

Limite de détection du THC dans les cheveux dans le contexte de l'aptitude à la conduite

Cut-off for THC in hair in context of driving ability

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RÉSUMÉ

En Allemagne, la consommation régulière de cannabinoïdes est incompatible avec la possession d'un permis de conduire. Ainsi, l'analyse des cheveux est régulièrement utilisée dans le contexte de l'examen d'aptitude à la conduite. Selon les recommandations de la Society of Hair Testing (SoHT), la Gesellschaft für Toxicologie und forensische Chemie (GTFCh) et la Société Française de Toxicologie Analytique (SFTA), une valeur limite de 0,1 ng/mg de THC dans les cheveux est appliquée. Dans cette présentation, les résultats de l'analyse des cheveux pour le THC obtenus à l'Institut de Médecine Légale de la Charité (Berlin, Allemagne) dans le contexte de l'examen de l'aptitude à la conduite ont été évalués. Parmi 625 échantillons de cheveux étudiés, 453 étaient négatifs. D'autre part, en utilisant une valeur limite de 0,1 ng/mg, 47% des 172 cas positifs avec une concentration de THC détectée sans ambiguïté, ont été rendus négatifs. Par comparaison avec les statistiques de la consommation de Cannabis en Allemagne et du fait que toutes les personnes testées avaient un historique de consommation de Cannabis, cette proportion est très grande. Par conséquent, il a été proposé d'abaisser la limite à 0,05 ng/mg. En outre, il a été montré que les dosages effectués sur des échantillons de cheveux plus courts que les 6 cm habituels n'avaient pas d'effet essentiel sur la proportion de résultats positifs.

SUMMARY

In Germany, habitual use of cannabinoids is incompatible with possession of a driving license. Therefore, hair analysis is regularly used in context of the driving ability examination. According to the recommendations of the Society of Hair Testing (SoHT), the Society of Toxicological and Forensic Chemistry (GTFCh) and the Société Française de Toxicologie Analytique (SFTA), a cut-off value of 0.1 ng/mg THC in hair is applied. In this presentation the results of the hair analysis for THC obtained in the Institute of Legal Medicine of the Charité Berlin in context of driving ability examination were evaluated. From 625 hair samples 453 were negative. In addition to that, using the cut-off of 0.1 ng/mg, 47 % of the 172 positive cases with unambiguously detected THC were also decided to be negative. As shown by comparison with the statistics of cannabis use in Germany and regarding the fact that all test persons had a cannabis history, this portion is very high. Therefore, it is proposed to decrease the cut-off to 0.05 ng/mg. Furthermore, it is shown that the investigation of hair samples shorter than the usual 6 cm has no essential effect on the portion of positive results.

Introduction

According to the actual cannabis legislation in Germany (1), the possession of a driving license is excluded in case of regular (habitual) cannabis use. In case of occasional cannabis use, a driving license can be issued if the applicant is able to separate (in time) between cannabis use and driving. The driving license is generally not issued if cannabis is consumed in combination with alcohol or other illicit drugs. A suspicion about driving inability because of cannabis abuse arises if there is evidence at the police that the applicant was in possession of cannabis products (two or more times small amounts or once large amounts), that he used cannabis without context to driving or that he was under the influence of cannabis whilst driving. In principle, three categories of cannabis use must be distinguished:

- Single or altogether a few times use (experimental use). This is without consequences to driving license.
- Occasional use. The frequency of use is not clearly defined, it is everything between experimental and regular use. If an occasional use is assumed, the applicant must undergo a Medical Psychological Investigation (in German "MPU") in order to find out whether he is able to abstain from driving if he has used the drug and vice versa.
- Regular use. There were several controversy opinions about this term (1). It means at least daily or almost daily or more than 200 doses per year. In this case, the driving license is suspended.

