Investigation of drugs of abuse and relevant metabolites in Dutch sewage water by liquid chromatography coupled to high resolution mass spectrometry

Lubertus Bijlsma[a,§], Erik Emke[b], Félix Hernández[a], Pim de Voogt[b,c,*]

[a] *Research Institute for Pesticides and Water, University Jaume I, Avda. Sos Baynat s/n, E-12071 Castellón, Spain.*

[b] *KWR Watercycle Research Institute, Chemical Water Quality and Health, P.O. Box 1072, 3430 BB Nieuwegein, the Netherlands.*

[c] *Institute for Biodiversity and Ecosystem Dynamics, University of Amsterdam, P.O. Box 94248, 1090 GE Amsterdam, the Netherlands*

[§]*Visiting scientist at KWR*

** Corresponding author: w.p.devoogt@uva.nl, Tel +31 20 5256565, Fax +31 20 5257431.*

ABSTRACT

An extensive study on the presence of illicit drugs and pharmaceuticals with potential for abuse in sewage waters was made for the first time in the Netherlands. A total number of 24 target drugs were investigated in influent and effluent wastewater using liquid chromatography coupled to a high resolution Orbitrap mass spectrometer. This powerful analyzer has allowed not only the detection and identification of the compounds under investigation, but also their quantification at very low levels, which is highly innovative in the field of drugs of abuse. Samples were taken from five sewage treatment plants (STPs) during a whole week. The selected STPs served four cities of different size and an international airport. Daily variances of drug loads were demonstrated and removal efficiencies calculated for each drug and STP individually. Twelve target compounds were found in at least one influent or effluent, and highest concentrations were observed in influents collected from more urbanized areas. The compounds more frequently detected were amphetamine, benzoylecgonine, cocaine and THC-COOH together with the pharmaceuticals codeine, oxazepam and temazepam. Established week trends in consumption of drugs showed distinct differences between individual drugs. A slightly different occurrence pattern was observed in wastewaters from the airport. Thus, methamphetamine was only detected at Schiphol, a fact that was interpreted to be caused by consumption of this drug by travelers. Despite the fact that the Netherlands has frequently been criticized for its liberal drug policy the results from this study did not reveal higher drug consumption than found elsewhere, with the exception of cannabis.

Keywords

Illicit drugs of abuse, accurate mass, linear ion trap (LTQ FT) Orbitrap mass spectrometry, sewage water, STP removal efficiency.

1. INTRODUCTION

Drugs of abuse (DOAs), either illicit or legal, are a special group of widely consumed drugs. DOAs may enter the sewage system, unaltered or as metabolites, after consumption and excretion. Data obtained from analysis of sewage water have been used to estimate consumption or to observe usage trends in communities [Van Nuijs et al., 2011; Zuccato et al., 2005]. Since manufacturers, distributors and consumers are normally unknown, reliable data on the consumption of these drugs is difficult to obtain. Studies on the presence of DOA in sewage waters (influent) have provided complementary insight to the information on consumption usually obtained through enquiries and inventories. In addition, environmental loads can be calculated, and by analyzing both influent and effluent sewage waters the removal efficiency of a sewage treatment plant (STP) can be evaluated, taking into account the residence time of water in the plant [Huerta-Fontela et al., 2008; Postigo et al., 2010].

The existing methods for determination of DOAs in water are mainly based on liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS), using triple quadrupole (QqQ) analyzers, due to its excellent characteristics for quantification at low levels, such as high sensitivity and selectivity. However, other MS analyzers are gaining interest at present. Thus, high resolution mass spectrometry (HRMS) has shown strong potential for target and non-target screening [Hernández et al., 2011a; Hogenboom et al., 2009], but it has also been proved to be an efficient tool for the simultaneous identification and quantification of DOAs at low concentrations in complex sewage water samples [Bijlsma et al 2012]. The satisfactory sensitivity in full-scan acquisition mode and high resolving power obtained by the latest generation of HRMS has facilitated its application not only to qualitative/elucidative purposes but also for quantification in the last few years [Kaufmann et al., 2011; Kellmann et al., 2009; Krauss and Hollender, 2008; Nurmi and Pellinen, 2011]. An attractive feature of HRMS is that a retrospective analysis can be made from full-scan accurate mass data generated, in order to search for additional compounds not included in the first screening. This can be made without the need of additional analysis, as demonstrated previously [Bijlsma et al., 2012; Hernández et al., 2011b; Hogenboom et al., 2009].

