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Original Article

Hair Analysis as a Diagnostic and Forensic Tool in a Social Support System for Families with Underage Children and Drug Abusing Parents: Four Year Experience

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Abstract

Between 2011 and 2014, 388 hair samples from children (age 1-14 years) and 594 hair samples from parents/caregivers were analyzed for methadone, opiates, cocaine, amphetamines, ecstasy, cannabinoids and benzodiazepines by LC-MS/MS and GC-MS. Hair testing was preferentially performed for parents/caregivers. Children were included in case of positive parents/caregivers results or in urgent cases after a court decision if the parents/caregivers declined the testing. A follow-up hair test was performed after 6-months (positive results) or 12-months (negative results).

Results: The children hair appeared to be a sensitive indicator of the handling of drugs in their environment and often showed a similar or the same drug profile as their parents. The annual statistics for the initial test of children were no drugs 22-33%, methadone 9-25%, heroin 7.5-28%, cocaine 46-57%, amphetamines and ecstasy 1.6-7.0%, cannabinoids 21-49% and benzodiazepines 0-5%. As a rule, concentrations in hair of younger children were higher than of their elder siblings. In severe cases and based on the entire information about the case, the children were immediately taken into custody, the family court was appealed, the children were admitted to children's homes or foster families, or the drug consuming adult was separated from the family and a withdrawal treatment was initiated. Follow-up tests showed an improvement in the situation of the children particularly in severe cases.

Conclusion: Hair analysis proved to be a very efficient working instrument for social authorities in the systematic improvement of child-welfare in drug abusing environments.

Key words: Child welfare, Drug abuse, Hair analysis, Social services, Methadone maintenance

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تحليل الشعر كأداة تشخيصية ودليل جنائي في نظام الرعاية الاجتماعية للأسر التي لديها أطفال دون السن القانونية وأولياء أمور متعاطين للعقاقير والمخدرات، عرض خبرة أربع سنوات

المستخلص

لقد تم تحليل 388 عينة شعر لأطفال تتراوح أعمارهم بين 1-14 عاماً، و 594 عينة شعر من الوالدين أو مقدمي الرعاية للأطفال وذلك خلال الفترة من 2011 - 2014. وقد تم فحص هذه العينات للكشف عن الميثادون والأفيونات والكوكايين والأمفيتامينات والاكستازي، والقنبيات والبنزوديازبينات باستخدام تقنيتي LC-MS/MS و

GC-MS. وتم إجراء اختبار الشعر التفضيلي للآباء والأمهات أو مقدمي الرعاية للأطفال. ولقد تم إدارج الأطفال في الاختبار في حالة كون نتائج اختبار أولياء الأمور أو مقدمي الرعاية إيجابية، أو في الحالات العاجلة بعد قرار من المحكمة عند رفض الاختبار من قبل أولياء الأمور أو مقدمي الرعاية . كما تم متابعة الحالات بإجراء اختبار مراقبة للشعر بعد ستة أشهر للحالات ذات النتائج الإيجابية أو اثني عشر شهرا للحالات ذات النتائج السلبية.

النتائج: إن عينات شعر الأطفال تبدي إمكانية بكونها مؤشر حساس عن البيئة المحيطة بهم والتي تتعامل مع العقاقير والمخدرات. وفي أغلب الحالات أظهرت هذه العينات نتائج مماثلة لفحوصات العقاقير والمخدرات مع نتائج الوالدين أو مقدمي الرعاية. ومن الإحصائيات السنوية التي تناولت الاختبارات الأولية للأطفال وجد عدم وجود مخدرات في 22-33% من الحالات، الميثادون 9-25%، والهيروين 7.5-28%، والكوكايين 46-57%، والأمفيتامينات والاكستازي 1.6-7.0%، والقنبيات 21-49%، والبنزوديازيبينات 0-5%. وكقاعدة عامة، كانت التراكيز في عينات شعر الأطفال الأصغر سناً أعلى مقارنة بعينات الأشقاء الأكبر سناً. في الحالات الخطيرة، واستناداً إلى المعلومات الكاملة حول مثل هذه القضايا، تم تحويل الأطفال على الفور إلى الوصاية، وتم اتخاذ قرار من قبل محكمة الأسرة بقبول الأطفال في دور رعاية الأطفال أو لدى أسرة كفيلة، أو اتخاذ قرار بفصل أو استبعاد فرد الأسرة الكبير والمتعاطي للعقاقير أو المخدرات، وكذلك البدء عند الطفل ببرنامج علاج الانسحاب، وأظهرت اختبارات المتابعة تحسن أوضاع الأطفال وخاصة في الحالات الشديدة.

الخلاصة: لقد أثبت تحليل الشعر أنه أداة عملية وفعالة جداً للسلطات الاجتماعية في التحسين المنهجي لرعاية الأطفال في بيئات إساءة استخدام العقاقير والمخدرات.

الكلمات المفتاحية: رعاية الأطفال، تعاطي المخدرات، تحليل الشعر، الخدمات الاجتماعية، المعالجة البديلة بالميثادون.

Introduction

Children of drug addicted parents are in permanent danger of poisoning as has been shown by several fatal cases involving methadone and other drugs [1-8]. However, the social and educational aspects are even more severe. The children are threatened by neglect, missed education, malnutrition, maltreatment, poverty and social discrimination. Besides the physical endangerment, parental drug abuse is also a serious developmental risk for the emotional and psychosocial adaptation of the child. According to estimations, this concerns between 30,000 and 60,000 children in Germany [9]. Families with illegal drug addicted parents often live on the verge of society. Life is often dominated by drug related crime and prostitution. In their family setting, children experience material distress, bad housing, illness, unemployment, conflicts and violence. The children are in a permanent atmosphere of unsteadiness and uncertainty, caused by frequent and drastic drug related mood changes of the parents.

Social help and legal decisions in favor of the children require information about the kind and extent of the parental drug abuse and about the exposure of the children to drugs. Since this information is often concealed, downplayed or repressed by the parents, objective test results are required. Hair analysis proved to be particularly suitable for this purpose because of the long time window of drug detection ranging from several months to years [10,11]. Therefore, this technique was chosen as the essential diagnostic tool in a widespread system of regulations which were implemented in the German Hanseatic city of Bremen (550,000 inhabitants) in 2011. Besides hair samples from parents, children's hair was also tested. The first results of this project were presented in a previous publication [12]. In the present paper, the findings after four years of this project are reported.

