consent, for future research uses of milk samples and associated data,
23 breastfeeding mothers completed an interview providing demographics, maternal and child

1 health history, and details regarding exposures to medications, alcohol, tobacco, and other
2 recreational substances. Participants recalled their exposures for the 14 days prior to milk sample
3 collection and provided additional information on exposure to herbal supplements, prescription
4 medications, or recreational substances since giving birth. Women were instructed to pump and
5 collect 50 mL of milk up to a full expression as close to the time of the scheduled interview as
6 possible and up to 24 hours prior. Any quantity of milk ≥ 1 mL was accepted. The HMB protocol
7 was approved by the institutional review board at the University of California San Diego, and a
8 National Institutes of Health (NIH) Certificate of Confidentiality was obtained.

9 *Assessment of Exposure*

10 For HMB participants who reported marijuana use at any time since giving birth, the
11 maternal questionnaire data included route of administration (inhaled, ingested, topical),
12 frequency of use, dose, and time since last use prior to sample collection. If mothers indicated a
13 dose unit of joints, puffs or grams, the route of administration was classified as “inhalation
14 only”; those who indicated a dose unit of drops, milligrams or servings were classified as “other
15 only”. Those who reported more than one route of administration were classified as “both”. The
16 frequency of marijuana use was determined by calculating the average number of uses per day
17 during the most recent exposure window within the 14-day recall. Hours since last use was
18 calculated using the end of the exposure interval for the most recent exposure up to the time of
19 milk sample collection.

20 *Collection and Preparation of Human Milk Samples*

21 Prior to sample collection, participants were instructed to clean the nipple and areola of
22 the breast with an alcohol wipe. Milk sampling occurred using one of two methods: 1) if at the
23 HMB research center, participants pumped milk with a hospital grade industrial breast pump

1 (either the Medela Symphony® or the Hygeia Enjoye®) using a sterile collection kit provided by
2 study staff; or 2) if a home collection, participants pumped milk into a personal milk collection
3 container specific to their own hand or electric breast pump. For home collections, expressed
4 milk was refrigerated at 0-4°C until either transported on ice to the HMB research center or
5 picked up by courier within 24 hours of collection and shipped overnight on ice to the HMB
6 facility. Upon receipt, samples were aliquoted into 1 – 15 mL cryovials or centrifuge tubes and
7 stored at -80°C.

8 ***Analytical Procedure***

9 Reference standards (Δ^9 -THC, 11-OH-THC, CBD, and cannabinal [CBN]) and
10 isotopically labeled internal standards (THC-d3, 11-OH-THC-d3, CBD-d3, CBN-d3) were
11 purchased from Cerilliant (Round Rock, TX). BondElut® certify (Varian, Lake Forest, CA) C-
12 18 cartridges were used for solid-phase extraction. HPLC grade water, 0.1% formic acid in
13 acetonitrile (ACN), and methanol (MeOH) were purchased from Fisher Scientific. Reagent grade
14 ammonium formate and pure sodium hydroxide pellets were purchased from Sigma-Aldrich.

15 We followed the Wei *et al* saponification-solid-phase extraction method for sample clean
16 up and extraction with minor modifications.¹⁵

17 Chromatographic separation was achieved on reverse-phase C-18 column (MAC-MOD Ace 5 C-
18 18, 15cm • 2.1mm). The mobile phase consisted of 5 mM ammonium formate in 0.05% formic
19 acid (Solvent A) and ACN in 0.1% formic acid (Solvent B). A gradient elution was performed
20 over 15 minutes at a flow rate of 200 μ L/min. Retention times for 11-OH-THC, CBD, THC
21 were 9.35, 10.62, and 11.70 minutes in positive ion mode, respectively. Quantitation of THC, 11-
22 OH-THC, and CBD and isotopically labeled internal standards was achieved on a triple

1 quadrupole mass spectrometer (API-4000, AB Sciex) in positive ion mode. CBN and CBN-d3
2 were detected in negative ion mode.

