# Fate and Behaviour of Drugs in the Environment

A Thesis Submitted in Partial Fulfilment of the Requirement of Nottingham Trent University for the Degree of Doctor of Philosophy.

By

**Aliru Olajide Mustapha**


School of Science and Technology
Clifton Lane, Nottingham
NG11 8NS

December, 2012

# Declaration

"I hereby declare that the dissertation submitted for the Degree of Doctor of Philosophy in Analytical/Environmental Chemistry at Nottingham Trent University, is my own original work and has not previously been submitted to any Institution or University or quoted as indicated and acknowledged by means of a comprehensive list of references."

## Copyright statement:



**A.O.Mustapha**

# Contents

**List of Tables:**

**Tables of Figures:**

## Abbreviations

| | |
|---|---|
| 6-MAM | 6-monoacetylmorphine |
| AE | anhydroecgonine |
| AEME | anhydroecgonine methylester |
| AM | amphetamines |
| APEO | alkylphenol polyethoxylate |
| BOD | biochemical oxygen demand |
| BSTFA | *N, O*, bis (trimethylsilyl) trifluoroacetamide |
| BZE | benzoylecgonine |
| CE | cocaethylene |
| COC | cocaine |
| COD | chemical oxygen demand |
| DTR | drug target residue |
| EDCs | endocrine disrupting substances |
| EME | ecgoninemethylester |
| GCMS | gas chromatography-mass spectrometry |
| HER | heroin |
| HRT | hydraulic retention time |
| HS | United Nations Office on Drugs and Crime |
| Kd | water-solid partition |
| $K_{ow}$ | octanol/water partition coefficient |
| LC-MS | liquid chromatography- mass spectrometry |
| LOD | limits of detection |
| LOQ | limits of quantification |
| LSD | lysergic acid diethylamide |
| M3G | morphine-3-β-glucuronide |
| M6G | morphine-6-β-glucuronide |
| MA | methamphetamine |
| MDE | methylenedioxyethylamphetamine |
| MDMA | methylenedioxymethamphetamine |
| MOR | morphine |
| MS | mass spectrometry |
| MSAF | mixed submerged aerated filter |
| MSD | mass selector detector |
| NC | norcocaine |
| NorD | nordazepam |
| NPEO | nonylphenol polyethoxylate |
| OC | organic carbon |
| PAHs | polycyclic aromatic hydrocarbons |
| PCBs | polychlorinated biphenyls |
| PS | primary sludge |
| RAF | royal air force |
| SAF-1 | submerged aerated filter |
| SPE | solid phase extraction |
| STW | sewage treatment work |
| THC | tetrahydrocannabinol |
| THC-COOH | tetrahydrocannabinol-9-carboxylic acid |
| TIC | total ion corresponding area |
| TSS | total suspended solid |
| TMCS | trimethylchlorosilane |
| UDP | uridinediphospho glucuronic acid |
| UNODC | united nations office on drugs and crime |

## Glossary of Technical Terms

| | |
|---|---|
| **Chromatography** | physical method of separation in which the components are distributed between the stationary and mobile phases that moves in a definite direction. |
| **Chromatogram** | a graphical presentation of detector response depicting concentration of analyte in the sample on a paper or layer with separated zones as in planar chromatography. |
| **Effluent** | aqueous sample in mobile phase leaving the column or treatment plant |
| **Influent** | aqueous sample in mobile phase entering the column or treatment plant |
| **Sample** | mixture consisting of a number of components/analytes. |
| **Sample components** | the chemically pure constituents of the sample |
| **Gas chromatography** | a separation technique in which the mobile phase is a gas |
| **Adsorption** | separation based mainly on differences between the adsorption affinities between the sample components and active solid. |
| **Sewage treatment work** | convectional site where wastes are treated and the most significant routes through which the drugs inadvertently enter the environment via sewage treatment systems |
| **Aquatic environment** | domestic, industrial influents and effluents which include rivers, surface waters, underground waters and ecosystems. |
| **Pollutants** | chemical substances that have potential toxicological consequences on the environment beyond a particular threshold. |
| **Residual analyte** | bio-transformed compound arising from degradation of residues that generates toxic by-products |
| **Metabolite** | the active ingredients of medicinal products with a wide range of chemical structures that are excreted from humans and animals after metabolism of dosed user producing more polar degradation products of which many complex modes of biochemical pathways are poorly understood. |
| **Matrix** | sample media where compounds can be extracted for chemical analysis. |
| **Pharmaceuticals** | drugs procured legally for medicinal or therapeutic use with or without prescription |
| **Illicit drugs** | drugs procured illegally for illicit use without prescription |
| **Sewage sludge** | complex association of wastes of industrial chemicals and human excreta containing mixtures of residues with several valuable properties which are agriculturally relevant. |
| **Drug stability** | conditions of temperature and pH that minimize degradation of drug |
| **Degradation pathways** | a typical interplay of complex physical, biochemical and transformational routes of pollutants in sewage treatment works |
| **Solid Phase Extraction** | multi-step extraction procedures of different protocols for improved recovery, and ability of delivering clean extracts using several adsorbents. |
| **Batch test** | experiment to monitor bio-degradation of compounds |
| **Partition coefficient** | distribution ratio of a compound between two partially miscible solvents in intimate contact (Kd). |
| **octanol/water partition coefficient** | the ($K_{ow}$) is a chemical concentration ratio in the octanol/aqueous phases. |

## Acknowledgement:

Firstly, I would like to express my sincere gratitude and appreciation to Almighty **ALLAH** for this work. Aliham-dulilah Robil-alamin!

My very deep appreciation goes to my Supervisors: **Dr Mike Coffey** (Nottingham Trent University) **Dr Jason W. Birkett** (Liverpool John Moore University) and **Dr Chris Lloydmills** (Nottingham Trent University) for their supervision through the various twists and turns that this work has taken. Their interests in my work and most especially their supervisions were beyond my wildest imagination. I wish them joy without limit in life.

Analytical Chemistry Department of NTU for the research facilities and The Federal Polytechnic Offa, Nigeria for the study permission in United Kingdom. Analytical Chemistry Laboratory Team: Mary, Dave and Nigel Mould**.** All your supports over the years at NTU are much appreciated and will be remembered fondly.

Many thanks to Dr. Jo Guy and Mr Jonathan Kendall, Works Managers of RAF Molesworth, Cambridge and Stoke Bardolph, Nottingham STWs for wastewater samples.

No one who achieves success does so without acknowledging the help of others. Special thanks to the following special people for very special supports: Alh. Ishola Motunrayo Abdulsalam (Gbuu); Mrs Ogunjobi; Alfa Hakeem Olateju; Imam Shuaib (Chief Imam of Pampo); Mr Taiye Mohammed-Bello Mustapha; Dr & Mrs Salaudeen (Newcastle); Mr & Mrs Sola Adewumi (Equatorial Energy); Mrs Ayo Adebola (NHS); Mr Lanre Alanamu (Abuja); Hon. Nimota Akanbi; Oba Mufutau Gbadamosi (Oloffa of Offa); Mr & Mrs Bashir Popoola (BapConsult); Engr Kamaldeen Mustapha; Alh. Yakub Bolaji Razak; Mr Larongbe Afolabi (Prof); Mr Ganiyu Waheed (SLT); Alfa Tapa; Mr Kayode Rewire; Mr Yinka Oladimeji, Naomi Okletey (Ghana), Mr Tajudeen Bello (Iyeru Okin Bank); Mr Folorunsho (CTCS, Offa), Mr Salman (Sincere Co-op) , Mrs Adeyemi (Wintech) & Alh. Laide Yusuf (Abuja), Mr Leo Adeyemi, Mr & Mrs Olagunju, friends/well-wishers, Olojokus & Oyedepos.

The darker the night, the brighter the stars, the deeper the grief, the closer is God. I pray all of you would be fulfilled in life beyond comprehensions. Whom God bless no man can curse.

Fellow PhD research colleagues: Salem (Libya), Dhu-khali (Libya), Anushuya (India), Biola (Nigeria), Khdija (Libya), Nasreedin (Libya), Reham (Saudi Arabia), David (England), Oli (England), David (Spain), Nora Balahmar (Saudi Arabia) & Fatma Mohammed (Libya). I appreciate you all.

Dedicated to late Mother (**Aminat Ajoke Hannafi Olojoku**, 13 April 1980) - the primal chemist of the universe with the incredible capacity that transformed me into life, late Father (**Hannafi Ayinde Olojoku**, 10 March 2000) enjoined me to always trust God and late Sister (**Silifat Amope Ahmed Omodele**, 14 April 2004) – whom I lost a confidant. ***Adieu!*** May their souls rest in Jannatul Fir'daus.

Finally to my family **- Halimat, Aminat, Aishat and Mohammed** – *I love you just the way you are*.

## Abstract

Sewage treatment works (STWs) are routes through which treated wastewater effluents often containing myriads of chemicals are passed into receiving waters due to incomplete removal processes as have been identified in several studies. The current work aimed to determine the levels of these chemicals in the effluents from Stoke Bardolph STW, Nottingham and to determine the fate and behaviour of compounds by conducting degradation batch studies under different treatment conditions. The selection of representative illicit compounds; cocaine and its metabolite benzoylecgonine, heroin and its metabolites 6-monoacetylmorphine and morphine and a pharmaceutical (diazepam) was based on their presence in the STW effluent.

The results obtained using solid phase extraction gas-chromatography technique (SPE-GCMS) showed thirteen compounds detected at concentrations between 1.9 and 3147 ng $L^{-1}$ in effluents from Stoke Bardolph STW. Procaine, bromacil, codeine, lidocaine, ibruprofen, caffeine, nicotine and diazepam were the most abundant compounds in the final effluent with concentrations of 99.2, 1806.8, 33.5, 71.8, 3147, 213.4, 252.5 and 105.2 ng $L^{-1}$, respectively. The percentage recoveries ranged from 74.5 – 109.6%, with the instrumental limits of detection (LODs) ranges of 0.2 – 12.7 ng $L^{-1}$, and relative standard deviation (RSD) values of 0.6 – 4.7% were achieved for all the compounds.

The batch tests enabled determination of the degradation of the compounds at different temperatures and times, using various sludge types after characterization. Removal rates of cocaine (91.0%), benzoylecgonine (90.6%), heroin (97.9%), morphine (99.7%), 6 monoacetylmorphine (93.3%) and diazepam (99.7%) were measured after 3 hours equilibration; partition coefficients ($K_d$) for these six substances ranged from 1.2 – 68.1 Kg $L^{-1}$. The degradation of compounds at 19 ± 0.5$^{o}$ C was relatively greater but it still occurred slowly at 4 ± 0.5$^{o}$C, at between 5 and 10%.

Mass balances for two STW (Molesworth, Cambridgeshire, U.K. and Stoke Bardolph) were constructed using the removal rate data from these batch studies. Final effluent concentrations of 110.0 ng $L^{-1}$ (cocaine), 690.0 ng $L^{-1}$ (benzoylecgonine), 10.0 ng $L^{-1}$ (morphine), 80.0 ng $L^{-1}$ (6-monoacetylmorphine), and 0.7 ng $L^{-1}$ (diazepam) were found in effluents after a total of 8 hour hydraulic times (8 HRT) from an initial influent concentration of 50 mg $L^{-1}$. Projected influent concentrations of cocaine (14, 471 ng $L^{-1}$) and benzoylecgonine (23, 907.1 ng $L^{-1}$) at Stoke Bardolph were derived from back-calculating measured final effluent concentrations using this same mass balance approach.

Work encompassed in this study directly measures illicit drug removal rates in laboratory studies for the first time. The application of removal rates in calculating mass balances in sewage works is an improvement over prior studies where assumptions on removal rates at STW were made.

# CHAPTER 1: Introduction & Literature review

Environmental occurrence of organic pollutants through interconnectedness of human actions and activities impacts the environment in many ways. These 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 individual use of these pharmacologically active substances generates 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.

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) is the preferred method 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. Use of sewage and wastewater in batch studies to investigate partitioning/degradation of selected drugs in such media are investigated because of the current insufficient information on their biodegradability and persistence after their disposal to lands or receiving waters. As a result, decisions and policy thrusts regarding the future practices of safe sewage-sludge disposal as well as complete removal of these contaminants from STWs effluent-waters becomes difficult.

Batch tests using sewage sludge grab samples obtained from two sewage treatment works have been conducted to determine the effects of the compounds physico-chemical properties and biological sludge characteristics on biodegradation. Degradation of selected illicit drugs such as cocaine and its active metabolite benzoylecgonine, heroin with 6-monoacetylmorphine and morphine and diazepam with nordiazepam (NorD) as metabolites respectively is examined together with the concomitant production of some other metabolites and other compounds. Degradation has been determined to involve both biotic and abiotic processes with the mixed liquor solids concentration involving both intracellular and extracellular enzyme activities which influenced compounds degradation. However, increased degradation of the drugs led to the accumulation of the related metabolites which were in turn degraded, but some showed possibilities of conjugation of residues that may result in their escape from complete removal from the sewage treatment processes to the receiving waters in a complex-interplay of interactions. The capability is also outlined of furthering our understanding of fate and

behaviour of drugs with particular reference to illicit drugs, abused pharmaceutical and environmental processes in our quest to understand the overall issues of drugs and make available exposure data for the aquatic realm.

Mass balance calculations to assess amount of drug degradation in the STWs is addressed as limited calculations have been carried out in the literature. To improve upon erroneous calculation of drug consumption based on analytes found in surface water, assessment of the existing reports in the literature can be compared to our typical design studies of drug degradation at four sampling points through the process at RAF Molesworth sewage treatment works (STW). The Molesworth base is manned 24hrs a day; the flow arrives to the works at a reasonably consistent rate (78 $m^3$/d). Possible applications of mass balance with respect to mass transfer in each sewage sample (processing unit) and to evaluate degradation-sorption variables in the overall removal efficiency of compounds are presented as more work is required in this area.

### 1.1 Sources 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.

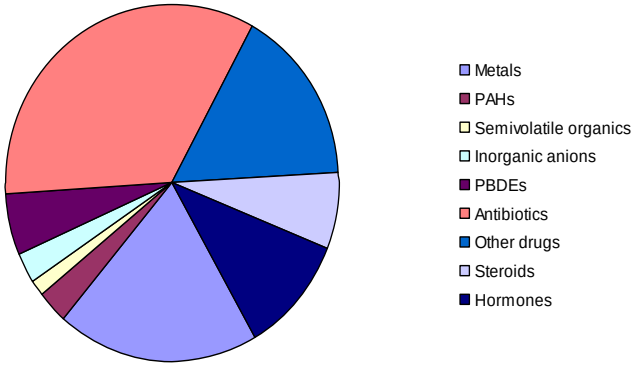



**Figure 1:** Chemical components in sewage sludge. Data from The U. S. Environmental Protection Agency. (Renewable Energy Venture, Austin, Texas) [4]

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 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 weak oestrogens on breast cancer cells have all 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.

**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 | Detergents | Linear alkylbenzene Sulphonates (LASs), | Sewage | 33-38 |
| Chlorobenzenes | Paint removers | chlorobenzenes | Sewage | 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 |

## 1.2 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. As shown in Fig. 2, the literature shows that many parent drugs escape biodegradation and possible metabolic conjugates of excreted metabolites are often revert back to their original parent form after microorganisms' cleavage and these may lead to increase in concentration of parent pharmaceutical in the sewage [89-93].

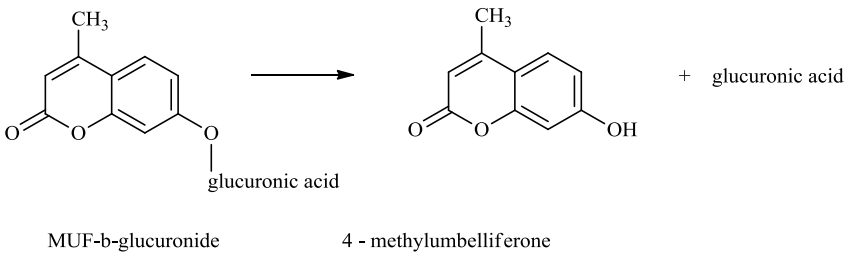



**Figure 2:** Microbial cleavage of excreted metabolic conjugates into unchanged parent drug [89]

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, $\beta_2$ –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].

In the following, a survey of different therapeutical classes of pharmaceuticals with chemical structures are discussed and their manifestation in the surface, drinking and underground waters.

### 1.2.1 Acidic pharmaceuticals and phenolic antiseptics

The antiseptic biphenylol, antiphlogistic ibuprofen, salicylic acid and bezafibrate (lipid regulator) are examples of acidic compounds because of their carboxylic and one or two phenolic hydroxyl group moieties. Different methods exist for the enrichment and derivatization of acidic drugs, but simultaneous determination by diazomethane methylation or trifluoroacetylation has been performed using different batches of solid phase extraction (SPE) with over 80% recoveries. Figure 3 show the chemical structures of four selected acidic drugs and antiseptics:

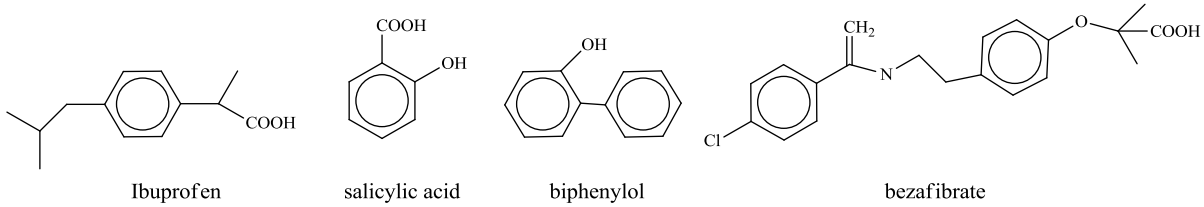



**Figure 3:** Chemical structures of four selected acidic drugs and antiseptics [89]

### 1.2.2 Betablocker and $ß_2$-sympathomimetics

Selected betablockers (sotalol, antenolol, metoprolol) and $\beta_2$-sympathomimetics (salbutamol) illustrate a secondary aminoethanol and several hydroxyl groups in the structures of the both medicinal classes. The functional groups make the compounds very polar; hence a gas chromatography quantitative analysis requires derivatization by silylation and trifluoroacetylation of hydroxyl and secondary amino groups respectively. Mean recoveries of over 70% has been recorded after extraction and derivatization. Figure 4 show the chemical structures of four selected betablocker and β2-sympathomimetics.

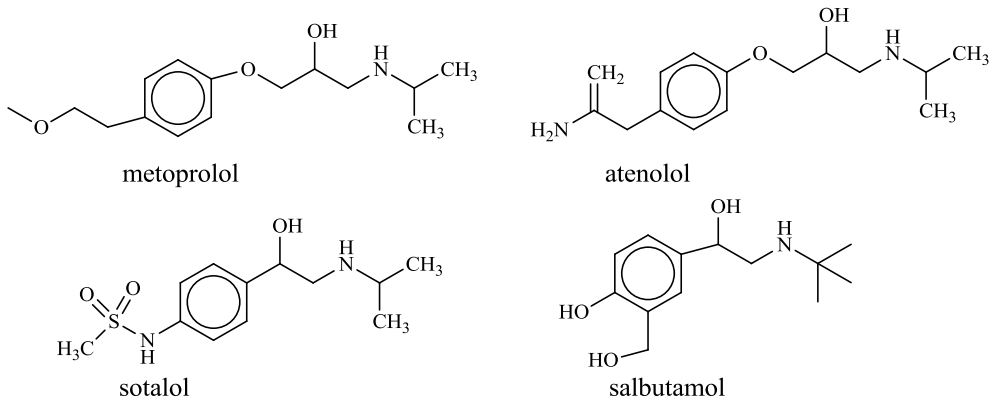



**Figure 4:** Structures of four selected betablocker and β2-sympathomimetics [89]

### 1.2.3 Neutral pharmaceuticals

Compounds with no acidic functional groups belong to 'neutral pharmaceuticals'. Antiphlogistics, vasodilators, lipid regulators, antiepileptic agents and psychiatric are different medicinal classes of drugs that are neutral or weakly basic and therefore require no derivatization when analysed by gas chromatography. With recoveries of over 70% in GCMS, they are also enriched in the reversed phased sorbent at neutral pH in HPLC conditions similar to betablockers and $\beta_2$-sympathomimetics. Figure 5 show the chemical structures of four selected neutral pharmaceuticals.

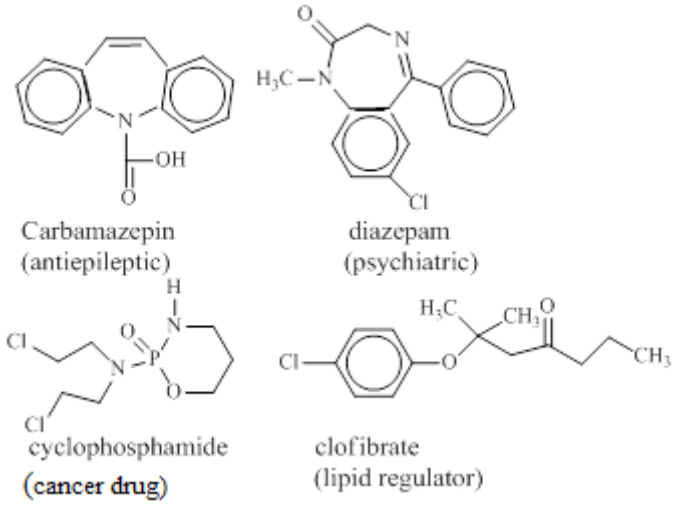



**Figure 5:** Structures of four selected neutral pharmaceuticals [89]

### 1.2.4 Antibiotics

In the literature, about 18 antibiotics belonging to different groups of penicillin, tetracyclines, sulphonamides and macrolide have been determined in waters with recoveries exceeding 80% with standard deviation between 5 – 26% using SPE ( 500 mg RP-$C_{18}$) [89, 113]. Similar SPE methods

using LC-MS with electrospray ionization and surrogate standards in water matrices has been described [114]. Figure 6 show the chemical structures of four selected antibiotics.

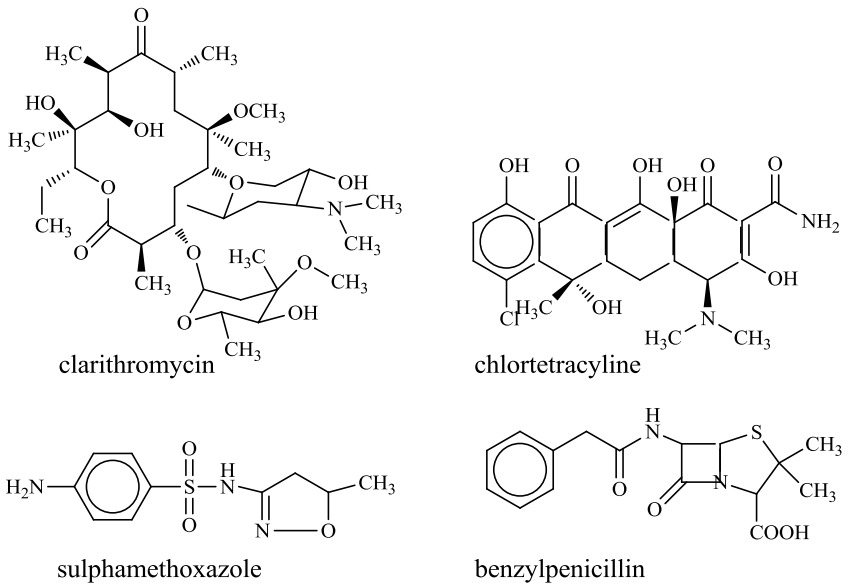



**Figure 6:** Structures of four selected antibiotics. [89]

### 1.2.5 Iodinated X-ray contrast media.

These medicinal compounds display high polarity and usually persistent to environmental degradation and against metabolism by organisms making the concentration of contrast media in ground water and surface water found at the lower range of 7- 10 ng $L^{-1}$ in the literature [115]. Figure 7 show the chemical structures of three selected Iodinated X-ray contrast media

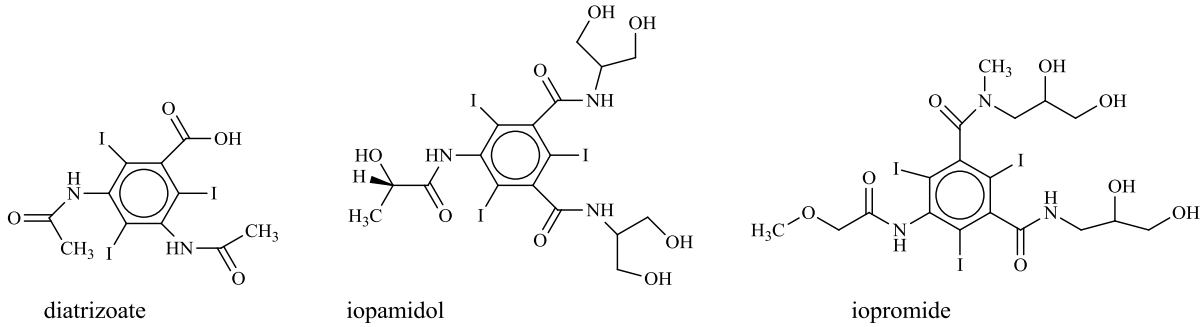



**Figure 7:** Structures of three selected Iodinated X-ray contrast media [89]

### 1.2.6 Estrogens

Natural (17β –estradiol) and synthetic estrogens (17α-ethinylestadiol) contains phenolic and aliphatic hydroxyl groups and due to their properties they can be analysed simultaneously. The excreted quantities compared to other pharmaceuticals are often low because of their high lipophilicity (log $P_{ow}$

3.5-4.6). But a literature source has given a method that determined estrogens in sewage samples and river waters to 0.5 ng $L^{-1}$ [80]. Figure 8 show the chemical structures of three selected estrogens

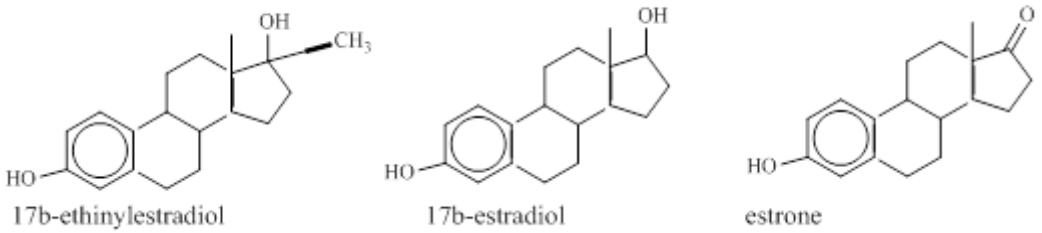



**Figure 8:** Structures of three selected estrogens [89]

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 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, 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].

