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Hair concentrations and self-reported abuse history of 20 amphetamine and ecstasy users

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Abstract

Hair samples of 20 volunteers of the techno-music scene, who more or less regularly consumed ecstasy tablets and speed and anonymously reported their abuse history, were analyzed in one to seven 3 cm segments for amphetamine (A), methamphetamine (MA), methylenedioxyamphetamine (MDA), methylenedioxymethamphetamine (MDMA), methylenedioxyethamphetamine (MDE) and *N*-methyl-1-(1,3-benzodioxol-5-yl)-2-butylamine (MBDB) by digestion in 1 M NaOH, subsequent extraction with C18 Bond Elut columns, derivatization with pentafluoropropionyl anhydride and GC/MS-SIM measurements using deuterated standards of A, MA, MDA and MDMA. The concentrations were in the regions 0.1 to 4.8 ng/mg for A (17 samples), 0.05 to 0.89 ng/mg for MDA (16 samples), 0.1 to 8.3 ng/mg for MDMA (16 samples), 0.12 to 15 ng/mg for MDE (13 samples) and 0.21 to 1.3 ng/mg for MBDB (2 samples). MA was not detected. For comparison the frequency and the concentration of these drugs in 124 different ecstasy tablets were determined by HPLC. The drug concentration in the hair segments were compared with the volunteers' reports. Despite the enormous interindividual differences qualitatively an increase of the total concentration of MDA, MDMA and MDE in the proximate 3 cm segments with increasing ecstasy abuse frequency during the last three month before sampling is recognized. In the individual comparison with the chronological consumer reports in most cases a longer interruption or a change of the abuse intensity is not clearly seen at the segment concentrations. As a reason the incorporation of the drugs from sweat into elder hair regions and the slow removal by washing are discussed. © 1997 Elsevier Science Ireland Ltd

Keywords: Amphetamines in hair; Ecstasy; Ecstasy abuse history (self reported); Hair analysis, 3,4-Methylenedioxyamphetamine derivatives in hair

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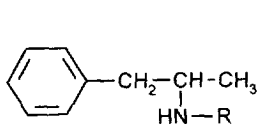
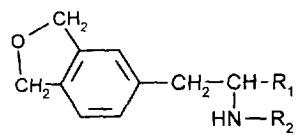
1. Introduction

In the last few years in Germany, the abuse of amphetamine and particularly of methylenedioxymethamphetamine (MDMA, "adam", "ecstasy") and of methylenedioxyethamphetamine (MDE, "eve") as well as to a minor extent also of *N*-methyl-1-(1,3-benzodioxol-5-yl)-2-butylamine (MBDB) has become a widespread habit of young people at techno and rave parties [1]. The structure of the compounds is shown in Fig. 1. Besides the desired psychedelic, recreational, "entactogenic", reinforcing and stimulating effects [2–4], the intake of these compounds is combined with severe health risks. Beside acute intoxications [5–14], serotonine neurotoxicity after chronic intake [3,15,16] and severe psychiatric disorders [2,17–20] were also observed in many cases.

Also, in cases without these severe adverse effects, the abuse of the amphetamine derivatives is regarded as a cause of unsuitability for driving because of mydriasis, restlessness, confusion, breaking down of inhibitions, venturesome or reckless behavior and perception disorders during the acute action of the drugs as well as because of exhaustion, tiredness, anxiety, mental confusion, lack of concentration and depressions after the acute phase [4,18].

Hair analysis has proved to be a suitable method in order to detect chronic abuse of opiates, cocaine, cannabinoides and amphetamines with medical or forensic implications, particularly also in cases of the suspension of the driving licence. The detection of amphetamines and 3,4-methylenedioxyamphetamine derivatives in hair was described by Kintz et al. [23], Nakahara et al. [24], Röhrich et al. [21], Skopp et al. [12], Suzuki et al. [25] and Weinmann et al. [22]. In such investigations hair concentrations of amphetamine between 0.96 and 15.8 ng/mg [23] or 0.5 and 0.9 ng/mg [25] (methamphetamine metabolite), methamphetamine between 0.6 and 56.5 ng/mg [24,25], MDA between 0.04 and 5.0 ng/mg [12,21,22], MDMA between 0.05 and 6.4 ng/mg [12,21] and MDE between 0.8 and 17 ng/mg [21,22] were measured. Nakahara et al. found in 9 from 11 cases of methamphetamine abuse a good coincidence of the sectional hair analysis results with the self reported drug history [24]. MBDB was not yet described as the analyte in hair analysis. A general problem in the interpretation of hair concentrations of illegal drugs is the lack of a dose–hair concentration relationship, since as a rule for the investigated samples information about the abuse dose and frequency were not available. Although in principle a good relationship cannot be expected because of the multiple





Amphetamine A, R = H
Methamphetamine MA, R = CH_3

3,4-Methylenedioxyamphetamine MDA, $R_1 = CH_3$, $R_2 = H$
3,4-Methylenedioxymethamphetamine MDA, $R_1 = R_2 = CH_3$
3,4-Methylenedioxyethamphetamine MDA, $R_1 = CH_3$, $R_2 = C_2H_5$
N-Methyl-1-(1,3-benzodioxol-5-yl)-2-butylamine MBDB, $R_1 = C_2H_5$, $R_2 = CH_3$

Fig. 1. Structure of the drugs contained in "speed" and in "ecstasy" tablets.

interindividual differences in e. g. pharmacokinetics, hair color and hair care, it is important to know, which region of the hair concentration may be expected after a certain degree of abuse, and how long the drug can be detected after the abuse has been stopped.

Therefore in this paper the analytical investigation of hair samples of 20 regular "speed" or "ecstasy" abusers of the techno scene is described, who anonymously reported their drug history in a drugs advice center. Since in these reports only the number of the ecstasy tablets without a characterization of the real drug content could be given, 124 different ecstasy tablets were analyzed in a drug checking program by HPLC for MDA, MDMA, MDE, MBDB, amphetamines and other drugs.

2. Materials and methods

2.1. Hair samples and supplementary information

From each of the volunteers a hair tuft of about 5 mm diameter was cut as close as possible above the skin in the vertex region, fixed with a string and stored in a plastic envelop in the darkness. In a questionnaire the following data were anonymously registered: age, sex, kind of the drugs, consumption history (cf. Table 2), kind, frequency and last date of the hair treatment (washing, perm, bleaching, dyeing), longer periods of sunbathing.

