

# Passive Cannabis Smoke Exposure and Oral Fluid Testing

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## Abstract

Oral fluid testing for  $\Delta^9$ -tetrahydrocannabinol (THC) provides a convenient means of detection of recent cannabis usage. In this study, the risk of positive oral fluid tests from passive cannabis smoke exposure was investigated by housing four cannabis-free volunteers in a small, unventilated, and sealed room with an approximate volume of 36 m<sup>3</sup>. Five active cannabis smokers were also present in the room, and each smoked a single cannabis cigarette (1.75% THC). Cannabis smoking occurred over the first 20 min of the study session. All subjects remained in the room for approximately 4 h. Oral fluid specimens were collected with the Intercept DOA Oral Specimen Collection Device. Three urine specimens were collected (0, 20, and 245 min). In addition, three air samples were collected for measurement of THC content. All oral fluid specimens were screened by enzyme immunoassay (EIA) for cannabinoids (cutoff concentration = 3 ng/mL) and tested by gas chromatography–tandem mass spectrometry (GC–MS–MS) for THC (LOQ/LOD = 0.75 ng/mL). All urine specimens were screened by EIA for cannabinoids (cutoff concentration = 50 ng/mL) and tested by GC–MS–MS for THCCOOH (LOQ/LOD = 1 ng/mL). Air samples were measured for THC by GC–MS (LOD = 1 ng/L). A total of eight oral fluid specimens (collected 20 to 50 min following initiation of smoking) from the four passive subjects screened and confirmed positive for THC at concentrations ranging from 3.6 to 26.4 ng/mL. Two additional specimens from one passive subject, collected at 50 and 65 min, screened negative but contained THC in concentrations of 4.2 and 1.1 ng/mL, respectively. All subsequent specimens for passive participants tested negative by EIA and GC–MS–MS for the remainder of the 4-h session. In contrast, oral fluid specimens collected from the five cannabis smokers generally screened and confirmed positive for THC throughout the session at concentrations substantially higher than observed for passive subjects. Urine specimens from active cannabis smokers also screened and confirmed positive at conventional cutoff concentrations. A biphasic pattern of decline for THC was observed in oral fluid specimens collected from cannabis smokers, whereas a linear decline was seen for passive subjects suggesting that initial oral fluid contamination is cleared rapidly and is followed by THC sequestration in the oral mucosa. It is concluded that the risk of positive oral fluid tests from passive cannabis smoke inhalation is limited to a period of approximately 30 min following exposure.

## Introduction

Cannabis is the most commonly used illicit drug in the United States. According to the National Survey on Drug Use and Health (1), there were 14.6 million past-month users in 2002, and about one third, or 4.8 million, used cannabis on 20 or more days in the past month. For the majority of users, the smoked route is preferred, although cannabis also is consumed by the oral route by mixing in various foodstuffs such as brownies. The fate of cannabinoids in cannabis is dependent upon conditions of usage. During the smoking process, 23–30% of the total  $\Delta^9$ -tetrahydrocannabinol (THC) content in cannabis is destroyed by pyrolysis and 20–37% is delivered in mainstream smoke to the user. The remaining 40–50% of THC is released to the environment in sidestream smoke (2).

Individuals in the immediate environment where cannabis is smoked are exposed to THC by passive inhalation. The extent of THC exposure depends upon a variety of factors, including the amount and potency of cannabis combusted, environmental conditions such as room size and ventilation conditions, and individual characteristics such as body size and metabolic rate. Several experimental studies have been conducted to determine the extent of THC exposure under passive conditions (3–6). Generally, the focus of these studies has been to determine whether passively exposed individuals produce positive urine tests following exposure. For example, under extreme conditions of exposure to the sidestream smoke from 16 cannabis cigarettes, urine concentrations of 11-nor- $\Delta^9$ -THC-9-carboxylic acid (THCCOOH) ranging from 15 to 87 ng/mL have been reported (3). Under less extreme conditions, however, it has been generally concluded that passive inhalation exposure does not result in positive urine tests at conventional cutoff concentrations (3,4).

Oral fluid testing for cannabis also has the potential to produce positive results not only from active use (7,8), but from passive exposure. However at present, there is limited data that addresses the risk of passive inhalation. In a controlled dosing study conducted by Niedbala et al. (7), two individuals who were passively exposed to the smoke from 10 cannabis cigarettes were shown to produce positive results in a screening assay, but all specimens failed to test positive by gas chromatography–tandem mass spectrometry (GC–MS–MS).

