

Hazard/Risk Assessment

Illicit Drugs as a Potential Risk to the Aquatic Environment of a Large Freshwater Lake after a Major Music Festival

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Abstract: The present study strengthens the view that residues of drugs of abuse may become widespread surface water contaminants following a local music festival. Overall, 10 illicit drugs were detected from the aquatic environment after the festival; cocaine and 3,4-methylenedioxyamphetamine were present in the highest concentrations. The presence of illicit drugs and their metabolites over 3 monitored festival yr suggested that consumption of these drugs was temporally linked with events. Weather conditions seriously influenced detection of contaminants deriving from events at the lakeshore. Most of the illicit drugs retained their pharmacological activities, with a potentially adverse impact on wildlife. *Environ Toxicol Chem* 2021;40:1491–1498. © 2021 The Authors. *Environmental Toxicology and Chemistry* published by Wiley Periodicals LLC on behalf of SETAC.

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INTRODUCTION

An obscure group of emerging pollutants identified in the aquatic environment are plant-derived and synthetic illicit drugs (Boleda et al. 2009; Kasprzyk-Hordern 2010). The rise in scientific interest in illicit drugs stems from the demonstrated adverse impact of these substances and their metabolites on aquatic ecosystems, in addition to their potential human health effects (Pal et al. 2013; dos Santos and Nardocci 2019). Recently, it has become evident that the use of alcohol and illicit drugs among large music festival participants is a major cause of public health problems because populations engaging in these activities may encourage others to consume alcohol and drugs. Several adverse events, including fatal and nonfatal drug-related overdoses, have been reported at numerous electronic dance music festivals (Lim et al. 2008; Lai et al. 2013; Mohr et al. 2018). Large outdoor festivals often continue for several days, lasting throughout the night; and attendees

frequently use illicit drugs to induce mind-altering and euphoric effects. The illicit drugs enhance their physical performance, mainly to overcome somnolence and fatigue and in general to increase their buzz and enliven the overall festival experience (Palamar et al. 2016). At such social events, conventional illicit drugs include compounds such as opiates, cannabis, amphetamines, other new “designer” drugs, and illegally used prescription drugs (e.g., opiate painkillers such as codeine; Fox et al. 2018). Consumption of most illicit drugs has a range of adverse health, social, and economic consequences for the individual consumer, while also imposing unwanted costs on society (Pavlukovic et al. 2017; Hoegberg et al. 2018). Presumably, only a fraction of the social, economic, and environmental problems associated with emerging drug abuse are recognized and reported (Miller et al. 2009; Mennis et al. 2016).

With the consumption of illicit drugs increasing and spreading rapidly worldwide, our study focused on the ecological consequences of these activities. Urine, saliva, and wastewater analyses are alternative ways of monitoring the population's drug use by measuring excreted drug residues (Lai et al. 2013). In this way, the consumption of conventional illicit drugs such as cocaine, amphetamines, ecstasy, cannabis, and heroin (Zuccato et al. 2008, 2011; van Nuijs et al. 2009; Prichard et al. 2012) has already been estimated at specific facilities (Panawennage et al. 2011; Postigo

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et al. 2011) and sporting events (Gerrity et al. 2011). Even though it has been reported that illicit drugs are present at fairly low environmental concentrations (nanograms per liter to milligrams per liter), it is still unclear whether such concentrations in surface water can cause undesirable physiological effects in wildlife (Pal et al. 2013). Rosi-Marshall and coworkers (2015) reviewed the available literature on the ecological effects of illicit drugs on the aquatic environment. Although the research is limited, recent studies suggest that aquatic organisms, including bacteria, algae, invertebrates, and fish, are all affected by these illicit drugs at environmentally relevant concentrations. To evaluate which compounds are more likely to pose a risk for aquatic organisms, ecological risk assessment can be performed. To undertake these evaluations, earlier ecotoxicological studies provide appropriate toxicology data. For example, ketamine and norketamine caused acute toxicity to *Daphnia magna*, with median lethal concentration (LC50) values of 30.93 and 25.35 mg/L, respectively, after 48 h of exposure (Li et al. 2017). Benzoylcegonine and cocaine toxicity were tested on zebrafish embryo/larva and fern spores, and at 1 mg/L no-observed-effect concentrations (NOECs) were determined for both drugs (García-Camero et al. 2015). We suspect that the consequences of illicit drugs causing sublethal toxicity on different physiological pathways at the molecular to cellular levels are highly underestimated.