2.2. Drug samples and reagents

The drug samples used for the calibration and the deuterated standard compounds were purchased from Sigma-Aldrich or from Radian. The illegal ecstasy tablets were obtained from the same drug advice center, where the hair samples were collected. The solvents as well as pentafluoropropionic anhydride and all other reagents used in this investigation were obtained from Merck. The solid-phase extraction of the hair solutions was carried out with C18 Bond Elut LCR columns purchased from Varian.

2.3. Sample preparation

In order to remove external impurities the whole hair tuft was washed for 5 min with 20 ml acetone in an ultrasocic bath and dried. The analysis was carried out in 3 cm sections beginning at the proximal end of the hair samples. Each section was cut into small pieces and between 20 and 50 mg were digested by treating with 1 ml 1 N NaOH for 30 min at 80°C. Then 200 ng of each amphetamine-D_5, methamphetamine-D_5, MDA-D_5 and MDMA-D_5 in 20 μl *n*-propanol as the internal standards were added. The solution was neutralized by adding 1 ml 1 N HCl, adjusted to pH 7.6 with 1 ml 0.1 M phosphate buffer and a small undissolved residue was removed by centrifugation. After that the solid-phase extraction with the C18 Bond Elut LCR columns was carried out in the following steps: conditioning by 6 ml methanol and 3 ml H_2O, extraction of the hair sample solution, washing with 3 ml H_2O, 3 ml 1 M $NaHCO_3$ and 3 ml H_2O, drying by

30 min suction of air through the column, elution with 2 ml CH_2Cl_2 /acetone (1:3) and evaporation under nitrogen at room temperature.

For the derivatization of the substituted amphetamines in literature acetic anhydride [26,27] trifluoroacetic anhydride [23,25], heptafluorobutyric anhydride [28] 4-carbethoxyhexafluorobutyryl anhydride [28] or perfluorooctanoyl anhydride [29] are described. In our investigation the residue was derivatized by 40 min heating with 100 μl pentafluoropropionyl anhydride (PFPA) at 80°C. Then the excessive PFPA was evaporated in a nitrogen stream and the residue dissolved in 50 μl ethyl acetate. Experiments to use this reagent as a mixture with pentafluoropropanol, which is a general derivatization reagent in drug analysis, were not successful, since the reaction was incomplete for the secondary amines MDMA, MDE and MBDB. In the case of undiluted PFPA obviously for steric reasons only the *N*-ethyl compound MDE had a derivatization yield of only about 70%, which could not be increased by a longer reaction time or by increasing the temperature. For the other compounds the reaction was complete. All derivatives proved to be stable at least 96 h in ethylacetate, in which they were dissolved for GC/MS measurement. For the GC/MS measurement 1 μl of this solution was injected.

2.4. Gas chromatography/mass spectrometry measurements

The GC/MS measurements were carried out with a Hewlett-Packard device consisting of an HP5890 series II gas chromatograph, an HP 5971 mass selective detector and an HP 7673 autosampler. An HP-1 capillary column (100% methyl silicone) was used for the chromatographic separation with the carrier gas helium at a flow-rate of 1 ml/min and a splitless injection. The following temperature program was applied: 2 min at 70°C, 15 K/min to 300°C, 10 min at 300°C. All compounds are well separated under these conditions, as shown in a typical chromatogram in Fig. 2.

For all PFP derivatives characteristic and reproducible mass spectra were obtained. As an example the spectra of the two isomeric compounds MDE-PFP and MBDB-PFP are shown in Fig. 3. The identification and quantitative determination of the amphetamines was carried out in the selected ion monitoring mode (SIM). From the mass spectra of the

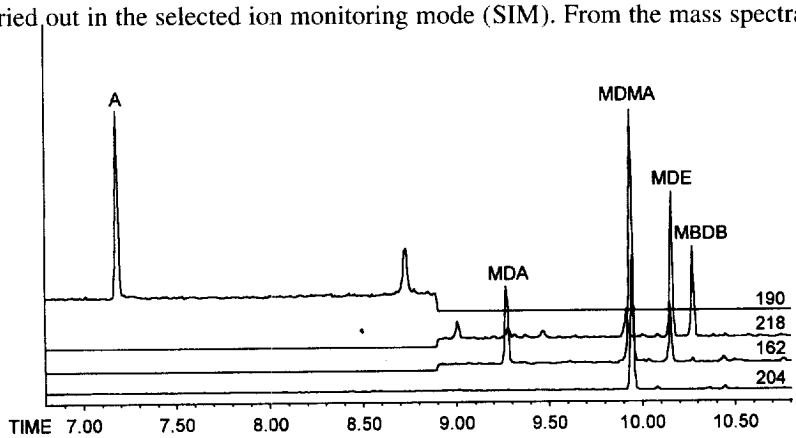


Fig. 2. GC/MS–SIM chromatogram of the hair extract of the volunteer 04, segment 3–6 cm.

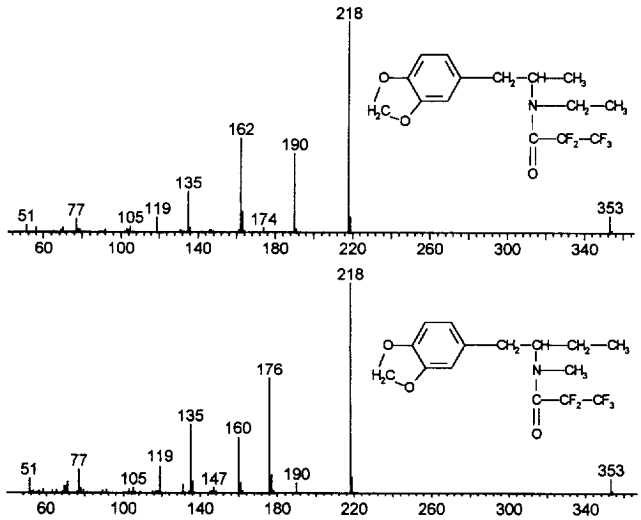


Fig. 3. Mass spectra of the pentafluoropropionyl derivatives of MDE and MBDB.

