Concentrations of Δ⁹-Tetrahydrocannabinol and 11-Nor-9-Carboxytetrahydrocannabinol in Blood and Urine After Passive Exposure to Cannabis Smoke in a Coffee Shop

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Abstract

Cannabinoid concentrations in blood and urine after passive exposure to cannabis smoke under real-life conditions were investigated in this study. Eight healthy volunteers were exposed to cannabis smoke for 3 h in a well-attended coffee shop in Maastricht, Netherlands. An initial blood and urine sample was taken from each volunteer before exposure. Blood samples were taken 1.5, 3.5, 6, and 14 h after start of initial exposure, and urine samples were taken after 3.5, 6, 14, 36, 60, and 84 h. The samples were subjected to immunoassay screening for cannabinoids and analyzed using gas chromatography–mass spectrometry (GC–MS) for Δ⁹-tetrahydrocannabinol (THC), 11-nor-hydroxy-Δ⁹-tetrahydrocannabinol (THC-OH), and 11-nor-9-carboxy-Δ⁹-tetrahydrocannabinol (THC-COOH). It could be demonstrated that all volunteers absorbed THC. However, the detected concentrations were rather small. None of the urine samples produced immunoassay results above the cutoff concentration of 25 ng/mL. THC-COOH concentrations up to 5.0 and 7.8 ng/mL before and after hydrolysis, respectively, were found in the quantitative GC–MS analysis of urine. THC could be detected in trace amounts close to the detection limit of the used method in the first two blood samples after initial exposure (1.5 and 3.5 h). In the 6 h blood samples, THC was not detectable anymore. THC-COOH could be detected after 1.5 h and was still found in 3 out of 8 blood samples after 14 h in concentrations between 0.5 and 1.0 ng/mL.

Introduction

The detection of drugs in a toxicological blood or urine screening is usually associated with severe personal consequences such as loss of the driving license, countermand of probation, or job loss. Moreover, driving under the influence of cannabis is sanctioned by the German Road Traffic Act. A fine is imposed on a driver if the THC concentration in serum exceeds 1 ng/mL. Thus, it is not surprising that after detection of Δ⁹-tetrahydrocannabinol (THC) or its main metabolite 11-nor-9-carboxy-Δ⁹-tetrahydrocannabinol (THC-COOH), the concerned persons frequently claim they did not actively smoke marijuana. The only explanation for the incorporation of cannabinoids other than active intake could be the passive inhalation during a stay in rooms filled with cannabis smoke.

Several studies demonstrated that passive THC inhalation from the environmental air is indeed possible, and the exposure to marijuana smoke may lead to considerable concentrations of THC and metabolites in blood and urine (1–6). A comprehensive review was given by Skopp and Pötsch (7). The limitations of most of these studies was that they were performed under extreme conditions, which rarely occur in real life. In the study by Mørland et al. (3), five volunteers were exposed to cannabis smoke in a small car with an available air volume of approximately 1650 L. This would correspond to a room with a 2.5 m ceiling height and a floorspace of 0.66 m^2. The exposure time was 30 min, and six marijuana cigarettes, each containing 7.5 mg THC, were burned down. The passive smokers had peak blood concentrations of THC ranging from 1.3 to 6.3 ng/mL. Cone et al. (1) exposed 5 men to sidestream smoke of 4–16 marijuana cigarettes (2.8 mg THC) for 60 min over 6 consecutive days. The room volume was about 12.2 m^3, which corresponds to an assumed floorspace of 4.88 m^2 (2.5 m height). They found daily mean plasma levels for THC in the range of 0.8 to 2.5 ng/mL (4 cigarettes) and of 2.4 to 7.4 ng/mL (16 cigarettes). Perez-Reyes et al. (4) found a maximum THC plasma concentration of 2.2 ng/mL after a two-time repeated exposure to marijuana smoke in a room with a volume of 15.5 m^3 (2.44 × 2.44 × 3.05 m; solid furniture was subtracted from the total room volume). The volumes of all of the rooms where the exposure did happen were extremely small with calculated areas in the range of approximately 0.7–6 m^2. This is much less than the dimension of locations where cannabis is usually smoked such as at parties, in pubs, or in discotheques.

The aim of the present study was to investigate to which ex-

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tent THC and THC metabolites appear in blood and/or urine after passive exposure to cannabis smoke under realistic conditions. The samples were analyzed with immunoassay for cannabinoids and with gas chromatography–mass spectrometry (GC–MS) for THC, 11-nor-hydroxy-Δ^9-tetrahydrocannabinol (THC-OH), and THC-COOH. A GC–MS method according to Becker et al. (8) and Roehrich et al. (9,10) was used for the analyses of serum and urine. This method is applied routinously in our laboratory.

Experimental

The study was designed as a field experiment. The basic approach comprised of the volunteers staying at a place where cannabis is used intensely to find out if THC is incorporated by passive inhalation under these circumstances. The study protocol was authorized by the ethics review board of the medical association of the German federal state of Rheinland-Pfalz. It was in accordance with the declaration of Helsinki in the version of the general assembly of World Medical Association (WMA) in Tokyo, Japan (2004).