Our aims were to 1) analyze water samples collected before, during, and after a music festival using a suspect screening approach; 2) elucidate the identity and provide a quantitative snapshot of the recreational substances used during the festival; 3) evaluate and assess the risks posed by the appearance of contaminants; and 4) discuss the environmental challenges of a temporary increase in psychostimulant concentrations in the aquatic environment.

MATERIALS AND METHODS

Study area and sample collection

Our study was carried out in Lake Balaton (Hungary), one of the largest shallow lakes (600 km², average water depth ~3 m) in central Europe, which can be divided into 4 basins (Istvanovics et al. 2007). The lake is extremely calcareous, with high magnesium calcite making up 50 to 60% of sediments. Turbidity is high particularly during summer because of both wind-induced sediment resuspension (polymictic) and slow sedimentation of the precipitating carbonates (Istvánovics et al. 2004). An annual large music festival was held on the shore of the lake, lasting for 5 to 6 d with 154 000, 165 000, and 172 000 attendees in July of 2017, 2018, and 2019, respectively. The festival area was approximately 25 ha, where 3000 m² of staging was created that included several stages in the water. Water samples were collected over consecutive years between 2017 and 2019 during April (3 mo prior to the festival), June (1 wk prior to the festival), July (1 d after the festival), August (1 mo after the festival), and November (4 mo after the festival). The samples were derived from 3 sites in the littoral region inside the festival area (proximate sites) and from 2 reference points (remote sites) located 6 and 8 km away from the festival area (Figure 1; Supplemental Data, Table S1). The average distance between (proximate) sampling sites was 400 m, and the distance from the shoreline was 35 m, where the average water depth was 120 cm. Duplicate samples were collected in borosilicate glass containers with Teflon-faced caps (Thermo Fisher Scientific) as grab samples (each sample 2 L), after they were rinsed 2 to 3 times. Each sample was immediately cooled in a closed container until it arrived at the laboratory in less than 4 h and extracted within 20 h; therefore, the sample was fully prepared within 24 h from the time of sampling.

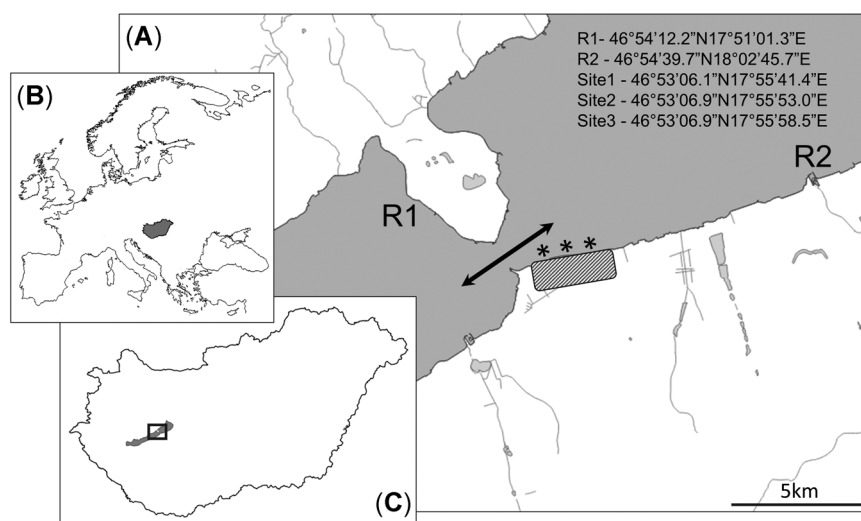


FIGURE 1: (A) Map showing the annual music festival area (gray-shaded box) and the 3 (proximate) sampling sites (asterisks). The water body is indicated in gray. The average distance between sampling sites was 400 m, and the distance from the shoreline was 35 m, where the average water depth was 120 cm. R1 and R2 indicate the reference points (remote sites). The festival area was approximately 25 ha, where 3000 m² of staging was created that included several stages in the water. The geographic position of Hungary in Europe (B) and the position of Lake Balaton in Hungary (C) are shown as insets.

