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Occurrence and Risk Assessment of Illicit Drugs in Wastewater Treatment Plants' influent and effluent in Halland County, Sweden:

Cocaine, MDMA, Amphetamine, Methamphetamine, and Cannabis

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Abstract

Recently, the presence of illicit drugs in effluents from Wastewater Treatment Plants (WWTPs) and the aquatic environment has raised concern over their possible negative effects on aquatic organisms. In this study, therefore ecotoxicological data was retrieved through a literature survey and by using the software ECOSAR. Predicted No Effect Concentrations (PNEC) of five types of illicit drugs and their metabolites including 1. Cocaine (COC) and its metabolite Benzoylecgonine (BE), 2. Amphetamine (AMPH), 3. Methamphetamine (METH), 4. Cannabis ((delta 9 tetrahydrocannabinol (THC), 11-Nor-9-carboxy-delta-9-tetrahydrocannabinol (THC-CCOH)) and 5. 3,4-Methylenedioxymethamphetamine (MDMA) for species of three trophic levels in aquatic ecosystems derived. Predicted Environmental Concentrations (PEC) of above mentioned drugs in twelve WWTPs' influent and two effluents in Halland County have been measured by Swedish Toxicology Research Center (SWETOX co). Acute and chronic Risk Quotients (RQ) of the mixture of illicit drugs based upon two novel approaches calculated for the effluents of two WWTPs. Wastewater treatment plant in Ängstorp found with better removal efficiency of above-mentioned illicit drugs and the lower total RQs of ($0.01<MRQ_{acute}<0.1$; $MRQ_{chronic} = 0.13$) in comparison with that of Västra Stranden with the total RQs of ($0.1<MRQ_{acute}<1$; $MRQ_{chronic} = 1.4$). Although the RQ of WWTPs' effluent was higher than 1 in Västra Stranden, there was no potential of risk on aquatic organisms in surface waters receiving that effluent. AMPH and Cannabis found as the most degradable substances through the WWTPs with (90-100%) of removal efficiency. Moreover, the results revealed that Cannabis (THC-COOH) was the most hazardous illicit drug on aquatic species in case of acute and chronic effects while, in terms of genotoxicology, a mixture of Cocaine metabolites found the most dangerous mixture of illicit drugs on zebrafish embryos.

Keywords:
Drugs of abuse, Environmental Risk Assessment (ERA), Predicted No Effect Concentration (PNEC), Sewage Treatment Plants (STPs), ECOSAR

Introduction

Background

Emerging pollutants are defined as synthetic or naturally occurring chemicals that are not commonly monitored in the environment. These includes pharmaceuticals, illicit drugs, personal care products, pesticides, industrial chemicals and endocrine disrupting substances which all may be mobile and reach the aquatic and terrestrial ecosystems. Once entered into the environment they may be persistent and even if they occur at low concentrations they may posing a threat to humans or other organisms. (Geissen et al., 2015 ; Gavrilescu et al. 2015 ; Repice et al., 2013 ; Parolini et al., 2016).

According to the office on drugs and crime of United Nations, about five per cent of the world's adult population in 2010, and around 275 million people in 2016 were estimated to have used an illicit drug at least once whereas, 70 per cent of the global burden of disease due to drug consumption disorders, were attributable to opioids in 2015. Also, the majority of 190,000 premature deaths that occurred were due to the use of opioids globally. (UNODC, 2012; UNODC, 2017; UNODC, 2018). Although, the threats of methamphetamine (METH) and other psychoactive drugs were the focus of attention recently, the production of cocaine (COC) and opioids is increasing and there are no signs of stopping their crippling effects. In particular, the use and abuse of illicit drugs has increased dramatically within Western counties so that Cannabis herb (THC/THC-COOH) previously produced locally in Europe, is now gaining entry into Europe from Morocco in Africa. Among amphetamines, major quantities of MDMA (ecstasy) are being seized in Europe. Also, laboratories manufacturing amphetamine stimulants, which were mostly manufacturing METH, are continuing to increase, thus METH use is spreading and there are growing concerns about methamphetamine use in North America, South-West Asia and parts of

Europe. (Binelli et al., 2012; UNODC, 2014; UNODC, 2017). However, together with illicit drugs consumption, other reasons such as, improper disposal of medicines, and incomplete removal in the wastewater treatment plants, resulted in the enhanced levels of detection of these type of substances in the environment. Consequently, in the last decade illicit drugs and their metabolites have received attention as they might be classified as emerging organic contaminants in aquatic ecosystems. They are continuously released into surface water and they have also been detected in ground water (due to artificial recharge using treated wastewater and surface water) (Nefau et al., 2013; Stuart & Lapworth, 2017; Campestrini & Jardim, 2017; Wang et al., 2016; Sangion & Gramatica, 2016).

Fate of illicit drugs in WWTPs and surface waters

The procedure of assessing the prevalence of drugs of abuse by interviewing, surveying consumers, prescriptions and production records, or seizure statistics are expensive or time-consuming to carry out and may not be accurate due to reporter bias. Analyzing the raw sewage in order to monitor drugs is considered as an alternative method by researchers to estimate illicit drugs consumption (Yadav et al., 2017; Zuccato, et al., 2005). Majority of pharmaceuticals or administered drugs excreted by humans via urine and feces as a primary compound or its metabolite from private households and hospitals discharging directly into the sewer network. Therefore, method of analysis of the wastewater entering municipal wastewater treatment plants (WWTPs) referred to as ‘wastewater analysis’ or ‘wastewater-based epidemiology’ applied by many researchers in order to monitor illicit drugs concentration levels in the aquatic ecosystems (Pal et al., 2013; Zuccato et al., 2008; Komori et al., 2013; Castiglioni et al., 2014). Hence, concentrations of drugs of abuse have been measured by several studies and it has been revealed that their presence is found in surface waters receiving wastewater effluent (Rosi-Marshall et al., 2015). MDMA and METH concentrations have been investigated in wastewater treatment plants by Jones-Lepp and coworkers, (2004) who stated that continuous exposure to these types of psychoactive drugs could have an adverse effect on biota. Also, Bartelt-Hunt et al. (2009) reported the presence of METH in WWTPs effluents and in streams receiving the effluent.

Cocaine and its metabolite Benzoylecgonine were detected in WWTPs and also in surface waters in two different studies conducted in Dublin, Ireland and river Po, Italy. Table 2 summarizes the

occurrence and fate of drugs of abuse in the wastewater treatment plants in different countries reviewed in this study.

Environmental Risk Assessment (ERA)

Risk assessment considered as a central theme in the control of chemicals playing a fundamental role for national and international regulatory guidelines. Environmental risk encompasses Risk impacts on the environment, and arises from exposure and hazard. ERA includes three steps of exposure assessment, effect assessment, and risk characterization. Exposure can be assessed by measuring exposure concentrations. Second step is to conduct laboratory experiments on aquatic species to come up with the amount of concentration which affect the representative aquatic organisms from three trophic levels (algae, daphnid, fish). The values obtaining from acute tests considered as Effect Concentration (EC) or, Lethal Concentration (LC) (when the effect is mortality) whereas, Chronic values (Chv) obtain from chronic tests. Risk characterization as the final step is the estimation of the adverse effects likely to occur in environmental compartment due to actual or predicted exposure to a substance with derivation of Risk Quotient (RQ) (Van Leeuwen & Vermeire, 2007).

Although, review attempts in this study revealed that in case of pharmaceuticals, sufficient toxicological data for calculating risk quotient are available, very little research has been carried out in which, the eventual eco-toxicological risk for drugs of abuse has been assessed. This was according to the report of Statens Forurensningstilsyn (2007) stating that in case of the illicit drugs there are very little occurrences of data or little aquatic toxicity data are available.

Lack of aquatic ecotoxicological data for psychoactive substances makes it difficult to conduct proper environmental risk assessment (Styszko et al., 2016) and there is limited information on the environmental risk of illicit drugs to other aquatic ecosystems in Europe due to scarcity of data on environmental concentration downstream in sewage treatment plants.

