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Short communication

Prevalence and distribution patterns of amphetamine and methamphetamine consumption in a federal state in southwestern Germany using wastewater analysis

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ABSTRACT

Background: Wastewater analysis is a new approach developed to estimate drug (of abuse) consumption in large communities, such as cities or even whole countries.
Aims: This paper presents data on the loads of amphetamine and methamphetamine measured in ten wastewater treatment plants in different parts of a German federal state. It provides an estimation of the intensity of the consumption and a comparison to other regions in Germany and Europe.
Methods: Consumption of amphetamine and methamphetamine was estimated by analysis of drug residues in composite 24 h samples of wastewater after mechanical treatment over one week by liquid chromatography-high resolution tandem mass spectrometry. Samples were collected from the inlet of ten wastewater treatment plants (WWTP) in the federal state of Saarland, representing bigger cities (>200,000 inhabitants), medium sized cities (>50,000 inhabitants), small cities (>25,000 inhabitants), and villages (<25,000 inhabitants). In each WWTP, samples were taken daily for seven consecutive days in July 2014.
Results: We observed differences of amphetamine versus methamphetamine loads (expressed as mg/day/1000 inhabitants): Amphetamine loads were much higher in all tested WWTPs indicating a low prevalence of methamphetamine abuse in the federal state of Saarland at the tested period. These findings are in line with previous reports about the distribution of amphetamine and methamphetamine in Germany and Europe.
Conclusions: The approach confirms that wastewater analysis can provide valuable data about the abuse pattern of drugs of abuse in cities and larger areas. It can be useful for planning interventions aimed at specific areas and substances.

1. Introduction

Support of public health and law enforcement services with data on patterns of drug use can be done by analysing the urinary excretion products of drugs and alcohol in wastewater influent (Castiglioni et al., 2008; Mastroianni et al., 2014; van Nuijs et al., 2011; Zuccato et al., 2008). Such data can be used to estimate the relative amount of drugs consumed by people living in a defined area and data can support drug precaution programmes. Wastewater analysis especially allows for monitoring temporal and spatial trends in drug use. It also provides highly frequent updates on estimates of drug use, identifies changing habits, and the use of new substances (Castiglioni et al., 2014; Ort et al., 2014). Ort et al. (2014) recently presented a work dedicated to the spatial differences and temporal changes in illicit drug use in Europe and concluded that wastewater analysis provided complementary evidence on illicit drug consumption and that it can measure total illicit drug use more quickly and regularly than surveys. They monitored amongst other the excretion pattern of amphetamine (AMP) and methamphetamine (METH). The highest AMP loads were found in Belgium, the Netherlands, western Germany, and northern Europe. In contrast, the locations with the highest METH loads were found in the Czech Republic, Slovakia, and eastern Germany. In Germany, Dülmen and Dortmund (West) and Dresden (East) were part of the study. This indicated an apparent geographical difference in the use of the amphetamine-like stimulants. However, as there were only two cities monitored in western Germany, data on this

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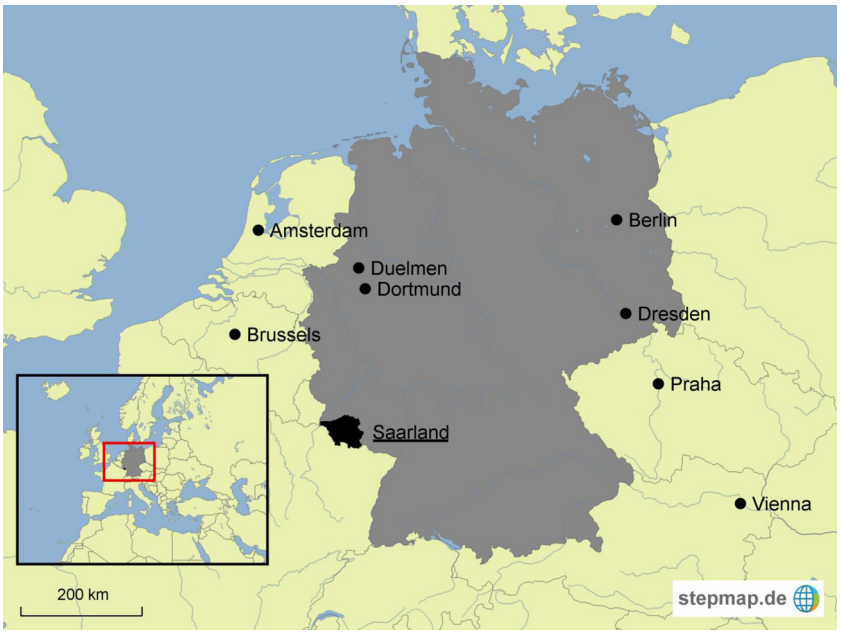

Fig. 1. Map indicating the sampling area (black) within central Europe.

phenomenon in the consumption pattern of AMP and METH within Germany needs further support. Therefore, the aim of this study was to analyse for differences in the loads of AMP and METH within one federal state including rural and urban areas in the southwest of Germany by wastewater analysis.

2. Methods and materials

2.1. Reference drugs, deuterated analogues and chemicals

The drugs and deuterated analogues were obtained from Promochem (Wesel, Germany). All other chemicals used were of analytical grade and obtained from Merck (Darmstadt, Germany).