Chemical analysis

A total of 34 drug residues (parent drugs and key metabolites) were analyzed. These included conventional illicit drugs (cocaine, benzoylecgonine, methamphetamine, 3,4-methylenedioxymethamphetamine [MDMA]), a psychedelic drug (ketamine), and an opioid (tramadol). The validation parameters and the utilized precursor-product transitions with the related collision energies in Supplemental Data, Table S2, were indicated. Details of the sample preparation process and instrument analysis have been reported previously (Maasz et al. 2019). Briefly, prior to sample filtration (GF/F 0.7 μm glass microfiber filter, 516-0345; VWR), the corresponding mass-labeled internal standard was added to the samples, which was used for quantification. Drug residues were concentrated on Strata X-CW (8B-S035-FCH; Phenomenex) solid-phase extraction (SPE) cartridges using an automated SPE system (AutoTrace 280; Thermo Scientific). The dried (under nitrogen gas) eluates were reconstituted with acetonitrile and transferred to ultra-performance liquid chromatography vials. The selected drug residues were analyzed and quantified using supercritical fluid chromatography (ACQUITY UPC2 system; Waters) coupled with tandem mass spectrometry (MS/MS; Xevo TQ-S Triple Quadrupole; Waters). Each sample was analyzed in triplicate using MassLynx software (Ver 4.1 SCN950) and evaluated with TargetLynx XS software. Separation of compounds was performed on an ACQUITY UPC2 BEH analytical column (3.0 \times 100 mm, 1.7 μm particle size, 186007607; Waters). The electrospray ionization source was operated in positive ion mode with a spray voltage of 3 kV and a cone voltage of 30 V. All MS/MS experiments were performed with an isolation window of 0.4 m/z . The observed ions were accepted and quantified if they had appropriate MS1 mass, retention time, MS2 masses, fragmentation pattern, and internal standard correction.

Environmental risk characterization

To estimate the harmful effects of illicit drugs on an aquatic ecosystem, a risk quotient is usually applied (US Environmental Protection Agency 1997a, 1997b), which is defined as the ratio of the maximum measured environmental concentration (MEC) to the predicted-no-effect concentrations (PNECs), where PNEC depends on aquatic toxicity data of the illicit drugs and the assessment factors (Grung and Schlabach 2007; van der Aa et al. 2013; Mendoza et al. 2014; Liu et al. 2015). Literature sources provide toxicity data (LC50, median effect concentration [EC50], or NOEC) for algae, cladocerans, and fish with the investigated drugs (Food and Drug Administration, Center for Drug Evaluation and Research 1996; Russom et al. 1997; Sanderson et al. 2004; Deo 2014; Mendoza et al. 2014; García-Camero et al. 2015; Li et al. 2017) for each element of the PNEC calculation. Predicted toxicity values from the US Environmental Protection Agency's Ecological Structure Activity Relationships Class Program (Ver 2.0) were used in cases for which no laboratory data were available. This database is fairly unreliable; therefore, the applicable assessment factor was 1000 (Zhang et al. 2017).

RESULTS AND DISCUSSION

Quantification and method validation

Concentrations of compounds were calculated using the standard calibration curve for water spiked with compounds before extraction, which were constructed using a detector response defined as the ratio of the peak ion (the specific product ion of the highest intensity as the qualifier ion) to the base peak ion of the related internal standard. Deuterated and isotope-labeled internal standards were added prior to SPE extraction to minimize matrix effects, compensate for losses or enhancement of compounds, and ensure that there were no analytical or sampling batch effects between sample and analysis batches and over time. The average absolute recovery of citalopram-d6, carbamazepine-d10, E2-13C3, and *N*-ethyloxazepam was 77.8 ± 15.0 , 86.3 ± 6.2 , 86.9 ± 30.2 , and 86.4 ± 31.8 , respectively. The mean (0.96 ± 0.02) correlation coefficient (R^2) of the calibration curves was typically >0.95 and showed linearity in the range of 0.1 to 1000 ng/L for the majority of illicit drugs. The average method accuracy was 88.7%. This method achieved simultaneous quantitative analysis of 34 illicit drugs, where the limit of detection and limit of quantitation values (Supplemental Data, Table S2) were 0.01 to 25.00 and 0.02 to 80.00 ng/L concentration range, respectively. Nonspiked samples were analyzed to measure the background concentrations simultaneously. Procedural blanks consisting of ultrapure water were analyzed as the controls for procedural contamination.

Occurrence of illicit drugs

The present study is the first survey conducted in a natural body of water after a major lakeside music festival using an exact analytical approach to detect illicit drugs. In the present study, 11 illicit drugs were identified and quantified during the investigated time period (Table 1; Supplemental Data, Figure S1). Blank areas in Table 1 correspond to nondetected values. In 2017, 6 drugs were detected after the music event (July, 1 d after the festival) that could not be detected earlier or at the reference points. One month later (August) the contamination levels for cocaine decreased by approximately 90%, for benzoylecgonine by approximately 75 to 85%, for MDMA by approximately 90 to 96%, and for ketamine by 50%; and all were undetectable by November (4 mo after the festival). Ecgonine methyl ester, 5,6-methylenedioxy-2-aminoindane (MDAI), 3,4-methylenedioxyamphetamine (MDA), and methamphetamine were not detectable by August (1 mo after the festival).