**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 |
| **$\beta_2$-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 |

## 1.3 Illicit drugs 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].

Until few years ago, nearly nothing was known about the environmental impact of the illicit drugs, whether the illicit drugs similarly exist and survive in the environment like other medicinal drugs [126-127]. 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 [128-129]. 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 [130]. 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. 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 $L^{-1}$. The relative concentration of benzoylecgonine for example is about 10 times higher than the parent drug.

**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 | [131] |
| | 5 STPs, Belgium | 22 -678 | - | 1.2 - 26 | [132] |
| | 37 STPs, Belgium | 32 – 753 | - | - | [131] |
| | 3 Rivers, Italy | - | - | 0.3 – 44 | [133] |
| | 5 STPs, Ireland, UK | 489 ± 117 | 25 - 248 ± 20 | 0 – 33 ± 11 | [134] |
| | Eastern Spain | 370 – 1000.24 | 30 – 560 | - | [135] |
| | 30 STPs, Belgium | 09 – 683 | - | - | [136] |
| | 2 STPs, Italy | 218.4 – 421.4 | 0.9 – 10.7 ± 3.2 | - | [137] |
| | 4 STPs; River Po. | 42 – 120 | - | - | [124] |
| | 42 STPs, NE Spain | 04 – 4700 | 01 – 100 | - | [138] |
| | Barcelona, Spain | 2.40 | - | - | [139] |
| **Benzoylecgonine** | 5 STPs, Spain | 2307.0 | - | 111 | [131] |
| | 5 STPs, Belgium | 82 – 1 898 | 928 | 44 - 191 | [132] |
| | 37 STPs, Belgium | 46 –2258 | - | - | [133] |
| | 3 Rivers, Italy | 2.2 – 183 | - | - | [133] |
| | 5 STPs, Ireland UK | 290 ± 11 | 22 | - | [134] |
| | Eastern Spain | 150 – 1000.5 | 22 ± 4 – 31 ± 18 | - | [135] |
| | 30 STPs, Belgium | 37 – 1550 | 6.0 – 7.9 | - | [136] |
| | 2 STPs, Italy | 547.4 -197.2 | - | - | [137] |
| | 4 STPs; River Po. | 420 - 750 | 0.92 – 100.3 ± 28.6 | - | [124] |
| | 42 STPs, NE Spain | 09 – 7500 | - | - | [138] |
| | Barcelona, Spain | 5.24 | 01 – 1500 | - | [139] |
| | 12 STPs, Germany | 65± 5 | 77± 9 | 71 | [140] |

**Table 4 (contd)** Survey of illicit drugs and pharmaceuticals concentration in wastewaters

| Analytes | Matrix | Influent ($ng\ L^{-1}$) | Effluent ($ng\ L^{-1}$) | SW ($ng\ L^{-1}$) | Ref |
|---|---|---|---|---|---|
| **Nor- BE** | 3 Rivers, Italy | - | - | 0.2 – 8.4 | [133] |
| | Eastern Spain | 150 - 430 | 30 – 170 | - | [135] |
| | 2 STPs, Italy | 18.8± 5.6 – 36.6 ± 7.8 | <LOQ – 7.5 ± 2.9 | - | [137] |
| **Cocaethylene** | 2 STPs, Italy | 5.9 ± 2.6 – 11.5 ± 5.1 | 0.2 ± 0.5 | - | [137] |
| | Barcelona, Spain | 77.5– 78.5±33.2 | 1.71– 4.2± 1.2 | 4.63 | [139] |
| | 3 Rivers, Italy | - | - | 0.07 – 0.2 | [133] |
| **Nor-cocaine** | 3 Rivers, Italy | - | - | 0.15 – 3.6 | [133] |
| | Eastern Spain | 0.15 – 0.43 | 0.03 - 0.17 | - | [135] |
| | 2 STPs, Italy | 4.3 ± 0.9 – 13.7 ± 5.3 | 0.7 ± 0.5 | - | [137] |
| **Amphetamines** | 3 Rivers, Italy | - | - | <0.65 | [133] |
| | Eastern Spain | 1400 | 110 – 210 | - | [135] |
| | 2 STPs, Italy | 5.4 – 14.7± 10.6 | 2.8 | - | [137] |
| | 42 STPs, NE Spain | 03 - 6880 | 04 - 2100 | - | [138] |
| | Barcelona, Spain | 20.8 – 41.1± 9.1 | 0.45– 2.2 ± 0.1 | 2.84 | [139] |
| | 5 STPs, Spain | 15 | <1.0 | <0.8 | [131] |
| **Metamphetamines** | 5 STPs, Nebraska USA | 1.3 ± 0.1 – 1.4 | 35.0± 7.3 | - | [141] |
| | 3 Rivers, Italy | 0.1 – 62.6 ± 13 | - | <0.41 – 1.7 | [133] |
| | Eastern Spain | - | <100 - 540 | - | [135] |
| | 2 STPs, Italy | <500 | <1.11 – 3.5 ± 2 | - | [137] |
| | 42 STPs, NE Spain | 3 - 277 | 3 - 90 | - | [138] |
| | 3 STPs, USA | 15 ± 2 – 66 ± 14 | 0.8 – 1.3 | - | [141] |
| | Barcelona, Spain | 4.8 – 18.2 ± 5.8 | 2.1 – 6.3 ± 0.6 | 2.87 | [139] |
| | Murray, USA | 6.0 - 34 | 03 - 7 | - | [142] |
| **MDA** | 42 STPs, NE Spain | 03 - 266 | 01 - 200 | - | [138] |
| | 3 Rivers, Italy | - | - | 3 ± 0.3 – 4 | [133] |
| | Eastern Spain | 500 - 1400 | 41.0 – 68.0 | - | [135] |
| | 2 STPs, Italy | 4.6 ± 7.3 – 8.7 | 0.9 ± 1.9 – 1.1± 1.5 | - | [137] |
| | 5 STPs, Spain | 03 - 266 | 1 - 200 | - | [131] |

**Table 4 (contd)** Survey of illicit drugs and pharmaceuticals concentration in wastewaters

| Analytes | Matrix | Influent ($ng\ L^{-1}$) | Effluent ($ng\ L^{-1}$) | SW ($ng\ L^{-1}$) | Ref |
|---|---|---|---|---|---|
| **MDMA** | 3 Rivers, Italy | - | - | 1.1 – 4.0 | [133] |
| | Eastern Spain | 326 – 2700.5 | 100 – 210.2 | - | [135] |
| | 2 STPs, Italy | 13.6 – 14.2 | 4.4 ± 3.7 – 5.1± 3 | - | [137] |
| | 5 STPs, Spain | 91 | 67 | 3.5 | [131] |
| | STP, Italy | 2 - 598 | 2 - 267 | - | [138] |
| | Barcelona, Spain | 133– 135.13 ± 29.8 | 8.2– 14.8 ± 2.2 | 129 | [139] |
| | Murray, USA | <1.0 – 10.0 | - | - | [142] |
| | 42 STPs, NE Spain | 2 - 598 | 2 - 267 | - | [138] |
| **MDEA** | 5 STPs, Spain | 27 | <2.1 | - | [131] |
| | 2 STPs, Italy | 4.19 – 1.5 ± 3.8 | <1.64 | - | [137] |
| | STP, Italy | 6 - 114 | 12 | - | [135] |
| | STP, Spain | <500 | <100 | - | [138] |
| | 42 STPs, NE Spain | 06 - 114 | 12 | - | [138] |
| **Opiates** | | | | | |
| **Heroin** | Barcelona Spain | 2.4 | 1.2 | - | [139] |
| | STP, Italy | 20.0 | <20.0 | <1.5 | [142] |
| **Morphine** | 5 STPs, NE Spain | 25.9 – 96.7 | 20.9 – 81.1 | - | [142] |
| | 3 Rivers, Italy | - | - | 3.5 - 38 | [133] |
| | 5 STPs, Ireland | 874 ± 86 | 452 | - | [134] |
| | 2 STPs, Italy | 83.3– 204.4 | 5.5 ± 11.1 | 1-2L | [137] |
| | Barcelona, Spain | 68.1 – 162.9 ± 20.0 | 21.8 ± 3.0 | 3.25 | [139] |
| | 12 STPs, Germany | 123 ± 6 | 9.0 ± 1.2 | 83 | [140] |
| | STP, Italy | 7.1 – 96.7 | 0.1 – 8.1. | 4.8 – 6.3 | [142] |
| **Nor-morphine** | 5 STPs, NE Spain | 30.7 | - | - | [142] |
| | 1 STP, Italy | <25 | <2.5 – 3.7 | <12..5 | [142] |
| **6 ACM** | 3 Rivers, Italy | - | - | 0.93 | [133] |
| | 2 STPs, Italy | 10.4± 4.8 – 11.8 ± 8.5 | - | - | [134] |
| | Barcelona, Spain | 8.4 – 12.8 ±3.1 | 2.5 – 3.6 ± 0.5 | - | [139] |

**Table 4.**

| 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 | [140] |
| | STP, Italy | 102 ± 14 | <3.1 | <0.9 – 3.4 | [143] |
| | | | | | [142] |
| **M3G** | 2 STPs, Italy | 2.5 ± 7.1 – 18.1 ± 30 | <0.48 | - | [137] |
| **Methadone** | 5 STPs, Spain | 4.0 – 239 | 4.0 – 24.7 | - | [142] |
| | 3 Rivers, Italy | - | - | 4.9 – 10.1 | [133] |
| | 2 STPs, Italy | 11.6± 1.7 – 49.7 ± 9.6 | 9.1 ± 0.5 – 36.2 ± 2.8 | - | [137] |
| | 12 STPs, Germany | 123 ± 6 | 9.0 ± 12 | 83 | [140] |
| | STP, Italy | 4 – 23.9 | 2 – 2.7 | 4.9 – 10.1 | [142] |
| **Codeine** | 5 STPs, NE Spain | 18.1 – 119.7 | 3.1 - 397 | - | [142] |
| | 3 Rivers, Italy | - | - | 1.0 - 51 | [133] |
| | 12 STPs, Germany | 80 ± 5 | 7.7 ± 8 | 90 | [143] |
| **Nor-codeine** | 5 STPs, NE Spain | 5 – 68.0 | 15.5 – 22.9 | - | [142] |
| **6 Acetyl codeine** | 3 Rivers, Italy | - | - | <0.31 | [133] |
| **EDDP** | 3 Rivers, Italy | - | - | 9.9 – 18.0 | [133] |
| | 5 STPs, Ireland UK | 9.0 – 206 ± 10 | - | - | [134] |
| | 2 ST1 STP, Italy | 19.8 ± 3.1 – 91.3 ± 19.2 | 22.6± 0.6 – 72.1± 8.7 | - | [137] |
| | STP, Italy | 4.5 – 41.3 | 4.9 – 56.7 | 9.61 – 17.5 | [142] |
| **THC** | 5 STPs, NE Spain | 11.3 – 31.5 | - | - | [142] |
| | 2 ST1 STP, Italy | 62.7 ± 5 – 91.2 ± 24.7 | <0.94 – 7.2 ± 3.7 | - | [137] |
| | Barcelona, Spain | 4.3– 21.03 ± 7.8 | 8.4 ± 3.8 – 11. 23 | 2.65 | [139] |
| | STP, Italy | 8.3 – 31.5 | <8.3 | <7.0 – 13.6 | [142] |
| **THC-COOH** | 3 Rivers, Italy | - | - | 0.48 -3.7 | [133] |
| | STP, Italy | 12.5 – 96.2 | 12.5 | 16.4 – 34.1 | [142] |
| | 5 STPs, NE Spain | 37.8 – 96.2 | 14.8 – 48.1 | - | [142] |
| **OH- THC** | Barcelona, Spain | 8.4 – 46.3 | 4.8 – 15.3 | 10.7 | [139] |

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. The need to critically review the present current development on the degradation processes (fate and behaviour) before and after sewage disposal from STWs to the environment and the current analytical methodologies that meet particular application should not be overlooked [145].

### 1.4 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.

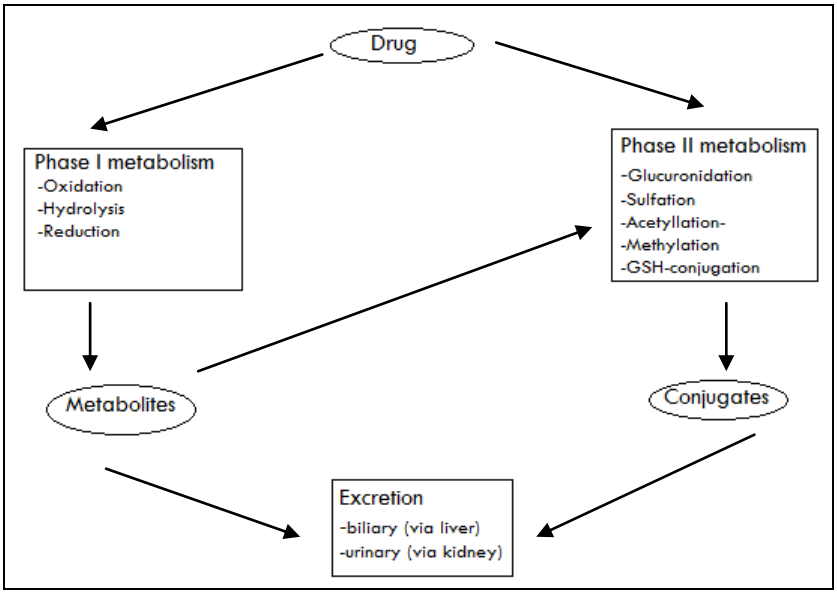



**Figure 9:** Simplified scheme of dug metabolism in the human body [92]

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.

### 1.4.1. 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 [94,146]. The street cocaine used by addicts is often mixed or cut with a number of diluants [147], 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 [148-151]. 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 [152, 153]. 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 [154, 155]. 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 [156]. When cocaine is smoked, anhydroecgonine methylester (AEME) is produced and through enzymatic hydrolysis get converted to anhydroecgonine (AE) or ecgonidine [157]. 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 [158]. 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 [159].

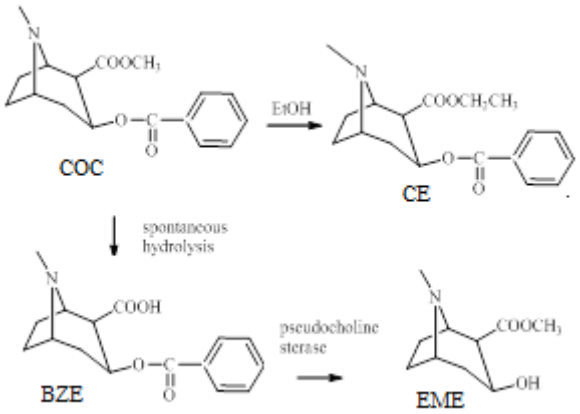



**Figure 10:** Degradation pathways of cocaine in the human body [145].

### 1.4.2. 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") [160].

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 [161, 162]

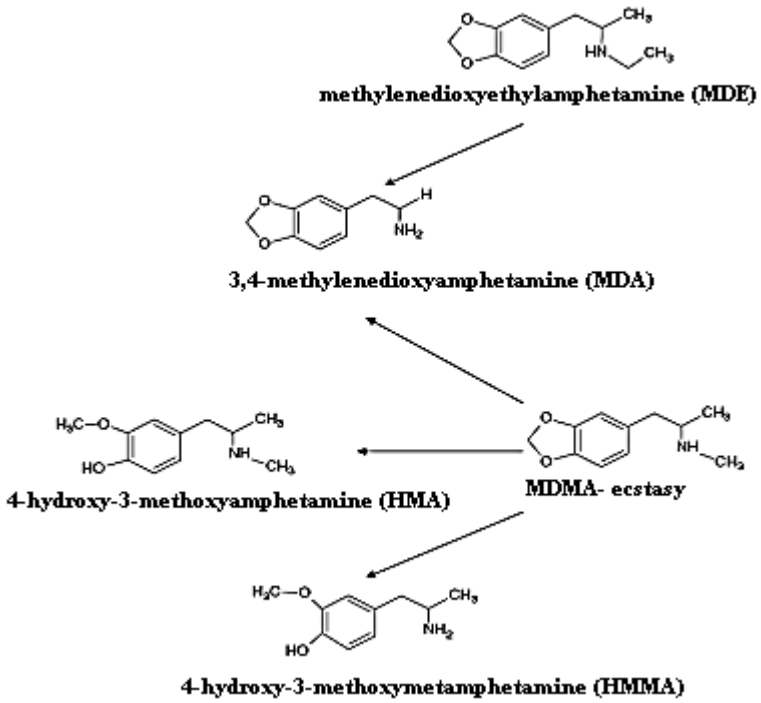



**Figure 11:** Main metabolites of 3, 4-methylenedioxymethamphetamine (MDMA) in urine [127].

### 1.4.3. 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 oly 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 [163]. 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 [164], 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 [165].

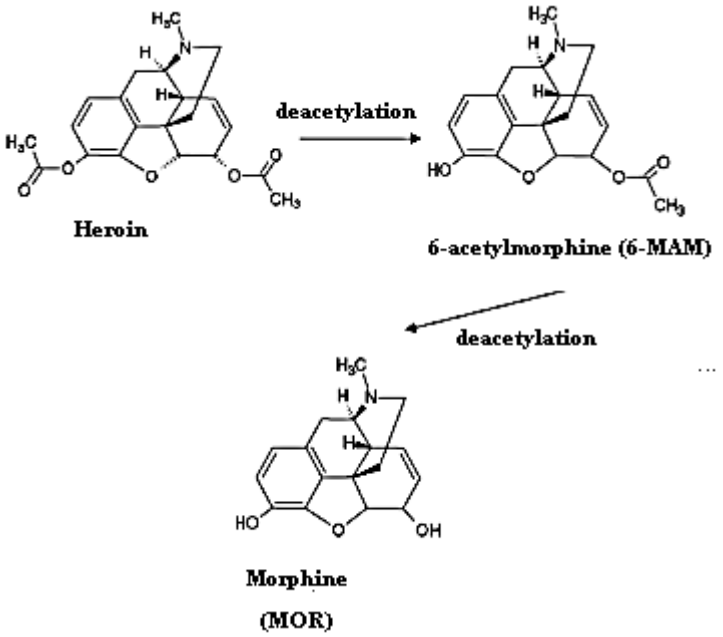



**Figure 12:** Degradation pathways of heroin and its main metabolites in living organisms. [168].

### 1.4.4. 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 [166]. 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 [167]. 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 [169]. In a review paper of Reuschel *et al* [217], 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 [170]. Additional metabolites have also been identified in the laboratory animals but are yet to be found in human fluids [171]. The LSD compounds were however not studied in the current work.

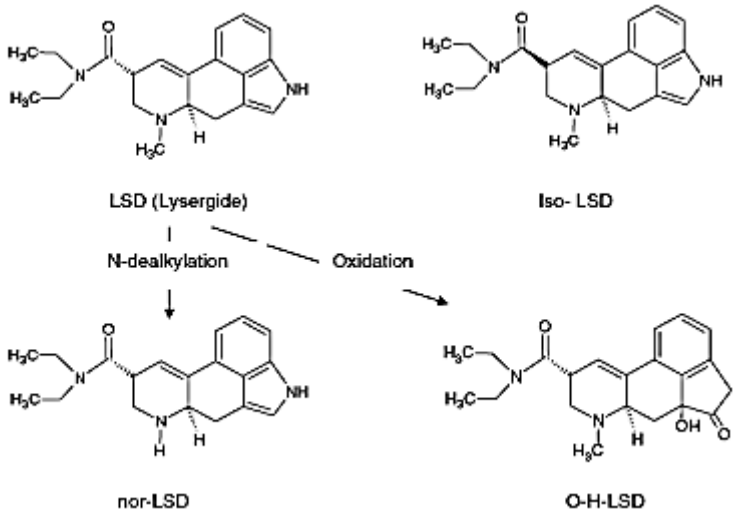



**Figure 13:** Lysergic Acid Diethylamide and metabolites in human fluids [168]

### 1.4.5. 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 [172]. 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 $\Delta^9$-THC is metabolized to 11-hydro-$\Delta^9$-THC, which is metabolized to 9-carboxy-THC [173], but the metabolism of THC is still not properly understood.

Figure 14 shows the structure of $\Delta^9$-tetrahydrocannabinol (THC) and its metabolites in human urine [127]. The main metabolite is $\Delta^9$-tetrahydrocannabinol-9-carboxylic acid (THC-COOH) and it is excreted as glucuronide-acid conjugate THC-COOH- glucuronide) [174], 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.

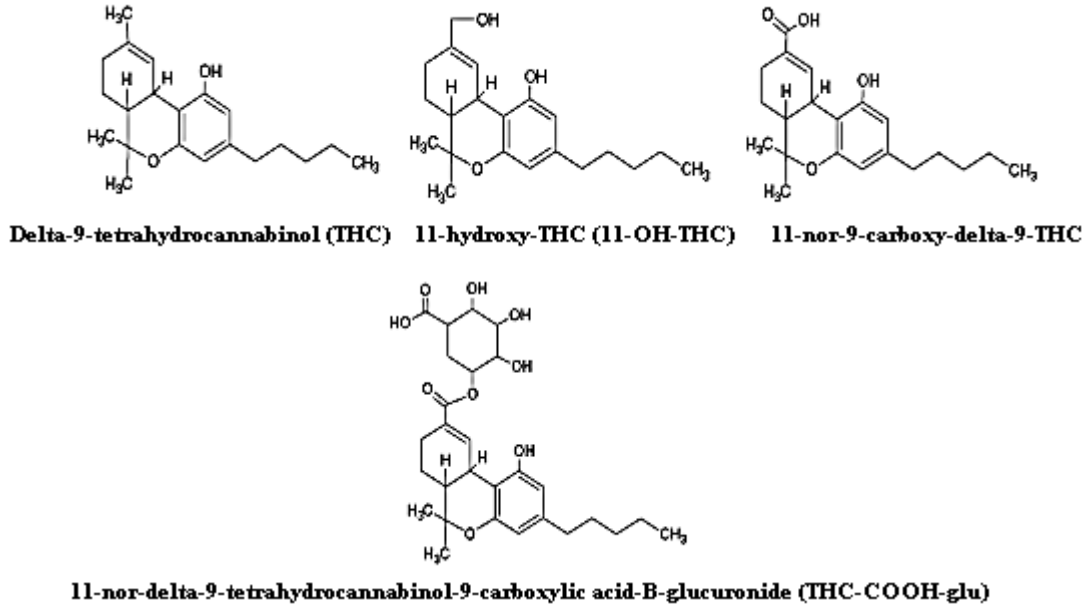



**Figure 14:** Major metabolites of Δ9-tetrahydrocannabinol (THC) in urine [168].

## 1.5. Sewage Water Treatment Operations

The operation of sewage water treatment includes the removal of contaminants from wastewater runoffs, domestic and industrial sewage before it can be safely returned to the environment. The removal of physical, chemical and biological contaminants involves the physical, chemical and biological process treatments with the main objective being to protect the public from diseases and produce environmentally safe water quality suitable for disposals. The three main sources of wastewater are *domestic wastewater* (human and household wastes, sinks, baths and showers), *industrial wastewater* (wastes from factories, industrial chemicals, airports, shopping centers and schools) and *storm runoff and ground water* (street drains and storm runoff via cracks into sewer and interceptor lines. Therefore to achieve the goal of cleaning used water, human intervention becomes necessary and the process goes through primary, secondary and tertiary treatment as shown in Figure 15.

- Primary treatment: The waste stream from homes and businesses (influent) are carried in sewers to the treatment works and enters through *bar screens* and this includes temporary holding of waste stream in a quiescent basin to remove the heavy solids and pollutants while the floating lighter matter, oils and grease may also be removed and the trash collected at the bar screen is disposed off. The waste stream is passed onto the *grit chambers*, where the wastewater slows down to allow solids such as sands, grits, plastics, broken glass and other particles to settle and be removed. The wastes stream flows from the grit chamber into a *primary clarifier* (sedimentation tank) which collects the smaller particules and dissolved solid matters (primary sludge), which includes scum, and floating grease, through special

devices or scrapers. About 40 – 50% of the solids are removed in the primary treatment process.