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The goal of the present study was to assess the potential risk of passive cannabis smoke inhalation by exposing individuals to more extreme smoke conditions than utilized in the earlier study. Considering the importance of room size and ventilation on exposure levels of THC (3), the present study was conducted in a small sealed room in which five cannabis users simultaneously smoked a cannabis cigarette in the presence of four cannabis-free volunteer subjects. Oral fluid and urine specimens were collected and analyzed by enzyme immunoassay (EIA) and GC-MS-MS. In addition, room air concentrations of THC were monitored periodically during and after the smoking period.

## Experimental

### Participants

The subjects participating in the study were nine healthy, caucasian, male volunteers. The ages of the cannabis smokers were from 21 to 25 years, and the ages of the passive subjects were from 37 to 49 years. The five cannabis smokers reported prior use of cannabis on an infrequent basis. The four passive subjects were cannabis-free at the time of the study based on self-reports and oral fluid and urine tests for cannabis conducted prior to the start of the study.

### Marijuana doses

Commercial cannabis cigarettes containing a mixture of cannabis and tobacco were purchased from a coffee house in the Netherlands. Analysis of THC content was performed by GC-MS (Pharmacy Academisch Ziekenhuis, Groningen, Netherlands). The cigarettes contained an average of 1.75% THC per cigarette (approximately 12.75 mg of THC).

### Study conditions, procedures, and specimen collections

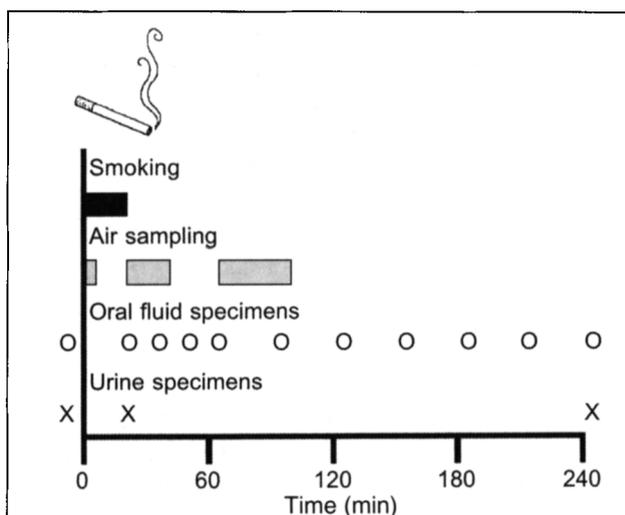
The study was conducted in a closed room with all door and window openings sealed. The dimensions of the room were approximately 3-m × 4-m × 3-m high with an approximate volume of 36 m<sup>3</sup>. Prior to beginning the study, all subjects provided oral fluid and urine specimens. A single cannabis cigarette was provided to each of five individuals who were instructed to smoke the entire cigarette according to their normal pace of smoking. The period of smoking lasted for approximately 20 min. Four subjects remained in the closed room with the smokers for the entire smoking period. The passive subjects were located approximately 1.5 m from the smokers. Research staff also remained in the room during the study and assisted in the collection of specimens. All participants remained in the sealed room for a period of approximately 4 h. Oral fluid specimens were collected from the four passive subjects at the following times (timed from beginning of cannabis smoking): -5, 20, 35, 50, 65, 95, 125, 155, 185, 215, and 245 min. The same collection times were employed for the active cannabis smokers with the exception that no oral fluid specimen was collected at 50 min. No drinking was allowed for the first 60 min. Limited consumption of liquids was allowed between later collections as long as it took place no less than 10 min before the next collection. Urine specimens were collected from all nine subjects before smoking (-5 min) and at 20 and

245 min. Figure 1 illustrates the timeline for smoking, air sample collection, and specimen collections.

Oral fluid specimens were collected with the Intercept DOA Oral Specimen Collection Device (OraSure Technologies, Bethlehem, PA) according to manufacturer's instructions. Briefly, the collection device consisted of a treated absorbent cotton fiber pad affixed to a nylon stick and a preservative solution (0.8 mL) in a plastic container. With this device, an average of 0.4 mL of oral fluid was collected. The collection device pad was placed between the lower gum and cheek for 2-5 min, and then placed in the preservative solution (0.8 mL). The specimen container was sealed and express shipped to the laboratory for testing. Urine specimens were collected in standard collection vials, sealed, and express shipped to the laboratory for testing.