N-pentafluoropropionyl derivatives the m/e values given in Table 1 were used. For amphetamine, methamphetamine, MDA and MDMA the sample concentration was determined from the peak area ratio with the corresponding deuterated standard.

Table 1
m/e values used in the GC/MS–SIM measurements and detection limits of the *N*-pentafluoropropionylamphetamine derivatives in hair samples

Compound	GC/MS–SIM analysis*		Detection limit,
	m/e values	peak area ratio	ng/mg hair**
Amphetamine-PFP	190, 118	100: 89	0.1
Amphetamine-D_5-PFP	194, 123	100: 41	-
Methamphetamine-PFP	204, 118	100: 23	0.2
Methamphetamine-D_5-PFP	208, 119	100: 31	-
Methylenedioxyamphetamine-PFP (MDA-PFP)	135, 162	100: 49	0.04
Methylenedioxyamphetamine-D_5-PFP (MDA-D_5-PFP)	136, 167	100: 29	-
Methylenedioxymethamphetamine (MDMA-PFP)	204, 162	100: 63	0.04
Methylenedioxymethamphetamine-D_5-PFP (MDMA-D_5-PFP)	208, 163	100: 64	-
Methylenedioxyethamphetamine-PFP (MDE-PFP)	218, 190	100: 27	0.1
N-methyl-1-(1,3-benzodioxol-5-yl)-2-butylamine-PFP (MBDB-PFP)	218, 176	100: 53	0.04

* The underlined m/e values were used for quantification. ** The data are related to a sample amount of 20 mg.

However, for MDE and MBDB, the deuterated standards of which were not available, a calibration curve with MDA-D5 as the internal standard was used for the quantitation. For all compounds a linear relationship between the sample concentration and the GC/MS response relative to that of the internal standards was found between 10 ng and 1 μg per sample. The absolute rate of recovery differed strongly from experiment to experiment, but related to the internal standards it was always between 90 and 110%. The detection limits (cf. Table 1) were found at significantly lower concentrations for the methylenedioxyamphetamines (2–5 ng/sample=0.1–0.25 ng/mg for a 20 mg sample) than for amphetamine and methamphetamine (5–10 ng/sample=0.25–0.5 ng/mg for a 20 mg sample).

2.5. HPLC analysis of the ecstasy tablets

The HPLC measurements were carried out on a Shimadzu HPLC device consisting of a pump LC-6A, an autosampler SIL-9A, a photodiode array detector SPD-M6A, a computer 386 DX33 and a printer HP Laser Jet 4. The SPD-M6A is operated by the software CLASS® and a UV spectra library of more than 1.700 toxic compounds. The HPLC-column was a Lichrosorb RP8, 5 μm, 250 x 4,0 mm, Merck Darmstadt. The isocratic mobile phase consisted of acetonitril/phosphate buffer pH 2.3 (20: 80 v/v) and was degassed by a helium stream. Before measurement the tablets were dissolved in the mobile phase and the insoluble constituents were removed by centrifugation. In some cases (e.g. in order to distinguish between MDE and MBDB) GC/MS was used for confirmation of the results.

3. Results and discussion

The self-reported data about the intake of ecstasy and speed as well as about the hair treatment by the subjects are given in Table 2. Besides these drugs 15 subjects reported a regular smoking of cannabis products, 7 an occasional sniffing of cocaine, 6 an occasional intake of LSD and one an irregular intake of diazepam and a seldom abuse of heroin.

By the name of "ecstasy" a wide variety of tablets is understood, which contain mostly MDMA, MDE, MBDB or mixtures between them. In a drug checking program 124 of these illegal tablets, which were all different in colour, weight, shape or stamping, were analyzed in 1995 and 1996 by HPLC. The results are shown in Table 3. MDMA was found in the tablets almost double as many (about 60%) as MDE (32%). MBDB was detected only in three tablets (2.4%). It is seen from this frequency of the substances in the pills that in the hair of ecstasy consumers mainly MDMA or MDE should be found, whereas MBDB is rather rare, and in no case MDA was present. "Speed" was abused mainly as a powder of different concentration and only to a smaller extent by intake of tablets.

Beside these uncertainties the retrospective reports are in many cases not very detailed. Nevertheless it was possible to assign the consumers to one of four degrees with respect to the abuse intensity of ecstasy within the last three month before sampling (cf. Table 2): 1=insignificant abuse (less than 1 tablet/month), 2=moderate abuse (1 to

Table 2
Self-reported ecstasy and speed consumption history and hair treatment of 20 volunteers of the techno scene

No.	Age/Sex	Date of sampling	Drug abuse history	Abuse intensity*	Hair length (l), colour (c), washing (w), other treatment**
01	20 m	13.03.96	*ecstasy*: since Jan 1994, Sep–Nov 95: 1 tablet weekly, Dec 95–Jan 96 no tablets, Feb 96: 3 pills. *speed*: Oct 93 to Jan 95: 250 mg per week.	1	l: 7 cm c: middle blond w: 4 x weekly
02	29 m	13.03.96	*ecstasy*: Jun 95 6 tablets, Jul 95 2 tablets, Aug–Dec 95 15–20 tablets per month, Jan 96 10 tablets, Feb 96 no tablets, Mar 96 4 tablets. *speed*: Aug 95–Jan 96 1–2 g per month, Feb 96 no intake, Mar 96 1 g.	4	l: 8 cm c: middle blond w: 3 x weekly
03	21 m	13.03.96	*ecstasy*: 0.5 to 1 tablet per week since Sep 95. *speed*: 150 mg every weekend since Dec 95, last 2 weeks in Dec 95 no intake, increasing amount in Feb 96.	2	l: 10 cm c: middle blond w: 3–4 x weekly
04	30 f	16.03.96	*ecstasy*: 2 tablets each second weekend since 92, at one weekend in autumn 95 2 tablets with the stamping '$'. *speed*: irregularly, much in 94, no in winter 95/96, 3 weeks ago 1 g.	3	l: 17 cm c: light brown w: 1 x weekly
05	32 f	16.03.96	*ecstasy*: altogether 10 tablets, irregularly since Jun 95. *speed*: altogether 0.5 g since Jan 95	1	l: 8 cm c: black w: 2 x weekly
06	19 m	18.03.96	*ecstasy*: altogether 3 tablets in Oct 95, Dec 95 and Jan 96. *speed:* 1 g in October 95.	1	l: 6 cm c: middle blond w: 6 x weekly
07	18 m	20.03.96	*ecstasy*: May–Jun 95 1 tablet every second week, Jun–Aug 95 2–3 tablets per week, Sep–Nov 95 no intake, Nov 95–Mar 96 1 tablet per month. *speed*: summer 95 1g per week.	1	l: 23 cm c: middle blond w: 3–4x weekly
08	19 m	20.03.96	*ecstasy*: May–Jun 95 1/2 tablet per week, Jul–Aug 95 2–3 tablets per week, Aug–Nov 95 1 tablet per week, Nov–Dec 95 no intake, Jan–Mar 96 2–3 tablets per week. *speed*: May–Aug 95 1 g per week, Aug to Jan 96 no intake, Jan to Mar 96 1 g per week.	4	l: 20 cm c: light brown w: 1 x weekly