2. Materials and Methods

2.1. Subjects and Hair Samples

Between March 2011 and December 2014, 388 hair samples from children (age 1-14 years), 22 hair samples from adolescents (age 15-17 years), and 572 hair samples from parents/caregivers (age 18-67 years) were analyzed upon the order of the Offices of Social Services at the Senate of the Hanseatic City of Bremen. These numbers include initial tests and control tests. The samples were collected from the vertex posterior region using the hair collection kit of the Institute of Legal Medicine of the University Hospital, Charité, Berlin, by cutting as close as possible above the skin. For longer hair, the proximal segment upto 6 cm was analyzed. Shorter hair was investigated in full length.

This study was conducted according to the Helsinki ethical principles for medical research involving human subjects of the World Medical Association. Hair sampling and analysis were performed either on the basis of a written informed consent or on decision of the local family court. In

the case of children, the informed consent was signed by the parents or other caregivers. The samples were anonymized, and only the age of the individual was recognizable from the code. For about 30 families, the connections between children and adults were disclosed if the family court or the social workers needed extended interpretation concerning the drug distribution between the family members.

2.2. Analysis of hair samples

The hair samples were analyzed for 31 drugs and metabolites [6-acetylmorphine (6-AM), 7-aminoflunitrazepam, acetylcodeine, alprazolam, amphetamine, anhydroecgonine methyl ester, benzoylecgonine (BE), bromazepam, cannabidiol (CBD), cannabinol (CBN), cocaethylene, cocaine, codeine, diazepam, dihydrocodeine, EDDP, flunitrazepam, heroin, α-hydroxyalprazolam, lorazepam, methylenedioxyamphetamine (MDA), methylenedioxyethamphetamine (MDEA), methylenedioxymethamphetamine (MDMA), methadone, methamphetamine, ecgonine methyl ester, morphine, norcocaine, nordiazepam, oxazepam and Δ9-tetrahydrocannabinol (Δ9-THC)]. The methods were fully validated according to international guidelines [13,14] and were described in detail in previous papers [15,16].

For basic drugs, benzodiazepines and metabolites the hair was washed once in water and twice in acetone. After drying, it was cut into 1–2 mm pieces and 20 mg were twice incubated for 18 hours with each 0.5 mL of a mixture of methanol/acetonitrile/2 mM of ammonium formate (25:25:50, v/v/v) under gentle shaking at 37°C. A mixture of 23 deuterated drugs or metabolites (each 5 ng) was used as internal standards. Both extracts were united and evaporated in a nitrogen stream to a residue of 0.5 mL. Five microliters of the residues were injected for analysis by LC-QTOF-MS or LC-MS/MS without further cleanup. The limits of detection (LOD) were between 0.001 and 0.005ng/mg and the limits of quantification (LOQ) between 0.003 and 0.01 ng/mg [15].

The cannabinoids Δ9-tetrahydrocannabinol (Δ9-THC), cannabinol (CBN), and cannabidiol (CBD) were determined in hair by derivative headspace solid phase microextraction (HS-SPME) and GC-MS with N,O-bis(trimethylsilyl) trifluoroacetamide (BSTFA) as a derivatization agent [16]. Between 15 and 30 mg of washed hair pieces were digested with 1 mL of 1N NaOH each containing 10 ng d3-THC, d3-CBN, and d3-CBD as internal standards for 20 minutes at 80°C. The solution was twice extracted with 2 mL iso-octane, the solvent evaporated, 10 μl BSTFA added, and the residue submitted to HS-SPME-GC-MS in selected ion monitoring mode. The LOD and LOQ were: THC 0.003 and 0.01 ng/mg, CBN 0.004 and 0.01 ng/mg, and CBD 0.004 and 0.1 ng/mg.

3. Results and Discussion

3.1. Structure of the social support system and the role of hair analysis

The social support system for families with drug addicted parents and underage children aims to protect children against the abovementioned endangerments of parental drug abuse. It is based on the German criminal code, the German social legislation and on a written agreement between parents and the local social welfare department for granting the child welfare. The local family court is called in case of insufficient cooperation of the parents. Confidentiality must be maintained, but the prevention of danger to the child outweighs medical secrecy in cases of emergency.

The structure of the case management in this support system is shown in Fig. 1. It consists of a widespread network of social and medical institutions and services under the coordination of the social welfare office of the federal state government and also includes the family court. New cases with young children become known from maintenance treatment or in case of admitted or suspected drug use. After determination of the initial status, the hair of the parents is investigated. If drugs are detected, the hair test of the children is performed in order to get an impression about the extent of drug exposure. Depending on all available information, including the hair results, measures are taken in severe cases such as the immediate removal of the child from the family, appeal of the family court, admission into children's homes or by foster families, separation of the drug consuming father or mother from the family, or initiation of withdrawal treatment.

In less severe cases, the children remain in the family, which can recieve different kinds of support. A prerequisite is to achieve drug abstinence. Follow-up hair tests of parents and children are scheduled after 6 months in case of positive results and after 12 months in case of negative results.

It must be emphasized that the measures mentioned above are not solely based on the hair results but are

