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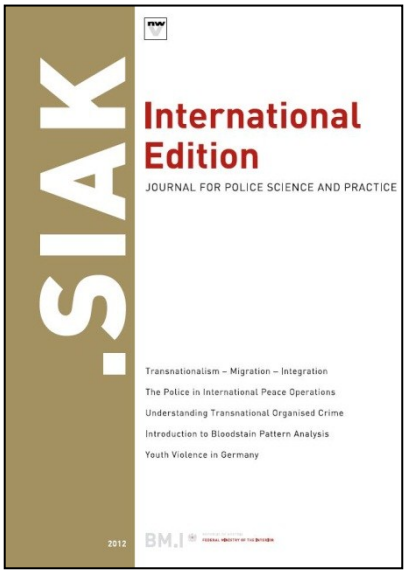

Sachs, Hans (2015):

Toxicological Hair Analysis

SIAK-Journal − Journal for Police Science and Practice (International Edition Vol. 5), 76-84.

doi: 10.7396/IE_2015_G

Please cite this articel as follows:

Sachs, Hans (2015). Toxicological Hair Analysis, SIAK-Journal − Journal for Police Science and Practice (International Edition Vol. 5), 76-84, Online: http://dx.doi.org/10.7396/IE_2015_G.

published online: 7/2015

Toxicological Hair Analysis

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The German legal system provides for the possibility of studying hair samples, in addition to urine samples, to check the abstinence of given offenders from drugs or alcohol as a condition of their probation. In addition, questions often arise during investigations about the consumption of drugs/alcohol in the history of a case, e.g. whether a suspect was under the influence of drugs at a given time in the past. It can also be important for investigators to know which substance or combination of substances were consumed during a certain period of time. After a while, such information can no longer be obtained from urine or blood samples, since the substances are metabolised in the body and the metabolites are excreted. Hair, by contrast, incorporates substances during growth and stores these for a relatively lengthy period. Special methods of analytical chemistry (mass spectrometry techniques) can detect minuscule quantities of alcohol/drugs or their breakdown products in hair samples, thereby enabling patterns of past alcohol or drug consumption to be identified by taking the rate of the growth of the hair into account. Details of the applications, methods and current limits of hair analysis are presented in this paper.

1. INTRODUCTION AND BACKGROUND

Current hair analysis draws largely on the period following the introduction of liquid chromatography in combination with tandem mass spectrometry (LC-MS-MS), which led to a dramatic rise after 2000 in the possibilities of hair analysis, extending to comprehensive screening and also, owing to massively improved sensitivity, the detection of a single consumption of certain substances affecting the central nervous system after weeks or months.

However, it was a long way to get there. The era of drug detection in hair using radioimmunological tests for morphine began in the early 1980s. Owing to non-specific reactions in immunochemical tests, hair analysis first really gained momentum in the second half of the 1980s, once individual substances could be specifically detected using gas chromatography with a mass spectrometer (GC-MS). For a long time attempts were simply made to apply proven blood and urine screening techniques to hair, by dissolving hairs as far as possible and then testing them as body fluids. Hydrolysis was also involved. It was precisely because the samples were treated in such a way that it was recognised only fairly late (around 1990) that for common drugs such as heroin, cocaine and cannabis, the parent compounds (or specific metabolites such as 6-acetylmorphine in the

case of heroin) were far easier to detect than the usual (hydrophilic) metabolites in blood and urine. However, excitement at the advances in sensitivity and therefore the increased ability to detect consumption did not last long. Detection of the parent compounds gave rise to an ongoing discussion about possible contamination of the hair with the detected drugs, with the intensity of the debate varying according to the legal situation in the various countries. What is known as "general unknown screening" was unsuccessful owing to low sensitivity using GC-MS.

The 1990s saw the development of new methods of detection for individual substances or relatively small groups of substances using GC-MS with selected ion monitoring. A real race was on, as manifested in academic publications by the sentence "To our knowledge this is the first time that substance XX could be detected in hair". That competition lead to curious phenomena such as substances being detected for the first time on more than one occasion, and even clean background levels being published as evidence of a positive finding.

Generally it takes one to two weeks for the substances circulating in the blood to appear in the hair sufficiently far beyond the scalp to be present in the hair sample taken. Scalp hairs grow fairly uniformly over a period of three to six years (anagen phase), but then undergo a stop of the growth phase of three to six months (telogen phase) before falling out. Following prolonged substance consumption, strands of hair still contain 10 to 20 % positive hairs that have stopped growing. Totally negative results only come back after around six months, depending on the sensitivity of the screening.

