Acta Poloniae Pharmaceutica – Drug Research, Vol. 71 No. 1 pp. 25–33, 2014 ISSN 0001-6837
Polish Pharmaceutical Society

ANALYSIS

AMPHETAMINES IN WASTEWATER OF THE CITY POZNAŃ (POLAND) - ESTIMATION OF DRUG ABUSE

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Abstract: The aim of the study was to determine the profile of amphetamines consumed by a community in Poland. Amphetamine, methamphetamine and MDMA (ecstasy) were detected in wastewater samples collected from the main Wastewater Treatment Plant in the city of Poznań (about 687 000 people) using liquid chromatography/tandem mass spectrometry (LC-MS-MS). Back-calculations used in the sewage epidemiology approach were applied to estimate the level of consumption of the drugs being analyzed. These types of studies were carried out for the first time in Poland for a considerable period – from June 2009 to December 2010. The analysis of variance (ANOVA) confirmed significant monthly differences in amphetamine consumption. The concentration of amphetamine, methamphetamine and MDMA in wastewater samples and the levels of their consumption were lower than reported in other European countries, but unexpectedly, the ratio of consumed methamphetamine to MDMA and the consumption level of methamphetamine were relatively high. This study shows that sewage epidemiology is a promising tool, especially when combined with classical methods, to estimate illicit drugs use in a particular population. Therefore, efforts should be made to monitor the profiles and consumption levels of drugs and to extend the scope of the research to other illicit substances, especially cannabinoids and cocaine.

Keywords: illicit drugs, amphetamines, wastewater, HPLC-MS/MS

Consumption of illicit drugs is increasing each year, with some changes in the profile of the drugs used. The available data concerning drug consumption are published in the national and European reports. Official figures of the prevalence of drug abuse are currently derived from population surveys integrated with crime statistics, medical records, drug production, and seizure rates. However, such an estimation of illicit drugs consumption only gives the general picture and is very subjective as it is based on information from the consumers themselves. Moreover, surveys are expensive to conduct and only a few European countries collect information every year, although there are some differences in their methodology, so results should be interpreted with caution.

According to the European Monitoring Centre for Drugs and Drug Addiction Annual Report 2010 (1), cannabis remains the most popular illicit drug in Europe, although levels of consumption differ considerably between countries. In the eastern countries, the prevalence level of cannabis consumption is increasing and often exceeds the level found in Western Europe. Cocaine is the second most commonly used illicit drug in Europe at a high and still rising level. The problem of amphetamine use is mainly reported by countries in northern Europe, although methamphetamine use remains largely restricted to the Czech Republic and Slovakia, as well as their neighboring countries. In many eastern countries amphetamine (or methamphetamine) remains the most commonly used stimulant drug. Based on surveys conducted in Poland in 2008, cannabis and amphetamine are the most commonly used illicit drugs (1, 2).

Within the last few years a new approach, termed ‘sewage epidemiology’, has been applied in order to estimate the consumption levels of illicit drugs recommended by EMCDDA. This method proposed by Daughton and Thernes (1999) was first implemented by the Zucatto research group (3-5) to estimate the level of consumption of cocaine in some Italian cities, and was based on an analysis of surface and wastewater samples. Such investiga-

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tions have been conducted in the last few years in other European countries, such as Belgium (6-8), United Kingdom (9), Italy - Florence (10), Spain (11-13), Croatia (14), and Switzerland (15), as well as in Canada (16) and the United States of America (17). Combined with a classical population survey approach, this will provide an integrated and powerful tool not only for studying drug use trends in the population, but also for assessing the effectiveness of various treatments applied for drug abuse prevention.

The new methodology provides the possibility of monitoring the consumption levels of illicit drugs in a specific area over a long period of time and for making comparisons with national reports based on the population surveys. The sewage approach was first applied in this area of Poland and is especially valuable because of the length of time required for such investigations.