### Immunoassay

Oral fluid specimens were analyzed with the Cannabinoids Intercept™ MICRO-PLATE enzyme immunoassay by OraSure Technologies following manufacturer's procedures. Briefly, the EIA procedure consisted of addition of 25 µL of specimen, calibrator, or control to each well of an anti-THC coated plate (immobilized sheep anti-cannabinoids polyclonal antibody), addition of 25 µL of buffer, and incubation for 60 min at room temperature (RT). After incubation, 50 µL of THC enzyme conjugate (horseradish peroxidase labeled with THC derivative) was added and the plate was incubated for an additional 30 min at RT. The plate was then washed six times with 0.3 mL of distilled water, followed by addition of 0.1 mL substrate reagent (tetramethylbenzidine) and incubation for 30 min at RT in the dark. After incubation, 0.1 mL of stopping reagent (2N sulfuric acid) was added. Absorbance was measured at 450 nm and 630 nm within 15 min of stopping the reaction. Quality control samples (below cutoff control containing 0.5 ng/mL THC; above cutoff control containing 2.0 ng/mL THC) were required to test appropriately. Responses of specimens were compared to the mean response of the calibrator (1 ng/mL, *N* = 2). Specimens with absorbance less than or equal to the calibrator



**Figure 1.** Time line of smoking cannabis, air sampling, and collection of oral fluid and urine specimens from active cannabis smokers and passive subjects. Specimen collection times are based on the start of cannabis smoking.

were considered positive, and specimens with responses greater than the calibrator were considered negative. THC concentrations were adjusted for dilution ( $\times 3$ ) and are reported based on estimated oral fluid concentration. Consequently, the EIA cutoff concentration for a neat oral fluid specimen was 3 ng/mL.

Urine specimens were analyzed on a Hitachi 911 Analyzer (Roche Diagnostic, Indianapolis, IN) following Dade Behring manufacturer's procedures with the Dade Behring Syva Emit II Plus Cannabinoid Assay Kit (Syva Company, Dade Behring, Inc., Cupertino, CA). The EIA cutoff concentration for urine was 50 ng/mL. Urine creatinine was measured on a Ortho Vitros 250 (Ortho-Clinical Diagnostics, Inc., Rochester, NY) using an enzymatic method.

### Confirmation methods

Quantitative analysis of THC in oral fluid specimens was performed by GC-MS-MS on a Saturn 2000 ion trap quadrupole (Varian, Walnut Creek, CA) equipped with a 5% phenyl methyl silicone capillary column (15 m  $\times$  0.25-mm i.d.). The capillary inlet system was operated in the splitless mode. Instrumental conditions were as follows: injection port, 275°C; GC temperature program, 80°C for 1 min, ramp 20°C/min to 300°C, hold 0.5 min; transfer line, 290°C; source, 250°C; manifold, 40°C. A total of 400  $\mu$ L of each oral fluid specimen was used in the extraction procedure. Initially, internal standard (THC- $d_3$ ) at a concentration of 20 ng/mL was added to each specimen, calibrator, and control sample. Each sample was treated with 3.6 mL of 1M acetic acid. The tubes were vortex mixed 30 s and then extracted by solid-phase extraction (SPE) using the Rapid-Trace SPE workstation (Caliper, Hopkinton, MA). The SPE column was SPEC C-18 (Varian, Walnut Creek, CA) which was conditioned with methanol, rinsed with 50% methanol, and eluted with 80:20 (v/v) hexane/ethyl acetate. The residue was derivatized with 40  $\mu$ L of heptafluoropropionic anhydride and 20  $\mu$ L of pentafluoroisopropanol in 500  $\mu$ L toluene at 90°C for 15 min. The derivatized extract was washed in 1 mL of 0.1M phosphate buffer (pH 6). The top layer was dried down and reconstituted in 50  $\mu$ L ethyl acetate. A calibration standard curve of THC was prepared for each batch at 0.5, 1, 2.5, 5, 10, and 20 ng/mL concentration in artificial saliva (certified blank matrix). The artificial saliva was prepared in-house and contained a surfactant, preservative, and salts that simulated the ionic strength and properties of oral fluid mixed with the Intercept diluent. Calibration was performed as a linear fit through zero. Control samples containing 0, 0.5, 1.0, and 2.0 ng/mL THC in oral fluid diluent were included in each batch. The following parent ions were selected for each compound to form product ions:  $m/z$  492 for THC and  $m/z$  495 for THC- $d_3$ . The following product ions were selected for quantitation:  $m/z$  238 for THC and  $m/z$  238 for THC- $d_3$ . For a specimen to be considered positive for THC, the product ions had to be present and within 2% of the retention time of the calibrator. In addition, the area of each ion had to be greater than the corresponding area of the ion in the calibration standard. The LOQ/LOD for THC was 0.25 ng/mL for a 0.4-mL extracted specimen. THC concentrations were adjusted for dilution ( $\times 3$ ) and are reported based on estimated oral fluid concentration. Consequently, the LOD/LOQ for THC in neat oral fluid was 0.75 ng/mL.