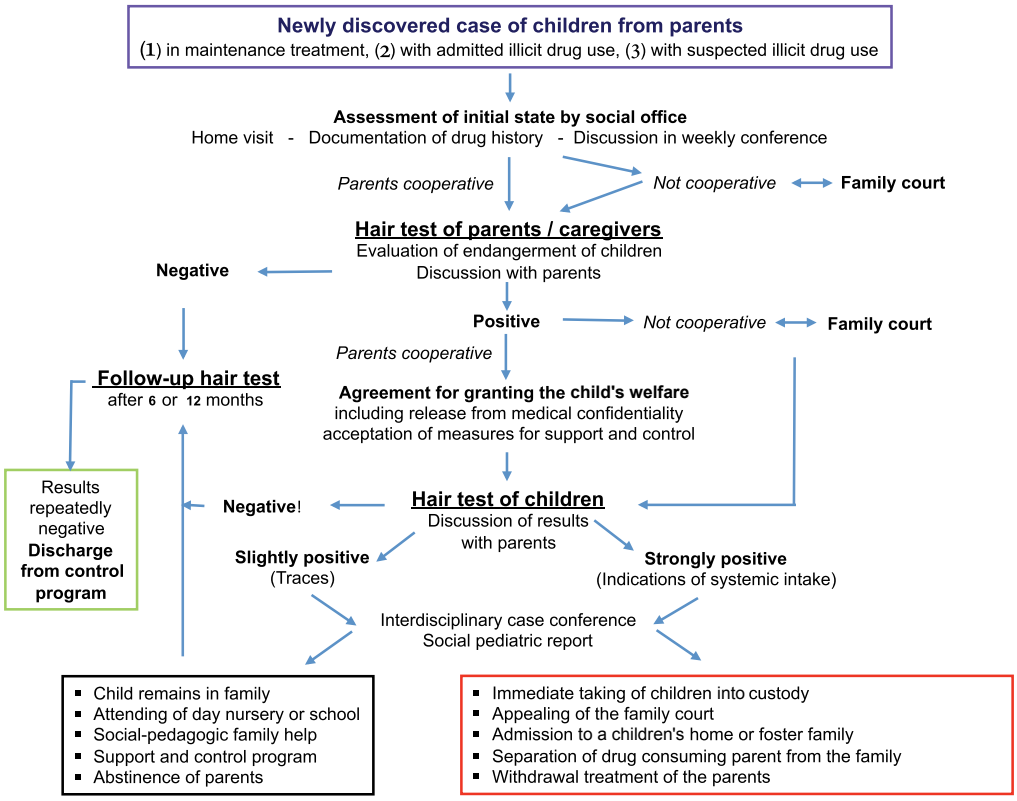

Figure 1- *Case management in the social support system for families with underage children and drug abusing parents.*

a consequence of all information available in each case. Despite tremendous progress in performance and interpretation of hair analysis for drugs in recent years, there remains a large biological variability of the concentrations, and false negative or false positive results cannot fully be excluded.

3.2. Children and parents/caregivers included in this study

This is an ongoing project that started in March 2011. This paper includes 982 hair samples which were collected until the end of 2014. The number of tested children and parents or caregivers who were tested between one and six times is given in Table 1. A small number of adolescents (age 15 to 17 years) were included in the group of adults since at this age, active drug exposure must be assumed.

It can be seen that until now the majority of the children and adults (80.7% and 79.4%) were tested only once and one to five follow-up tests were only performed for the remaining 19.7 and 20.6%. However, as shown in Fig. 2, there is a steady increase of the portion of follow-up tests from 2011 to 2014 (from 2.4% to 38.7% for children and from 0% to 35.8% for adults). The high portion of new cases, even in the fourth year of the project, can be explained by newly discovered families with drug problems, the birth of children in such families or changes of residence. Generally, there is a large residential fluctuation in this clientele. For these reasons, and since social support and hair testing require a high degree of cooperation from the parents, a consequent realization of the project including all follow-up tests cannot be expected.

Fig. 3 shows the distribution of age among the children and adults included in the study. Children were investigated only above the age of one since it is difficult in cases of younger children to distinguish between in-utero incorporation into hair and post-natal incorporation. There is a steady decrease of the sample number with increasing age, and the majority of samples were collected from toddlers and preschool children. For the adult group, most samples were from individuals between 20 and 40

Table 1- *Tested children and parents or caregivers and number of tests between March 2011 and December 2014*

Frequency of testing	Number of children	Number of parents / caregivers
1 x	239	358
2 x	34	55
3 x	13	28
4 x	8	9
5 x	2	0
6 x	0	1
Total number of individuals	296	451
Total number of hair samples	388	594

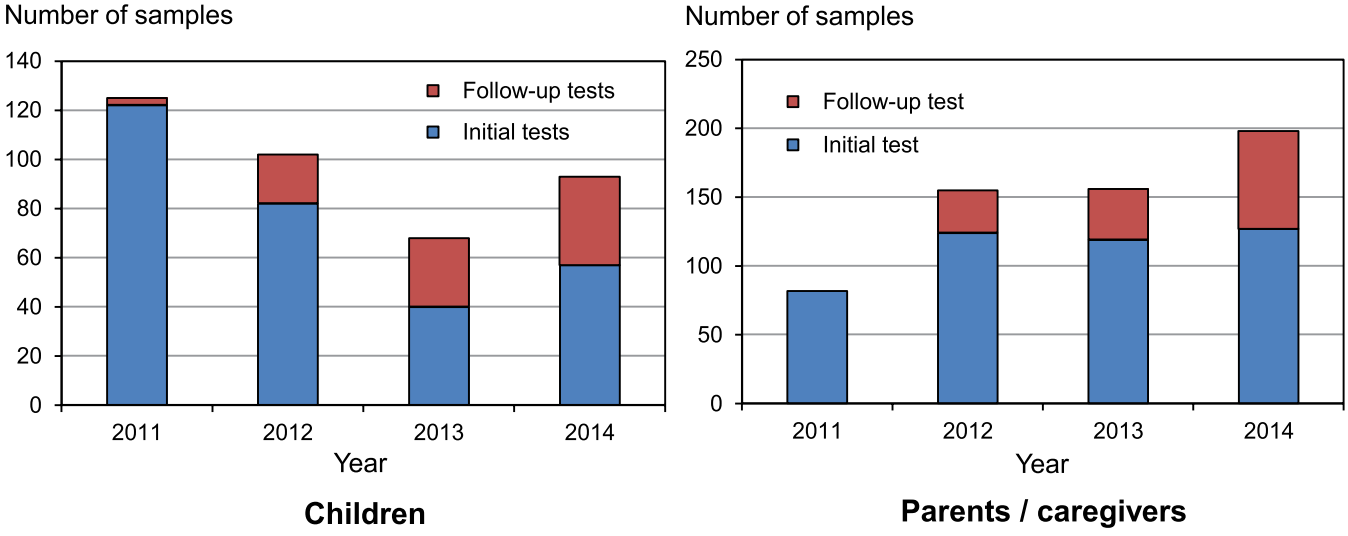

Figure 2- *Numbers of initial and follow-up hair tests of children and parents/caregivers between 2011 and 2014.*

years old, but there were also some adolescents between 15 and 18 years and some persons up to 67 years old in the environment of the children.

For reasons of confidentiality, further information such as gender and links to samples from other family members were not generally disclosed and were only known to the laboratory regarding about 30 families when the distribution of the drugs between the family members had to be interpreted to the court or the social workers for a more profound decision. Examples are given in section 3.3.3.