Genotoxic effects on the aquatic species of different trophic levels

Genotoxicity refers to potentially harmful effects on genetic material. it serves the purpose of establishing whether or not substances have the potential to induce heritable germ cell mutations at the gene or chromosome level (Van Leeuwen & Vermeire, 2007). Although, some researchers developed the methods in order to measure illicit drug concentrations in aquatic environments, a

few studies investigated changes in the protein profile of aquatic non-target organisms in response to illicit drug exposure (Binelli et al., 2012; Parolini et al., 2018a). The first attempt to investigate the cyto-genotoxic effects of cocaine on *Dreissena polymorpha* (Zebra mussel) was made by Binelli et al. (2012). DNA damage, imbalance in oxidative status, and significant rise in apoptosis (cell death) observed as adverse effects of zebra mussel exposure to COC, BE, AMPH, and MDMA (Parolini et al., 2016; Binelli et al., 2012). DNA fragmentation and imbalance in the oxidative status of embryos of *Danio rerio* (Zebrafish) exposed to COC and its metabolites showed in a study by Parolini et al. (2017). Also, there are a few studies investigated the adverse effects of drugs of abuse on *Oryzias latipes* (Medaka fish) larvae and eel (Gay et al., 2013; Liao et al., 2015; Capaldo et al., 2012).

Recently, monitoring data from the County Board of Halland and other places in Sweden has become available and consequently an environmental risk assessment (ERA) can be conducted following EU guidelines for deriving Predicted Non Effect Concentration (PNEC) and using the Hazard Quotients (HQ) approach (Mastroianni, et al., 2016) to know the eco-toxicological effects of illicit drugs. Consequently, the aim of this study is to answer the following questions:

1. Which of the illicit drugs (COC, BE, AMPH, METH, MDMA, THC/THC-COOH) are the most degradable ones through the WWTP systems and which are not easily removed? 2. Which of the above mentioned illicit drugs are the most hazardous to the aquatic environment (ecotoxic effects)? and 3. Is there a potential of environmental risk (RQ>1) in the WWTPs' effluents or their recipient waters in Angstorp and Vastra Stranden? 4. In case of genotoxic effects which of the above mentioned illicit drugs are the most dangerous to the aquatic organisms?

Materials and Methods

Target compounds

The concentration of five illicit drugs belonging to three different chemical classes (Cannabinoids, Amphetamine Type Stimulants (ATSs), and Cocainics (UNODC, 2016; Mendoza et al., 2014)) was measured in the samples collected. Considering the molecular structure of COC and BE, consist of "Esters" and respectively have two and three attachments of carbon to nitrogen placed in "Aliphatic Amine" class based on ECOSAR classification. AMPH and METH with one and two attachments of carbon to nitrogen are among "Aliphatic Amine" class (Table 1). This study

considered the lowest LC50/EC50 of those substances with more than one type of chemical structure.

Table 1 Physical and chemical characteristics of cocaine (COC), benzoylecgonine (BE), amphetamine (AMPH), methamphetamine (METH), Cannabis, and MDMA.

Group[1]	Illicit drugs	CAS	Molecular Weight	Classification [a]	Water solubility [2](mg/l)	LogKow Measured[3]	LogKow Estimated[3]
Cocainics	COC	50362	303.36	Aliphatic Amine/Esters	*1800*	2.3	2.17
	BE	519095	289.33	Aliphatic Amine/Esters	**88311.88**	[n.a]	-1.32
Cannabinoids	THC	1972083	314.47	Phenol	**0.04**	6.97	7.73
	THC-COOH	56354064	344.45	Phenol	*2.8*	6.97	7.6
ATS	AMPH	300629	135.21	Aliphatic Amine	**35306.08**	1.76	1.76
	METH	537462	149.24	Aliphatic Amine	**13293.77**	2.07	2.22
	MDMA	42542109	193.25	Aliphatic Amine/Bezodioxoles	**991.55**	2.15	2.28

[1] from Mendoza et al. (2014)

[n.a] not available

[a] from ECOSAR version 2.0

[2] in italics measured values; in bold estimated values

[3] see section "effect assessment (derivation of PNEC)"

Ecological Risk Assessment (ERA)

In order to calculate the risk quantities for these kinds of chemicals, different phases of environmental risk assessment have been defined as follows:

Exposure assessment in WWTPs' effluents and surface waters (measuring MEC values)

Predicted Environmental Concentration (PEC) can be estimated by integrating information on predicted amounts of emission of chemicals and specific removal efficiencies in a sewage treatment plant or surface waters. Measuring the real concentrations of chemical substances such as pharmaceuticals in the different compartments of the environment which is called Measured Environmental Concentration (MEC) is considered as an alternative for PEC. According to the EU

EMEA (European Agency for the Evaluation of Medicinal Products, 2003) cited by Hoyett et al. (2016), If PEC value < 10 ng/L, it may be assumed that the compound presents no environmental risk. If the PEC >10 ng/L the compound is known to pose special ecotoxic effects. (Hoyett et al., 2016; Van Leeuwen & Vermeire, 2007; Rudden, 2006).

In this study MEC values measured from sample analyzing by SWETOX (ngL^{-1}) for each substance in twelve WWTPs (inflow/outflow) of Halland County (Table 2). The values were converted from ($\mu g/m^3$) to (µg/l) and (ng/l).

In order to estimate the chemical concentrations in surface waters, this study used a simple mathematical equation defined by Van Leeuwen & Vermeire (2007). According to Van Leeuwen & Vermeire (2007), the concentration of a chemical in a surface water, is estimated from the concentration of that chemical in a WWTPs' effluent divided by a specified Dilution Factor (DF). Assuming the homogeneous distribution of the chemical in surface water, a DF of 10 is applied (Van Leeuwen & Vermeire, 2007). So, the concentration of each of the illicit drugs in WWTPs' effluent was divided by 10 to estimate their concentrations in surface water receiving that effluent.

WWTPs' sampling in Halland County (MEC values)

The method of analysis was based on liquid chromatography tandem mass spectrometry (LC-MS/MS). This is a method used very frequently in studies to detect drugs of abuse in environmental samples due to its high sensitivity, versatility and selectivity features. (Zuccato et al., 2005 ; Andres-Costa et al., 2014 ; Östman et al., 2014 ; Postigo et al., 2010 ; Baker & Kasprzyk-Hordern 2013 ; Al Aukidy et al., 2012 ; Bueno et al., 2011 ; Campestrini & Jardim, 2017).

A 2017 sampling plan organized for twelve municipalities in Halland County (Ängstorp, Västra Stranden, Åled, Simlångsdalen, Veinge, Laholm, Hedhuset, Knäred, Busör, Getinge, Oskarström and neighboring Skåne County (Båstad)) of influent wastewater on specific occasions, Tuesday 12/9/2017, Saturday 26/ 11/2017 (and 25 / 12/2017, which were then removed from the plan). The concentrations of illicit drugs were calculated in accordance with the guidelines for the EMCDDA.

Effect assessment (Derivation of PNEC)

Effect assessment is an evaluation process consists of deriving the "Predicted No Effect Concentrations (PNECs)" using endpoints for at least three different trophic levels of species

(algae, daphnia and fish). Standard acute toxicity tests are performed on algae, daphnia and fish and considering the worst case scenario, the lowest values of EC50 effect concentration or Lethal concentration (LC50) or the chronic "No Observed Effect concentration (NOEC)" available for these tests is used to calculate a PNEC (Hoyett et al., 2016; Van Leeuwen & Vermeire, 2007; Rudden, 2006). In order to derive PNECs through literature this study reviewed articles applying keywords "PNEC", "drugs of abuse", on databases "Science Direct" (64 results) and "drugs of abuse" on database "Web of Science" refined by "environmental risk assessment" (88 results). In case of genotoxic effects of drugs of abuse, keywords "genotoxicity" and "illicit drugs" on database "Web of Science" (12 results) and also, keywords "illicit drugs", "genotoxic" and "aquatic species" on database "Science Direct" (96 results) applied. Articles containing data of five illicit drugs studied in this thesis were collected.