2.2. Sewage-sample collection and treatment

Samples were collected at ten different wastewater treatment plants (WWTP) spread around the federal state of Saarland, Germany. The area is shown in Fig. 1. The characteristics of the individual WWTP can be found in Table 1. An Endress + Hauser stationary water sampler type asp-station 2000 was used to collect 24-h (time proportional) composite sewage samples from a point located immediately after the grit chamber. The total sample volume was 600 mL (made up of twelve 50 mL two hourly grab samples) with a sample interval from 8 am to 8 am throughout the course of the weeks 29 and 30 in July 2014. Immediately after sample collection, 10 mL aliquots were prepared, fortified with internal standard solutions (AMP-d_5 and METH-D_5, final concentration 50 pg/mL), acidified with acetic acid (100 μL), and stored at −20 °C until final sample preparation.

2.3. Sample preparation and instruments

Sample cleanup (10 mL of wastewater) was first done via filtration using Phenex-GF/CA 28 mm syringe filters (Phenomenex, Aschaffenburg, Germany) to remove particles and afterwards via solid phase extraction (SPE) on Isolute 200 mg/3 mL HCX cartridges (Biotage, Uppsala, Sweden). Cartridges were primed with methanol (1 mL) and then water (1 mL). Samples were loaded and washed with water (1 mL), HCl (0.1 M, 1 mL) and methanol (1 mL). Analytes were finally eluted using a methanol/NH_3 mixture (98/2, v/v, 1.25 mL). Eluents were evaporated to dryness under a stream of nitrogen (40 °C) and reconstituted using 200 μL of a mixture of water/methanol/formic acid (50/49/1, v/v/v) prior to analysis via liquid chromatography–high resolution tandem mass spectrometry (LC–HR-MS/MS).

Chromatographic separation of the analytes was carried out on a Dionex Ultimate UHPLC System (Thermo Fisher, Dreieich, Germany) using a Kinetex Phenyl–Hexyl (Phenomenex, Aschaffenburg, Germany) column (2.6 μm, 100 mm × 2.1 mm) guarded by a SecurityGuard ULTRA cartridge (Phenomenex, Aschaffenburg, Germany) both tempered at 40 °C. The mobile phases consisted of 2 mM aqueous ammonium formate plus 0.1% formic acid (pH 3, eluent A) and 2 mM aqueous ammonium formate with acetonitrile:methanol (50:50, v/v; 1% water) plus 0.1% formic acid (eluent B). The flow rate was set to 0.5 mL/min for 0–1 min, increasing to 0.6 mL/min from 1–6 min, increasing to 0.8 mL/min from 6 to 7 min, hold at 0.8 mL/min from 7 to 8.5 min, and decreased from 0.8 to 0.5 mL/min from 8.5 to 10 min. The gradient was as follows: 0–1.0 min 99.5% A, 1–6 min to 40% A, 6–7 min to 0.5% A, 7–8.5 min hold 0.5% A, 8.5–10 min hold 99.5% A. The pump curve was set to a value of 8 for 1–9 min and to a value of 5 for 0–1 and 9–10 min.

Detection of analytes was achieved via HR-MS/MS on a Thermo Fisher Q Exactive plus system (Thermo Fisher, Dreieich, Germany) using positive electrospray ionization. The settings of the instrument were as follows: sheath gas, 50 arbitrary units (AU); auxiliary gas, 10 AU; spray voltage, 3.00; heater temperature, 320 °C; ion transfer capillary temperature, 320 °C; and S-lens RF level, 60.0. Mass spectrometry was performed using positive full scan (FS) data and a product reaction monitoring (PRM) mode. The settings for FS data acquisition were as follows: resolution, 70,000; microscans, 1; automatic gain control (AGC) target, 3e6; maximum injection time (IT), 200 ms; and scan range, 50–750 *m/z*. The settings for PRM were as follows: resolution, 17,500; microscans, 1; AGC target, 2e5; maximum IT, 100 ms; isolation window, 1.0 *m/z*, HCD with stepped normalized collision energy (NCE), 35%; spectrum data type, profile; and an inclusion list on the *m/z* of interest (136.1120, 141.1435, 150.1277, 155.1591).

2.4. Method validation

The method used was validated in accordance to the Guideline on bioanalytical method validation published by the European Medicine Agency (EMA, 2011). Briefly, the method was tested for selectivity using tap water and surface water, carry-over (using blank samples following the high QC), lower limit of quantification (LLOQ) defined as lowest calibration standard, within-run and between-run accuracy using five samples per level at four concentration levels (LLOQ, low QC, medium QC and high QC), within-run and between-run precision, matrix effect using six lots of wastewater, tap water, and surface water, and stability (freeze-thaw stability for three cycles, short term stability for up to 12 h at room temperature, sample processing temperature stability for up to 48 h at room temperature). The analytical runs consisted of a blank sample, a zero sample, six calibration standards (5–1000 pg/mL), three levels of QC samples (low, medium and high) in duplicate and the study samples. Data evaluation and statistical analysis were made by GraphPad Prism 5.00 (GraphPad Software, La Jolla, CA, USA). *T*-test conditions were unpaired and two-tailed and ANOVA conditions were nonparametric (Kruskal–Wallis) using a Dunns post hoc ($P < 0.05$).

3. Results and discussion

3.1. Method validation

The analytical method was successfully validated in accordance with the criteria of the European Medicine Agency (EMA, 2011). Using the product ion mass spectra (MS^2) of the analytes, the method was selective enough down to the LLOQ. Using only the accurate masses of the protonated molecule (MS1), interferences