- Secondary treatment refines and removes 85 – 90% of the contaminants from the waste stream passed to it from the primary clarifier and flows into the *aeration tank* which supplies air to the wastewater to speed up the microorganisms and oxidizes the harmful organic matters. The aeration process reduces the organic matter and the waste stream flows into the *secondary clarifier* (secondary sedimentation) where the dispesed solids and flocs of bacteria (from activated sludge plant) or humus sludge (from trickling filter works) settles. The activated sludge is usually re-circulated back into the aeration basin to provide more bacterial population to aid degradation/decomposition of incoming matters.
- Tertiary treatment consists of chemical disinfection of waste stream carried out by disinfectants, ozone, UV, or chlorine to kill the disease-causing organisms before the treated waste stream (effluents) finally leaves the sewage treatment works to a local stream or river (receiving waters). Typically, the final effluent is sufficiently clean to be discharged to the receiving river while the sludge (biosolids) is continuously being removed, stabilized, dewatered and utilized for agricultural applications [349].

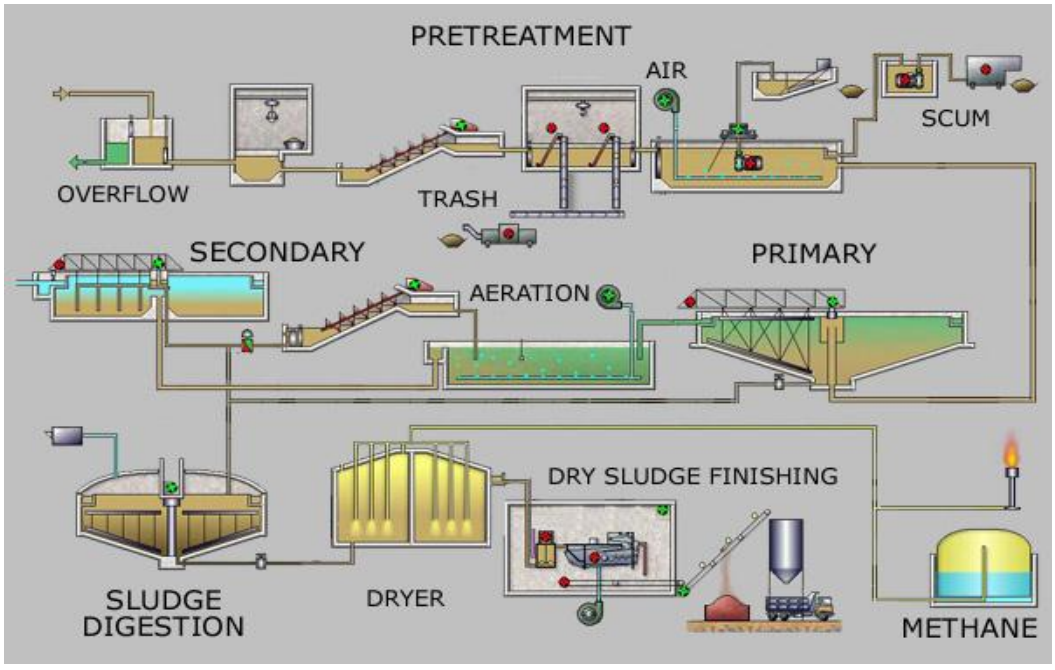



**Figure 15**: Process flow diagram for a typical large-scale treatment plant [350]

### 1.6. 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, 131, 137, 141, 243-249]. 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 [250-252] and membrane bioreactors (MBR) [253, 254] 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 [255]. 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 [255-261].

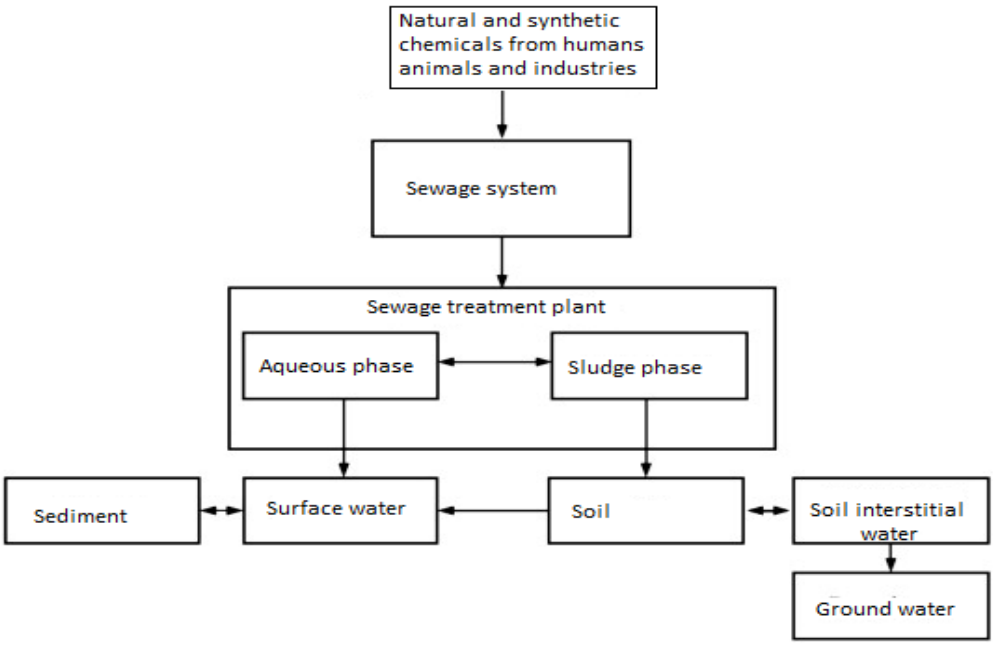



**Figure 16:** Organic contaminant fate and distribution in the environment [291]

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 [258-260]. In 2009, the understanding of the STWs systems and the degradation processes involved were observed by Kasprzyk-Horden *et al* [261] 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 [126]. 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 [262], 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.

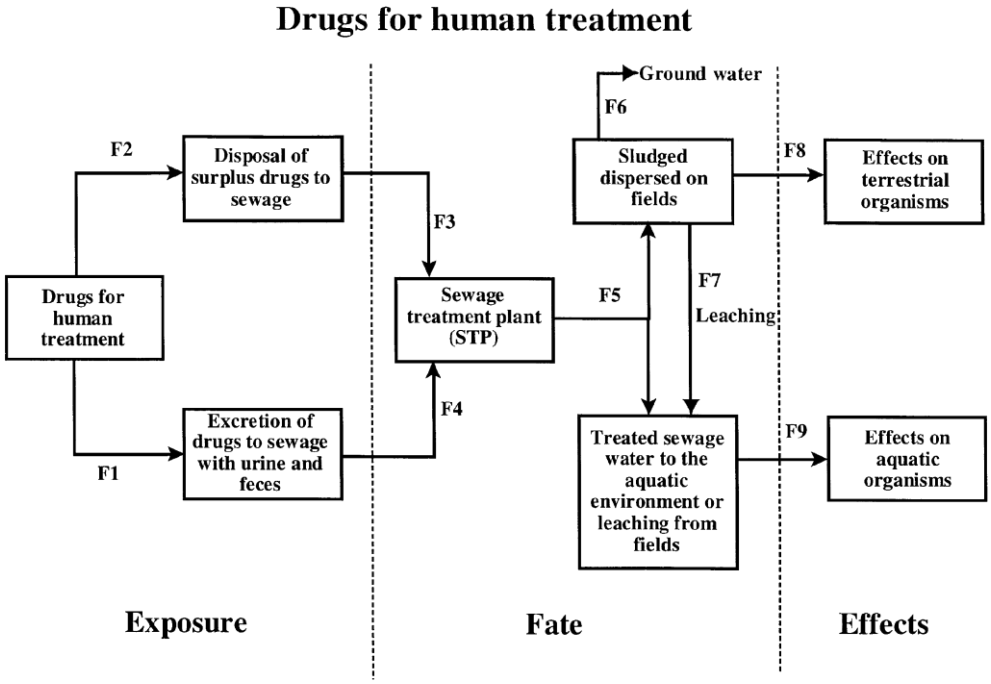



**Figure 17**. Anticipated exposure routes of drugs for human treatment in the environment [120]

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.

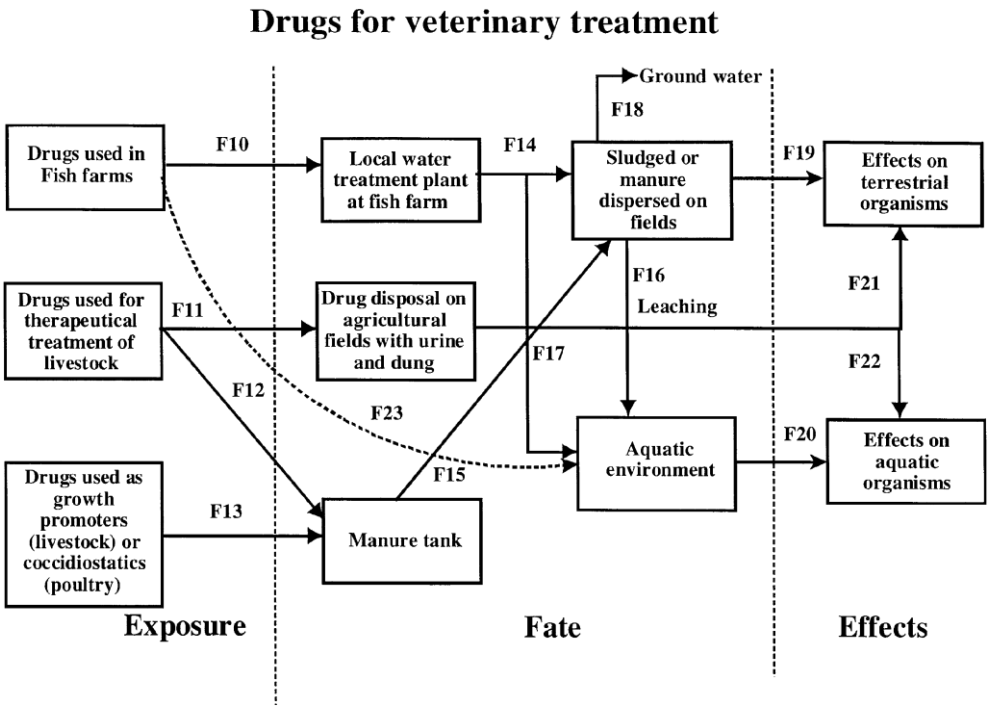



**Figure 18**. Anticipated exposure routes of drugs for veterinary treatment in the environment [120].

### 1.7 Microbial degradation in the aquatic environment.

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 [175-177]. 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* [178], 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.

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 [179-181]. Biodegradability studies of organic priority pollutants and reduction in toxicity of these pollutants in wastewaters treatment processes have also been carried out [182, 183]. 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, 182]. About 80-99% removal efficiency was recorded from the parallel and spiked treatments of between 50-150 µg $L^{-1}$ 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.

**Table 5:** Sewage sludge disposal in England, 2008-2011 [184]

| 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

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* [185] 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 [186-190]. 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 [191-193]. 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 [194]. The presence of intestinal pathogenic bacteria and animal parasites in sewage sludge has been confirmed from several investigations [195,196]. 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 [197]. The potential health risk imposed due to the presence of organic and inorganic compounds found in sewage sludge is of concern in humans [198,199] if they are delivered at high enough doses to cause effects through the consumption of products derived from animals grazing on contaminated pastures [200]. 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 [201-206].

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* [132] 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 [207]. Four relatively new metabolites of cocaine: ecgonidine, norecgonidine methylester, *p*- hydroxyl-benzoylecgonine, and *m*- hydroxyl-benzoylecgonine [208], and two conjugates of metamphetamines: *p*-hydroxy-metamphetamine (*p*-OHMA-sulfate), (*p*-OHMA-Sul) and (*p*-OHMA) (*p*-OHMA-glucuronide) [207] 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* [168] 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 [234,235]. 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 [236]. 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 [175-177].

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

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

| Compound | Human metabolites identified in biological fluids [214-233] | Human metabolites identified in the aquatic environment [116, 131, 134, 136, 139, 142, 208-212] |
|---|---|---|
| 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 | $\Delta^9$-tetrahydrocannabinol (THC) | Detected |
| | $\Delta^9$-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

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, 178, 237].

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 [238]. 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 [236]. 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 [239]. 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 [234]

**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 |

Elimination of selected acidic pharmaceuticals from municipal wastewater using activated sludge systems and membrane bioreactors [240], modelling versus measurement experiment of effluent from hospitals and private households to the total loads of diclofenac and carbamazepine in municipal sewage effluent [241] and identification of microbial degradation of trimethoprim in nitrifying activated sludge batch studies have been reported in the literature.

### 1.8. 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 [131]. 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 [132]. 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 [134, 137, 242]. Stability of other drugs of abuse like heroin, amphetamines-like substances and lysergic acid and their metabolites were not found in the literature.

### 1.9. 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].

### 1.9.1 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 [142,210]. 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 [132,131].

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 [271-274] especially urine [275-277], oral fluid [214, 215], and blood [216] 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 [277]. 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.

**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 (LOD/LOQ) | Ref. |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Volume (mL) | Extraction | Method Recovery (%) | C'graphic Column | Mobile Phase | Detector (Interface) | Acq. Mode | (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 | $2\text{-}4^{WW}$ $20^{SW}$ | [132] |
| 1Cocaine 1 Opoiod. 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 | - | [269] |
| 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 | [131] |
| 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 | [131] |
| 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 (LOD/LOQ) | Ref. |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Volume (mL) | Extraction | Method Recovery (%) | Chromatographic Column | Mobile Phase | Detector (Interface) | Acq. Mode | (ng $L^{-1}$) | |
| 3 Cocaine<br>1ALC<br>3 Opiods<br>1LSD | WW | 500 mL | SPE (Strata-XC, 200mg) | 50-65 | HPLC: Phenomenex Onyx $C_{18}$ (200 x 3.0mm | | | | 36-120 | [134] |
| 5 ALCs<br>5 Cocaine<br>5 Opiods<br>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 $pg/L^{wwinf}$<br>1 $ng/L^{wweff}$ | [209] |
| 2 Cocaine | SW<br>WW | 100 mL<br>500 mL | SPE (Oasis, HLB, 500 mg) | - | Zorbax Rx-SIL (2.1 x 150mm, 5µm) | - | ITMS | MRM ($ESI^+$) | 20 | [270] |
| 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 | [142] |
| 5 ALCs<br>2 Cocaine.<br>1LSD<br>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 | [138] |

**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 | Method (LOD/LOQ) | Ref. |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Volume (mL) | Extraction | Method Recovery (%) | Chromatographic Column | Mobile Phase | Detector (Interface) | Acq. Mode | (ng $L^{-1}$) | |
| 1 ALC<br>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 | [266] |
| 8 Opiods<br>2Cannabiniod | SW<br>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 | [135] |
| 8 Opiods<br>2 Cannabiniod | SW<br>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 | - | [142] |

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.

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.

### 1.9.2. 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 [124,137]. 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 [266, 269] 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* [214] and Miltona *et al* [275] 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].

**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 | [132] |
| 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 | [137,266, 124,269, 133] |
| 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% $NH_3$ | [214,215] |
| 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 | [135] |
| 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 | [134] |
| Strata-XC™ (200mg/6mL) | - | -same- | -same- | -same- | [134] |

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

| | | 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. | [132] |
| 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 | [132,270] |
| 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 | [132, 278] |
| 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 | [83,278] |
| 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 | [132,279] |
| 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. | [266] |

**Table 9: Solid Phase Extraction (SPE) protocols in wastewater pre-treatment (contd)**

| | 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 | [266] |
| Oasis, WAX (60 mg) | Weak anion-exchange mixed mode polymeric sorbent built upon HLB copolymer (application: strong acids). | -same- | -same- | -same- | [266] |
| Chromabond, $C_{18}$ (200 mg). | Silical-based, endcapped sor-bent (non-polar compounds). | -same- | -same- | -same- | [266] |
| Isolute, HCX (200 mg) | Weak anion-exchange mixed mode (non-polar and basic analyte). | -same- | -same- | -same- | [266] |

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) [137], Isolute ENV+® (500mg/6 mL) and Isolute PH® (1000mg/6 mL) adsorbents [136], and Bond Elut Certify® adsorbent [214] were compared with other adsorbents by Gheorge *et al* [132] 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.

## 1.10. PhD Research Objectives:

To date, there is paucity of information, to our knowledge in the literature on the fate and behaviour of illcit drugs in the aquatic environment. Also not very clear was the use of simple mass balance calculations to assess degradation pattern and the removal rate of the compounds from municipal STW as outlined in the following research objectives.

- To determine trace drugs analysis in environmental media and quantification using GCMS technique.
- To analyse actual influents wastewater, effluents and sludge from sewage treatment works (STWs) to assess licits and illicit drugs content.
- Use of sewage sludge in batch studies to investigate degradation of selected drug materials in such media.
- Use of mass balance calculations to assess drugs mass-flow, their removal rates and estimate influents from effluent concentrations using the batch studies data.

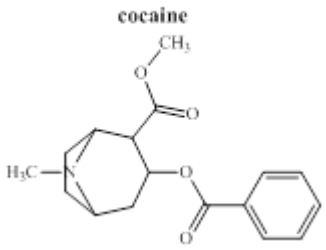
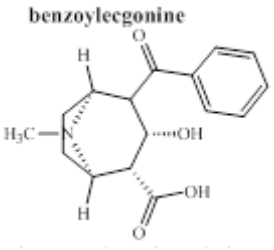
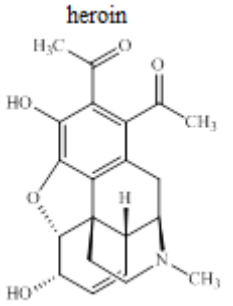
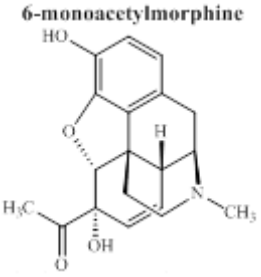
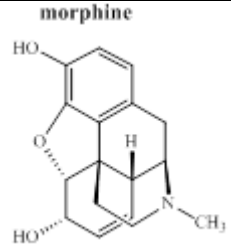
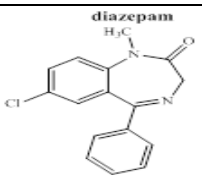
## 1.11. Criteria for selection of compounds used in sewage batch tests

The literature search showed that many classes of illicit drugs exist and survive in the environment like other pharmaceutical/medicinal drugs, but while many of these drugs break down rapidly, others show some degree of resistance to degradation in the environment [275, 281].

In this current study, simple and systemic random samplings of wastewaters on different days showed the presence of 13 different compounds: cocaine, benzoylecgonine, codeine, diazepam, morphine, ephedrine, lidocaine, diacetylmorphine, ibuprofen, procaine, amphetamine, ecgonine methylester and bromacil (herbicide) as detected at the river outflow of Stoke Bardolph STW, Nottingham. The concentrations of these drugs were analysed in wastewaters at ng $L^{-1}$ to µg $L^{-1}$ levels. Of these compounds; 6 compounds selected for experimental batch studies were: cocaine and its metabolite benzoylecgonine; heroin and its metabolites 6-monoacetylmorphine and morphine and diazepam representing two classes of illicit drugs and abused pharmaceutical based on their presence in Stoke Bardolph STW's effluent which allowed some presence of residues in detectable concentration (Table 10). Moreover, examination of the literature indicates either there is no work, or no UK research was found on the degradation studies of these drugs having been carried out. But continuous exposure of these chemicals to the aquatic environment may have only imperceptible consequences to aquatic environment [101].

The selection of these 6 compounds for experimental batch studies therefore aims to make available exposure data for the aquatic realm on the behaviour of selected compounds within the UK sewage works.

**Table 10:** Data of illicit compounds and a pharmaceutical used in batch studies [134].

| Class of drugs | Compounds | Log Kow | Structures |
|---|---|---|---|
| **Cocaine** | Cocaine | 2.3 |   |
| | Benzoylecgonine | 1.3 |   |
| **Opiates** | Heroin | 1.7 |   |
| | 6-monoacetylmorphine | 1.6 |   |
| | Morphine | 0.9 |   |
| **Benzodiazepine** | Diazepam | 2.9 |   |

# CHAPTER 2: Materials and Methods

## 2.1. Experimental preparation

A simple experiment to simulate conditions in actual STWs was designed to use raw sewage in batch studies to provide natural bacterial species and population that can allow continuous degradation of metabolites slowly and naturally in contrast to utilising synthetic activated sludges. Three hours duration for the degradation studies was chosen as this permitted an intensive subsampling and processing allowing data-intensive assessments for an anticipated fast (min $hr^{-1}$) degradation rate. 13 different compounds, including cocaine, benzoylecgonine, codeine, diazepam, morphine, ephedrine, lidocaine, diacetylmorphine, ibuprofen, procaine, amphetamine, ecgonine methylester and bromacil (herbicide) as detected in random wastewater samplings from Nottingham STW effluents. But only 6 compounds were used in spiking (batch) studies and these are cocaine and its metabolite benzoylecgonine; heroin and its metabolites 6-monoacetylmorphine and morphine and diazepam as shown below in Table 10.

## 2.2. Chemicals and materials

Standard compounds of cocaine, benzoylecgonine, heroin, 6-acetylmorphine, morphine, diazepam, ephedrine, lidocaine, codeine, ibuprofen, procaine, amphetamine, ecgonine methylester, cocaethylene, nordazepam, caffeine, nicotine and bromacil (herbicide) were purchased under license from both Sigma Aldrich (Gillingham Dorset, UK) and LGC standards (Teddington Middlesex, UK). Analar grade hydrochloric acid (HCl), ammonium hydroxide ($NH_4OH$) and methanol (MeOH) used for pH adjustment and sample preparations were obtained from Aldrich. A derivatizing agent, *N*, *O*, bis (trimethylsilyl) trifluoroacetamide (BSTFA with 1% trimethylchlorosilane, TMCS) was purchased from Cerrilliant (Round Rock, TX, USA). The choice of BSTFA as a silylating agent for derivatizaion is due to its faster reaction and volatility of its by-products. Pyridine was also purchased from Aldich and was used to provide appropriate derivatization reaction medium. Reagent water was from a Millipore milliQ water purification system (ELGA labwater, UK). Stock solutions of each chemical at 100 µg $L^{-1}$ were prepared in methanol and were stored at -20 $^{o}$C in the dark at pH = 2 with 37% HCl until analysis [132], while working solutions were prepared from appropriate dilutions. Oasis HLB® sorbent in a 47mm SPE disc format and disc holder were purchased from Waters (Elstree Herts, UK). A Phenomenex SPE Vacuum Manifold (Macclesfield Cheshire, UK) with 12 ports and a self-cleaning and drying vacuum was used for loading and elution of samples with appropriate solvent mixtures

## 2.3 Description of the STWs studied with sampling location

### 2.3.1. RAF Molesworth Sewage Treatment Work

Royal Air Force (RAF) base Molesworth is located in Molesworth, Suffolk, approximately 20 miles from Cambridge. There are no residents on the base. However, the base operates 24 hours per day

with approximately 1,200 personnel (over 2 shifts), with an overnight staffing around 400 personnel. The STW is located within the RAF base and utilises activated sludge for secondary biological treatment. The plant is consented to discharge a maximum of 360 $m^3day^{-1}$ (0.1Mgal $day^{-1}$). The average volume treated by the works is approximately 78.4 $m^3$ $day^{-1}$ (0.02 Mgal $day^{-1}$). RAF Molesworth has separate wastewater and surface water drainage networks and wastewater is pumped from across the base to a biological treatment works where it is treated prior to discharge to a tributary of 'Cock Brook'. The existing works which is shown schematically in the diagram below in Fig. 19 has four different stages: pre-treatment, primary treatment, secondary treatment and reed beds. The sewage plant process starts from terminal pumping station where sewage is pumped to a raised inlet works and screened. A storm overflow diverts excess flow to the storm tanks and settled material accumulates in the storm tanks which are always manually cleared. Wastewater gravitates to a primary tank which removes coarse materials and a recently installed the submerged aerated filter (SAF process) removes biochemical oxygen (BOD), ammonia and finely dispersed solids. This new process replaced the previous plastic media filter system. Humus tanks are used to remove any secondary settleable material. The recirculation pumping system has not been used as it was installed to ensure the required wetting rate of the old plastic media filter was maintained. The reed beds polish the final effluent to required quality standards before being finally discharged to the neighbouring rivers.

The supernatant constitutes the effluents that are passed forward. The excess secondary sludge, the solids from primary sedimentation and sedimentation of solid wastes and liquid stream in humus tanks are recycled back into the inlet of the plant. Co-settled sludge is pumped from the primary sludge well to a sludge storage tank. Decant liquors can be removed from the tank by an adjustable decant arm. The sampling points for the analysis are: (i) inlet to the grit removal unit (influent wastewater), (ii) inlet to primary sedimentation tank (primary sludge), (iii) inlet to submerged aerated filter reactor (SAF-1), (iv) mixed SAF, (v) secondary sedimentation unit (humus sludge) and (vi) outlet of sedimentation unit (effluent) [292].