In spite of extensive search of the literature, ecotoxicological endpoints from laboratory studies for relevant organisms has not been found for every illicit drug. Hence, in this study ecotoxicological endpoints were also predicted using the Quantitative Structure Activity Relationships (QSAR) using the Ecological Structure Activity Relationships (ECOSAR) model (Sanderson et al., 2003).

The first version of ECOSAR was developed in the early eighties and it can be used to predict the toxicity of chemicals to aquatic organisms such as fish, invertebrates and algae for example when experimental data is not available. This software works based on the similarity of chemicals' molecular structures to other compounds for which there is sufficient aquatic toxicity information and similarity in measured effect levels from aquatic toxicity data (Jones et al., 2002; Sanderson et al., 2003; ECOSAR, 2017). Using the measured aquatic toxicity values and regression equations (currently 709 for more than 111 chemical classes) acute and chronic endpoint estimates of toxicity can be calculated (Sanderson et al., 2003; ECOSAR, 2017). According to Nabholz (2001) cited by Sanderson et al. (2003), the validity of the models have been tested and an 87.9 % agreement between predictions of the ECOSAR model and measured data for more than 2000 different chemicals was estimated. In order to characterize the potential aquatic hazard, the ECOSAR program can calculate ecotoxicological endpoints of acute effects (Fish, 96-hr LC50; Daphnid, 48-hr EC50; Algae, 72- or 96-hr EC50) and chronic effects (Fish, ChV; Daphnid ChV; Algae, ChV). The ChV, or Chronic Value, is defined as the geometric mean of the no-observed-effect

concentration (NOEC) and the lowest-observed-effect concentration (LOEC). ECOSAR derives toxicity values for three general types of chemicals: Neutral Organics, Organic Chemicals with Excess Toxicity, and Surfactant (Surface-Active) Organic Chemicals. Drugs of abuse belong to the chemical substances with excess toxicity. This class of chemicals are more toxic than predicted by baseline toxicity equations to one or more aquatic organisms and therefore, separate Quantitative Structure-Activity Relationships (QSARs) have been developed for these types of chemicals. Considering uncertainties such as inconsistent laboratory test conditions, inaccurate measurements for chemicals with high Kow values, in conditions where pH can affect a chemical partitioning or where log Kow values were not available for a chemical substance QSARs were derived using predicted log Kow values. Hence, using estimated log Kow is recommended, ECOSAR will accept user-entered log Kow values though. Having log Kow and valid toxicity data, ECOSAR derives mathematical relationships between log Kow and toxicity applying regression techniques. In addition, the ECOSAR program considers the toxicity measured at, pH 7 (approximating environmental conditions), the total organic carbon content should not exceed 2 mg/L, and the water hardness should be approximately 150 mg/L $CaCO_3$. Moreover, when measured data are lacking within a class to derive a chronic value (ChV) (e.g., there is no actual toxicity data for algae) ECOSAR applies a method named Acute Chronic Ratio (ACR) which is a ratio of acute values to chronic values (acute values/chronic values) and can range from 1 to 26 depending on species, chemical class, and available measured data. If a measured ACR is known for a class, then the ECOSAR uses it and If not (in case of illicit drugs class), then an ACR of 10 is generally applied for fish and daphnid, and an ACR of 4 is used for green algae to derive chronic values. To estimate the toxicity to aquatic organisms, when the log Kow ≤ max log Kow, ECOSAR provides reliable quantitative (numeric) toxicity estimates for acute effects. If the log Kow exceeds those general limits, means that there is “no effects at saturation” during a 48 to 96-hour test because the solubility of the chemical decreased. Tolls et al. (2009) cited by ECOSAR (2017) stated that in terms of chronic exposures, If the log Kow >8 “no effects at saturation” are expected in saturated solutions even with long-term exposures. (ECOSAR, 2017)

Risk characterization for derivation of RQs

In order to modify the PNEC value to account for the uncertainty involved in extrapolating from laboratory conditions into the real environmental conditions an Assessment Factor (AF) is applied

to the PNEC value through the formula: PNEC/AF whereas: AF=1000 for acute data (EC50/LC50); AF=100 when long term or chronic value (Chv) is available; (Kosma et al., 2014; Van Leeuwen & Vermeire, 2007). The assessment factor of 1000 considered as a standard AF by European Commission (2003) according to Kim et al. (2017) and applied in other reports (Thomaidi et al. 2015) on the lowest EC50/LC50. In this study, the risk assessment method based upon the legislation in ECHA (2008) in a way that, the AF of 1000 and 100 applied on the lowest LC50/EC50 and ChV repectively.

Environmental risks are often expressed as PEC/PNEC or MEC/PNEC ratios as risk quotients in many international regulatory frameworks. There are three levels of Risk Quotients frequently defined in literatures: when $0.01 < RQ < 0.1$ they considered the low risk of pharmaceuticals; $0.1 < RQ < 1$ defined as a "medium risk"; and $RQ>1$ shows the "high risk" (Hoyett et al., 2016; Kosma et al. 2014; Santos et al., 2007; Afonso-Olivares et al., 2017; Chen et al., 2016; Pereira et al., 2017; Al Aukidy et al., 2012). Hence, the RQ values for the mixture of five illicit drugs in the effluents of two WWTPs calculated in this study interpreted based upon above mentioned criteria. In order to interpret the toxicity data Hernando et al., (2006) cited a directive of Commission of the European Communities (EC, 1996) which classifies substances according to EC50 values. Substances with $EC50 < 1$ mg/L classified as: "very toxic to aquatic organisms"; from 1 through 10 mg/L "toxic" and from 10 through 100 mg/L "harmful to aquatic organism". According to these values the results of EC50 for every single substance obtained in this study can be identified as the level of toxicity for each illicit drug.

In order to measure the risk quantities of these kind of chemicals, Predicted No Effect Concentration (PNEC) derived from compiled data (Table2) in a way that, the lowest value of acute or chronic data divided respectively by an AF of 1000 and 100 considering the worst case scenario. In case of COC and cannabis and their metabolites for which the exact concentration were not available in SWETOX report this study takes the lowest PNEC among both compound and its metabolite to derive the PNEC of minimum.

Calculating the Risk Quotients (RQ values)

Environmental risks for each illicit drug according to above mentioned explanations calculated by the following formula.

$$RQi = \left(\frac{MEC}{PNECi}\right)$$

Where, (RQ_i) is the risk quotient of each drug and (MEC_i) and ($PNEC_i$) are Measured Environmental Concentration and Predicted No Effect Concentration of each drugs of abuse in the influent or effluent of the WWTPs of Halland county or surface waters receiving those effluents. According to Backhaust and Faust (2012), the calculation of an "ecosystem risk quotient" for mixtures involves two extrapolation steps: a) the extrapolation from experimental toxicity data for single substances by calculating the PNEC using assessment factors; and b) the extrapolation from single substances to the mixture of chemicals which can be calculated by equation 1 and 2.

So, in order to calculate the RQs of chemical mixtures two approaches suggested by Backhaust and Faust (2012) and cited by Liu et al. (2015): A) the PNECs of the individual compounds are calculated first and then extrapolates from single substance to the mixture by summing up the MEC/PNEC values of each compound (eq. 1); B) in the second approach the order of the two extrapolation steps needs to be reversed in a way that, the sum of toxic units calculated for each trophic level and then the risk quotient calculated based on the most sensitive organism group (eq. 2). EC50 in this equations represents EC50 or LC50 and AF of 1000 are used (Backhaust and Faust, 2012; Liu et al., 2015).

$$MRQ_{MEC/PNEC} = \sum_{i=1}^{n}\left(\frac{MECi}{PNECi}\right) = \sum_{i}^{n}\frac{MECi}{\min(EC50algae, EC50fish, EC50daphnid)\times(\frac{1}{AFi})} \quad (1)$$

(2)

$$MRQ_{stu} = \max(STUalgae, STUfish, STUdaphnid) \times AF =$$

$$max(\sum_{i}^{n}\frac{MECi}{EC50i,algae}, \sum_{i}^{n}\frac{MECi}{EC50i,fish}, \sum_{i}^{n}\frac{MECi}{EC50i,daphnid}) \times AF$$

Where ($MRQ_{MEC/PNEC}$) is the "Mixture Risk Quotient" and (MECi/PNECi) are the risk quotient of each drug. Also, TU and STU are the "toxic unit (MEC/EC50)" and the "sum of toxic unit", respectively (Backhaust and Faust, 2012; Liu et al., 2015).