In 2018, no drug residues were observed (except tramadol) in the lake until the festival. However, cocaine, MDMA, and MDAI were detectable after the festival (July 2018, 1 d after the festival). Tramadol did not show any correlation with the event and was observed throughout the years at almost the same concentration range (Table 1; Supplemental Data, Figure S1). The reason for the appearance of MDA in August 2018 is uncertain. However, the shoreline of Lake Balaton is a popular tourist site in the summer period, and this can be why this illicit

drug appeared in our samples. On the last 2 d of the festival, a north wind blew, which usually causes strong waves on the south shore of the lake. Because of the strong winds and waves, presumably fewer festival visitors bathed in the lake.

In 2019, the same contamination profile was detected as in 2017, except that drugs were present at much higher concentrations and amphetamine was present instead of ecgonine methylester in the samples. This can be explained by the weather conditions being similar in these 2 yr (2017, 2019); for example, as in 2018, a north wind blew for the last 2 d of the festival, which caused heavy waves along the southern shoreline. The heavy waves may have decreased the number of bathers but may have also contributed to the dilution and diffusion of illicit drugs.

The frequency of occurrence of tramadol was 92%, and the average concentration was 0.30 ± 0.08 ng/L. None of the illicit drugs were detected at the reference points except tramadol, which was observed at a 0.7 to 0.8 ng/L; therefore, measured values at the reference points are not shown in Table 1 and Supplemental Data, Figure S1.

Overall, in the investigated years, the number of detected illicit drugs peaked immediately after the event (July, 1 d after the festival; Table 2). The occurrence of cocaine and MDMA in the samples collected over 3 festival yr suggested that consumption of these drugs was consistent, and these illicit drugs were present in all samples 1 d after the events. An earlier study demonstrates that the abuse of some illicit drugs is closely associated with specific music preferences (Mackulak et al. 2019). In 2018, rain and a strong north wind occurred during the festival; thus, not all illicit drugs were observed in the samples. The assessment of illicit drugs in the aquatic environment could be influenced by several factors such as weather conditions (rain, wind), dilution (streams, wave-driven currents and rain), and sequestration in the sediment. The lake has 2 basins separated by the Tihany Narrows close to the festival area, where the water current flows with a speed of up to 2 m/s (Figure 1). The water current flow and weather conditions therefore can easily affect the concentrations of illicit drugs at the sampling sites. Most of the illicit drugs were measured in low concentrations in surface water at the festival area, but some of them persisted for up to 3 mo. It is therefore important to report on the fate of both parent molecules as well as their metabolites in surface waters to evaluate their possible negative effects in environmental waters in the future. Our data agree well with the observation that the intact amphetamine compound decreased in the artificial streams from $<1 \mu\text{g/L}$ on day 1 to $0.11 \mu\text{g/L}$ on day 22. Nevertheless, it was relatively persistent, with half-life values in soil of >500 d (Pal et al. 2011).

We have no information on whether the contamination was derived from a direct or an indirect load because of inappropriate wastewater drainage from the festival area and toilet infrastructure conditions. One must consider that in the festival area the contamination burden of illicit drugs is expected to be much higher than we observed because terrain sources (including fixed and mobile toilets, bushes) were not included in the present study. Moreover, any accumulation of sediment in the lake is also unknown; therefore, measured drug

TABLE 2: Frequency of occurrence of detected illicit drugs per sampling campaign

Sampling period	Cocaine	Benzoyllecgonine	Ecgonine methyl ester	Amphetamine	Methamphetamine	MDAI	MDA	MDMA	Ketamine	Norketamine	Tramadol
April (n = 6)			17%								100%
June (n = 9)											89%
July (n = 9)	100%	67%	11%	11%	56%	11%		100%	67%	33%	100%
August (n = 6)	11%	33%					11%	22%	33%		56%
November (n = 6)							3%	31%	25%		67%
Σ (n = 36)	28%	25%	6%	3%	14%	3%	3%	31%	25%	8%	94%

Bold indicates contamination levels 1 d after the music event.
MDAI = 5,6-methylenedioxy-2-aminoindane; MDA = 3,4-methylenedioxyamphetamine; MDMA = 3,4-methylenedioxy-methamphetamine.

concentrations could be much lower than actual concentrations and cannot serve as an estimation of total drug consumption because the usage level may be underestimated. Beyond the festival area, wastewater seepage is not relevant because this pollution is removed from the catchment area by the sewage transfer conduction system (Maasz et al. 2019). The survey strategy used cannot estimate the actual drug consumption levels, but it provides valuable information on whether these drugs are definitely present during the festival period and that they temporarily contaminate the surface water. For a more exact estimate, wastewater analysis should also be performed by considering the strategy of Lai and coworkers (2013). The overall results of the present study, however, reliably complement traditional questionnaire surveys from such events, with additional advantages of giving direct evidence of drug abuse and being more objective while avoiding major ethical issues.

