Available online at www.elixirpublishers.com (Elixir International Journal)

Applied Chemistry

Elixir Appl. Chem. 69 (2014) 22974-22999

ISSN: 2229-712X

Fate of Drugs and their Metabolites in the Environment

Mustapha A Olajide[1], Michael Coffey[2] and Jason W. Birkett[3]

[1]Department of Science Laboratory Technology, Federal Polytechnic Offa, PMB 420, Offa, Kwara State, Nigeria.

[2]Department of Analytical Chemistry, School of Science & Technology, Eramus Darwin Building, Nottingham Trent University, Nottingham, NG11 8NS.

[3]Pharmacy and Biomolecular Sciences, Faculty of Science, James Parsons Building, Byrom Street, Liverpool John More University, Liverpool, L3 3AF.

ARTICLE INFO

Article history:

Received: 20 January 2014;

Received in revised form:

20 March 2014;

Accepted: 4 April 2014;

Keywords

Drug use,

Illicit drugs,

Pharmaceuticals,

HPLC-MS, GCMS,

Wastewater analysis.

ABSTRACT

The individual use of these pharmacologically active substances which generate great but underappreciated levels of other toxicologically potent and associated bioactive metabolites through purposeful and inadvertent discharge to the environment via excreta and by illegal disposal has become a global issue. This work reviews aspects of drugs occurrence, metabolism, transport routes, stability, analysis and environmental distribution of these emerging contaminants and highlights current developments in investigating and monitoring their fate and potential effects in aquatic environments. Gas chromatography-mass spectrometry (GCMS) and high performance liquid chromatography are the preferred methods for trace drugs analysis in wastewaters as their measurements depend largely on successful application of a fast and reliable method for qualitative and quantitative determination. The application of this method to the actual influents, effluents, sludge and environmental sediments from sewage treatment works (STWs) allows the assessment of drugs content and the extent at which STW helps in the transport of these pollutants (via different media) into the environment. The capability is also outlined for furthering our understanding of fate and behaviour of drugs with particular reference to illicit drugs, abused pharmaceuticals and environmental processes in our quest to understand the overall issues of drugs and make available exposure data for the aquatic realm.

Introduction

Environmental occurrence of organic pollutants through interconnectedness of human actions and activities impacts the environment in many ways. The impacts arising from the potential of global warming, deforestation and deposition of drugs comprising myriads of chemical and therapeutic classes harbours risks to our daily lives. The high consumption of drugs have significant worldwide consequences and the comprehensive assessment to provide updates regarding the occurrence, metabolism, measurement as well as removal of these contaminants from STWs effluent-waters have therefore become important for humans and aquatic environment.

Occurrence of chemical substances in the aquatic environment

Heavy metals, solvents, dyes, pesticides etc. are some of the chemicals that enter the aquatic environment in several ways causing chemical pollution. Some are either from sewage treatment works (STWs) or are dumped directly from industrial effluents. Other sources include the use of herbicides and fertilizers in agriculture. Apart from phytoestrogens that come from plants; humans and animals also excrete natural hormones which are disrupting chemicals in the environment [1]. In effluents, bisphenol A (BPA), nonylphenol, nitrates found in fertilizers as well as animal excrements and industrial chemicals occur [2]. Figure 1, shows also the presence of polycyclic-aromatic hydro carbons (PAHs), heavy metals and phthalates are shown.

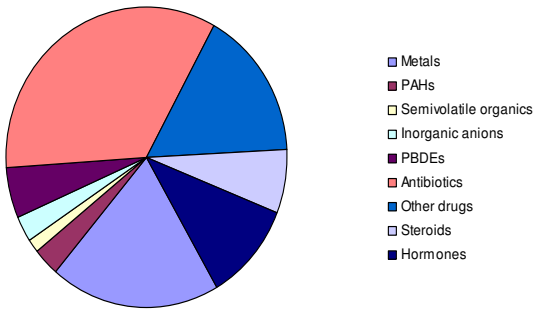

Figure 1: Chemical components in sewage sludge. Data from The U. S. Environmental Protection Agency. (Renewable Energy Venture, Austin, Texas) [4]

Other classes of endocrine disrupting compounds (EDCs), which includes multitudes of chemicals are considered in the studies of accumulation of potential toxic elements exposed to sheep grazed on grassland with repeated applications of sewage sludge and its exposure effects on sheep foetal testis development at different gestation periods [3, 4] have been reported. The chemicals used in the plastic industry includes phthalate esters and other major environmental pollutants [5] and Koppe *et al* [6] studied the metabolism of the parent phthalate and argued that a very active glucuronidated metabolite (monoester) was excreted, while in the digestive system their higher monomers has been detected [7].

Table 1 shows most of the reported data of pollutants and residual analytes in sewage samples showing the sources and the

Tele:

E-mail addresses: jayswing2007@yahoo.com

analytes found. In foetus studies, high bioaccumulation of phthalates due to easy placental transfer [8] has been observed and the effects of high doses of phthalates on male's reproductive organs have been shown but in most organ systems, they are relatively non-toxic. A reduction in testosterone production in rats exposed to phthalates confirmed extensive studies of phthalates with the increased high levels of human exposure in human spermatozoa increased damage to DNA [9-14].

Activity effects of dibutyl phthalate (DBP) [62], di-ethylhexyl phthalate (DEHP) induced 'anti-androgenic' on humans testicular dysgenesis syndrome [63], multinucleate gonocytes in rats [64-66] and occurrence of oestrogens on breast cancer cells have been linked to phthalate exposures [67].

Polychlorinated biphenyls (PCBs) are (unreactive) organic compounds, which constitute a class of 209 congener groups. The commercial production of pulp bleaching, herbicides, metal smelting, by-products in combustion processes of incineration, chlor-alkali and coal-fired power stations or processes are main sources of PCBs stable compounds. Rudel *et al* [68] had listed their uses as electronic components, pesticides extenders, cutting oils, sealants, adhesives, stabilizing additives in flexible PVC coatings of electrical wiring, wood floor production, finishers, flame retardants, hydraulic fluids, paints, de-dusting agents coolants, insulating fluids for transformers, capacitors, and in carbonless copy paper. PCBs are stable, very resistant to oxidative degradation, only degrade anaerobically and readily persist longer [69]. On human health effects, anemia, thyroid gland injuries, impaired reproduction, stomach and liver injuries have all been reported [70, 71]. Exposures of PCBs can interfere with oestrogen levels of animals [72]. Impairments of immune system, lowering of testosterone levels in males, elevating the levels of progesterone in females and disruption of thyroid hormone function [73]. Bioaccumulation of PCBs induces oestrogenic effects in animal tissues [74]. Additional studies from Whyatt *et al* [75], Lilienthal [76] and Korach [77] have all further confirmed that the chlorinated congeners are more stable and persistent longer than less chlorinated compounds.

Pharmaceuticals in aqueous environment

Pharmaceutical substances are pollutants that are steadily increasing in wide variety in the aquatic environment apart from the traditional pollutants like polychlorinated biphenyls (PCBs), pesticides and polycyclic aromatic hydrocarbons (PAHs) in recent years [78-83]. Despite the rapid rise and continuous discharge of these chemicals of which some are carcinogenic, reproductive toxic and mutagenic in environmental matrices [84-87], studies have indicated that their removals have been found to be incomplete and inadequate attention on the fate and behaviour during the transport of many drugs after their intended use have increased the risks of possible environmental effects [88-92]. The active ingredients of medicinal products with a wide range of chemical structures are excreted as parent drugs with associated metabolites after metabolism by dosed user and these are further subjected to biotransformation in the sewage treatment processes producing more polar degradation products of which many complex modes of biochemical pathways are poorly understood. This has led environmental research's increasing attention to pharmaceuticals and their corresponding metabolites considering the production of large number of registered pharmaceuticals and those procured illegally for illicit use or without prescription [89]. Yet, large quantities of different chemical classes of new pharmaceuticals enter the already saturated marketplace and these are disposed through agrochemicals runoff and the sewage systems to the aquatic environment

One of the major sources is excreta and urine containing the unmetabolized drug residues and its active metabolites being flushed down in the toilets, many unwanted and expired prescription drugs are deliberately disposed of via drains [95-97]). Also, Richardson & Bowron [93] reported that most of the drugs like antiseptics and lotions are assumed acceptable to be diluted to low levels in crude sewage when sluiced away. Numerous papers have reported the distribution of different chemicals belonging to different therapeutic classes such as antibiotics, anti-inflammatory drugs, lipid regulators, beta-blockers, β_2 –sympathomimetics, antiepileptics, antidepressants, antineoplastics, contraceptives, tranquilizers, diagnostic contrast media, preservatives and sunscreen agents in different media of the environment at the specific levels ranging from ngL^{-1} to $\mu g\ L^{-1}$ [98-101]. Also reported at microgram levels in rivers were theophylline, erythromycin and tetracycline and some amounts of oestrogen from oral contraceptive in sewage systems excreted by human population [102]

In Switzerland, about 4 tonnes/year of fluoroquinolones (antibacterial drug) are sold and 14 tonnes/year in Italy [90, 103, 104], while 100 tons of annual drug prescription in Germany alone does not include several other pharmaceuticals that have been reported in aquatic samples in numerous papers ranging from ngL^{-1} to $\mu g\ L^{-1}$ levels [87, 90, 104-106]. The recent analytical studies in UK further show that some pharmaceuticals are incompletely removed from sewage treatment works and surface waters such as lakes, rivers and seas have some detectable pharmaceuticals present [84, 105, 107-110].

Table 2 lists data of the main pharmaceuticals monitored in German STWs as well as German rivers and streams with 10,11-dihydro-10,11-dihydro-carbamazepine (DHH), a metabolite of antiepileptic carbamazepine having highest influent and effluent concentration of 4100 and 2600 ng L^{-1}.

Table 3, shows antiepileptic carbamazepine has highest concentration of 6300 ng L^{-1}, X-ray contrast media were between 11, 000-15, 000 ng L^{-1} [117]. About 31 pharmaceuticals and five metabolites were found in at least one sample of 40 German rivers. Out of 69 target compounds, only 10 were found in drinking water [119]. The survey of exposure effects and other environmental relevance is in the literature reviews [83, 120].

Commonly used illicit drugs and their metabolites in aqueous environment

The term "illicit drug or drug of abuse" is normally used to describe those drugs that are controlled under the Misuse of Drugs Act, 1971. The legislation regulates controlled drugs into classes depending on the harm they cause, and there are various offences including the unlawful possession of a controlled substance [121]. The emerging risks with the prevalence and trends in the illegal production and abuse of illicit drugs have prompted the establishment of many International Agencies [122, 123] to monitor and conduct the risk assessments of the social, economic and environmental impacts the menace are eliciting, particularly in the consumer countries. The common classes of illicit drugs are cocaine, amphetamine, opioid, lysergic acid diethylamide (LSD), hallucillogen and cannabinoid, and by the hidden activity of their users, it has helped its purported widespread and continual escalating use [83]. The idea of Daughton [101] to use a non – intrusive approach to approximate the level of illicit drugs consumption at community level which was later demonstrated by Zuccato *et al*

[124] determined the levels of cocaine in waters and related the quantity to the amount of drug consumed by a local population. The approach apparently provided information needed by environmental scientists and appropriate authorities involved in the fight against the drug menace. It has been argued that the sewage systems constitute one of the potential routes those drugs enter the environment; other highly dispersed sources include disposals by drug and manufacturing laboratories [125].

Human metabolism of environmentally relevant drugs.

In the human body, drugs are bio-transformed into one or more metabolites and after the loss of pharmacological activity the metabolites and unchanged parent drugs are eliminated from the body systemic circulation via urine or faeces. A number of parameters which include age, gender, ethnicity, patient and the time of administration have been associated to degree of metabolism. In Figure 9, the metabolism of drugs in the human body shows Phase I and Phase II reactions. The phase I comprises of oxidation reaction such as in aliphatic hydroxylation of ibuprofen and diclofenac, epoxidation of carbamazepine and ring oxidation of propranolol, while reductions, alkylations and dealkylations are other reactions.

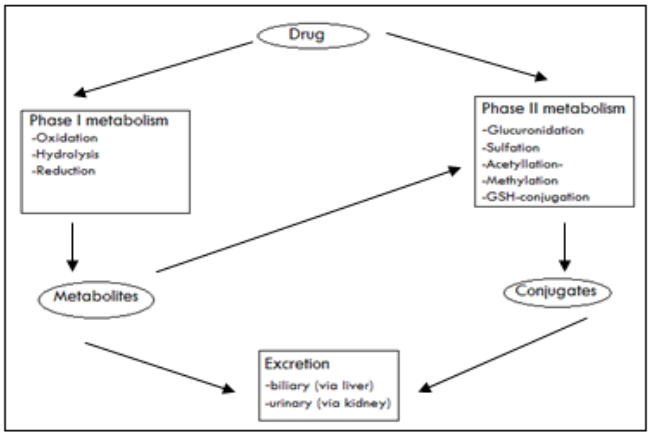

Figure 2: Simplified scheme of dug metabolism in the human body [92]

The conjugation reaction type occurs when polar molecules in Phase I transfer to the metabolites in Phase II such as the transfer of glucuronic acid to phenols, hydroxyls, caroxyls, thiols, amines and hydroxylamino groups. [92]. There is therefore interest in identifying the metabolites that may pass on to the sewage, and those that might stay longer in the STWs and enter the environment through untreated water effluents or sewage biosolids.

In the following sections, the metabolisms of the five major classes of illicit drugs found in various wastewaters are discussed.

Human metabolism of cocaine

Cocaine is extracted from the leaves of two species of coca: Erythroxylum coca and Erythroxylum novogranatense. The cocaine hydrochloride is normally formed after the alkaloids are precipitated with sodium carbonate and then dissolved in dilute HCl containing about 40% of cocaine, but when cocaine hydrochloride is extracted with ether in aqueous alkaline solution, it produces "free base" which contains 85-90% of pure cocaine [94,126]. The street cocaine used by addicts is often mixed or cut with a number of diluants [127], and these adulterants are sometimes the cause of poisoning. Cocaine is a powerful addictive stimulant drug with three common routes of administration: smoking, intravenously and intranasally (through the nose). Figure 10 shows only the compounds we determined in the results, however, cocaine is spontaneously metabolized by the action of pseudo cholinesterase and hepatic esterase to give ecgonine methylester (EME) with the loss of benzoyl group [128-131]. A non-enzymatic hydrolysis at pH above 6 converts cocaine to benzoylecgonine (BZE) by demethylation as its main metabolite. BZE can be detected in the urine 48 hours after cocaine administration with a urinary excretion half-life of 6-8 hours [132-133]. The N-demethylation of cocaine leads to norcocaine (NC) (the most toxic metabolite) by P450 enzymes and then metabolized to N-hydroxynorcocaine by brain FAD –containing mono- oxygenases [134-135]. Norcocaine can further be hydrolysed to benzoylecgonine. Cocaine undergoes trans-esterification by enzymatic reaction in the liver in the presence of alcohol to form cocaethylene (CE); which has been reported to be more toxic than cocaine [136]. When cocaine is smoked, anhydroecgonine methylester (AEME) is produced and through enzymatic hydrolysis get converted to anhydroecgonine (AE) or ecgonidine [137]. The other metabolites of cocaine (ecgonidine, norecgonidine methylester, *p*- hydroxyl-benzoylecgonine, and *m*- hydroxyl-benzoylecgonine) found in human urine have minor metabolic-pathways that involve the aromatic meta- and para-hydroxylation of cocaine followed by partial hydrolysis to the corresponding HO-Be isomers [138]. About 1-9% of cocaine has been excreted unchanged in the urine with much higher proportion in acid urine; its metabolites are recovered in variable proportions which depend on the route of administration [139].

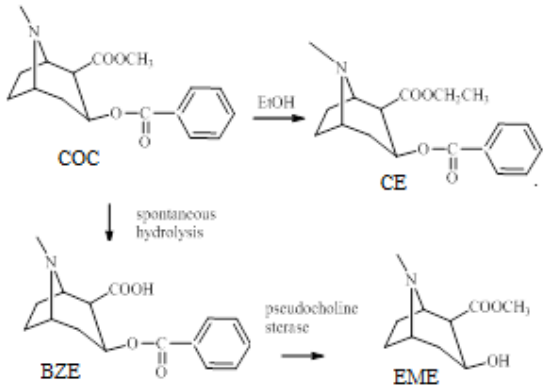

Figure 3: Degradation pathways of cocaine in the human body [140].

Human metabolism of amphetamine

Among the drugs classified as amphetamines are amphetamines (AM), methamphetamines (MA, "speed") and methylenedioxymethamphetamine (MDMA, "ecstasy or Adam"). They are usually taken orally but can be snorted, smoked or injected. They are addictive stimulant drugs that affect the central nervous systems among other risks of dependence and abuse. Other designer drugs are methylenedioxyethylamphetamine (MDE, "eve") and 3, 4-methylenedioxyamphetamine (MDA, "love pills") [141].

The major metabolic pathway involves deamination of cytochrome P450 to para- hydroxyl amphetamine and phenylacetone, this later compound is oxidised to benzoic acid and excreted as glucuronide or glycine (hippuric acid) conjugates. Smaller amounts of amphetamine are also converted to norepheridine by oxidation. Although most enzymes involved in amphetamine metabolism have not been clearly defined, CYP2D6 is known to be involved with the formation of 4-hydroxylamphetamine [142-143]

Table 1: Organic contaminants in sewage sludge [15 – 61].