Table 2. Continued

No.	Age/Sex	Date of sampling	Drug abuse history	Abuse intensity*	Hair length (l), colour (c), washing (w), other treatment**
09	18 m	20.03.96	*ecstasy*: Jan 94 to summer 95: 2 tablets monthly, summer 95 to Jan 96 1 tablet weekly, since Feb 96 no intake. *speed*: 0.5 g weekly since summer 95.	2	l: 26 cm c: light brown w: 3 x weekly
10	18 m	01.04.96	*ecstasy or speed*: 3–4 days per week, at these days 4–5 times.	4	l: 10 cm c: reddish w: 5–6 x weekly
11	25 f	29.06.96	*ecstasy*: 1 tablet per month from Feb–Dec 95, in the first half year of 1996 only 2–3 tablets. *speed*: 50 mg per month from Mar–Dec 95, 50 mg every 2–3 weeks from Jan to Jun 96.	1	l: 7 cm c: red-brown w: 2–3 x weekly
12	24 f	06.07.96	*ecstasy*: since Nov. 94 1 tablet every 2–3 month, since Jan 96 1 tablet per month. *speed*: since Mar 96 50 mg per month.	1	l: 11 cm c: light brown w: 2–3 x weekly shading once every 6 month
13	20 m	06.07.96	*ecstasy*: 2 tablets about every third week since Sep 95. *speed:* 100–200 mg per week since Dec 95.	2	l: 4 cm c: middle blond w: 1–2 x weekly
14	22 m	19.07.96	*ecstasy*: 1–2 tablets about every second week since Oct 95. *speed*: 50–100 mg almost every weekend since December 1995.	3	l: 10 cm c: dark brown w: 3–4 x weekly
15	29 m	19.07.96	*ecstasy:* 2–4 pills per week since Apr 95 with an interuption in Jun 96. *speed*: 100–200 mg per week since Summer 95 with an particularly intense phase in Jun 96.	4	l: 7 cm c: dark brown w: 5–7 x weekly
16	22 m	12.08.96	*ecstasy*: 1 tablet between weekly and monthly from Nov 93 to Oct 95, after that only 1 tablet in summer 96. *speed*: no data.	1	l: 8 cm c: light brown w: 2–3 x weekly last bleaching in Nov 94.
17	21 m	15.08.96	*ecstasy*: 1 tablet every 6 to 8 weeks since beginning of 95. Mar to May 96 2–3 tablets per weekend. *speed*: no intake.	2	l: 17 cm c: middle blond w: 1–2 x weekly dyeing last time in Jan 96.
18	27 m	19.08.96	*ecstasy*: 1–2 tablets weekly from Apr to Jul 96. *speed*: no data.	3	l: 11 cm c: black w: 3 x weekly

Table 2. Continued

No.	Age/Sex	Date of sampling	Drug abuse history	Abuse intensity*	Hair length (l), colour (c), washing (w), other treatment**
19	20 f	21.08.96	*ecstasy*: 1–3 tablets weekly since Oct 94 with increasing tendency, particularly intensely two weeks before sampling. *speed*: about once every two weeks.	4	l: 9 cm c: middle blond w: 3 x weekly
20	19 m	06.09.96	*ecstasy*: 1 pill weekly since 1993 with an interruption between 12 and 4 weeks before hair sampling. *speed*: 0.5 g weekly, interruption as above.	2	l: 14 cm c: black w: 4 x weekly,

* Reported abuse intensity in the last three month before sampling: 1=insignificant abuse (<1 tablet/month), 2=moderate abuse (1 to 3 tablets/month), 3=strong abuse (>3 to 8 tablets/month), 4=very strong abuse (>8 tablets/month). ** Perm, dyeing, shading or bleaching, in most cases such treatment was denied.

3 tablets per month), 3=strong abuse (more than 3 to 8 tablets per month), 4=very strong abuse (more than 8 tablets per month). A similar classification for speed (amphetamine) on the basis of the reported data was not possible.

Depending on their length, all hair samples were analyzed in 1 to 7 segments of 3 cm. A typical SIM-chromatogram is shown in Fig. 2. For a clear illustration only one m/e value for each substance is shown, and the deuterated standards are omitted in this figure. The concentrations are given in Table 4. In no case MA was found. But almost in all positive cases A, MDA, MDMA and MDE were detected confirming the mixed consumption of speed and the ecstasy substances. The concentrations are 0.1 to 4.8

Table 3
Results of the HPLC investigation of 124 illegal 'ecstasy' tablets in a drug checking program

Drug*	Number of tablets		mg/tablet	
	number	%	range	medium
MDA	0	0	-	
MDMA	74	59,7	43–160	105
MDE	40	32,3	50–134**	106
MBDB	3	2,4	184–207	197
A	9	7,3	5,3–76	34
MA	1	0,8	67	-

* MDMA and MDE were combined in 10 pills and A was present in 3 MDMA tablets. Furthermore the following drugs were found either alone or in mixture with the amphetamines: bromhexine (1 x, 8 mg), coffeine (12 x, 2.5–160 mg), 2,5-dimethoxy-4-bromophenylethylamine (2 x, 4,6 and 4,7 mg), ephedrine (3 x, 1–18 mg), flunitrazepam (1 x, 2 mg), quinine (5 x, 6–35 mg), paracetamol (2 x, 3 and 42 mg). No other illegal drugs were detected.