Environmental risk assessment

Generally, pharmaceuticals are present at very low concentrations in the aquatic environment. Although a huge database of the contamination levels of these types of pollutants in water systems exists globally, there is still a lack of correlation in the levels of these pollutants with possible long-term impacts in humans and wildlife.

Acute data for ecgonine methyl ester, MDAI, and MDA are not available; therefore, risk quotient analysis of these drugs was not carried out. Moreover, no chronic data with the relevant environmental concentrations were available for any studied compounds. Considering the detected concentration levels of tramadol and methamphetamine, no risk to the aquatic environment could be suggested. Of the 34 compounds measured, only ketamine, cocaine, and MDMA yielded risk quotient values >0.01, thus indicating that low, medium, or high risk is probable (Table 3). The risk quotient value for amphetamine, norketamine, and benzoylecgonine was found to be <0.01, suggesting negligible environmental effects. These results should, however, be interpreted with caution because the detected MEC values may be underestimated and do not provide a real picture of the chronic exposure of organisms present in the ecosystem. Occasionally, there may be much higher concentrations; furthermore, the restricted experimental data and the use of high assessment factors can seriously affect the risk quotient values. Thus, the possible adverse effects of illicit drugs on human health and ecosystem functioning should not be neglected (Zhang et al. 2017). Recent studies confirm that amphetamine, methamphetamine, cocaine, and morphine may have ecological consequences even at environmentally relevant concentrations. For example, in artificial streams treated with amphetamine, lower biofilm chlorophyll and gross primary production, along with decreased seston respiration, were observed (Lee et al. 2016). The toxicity of amphetamine to rainbow trout (*Oncorhynchus mykiss*) hepatocytes and water flea (*D. magna*) was also observed (Lilius et al. 1994). Sublethal doses of cocaine and benzoylecgonine on zebra mussel (*Dreissena polymorpha*) elicited marked DNA damage, an increase in the number of micronucleated cells,

TABLE 3. Risk quotient (RO) for identified drugs calculated from maximum measured environmental concentration (MEC), predicted no-effect concentration (PNEC) for the lowest available toxicological data (LC50, EC50, or NOEC, in nanograms per liter) and the assessment factor (AF) applied for PNEC calculation

Compound	MEC	Ecotoxicological data	Experiment information	AF	PNEC	RQ	Risk	References
Ketamine	3454.1	30 930 000	LC50— <i>Daphnia magna</i>	1000	30 930	0.112	Medium	Li et al. (2017)
Cocaine	269.6	1 000 000	NOEC ^a — <i>Danio rerio</i>	100	10 000	0.027	Low	García-Camero et al. (2015)
MDMA (Ecstasy)	90.4	216 000	EC50—daphnids (ECOSAR model)	1000	216	0.419	Medium	Sanderson et al. (2004)
Tramadol HCl	0.8	73 000 000	EC50— <i>Daphnia</i> spp.	1000	73 000	0.000	No	Food and Drug Administration, Center for Drug Evaluation and Research (1996)
Amphetamine sulfate	14.9	28 800 000	EC50— <i>Pimephales promelas</i>	1000	28 800	0.001	Negligible	Russom et al. (1997)
Norketamine	219.4	25 350 000	LC50— <i>Daphnia magna</i>	1000	25 350	0.009	Negligible	Li et al. (2017)
Benzoylecgonine	8.2	1 000 000	NOEC— <i>Danio rerio</i>	100	10 000	0.001	Negligible	García-Camero et al. (2015)
Methamphetamine	2.7	110 000 000	Chronic toxicity—fish	100	1 100 000	0.000	No	Deo (2014)

^aDefault PNEC, set at the same level as a related compound with similar metabolic pathway (benzoylecgonine).