Quantitative analysis of THCCOOH in urine specimens was performed by GC-MS-MS on a Varian 1200 triple-stage quadrupole (Varian, Walnut Creek, CA) equipped with a 5% phenyl methyl silicone capillary column (15 m  $\times$  0.25-mm i.d.). The capillary inlet system was operated in the splitless mode. Instrumental conditions were as follows: injection port, 275°C; GC temperature program; 130°C for 1 min, ramp 20°C/min to 300°C; transfer line, 280°C and source temperature, 200°C. A total of 1 mL of each urine specimen was used for the extraction. Initially, internal standard (THCCOOH- $d_3$ ) at a concentration of 15 ng/mL was added to each specimen, calibrator, and control sample. Each sample was treated with 100  $\mu$ L of 10M NaOH. The tubes were hydrolyzed for 20 min at room temperature. The samples were acidified with 3000  $\mu$ L of 1M acetic acid and vortex mixed. The samples were extracted the same as the oral fluid samples. The eluent was evaporated to dryness at 40°C and derivatized with 100  $\mu$ L of BSTFA (1% TMCS) at 70°C for 20 min. A calibration standard of THCCOOH was prepared for each batch at 15 ng/mL concentration in certified blank urine. Calibration was performed as a linear fit through zero. Control samples containing THCCOOH in urine at concentrations  $\pm$  25% of cutoff were included in each batch. The following parent ions were selected for each compound to form product ions:  $m/z$  488 for THCCOOH and  $m/z$  491 for THCCOOH- $d_3$ . The following product ions and qualifier ions (shown in parenthesis) were selected for quantitation:  $m/z$  297 (371) for THCCOOH and  $m/z$  300 (374) for THCCOOH- $d_3$ . For a specimen to be considered positive for THCCOOH, the product ions had to be present and within 2% of the retention time of the calibrator. In addition, the area of each ion had to be greater/equal than the corresponding area of the ion in the calibration standard. In addition, qualifier ion ratios had to be within  $\pm$  20% of the calibration standard. The assay LOQ/LOD for THCCOOH was 1.0 ng/mL for a 1-mL extracted specimen.

### Analysis of air samples

Room air samples were collected with equipment provided by Draeger Safety (Lubeck, Germany). An air sampling pump (Draeger Quantimeter 1000) was fitted with a thermodesorption tube (TDS) filled with Tenax TA. The pump sampled air at a constant rate to a maximum volume of 5 L. The air sampling system was located at an equal height and within 1.0-1.5 m of the passive participants. Air samples were taken at periodic intervals during the smoking session. The timeline for the air sample collections (based upon cannabis smoking starting at 0 min and lasting for 20 min) were as follows: 0-5 min, 20-40 min, and 65-100 min. Following collection, TDS tubes were removed, sealed, and stored for later analysis. Blank air samples (TDS tube not attached to pump) were also collected. For analysis, the tubes were analyzed according to established methods [MDSH 80 (HSE) and DIN/ISO16000-6]. THC- $d_9$  was used for internal standardization. Briefly, THC was thermally desorbed and a helium gas stream transferred material to a "cryo-trap". Analysis of trapped THC was performed by GC-MS (HP-5-MS; GC temperature program, 40°C to 290°C). THC identification was made by comparison of full scan data to reference mass spectra. Quantitation was performed by mass selective detection. The limit of quantitation (LOQ) for THC was 1 ng/L of air.

### Pharmacokinetics

THC oral fluid data were fitted by regression analysis with WinNonlin™ Professional, version 4.1 (Pharsight Corporation, Mountain View, CA) software. Pharmacokinetic parameters were obtained by use of model-independent methods. The distribution and elimination rate constants ( $\lambda_z$ ) were estimated by linear regression of oral fluid data points from apparent initial and elimination phases. The half-life estimates ( $T_{1/2}$ ) were calculated from the relationship  $T_{1/2} = 0.693/\lambda_z$ .

### Results

#### Exposure conditions

Room air concentrations of THC present during passive inhalation were measured at three selected intervals during and after smoking (Table I). THC concentrations were highest in the first air sample (0.19  $\mu\text{g/L}$ ) collected during smoking. The second air sample, collected immediately after smoking contained 0.082  $\mu\text{g/L}$  THC. The third air sample, collected starting

**Table I. THC Concentrations in Room Air and Estimated Total THC Dose Inhaled by Passive Subjects\***

Session Interval (min)	Method of THC Determination	Room Air THC During Interval ( $\mu\text{g/L}$ )	Minutes per Interval	Estimated Total Air Volume Inhaled During Interval (L)	Estimated Total THC Inhaled per Subject Over Interval (mg)	% Total THC Intake
0-5	Air sample measurement	0.190	5	35	0.0067	14.4
5-20	Interpolated	0.136	15	105	0.0143	30.9
20-40	Air sample measurement	0.082	20	140	0.0115	24.9
40-65	Interpolated	0.0435	25	175	0.0076	16.5
65-100	Air sample measurement	0.005	35	245	0.0012	2.7
100-240	Assumed from last air sample	0.005	140	980	0.0049	10.6
<b>Total</b>					0.0461	100.0

\* THC in room air was measured at designated interval and interpolated between intervals. THC in air in the remaining time in the session after the last measured sample was assumed to be equivalent to the last measurement. Amount of THC inhaled by each subject was estimated based on the assumption of 14 respirations/min and a respiratory volume of 0.5 L.