Instrumentation and reagents

Instrumentation consisted of an HP 6890 GC with an autosampler (Agilent, Waldbronn, Germany), HP 5973 MS (Agilent), and HP-5 MS capillary column (30 m × 0.25-mm i.d., 0.25-µm film thickness, Agilent). Rapid Trace SPE workstation (Caliper Life Sciences, Ruesselsheim, Germany) was also employed. All solvents and reagents were analytical grade. Methanol, acetone, hexane, ethyl acetate, dichloromethane, isooctane, acetic acid, tetrabutylammonium hydroxide, and dimethylsulfoxide were purchased from Merck (Darmstadt, Germany), methyl iodide from Sigma Aldrich (Deisenhofen, Germany), solid-phase extraction columns from Mallinckrodt Baker (Griesheim, Germany), and all drug standard solutions as well as deuterated compounds from Cerilliant (Austin, TX) via LGC Standards (Wesel, Germany). Cannabinoid and creatinine assays for urine included a Cedia Multi-Level THC and DRI Creatinine-Detect Test (Microgenics, Thermo Fisher Scientific, Passau, Germany) with a Hitachi 912 analyzer (Microgenics). Cannabinoid assay for serum ran on an Inspec II-THC-EIA (Mahsan Diagnostika, Reinbeck, Germany) with Bio-Lab 200 analyzer (Mahsan Diagnostika).

Subjects

Eight healthy volunteers (four female and four male) participated in this study. None had a history of cannabis use nor came in contact with hashish, marijuana, or cannabis smoke within the last month before the study. Details are given in Table I.

Passive smoke exposure conditions

The field study took place in a well-attended coffee shop in Maastricht in the Netherlands. Coffee shops in the Netherlands are pubs where cannabis can be consumed legally. The coffee shop was located on a river in a former freight ship. The room in which the exposure occurred was below deck. This room was entered through a hatch on the deck and had a surface area of about 12 × 7 m and a height of approximately 2.5 m, leading to an estimated room volume of about 200 m^3. It had no windows but relatively efficient ventilation and, therefore, was not very smoky during the exposure period.

The duration of stay in the room was 3 h. The eight volunteers sat together at a table almost in the middle of the room. They were seated the entire time, except two or three times when one of them stood up to get a drink. Each volunteer consumed two to three cans (0.33 L) of soft drinks during the exposure period, but they did not eat anything. The passive subjects remained in the room the entire time, except for when they had blood sampling from the permanent venous catheter at t1. Blood sampling took place in a restroom located directly near the room where the exposure occurred. The blood sampling in the restroom lasted only a short time, approximately 5 min.

The other guests entered the room over the stairs, sat down at different tables, and smoked cannabis. After consumption, they left the room. Most of the smokers stayed in the pub only a short time of about 15 min; a few remained longer and stayed approximately for 1 h. Because of the high fluctuation, the total number of active cannabis consumers can only be roughly estimated, and it is also impossible to indicate how often individuals went in or out of the room. The smallest number of cannabis smoking guests simultaneously present during the exposure time was 8, and the largest number was 25. Because of the high fluctuation and the varying places where the guests sat, the distances between active smokers and the passive subjects were continuously changing. Thus, it is not possible to specify the exact distance between active and passive subjects. But none of the smokers sat closer than 1 m to the passive subjects, and the most distant smokers were about 5 to 6 m away.

The coffee shop owners allowed the investigations inside their pub under the restriction that none of the guests be annoyed. Therefore, interviewing the smokers or attempting to influence their smoking habits were undesireable, and the passive volunteers had no contact with the active smokers at all. Thus, no information concerning the amount of the smoked marijuana or hashish during the 3-h period about the potency of the used cannabis or if it was mixed with tobacco was obtained. The smokers used different consumption

Table I. Characteristics of the Volunteers

Subject	Sex	Age (years)	Weight (kg)	Height (m)	BMI (kg/m^2)
P1	Female	28	60	1.64	22.3
P2	Female	30	68	1.79	21.2
P3	Male	59	82	1.64	30.5
P4	Male	46	85	1.90	23.5
P5	Female	29	58	1.60	22.7
P6	Male	41	100	1.88	28.3
P7	Male	49	85	1.84	25.1
P8	Female	27	57	1.66	20.7

techniques. Cigarettes were preferred, but some smokers also used hashish pipes or water pipes ("bong"). However, it is not known how deep or how frequently the smoke was inhaled during consumption. The exact number of cigarettes smoked during the 3-h period and the average content of THC of the cannabis cigarettes is also not known. Additionally, 8 g hashish and marijuana was burned down by the volunteers in an ashtray about 1.5 m away from them. The burning led to a bit more cannabis smoke in the room over a short period. The THC concentration in room air was not measured for lack of appropriate technical equipment. Also, no attempt was made to measure CO levels in the air or on the breath.

Sample collection

Initially, blood and urine sample were taken from each volunteer before exposure to cannabis smoke (t0). Blood was collected 1.5 (t1), 3.5 (t2), 6 (t3), and 14 (t4) h after start of the exposure. Urine samples were taken after 3.5 (t2), 6 (t3), 14 (t4), 36 (t5), 60 (t6), and 84 (t7) h. The blood sampling during the sojourn in the coffee shop at t1 (1.5 h) was carried out by using a permanent venous catheter that was hidden under the shirt sleeve. The samples were refrigerated and transferred to the laboratory of the Institute of Legal Medicine. The blood samples were centrifuged, and the serum was separated. Urine and serum samples were deep frozen and stored at –23°C until analysis.