** 1 tablet with an extreme content of 250 mg MDE is not included.

Table 4
Concentrations of amphetamine A, 3,4-methylenedioxyamphetamine MDA and its derivatives MDMA, MDE and MBDB in hair samples of 20 volunteers of the techno scene

Sample No.	Segment (cm)	Hair concentration (ng/mg)*				
		A	MDA	MDMA	MDE	MBDB
01	0–3	n. d.	0.43	n. d.	n. d.	n. d.
	3–6	n. d.	0.10	n. d.	n. d.	n. d.
02	0–3	0.13	0.36	3.8	n. d.	n. d.
	3–6	0.14	0.50	1.1	n. d.	n. d.
03	0–3	0.30	0.07	0.50	0.24	n. d.
	3–6	0.25	0.05	0.69	0.29	n. d.
	6–9	0.27	0.08	n. d.	n. d.	n. d.
04	0–3	3.4	2.1	8.3	4.1	n. d.
	3–6	2.9	2.1	4.2	4.9	1.3
	6–9	3.7	2.1	4.5	1.7	0.92
	9–12	3.6	0.89	5.7	1.7	0.56
	12–15	3.6	0.61	4.3	2.0	0.50
05	0–3	0.08	0.24	1.5	3.1	n. d.
	3–6	0.11	0.17	1.4	2.3	n. d.
06	0–6 (2 S)	n. d.	n. d.	n. d.	n. d.	n. d.
07	0–12 (4 S)	n. d.	n. d.	n. d.	n. d.	n. d.
08	0–3	0.24	0.20	1.3	0.26	n. d.
	3–6	0.21	0.22	0.11	2.8	1.0
	6–9	n. d.	0.28	0.71	2.1	0.10
	9–12	0.13	0.17	0.23	0.52	0.81
	12–15	0.15	0.11	0.15	0.66	0.21
09	0–3	0.18	0.39	2.3	1.2	n. d.
	3–6	n. d.	0.11	0.51	0.52	n. d.
	6–9	n. d.	0.08	n. d.	0.23	n. d.
	9–12	n. d.	n. d.	n. d.	n. d.	n. d.
	12–21 (7 S)	n. d.	n. d.	n. d.	n. d.	n. d.
10	0–3	0.52	0.05	n. d.	n. d.	n. d.
	3–6	0.39	0.05	0.34	n. d.	n. d.
	6–9	0.53	0.11	0.52	0.59	n. d.
11	0–3	0.76	n. d.	0.34	n. d.	n. d.
	3–6	1.3	n. d.	1.1	n. d.	n. d.
12	0–3	3.9	n. d.	0.18	n. d.	n. d.
	3–6	4.3	n. d.	0.41	1.3	n. d.
	6–9	1.8	0.54	0.71	2.8	n. d.
13	0–3	4.0	n. d.	0.12	11	n. d.
14	0–3	1.7	0.31	0.33	1.1	n. d.
	3–6	4.8	0.09	0.86	0.23	n. d.
	6–9	3.5	0.05	0.20	0.22	n. d.
15	0–3	0.92	0.47	1.1	4.7	n. d.
	3–6	0.37	0.15	0.60	0.95	n. d.
16	0–3	0.09	0.04	0.36	n. d.	n. d.
	3–6	0.11	0.07	0.66	n. d.	n. d.
17	0–3	n. d.	0.14	0.12	1.3	n. d.
	3–6	0.09	0.43	n. d.	3.6	n. d.
	6–9	0.08	0.27	0.13	2.4	n. d.
	9–12	n. d.	0.18	n. d.	1.7	n. d.
	12–15	0.13	0.21	n. d.	1.5	n. d.
18	0–3	0.10	0.35	n. d.	4.7	n. d.
	3–6	n. d.	n. d.	n. d.	0.93	n. d.
	6–9	0.13	n. d.	n. d.	2.7	n. d.
19	0–3	n. d.	0.55	1.1	15	n. d.
	3–6	0.11	0.38	0.16	4.9	n. d.
	6–9	0.17	0.30	0.28	2.1	n. d.
20	0–3	0.74	0.23	0.20	2.7	n. d.
	3–6	0.43	0.20	0.11	2.5	n. d.
	6–9	1.3	0.17	0.14	2.2	n. d.
	9–12	2.1	0.21	0.32	3.1	n. d.

ng/mg for A, 0.05 to 0.89 ng/mg for MDA, 0.1 to 8.3 ng/mg for MDMA and 0.12 to 15 ng/mg for MDE. These regions are in agreement with the data described in literature [12,21–25]. MBDB was found only in two cases between 0.21 and 1.3 ng/mg.

Since MDA was present in no instance in the tablets it should originate, in most cases, from the metabolic *N*-dealkylation of MDMA or MDE. Only in sample 1, an intake of MDA cannot be excluded. Apart from some extreme values the concentration ratio MDA/(MDMA+MDE) is between 0.03 and 0.20. This ratio, which was also found in urine samples of ecstasy-consumers [30], could be a criterion for the deposition in the hair originating from the drug abuse and not from external contamination. An exception seems to be case 13, where MDA is missed despite a very high MDE concentration and the regular intake of the drug reported by the subject.

In Fig. 4 the concentrations of MDA, MDMA and MDE in the proximate 3 cm segments are shown for the 4 abuse intensity degrees in the order of the increasing total concentration of these substances. MBDB was omitted, since it was in no case detected in this segment. The hair analysis proves to be a very sensitive method for the detection of ecstasy abuse, since less than 1 tablet/month in most cases leads to a measurable concentration. For example, volunteer 16 reported the intake of only 1 tablet during the last 9 month. That means that the total amount of MDMA (0,36 ng/mg and 0.66 ng/mg) in both sections of the 8 cm long sample should origin from this one tablet.