Pollutants	Sources/usages	Analytes	Matrix	Ref
Organochlorine Pesticides and PCBs	Agricultural control of pests, transformer fluids, plasticisers PVCs and artificial Rubbers	g-HCH, Aldrin, Endrin, PCBs, Dieldrin	sewage	15, 16
Chlorophenols & chlorophenoxy acids	Herbicides	4-chlorophenol 2-chlorophenol, 2-chloro-6-MP MCPA; 2,4-D	Sewage	16-19
Organophosphorus Compounds	Pesticides	residues	Sewage	20, 21
Nitrosamines & Nitroaromatics	Control nematodes	Dimethylnitrosamine; NDMA; NDEA; NPYR; NMOR.	Sewage	21, 22
Mineral oils	Engine oils, paints	Paraffine, alkybenzene cycloparaffine	Sewage	23, 24
Alkylphenols	Detergents, surfactants	4-alkylphenol; polyetho- xylates; 4-nonylphenol (NP); Monoethoxylates (NP1EO); (NP2EO)	Sewage	25, 26
Lipids	Petroleum hydrocarbon	Phosphatidyl serine, Phosphatidyl ethanolamine, Phosphatidyl choline	Sewage	24, 27
Acrylamide Monomer	Coagulants	Polyacrylamide	Sewage	28
Phthalates esters	Plasticisers	bis-(2-ethylhexyl)phthalate	Sewage	29
Organotin compounds	Stabilisers in PVCs biocides, foams	Tributyltin oxide	Sewage	30-32
Surfactants & Related residues Chlorobenzenes	Detergents Paint removers	Linear alkylbenzene Sulphonates (LASs), chlorobenzenes	Sewage Sewage	33-38 39-46
Polychlorinated dibenzodioxins (PCDD)	Pulp bleaching	Congener group	Sewage	47-54
Polycyclic-aromatic hydro carbons (PAHs)	Pyrolysis of organic materials.	Naphthalenes	Sewage	55-61

Table 2: Occurrence of psycho-active drugs and beta blockers in STWs [116]

Substances	Influent			Effluent		
	LOQ [ng L^{-1}]	No of samples	Max [ng L^{-1}]	LOQ [ng L^{-1}]	No of samples	Max [ng L^{-1}]
Antiepileptics						
Carbamazepine	200	9	1000	100	9	1200
DH-CBZ	100	7	30	50	8	30
DHH	200	9	4100	10	9	2600
Primidone	200	9	420	10	9	250
Antidepressants						
Doxepin	200	9	100	10	9	190
Opioids						
Oxycodon	200	0	-	10	0	-
Dihydrocodeine	200	9	140	10	9	70
Codeine	200	9	160	10	9	30
Morphine	200	9	440	10	9	29
Methadone	100	9	130	5	9	120
Tramadol	200	6	470	10	6	370
Tranquilizers						
Diazepam	200	0	-	10	0	-
Nordiazepam	200	0	-	10	0	-
Oxazepam	200	6	190	10	6	180
Beta blockers						
Atenolol	100	9	910	5	9	370
Sotalol	100	9	1300	5	9	1200
Metoprolol	100	9	1200	5	9	1100
Propranolol	5	9	70	3	9	60
Bisoprolol	100	9	380	5	9	270
Celiprolol	100	9	160	5	9	160
Betaxolol	5	4	10	3	1	-

Note: DH-CBZ (10, 11-dihydrocarbamazepine

Table 3: Pharmaceuticals in German STW effluents, rivers and streams [81, 105, 116, 117]

Analyte	STWs			Rivers/streams	
	LOQ (ng L^{-1})	Number STWs	Maximum (ng L^{-1})	LOQ (ng L^{-1})	Maximum (ng L^{-1})
Lipid regulator					
Bezafibrate	250	49	4600	25	3100
Gemfibrozil	50	49	1500	10	510
Clofibric acid	50	49	1600	10	550
Fenofibric acid	50	49	1200	10	280
Antiphlogistics					
Diclofenac	50	49	2100	10	1200
Ibuprofen	50	49	3400	10	530
Indomethacin	50	49	600	10	200
Naproxen	50	10	520	10	390
Ketoprofen	50	49	380	10	120
Phenazon	100	30	410	20	950
Acetylsalicylic acid	100	49	1500	20	340
Salicylic acid	50	36	140	10	4100
Betablocker					
Metoprolol	25	29	2200	10	2200
Propranolol	25	29	290	10	590
Betaxolol	25	29	190	10	30
Bisoprolol	25	29	370	10	2900
β_2-Sympathomimetics					
Terbutalin	50	29	120	10	<LOQ
Salbutamol	50	29	170	10	35
Psychiatric drug					
Diazepam	30	20	40	30	<LOQ
Antiepileptic					
Carbamazepine	50	30	6300	30	1100
Antibiotics					
Clarithromycin	20	8	260	20	260
Roxithromycin	20	10	1000	20	560
Chloramphenicol	20	10	560	20	60
Sulfamethoxazol	20	10	2000	20	480
Trimethoprim	20	10	660	20	200
Dehydrato-erythromycin	20	10	6000	20	1700
X-ray contrast media					
Iopamidol	10	25	15000	10	2800
Iopromide	10	24	11000	10	910
Diatrizoate	10	25	8700	10	ca.100
Iomeprol	10	12	3800	10	890
Estrogens					
Estrone	1	38	70	0.5	1.6
17β-Estradiol	1	38	3	0.5	<LOQ
17β-Estradiol-17-valerate	4	38	<LOQ	2	<LOQ
17α-Ethinylestradiol	1	38	15	0.5	<LOQ
16α-Hydroxyestrone	1	15	5	0.5	<LOQ

Table 5: Sewage sludge disposal in England, 2008-2011 [196]

Mode of disposal	% Total disposed							
		2008/2009		2009/2010		2010/2011		Jan-Dec 2011	
	Tonnes		%	Tons	%	Tons	%	Tons	%
Land fill	13784		50	12490	47	11391	43	10135	39
Incineration with EfW*	3325		12	3610	14	3975	15	4577	18
Inicineration without EfW	6		0	6	0	5	0	4	0
Recycled/Composted/Reused	10082		37	10275	39	10588	4	10844	42
Other	198		1	255	1	356	1	404	2

*EfW = Energy from waste

Table 6: Illicit drug metabolites of human origin detected in the environment*

Compound	Human metabolites identified in biological fluids [150, 225-242]	Human metabolites identified in the aquatic environment [116158, 221, 243-251]
Amphetamine	Amphetamine (AM)	Detected
	3, 4-methylenedioxyamphetamine (MDA)	Detected
	Methylenedioxymethamphetamine (MDMA)	Detected
	Methylenedioxyethylamphetamin (MDEA)	Detected
	Methylbenzodioxolylbutanamine (MBDB)	Detected

	Metamphetamine (MA)	Detected
	p-hydroxy-metamphetamine (*p*-OHMA)	-
	p-OHMA-glucuronide (*p*-OHMA-Glu)	-
	p-OHMA-sulfate (*p*-OHMA-Sul)	-
Cocaine	Cocaine (Cocaine)	Detected
	Benzoylecgonine (BE)	Detected
	Ecgonine methyl ester (EME)	Detected
	Cocaethylene (CE)	Detected
	Norcocaie (Nor- COC)	Detected
	Ecgonidine	-
	nor-ecgonidine	-
	nor- ergonine methylester	-
	m-OH-benzoylegonine	-
	ecgonine	-
	ecgonidine methylester	-
Opiates	Heroin	Detected
	Morphine	Detected
	Nor-morphine	Detected
	6-monoacetylmorphine (6-ACM)	Detected
	Morphine -3- glucuronide (M3G)	Detected
	Methadone	Detected
	2-ethylene-1,5-dimethyl 1-3,3-diphenylpyrolidene (EDDP)	Detected
	Ethyl morphine	-
LSD	Lysergicdiethylamide (LSD)	Detected
	Hydroxyl Lysergicdiethylamide (OH-LSD)	Detected
	Nor - Lysergicdiethylamide (Nor-LSD)	Detected
	Iso - Lysergicdiethylamide (Iso-LSD)	Detected
	2-oxo-3-hydroxy-LSD (2-Oxo-3-OH-LSD)	-
Cannabinoids	Δ^9-tetrahydrocannabinol (THC)	Detected
	Δ^9-tetrahydrocannabinol-9-carboxylic acid (THC-COOH)	Detected
	THC-COOH- glucuronide (THC-COO gluc.)	Detected
	Hydroxyl -THC- conjugate (OH-THC)	Detected

* No identified microbial degradates in the literature

Table 7: Assessment of the biodegradability of pharmaceutical chemicals [93, 111]

Compound	Test result
Amitriptyline	Non-biodegradable
Ampicillin	Biodegradable
Aspirin	Readily biodegradable
Caffeine	Readily biodegradable
Chlorhexidine	Non-biodegradable
Clofibrate	Non-biodegradable
Codeine phosphate	Non-biodegradable
Dextropropoxyphene	Non-biodegradable
Ephedrine	Readily biodegradable
Erythromycin	Non-biodegradable
Ibuprofen	Biodegradable
Menthol	Readily biodegradable
Meprobamate	Non-biodegradable
Methyldopa	Non-biodegradable
Metronidazole	Non-biodegradable
Naproxen	Non-biodegradable
Paracetamol	Readily biodegradable
Phenylpropanolamine	Readily biodegradable
Sulphamethoxazole	Non-biodegradable
Sulphasalazine	Non-biodegradable
Tetracycline	Non-biodegradable
Theobromine	Readily biodegradable
Theophylline	Readily biodegradable
Tolbutamide	Non-biodegradable

Table 8 Chromatographic (LC-MS/MS) methods for the determination of illicit drugs and human metabolites in water (2000-2011)

Analytes	Matrix	Sample Preparation			LC		MS		Method Ref.
		Volume (mL)	Extraction	Method Recovery (%)	C'graphic Column	Mobile Phase	Detector (Interface)	Acq. Mode	(LOD/LOQ) (ng L^{-1})
3 Cocaine: (CO, BE, EME)	WW SW	100	SPE (Oasis, HLB, 500 mg)	73-96	Zorbax Extended C_{18}(2.1mm x 50mm x 3.5µm) HILIC: Rx-SIL (2.1 x 150mm,5µm)	250 µL/min. A:H_2O/AcN (92:2),10mM NH_4HCO_2 (pH 3). B: ACN.	ITMS (ESI^+)	MRM	2-4 ^{WW} 20 ^{SW}	[219]
1Cocaine 1 Opioid. 1Cannabiniod 3 ALC.	WW	-	SPE (Oasis, MCX, 200mg)	-	HPLC: XTerraMS C_{18} (100mm x 2.1mm,3.5µm)	250 µL/min. A: 0.1% in H_2O. B: AcN	QqQ (ESI^+)	SRM	-	[270]
5 Cocaine 4 ALCs 3 Opiods 1Cannabiniod	SW	250 mL	SPE (Oasis, MCX,60mg)	96-105 97 10 85-90 69-84	HPLC: XTerraMS C_{18}(100mm x 2.1mm,3.5µm)	250 µL/min. A: 0.1% in H_2O. B: AcN	QqQ (ESI^+)	SRM	0.02-0.05 0.01-0.35 0.02-0.28 0.14-0.36	[158]
2 Cocaine	SW WW	100 mL 500 mL	SPE (Oasis, HLB, 500 mg)	-	HILIC: Zorbax Rx-SIL (2.1 x 150mm, 5µm)	-	ITMS (ESI^+)	MRM	≤ 20	[158]
2 Cocaine	SW	500 mL	SPE (Oasis, MCX, 60mg)	90	HPLC: A Luna C_{18} (50mm x 2mm i.d, 3 µm)	250 µL/min A: 0.1% in H_2O. B: AcN	-	MRM	-	[124]

Analytes	Matrix	Sample Preparation			LC		MS			Method Ref.
		Volume (mL)	Extraction	Method Recovery (%)	Chromatographic Column	Mobile Phase	Detector (Interface)	Acq. Mode		(LOD/LOQ) (ng L^{-1})
3 Cocaine 1ALC 3 Opiods 1LSD	WW	500 mL	SPE (Strata-XC, 200mg)	50-65	HPLC: Phenomenex Onyx C_{18} (200 x 3.0mm				36-120	[244]
5 ALCs 5 Cocaine 5 Opiods 1Cannabiniod	WW	50 mL	SPE (Oasis, MCX, 60mg)	50-105	HPLC: XTerraMS C_{18}(100mm x 2.1mm, 3.5µm)	250 µL/min A:CH_3COOH/ H_2O. B: AcN A_2: 0.05% TEA/ H_2O	QqQ (ESI^+)	MRM	300 pg/L^{wwinf} 1 ng/L_{wweff}	[248]
2 Cocaine	SW WW	100 mL 500 mL	SPE (Oasis, HLB, 500 mg)	-	Zorbax Rx-SIL (2.1 x 150mm, 5µm)	-	ITMS	MRM (ESI^+)	20	[271]
1 ALC	WW	250 mL	SPE (Oasis, HLB, 200 mg)	36-49	HPLC:Varian Pursuit XRs C_{18} (100mm x 2.0mm, 3µm)	A:water/0.5% HCOOH B: 82% CH_3OH/ 18% AcN/0.5% HCOOH	-	Scan (CID)	0.25-5.0	[247]
5 ALCs 2 Cocaine. 1LSD 1Opiod	WW	100 mL	SPE (Oasis HLB, 200 mg)	70-110	UPLC:Acquity BEH C_{18} (100mm x 2.1mm, 1.7 µm)	A: AcN/0.1% HCOOH. B: 30mM HCOOH/ NH_4HCO_2	QqQ (ESI^+)	-	5 - 850	[272]

Table 8 LC-MS/MS methods for the determination of illicit drugs and human metabolites in water (2000-2011)

Analytes	Matrix	Sample Preparation			LC		MS			Ref.
Method		Volume (mL)	Extraction Method	Recovery (%)	Chromatographic Column	Mobile Phase	Detector (Interface)	Acq. Mode	(LOD/LOQ) (ng L^{-1})	
1 ALC 2 Cocaine.	SW	100 mL	SPE (Oasis, MCX,60mg)	65-106	UPLC: Acquity BEH C_{18} (1.7µm, 1 mm x 100mm)	A:94.5% H_2O. 5% MeOH, 5% CH3COOH (pH 2.8) B:99.5% MeOH + 0.5% Acetic	QqQ (ESI^+)	MRM	0.3-50	[259]
8 Opiods 2Cannabiniod	SW WW	200 mL	SPE (Oasis, HLB, 200 mg)	40-70	UPLC: Acquity BEH C_{18} (1.7µm, 1 x 100mm)	A: MeOH B: 5 mM NH_4HCO_2	QqQ (ESI^+)	SRM	0.1-25	[258]
8 Opiods 2 Cannabiniod	SW WW	50 mL	SPE (Oasis, MCX,(150mg)	69-94%	UPLC: Acquity BEH C_{18} (1.7µm, 2. 1 mm x50mm)	A: MeOH B: 5 mM NH_4HCO_2 + 1% formic acid	QqQ (ESI^+)	SRM	-	[247]

Acq. Mode - Acquisition mode- SRM, Selected reaction monitoring; CID, Collision-induced dissociation.
Detector and Interface used – QqQ, Triple quadrupole; ITMS, Ion Trap mass spectrometry, ESI, Electrospray ionization.
MeOH – Methanol; TEA, Triethylamine; NH_4HCO_2, Ammonium acetate; AcN, Acetonitrile, H_2O, Water.
WW, Wastewater; SW, Surface water; WW^{inff}, Waste water influent; WW^{eff}, Waste water effluent.
RPLC, Reversed-phase liquid chromatography; UPLC, Ultra- performance liquid chromatography; HILIC, Hydrophilic interaction chromatography.

Table 9. Solid Phase Extraction (SPE) protocols in wastewater pre-treatment

Protocols					
Types	Sorbent materials	Conditioning	Washing	Elution	Ref
Isolute, pH® (1000 mg/6 mL)	Silical treated with phenyl groups in which silanol group are end-capped.	2 mL of MeOH and 6 mL of milli-Q water, sample loading at (pH 6)	6mL of 5% MeOH in water, drying in in vacuum for 15 min	2 x 4 mL of 5% NH_3 in acetone	[219]
Oasis, MCX® (500 mg/6 mL)	Polymeric sorbent with strong cation –exchange sulfonic group located on surface of poly(Divinyl benzene-Co-N-vinyl py rrolidone) copolymer .	6 mL of MeOH , 3mL of milli-Q water and 3 mL of water at pH 2, sample load-ing at pH 2.	3 mLof milli-Q water at pH 2, dryingfor 15 min. under vacuum	6 mL of MeOH and 6 mL of 5% NH_3 in MeOH	[124, 159, 186, 259, 270]
Bond Elut Certify® (300mg/6 mL)	Lipophilic and strongly cationic properties	3 mL of MeOH and 3 ml of milli-Q water, sample Loading at pH 6.	2 mL of of milli-Q H_2O at at pH 2, and 3 mL of MeOH, drying for 15 min under vacuum	2 x 4 mL of 80: 20 DCM/isopropanol mixture with 2% NH_3	[225,226]
SCX® (500 mg/6 mL)	-	2 mL of MeOH, I mL of milli-Q water and 1 mL of 0.25 M phosphate buffer (pH 3), loading at pH 3	I mL of 0.25 M phosphate (pH 3), 0.5 mL of 0.1M acetic acid and 1 mL of MeOH, drying for 30 min	1.5 mL of 3% NH_4OH in 1.5 mL of MeOH	[258]
Phenomenex Strata-X™ (200 mg/6 mL)	-	2 x 6 mL of MeOH and 2 x 6 mL of H_2O, sample loading at pH 6.	50 mL of 10% MeOH in 100 mM formic acid + 500 µL of acetic acid, drying for 30	10 mL of 5% v/v NH_4OH in 1:1 acetone: ethyl acetate	[244]
Strata-XC™ (200mg/6mL)	-	-same-	-same-	-same-	[244]

Protocols					
Types	Sorbent materials	Conditioning	Washing	Elution	Ref
Chrolut, ENV® (500 mg/6 mL)	Hyper-crosslinked polystyrene-divinyl benzene polymer based.	3 mL of MeOH and 3 mL of milli-Q water, sample loading.	air through the column for 1 hr.	5 mL of MeOH	[82]
Isolute, ENV® (500 mg/6 mL)	Hydrophobic sorbent with hydroxylated polystyrene divinyl benzene copolymer	2 mL of MeOH and 6 mL of milli-Q water, sample loading at pH 6	6 mL of 5% MeOH in water, drying under vacuum for 15 min.	2 x 4 mL of MeOH.	[219]
Chromabond, Easy (500 mg/6 mL)	Bifunctional polystyrene divinyl benzene copolymer	5 mL of hexane, 5 mL of ethyl acetate, 10 mL of MeOH and 1 mL of Milli- Q water.	5 mL of milli-Q water drying under vacuum for 15 min.	2 x 4 mL of MeOH	[219,271]
Oasis, HLB® (500 mg/6 mL)	Divinylbenzene/N-vinyl pyrrolidone) copolymer with hydrophilic/lipo philic properties	3 mL of MeOH and 3 mL of milli-Q water, sample loading at pH 6	3 mL of 5% MeOH in milli-Q water drying under vacuum for 15 min	2 x 4 mL of MeOH	[219,243]
Oasis, HLB® (500 mg/6 mL)	-same-	5 mL of hexane, 5 mL of ethyl acetate, 10 mL of MeOH and 1 mL of Milli-Q water	5 mL of milli-Q water drying under vacuum for 15 min.	2 x 4 mL of MeOH	[83,243]
Isolute ,C_{18} (EC)® (500 mg/6 mL)	Strongly apolar and lipo- philic based on octadecyl silica with end capping of free silanol group.	2 mL of MeOH and 6 mL of milli-Q water, sample loading at pH 6	6 mL of 5% MeOH in milli-Q water drying under vacuum for 15 min.	2 x 4 mL of 5% NH_3 in acetone	[219,272]
Oasis, Max (60 mg)	Strong anion-exchange mixed mode polymeric	2 mL of MeOH and 2 mL of 2% HCOOH (pH 2.1)	2 mL of 2% HCOOH/ H_2O, wrapped in aluminium	1 mL of MeOH and 2 mL of 5% NH_4OH in MeOH.	[259]

Protocols					
Types	Sorbent materials	Conditioning	Washing	Elution	Ref
	sorbent built upon HLB copolymer (application: acids)		foil and stored in a freezer until eluted.		
Oasis, WCX (60 mg)	Weak cation-exchange mixed mode polymeric sorbent built upon HLB copolymer (application: strong bases).	2 mL of MeOH and 2 mL of 2% HCOOH (pH 2.1)	-same-	1 mL of MeOH and 2 mL of 5% NH_4OH in MeOH	[259]
Oasis, WAX (60 mg)	Weak anion- exchange mixed mode polymeric sorbent built upon HLB copolymer (application: strong acids).	-same-	-same-	-same-	[259]
Chromabond, C_{18} (200 mg).	Silical-based, endcapped sor-bent (non-polar compounds).	-same-	-same-	-same-	[259]
Isolute, HCX (200 mg)	Weak anion- exchange mixed mode (non-polar and basic analyte).	-same-	-same-	-same-	[259]

Table 4 Survey of illicit drugs and pharmaceuticals concentration in wastewaters.

