

Hair analysis for THCA-A, THC and CBN after handling cannabis plant material

B. Moosmann¹, N. Roth¹, A. Jacobsen-Bauer² and V. Auwärter¹

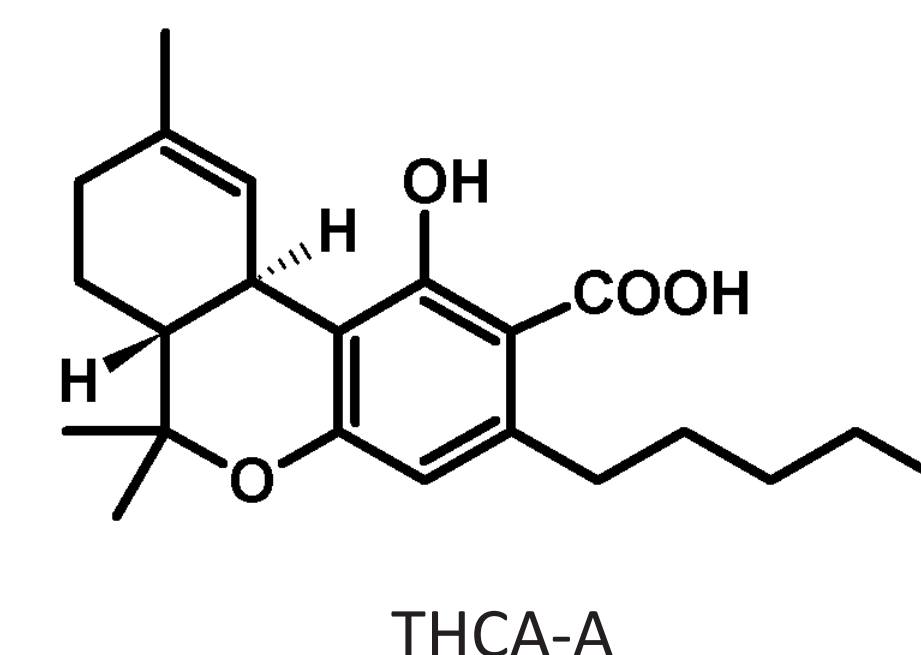
¹Institute of Forensic Medicine, Forensic Toxicology, University Medical Center Freiburg, Germany

²State Office of Criminal Investigation Baden-Württemberg, Stuttgart, Germany

**Institute of Forensic Medicine
Forensic Toxicology**

Introduction

A previous study has shown that Δ^9 -tetrahydrocannabinolic acid A (THCA-A), the non psychoactive precursor of Δ^9 -tetrahydrocannabinol (THC) in the cannabis plant does not get incorporated into the hair through the bloodstream after repeated oral intake (applied limit of detection: 50 pg/mg) [1]. However, THCA-A can be measured in forensic hair samples in concentrations often exceeding the detected THC concentrations and may therefore act as a marker for an external contamination. Recently, another study demonstrated that a contamination through side-stream marijuana smoke can be ruled out as a source for high THCA-A concentrations in hair [2] and proposed that transfer through contaminated fingers may be an explanation for the findings in forensic hair samples. As a consequence, a study was carried out to analyze whether the handling of cannabis plant material prior to consumption is a contributing factor for THC positive hair results and to evaluate if THCA-A can act as a marker for such a contamination.



Materials and methods

Nine subjects (hair lengths: 5.5 - 36 cm) rolled a marijuana joint containing 500 mg marijuana flowers (8.9% THCA-A & 1.3% THC) as well as 500 mg tobacco on five consecutive days. Afterwards the participants were instructed not to wash their hands for at least three hours. Three hair samples of each participant were obtained. One prior to the study, one at the end of the five day period and one sample one month after the first exposure. In addition to the hair samples urine samples were obtained to exclude cannabis consumption prior to or during the study period. The concentrations of THC, THCA-A and cannabinol (CBN) were measured in the segmented hair (3 cm segments of the first 12 cm hair and 6 cm segments for the remaining hair) after methanolic extraction using a validated LC-MS/MS method with a lower limit of quantification of 2.5 pg/mg for THCA-A and 20 pg/mg for THC and CBN [3].

Results and discussion

Concentrations

n = 9

5 days after first exposure

	THCA-A [pg/mg]	THC [pg/mg]	CBN [pg/mg]	Ratio THCA-A : THC
Mean	395	53.9	31.3	5.5
SD	565	34.4	19.5	5.6
Median	216	46.5	28.3	4.6
Range	15.3 - 1820	~14.3 - 92.5	~9.6 - 49.1	1.1 - 19.9

1 month after first exposure

	THCA-A [pg/mg]	THC [pg/mg]	CBN [pg/mg]	Ratio THCA-A : THC
Mean	25.8	6.6	7.3	3.6
SD	20.7	6.6	10.6	1.8
Median	19.3	9.3	0.0	4.0
Range	0 - 57.1	0 - ~17	0 - 22.4	max 6.0

THCA-A, THC and CBN could be detected in the hair samples from all nine participants taken at the end of the exposure period, whereas the three participants with the shortest hair (5.5 cm - 12 cm) showed the highest degree of contamination. However, with shorter hair the analytes seem to be washed out more easily and as a consequence of this, these participants show the largest decrease of the cannabinoid concentrations in the third sample. One month after the exposure, THCA-A could still be detected in the hair samples of eight participants with a decline of the THCA-A concentration from sample 2 to sample 3 ranging from 97% to 77%. Furthermore, THC could be detected in the hair samples of five participants. The concentrations of THC in these samples were below the cut-off applied in Germany for driving license issues (20 pg/mg). However, when applying alkaline hydrolysis, THCA-A will be partly decarboxylized and detected as THC.

