In Vitro Contamination of Hair by Marijuana Smoke

JANA THORSPECKEN,1 GISELA SKOPP,1,* AND LUCIA PÖTSCH2

Background: The deposition of cannabinoids on/into hair from environmental smoke can be considered as a potential source of drug findings in hair. We studied external uptake of cannabinoids from marijuana smoke, investigating possible influencing factors on drug uptake and the efficiency of decontamination procedures.

Methods: Strands of a natural hair sample were moistened with water, greased with sebum or sebum/sweat, or bleached or permed. Treated and untreated samples were exposed to marijuana smoke for 60 min. Aliquots of each hair strand were either kept unwashed or were washed with methanol, dichloromethane, or 5 g/L dodecyl sulfate in water. Cannabinoid concentrations in unwashed and washed hair samples, as well as in air samples collected from the exposure chamber and in the marijuana sample being combusted, were quantified by gas chromatography–mass spectrometry or gas chromatography.

Results: Cannabinoids were deposited on the hair fibers from marijuana smoke. Cannabinoid concentrations were dependent on air concentration and hair pretreatment. Uptake was less in untreated than in pretreated hair. Concentrations were increased in damp hair, but were even higher in greased hair. There was no significant difference in concentration between bleached and permed strands. External contaminants were completely removed by washing with methanol and dichloromethane in untreated hair only. Washing with dodecyl sulfate in water was insufficient in all cases.

Conclusions: Exposures of hair to marijuana smoke yields detectable cannabinoids depending on concentrations in the air, hair care habits, and cosmetic treatment.

Environmental marijuana smoke exposure may produce false-positive or falsely increased test results in hair.

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Hair analysis has shown great potential in the detection and control of drug use (1–5) because drugs become entrapped during hair fiber formation and stabilized in the keratin matrix. The accurate interpretation of analytical results continues to be difficult because many questions concerning drug incorporation into hair remain unanswered. This applies particularly to cannabinoid findings in hair. Incorporation of cannabinoids into hair is weak compared with basic substances such as cocaine or methylendioxymethamphetamine (6–8). The concentration of Δ⁹-tetrahydrocannabinol (THC)³ that can be detected in hair after active drug use is typically far less than 5 μg THC/g of hair (7). The analysis of hair to identify cannabis use is often restricted to the identification of THC, because 11-nor-9-carboxy-Δ⁹-THC concentrations, reported to be <0.1 μg/g of hair, cannot be determined by routine gas chromatography–mass spectrometry (GC/MS) procedures (9, 10). Consequently, for cannabinoids, the use of the metabolite-to-parent drug ratio, proposed to differentiate active use from passive exposure, cannot be applied to routine cases (11, 12).

Although detectable amounts of THC in the blood of nonsmokers have been demonstrated after exposure to marijuana smoke, the low concentrations in blood in these circumstances make it unlikely that the amounts of THC in hair would be measurable (13). In addition to inhalation of smoke, the deposition of a drug on/into the keratin matrix of hair from the environment has been proposed as a further source of drug findings in hair. Romano and coworkers (14, 15) showed that manual contamination with powdered cocaine or heroin hydrochloride and hashish or marijuana produced positive drug findings and that decontamination procedures were not sufficient to remove drugs that had penetrated into hair fibers.

³ Nonstandard abbreviations: THC, Δ⁹-tetrahydrocannabinol; GC/MS, gas chromatography–mass spectrometry; CBN, cannabinol; and CBD, cannabidiol.
Several authors (16–18) have investigated the deposition of cocaine from the vapor produced by crack smoking, but similar results for cannabis are not yet available.

The objective of the present study was to answer the question of whether marijuana smoke can produce positive cannabinoid findings in hair. Possible influencing factors of drug deposition on the keratin matrix, such as moisture, sebum, and bleaching or perming procedures, were investigated. In addition, commonly used washing procedures were applied to determine whether they are sufficient to remove drugs attributable to environmental uptake.

**Materials and Methods**

**STUDY DESIGN**

A sample of natural dark blonde hair was divided into several strands. One set of samples was not treated (control samples), whereas the others were moistened with tap water (series 1), greased with artificial sebum or (control samples), whereas the others were moistened for several strands. One set of samples was not treated with artificial sebum or a mixture of sebum and artificial sweat (series 2), or bleached or permed (series 3). Before exposure to smoke, the cut ends of all hair fibers were sealed with nail polish to avoid drug uptake by the cross-sections. The proximal sites of untreated (control samples) and treated hair strands of series 1–3 were fixed with a hairgrip, which was attached on a grid at the bottom of a vacuum desiccator. The loose ends of the hairs in each sample did not touch each other, the walls of the desiccator, or the grid. The desiccator was evacuated, and marijuana smoke was delivered by a beekeeper pipe (0.5 g of marijuana mixed with ~1 g of tobacco; Bienen-Meier) through a tubing (50 cm) that was inserted into the desiccator (6.11 L). The samples were kept in the closed chamber for 60 min. The tubing was replaced, and the desiccator was cleaned between uses.