Analytes	Matrix	Influent ($ng\ L^{-1}$)	Effluent ($ng\ L^{-1}$)	Surface Water ($ng\ L^{-1}$)	Ref
Cocaine	5 STPs, Spain	225.0	47	10	[158]
	5 STPs, Belgium	22 -678	-	1.2 - 26	[219]
	37 STPs, Belgium	32 – 753	-	-	[158]
	3 Rivers, Italy	-	-	0.3 – 44	[186]
	5 STPs, Ireland, UK	489 ± 117	25 - 248 ± 20	0 – 33 ± 11	[244]
	Eastern Spain	370 – 1000.24	30 – 560	-	[258]
	30 STPs, Belgium	09 – 683	-	-	[245]
	2 STPs, Italy	218.4 – 421.4	0.9 – 10.7 ± 3.2	-	[159]
	4 STPs; River Po.	42 – 120	-	-	[124]
	42 STPs, NE Spain	04 – 4700	01 – 100	-	[272]
	Barcelona, Spain	2.40	-	-	[246]
Benzoylecgonine	5 STPs, Spain	2307.0	-	111	[158]
	5 STPs, Belgium	82 – 1 898	928	44 - 191	[219]
	37 STPs, Belgium	46 –2258	-	-	[186]
	3 Rivers, Italy	2.2 – 183	-	-	[186]
	5 STPs, Ireland UK	290 ± 11	22	-	[244]
	Eastern Spain	150 – 1000.5	22 ± 4 – 31 ± 18	-	[258]
	30 STPs, Belgium	37 – 1550	6.0 – 7.9	-	[245]
	2 STPs, Italy	547.4 -197.2	-	-	[159]
	4 STPs; River Po.	420 - 750	0.92 – 100.3 ± 28.6	-	[124]
	42 STPs, NE Spain	09 – 7500	-	-	[272]
	Barcelona, Spain	5.24	01 – 1500	-	[246]
	12 STPs, Germany	65± 5	77± 9	71	[276]

Analytes	Matrix	Influent ($ng\ L^{-1}$)	Effluent ($ng\ L^{-1}$)	SW ($ng\ L^{-1}$)	Ref
Nor- BE	3 Rivers, Italy	-	-	0.2 – 8.4	[186]
	Eastern Spain	150 - 430	30 – 170	-	[258]
	2 STPs, Italy	18.8± 5.6 – 36.6 ± 7.8	<LOQ – 7.5 ± 2.9	-	[159]
Cocaethylene	2 STPs, Italy	5.9 ± 2.6 – 11.5 ± 5.1	0.2 ± 0.5	-	[159]
	Barcelona, Spain	77.5– 78.5±33.2	1.71– 4.2± 1.2	4.63	[246]
	3 Rivers, Italy	-	-	0.07 – 0.2	[186]
Nor-cocaine	3 Rivers, Italy	-	-	0.15 – 3.6	[186]
	Eastern Spain	0.15 – 0.43	0.03 - 0.17	-	[258]
	2 STPs, Italy	4.3 ± 0.9 – 13.7 ± 5.3	0.7 ± 0.5	-	[159]
Amphetamines	3 Rivers, Italy	-	-	<0.65	[186]
	Eastern Spain	1400	110 – 210	-	[258]
	2 STPs, Italy	5.4 – 14.7± 10.6	2.8	-	[159]
	42 STPs, NE Spain	03 - 6880	04 - 2100	-	[272]
	Barcelona, Spain	20.8 – 41.1± 9.1	0.45– 2.2 ± 0.1	2.84	[246]
	5 STPs, Spain	15	<1.0	<0.8	[158]
Metamphetamines	5 STPs, Nebraska USA	1.3 ± 0.1 – 1.4	35.0± 7.3	-	[160]
	3 Rivers, Italy	0.1 – 62.6 ± 13	-	<0.41 – 1.7	[186]
	Eastern Spain	-	<100 - 540	-	[258]
	2 STPs, Italy	<500	<1.11 – 3.5 ± 2	-	[159]
	42 STPs, NE Spain	3 - 277	3 - 90	-	[272]
	3 STPs, USA	15 ± 2 – 66 ± 14	0.8 – 1.3	-	[160]
	Barcelona, Spain	4.8 – 18.2 ± 5.8	2.1 – 6.3 ± 0.6	2.87	[246]
	Murray, USA	6.0 - 34	03 - 7	-	[247]
MDA	42 STPs, NE Spain	03 - 266	01 - 200	-	[272]
	3 Rivers, Italy	-	-	3 ± 0.3 – 4	[186]
	Eastern Spain	500 - 1400	41.0 – 68.0	-	[258]
	2 STPs, Italy	4.6 ± 7.3 – 8.7	0.9 ± 1.9 – 1.1± 1.5	-	[159]
	5 STPs, Spain	03 - 266	1 - 200	-	[158]

Analytes	Matrix	Influent (ng L^{-1})	Effluent (ng L^{-1})	SW (ng L^{-1})	Ref
MDMA	3 Rivers, Italy	-	-	1.1 – 4.0	[186]
	Eastern Spain	326 – 2700.5	100 – 210.2	-	[258]
	2 STPs, Italy	13.6 – 14.2	4.4 ± 3.7 – 5.1± 3	-	[159]
	5 STPs, Spain	91	67	3.5	[158]
	STP, Italy	2 - 598	2 - 267	-	[272]
	Barcelona, Spain	133– 135.13 ± 29.8	8.2– 14.8 ± 2.2	129	[246]
	Murray, USA	<1.0 – 10.0	-	-	[247]
	42 STPs, NE Spain	2 - 598	2 - 267	-	[272]
MDEA	5 STPs, Spain	27	<2.1	-	[158]
	2 STPs, Italy	4.19 – 1.5 ± 3.8	<1.64	-	[159]
	STP, Italy	6 - 114	12	-	[258]
	STP, Spain	<500	<100	-	[272]
	42 STPs, NE Spain	06 - 114	12	-	[272]
Opiates	Barcelona Spain	2.4	1.2	-	[246]
Heroin	STP, Italy	20.0	<20.0	<1.5	[247]
	5 STPs, NE Spain	25.9 – 96.7	20.9 – 81.1	-	[247]
Morphine	3 Rivers, Italy	-	-	3.5 - 38	[186]
	5 STPs, Ireland	874 ± 86	452	-	[244]
	2 STPs, Italy	83.3– 204.4	5.5 ± 11.1	1-2L	[159]
	Barcelona, Spain	68.1 – 162.9 ± 20.0	21.8 ± 3.0	3.25	[246]
	12 STPs, Germany	123 ± 6	9.0 ± 1.2	83	[246]
	STP, Italy	7.1 – 96.7	0.1 – 8.1.	4.8 – 6.3	[247]
	5 STPs, NE Spain	30.7	-	-	[247]
Nor-morphine	1 STP, Italy	<25	<2.5 – 3.7	<12..5	[247]
	3 Rivers, Italy	-	-	0.93	[186]
6 ACM	2 STPs, Italy	10.4± 4.8 – 11.8 ± 8.5	-	-	[244]
	Barcelona, Spain	8.4 – 12.8 ±3.1	2.5 – 3.6 ± 0.5	-	[246]

Analytes	Matrix	Influent (ng L^{-1})	Effluent (ng L^{-1})	SW (ng L^{-1})	Ref
	12 STPs, Germany	8.4 – 12.8 ±3.1	0.9 ±1.2	83	[246]
	STP, Italy	102 ± 14	<3.1	<0.9 – 3.4	[277]
					[247]
M3G	2 STPs, Italy	2.5 ± 7.1 – 18.1 ± 30	<0.48	-	[159]
Methadone	5 STPs, Spain	4.0 – 239	4.0 – 24.7	-	[247]
	3 Rivers, Italy	-	-	4.9 – 10.1	[186]
	2 STPs, Italy	11.6± 1.7 – 49.7 ± 9.6	9.1 ± 0.5 – 36.2 ± 2.8	-	[159]
	12 STPs, Germany	123 ± 6	9.0 ± 12	83	[277]
	STP, Italy	4 – 23.9	2 – 2.7	4.9 – 10.1	[247]
Codeine	5 STPs, NE Spain	18.1 – 119.7	3.1 - 397	-	[247]
	3 Rivers, Italy	-	-	1.0 - 51	[186]
	12 STPs, Germany	80 ± 5	7.7 ± 8	90	[277]
Nor-codeine	5 STPs, NE Spain	5 – 68.0	15.5 – 22.9	-	[247]
6 Acetyl codeine	3 Rivers, Italy	-	-	<0.31	[186]
EDDP	3 Rivers, Italy	-	-	9.9 – 18.0	[186]
	5 STPs, Ireland UK	9.0 – 206 ± 10	-	-	[244]
	2 ST1 STP, Italy	19.8 ± 3.1 – 91.3 ± 19.2	22.6± 0.6 – 72.1± 8.7	-	[159]
	STP, Italy	4.5 – 41.3	4.9 – 56.7	9.61 – 17.5	[247]
THC	5 STPs, NE Spain	11.3 – 31.5	-	-	[247]
	2 ST1 STP, Italy	62.7 ± 5 – 91.2 ± 24.7	<0.94 – 7.2 ± 3.7	-	[159]
	Barcelona, Spain	4.3– 21.03 ± 7.8	8.4 ± 3.8 – 11. 23	2.65	[246]
	STP, Italy	8.3 – 31.5	<8.3	<7.0 – 13.6	[247]
THC-COOH	3 Rivers, Italy	-	-	0.48 -3.7	[186]
	STP, Italy	12.5 – 96.2	12.5	16.4 – 34.1	[247]
	5 STPs, NE Spain	37.8 – 96.2	14.8 – 48.1	-	[247]
OH- THC	Barcelona, Spain	8.4 – 46.3	4.8 – 15.3	10.7	[246]

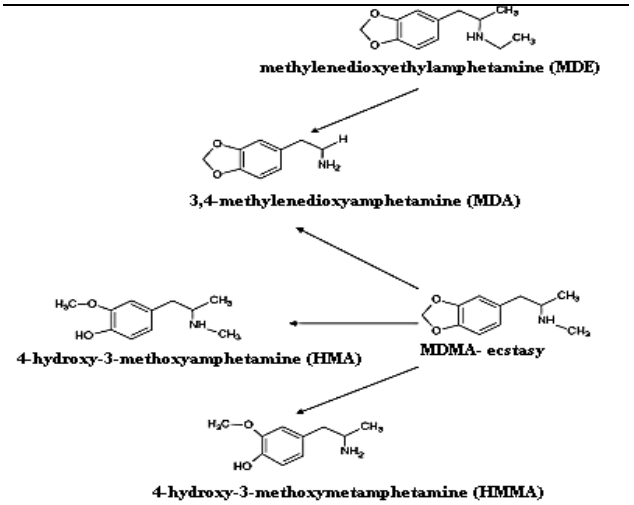

Figure 4: Main metabolites of 3, 4-methylenedioxymethamphetamine (MDMA) in urine [144].

Human metabolism of opiates.

Opium comes from the opium poppy (papaver somniferum), a conjugated juice from the unriped capsule. It is an ingredient in morphine, codeine and theobaine. Several illegal drugs are produced from the opium poppy and the common ones are morphine and heroin, while 6-monoacetylmorphine/ and morphine are their related metabolites. The phenolic hydroxyl at position 3, the alcoholic hydroxyl at position 6 and the nitrogen atom plays important roles in morphine metabolism.

Figure 12 only show how heroin (diacetylmorphine) degradation pathways to produce main metabolites that we determined in the current work. But different morphine conjugates may arise from the actions of different enzymes, this emphasises the complexity of morphine metabolism [145]. Approximately 90% of an administered dose of morphine is excreted in the urine only about 10% is excreted as unchanged morphine. Morphine -3- glucuronide (M3G) is the major metabolite, while Morphine -6- glucuronide (M6G) is a minor one [146], and nor- morphine and nor-morphine-6-glucuronide have also been found in human urine and detected in wastewaters [Table 4]. Other minor metabolites like codeine (3-O-methylmorphine) and morphine- N- oxide have been identified in the urine of chronic users [147].

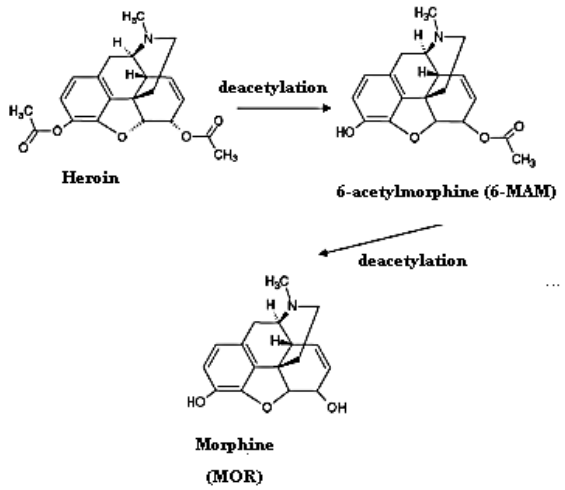

Figure 5: Degradation pathways of heroin and its main metabolites in living organisms. [150].

Human metabolism of Lysergic Acid Diethylamide (LSD)

Lysergic acid diethylamide is a compound derived from ergot alkaloids, a powerful hallucinogenic drug commonly sold as "acid" on the street as a drug of abuse. It is a non-addictive drug that comes in tablets or blotting paper, though liquid LSD is also available [148]. The drug is quickly metabolized in the body, where it is dispersed in the biological fluids in very low concentration and very small amount of the original dose is eliminated in the human urine [149]. In Figure 13, the following LSD metabolites have been identified in human biological fluids: 13-hydroxy-LSD, 14-hydroxy-LSD, N-demethyl LSD, 2-oxo-LSD, and 2-oxo-3-hydroxy-LSD [121-123]. The main metabolite of LSD is 2-oxo-3-hydroxy-LSD and 13- and 14-hydroxyl-LSD are excreted as glucuronide conjugates in urine [151]. In a review paper of Reuschel *et al* [152], evidences supporting a much higher concentration of 2-oxo-3-hydroxy-LSD in human urine of LSD users than the parent drug and 2-Oxo-LSD concentrations were reported. The iso-LSD and LSD exist as stereoisomers in illicit preparations and therefore iso-LSD is not a metabolite, it's frequently found in urine as a main contaminant of LSD [153]. Additional metabolites have also been identified in the laboratory animals but are yet to be found in human fluids [154]. The LSD compounds were however not studied in the current work.

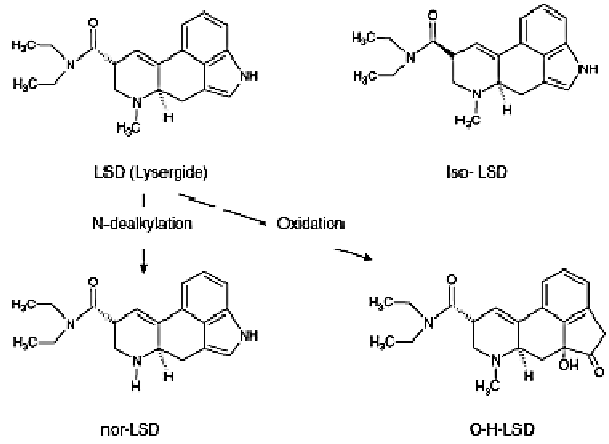

Figure 6: Lysergic Acid Diethylamide and metabolites in human fluids [168]

Human metabolism of Cannabinoids

The cannabinoids, of which the most important one is tetrahydrocannabinol (THC) is the active chemical in *cannabis sativa L*, other active constituents are cannabidiol and cannabinol. Cannabis is commonly known as the source of the 'marijuana' drug and for centuries, this plant has been widely cultivated around the world for its fibres. The cannabinoids are non-polar compounds with low solubility in water but are soluble in fat, alcohol and many organic solvents, they are self-administered by smoking. The volatilized fractions are inhaled to give physiological effects. It is non-addictive and there are no withdrawal symptoms but one of the common side-effects of its use is making the user drowsy with reduced concentration and short term memory [155]. About 66 types of cannabinoids have been isolated from the cannabis plant but three of them have received most attention from researchers as a result of their natural prevalence. These are: phytocannabinoids (obtained from cannabis plant), synthetic cannabidiols (prepared from laboratory) and endogenous cannabinols (obtained from the body of humans and animals).

On ingestion, the cannabinoids are metabolized in the liver, especially by cytochrome P450 mixed-function oxidase, mainly CYP2C9. It is stored in the fat where Δ^9-THC is metabolized to 11-hydro-Δ^9-THC, which is metabolized to 9-carboxy-THC

[156], but the metabolism of THC is still not properly understood.

Figure 14 shows the structure of Δ^9-tetrahydrocannabinol (THC) and its metabolites in human urine [144]. The main metabolite is Δ^9-tetrahydrocannabinol-9-carboxylic acid (THC-COOH) and it is excreted as glucuronide-acid conjugate THC-COOH- glucuronide) [157], the metabolites can be detected in the body after weeks. It appears that the illegal status of the plant in most countries affected its systematic studying.

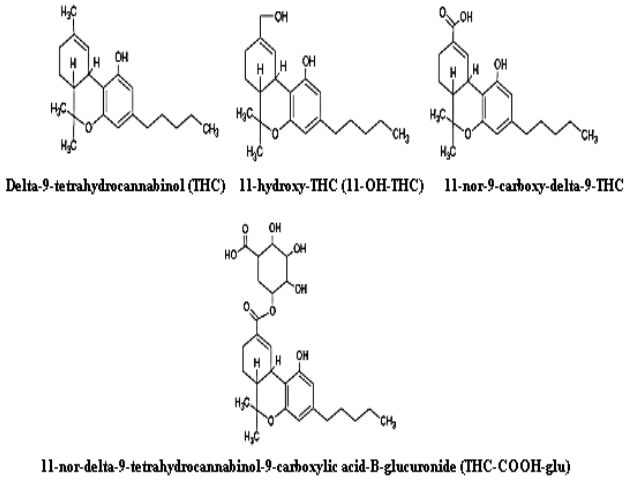

Figure 7: Major metabolites of Δ9-tetrahydrocannabinol (THC) in urine [168].

Sewage Treatment Works as transport routes of pollutants.

Conventional sewage treatment works are the most significant routes through which the drugs enter the environment via untreated sewage and domestic sewage treatment systems. The ingested chemicals and associated metabolites are excreted via faeces and urine and passed onto sewage treatment systems. Discharges from manufacturers, commercial, domestic and run-off areas of unwanted and unused chemicals to the domestic sewage system are other major sources. Sewage sludge is the remaining residues after sewage treatment and the treated sewage sludge has several valuable properties which are agriculturally relevant; these include soil building potential giving it a strong hold, availability of nutrients and valuable trace elements essential to animals and plants, an efficient and sustainable alternative source to inorganic fertilisers and mineral fertilisers such as phosphate, and soil nutrient recovery through slow release of nitrogen.

Residues of pharmaceutical and illicit compounds have been found in surface waters in concentrations from ngL^{-1} to ugL^{-1} in many countries with the levels and distribution of these illegal compounds as found in wastewaters reported in Spain, Belgium, Italy, Germany, UK and USA [124, 158-167]. Apart from the active sludge processes, percolating filters, nitrification and de-nitrification facilities, investigations into the treatment technologies for the potential removal of drug residues and other organic compounds from the effluents of STWs have additionally identified ozonation [168-170] and membrane bioreactors (MBR) [171, 172] as biological means to provide improved potential in removing trace pollutants from the urban wastewaters. Microbial degradation has been suggested as the most important removal process in the sewage treatment works and with the continuing extensive studies on the metabolism and transformation of pharmaceuticals and other organics in humans and mammals, the microbial biodegradation pathways of some these chemicals, the persistence of their products and likely toxicity would largely be known [173]. Figure 16 below illustrates a typical interplay of complex physical, biochemical and transformational routes of pollutants in STWs and each transport route depends on the nature of influents [173-179].