3.3. Drug concentrations in hair of parents and children

All samples were analyzed for the 31 drugs and metabolites as described in section 2.2. For each sample, a detailed report was written considering all findings. In this section, only the key substances (parent compound or metabolite) of each drug shall be considered. More details were described previously [12]. Furthermore, this section is limited to the initial test of all subjects. The development in the follow-up tests is described in section 3.5. In this child protection project, all concentrations above LOQ and with unambiguous identification of the drug were given. No cut-offs recommended by national and international guidelines were used [17-19].

3.3.1. Initial testing results of children

The numbers and percentages of positive results and

Table 2- *Illegal drug concentrations found in the initial hair test of 296 children and frequency of positive results between 2011 and 2014. Only parent drugs or key metabolites are shown.*

Substance*	Number of positive results	Concentration, (ng/mg)			Frequency of positive results, %			
	2011-2014 (N=296)	Range	Mean	Median	2011 (N=122)	2012 (N=79)	2013 (N=39)	2014 (N=56)
Methadone	43 (14.5%)	0.01-2.16	0.27	0.080	24.6	8.9	0	10.5
6-AM (Heroin)	58 (19.6%)	0.01-11.1	0.48	0.069	27.9	15.2	7.7	15.8
Cocaine	148 (50.0%)	0.01-17.8	0.68	0.079	46.7	48.1	56.4	54.4
Amphetamine	14 (4.7%)	0.025-5.96	1.15	0.34	3.3	6.3	2.6	7
Methamphetamine	0 (0%)	0	0	0	0	0	0	0
MDMA (Ecstasy)	5 (1.7%)	0.03-0.52	0.15	0.056	1.6	2.5	0	1.8
MDE (Ecstasy)	0 (0%)	0	0	0	0	0	0	0
Δ9-THC (Cannabis)	111 (37.5%)	0.01-2.58	0.16	0.056	38.5	43	48.7	21.1
Benzodiazepines**	12 (4.1%)	0.012-0.18	0.043	0.023	4.1	5.1	0	5.2
No drugs	80 (27%)	----	----	---	22.1	32.9	20.5	33.3

*6-AM = 6-acetylmorphine, MDMA = methylenedioxymethamphetamine, MDE = methylenedioxyethamphetamine, Δ9-THC = Δ9-tetrahydrocannabinol

** From the tested benzodiazepines only diazepam and nordazepam were found in children hair.

the ranges, means and medians of the concentrations of the drugs found in the first test of the 296 children for the whole time period are shown in Table 2. Furthermore, the positive percentages for each year are also given. In 80 children (27%), no drugs were found. There was a high frequency of multiple drug exposure in the remaining children. Out of 216 children, 99 samples were positive for one drug, 74 samples for two drugs, 29 samples for three drugs, 13 samples for four drugs and one sample for five drugs. The most frequently detected drugs were cocaine (50%) and Δ9-THC (37.5%) followed by 6-acetylmorphine (6-AM, a key metabolite of heroin, 19.6%) and methadone (14.4%). There is no clear trend in the percentage of positive results within the four years, with the exception of methadone and 6-AM wich exhibited a particularly high frequency in 2011. This can be explained by the fact that in the first

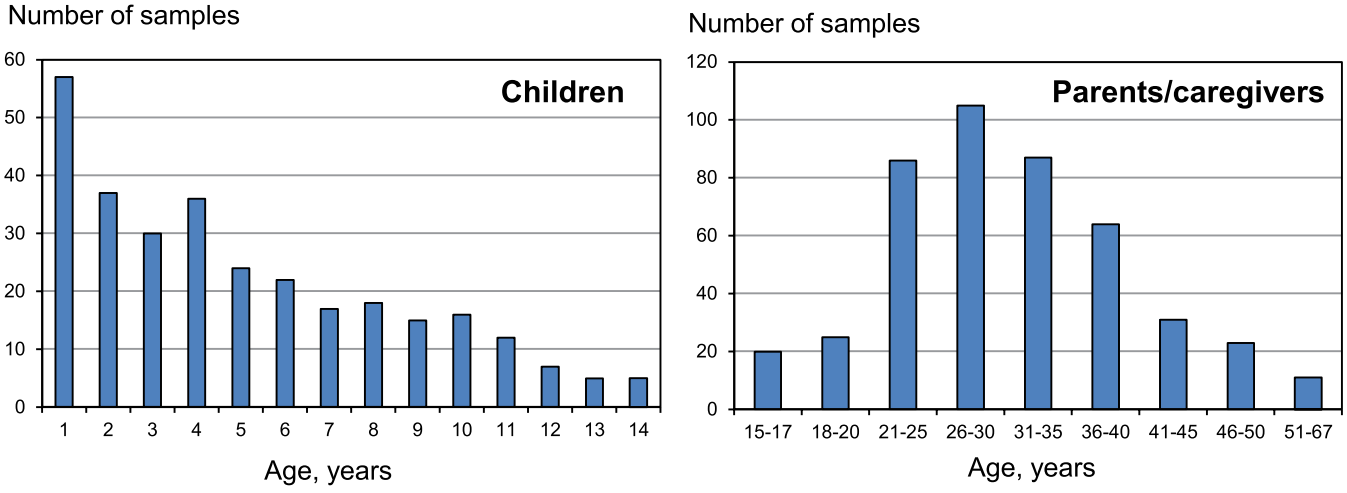

Figure 3- *Frequency of hair samples at different ages of the tested children and parents/caregivers.*

Table 3- *Illegal drug concentrations found in the initial hair test of parents or caregivers and frequency of positive results between 2011 and 2014. Only parent drugs or key metabolites are shown.*

Substance*	Number of positive results	Concentration, ng/mg			Frequency of positive results, %			
	2011-2014	Range	Mean	Median	2011 (N=122)	2012 (N=79)	2013 (N=39)	2014 (N=56)
Methadone	57 (12.6%)	0.024-25.7	7.48	6.49	26.8	12.4	6.9	9.0
6-AM (Heroin)	59 (13.1%)	0.01-11.2	1.49	0.28	24.4	13.2	9.5	9.0
Cocaine	200 (44.3%)	0.01-36.8	2.39	0.24	52.4	43.0	38.8	45.1
Amphetamine	35 (7.8%)	0.02-20.5	0.99	0,13	8.5	7.4	4.3	10.5
Methamphetamine	1 (0.2%)	0.02	0.02	0.02	0.0	0.0	8.6	0.0
MDMA (Ecstasy)	22 (4.9%)	0.02-18.5	1.33	0.24	6.1	4.1	3.4	6.0
MDE (Ecstasy)	3 (0.7%)	0.13-1.09	0.47	0.20	3.7	0.0	0.0	0.0
Δ9-THC (Cannabis)	196 (43.5%)	0.01-8.26	0.63	0.21	45.1	47.1	44.8	37.6
Benzodiazepines**	31 (6.9%)	0.01-1.90	0.36	0.15	7.3	4.1	4.3	11.3
No drugs	127 (28.2%)	----	----	----	19.5	27.3	32.8	30.1

*6-AM = 6-acetylmorphine, MDMA = methylenedioxymethamphetamine, MDE = methylenedioxyethamphetamine, Δ9-THC = Δ9-tetrahydrocannabinol

** From the tested benzodiazepines found in parents / caregivers hair: diazepam (19x), nordazepam (16x), oxazepam (12x), flunitrazepam (2x) and lorazepam (1x).