The course of events in a case of doubt about driving ability because of cannabis and the role of hair analysis in this process in Berlin is shown in Fig. 1. There is no uniform procedure in the different states of Germany (2,3). The information about a cannabis offence (possession or use with or without context to driving) is forwarded from the police to the public affairs office which is responsible also for issuing or suspension of the driving licenses. In case of a valid license, after 6 months to 2 years a hair expertise is requested. In case of application for a new driving license or for extension of an old one, this is requested without delay. The expertise must be submitted within 1-3 months after request. Usually, the applicant appears as late as possible for hair sampling in the laboratory which performs the analysis and writes the expertise within two weeks. For the decision whether a result is positive or negative, the cut-off of 0.1 ng/mg Δ^9 - tetrahydrocannabinol (THC) in hair is applied. A negative result means that no or only experimental use is assumed but occasional use cannot completely be excluded. This is without further consequences to the driving license. A positive result proves, that the person had contact to cannabis products. The

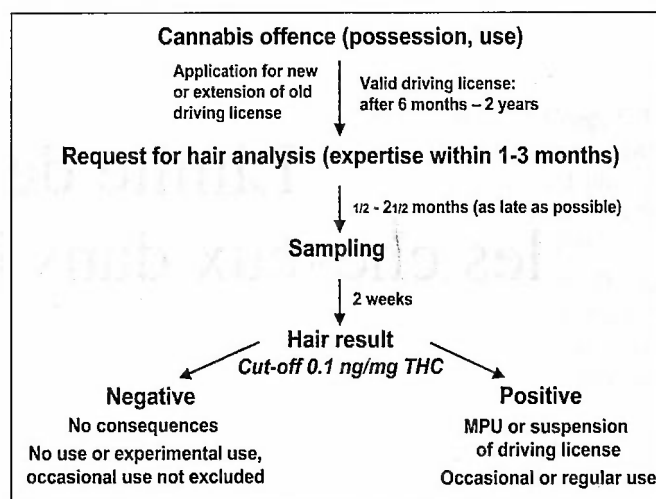


Figure 1.: Course of events in hair testing for THC in context of driving ability examination in Berlin.

concentrations correspond to occasional or even regular use. In this case an MPU is requested or the license is suspended if additional evidence proves regular use.

The cut-off value of 0.1 ng/mg THC is recommended by the Society of Hair Testing (SoHT) (4), by the Society of Toxicological and Forensic Chemistry (GTFCh) (5) as well as by the Société Française de Toxicologie Analytique (SFTA) (6). In this paper, the hair results of 625 driving ability cases investigated in the Institute of Legal Medicine of the Charité Berlin are compared with the statistics of the marijuana and hashish use in Germany in order to find out whether the cut-off of 0.1 ng/mg THC in hair is suitable for the described task.

Materials and methods

Hair samples

Hair samples were obtained from 625 test persons who were asked for a hair test by the authorities because of doubtful driving ability after a cannabis offence. 580 (93 %) were male and 45 (7 %) female. The age ranged from 17 to 59 years, mean 26 years, 63 % being between 18 and 27 years. Two scalp hair samples (one for analysis and the second in reserve for cases of objection) were collected from each person by cutting directly above the skin from the vertex posterior site. Both samples were separately stored in paper envelopments at room temperature until analysis. Samples longer than 6 cm were fixed by a string and only the proximal segment 0-6 cm was investigated. Samples shorter than 6 cm were analyzed in full length.

During the time of this study, the method for determination of cannabinoids from hair was changed. Therefore, both methods are briefly described.

1. Determination of THC by extractive derivatization with CH₃I

This procedure was adapted to a method described by Moeller et al. for serum (7,8). After washing with water and acetone and cutting to small pieces, 30-50 mg hair were digested in 1 ml 1 N NaOH + 20 ng D₃-THC (internal standard, 20 µl of a 1 µg/ml solution in methanol) for 20 min at 80 °C. The solution was twice extracted with n-hexane/ethyl acetate (9:1 v/v, 2 ml and 1 ml). After evaporation of the solvent at 40 °C in nitrogen stream, 200 µl dimethyl sulphoxide, 50 µl tetrabutylammonium hydroxide (20 % in H₂O) and 50 µl CH₃I were added to the residue. The mixture was vortexed and let react for 15 min. Then, 200 µl 0.1 N HCl and 1 ml i-octane were added and the mixture was shaken for 10 min. After that, the i-octane phase was separated and evaporated in the nitrogen stream. The residue was dissolved in 40 µl ethyl acetate and 2 µl were injected for GC-MS-SIM. The following m/z were measured: THC 313, 328 and 345, D₃-THC 316, 331, 248 (quantifier ions printed *italic*). The LOD and LOQ were 0.01 and 0.03 ng/mg respectively.