3 *Statistical Analysis*

4 Demographic and clinical variables for mothers and infants were summarized using
5 frequencies and percentages. For each cannabis metabolite, concentrations were categorized as
6 either above or below the quantification limit which was defined as 1 ng/mL. Summary
7 statistics for those samples with concentrations above the limits of quantification for each
8 metabolite were computed. Concentrations of Δ 9-THC were log transformed. Linear regression
9 models were used to estimate associations between the log concentrations of Δ 9-THC and time
10 since last cannabis exposure, frequency of use, dose, and route of administration. Other
11 covariates that were considered included maternal body mass index, age and sex of the infant,
12 time from sample collection to analysis (sample age), time of day of sample collection, time
13 since last feed or pump, and feeding frequency. Effect estimates and 95% confidence intervals
14 (CIs) as well as p-values were reported.

15 To estimate potential infant exposure and infant plasma concentrations, we used the Δ 9-
16 THC concentrations from the linear regression models to compute the mean cumulative dose of
17 Δ 9-THC hypothetically ingested over a 24-hour period by a nursing 3 month old infant that
18 weighs 6.1 kg, assuming 3.1875 ounces of milk was consumed at each feeding over eight
19 equally-spaced feedings with 6% oral bioavailability.¹⁶ All statistical analyses were performed
20 using R version 3.4.1 with a two-sided p-value below 0.05 judged as significant.

21

22 **Results**

1 Between 2014 and 2017, 50 mothers who participated in the HMB research repository
2 reported recent marijuana use and provided a breast milk sample. Four mothers provided two
3 samples at different time points. Characteristics of the participants are shown in Table 1. Two-
4 thirds of the women were breastfeeding a child <1 year of age. The most common route of
5 administration was by inhalation only (64%) and most women in the sample (88%) reported at
6 least daily marijuana use.

7 As shown in Table 2, $\Delta 9$ -THC was detectable in 34 (63%) of 54 samples; among these,
8 the median concentration of $\Delta 9$ -THC was 9.47 ng/mL (range 1.01, 323.00). Only 5 (9%) of the
9 54 samples had measurable concentrations of 11-OH-THC. Similarly, 5 (9%) of the 54 samples
10 had measurable concentrations of CBD. Only one sample had measurable levels of all three
11 cannabinoids ($\Delta 9$ -THC, 11-OH-THC, CBD); in this sample, the concentration of $\Delta 9$ -THC was
12 the highest measured in the series (323 ng/mL). Similarly, only one other sample had measurable
13 levels of two of the cannabinoids (11-OH-THC and CBD but not $\Delta 9$ -THC); in that sample, the
14 concentration of CBD was the highest measured in the series (8.56 ng/mL). There were no
15 samples with detectable levels of CBN.

16 As shown in Table 3 and in Figure 1 a there was a significant association between log
17 $\Delta 9$ -THC concentrations and hours since last marijuana use in the fitted regression model. On
18 average, for each additional hour between last exposure to marijuana and the milk sample
19 collection, there was a reduction of 0.03 in the log concentration of $\Delta 9$ -THC (95% CIs -0.04, -
20 0.01, $p=0.005$). On the unlogged scale, this corresponds to a reduction in $\Delta 9$ -THC concentration
21 of 3% per hour after last exposure, which can be used to estimate a half-life of approximately 27
22 hours for $\Delta 9$ -THC in human milk. The longest duration between last use of marijuana and
23 measurable $\Delta 9$ -THC was approximately 140 hours or six days (Figure 1).

1 As shown in Table 3, in each of the separate regression models, hours since last use was a
2 significant negative predictor of log $\Delta 9$ -THC concentration. Number of uses per day was also
3 significantly associated with increasing concentrations of log $\Delta 9$ -THC (0.51 95% CI 0.03, 0.99,
4 $p=0.039$), as was sample age in months (0.08, 95% CI 0.00, 0.15, $p=0.038$). No other covariates
5 among those considered were significant predictors of log $\Delta 9$ -THC concentration.