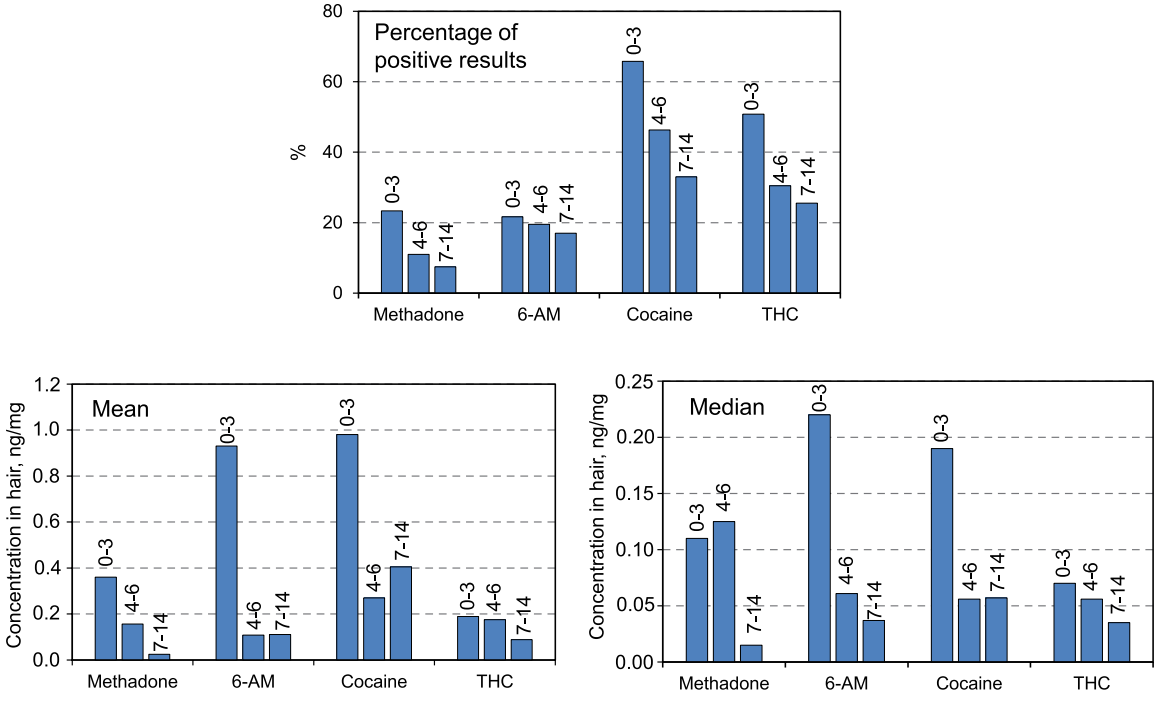

Figure 4- *Percentage of positive results, mean concentrations and median concentrations of methadone, 6-acetylmorphine (6-AM), cocaine and THC in the initial hair test of children in the age groups 1-3 years (N=120), 4-6 years (N=82) and 7-14 years (N=94).*

year of the project, children from parents in methadone maintenance programs who frequently co-abuse heroin were preferentially included, and that in the following years, less new cases of children with parents in methadone maintenance treatment were observed.

The concentrations vary strongly with the majority below the usual values of regular drug users. However, there are several samples with concentrations typical for occasional or regular abuse. There is a clear effect of children's age on the frequency of positive results and on the drug concentrations as shown in Fig. 4 for methadone, 6-AM, cocaine and Δ9-THC. Toddlers aged 1-3 years always displayed the highest portion of positive results and almost always the highest mean and median

Table 4- *Concentrations in hair (ng/mg) of families with positive methadone, heroin, cocaine and cannabinoid results*.*

	Family 1			Family 2		Family 3		Family 4		
Family member	**Mother**	**Child 1**	**Child 2**	**Mother**	**Child**	**Mother**	**Child**	**Mother**	**Child 1**	**Child 2**
Age, years	**34**	**5**	**1**	**29**	**2**	**35**	**7**	**34**	**13**	**7**
Methadone	12.8	0.024	0.16	15.6	0.29	41.0	1.65	n.d.	n.d.	n.d.
EDDP	0.53	n.d.	0.012	0.14	n.d.	0.39	0.037	n.d.	n.d.	n.d.
Morphine	1.94	n.d.	n.d.	0.24	n.d.	0.21	n.d.	n.d.	n.d.	n.d.
6-Acetylmorphine	5.63	0.11	0.59	pos.	0.085	0.31	0.04	n.d.	n.d.	n.d.
Heroin	pos.	n.d.	pos.	n.d.	pos.	n.d.	n.d.	n.d.	n.d.	n.d.
Codeine	0.43	n.d.	0.006	n.d.	n.d.	0.21	n.d.	n.d.	n.d.	n.d.
Cocaine	1.57	0.036	0.24	1.68	0.073	12.54	1.06	1.06	0.08	0.27
Norcocaine	pos.	n.d.	pos.	pos.	n.d.	0.42	0.014	pos.	n.d.	pos.
Benzoylecgonine	0.7	0.021	0.063	0.82	0.023	1.07	0.05	0.19	n.d.	0.03
Ecgonine methyl ester	n.d.	n.d.	n.d.	n.d.	n.d.	0.46	n.d.	n.d.	n.d.	n.d.
Cocaethylene	n.d.	n.d.	n.d.	0.35	n.d.	n.d.	n.d.	0.04	n.d.	n.d.
Δ9-Tetrahydrocannabinol (THC)	1.87	n.d.	0.05	n.d.	n.d.	n.d.	n.d.	1.60	0.39	1.36
Cannabinol (CBN)	0.25	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.18	0.09	0.36
Cannabidiol (CBD)	0.05	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

** Only drugs or metabolites with positive results are shown. n.d. = not detected, pos. = detected but not quantified.*

concentrations followed by the age groups 4-6 years and 7-14 years. This is in agreement with previous findings and can be explained by the closer contact of younger children with the drug consuming parents and their environment indoors, whereas elder children spend more time outdoors or at school [20,21]. Furthermore, small children tend to put contaminated objects into their mouth and also have a higher respiratory rate than older children or adults, which increases inhalation of smoke and dust.