In most cases, scalp hair grows at a rate of between 0.8 and 1.4 cm per month. Lower growth rates can occur in elderly people or people with hair growth disorders. There are, however, also cases when a growth rate of more than 2.0 cm per month is observed. For the purposes of simplification and to gain a rough estimate of the period of consumption, a rate of 1.0 or 1.1 cm/month is typically assumed. If the growth rate is of particular importance in a case, it can be determined most easily by taking another sample from the same place weeks after the first sample was taken and measuring the hair length.

The irregularity of hair growth, however, also provides for the possibility of detecting regular substance consumption that lies further back than would be possible based on calculation merely according to hair length. Such calculations show to what extent considerable concentrations can still be detected even after the start of abstinence (see Figure 1, page 78).

Despite the complexity of hair growth, it is usually possible to gain an impression of the timeline of consumption using segmental hair analysis.

Hair colour and hair strength, as well as the condition of the hair structure after months of cosmetic treatment and environmental influences (sunshine etc.) can lead to different concentrations. Aggressive bleaching in particular leads to destruction of the cuticle (the outer protective layer of the hair), which means foreign substances are washed out more easily.

Body hair can also be taken as a sample instead of or in addition to scalp hair. Pubic hair, underarm hair and other body hair grows more slowly than scalp hair and has an anagen phase of approx. 44 to 77 weeks (40 to 60 % of the hair) and a long telogen phase of approx. 48 to 73 weeks. A body hair sample represents the period of the total growth cycle (which can be over two years). However, it is very important to check the sample for natural tips (to exclude shaving). Body hair can be used as a

Source: modified from Sachs 1995 and Pragst 2004

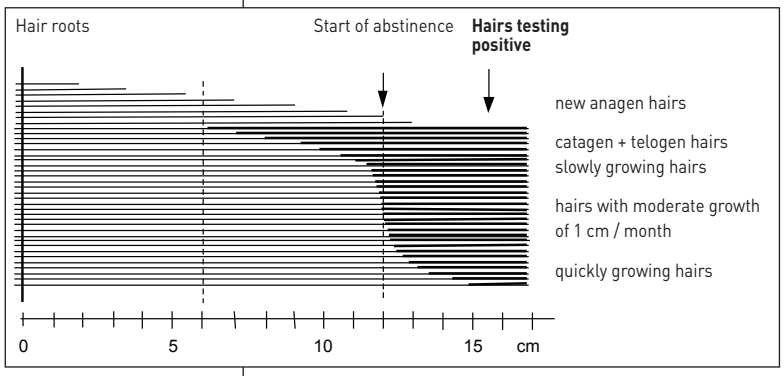

Figure 1: Following intensive substance consumption, the regrowing segment of scalp hair (left) is only substance-free after several months

substitute for head hair to check for consumption of a substance or abstinence, for example ahead of a medical-psychological assessment. However, owing to the long telogen phase, it does not allow for an estimate of consumption patterns over time using segmental analysis in the same way as scalp hair does. In any case, it is generally not possible to take a sample of body hair in a sufficiently orderly way for such purposes.

Detection of foreign substances in hair consists of various steps (decontamination, hair digestion/extraction, clean-up and then analysis). When interpreting the results, it should be noted that different techniques can produce different findings. Direct comparison of quantitative results from different measurement techniques is therefore problematic.

2. APPLICATION OF HAIR ANALYSIS IN VARIOUS COUNTRIES AND HOW IT DIFFERS FROM URINE ANALYSIS

Hair analysis studies a completely different period of time compared to urine analysis. Ideally a hair sample looks like in Figure 2. It enables consumption over several months to be studied retrospectively. A urine test, by contrast, examines only a relatively short period rarely exceeding 48 hours. Meaningful checks using urine samples are therefore only possible if the person being tested is taken by surprise, i.e. the sample is taken on the same day as the summons or on the following day. However, since that requires considerable organisation, in countries where abstinence checks are carried out on a large scale, i.e. to assess fitness to drive or as a condition of probation, either a urine sample program or retrospective hair screening is performed. An example is the programme established in Germany as part of the assessment criteria for fitness to drive (Schubert/Dittmann 2013). In Germany an estimated 20,000 and 50,000 hair samples are taken each year to test for abstinence from drugs and alcohol respectively as part of the programme.