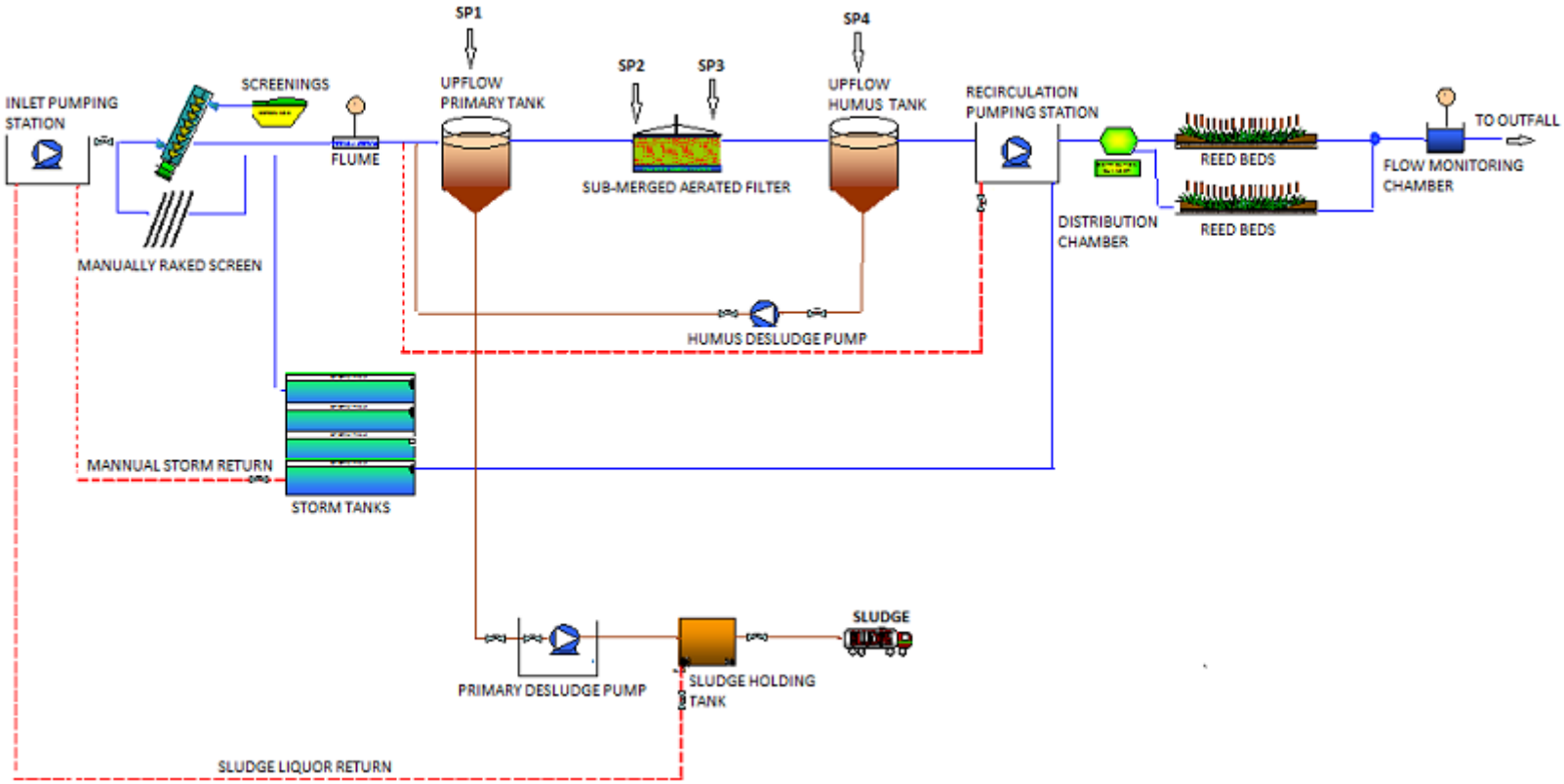



**Figure 19:** Diagram of the Molesworth sewage treatment work at RAF showing location of the sampling points (SP1=primary sludge tank; SP2 = submerged aerated filter tank; SP3 = mixed submerged aerated filter tank and SP4 = humus tank [292]

### 2.3.2. Stoke Bardolph STW, Nottingham Sewage Treatment Work (STW)

The Stoke Bardolph STW is located just outside Nottingham and it is a total distance of about 10 miles from Nottingham Trent University, Clifton Campus. The STW has serviced large increases in population in the last decades, the largest sewage treatment works in Nottinghamshire. Nottingham STW at Stoke Bardolph is also the largest works in the East Midlands serving half a million people and an additional 200 000 'population equivalent' from trade effluents. The treatment plant employs both primary and secondary treatment using activated sludge and on average it handles 170 million litres of sewage per day. The inlet removes 2000 tonnes of 'road' grit a year and a further 330 tonnes of paper and plastics. It takes a total of 16 hours (including recycling stages) to completely treat the liquid phase before returning it as a high quality final effluent to the River Trent [289]. The influent wastewater goes through several treatment stages, shown schematically below (Figure 20).

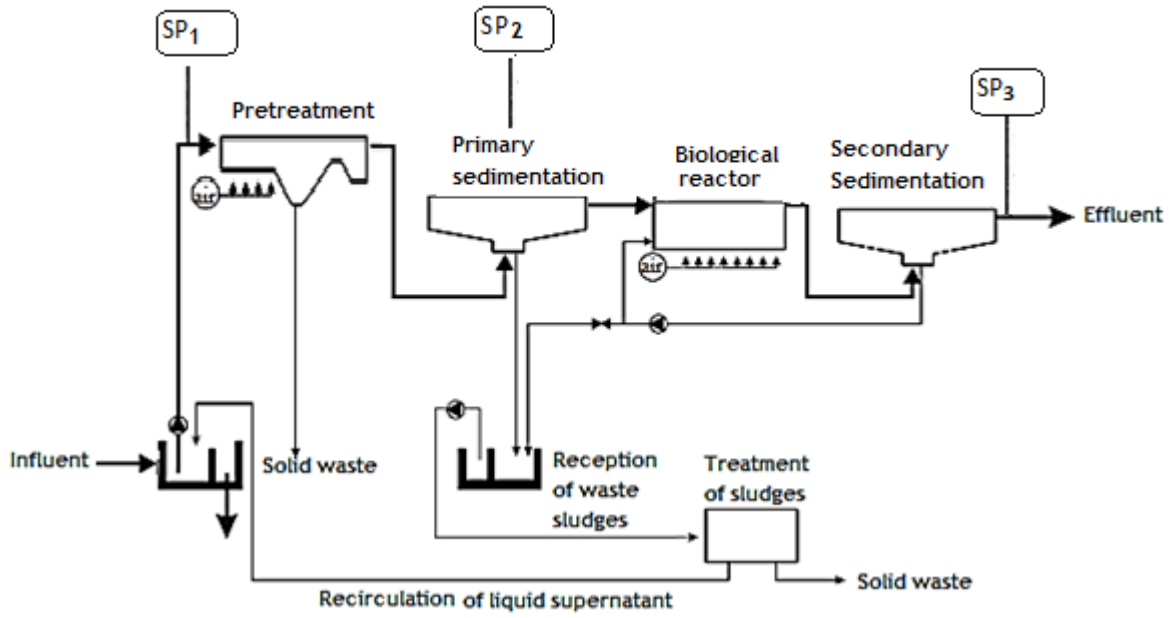



**Figure 20**: Illustration diagram of Stoke Bardolph STW showing location of the sampling points ($SP_1$ = influent waste water tank; $SP_2$ = primary sedimentation tank and $SP_3$ = secondary sedimentation tank) [289]

At Stoke Bardolph, the treatment process proceeds as follows:

- Primary Treatment: Coarse solids are removed in the bar rack and the grit is removed in a grit chamber. The heavier organic matters are settled in primary settlement to form a primary sludge. The sludge is removed by pumps and transferred to another part of the site for treatment. The heavy organic solids are collected at the primary clarifier where 50 to 70% of the suspended solids and 25 to 40% of the biochemical oxygen demand (BOD) are removed.
- Secondary Treatment: In the aeration tanks, the aerated biological process utilises useful micro-organisms. The influent of the aeration tank is mixed with activated sludge and the mixed liquor is aerated and the aerobic processes stimulated with the growth rate of bacteria

becoming faster. Flocculation takes place as a result of bacteria depletion of biomass to form insoluble substrate which becomes a solid biomass and these flocs sediment in the secondary clarifier.

- Tertiary Treatment: Chemical disinfection of waste stream is carried out here by disinfectants such as ozone, UV, or chlorine to kill the disease-causing organisms. Finally, the final effluent water is discharged to the receiving waters.
- Sludge Treatment: Sludge from primary treatment, surplus activated sludge and activated sludge from outlying smaller treatment works is treated at Stoke Bardolph. Digestion is carried out in 4 primary digesters before being transferred to 4 secondary digesters. Sludge can then be pumped to land but is usually dewatered to produce a cake which can also be applied to agricultural land.
- Recycling: A series of underground pipes allow sludge to be distributed to fields on the Stoke Bardolph and Bulcote estate. A tractor pulling specially designed equipment injects the sludge into the soil. This provides nutrients for a range of crops which are grown on the farm [288, 289].

## 2.4. Sampling section.

The sampling plan we adopted was identification of the appropriate sites at both Stoke Bardolph STW Nottingham STW and RAF Molesworth STW at Cambridge to collect samples. Sampling operations started with the collection of wastewaters and sewage samples between 22nd February 2010 to 18$^{th}$ June 2011. Final effluents were sampled and analysed to assess the levels of pollutant arising from the STWs discharges. No samples were collected during heavy rain or storm weather to prevent dilution and possible analyte losses due to overflow. Plastic containers were used to collect the grab waste water samples and Winchester bottles used to collect and store sewage samples from the locations.

Wastewaters (effluent only) were filtered over Whatman GF-C glass microfiber filter (~ 30 µm) to remove solid particles and transfered into pre-cleaned Winchester glass bottles, adjusted to pH = 2 with 37% HCl and stored at 4$^{o}$C until analysis to minimise bacterial activity and prevent degradation of drugs and its metabolites arising during storage; which was carried out within 24hrs in the laboratory [178, 296]. Sewage sludge samples were directly collected in pre-cleaned Winchester glass bottles and stored at -20 $^{o}$C to preserve samples. Sampling expeditions are shown in Table 11 below.

**Table 11:** Sampling expeditions, dates and locations

| Sampling dates | **Samples collected** | **Locations** |
|---|---|---|
| Mon. 22-2-2010 | Effluent wastewaters | Nottingham STW at Stoke Bardolph |
| Thur. 25-2-2010 | Effluent wastewaters | Nottingham STW at Stoke Bardolph |
| Thur. 4-3-2010 | Effluent wastewaters | Nottingham STW at Stoke Bardolph |
| Thur. 11-3-2010 | Effluent wastewaters | Nottingham STW at Stoke Bardolph |
| Mon. 15-3-2010 | Effluent wastewaters | Nottingham STW at Stoke Bardolph |
| Thur. 18-3-2010 | Effluent wastewaters | Nottingham STW at Stoke Bardolph |
| Fri. 23-4-2010 | Effluent wastewaters | Nottingham STW at Stoke Bardolph |
| Sat. 24-4-2010 | Effluent wastewaters | Nottingham STW at Stoke Bardolph |
| Tue. 4-5-2010 | Effluent wastewaters | Nottingham STW at Stoke Bardolph |
| Wed. 5-5-2010 | Effluent wastewaters | Nottingham STW at Stoke Bardolph |
| Thur. 6-5-2010 | Effluent wastewaters | Nottingham STW at Stoke Bardolph |
| Fri. 7-5-2010 | Effluent wastewaters | Nottingham STW at Stoke Bardolph |
| Sat. 8-5-2010 | Effluent wastewaters | Nottingham STW at Stoke Bardolph |
| Sun. 9-5-2010 | Effluent wastewaters | Nottingham STW at Stoke Bardolph |
| Mon. 10-5-2010 | Effluent wastewaters | Nottingham STW at Stoke Bardolph |
| Fri. 29-10-2010 | Sewage sludge, influent & effluent wastewaters | Molesworth STW at RAF base |
| Thur. 5-5-2011 | Sewage sludge, influent & effluent wastewaters | Molesworth STW at RAF base |
| Tue. 7-6-2011 | Sewage sludge, influent & effluent wastewaters. | Nottingham STW at Stoke Bardolph |
| Sat. 18-6-2011 | Effluent wastewaters | Nottingham STW at Stoke Bardolph |

Initially, a simple random sampling approach was used in the 1st sampling session at the two STWs to minimise the possibility of bias [343]. Systemic sampling approach was adopted in the 2nd sampling session of wastewater at Stoke Bardolph STW, Nottingham from Tuesday, 04 May – Monday, 10 May 2010 (Table 10), where the first sample taken was random and the subsequent sample collections every 24 hrs to monitor daily variation. As with analytical processes, analytical measurement can introduce errors due to:

1. Variations inherent in the bulk sample i.e. collection at various points can introduce variation

2. Variation due to the sampling process.

In this current work, representative samples were taken with the following measures taken to ensure the impacts of variation were minimal:

1. Ensured adequate sample storage at appropriate temperature and pH, handling and proper samples labelling.
2. Avoided cross-contamination in the laboratory environment
3. Ensured potential cross-contamination by instruments were considered and avoided.

## 2.5. Experimental programme

During primary and secondary wastewater processes, the fate of cocaine, benzoyecgonine, heroin, 6-monoacetylmorphine, morphine and diazepam were determined under different conditions in a series of jar tests. In a series of experiments with 250 mL of the aliquots of raw sewage in the conical flasks spiked with 1 mL of 12.5 mg of separate standard of drugs (not a mixture). Background concentrations of the six analytes were measured and none of the compounds under investigation were detected in the original sludge samples. The initial concentration was thus 50 mg $L^{-1}$ equivalents for each of the six drugs of interest and each batch test was initially 0.5 mg of drug in 10 mL of raw wastewater or sewage samples.

The choice of 12.5 mg/250 mL of sample for each of the selected compounds was used to achieve optimal experimental strategy that would provide evidence of the presence of residual compounds at concentration in the lower ng $L^{-1}$ range for the batch test after 3h duration. Therefore adequate spiking concentration for batch eperiments was selected after initial trial experiment to determine adequate concentration that would allow measurable concentration of parent drug and associated degradation products. No possible evaporation occurred during the batch studies as aluminium foil caps were securely placed on the flasks with the jar tests carried out in the incubator to maintain a typical activated sludge plant constant temperature. Batch experiments were conducted at two temperatures of 19 ± 0.5$^{o}$C and lower abiotic temperature of 4±0.5$^{o}$C. Sample were agitated using magnetic stirrer throughout to obtain homogenous sample of which 10 mL of batch sample was removed at every 15 min intervals. These subsamples were centrifuged (1500 rpm for 5 min) and filtered using 0.45 µm cellulose acetate Whatman and the aqueous phase was extracted by solid phase extraction using Oasis HLB (500mg/6 mL) adsorbent (made up of a divinylbenzene/*N*-vinylpyrrolidone copolymer with both hydrophilic and hydrophilic properties) with very good recovery data [278] compared to other adsorbents such as MCX® (500mg/6mL) [137], Isolute ENV+® (500mg/6 mL) and Isolute PH® (1000mg/6 mL) adsorbents [136], and Bond Elut Certify® adsorbent [214]. The choice was based on the use of Oasis HLB® (500mg/6 mL) in the extraction of cocaine and its metabolites in waste and surface water in the literature, and it was recommended as most suitable adsorbent for organic

compounds because of its lower solvents usage, time, stability to pH range and over 75% recovery for most analytes in aquatic medium was achieved [132]. The solid phase was extracted by shaker extraction for 15 min after initial drying with about 2 g of anhydrous sodium sulphate followed by addition of 2 x 5 mL each of ethyl acetate and dichloromethane.

### 2.5.1. Spike recovery studies of compounds from sewage sludge samples.

Recovery rates of the drugs depended on the nature of the samples collected from each RAF sampling point: primary sludge (PS), submerged aerated filter -1 (SAF-1), mixed secondary aerated filter (MSAF) and humus sludge (HS) and Stoke Bardolph STW sampling points: influent wastewater -1, influent wastewater – 2, primary effluent, secondary effluent –North, secondary effluent –South, and secondary effluent –New. Recovery experiment for 1L of wastewater were spiked at mixed concentrations of 100 ug $mL^{-1}$ , while the liquid phase of the raw sewage samples were spiked with known concentration of drugs (50 mg $L^{-1}$) and analysed in exactly similar ways as in the full batch studies. There were only one recovery values for liquid phase (re-computed into the data) as presented in Table 13 and 14 (Result section- Chapter 3.1.1 ad 3.1.2). The recovery experiment for solid phase could not be completed in particular because the drugs were not enough for batch studies - our most suitable options in the situation. However, this may introduce uncertainties in the data.

### 2.5.2. GCMS operation and quantitation of compounds in Nottingham STW effluents.

**GCMS Instrumentation:**

The two major building blocks in gas chromatography-mass spectrometry are gas chromatography (GC) and mass spectrometer (MS). The GC separates mixtures into different components depending on the chemical properties of molecules, column's phase properties and dimensions (length, diameter, film thickness) as the sample travels in the capillary column of the gas chromatograph. The interactions between the column and the molecules allow the molecules to be retained and come off (elute) at different retention times from the column. These molecules are now captured, ionized into fragments and are detected separately by MS downstream. The electrical signals are produced from the ionized fragments detected usually by an electron multiplier diode. Single ion mode (SIM) scan was used in the instrument method to target a range of mass fragments. SIM analysis allows for a smaller quantity of a compound to be detected and measured using Excel software for quantification. A quadrupole mass spectrometer (Mass Selective Detector) is a popular detector and others that can be encountered include: ion trap mass spectrometer (IoT), tandem quadrupoles (MS-MS) and time of flight (TOF). The standard ionization technique is electron impact (EI), where a high energy electron typically 70 eV (electron Volts) produced by a /filament (quadrupole source) bombards the molecules into characteristic and reproducible fragments [339]. The electron ionization process in gas phase is described as: $M + e^- \rightarrow M^{+\cdot} + 2e^-$

[Where M = analyte molecule being ionized, $e^-$ = electron and M = $M^{+\bullet}$ is the resulting ion]. The generated spectra can be compared with the in-built library spectra software supplied by National Institute of Standards (NIST-USA) employing matching algorithms such as Probability Based Matching [340] and dot-product [341] for compound identification.

**GCMS Operation and quantitation of compounds:**

In the current work, gas chromatography mass spectrometry analyses were performed with an Agilent 6890 GC coupled to Agilent 5975 inert XL mass selector detector (MSD: 2564.7 eV), using a capillary column (HP5-MS) (30.0 x 0.25mm x 0.25 um film thickness) with helium as carrier gas (1 mL $min^{-1}$). With sample injection in splitless mode, the analyte separation was achieved with the temperature programming: 50$^o$C (hold 2min), rise to 300$^o$C at 10$^o$C $min^{-1}$ and then held at 300$^o$C for 3 min. The standard software supplied by the Agilent Chemstation (manufacturer) was used for data acquisition and analysis. The mass spectrometer was in electron impact (EI) ionization mode at 70eV. Quantitation was determined using total ion corresponding area (TIC) with the EI mass spectra recorded in scan mode (scan range 45- 550*m/z)* with GCMS method total run time of 30 min.

The linear calibration curves using five point curves from a spiked water concentration range of 2-10 $ugL^{-1}$ were obtained after appropriate dilutions from 100 $ugmL^{-1}$ and all the compounds (ibuprofen, benzocaine, caffeine, lidocaine, cocaine, codeine, amphetamine, metamphtamine, ecgonine methylester, methadone, heroin, benzoylecgonine, cocaethylene, ecgoninemethylester, morphine, nicotine, 6-acetylmorphine, 6-acetylcodeine, diacetylmorphine, diazepam and procaine) prepared in methanol (blank). The *m/z* major characteristic mass fragments and retention times of compounds identification were presented in Table 12. The characteristic mass fragments and retention times of analytes were used for both qualitative and quantitative determinations with the mass spectrometer in electron impact (EI) ionization mode at 70eV and quantitation was determined in single ion mode.

**Table 12:** *m/z* ions selected for substances identification

| **Compound** | **Major ions** (molecular & two product ions) | **Retention time** (min) |
|---|---|---|
| Amphetamine | *m/z* **206**, 116, 73 | 10.0 |
| Ecgonine methyl ester | *m/z* **199**, 96, 82 | 11.7 |
| Nicotine | *m/z* 162, 133, 84 | 12.9 |
| Ibuprofen-*O*-TMS | *m/z* **278**, 160, 73 | 15.6 |
| Benzoylecgonine-*N*-TMS | *m/z* **237**, 222, 192 | 17.3 |
| Caffeine | *m/z* **194**, 109, 67 | 18.2 |
| Lidocaine | *m/z* **234**, 86, 58 | 18.7 |
| Bromacil | *m/z* 205, 190, 162 | 19.4 |
| Procaine | *m/z* **235**, 99, 88 | 20.2 |
| Cocaine | *m/z* **303**, 182, 82 | 21.9 |
| Codeine-*O*-TMS | *m/z* **371**, 178, 73 | 24.0 |
| Diazepam | *m/z* **283**, 256, 221 | 24.1 |
| 6-MAC | *m/z* **341**, 282, 229 | 24.6 |
| 6-MAM | *m/z* **327**, 268, 215 | 24.9 |
| Heroine | *m/z* **369**, 310, 268 | 26.3 |

#### 2.5.2.1 Instrumental detection limits.

Detection limits which were obtained from injection of low spiked mixed concentrations of each analyte were used to calculate the limits of detection; LOD and quantification; LOQ using the formula:

LOD = 3 x *h*max x *R* [eqn. 1]

(where *h*max = maximum amplitude of the noise; *R* = concentration of compound (in ng $L^{-1}$)/peak heights).

Example*: h*max (maximum amplitude of the noise for cocaine) = 50 (TIC value)

*R* (concentration of cocaine) = 2000 $ngL^{-1}$

Peak height for cocaine = 382576 (as presented in Appendix 4):

$$LOD = 3 \times 50 \times 2000 \div 382576 = 0.78 \text{ ng } L^{-1}$$

The LODs for the remaining compounds are presented in Table 13

All the calculated LODs, expressed in ng $L^{-1}$ were significantly lower than the concentrations of analyte deteted in Stoke Bardolph STW, Nottingham effluents. The empirical values of both LOD presented greater probability to real values and not just some random fluctuations of the blank reading [97,132]. (Result section – Chapter 3).

### 2.5.3. The physico-chemical characterization of the wastewaters

Total suspended solids (TSS), chemical oxygen demand (COD), organic carbon (OC), ash and pH were determined by standard methods [282-284] as outlined below. Results are presented in Table 15 (Result section – Chapter 3)

#### 2.5.3.1. Total suspended solids (TSS) determination.

The TSS in the sludge samples, influent (raw) and effluent (treated) wastewater were determined after filtration through GF/C (1.2 µm) using equation 2:

TSS conc. ($mgL^{-1}$) = [$W_{sand+silt+clay}$ (g) / $V_{sample}$ (mL) x $10^6$] [eqn. 2]

Example: Total suspended solids (TSS) determination of influent sample (InfWW-1) collected on Saturday, 18 June 2012 from Stoke Bardolph Nottingham STW:

Influent-1 wastewater (InfWW-1) = 1000 mL

Weight of solid (Wsand+silt+clay) = 0.7979 mg

TSS conc. = [0.7979 mg/1000 mL x$10^6$] = 797.9 ($mgL^{-1}$) of influent sample

#### 2.5.3.2. The pH measurement.

The unfiltered wastewaters and sludge samples were measured directly with pre-calibrated pH meter (HANNA HI 4212 model).

#### 2.5.3.3. The ash content.

Organic carbon was determined by combustion method. 1.0 g of sewage was placed in a crucible and put into a furnace at 350$^{o}$C for an hour, after which the temperature was raised to 550$^{o}$C and left for 24 hours. The ash content is the measure of inorganic component of the sewage samples and the equation below worked the calculation of ash content using 1.0 g of sewage.

Total ash content (g) = [$Wc_1$+ $W_{ash}$] (g) – [$Wc_1$] (g) [eqn. 3]

(Where $Wc_1$ = initial weight of crucible; $W_{ash}$ = weight of ash)

Example: The ash content determination of HS sludge sample collected on Friday, 29 October 2010 from Molesworth STW:

Wc = weight of crucible (33.7535 g)

$Wsample_1$ = initial weight of sample (1.0 g)

$Wsample_2$ = final weight of sample

Total ash content = [34.7535 – 34.8172] = 0.0637 (g/g).

#### 2.5.3.4. Organic carbon content.

Organic carbon was determined by combustion method. 1.0 g of sewage was placed in a crucible and put into a furnace at 350$^{o}$C for an hour, after which the temperature was raised to 550$^{o}$C and left for 24 hours. The remaining ash was weighed and organic C was calculated from the loss in weight during ashing.

Organic C (g) = $W_{sample}$ (g) - $W_{ash}$ (g) [eqn. 4]

Example: Organic carbon content determination of MSAF sludge sample collected on Friday, 29 October 2010 from Molesworth STW:

$W_{sample}$ (g) = 1.0 g

$W_{ash}$ (g) = 0.0637

Organic C = [1.0 – 0.0637] = 0.9363 (g/g)

#### 2.5.3.5 The Chemical Oxygen Demand (COD) determination.

The COD was measured after appropriate dilution of 1mL of each sample with distilled water:

1. <u>RAF Molesworth STW wastewaters:</u>

Raw influent (dilution factor of 200)

Treated effluent (dilution factor of 10)

Humus & SAF sludges (dilution factor of 100)

PS & MSAF sludges (dilution factor of 150)

2. <u>Nottingham STW wastewaters:</u>

Raw influent (dilution factor of 100)

Treated effluent (dilution factor of 10)

After 2 ml of each of the pre-diluted sample was dispensed into a COD tube in triplicate, 1 mL of potassium dichromate (0.0083 M) was added while a fourth tube with 2 mL of distilled water acted as

a control (blank). In a fume-cupboard, 2 mL COD sulphuric acid (a mixture of sulphuric acid and silver sulphate) and 2 mL of acidified 5% mercurous sulphate solution (masking of chloride) were added. After the tubes were placed in the heating block and reflux for 1 hr. The sample and blank were finally titrated with the standardized ferrous ammonium sulphate, FAS (0.005 M) using a drop of ferroin indicator with a colour change from blue-green to grey orange. The COD was calculated using the following equation 5:

$$COD\ (mgdm^{-3}O_2) = 800 \times M \times [V_{blank} - V_{sample}] \div 50 \qquad \text{[eqn. 5]}$$

Example: The chemical oxygen demand (COD) determination of HS sludge sample collected on Friday, 29 October 2010 from Molesworth STW:
Sample of humus wastewater taken = 1 mL each (diluted to 100 mL with distilled water in triplicate)

$V_{blank}$ = blank titration reading volume of FAS used (average of triplicate readings) = 13.75

$V_{sample}$ = sample titration reading volume of FAS used (average of triplicate readings) = 7.20 mL

Standardization of FAS (mL) against 20 mL of potassium dichromate (0.0083 M):

Volume of FAS used after titration = 32.50 mL.