According to the criteria given in section 3.4.2., 68 of the 296 initially tested children (23%) presented very serious results, 55 cases for 1 drug, 8 cases for two drugs and 5 cases for three drugs. These results included methadone (13 times), heroin (22 times), cocaine (35 times), amphetamine (11 times) and twice MDMA.

3.3.2. Initial testing results of parents or caregivers

The results of the initial hair test of 451 parents or caregivers are given in Table 3. The frequencies of the different drugs are very similar to those of the children. In 127 samples (28.2%) no drug was detected. From the remaining 324 samples with positive results, there were

155 samples positive for one drug, 97 samples positive for two drugs, 42 samples positive for three drugs, 22 samples positive for four drugs, 7 samples positive for five drugs and one sample positive for six drugs. Cocaine (44.3%) and Δ9-THC (43.5%) occurred most frequently followed by 6-AM (13.1%) and methadone (12.6%). In the same way, the frequency of methadone and of 6-AM was much higher in 2011 than in the following years. This is caused by the fact that from 2012 parents in maintenance treatment (but not their children) were mainly tested in another laboratory and the data were not available for this study.

The concentrations also vary strongly in this group, but the means and medians are clearly higher than the children and are in the typical ranges as described in in the literature [22]. There were 39 parents or caregivers (8.6%) with methadone in the range of maintenance treatment. On using the criteria given in section 3.4.1., a further 65 parents or caregivers (14.4%) displayed concentrations of illegal hard drugs in the range of regular and frequent use (56 times one drug and 9 times two drugs). Between them, heroin (6-AM) occurred 22 times, cocaine 22 times, amphetamine 4 times, and ecstasy (MDMA or MDE) 4 times.

3.3.3. Comparison of concentrations within families

The comparison of hair results between children and parents within a family is often very helpful for the assessment of the domestic drug situation. This was demonstrated for several examples in the previous paper about the project [12] and is shown in Table 4 for four further families (mothers with one or two children). In family 1, according to the hair findings, the mother was on methadone maintenance treatment but continued to abuse heroin, cocaine and cannabis. This is also clearly reflected in the hair sample of the one year old child with one to two orders of magnitude lower concentrations. The concentrations are even lower in the hair of the five year old child, and cannabis was not detected. The situation is similar in families 2 and 3 with the exception of cannabis and the additional detection of cocaethylene as a proof of co-consumption of cocaine and alcohol by the mother of family 2. Only cocaine and cannabis were detected in family 4 with the THC and CBN concentration of the seven year-old child in the same order as of the mother. The concentrations of the 13-year-old child were clearly lower the mother.

From the family examples as a whole, it can be concluded that the drug situation of the parents is often very well mirrored by the hair results of the children. Usually, the concentrations in children's hair are much lower than those in the hair of their consuming parents. However, there are also examples, e.g. for Δ9-THC, where the concentration in children's hair was in the same range or even higher than in the hair of their parents. As a rule, the concentrations in hair of younger children are higher than of their elder sisters or brothers. If the tested parent (e.g. the mother) displays negative or very low results, despite clearly positive findings for the children, there must be another drug consuming person (e.g. the father) in the environment of the children who was not tested.

3.4. Interpretation of hair results

A detailed report was written for each tested sample including characterization of the hair sample, information about hair cosmetics used, a short description of the analytical method, a table of the measured concentration and interpretation. In case of follow-up testing, the change in comparison to all previous tests was also interpreted. It is very important that any decision taken by the social offices does not solely depend on the hair report but must be based on entire body of evidence known in a specific case. The interpretation is different for adults and children.

3.4.1. Interpretation for parents or caregivers

Physiological basis, principles, and limitations in interpretation of hair results have been comprehensively described in the literature [11]. In case of the parents or caregivers, it had to be distinguished as far as possible between active drug consumption and external contamination of the hair. This is mainly based on the detection and concentrations of metabolites. The concentrations were compared with the whole database of the corresponding drug and were assigned to the lower range (below 25 percentile), medium range (25 to 75 percentile) and upper range (above 75 percentile). The values described by Tsanaclis and Wicks for a large population appeared to be very helpful for this purpose [22]. In this child protection project, a differentiation of the exposure/consumption into experimental, occasional (<25 percentile) and regular / frequently (≥25 percentile) was performed. The corresponding approximate concentration ranges for the drugs are given in Table 5. Parents with regular and frequent use are, with a high degree of probability, addicted to the drug and are a serious risk to the child's welfare. The

Table 5- *Interpretation of drug concentrations in hair (ng/mg) of parents / caregivers.*

Concentration range	Traces	Lower range	Medium and upper range
Exposure or consumption	Experimental or contamination	Occasional	Regular, frequently
Methadone	<0.1	0.1 to ≤2.0	>2.0*
6-Acetylmorphine (Heroin)	<0.1	0.1 to ≤1.0	>1.0
Cocaine	<0.2	0.2 to 2.0	>2.0
Amphetamine	<0.1	0.1 to 2.0	>2.0
MDMA (Ecstasy)	< 0.1	0.1 to 1.0	>1.0
Δ9-THC	<0.02	0.02 to 0.7	≥0.7

* *Methadone maintenance treatment*

frequency of such cases in the initial and follow-up tests is described in section 3.5.

Finally, also an estimation of the time period represented by the hair sample was given in the expertise. As the minimum period, the length of hair above the hair root is equal to the number of months before sampling. That means a proximal hair segment of 6 cm represents 6 months before sampling. Caused by telogen and slow growing hair, this period can be up to 6 months longer.