Immunoassay screening of urine

For the immunoassay testing of urine, the Cedia Dau cannabinoid assay (Microgenics) was applied. The assay was performed on a Hitachi 912 analyzer using 200 µL of urine. The cutoff concentration was set to 25 ng/mL, and the detection limit of 1.3 ng/mL for free THC-COOH was given by the manufacturer.

Immunoassay screening of serum

The Inspec cannabinoid assay (Mahsan Diagnostika) was used for the immunoassay testing of serum. Serum (400 µL) was assayed using a Bio-Lab 200 analyzer. The cutoff concentration was 3 ng/mL, and the detection limit for free THC-COOH was 1 ng/mL (specifications of the manufacturer).

Alkaline hydrolysis of urine

Urine was analyzed directly as well as following hydrolysis. A portion of each urine sample was hydrolyzed for conjugate cleavage of THC-COOH-glucuronide (11-nor-Δ^9-tetrahydro-

Table II. Method Validation Data

	LOD (ng/mL)	LLOQ (ng/mL)	Accuracy (Low) (%)	Accuracy (High) (%)	Intraassay (Low) (%)	Intraassay (High) (%)	Interassay (Low) (%)	Interassay (High) (%)	Recovery (Low) (%)	Recovery (High) (%)
THC (serum)	0.1	0.5	0.0	–1.5	10.8	5.1	13.0	6.9	78	83
THC-OH (serum)	0.2	0.5	–6.5	–0.4	5.8	2.0	8.8	5.5	97	96
THC-COOH (serum/urine)	0.4	0.6	–0.5	2.8	2.5	1.2	6.9	3.5	82	85
THC-COOH (hydrolyzed urine)	0.7	1.0	–10.4	–10.9	2.5	3.1	4.1	7.4	52	66

Table III. Results of Immunoassay Testing (CEDIA DAU) of urine

	t0 (0 h)		t2 (3,5 h)		t3 (6 h)		t4 (14 h)		t5 (36 h)		t6 (60 h)		t7 (84 h)	
Subject	Creatinine (mg/dL)	Cannabinoid equivalents (ng/mL)	Creatinine (mg/dL)	Cannabinoid equivalents (ng/mL)	Creatinine (mg/dL)	Cannabinoid equivalents (ng/mL)	Creatinine (mg/dL)	Cannabinoid equivalents (ng/mL)	Creatinine (mg/dL)	Cannabinoid equivalents (ng/mL)	Creatinine (mg/dL)	Cannabinoid equivalents (ng/mL)	Creatinine (mg/dL)	Cannabinoid equivalents (ng/mL)
P1	275	9	152	14	201	17	293	14	33	11	134	10	–	–
P2	93	11	33	11	185	20	179	17	114	13	91	9	136	0
P3	126	0	105	8	67	13	199	15	225	10	286	13	278	12
P4	195	0	155	9	118	15	159	15	166	10	117	8	294	0
P5	154	8	235	16	80	12	220	16	88	13	93	13	112	9
P6	207	11	270	15	190	19	282	20	240	11	223	11	284	0
P7	306	0	336	10	294	14	224	13	–	–	396	8	234	0
P8	225	0	101	11	176	15	201	15	64	11	220	14	202	3
Mean	198	5	173	12	164	16	220	16	133	11	195	11	220	3
Range	93–306	0–11	33–336	8–16	67–294	12–20	159–293	13–20	33–240	10–13	91–396	8–14	112–294	0–12

cannabinol-9-carboxylic acid β-glucuronide) prior to GC–MS analysis. Potassium hydroxide solution (10 M, 300 µL) and 50 µL of the internal standard mixture (methanolic solution composed of 0.1 ng/µL of THC-d_3 and THC-OH-d_3 as well as 1 ng/µL THC-COOH-d_9) were added to 1 mL urine. The mixture was vortex mixed and heated to 60°C for 15 min. Afterwards, the solution was cooled down to 0°C in an ice bath. Glacial acetic acid (400 µL) and 3 mL 0.05 M phosphorus acid were added, and the mixture was adjusted to pH 4–5 by dropwise addition of further phosphorus acid. The solution was replenished to a final volume of 7 mL with phosphate buffer (0.1 M, pH 6).

Solid phase extraction and derivatization

A 1-mL aliquot of serum or urine was diluted with 6 mL of phosphate buffer (0.1 M, pH 6), and 50 µL of the internal standard solution was added. In the case of the hydrolyzed urine samples, the total amount of 7 mL urine phosphate buffer mixture (preparation described earlier) was used. The mixture was applied to a solid-phase extraction column (Bakerbond SPE C_{18}, 500 mg), which had been conditioned by flushing with 2 × 3 mL of methanol and 2 mL of water. The column was rinsed with 2 × 2 mL water, 2 × 2 mL water/methanol (80:20, v/v), and 1 mL of 0.1 M acetic acid. The column was dried for 10 min. The cannabinoids were eluted with 3 mL dichloromethane/acetone (50:50, v/v). The extract was evaporated under a slight stream of nitrogen at 40°C. The solid-phase extractions can be carried out either automatically using the Caliper Rapid Trace SPE workstation or manually with a vacuum manifold. THC, THC-OH, and THC-COOH were derivatized with methyl iodide. First 200 µL of a mixture of dimethylsulfoxide and 60% aqueous tetrabutylammonium hydroxide solution (98:2, v/v) was added to the extract. Subsequently, 50 µL methyl iodide was added, and the mixture was vortex mixed. After 5 min at room temperature, 200 µL 0.1 N hydrochloric acid was added. The methylated cannabinoids were then extracted with 2 portions of 1 mL isooctane. The organic layer was separated, and the solvent evaporated at 30°C in a slight nitrogen stream. For GC–MS analysis, the dry residue was dissolved in 50 µL of anhydrous ethyl acetate.