Therefore, concentration of FAS = 0.0083 x 20 ÷ 32.50 = 0.0051M.

Substitution in equation 5 gives:

$$COD = 800 \times 0.005\ [(13.75 - 7.20) \div 50]$$

$$= 0.524\ (mgdm^{-3}O_2)$$

Multiplying by dilution factor (100) then;

$$COD = 52.4\ (mgdm^{-3}O_2)$$

The explanation of calculations with worked examples of LOD, ash, organic carbon, TSS and COD contents are discussed in Table 15 (Results - Section 3.1.4).

## 2.6 Wastewater analysis

Buchner bottles were used to collect the grab samples and were all stored in glass bottles, adjusted to pH of 2 with 37% HCl and stored at 4$^{o}$C for preservation. The pH adjustment was to prevent degradation of drugs and metabolites arising during storage. Water samples (1L) in triplicates, before extraction, were filtered over a glass Whatman GF-C glass microfiber filter (1.2 µm) to remove solid particles. Afterwards, the filtrates were adjusted to pH of 6 using HCl and $NH_4OH$ as appropriate and were extracted by solid phase extraction using Oasis HLB (500mg/6 mL) made up of a divinylbenzene/*N*-vinylpyrrolidone copolymer with both hydrophilic and hydrophobic properties [132]. The cartridge was soaked and conditioned with 5 mL of methanol without vacuum for 5 min

and 10 mL methanol followed by 10 mL water with vacuum, respectively. Wastewater samples (1L) at 100 ml per minute (c.a.10 psi pressure) were introduced and the cartridge was then washed with 10 mL reagent water, air-dried under vacuum for 15 min. Elution was performed in three steps: (i) 5 mL of methanol using no vacuum was added and after 2 minutes vacuum was turned on to collect first eluates. (ii) 12 mL of 12% ammonia in methanol was added to collect second eluates and (iii) 6 mL of 50:50 acetone/methanol was finally added and the combined eluents were evaporated to dryness using a nitrogen stream and a sand bath set to 70$^{o}$ C [283]. The mixed-eluates were added with 50 µL BSTFA + 1% TMCS in 50 uL of pyridine as solvent added, incubated at 70$^{o}$ C for 20 min (~ total volume 100µL ) as chemical derivatization step to improve the analysis of primary and secondary amines/alcohols containing compounds as their trimethylsilyl derivatives [215]. The derivatized extracts were filtered (nylon membrane 0.45µm) and centrifuged for 5 min at 1000 rpm. The clean filtrates were then transferred to glass 250 mL vials and placed in an auto sampler for gas chromatography mass spectrometry analysis.

**Chemistry of derivatization (silylation) of analytes using BSTFA with 1% TMCS:**

A derivatizing agent, *N, O,* bis (trimethylsilyl) trifluoroacetamide (BSTFA with 1% trimethylchlorosilane, TMCS) is an effective trimethylsilyl donor and it is most versatile when used to enhance GC performance by blocking protic sites, thereby reducing dipole-dipole interactions and hydrogen bonding, and increasing volatility. Silyl groups, for example, trimethylsilyl (-$SiMe_3$) are introduced as protecting groups to replace acidic hydrogen on the compound:

$$RH \rightarrow R^{-} + H^{+}$$

$$R^{-} + R'_3SiCl \rightarrow R\text{-}SiR'_3 + Cl^{-}$$

It has advantage of the volatility of its by-products, mono-(trimethylsilyl) trifluoroacetamide and trifluoroacetamide over many others because they elute with the solvent front which rarely interfere with analyte peaks in chromatograms. The use of large excess of derivatising agent and solvent (where necessary) can help to minimise problems of interference by moisture or other sample impurities [342].

## 2.7 Sewage sludge batch tests

For batch studies, 250 ml of each unfiltered sludge sample were measured in a conical flask and spiked with 12.5 mg of standard drugs (Sigma Aldrich analar grade: cocaine, benzoylecgonine, heroin, 6 – monoacetylmorphine, morphine and diazepam). Blanks of deionised water after extraction and concentration were treated with similar solvents (pyridine and BSTFA + 1% TMCS) used in batch studies and run in GC. Samples for the determination of drugs were taken at 15 min intervals over a period of up to 3h. The analytical method used was a modified published analytical method [238] and is described below. Samples (10 mL) were centrifuged (Jouan C31, VWR, UK) at 1500 rpm for 5 min to separate the solid and aqueous phase. After the addition of 2g oven dried sodium sulphate (VWR, Lutterworth, UK), extraction of the solid phase was by shaker extraction for 15 min each with

5 ml ethyl acetate twice and finally 5 mL dichloromethane (DCM). Fractions were combined and dried under nitrogen and reconstituted with 50 µL each of pyridine and BSTFA + 1% TMCS. Supernatant aqueous sample after separation from solid sample was put for SPE enrichment and analysed on GCMS as described in wastewater analysis above.

### 2.8. Operational issues encountered with batch studies.

Municipal STWs contain lot of different trace polluting substances received from many sources as earlier mentioned, therefore carrying out degradation studies of drugs and the analysis of their metabolites using solid phase extraction (SPE), derivatization, detection and confirmation by gas chromatography mass spectrometry (GCMS) in highly contaminated sewage samples would normally be met with some operational issues. To guarantee accurate and reproducible data in highly polluted sewage, some operational issues and treatment options were anticipated (see below). Considerable efforts were made to enable a simple, robust and complete picture of the degradation studies to model a chemical fate in the STW and our most suitable options in the situation are presented:

1. On the basis of precautionary measures after ensuring none of the compounds under investigation were detected in the original sludge, the samples from different processing units of RAF Molesworth STW in Cambridge were obtained to minimise unnecessary drug-drug interferences that may influence some chemical properties of drugs or inhibit their biodegradability potential

2. Studies on samples from Stoke Bardolph STW, Nottingham were also conducted to see expected variability since the configuration of municipal treatment works vary in design capacity and location but both are considered for effects of degradation in different treatment facilities or sludge types.

3. The spiking concentration of 12.5 mg of each drug in 250 mL flasks was considered adequate after initial random degradation studies to allow measurable concentration in the lower $ugL^{-1}$ range after 3h period of exposure.

4. Pre-selection of environmentally relevant compounds for batch studies were based on the initial results of the analysis of survey of water effluents from Stoke Bardolph, Nottingham STW which showed the presence of these drugs in the samples collected as well as their concentrations in other UK wastewater as reported in the literature.

5. Heroin was purchased under license from Sigma Aldrich (Gillingham Dorset, UK). As a supplement to the commercial heroin (Sigma Aldrich), additional heroin was required for batch experiments. Pure heroin was not enough for batch studies, but street heroin was used as a substitute.

6. The street heroin GC chromatogram (Figure 21) was analysed for its percentage purity to estimate the right quantity to conclude batch experiment. From this, 0.5mg of street heroin was dissolved in 10 mL of chloroform with triplicate samples run on GC to estimate the concentration of heroin below:

Calculation to derive 12.5 mg of pure heroin from street heroin samples for batch studies:

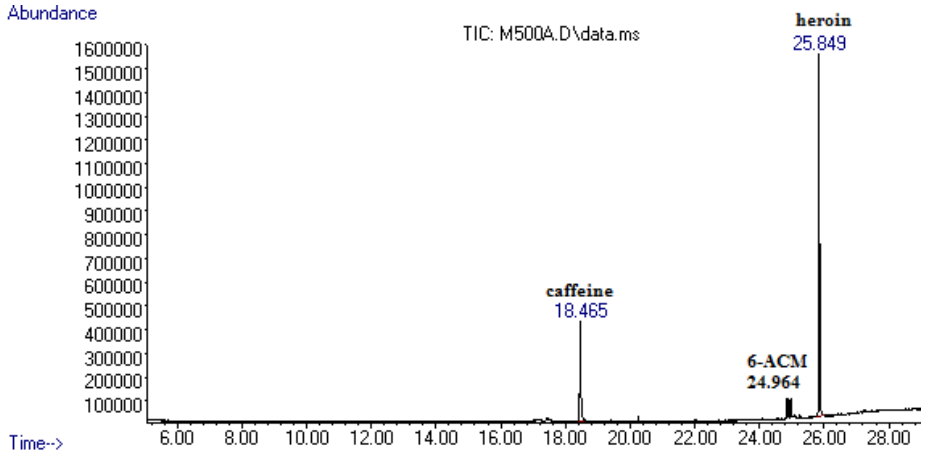



**Figure 21**: Chromatogram of street heroin showing other adulterants

TIC data A = 29136226
TIC data B = 28766483
TIC data C = 28050152
TIC data (mean) = 28650954
Heroin standard calibration equation **(y) = 106632x** [eqn. 3]

Substitution gives = 28650954/106632 = 268.690 µg $mL^{-1}$

Therefore, the amount of heroin in 0.5 mg of street sample = 268.690 (0.269 mg)

% purity = 268.690/500 = 53.738

If 0.269 mg of actual heroin was found in 0.5 mg of street heroin.

To calculate the amount of street heroin that would give 12.5 mg of actual heroin:

0.269 mg of actual heroin was in 0.5 mg of street heroine.

Amount of street heroin required to give12.5 mg of actual heroin = 0.5 x 12.500 ÷ 0.269 = 23.261 mg of street heroin

The **23.261 mg** of street heroin would contain exactly 12.5 mg of actual heroin needed

7. In Stoke Bardolph STW Nottingham, sampling consist of only (i) inlet to the grit removal unit, (ii) primary sedimentation tank and (iii) outlet of the secondary sedimentation tank leading to outflow which discharges to River Trent. Site constraints did not allow collection

of raw sewage samples at different processing units and this made it difficult to assess the total performance of the system using only 3 sample points. However, the sampling regime has been produced to make the best use of the available resources as complete comparison of samplings of various units with that of Molesworth STW units were impossible in the circumstances of this study.

8. In RAF Molesworth STW, samples were from four sampling units for batch studies and the results were used to assess plant efficiency at each of the selected process stages.
9. In the scope of this current work, 6 representative drug: cocaine and benzoylecgonine (cocaine group), heroin, 6-monoacetylmorphine, morphine (opiates) and diazepam (pharmaceutical) were used for degradation tests using activated sludge as inoculum; a 3h degradation time as a model system for municipal sewage treatment was chosen in excess of normal 2h typical hydraulic retention time (HRT) for unit treatment in many STWs. The high HRT and sludge age of some works may contribute to increased biodegradation rates.

# CHAPTER 3: Results & Discussion

## 3.1 Effluent evaluation of Stoke Bardolph, Nottingham STW

In this chapter, the analysis of the recovery data is summarised for the spiked wastewaters and the sewage samples collected from Stoke Bardolph, Nottingham and Molesworth STWs. The data obtained includes only the recovery data for the compounds in aqueous phase, quantitation of compounds and characterization of wastewaters with the measurements of pH, total suspended solids (TSS), ash content, organic carbon content and the chemical oxygen demand (COD).

Data from real-water effluent is presented with their respective limits of detection. The results found in the Nottingham STW effluent generally compares well with results obtained from other wastewaters treatment sites around the world (Table 4).

### 3.1.1. Recovery data and quantitation of compounds in spiked waters.

Standard compounds and sources used for recovery experiment were already mentioned in the Method Section 2.2. All the selected 16 compounds in their underivatized form and as their trimethylsilyl derivatives are shown in the representative chromatograms Fig 22 showing the separation of drugs were used for standard calibrations and recovery calculations. Preliminary tests indicated no background contaminations were present as interferences with our recovery experiments

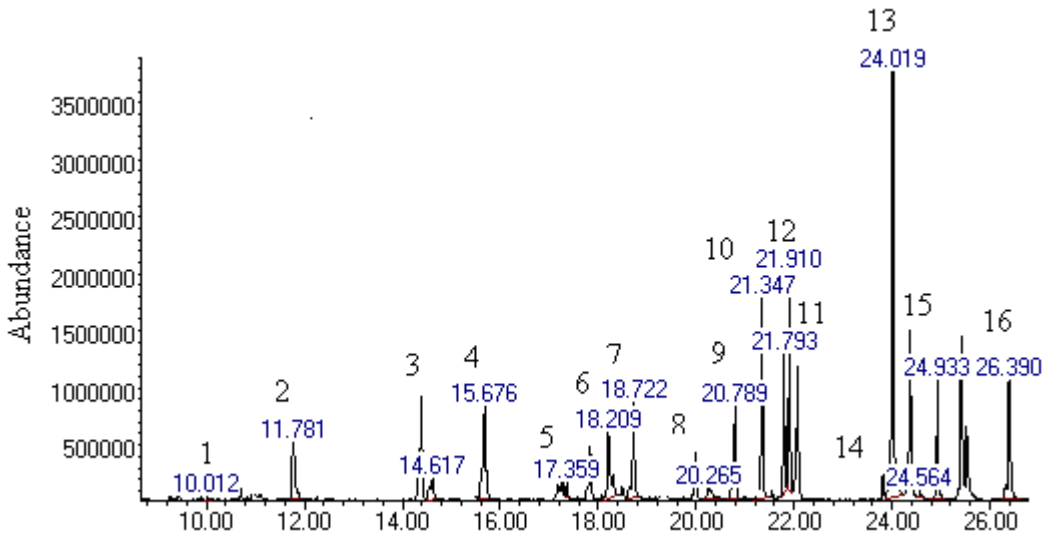



**Figure 22:** Total ions chromatogram of compounds their underivatized forms/trimethylsilyl derivatives obtained from extraction of de-ionised water sample spiked with standard analytes concentration of 4ug $L^{-1}$ (1 = Amphetamine-*N*-TMS; 2 = Ecgonine methyl ester-*O*-TMS; 3 = Metamphetamine; 4 = Aspirin-*N*-TMS; 5 = Ibuprofen-*O*-TMS; 6 = Benzocaine-*N*-TMS; 7 = Caffeine-*N*-TMS; 8 =Lidocaine; 9 = Procaine-*N*-TMS; 10 = Methadone; 11 = Cocaine; 12 = Codeine-*O*-TMS; 13 = Diazepam; 14 = 6-acetylcodeine-*O*-TMS; 15 = 6-acetylmorphine-*O*-TMS; 16 = Diacetylmorphine)

The few examples of reference mass spectra of above selected compounds from the NIST library and sample spectra are shown in Appendix 1, which also includes the fragmentation ions, retention data

and the GC TIC traces of all analytes for identification as shown in Table 13. Mean recoveries were evaluation of triplicates measurements of mixed drugs at 2 and 4 ug $L^{-1}$ with standard deviations.

**Table 13**: Performance characteristic of SPE –GCMS with spiked de-ionised water (n=3).

| Analyte | LOD | % Recovery (mean ± STD) | |
|---|---|---|---|
| | (ng/L) | 2000 (ng/L) | 4000 (ng/L) |
| **Substances studied in batch experiments and their matabolites** | | | |
| Cocaine | 0.8 | 83.0 ± 2.4 | 85.9 ± 2.1 |
| Benzoylecgonine | 4.6 | 109.6 ± 9.7 | 90.2 ± 2.3 |
| Ecgonine ME | 6.9 | 92.5 ± 1.2 | 92.2 ± 1.7 |
| Cocaethylene | 0.9 | 85.2 ± 1.8 | 90.8 ± 1.3 |
| Heroine | 4.3 | 74.5 ± 4.7 | 82.7 ± 1.9 |
| 6- acetylmorphine | 4.6 | 107.5 ± 8.6 | 87.6 ± 2.1 |
| Morphine | 0.9 | 88.5 ± 1.0 | 79.2 ± 2.1 |
| Diazepam | 1.9 | 76.6 ± 2.6 | 86.0 ± 4.7 |
| **Other compounds analysed in wastewaters** | | | |
| Nicotine | 12.7 | 101.6 ± 2.6 | 98.4 ± 1.9 |
| Codeine | 0.2 | 78.4 ± 2.8 | 81.9 ± 2.3 |
| Lidocaine | 1.5 | 83.6 ± 2.3 | 91.7 ± 1.4 |
| Caffeine | 8.6 | 86.0 ± 2.4 | 91.0 ± 1.4 |
| Procaine | 9.2 | 88.8 ± 1.6 | 86.7 ± 0.6 |
| Ephedrine | 0.1 | 81.0±1.4 | 83.7± 4.3 |
| Ibuprofen | 5.9 | 89.6 ± 4.3 | 75.3 ± 3.4 |
| Amphetamine | 3.3 | 83.9 ± 1.6 | 70.6 ± 1.7 |

Reproducibility/repeatability is a measure of consistency of the results obtained and this was determined by measuring the two levels of drug concentration at three separate GCMS runs.

The percentage recoveries ranged from 74.5 – 109.6% for all 18 compounds extracted were achieved and the values compare well with the recoveries values in Table 4 for many illicit compounds, such as cocaine (69 – 105%), opiates (85 – 90%) and cannabinoids (69 – 84) [131].

The coefficient of variation ($R^2$) indicates the linearity of the calibration graphs used to derive concentration values and these are higher than 0.99 in most cases ($0.9534<r^2<0.9998$).

### 3.1.2. Recovery data from different sewage matrices (aqueous)

The recoveries for the compounds were only done for the liquid phase of the sewage and since these recovery data were used for both liquid and solid phases it would introduce uncertainty. However, the aqueous phase data ranged between 75.8 – 96.2 % from different sewage matrices and are presented in Table 14 and the final results were adjusted for each drug accordingly to reflect the recovery rates. The results compares well with other discovery experiments carried out in other places, like the

average levels of cocaine and its metabolites (benzoylecgonine, BZE) in the River Po which have shown recoveries of > 90% for the two compounds [132].

**Table 14:** Recovery data for the compounds in aqueous phase **(%) (n=3,** mean ± STD**).**

| **Drug** | **Aqueous matrix** | | | | |
|---|---|---|---|---|---|
| | PS | SAF-1 | MSAF | HS | SB |
| Cocaine | 91.5±1.8 | 90.1±1.3 | 86.6±6.5 | 95.4±1.2 | 94.2±0.7 |
| Benzoylecgonine | 96.2±0.8 | 88.1±2.7 | 85.3±2.4 | 92.5±3.1 | 93.4±7.5 |
| Heroin | 86.3±2.9 | 83.4±1.8 | 87.2±6.4 | 87.4±6.8 | 82.6±2.5 |
| 6-monoacetylmorphine | 94.5±1.0 | 89.3±1.2 | 86.4±6.2 | 94.2±2.0 | 93.3±0.8 |
| Morphine | 78.4±4.4 | 76.1±3.1 | 75.8±3.6 | 79.2±2.3 | 80.2±4.1 |
| Diazepam | 92.1±3.4 | 84.2±1.0 | 82.5±3.2 | 92.7±2.6 | 93.7±1.3 |

Note: PS (primary sludge); SAF-1 (submerged aerated filter-1); MSAF (mixed SAF); HS (humus sludge) and SB (Stoke bardolph primary influent)

In a related investigation, 500mL of wastewater samples were filtered through micro-fibre filters and extracted by Strata- XC™ SPE cartridge, the filtrate was adjusted to pH 6 with HCl with recovery for most analytes found in the region of 50-65% [134]. Cocaine (COC), benzoylecgonine (BE) and ecgonine methylester (EME) have been determined on 100mL and 500mL in wastewater and surface water by a method that involved the use of SPE and LC-MS analysis [134] and the overall method variability was ≤10% for the influents, ≤ 5% for effluents and recoveries in wastewater were ≥ 80%.

At the Toxicological Centre, University of Antwerp, Belgium, researchers have carried out the measurement of spatial and temporal variations in the occurrence of cocaine and benzoylecgonine in waste and surface water with overall removal efficiency of the compounds from the STWs of ≥93%. In addition to the same analytical procedure, the determination of illicit drugs in wastewater effluents using Polar Organic Chemical Integrative Samplers (POCIS) methodology for sampling was introduced [141]. The estimated detection limits for most compounds were less than 1pg $\mu L^{-1}$ while the averaged recovery was 123±30%; however, the determination of the uptake rates for the compounds of interest for quantitative analysis is the limitation of POCIS.

In other developments, the occurrence of psychoactive drugs in wastewaters and recoveries have been reported between 70 - 101% from simultaneous determination of amphetamines, cocaines, cannabis and their metabolites in wastewaters after SPE enrichment of the analytes, while recoveries of 70 -120% were reported with precision of ≤ 20% for other substances, such as nicotine, cotinine, caffeine, paraxanthine, methadone and ketamine [138]

### 3.1.3. Match probability data of analytes from Stoke Bardolph, Nottingham wastewaters

The extract match probability data for selected analytes obtained from the GC NIST Search library are in Table 15 with our defined terms of likelihood in percentages into unlikely, possible match,

probable match and highly probable match for qualitative aspects of our findings. The probability match groups: ibuprofen, caffeine, procaine, ephedrine, dihydrocodeine (as highly probable match); cocaine, benzoylecgonine, diazepam, lidocaine, bromazil and nicotine (as probable match); codeine (as possible match) and morphine (as unlikely match).

**Table 15:** Match probability data table for selected compounds obtained from the GC NIST Search Library

| Compound | Library Search | Analyte Match | Reference Match | Probability (%) | Analyte Retention Time | Reference Retention Time | Remarks (Terms of Likelihood) |
|---|---|---|---|---|---|---|---|
| Procaine | R | 846 | 904 | 71.5 | 20.25 | 20.27 | H. probable |
| Ephedrine | M | 599 | 692 | 88.4 | 15.21 | 15.09 | H. probable |
| Dihydrocodeine | M | 585 | 740 | 85.4 | 23.50 | 23.50 | H. probable |
| Benzoylecgonine | ni | 747 | 864 | 45.1 | 16.38 | 16.42 | Probable |
| Diazepam | ni | 751 | 841 | 56.4 | 24.10 | 24.04 | Probable |
| Morphine | M | 731 | 763 | 22.7 | 24.50 | 24.49 | Unlikely |
| Lidocaine | R | 758 | 834 | 58.0 | 18.76 | 18.72 | Probable |
| Bromazil | ni | 777 | 852 | 56.4 | 19.41 | 19.49 | Probable |
| Codeine | R | 688 | 772 | 38.2 | 24.05 | 24.04 | Possible |
| Nicotine | M | 841 | 940 | 53.0 | 12.99 | 12.96 | Probable |
| Ibuprofen | M | 859 | 878 | 90.7 | 16.13 | 15.68 | H. probable |
| Paracetamol | M | 836 | 877 | 92.6 | 17.56 | 17.55 | H. probable |
| Caffeine | M | 876 | 889 | 92.3 | 18.65 | 18.26 | H. probable |
| Cocaine | M | 758 | 686 | 52.4 | 21.89 | 21.99 | Probable |

Terms of likelihood in percentages:

Highly probable match = 70% above

Probable match = 50 – 69%

Possible match = 30 – 49%

Unlikely match = ≤ 30%

Further comparison of reference compound retention time with the analytes provided valuable confirmatory processes in the matching probabilities of compounds with lower percentages.

### 3.1.4. Characterization of wastewaters from Stoke Bardolph, Nottingham and Molesworth STWs

Due to variability in the overall composition of wastewaters as a result of continuous discharges and potential matrix effects on degradation studies, the sewage sludge characterizations for wastewaters and sewage samples were analysed for total suspended soilds (TSS), organic carbon (organic C), ash and chemical oxygen demand (COD) and results are shown below in Table 16.

**Table 16**. Characterization of wastewaters from RAF Molesworth and Nottingham STWs (June 2011)

| Matrix | pH (Temp; $^{o}$C) | TSS (mg/L) | Organic C (g) | Ash content (g) | COD ($mgdm^{-3}O_2$) |
|---|---|---|---|---|---|
| Molesworth STW | | | | | |
| InfWW | 8.5 (17.1) | 36243.0 | | | 216.8 |
| EffWW | 7.8 (18.7) | 5.5 | | | 1.8 |
| PS | 5.7 (14.4) | 77386.5 | 0.95 | 0.07 | 162.6 |
| MSAF | 5.7 (14.4) | 8465.0 | 0.93 | 0.05 | 138.0 |
| SAF-1 | 7.1 (14.6) | 70793.5 | 0.95 | 0.07 | 82.0 |
| HS | 7.1 (14.2) | 3563.5 | 0.94 | 0.06 | 52.4 |
| Nottingham STW | | | | | |
| InfWW- 1 | 8.4 (12.7) | 797.9 | | | 36.0 |
| InfWW- 2 | 8.1 (12.1) | 599.1 | | | 28.6 |
| 1$^{o}$EffWW | 7.7 (12.2) | 897.0 | | | 48.0 |
| 2$^{o}$EffWW (north) | 7.7 (12.3) | 9.7 | | | 6.2 |
| 2$^{o}$EffWW (south) | 7.9 (12.2) | 9.9 | | | 5.1 |
| 2$^{o}$EffWW (new) | 8.0 (12.4) | 8.6 | | | 3.6 |

Note: InfWW = influent wastewater; EffWW = effluent wastewater; PS = primary sludge; MSAF = mixed SAF; SAF-1 = secondary aeration filter; HS = Humus sludge; InfWW-1= primary influent -1; InfWW = primary influent -2; 1$^{o}$EffWW = primary effluent; 2$^{o}$EffWW = primary influent (north); 2$^{o}$EffWW = primary influent (south); 2$^{o}$EffWW = primary influent (new).