2. Determination of THC, CBD and CBN by headspace solid phase microextraction (HS-SPME) and GC-MS

The experimental details of this method which was developed during the study were described in a previous paper (9). In brief: The hair sample was washed with water and acetone and cut to pieces of 1-2 mm length. 1 ml 1 N NaOH and 10 ng D₃-THC (internal standard) in 10 µl methanol were added to 15-30 mg of the sample. After hydrolysis for 20 min at 80 °C, the analytes were isolated by extraction with 2 x 2 ml i-octane. The solvent was evaporated in a 10 ml headspace vial at 40 °C in a nitrogen stream. 10 µl bis(trimethylsilyl)trifluoroacetamine (BSTFA) were added to the residue for derivatization. The automatic HS-SPME was performed directly from this mixture for 20 min at 125 °C using a 100 µm PDMS fiber and a MultiPurposeSampler MPS 2 (Gerstel GmbH, Mühlheim, Germany). The desorption in the injection port of the GC occurred at 280 °C for 10 min. In the GC-MS-SIM measurement at a HP5-MS capillary column the following m/z were used: CBD 351, 390 and 458, THC 303, 371 and 386, D₃-THC 306, 374 and 389 and CBN 310, 367 and 382 (the quantifier ion printed *italic*). In the validation of the method the following LOD and LOQ were determined: THC 0.010 and 0.032 ng/mg, CBD 0.013 and 0.038 ng/mg and CBN 0.016 and 0.048 ng/mg.

In a comparison between both methods including 70 positive samples, a good conformity of the results was found.

Results and Discussion

In this paper only THC will be considered. CBD and CBN were detected in many of the cases and were used as a qualitative confirmation of the results. From the 625 samples 453 were negative (72 %, THC below LOD) whereas the THC concentrations of 172 samples were between > LOD and 7.72 ng/mg. The statistics is shown in Table I. According to the cut-off 0.1 ng/mg, 47 % of the unambiguously positive results were declared to be negative in the expertise's. Furthermore, the division of the positive samples in Table II into a lower range from LOD to percentile 25, a medium range from percentile 25 to percentile 75 and an upper range from percentile 75 to maximum shows that the complete lower range is below the cut-off and the median (0.11 ng/mg) is only slightly above the cut-off.

The hair length required for representation of at least 6 months and at maximum 12 months is 6 cm. However, due to the present short hair fashion for male, many of the test persons had hair shorter than 6 cm. Furthermore, it was suspected that in some cases the head was shaved after receiving the request for the test in order to remove positive hair from previous drug use and then let it grow until sampling. In order to use hair analysis still in such cases, it was agreed with the authorities to accept also hair samples shorter than 6 cm. In order to examine whether shorter hair samples have a higher proportion of negative results, the analytical outcome were statistically evaluated with respect

Table I : Distribution of THC concentrations in hair samples.

	No. of samples	Percentage
Total	625	100
Negative	453	72
Positive	172	28
THC, ng/mg	No. of samples	Percentage from pos. samples
0.020-0.050	44	25.5
0.051-0.099	37	21.4
0.10-0.50	57	33.1
0.51-1.00	24	14.9
> 1.00	9	5.1

Table II : Statistics of THC concentrations in 172 positive hair samples investigated driving ability examination.

Minimum	Positive (LOD 0.01 ng/mg)	↑	Lower range
Percentile 25	0.04 ng/mg		
Median	0.11 ng/mg	↑	Medium range
Percentile 75	0.32 ng/mg		
Maximum	7.72 ng/mg	↑	Upper range

to the sample length. As shown in Fig. 2, there is no preference of negative results in the shorter samples. The portion of the positive results is between 24 and 33 % without a noticeable correlation to sample length. Therefore, analysis of samples shorter than 6 cm is still efficient in driving ability investigation.

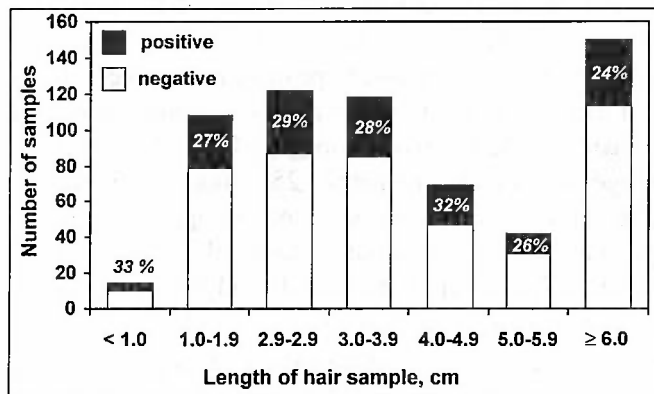


Figure 2 : Influence of hair sample lengths on the outcome of the hair analysis for THC in context driving ability examination in Berlin.