Results and Discussion

Occurrence of illicit drugs in WWTPs of different countries (MEC values)

This study provided the concentrations (ng /l) of the groups of illicit drugs in the influent and effluent of the wastewaters, as reported in literatures mostly from European countries and also, the results of the Halland county WWTPs compiled in Table 2. As reported in previous studies sampling has been conducted in different weather conditions such as wet or dry seasons. In most of the cases median or mean values are considered as the influent or effluent concentrations. The concentrations of drugs of abuse in the influents indicate patterns of drugs consumption in the local community, while that in effluent reflects the potential for the contamination of the receiving water bodies. As a snapshot, table 1 provided data of illicit drugs occurrence in WWTPs in Spain, the Netherland, Italy, Switzerland, UK, Sweden, USA, and Belgium). In Halland County sampling was conducted twice in cold season.

Table 2 Concentration of cocaine (COC), benzoylecgonine (BE), amphetamine (AMPH), methamphetamine (METH), Cannabis, and MDMA (ngL-1) in the influent and effluent of Wastewater Treatment Plants (WWTPs).

WWTPs/Substances	**COC (ngL-1)**		**BE (ngL^{-1})**		**AMPH (ngL^{-1})**		**METH (ngL^{-1})**		**Cannabis group(ngL^{-1})**		**MDMA (ngL^{-1})**	
	Inf.	Eff.	Inf.	Eff.	Inf.	Eff.	Inf.	Eff.	Inf.	Eff.	Inf.	Eff.
Britain{1}	56.8	14.8	196.4	61.8	134.9	8.5	2.3	0.8	-	-	10.3	13.4
Southeastern Spain{2}	474	171	2541	101	496	225	614	-	-	-	-	-
Eindhoven, Netherland {3}	118	<6 [a]	862	21	682	6.9	<15 [a]	<5 [a]	131	<7 [a]	92	107
Netherlands {10}	363	3	1463	20	310	nd	151	33	378	nd	102	56
Amsterdam, Netherland{3}	-	-	434	35	88	<4 [a]	<15	<5	375	22	140	138
Milan, Italy (Nosedo){4}	421.4	<0.99 [a]	1132	<0.92[a]	14.7	<2.8 [a]	16.2	3.5	62.7	<1.75 [a]	14.2	4.4
Switzerland, Lugano{4}	218.4	10.7	547.4	100.3	<2.8	<2.8	<3.5	<3.5	91.2	7.2	13.6	5.1
Switzerland{5}	248	15	604	96	7	<20	<20	7	<100	<100	26	11

Antwerpen-Noord, Belgium{6}	167	<1 a	465	7	-	-	-	-	-	-	-	-
Aalst, Belgium{6}	92	<1 a	322	18	-	-	-	-	-	-	-	-
Cookeville, USA{7}	-	-	-	-	86.4	0.639	60.3	0.4	-	-	-	-
Northeastern, Spain{8}	200	10	1100	90	207	28	6	6	-	-	43	56
Valencia, Spain 1{9}	748.5	nd	135.6	58.3	39.8	nd	4.7	nd	222	nd	39.4	57.1
Valencia, Spain 2{9}	1269.5	nd	1393.1	17.2	21.2	nd	4.1	14.3	236.9	nd	25	26.7
Valencia, Spain 3{9}	1696.6	nd	3050.1	158	59.7	nd	4.3	nd	484.9	nd	52	46.7
Ängstorp, Halland, Sweden{11}	114.6 inf. Of COC+BE		12.5 Eff. Of COC+BE		682.2	0	0.3	1.5	100.5	1.4	3.5	4.3
Västra Stranden Halland, Sweden {11}	122.5 inf. Of COC+BE		48.5 eff. Of COC+BE		504.8	0	4.3	6.1	163.3	15.4	12	24.2
Åled, Halland, Sweden {11}	43.9 inf. Of COC+BE		Eff.: na		354.9	na	0.6	na	62.5	na	0	na
Simlångsdalen ,Halland, Sweden {11}	6.5		Eff.: na		347.6	na	0.5	na	60.2	na	17.8	na
Veinge , Halland, Sweden {11}	169.7		Eff.: na		1850.3	na	0.3	na	101.4	na	8.3	na
Laholm , Halland, Sweden {11}	135.8		Eff.: na		310	na	5.3	na	25	na	2.9	na
Hedhuset, Halland, Sweden {11}	17.6		Eff.: na		0	na	4.6	na	0	na	19.2	na
Knäred , Halland, Sweden {11}	16.3		553.2	na	1	na	123.4	na	7.5	na		
Busör Halland, Sweden {11}	115.8		Eff.: na		159.3	na	1.2	na	19.9	na	2	na
Getinge , Halland, Sweden {11}	3.1		Eff.: na		102.5	na	0.5	na	73.1	na	2.3	na
Båstad , Sweden {11}	103.3		Eff.: na		304.1	na	3.8	na	63.9	na	0.5	na
Oskarström, Halland, Sweden {11}	14.6		Eff.: na		227.4	na	3.4	na	43.4	na	1.6	na

a limit of quantification (LOQ)

nd not detected

na not available

{1} (Baker & Kasprzyk-Hordern, 2013); {2} (Bueno, et al., 2011); {3} (Bijlsma, et al., 2012); {4} (Castiglioni, et al., 2006); {5} (Berset et al., 2010);); {6} (van Nuijsa, et al., 2009); {7} (Boles & Wells, 2016); {8} (Huerta-Fontelaa, et al., 2008); {9} (Andres-Costa et al., 2014); {10} (Van der Aa et al., 2013); {11} SWETOX (this study), 2017)

It can clearly be seen that, Spain showed the highest concentration of COC and its metabolite BE in the influent of the treatment plants. Baz-Lomba et al. (2016) stated that the country was among the ones with the most cocaine seizures in 2013. On the other hand, the lowest amount of COC concentrations were recorded in Sweden. Although, in terms of AMPH consumption, Halland, Sweden took the highest rank following by the Netherlands, removal efficiency in WWTPs was 100% and 98.9% for Sweden and Netherlands respectively. In addition, in agreement with other studies (Huerta-Fontela et al., 2008; Postigo et al., 2010) AMP, COC and cannabis were among the narcotics with the highest removal efficiency in WWTPs in Halland region in Sweden. In most of the regions in Halland, Sweden, MDMA (ecstasy) concentration in the influent was below the level of a potential environmental risk ($<10 ngL^{-1}$). However, this substance, has the lowest removal efficiency in WWTPs in compare with other drugs of abuse (Andres-Costa et al., 2014). Actually the removal rate was negative. This negative removal rates according to Postigo et al. (2010) and Andres-Costa et al. (2014) was due to the lower residence times and desorption during wastewater treatment system. Although, physical and technical characteristics of WWTPs could contribute to the removal efficiency of chemical substances, the substantially lower concentrations of most of the compounds in effluents than influents are due to the extensive degradation or sorption of those drugs during the wastewater treatment processes (Castiglioni et al., 2006). Although, the ratio of COC to its metabolite BE could be due to the environmental conditions (temperature) and WWTPs' parameters such as residence time (Andres- Costa et al., 2014), a research conducted on occurrence of illicit drugs in the influent and effluent of 25 WWTPs in France (Nefau et al., 2013) declared that variable trends of removal were between the compounds and to a lesser extent between different treatment technologies of WWTPs.