EXPERIMENTAL

Wastewater samples

The study was performed in Poznań, Poland's fifth largest city. The samples were collected from the central wastewater treatment plant, which, at the time served almost the whole city including its suburbs, in total about 687 000 people. Two wastewater samples (10 L each) were collected twice a week, on Monday and on Wednesday, from June 2009 to December 2010. All samples were collected at the same point before any chemical and physical treatment, with the exception of sedimentation, and the mean flow rate was 130 000 m^3/day. Analysis of the samples was performed the same day immediately after collection.

Reagents

All pure standards: amphetamine, methamphetamine, 3,4-methylenedioxyamphetamine (MDA), 3,4-methylenedioxymethamphetamine (MDMA or ecstasy), 3,4-methylenedioxyethylamphetamine (MDEA) and their deuterated molecules used as internal standards: amphetamine-d_6, methamphetamine-d_9, MDA-d_5, MDEA-d_5 and MDMA-d_5 were purchased from Certilliant, a Sigma-Aldrich Company. The standards (solutions in methanol (1 mg/mL)) were diluted to 10 ng/µL with methanol and stored in the dark at -20°C. All other reagents were acquired from J.T. Baker (USA).

Sample treatment and analysis

Samples were filtered on a glass microfiber filter GF/A 1.6 µm (Whatman, Kent, U.K.), prior to extraction were spiked with 15 ng of each internal standard and the pH was adjusted to 7.0 ± 0.4 with phosphate buffer (pH = 7.0). Solid-phase extraction of the substances being analyzed was performed using Bakerbond Narc-2 mixed mode cartridges, which were conditioned with methanol (2 mL) followed by deionized water (2 mL) and then by phosphate buffer (2 mL, 0.1 M, pH 7.0). Next, the sample was passed through the cartridges under a vacuum at a flow rate of 10 mL/min. When the sample was eluted under gravity, the column was washed with deionized water (2 mL) followed by hydrochloric acid (0.1 M, 0.5 mL) and then by methanol (0.5 mL). A vacuum was applied and the cartridges were dried for 20-30 min. The analytes were eluted into a vial with a mixture of chloroform : isopropanol : ammonium hydroxide (80 : 30 : 3.2 mL). The eluates of two samples (each 10 L) were pooled and dried under a nitrogen stream.

Liquid chromatography – tandem mass spectrometry

The pooled and dried samples were redissolved in 200 µL of mobile phase, centrifuged and transferred into glass vials for instrumental analysis. Twenty microliters of the solution were injected in the LC-MS spectrometer (Agilent HPLC 1200 series, 6410B Triple Quad LC/MS System). Chromatographic separation was performed using a capillary column (Agilent Zorbax XDBC18, 4.6 × 50 mm × 1.8 µm) at a flow rate of 0.45 mL/min. The mobile phase were solutions: water with formic buffer (pH~3.2) and acetonitrile with a gradient from 10 to 70% of acetonitrile (6.5 min.). The capillary voltage was 4000 V, the temperature was 300°C, the auxiliary and collision gas was N_2. The collision energy and tube lens were optimized separately for each analyte and standards. All selected analytes were analyzed in positive ionization mode (ESI+). Identification and quantification were performed using two characteristic transitions in multiple-reaction monitoring (MRM) mode for the fragmentation products of the protonated or deprotonated pseudomolecular ions of each substance and each deutered analogue (Tab. 1).

A 8-point calibration curve was constructed at 4, 8, 12, 16, 20, 24, 28 and 32 ng for amphetamine, methamphetamine, MDA and MDMA and the solutions were spiked with 30 ng of all internal standards. Validation was carried out according to the Funk methodology (18), including testing homogeneity, linearity, homogeneity of variances (precision), outliers and securing the lower range limit. The matrix effect was determined by analyzing 50

Table 1. Conditions for MRM determination of illicit drugs.