**Table II. GC-MS-MS and EIA Results for THC in Oral Fluid and for THCCOOH in Urine of Four Passive Subjects in the Presence of Five Cannabis Smokers\***

Minutes <sup>†</sup>	Passive #1		Passive #2		Passive #3		Passive #4		Mean (N = 4)	
	OF <sup>‡</sup> GC-MS-MS (ng/mL) (EIA)	Urine GC- MS-MS (ng/mL) (EIA)	OF GC-MS-MS (ng/mL) (EIA)	Urine GC- MS-MS (ng/mL) (EIA)	OF GC-MS-MS (ng/mL) (EIA)	Urine GC- MS-MS (ng/mL) (EIA)	OF GC-MS-MS (ng/mL) (EIA)	Urine GC- MS-MS (ng/mL) (EIA)	OF GC-MS-MS (ng/mL) (SEM)	Urine GC- MS-MS (ng/mL) (SEM)
-5 (-25)	0 (-)	0 (-)	0 (-)	0 (-)	0 (-)	0 (-)	QNS (-)	0 (-)	0 (0)	0 (0)
20 (0)	6.3 (+)	0 (-)	8.4 (+)	0 (-)	12.3 (+)	0 (-)	26.4 (+)	0 (-)	13.4 (3.9)	0 (0)
35 (15)	0 (-)	NS	6.9 (+)	NS	5.1 (+)	NS	7.2 (+)	NS	4.8 (1.4)	NS
50 (30)	0 (-)	NS	0 (-)	NS	3.6 (+)	NS	4.2 (-)	NS	2.0 (1.0)	NS
65 (45)	0 (-)	NS	0 (-)	NS	0 (-)	NS	1.1 (-)	NS	0.3 (0.2)	NS
95 (75)	0 (-)	NS	0 (-)	NS	0 (-)	NS	0 (-)	NS	0 (0)	NS
125 (105)	0 (-)	NS	0 (-)	NS	0 (-)	NS	0 (-)	NS	0 (0)	NS
155 (135)	0 (-)	NS	0 (-)	NS	0 (-)	NS	0 (-)	NS	0 (0)	NS
185 (165)	0 (-)	NS	0 (-)	NS	0 (-)	NS	0 (-)	NS	0 (0)	NS
215 (195)	0 (-)	NS	0 (-)	NS	0 (-)	NS	0 (-)	NS	0 (0)	NS
245 (225)	0 (-)	0 (-)	0 (-)	0 (-)	0 (-)	3.4 (-)	0 (-)	0 (-)	0 (0)	0.9 (0.7)

\* EIA results are shown in parentheses. The EIA cutoff concentrations were 3 ng/mL for oral fluid and 50 ng/mL for urine. The GC-MS-MS LOQ/LOD for THC was 0.75 ng/mL and for THCCOOH was 1.0 ng/mL. Oral fluid concentrations are multiplied x3 to correct to neat oral fluid.

<sup>†</sup> Abbreviations: OF, oral fluid; SEM, standard error of the mean; -, negative; +, positive; NS, no sample; and QNS, quantity not sufficient.

<sup>‡</sup> Timed from start of smoking. Time shown in parentheses indicates time following cessation of cannabis smoking.

Table III. GC-MS-MS and EIA Results for THC in Oral Fluid and for THCCOOH in Urine of Five Cannabis Smokers\*