Both ends of the exposed hair fibers were cut (~3 mm), and hair samples were further divided into eight strands and weighed (~250 mg/strand). In a particular series, two samples of each hair strand were kept unwashed, whereas two samples at a time were treated with 5 mL of one of the following wash solutions at room temperature for 10 min: (a) methanol (three times), (b) dichloromethane (three times), or (c) aqueous dodecyl sulfate (5 g/L) followed by distilled water (three times). The dried samples were pulverized in a ball mill (Retsch) for 4 min.

For the determination of air cannabinoid concentrations in the exposure chamber, we collected 4 mL of air directly after combustion of the marijuana/tobacco mixture with a gas-tight syringe (Hamilton) through a GC septum for autosampler use, which was attached to the desiccator by a three-way stopcock. The gas was then passed through 2 mL of n-hexane containing 100 ng of deuterated THC as internal standard in a head space vial. The n-hexane phase was taken to dryness under nitrogen, and the residue was further processed as reported for the hair samples. In each series, four air samples were collected.

In addition, two aliquots of each wash solution (0.1 or 1.0 mL) were analyzed for cannabinoids.

Determination of THC, cannabidiol (CBD), and cannabidiol (CBD) in the marijuana sample being combusted involved extraction with methanol (50 mg of ground plant material/5 mL of methanol; duplicate measurement) and analysis by GC with a nitrogen–phosphorous detector (Shimadzu).

**MATERIALS**

Kerling International Haarfabrik supplied the hair sample. It had been pooled from natural hair strands of Caucasian origin and mixed to homogeneity as far as possible. This hair sample was used for the experiments as well as for preparation of calibrators. All chemicals were of the highest purity available. NaCl, NaOH, HCl, NH₄Cl, acetic acid, lactic acid, squalene, and cholesterol were from Merck, and n-hexane, methanol, and dichloromethane were purchased from Roth. CBN and CBD were obtained from Lipomed, and THC and THC-d₃ were from Cerilliant. For derivatization, N,O-bis(trimethylsilyl) trifluoroaceticamide containing 10 g/L trimethylchlorosilane (Fluka) was used. Cacao butter, purified wool wax, and Vaseline were obtained from a local pharmacy.

Artificial sweat was made according to the formula proposed in the 3160/2 ISO standard (19), and sebum was prepared as reported previously (20). Two parts of artificial sweat were thoroughly mixed with one part of sebum at 40 °C. Approximately 200 mg of sebum or sebum/sweat was applied to 1 g of hair fibers.

For bleaching and perming, commercially available formulas for home use (Poly Blonde; Poly Lock) were applied to the hair strands according to the manufacturer’s instruction.

**GC/MS ANALYSIS**

The cannabinoid concentrations in unwashed and washed hair samples of series 1–3, in wash solutions, and air samples were determined by GC/MS. Approximately 50 mg of the hair powder was weighed, 250 ng of THC-d₃ was added as internal standard, and the sample was dissolved in 1 mL of 0.1 mol/L NaOH (100 °C for 30 min). The solution was adjusted to a pH of 5.5 with 1.0 mol/L HCl, and THC, CBN, and CBD were extracted into n-hexane and quantified by GC/MS after silylation, as described elsewhere (21). Measurements were performed in duplicate for each hair sample, and values are presented as the mean.

THC-d₃ was added as internal standard to the wash solutions, and aqueous wash solutions were extracted into n-hexane at pH 5.5 (0.1 mol/L HCl), whereas methanol and dichloromethane washings were taken directly to dryness (nitrogen and 40 °C). All residues were processed as reported for hair extracts.