Substance	Retention time (min)	Fragmentor voltage	Precursor ion m/z	Product ion I m/z and collision energy (eV)	Product ion II m/z and collision energy (eV)
Amphetamine-d_6	3.4	60	142.1	125.1 (8)	93.1 (17)
Amphetamine	3.4	70	136	119.1 (5)	91.1 (17)
MDA-d_5	3.6	70	185.1	168.1 (5)	110.1 (21)
MDA	3.6	60	180.1	163 (5)	105.1 (21)
Methamphetamine-d_9	3.8	80	159.2	125.1 (5)	93.05 (17)
Methamphetamine	3.8	80	150.1	119.1 (8)	91 (17)
MDMA-D5	3.9	80	199.1	165 (9)	107.1 (25)
MDMA	3.9	80	194.1	163.1 (5)	105.1 (25)

Table. 2. Validation according to Funk (DIN 32645).

Substance	a	b	Residual standard deviation	Process standard deviation	Process variation coefficient (%)	Decision limit DL (ng/L)	Detection limit LOD (ng/L)	Quantification limit (LOQ) (ng/L)
	y = bx + a					(DIN 32645)		
Amphetamine	531	1270	2.57	0.20	1.12	0.36	0.71	1.07
						range 1 - 2.4		
Methamphetamine	15555	126.7	6.96	0.55	3.06	0.81	1.65	2.32
						range 3.5 – 7		
MDMA	40.28	1143	6.09	1.11	6.09	0.89	1.77	2.66
						range 3 - 4.8		

mL of wastewater samples spiked with internal standards. The recoveries for the whole process of sample preparation, filtration and extraction were set within the range 0.80-0.93. The detection limits (LOD) and quantification limits (LOQ) for the whole method were calculated by spiking wastewater samples with different amounts of the substances analogously, just as on the calibration curve. The results of all validation activities are shown in Table 2.

Back-calculation of community drug use

Estimation of community drug use was done according to the method described by Zuccato et al. [3] (Fig. 1). Because surveys conducted in Poland show that amphetamine is a commonly used illicit drug, a group of amphetamine-like stimulants was chosen for analysis (amphetamine, methamphetamine, MDA, MDMA, MDEA). In the case of amphetamines, the substances which are used as drug target residues (DTR) are the parent drugs, because all are excreted mainly as unchanged compounds. The concentrations of these substances were very low, and therefore the dried residues of two untreated wastewater samples (each 10 L) after filtration and SPE extraction were pooled and combined by redissolving them in a mobile phase to perform HPLC-MS-MS analysis. The mean concentrations of DTR in ng/L of all samples collected in one month were multiplied by the mean flow monthly rate in the Wastewater Treatment Plant (WWTP) to give the amount of DTR (grams) discharged per month. This value was then divided by the number of people served by the WWTP to estimate the grams of DTR excreted in wastewater per person per month and finally normalized to a value of grams per month per 1000 people. Cocaine consumption was originally estimated by Zuccato from the data for its major metabolite, benzoylecgonine (BE), so a molar ratio of 1.05 was applied to compensate for the higher molecular weight of BE compared with cocaine. In the case of amphetamines the parent drug is determined and therefore the molar ratio is 1, so the correction factor for the estimation takes into consideration only the percentage of the drug dose excreted as DTR (for amphetamine it is 30, for