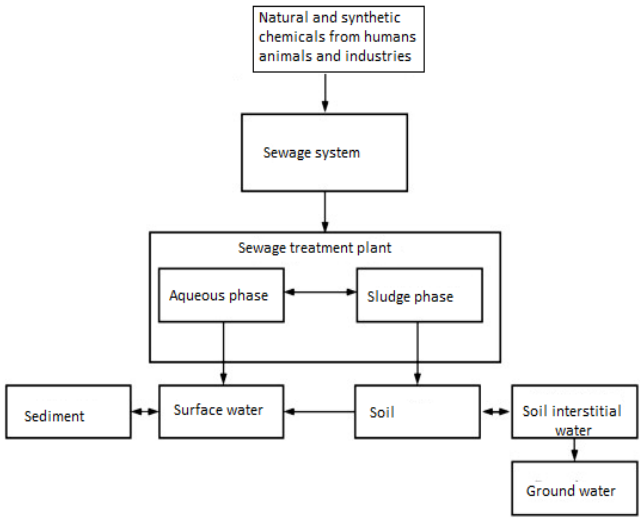

Figure 16: Organic contaminant fate and distribution in the environment [183]

The microbial degradability of the illicit drugs in the sewage as well as their degradation pathways has not been reported. However, small studies on selected pharmaceuticals with the identification of some microbial degradates suggest that similar processes are likely to affect the illicit drugs [176-178]. In 2009, the understanding of the STWs systems and the degradation processes involved were observed by Kasprzyk-Horden *et al* [179] on selected pharmaceuticals and illicit drugs (cocaine, benzoylecgonine and amphetamine) where the differences in the performance of activated sludge and trickling filter on a 5-month monitoring program was undertaken from two different STWs in South Wales, UK. However, the choice of sampling points was just to verify the removal efficiency of the two contrasting STWs and the work recorded over 85% removal efficiency of most drugs with STW utilising activated sludge compare to less than 70% reported for trickling filter.

However, for the first time, direct measurement of the illicit drug removal rates in laboratory (batch) studies would carried out to improve upon the understanding of the degradation rates of cocaine (COC); benzoylecgonine (BZE); heroin (HER); 6-monoacetylmorphine (6-MAM); morphine (MOR) and diazepam (Diaz) under different conditions to obtain removal rates. The capabilities of the current experimental batch data in generating removal rates of drugs would be applied in mass balance calculation to improve influent measurement.

Also, no publication to our knowledge has been found on the ecotoxicological impacts of chronic exposure of illicit drugs and their metabolites as the STWs procedures cannot effectively remove all the drugs or polar compounds due to their hydrophobic/lipophilic character [111]. Apart from volatilisation, hydrolysis (abiotic) and biodegradation (biological processes), physical-chemical adsorption of polar compounds onto the biosolids surfaces also occurs. The interaction of compounds with high adsorption coefficients in particular determines the extent of the removal. Natural solids like clay, sediment and micro-organisms and added solids (e.g. active carbon, coagulants) facilitate STWs removal processes [184]. Those adsorbed on solids and passed as sludge enter the environment when spread on agricultural lands as manure and the compounds continue in the ecosystems or are possibly leached into underground waters; while those with low adsorption coefficients are released as effluents into the receiving waters. The removal of organic compounds is often

incomplete in most municipal STWs, the sewage-sludge and effluent waters are therefore the primarily routes at which these chemicals enter the environment. Apart from the biodegradation, chemical degradation and sorption processes in typical STW details of which are not well understood because of the complex mixtures present are the other main removal processes during the wastewater treatment. The physicochemical properties of the contaminants ultimately determines their extent of persistence, toxicity and potential environmental effects after the sewage-sludge disposal to agricultural lands or effluents waters disposed of to seas.

The existing priority substance classifications by the European Communities Priority Substances Directive notwithstanding [185], the emerging priority contaminants groups like 'illicit drugs and their metabolites' have no safe-levels because of insufficient information on their biodegradability and persistence after their disposal to lands or receiving waters. Insufficient information, decisions and policy thrusts regarding the future practices of safe sewage-sludge disposal mean that complete removal of contaminants from STWs effluent-waters becomes difficult.

Existence of uncontrolled discharges of different types of compounds from humans and from veterinary treatment into the environment via STWs is shown in the anticipated exposure in Figures 17 and 18. Drugs for human treatment are primarily exposed to the environment from routes Fig 17 (F1 & F2) and enter different treatment fate processes at points F3 & F4 and terminate at F8 and F9.

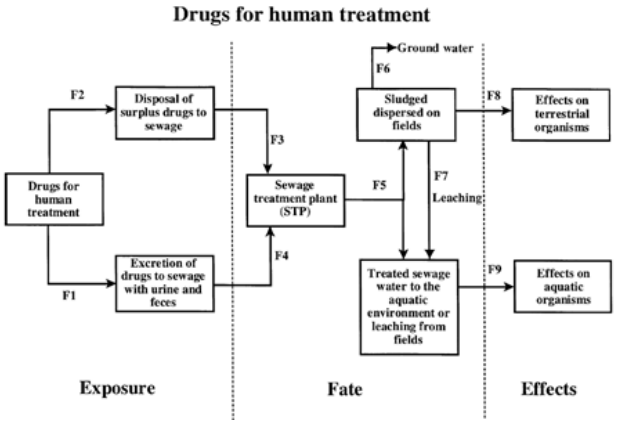

Figure 17. Anticipated exposure routes of drugs for human treatment in the environment [186]

The effects on terrestrial and aquatic organisms continue with drugs from veterinary treatment in Fig. 18 (F10-F13) in another complete process of bio-chemical reactions and mechanisms with anticipated toxicity impacts on the ecosystems not yet understood.

Studies in the literature have confirmed the enrichment of the sewage sludge partitioning of chemicals onto sludge solids or suspended in solution is due to their hydrophilicity/lipophilicity properties compared to influent sewage [187-189]. Understanding of the fate and behaviour of pollutants during sewage treatment will show the degradation possibility of compounds that are completely or partially degraded in aqueous and solid phases, sorbed to sludge solids or mineralised. In a study reported by Strachan *et al* [190], organic contaminants are located within the fraction of large organic wastes (biomass) which are repository of living and dead micro-organisms required for degradation processes.

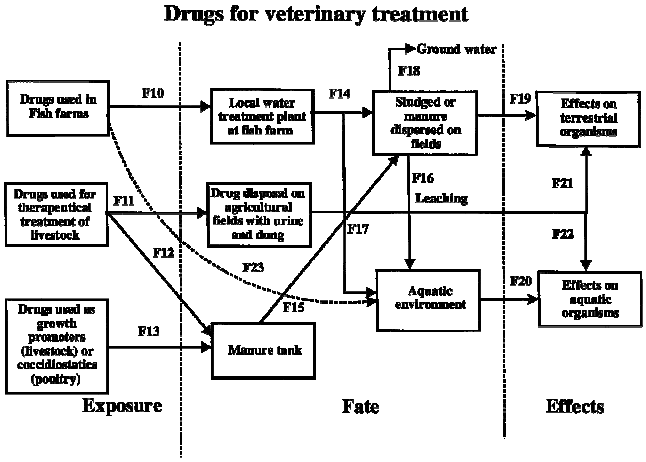

Figure 18. Anticipated exposure routes of drugs for veterinary treatment in the environment [186]

Microbial degradation in the aquatic environment

Investigations on the levels of removal of organic residues from a wastewater plant studies have shown toxicity correlation of wastewater effluents on aquatic organisms to determine the response levels with degree of contamination [191-193]. Biodegradability studies of organic priority pollutants and reduction in toxicity of these pollutants in wastewaters treatment processes have also been carried out [194-195]. Also evaluated were 22 priority pollutants belonging to the class of phthalates, pesticides, polycyclic aromatic hydrocarbons and phenols in an activated sludge pilot plant [111, 194]. About 80-99% removal efficiency was recorded from the parallel and spiked treatments of between 50-150 $\mu g\ L^{-1}$ concentrations. The results indicated a degradation of phenols, enrichment of PAHs to about 64% from the mass balance calculations while pentachlorophenol was associated with the solid phases. Table 5 presents percent sewage sludge disposal in the last 3 years, and the subsequent transport of these residual organic pollutants in the sewage which enters the environment and becomes the issue of current concern.

Sewage is a complex association of wastes of human excreta containing mixtures of fats, sugars, lignin, protein, cellulose, humid materials, amino acids and fatty acids. Wang *et al* [197] studied the partitioning mechanism of the organic residues within the biomass and sorption onto the sludge surface as a two-stage process. Determination and prioritisation of typical sewage sludge can be a complex task because of many synthetic organic materials with various residues of diverse origins due to 1) interferences of co-contaminants in complex matrices, sample extraction and clean ups, sensitive techniques needed to determine low concentrations 2) the fate and behaviour of sludge-derived residues after disposals requires investigation to monitor persistence and environmental impacts and 3) bio-transformation arising from degradation of residues generates toxic by-products, but unavailability of some compounds, sorped onto sewage solids to bacteria for degradation can be significant as little is known about the final fate of these organics [198-202]. Difficult isolation of sludge samples arises also from non-uniformity of extraction procedures and variability in obtaining grab samples as a representative of all various genotoxins in the sludge matrix [203-205]. Different processes or techniques are often adopted for specific effluents depending on the origins of the contaminants. Generally factors often considered, though contaminants can be lost during treatments in a complex variety of ways are: 1) sorption/association with sewage solid surfaces 2) abiotic processes/hydrolysis involving chemical degradation 3) volatilisation and 4) biodegradation [111].

Humans are typically exposed to numerous organic and inorganic pollutants, as by-products from treatment of waste water from domestic, agricultural and industrial sources which constitutes sewage [206]. The presence of intestinal pathogenic bacteria and animal parasites in sewage sludge has been confirmed from several investigations [207, 208]. However, sewage sludge may contain relatively large amounts of heavy metals as well as organic pollutants such as phthalates, polychlorinated biphenyls (PCBs), alkyphenols, and organoclorine pesticides compared to normal environmental levels in soil, water, and air [2]. Increasing amounts of sewage sludge are used for land filling and agricultural land including pastures grazed by ruminants following the ban on ocean dumping of sludge [209]. The potential health risk imposed due to the presence of organic and inorganic compounds found in sewage sludge is of concern in humans [210, 211] if they are delivered at high enough doses to cause effects through the consumption of products derived from animals grazing on contaminated pastures [212]. Adverse effects which have been reported in humans include perturbation of male reproductive tract, certain male and female cancers, declined fertility, thyroid dysfunction and ill impacts on the central nervous system, gastroenteritis, damage to liver, kidneys and blood, hepatitis, occupational asthma, infection of skin or eyes and inflammation of the lung following sewage sludge exposure. Different groups of environmental chemicals with a variety of mechanisms and disrupting activities have been identified and discussed [213-218].

In the literature, degradation studies of pharmaceuticals have identified degradates of anti-inflammatory, analgesics and blood-lipid regulators. In batch studies of acetylsalicylic acid with suspended activated sludge, the decrease of about 70-99% in concentration after 6, 24 and 72 h was observed but no metabolites were detected using GCMS [89]. The degradation studies of anti-inflammatory and blood-lipid regulators such as bezafibrate, diclofenac, naproxen and ketoprofen in activated sludge were carried out, but only ketopofen biotransformed into [3-(hydroxyl-carboxy-methyl) hydratropic acid and [3-(keto-carboxyl- methyl) hydratropic acid [89]. Biodegradation of trimethoprim showed resistance to degradation in a reactor filled with activated sludge, but its degradation in a nitrification process was completed in 3 days. In a similar study, Ternes et al [89] investigated degradability of estrogens in aerobic batch reactors at two different concentrations using GCMS. The 17β-estradiol was oxidised to estrone without any detectable degradates. Also, 16α-hydoxy-estrone was similarly degraded without degradation products. In a subsequent work, the biodegradation studies of trimethoprim, anti-tumorals cisplatin, cyclophosphamide, cytarabine, X-ray contrast agents, iopromide and diatrizoate has been carried out but not all the details of metabolites identification were reported [89].

Concerning the degradability of illicit drugs, apart from sample degradation, biodegradation is a natural process that has been reported by the stability experiment conducted by Georghe *et al* [219] and which observed that the concentration of cocaine and ecgonine methylester changed in surface water by 40 and 95% after 5 and 24 h test period respectively. However, benzoylecgonine level was constant or increased in the study. Photodegradation is another abiotic process involving complex reactions and pathways that could affect the aquatic fate of compounds, particularly when degradates are resistance to hydrolytic processes [220]. Four relatively new metabolites of cocaine: ecgonidine, norecgonidine methylester, *p*- hydroxyl-benzoylecgonine, and *m*- hydroxyl-benzoylecgonine [221], and two conjugates of metamphetamines: *p*-hydroxy-metamphetamine (*p*-OHMA-sulfate), (*p*-OHMA-Sul) and (*p*-OHMA) (*p*-OHMA-glucuronide) [220] have been identified in human urine.

The identification of this phase-II degradates and other metabolites in urine indicate likelihood of their presence in wastewater samples, unless they are further degraded in the sewage treatment works.

In a study, Pizzolato *et al* [150] observed the 40-80% degradation of cocaine and its metabolites in river waters under sunlight and pseudo-sunlight after 11 days of exposures as compared to HPLC grade water. Degradation was about 80% faster in river water as cocaine degraded to benzoylecgonine confirming the effects of both biodegradation and photodegradation.

Identification of microbial metabolites of ibuprofen has been found to be identical with the compound human metabolites [222-223]. During wastewater treatment, apart from the sorption behaviour of potential organic contaminants to the sludge solids, the removal of organic residues and associated metabolites are through microbial degradation as earlier reported as part of the removal mechanism of some pharmaceuticals and endocrine disrupting compounds (EDCs) in the sludge [80-83]. Hydrolysis (abiotic process) is the most important mechanism in the chemical degradation pathways through which compounds are removed [234]. The enrichment of the sewage sludge partitioning of chemicals onto sludge solids or suspended in solution is due to their hydrophilicity/lipophilicity properties compared to influent sewage [187-189].

Table 6 summarises the drugs and their metabolites identified from both human biological fluids and aquatic environments.

Appreciation of the degradation possibility of compounds whether they be completely or partially degraded in aqueous and solid phases, sorbed to sludge solids or mineralised is an important step in understanding the fate and behaviour of pollutants during sewage treatment. Within the large organic wastes in sewage is biomass of living and dead micro-organisms required for degradation processes within which some fractions of organic contaminants could be found. Sequential biological processes in alternating oxidative and reductive conditions for recalcitrant organic compounds plays a major role in removal mechanism [111, 190, 252].

In the degradation studies of alkylphenol polyethoxylate (APEO) surfactants, the recalcitrant and estrogenically active alkylphenols (APs) were produced from commercial NPEO using synthetic activated sludge in batch tests. The levels and distribution of the short chain compounds after NPEOs degradation confirmed in many ways these routes by which pollutants are discharged to the aquatic environment due to incomplete removal from treatment processes [253]. In activated sludge, viable and diverse bacterial population is maintained when the biological sludge is re-cycled from settling tank back to the aeration tank to produce high quality effluent, reduced biomass, maximised conversion of substrate and less production of waste sludge The oxidation of organic matter in an biological aerobic process generates carbon dioxide and water with the new but reduced biomass and dissolved residual organic matter in the effluent [224]. In related studies, Richardson and Bowron [93] assessed the biodegradability of some specific chemicals as presented in Table 7, but yet to be investigated are the degradation processes as well as the extent of transformations in producing different chemical metabolites [111]. Pathways of microbial degradation of selected acidic pharmaceuticals and their occurrence in municipal wastewater treated by a membrane bioreactor have been reported [254]. To further understand the behaviour of compounds in sewage plants, studies of metabolites from the biodegradation of pharmaceutical residual

of ibuprofen in biofilm reactor also confirmed the effects of biodegradations [222]

Elimination of selected acidic pharmaceuticals from municipal wastewater using activated sludge systems and membrane bioreactors [255], modelling versus measurement experiment of effluent from hospitals and private households to the total loads of diclofenac and carbamazepine in municipal sewage effluent [256] and identification of microbial degradation of trimethoprim in nitrifying activated sludge batch studies have been reported in the literature.

Stability of drugs and metabolites

The stability of drugs and their metabolites in the aqueous environment depends on some conditions of temperature and pH to minimise degradation of analytes. Studies recommended the acidification of samples to pH 2 and - 20^{o} C for storage in a stability study of cocaine and its metabolites (e.g. benzoylecgonine and ecgoninemethylester) where a pond free of drugs was spiked with different concentration of cocaine and benzoylecgonine at modified pH values of 2 and 6 and temperatures (-20^{o}C, +4^{o}C and +20^{o}C) for 5day stability tests. The 22% degradation of cocaine after 3 days and 35% after 5 days at pH 6 and +4^{o}C were observed. Also, ca.75% degradation was observed at +20^{o}C at pH > 6 for 1 day [158]. Using different preservation conditions, some decreases in the concentration of cocaine (36%), cocaethylene (13%), nor-cocaethylene (15%) and M3G (96%) and changes led to corresponding changes in the levels of metabolites (BZE, nor-BZE and MOR) respectively with the optimal conditions for storage similar to that observed for Cocaine, BZE and EME [219]. Similar works have also shown preserved samples at -20^{o}C with addition of HCl (pH 2) stopping bacterial action. Cocaine stability in wastewater at 4^{o} C for 48 hours was investigated but no changes were observed. Storage experiments with methanolic extracts for 7 days at different temperatures observed degradation of up to 15% with extracts stored at +4^{o}C but no changes with those stored at -20^{o}C [244, 159, 257]. Stability of other drugs of abuse like heroin, amphetamines-like substances and lysergic acid and their metabolites were not found in the literature.

Analytical Methodologies

In recent years, important advances in the development of chromatographic and mass spectrometric methods have been made, particularly in the detection and quantitative measurement of illicit drugs and their metabolites in various biological and aquatic matrices. The techniques based on liquid chromatography- mass spectrometry (LC-MS) or liquid chromatography tandem mass spectrometry (LC-MS^2) and gas chromatography-mass spectrometry (GC-MS) or gas chromatography tandem mass spectrometry (GC-MS^2) are very popular primarily because of their ability to detect and measure chemical substances at very low concentration. In addition to analytical methods for tracing pharmaceuticals residues in water and wastewaters that have been extensively used [93, 97-101], other analytical procedures for quick screening of drugs residue in aqueous environments including several inexpensive immunochemical approaches, as an alternative method to the chromatographic techniques for the efficient analysis of pharmaceuticals have also been published [96].

Chromatographic techniques

Table 8 shows the survey of chromatographic techniques from peer-reviewed literature in the determination of illicit drugs and human metabolites in waters. The review covers the extraction volumes, mobile phases, detectors (interfaces) and acquisition modes used by different scientists to provide sensitivity and selectivity. Also included are limits of quantifications depending on matrices for quantification and confirmation of drugs. The HPLC separation procedures rely on the principles of reversed-phase columns with different solvent gradients depending on applications [247-249]. Recently, variations over conventional LC-MS method have appeared in the literature eg. Ultra-performance liquid chromatography (UPLC-ESI-MS/MS), the ultra-fast UPLC-MS^2 is unique for its short columns packed with small particles sizes and stability at different pH range [135, 266 -268]. With the development of this relatively new technology, a shorter analysis time as well as gain in separation efficiency, resolution and sensitivity has been reported. To minimised the effects of ion suppression on the analytical signal, a relatively new HILIC; Hydrophilic interaction chromatography technique was also carried out in some experiments. Analytes were better retained on HILIC column, unaffected by ion suppression and a reduction in analytical signal was minimised [158, 219].