GC–MS analysis

For GC–MS analysis of cannabinoids, a HP-5 MS capillary column was used. The carrier gas was He (constant flow: 1 mL/min), the injection volume 1 µL (splitless injection), the injector temperature 250°C, and the transfer line temperature 280°C. The oven temperature program was 2 min isothermally at 60°C, 40°C/min to 170°C, 8°C/min to 270°C, 7.75 min isothermally at 270°C, 30°C/min to 300°C, and 5 min isother-

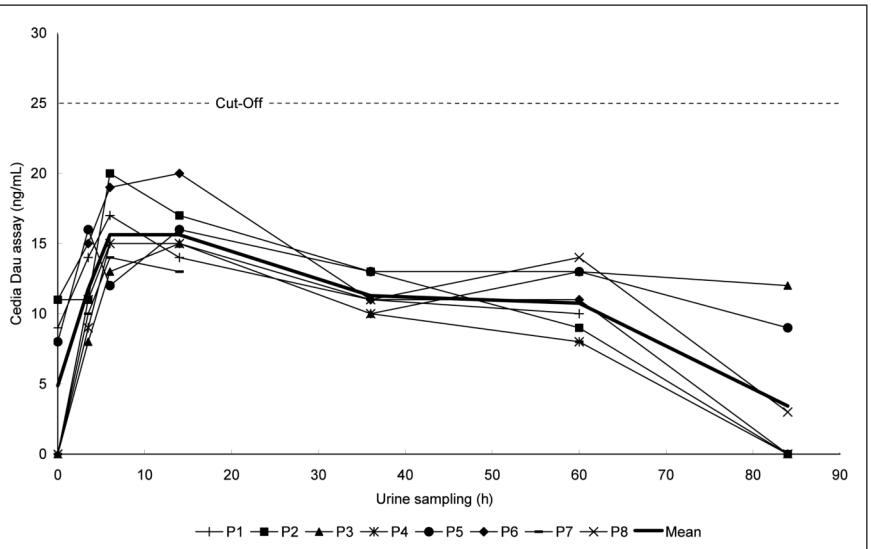

Figure 1. Immunoassay testing (Thermo Fisher Cedia Dau assay) of urine.

Table IV. Results of GC–MS Analyses of Urine Without and After Alkaline Hydrolysis

	t0 (0 h)		t2 (3.5 h)		t3 (6 h)		t4 (14 h)		t5 (36 h)		t6 (60 h)		t7 (84 h)	
Subject	THC-COOH (ng/mL)	THC-COOH hydrolysis (ng/mL)	THC-COOH (ng/mL)	THC-COOH hydrolysis (ng/mL)	THC-COOH (ng/mL)	THC-COOH hydrolysis (ng/mL)	THC-COOH (ng/mL)	THC-COOH hydrolysis (ng/mL)	THC-COOH (ng/mL)	THC-COOH hydrolysis (ng/mL)	THC-COOH (ng/mL)	THC-COOH hydrolysis (ng/mL)	THC-COOH (ng/mL)	THC-COOH hydrolysis (ng/mL)
P1	0	0	0	0	0.6	2.9	(0.5)*	2.4	0	0	0	0	–	–
P2	0	0	0	0	5.0	7.8	2.9	4.8	0.6	1.2	0	0	0	0
P3	0	0	0	0	(0.5)	1.0	2.3	4.1	0	2.1	0	1.6	0	1.1
P4	0	0	(0.5)	1.7	1.9	3.7	4.2	4.7	1.3	2.0	(0.4)	(0.9)	0	(0.8)
P5	0	0	1.0	1.7	0.8	1.5	1.4	2.6	0	0	0	0	0	0
P6	0	0	1.7	2.6	3.4	6.1	4.8	7.3	0.6	(0.9)	0.6	(0.8)	0	(0.7)
P7	0	0	(0.5)	1.6	1.6	2.5	1.0	1.3	–	–	0	0	0	0
P8	0	0	0	0	1.6	2.3	1.6	3.0	0	0	0	0	0	0
Mean	0	0	0.5	1.0	1.9	3.5	2.3	3.8	0.4	0.9	0.1	0.4	0	0.4
Range	0	0	0–1.7	0–2.6	(0.5)–5.0	1.0–7.8	(0.5)–4.8	1.3–4.8	0–1.3	0–2.1	0–0.6	0–1.6	0	0–1.1

* Approximate values below the LLOQ are given in parentheses.

mally at 300°C. EI ionization (70 eV) was used, ion source temperature 230°C, quadrupole temperature 150°C. The following ions (methyl derivatives of THC, THC-OH, and THC-COOH) were measured in selected ion monitoring (SIM) mode (dwell time per ion: 30 ms): *m/z* 248, 316, 331-target for THC-d_3 [internal standard (IS), Rt: 15 min]; *m/z* 245, 285, 313, 328-target for THC (Rt: 15 min); *m/z* 316-target, 317, 361 for THC-OH-d_3 (IS, Rt: 17 min); *m/z* 284, 313-target, 314, 358 for THC-OH (Rt: 17 min); *m/z* 322-target, 363, 381 for THC-COOH-d_9 (IS, Rt: 18.5 min); and *m/z* 313-target, 341, 357, 372 for THC-COOH (Rt: 18.5 min). Ion ratios and retention time were used as identification criteria for THC and THC-COOH. For quantification, the peak areas of the ions specified as "target" were used. Quantification was based on peak-area ratios relative to the respective IS.