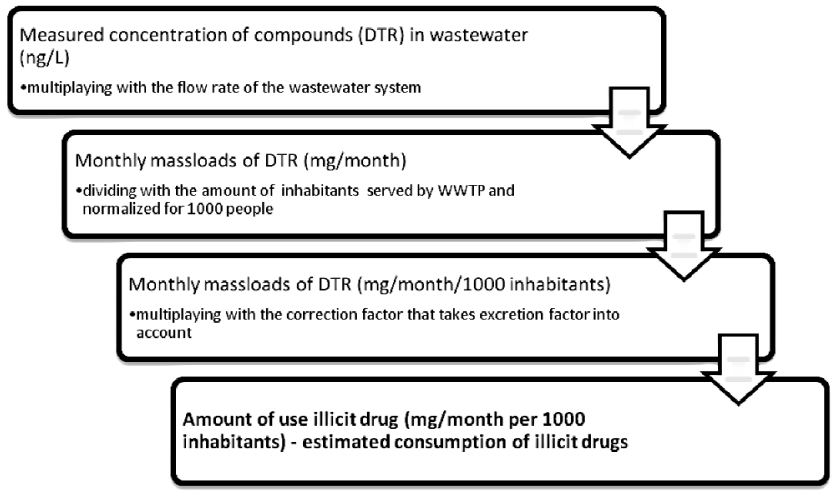

Figure 1. Scheme of the overview of applied back-calculations in the sewage epidemiology approach

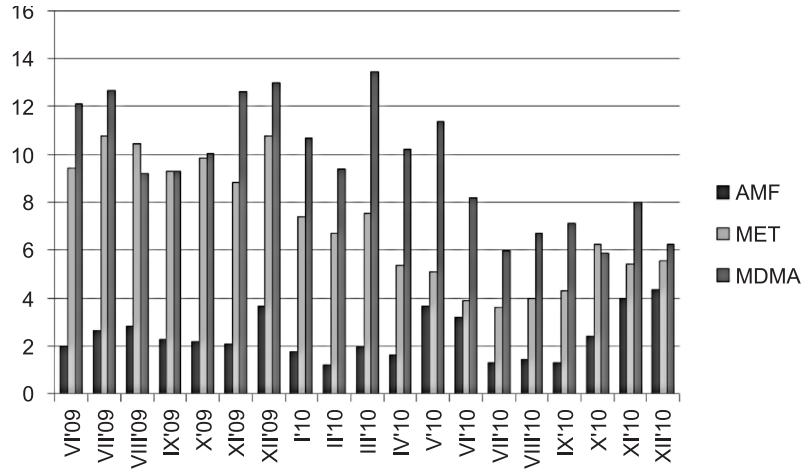

Figure 2. Mean loads DTR/mg/month/1000 people from June 2009 to December 2010 for amphetamine (AMF), methamphetamine (MET) and MDMA (ecstasy)

methamphetamine 43 and for MDMA 65). Correction factors (the fraction of the consumed parent drug extracted as DTR in urine and the parent drug-to-DTR molar mass ratio) were 3.3 for amphetamine, 2.3 for methamphetamine and 1.5 for ecstasy. Finally, the amount of illicit drugs consumed monthly by 1000 people was estimated.

Because of the procedure and the low concentrations of compounds analyzed (DTR), only monthly levels of consumption could be compared and the significance of monthly differences was checked by ANOVA analysis of variance. There was no possibility to observe the daily profile of illicit drugs consumed.

It is also possible to estimate the number of doses consumed by the local community (1000 people) per day or month by dividing the consumption value by a single typical dose for each illicit drug. The typical oral dose for amphetamine and methamphetamine is 30 mg, but for MDMA (ecstasy) it is 100 mg. However, it is recommended to focus on the amount of illicit drugs estimated for 1000 people per day or the amount of DTR excreted per day per 1000 people and these results should be monitored for a period of time to give an objective insight into the level and profile of illicit drugs consumed by the local community.

Table 3. Results of determination of amphetamine (AMF), methamphetamine (MET) and MDMA (ecstasy) and estimated consumption.