The procedure is precisely regulated in the assessment criteria, which cover the accreditation of laboratories, as well as sample collection and permitted analysis techniques. As a rule, each of the substances

Source: Case material of the Forensic Toxicological Center in Munich

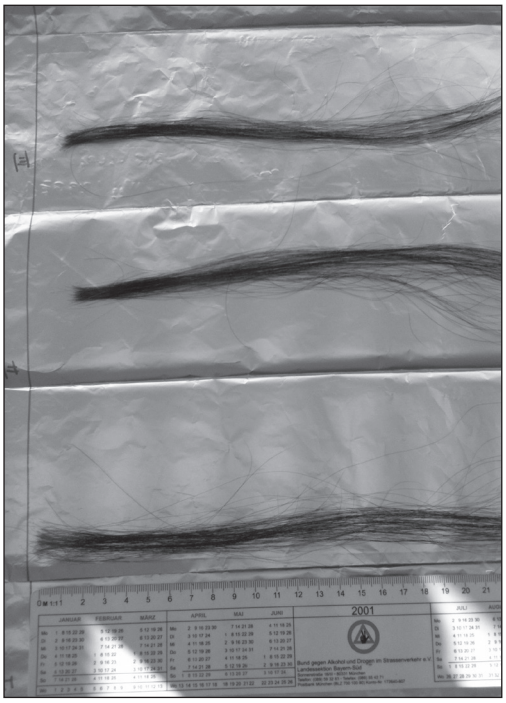

Figure 2: Hair sample taken to check abstinence from drugs and alcohol

separately listed in the assessment criteria is screened for. The cut-offs are very low, requiring laboratories to perform highly sensitive assays, which is confirmed during accreditation. Generally between 15 and 20 % of the hair samples come back positive in the abstinence check. The rate is significantly lower when six urine samples are taken over a period of twelve months, even if those concerned receive an irregular and unpredictable telephone summons to provide a urine sample on the following day.

The situation in Switzerland regarding fitness to drive is similar, and is to be further standardised by the middle of 2015. A significant difference compared to Germany is that checks for cannabis consumption are only possible using urine samples.

Source: Schubert/Dittmann 2013

Substance class Target analyte	**Cut-off in hair** [ng/mg]
Cannabinoids THC-COOH THC	 0.02
Opiates Morphine (codeine, dihydrocodeine and MAM in hair)	 0.1
Cocaine Benzoylecgonine Cocaine	 0.1
Amphetamines Amphetamine and designer amphetamines	 0.1
Methadone EDDP Methadone	 0.1
Benzodiazepine Diazepam Nordazepam Oxazepam Alprazolam (OH-Alprazolam) Bromazepam Flunitrazepam (7-amino-flunitrazepam) Lorazepam	 0.05 0.05 0.05 0.05 0.05 0.05 0.05
Ethyl glucuronide	0.007 (7 pg/mg)

Table 1: Polytoxicological screening substances in abstinence checks to assess fitness to drive in Germany

There is some justification for that, since in both countries screening is usually performed for the principal psychoactive constituent of cannabis, THC, rather than for the metabolite tetrahydrocanabinol carboxylic acid (THC-COOH), though ultimately consumption can only be proven by positive detection of the latter. Aside from that, hair analysis is used just as frequently in Switzerland as in Germany in criminal cases, such as child abuse cases in which a child is suspected of having been given sleep aids.

In Great Britain hair analysis is used above all in child custody cases. The question is always whether children are allowed to stay with parents who are suspected of having a drug or alcohol addiction.

In the USA hair analysis has centered around workplace checks from the start. Thousands of hair analyses are performed daily for that purpose. The legal possibilities of large companies to perform such checks when hiring are completely different from in Europe, which again shows that the use of hair analysis largely depends on the given legal situation.

3. CURRENT STATUS OF DETECTION OF ALCOHOL CONSUMPTION

After ingestion, alcohol is converted into the minor metabolites fatty acid ethyl esters (FAEEs) and ethyl glucuronide (EtG) (see Figure 3, page 80). These metabolites circulate in the blood and are also incorporated in growing hair. That means they can serve as long-term markers of alcohol consumption.