The use of MS/MS with triple quadrupole (QqQ) analyzers with electrospray ionization (ESI$^+$) were mostly used in selected reaction monitoring mode (SRM) to minimize the matrix interferences. The choices of ionization in ESI positive-ion mode were to have achieved ionizations and simultaneous determinations of analytes.

The HPLC-MS methods are also used in the analysis of illicit substances in the literature [208,214]. Both HPLC and GC-MS have been applied in the determination of pharmaceuticals in different matrices of biological fluids [262-265] especially urine [266-269], oral fluid [225, 226], and blood [227] samples. The advantage of HPLC-MS in the determination of the main illicit drug classes including cocaine, amphetamines, opiates and synthetic opiods, cannabinoids and their metabolites is due to its no hydrolysis, no derivatization, one- step extraction and with the introduction of atmospheric pressure ionisation (API) interfaces, the technique has been popular [269]. HPLC is a good and popular technique for highly polar, high molecular weight and thermolabile compounds. Its reproducibility, sensitivity and overall lower costs have therefore made it a convenient method. The use of GCMS is very rapid, faster and highly specific with in-built NIST library softwares for compound identification and elimination of matrix effects.

Principle of the choice of method (SPE-GC-MS): The trace analysis in wastewater can be captured as simple liquid chromatographic process where the SPE sorbent acts as the stationary phase and water constitutes the mobile phase during the extraction. During the percolation step, analytes that are trapped and cannot elute constitute the sample matrix. The enrichment of analytes from a large volume of aqueous sample on sorbent depends on how strongly the analytes are retained while allowing low retention during elution with organic solvents.

The method of coupling of SPE to GC-MS can be directly integrated as an online analytical system or off-line where subsequent chromatographic analysis is completely separated from the sample treatment. As long as the compounds are sufficiently thermally stable and volatile enough, gas chromatography (GC) allows a broad variety of samples to be analysed. As for all other chromatographic techniques, a mobile (carrier gas e.g. helium, argon, nitrogen, etc.) and a stationary phase (packed column or solid support coated with the liquid stationary phase of high boiling polymer e.g capillary columns of a small-diameter tube like 0.25 mm film in a 0.32 mm tube) are required . Different compounds are separated due to the interaction of the compound with the stationary phase (“like-dissolves-like”-rule). The stronger the interaction is, the longer the compound remains attached to the stationary phase, and the more time it takes to go through the column (longer retention time). GC-MS is a good combination of coupled analytical

systems as GC separates the compounds then MS identifies them based on their fragmentation pattern.

Solid Phase Extraction (pre-concentration)

Table 9 shows the multi-step extraction procedures of different protocols which have been reported in the peer-reviewed literature, to eliminate the influence of matrices [124,159]. Apart from matrix effect, improved recovery, stability under pH and ability of delivering clean extracts have resulted into various tests of several SPE adsorbent to determine suitable parameters relevant to a particular application need. Several SPE methods and adsorbents have been developed and used in conjunction with LC-MS^2 or GC-MS^2 in the determination of illicit drugs and their metabolites in aquatic media at very low concentrations (ng L^{-1} levels). Recently, Oasis MCX® (500mg/6mL) adsorbent, a polymeric sorbent with mixed–reversed/strong cation-exchange sulfonic acid group located on the surface of a (divinylbenzene-co-N-vinyl pyrrolidone) has been used [259, 270] to extract drug analytes from aqueous samples. After the samples were adjusted to pH 2 with 37% HCl or 0.01NHCl, the cartridge was pre-conditioned with 6ml of MeOH, 3mL of milli-Q water and 3mL water at pH 2. Samples were loaded into the cartridges at flow rate between 5-20mL min^{-1}, vacuum-dried for 5min and eluted with 6mL of MeOH and 6ml of 5% NH_3 in MeOH. The cartridges were found to be stable perhaps because of its two phases that were assumed could retain all compounds investigated. In related development Wylie *et al* [225] and Miltona *et al* [266] have used Bond Elut Certify®, a lipophilic and strongly cationic-adsorbent with similar conditioning and washing steps as used with Oasis MCX® adsorbent, the only difference was 2 x 4 mL of 80:20 DCM/isopropanol mixtures with 2% NH_3 in elution step. Traditional SPE materials such as the modified silica's e.g. C_8, (octyl), C_{18} (octadecyl) or CN (cyanopropyl) materials have low pH range, poor selectivity and residual silanol group which often leads to low recoveries in aqueous sample [82,90].

Bones et al [94] investigated the use of three sorbents: Phenomenex Strata- X™, Strata- XC™ and Strata- XCW™, all in 200mg sorbent mass pre-packed in to 6mL cartridges, but Strata- XC™ provided the highest analyte recovery. In other experiments, the Oasis HLB® (500mg/6 mL) adsorbent [278], MCX® (500mg/6mL) [159], Isolute ENV+® (500mg/6 mL) and Isolute PH® (1000mg/6 mL) adsorbents [245], and Bond Elut Certify® adsorbent [225] were compared with other adsorbents by Gheorge *et al* [219] in the extraction of cocaine and its metabolites in waste and surface water and the authors recommended the use of Oasis HLB® (500mg/6 mL, protocol 1) as most suitable adsorbent for organic compounds because of its lower solvent usage, time, stability to pH range and over 75% recovery for most analytes in aquatic medium.

Results and conclusions of survey of drugs in wastewaters

Generally, the illicit drug detection has been limited to the continuous screening of individual's biological fluids (urine, blood, oral-fluids and sweat), population survey with crime, drug production data, drug seizures and medical records [273,274]. The official estimates of the community consumption of illicit drugs from these exercises can be very unreliable because of the hidden nature and network of manufacture, importation, supply and usage without authorisation. Globally, United Nation Office of Drugs and Crime, (UNODC) estimates that between 149 and 272 million people, or, 3.3% to 6.1% of the population aged 15-64 used illicit substances at least once in the previous year [275]. Drugs are used in many ways and in many combinations by prescription for medical purposes, some illicit drug users often utilise therapeutic pharmaceuticals to supplement their illicit drug use by diverting common pharmaceuticals for illicit personal use and this illegal practice have affected societies in a myriad of ways. However, with the continuing pattern of escalation in use of illicit drugs and the discharge of their bioactive metabolites to sewage systems, and the present mode of sewage disposal (e.g. to grassland, landfills, incineration, horticulture, land reclamation) as complex mixtures so the processes involved in drugs removal at various STWs are not fully understood. Table 4 therefore summarises and compares the levels and distribution of the drugs from different STWs as reported in the literature in the last ten years. Also in Table 4, it was observed that the relative concentrations of drugs influents were higher compare to the effluents indicating the degree of removals. For example in 5 STWs in Spain, cocaine and benzoylecgonine in the influents were 225 and 2307 ng L^{-1} compare to only effluent cocaine concentrations of 47 ng L^{-1}. **T**he relative concentration of benzoylecgonine for example is about 10 times higher than the parent drug.

The removal of organic compounds is often incomplete in most municipal WWTPs; the sewage-sludge and effluent waters are therefore the primarily routes at which these chemicals enter the environment. Apart from the biodegradation, chemical degradation and sorption processes in typical WWTP of which details are not well understood because of the complex mixtures present during the wastewater treatment. The physicochemical properties of the contaminants ultimately determines their extent of persistence, toxicity and potential environmental effects after the sewage-sludge disposal to agricultural lands or effluents waters disposed of to seas. The existing priority substance classifications by the European Commission and U.S. Environmental Protection Agency notwithstanding [185]; the emerging priority contaminants groups like 'illicit drugs and their metabolites' have no safe-levels because of insufficient information on their biodegradability and persistence after their disposal to lands or receiving waters. As a result of insufficient information, decisions and policy thrusts regarding the future practices of safe sewage-sludge.

The illicit drugs and their metabolites are mainly from faeces and urination, the pattern of lavatory use fluctuates between individuals, certain periods of work and the population of residents in a particular environment, the load pattern of illicit substances would likely fluctuate in similar way [144]. Since many active researches have been on detection of illicit drugs and related products, studies on their fate and behaviour are therefore most warranted.

We have therefore critically reviewed the current development on the occurrence, metabolism, treatment processes, measurement before and after discharges from STWs to the environment with the current analytical methodologies that meet particular application needs such as fate of drugs monitoring.

With the developments on different aspects of drug's transformations in the environment recently published in the chemical literature which includes occurrence and fate, treatability by conventional and non-conventional processes, and several miscellaneous others [276-293]. The pharmaceutical and illicit markets will continue to grow to provide numerous commercial and therapeutic purposes. Today, large numbers of new drugs are introduced into the market and the number of patents granted keeps increasing accordingly. As these products reach the market with already large number of pharmaceutical companies involve in their development continue to grow. Information in the literature on the distribution levels, laboratory studies of fate and behaviour of some classes of illicit and pharmaceuticals drugs and their environmental assessment are rapidly changing. It is not very clear if current chemical pollution approaches in terms of effective monitoring and control of chemical discharges into the aquatic environment

would achieve desired effect and environmental scientist will have to be ready for new challenges ahead.

References

[1] Rhind SM. Endocrine disrupting compounds and farm animals: their properties, actions and of exposure. Domestic Animal Endocrinology. 2002; 23 (Special Issue): 179-187.

[2] Rhind SM. Are endocrine disrupting compounds a threat to farm animal health, welfare and productivity. Reproduction in Domestic Animals. 2005; 40: 282-290.

[3] Wilkinson JM, Hill J, Livesey CT. Accumulation of potentially toxic elements by sheep grazed on grassland given repeated applications of sewage sludge. Animal Science. 2001; 72, pp. (179-190).

[4] Egbowon BF. Sewage sludge exposure affects sheep fetal testis development at different periods of gestation. 2010 MPhil Thesis, School of Biosciences and Division of Animal Science, University of Nottingham, UK

[5] Sharman M, Read WA, Castle L, Gilbert J. Levels of di-(2-ethylhexyl) phthalate and total phthalate esters in milk, cream, butter and cheese. Food Addit Contamination. 1994; 11 (3): 375-385.

[6] Koppe JG, Bartonova A, Bolte G, Bistrup ML, Busby C, Butter M et al. Exposure to multiple environmental agents and their effect. Acta Paediatr Suppl. 2006*;* 95: 106–113.

[7] Heindel JJ, Gulati DK, Mounce RC, Russell SR, Lamb JC. Reproductive toxicity of three phtalic acid esters in a continuous breeding protocol. *Fundam Appl Toxicology. 1989;* 12: 508-518.

[8] Lottrup G, Andersson AM, Leffers H, Mortensen GK, Toppari J, Skakkebaeek NE et al. Possible impact of phthalates on infant reproductive health. International Journal of Andrology. 2006; 29: 172–180.

[9] Akingbemi BT, Sottas CM, Koulova AI, Klinefelter GR, Hardy M.P. Inhibition of testicular steroidogenesis by the xenoestrogen bisphenol A is associated with reduced pituitary luteinizing hormone secretion and decreased steroidogenic enzyme gene expression in rat Leydig cells. *Endocrinology*. 2004; 145: 592–603.

[10] Duty SM, Singh NP, Silva MJ, Barr DB, Brock JW, Ryan L. The relationship between environmental exposure to phthalates and DNA damage in human sperm. *Environ Health Perspect*ive. 2003; 111: ++1164–1169.

[11] Davis BJ, Maronpot RR, Heindel JJ. Di-(2-ethylhexyl) phthalate suppresses estradiol and ovulation in cycling rats. *Toxicology and Applied Pharmacology. 1994;* 128: 216–223.

[12] Mylchreest E, Wallace DG, Cattley RC, Foster PMD. Dose-dependent alterations in androgen-regulated male reproductive development in rats exposed to di(n-butyl) phthalate during late gestation. *Toxicology Science*. 2000; 55: 143-151.

[13] Li LH, Jester WF, Laslett AL, Orth J.M. A single dose of di-(2-ethylhexyl) phthalate in neonatal rats alters gonocytes, reduces Sertoli cell proliferation, and decreases cyclin D2 expression. *Toxicology & Applied Pharmacology*. 2000; 166 (3): 222-229.

[14] Gray LE Jr, Ostby J, Monosson E, Kelce WR. Environmental antiandrogens: low doses of the fungicide vinclozolin alter sexual differentiation of the male rat", Toxicology Industrial. Health. 1999; 15: 48–64.

[15] Garcia-Gutierrez A, McIntyre AE, Lester JN. Analysis of aldrin and endrin in sewage sludges. *Environ. Technol. Letter. 1992;* (12): 541-544.

[16] McIntyre AE, Lester JN. Occurrence and distribution of persistent organochlorine compounds in UK sewage sludges. *Water Air Soil Pollution. 1984;* 23 (4): 397-415.

[17] Hill DW, McCarty PL. Anaerobic degradation of selected chlorinated hydrocarbon pesticides. *Journal of Water Pollution Cont. Fed. 1967;*.39 (8): 1259-1277.

[18] Hill NP, McIntyre AE, Perry R, Lester JN. Development of a method for the analysis of chlorophenoxy herbicides in waste waters amd waste water sludges. *International Journal of Environment. Anal. Chem*. 1983; 15 (2): 107-130.

[19] Folke J. Organic micropollutants in the sewage from a phenoxy-alkanoic acid production plant after biological treatment in a municipal trickling filter plant. J. High Resol. *Chromatogr. Chromatogr. Commun*. 1984; 7 (l): 25-32

[20] McIntyre AE, Perry R, Lester JN. Analysis and incidence of organophosphorus compounds in sewage sludges. *Bull. Environ. Contam. Toxicol. 1981;* 26: 116-123.

[21] Mumma RO, Raupach DC, Waldman JP, Tong SSC, Jacobs ML, Babish JG. National survey of elements and other constituents in municipal sewage sludges. *Arch. Environ. Contam. Toxicology. 1984;* 13 (l): 75-83.

[22] Brewer WS, Draper AC, Wey SS. The detection of dimethylnitrosamine and diethylnitrosamine in municipal sewage sludge applied to agricultural soils. *Environmental. Pollution* (Ser. B). 1980; 1: 37-43.

.[23] Britcher H, Waggott A. Determination of pollutants in EfBuents. A Survey of Concentrations of Mineral Oils in UK Sewages, Eflluents and Sludges, *Water Research Centre report*, DoE 233-S, MPC 4332CIPES 1332C.

[24] Liu DL. Measuring petroleum hydrocarbons in digested sewage sludges. *Water Sewage Works. 1978;* 125 (3): 40-44.

[25] Giger W, Brunner PH, Schaffner C. 4 Nonylphenol in sewage sludge: accumulation of toxic metabolites from non-ionic surfactants. *Science. 1984;* 225 (4662): 623-625.

[26] Tschui M, Brunner PH, Giger W. Accumulation of Refractory Cnonylphenol During Mesophilic Anaerobic Sludge Stabilisation. COST 68 Working Party. 1985; (1) Workshop, Utrecht, 22 April.

[27] Stott DE, Tabatabai MA. Identification of phospholipids in soils and sewage sludges by high performance liquid chromatography. *Journal of Environmental Quality. 1985;* 14 (l): 107-110.

[28] Brown L, Bancroft KCC. Rhead, Laboratory studies on the adsorption of acrylamide monomer by sludge, sediments, clays, peat and synthetic resins. *Water Research*. 1980; 14: 779.

[29] O'Grady DP, Howard PH, Werner AF. Activated sludge biodegradation of 12 commercial phthalate esters. *Appl. Environ. Microbialogy. 1985;* 49 (2): 443-445.

[30] Laughlin RB, Linden O. Fate & effects of organotin compounds. *Ambio. 1985;* 14 (2): 88-94.

[31] Vrijhof H. Organotin compounds and international treaties on the pollution of water by dangerous substances: black or grey list substances. *Science of Total Environ*. 1985; 43 (3): 221-233.

[32] Craig PJ, Moreton PA, Rapsonanikis S. Methylation of mercury, tin and lead in aqueous and sediment environments, in Heavy Metals in the Environment. International Conference Proceedings, 1983; Heidelberg.

[33] McEvoy J, Giger W. Accumulation of linear alkylbenzene sulphonate surfactants in sewage sludges. *Naturwissenschaften*. 1985; 72: S429.

[34] Motschi H, McEvoy J. Influence of metaliadsorbate interactions on the adsorption of linear alkylbenzene sulphonates to hydrous surfaces. *Naturwissenschaften*. 1985; 72: S654.