Month	Concentration (ng/L)			DTR mass loads mg/month/1000 ppl			Drug consumption mg/month/1000 ppl			Number of doses dose/month/1000 ppl		
	AMF (± 0.03)	MET (± 0.07)	MDMA (± 0.07)	AMF	MET	MDMA	AMF	MET	MDMA	AMF	MET	MDMA
Jun'09	0.25	1.22	1.56	1.99	9.42	12.12	6.573	21.66	18.18	0.22	0.72	0.14
Jul'09	0.32	1.35	1.54	2.64	10.78	12.64	8.70	24.78	18.96	0.29	0.82	0.14
Aug'09	0.40	1.46	1.29	2.84	10.42	9.21	9.37	23.98	13.82	0.31	0.80	0.10
Sep'09	0.34	1.38	1.38	2.27	9.30	9.27	7.49	21.40	13.91	0.25	0.71	0.10
Oct'09	0.30	1.37	1.39	2.18	9.83	10.04	7.22	22.61	15.06	0.24	0.75	0.11
Nov'09	0.30	1.26	1.82	2.18	8.84	12.61	7.22	20.34	18.92	0.24	0.68	0.14
Dec'09	0.48	1.41	1.71	3.64	10.76	13.00	12.02	24.76	19.50	0.40	0.82	0.15
Jan'10	0.34	1.41	2.03	1.78	7.42	10.69	5.89	17.06	16.04	0.19	0.57	0.12
Feb'10	0.25	1.37	1.92	1.24	6.70	9.39	4.09	15.40	14.08	0.14	0.51	0.11
Mar'10	0.27	1.03	1.83	1.97	7.56	13.44	6.52	17.38	20.16	0.22	0.58	0.15
Apr'10	0.29	0.96	1.83	1.63	5.38	10.21	5.40	12.38	15.31	0.19	0.41	0.12
May'10	0.59	0.81	1.82	3.68	5.08	11.39	12.13	11.68	17.01	0.40	0.39	0.13
Jun'10	0.69	0.83	1.75	3.22	3.90	8.20	10.63	8.97	12.31	0.35	0.30	0.09
Jul'10	0.25	0.69	1.14	1.32	3.60	5.97	4.38	8.28	8.96	0.15	0.27	0.07
Aug'10	0.24	0.66	1.12	1.43	3.99	6.72	4.73	9.19	10.08	0.16	0.31	0.08
Sept'10	0.23	0.76	1.27	1.32	4.30	7.14	4.36	9.89	10.71	0.14	0.33	0.08
Oct'10	0.48	1.24	1.17	2.43	6.24	5.90	8.02	14.36	8.85	0.27	0.48	0.06
Nov'10	0.64	0.87	1.29	3.97	5.41	8.02	13.11	12.44	12.02	0.44	0.41	0.09
Dec'10	0.71	0.91	1.02	4.34	5.58	6.27	14.32	12.83	9.41	0.47	0.43	0.07

RESULTS

The results of a nineteen-month study of wastewater concerning amphetamine, methamphetamine and MDMA (ecstasy) are reported in Table 3 and Figure 2. The bar graph presents population-standardized DTR mean monthly loads collected from June 2009 to December 2010. As far as analysis of very low concentration is concerned, in this case it was necessary to perform validation of the analytical process. Table 2 contains basis figures related to the quality assurance of quantification. Table 3 presents figures calculated according to the method given in the calculation chapter, i.e., monthly DTR loads, drug consumption of amphetamines (also referred to as collective drug consumption rates) and estimated doses consumed per 1000 people. For the correct interpretation of increasing or decreasing tendencies, data grouped by month were analyzed by a one-way ANOVA and Multiple Comparison Test. The results of this analysis are presented in Table 4 and in Figure 3. ANOVA shows significant differences between the means of monthly DTR loads (mg/month/1000 people) except for February-July-September 2010 and June 2009-March 2010 in the case of amphetamine, and April-September 2010 and August-December 2009 in the case of methamphetamine.

As far as amphetamine is concerned, an increase was noted in December 2009, May and June 2010 and another increase in the monthly load of amphetamine was noticeable in November and December 2010. A decrease in amphetamine use was observed in February 2010 and from June to September 2010.

As far as methamphetamine is concerned, there is a visible decreasing tendency in monthly loads from June 2009 to July 2010 with the exception of December 2009 when a single increase was observed. There was an increase in DTR mass loads from August 2010 to the end of research but this was significantly smaller than in the initial period of monitoring.

MDMA DTR mass loads showed the highest values in June and July 2009 compared to November to December 2009. Two increases were recorded in 2010, namely in March and May. From June 2010 to the end of the research, a downward trend was observed.