Validation of the GC–MS method

The GC–MS method was validated according to current standards (11–13). The method validation was performed by using a Microsoft Excel-based validation program Valistat (14). Drug-free serum and urine were used as a blank matrix for the validation measurements. A six-point calibration curve was used for each compound. The different calibration levels were obtained by spiking the blank matrix with 50 µL of methanolic solutions containing appropriate amounts of the analytes. The calibration levels were 0.5, 1, 2, 3, 4, and 5 ng/mL for THC and THC-OH as well as 5, 10, 20, 30, 40, and 50 ng/mL for THC-COOH. Each calibration level was measured in six repetitions. The calibrations were linear in the range tested. Accuracy and precision were calculated from the results of two analyses in a series performed on eight different days at two concentration levels (low, high). The recovery rates were determined in six repetitions low and high concentration levels. At the low level, the concentration of THC and THC-OH was 1 ng/mL and 10 ng/mL for THC-COOH. In the high level, THC and THC-OH was 5 ng/mL and 50 ng/mL for THC-COOH. Validation data are given in Table II. The validation data for THC-COOH in hydrolyzed urine were obtained in the same way as for free THC-COOH but was done using urine samples spiked with equivalent amounts of THC-COOH-glucuronide instead. Therefore, the completion of hydrolysis is included in the recovery rates determined for the hydrolyzed urine samples (Table II). The limit of detection (LOD) and the lower limit of quantification (LLOQ) were calculated statistically from the calibration data and by means of signal-to-noise ratios (S/N). For the LLOQ, the S/N is required to be 10:1, and for the LOD, the S/N is 3:1. Positive results that were less than the LLOQ were given as approximate values.

Results and Discussion

The results of the immunoassay screening of the urine samples applying the Cedia Dau assay are presented in Table III and Figure 1. The concentrations increased between t0 and t2 and reached a maximum average of 16 ng/mL between 6 and 14 h after starting the exposure to cannabis smoke. None of the urine samples produced immunoassay results higher than the cutoff concentration of 25 ng/mL. Hence, none of the passive smokers would test positive for cannabis use, and no further

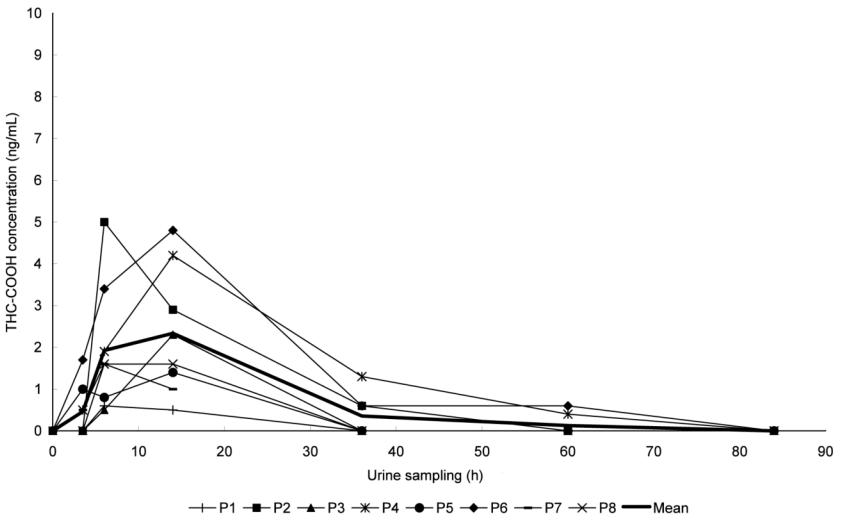

Figure 2. Concentrations of THC-COOH in urine analyzed with GC–MS.

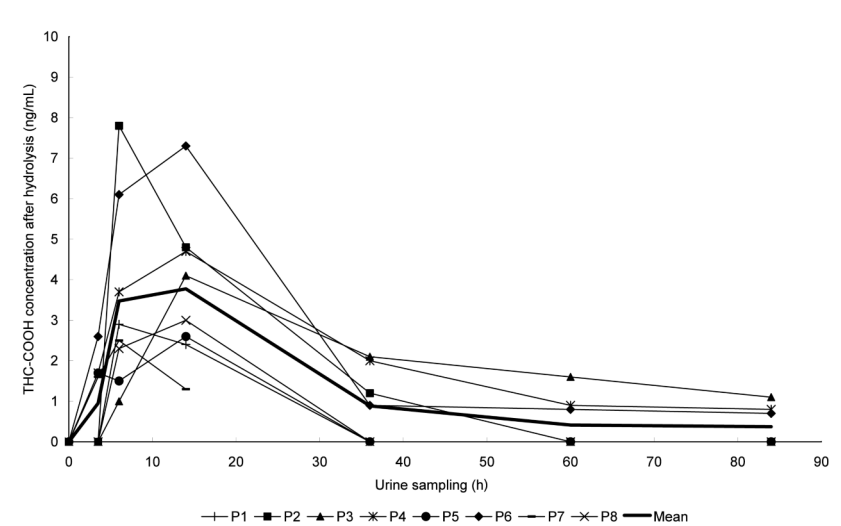

Figure 3. Concentrations of THC-COOH in urine after alkaline hydrolysis analyzed with GC–MS.