6 Based on our assumptions regarding breastfeeding frequency, quantity of milk ingested,
7 and 6% oral bioavailability, the estimated plasma concentration of $\Delta 9$ -THC in a hypothetical 3
8 month old infant weighing 6.1 kg was 0.040 ng/ mL. Compared to the plasma concentration of
9 an adult who consumed 10 mg of $\Delta 9$ -THC, the estimated infant dose ingested via breastmilk
10 would be approximately 1000 times lower.²⁴

11 $\Delta 9$ -THC was not detectable in 20 (37%) of 54 samples. Fewer women with undetectable
12 levels of $\Delta 9$ -THC reported more than daily use compared to women with measurable quantities
13 (41.2% vs. 21.1%). In addition, the mean number of hours since last use was higher among
14 those women with levels below limits of detection compared to those above, with wide
15 variability across samples (53.06 hours [SD 76.54] vs. 24.48 hours [SD 32.87]). However, the
16 only statistically significant factor differentiating the two groups was route of administration.
17 The majority of women with quantifiable levels of $\Delta 9$ -THC used marijuana exclusively via
18 inhalation (76.5%) compared to only 36.8% in the group with levels of $\Delta 9$ -THC below limits of
19 detection ($p=0.010$) (data not shown).

20

21 **Discussion**

22 This is the first study to quantify levels of specific cannabinoids detectable in human
23 milk in a relatively large sample of mothers with detailed and varied histories of recent

1 marijuana use of currently available products. Δ 9-THC, 11-OH-THC, and CBD were all
2 detectable at 1 ng/mL or above in at least one human milk sample. Δ 9-THC was detected in 34
3 (63%) of the 54 samples analyzed. This finding is consistent with the few previously published
4 case reports in which Δ 9-THC was measurable in human milk.

5 Cannabinoids are highly lipophilic compounds.^{16,17} Therefore, it is not surprising that
6 metabolites would be detected in human milk which is comprised of 3-5% fat.¹⁸ However, the fat
7 content of human milk is the most variable of its macronutrient components; hindmilk may
8 contain two to three times the concentration of milk fat found in foremilk.^{19,20} As a result, we
9 might have expected that women in this study who did not provide a full expression of the breast
10 would have lower concentrations of cannabinoids; however, 80% of the samples were collected
11 from a full expression (Table 1), and sample collection type as a covariate was not significant in
12 any of our models (Table 3). Further, 11-OH-THC is less lipophilic than Δ 9-THC, which might
13 explain why only five of the milk samples had measurable concentrations for this
14 cannabinoid.^{16,17}

15 The number of times a woman used marijuana per day was a positive predictor of log Δ 9-
16 THC concentrations in milk. This finding was expected and consistent with the few previous
17 case reports in which higher concentrations were found in one mother who smoked marijuana
18 seven times per day compared to another who smoked one time per day.⁸

19 In our study, the sample age was also a significant predictor of log Δ 9-THC
20 concentrations in milk, which was unexpected. Cannabinoids are stable with minimal
21 degradation long-term when frozen, and there is no evidence that more metabolites are formed
22 during storage.¹⁵ However, sample age was also positively correlated with the number of times a

1 woman used marijuana per day, i.e., dose (eFigure1). Thus, the association between sample age
2 and log $\Delta 9$ -THC may be explained by this artifact in the data.

3 In this study, we did not measure infant plasma concentrations. However, based on our
4 assumptions, we estimated the mean infant plasma concentration of $\Delta 9$ -THC obtained from
5 breastfeeding to be about 1000 times lower than the concentration in an adult after a single dose
6 of 10 mg of $\Delta 9$ -THC²⁵. A previous case report showed that a nursing infant exposed to marijuana
7 via breast milk had high concentrations of $\Delta 9$ -THC, 11-OH-THC and $\Delta 9$ -carboxy-THC in feces
8 indicating that the child absorbed, metabolized and excreted marijuana metabolites despite the
9 lower $\Delta 9$ -THC levels present in the mother's milk⁸. If a child is exposed to low levels of $\Delta 9$ -
10 THC in milk daily, there is a concern for accumulation of the various cannabinoids in the nursing
11 infant due to slow elimination from body fat stores and continuous daily exposure.¹⁶