Minutes†	Smoker #1		Smoker #2		Smoker #3		Smoker #4		Smoker #5		Mean (N = 5)	
	GC-MS-MS (ng/mL) (EIA)	Urine GC-MS-MS (ng/mL) (EIA)	GC-MS-MS (ng/mL) (EIA)	Urine GC-MS-MS (ng/mL) (EIA)	GC-MS-MS (ng/mL) (EIA)	Urine GC-MS-MS (ng/mL) (EIA)	GC-MS-MS (ng/mL) (EIA)	Urine GC-MS-MS (ng/mL) (EIA)	GC-MS-MS (ng/mL) (SEM)	Urine GC-MS-MS (ng/mL) (SEM)	GC-MS-MS (ng/mL) (SEM)	Urine GC-MS-MS (ng/mL) (SEM)
-5 (-25)	0 (-)	22.0 (+)	0 (-)	0 (-)	0 (-)	11.0 (-)	0 (-)	70.0 (+)	0 (0)	20.6 (13.0)	20.6 (13.0)	20.6 (13.0)
20 (0)	255.0 (+)	30.0 (+)	390.0 (+)	46.0 (+)	252.0 (+)	8.0 (-)	150.0 (+)	72.0 (+)	330.0 (+)	275.4 (40.5)	33.8 (11.7)	33.8 (11.7)
35 (15)	153.0 (+)	NS	258.0 (+)	NS	300.0 (+)	NS	105.0 (+)	NS	219.0 (+)	207.0 (35.2)	NS	NS
65 (45)	45.0 (+)	NS	141.0 (+)	NS	72.0 (+)	NS	19.2 (+)	NS	24.3 (+)	60.3 (22.2)	NS	NS
95 (75)	21.6 (+)	NS	102.0 (+)	NS	81.0 (+)	NS	6.0 (+)	NS	22.2 (+)	46.6 (18.9)	NS	NS
125	11.4 (+)	NS	132.0 (+)	NS	99.0 (+)	NS	12.6 (+)	NS	8.4 (+)	52.7 (26.2)	NS	NS
(105)	11.1 (+)	NS	120.0 (+)	NS	42.0 (+)	NS	4.5 (+)	NS	5.4 (+)	36.6 (22.0)	NS	NS
155	6.0 (+)	NS	69.0 (+)	NS	72.0 (+)	NS	4.8 (-)	NS	11.1 (+)	32.6 (15.5)	NS	NS
(135)	4.2 (+)	NS	78.0 (+)	NS	33.0 (+)	NS	6.9 (+)	NS	7.8 (+)	26.0 (14.0)	NS	NS
185	4.5 (-)	81.0 (+)	87.0 (+)	82.0 (+)	42.0 (+)	23.0 (+)	4.8 (+)	168.0 (+)	7.5 (+)	29.2 (16.1)	75.2 (26.7)	75.2 (26.7)
(165)												
215												
(195)												
245												
(225)												

\* EIA results are shown in parentheses. The EIA cutoff concentrations were 3 ng/mL for oral fluid and 50 ng/mL for urine. The GC-MS-MS LOQ/LOD for THC was 0.75 ng/mL and for THCCOOH was 1.0 ng/mL. Oral fluid concentrations are multiplied x3 to correct to neat oral fluid.

† Abbreviations: OF, oral fluid; SEM, standard error of the mean; -, negative; +, positive; and NS, no sample.

‡ Timed from start of smoking. Time shown in parentheses indicates time following cessation of cannabis smoking.

45 min after the end of smoking, contained 0.005 µg/L THC. A plot (not shown) of THC room air concentrations versus time indicated that levels declined in an apparent linear fashion over the passive exposure session. All blank air sample measures were negative for THC (LOQ = 1 ng/L air).

For purposes of estimating the total possible intake of THC by passive subjects, room air THC concentrations were estimated between the three measured intervals by interpolation between measurements. From 100 min to 240 min, room THC concentrations were assumed to remain at the concentration measured at 100 min. The estimated total air intake by passive subjects was based on the assumption of 14 respirations/min and 500-mL respiratory volume. Based on these estimates, passive subjects inhaled a total of approximately 0.05 mg THC during the passive exposure session. Approximately 70% of the total exposure to THC occurred during the first 40 min of the session. Table I summarizes the air measurements and estimations of total THC intake by passive subjects.

#### Screening and confirmation testing for cannabis in passive inhalation subjects

EIA and GC-MS-MS results for oral fluid and urine specimens collected from the four passive participants are shown in Table II. All oral fluid and urine specimens collected prior to the start of the exposure session tested negative by EIA and GC-MS-MS. A total of eight oral fluid specimens, collected from the four subjects during and after smoking, screened positive for cannabis at a cutoff concentration of 3 ng/mL and were confirmed positive for THC by GC-MS-MS at concentrations ranging from 3.6 to 26.4 ng/mL. The collection times for these specimens ranged from 20 min to 50 min following initiation of smoking (0-30 min after cessation of smoking). One subject, (Passive #4), had two oral fluid specimens, collected at 50 and 65 min, tested negative by EIA, but tested positive for THC by GC-MS-MS at 4.2 and 1.1 ng/mL, respectively. All subsequent specimens for passive participants tested negative by EIA and GC-MS-MS for the remainder of the session. All urine specimens collected during the session tested negative by EIA (cutoff concentration = 50 ng/mL) and GC-MS-MS (cutoff concentration = 15 ng/mL) for THCCOOH. One subject (Passive #3) had traces of THCCOOH in urine at 245 min by GC-MS-MS (3.4 ng/mL).