[35] Sweetman AJ, Rogers HR, Crathome B. Monitoring of organic contaminants in sewage sludge with differing catchments and assessment of their potential for phytotoxic effects using a barley seedling bioassay, in J.E. Hall, D.R. Sauerbeck and P. L'Hermite (Eds), "Effects of Organic Contaminants in Sewage Sludge on Soil Fertility. Plants and

Animals, Proceedings of a meeting held at Braunschweig, 6-8 June 1990, Commission of the European Communities.
[36] Takada H, Ishiwatari R. Quantitation of longchain alkylbenzenes in environmental samples by silica gel column chromatography and high resolution gas chromatography. *Journal of Chromatography*. 1985; 346: 28 l-290.
[37] Takada H, Ishiwatari R. Linear alkylbenzenes in urban riverine environments in Tokyo: distribution, source and behaviour. *Environ. Sci. Technology*. 1987; 21 (9): 875-883.
[38] Takada H, Ishiwatari R. Biodegradation experiments of linear alkylbenzenes (LABS): isomeric composition of Cl2 LABS as an indicator of the degree of LAB degradation in the aquatic environment. Environ. *Science Technology. 1990;* 24: 86-91.
[39] Verschueren K. Handbook of Environmental Data on Organic Chemicals", 2nd edn., Van Nostrand Reinhold, New York, **1983**.
[40] Muller MD. Hexachlorobenzol in der Schweiz- Aussmass und Hintergrunde der Umweltkontamination. *Chimia. 1982;* 11: 437-435.
[41] Gaffney PE. Carpets and rugs industry 1: water and wastewater treatment plant operation. *Journal of Water Polluion. Cont. Fed.* 1986; 48: 2590.
[42] Jacobs LW, Zabik MJ. Importance of sludgeborne organic chemicals for land application programs", 6th Annual Madison Conf. on Applic. Res. Pratt. Munic. and Indust. Waste, Univ. Wisconsin. 1983: 418-426.
[43] Topping B. The biodegradability of paradichlorobenzene and its behaviour in model activated sludge plants. *Water Research*. 1987; 21: 295-300.
[44] Spain JC, Nishino SF. Degradation of 1,4- dichlorobenzene by a Pseudomonas sp. Appl. Environ. *Microbiol. 1987;* 53: 1010-1019.
[45] Schraa G. Anaerobic degradation, processes and test methods, in G. Angeletti and A. Bjorseth (Ed@, Organic Micropollutants in the Aquatic Environment, Kluwer, Dordrecht.
[46] Kirk PWW, Rogers HR, Lester JN. The fate of chlorobenzenes and permethrins during anaerobic sewage sludge digestion. *Chemosphere*. 1989; 18 (9/10): 1771-1784.
[47] Weerasinghe NCA, Gross ML, Lisk DJ. Polychlorinated dibenzodioxins and polychlorinated dibenzofurans in sewage sludges. *Chemosphere. 1985;* 14 (5): 557-564.
[48] Hagenmaier H, Brunner H, Haig R, Berchtoid A. PCDDs and PCDFs in sewage sludge, river and lake sediments from South West Germany. *Chemosphere. 1986;* 15: 1421-1428.
[49] Hagenmaier H, Brunner B. Isomer specific analysis of pentachlorophenol and sodium pentachlorophenate for 2,3,7,8-substituted PCDD and PCDF at sub ppb levels. *Chemosphere*. 1987; 16: 1759-1764.
[50] Oberg LG, Andersson R, Rappe C. De-novo formation of hepta- and octachlorodibenzo-p-dioxins from pentachlorophenol in municipal sewage sludge in Proceedings of 'DIOXIN '92', Tampere, Finland.
[51] Rappe C, Andersson R. Analyses of PCDDs and PCDFs in wastewater from dishwashers and washing machines. DIOXIN '92', Tampere, Finland, Extended Abstracts. *Organohalogen Compounds*. 1992; 9: 191-194.
[52] Rappe C, Andersson R.Levels of PCDDs and PCDFs in human faeces", 'DIOXIN '92' Tampere, Finland, Extended Abstracts. *Organohalogen Compounds. 1992*; 9: 195-198.
[53] Broman D, Naf C, Rolff C, Zebuhr Y. Analysis of polychlorinated dibenzo-p-dioxins (PCDD) and polychlorinated dibenzofurans (PCDF) in soil and digested sewage sludge from Stockholm, Sweden. *Chemosphere*. 1990; 21: 1213-1220.
[54] Rogers HR, Ambridge P, Stenhouse I. The Occurrence of PCDDs and PCDFs in Some UK Sewage Sludges - A Preliminary Investigation, WRc report for the Department of the Environment, 1989, DOE 2093-M.
[55] Neff JM. Polycyclic Aromatic Hydrocarbons in the Aquatic Environment - Sources, Fates and Biological Effects. Applied Science Publishers, 1979; London.
[56] McIntyre AE, Perry R, Lester JN. Analysis of polynuclear aromatic hydrocarbons in sewage sludges. *Analytical Letter. 1981;* 14: 291-309.
[57] Wszolek PC, Wachs T. Occurrence of polycyclic aromatic hydrocarbons in municipal sewage sludge ashes. *Arch. Environmental. Contamination Toxicology. 1982*; 11: 69-72.
[58] Aichberger K, Reifenauer D. Determination of polycyclic aromatic hydrocarbons in sewage sludge", in P. L'Hermite and H. Ott (Eds), Proceedings of 3rd International Symposium on Processing and Use of Sewage Sludge, 1983; Brighton, Reidel, Dordrecht.
[59] Nicholls P, Perry R, Lester JN. The influence of heat treatment on the metallic and PAH content of sewage sludge", *Science Total Enviroment. 1979;* 12: 137-150.
[60] Hagenmaier H, Kaut H, Krauss P. Analysis of polycyclic aromatic hydrocarbons in sediments, sewage sludges and composts from municipal refuse by HPLC, in J. Albaiges and R.W. Frei (Eds), Fate of Hydrocarbons in the Environment, An Analytical Approach. Current Topics in Environmental and Toxicological Chemistry, 10, Gordon and Breach, New York.
[61] Bridle TR, Webber MD. A Canadian perspective on toxic organics in sewage sludge. Proc. Conf. Work Party 5, CEC, Concerted Action on the Characterisation, Treatment and Use of Sewage Sludge. 1982; WRc report 2-S.
[62] Gray LE, Ostby J, Furr J, Wolf CJ, Lambright C, Parks L et al. Effects of environmental antiandrogens on reproductive development in experimental animals. Human Reproduction Update. 2001 (7) 248-264.
[63] Ema M, Miyawaki E. Adverse effects on development of the reproductive system. *Reprod Toxicol*ogy. 2001; 1 (5): 189-194.

[64] Couse JF, Korach K.S. Estrogen receptor-alpha mediates the detrimental effects of neonatal diethylstilbestrol (DES) exposure in the murine reproductive tract. *Toxicology. 2004;* 205: 55–63.
[65] Johnston H, Baker PJ, Abel M, Charlton HM, Jackson G, Fleming L et al. Regulation of Sertoli cell number and activity by follicle-stimulating hormone and androgen during postnatal development in the mouse. *Endocrinology*. 2004; 145: 318-29.
[66] Jobling S, Reynolds T, White R, Parker MG, Sumpter JP et al. A variety of environmentally persistent chemicals, including some phthalate plasticizers, are weakly estrogenic. *Environ Health Perspect*. 1995; 103: 582-587.
[67] Soto AM, Sonnenschein C, Chung K.L, Fernandez MF, Olea N, Olea SF et al. The e-screen assay as a tool to identify estrogens: an update on estrogenic environmental pollutants. *Environ Health Perspect*. 1995; 103 (Suppl3): 113-122.
[68] Rudel RA, Seryak LM, Brody JG. PCB-containing wood floor finish is a likely source of elevated PCBs in residents' blood, household air and dust: a case study of exposure. *Environmental Health*. 2008; 7: 2.
[69] Safe SH. Polychlorinated biphenyls (PCBs), dibenzo-p-dioxins (PCDDs), dibenzofurans (PCDFs), and related compounds: environmental and mechanistic considerations which support the development of toxic equivalency factors (TEFs). *Crit Rev Toxicology. 1990;* 21: 51-88.
[70] Brouwer A, Reijnders PJH, Koeman JH. Polychlorinated biphenyl (PCB)-contaminated fish induce vitamin A and thyroid

hormone deficiency in the Common Seal. (*Phoca vitulina*). *Aquatic Toxicology. 1989;* 15: 99–106.
[71] Reijnders JH, Koeman JH. Polychlorinated biphenyls (PCB)-contaminated fish induces vitamin A and thyroid deficiency in the common seal Phoca vitulina. *Aquatic Toxicology. 1989;* 15: 99-106.
[72] Venners SA, Korrick S, Xu X, Chen C, Guang W, Huang A *et al.* Preconception serum DDT and pregnancy loss: a prospective study using a biomarker of pregnancy. *America Journal of Epidemiol*ogy. 2005; 162: 709–716.
[73] Zimmer K., Gutleb AC, Lyche JL, Dahl E, Oskam I, Krogenas A. Altered stress induced cortisol levels in goats exposed to polychlorinated biphenyls (PCB 126 and PCB 153) during foetal and postnatal development *Journal of Toxicology Enviromental Health* A. 2008; (272) 164–172.
[74] Krishman V, Safe S. Polychlorinated biphenyls (PCBs), dibenzo-p-dioxins (PCDDs) and dibenzofurans (PCDFs), as antiestrogens in MCF-7 human breast cancer cells: quantitative structure-activity relationships. *Toxicology Appl Pharmacol*. 1993; (120): 55-61.
[75] Whyatt RM, RauhV, Barr DB, Camann DE, Andrews HF, Garfinkel R *et al.* Prenatal insecticide exposures and birth weight and length among an urban minority cohort", *Environ Health Perspect*ive. 2004; 112: 1125–1132.
[76] Lilienthal H, Winneke G. Sensitive periods for behavioural toxicity of polychlorinated biphenyls: determination by cross fostering in rats. Fundam Appl Toxicology. 1991; 17: 368-375.
[77] Korach K.S, Sarver P, Chae K, McLachlan JA, McKinney JD et al. Estrogen receptor-binding activity of polychlorinated hydrobiphenyls conformationally restricted structural probes. Mol Pharmacology. 1988; 33: 130-126
[78] Jones OAH, Voulvoulis N, Lester JN. The occurrence and removal of selected pharmaceutical compounds in a sewage treatment works utilising activated sludge treatment. *Environmental* Pollution. 2007; 145 (3): 738-744
[79] Loffler D, Rombke J, Meller M, Ternes TA. Environmental fate of pharmaceuticals in water/sediment systems. *Environmental science & technology. 2005;* 39 (14): 5209-5218**.**
[80] Ternes TA, Stumpf M, Mueller J, Haberer K., Wilken RD, Servos M. Behaviour and occurrence of estrogens in municipal sewage treatment plants--I. Investigations in Germany, Canada and Brazil",*The Science of the total environment. 1999;* 225 (1-2): 81-90.
[81] Ternes TA. Occurrence of drugs in German sewage treatment plants and rivers. *Water research. 1998;* 32 (11): 3245-3260.
[82] Jones OAH, Voulvoulis N, Lester JN. The occurrence and removal of selected pharmaceutical compounds in a sewage treatment works utilising activated sludge treatment. *Environmental Pollution. 2007;* 145 (3): 738-744.
[83] Daughton CG, Ternes TA. Pharmaceuticals and personal care products in the environment: agents of subtle change. *Environmental health perspectives. 1999;* 107 Suppl 6: 907-938.
[84] Zhang ZL, Zhou JL. Simultaneous determination of various pharmaceutical compounds in water by solid-phase extraction–liquid chromatography–tandem mass spectrometry. *Journal of Chromatography A, 2007;* 1154 (1–2): 205-213.
[85] Richardson SD, Ternes TA. Water analysis: emerging contaminants and current issues. *Analytical Chemistry. 2005;* 77 (12): 3807-3838.
[86] Ashton D, Hilton M, Thomas KV. Investigating the environmental transport of human pharmaceuticals to streams in the United Kingdom. *Science of The Total Environment. 2004;* 333 (1–3): 167-184.
[87] Tixier C, Singer HP, Oellers S, Muller SR. Occurrence and fate of carbamazepine, clofibric acid, diclofenac, ibuprofen, ketoprofen, and naproxen in surface waters. *Environmental science & technology. 2003;* 37 (6): 1061-1068.
[88] Zuccato E, Calamari D, Natangelo M, Fanelli R. Presence of therapeutic drugs in the environment. *Lancet.2000;* 355 (9217): 1789-1790.
[89] Ternes TA. Analytical methods for the determination of pharmaceuticals in aqueous environmental samples", *TrAC Trends in Analytical Chemistry. 2001;* 20 (8): 419-434.
[90] Castiglioni S, Bagnati R, Calamari D, Fanelli R, Zuccato E. A multiresidue analytical method using solid-phase extraction and high-pressure liquid chromatography tandem mass spectrometry to measure pharmaceuticals of different therapeutic classes in urban wastewaters. *Journal of Chromatography A. 2005;* 1092 (2): 206-215
[91] Hernando MD, Gómez MJ, Agüera A, Fernández-Alba AR. LC-MS analysis of basic pharmaceuticals (beta-blockers and anti-ulcer agents) in wastewater and surface water. *TrAC Trends in Analytical Chemistry. 2007;* 26 (6): 581-594.
[92] Pérez S, Barceló D. Application of advanced MS techniques to analysis and identification of human and microbial metabolites of pharmaceuticals in the aquatic environment. *TrAC Trends in Analytical Chemistry. 2007;* 26 (6): 494-514.
[93] Richardson ML, Bowron JM. The fate of pharmaceutical chemicals in the aquatic environment. *J. Pharm. Pharmacol*. 1985; 37: l-12.
[94] Ternes TA, Kreckel P, Mueller J. Behaviour and occurrence of estrogens in municipal sewage treatment plants--II. Aerobic batch experiments with activated sludge. *The Science of the total environment. 1999;* 225 (1-2): 91-99
[95] Buchberger WW. Novel analytical procedures for screening of drug residues in water, waste water, sediment and sludge. *Analytica Chimica Acta. 2007;* 593 (2): 129-139.
[96] Petrovic M, Petrovic M, Barceló D. LC-MS for identifying photodegradation products of pharmaceuticals in the environment. *TrAC Trends in Analytical Chemistry. 2007;* 26 (6): 486-493.
[97] Fatta D, Achilleos A, Nikolaou A, Meriç S. Analytical methods for tracing pharmaceutical residues in water and wastewater. *TrAC Trends in Analytical Chemistry. 2007;* 26 (6): 515-533.
[98] Rahman F, Langford KH, Scrimshaw MD, Lester JN. Polybrominated diphenyl ether (PBDE) flame retardantS. *The Science of the total environment. 2001;* 275 (1-3): 1-17.
[99] O'Connor S, Aga DS. Analysis of tetracycline antibiotics in soil: Advances in extraction, clean-up, and quantification. *TrAC Trends in Analytical Chemistry. 2007,* 26 (6): 456-465.
[100] Bajad S, Shulaev V. Highly-parallel metabolomics approaches using LC-MS2 for pharmaceutical and environmental analysis. *TrAC Trends in Analytical Chemistry. 2007;* 26 (6): 625-636.
[101] Daughton CG. Non-regulated water contaminants: emerging research. *Environmental Impact Assessment Review. 2004;* 24 (7-8): 711-732.
[102] Watts CD, Crathome B, Fielding M,Steel CP. Identification of non-volatile organics in water using field desorption mass spectrometry and high performance liquid chromatography in G. Angeletti and A. Bjorseth (Eds), Analysis of Organic Micropollutants in Water. Report of the 3rd European Symposium on Organic Micropollutants, Oslo, Norway, September 19-21, 1983; Reidel, Dordrecht.
[103] Golet EM, Alder AC, Hartmann A, Ternes TA, Giger W. Trace determination of fluoroquinolone antibacterial agents in urban wastewater by solid-phase extraction and liquid chromatography with fluorescence detection. *Analytical Chemistry. 2001;* 73 (15): 3632-3638.

[104] Calamari D, Zuccatto E, Castiglion S, Bagnat R, Fanalli R. Strategic Survey of Therapeutic Drugs in the Rivers Po and Lambro in Northern Italy. *Environ. Sci. Technol. 2003;* 37: 1241-1248
[105] Hirsch R, Ternes T, Haberer K., Kratz K.. Occurrence of antibiotics in the aquatic environment. *The Science of the total environment. 1999;* 225 (1-2):109-118.
[106] Miao X, Koenig BG, Metcalfe CD. Analysis of acidic drugs in the effluents of sewage treatment plants using liquid chromatography–electrospray ionization tandem mass spectrometry. *Journal of Chromatography A. 2002;* 952, (1-2): 139-147.
[107] Ternes T, Bonerz M, Schmidt T. Determination of neutral pharmaceuticals in wastewater and rivers by liquid chromatography–electrospray tandem mass spectrometry. *Journal of Chromatography A. 2001;* 938 (1–2): 175-185.
[108] Vieno NM, Tuhkanen T, Kronberg L. Analysis of neutral and basic pharmaceuticals in sewage treatment plants and in recipient rivers using solid phase extraction and liquid chromatography–tandem mass spectrometry detection. *Journal of Chromatography A. 2006;* 1134 (1–2): 101-111.
[109] Barceló D. Pharmaceutical-residue analysis. *TrAC Trends in Analytical Chemistry. 2007;* 26, (6): 454-455.
[110] Beausse J. Selected drugs in solid matrices: a review of environmental determination, occurrence and properties of principal substances. *TrAC Trends in Analytical Chemistry. 2004;* 23 (10-11): 753-761.
[111] Rogers H.R. Sources, behaviour and fate of organic contaminants during sewage treatment and in sewage sludges. *Science of The Total Environment. 1996;* 185 (1-3): 3-26.
[112] Petrovic M, Gonzalez S, Barceló D. Analysis and removal of emerging contaminants in wastewater and drinking water. *TrAC Trends in Analytical Chemistry. 2003;* 22 (10): 685-696.
[113] Hirsch R, Ternes TA, Haberer K, Mehlich A, Ballwanz F, Kratz KL. Determination of antibiotics in different water compartments via liquid chromatography-electrospray tandem mass spectrometry. *Journal of chromatography.A. 1998;* 815, 2: 213-223.
[114] Alder AC, McArdell CS, Gole EM, Ibric S, Molnar E, Nipales NS, Giger W. in: Ch. Daughton, T. Jones-Lepp (Editors), Pharmacutical and personal care products in the environment: Scientific and regulatory Issue, ACS-Symposium series, 2001, in press.
[115] Putschew A, Wischnack S, Jekel, M. Occurrence of triiodinated X-ray contrast agents in the aquatic environment. *The Science of the total environment. 2000;* 255 (1-3): 129-134.
[116] Wick A, Fink G, Joss A, Siegrist H, Ternes TA. Fate of beta blockers and psycho-active drugs in conventional wastewater treatment. *Water research. 2009;* 43 (4): 1060-1074.
[117] Ternes TA., Hirsch R. Occurrence and Behavior of X-ray Contrast Media in Sewage Facilities and the Aquatic Environment. Environ. Sci. Technol. 2000; 34: 2741-2748
[118] Ternes TA. Pharmaceuticals and metabolites as contaminants of the aquatic, in: Ch, Daughton, T. Jones-Lepp (Editors), Pharmacutical and personal care products in the environment: Scientific and regulatory Issue, ACS-Symposium series, 2001; in press.
[119] Halling-Sørensen B, Nors Nielsen S, Lanzky PF, Ingerslev F, Holten Lützhøft HC, Jørgensen SE et al. Occurrence, fate and effects of pharmaceutical substances in the environment- A review. *Chemosphere. 1998;* vol. 36, no. 2, pp. 357-393.
[120] Jørgensen SE, Halling-Sørensen B. Drugs in the environment. *Chemosphere. 2000;* 40 (7): 691-699.
[121] The Misuse of drugs Act 1971 (modification) order 2001. Available from: www.opsi.gov.uk/si/si2001/20013932. html.
[122] European Monitoring Centre for Drugs and Drug Addition (EMCDDA), 2002. EMCDDA project CT. 99. EP. 08. B. Handbook for surveys on drug use among the general population. EMCDDA: Lisbon, Portugal, August, 2002. Available from: www.emcdda.europa.eu.
[123] European Monitoring Centre for Drugs and Drug Addition (EMCDDA), 2007. The state of the drug problem in the European Union and Norway. Annual report 2007. EMCDDA: Lisbon, Portugal. Available from: http://www.emcdda.europa.eu/attachments.4m/att-42156-EN-TDA (07001ENG.pdf).
[124] Zuccato E, Chiabrando C, Castiglioni S, Calamari D, Bagnati R, Schiarea S. Cocaine in surface waters: a new evidence-based tool to monitor community drug abuse. *Environmental health: a global access science source. 2005;* 4: 14.
[125] Hilton MJ, Thomas KV. Determination of selected human pharmaceutical compounds in effluent and surface water samples by high-performance liquid chromatography–electrospray tandem mass spectrometry. *Journal of Chromatography A. 2003;* 1015 (1-2): 129-141.
[126] Ellenhorn MJ, ed, Barceloux DG**.** New York Elsevier Sc Publishing Company, Inc, 1998; 644-661.
[127] Van Viet H, Chevalier P, Sereni C, Bornet P, Bautier P, Degos C.F. Neurologic complications of cocaine abuse. *Presse medicale (Paris, France : 1983). 1990;* 19 (22): 1045-1049.
[128] Kolbrich EA, Barnes AJ, Gorelick DA, Boyd SJ, Cone EJ, Huestis MA. Major and minor metabolites of cocaine in human plasma following controlled subcutaneous cocaine administration. *Journal of analytical toxicology. 2006;* 30 (8): 501-510.
[129] Matsubara K., Kagawa M, Fukui Y. In vivo and in vitro studies on cocaine metabolism: Ecgonine methyl ester as a major metabolite of cocaine. *Forensic science international. 1984;* 26 (3): 169-180.
[130] Cone EJ. Pharmacokinetics and pharmacodynamics of cocaine. *Journal of analytical toxicology. 1995;* 19, (6): 459-478.
[131] Stewart DJ, Inaba T, Lucassen M, Kalow W. Cocaine metabolism: cocaine and norcocaine hydrolysis by liver and serum esterases. *Clinical pharmacology and therapeutics. 1979;* 25 (4): 464-468.
[132] Saxon AJ, Calsyn DA, Haver VM, Delaney CJ. Clinical evaluation and use of urine screening for drug abuse. *The Western journal of medicine; 1988;* 149 (3): 296-303.
[133] Ambre J. The urinary excretion of cocaine and metabolites in humans: a kinetic analysis of published data. *Journal of analytical toxicology. 1985;* 9 (6): 241-245.
[134] Kloss MW, Rosen GM, Rauckman EJ. Cocaine-mediated hepatotoxicity. A critical review. *Biochemical pharmacology. 1984;* 33 (2): 169-173.
[135] Kloss MW, Rosen GM, Rauckman EJ. Biotransformation of norcocaine to norcocaine nitroxide by rat brain microsomes. *Psychopharmacology. 1984;* 84 (2): 221-224.
[136] Hearn WL, Flynn DD, Hime GW, Rose S, Cofino JC, Mantero-Atienza E et al. Cocaethylene: a unique cocaine metabolite displays high affinity for the dopamine transporter. *Journal of neurochemistry. 1991;* 56 (2): 698-701.
[137] Scheidweiler KB, Shojaie J, Plessinger MA, Wood RW, Kwong TC. Stability of methylecgonidine and ecgonidine in sheep plasma in vitro. *Clinical chemistry. 2000;* 46 (11): 1787-1795.
[138] Maurer HH, Sauer C, Theobald DS. Toxicokinetics of drugs of abuse: current knowledge of the isoenzymes involved in the human metabolism of tetrahydrocannabinol, cocaine,