Table 16 above shows the mean results as presented and explained in the worked calculation above to illustrate the total COD, organic C and TSS contents achieved from different samples collected from the two different STWs. The highest effluent COD concentration from both STWs was 6.2 $mgdm^{-3}O_2$, and this was below the consent limit of 15 mg $L^{-1}$ BOD shown in Table 16 [292], while the highest concentration of COD observed for influents were 216.8 $mgdm^{-3}O_2$ (RAF Molesworth STW) and 48 $mgdm^{-3}O_2$ (Nottingham STW), indicating the nature of sewage and different treatment processes can influence the kinetics of degradation [281]. The COD values obtained from the influent and effluent samples collected from Stoke Bardolph Nottingham and Molesworth STWs therefore compares well with the values reported for primary effluent (309 $mgdm^{-3}O_2$ COD), food processing sewage (7249 $mgdm^{-3}O_2$ COD), swine waste (67,444 $mgdm^{-3}O_2$ COD), secondary effluent (35 $mgdm^{-3}O_2$ COD ), lagoon (27089 $mgdm^{-3}O_2$ COD) and effluent (71 $mgdm^{-3}O_2$ COD) in a study [294]. This would indicate that the values produced in this work are in line with typical STW [294], ensuring sewage characerisics in the current work batch studies can be assumed to be representative of most municipal sewage types.

Similar studies carried out in two other places have also reported a range of 65 – 686 $mgdm^{-3}O_2$ [295] and 86 – 2852 $mgdm^{-3}O_2$ of COD [287] in sewage. In this current work, the difference in influent and effluent results as reported may form a basis of empirical relationship between COD

removal and the quality of treatment processes of sewage or wastewaters and these functions may be used to develop a gauge for sewage treatment to confirm adequate efficiency so that effluent quality is sufficiently good.

Treatment of organic matter in an activated sludge plant has also produced 22.7 – 253 mg $L^{-1}$ TSS [275] in a study, but in Nottingham and Molesworth STWs, the remaining suspended particulates after treatments represent the final effluent (TSS) concentrations found to be 5.5 mg $L^{-1}$ (Molesworth STW) and 10.0 mg $L^{-1}$ (Stoke Bardolph STW, Nottingham) compared to influent (TSS) concentrations of 797.9 mg $L^{-1}$ (Stoke Bardolph, Nottingham) and 36243.0 mg $L^{-1}$ (Molesworth STW) while 897.0 - 77386.5 mg $L^{-1}$ of TSS were observed for sewage sludges from both STWs. However, it is important to note the large difference in the levels of TSS from influent and effluent from the two STWs as quoted as this was evidence of extent of removals. Since domestic sewage differs in strength due to strong temporal variation, function controlling sludge characteristic can only be measured when effluent performance is compared to influent data and this was satisfactory with respect to our experimental results of TSS from both STWs. In addition, the TSS found in the effluents of the two STWs was significantly less than the 30 mg $L^{-1}$ consent limit set by UK Environment Agency Compliance - Discharge Consent as presented in Table 17 [292].

**Table 17**: UK Department for Environment, Food and Rural Affairs [292]

| Parameter | Consent Limit ($mgL^{-1}$) |
|---|---|
| Biochemical Oxygen Demand (BOD) | 15 |
| Ammoniacal Nitrogen expressed as N ($NH_4$-N) | 5 |
| Total Suspended Solids (TSS) | 30 |

In our current work, the average concentration of organic carbon found in all the four sewage types collected from Molesworth STW was 0.94 g per 1.0 gram of sewage samples. The levels of organic carbon were expectedly higher in the sludge due to myriads of materials as they facilitate compound degradation and removal, since the extent of drug removal to particulates/solids depends on the amenability of drugs to degradation on association to biological solids and possible volatilisation [297]. Unfortunately, direct comparison and evaluation of the organic carbon from sewage sample from the Stoke Bardolph STW Nottingham was impossible due to inability to obtain raw sewage due to site restrictions.

### 3.1.5. Occurrence of compounds in Stoke Bardolph STW Nottingham effluent.

The 12 compounds, including cocaine, benzoylecgonine, codein, diazepam, morphine, ephedrine, lidocaine, diacetylmorphine, ibuprofen, procaine, amphetamine, ecgonine methylester and an herbicide (bromacil) were detected in random wastewater sampleings from Nottingham STW effluents.

**Example of calculations of wastewaters concentration values for codeine from Stoke Bardolph STW Nottingham effluents collected on Monday, 22 February 2010:**

For example, using codeine GC TIC and calibration data **(**presented in Appendix 2A**).**

Linear calibration equation for codeine: **y** = 26382267.9**x**

Codeine TIC area: 2843877 **(**presented in Appendix 3)

Substitution gives, 2843877 / 26382267.9 = 0.1077 ng/ uL = **x**

**(Note:** Detector concentration in **ng/µL** (equivalent to **µg/mL** of real solution concentration**)**

Therefore, 0.1077 (ng/µL) **is equivalent to** 0.1077 (µg/mL) = **x**

Volume presented to detector: 100 uL

100uL (0.1 mL) represents 1000 mL of wastewater from which codeine was actually concentrated.

Since 0.1077 µg of codeine is contained in 1mL from calibration

Therefore, 0.1 mL will contain: 0. 01077 µg/mL = **x**

Conversion to ng (multiply by 1000):

0.01077 (µg/mL) x 1000 = **10.8 ng/mL**

The concentration values presented in Table 18 and 19 were raw values of the analytes, as their various percentage recoveries were not re-computed back into the calculations. However, it can be seen that our results can still be in line with the results obtained previously using different techniques. In the current work, the occurrence of drugs in wastewaters with the mass spectrometer in electron impact (EI) recorded in scan mode (scan range 45-550 *m/z*) gave abundant molecular ion of each compound and two precursor ions: cocaine (303>182; 303>82), benzoylecgonine (290>168; 290>150)codeine (371>178; 371>73), diazepam (285>256; 283>221), morphine (181>124; >181>96), ephedrine (230>179; 230>58), lidocaine (234>86; 234>58), diacetylmorphine (369>310; >369>268), ibuprofen (278>160; >278>73), procaine (235>99; >235>88), amphetamine (206>116; 206>73), ecgonine methyl ester (199>96; 199>82), bromacil (270>205; 270>187), caffeine (194>109; 194>67), and nicotine (161>84; 161>131). The fragmented ions produced the characteristic and reproducible *m/z* signals used for individual compound identification/quantification.

**Effluent at Stoke Bardolph Nottingham compared to other sites:**

Literature has shown many determinations of various illicit drugs and pharmaceuticals and their metabolites in different matrices of the aquatic environment [131-142, 298 -307]. In this current work, the effluents from RAF Molesworth didn't contain any drug concentrations and so is not discussed here.

**Table 18**: Analyte concentration of wastewater from Nottingham STW effluents (February- April 2010). (mean ± STD, n = 3)

| Compound (ng/L) | Sampling days | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Mon 22/02/10 | Thur 25/02/10 | Thur 04/03/10 | Thur 11/03/10 | Mon 15/03/10 | Thur 18/03/10 | Fri 23/04/10 | Sat 24/04/10 |
| Cocaine | 1.9 ± 0.02 | < 0.8 | < 0.8 | < 0.8 | < 0.8 | < 0.8 | < 0.8 | < 0.8 |
| Benzoylecgonine | < 4.6 | < 4.6 | < 4.6 | 32.9 ± 1.4 | 23.7 ± 4.7 | 12.2 ± 4.2 | < 4.6 | < 4.6 |
| Ecgonine methyl E | < 6.9 | < 6.9 | < 6.9 | < 6.9 | < 6.9 | < 6.9 | 8.1 ±2.04 | 17.3 ± 0.2 |
| Diacetylmorphine | < 4.3 | < 4.3 | < 4.3 | 49.1 ± 0.03 | < 4.3 | < 4.3 | < 4.3 | < 4.3 |
| Morphine | < 4.6 | < 4.6 | < 4.6 | 42.5 ± 2.0 | < 4.6 | < 4.6 | < 4.6 | < 4.6 |
| Diazepam | 3.9 ± 0.03 | < 1.9 | < 1.9 | 41.7 ± 1.3 | 58.9 ± 0.5 | 105.2 ± 0.5 | < 1.9 | < 1.9 |
| Amphetamine | < 3.3 | < 3.3 | < 3.3 | < 3.3 | < 3.3 | < 3.3 | 3.9 ±1.32 | < 3.3 |
| Ephedrine | < 0.1 | < 0.1 | 15.8 ± 1.8 | < 0.1 | < 0.1 | < 0.1 | < 0.1 | < 0.1 |
| Lidocaine | 37.4 ± 1.0 | 76.4 ± 3.5 | < 1.5 | 22.7 ± 0.6 | 21.2 ± 0.2 | 15.8 ± 0.1 | 70.5 ± 0.9 | 43.5 ± 1.4 |
| Ibuprofen | < 5.9 | < 5.9 | < 5.9 | 84.6 ± 3.8 | < 5.9 | < 5.9 | < 5.9 | < 5.9 |
| Procaine | 161.9 ± 13.4 | < 9.2 | < 9.2 | < 9.2 | 99.2±0.5 | < 9.2 | < 9.2 | < 9.2 |
| Amphetamine | < 3.3 | < 3.3 | < 3.3 | < 3.3 | < 3.3 | < 3.3 | 3.9 ±1.3 | < 3.3 |
| Bromacil (herbicide) | 1140.1 ± 32.8 | < 0.9 | 140.9 | < 0.9 | 338.0 | 196.7 | 1090.5 ± 14.4 | 1806.8 ± 5.7 |

Note: < detection limits (LOD)

**Table 19**: Analyte concentration of wastewater from Nottingham STW effluent (May-June 2011) (mean ± STD, n = 3)

| Compound (ng/L) | Sampling days | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Tue 4/05/10 | Wed 5/05/10 | Thur 6/05/10 | Fri 7/05/10 | Sat 8/05/10 | Sun 9/05/10 | Mon 10/05/10 | Sat 18/06/11 |
| Bromacil (herbicide) | 124.4 ± 15.6 | 995.6±71.7 | 1187.9±122.4 | 1052.4±110.6 | 1218.1±75.9 | 1191.8±110.1 | 1230.2±120.3 | < 0.9 |
| Lidocaine | < 1.5 | 781.8±132.9 | 358.2±63.2 | 211.9 ± 8.7 | 182.2± 10.9 | 75.7± 6.9 | 59.4±6.9 | < 1.5 |
| Codeine | < 0.2 | < 0.2 | < 0.2 | 5.8± 2.4 | 29.6 ± 2.9 | 14.4 ± 1.2 | 12.1 ± 0.5 | < 0.2 |
| Caffeine | < 8.6 | < 8.6 | < 8.6 | < 8.6 | < 8.6 | < 8.6 | < 8.6 | 213.4±13.0 |
| Nicotine | <12.7 | <12.7 | <12.7 | <12.7 | <12.7 | <12.7 | <12.7 | 252.5±2.2 |
| Ibuprofen | < 5.9 | < 5.9 | < 5.9 | < 5.9 | < 5.9 | < 5.9 | < 5.9 | 3147±3.5 |

Note: < detection limits (LOD)

It was expected because it's an RAF base so it is very unlikely any illicit drug metabolites will be detected in that effluent. The results of simple and random sampling operations of only effluents collected from the Stoke Bardolph STW Nottingham are discussed below:

**Cocaine compounds:**

As presented in Table 18 and 19, cocaine concentration was found to be 1.9 ng $L^{-1}$ with LOD of 0.8 ng $L^{-1}$ and this was within the range of 0.9-10.7 ng $L^{-1}$ reported from a site in Italy [137] and 01-100 ng $L^{-1}$ from 42 STWs in North East Spain [138]. However, the value of 1.9 ng $L^{-1}$ reported here was lower than 47 ng $L^{-1}$ detected from the other STWs effluents in Spain [131] and 10 ng $L^{-1}$ of cocaine in surface water had been from in Belgium [94], but cocaine levels between 1.2-26.0 ng $L^{-1}$ were also detected from three rivers in Italy by the Department of Environmental Health Sciences, Mario, Negri Institute for Pharmacological Research, Milan [132]. Illicit drugs and their metabolites analyses in urban waters have also reported 0.2-1 µg $L^{-1}$ for cocaine [137]. Cocaine was also found in the range of 25 – 248 ng $L^{-1}$ in 70% of the river water samples collected in Dublin, Ireland [134].

Benzoylecgonine, a major metabolite of cocaine was found in our result in the range of 12.2-32.9 ng $L^{-1}$ and this compares well with the values of 25 ± 5 ng $L^{-1}$ from River Po, Italy with total recoveries of ≥ 90% [124] but our value was much lower than 77 ng $L^{-1}$ previously reported from Germany STP effluents [140]. Similarly, samples of river water in Dublin detected benzoylecgonine in the range of 22 – 290 ng $L^{-1}$ [134]. Perhaps the strict legislation for unlawful possession of controlled substances in United Kingdom under the Misuse of Drugs Act, 1971 [121] could generally be the reason for the low detection of illicit drugs in the effluents.

Ecgonine methyester was 8.1 ng $L^{-1}$ with LOD of 6.9 ng $L^{-1}$ as detected in the current analysis. Interestingly, cocaine, benzoylecgonine, ecgonine methylester and amphetamine were all recorded on Monday and Friday sampling periods and the occurrence can be explained as being recreational drugs that are mostly used on weekends but this may also depend on HRT or delay [221].

**Opiates:**

Identification and measurement of illicit drugs and their metabolites in urban waters which included 80-200 ng $L^{-1}$ for morphine and 10 ng $L^{-1}$ for 6-acetylmorphine have also been carried out [137], whereas morphine, codein, diacetylmorphine and ephedrine from current study were 42.5 ng $L^{-1}$, 3.3 - 33.5 ng $L^{-1}$, 49.1 ng $L^{-1}$ and 15.8 ng $L^{-1}$ with their corresponding LODs as presented in Table 13, respectively. Also, the determination of illicit drugs using a triple quadrupole mass spectrometry equipped for water samples has reported 0.93 ng $L^{-1}$ for 6-acetylmorphine [133].

**Amphetamines**:

Amphetamine was found in Stoke Bardolph STW Nottingham effluent to be 8.1 ng $L^{-1}$ with LOD of 0.7 ng $L^{-1}$ but this was observed to be very much lower than the range of 110-210 ng $L^{-1}$ previously found in surface and urban wastewaters in the literature [135]. Also, 20 ng $L^{-1}$ for amphetamines has been reported in another measurement of illicit drugs and their metabolites in urban wastewaters

[137], but the value obtained from the current study falls within 0.4-2100 ng $L^{-1}$ recorded from experimental determination of psychoactive stimulatory drugs in wastewaters in north-eastern Spain [138].

**Other substances detected in effluents:**

High levels of procaine (161.9 ng $L^{-1}$) with LOD of 9.1 ng $L^{-1}$ in wastewater may be explained as due to its local use as anesthetic and its presence in wastewater could be explained since comparable concentration data in the chemical literature could not be found, we may as well assume that this is the first time procaine presence would be reported which make Nottingham STW effluent analysis significantly different.

Diazepam concentrations in Stoke Bardolph STW Nottingham were between 3.9-105.2 ng $L^{-1}$ and the results compares well with an investigation of levels of community consumption of illicit drugs and abused pharmaceuticals with 38 ng $L^{-1}$ - 127 ng $L^{-1}$ of diazepam estimated in the analysis of 500mL of wastewater samples in Dublin, Ireland. Morphine, methadone and tempazepam were the other substances detected, while analytical sensitivity of the LC-MS-MS to test the sample matrix was in the range of 1 – 10 g $L^{-1}$ in the water [134].

Ibuprofen was initially detected in effluent as 84.6 ng $L^{-1}$ with LOD of 5.9 ng $L^{-1}$ in March 2010 and this was lower than 5.8 ug $L^{-1}$ found in Spain STWs in 2004 [263]. But further sampling of wastewaters in June 2011 showed the presence of ibuprofen (3147.0 ng $L^{-1}$), caffeine (213.4 ng $L^{-}1$) and nicotine (252.5 ng $L^{-1}$) with LODs of 5.9, 8.6 and 6.4 ng $L^{-1}$, respectively.These values are higher than the ones previously reported in a Spain STW influent with concentration between 2.6 and 5.7ng $L^{-1}$, respectively [116] and the 175 – 198 ng $L^{-1}$ of nicotine also found in three rivers in Spain [351]. However, a range of 428 – 3786 ng $L^{-1}$ of nicotine detected in the simultaneous analysis of hospital effluents in Spain using GCMS which was higher than the levels found in our study[352]. Stumpf *et al* [286] has also reported ibuprofen concentration of 0.3 ug $L^{-1}$ in Brazilian STW influent. Since these drugs are common analgesic and antipyretic drugs commonly used for the relief of fever, headaches and other minor aches and pains, their presence in wastewaters may be as a result of medicinal and therapeutic use rather than abused or illicit consumption [134].

Nicotine, likely from tobacco sources and its high concentration in effluent may suggest possibility of its higher concentration in influents, but a variety of processes during wastewater treatment, culminating in transformation of organic contaminants leading to a reduction in concentration of significant amounts of synthetic contaminants, during sewage treatment and in effluent would have drastically reduced its concentration [111].

The systematic sampling approach was adopted in the second sampling section to monitor possible daily variation in the concentration of analytes between 04 May – 10 May 2010 as presented in Table 19 showed bromacil, codeine and lidocaine mostly detected in all the seven daily samples collected with varying concentration ranges from bromacil (124.4 -1230.2ng $L^{-1}$), codeine (5.8 – 29.6 ng $L^{-1}$)

and lidocaine (59.4 -781.8 ng $L^{-1}$) with corresponding LODs as presented in Table 13. Lidocaine and codeine levels in dissimilar distribution pattern in the river outflow were also observed, but since samplings were systematic, the dilution within the receiving water was another possibility of concentration gradient, since the levels detected would be expected to be significantly decreased with increasing distance from the discharge point. Like procaine, there is paucity of information in the literature on the level of lidocaine in wastewaters to compare results.

Bromacil is an herbicide used in agriculture, its large presence in effluent could not be explained as there was no rainfall during the sampling periods to show the possibility of run–off of neighbouring farmlands. However, the herbicide's spread could possibly be explained in terms of delayed-run-off at the STW which sometimes leads to particular pattern that cannot be explained as the sampling was executed for 7 days. Deliberate deployment of herbicides on farmlands could possibly be another reason for such high concentration of bromacil in the effluent. Bromacil could increasingly become a major pollutant since it has been reported previously in 14-36% of wastewater samples collected weekly in a typical South Florida watershed where evaluation of its concentration ranged from 0.5 – 0.6 ng $L^{-1}$ [285]. This may also apply to UK going by the spread and very high concentrations of bromacil found in the Nottingham wastewater effluent as reported in the current work. Unfortunately, there was no UK reference of bromacil concentrations in wastewaters.

Similar residues of pharmaceutical and illicit compounds were reported comprehensively in the survey of published data from EU countries and USA with different levels and distribution found in different STWs as presented in Table 4 [131 - 142]. The results from our current work further confirmed conventional sewage treatment works as the most significant routes through which the drugs enter the environment but at different rates compared to other sites. Though these substances may have possibly escaped detection due to our random sampling strategy, nonetheless the presence of some illicit compounds in the results we obtained confirmed them as new challenges of pollutants in wastewaters unlike other pharmaceutical compounds that are coming from domestic sewage, industrial, hospital and accidental sources and limited data available for most illicit chemicals have made treatability in STWs difficult to assess and therefore information from experimental data on degradation studies might help STWs upgrade treatment capability. Systemic sampling strategy at appropriately determined intervals may allow the evaluation of the pollution in the levels of the new analytes that discharge through STWs to the environment to be monitored and appropriately evaluated.

Unfortunately, despite the efforts of Stoke Bardolph STW Nottingham in the reduction of the concentrations of many compounds from reaching surface waters, some of these compounds were still detected in the effluents.

The purpose of Table 20 facilitates comparison of our SPE GCMS method limits of detection with percent recoveries that were generally > 70% (Table 13) with the values reported in Dublin, Republic of Ireland [133] and Italy [134], using SPE LC-MS-MS techniques.

**Table 20**: Method validation for the analysis of illicit drugs and their metabolites in waters from Italy & UK

| **Analyte** | **Zuccato *et al* [ 133]** | | | **Bones *et al* [ 134]** | | |
|---|---|---|---|---|---|---|
| | % recovery (mean ±SD) | LOD (ng $L^{-1}$) | LOQ ($ngL^{1}$ | % recovery (mean ± SD) | LOD (ng $L^{-1}$) | LOQ (ng $L^{-1}$) |
| Benzoylecgonine | 96 ± 6.7 | 0.03 | 0.10 | 53 ± 3 | 1 | 2 |
| Cocaine | 105 ± 1.9 | 0.04 | 0.13 | 56 ± 2 | 1 | 2 |
| Cocaethylene | 105 ± 0.2 | 0.02 | 0.07 | 65 ± 3 | 1 | 5 |
| Amphetamine | 101 ± 4.5 | 0.19 | 0.65 | 52 ± 1 | 7 | 22 |
| Morphine | 85 ± 1.2 | 0.16 | 0.55 | 4 ± 0 | 257 | 856 |
| 6-acetylmorphine | 87 ± 3.5 | 0.28 | 0.93 | | | |
| Codeine | 107 ± 8.6 | 0.19 | 0.62 | | | |
| 6-acetylcodeine | 114 ± 8.3 | 0.09 | 0.31 | | | |
| Methadone | 104 ± 10 | 0.02 | 0.07 | 55 ± 0 | 4 | 14 |

# CHAPTER 4: Data analysis in batch studies

Batch studies data of each of the six compounds (cocaine, benzoyecgonine, heroin, 6-monoacetylmorphine, morphine and diazepam) to evaluate their biodegradability under different experimental conditions is presented. The experimental parameters for the batch studies for each drug include:

- Evaluation of the degradation and removal of drugs in both primary and secondary sewage samples at 19 ± 0.5$^{o}$C.
- Evaluation of biotic (19 ± 0.5$^{o}$C) and abiotic (4 ± 0.5$^{o}$C) degradation processes of compounds in sewage.
- Evaluation of the effect of suspended solids on the removal of compounds.

Sorption and degradation are two important removal mechanisms of compounds from the Molesworth sewage treatment works, explaining the detailed calculation of data in each column of Tables 21-33.

### 4.2.1 Possible uncertainties/ sources of errors in the experimental data.

This section acknowledges some steps that may have introduced some possible uncertainties/errors in the quantification of the data as highlighted below.

- Quantification and recovery data: The highest calibration concentrations of compounds used in Appendix 2B for the quantification and recovery experiment were lower than the spiked concentrations of 500 ug/ mL, and we recognised these could introduce some possible error on quantification of those recoveries. However, linearity with very good coefficient of variations for all compounds were produced from the lower concentrations used for quantification.

- Recoveries from solid phase: No recovery values from solid phase were used as we used only the recovery obtained from the liquid phase for both phases and this may introduce uncertainty in the data. A poor recovery could significantly underestimate the amount of illicit drug sorbed to the particulate phase and hence ensuing calculations could overestimate degradation rates

- Detection limits: Absence or low detection of some compounds in analysed wastewaters were reported as < detection limits values (lower than detection limits) of corresponding drugs.

- Calculation of water-solid partition coefficient (Kd): If degradation is taking place as well as sorption, the estimated (measured) Kd is subject to error and may not accurately reflect the actual physical Kd.

**Worked example of cocaine degradation data in Molesworth primary sludge in Table 21 below:**

To obtain degradation data of cocaine in primary sludge batch tests at 19 ± 0.5$^{o}$C:

Initial spiked cocaine concentration = 12.500 mg in 250 mL flask (50 mg $L^{-1}$ of sewage).

Each batch of sample contained 0.5 mg (500 µg in 10 mL).

Every 15 min, 20 mL of aliquot (2 x10 mL) were removed out and analysed over a total period of 3h.

The duplicate samples 1 and 2 had cocaine distributed in both aqueous and solid phases as calculated in the following steps using calibration graphs in Appendix 2B:

Cocaine Calibration Equation (y) =13223636.07**x**

**(Note:** Detector concentration of **ng/µL** (equivalent to **µg/mL** of sample solution concentration**)**

**Step 1:**

Cocaine **TIC area** for sample 1 (aqueous) = 282496537
Cocaine **TIC area** for sample 2 (aqueous) = 285194159

Cocaine **TIC area** for sample 1 (solid) = 69133169
Cocaine **TIC area** for sample 2 (solid) = 70759677

**Step 2:**

Substitution into above calibration equation of cocaine gives:
sample 1 (aqueous): 282496537 = 13223636.07**x**
sample 2 (aqueous): 285194159 = 13223636.07**x**

sample 1 (solid): 69133169 = 13223636.07**x**
sample 2 (solid): 70759677=13223636.07**x**

**Step 3:**

Since duplicate of 10 mL of aliquot were concentrated by drying under nitrogen into 1 mL for measurement, therefore:
Sample 1 (aqueous): **x** = 21.363 µg in 10 mL
Sample 2 (aqueous): **x** = 21.567 µg in 10 mL
Mean = 21.466 µg in 10 mL

Sample 1 (solid): **x** = 5.228 µg in 10 mL
Sample 2 (solid): **x** = 5.351 µg in 10 mL
Mean = 5.29 µg in 10 mL

**Step 4:**

The concentrations in 250 mL flask with % recovery of cocaine in PS (Table 14) re-computed back:

Sample 1 (aqueous) = (21.363 µg x 25) = 534.075 µg in 250 mL
Sample 2 (aqueous) = (21.567 µg x 25) = 539.175 µg in 250 mL
**Mean** = 536.625 µg in 250 mL ÷ 91.5% (cocaine recovery in PS) = 586.5 µg in 250 mL

Sample 1 (solid) = (5.228 µg x 25) = 130.700 µg in 250 mL
Sample 2 (solid) = 5.351 µg x 25) = 133.775 µg in 250 mL
**Mean** = 132.238 µg in 250 mL ÷ 91.5% (cocaine recovery in PS) = 144.522 µg in 250 mL

The remaining sewage sample volume in the flask would be 230 mL.
After 30 min, another 20 mL of aliquot (2 x10 mL) were also removed and analysed as above.
The concentrations would now be multiplied by 23 to give the cocaine concentration left in 230 mL
After 45 min, another 20 mL of aliquot (2 x10 mL) was also removed and analysed as above.
The concentrations would now be multiplied by 21 to give the cocaine concentration left in 210 mL

Duplicate samples were taken at every time scale (15 min) and cocaine concentrations in each of the successive 190, 170, 150, 130, 110, 90, 70, 50, 30 mL of sewage samples were calculated for 3hrs.