MDMA = 3,4-methylenedioxymethamphetamine; LC50 = median lethal concentration; NOEC = no-observed-effect concentration; EC50 = median effect concentration; ECOSAR = Ecological Structure Relationships.

and a rise in apoptosis (Binelli et al. 2012, 2013; Parolini et al. 2013). Changes in behavioral activity were observed in zebrafish (*Danio rerio*), induced by cocaine via dopaminergic signaling (Darland and Dowling 2001). In addition, skeletal muscle of cocaine-exposed European eel (*Anguilla anguilla*) at an environmental concentration (20 ng/L) showed evidence of serious injury, including swelling and muscle breakdown. These changes were detectable 10 d after the end of cocaine exposure (Capaldo et al. 2018). It was shown that methamphetamine significantly attenuates long-term memory formation in *Lymnaea stagnalis* (Kennedy et al. 2010; Rosi-Marshall et al. 2015). Morphine decreased the immune response of freshwater mussel (*Elliptio complanata*), displaying decreased cell adherence, lipid peroxidation, activity of intracellular esterase, and phagocytic activity (Gagne et al. 2006; Pal et al. 2013). All these studies demonstrate that illicit drugs present in streams and lakes have the potential to affect both the structure and function of ecological communities. To that end, further attention should be paid to them by performing additional monitoring surveys that could be customized to events occurring at the lakeshore.

CONCLUSION

Even though sanitation protocols are implemented in festival areas, lakeside music festivals may pollute lake water with several kinds of illicit drugs. The duration of illicit drug loads in the aquatic environment, however, is case-dependent; and illicit drugs often disappear within a short period. Nevertheless, the acute effects of these drug “cocktails” on aquatic wildlife are unknown.

Supplemental Data—The Supplemental Data are available on the Wiley Online Library at <https://doi.org/10.1002/etc.4998>.

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Data Availability Statement—Data, associated metadata, and calculation tools are available from the corresponding author (maasz.gabor@okologia.mta.hu).

REFERENCES

- Binelli A, Marisa I, Fedorova M, Hoffmann R, Riva C. 2013. First evidence of protein profile alteration due to the main cocaine metabolite (benzoylecgonine) in a freshwater biological model. *Aquat Toxicol* 140:268–278.
- Binelli A, Pedriali A, Riva C, Parolini M. 2012. Illicit drugs as new environmental pollutants: Cyto-genotoxic effects of cocaine on the biological model *Dreissena polymorpha*. *Chemosphere* 86:906–911.
- Boleda MA, Galceran MA, Ventura F. 2009. Monitoring of opiates, cannabinoids and their metabolites in wastewater, surface water and finished water in Catalonia, Spain. *Water Res* 43:1126–1136.
- Capaldo A, Gay F, Lepretti M, Paoletta G, Martucciello S, Lionetti L, Caputo I, Laforgia V. 2018. Effects of environmental cocaine concentrations on the skeletal muscle of the European eel (*Anguilla anguilla*). *Sci Total Environ* 640:862–873.
- Darland T, Dowling JE. 2001. Behavioral screening for cocaine sensitivity in mutagenized zebrafish. *Proc Natl Acad Sci USA* 98:11691–11696.
- Deo RP. 2014. Pharmaceuticals in the surface water of the USA: A review. *Curr Environ Health Rep* 1:113–122.