3.1 Ethyl glucuronide

Only testing for ethyl glucuronide (EtG) is permitted in abstinence controls in Germany to assess fitness to drive, and the whole procedure is specified in the chemical toxi-

Source: Pragst 2006

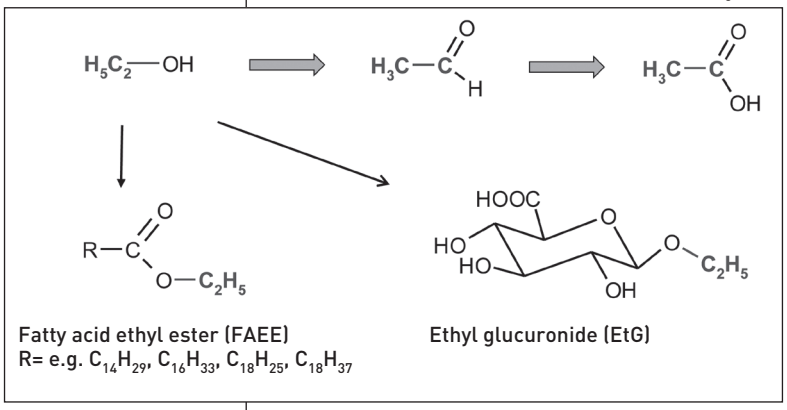

Figure 3: Fatty acid ethyl ester and ethyl glucuronide as minor metabolites of alcohol

cological testing criteria of the assessment criteria (see above). However, in order to judge intensity of consumption, sometimes a test for FAEE is also performed, especially when it is necessary to distinguish between excessive and moderate consumption, for example, when assessing criminal responsibility.

When performing retrospective abstinence screening by testing hair for EtG, a month can be assumed for each centimetre of hair length, up to a maximum of 3 cm. Testing for EtG as proof of abstinence according to the assessment criteria is not suitable for a longer period. That rule is disputed. While routine, segmental tests repeatedly show that concentrations following allegedly regular consumption are higher in the 0 to 3 cm segments near the roots than in the next 3 to 6 cm, with a further decrease toward the tips of the hair, corroborating the rule, other researchers (Ägius/Ferreira et al. 2012) cannot observe any significant differences in segments up to 6 cm long.

A concentration of less than 7 pg/mg counts as proof of abstinence. There are no false positive results above that. Our experiences to date suggest that the cut-off of 7 pg/mg for EtG is very high, i.e. it benefits those being screened. Genuine abstinence is closer to 1 to 3 pg/mg.

3.2 Fatty acid ethyl ester

Since FAEEs are present in the blood, they can be transported to the hair (Pragst/Yegles 2006). According to current knowledge, that takes place primarily via the sebaceous glands and the sebum that is excreted into the hair roots.

FAEEs are detected using headspace solid-phase micro extraction in combination with GC-MS. Although a large number of fatty acids are known and could therefore be selected, only four FAEEs are used for the quantitative analysis and assessment of alcohol consumption:

- Ethyl myristate,
- Ethyl palmitate,
- Ethyl oleate,
- Ethyl stearate.

The detection limits for these esters are, at less than 0.1 ng/mg, within the range of the FAEEs produced naturally by the body, i.e. in a range that is also present in the case of abstinence from alcohol. Generally, the proximal 3 and 6 cm segments are tested, since the statistical findings apply to those.

The assessment criteria were established by studying the hair samples of a large number of subjects with known drinking behaviour (teetotalers, moderate drinkers, alcoholics undergoing withdrawal treatment and fatalities involving known alcohol abuse). Accordingly, in line with the recommendations of the Society of Hair Testing (SoHT), the values set out in Table 2 (see page 81) are applied to hair in the 0 to 3 cm segment from the scalp.

4. CURRENT STATUS OF TESTING FOR DRUGS AND MEDICATION

While GC-MS (-MS) is also used to test hair for alcohol markers with high sensitivity, LC-MS-MS tends to be used for detecting drugs. The LC-MS technique benefits from the fact that the vast majority of drugs and medication affecting the

central nervous system contain nitrogen in amine or amide groups, which are readily ionised during electrospray ionisation. An overview of the possibilities can be found in the books of Kintz (Kintz 2006, 187–200) and Madea/Musshoff (Madea/Musshoff 2004). The techniques used, however, have progressed since. The examples below will be used to illustrate how further developments since then can also affect forensic toxicological assessments.