**Spiked drugs removal rates calculations:**

$C_{degrad} = C_{initial} - C_{aqueous} - C_{solids}$ [eqn 6]

'C' represents concentrations of each drug in different media.

Therefore, COC degraded ($C_{degrad}$) = [12500 – 586.5 – 144.5]

=11769.0 µg (in 250 mL flask).

The sorption behaviour using apparent solid-water partition coefficient ($K_d$, in Kg $L^{-1}$) in treatment units was calculated from eqn 7 below [353]:

$$K_d = C_{sorbed}/SS.\ C_{soluble} \quad \text{[eqn 7]}$$

Where $C_{sorbed}$ = chemical concentration in sorbed phase (µg $L^{-1}$) and $C_{soluble}$ is concentration in aqueous (µg $L^{-1}$) and SS = suspended solids concentration (Kg $L^{-1}$) using data from Table 16.

So, for cocaine in PS, we have 21.5 µg/10 mL, or 2,150 µg/L in aqueous phase and 5.29 µg in the solids from 10 mL .

From Table 15, the PS has 77, 387 mg/L solids and so in 10 mL sample, 773 mg.

We have 5.29/773 µg/mg = 6835 µg/kg

Therefore, $K_d$ = 6835/2150 = 3.17 Kg/L

Any difference in the concentration between original drug concentration in the raw samples and concentration in filtered aqueous phase is accounted for by association with solids or degradation. Drug concentration as percent removals were therefore expressed using the equation 8 below [281]:

$$\% \text{ removal} = \frac{100\ [\text{initial drug}] - [\text{final drug in aqueous phase}]}{[\text{Initial drug}]} \quad \text{[eqn 8]}$$

$$= 100 \times [12500 - 586.5] \div [12500]$$

$$= 95.3\%$$

Concentration data in the batch experiment are contained in Appendix 4 expressed in duplicate measurement and presented below in Tables 21 – 40.

### 4.2.2 Evaluation of degradation and removal of drugs in primary and secondary sludge treatment samples.

For our batch tests, a 3h contact time was selected (though 2h was average period of a process unit in RAF Molesworth STW under the hydraulic conditions) to extensively monitor the degradation processes. Samples of primary sludge and SAF-1 (250 mL) at 19± 0.5$^{o}$C were spiked with 12.5 mg of each drug (cocaine, benzoylecgonine, heroin, morphine, 6-monoacetylmorphine and diazepam). The samples were mixed thoroughly and 2 x 10 mL aliquots were removed at timed interval (15 min) over a period of 3h. Table 21 shows the degradation of cocaine in both primary and secondary aerated sludge increased with exposure time with corresponding increment in their degradation products.

A very rapid abiotic removal was observed in the first 15 min of cocaine degradation in primary sludge in batch sudies. This observation was rather different from generally accepted positions from the literature that no significant degradation occurs in primary sedimentation [354]. But over a period of 3h, the cocaine showed decline in concentration with its uneven distribution between both solid and aqueous phases in a pattern that reflects their hydrophobic/hydrophilic nature. As compounds become lesser in concentration, the degradation products such as ecgoine methyl ester and cocaethylene were simultaneously identified and quantified. As degradation progressed the degradation products pattern could not be properly followed due to their diffuse nature of being further degraded as they are formed. The benzoylecgonine (a principal hydrolysis product) could not be detected possibly due non – hydrolysis as cocaine has been reported to be stable in pond water in pH = 2 at 25$^{o}$C for 5 days [132] or low detection. But the presence of ecgonine methyl ester and cocaethylene (other bio-degradation products of cocaine) were observed as presented in Appendix 4 (Table 1B, 1C, 2B and 2C) confirming the presence of bio-degradation processes in primary sludge.It must however be generally emphasised that compound removal from aqueous phase in sewage may not indicate their complete degradation.

However, the partition fractions of each batch test with drug and their related metabolites were analysed and related to sludge types under the same condition. Similar degradation was observed in both sludges showing that biological aeration sludge in secondary treatment had little influence. The sorption behaviour of compounds was assessed by solid-water partition coefficient ($K_{d,}$ L/Kg), as calculated by $C_{sorbed}/SS.C_{soluble}$ (where $C_{sorbed}$ = chemical concentration in sorbed phase, $C_{soluble}$ is concentration in aqueous SS = suspended solids). The $K_d$ values were calculated for each time period from batch studies data, the 2-3 HRT is the usual processing time in the Molesworth STW. The observed low solid-water partition coefficient for cocaine ($K_d$ < 14) in both sludges indicated low sorption as the removal mechanism but since recovery experiment for the solid could not completed, further experiment is needed fo confirmation. From the results presented here, the $K_d$ values for cocaine and other drugs (Table 20- 25) ranged from 1.2 – 68.1 were however generally very much lower than the range of 12300 – 37700 L/Kg calculated for detected ten quinolone and fluoroquinolone antibiotics in a municipal SWT [290].

**Table 21**: Degradation of cocaine in Primary and SAF-1 sludge batch tests at 19 ± 0.5°C (Data derived from, Table 2A & 5A in Appendix 5)

| Time (min) | Conc. of cocaine (µg in 250 ml of primary sludge) | | | | | Conc. of cocaine (µg in 250 ml of SAF-1 sludge) | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | COC soluble | COC sorbed | COC degraded | $K_d$* | Removal (%) | COC soluble | COC sorbed | COC degraded | $K_d$* | Removal (%) |
| 15 | 586.5 | 144.5 | 11769.0 | 3.2 | 95.3 | 4619.4 | 3983.6 | 3897.0 | 12.2 | 63.0 |
| 30 | 433.3 | 186.2 | 11880.5 | 5.6 | 96.5 | 4198.6 | 3635.0 | 4666.4 | 12.2 | 66.4 |
| 45 | 435.0 | 94.5 | 11970.5 | 2.8 | 96.5 | 3367.1 | 3151.9 | 5981.0 | 13.2 | 73.1 |
| 60 | 425.8 | 87.9 | 11986.3 | 2.7 | 96.6 | 3405.2 | 2384.7 | 6710.0 | 9.9 | 72.8 |
| 75 | 371.6 | 110.6 | 12017.8 | 3.8 | 97.0 | 3003.1 | 2109.6 | 7387.3 | 9.9 | 76.0 |
| 90 | 225.3 | 80.8 | 12193.9 | 4.6 | 98.2 | 2553.9 | 1391.4 | 8554.7 | 7.7 | 79.6 |
| 105 | 221.3 | 63.3 | 12215.5 | 3.7 | 98.2 | 1722.5 | 853.9 | 9923.6 | 7.0 | 86.2 |
| 120 | 195.8 | 50.3 | 12253.8 | 3.3 | 98.4 | 1413.1 | 1018.8 | 10068.1 | 10.2 | 88.7 |
| 135 | 158.1 | 53.2 | 12288.6 | 4.4 | 98.7 | 1155.2 | 659.1 | 10685.8 | 8.1 | 90.8 |
| 150 | 122.1 | 38.0 | 12339.9 | 4.0 | 99.0 | 658.3 | 579.1 | 11262.6 | 12.4 | 94.7 |
| 165 | 82.6 | 22.8 | 12394.6 | 3.6 | 99.3 | 502.4 | 311.1 | 11686.6 | 8.8 | 96.0 |
| 180 | 46.0 | 9.5 | 12444.5 | 2.7 | 99.6 | 306.2 | 229.1 | 11964.6 | 10.6 | 97.6 |

*$K_d$ is solid-water partition coefficient (Kg $L^{-1}$).

Over 98% removal of cocaine was achieved in 3h. Comparing removal efficiencies from concentration of cocaine in raw pimary sewage and SAF-1 gave about 10% difference in 2h. Also in every 15 min, empirical relationship exists between cocaine dissolved, sorbed and degraded as they all relate to each other and added up to 12500 ug. This approach was later applied in mass balance calculations to capture the degradation process and transport of compounds in STW units.

The pattern of degradation observed for cocaine was the same for benzoylecgonine (BZE) but with relatively higher degradation in primary sludge (Table 22). As one of the major human metabolites of cocaine, its biodegradation studies in aquatic environment become important to understand the final fate of cocaine. In the current study, its sorption potential ($K_d$ < 2.5) indicated biodegradation as predominant removal process compare to sorption to solids. But the removal efficiencies of BZE in both PS and SAF-1 also showed 10% difference in 2h.

Also, morphine is one of the metabolites of heroin; it was selected primarily to monitor its biodegradability and its final fate. Therefore, Table 23 show the result of a relative higher degradation of morphine within a biological secondary aerated sludge compare to primary sludge as observed from our batch studies, but like cocaine and BZE, a lower sorption potential of 0.1 – 1.3 made degradation a significant removal process with 85 – 99% and 94 – 100% removal of morphine.

**Table 22**: Degradation of BZE in PS and SAF-1 sludge batch tests at 19 ± 0.5°C (Data derived from Table 15 & 17 in Appendix 5)

| Time (min) | Conc. of BZE (µg in 250 ml of primary sludge) | | | | | Conc. of BZE (µg in 250 ml of SAF-1 sludge) | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | BZE soluble | BZE sorbed | BZE degraded | $K_d$ | Removal (%) | BZE soluble | BZE sorbed | BZE degraded | $K_d$ | Removal (%) |
| 15 | 406.3 | 89.8 | 12003.9 | 2.9 | 96.7 | 6117.5 | 2824.4 | 3558.1 | 6.5 | 51.1 |
| 30 | 355.4 | 110.1 | 12034.5 | 4.0 | 97.2 | 3919.4 | 1755.7 | 6824.9 | 6.3 | 68.6 |
| 45 | 319.4 | 88.4 | 12092.2 | 3.6 | 97.4 | 3112.9 | 1175.1 | 8211.9 | 5.3 | 75.1 |
| 60 | 288.7 | 88.8 | 12122.5 | 4.0 | 97.7 | 2615.1 | 1656.0 | 8228.8 | 9.0 | 79.1 |
| 75 | 240.9 | 141.8 | 12117.3 | 7.6 | 98.1 | 2495.2 | 1443.9 | 8561.0 | 8.2 | 80.0 |
| 90 | 206.2 | 126.7 | 12167.2 | 7.9 | 98.4 | 2069.2 | 1183.1 | 9247.7 | 8.1 | 83.4 |
| 105 | 166.3 | 136.4 | 12197.2 | 10.6 | 98.7 | 1746.9 | 1033.7 | 9719.4 | - | 86.0 |
| 120 | 119.4 | 119.3 | 12261.3 | 12.9 | 99.0 | 1505.9 | 856.9 | 10137.2 | - | 88.0 |
| 135 | 101.9 | 123.2 | 12274.9 | 15.6 | 99.2 | 1256.0 | 692.7 | 10551.4 | - | 90.0 |
| 150 | 77.3 | 113.0 | 12309.8 | 18.9 | 99.4 | 912.6 | 519.9 | 11067.5 | - | 92.7 |
| 165 | 43.3 | 86.1 | 12370.6 | 25.7 | 99.7 | 600.8 | 368.3 | 11530.9 | 8.7 | 95.2 |
| 180 | 25.6 | 63.3 | 12411.1 | 31.9 | 99.8 | 364.8 | 211.3 | 11923.8 | 8.2 | 97.1 |

Though many of the biological wastewater units have different characteristics, yet most compounds tend to exhibit general tendencies to accumulate to solids and sediments. But the removal rate of morphine appears to be related to degradation processes and less of sorption. Gradual removal rate of morphine in both primary and secondary sewage with the decline in the concentration of morphine may be due to bio-degradation mechanism.

In this current work, comparable removals of benzoylecgonine with cocaine infer that sorption may not be the only removal mechanisms. Gradual decline in the concentration profile of benzoylecgonine (Table 22), morphine (Table 23), 6-MAM (Table 24) and diazepam (Table 26) were apparent but degradation producs of the two compounds could not be identified in both primary and SAF-1. However, the measurement of the degradation products of heroin (morphine and 6-MAM) provided evidence of degradation as presented in the Appendix 4 (Table 36b, 36c, 37b, 37c and 38b) in PS and SAF-1. Again, degradation products occurring simultaneously limited the accurate measurements of all metabolites as the degradation was going on.

**Table 23**: Degradation of Morphine in PS and SAF-1 sludge batch tests at 19 ± 0.5°C (Data derived from Table 22 & 24 in Appendix 5)

| Time (min) | Conc. of MOR (µg in 250 mL of primary sludge) | | | | | Conc. of MOR (µg in 250 mL of SAF-1 sludge) | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | MOR soluble | MOR sorbed | MOR degraded | $K_d$ | Removal (%) | MOR soluble | MOR sorbed | MOR. degraded | $K_d$ | Removal (%) |
| 15 | 1901.8 | 429.3 | 10168.9 | 3.1 | 84.8 | 698.7 | 98.6 | 11702.7 | 3.3 | 94.4 |
| 30 | 1678.9 | 430.0 | 10391.1 | 3.9 | 86.6 | 411.2 | 128.4 | 11960.4 | 4.2 | 96.7 |
| 45 | 1434.8 | 361.7 | 10703.5 | 3.7 | 88.5 | 363.2 | 116.0 | 12020.8 | 4.0 | 97.1 |
| 60 | 1178.4 | 355.8 | 10965.8 | 3.3 | 90.6 | 329.0 | 91.0 | 12080.0 | 3.6 | 97.4 |
| 75 | 1029.2 | 398.2 | 11072.6 | 5.1 | 91.8 | 248.9 | 68.6 | 12182.5 | 5.6 | 98.0 |
| 90 | 843.7 | 304.1 | 11352.2 | 6.6 | 93.3 | 210.5 | 62.2 | 12227.4 | 7.2 | 98.3 |
| 105 | 641.7 | 283.1 | 11575.2 | 6.2 | 94.9 | 171.2 | 63.3 | 12265.5 | 7.4 | 98.6 |
| 120 | 486.8 | 192.3 | 11820.9 | 6.4 | 96.1 | 145.3 | 60.5 | 12294.1 | 7.9 | 98.8 |
| 135 | 399.3 | 207.9 | 11892.8 | 5.0 | 96.8 | 87.7 | 40.7 | 12371.6 | 5.5 | 99.3 |
| 150 | 287.4 | 146.2 | 12066.4 | 7.4 | 97.7 | 41.7 | 36.1 | 12422.2 | 8.1 | 99.7 |
| 165 | 163.7 | 125.3 | 12211.0 | 6.7 | 98.7 | 24.1 | 25.9 | 12449.9 | 7.3 | 99.8 |
| 180 | 58.3 | 73.8 | 12367.9 | 4.6 | 99.5 | 13.7 | 15.3 | 12471.0 | 5.1 | 99.9 |

Another human metabolite of heroin studied in both primary and secondary treatment sludge was 6-monoacetylmorphine (6-MAM) and results presented in Table 24. The idea was to observe the 6-MAM trend of degradation to know its final fate. Unlike the four compounds studied earlier, i 6-ACM showed similar degradation in the primary sludge with corresponding low sorption potential ($K_d$ < 9) and removal efficiency of between 98 – 100 % in PS, but removal rates were relatively slower after 2h of exposure in SAF-1 (19 – 86 %) from the result shown. In real time, after 2h treatment there are possibilities of most compounds in aqueous phase to pass on to the next treatment tank due to the average 2 HRT for RAF Molesworth. However, the observed variability in degradationpattern may likely make the removal of 6-MAM faster in primary sludge than in SAF-1. Heroin and diazepam in Table 25 and 26 follow the pattern observed in cocaine. The heroin has over 96% removal in both sludges, but in Table 26, diazepam showed more removal in primary sludge.

**Table 24**: Degradation of 6-MAM in PS and SAF-1 sludge batch tests at 19 ± 0.5°C (Data derived from Table 29 & 31 in Appendix 5)

| Time (min) | Conc. of 6-ACM (µg in 250 mL of primary sludge) | | | | | Conc. of 6-ACM (µg in 250 mL of SAF-1 sludge) | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 6MAM soluble | 6MAM sorbed | 6MAM. degraded | $K_d$ | Removal (%) | 6MAM soluble | 6MAM sorbed | 6MAM. degraded | $K_d$ | Removal (%) |
| 15 | 213.7 | 14.9 | 12271.4 | 1.6 | 98.3 | 10135.8 | 1693.6 | 670.6 | 2.3 | 18.9 |
| 30 | 160.8 | 13.4 | 12325.8 | 1.3 | 98.7 | 7091.8 | 1045.0 | 4363.2 | 2.7 | 43.3 |
| 45 | 123.8 | 11.1 | 12365.1 | 1.2 | 99.0 | 6023.9 | 1043.5 | 5432.6 | 2.5 | 51.8 |
| 60 | 101.6 | 9.1 | 12389.3 | 1.2 | 99.2 | 6195.8 | 916.8 | 5387.4 | 2.6 | 50.4 |
| 75 | 70.3 | 9.2 | 12420.6 | 2.5 | 99.4 | 5234.1 | 668.1 | 6597.8 | 1.8 | 58.1 |
| 90 | 60.1 | 8.4 | 12431.5 | 1.9 | 99.5 | 3724.6 | 663.9 | 8111.5 | 2.5 | 70.2 |
| 105 | 59.8 | 10.0 | 12430.2 | 3.3 | 99.5 | 2302.7 | 896.1 | 9301.3 | 5.2 | 81.6 |
| 120 | 47.0 | 7.9 | 12445.0 | 3.0 | 99.6 | 1750.4 | 754.9 | 9994.7 | 6.3 | 86.0 |
| 135 | 24.8 | 7.6 | 12467.6 | 2.2 | 99.8 | 1360.5 | 606.9 | 10532.6 | 5.4 | 89.1 |
| 150 | 18.9 | 7.1 | 12474.0 | 3.1 | 99.8 | 1042.2 | 517.3 | 10940.4 | 7.0 | 91.7 |
| 165 | 13.4 | 5.1 | 12481.5 | 5.1 | 99.9 | 799.2 | 292.4 | 11408.4 | 5.3 | 93.6 |
| 180 | 6.2 | 3.3 | 12490.5 | 7.1 | 100.0 | 394.6 | 148.8 | 11956.6 | 5.2 | 96.8 |

**Table 25**: Degradation of Heroin in PS and SAF-1 sludge batch tests at 19 ± 0.5°C (Data derived from Table 36A & 38A in Appendix 5)

| Time (min) | Conc. of HER (µg in 250 mL of primary sludge) | | | | | Conc. of HER (µg in 250 mL of SAF-1 sludge) | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | HER soluble | HER sorbed | HER. degraded | $K_d$ | Removal (%) | HER soluble | HER sorbed | HER. degraded | $K_d$ | Removal (%) |
| 15 | 480.0 | 677.0 | 11343.0 | 18.2 | 96.2 | 55.4 | 80.0 | 12364.6 | 9.7 | 99.6 |
| 30 | 414.1 | 652.6 | 11433.3 | 20.4 | 96.7 | 46.0 | 72.5 | 12381.4 | 26.1 | 99.6 |
| 45 | 286.3 | 459.4 | 11754.3 | 20.8 | 97.7 | 47.1 | 64.8 | 12388.1 | 19.4 | 99.6 |
| 60 | 276.4 | 0.0 | 12223.6 | 0.0 | 97.8 | 36.3 | 62.4 | 12401.3 | 26.9 | 99.7 |
| 75 | 204.2 | 0.0 | 12295.8 | 0.0 | 98.4 | 33.4 | 38.0 | 12428.6 | 16.1 | 99.7 |
| 90 | 167.7 | 0.0 | 12332.3 | 0.0 | 98.7 | 27.8 | 38.9 | 12433.3 | 19.8 | 99.8 |
| 105 | 140.3 | 0.0 | 12359.7 | 0.0 | 98.9 | 21.6 | 28.6 | 12449.9 | 18.7 | 99.8 |
| 120 | 80.4 | 0.0 | 12419.6 | 0.0 | 99.4 | 17.7 | 17.3 | 12465.0 | 12.0 | 99.9 |
| 135 | 61.0 | 0.0 | 12439.0 | 0.0 | 99.5 | 16.2 | 21.4 | 12462.4 | 18.7 | 99.9 |
| 150 | 34.7 | 0.0 | 12465.3 | 0.0 | 99.7 | 9.2 | 15.9 | 12474.9 | 24.4 | 99.9 |
| 165 | 24.0 | 0.0 | 12476.0 | 0.0 | 99.8 | 7.0 | 6.3 | 12486.7 | 12.8 | 99.9 |
| 180 | 14.7 | 0.0 | 12485.3 | 0.0 | 99.9 | 4.6 | 3.4 | 12492.0 | 15.1 | 100.0 |

**Table 26**: Degradation of Diazepam in PS and SAF-1 sludge batch tests at 19 ± 0.5°C (Data derived from Table 43A & 45 in Appendix 5)

| Time (min) | Conc. of DIAZ (µg in 250 mL of primary sludge) | | | | | Conc. of DIAZ (µg in 250 mL of SAF-1 sludge) | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | DIAZ soluble | DIAZ sorbed | DIAZ. degraded | $K_d$ | Removal (%) | DIAZ soluble | DIAZ sorbed | DIAZ. degraded | $K_d$ | Removal (%) |
| 15 | 2015.7 | 4919.3 | 5565.1 | 10.5 | 83.9 | 131.2 | 447.1 | 11921.7 | 26.5 | 99.0 |
| 30 | 1547.0 | 4504.2 | 6448.8 | 12.6 | 87.6 | 120.7 | 400.3 | 11979.0 | - | 99.0 |
| 45 | 1401.2 | 2933.2 | 8165.6 | 10.0 | 88.8 | 106.5 | 349.3 | 12044.1 | 46.4 | 99.1 |
| 60 | 1093.9 | 3229.7 | 8176.4 | 10.7 | 91.2 | 93.8 | 281.6 | 12124.6 | 36.8 | 99.2 |
| 75 | 979.9 | 3028.4 | 8491.7 | 13.3 | 92.2 | 83.3 | 254.8 | 12161.8 | 31.8 | 99.3 |
| 90 | 653.6 | 2690.2 | 9156.2 | 17.6 | 94.8 | 71.0 | 208.8 | 12220.2 | 41.6 | 99.4 |
| 105 | 597.8 | 2104.9 | 9797.3 | 15.2 | 95.2 | 60.5 | 168.8 | 12270.7 | 51.5 | 99.5 |
| 120 | 439.4 | 1887.4 | 10173.2 | 18.6 | 96.5 | 50.5 | 142.3 | 12307.2 | 46.0 | 99.6 |
| 135 | 307.7 | 1506.1 | 10686.2 | 21.2 | 97.5 | 36.8 | 112.8 | 12350.4 | 43.4 | 99.7 |
| 150 | 190.8 | 1301.3 | 11008.0 | 29.5 | 98.5 | 28.6 | 84.8 | 12386.6 | 46.1 | 99.8 |
| 165 | 118.8 | 883.8 | 11497.4 | 32.2 | 99.0 | 11.8 | 58.4 | 12429.8 | 69.9 | 99.9 |
| 180 | 37.0 | 508.1 | 11954.9 | 66.7 | 99.7 | 6.9 | 33.1 | 12460.0 | 68.1 | 99.9 |

#### 4.2.2.1. Removal of drugs during primary sewage treatment:

Figure 23 further demonstrates the degradation as a removal mechanism for all drugs at different rates with decline in various compounds concentrations distributed between aqueous and solid phases.

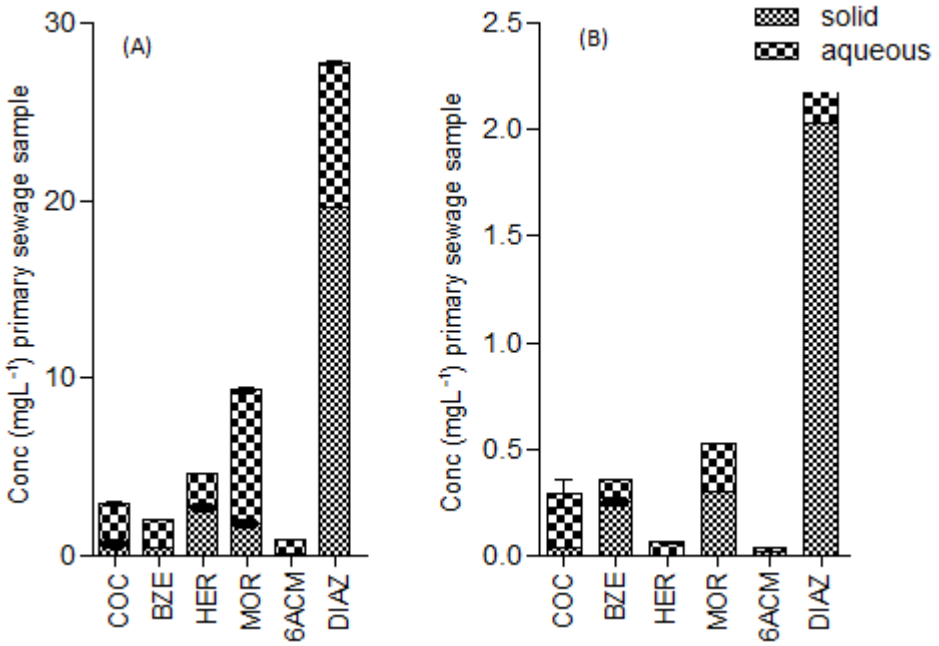



**Figure 23:** Degradation of compounds in primary sludge at time (A) 15 min and (B) 3h, demonstrating the change in distribution/partitioning as drugs degrade at 19± 0.5°C. (Data derived from Table 19 - 24)

In Figure 24, it can also be seen that all drug concentrations falls rapidly within the first hour of exposure to sewage with significant removals within the time of contact but slows down as biodegradation advances. Diazepam showed slower degradation but greater pattern of distribution in the aqueous phase as biodegradation progressed over 3 hour periods.