Besides nicotine and alcohol, cannabis is the most abused substance in Germany (10,11). 31 % of the juveniles (age 12-25) have experience with cannabis. 3.4 million persons used this drug within the last 12 months. 270,000 persons are habitual consumers, and 14,300 persons are in psychiatric treatment because of cannabis abuse. 10,762 confiscation cases were reported by the German police in 2004. The statistics of the frequency of cannabis use in West Germany in 2000 is shown in Fig. 3. This statistics should approximately also meet the situation in Berlin. About 47 % of the consumers used the drug only between one and five times and can be classified as experimental consumers. 42 % used the drug between 6 and 199 times corresponding to the typical range for occasional consumption. About 11 % had a consumption of more than 200 doses/year and that means regular (daily or almost daily) use.

All of the 625 applicants involved in the present study had a cannabis history and therefore, in a first approximation, should represent the statistics with respect to consumption frequency as it is given in Fig. 3. Therefore, in Fig. 4 the distribution of the hair concentrations of the present study is compared with the general frequency statistics of cannabis use. Although a strong inter-individual correlation between cumulative dose and hair concentration is generally not found, it is assumed in this comparison that, as a mean, very frequent use leads to the highest concentrations whereas experimental use results in the lowest (not detectable) values. It follows from this comparison that by the cut-off of 0.1 ng/mg only the regular and a relatively small

portion of the occasional consumers are detected whereas the overwhelming portion of the occasional consumers remains undiscovered. This was confirmed by several test persons who were surprised by the negative results. This situation could at least partially be improved by lowering the cut-off to 0.05 ng/mg.

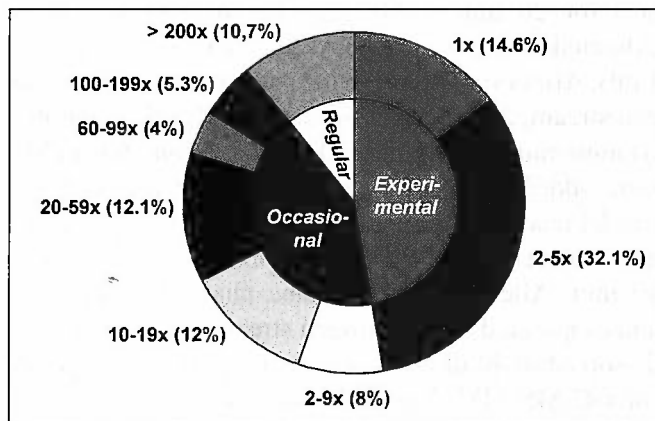


Figure 3 : Statistics of the frequency of cannabis use per year in West Germany in 2000 according to (11) and attribution to experimental, occasional and regular consumption.

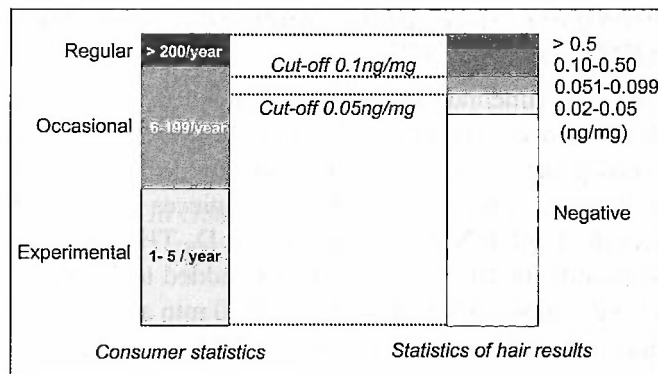


Figure 4 : Comparison of consumer statistics and THC results in hair.

Conclusion

In driving ability examination, regular and occasional cannabis use should lead to positive hair results. The cut-off value should eliminate single (experimental) use and analytically false positive results. Until now, there are no reliable (e. g. prospective) studies known for evaluation of the consumption frequency with respect to establishing a cut-off. As shown in this paper, it follows from statistical considerations of consumption behavior and hair concentrations that a considerable portion of occasional users is not detected with a cut-off of 0.1 ng/mg THC in hair. Therefore, it is proposed to decrease the cut-off to 0.05 ng/mg THC in hair. A prerequisite is that the method is sufficiently sensitive. The detection of CBD and CBN should be used for confirmation of the results.

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