4.1 Screening using multi-target analyses

In the case of a multi-target analysis, transitions of individual specific fragments that have previously been determined by an optimisation programme after injection of the pure substance are scanned in a targeted way during an LC-MS-MS analysis. One of the advantages is that the transitions are so specific that a large number of substances can be detected in a single analysis with high sensitivity. Since the analytes do not need to be fully separated, four to six analyses per hour can be performed. In Munich, in the case of serious crimes, especially where drugs are involved, blood, urine and hair samples are taken as a rule. The blood sample is taken to assess whether the person was under the influence and, together with the urine sample, to assess recent consumption, while the hair sample is used to assess regular consumption and a possible addiction.

It is not uncommon for hair analysis to provide an overall picture of drug and medication consumption, as indicated by Table 3 (see page 82). It shows the test results of an evidently polytoxicomanic offender, who took a total of 18 different psychoactive substances, in some cases in considerable quantities, during the period of growth of the 6 cm hair segments tested.

During these studies it was also found that only a minority of 25 % consumed just one type of drug. Consumption of multiple drugs, which can only be detected by hair analysis, because consumption does not need to be close in time to sample collection, is the rule. Two percent of this selected group took more than seven substances.

Source: Society of Hair Testing

Drinking behaviour	FAEE (ng/mg)	EtG (pg/mg)
Teetotalers	< 0,20 ng/mg	< 7 pg/mg
Moderate drinkers	< 0,50 ng/mg	< 30 pg/mg
Alcohol abuse	≥ 0,50 ng/mg (> 1,0 ng/mg for 0-6 cm)	≥ 30 pg/mg

Table 2: Cut-offs for FAEEs and EtG in hair

Such screening is also used when there is suspicion of drugging being used to sedate a victim for whatever reason. It is naturally important in such cases that even a single ingestion/instance of drugging can be detected. That is certainly not possible for all drugs or medication. However, the chromatogram of a hair sample that was taken four weeks after an operation during which fentanyl was given, following pre-operative administration of 10 mg of diazepam the previous night, shows that there are grounds for optimism (see Figure 4, page 82). Technology has further improved since that study, and sensitivity has increased five-fold to ten-fold for some substances, so there is definitely hope of soon being able to detect a single consumption, and, vice-versa, the possibility of being able to prove abstinence.

4.2 Confirmation of repeated drugging using single hair analysis

Even if there is a positive finding in a strand of hair, the exact number of times that the drug was consumed/given and the precise date cannot be established. That is a failing that often leads to the case being dropped in the absence of sufficient other indications or evidence. However, there is a solution in certain cases.

Source: Case material of the Forensic Toxicological Center in Munich

	Segment A [ng/mg]	Segment B [ng/mg]
Cocaine	1,4	3,5
Benzoylecgonine	0,13	0,51
Norcocaine	Trace (<0,005)	0,01
Diacetylmorphine (heroin)	0,26	0,45
6-acetylmorphine (MAM)	3,3	9,3
Morphine	0,82	1,0
Codeine	0,76	0,71
Hydrocodone	0,017	0,009
Hydromorphone	0,018	0,007
Tilidine	0,20	0,63
Nortilidine	0,18	0,66
Tramadol	6,1	12,2
Nortramadol	1,4	4,6
Diazepam	0,036	0,14
Nordazepam	0,11	0,25
Oxazepam	0,013	0,024
Lorazepam	0,019	0,019
Amphetamine	0,58	1,1
Methylendioxymethyl-amphetamine (MDMA)	Trace (<0,005)	0,10
Tetrahydrocannabinol (THC)	0,03	0,10
Doxylamine	0,13	0,041
Carbamazepine	0,12	0,86
Citalopram	0,27	0,59
Doxepin	0,33	0,64
Mirtazapin	Trace (<0,005)	0,029
Amitriptylin	not detectible	0,006
Nortriptylin	0,015	0,038
Risperidon	0,006	0,018
Clozapin	not detectible	0,010

Table 3: Hair sample of a polytoxicomanic subject who came to the attention of investigators because of a crime