### Screening and confirmation testing of cannabis smokers

EIA and GC-MS-MS results for oral fluid and urine specimens collected from the five cannabis smokers are shown in Table III. All oral fluid specimens collected prior to the start of the exposure session tested negative by EIA and GC-MS-MS. Urine specimens collected prior to the session for Smokers #1 and #5 were positive for THCCOOH by EIA and GC-MS-MS at conventional cutoff concentrations. Smoker #4's urine specimen screened negative, but contained 11 ng/mL of THCCOOH by GC-MS-MS. All oral fluid specimens, collected at 20 min following initiation of smoking, screened positive and contained a mean THC concentration of 275.4 ng/mL (range 150.0–390.0 ng/mL). Concentrations of THC declined to a mean of 207.0 ng/mL at 35 min and to a mean of 60.3 ng/mL at 65 min. After 65 min, there was a slower decline in THC concentrations throughout the remainder of the 245 min session. Two oral fluid specimens, collected toward the end of the session, screened negative (Smoker #1, 245 min; Smoker #4, 185 min), but tested positive by GC-MS-MS at concentrations of 4.5 ng/mL and 4.8 ng/mL.

THC concentrations in smoker's oral fluid specimens appeared to decline in a biphasic manner (Figure 2). Estimates of the initial half-life of THC ranged from 11.5 min to 31.1 min with a mean of 20.8 min. From three to five data points were utilized in these calculations. Estimates of the terminal half-life of THC ranged from 73.5 min to 221.1 min with a mean of 121.6 min. From seven to nine data points were utilized in these calculations. A plot of mean concentrations of THC in oral fluid for the five cannabis smokers and the four passive subjects is shown in Figure 2.

Four of the five urine specimens collected at 20 min screened positive, and three were confirmed for THCCOOH at the 15 ng/mL conventional cutoff concentration. Urine specimens for Smokers #3 and #4 contained THCCOOH at 13 ng/mL and 8 ng/mL at 20 min, respectively. At 245 min, all five smokers' urine specimens screened and confirmed positive. The mean

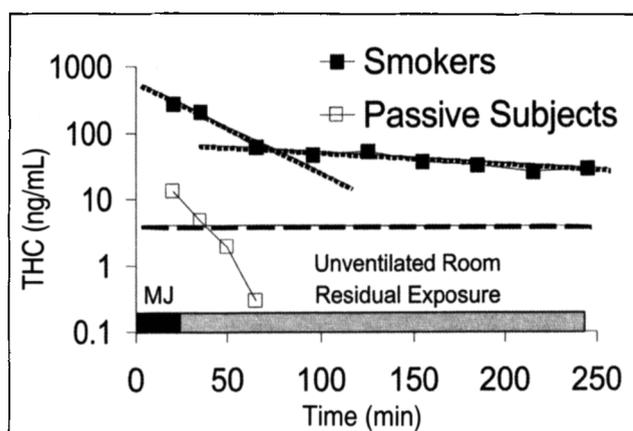
THCCOOH concentration at 245 min for the five subjects was 75.2 ng/mL.

### Discussion

This study demonstrated that oral fluid specimens, collected from individuals passively exposed to smoke from five cannabis cigarettes in a small unventilated room, tested positive for THC for up to 50 min after initiation of cannabis smoking (30 min after cessation). THC continued to be detectable by GC-MS-MS in one passive subject at 65 min, but failed to screen positive. All specimens collected after 65 min tested negative in screening and confirmation assays. The cutoff concentrations for THC utilized in this study were 3 ng/mL for screening and 0.75 ng/mL (LOD/LOQ) for confirmation. These cutoff concentrations take into account the dilution factor ( $\times 3$ ) and recovery of THC from the Intercept collection device. It should be noted that these cutoff concentrations for THC are somewhat lower than those recommended in the recently reported proposed guidelines by SAMHSA of 4 ng/mL for screening and 2 ng/mL for confirmation (9).

In contrast to passively exposed individuals, oral fluid specimens from the active cannabis smokers generally tested positive throughout the 4-h study session. As seen in Figure 2, mean THC concentrations following passive cannabis exposure were extremely low in comparison to active smokers. Oral fluid THC in passive subjects appeared to decline in a linear fashion. By 30 min after cessation of smoking, THC concentrations were near or below cutoff concentrations proposed by the Department of Health and Human Services (9). These results are generally consistent with the study by Niedbala et al. (7) who reported oral fluid results for two passive inhalation subjects who were in the presence of ten active smokers. In that study, three positive screening results were found (two specimens collected at approximately 30 min after smoking and one specimen collected 3.5 h), but all failed to be positive by GC-MS-MS (THC cutoff concentration = 0.5 ng/mL). The lack of confirmed positive THC findings was most likely due to differences in environmental study conditions between the current and previous study. In the present study, the room volume was approximately 36 m<sup>3</sup> and the room was sealed with no ventilation. In the earlier study, the room volume was approximately 136 m<sup>3</sup> and windows were opened during the study. The importance of ventilation on THC room air content is a known factor in determining the extent of passive exposure (3).