12 Since the brain rapidly develops during the time period when, ideally, a child's main
13 source of nutrition is human milk, brain development may be altered by $\Delta 9$ -THC exposure.
14 Previous studies have suggested that *prenatal* exposure to cannabis may interfere with brain
15 development resulting in deficits in cognitive and behavioral function.²¹⁻²³ It is reasonable to
16 speculate that $\Delta 9$ -THC, 11-OH-THC or CBD exposure during breastfeeding, depending on the
17 dose and timing, could influence normal brain development of a child.

18 This study had several limitations as well as strengths. Samples were collected under
19 different conditions, not all breast milk collections were directly observed, and we relied on
20 maternal report of marijuana exposure. However, all participants completed a 14-day recall
21 guided by trained study staff who prompted for specific daily use with the aid of a calendar. We
22 had detailed information on timing and dose as well as information on a wide variety of other
23 relevant covariates. Furthermore, the samples were collected from mothers who were using

1 currently available cannabis products. In contrast, previously published data were collected at a
2 time when $\Delta 9$ -THC concentrations in marijuana were far less than is typical today.²⁴

3 We did not have infant plasma samples; instead we estimated potential infant $\Delta 9$ -THC
4 exposure based on several assumptions. Our infant exposure estimates may be an under- or
5 overestimation depending on infant age and other factors. Future studies are required to better
6 characterize the distribution of cannabinoids in human milk through more intensive and paired
7 (milk/plasma) sampling and with more detailed data about the cannabinoid content of the
8 product and exact route used by the mothers. In addition, longitudinal sampling of plasma of
9 breastfeeding infants with either single-dose exposure (mothers who are occasional users) or
10 steady-state (mothers who are frequent users) is needed to determine the extent to which
11 cannabinoids accumulate in the breastfeeding infant.

12 Our findings also highlight a critical need for further research on neurodevelopmental
13 outcomes in infants breastfed by mothers using marijuana. The one previous study that found
14 psychomotor deficits in 12-month old infants breastfed by mothers using cannabis was
15 conducted in an era when $\Delta 9$ -THC concentrations in marijuana were estimated to be one third of
16 what they are today.^{11,24}

17 In summary, $\Delta 9$ -THC was measurable in highly variable concentrations in the breast milk
18 of approximately two-thirds of samples from women who reported marijuana use during
19 breastfeeding and up to approximately six days since last reported dose. While the estimated
20 median daily dose of $\Delta 9$ -THC ingested by the infant is very low compared to adult doses, the
21 high variability in breastmilk concentrations means that some infants may be exposed to daily
22 amounts of cannabinoids closer to (but still lower than) typical adult amounts. Furthermore, the
23 extent of oral absorption in breastfeeding infants, metabolism and accumulation patterns, and

1 pharmacologic effects of even low levels of cannabinoids on neurodevelopment in infants are
2 unknown and require further study. As marijuana is the most commonly used recreational drug
3 among breastfeeding women, information regarding risks to breastfeeding infants is urgently
4 needed.

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Table 1. Selected characteristics of maternal and infant participants, n = 54, 2014-2017