- dos Santos CEM, Nardocci AC. 2019. Prioritization of pharmaceuticals in drinking water exposure based on toxicity and environmental fate assessment by in silico tools: An integrated and transparent ranking. *Comput Toxicol* 9:12–21.
- Food and Drug Administration, Center for Drug Evaluation and Research. 1996. Retrospective review of ecotoxicity data submitted in environmental assessments. Docket 96N-0057. Rockville, MD, USA.
- Fox J, Smith A, Yale A, Chow C, Alaswad E, Cushing T, Monte AA. 2018. Drugs of abuse and novel psychoactive substances at outdoor music festivals in Colorado. *Subst Use Misuse* 53:1203–1211.
- Gagne F, Blaise C, Fournier M, Hansen PD. 2006. Effects of selected pharmaceutical products on phagocytic activity in *Elliptio complanata* mussels. *Comp Biochem Physiol C Toxicol Pharmacol* 143:179–186.
- García-Camero JP, García-Cortés H, Valcárcel Y, Catalá M. 2015. Environmental concentrations of the cocaine metabolite benzoylecgonine induced sublethal toxicity in the development of plants but not in a zebrafish embryo-larval model. *J Hazard Mater* 300:866–872.
- Gerrity D, Trenholm RA, Snyder SA. 2011. Temporal variability of pharmaceuticals and illicit drugs in wastewater and the effects of a major sporting event. *Water Res* 45:5399–5411.
- Grung M, Schlabach M. 2007. Human and veterinary pharmaceuticals, narcotics, and personal care products in the environment: Current state of knowledge and monitoring requirements. Norwegian Pollution Control Authority, Oslo, Norway.
- Hoegberg LCG, Christiansen C, Soe J, Telving R, Andreassen MF, Staerk D, Christrup LL, Kongstad KT. 2018. Recreational drug use at a major music festival: Trend analysis of anonymised pooled urine. *Clin Toxicol (Phila)* 56:245–255.
- Istvanovics V, Clement A, Somlyódy L, Specziar A, G-Toth L, Padisak J. 2007. Updating water quality targets for shallow Lake Balaton (Hungary), recovering from eutrophication. *Hydrobiologia* 581:305–318.
- Istvánovics V, Osztoics A, Honti M. 2004. Dynamics and ecological significance of daily internal load of phosphorus in shallow Lake Balaton, Hungary. *Freshw Biol* 49:232–252.
- Kasprzyk-Hordern B. 2010. Pharmacologically active compounds in the environment and their chirality. *Chem Soc Rev* 39:4466–4503.
- Kennedy CD, Houmes SW, Wyrick KL, Kammerzell SM, Lukowiak K, Sorg BA. 2010. Methamphetamine enhances memory of operantly conditioned respiratory behavior in the snail *Lymnaea stagnalis*. *J Exp Biol* 213:2055–2065.
- Lai FY, Thai PK, O'Brien J, Gartner C, Bruno R, Kele B, Ort C, Prichard J, Kirkbride P, Hall W, Carter S, Mueller JF. 2013. Using quantitative wastewater analysis to measure daily usage of conventional and emerging illicit drugs at an annual music festival. *Drug Alcohol Rev* 32:594–602.
- Lee SS, Paspalof AM, Snow DD, Richmond EK, Rosi-Marshall EJ, Kelly JJ. 2016. Occurrence and potential biological effects of amphetamine on stream communities. *Environ Sci Technol* 50:9727–9735.
- Li SW, Wang YS, Lin AY-C. 2017. Ecotoxicological effect of ketamine: Evidence of acute, chronic and photolysis toxicity to *Daphnia magna*. *Ecotoxicol Environ Saf* 143:173–179.
- Lilius H, Isomaa B, Holmström T. 1994. A comparison of the toxicity of 50 reference chemicals to freshly isolated rainbow trout hepatocytes and *Daphnia magna*. *Aquat Toxicol* 30:47–60.
- Lim MS, Hellard ME, Hocking JS, Aitken CK. 2008. A cross-sectional survey of young people attending a music festival: Associations between drug use and musical preference. *Drug Alcohol Rev* 27:439–441.