Some exploratory studies [Hogenboom et al., 2009; Van der Aa et al., 2010] have demonstrated the presence of DOAs in Dutch waters. Nevertheless, a detailed quantitative study on wastewater influents and effluents in the Netherlands has not been performed until now. This seems interesting, since the Dutch liberal drug policy on soft drugs might lead to higher consumption of

cannabis, which would result in higher concentrations in wastewater.. In the present work, a recently developed and validated methodology, based on solid phase extraction (SPE) followed by LC-HRMS, has been used [Bijlsma et al. 2012]. The study assesses the behavior of 24 DOAs and metabolites in five different sewage water treatment plants (STPs) from four Dutch cities and an international airport. To the best of our knowledge, this is one of the few studies reporting the monitoring of this number of DOAs during one whole week, and one of the first in this field exploiting the quantitative capabilities of the Orbitrap mass analyzer. Results contribute among others to the evaluation of the daily variances of DOAs loads at different location of the Netherlands and the removal efficiencies of STPs with highly similar treatment steps.

2. MATERIALS AND METHODS

2.1. Sample collection

In total, 32 influent and 32 effluent sewage water samples were collected from five STPs. The five STPs were located in the Netherlands (**Figure S1** of the Supplementary information, SI) serving four cities (Utrecht, Eindhoven, Apeldoorn and Amsterdam) and the international airport of Amsterdam (Schiphol). The main characteristics of each STP are summarized in **Table S1** (SI). As can be seen in the table, all STPs investigated are equipped with conventional activated sludge secondary treatment and tertiary nitrogen and phosphate removal. Main differences among them refer to their water treatment capacity and the lag-time of the water in each STP.

Samples (24 hour flow dependent, starting and finishing time (8:30 am to 8:30 am)) were taken during the third and fourth week of February 2010 (Wednesday to Wednesday). For Schiphol during the weekend only a composite sample reflecting an average concentration of the analyte over a 72 h time period could be collected. All 64 sewage water samples were collected in polyethylene high density bottles and stored in the dark at 4 °C to be transported to the laboratory within 24 h maximum. Upon reception in the laboratory, samples were immediately stored in the dark at -18 °C until analysis to minimize degradation of analytes. No additional measures to delay decomposition of unstable compounds (e.g. cocaine) [Gheorghe et al., 2008] were taken.

2.2. Analytical methodology

Sample treatment and specific information on instrument operating conditions, both chromatographic and spectrometric, and on method validation can be found elsewhere [Bijlsma et al., 2012]. The list of 24 target compounds investigated in this work is shown in SI. Briefly, 200 mL effluent or 100 mL influent sewage water samples were spiked with a mixed isotope labelled internal standard (ILIS) solution and extracted using SPE cartridges (Oasis HLB). After elution with methanol, extracts were evaporated and reconstructed in 500 µL of 10% methanol aqueous solution. The sample extract (20 µL) was injected directly into the LC - linear ion trap (LTQ) FT Orbitrap system. Chromatographic separation of the compounds was achieved using an XBridge C_{18} column and an optimized gradient using water:methanol, both with 0.05% formic acid. The mass

spectrometer operated under data-dependent-acquisition (DDA) mode during the complete chromatographic run, in which both MS and MS^n spectra were acquired. Full-scan accurate mass spectra from 50 to 600 Da were obtained at a resolution of 30,000 FWHM. All data were acquired and processed using Xcalibur 2.1 software.

2.3. Calculation of elimination rates

Removal efficiencies were calculated by comparing effluent concentrations (C_E) from day (x+1) with influent concentrations (C_I) from day (x), thus assuming an average residence time of 24 h (see also **Table S1**, SI). Efficiencies (E) were calculated as $E = (1 - (C_E/C_I))\ 100\%$. For each STP this resulted in 6 values (for Schiphol only 3), the average of which was then calculated.

3. RESULTS AND DISCUSSION

3.1. Occurrence of drugs of abuse and metabolites in Dutch wastewater

The overall frequency of detection of target compounds in the influent and effluent sewage water samples analyzed is shown in **Table 1**. The mean concentrations and the concentration ranges (in ng/L) for each compound per location are also displayed.

Twelve DOAs were found in at least one of the influents analyzed. The samples from Amsterdam showed the highest mean concentrations in influents for benzoylecgonine (2306 ng/L), cocaine (434 ng/L), the cannabis metabolite (THC-COOH, 375 ng/L) and MDMA (140 ng/L). This might be explained by the fact that Amsterdam is the largest city selected, in line with other studies where higher MDMA and cocaine consumption was related to more urbanized areas or large cities [Banta-Green et al., 2009; Van Nuijs et al., 2011]. However, the highest levels of amphetamine were found in influents from Eindhoven, with concentrations ranging from 266 to 1779 ng/L, and an average of 682 ng/L.