Table V. Results of Immunoassay Testing (Inspec ELISA) and GC–MS Analyses of Serum

	t0 (0 h)			t1 (1.5 h)			t2 (3.5 h)			t3 (6 h)			t4 (14 h)		
	ELISA	GC–MS		ELISA	GC–MS		ELISA	GC–MS		ELISA	GC–MS		ELISA	GC–MS	
Subject	Cannabinoid equivalents (ng/mL)	THC (ng/mL)	THC-COOH (ng/mL)	Cannabinoid equivalents (ng/mL)	THC (ng/mL)	THC-COOH (ng/mL)	Cannabinoid equivalents (ng/mL)	THC (ng/mL)	THC-COOH (ng/mL)	Cannabinoid equivalents (ng/mL)	THC (ng/mL)	THC-COOH (ng/mL)	Cannabinoid equivalents (ng/mL)	THC (ng/mL)	THC-COOH (ng/mL)
P1	2	0	0	1	0.5	0	2	(0.4)*	(0.5)	–	–	–	–	–	–
P2	1	0	0	1	0.6	0	2	0.6	0.7	2	0	(0.4)	1	0	0
P3	1	0	0	1	0.5	(0.4)	2	(0.4)	0.7	2	0	(0.4)	2	0	0.6
P4	1	0	0	2	0.5	0.6	2	0	1.1	2	0	0.7	2	0	(0.5)
P5	1	0	0	2	0.7	0	2	(0.4)	0.7	2	0	0	2	0	0
P6	2	0	0	2	0.7	0.8	4	0.7	1.7	4	0	1.5	3	0	1.0
P7	1	0	0	–	–	–	2	0	0.6	1	0	(0.4)	1	0	0
P8	2	0	0	5	0	0	7	0	(0.5)	2	0	(0.4)	1	0	0
Mean	1	0	0	2	0.5	0.3	3	0.3	0.8	2	0	0.5	2	0	0.3
Range	1–2	0	0	1–5	0–0.7	0–0.8	2–7	0–0.7	0.5–1.7	1–4	0	0–1.5	1–3	0	0–1.0

* Approximate values below the LLOQ are given in parentheses.

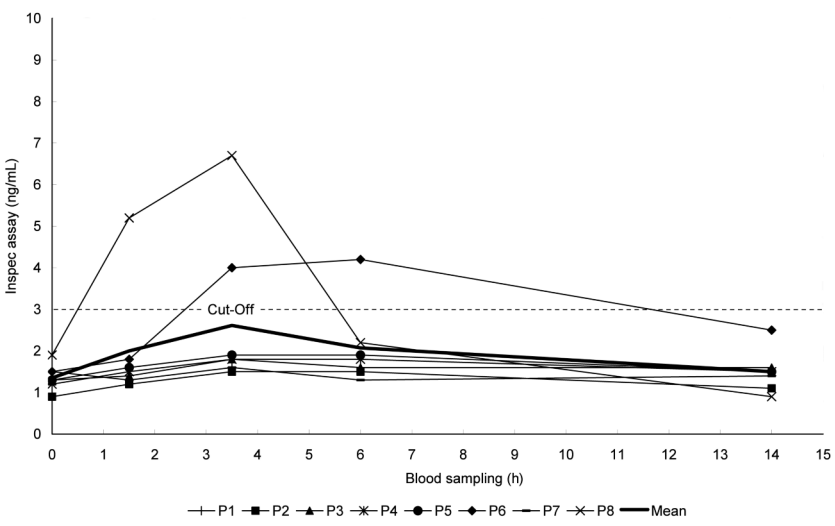

Figure 4. Immunoassay testing (Mahsan Inspec assay) of serum.

chromatographic analyses would typically be done routinely. The highest assay results were observed in the urine samples of subject P2 at 6 h (20 ng/mL) and subject P6 at 6 and 14 h (19 and 20 ng/mL). If the cutoff were to be lowered to 10 ng/mL, 37 urine samples would be evaluated as being positive for cannabis.