The GC/MS system consisted of a HP 6890 gas chromatograph and an HP 5973 mass spectrometer (Agilent), and the compounds were eluted from a CP-Sil 5 column.
[12.5 m × 0.53 mm (i.d.); Chrompack]. Detection was by
ionization in the electron impact mode (70 eV), with the
scan mode set at single-ion monitoring (THC, m/z 299,
314, and 271; THC-d3, m/z 302, 317, and 274; CBN, m/z 295,
310, and 298; CBD, m/z 231, 246, and 314). For quantification,
the peak-area ratio for THC (m/z 299) and its internal
standard (m/z 302) was determined; for CBN and CBD,
the response factors were calculated against THC-d3 (m/z
302:314 for CBD and m/z 302:295 for CBN) and referenced
to the particular calibration curves. A separate calibration
curve constructed with a stock solution of the analytes in
n-hexane was prepared for the air samples. The seven-
point calibration curves covered a concentration range of
0.5–50 μg/g of hair, 0.5–50 μg/L of air, and 1–250 μg/L of
wash solution. The limits of detection and quantification
were determined from the calibration curves according to
DIN 32645 (22).

The within-run imprecision (CV) was determined by
extracting and assaying five aliquots of hair to which the
cannabinoids had been added at the lowest and highest
calibrator concentrations in a single batch. Between-run
imprecision was determined by testing two single ali-
quots (lowest and highest calibrator concentrations) in
five consecutive assays. The recovery of analytes from
n-hexane was calculated from samples to which cannabi-
noids had been added (10 μg/L) with the internal stan-
dard added before evaporation (n = 5) of the solvent or
directly to the residue (n = 5). A similar procedure was
applied to estimate the recovery of the analytes from
aqueous wash solutions (n = 5).

Results

The marijuana sample used for the experiments contained
86 mg/g THC and 0.4 mg/g CBN. CBD was not detect-
able at a detection limit of 0.01 mg/g and could not be
identified in any air or hair sample. The mean air concen-
trations of THC and CBN (n = 4) determined in series 1–3
are given in Table 1. Mean (SD) recovery of the analytes in
air samples from n-hexane was 93 (8)% for THC, and 71
(8)% for CBN (n = 5). In air samples that were collected
near the end of smoke exposure, THC and CBN concentra-
tions had decreased to as little as 20% of the initial
values (data not shown). The concentration of CBN rela-
tive to THC was considerably higher in air specimens
than in the marijuana sample, whereas the ratio of CBN to
THC in smoke was similar to that deposited on hair fibers.

Substantial amounts of both compounds were detect-
able in hair fibers; amounts were dependent on air con-
centrations as well as on the pretreatment of the hair
sample (Table 1). Duplicate measurements agreed within
5% of the mean. Intraassay CV ranged from 2.6 (3.3)% to
7.9 (8.2)%; whereas interassay CV were between 4.1 (3.9)%
and 8.3 (8.4)% at the highest and lowest THC (CBN)
calibrator concentrations, respectively. In all series, the
concentrations of THC and CBN were always less in
untreated hair than in pretreated hair. Cannabinoid con-
centrations were higher in hair moistened with tap water
or a mixture of sebum and sweat than in apparently dry
hair, and were still higher in greased, dry hair. We found
no significant difference in THC or CBN uptake from
marijuana smoke among bleached and permed hair sam-
ples.

The efficiency of the various wash procedures for
removal of cannabinoids was dependent on hair pretreat-
ment as well as on the particular wash solution. Methanol
dichloromethane removed both THC and CBN com-
pletely from untreated hair. Methanol was superior to
dichloromethane when applied to damp hair, whereas
dichloromethane completely removed cannabinoid resi-
dues from bleached hair strands or hair treated with
sebum or sebum/sweat. After shampooing (dodecyl sulfate)
and three wash cycles with water, significant
amounts of THC and CBN remained regardless of
whether the hair was pretreated. As for methanol and
dichloromethane, this wash procedure was more effective
when applied to untreated hair.

The highest concentrations of THC and CBN were
always observed in the first wash for procedures a through c, and concentrations in wash solutions from procedure c were generally lower than in procedures a or b. Mean (SD) recoveries of THC and CBN from aqueous wash solutions were 87.2 (3.6)% and 86.8 (5.8)%, respectively. In series 1 (individual data not shown), total concentrations of THC and CBN did not differ significantly between methanol and dichloromethane washes 1–3. In apparently dry hair, total cannabinoid concentrations in the methanol washes were 1634 μg/L THC and 131 μg/L CBN, and the total concentrations in the dichloromethane washes were 1689 μg/L THC and 286 μg/L CBN. For damp hair, the total concentrations in the methanol washes were 1657 μg/L THC and 132 μg/L CBN, and the total concentrations in the dichloromethane washes were 1641 μg/L THC and 256 μg/L CBN. However, concentrations were considerably lower in the wash solutions of procedure c. In solutions from apparently dry hair, the THC and CBN concentrations were 424 and 44 μg/L, respectively, and in solutions from damp hair, the concentrations were 475 and 45 μg/L, respectively.