Characteristic	N= 50 mothers, No. (%)	N=4 mothers who gave a repeat sample, No. (%)
Maternal Age (yrs)		
22-25	7 (14)	
25-30	17 (34)	
30-35	18 (36)	
35-41	8 (16)	
Maternal Ethnicity		
Hispanic	9 (18)	
Non-Hispanic	41 (82)	
Maternal Race		
Caucasian	44 (88)	
Black	1 (2)	
Asian	2 (4)	
Native American	3 (6)	
Maternal Education (yrs)		
Partial High School	1 (2)	
High School Graduate/GED	4 (8)	
Some College/ Specialization	27 (54)	
College Graduate	14 (28)	
Post-Graduate	4 (8)	
Maternal Body Mass Index (BMI)^a		
<18.5	0 (0)	
18.5-24.99	17 (34)	
25-29.99	17 (34)	
>30	9 (18)	
Parity		
1	21 (42)	
>1	29 (58)	
Route of Marijuana Exposure^b		
Inhalation Only	32 (64)	2 (50)
Other Only	7 (14)	0 (0)
Both	11 (22)	2 (50)
Frequency of Marijuana Use		
<1 use/day	6 (12)	1 (25)
1 use/day	23 (46)	3 (75)
>1 use/day	21 (42)	0 (0)
Full Expression of the Breast^c		
No	9 (18)	0 (0)
Yes	41 (82)	4 (100)
Infant Age (months)^d		
0-3	3 (6)	0 (0)
3-6	20 (40)	0 (0)

6-12	11 (22)	0 (0)
>12	16 (32)	4 (100)
Infant Sex		
Female	22 (44)	
Male	28 (56)	

- 1 ^a Body Mass Index (BMI)= bodyweight in kilograms divided by height in meters squared
- 2 ^b Inhalation only was defined as a dose unit of joints, puffs, or grams. Other only was defined as a dose unit of drops,
- 3 milligrams or servings. Both was defined as a dose unit from the both the inhalation only and the other only groups
- 4 ^c A full expression of milk was defined as emptying the breast of milk entirely during one pump session
- 5 ^d If the mother of the baby was breastfeeding >1 child, only data from child 1 was included
- 6

1 **Table 2: Concentrations of cannabinoids in human milk samples, n= 54**

2

	Min.	1st Quartile	Median	3rd Quartile	Max.	AQL	BQL
Delta-9-Tetrahydrocannabinol (ng/mL)	1.01	2.29	9.47	46.78	323.00	34	20
11-hydroxy-tetrahydrocannabinol (ng/mL)	1.33	1.35	2.38	5.45	12.80	5	49
Cannabidiol (ng/mL)	1.32	2.92	4.99	5.97	8.56	5	49

3 *AQL, above quantification limits, was defined as ≥ 1 ng/ mL; BQL, below quantification limits, was defined as < 1