In general, amphetamine and MDMA were at comparatively lower concentration levels than cocainics. The levels of codeine and the benzodiazepines, oxazepam and temazepam, in influents were in the same order of magnitude in all four cities, with mean concentrations ranging from 240 to 372 ng/L, 356 to 677 ng/L and 208 to 297 ng/L, respectively. Influent samples from the STP of Schiphol airport contained mean concentrations for oxazepam and temazepam slightly lower (153 and 164 ng/L, respectively), whereas codeine (536 ng/L) and cocaine (559 ng/L) were relatively higher compared to influents of municipal STPs. Two influents samples from Schiphol airport were found positive for methamphetamine (17 ng/L), while no methamphetamine was detected in any of the 4 Dutch cities. The reason for the occurrence of this drug might be related to international passengers travelling to or via this airport. The consumption and abuse of methamphetamine is not very popular in the Netherlands, yet much more so in East and South-East Asia [UNODC, 2010] and North-East Europe [EMCDDA, 2010].

It is interesting to notice that one influent sample collected from Schiphol airport and one collected in Apeldoorn showed unexpectedly high cocaine/benzoylecgonine ratios (0.85 and 2.20, respectively). Based on their molar mass relation and on the excretion rate limits as unchanged cocaine and as benzoylecgonine, cut-off values of 0.75 and 0.27 for the cocaine/benzoylecgonine

ratio have been proposed [Postigo et al., 2010; Van Nuijs et al., 2009]. A value above this ratio suggests that not all measured cocaine results from human consumption. Our data by far exceed these cut-off ratios, which might indicate disposal of non-consumed cocaine into the sewage water system. For Apeldoorn no clear explanation was available. However, the exceeded ratio at Schiphol airport might be related with the presence of drug traffickers, who for example due to a (sudden) surge of anxiety might unload their 'goods' into the sanitary facilities either on board the aircraft or at the airport before passing customs control. However, an extensive study, including more data and additional information (e.g. flight schedules) would be necessary to sustain this hypothesis.

Thirteen DOAs were present in at least one of the effluent sewage waters analyzed. In general, concentrations of DOAs in effluents were lower than those of influents, suggesting removal by degradation or sorption of these substances in STPs. However, for some target compounds the opposite occurred. Thus, methadone and ritalin were exclusively detected in effluents, albeit at low concentrations (maximum concentrations of 58 ng/L and 6.2 ng/L, respectively). This might be due, especially for ritalin, to the difficulties for detection and quantification of low analyte levels in influents, as a consequence of lesser pre-concentration along sample treatment step and stronger matrix ionization suppression in this type of samples.

Ketamine was mainly detected in effluents, with the exception of Eindhoven, where it was also found in influent samples (17 ng/L), however at lower concentrations than in effluent (44 ng/L). Concentrations for benzodiazepines in effluents were nearly always higher than those found in their corresponding influents. Codeine and MDMA were found occasionally at higher concentration in the effluent sewage water. In the case of codeine, this is in agreement with the results published by others [Boleda et al., 2007].

It is also worth mentioning that the benzodiazepine diazepam was detected neither in influent nor in effluent sewage water. According to Löffler et al. (2005) diazepam undergoes fast and extensive sorption onto sediments and is highly stable in soils and during sewage water treatment. Diazepam is considered highly persistent, while oxazepam is moderately persistent in water/sediment systems. Differences in behavior of benzodiazepines are associated with differences in functional substituent groups, and - in agreement with our study - only the hydroxylated tranquilizers, oxazepam and temazepam, were reported to be present in influents and effluents [Hummel et al., 2006]. In addition, oxazepam is one of the main metabolites of nordazepam and diazepam [Besse et al., 2008], and therefore it could result from oxazepam use but also from other benzodiazepines. This

might also be another reason that diazepam was not detected in any sewage water, as part is excreted as oxazepam [Löffler et al., 2005]. For this reason, Besse et al. (2008) suggest that oxazepam could be used as an indicator of contamination of the aquatic environment by benzodiazepines.