The finding of positive tests for THC in oral fluid specimens that were collected from passive subjects shortly after cannabis smoke exposure was not surprising. Sidestream smoke from cannabis contains up to 50% of the total THC content in cannabis (2). Depending upon environmental conditions, individuals in the vicinity where cannabis is combusted can inhale significant amounts of THC from sidestream smoke exposure. Passive exposure studies have shown that inhaled THC is absorbed, metabolized and subsequently excreted in urine in a similar fashion as active cannabis smokers. Cone et al. (3) reported that passive exposure to the smoke of four cannabis



**Figure 2.** Mean THC concentrations (GC-MS-MS) in oral fluid specimens of five cannabis smokers and four passive subjects. The heavy dotted line indicates the DHHS-proposed confirmatory cutoff concentration of 2 ng/mL. The two dotted lines illustrate the biphasic nature of THC disposition in oral fluid of active smokers. MJ = period of marijuana smoking. Unventilated room residual exposure indicates the period in which subjects remained in the unventilated room following marijuana smoking.

cigarettes resulted in individuals inhaling approximately 0.54 mg of THC with subsequent urinary excretion of THCCOOH in amounts up to 12 ng/mL. Under more extreme conditions (exposure to the smoke of 16 cannabis cigarettes), as much as 1.72 mg of THC was inhaled with subsequent urinary excretion of up to 87 ng/mL of THCCOOH. In the current study, only limited air sampling occurred. Based on these data, it was estimated that approximately 0.05 mg of THC was inhaled (Table I). Again, these different amounts of inhaled THC are most likely due to differences in environmental study conditions. In the study by Cone et al. (3), the room size (12.6 m<sup>3</sup>) was approximately one-third the size utilized in the current study. Thus, all available evidence suggests that the degree of passive cannabis exposure is highly dependent not only upon the amount of THC combusted, but the environmental conditions present at the time of the exposure.

Oral fluid specimen tests from the active smokers in this study revealed considerable variability in concentration over the four hour session. Although all subjects initially tested highly positive at 20 min (end of smoking), THC concentrations for Smokers #1, #4, and #5 declined within 125 min to approximately 10 ng/mL, whereas Smokers #2 and #3 remained near or above 100 ng/mL. The decline in THC concentrations after 125 min for all subjects slowed, possibly indicating that the initial high THC contamination in oral fluid had reached equilibrium and that disposition was governed by different processes.

In most cannabis smokers, a biphasic decline in THC concentrations appeared to occur after the smoking period (Figure 2). The estimated mean half-life for THC during the initial phase was 20.8 min, whereas the terminal phase half-life was 121.6 min. Huestis and Cone (10) have postulated that the initial phase represents direct "contamination" of oral fluid by THC immediately after exposure. Clearance of THC from oral fluid during this phase most likely occurs by swallowing and by formation of a "depot" in the oral mucosal cavity. The second phase likely represents THC that is bound as a "depot" in the mucosa. With active cannabis use, the amount of depot THC clearly is several orders of magnitude higher than amounts found after passive exposure. Only one "phase" was apparent following passive exposure (Figure 2). Once THC becomes sequestered after active cannabis use, both transmucosal absorption into the bloodstream and "leakage" of THC from the depot into oral fluid occurs over a period of 6 to 12 hours (or longer depending on dose). This interpretation would account for the observed patterns of THC measured in oral fluid specimens collected after controlled dosing studies. Consistent with this interpretation, the amounts of THC stored in oral mucosal depots would be expected to be subject dependent and would account for the variability in THC concentrations observed in this study.

## Conclusions

The risk of positive oral fluid tests for THC resulting from passive cannabis smoke was limited to approximately 30 min after exposure. THC concentrations in oral fluid of passive subjects were highest at the conclusion of smoking and declined in a linear fashion within 45 min below the cutoff concentration. In contrast, THC concentrations in cannabis smokers were considerably higher and generally remained positive through the 4-h session at the proposed DHHS cutoff concentration of 4 ng/mL for oral fluid. It is concluded that the risk of positive oral fluid tests from passive cannabis smoke inhalation is limited to a period of approximately 30 min following exposure.

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