3.4.2. Interpretation for children's hair results

There are several aspects to consider in the interpretation of children's hair results. A positive result means in every case that there was a handling of the drug in the environment of the child. This means there is always a risk to the child as described in the introduction. However, it can generally be assumed that in cases of high concentrations the degree of risk was more severe than if only traces of the drug were found. Therefore, criteria for assessment of the drug concentrations were chosen in this project in the categories "detected", "serious exposure" and "very serious exposure" and are given in Table 6. The frequency of very serious exposure cases in the initial and follow-up tests is discussed in section 3.5.

It is also important to distinguish between systemic incorporation of the drug in hair after oral intake or inhalation by the child and external deposition into hair from dust, smoke or contaminated hands or surfaces. In this project, the detection and concentrations of "non-hydrolytic" metabolites were used as criteria for a systemic incorporation since they are not formed by hydrolysis outside of the body but only enzymatically within the body. This concerns EDDP from methadone, norcocaine from cocaine and MDA from MDMA or MDE. In the case of heroin, the concentration ratio of morphine and 6-acetylmorphine, which can both be formed by hydrolysis from heroin, was assessed. More details for children's hair were previously described [12]. Due to the absence of a suitable non-hydrolytic metabolite, no distinction between systemic and external incorporation was possible for amphetamine. In the case of cannabis, mainly external deposition of Δ9-THC can be assumed. However, in some cases systemic uptake by inhalation of smoke was probable by the detection of the THC-COOH [12].

It can also not totally be excluded that a non-hydrolytic metabolite is transferred from the consuming parent to the hair of the child. Such cases were probable for EDDP, which could be incorporated from the sweat of the mother into the hair of the child [12]. Heavy sweating is one of the most common adverse effects of methadone.

The important question whether a systemic intake of the drug occurred by intentional administration or accidentally cannot be answered by hair analysis but is a question of criminological investigation. There are lethal and survived poisoning cases known in which the mothers tried to calm their children by administration of a methadone overdose [5,23]. Also, the question whether in the case of a positive result the handling or consumption of the drug occurred in presence of the child cannot unambiguously be answered. Frequent contamination of the room in absence of the child or frequent touching of the child's hair with contaminated hands after preparing and smoking cannabis joints in another place can be sufficient for a positive result [24].

There are also differences between adults and children with respect to the hair growth rate and presence of telogen hair [11,25,26]. However, this concerns mainly the perinatal period up to the age of one year. Since in this project only samples from children above the age of one year were included, in a first approximation the same chronological interpretation as for adults was applied (growth rate ≈1cm/month).

3.5. Development from initial to follow-up tests

The success of measures and support provided by the social offices are evident from the results of the follow-up tests. Therefore, the development of the cases as it appears from the comparison of initial and follow-up hair results was evaluated for the 57 children and 93 adults for whom follow-up tests were performed.

As examples, the results from the initial and three follow-up tests for a child and a mother are shown in Table 7. In the initial test, the 15 month old child exhibited very high concentrations of methadone, 6-acetylmorphine and cocaine and a positive diazepam result. Systemic drug intake was probable from the detection of EDDP and norcocaine. Eight months later, only a small cocaine value remained, which could be explained to be older residues in telogen hair. Obviously, the measures of the social authorities were successful. However, it can be seen from the third and fourth test two years later that again heroin and cocaine were handled in the surroundings of the child, and that diazepam was administered.

Table 6- *Interpretation of drug concentrations (ng/mg) in hair of children*

Degree of exposure	Detected	Serious	Very serious
Methadone	<0.10, no EDDP	0.10 to 0.49, no EDDP	≥0.5, no EDDP ≥0.1 ng/mg + EDDP
6-Acetylmorphine (Heroin)	< 0,05	< 0,1	≥ 0,1
Cocaine	<0.1, no norcocaine	0.1 - 0.5, no norcocaine	≥0.1 + norcocaine ≥0.5, no norcocaine
Amphetamine	<0.05	0.05 - 0.1	≥ 0,1
MDMA (Ecstasy)	<0.05	0.05 - 0.1	≥ 0,1
Δ9-THC	<0.1	≥0.1	----

** Methadone maintenance treatment*

The mother in Table 7 from another family was on methadone maintenance treatment and all four hair tests showed an occasional but steady abuse of heroin (6-AM). In addition to that, new abuse of cocaine became evident in test 4 with a relatively high concentration. Obviously, the situation worsened during the testing period.

The statistic evaluation of the cases was separately performed for so-called hard drugs (heroin, cocaine, amphetamine and ecstasy) and for cannabinoids. Methadone was only considered as a "hard drug" for children since its presence in the hair of adults in the medium and upper concentration ranges was due to maintenance treatment. According to the outcome of initial and follow-up tests, the cases were subdivided into groups: (1) no drugs in initial and follow-up tests, (2) improvement by strong decrease of concentrations or negative results, (3) no essential change of drug exposure and (4) worsening by increased exposure or new exposure to additional drugs. Furthermore, for children, the number of very serious cases according to Table 6 was compared between the initial test and the last follow-up test. For adults, the number of cases in the medium and upper concentration range (criteria in Table 5) was compared accordingly. The results are shown in Figure 5. It can be seen from the four graphs that a favorable development (negative in all tests or improvement) dominates over worsening or unchanged drug use. However, this favorable outcome is more pronounced for children and for hard drugs than for parents and cannabis. Whereas in the case of children the number of severe cases (only with hard drugs) decreases from 21 in the initial test to 5 in the last follow-up test, it falls only from 17 to 12 cases for the parents.