A detailed comparison of data obtained in this work with drug concentrations reported in the literature is problematic due to the uncertainties associated to the different steps of this type of works (e.g. sampling, stability of compounds, analytical measurements, etc). However it seems clear that the levels of drugs and metabolites found in Dutch sewage waters are roughly of the same order of magnitude as those observed in other countries worldwide (e.g. Australia [Irvine et al., 2011], Belgium [van Nuijs et al., 2009], Italy [Castiglioni et al., 2006; Zuccato et al., 2005], Ireland and UK [Bones et al., 2007; Kasprzyk-Hordern et al., 2009], Spain [Bijlsma et al., 2009; Huerta-Fontela et al., 2008; Postigo et al., 2010] USA [Chiaia et al., 2008; Jones-Lepp et al., 2004] An exception is the relatively high concentration found for THC-COOH in influents of Dutch cities, as deduced from data shown in **Table S2** (SI). This fact might be related with the Dutch drug policy on cannabis (marijuana and hashish) usage, which is permitted for every citizen over age eighteen. In addition, the mean concentrations of MDMA in influents and effluents observed in the present study are relatively high, yet similar to those found in Barcelona and Valencia [Postigo et al., 2008], but about 10 times higher than those measured in Milan [Castiglioni et al., 2006]. Morphine could not be quantified in Dutch sewage waters, surely due to the low sensitivity of the method for this compound (limits of quantification were 360 and 125 ng/L for influents and effluents, respectively). On the contrary, significant levels (approximately 80 ng/L in influents) were found elsewhere [Boleda et al., 2007; Castiglioni et al., 2006]. Nevertheless, precaution on the interpretation of the data is required, as a one-to-one comparison is difficult to make. For a correct comparison of data, various factors such as weather conditions at time of sampling, treatment, capacity and lag-times of the STPs, etc, need to be taken into account. This would implicate a much more extensive study, organized and coordinated at the international level.

3.2. Daily variations of drug loads over one week

Four of the selected STPs serve large cities, which were considered important for studying DOAs consumption at the community level. Amsterdam is the capital city with a lot of tourists and

students (~10%); Utrecht and Eindhoven are typical province towns with large student populations (>20% and 10% respectively); Apeldoorn is a town in a more 'rural' area with hardly any students (<3%). In addition, the STP from the international airport of Amsterdam (Schiphol) was selected to study consumption behaviour of travellers. By plotting drug loads of influents against the days of the week, an indication can be given on the behaviour of DOAs consumption of each location. This approach seems appropriate, as loads of DOAs (g/day) are calculated using concentrations (ng/L), taking into account the amount of water (m^3) processed by the STP during the corresponding day [Zuccato et al., 2005]. The latter is important, since the flow rate of the water stream can vary considerably (up to a factor of 3 in between days).

Daily variations of drug loads along a whole week in each STP are illustrated in **Figure 1**. In general, loads of cocaine and its main metabolite benzoylecgonine were highest on Saturday, Sunday or Monday, suggesting a preference of cocaine consumption on weekend days. On the contrary, codeine, ketamine, benzodiazepines (e.g. oxazepam, temazepam and nordiazepam) show a continuous load throughout the week, implying a different pattern of use. The daily variances of the STP from Amsterdam, where the highest overall drug loads found in the present study are observed, also suggest increased consumption of MDMA and cannabis during weekends. These general tendencies are consistent with reported results of monitoring studies performed in other countries over several consecutive days [Berset et al., 2010; Bijlsma et al., 2009; Huerta-Fontela et al., 2008; Terzic et al., 2010].

At the STP of Schiphol airport, loads of DOAs were significantly lower. However, when comparing the ratio of served population (e.g. ratio Amsterdam:Schiphol, approx. 20 (**Table S1**, SI)) with loads ratio (also ~20) the consumption seemed about equal. Our results also suggest similar consumption behaviour for cocaine, codeine and benzodiazepines. An interesting load pattern for cocainics was observed for the Utrecht STP, where highest loads were found on Friday and Tuesday, corresponding to the consumption of cocaine on Thursday and Monday, as the samples were collected from 8:30 am of the day before to 8:30 am of the day of sampling. In addition, oxazepam shows similar daily dynamics. These results are not coherent with those observed elsewhere and an unambiguous explanation for these distinctive patterns cannot be proposed. Another unexpected consumption pattern was observed for amphetamine in Eindhoven, where relatively high loads were detected at the start of the sampling week after which the loads decreased. This may have been caused by an incidental dump or by an atypical use pattern. The actual sampling

week happened to be directly after the termination of the carnival period (that ended on Tuesday) that is extensively celebrated in the city of Eindhoven. In a new European monitoring campaign started in 2011 the findings reported here will be compared.