Only 27 of the 37 immunoassays that resulted more than 10 ng/mL in urine could be confirmed by GC–MS with or without hydrolysis, suggesting that 10 assay results have to be considered false positive. The concentration of THC-COOH in urine analyzed with GC–MS are presented in Table IV and Figures 2 and 3. THC-COOH was detectable in the urine samples of four of the volunteers at t2 (3.5 h), 30 min after the end of the exposure. The concentrations ranged from approximately 0.5 to 1.7 ng/mL and from 1.6 to 2.6 ng/mL after hydrolysis. The mean concentration was 0.5 ng/mL (1.0 ng/mL after hydrolysis). The concentration increased on average to 1.9 ng/mL (3.5 ng/mL after hydrolysis) 6 h after start of the experiment, reached a maximum of 2.3 ng/mL (3.8 ng/mL after hydrolysis) after 14 h, and declined continuously at t5 and t6. Free THC-COOH was no longer detectable with GC–MS in the urine samples obtained 84 h after the start of the exposure, but minimal traces of THC-COOH could be detected in the urine samples of subjects P3 (1.1 ng/mL), P4 (approximately 0.8 ng/mL), and P6 (approximately 0.7 ng/mL) after alkaline hydrolysis. In general, the concentrations of THC-COOH in urine were considerably increased after hydrolysis. The increase in THC-COOH concentration was mostly in a range of about 50–100% and in two samples nearly 400% (P1 at t3, 0.6 to 2.9 ng/mL and at t4, 0.5 to 2.4 ng/mL), suggesting that THC-COOH was intensely metabolized to conjugates. However, the reason for the comparatively high portion of free THC-COOH in the urine samples is not evident. Perhaps this could be an indication for hydrolysis of the glucuronide prior to analysis caused by storage conditions. It has to be noted that in none of the samples did the THC-COOH concentration exceed 10 ng/mL, even after alkaline hydrolysis. The highest concentrations observed were 7.8 ng/mL and 7.3 ng/mL (P2 at 6 h and P6 at 14 h, respectively, both after hydrolysis). Hence, passive inhalation of THC smoke seems incapable producing the THC-COOH levels in urine that are typically related to recent active use of cannabis.

The results of the serum analyses are summarized in Table V and shown in Figures 4–6. Four immunoassay results that were above the cutoff concentration of 3 ng/mL were found at 1.5 h (P8), 3.5 h (P6, P8), and 6 h (P6). However, the corre-

sponding amounts of THC and THC-COOH measured with GC–MS in these samples were either very small or negative. The immunoassay result in the sample of volunteer P8 at t1 was obviously a false positive, and in the sample taken at t2, traces of THC-COOH (approximately 0.5 ng/mL) were found, which would be judged as being negative in a routine forensic investigation. In contrast, positive assay results in the samples of volunteer P6 could be confirmed at t2 (THC 0.7 ng/mL and THC-COOH 1.7 ng/mL) and at t3 (THC-COOH 1.5 ng/mL). THC could be detected in the blood of volunteers P1 to P6 during the exposure to cannabis smoke at t1 (1.5 h). The THC concentrations ranged from approximately 0.5 to 0.7 ng/mL (mean 0.5 ng/mL). At t2 (3.5 h after start), which was half an hour after the end of exposure, the serum of five of the eight volunteers was still positive for THC in concentrations between approximately 0.4 and 0.7 ng/mL. It has to be pointed out that none of the subjects reported a personal feeling of cannabis influence, even though THC was present in the blood. THC was no longer detectable in the blood sampled 6 h after beginning of the smoke exposure. The primary THC metabolite THC-OH, which is known to be pharmacologically active, was not detected in any sample. The inactive main metabolite THC-COOH was found in the samples collected during exposure at 1.5 h in an average concentrations of 0.3 ng/mL (range 0–0.8 ng/mL). The average THC-COOH concentration shortly after ending the exposure (3.5 h) was 0.8 ng/mL and then declined to a mean concentration of 0.5 ng/mL after 6 h. After 14 h, THC-COOH could only be detected in three serum samples in concentrations of approximately 0.5, 0.6, and 1.0 ng/mL, whereas all other samples were negative at this time.

The THC serum concentrations in the recent study were much smaller than the concentrations found in the investigations of Mørland et al. (3) and Cone et al. (1,2), who observed THC levels up to 6.3 and 7.4 ng/mL. Cone et al. (1,2) found THC-COOH concentrations in urine samples analyzed with GC–MS in the range of 10–87 ng/mL with an average of 30 ng/mL after exposure to the smoke of 16 marijuana cigarettes. This is more than 10 times higher than the urine concentrations of free and hydrolyzed THC-COOH in this particular investigation, which were in the range of approximately 0.4–7.8 ng/mL (on average around 2 ng/mL). Similar THC-COOH concentrations in a range of 0–6 ng/mL were found by Cone et al. (1) in hydrolyzed urine samples after the first exposure to the smoke derived from four 2.8%-THC marijuana cigarettes (1,2). These conditions are highly comparable to the current study. After the second exposure to the smoke of four marijuana cigarettes on the following day, Cone et al. (1) found a maximum THC-COOH level of 12 ng/mL, which is considerably higher than the maximum concentration of 7.8 ng/mL in the present study. This means that the exposure to cannabis smoke during the single 3-h stay in a coffee shop led to cannabinoid concentrations in urine comparable to those after single exposure to four 2.8%-THC marijuana cigarettes. Therefore, it seems that higher urine concentrations might only be caused by passive inhalation under intensive or repeated exposure conditions. These findings are reflecting the more realistic setting of the present study. Even if all 25 guests would have smoked cannabis at the same time, it would have been impossible to reach THC air concentrations in a room volume of about 200 m^3 in the magnitude of the THC air concentrations found in the tiny rooms of previous