4 ng/mL

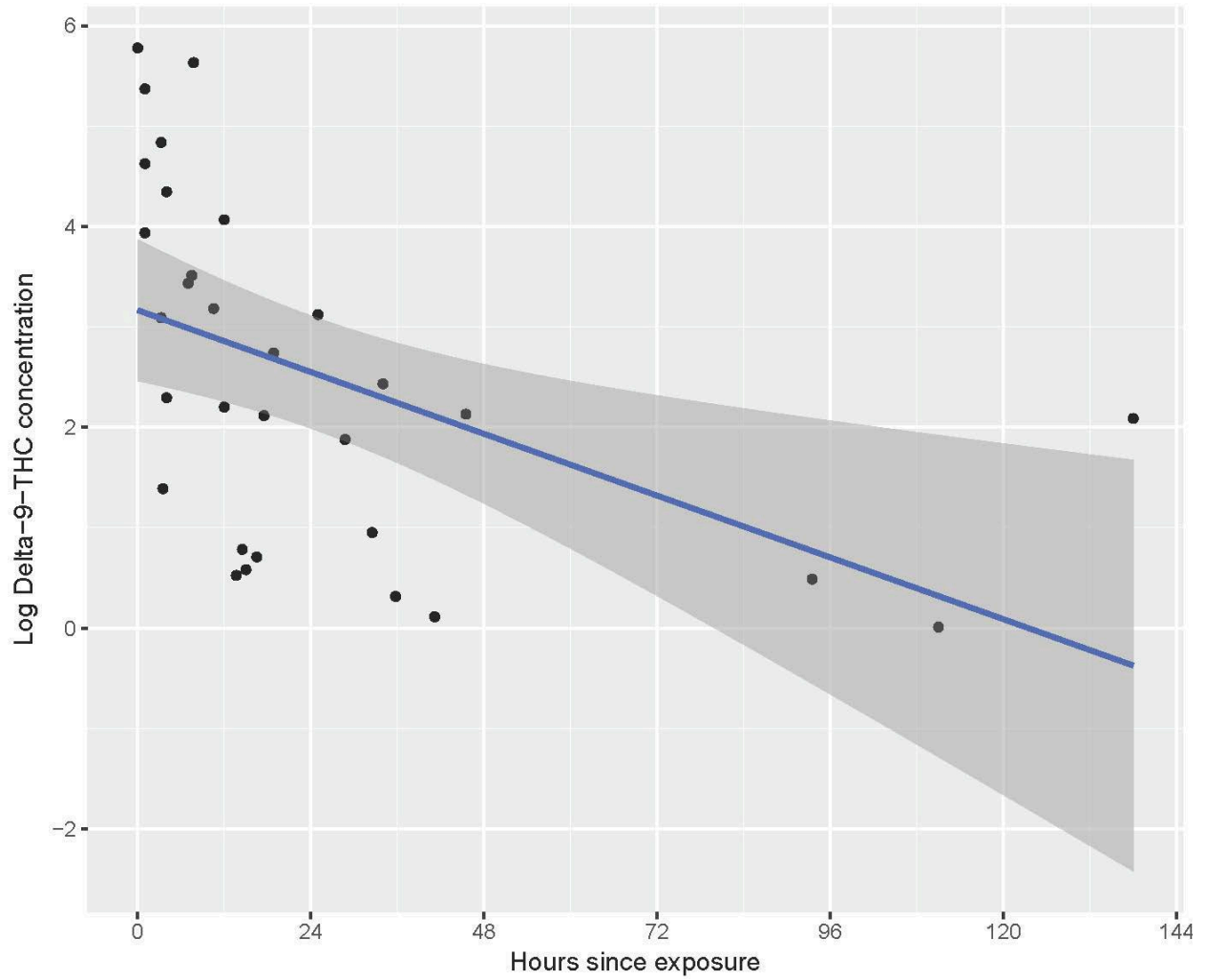
5 ** The concentration of CBN was BQL in all 54 samples

6

1 **Table 3. Log Δ -9-THC concentration by characteristics of maternal cannabis use and**
 2 **sample collection, n=34 samples**
 3

	Estimate	95% CI	p-value
Hours Since Last Use			
Hours ^a	-0.02	(-0.04, 0.00)	0.005
Uses per Day			
Hours	-0.02	(-0.04, 0.00)	0.032
Uses/Day ^b	0.51	(0.03, 0.99)	0.039
Route^c			
Hours	-0.02	(-0.04, -0.01)	0.008
Route: Other Only	-0.69	(-3.06, 1.67)	0.553
Route: Both	-1.11	(-2.64, 0.41)	0.146
Puffs^d			
Hours	-0.02	(-0.04, 0.00)	0.031
Puffs	-0.04	(-0.34, 0.26)	0.785
Heavy Use^e			
Hours	-0.02	(-0.04, 0.00)	0.062
Heavy Use	1.13	(-0.28, 2.53)	0.110
Sample Age^f			
Hours	-0.02	(-0.04, 0.00)	0.044
Sample Age (months)	0.08	(0.00, 0.15)	0.038
Time of Sample Collection^g			
Hours	-0.03	(-0.04, -0.01)	0.008
Time of Collection (PM)	0.27	(-0.97, 1.51)	0.662
Time Since Last Feed/ Pump^h			
Hours	-0.03	(-0.04, -0.01)	0.005
Time Since Last Feed./Pump (Hours)	-0.05	(-0.18, 0.09)	0.470
Feeding Frequencyⁱ			
Hours	-0.02	(-0.04, 0.00)	0.019
Feeding Frequency (Feeds/Day)	0.15	(-0.04, 0.34)	0.117