It follows from the comparison in Fig. 5 that the efforts to minimize the drug exposure of the children were more

Table 7- *Two examples from different families of the results (ng/mg) of the initial and three follow-up hair tests. Analyzed hair segment upto 6 cm.*

Child, born in October 2010				
Test No.	1	2	3	4
Date of testing	Feb. 2012	Oct. 2012	May 2014	Dec. 2014
Methadone	0.47	n.d.	n.d.	n.d.
EDDP	0.017	n.d.	n.d.	n.d.
Morphine	0.41	n.d.	0.01	0.03
6-Acetylmorphine	3.30	n.d.	0.07	0.14
Heroin	Pos.	n.d.	n.d.	0.02
Codeine	n.d.	n.d.	n.d.	n.d.
Acetylcodeine	0.27	n.d.	n.d.	0.01
Cocaine	2.52	0.096	0.34	0.09
Norcocaine	Pos.	n.d.	n.d.	n.d.
Benzoylecgonine	0.52	Pos.	0.06	0.04
Ecgonine methyl ester	n.d.	n.d.	n.d.	n.d.
Cocaethylene	n.d.	n.d.	n.d.	n.d.
Diazepam	0.078	n.d.	n.d.	0.02
Mother, born in May 1979				
Test No.	1	2	3	4
Date of testing	Dec. 2011	Mar. 2012	Feb. 2014	Aug. 2014
Methadone	14.2	14.83	9.88	41.0
EDDP (Methadone-Metabolite)	0.28	0.32	0.32	0.39
Morphine	0.22	0.19	0.24	0.21
6-Acetylmorphine	0.28	0.28	0.31	0.31
Heroin	n.d.	n.d.	n.d.	n.d.
Codeine	0.076	0.089	0.28	0.21
Acetylcodeine	0.017	0.033	0.03	0.04
Cocaine	n.d.	n.d.	n.d.	12.54
Norcocaine	n.d.	n.d.	n.d.	0.42
Benzoylecgonine	n.d.	n.d.	n.d.	1.07
Ecgonine methyl ester	n.d.	n.d.	n.d.	0.46

n.d. = not detected.

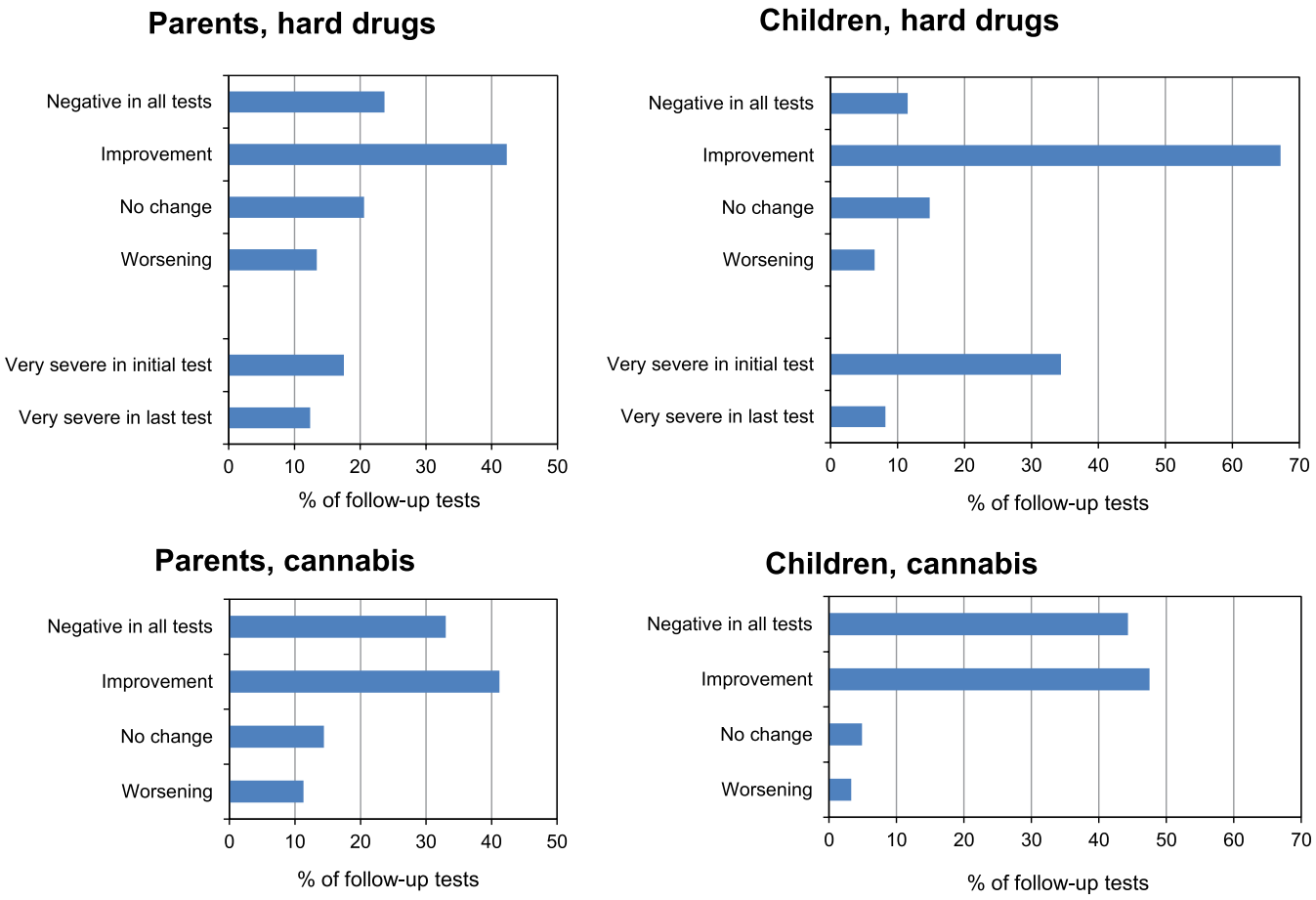

Figure 5- *Change of hair test results from initial test to last follow-up test of 93 parents or caregivers and 57 children.*

successful than to overcome the drug addiction of the parents. The lower improvement concerning cannabis in children as well as parents can be explained by the general more moderate opinion about this drug, as is obvious in the ongoing debate about its legalization.

4. Conclusions

After the experience of four years with this continuous project, hair analysis proved to be a very efficient working instrument for social authorities in the systematic improvement of child-welfare in a drug abusing environment. Because of the long time window and the high performance of applied chromatographic and mass spectrometric techniques, hair testing of parents reveals their use of illegal drugs with high sensitivity and specificity. Additional investigation of children's hair gives evidence about the degree of their exposure to these drugs.

However, it must be cautioned against the uncritical and schematic interpretation of hair results. Drug concentrations in hair can arise from different ways of incorporation, depend strongly on variable physiology of hair growth and many other individual parameters, and are decreased to a different degree by hair care and hair cosmetics. Therefore, the interpretation should be performed by experienced hair analysts who know the pitfalls and the up-to-date literature of drug analysis in hair. Consequences such as the removal of the child from the family and admission into children's homes or by foster families, or separation of the drug consuming father or mother from the family should never be based solely on the hair analysis result but must include all other sources of information. This appeared to be ensured by the principles of this social support system as described in section 3.1 and in Fig. 1.

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