DISCUSSION

Monitoring of any figures connected with DTR extraction loads or estimated local community consumption of the drugs analyzed showed that this method can detect any fluctuations and trends in consumption that occur during the course of a week, month or year (depending on the methods of sampling). The analysis of profiles of drugs consumed which are summarized in Table 3 or in a bar graph (Fig. 2) showed an increase in amphetamine and methamphetamine consumption during the traditional periods of examinations at high schools and universities and institutions at higher education level i.e., November-December and May-June. As for ecstasy, the increase in consumption is visible in November, December, May, June as well as in the

Table 4. Results of analysis of variance ANOVA.

	Sums of squares (SS)	Degree of freedom (dF)	Mean squares (SS/df) (MS)	F-statistics	Prob > F (p)
	Amphetamine				
Columns	65.9156	18	3.66198	13902.48	5.24361e-097
Error	0.015	57	0.00026		
Total	65.9307	75			
	Methamphetamine				
Columns	449.216	18	24.9564	106918.78	2.97059e-122
Error	0.013	57	0.0002		
Total	449.229	75			
	MDMA				
Columns	452.88	18	25.16	187006.79	3.57393e-129
Error	0.008	57	0.0001		
Total	452.887	75			

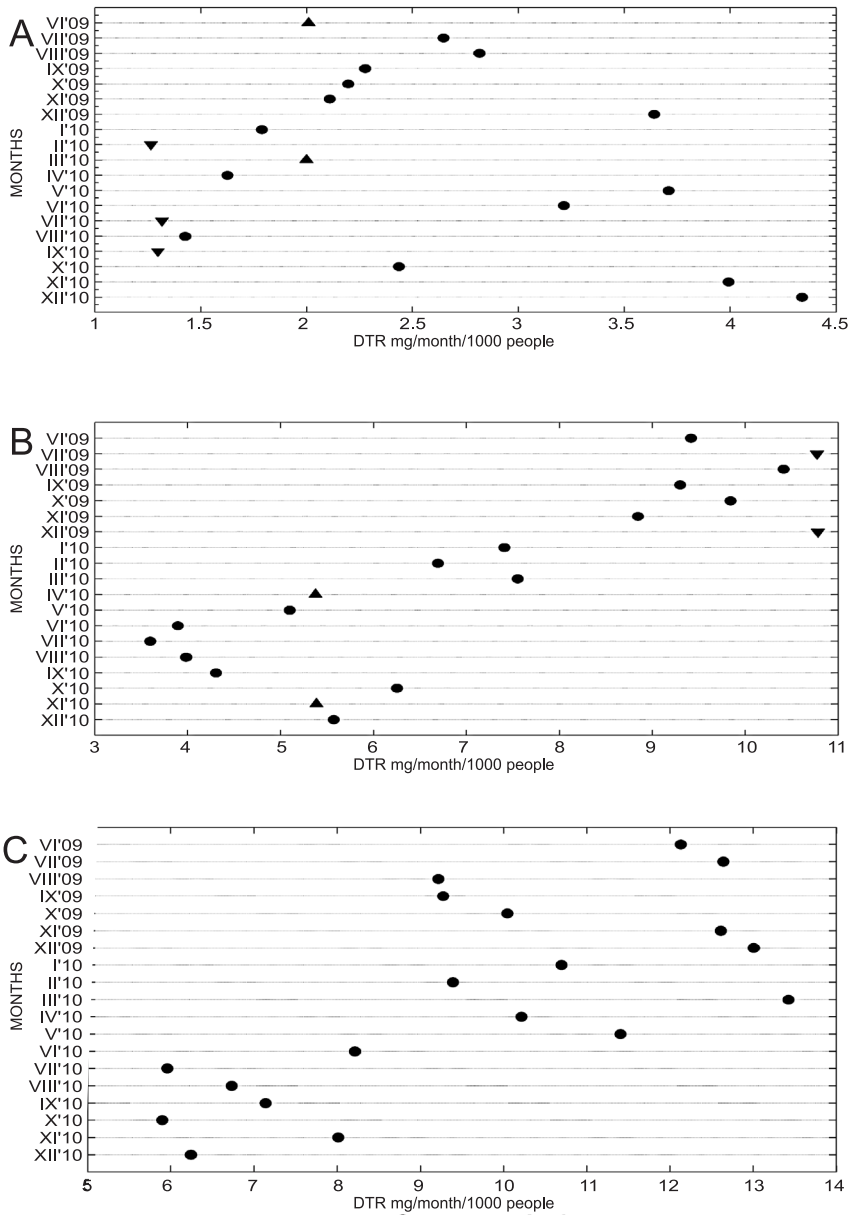

Figure 3. **A**. Amphetamine. **B.** Methamphetamine. **C.** MDMA (ecstasy). Multiple comparisons. Visualization of the difference between group means (DTR loads/mg/month/1000 people). Triangle in the vertical line - no significant difference, circle – significant difference

June-July holiday period. In the case of all three amphetamines, there is a noticeably rapid decrease (April 2010 to July) followed by increasing consumption to December 2009-end of research. It is hard to explain this phenomena but the authors connect this information with the closing down of all designer drug shops in Poland in October 2010. Following this, the small increase can be related to the lack of availability of designer drugs on the market.

Our data prove that amphetamines are commonly present in wastewater and they can be used as a valuable and exact parameter to estimate profile, tendency and consumption figures. These parameters are good tools for evaluating the efficiency of anti-drug activities and the validity of expenditures on individual projects. Comparisons of the estimated values of doses consumed per 1000 people with the results from other European countries reveal that there is a low level of daily consumption of amphet-