Furthermore it can be seen from Fig. 4, that on average the total concentration increases with the increasing number of tablets per month. However, it follows from the large variation in each group, that in a single case even a qualitative conclusion from the

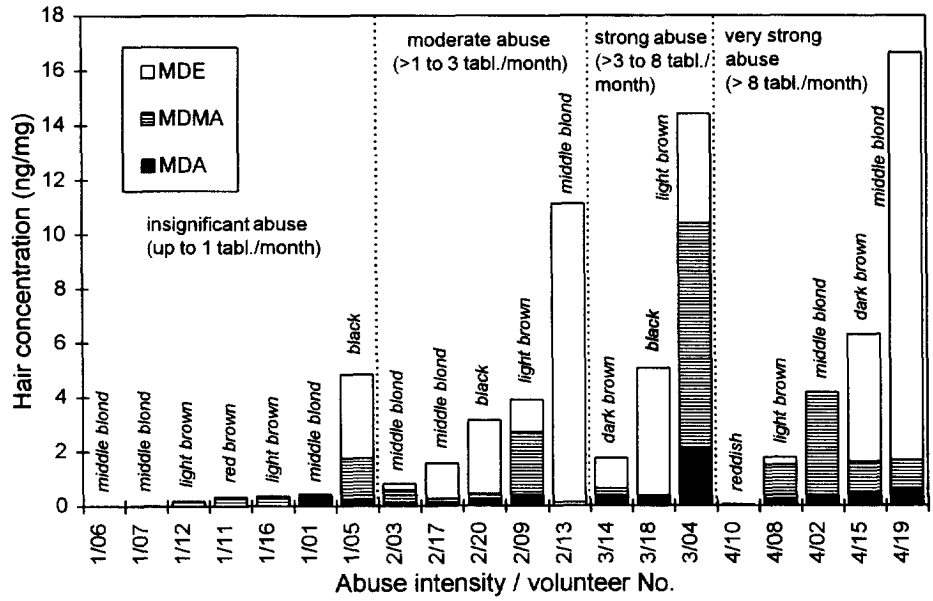


Fig. 4. Hair concentrations of MDA, MDMA and MDE and hair colour of the 20 volunteers arranged in 4 groups according to their reported abuse frequency in the last three month before sampling.

hair concentration about the abuse intensity is not possible. Since the melanin concentration has a strong effect on the deposition of many drugs in hair [31–33], it was tried to explain the large differences in each group by a different hair colour, which is also given in Fig. 4, but there is no unambiguous effect. Typical differences in the hair treatment between the volunteers are not obvious from the reports (Table 2).

In all cases the analytical results of all hair segments were compared with the consumption history of the volunteers' reports. Generally the attribution of a certain hair length region to a particular time period, in which this region should have been formed, is difficult because of the interindividual quite different hair growing rate and because of the inhomogeneity in age of this region due to the growth arrest of a part of the hair in the cathagenic and telogenic phases. It was shown by Poetsch et al. [34] at 41 individuals that the hair growing rate in the anagenic phase with a probability of 82% is between 9 and 13 mm in 28 days, and that as a rule the proximate end of a hair sample represents the time about three weeks before sampling. Since in our investigation the individual determination of the hair growing rate was not possible, for comparison with the drug consumption history, a medium growing rate of 1.15 cm/month (30 days) was assumed and the proximate end of the hair sample was attributed to three weeks before the sampling date. Particularly for the long hair samples 8, 9, and 17, for which 4 to 7 segments were investigated and a strong change of the consumption habits is reported, it was tried to vary the assumed growing rate within the limits of 9 and 13 mm/28 days, but there was no better coincidence. In addition to a chronological elucidation of the drug consumption in the case of the ecstasy abuse it is also possible to recognize, which of the ecstasy drugs is preferentially ingested in the corresponding time periods. For example, a change from MDMA to MDE or conversely is obvious in the cases 08 or 14.

Volunteer 04 reported a regular intake of 2 ecstasy tablets each second weekend since 1992 as well as an irregular consumption of speed with an interruption in winter 95/96 and 1 g intake about 3 weeks before sampling. In this case the hair was investigated in 3 cm segments and in 1 cm segments as well. The results are shown in Fig. 5. A, MDA, MDMA and MDE were found in all segments along the full hair length. MBDB is missed in the proximate 3 cm and 1 cm segments, but is present in all other segments with a maximum in the second 3 cm and the fourth and fifth 1 cm segments. The concentration of the ecstasy drugs decreases in almost all segments in the order MDMA>MDE>MBDB. Only in the second 3 cm and in the fourth 1 cm segment MDE dominates. Obviously tablets with this substance were more frequently taken in the last three to four month before sampling with a maximum about 4 month before sampling.

Since MBDB was rather rare in the tablets in comparison to MDMA and MDE (c.f. Table 3), it is improbable that volunteer 4 regularly ingested it over a time period of more than 1 year. Instead of that it must be assumed that MBDB was only consumed in the time period corresponding to the fourth and fifth 1 cm segments and was incorporated into the elder hair segments by sweat with a decreasing concentration in the distal parts of the hair. This is in agreement with the information of this volunteer, that he took only 2 tablets with the stamping "$" (substance MBDB according to our analysis) in autumn 1995. The minor MBDB concentration in the second and third 1 cm segments could be explained by the catagenic or telogenic parts of the hair sample since a drug transport from elder to younger hair segments by sweat is improbable.