In most of the case studies including twelve regions in Sweden, the most degradable illicit drugs were AMPH and Cannabis with (90-100%) of removal efficiency followed by, COC and its metabolite, BE (60-98%). METH found with the removal rate of (60-80%) while MDMA, showed the lowest removal efficiency through the WWTPs. Thus, other WWTPs in Halland County could have the same removal efficiency for illicit drugs as Ängstorp and Västra Stranden. Moreover, the

WWTP in Angstorp showed a better function in terms of removing illicit drugs in compare with that of Västra Stranden.

Effect assessment (PNEC values)

In table 3 – 5 PNEC values derived from literature or by applying the ECOSAR software for three groups of illicit drugs and different trophic level of aquatic species are presented. Moreover, this study provided equations based upon the lowest PNEC data reported in literature and those obtained by ECOSAR application (eq. 3-7). In case of having the influent or effluent concentrations of COC or COC+ its metabolite (BE), THC, AMPH, METH, and MDMA, in other regions of Halland County, the total RQs could be calculated by applying these equations. There is a lack of information in terms of PNEC values for illicit drugs in literatures. Hence, collecting data from scientific articles and the results of computer programs (ECOSAR) which have been carried out in this study would be beneficial in order to further characterize the environmental hazards of illicit drugs in aquatic environments.

Cocaine and its metabolite Benzoylecgonine

Some studies calculated the PNECs using the older version of ECOSAR program so, values calculated by the most recent version (2.0) in this study, were selected. Considering that the exact concentration of COC and its metabolite BE have not been reported by SWETOX, this study took the most sensitive endpoints of COC and BE as the worst case scenario. ECOSAR program calculated data for two sub-classifications of COC and BE (Aliphatic Amine and Esters) based on similarity of structure. COC and BE could be in aliphatic amine and Esters classes due to their molecular structure (ECOSAR, 2017). So, both of QSARs reviewed to find the minimum toxicity values in accordance with other studies such as Mendoza et al. (2014). The PNEC values derived for COC and BE in this study was (4.35 μg/l and 6805.17 μg/l respectively) (Table 3).

Cannabis (THC/THC-COOH)

In terms of THC and THC-COOH, “selected Log Kow” of each illicit drug applied by ECOSAR program was higher than “max Log Kow”. Therefore, the PNECs is not considered reliable by the

program and it is therefore replaced by other data ($PNEC_{zebrafish}$ = 2.0) from an experimental survey conducted by Thomas, (1975) cited by Mendoza et al. (2014) (Table 4). Moreover, ECOSAR program reported that THC-COOH may not be soluble enough to measure the predicted effect for fish and daphnid so, PNEC for algae (0.05 µg/Lit) were selected.

Amphetamine Type Stimulants (AMPH, METH, MDMA)

In order to derive PNEC for AMPH the literature survey revealed that all data was derived from another study by Lilius et al. (1994) and the most sensitive specie was algae with EC_{50} of (3.8 µg/Lit) in accordance with the results of ECOSAR in this study (Table 5). Regarding PNEC derivation for METH, ECOSAR program have been applied in all studies. Hence, values from ECOSAR v.2.0 (this study) was used to derive the PNEC of (1.97 µg/Lit). A PNEC of 0.22 µg/Lit was derived for MDMA. Considering QSARs, MDMA belongs to both benzodioxoles and aliphatic amine class due to the structure (1,3-benzodioxol) and (two carbon attachments to nitrogen) (ECOSAR, 2017). Hence, both of QSARs reviewed to find the minimum toxicity values in accordance with other studies such as Mendoza et al. (2014).

The most sensitive species for COC, AMPH, METH, and THC-COOH was algae, whereas fish was the most sensitive for THC and BE and daphnids were the most sensitive for MDMA. In addition, Cannabis found as the most hazardous illicit drug considering both acute and chronic effects with the PNEC values of (0.05µg/l) and (0.011 µg/l) respectively.

Table 3 Predicted Non Effect Concentration (PNEC) data for cocaine (COC) and benzoylecgonine (BE) derived from ECOSAR and literature.

Cocaine (Aliphatic Amine class)						
Organisms	Duration	End point	Concentration (mg/Lit)	Max Log Kow [1]	PNEC[2] (µg/Lit)	Reference (Software/Literature)
Fish	96h	LC50	45.09	5	45.09	ECOSAR V2.0
Daphnid	48h	LC50	5.48	5	5.48	ECOSAR V2.0
Green Algae	96h	EC50	4.35	6.4	4.35	ECOSAR V2.0
Fish		ChV	2.47	8	24.7	ECOSAR V2.0
Daphnid		ChV	0.46	8	4.6	ECOSAR V2.0
Green Algae		ChV	1.46	8	14.6	ECOSAR V2.0
n.a			4.9[a]		4.9	Van der Aa et al. 2013
n.a			4.91[a]		4.91	Statens forurensningstilsyn (SFT) 2007
Fish			13.0[a]		13.0	Mendoza et al. 2014
Cladocerans			4.9[a]		4.9	Mendoza et al. 2014
Algae			2.28[a]		2.28	Mendoza et al. 2014
Cocaine (Esters class)						
Fish	96h	LC50	32.29	5	32.29	ECOSAR V2.0
Daphnid	48h	LC50	65.92	5	65.92	ECOSAR V2.0
Green Algae	96h	EC50	27.31	6.4	27.31	ECOSAR V2.0
Fish		ChV	2.34	8	23.4	ECOSAR V2.0
Daphnid		ChV	42.93	8	429.3	ECOSAR V2.0
Green Algae		ChV	7.4	8	74	ECOSAR V2.0
Benzoylecgonine (Aliphatic Amine class)						
Organisms	Duration	End point	Concentration (mg/Lit)	Max Log Kow [2]	PNEC[2] (µg/Lit)	Reference (Software/Literature)
Fish	96h	LC50	83953.16	5	83953.16	ECOSAR V2.0
Daphnid	48h	LC50	6805.17	5	6805.17	ECOSAR V2.0
Green Algae	96h	EC50	12041.68	6.4	12041.68	ECOSAR V2.0
Fish		ChV	15834.07	8	158340.7	ECOSAR V2.0
Daphnid		ChV	384.22	8	3842.2	ECOSAR V2.0
Green Algae		ChV	3027.3	8	30273	ECOSAR V2.0
n.a			4.9[c]		4.9	Van der Aa et al. 2013
Cladocerans			6805.16[b]		6805.16	Mendoza et al. 2014
Algae			12041.67[b]		12,041.67	Mendoza et al. 2014
Benzoylecgonine (Esters class)						
Fish	96h	LC50	33458.82	5	33458.82	ECOSAR V2.0
Daphnid[3]	48h	LC50	99206.92	5	99206.92	ECOSAR V2.0
Green Algae	96h	EC50	71406.49	6.4	71406.49	ECOSAR V2.0
Fish		ChV	5050.13	8	50501.3	ECOSAR V2.0
Daphnid[3]		ChV	176080.09	8	1760800.9	ECOSAR V2.0
Green Algae		ChV	3027.3	8	30273	ECOSAR V2.0
Fish			33458.81[b]		33,458.81	Mendoza et al. 2014

n.a not available

[a] Authors applied ECOSAR program version (1.11)

[b] Authors applied ECOSAR program version (1.11)

[c] Van der Aa et al. 2013 used data from cocaine due to a structural similarity

1 Selected logKow by ECOSAR program version (2.0) = 2.17

2 Selected logKow = -1.32 by ECOSAR program version (2.0)

3 a comment by ECOSAR program: chemical may not be soluble enough to measure the predicted effect level.

Table 4 Predicted Non Effect Concentration (PNEC) data for cannabis (THC and THC-COOH) derived from ECOSAR and literature.