- Liu JC, Lu GH, Xie ZX, Zhang ZH, Li S, Yan ZH. 2015. Occurrence, bioaccumulation and risk assessment of lipophilic pharmaceutically active compounds in the downstream rivers of sewage treatment plants. *Sci Total Environ* 511:54–62.
- Maasz G, Mayer M, Zrinyi Z, Molnar E, Kuzma M, Fodor I, Pirger Z, Takács P. 2019. Spatiotemporal variations of pharmacologically active compounds in surface waters of a summer holiday destination. *Sci Total Environ* 677:545–555.
- Mackulak T, Brandeburova P, Grencíková A, Bodík I, Vojs Stanova VA, Golovko O, Koba O, Mackulaková M, Spalková V, Gál M, Grabic R. 2019. Music festivals and drugs: Wastewater analysis. *Sci Total Environ* 659:326–334.
- Mendoza A, Rodriguez-Gil JL, Gonzalez-Alonso S, Mastroianni N, de Alda ML, Barcelo D, Valcárcel Y. 2014. Drugs of abuse and benzodiazepines in the Madrid region (central Spain): Seasonal variation in river waters, occurrence in tap water and potential environmental and human risk. *Environ Int* 70:76–87.
- Mennis J, Stahler GJ, Mason MJ. 2016. Risky substance use environments and addiction: A new frontier for environmental justice research. *Int J Environ Res Public Health* 13:607.
- Miller BA, Holder HD, Voas RB. 2009. Environmental strategies for prevention of drug use and risks in clubs. *J Subst Use* 14:19–38.
- Mohr ALA, Friscia M, Yeakel JK, Logan BK. 2018. Use of synthetic stimulants and hallucinogens in a cohort of electronic dance music festival attendees. *Forensic Sci Int* 282:168–178.
- Pal R, Megharaj M, Kirkbride KP, Heinrich T, Naidu R. 2011. Biotic and abiotic degradation of illicit drugs, their precursor, and by-products in soil. *Chemosphere* 85:1002–1009.
- Pal R, Megharaj M, Kirkbride KP, Naidu R. 2013. Illicit drugs and the environment—A review. *Sci Total Environ* 463–464:1079–1092.
- Palamar JJ, Acosta P, Ompad DC, Cleland CM. 2016. Self-reported ecstasy/MDMA/“Molly” use in a sample of nightclub and dance festival attendees in New York City. *Subst Use Misuse* 50:82–91.
- Panawennage D, Castiglioni S, Zuccato E, Davoli E, Chiarelli MP. 2011. Measurement of illicit drug consumption in small populations: Prognosis for noninvasive drug testing of student populations. In *Illicit Drugs in the Environment: Occurrence, Analysis, and Fate Using Mass Spectrometry*. John Wiley & Sons, Hoboken, NJ, USA, pp 321–331.
- Parolini M, Pedriali A, Riva C, Binelli A. 2013. Sub-lethal effects caused by the cocaine metabolite benzoylecgonine to the freshwater mussel *Dreissena polymorpha*. *Sci Total Environ* 444:43–50.
- Pavlukovic V, Armenski T, Alcantara-Pilar JM. 2017. Social impacts of music festivals: Does culture impact locals' attitude toward events in Serbia and Hungary? *Tour Manag* 63:42–53.
- Postigo C, Lopez de Alda M, Barcelo D. 2011. Evaluation of drugs of abuse use and trends in a prison through wastewater analysis. *Environ Int* 37:49–55.
- Prichard J, Lai FY, Kirkbride P, Bruno R, Ort C, Carter S, Hall W, Gartner CE, Phong TK, Mueller J. 2012. Measuring drug use patterns in Queensland through wastewater analysis. In *Trends and Issues in Crime and Criminal Justice*, 442. Australian Institute of Criminology, Canberra, ACT, Australia.
- Rosi-Marshall EJ, Snow D, Bartelt-Hunt SL, Paspalof A, Tank JL. 2015. A review of ecological effects and environmental fate of illicit drugs in aquatic ecosystems. *J Hazard Mater* 282:18–25.
- Russom CL, Bradbury SP, Broderius SJ, Hammermeister DE, Drummond RA. 1997. Predicting modes of toxic action from chemical structure: Acute toxicity in the fathead minnow (*Pimephales promelas*). *Environ Toxicol Chem* 16:948–967.
- Sanderson H, Johnson DJ, Reitsma T, Brain RA, Wilson CJ, Solomon KR. 2004. Ranking and prioritization of environmental risks of pharmaceuticals in surface waters. *Regul Toxicol Pharmacol* 39:158–183.
- US Environmental Protection Agency. 1997a. Terms of environment: Glossary, abbreviations and acronyms. EPA 175-B-97-001. Washington, DC.
- US Environmental Protection Agency. 1997b. Ecological risk assessment guidance for Superfund: Process for designing and conducting ecological risk assessments—Interim final. EPA 540-R-97-006. Washington, DC.
- van der Aa M, Bijlsma L, Emke E, Dijkman E, van Nuijs ALN, van de Ven B, Hernández F, Versteegh A, de Voogt P. 2013. Risk assessment for drugs of abuse in the Dutch watercycle. *Water Res* 47:1848–1857.
- van Nuijs ALN, Pecceu B, Theunis L, Dubois N, Charlier C, Jorens PG, Bervoets L, Blust R, Meulemans H, Neels H, Covaci A. 2009. Can cocaine use be evaluated through analysis of wastewater? A nation-wide approach conducted in Belgium. *Addiction* 104:734–741.
- Zhang Y, Zhang TT, Guo CS, Lv JP, Hua ZD, Hou S, Zhang Y, Meng W, Xu J. 2017. Drugs of abuse and their metabolites in the urban rivers of Beijing, China: Occurrence, distribution, and potential environmental risk. *Sci Total Environ* 579:305–313.
- Zuccato E, Castiglioni S, Bagnati R, Chiabrando C, Grassi P, Fanelli R. 2008. Illicit drugs, a novel group of environmental contaminants. *Water Res* 42:961–968.
- Zuccato E, Castiglioni S, Tettamanti M, Olandese R, Bagnati R, Melis M, Fanelli R. 2011. Changes in illicit drug consumption patterns in 2009 detected by wastewater analysis. *Drug Alcohol Depend* 118:464–469.