amine and MDMA in our city. It should be indicated that the ratio of methamphetamine to MDMA consumed is much higher than in other countries and there is unexpected and relatively high level of consumption of methamphetamine compared with other amphetamines. Such a discrepancy in the level of consumption is hard to explain, because the general consensus is that amphetamine is the far more widely consumed drug. It is important to remember that this research was carried out during a period when the designer drug trade flourished in Poland. Furthermore, according to the National Bureau for Drug Prevention, there has recently been a significant increase in the production and consumption of methamphetamine in Czech Republic due to the liberal laws there. Moreover, the latest police reports of the discovery of an alleged methamphetamine manufacturing laboratory in Poznań seem to confirm the results of this study.

There is also an agreement between surveys conducted by the National Bureau in 2009 (2) and our research, namely that amphetamines are among the most commonly used illicit drugs in Poland. However, the profile of amphetamines consumed was investigated here for the first time. It will be interesting to determine the level of illicit drugs in wastewater samples over a long period of time to monitor the changes in profiles and the levels of drug consumption and to extend the scope of the research to other substances, especially to cannabinoids and cocaine.

CONCLUSIONS

"Sewage epidemiology" is a powerful tool to monitor the levels and profile of illicit drugs consumed by the local community and it has been already applied successfully by research groups in many countries. Compared with surveys, this methodology gives an objective picture of the drug abuse and constitutes a real-time approach to estimate the consumption of illicit drugs in a given area. For the first time in Poland, such an investigation was carried out over a relatively long period of time, from June 2009 to December 2010 and the results are published. The concentrations of three amphetamines were determined in a wastewater samples from a large Polish city, Poznań. The levels of consumption of amphetamine, methamphetamine and MDMA were lower than in other European countries, but the levels of methamphetamine and MDMA were relatively higher compared with the level of amphetamine consumption. Our study proved that the profile and the levels of consumption of illicit drugs in our country might be quite different from those in the Western Europe.

Acknowledgments

This study was supported by Polish Ministry of Science and Higher Education and National Bureau for Drug Prevention. The authors are grateful to the staff of the central wastewater treatment plant of the city of Poznań in Koziegłowy for helping with wastewater sampling and providing data on influent flow rates.

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Received: 26. 03. 2013