THC						
Organisms	Duration	End point	Concentration (mg/Lit)	Max Log Kow [1]	PNEC[2] (µg/Lit)	Reference (Software/Literature)
Fish	96h	LC50	0.0061	7	0.0061	ECOSAR V2.0
Daphnid	48h	LC50	0.033	7	0.033	ECOSAR V2.0
Green Algae	96h	EC50	0.00066	6.4	0.00066	ECOSAR V2.0
Fish		ChV	0.0011	8	0.011	ECOSAR V2.0
Daphnid		ChV	0.0096	8	0.096	ECOSAR V2.0
Green Algae		ChV	0.0053	8	0.053	ECOSAR V2.0
Zebrafish			2[a]		2	Mendoza et al. 2014
Cladocerans			0.2 [b]		0.2	Mendoza et al. 2014
Algae		EC50	0.2 [b]		0.2	Mendoza et al. 2014
Zebrafish	96h	LC50	3.65[c]		3.65	Carty et al. 2018
n.a			0.016[b]		0.016	Statens forurensningstilsyn (SFT) 2007
THC-COOH						
Organisms	Duration	End point	Concentration (mg/Lit)	Max Log Kow [3]	PNEC[2] (µg/Lit)	Reference (Software/Literature)
Fish[2]	96h	LC50	0.47	7	0.47	ECOSAR V2.0
Daphnid[2]	48h	LC50	1.46	7	1.46	ECOSAR V2.0
Green Algae	96h	EC50	0.05	6.4	0.05	ECOSAR V2.0
Fish		ChV	0.07	8	0.7	ECOSAR V2.0
Daphnid[2]		ChV	0.34	8	3.4	ECOSAR V2.0
Green Algae[2]		ChV	0.29	8	2.9	ECOSAR V2.0
Fish		LC50	0.03 [b]		0.03	Mendoza et al. 2014
Cladocerans		LC50	0.03 [b]		0.03	Mendoza et al. 2014
Algae		EC50	0.11 [b]		0.11	Mendoza et al. 2014

[a] values from another survey conducted by Thomas (1975) cited by Mendoza et al. (2014). Article was not accessible

[b] Authors applied ECOSAR program version (1.11)

[c] actual data from an experiment

1 Selected logKow = 7.6 by ECOSAR program version (2.0)

[2] ECOSAR program comment: chemical may not be soluble enough to measure the predicted effect level.

[3]Selected logKow = 6.36 by ECOSAR program version (2.0)

Table 5 Predicted Non Effect Concentration (PNEC) data for amphetamine (AMPH), methamphetamine (METH), and MDMA derived from ECOSAR and literature.

AMPH						
Organisms	Duration	End point	Concentration (mg/Lit)	Max Log Kow [1]	PNEC[2] (µg/Lit)	Reference (Software/Literature)
Fish	96h	LC50	37.6	5	37.6	ECOSAR V2.0
Daphnid	48h	LC50	4.36	5	4.36	ECOSAR V2.0
Green Algae	96h	EC50	3.8	6.4	3.8	ECOSAR V2.0
Fish		ChV	2.39	8	23.9	ECOSAR V2.0
Daphnid		ChV	0.35	8	3.5	ECOSAR V2.0
Green Algae		ChV	1.24	8	12.4	ECOSAR V2.0
Fish			28.8[a]		28.8	Mendoza et al. 2014
Cladocerans			2.22[a]		2.22	Mendoza et al. 2014
Algae			3.8[a]		3.8	Mendoza et al. 2014
Fish			28.8[a]		28.8	Zhang et al. 2017
Cladocerans			2.22[a]		2.22	Zhang et al. 2017
Algae			3.8[a]		3.8	Zhang et al. 2017
n.a			3.8[b]		3.8	Statens forurensningstilsyn (SFT) 2007
METH						
Organisms	Duration	End point	Concentration (mg/Lit)	Max Log Kow [2]	PNEC[2] (µg/Lit)	Reference (Software/Literature)
Fish	96h	LC50	20.51	5	20.51	ECOSAR V2.0
Daphnid	48h	LC50	2.51	5	2.51	ECOSAR V2.0
Green Algae	96h	EC50	1.97	6.4	1.97	ECOSAR V2.0
Fish		ChV	1.1	8	11	ECOSAR V2.0
Daphnid		ChV	0.21	8	2.1	ECOSAR V2.0
Green Algae		ChV	0.67	8	6.7	ECOSAR V2.0
Fish		LC50	20.51[b]		20.51	Mendoza et al. 2014
Cladocerans		LC50	2.51[b]		2.51	Mendoza et al. 2014
Algae		EC50	1.97[b]		1.97	Mendoza et al. 2014
Fish		LC50	20.51[b]		20.51	Zhang et al. 2017
Cladocerans		LC50	2.51[b]		2.51	Zhang et al. 2017

Algae		EC50	1.97[b]		1.97	Zhang et al. 2017
n.a			2.3[a]		2.3	Van der Aa et al. 2013
n.a			2.26[a]		2.26	Statens forurensningstilsyn (SFT) 2007
MDMA (Aliphatic Amine class)						
Organisms	Duration	End point	Concentration (mg/Lit)	Max Log Kow [3]	PNEC[2] (µg/Lit)	Reference (Software/Literature)
Fish	96h	LC50	24.19	5	24.19	ECOSAR V2.0
Daphnid	48h	LC50	2.98	5	2.98	ECOSAR V2.0
Green Algae	96h	EC50	2.3	6.4	2.3	ECOSAR V2.0
Fish		ChV	1.27	8	12.7	ECOSAR V2.0
Daphnid		ChV	0.25	8	2.5	ECOSAR V2.0
Green Algae		ChV	0.78	8	7.8	ECOSAR V2.0
Fish		LC50	24.18[b]		24.18	Mendoza et al. 2014
Algae		EC50	2.3[b]		2.3	Mendoza et al. 2014
n.a		LC50	0.216[b]		0.216	Styszko et al. 2016
n.a			2.7[b]		2.7	Van der Aa et al. 2013
n.a			2.7[b]		2.7	Statens forurensningstilsyn (SFT) 2007
MDMA (Benzodioxoles class)						
Fish	96h	LC50	186.74	5	186.74	ECOSAR V2.0
Daphnid	48h	LC50	0.22	5	0.22	ECOSAR V2.0
Fish		ChV	0.86	8	8.6	ECOSAR V2.0
Daphnid		ChV	0.22	8	2.2	ECOSAR V2.0
Cladocerans		LC50	0.22[b]		0.22	Mendoza et al. 2014

[a] values from another survey conducted by Lelius et al. (1994) cited by Mendoza et al. (2014) and Zhang et al. (2014). Article was not accessible

[b] authors applied ECOSAR program version (1.11)

[1] Selected logKow = 1.76 by ECOSAR program version (2.0)

[2] Selected logKow = 2.22 by ECOSAR program version (2.0)

[3]Selected logKow = 2.28 by ECOSAR program version (2.0)

Risk Characterization (RQ values)

In (Table 6 and 7) RQ values calculated for the effluents of WWTPs for two regions in Halland is presented. However, RQs of the influent of all twelve regions can be calculated using equations (S3.1-S7.2 approach A) in appendix (fig.1). Risk quotients for the surface waters receiving effluents, calculated by applying a DF of (10) to the concentration of each of the illicit drugs in the effluents. So, RQ values of (0.013) and (0.142) obtained for surface waters in Ängstorp and Västra Stranden respectively (fig. 1).

It is obvious that, chronic values are lower than the acute ones in most of the cases. The RQs for the acute and chronic data calculated for the effluent of WWTPs in two regions is presented in Tables (6-7).

Table 6 Risk Quotient **(**RQ) values for the effluent of Wastewater Treatment Plants (WWTPs) in Ängstorp and Västra Stranden based on acute and chronic effects and (equation 1; approach A).