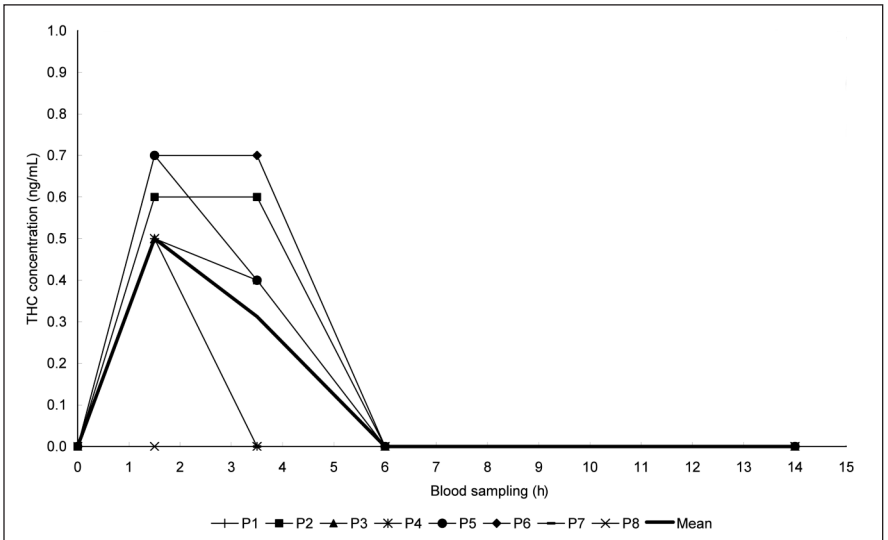

Figure 5. Concentrations of THC in serum analyzed with GC–MS.

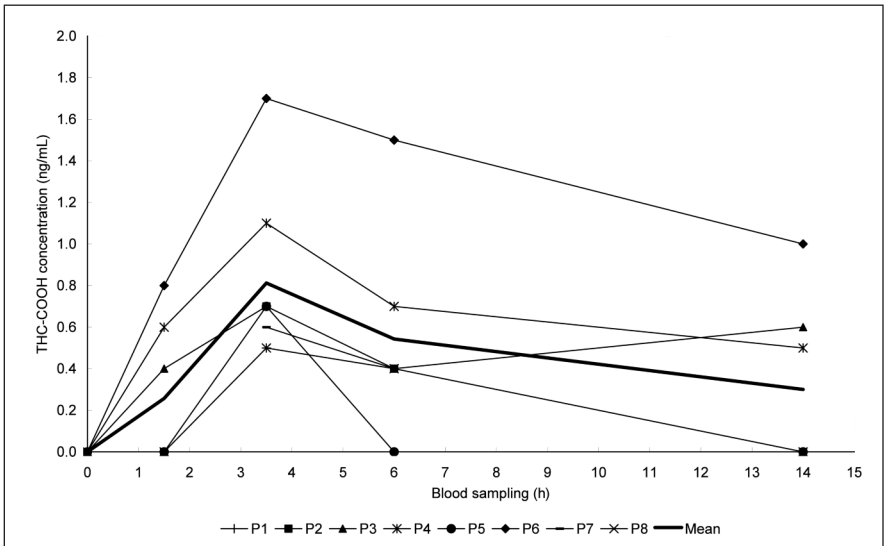

Figure 6. Concentrations of THC-COOH in serum analyzed with GC–MS.

studies. In these studies, extreme conditions far from common settings were chosen. Our investigations lead to the assumption that passive inhalation of cannabis smoke can only produce THC plasma levels considerably below 1 ng/mL during exposure or shortly afterwards. Already 3 h after exposure, THC is apparently no longer present in blood, and only trace amounts of the major metabolite THC-COOH can be found.

The experimental conditions in the studies of Law et al. (15) and Mulé et al. (16) seem to be closer to reality than the investigations mentioned earlier (1–3). Law exposed four passive inhalers to the smoke of six cannabis cigarettes containing 17.1 mg THC for three hours. The cigarettes were each smoked simultaneously by six volunteers in a small unventilated room of a volume of about 28 m^3. Blood and urine samples were taken three and six hours after the start of exposure, respectively. In the blood samples, no cannabinoids were detected, whereas in urine cannabinoid levels less than or equal to 6.8 ng/mL could be measured with radioimmunoassay. In the study of Mulé et al. (16), urine samples were taken 20–24 h after passive inhalation of cannabis smoke in a room of a volume of 22 m^3. The cannabinoid concentrations determined with immunoassay in all of the urine samples were less than 10 ng/mL. Because the exposure to cannabis smoke in the investigations of Law et al. (15) and Mulé et al. (16) occurred in rooms of reasonable dimensions, their findings seem to be in good agreement to the results of our study.

Conclusions

This study clearly demonstrated that all volunteers absorbed THC after passive exposure to cannabis smoke under real-life conditions. However, the resulting blood and urine concentrations were only very small. Because none of the urine samples produced immunoassay results that were more than the cutoff concentration of 25 ng/mL, none of the passive inhalers would be misjudged for cannabis use in a routine drug screening. If GC–MS testing would be done nevertheless, the corresponding THC-COOH concentration were below 10 ng/mL, even after hydrolysis. Furthermore, the results of this study indicate that passive exposure to cannabis smoke may only lead to trace amounts of THC in serum. Apparently, the THC serum concentrations are considerably below 1 ng/mL during or for a short time after exposure, and the concentrations of THC-COOH in serum do not exceed levels of about 2 ng/mL.

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