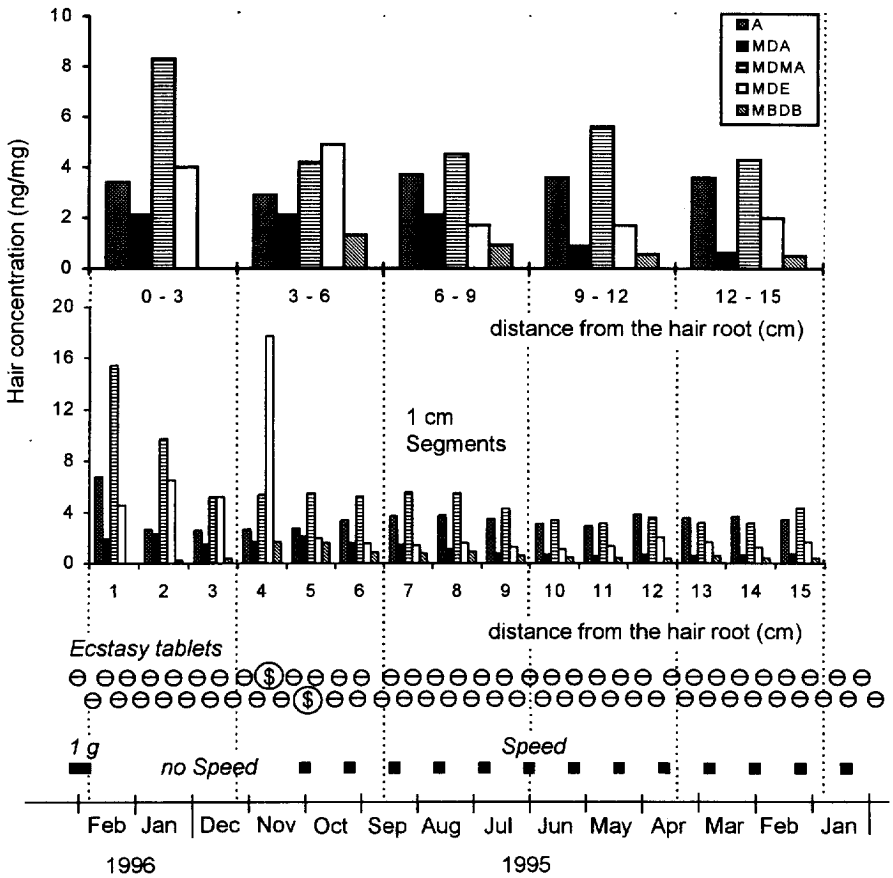


Fig. 5. Hair concentrations of Amphetamine, MDA, MDMA, MDE and MBDB in the 3 cm and in the 1 cm segments of volunteer 04 compared to the reported intake of ecstasy tablets and speed. In autumn 1995 the consumption of 2 tablets with the stamping "S" (substance MBDB) is reported. According to [34] a medium growing rate of 1.15 cm/month is assumed and the proximal end of the sample is dated to 3 weeks before sampling.

There is no strong difference in the amphetamine concentration between the segments, and the reported interruption of the speed uptake in winter 95/96 is only indicated by a small decrease of the amphetamine concentration in the corresponding hair segments. Since volunteer 4 reported a relatively high intake of speed three weeks before sampling, also amphetamine could have been incorporated into these segments by sweat.

It follows from the comparison between the 3 cm and the 1 cm segment analysis (Fig. 4) that despite the broadening effect of the catagenic and telogenic hair portion and of the incorporation via sweat more information can be obtained, if more and shorter segments are investigated.

Also for volunteer 08 (Fig. 6), all four drugs A, MDMA, MDE and MBDB as well as the metabolite MDA were detected, but in smaller concentrations. In this case periods

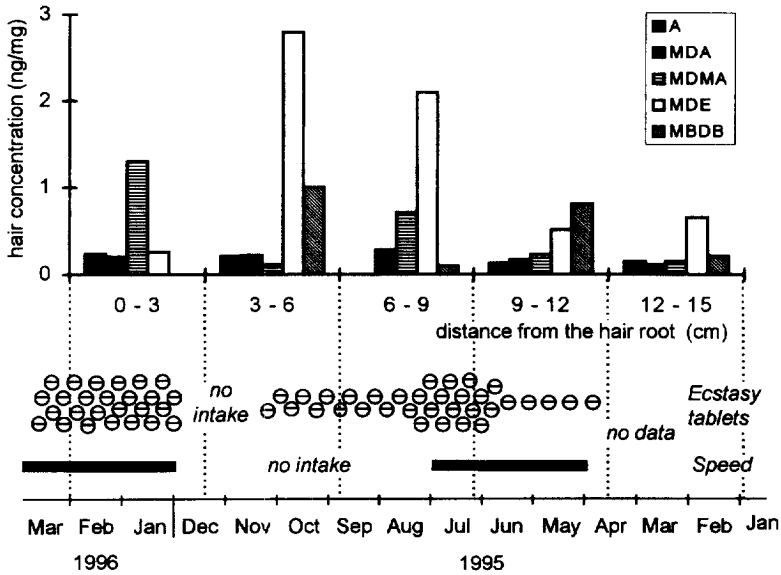


Fig. 6. Hair concentrations of amphetamin, MDA, MDMA, MDE and MBDB in the 3 cm segments of the volunteer 08 and reported intake of ecstasy tablets and speed. Time conditions as in Fig. 5.

with a very high and with a rather moderate intake were reported. It is seen from the comparison in Fig. 6 that these periods do not well coincide with the hair concentrations in the corresponding 3 cm hair segments. This cannot be improved by changing the time scale of the growing rate within the limits described by Poetsch et al [34]. If the reported data are correct a possible explanation could be that the more lipophilic compounds MDE and MBDB are more effectively incorporated into hair or less effectively removed by washing than MDMA, and that in 1996 a change in the intake from MDE and MBDB to MDMA occurred.

Volunteer 17 (Fig. 7) consumed mainly MDE as is obvious from the high concentrations of this substance in comparison to the small concentrations of MDMA, which is only just above the detection limit in segments 1 and 3. Despite the denied intake of speed a small concentration of amphetamine is found in segments 2, 3 and 5, which possibly originates from the amphetamine content of some of the tablets. In this case reduced consumption in the last 2 1/2 month before sampling is clearly recognized in a concentration decrease of MDE and its metabolite MDA in the first segment. On the other hand, the high concentrations in segments 3 to 5 do not correlate with the reported intake of only 1 tablet every 6 to 8 weeks and can again only be explained by the later deposition from sweat during the intense consumption period between February and May.