Acute effects							
Drug of abuse	Lowest LC/EC50 (µg/lit)	Most sensitive species	Lowest PNEC (µg/lit)	$MEC_{eff.}$ (µg/lit) WWTP Ängstorp	RQ_i $WWTP_{eff.}$ (Ängstorp)	$MEC_{eff.}$ (µg/lit) WWTP Västra Stranden	RQ_i $WWTP_{eff.}$ (Västra Stranden)
COC	4.35	Green Algae	4.35	0.0125	0.0028	0.0485	0.011
BE	6805.17	Daphnid					
THC	2	Zebrafish embryo	0.05	0.0014	0.028	0.0154	0.308
THC-COOH	0.05	Green Algae					
AMPH	3.8	Green Algae	3.8	0	0	0	0
METH	1.97	Green Algae	1.97	0.0015	$7.6*10^{-4}$	0.0061	0.003
MDMA	0.22	Daphnid	0.22	0.0043	0.02	0.0242	0.11
$MRQ_{MEC/PNEC}$					0.051		0.432
Chronic effects							
Drug of abuse	Lowest ChV (µg/lit)	Most sensitive species	Lowest PNEC (µg/lit)	$MEC_{eff.}$ (µg/lit) WWTP Ängstorp	RQ_i $WWTP_{eff.}$ (Ängstorp)	$MEC_{eff.}$ (µg/lit) WWTP Västra Stranden	RQ_i $WWTP_{eff.}$ (Västra Stranden)
COC	4.6	Daphnid	4.6	0.0125	0.0027	0.0485	0.0105
BE	3842.2	Daphnid					
THC	0.011	fish	0.011	0.0014	0.127	0.0154	1.4
THC-COOH	0.7	fish					
AMPH	3.5	Daphnid	3.5	0	0	0	0
METH	2.1	Daphnid	2.1	0.0015	$7.1*10^{-4}$	0.0061	0.003
MDMA	2.2	Daphnid	2.2	0.0043	0.00195	0.0242	0.01
$MRQ_{MEC/PNEC}$					0.13		1.4

Table 7 Risk Quotient **(**RQ) values for the effluent of Wastewater Treatment Plants (WWTPs)) in Ängstorp and Västra Stranden based on acute and chronic effects and (equation 2; Approach B).

Acute effects									
Drug of abuse	Fish (µg/lit)	daphnid (µg/lit)	Algae (µg/lit)	$MEC_{eff.}$ (µg/lit) WWTP Ängstorp	STU_i (Ängstorp)	MRQ_{stu} Ängstorp	$MEC_{eff.}$ (µg/lit) WWTP Västra Stranden	STU_i Västra Stranden	MRQ_{stu} Västra Stranden
COC	45090	5480	4350	0.0125	STUfish = 1.22E-06	0.031	0.0485	STUfish = 1.007E-05	0.322
BE	33458.82 *1000	6805.17 *1000	12041.68 *1000						
THC	2000	-	-	0.0014	STUdaphnid = 2.9E-06		0.0154	STUdaphnid = 1.14E-05	
THC-COOH	-	-	50						
AMPH	37600	4360	3800	0			0		
METH	20510	2510	1970	0.0015	STUalgae= 3.16E-05		0.0061	STUalgae= 0.000322	
MDMA	186740	220	-	0.0043			0.0242		
Chronic effects									
Drugs of abuse	Fish (µg/lit)	daphnid (µg/lit)	Algae (µg/lit)	$MEC_{eff.}$ (µg/lit) WWTP Ängstorp	STU_i (Ängstorp)	MRQ_{stu} Ängstorp	$MEC_{eff.}$ (µg/lit) WWTP Västra Stranden	STU_i Västra Stranden	MRQ_{stu} Västra Stranden
COC	2470	460	1460	0.0125	STUfish= 0.0013	0.13	0.0485	STUfish = 0.014	1.4
BE	15834.07 * 10^3	384220	3027.3* 10^3						
THC	1.1	9.6	5.3	0.0014	STUdaphnid = $5.3*10^{-5}$		0.0154	STUdaphnid= 0.0002	
THC-COOH	70	-	-						
AMPH	2390	350	1240	0			0		
METH	1100	210	670	0.0015	STUalgae= 0.0002		0.0061	STUalgae= 0.003	
MDMA	860	220	-	0.0043			0.0242		

The results showed that the RQ of the mixture of illicit drugs (MRQ) in case of acute effects in the effluents of both regions (Ängstorp and Västra Stranden) was less than 1 (MRQ<1). In terms of chronic effects there is a potential of high environmental risk on WWTPs' effluent in Västra Stranden (MRQ>1). However, MRQ of an effluent of WWTPs could not be considered as the risk characterization of surface waters in which the effluent ended up as, dilutions in the recipients can be expected. In addition, it is very important to note that this study only provided a snapshot of illicit drug concentrations in inflow and outflow of WWTPs in Halland during one weekend of a holiday occasion as representing a worst case scenario. So, it could not be generalized to other seasons or considered as a constant disposal amount of illicit drugs to the aquatic environment.
Also, the characteristics of the receiving water body in terms of flow rate or dilution factor will determine the extent to which it can tolerate the release of pharmaceuticals without perceptible adverse effects (Al Aukidy et al., 2012). So, applying a dilution factor of 10 consequently resulted in a ten times reduction of the RQ values in surface waters (fig. 1) which can be interpreted as there is no environmental risk on those aquatic compartments. This might be a realistic assumption in Swedish aquatic ecosystems but Pereira et al. (2017) stated that dilution factor might be much smaller especially in arid or semi-arid regions. Hence, further surveys on the effluent of WWTPs in Halland and their receiving surface waters during warm and cold seasons are needed in order to estimate the environmental hazards of drugs of abuse in more realistic conditions. Moreover, it can clearly be seen that (fig. 1) to what extent RQs of chronic data could be higher than those of acute ones. Therefore, it is important to take the environmental risk potential of illicit drugs during longer periods. In addition, using the fresh water species to estimate the environmental risk quotients can be considered as uncertainties since the recipient of WWTPs' effluent would be marine water.