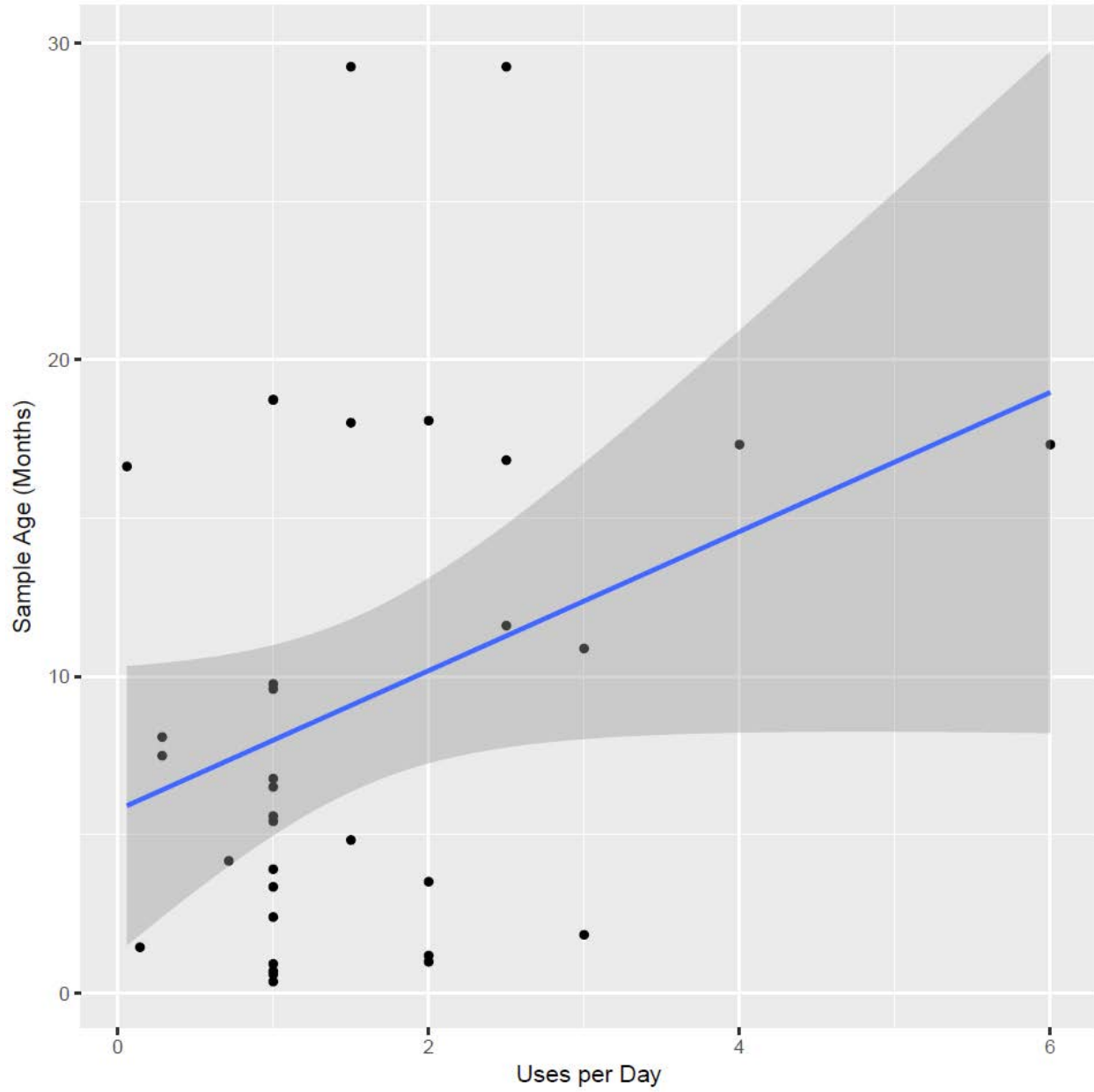
4 Separate multivariable linear regression models for each covariate with log-transformed Δ 9-THC as the outcome
 5 ^a Hours was defined as the number of hours between the most recent exposure end date and the date of sample collection
 6 ^b Uses per day was defined as the average number of uses of marijuana during the most recent exposure period
 7 ^c Route was defined as categorical variable with three levels: Inhalation, Other, and Both, with Inhalation as the reference
 8 category. Inhalation only was defined as a dose unit of joints, puffs, or grams. Other only was defined as a dose unit of drops,
 9 milligrams or servings. Both was defined as a dose unit from both the inhalation only and the other only groups
 10 ^d For the covariate puffs, the dataset was restricted to samples where the dose unit was indicated as “puffs” (n=27). The covariate
 11 “puffs” was reported as the number of puffs taken per use of marijuana
 12 ^e For the covariate Heavy Use, the dataset was restricted to samples where the dose unit was indicated as “Puffs” (n=27). The
 13 covariate “puffs” was reported as the number of puffs taken per use of marijuana. Heavy use was defined as women who reported
 14 uses per day and puffs greater than the median values in the sample (1 use/ day and 3 puffs per use, respectively)
 15 ^f Sample age was defined as the length of time between the date of milk collection and the date the sample was analyzed,
 16 measured in months.
 17 ^g Time of collection was defined as the time of day at which the sample was expressed, measured in number of hours after
 18 midnight and dichotomized into before noon (AM) and after noon (PM).
 19 ^h Time since last feed/pump was defined as the difference between the time of the sample collection and the time of the most
 20 recent feed or pump, measured in hours.
 21 ⁱ Feeding frequency was defined as the number of feedings per 24 hours.