The cannabinoid concentrations found in wash solutions from series 2 and 3 are shown in Tables 2 and 3. In series 2, cannabinoid concentrations in washings from hair samples always decreased in the following order: untreated hair > hair treated with sebum/sweat > hair treated with sebum. We observed very rapid decreases in the amounts of THC and CBN after the first wash for procedures a and b compared with washes from series 1 or 3. The highest analyte concentrations in wash solutions from series 3 resulted from bleached hair for procedures a–c. There was no difference between the methanol and dichloromethane washings from untreated and bleached hair.

Discussion

Although cannabis is widely used and smoking is the most popular route of drug administration, contamination of hair from external marijuana smoke has been minimally investigated. Strano-Rossi and Chiarotti (23) exposed a hair sample to marijuana smoke for evaluation of sample decontamination procedures. Data for cannabinoid concentrations of exposed hair and/or in wash solutions were not reported. Hair samples from individuals exposed to marijuana or hashish smoke for 15 min in a small room tested negative for THC after four to five successive washings with dichloromethane (24).

The present study showed that a single exposure to marijuana smoke may produce a positive cannabinoid finding in hair. The THC concentration in the desiccator was close to the concentrations in air reported in a study of passive exposure by Cone et al. (25), in which individuals had been exposed to the smoke of 16 marijuana cigarettes and air concentrations of THC were up to 10.9 μg/L. In the present study, the deposition of cannabinoids was found to be influenced by hair care habits and cosmetic treatments.

A sufficient amount of a fairly homogeneous hair sample was necessary to perform the experiments. Dark blonde is the most common hair color in Germany; we therefore chose a pooled, dark blonde hair sample to avoid possible effects attributable to interindividual differences in hair morphology, as indicated by the study of Zahlsen et al. (26) on environmental tobacco smoke exposure. The influence of melanin on the drug content of hair has been hypothesized to be much lower for environmental contamination than for systemic incorporation via the hair follicle. On the basis of the findings of Zahlsen et al. (26), that uptake of air nicotine was not influenced by hair pigmentation, in our study, hair color should not affect the results. After diffusion into the keratin fiber, a drug–melanin association can occur only on the surface of the pigment granules for basic drugs such as nicotine. As already shown for various acidic drugs (4, 7), THC is not presumed to exhibit a high melanin affinity. We also found no reports in the literature that indicated that the

### Table 2. THC and CBN concentrations in wash solutions in series 2.

<table>
<thead>
<tr>
<th>Cannabinoid</th>
<th>Concentration, μg/L</th>
<th>First wash</th>
<th>Second wash</th>
<th>Third wash</th>
<th>Fourth wash</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wash procedure a&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Untreated THC</td>
<td>241.4</td>
<td>8.5</td>
<td>4.1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CBN</td>
<td>33.1</td>
<td>1.0</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Sebum THC</td>
<td>145.7</td>
<td>4.6</td>
<td>1.1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CBN</td>
<td>24.1</td>
<td>0.7</td>
<td>0.4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Sebum/Sweat THC</td>
<td>175.8</td>
<td>3.3</td>
<td>1.1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CBN</td>
<td>20.8</td>
<td>0.4</td>
<td>ND&lt;sup&gt;e&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Wash procedure b&lt;sup&gt;d&lt;/sup&gt;</td>
<td>Untreated THC</td>
<td>298.8</td>
<td>10.0</td>
<td>4.6</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CBN</td>
<td>36.9</td>
<td>1.9</td>
<td>ND&lt;sup&gt;e&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Sebum THC</td>
<td>137.8</td>
<td>3.2</td>
<td>0.6</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CBN</td>
<td>23.6</td>
<td>1.0</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Sebum/Sweat THC</td>
<td>178.6</td>
<td>3.7</td>
<td>0.5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CBN</td>
<td>21.0</td>
<td>0.3</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>Wash procedure c&lt;sup&gt;f&lt;/sup&gt;</td>
<td>Untreated THC</td>
<td>113.8</td>
<td>5.2</td>
<td>2.3</td>
<td>1.5</td>
</tr>
<tr>
<td></td>
<td>CBN</td>
<td>11.5</td>
<td>0.7</td>
<td>0.3</td>
<td>0.4</td>
</tr>
<tr>
<td></td>
<td>Sebum THC</td>
<td>30.4</td>
<td>1.0</td>
<td>1.0</td>
<td>0.3</td>
</tr>
<tr>
<td></td>
<td>CBN</td>
<td>4.1</td>
<td>ND&lt;sup&gt;f&lt;/sup&gt;</td>
<td>~1.0&lt;sup&gt;c&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Sebum/Sweat THC</td>
<td>37.7</td>
<td>5.5</td>
<td>2.1</td>
<td>1.4</td>
</tr>
<tr>
<td></td>
<td>CBN</td>
<td>6.4</td>
<td>2.7</td>
<td>~0.2&lt;sup&gt;e&lt;/sup&gt;</td>
<td>~0.1&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup> Based on 50 g of hair per L of washing solution.