Comparison to forensic samples

Comparing the detected ratio of THCA-A to THC of 1.1 : 1 to 19.9 : 1 (Median: 4.6 : 1) in the study samples with the ratios found in forensic samples of alleged cannabis consumers (n = 42) which ranged from 0.9 : 1 to 28 : 1 (Median: 3.8 : 1), the findings suggest that transfer through contaminated fingers is probably the main reason for the high concentrations of THCA-A detected in routine samples. The decline of the ratios from sample 2 to sample 3 can be explained by slow decarboxylation of THCA-A due to light and heat exposure.

Conclusion

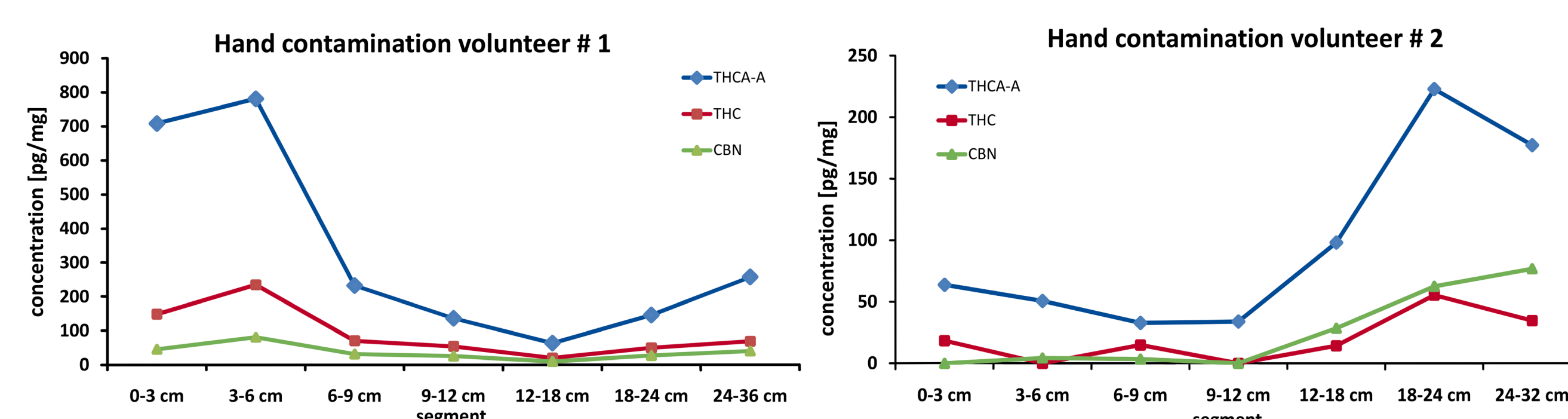
It can be concluded that at least parts of the THC and CBN as well as almost all of THCA-A found in routine hair analysis derive from external contamination caused by direct transfer through e.g. contaminated fingers. The three tested cannabinoids with their high lipophilicity can be transferred onto the hair and incorporated into it. Taking THC and CBN contamination caused by side-stream smoke [2], transfer of THCA-A, THC and CBN through contaminated fingers and potential decarboxylation of THCA-A in the analytical process into account, interpretation of consumption habits / frequency based on the measured THC concentration has to be strongly questioned. Additionally, the above findings may be an explanation for cases where high THC/CBN concentrations are measured in hair without a detectable presence of 11-nor-9-carboxy-THC. This finding is of particular interest in interpreting THC positive hair results of children or non-consuming partners of cannabis users, as the presence of THCA-A might indicate a possible incorporation of cannabinoids into the hair after 'passive' transfer.

References

- [1] Auwärter et. al. Hair analysis for Δ^9 -tetrahydrocannabinolic acid A – new insights into the mechanism of drug incorporation of cannabinoids into hair. *Forensic Sci Int* 196: 10-13, **2010**
- [2] Moosmann et. al. Hair analysis for THCA-A, THC and CBN after passive in vivo exposure to marijuana smoke. *Drug Test Anal* DOI: 10.1002/dta.1474
- [3] Roth et. al. Development and validation of an LC-MS/MS method for quantification of Δ^9 -tetrahydrocannabinolic acid A (THCA-A), THC, CBN and CBD in hair. *J Mass Spectrom* 48: 227-233, **2013**
- [4] Skopp et. al. Stability of cannabinoids in hair samples exposed to sunlight. *Clin Chem* 46: 1846-1848, **2000**

Further aspects

Distribution



Regarding the distribution of the contamination over the hair strand, it could be observed that the highest concentrations could either be found in the proximal (n = 3) or the distal (n = 6) segments, while the middle of the hair shaft seemed to be affected less. The high proximal concentrations are particularly critical as they may be misinterpreted as a result of heavy recent cannabis consumption.