3.3. Removal efficiency of sewage treatment plant

The analyses of sewage waters are of importance as they allow calculating removal rates for each DOA in a given STP. Good removal efficiency is essential, since Dutch STPs discharge their effluents into surface/river waters, which are important resources for drinking water production. In addition, contaminated effluents may have a potential impact on the aquatic ecosystem of the receiving water bodies.

Average removal rates, expressed as percentages, were calculated using the dissolved aqueous phase concentrations of the analytes in influent sewage waters and in their corresponding effluents. Lower levels in effluents might be a result of removal in the STP, due to microbial degradation, other transformation processes and/or sorption to the solid matter. In this work, the removal efficiencies varied significantly from 100% elimination, when analytes were detected in the influent but were absent in the effluent, to 0% elimination, when analytes were present in influents and effluents at around the same level or when analytes were not detected in influents. "Negative" elimination rates were considered when analyte concentrations were higher in the effluent.

Estimated average removal rates for each analyte and STP are shown in **Figure 2**. The highest removal efficiencies were observed for amphetamine (100%), methamphetamine (100%) and THC-COOH (98%), independently of the STP under study. Benzoylecgonine (90%) and cocaine (79%) also seemed to be efficiently removed in the STPs, which is in agreement with other reports [Huerta-Fontela et al., 2008; Postigo et al., 2010]. However, in the STP from Apeldoorn, the elimination of cocaine and benzoylecgonine was found to be much lower (25 and 73%, respectively), and similar to the results obtained by Terzic et al. (2010). Highly variable values and even negative elimination rates of amphetamine-like drugs and THC-COOH have been reported [Boleda et al., 2007; Postigo et al., 2010]. In our study, amphetamine and THC-COOH appeared to be efficiently removed in all STPs, as well as methamphetamine in the only STP where it was detected (Schiphol). Loganathan et al. 2009 reported less than 100% removal efficiency of methamphetamine, but this difference could be accounted for due to differences in STP treatment

process´s and the amount of methamphetamine coming into the STP. MDMA showed highest variability, but in general with poor elimination, ranging from -12 to 26%. High differences and lower removal efficiencies were also found among opiates, with average values of 23 and 37% for 6-MAM and codeine, respectively. Kasprzyk-Hordern et al. (2009) found similar results and related a more effective removal of amphetamine, cocaine and benzoylecgonine to activated sludge treatment, whereas a lower removal efficiency (42%) was found for codeine using the same technology.

As mentioned earlier, benzodiazepines were found nearly always at higher concentrations in effluents than in influents, resulting in "negative" removal rates (**Figure 2**). Accordingly, average removal efficiencies for oxazepam, temazepam and nordazepam were -46, -38 and -18%, respectively. This might be related to the cleavage of the conjugated molecules in influent sewage water, as demonstrated for estrogens [D'Ascenzo et al., 2003; Ternes et al., 1999]. Deconjugation of glucoronides that can occur during sewage water treatment, can also play an important role for other compounds, such as codeine and benzodiazepines, involving deconjugation of codeine-6-glucuronide, temazepam- and oxazepam-glucuronide during the treatment process [Boleda et al., 2007].

The application of Principal Component Analysis (PCA) using Statgraphics 7.0 helped us to evaluate the removal efficiency of the compounds investigated for each STP. Data were converted to Log (concentrations + 1) to correct for the dependence between arithmetic means and standard deviations. Homoscedasticity of variances was tested by means of Barlett's test ($P< 0.05$). The plot of the two first components explains 92% of the total variance (**Figure S2, SI**). The distance among variables, clearly separated by component 2, could be interpreted as the removal efficiency of the contaminants within each STP. The poorest removal efficiencies were observed for the treatment plants from Utrecht (u) and Apeldoorn (a). This might be related to their lower lag-times, i.e. the time it takes water to enter and leave the STP. Low lag-time was also related to poor elimination in the study of Postigo et al. (2010).

The analyses of both influents and effluents also allowed us to estimate the lag-time of the plant, demonstrating another applied issue of analysing chemicals in sewage waters. Lag-times can be estimated by using a marker compound, which is present in both influent and effluent sewage water. In our case, we used benzoylecgonine as a marker. Provided that the lag-time and removal efficiency are constant, the week profile of benzoylecgonine in influents and effluents can roughly be

overlapped; the difference in time between influent and effluent indicates the lag-time of the treatment plant. As an example, **Figure 3** shows the estimated lag-time for Apeldoorn (13 hours) and Amsterdam (24 hours), which are comparable with the data put at our disposal by the STP managers (10 and 24 hours, respectively).