The incorporation via sweat should also be the reason for the very high MDE concentration in the first segment of the sample 19. In this case a particularly intense consumption about two weeks before sampling and a regular intake of up to 3 tablets per week all the time since October 1994 was reported (cf. Tables 2 and 4). According to

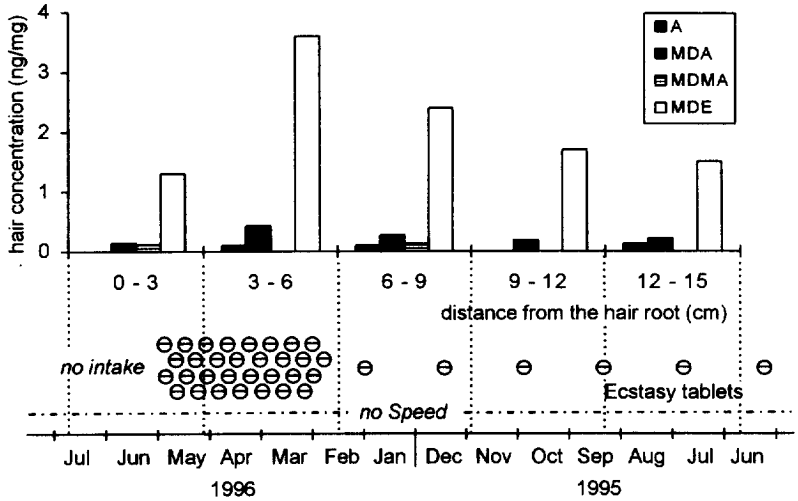


Fig. 7. Hair concentrations of amphetamine, MDA, MDMA and MDE of the 3 cm segments of the volunteer 17 and reported intake of ecstasy tablets. Time conditions as in Fig. 5.

Poetsch et al. [34] the hair section formed during the event two weeks before sampling should not be contained in the sample. Nevertheless the extreme MDE concentration in the first segment confirms this intense consumption.

In contrast to the examples discussed above there are two cases with a negative analytical result despite a reported ecstasy intake. In case 06 altogether 3 tablets were ingested during the growing period of the 6 cm long hair sample, but no ecstasy drugs were found. Also in the 23 cm long sample 07, which was investigated in 7 segments, neither the intense consumption period (altogether about 30 tablets between 8 and 6 month before sampling) nor the later intake of about 1 tablet per month are detected. It must be assumed that the drugs were removed by the usual washing of the hair (6 times or 3–4 times per week), since no other hair treatment was reported.

The strong decrease of the MDA, MDMA and MDE concentrations from segment 1 to segment 3 and the total absence of these drugs in the other 4 segments of sample 09 (Fig. 8) despite the strong or moderate consumption habit of this volunteer for more than two years before sampling is another example for the slow removal of the compounds by washing. Similarly the decrease of the concentrations from the first to the second segment in sample 15 can be explained. Obviously this removal depends strongly on the properties of the hair since in sample 20 with similar consumption habits and hair washing frequency the MDA and MDE concentrations of all 4 segments do not change very much. The amphetamine concentration of this sample is even three times higher in the distal segment 4 than in the proximate segment.

From the results described above it is difficult to estimate the time after the intake, during which in general these substances are detectable in hair. In some of the samples a detection time of at least a half to one year can be concluded from the elevated concentration of one or more of the substances in the corresponding segments in

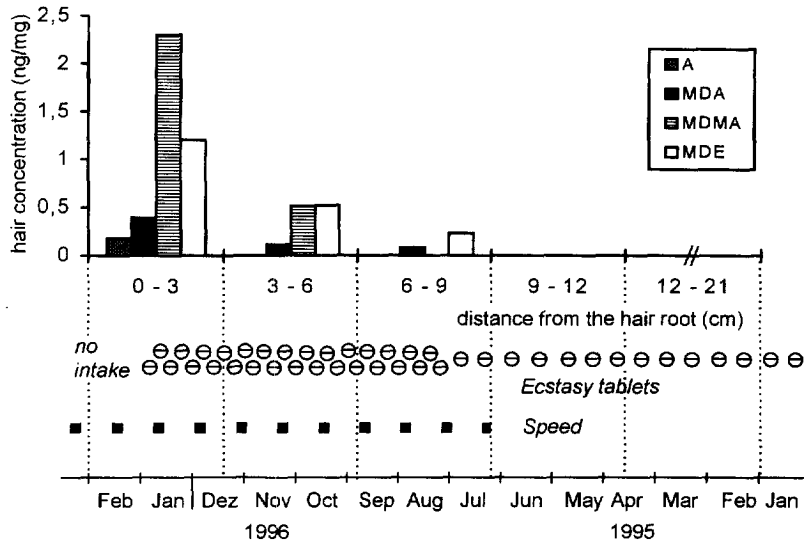


Fig. 8. Hair concentrations of amphetamin, MDA, MDMA and MDE of the 3 cm segments of the volunteer 19 and reported intake of ecstasy tablets and speed. Time conditions as in Fig. 5.

comparison to the younger segments (MBDB in sample 08, MDMA and MDE in sample 10, MDA, MDMA and MDE in sample 12, A in sample 20). In other cases an apparently long detection time could be simulated by the distal incorporation of the drugs from sweat as described above. This is particularly in the case of participants of techno and rave parties a very important restriction for the chronological interpretation of hair analysis results, since a loss of up to 5 l water (mostly as sweat) during one event is described. These findings are different from the results of Nakahara et al. [24], who found a good coincidence between hair analysis results and self-reported drug history for 9 of 11 methamphetamine abusers. Obviously in their quite different clientele the deposition from sweat is not as important as in our cases.

4. Conclusions

With respect to the practical application of the hair analysis of amphetamine and methylenedioxyamphetamine derivatives and the interpretation of the results in forensic cases the following conclusions can be deviated from these investigations:

1. Amphetamines and the methylenedioxyamphetamines MDA, MDMA, MDE or MBDB can very sensitively be detected in most of the abuse cases. In cases of ecstasy abuse the intake of 1 tablet may lead to a hair concentration in the order of 1 ng/mg. In general the hair concentration increases with increasing abuse intensity, but there are very large interindividual differences.

2. Particularly for participants of techno or rave parties the deposition from sweat is a predominant incorporation mechanism and seems to be the reason for the high concentration also in hair segments, which were formed before the abuse. The extend of the deposition from sweat decreases with increasing distance from the hair root and is interindividually quite different.
3. Because of the substantial effect of the deposition from sweat the chronological interpretation of results from the segment hair analysis is even more difficult. A positive analytical result in a distal hair segment may origin from a later abuse and therefore is no prove that in the corresponding time region the drug really was taken.
4. A positive result in the 3 cm proximal segment of anagen hair proves the intake of the drug within the last 3–4 month. False positive results in this hair segment should be caused by telogenic hair and can be avoided by comparison with the second segment. A transport of the drugs from more distal to more proximal segments by sweat was not observed.

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