heroin, morphine, and codeine. *Therapeutic drug monitoring. 2006;* 28 (3): 447-453.
[139] Schraa G. Anaerobic degradation, processes and test methods, in G. Angeletti and A. Bjorseth (Ed@, Organic Micropollutants in the Aquatic Environment, Kluwer, Dordrecht
[140] European Monitoring Centre for Drugs and Drug Addition (EMCDDA), Insight series 9, 2008. Assessing illicit drug in wastewater ISBN 978-92-9168-317-8.
[141] European Monitoring Centre for Drugs and Drug Addition (EMCDDA), 2007. The state of the drug problem in the European Union and Norway. Annual report 2007. EMCDDA: Lisbon, Portugal. Available from: http://www.emcdda.europa.eu/attachments.4m/att-42156-EN-TDA (07001ENG.pdf).
[142] Ensslin HK., Kovar K, Maurer HH. Toxicological detection of the designer drug 3,4-methylenedioxyethylamphetamine (MDE, "Eve") and its metabolites in urine by gas chromatography — mass spectrometry and fluorescence polarization immunoassay. *Journal of Chromatography B: Biomedical Sciences and Applications. 1996;* 683 (2): 189-197.
[143] Dollery, C.T. Clinical pharmacology of calcium antagonists", *American journal of hypertension. 1991;* 4 (2) pt 2: 88S-95S
[144] Pizzolato TM, de Alda MJL, Barceló D. LC-based analysis of drugs of abuse and their metabolites in urine. *TrAC Trends in Analytical Chemistry. 2007;* 26 (6): 609-624.
[145] Andrew R, Tasker R, Nakatsu, K.**.** Evaluation of 3,6-dibutanoylmorphine as an analgesic : Comparison with morphine and 3,6-diacetylmorphine. *Life Sciences. 1984;* 34 (17): 1659-1667.
[146] Knodell RG, Farleigh RM, Steele NM, Bond JH. Effects of liver congestion on hepatic drug metabolism in the rat. *The Journal of pharmacology and experimental therapeutics. 1982;* 221 (1): 52-57.
[147] Hand CW, Moore RA, McQuay HJ, Allen MC, Sear JW. Analysis of morphine and its major metabolites by differential radioimmunoassay. *Annals of Clinical Biochemistry. 1987;* 24 (Pt 2): 153-160.
[148] Canezin J, Cailleux A, Turcant A, Le Bouil A, Harry P, Allain, P. Determination of LSD and its metabolites in human biological fluids by high-performance liquid chromatography with electrospray tandem mass spectrometry. *Journal of Chromatography B: Biomedical Sciences and Applications. 2001;* 765 (1): 15-27.
[149] Reuschel SA, Eades D, Foltz RL. Recent advances in chromatographic and mass spectrometric methods for determination of LSD and its metabolites in physiological specimens. *Journal of Chromatography B: Biomedical Sciences and Applications. 1999;* 733 (1-2): 145-159.
[150] Pizzolato TM, de Alda MJL, Barceló D. LC-based analysis of drugs of abuse and their metabolites in urine. *TrAC Trends in Analytical Chemistry.2007;* 26 (6): 609-624.
[151] Neff JM. Polycyclic Aromatic Hydrocarbons in the Aquatic Environment - Sources, Fates and Biological Effects. Applied Science Publishers, 1979; London.
[152] Mueller CA, Weinmann W, Dresen S, Schreiber A, Gergov M. Development of a multi-target screening analysis for 301 drugs using a QTrap liquid chromatography/tandem mass spectrometry system and automated library searching. *Rapid communications in mass spectrometry : RCM. 2005;* 19 (10): 1332-1338.
[153] Postigo C, de Alda MJ, Barcelo D. Fully automated determination in the low nanogram per liter level of different classes of drugs of abuse in sewage water by on-line solid-phase extraction-liquid chromatography-electrospray-tandem mass spectrometry. *Analytical Chemistry. 2008;* 80 (9): 3123
[154] Nelson CC, Foltz RL in: R. C. Baselt (ed). Advances in analytical Toxicology, Vol. II, Year book Medical Publishers, Chicago, IL. 1989: 140.
[155] Lambert DM, Fowler CJ. The endocannabinoid system: drug targets, lead compounds, and potential therapeutic applications. *Journal of medicinal chemistry. 2005;* 48 (16): 5059-5087.
[156] Burns TL, Ineck JR. Cannabinoid analgesia as a potential new therapeutic option in the treatment of chronic pain. *The Annals of Pharmacotherapy. 2006;* 40 (2): 251-260.
[157] ElSohly MA, Slade D. Chemical constituents of marijuana: The complex mixture of natural cannabinoids. *Life Sciences. 2005;* 78 (5): 539-548.
[158] van Nuijs ALN, Pecceu B, Theunis L, Dubois N, Charlier, C, Jorens, P.G et al. Cocaine and metabolites in waste and surface water across Belgium. *Environmental Pollution. 2009;* 157 (1): 123-129
[159] Castiglioni S, Zuccato E, Crisci E, Chiabrando C, Fanelli R, Bagnati R. Identification and measurement of illicit drugs and their metabolites in urban wastewater by liquid chromatography-tandem mass spectrometry. *Analytical Chemistry. 2006;* 78 (24): 8421-8429.
[160] Bartelt-Hunt SL, Snow DD, Damon T, Shockley J, Hoagland K.. The occurrence of illicit and therapeutic pharmaceuticals in wastewater effluent and surface waters in Nebraska. *Environmental Pollution. 2009;* 157 (3): 786-791.
[161] Venners SA, Korrick S, Xu X, Chen C, Guang W, Huang A *et al.* Preconception serum DDT and pregnancy loss: a prospective study using a biomarker of pregnancy. *America Journal of Epidemiol*ogy. 2005; 162: 709–716.
[162] Zimmer K., Gutleb AC, Lyche JL, Dahl E, Oskam I, Krogenas A. Altered stress induced cortisol levels in goats exposed to polychlorinated biphenyls (PCB 126 and PCB 153) during foetal and postnatal development. *Journal of Toxicology Enviromental Health* A. 2008; (272): 164–172.
[163] Krishman, V, Safe S. Polychlorinated biphenyls (PCBs), dibenzo-p-dioxins (PCDDs) and dibenzofurans (PCDFs), as antiestrogens in MCF-7 human breast cancer cells: quantitative structure-activity relationships. *Toxicology Appl Pharmacol*. 1993; 120: 55-61.
[164] Whyatt RM, Rauh V, Barr DB, Camann DE, Andrews HF, Garfinkel R. *et al.* Prenatal insecticide exposures and birth weight and length among an urban minority cohort", *Environ Health Perspect*ive. 2004; 112: 1125–1132.
[165] Lilienthal H, Winneke G. Sensitive periods for behavioural toxicity of polychlorinated biphenyls: determination by cross fostering in rats. Fundam Appl Toxicology. 1991;17: 368-375.
[166] Korach KS, Sarver P, Chae K, McLachlan JA, McKinney JD. Estrogen receptor-binding activity of polychlorinated hydrobiphenyls conformationally restricted structural probes. Mol Pharmacology. 1998; 33: 130-126
[167] Carballa M, Omil F, Lema JM, Llompart M, García-Jares C, Rodríguez I. Behavior of pharmaceuticals, cosmetics and hormones in a sewage treatment plant. *Water research. 2004;* 38 (12): 2918-2926.
[168] Quintana JB, Weiss S, Reemtsma T. Pathways and metabolites of microbial degradation of selected acidic pharmaceutical and their occurrence in municipal wastewater treated by a membrane bioreactor. *Water Research*. 2005; 39: 2654-2664.
[169] Ternes TA, Stueber J, Herrmann N, McDowell D, Ried A, Kampman T.M et al. Ozonation: a tool for the removal of

pharmaceuticals, contrast media and musk frangrances from wastewater. *Water Research. 2003;* 37: 1976-1982.
[170] Huber MM, Canonical S, Park GY, von Gunten U. Oxidation of pharmaceuticals during ozonation and advanced oxidation processes. *Environ. Sci. Technol.*2003; 37: 1016-1024.
[171] Kloepler A, Gnirss R, Jekel M, Reemtsma T. Occurrence of benzothiazoles in municipal wastewater and their fate in biological treatment. *Water Sci. Technol*. 2004; 50 (5): 203-208.
[172] Zuehlke S, Duennbier U, Lesjean B, Gniss R, Buisson H. Longtime comparison of trace organics removal performance between convectional and membrane activated sludge processes. Poceedings of the WEFTEC 2003 Conference, Los Angeles, October 2003.
[173] Metcalf and Eddy. Wastewater Engineering: Treatment Disposal Reus, McGraw-Hill, Inc. 1991.
[174] Chen HC, Wang PL, Ding WH. Using liquid chromatography-ion trap mass spectrometry to determine pharmaceutical residues in Taiwanese rivers and wastewaters. *Chemosphere*. 2008; 72: 863-869.
[175] Perez S, Eichhorn P, Celiz MD, Aga DS. Structural characterization of metabolites of the X-ray contrast agent iopromide in activated sludge using ion trap mass spectrometry. *Analytical Chemistry. 2006;* 78 (6): 1866-1874.
[176] Steger-Hartmann T, Lange R, Schweinfurth H, Tschampel M, Rehmann I. Investigations into the environmental fate and effects of iopromide (ultravist), a widely used iodinated X-ray contrast medium. *Water research. 2002;* 36 (1): 266-274.
[177] Eichhorn P, Ferguson PL, Perez S, Aga DS. Application of ion trap-MS with H/D exchange and QqTOF-MS in the identification of microbial degradates of trimethoprim in nitrifying activated sludge. *Analytical Chemistry. 2005;* 77 (13): 4176-4184.
[178] Schreiber F, Szewzyk U. Environmentally relevant concentrations of pharmaceuticals influence the initial adhesion of bacteria. *Aquatic Toxicology. 2008;* 87 (4): 227-233.
[179] Kasprzyk-Hordern B, Dinsdale RM, Guwy AJ. Multi-residue method for the determination of basic/neutral pharmaceuticals and illicit drugs in surface water by solid-phase extraction and ultra-performance liquid chromatography–positive electrospray ionisation tandem mass spectrometry. *Journal of Chromatography A. 2007;* 1161 (1-2): 132-145.
[180] Daughton CG. Illicit drugs: contaminants in the environment and utility in forensic epidemiology. *Reviews of environmental contamination and toxicology. 2011;* 210: 59-110.
[181] Escher BI, Fenner K. Recent advances in environmental risk assessment of transformation products", *Environmental science & technology. 2011;* 45 (9): 3835-3847.
[182] Fatta-Kassinos D, Meric S, Nikolaou A. Pharmaceutical residues in environmental waters and wastewater: current state of knowledge and future research. *Analytical and bioanalytical chemistry. 2011;* 399 (1): 251-275.
[183] Ze-hua Liu**,** Yoshinori Kanjo, Satoshi Mizutani. Removal mechanisms for endocrine disrupting compounds (EDCs) in wastewater treatment — physical means, biodegradation, and chemical advance oxidation: A review. *Science of The Total Environment*. 2009; 407 (Issue 2): 731–748
[184] Berg T, Lundanes E, Christophersen AS, Strand DH. Determination of opiates and cocaine in urine by high pH mobile phase reversed phase UPLC–MS/MS. *Journal of Chromatography B. 2009;* 877 (4): 421-432.
[185] European Community, European Communities List of 129 Priority Substances, Direcive 76/464/EEC, Commission of the European Community.
[186] Zuccato E, Castiglioni S, Bagnati R, Chiabrando C, Grassi P, Fanelli R. Illicit drugs, a novel group of environmental contaminants. *Water research. 2008;* 42 (4-5): 961-968.
[187] Petrasek AC, Kugelman IJ, Austem M, Pressley TA, Winslow LA, Wise RH. Fate of toxic organic compounds in wastewater treatment plants. *Journal Water Pollut*ion Cont. Fed. 1983; 55 (10): 1286-1296.
[188] Hannah SA, Austem BM, Eralp AE, Wise RH. Comparative removal of toxic pollutants by six wastewater treatment processes. *Journal of Water Pollution* Cont. Fed. 1986;58: 27-34.
[189] Hannah SA, Austem BM, Eralp AE, Dobbs RA. Removal of organic toxic pollutants by trickling filter and activated sludge. J. Water Pollut. Cont. Fed. 1988;60: 1281-1283.
[190] Strachan GW, Nelson DW, Sonuners LE. Sewage sludge components extractable with nonaqueous solvents. J. Environ. Qual. 1983; 12 (l): 69-74.
[191] Petrasek AC, Kugelman IJ, Austem BM, Pressley TA, Winslow LA, Wise RH. Fate of toxic organic compounds in wastewater treatment plants. *J. Water Pollut.* Cont. Fed. 1983; 55 (10): 1286-1296.
[192] Hultmann B. Elimination of organic micropollutants. *Water Sci. Technol 1982;*14: 73.
[193] Van Rensberg JFT, Hassett A, Theron S, Wiechers SG. The fate of organic micropollutants through an integrated wastewater treatment/water reclamation system. *Water Technol. 1980;* 12: 537-552.
[194] Tabak, Quave, Mashi CI, Barth EF. Biodegradability studies with organic priority pollutant compounds", *J. Water Pollut*. Cont. Fed., 1981; 5 (3): 1503.
[195] Horning WB, Robinson EL, Petrasek AC. Reduction in toxicity of organic priority pollutants by pilot-scale conventional wastewater treatment process. *Arch. Environ. Contam. Toxicol.1984;* 13 (2): 191-196
[196] Department for Environment, Food and Rural Affairs (Defra), Waste Statistics Regulation Return to Eurostat http://www.defra.gov.uk/statistics/environment/waste/wrfg22-wrmswqtr 2012
[197] Wang L, Govind R, Dobbs RA. Sorption of toxic organic compounds on wastewater solids: mechanism and modelling. *Environ. Sci. Technol.,* 1993; 27: 1.
[198] Naylor LM, Loehr RC. Priority pollutants in municipal sewage sludge. Part I. *Biocycle. 1982;* 23: 18-22.
[199] Naylor LM, Loehr RC. Priority pollutants in municipal sewage sludge. Part II. *Biocycle*. 1982; 23 (6): 37-42.
[200] Lester JN. Presence of organic micropollutants in sewage sludges in Processing and Use of Sewage Sludge, Third International Symposium. Brighton, pp.27-30 September 1983.
[201] Taylor D. Managing organ& in sludge reuse programs. *Biocycle. 1984;* 25 (6): 20-22.
[202] Davis RD, Howell K, Oake RJ, Wilcox P. Significance of organic contaminants in sewage sludges used on agricultural land", in International Conference on Environmental Contamination, London, July, EP, Edinburgh, 1984.
[203] Hopke PK, Plewa MJ, Stapleton PL, Weaver DL. Comparison of the mutagenicity of sewage sludges. *Environ. Sci. Technol. 1984;* 18: 909-916.
[204] Babish JG, Johnson B, Brooks BO, Lisk DJ. Acute toxicity of organic extracts of municipal sewage sludge in mice", Bull. *Environ. Contam. Toxicol. 1982;* 29: 379-384.
[205] Clevenger TE, Hemphill DD, Roberts K, Mullins WA. Chemical composition and possible mutagenicity of municipal sludges. J. Water Pollut. Cont. Fed., 2007; 55 (12): 1470-1475.
[206] Lind PM, Öberg D, Larsson S, Kyle CE, Örberg J, Rhind SM. Pregnant ewes exposed to multiple endocrine disrupting pollutants through sewage sludge-fertilized pasture show an