<sup>b</sup> Three washes with 5 mL of methanol (10 min/washing step).

<sup>c</sup> CBN concentration above the limit of detection but below the limit of quantification (~0.25 μg/g of hair).

<sup>d</sup> Three washes with 5 mL of dichloromethane (10 min/washing step).

<sup>e</sup> ND, CBN not detectable (~0.06 μg/g of hair).

<sup>f</sup> One wash with 5 mL of 5 g/L dodecyl sulfate followed by three washes with 5 mL of water (10 min/washing step).
The ratio of THC and CBN deposited on hair was related to the ratio of both compounds in environmental smoke. Similar observations have been reported from controlled 72-h exposure of hair samples to environmental nicotine smoke (32). In this investigation, the chamber experiments revealed a linear increase in hair nicotine concentration with increasing chamber exposure up to 4.8 μg nicotine/L of air, suggesting the uptake was governed by an equilibrium between nicotine in air and on the hair surface. For chemicals such as polychlorinated dibenzo-p-dioxins, rapid adsorption equilibrium with hair occurred within <1 h (33).

At present, the detailed mechanisms and major influences governing drug uptake from environmental gaseous or particulate matter are largely unknown. For cocaine, concentrations in hair samples soaked in aqueous solutions of cocaine were far higher than those measured in hair samples exposed to crack smoke (18). A 5-min exposure of hair to an aqueous solution containing 1 mg/L cocaine hydrochloride was sufficient to produce measurable cocaine concentrations of 0.2 μg/g of hair (34). From experiments involving dyestuffs (35–37) and drugs such as opiates (38), it can be assumed that, in intact hair, diffusion via the cuticle cell edges along the endocuticle and the cell membrane complex proceeds rapidly. From there, solutes are probably further distributed throughout the other morphologic components of hair by a slower process. Diffusion is largely promoted by water molecules, which allows the swelling of nonkeratin tins in hair, thus enlarging the “gaps” on the hair surface and facilitating drug entry into hair. This may explain the higher cannabinoid concentrations in hair samples moistened with tap water or treated with sweat/sebum compared with untreated, apparently dry hair specimens. In series 2, trapping of cannabinoids by the greasy film on the hair surface can be hypothesized because THC and CBN are highly lipophilic and essentially water insoluble (27). Indeed, a higher concentration was found in hair treated solely with sebum.

From experiments on diffusion of dyestuffs and drugs, there is also evidence that adsorption and diffusion of foreign matter is strongly influenced by the hair morphology (35–38). Scalp hair plays an important role in individual appearance and, therefore, is frequently subjected to cosmetic treatments such as perming or bleaching. Both treatments lead to degradation of hair proteins and lipids and damage the nonkeratin layers of the cuticle, which represent the regions most vulnerable to these chemical interactions (39). Accordingly, as we expected, cannabinoid adsorption onto, or diffusion into, hair was increased in cosmetically treated hair compared with the untreated specimen (series 3).

Hair samples are generally cleaned before analysis to remove foreign matter from the hair surface. For cannabinoids, various wash procedures have been proposed, such as with washing with dichloromethane, methanol, Tween 80, phosphate buffer, or petroleum ether (9, 10, 21, 23, 24, 29).