Plant material

Marijuana (n = 458)

As different cannabis products show significant differences in cannabinoid content, conclusions regarding an exclusive transfer through contaminated fingers or surfaces based on the ratio of THCA-A and THC without knowledge of the product consumed is not possible.

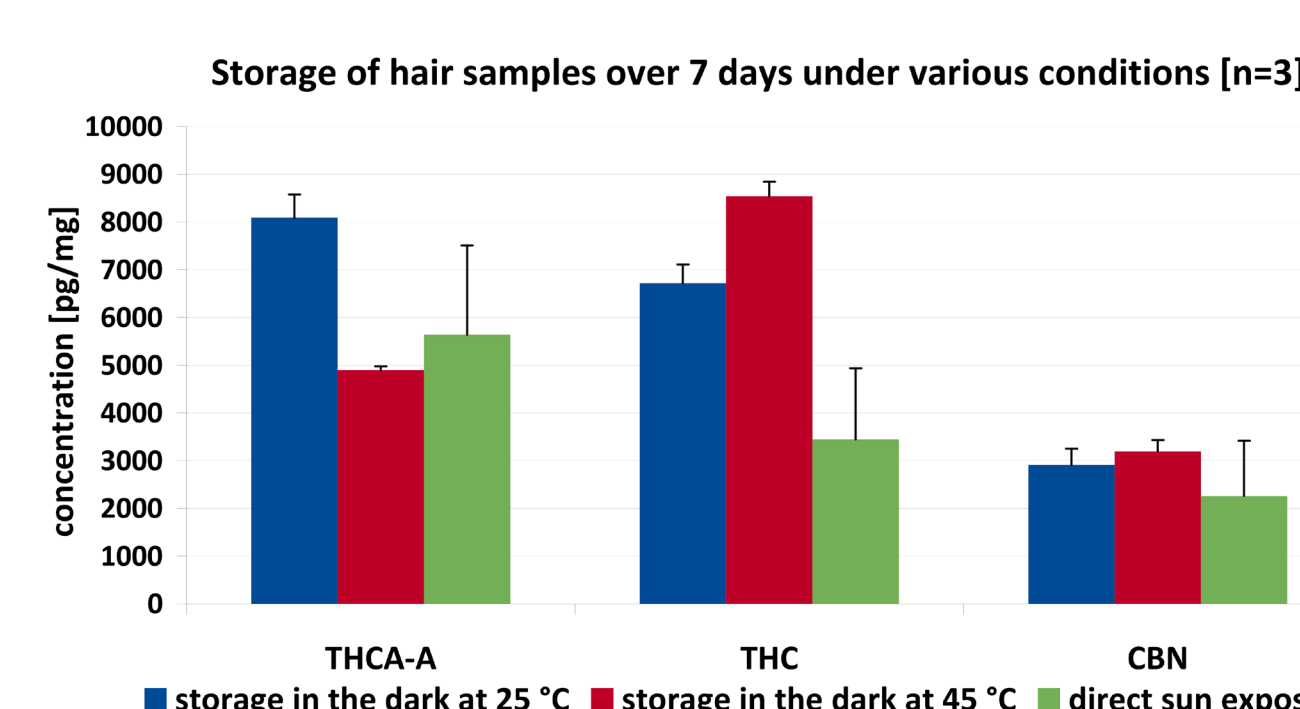
	THCA-A [%]	THC [%]	CBN [%]	Ratio THCA-A : THC
Mean	6.7	1.1	0	11.0
SD	4.8	1.4	0.1	11.6
Median	6.3	0.7	0	8.3
Range	0 - 17.8	0 - 10.9	0 - 1.3	0 - 119

Hashish (n = 180)

	THCA-A [%]	THC [%]	CBN [%]	Ratio THCA-A : THC
Mean	4.6	2.1	0.4	2.8
SD	3.8	1.5	0.4	3.4
Median	4.1	2.0	0.2	2.1
Range	0 - 15.5	0 - 9.2	0 - 2.0	0 - 29.9

Sun and heat exposure of the hair

As head hair is exposed to sunlight and heat, an additional experiment was carried out to evaluate the influence of these factors on the analytical results.



While both sunlight and heat exposure decrease the THCA-A concentration, only the latter seems to increase the THC concentration thus lowering the THCA-A : THC ratio. Furthermore, the observed decline of the THC and CBN concentrations when exposed to sunlight are in good accordance with the studies from Skopp et. al. [4].

Acknowledgement

The authors would like to thank the **German Academic Exchange Service (DAAD)** for covering the travel expenses to the 51st annual meeting of the TIAFT in Funchal, Portugal.

The project was funded by the **Deutsche Forschungsgemeinschaft (DFG-AU: 324/3-1)**.

Bjoern Moosmann
Institute of Forensic Medicine
Forensic Toxicology
Albertstraße 9
79104 Freiburg, Germany
bjoern.moosmann@uniklinik-freiburg.de