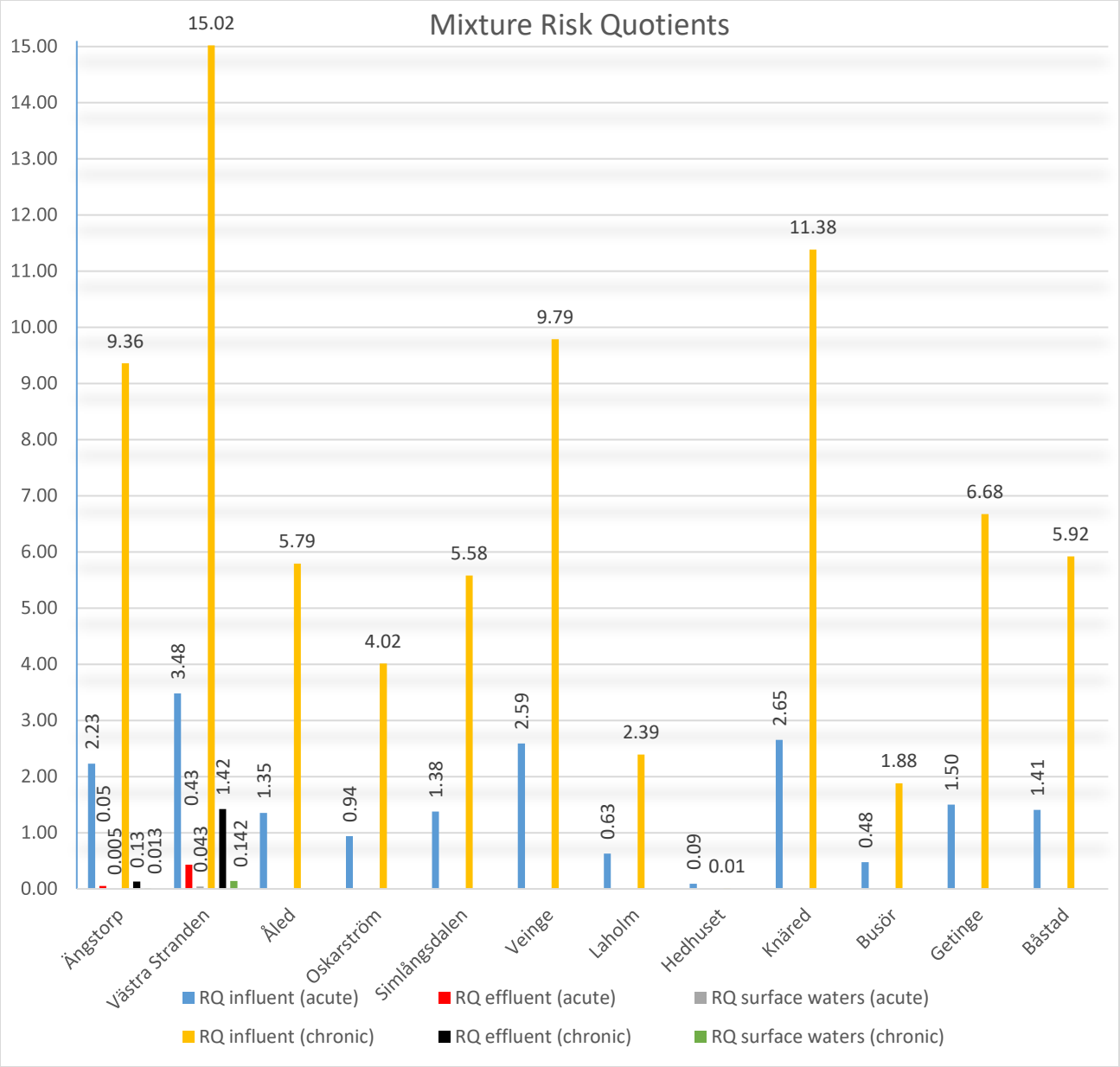

Fig.1 Risk Quotients (RQs) of influent and effluent of 12 wastewater treatment plants and surface waters receiving the effluents in Halland, Sweden considering acute and chronic Predicted non Effect Concentration (PNEC) values.

Genotoxic effects of illicit drugs on Mussels (experimental data)

In a study by Binelli et al., (2012) an increase of micronucleated cells of *Dreissena polymorpha* (Zebra mussel) and a significant rise in their apoptosis was observed after a short term (96 hours) exposure to a concentration of (10μg/l) of cocaine. Also, a dose of 5 μg/l of AMPH resulted in DNA and oxidative damage in these mussels after a 14-day exposure (Parolini et al., 2016b). Moreover, exposure to a range of (0. 5-1μg/l) of BE for 14-days resulted in oxidative stress and DNA damage on zebra mussels (Parolini et al., 2013). Although, the concentrations tend showing genotoxic effects are higher than those found in surface waters for a single substance, a realistic mixture of the above mentioned illicit drugs along with, morphine and MDMA after a period of 14-day exposure could increase of DNA fragmentation in Zebra mussel (Parolini et al., 2016a). In a study by Maranho et al. (2017) Cyto-genotoxic effects were evidenced in mussels exposed to crack cocaine concentrations ranging from (5 to 500 μg/l).

Genotoxic effects of illicit drugs on Fishes (experimental data)

Among vertebrates, *Danio rerio* (Zebrafish) is considered as a primary model offering numerous advantages to exam substance toxicity on vertebrate development in toxicology research, particularly on cardiac development. Many laboratories have used the zebrafish to study the effects of chemical compounds in the environment (Sarmah & Marrs, 2016). Zebrafish embryos exposure to a range of (0.01 μg/l -10 μg/l) of COC and its metabolites BE and ecgonine methyl ester during 96 h post fertilization increased cell mortality of embryos. (Parolini et al., 2017). While at the same time, 0.3 μg/l of each COC, BE, and EME could altered protein profile in embryos (Parolini et al., 2018a). *Oryzias latipes* (Medaka fish) larvae and eel exposure to METH (0.6 μg/l) and cocaine respectively resulted in hypoactivity and a clockwise swimming direction in Medaka and cocaine accumulation into the eel tissues and moreover, behaved like an endocrine disruptor which played a key role in the metabolic and reproductive processes of the eel (Gay et al., 2013; Liao et al., 2015; Capaldo et al., 2012).

Genotoxic effects of illicit drugs on Daphnids (experimental data)

Benzoylecgonine concentrations (1μg/l) similar to those found in aquatic ecosystems induced oxidative stress and affecting swimming behavior and the reproduction of *Daphnia magna* (Parolini, et al., 2018b).

Genotoxic and Ecotoxic data

A dose of (0.01μg/l) of the mixture of benzoylecgonine and ecgonine methyl ester found as the most genotoxic mixture of illicit drugs and zebra fish embryos considered as the most sensitive species to those concentrations in a short term experiment (acute effects).

However, the ecotoxicological data showed that cannabis (THC-COOH) was the most ecotoxic of the illicit drugs studied and green algae found as the most sensitive specie with estimated acute endpoint of 0.05 μg/l. In terms of chronic values, the lowest PNEC estimated for cannabis (THC) with (0.001μg/l) with fish as the most sensitive species.

Conclusion

Answering question 1, cannabis was the most degradable illicit drug efficiently removed through the treatment systems whereas, MDMA found with the lowest removal efficiency. Answering question 2 in terms of ecotoxicology, cannabis with the lowest PNEC value was the most hazardous substance in compare with other illicit drugs studied in this thesis. Answering question 3, the RQ of the effluent of WWTP in Västra Stranden was higher than 1 which can be interpreted as the high potential of environmental risk in that effluent. However, there was no potential of risk on aquatic organisms in surface waters receiving the WWTPs' effluents in Västra Stranden and Ängstorp. Answering question 4 in terms of genotoxicology, a mixture of Cocaine metabolites was the most hazardous substance when zebrafish embryos were exposed to that substance.

In this study a comprehensive literature review was made to find ecotoxicological endpoints for aquatic organisms in order to derive PNEC values for 5 illicit drugs and to estimate their risk. Very few experimental data were found and consequently there is an urgent need to study the effects of illicit drugs on aquatic organisms at large. A predictive tool, the software ECOSAR, therefore had to be used in order to derive PNEC-values and to conduct a preliminary ecological risk assessment. The assessment revealed that when taking dilution into account there is no emergent risk to aquatic organisms for single substances or for mixture effects. It is therefore important to continue to monitor the presence of illicit drugs in the environment. However, as there is a lack of experimental data and the model data for single species obtained by using ECOSAR has to be extrapolated to

ecosystem effects the uncertainty of the risk assessment is high and it can´t be ruled out that ecosystem effects might occur. In effect, decision makers may need to put forward new legislations in order to prevent more consumption of illicit drugs particularly in terms of cannabis with the highest RQ value and MDMA with the negative removal efficiency. The results of the study can motivate the engineers to develop and suggest new technologies to remove these substances more efficiently through the wastewater treatment plants specially in regions such as Västra Stranden with the treatment plant with lower removal efficiency of illicit drugs and high potential of environmental risk in its effluent.

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Appendix

Cocaine and its metabolite Benzoylecgonine $RQi = \left(\frac{MECi}{4.35}\right)$ for acute data (S3.1)

Cocaine and its metabolite Benzoylecgonine $RQi = \left(\frac{MECi}{4.6}\right)$ for chronic data (S3.2)

THC and THC-COOH $RQi = \left(\frac{MECi}{0.05}\right)$ for acute data (S4.1)

THC and THC-COOH $RQi = \left(\frac{MECi}{0.011}\right)$ for chronic data (S4.2)

AMPH $RQi = \left(\frac{MECi}{3.8}\right)$ for acute data (S5.1)

AMPH $RQi = \left(\frac{MECi}{3.5}\right)$ for chronic data (S5.2)

METH $RQi = \left(\frac{MECi}{1.97}\right)$ for acute data (S6.1)

METH $RQi = \left(\frac{MECi}{2.1}\right)$ for chronic data (S6.2)

MDMA $RQi = \left(\frac{MECi}{0.22}\right)$ for acute data (S7.1)

MDMA $RQi = \left(\frac{MECi}{2.2}\right)$ for chronic data (S7.2)

I was born in 1977 in Iran. Master's program in Applied Environmental Science in Sweden really motivated me to work on the environmental issues. I am currently doing a research in the field of greenhouse gas emission as a Ph.D. student.

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