1
2 *The fitted regression line is shaded with 95% confidence limits around the regression line.

3 **Figure 1.** Scatterplot and fitted regression line of log concentration of Δ -9-THC by hours since
4 last use of marijuana, N=34

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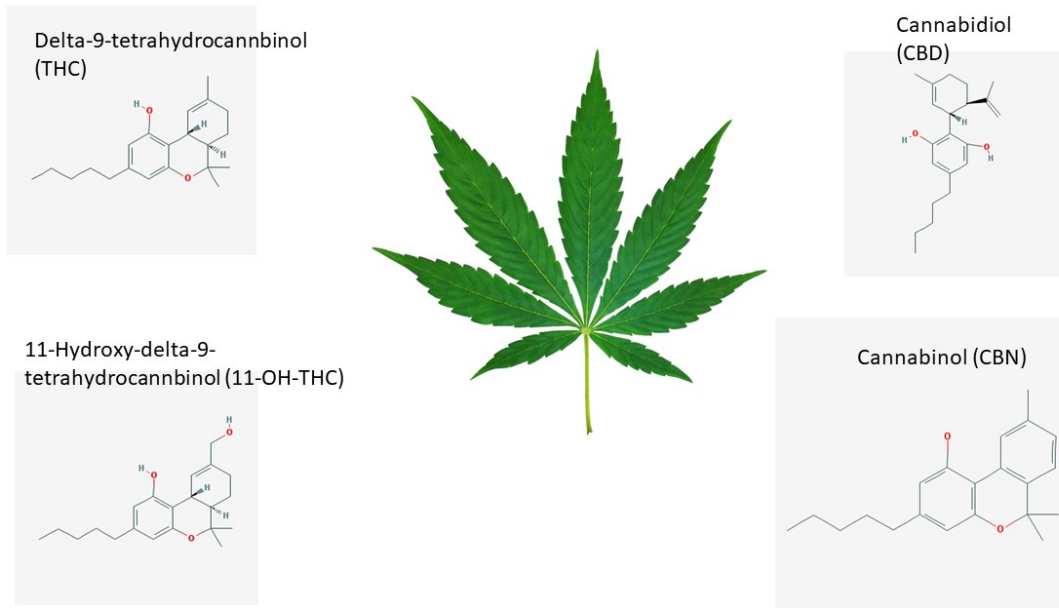


1

2 **eFigure 1.** Distribution of sample age in months by number of times used per day, n=34 human
3 milk samples

4

The primary cannabinoids derived from Marijuana



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5

eFigure 2. The four primary cannabinoids, Delta-9-THC, 11-OH-THC, CBD, and CBN, derived from Cannabis