Source: Case material of the Forensic Toxicological Center in Munich

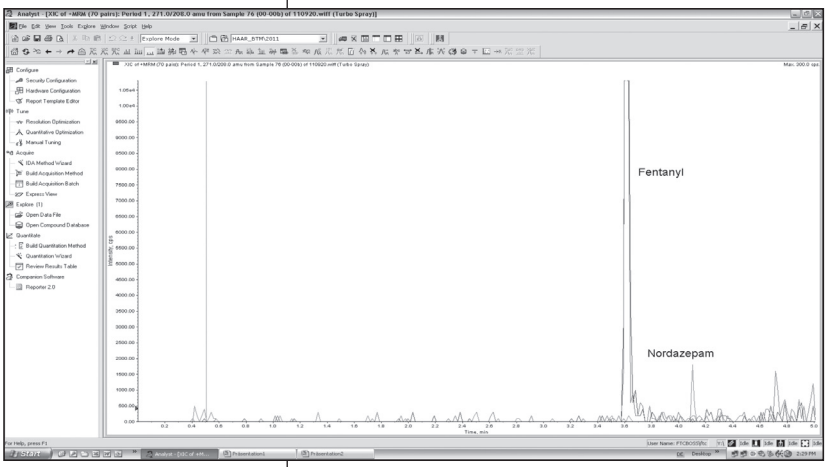

Figure 4: Chromatogram of a hair test following pre-operative use of diazepam and operative use of fentanyl

In a case of sexual abuse of a child by the father, traces of the sleep aid doxylamine were found in the hair sample of the child corresponding roughly to the period of the suspected incident. During the trial the father claimed that the child had once accidentally ingested a fluid containing doxylamine. The court then asked whether it could be proven that the child had ingested doxylamine more than once. Only the segmental analysis of a single hair could help. In fact several peaks in 5 mm long segments could be observed at intervals of several weeks that were not consistent with a single ingestion (see Figure 5, page 83).

4.3 Screening using high-resolution mass spectrometry

In the cases referred to above, a multi-target analysis was sufficient. It helped that the reference substances were at least available to toxicologists. The situation has changed dramatically in recent years with the emergence of synthetic cannabinoids and other designer drugs ("bath salts" and "research chemicals"). These substance classes are expanding daily and spread rapidly via the internet. A detection method was required that is highly sensitive and allows for identification even without the reference material. A solution was found in the form of LC-MS-TOF (liquid chromatography/time-of-flight mass spectrometry). For example, the synthetic cannabinoid JWH-200, which at that time was not available as a reference material, could be positively detected in a hair sample. The triple TOF method is not as sensitive as the latest-generation tandem MS instruments, but the fact that synthetic cannabinoids and most "bath salts" are readily ionised means that the procedure is promising in cases where experienced, previous drug users need to be tested.

Source: Case material of the Forensic Toxicological Center in Munich

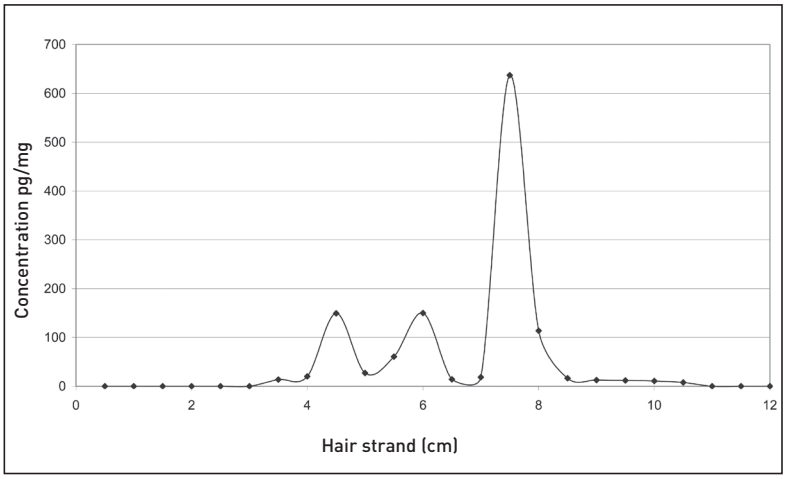

Figure 5: Doxylamine in the single hair of a child following sexual abuse by the father

CONCLUSION

Following targeted screening for individual substances in hair strands in the early period of hair analysis, the great efforts of working groups in Europe especially have led to the development of techniques for comprehensive and highly sensitive substance screening. In parallel with that, methods have been developed that enable the detection of selected analytes using the segmental study of single hairs, thereby allowing a more precise picture of consumption to be obtained. The aim is to be able to perform a general unknown screening on a single hair to detect a single ingestion of every substance in a therapeutic dose or the ingestion of a single unit of consumption.

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