<table>
<thead>
<tr>
<th>Table 3. THC and CBN concentrations in wash solutions in series 3.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cannabinoid</strong></td>
</tr>
<tr>
<td>-----------------------------------------------</td>
</tr>
<tr>
<td>THC</td>
</tr>
<tr>
<td>CBN</td>
</tr>
<tr>
<td>Bleached THC</td>
</tr>
<tr>
<td>CBN</td>
</tr>
<tr>
<td>Permed THC</td>
</tr>
<tr>
<td>CBN</td>
</tr>
</tbody>
</table>
| Wash procedure | a  
Untreated THC | 463.9 | 17.3 | 4.6 | 3.2 |
| CBN | 34.2 | 1.9 | 0.5 | 0.3 |
| Bleached THC | 767.9 | 57.0 | 22.4 | 14.9 |
| CBN | 59.3 | 5.7 | 2.2 | 1.7 |
| Permed THC | 575.5 | 19.9 | 6.9 | 3.2 |
| CBN | 41.0 | 1.6 | 0.9 | 0.3 |
| Wash procedure | b  
Untreated THC | 181.3 | 14.3 | 8.7 | 8.2 |
| CBN | 3.5 | 0.4 | 0.3 | 0.2 |
| Bleached THC | 167.8 | 3.2 | 3.3 | 3.0 |
| CBN | 3.5 | ~0.2 | ~0.2 | ~0.2 |
| Permed THC | 159.2 | 4.2 | 3.8 | 5.8 |
| CBN | 3.0 | ~0.2 | ~0.2 | ~0.2 |

*a Based on 50 g of hair per L of washing solution.  
*b Three washes with 5 mL of methanol.  
*c Three washes with 5 mL of dichloromethane.  
*d One wash with 5 mL of 5 g/L dodecyl sulfate followed by three washes with 5 mL of water.  
*e CBN concentration above the limit of detection but below the limit of quantification (<0.25 μg/g of hair).
28, 40). In the present study, dichloromethane and methanol were chosen because cannabinoids are slightly soluble in short-chain alcohols and chlorinated hydrocarbons. Methanol allows swelling of the hair, whereas dichloromethane does not. Typical hygienic practices were mimicked by wash procedure c, which used dodecyl sulfate in water. Water promotes swelling of the hair but poorly solubilizes cannabinoids. This wash procedure was insufficient to remove cannabinoids completely from any hair sample. Both methanol and dichloromethane successfully removed cannabinoids from untreated hair, suggesting that diffusion of THC and CBN into hair had not occurred. In the case of hair strands moistened with tap water, penetration of drugs into the fiber probably occurred during smoke exposure and/or washing with dichloromethane was insufficient. Although on gross inspection methanol appeared to successfully remove sebum or sebum/sweat, small amounts of THC and CBN were still detectable in hair after the three washes. These results demonstrate that it is important to choose the most favorable solvent for decontamination, with consideration of both the solubility of a particular compound and the swelling behavior of hair. In “wash” procedures used to clean the surface of the hair before sample processing, it should be noted that although drugs may be removed from the outside, they may have also penetrated into the keratin matrix. Interestingly, in the present study a positive cannabinoid finding in the last wash solution did not imply a positive cannabinoid finding in hair. In contrast, a negative result in the last wash solution did not always entail a negative result in hair. Therefore, the criterion often used in hair analysis, that a negative wash solution assures complete removal of external contamination, does not seem universally valid.

Although cannabinoids initially were higher in bleached hair, there was no difference in total removal of cannabinoids from untreated and bleached hair strands by dichloromethane washes. Removal of THC and CBN from permed hair by methanol was more effective, which could be attributable to less damage of the keratin matrix from permanent waving. Overall, dichloromethane seemed superior to methanol when applied to apparently dry hair. However, it should be noted that dichloromethane reduced the measured concentration of THC in hair from cannabis users, whereas methanol washes did not appear to affect quantitative results compared with unwashed samples (41).

In conclusion, our study has demonstrated that short-term exposure to marijuana smoke may lead to detectable amounts of THC and CBN in hair. A positive finding may result from major cannabis components in vapor and may also be influenced by hair care habits and cosmetic treatment of hair. The measured cannabinoid concentration was also influenced by the decontamination procedure. These findings should be considered when the frequency of cannabinoid consumption is estimated by hair analysis. Certainly the scalp hair of a marijuana smoker is exposed to environmental smoke and may be contaminated. It is therefore very likely that the same process that occurred in the in vitro experiments could occur in real life. High concentrations of cannabinoids in a hair sample do not unequivocally indicate frequent or heavy smoking habits. At present, there is growing consensus that identification of 11-nor-9-carboxy-Δ<sup>9</sup>-THC is a requirement for reporting a positive hair test result. We appreciate that the analytical requirements for identification of this metabolite are beyond the capability of the instrumentation used in this study (41, 42), but identification of this metabolite is considered a solution to the problems posed by the results of this study.

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References
14. Romano G, Barbera N, Lombardo I. Hair testing for drugs of