4. CONCLUSIONS

Advanced analytical methodology based on the use of LC-LTQ FT Orbitrap MS has been applied in this work for the simultaneous quantification and confirmation of 24 target drugs of abuse in sewage water. The results of an extensive week monitoring contributed to a better insight on drugs of abuse in the Netherlands and their presence in Dutch influent and effluent sewage water. Data of this work allowed evaluating removal efficiencies of the selected STPs, which were generally satisfactory except for benzodiazepines and MDMA. Week monitoring of analytes in both influents and effluents also allow estimating lag-times of each STP. The inclusion of the STP of the international airport of Schiphol, the size of which is equivalent to a small town but presumably reflecting differences in drug consumption compared to common townships, is innovative in this type of work and, to the best of our knowledge, has not been previously reported in the literature.

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SUPPLEMENTARY INFORMATION

In this section, a list of compounds investigated, and a table with the main characteristics of the STPs under study (Table S1) are included. Furthermore, two figures, one including the locations of the investigated STPs in the Netherlands (Figure S1), and another showing a PCA plot for removal efficiencies of different STPs (Figure S2), are added to provide supplementary information to the written text.

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Table 1: Overall frequency of detection[a] and mean concentrations[b] of drugs of abuse in influent and effluent sewage water samples from five STPs in the Netherlands.

Compounds	FD[a] (%)		Utrecht		Eindhoven		Apeldoorn		Amsterdam		Schiphol	
	Influent	Effluent		Concentration (ng/L)		Concentration (ng/L)		Concentration (ng/L)		Concentration (ng/L)		Concentration (ng/L)
	(n=32)	(n=32)		Influent	Effluent		Influent	Effluent		Influent	Effluent	
Amphetamine	100	13		98[b] (41-225)	< 4		682 (266-1779)	6.9 (4.5-12)		89 (40-93)	< 4	
Methamphetamine	6	-		< 15	< 5		< 15	< 5		< 15	< 5	
MDMA	75	100		87 (61-132)	94 (62-131)		92 (49-142)	107 (36-222)		< 12	30 (19-49)	
Cocaine	94	47		193 (142-307)	29 (10-56)		118 (99-134)	< 6		222 (87-571)	159 (103-235)	
Benzoylecgonine	100	75		1079 (432-1560)	196 (99-351)		862 (335-1413)	21 (14-37)		409 (260-568)	102 (83-117)	
6-MAM	9	28		27 (27)	13 (12-14)		< 19	< 7		< 19	< 7	
Methadone	-	100		< 45	38 (29-45)		< 45	8.7 (6.0-9.9)		< 45	22 (14-25)	
Codeine	100	100		240 (73-347)	245 (121-310)		280 (119-366)	242 (97-599)		251 (113-355)	180 (89-232)	
THC-COOH	88	9		183	13		131	< 7		91	< 7	

				(140-238)	(11-15)		(87-166)			(73-117)		
Ketamine	22	88		< 10	8.0 (4.5-11)		17 (10-34)	44 (21-61)		< 10	7.3 (2.2-14)	
Ritalin	-	44		< 20	5.0 (2.3-6.2)		< 20	2.1 (2.0-2.3)		< 20	< 2	
Oxazepam	100	100		677 (231-915)	852 (445-994)		377 (177-494)	486 (237-586)		589 (301-882)	778 (439-908)	
Temazepam	97	100		297 (99-414)	406 (208-508)		208 (92-279)	271 (133-314)		250 (209-300)	309 (159-371)	
Nordazepam	41	100		9.6 (4.2-21)	9.5 (7.1-11)		4.7 (4.0-7.1)	7.1 (3.6-8.0)		< 4	7.8 (4.7-9.7)	

[a]: Frequency of detection in influent and effluent sewage water samples.

[b]: Concentrations of drugs of abuse are the average of seven (one week) 24 h composite influent or effluent wastewater samples, except for Schiphol (average of three 24 h and one 72 h composite wastewater samples). Top: mean concentration; bottom: concentration range (in brackets).

FIGURE CAPTIONS

Figure 1: Daily variations of drug loads over the duration of a whole week in each STP.

Figure 2: Average removal efficiencies of drugs of abuse and metabolites in the investigated STPs.

Figure 3: Time plots vs. concentration of benozylecgonine (BE) demonstrating estimation of lag-time for two STPs: Apeldoorn (top) and Amsterdam (bottom).

Figure 1

Figure 2

Figure 3