anti-estrogenic effect in their trabecular bone. *Science of The Total Environment*. 2009; 408, (11): 2340-2346.
[207] Sahlstrom L, De Jones B, Aspan A. Salmonella isolated in sewage sludge trace back to human cases of Salmonellosis. Letters in applied microbiology. 2006; 43: 46-52.
[208] Gale P. Land application of treated sewage sludge: quantifying pathogen risks from consumption of crops. *J. Appl Microbiol*.. 2005; 98: 380-96
[209] Swanson RL, Bortman ML, O`Connor TP, Stanford HM. Science, policy and the management of sewage materials. The New York City experience. *Marine Pollution Bulletin*. 2004; 49: 679–87.
[210] Rhind SM**.** Anthropogenic pollutants – a threat to ecosystem sustainability? *Philosophical_Transactions of the Royal Society, B., 2009; 364: 3391-3401.*
[211] Evers JHL. Female subfertility. *Lancet*. 2002; 360: 151-159.
[212] Hallikainen A, Vartiainen T. Food control surveys of polychlorinated dibenzo-*p*-dioxins and dibenzofurans and intake estimates. *Food Addit Contam. 1997;* 14: 355-366.
[213] AmaralMendes JJ. The endocrine disrupters: a major medical challenge. *Food and Chemical Toxicology*. 2002; 40 (6): 781–788.
[214] Panzica GC, Viglietti-Panzica C, Mura E, Quinn Jr MJ, Lavoie E, Palanza P et al. Effects of xenoestrogens on the differentiation of behaviorally-relevant neural circuits. *Frontiers in Neuroendocrinology*. 2007; 28: 179–200.
[215] Ekelund R, Bergman A, Granmo A, Berggren M. Bioaccumulation of 4-nonylphenol in marine animals—a re-evaluation. *Environ Pollut*. 1990; 64: 107–20.
[216] Ahel M, McEvoy J, Giger W. Bioaccumulation of the lipophilic metabolites of nonionic surfactants in freshwater organisms. *Environ. Pollut*. 1993; 79: 243–248.
[217] Bigbsy RM, Caperell-Grant A, Madhukar BV. Xenobiotics released from fat during fasting produce estrogenic effects in ovariectomized mice. *Cancer Res. 1997;* 57: 865–869.
[218] Southworth GR, Keller JL. Mobilization of azaarenes from wastewater treatment plant biosludge. *Bulletin of environmental contamination and toxicology. 1984;* 32 (4): 445-452.
[219] Gheorghe A, van Nuijs A, Pecceu B, Bervoets L, Jorens PG, Blust R et al. Analysis of cocaine and its principal metabolites in waste and surface water using solid-phase extraction and liquid chromatography-ion trap tandem mass spectrometry. *Analytical and bioanalytical chemistry. 2008;* 391(4): 1309-1319.
[220] Shima N, Tsutsumi H, Kamata T, Nishikawa M, Katagi M. Miki A. Direct determination of glucuronide and sulfate of p-hydroxymethamphetamine in methamphetamine users' urine. *Journal of Chromatography B, 2006;* 830 (1): 64-70.
[221] Zhang JY, Foltz RL. Cocaine metabolism in man: identification of four previously unreported cocaine metabolites in human urine. *Journal of analytical toxicology. 1990;* 14 (4): 201-205.
[222] Zwiener C, Seeger S, Glauner T, Frimmel FH. Metabolites from the biodegradation of pharmaceutical residues of ibuprofen in biofilm reactors and batch experiments. *Analytical and bioanalytical chemistry* 372 (4): 569-575.
[223] Stumpf M, Ternes TA, Wilken RD, Rodrigues SV, Baumann W. Polar drug residues in sewage and natural waters in the state of Rio de Janeiro, Brazil. *The Science of the total environment. 1999;* 225, (1-2): 135-141.
[224] Langford KH, Lester JN. Fate and behaviour of endocrine disrupters in wastewaters treatment processes". In: Birkett, J.W., Lester, J.N. (Eds), Endocrine disrupters in wastewaters and sludge treatment processes. 2002; CRC Press, Florida, USA.
[225] Wylie FM, Torrance H, Seymour A, Buttress S, Oliver JS. Drugs in oral fluid: Part II. Investigation of drugs in drivers. *Forensic Science International. 2005a;* 150 (2-3): 199-204.
[226] Wylie FM, Torrance H, Anderson RA, Oliver JS. Drugs in oral fluid Part I. Validation of an analytical procedure for licit and illicit drugs in oral fluid. *Forensic science international. 2005b;* 150 (2-3): 191-198.
[227] Jones AW, Holmgren A, Kugelberg FC. Concentrations of cocaine and its major metabolite benzoylecgonine in blood samples from apprehended drivers in Sweden. *Forensic science international. 2008;* 177 (2-3): 133-139.
[228] Dams R, Murphy CM, Lambert WE, Huestis MA. Urine drug testing for opioids, cocaine, and metabolites by direct injection liquid chromatography/tandem mass spectrometry. *Rapid communications in mass spectrometry : RCM. 2003;* 17 (14): 1665-1670.
[229] Johansen SS, Jensen JL. Liquid chromatography–tandem mass spectrometry determination of LSD, ISO-LSD, and the main metabolite 2-oxo-3-hydroxy-LSD in forensic samples and application in a forensic case. *Journal of Chromatography B. 2005;* 825 (1): 21-28.
[230] Horn CK, Klette K.L, Stout PR. LC-MS analysis of 2-oxo-3-hydroxy LSD from urine using a Speedisk positive-pressure processor with Cerex PolyChrom CLIN II columns. *Journal of analytical toxicology. 2003;* 27 (7): 459-463.
[231] Stout PR, Horn CK., Klette KL. Rapid simultaneous determination of amphetamine, methamphetamine, 3,4-methylenedioxyamphetamine,3,4-methylenedioxymethamphetamine, and 3,4-methylenedioxyethylamphetamine in urine by solid-phase extraction and GC-MS: a method optimized for high-volume laboratories. *Journal of analytical toxicology. 2002;* 26 (5): 253-261.
[232] Dams R, Benijts T, Lambert WE, De Leenheer AP. Simultaneous determination of in total 17 opium alkaloids and opioids in blood and urine by fast liquid chromatography–diode-array detection–fluorescence detection, after solid-phase extraction. *Journal of Chromatography B: Analytical Technologies in the Biomedical and Life Sciences. 2002;* 773 (1): 53-61.
[233] Shima N, Tsutsumi H, Kamata T, Nishikawa M, Katagi M, Miki A. Direct determination of glucuronide and sulfate of p-hydroxymethamphetamine in methamphetamine users' urine", *Journal of Chromatography B. 2006;* 830 (1): 64-70.
[234] Weinmann W, Vogt S, Goerke R, Müller C, Bromberger A. Simultaneous determination of THC-COOH and THC-COOH-glucuronide in urine samples by LC/MS/MS. *Forensic science international. 2000;* 113 (1-3): 381-387.
[235] Maralikova B, Weinmann W. Confirmatory analysis for drugs of abuse in plasma and urine by high-performance liquid chromatography–tandem mass spectrometry with respect to criteria for compound identification. *Journal of Chromatography B. 2004;* 811 (1): 21-30.
[236]http://www.drugabuse.gov/researchreports/cocaine/cocaine 2.html. **2012**
[237] Needham SR, Jeanville PM, Brown PR, Estape ES. Performance of a pentafluorophenylpropyl stationary phase for the electrospray ionization high-performance liquid chromatography–mass spectrometry–mass spectrometry assay of cocaine and its metabolite ecgonine methyl ester in human urine. *Journal of Chromatography B: Biomedical Sciences and Applications. 2000;* 748 (1): 77-87.
[238] Jeanville PM, Estape ES, Needham SR, Cole, M.J. Rapid confirmation/quantitation of cocaine and benzoylecgonine in urine utilizing high performance liquid chromatography and

tandem mass spectrometry. *Journal of the American Society for Mass Spectrometry. 2000;* 11 (3): 257-263.
[239] Cone EJ, Tsadik A, Oyler J, Darwin WD. Cocaine metabolism and urinary excretion after different routes of administration. *Therapeutic drug monitoring. 1998;* 20 (5): 556-560.
[240] Canezin J, Cailleux A, Turcant A, Le Bouil A, Harry P, Allain, P. Determination of LSD and its metabolites in human biological fluids by high-performance liquid chromatography with electrospray tandem mass spectrometry. *Journal of Chromatography B: Biomedical Sciences and Applications. 2001;* 765 (1): 15-27.
[241] Dams R, Huestis MA, Lambert WE, Murphy CM. Matrix effect in bio-analysis of illicit drugs with LC-MS/MS: influence of ionization type, sample preparation, and biofluid. *Journal of the American Society for Mass* SpectrometrY. 2003; 14 (11): 1290-1294.
[242] Ternes TA, Joss A, Siegrist H. Scrutinizing pharmaceuticals and personal care products in wastewater treatment. *Environmental science & technology. 2004,* 38 (20): 392A-399A
[243] Akre C, Fedeniuk R, MacNeil JD. Validation of a simple, sensitive method for the determination of beta-estradiol in bovine urine using gas-chromatography negative-ion chemical ionization mass spectrometry. *The Analyst. 2004;* 129 (2): 145-149.
[244] Bones J, Thomas KV, Paull, B. Using environmental analytical data to estimate levels of community consumption of illicit drugs and abused pharmaceuticals. *Journal of environmental monitoring : JEM. 2007;* 9 (7): 701-707.
[245] van Nuijs ALN, Pecceu B, Theunis L, Dubois N, Charlier C, Jorens PG. Spatial and temporal variations in the occurrence of cocaine and benzoylecgonine in waste- and surface water from Belgium and removal during wastewater treatment. *Water research. 2009;* 43 (5): 1341-1349.
[246] Postigo C, de Alda MJ, Barcelo, D. Fully automated determination in the low nanogram per liter level of different classes of drugs of abuse in sewage water by on-line solid-phase extraction-liquid chromatography-electrospray-tandem mass spectrometry. *Analytical Chemistry. 2008;* 80 (9): 3123-3134.
[247] Hearn WL, Flynn DD, Hime GW, Rose S, Cofino JC, Mantero-Atienza E. Cocaethylene: a unique cocaine metabolite displays high affinity for the dopamine transporter. *Journal of neurochemistry. 1991;* 56 (2): 698-701.
[248] Castiglioni S, Zuccato E, Crisci E, Chiabrando C, Fanelli R, Bagnati, R. Identification and measurement of illicit drugs and their metabolites in urban wastewater by liquid chromatography-tandem mass spectrometry. *Analytical Chemistry. 2006;* 78 (24): 8421-8429
[249] Kasprzyk-Hordern B, Dinsdale RM, Guwy AJ. The occurrence of pharmaceuticals, personal care products, endocrine disruptors and illicit drugs in surface water in South Wales, UK. *Water research. 2008;* 42 (13): 3498-3518.
[250] Berg T, Lundanes E, Christophersen AS, Strand DH. Determination of opiates and cocaine in urine by high pH mobile phase reversed phase UPLC–MS/MS. *Journal of Chromatography B. 2009;* 877 (4): 421-432.
[251] Marais AAS, Laurens JB. Rapid GC–MS confirmation of amphetamines in urine by extractive acylation. *Forensic science international. 2009;* 183 (1-3): 78-86.
[252] Hunter M, Stephenson T, Kirk PWW, Perry R, Lester JN. Effects of salinity gradients and heterotrophic microbial activity on biodegradation of nitrolotriacetic acid in laboratory simulations of the estuarine environment. *Appl. Environ, Microbiol*. 1986; 51: 919-925.
[253] Langford KH, Scrimshaw MD, Birkett JW, Lester JN. Degradation of nonylphenolic surfactants in activated sludge batch tests. *Water Research*. 2005; 39: 870-876.
[254] Quintana JB, Weiss S, Reemtsma T. Pathways and metabolites of microbial degradation of selected acidic pharmaceutical and their occurrence in municipal wastewater treated by a membrane bioreactor. *Water research. 2005;* 39 (12): 2654-2664.
[255] Kimura K., Hara H, Watanabe Y. Elimination of selected acidic pharmaceuticals from municipal wastewater by an activated sludge system and membrane bioreactors. *Environ. Sci .Technol*. 2007; 41: 3708-3714
[256] Heberer T, Feldmann D. Contribution of effluents from hospitals and private households to the total loads of diclofenac and carbamazepine in municipal sewage effluents—modeling versus measurements. *Journal of hazardous materials. 2005;* 122 (3): 211-218.
[257] Postigo C, Lopez de Alda MJ, Barceló, D. Analysis of drugs of abuse and their human metabolites in water by LC-MS2: A non-intrusive tool for drug abuse estimation at the community level. *TrAC Trends in Analytical Chemistry. 2008;* 27 (11): 1053-1069.
[258] Bijlsma L, Sancho JV, Pitarch E, Ibanez M, Hernandez F. Simultaneous ultra-high-pressure liquid chromatography-tandem mass spectrometry determination of amphetamine and amphetamine-like stimulants, cocaine and its metabolites, and a cannabis metabolite in surface water and urban wastewater. *Journal of chromatography.A. 2009;* 1216 (15): 3078-3089.
[259] Kasprzyk-Hordern B, Dinsdale RM, Guwy AJ. Multi-residue method for the determination of basic/neutral pharmaceuticals and illicit drugs in surface water by solid-phase extraction and ultra-performance liquid chromatography–positive electrospray ionisation tandem mass spectrometry. *Journal of Chromatography A. 2007;* 1161 (1-2): 132-145.
[260] Wood M, Laloup M, Fernandez MDMR, Jenkins KM, Young MS, Ramaekers JG. Quantitative analysis of multiple illicit drugs in preserved oral fluid by solid-phase extraction and liquid chromatography–tandem mass spectrometry. *Forensic science international. 2005;* 150 (2-3): 227-238.
[261] Huerta-Fontela M, Galceran MT, Martin-Alonso J, Ventura F. Occurrence of psychoactive stimulatory drugs in wastewaters in north-eastern Spain. *Science of The Total Environment. 2008a;* 397 (1-3): 31-40.
[262] Phillips DL, Tebbett IR, Bertholf RL. Comparison of HPLC and GC-MS for measurement cocaine and metabolites in human urine. *Journal of analytical toxicology. 1996;* 20 (5): 305-308.
[263] Drummer OH, Gerostamoulos D, Chu M, Swann P, Boorman M, Cairns I. Drugs in oral fluid in randomly selected drivers. *Forensic science international.* 2007; 170 (2-3): 105-110.
[264] Concheiro M, de Castro A, Quintela O, López-Rivadulla M, Cruz, A. Determination of MDMA, MDA, MDEA and MBDB in ora)l fluid using high performance liquid chromatography with native fluorescence detection. *Forensic science international. 2005;* 150 (2-3): 221-226.
[265] Pujadas M, Pichini S, Civit E, Santamariña E, Perez K, de la Torre R**.** A simple and reliable procedure for the determination of psychoactive drugs in oral fluid by gas chromatography–mass spectrometry. *Journal of pharmaceutical and biomedical analysis. 2007;* 44 (2): 594-601.
[266] Dignac MA, Ginestet P, Rybacki D, Bruchet A, Urbain V, Scribe, P. Fate of wastewater organic pollution during activated sludge treatment: Nature of residual orgnic matter. *Water Research*. 2000; 34 (17): 4185-4194.

[267] Waumans D, Steven P, Braneel N, Tytgat J. Lab. of Toxicology, Campu,Gasthuisberg,O&N2,Leuven Belgium (jan.tytgat@pharm.kuleuven.be.
[268] Van Bocxlaer JF, Clauwaert KM, Lambert WE, Deforce DL, Van den Eeckhout EG, De Leenheer AP. Liquid chromatography-mass spectrometry in forensic toxicology. *Mass spectrometry reviews. 2000;* 19 (4): 165-214.
[269] Postigo C, de Alda ML, Barceló D. Evaluation of drugs of abuse use and trends in a prison through wastewater analysis. *Environment international. 2011;* 37 (1): 49-55.
[270] Zuccato E, Chiabrando C, Castiglioni S, Bagnati R, Fanelli R. Estimating Community Drug Abuse by Wastewater Analysis. *Environmental health perspectives. 2008a;* 116 (8): 1027-1032.
[271] van Nuijs ALN, Pecceu B, Theunis L, Dubois N, Charlier C, Jorens PG. Spatial and temporal variations in the occurrence of cocaine and benzoylecgonine in waste- and surface water from Belgium and removal during wastewater treatment. *Water research. 2009b;* 43 (5): 1341-1349.
[272] Huerta-Fontela M, Galceran MT, Martin-Alonso J, Ventura, F. Occurrence of psychoactive stimulatory drugs in wastewaters in north-eastern Spain. *Science of The Total Environment. 2008;* 397 (1-3): 31-40.
[273] Dickson S, Park A, Nolan S, Kenworthy S, Nicholson C, Midgley J et al. The recovery of illicit drugs from oral fluid sampling devices. *Forensic science international.* 2007; 165 (1): 78-84.
[274] Beyer J, Drummer OH, Maurer, HH. Analysis of toxic alkaloids in body samples. *Forensic science international. 2009;* 185 (1-3): 1-9.
[275] United Nations Office on Drugs and Crime (UNODC), **2011.** Commission in Narcotic Drugs, Draft Resolution http://www.unodc.org/unodc/document/data-and-analysis/WDR2011/global_and regional_ overview
[276] Wilson PC, Boman BJ. Characterization of selected organo-nitrogen herbicides in South Florida canals: Exposure and risk assessment. *Sci. of Total Environment*. 2011; 412-413: 119-26
[277] Ai Jia, Yi Wan, Yang Xiao, Jianying Hu. Occurrence and fate of quinolone and fluoroquinolone antibiotics in a municipal sewage treatment plant. *Water Research. 2002;* 46: 387 -394
[278] Baker DR, Kasprzyk-Hordern B. Critical evaluation of methodology commonly used in sample collection, storage and preparation for the analysis of pharmaceuticals and illicit drugs in surface water and wastewater by solid phase extraction and liquid chromatography–mass spectrometry. *Journal of Chromatography A. 2011;* 1218 (44): 8036-8059.
[279] Baker DR, Kasprzyk-Hordern B. Multi-residue analysis of drugs of abuse in wastewater and surface water by solid-phase extraction and liquid chromatography–positive electrospray ionisation tandem mass spectrometry. *Journal of Chromatography A. 2011;* 1218 (12): 1620-1631.
[280] Harman C, Reid M, Thomas KV. In situ calibration of a passive sampling device for selected illicit drugs and their metabolites in wastewater, and subsequent year-long assessment of community drug usage. *Environmental science & technology. 2011;* 45 (13): 5676-5682.
[281] Kasprzyk-Hordern B, Baker DR. Enantiomeric profiling of chiral drugs in wastewater and receiving waters. *Environmental science & technology. 2012;* 46 (3): 1681-1691.
[282] Kasprzyk-Hordern B, Baker DR. Estimation of community-wide drugs use via stereoselective profiling of sewage. *Science of The Total Environment. 2012;* 423 (0): 142-150.
[283] Lai FY, Ort C, Gartner C, Carter S, Prichard J, Kirkbride P. Refining the estimation of illicit drug consumptions from wastewater analysis: Co-analysis of prescription pharmaceuticals and uncertainty assessment. *Water research. 2011,* 45 (15): 4437-4448.
[284] Ra JS, Lee SH, Lee J, Kim HY, Lim BJ, Kim SH. Occurrence of estrogenic chemicals in South Korean surface waters and municipal wastewaters. *Journal of environmental monitoring : JEM. 2011;* 13 (1): 101-109
[285] van Nuijs ALN, Mougel J, Tarcomnicu I, Bervoets L, Blust R, Jorens PG. Sewage epidemiology — A real-time approach to estimate the consumption of illicit drugs in Brussels, Belgium. *Environment international. 2011;* 37 (3): 612-621.
[286] Brausch JM, Connors KA, Brooks BW, Rand GM. Human pharmaceuticals in the aquatic environment: a review of recent toxicological studies and considerations for toxicity testing. *Reviews of environmental contamination and toxicology. 2012;* 218: 1-99.
[287] Ra JS, Lee SH, Lee J, Kim HY, Lim BJ, Kim SH. Occurrence of estrogenic chemicals in South Korean surface waters and municipal wastewaters. *Journal of environmental monitoring JEM. 2011;* 13 (1): 101-109.
[288] Rujiralai T, Bull ID, Llewellyn N, Evershed RP. In situ polar organic chemical integrative sampling (POCIS) of steroidal estrogens in sewage treatment works discharge and river water. *Journal of environmental monitoring : JEM. 2011;* 13 (5): 1427-1434.
[289] Schilling K., Zessner M. Foam in the aquatic environment. *Water research. 2011;* 45 (15): 4355-4366.
[290] Ulrich EM, Morrison CN, Goldsmith MR, Foreman WT. Chiral pesticides: identification, description, and environmental implications. *Reviews of environmental contamination and toxicology. 2012;* 217: 1-74.
[291] Yu Y, Huang Q, Wang Z, Zhang K., Tang C, Cui J. Occurrence and behavior of pharmaceuticals, steroid hormones, and endocrine-disrupting personal care products in wastewater and the recipient river water of the Pearl River Delta, South China. *Journal of environmental monitoring : JEM. 2011;* 13 (4): 871-878.
[292] Ziylan A, Ince NH. The occurrence and fate of anti-inflammatory and analgesic pharmaceuticals in sewage and fresh water: treatability by conventional and non-conventional processes. *Journal of hazardous materials. 2011;* 187 (1-3): 24-36.
[293] Martı´nez MJ, Bueno S, Ucle´s MD, Hernando E, Da´voli AR, Ferna´ndez-Alba. Evaluation of selected ubiquitous contaminants in the aquatic environment and their transformation products. A pilot study of their removal from a sewage treatment plant. *Water research. 2011;* 45: 2 3 3 1 - 2 3 4 1