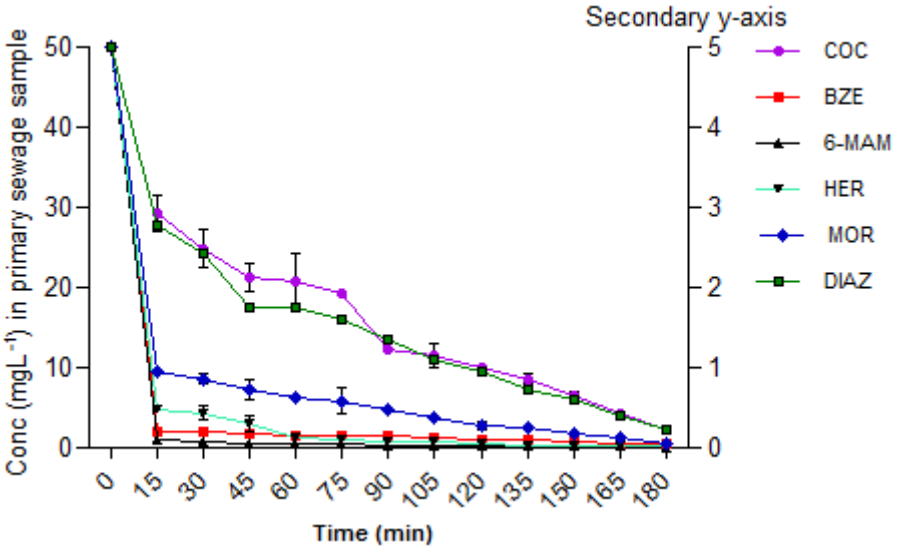



**Figure 24:** Plot showing combined concentrations of drugs in aqueous and solid phases in primary sewage sludge at 19± 0.5°C. (Data derived from Table 19 - 24)

The concentration of compounds decreases with concurrent accumulation of metabolites such as ecgoninemethylester, benzoyecgonine and cocaethylene for cocaine, morphine and 6-monoacetylmorphine for heroin and nordiazepam for diazepam which were in turn being further biodegraded. A second experiment to evaluate the significance of adsorption or degradation on retention time and its effects on removal rates was carried out. The results in Figure 25 below shows the removal rates of different compounds due to their different association with primary sewage solids (suspended solids concentration of 77387 mg $L^{-1}$) and different degradation rates. The two mechanisms were significant in the removal of compounds in the primary sedimentation unit over the range of retention times with more than 80% removal achieved within the first hour of contact.

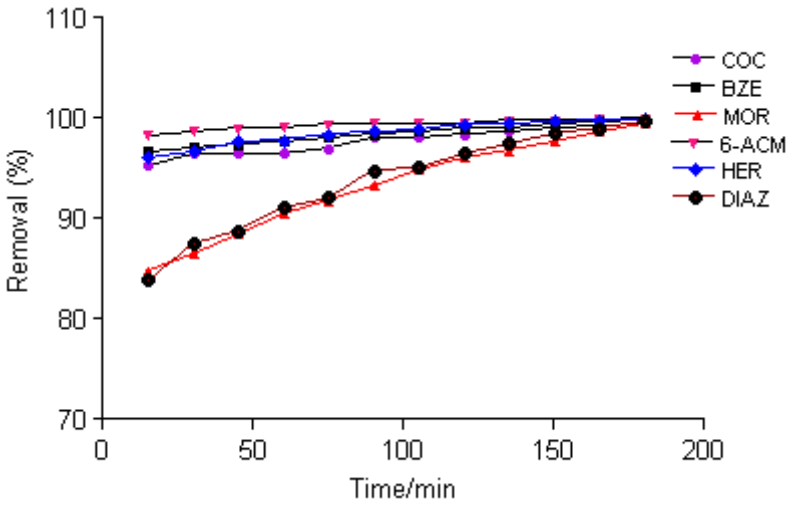



**Figure 25:** The effect of retention time on the removal of compounds during primary treatment processes. (Data derived from Table 19 - 24)

**4.2.2.2. Removal of drugs during secondary sewage treatment:** All six compounds exhibited significant but different removal rates over 3h retention time and the results are presented in Figure 26.

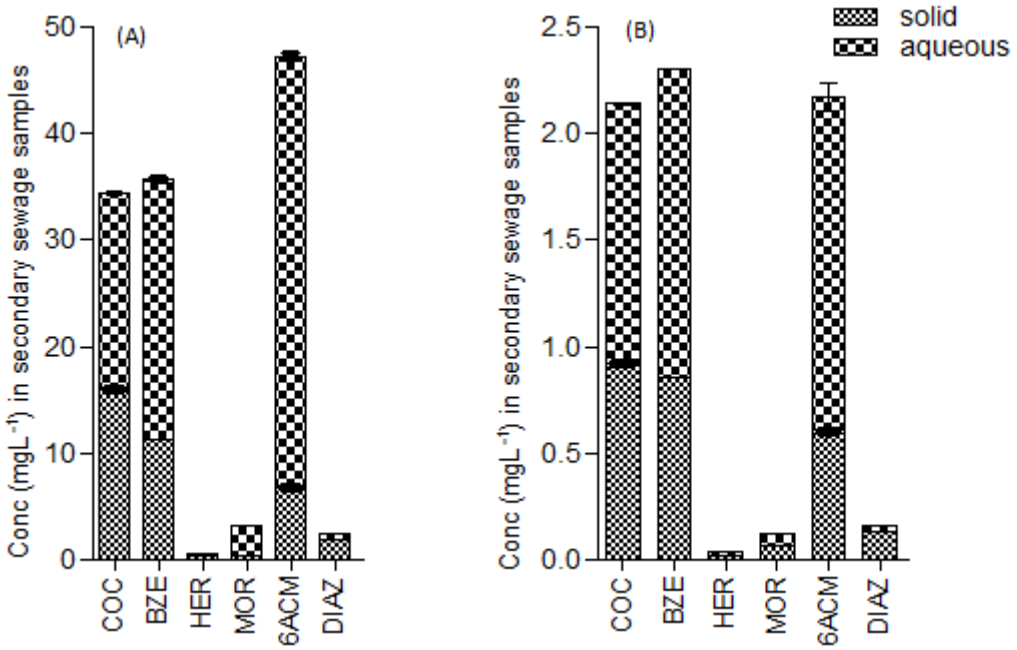



**Figure 26:** Degradation of compounds in SAF-1 sludge at time (A) 15 min and (B) 3h, demonstrating the change in distribution/partitioning as drugs degrade at 19± 0.5°C. (Data derived from Table 19 - 24)

The illustration of combined concentrations of drugs in aqueous and solid phases in the same secondary sewage sample at 19± 0.5°C is shown in Figure 27. Most compounds expectedly showed greater degradation in biological secondary sewage compared to the pattern observed in primary sludge in Figure 24.

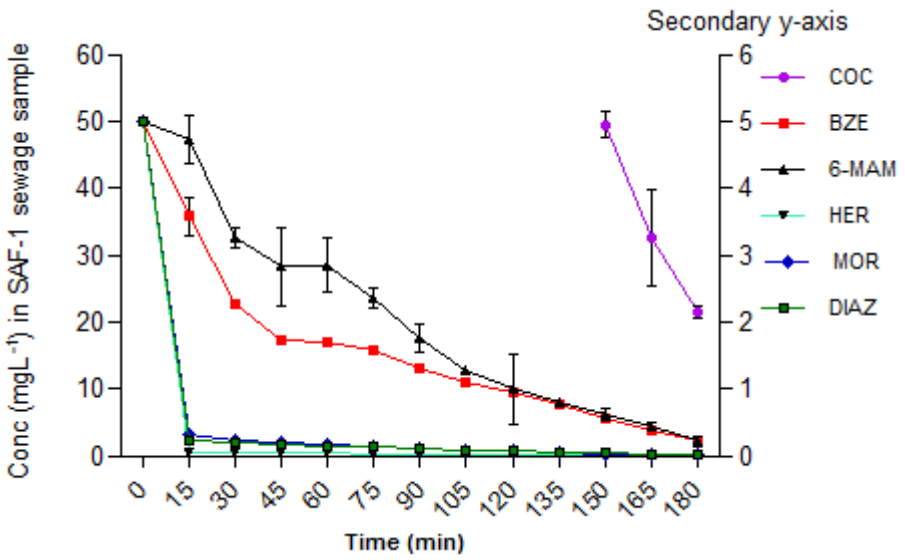



**Figure 27**. Plot showing combined concentrations of drugs in aqueous and solid phases in secondary sewage sludge (SAF-1) at 19± 0.5$^{o}$C.

In Figure 28, the effect of retention time on the removal of compounds during the secondary sewage processes can be seen with 6-monacetylmorphine, benzoylecgonine and cocaine showing average removal rate in the first hour of contact. Since others exhibited maximum removals at the same period of exposure, it was found that the removal of compounds at 19± 0.5$^{o}$C was favourable being the normal operational temperature of most STWs.

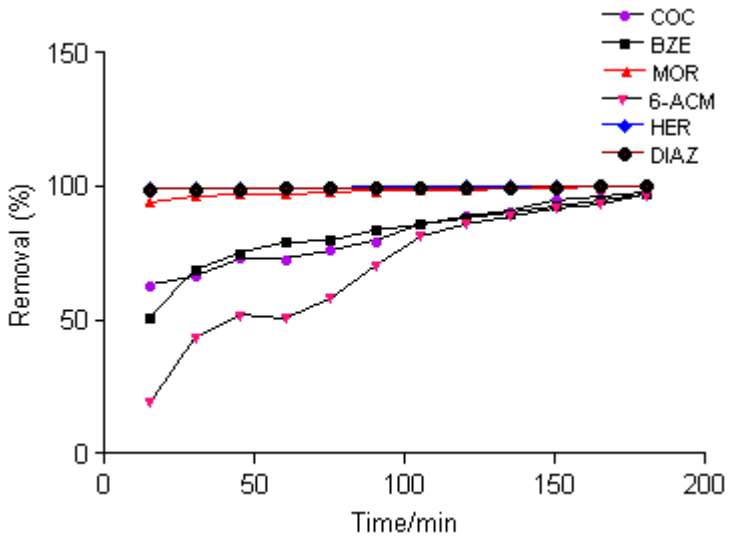



**Figure 28:** The effect of retention time on the removal of compounds during secondary treatment. (Data derived from Table 19 - 24)

### 4.2.3. Comparison of Kd values to LogKow data.

The octanol-water partition coefficient (Log Kow) is a laboratory-measured property of a substance that is recognized and are used extensively in environmental chemistry as it provides a thermodynamic measure of the tendency of the substance to prefer a non-aqueous or oily phase rather

than water (i.e. its hydrophilic/lipophilic balance). But the water-solid partition coefficient (Kd) measured for the selected illicit drugs and abused pharmaceuticals in the current work were generally higher compare with literature Log Kow data range of 1.6 – 2.9 in Table 10 as against 1.2 – 69.9. in Table 27 for the drugs. Again, calculating of Kd changes at the timed interval of 15 minutes for 3h could introduce uncertainties as the values would be higher with higher recoveries from the sludge types. However, Table 27 below generally show the relatively higher Kd values in secondary sludge compare to primary sludge with diazepam showing greater values in both sludge types.

**Table 27**: Data of water-solid distribution coefficients of drugs in both PS and SAF at 19± 0.5°C ($K_{d,}$ Kg/L).

| | Primary sludge (PS) | | | | | | Submerged aerated filter (SAF) | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Time (min) | COC | BZE | HER | 6MAM | MOR | DIAZ | COC | BZE | HER | 6MAM | MOR | DIAZ |
| 15 | 3.2 | 2.9 | 18.2 | 1.6 | 3.1 | 10.5 | 12.2 | 6.5 | 9.7 | 2.3 | 3.3 | 26.5 |
| 30 | 5.6 | 4.0 | 20.4 | 1.3 | 3.9 | 12.6 | 12.2 | 5.3 | 26.1 | 2.7 | 4.2 | - |
| 45 | 2.8 | 3.6 | 20.8 | 1.2 | 3.7 | 10.0 | 13.2 | 9.0 | 19.4 | 2.5 | 4.0 | 46.4 |
| 60 | 2.7 | 4.0 | 0.0 | 1.2 | 3.3 | 10.7 | 9.9 | 8.2 | 26.9 | 2.6 | 3.6 | 36.8 |
| 75 | 3.8 | 7.6 | 0.0 | 2.5 | 5.1 | 13.3 | 9.9 | 8.1 | 16.1 | 1.8 | 5.6 | 31.8 |
| 90 | 4.6 | 7.9 | 0.0 | 1.9 | 6.6 | 17.6 | 7.7 | - | 19.8 | 2.5 | 7.2 | 41.6 |
| 105 | 3.7 | 10.6 | 0.0 | 3.3 | 6.2 | 15.2 | 7.0 | - | 18.7 | 5.2 | 7.4 | 51.5 |
| 120 | 3.3 | 12.9 | 0.0 | 3.0 | 6.4 | 18.6 | 10.2 | - | 12.0 | 6.3 | 7.9 | 46.0 |
| 135 | 4.4 | 15.6 | 0.0 | 2.2 | 5.0 | 21.2 | 8.1 | - | 18.7 | 5.4 | 5.5 | 43.4 |
| 150 | 4.0 | 18.9 | 0.0 | 3.1 | 7.4 | 29.5 | 12.4 | 8.7 | 24.4 | 7.0 | 8.1 | 46.1 |
| 165 | 3.6 | 25.7 | 0.0 | 5.1 | 6.7 | 32.2 | 8.8 | 8.2 | 12.8 | 5.3 | 7.3 | 69.9 |
| 180 | 2.7 | 31.9 | 0.0 | 7.1 | 4.6 | 66.7 | 10.6 | 6.3 | 15.1 | 5.2 | 5.1 | 68.1 |

### 4.2.4. Evaluation of biotic versus abiotic degradation.

Further batch tests to evaluate biotic and abiotic degradation were carried out with a 250 mL of unfiltered primary sludge samples of the same suspended solids concentration (77387 $mgL^{-1}$) measured in a flask, refrigerated at 4 ± 0.5°C for 30 min to inhibit biological activity before the sample was spiked with drugs. Both biologically active and inactive samples were taken at 15 min for 3h. Table 28 below shows the cocaine faster degradation process at biotic temperature of 19 ± 0.5°C and a relatively slowly abiotic process at 4± 0.5°C were observed. The low temperature inhibited the biological process as gradual transformation of initial compound concentration to degradation products at the low temperature have demonstrated biodegradation process and chemical hydrolysis as two major removal mechanism, as there is no loss through volatilisation [188]. Since gradual decline in the levels of cocaine were observed in spite of microbial inhibition, therefore it was correct to

assume that bio-degradation was part of the removal processes and not only chemical degradation and reported in the performance differences of both activated sludge and trickling filters experiment [261]. Several reports of biological degradations such as the fate and behaviour of endocrine disrupters in wastewaters treatment processes in which the degradation of nonylphenolic surfactants in activated sludge batch tests and their removals were not by biological processes alone have been shown [238]. In the behaviour of the s-triazine herbicides, atrazine and simazine, during primary and secondary biological waste water treatment [281], the removal of *s*-triazine was also not by bio-degradation as the primary degradation products in the two experiments were not obvious hence their removal was sorption to the solids. However, the observed rapid abiotic removal of cocaine was in contrast to the observation of Gheorghe et al (132) in their "analysis of cocaine and its principal metabolites in waste and surface water using solid-phase extraction and liquid chromatography-ion trap tandem mass spectrometry" where cocaine was reported to be stable for 5 days at 20 $^{o}$C. Stability of cocaine and benzoylecgonine may be influenced by matrices due to difference in bacteria population that may aid bio-degradation. Apart from chemical hydrolysis of cocaine to benzoylecgonine, another bio-degradation product of cocaine is ecgonine methylester at pH = 2 and its formation may or may not be as rapid depending on the matrice composition. However, significant removal of cocaine and other compounds may still not indicate complete degradation as some might undergo partitioning into solid phase. Since the recovery experiment for the solid phase was not separately conducted and this may have introduced uncertainty. Further study is therefore needed to confirm set conclusions to fully understand the partition of cocaine especially in primary sludge [261]".

**Table 28**: Degradation of cocaine in PS at biotic (19 ± 0.5$^{o}$C) and abiotic (4 ± 0.5$^{o}$C) temperature (Data derived from Table 1A & 2A in Appendix 5)

| Time (min) | Conc. of cocaine (µg in 250 mL of PS) at 19 ± 0.5$^{o}$C | | | | | Conc. of cocaine (µg in 250 mL of PS) at 4 ± 0.5$^{o}$C | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | COC soluble | COC sorbed | COC degraded | $K_d$ * | Removal (%) | COC soluble | COC sorbed | COC degraded | $K_d$ * | Removal (%) |
| 15 | 586.5 | 144.5 | 11769.0 | 3.2 | 95.3 | 3430.2 | 205.9 | 8863.9 | 0.8 | 72.6 |
| 30 | 433.3 | 186.2 | 11880.5 | 5.6 | 96.5 | 3114.0 | 193.3 | 9192.7 | 0.8 | 75.1 |
| 45 | 435.0 | 94.5 | 11970.5 | 2.8 | 96.5 | 2924.7 | 175.2 | 9400.1 | 0.8 | 76.6 |
| 60 | 425.8 | 87.9 | 11986.3 | 2.7 | 96.6 | 2235.4 | 165.0 | 10099.6 | 1.0 | 82.1 |
| 75 | 371.6 | 110.6 | 12017.8 | 3.8 | 97.0 | 1689.5 | 148.0 | 10662.4 | 1.1 | 86.5 |
| 90 | 225.3 | 80.8 | 12193.9 | 4.6 | 98.2 | 1442.8 | 177.5 | 10879.7 | 1.6 | 88.5 |
| 105 | 221.3 | 63.3 | 12215.5 | 3.7 | 98.2 | 1197.9 | 191.4 | 11110.6 | 2.1 | 90.4 |
| 120 | 195.8 | 50.3 | 12253.8 | 3.3 | 98.4 | 941.1 | 140.6 | 11418.3 | 1.9 | 92.5 |
| 135 | 158.1 | 53.2 | 12288.6 | 4.4 | 98.7 | 733.4 | 165.0 | 11601.6 | 4.3 | 94.1 |
| 150 | 122.1 | 38.0 | 12339.9 | 4.0 | 99.0 | 518.7 | 126.23 | 11885.1 | 6.0 | 95.9 |
| 165 | 82.6 | 22.8 | 12394.6 | 3.6 | 99.3 | 365.5 | 132.7 | 12001.8 | 4.7 | 97.1 |
| 180 | 46.0 | 9.5 | 12444.5 | 2.7 | 99.6 | 125.2 | 74.2 | 12300.6 | 5.3 | 99.0 |

Nevertheless, treatment processes may use temperature strategy to optimise the removal of compounds from convectional sewage works during degradation.

**Table 29**: Degradation of BZE in PS at biotic (19 ± 0.5°C) and abiotic (4 ± 0.5°C) temperature (Data derived from Table 14 & 15 in Appendix 5)

| Time (min) | Conc. of BZE (µg in 250 mL of PS) at 19 ± 0.5°C | | | | | Conc. of BZE (µg in 250 mL of PS) at 4 ± 0.5°C | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | BZE soluble | BZE sorbed | BZE. degraded | $K_d$ | Removal (%) | BZE soluble | BZE sorbed | BZE. degraded | $K_d$ | Removal (%) |
| 15 | 406.3 | 89.8 | 12003.9 | 2.9 | 96.7 | 6101.6 | 736.7 | 5661.8 | 1.6 | 51.2 |
| 30 | 355.4 | 110.1 | 12034.5 | 4.0 | 97.2 | 2914.3 | 902.6 | 8683.2 | 4.0 | 76.7 |
| 45 | 319.4 | 88.4 | 12092.2 | 3.6 | 97.4 | 2619.1 | 725.0 | 9155.8 | 3.6 | 79.0 |
| 60 | 288.7 | 88.8 | 12122.5 | 4.0 | 97.7 | 2367.2 | 728.2 | 9404.6 | 4.0 | 81.1 |
| 75 | 240.9 | 141.8 | 12117.3 | 7.6 | 98.1 | 1975.5 | 1162.9 | 9361.6 | 7.6 | 84.2 |
| 90 | 206.2 | 126.7 | 12167.2 | 7.9 | 98.4 | 1690.4 | 1038.7 | 9770.9 | 7.9 | 86.5 |
| 105 | 166.3 | 136.4 | 12197.2 | 10.6 | 98.7 | 1364.0 | 1118.8 | 10017.1 | 10.6 | 89.1 |
| 120 | 119.4 | 119.3 | 12261.3 | 12.9 | 99.0 | 979.1 | 978.5 | 10542.4 | 12.9 | 92.2 |
| 135 | 101.9 | 123.2 | 12274.9 | 15.6 | 99.2 | 835.9 | 1009.9 | 10654.2 | 15.6 | 93.3 |
| 150 | 77.3 | 113.0 | 12309.8 | 18.9 | 99.4 | 633.5 | 926.5 | 10940.0 | 18.9 | 94.9 |
| 165 | 43.3 | 86.1 | 12370.6 | 25.7 | 99.7 | 354.8 | 705.9 | 11439.3 | 25.7 | 97.2 |
| 180 | 25.6 | 63.3 | 12411.1 | 31.9 | 99.8 | 210.1 | 518.8 | 11771.1 | 31.9 | 98.3 |

**Table 30**: Degradation of Morphine in PS at biotic (19 ± 0.5°C) and abiotic (4 ± 0.5°C) temperature (Data derived from Table 21 & 22 in Appendix 5)

| Time (min) | Conc. of MOR (µg in 250 mL of PS) at 19 ± 0.5°C | | | | | Conc. of MOR (µg in 250 mL of PS) at 4 ± 0.5°C | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | MOR soluble | MOR sorbed | MOR. degraded | $K_d$ | Removal (%) | MOR soluble | MOR sorbed | MOR. degraded | $K_d$ | Removal (%) |
| 15 | 1901.8 | 429.3 | 10168.9 | 3.1 | 84.8 | 9716.4 | 137.0 | 2646.6 | 0.2 | 22.3 |
| 30 | 1678.9 | 430.0 | 10391.1 | 3.9 | 86.6 | 8764.9 | 121.6 | 3613.5 | 0.1 | 29.9 |
| 45 | 1434.8 | 361.7 | 10703.5 | 3.7 | 88.5 | 7773.9 | 120.5 | 4605.6 | 0.2 | 37.8 |
| 60 | 1178.4 | 355.8 | 10965.8 | 3.3 | 90.6 | 5801.2 | 108.5 | 6590.2 | 0.5 | 53.6 |
| 75 | 1029.2 | 398.2 | 11072.6 | 5.1 | 91.8 | 6057.4 | 108.9 | 6333.7 | 1.9 | 51.5 |
| 90 | 843.7 | 304.1 | 11352.2 | 6.6 | 93.3 | 3927.8 | 91.1 | 8481.1 | 1.7 | 68.6 |
| 105 | 641.7 | 283.1 | 11575.2 | 6.2 | 94.9 | 4158.5 | 80.3 | 8261.2 | 0.8 | 66.7 |
| 120 | 486.8 | 192.3 | 11820.9 | 6.4 | 96.1 | 3504.3 | 66.0 | 8929.7 | 0.5 | 72.0 |
| 135 | 399.3 | 207.9 | 11892.8 | 5.0 | 96.8 | 2835.5 | 62.0 | 9602.5 | 0.4 | 77.3 |
| 150 | 287.4 | 146.2 | 12066.4 | 7.4 | 97.7 | 1976.5 | 49.6 | 10473.9 | 1.1 | 84.2 |
| 165 | 163.7 | 125.3 | 12211.0 | 6.7 | 98.7 | 1485.7 | 35.8 | 10978.5 | 1.4 | 88.1 |
| 180 | 58.3 | 73.8 | 12367.9 | 4.6 | 99.5 | 804.6 | 23.1 | 11672.3 | 1.6 | 93.6 |

**Table 31**: Degradation of 6-MAM in PS at biotic (19 ± 0.5°C) and abiotic (4 ± 0.5°C) temperature (Data derived from Table 28 & 29 in Appendix 5)

| Time (min) | Conc. of 6-MAM (µg in 250 mL of PS) at 19 ± 0.5°C | | | | | Conc. of 6-MAM (µg in 250 mL of PS) at 4 ± 0.5°C | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 6MAM soluble | 6MAM sorbed | 6MAM. degraded | $K_d$ | Removal (%) | 6MAM soluble | 6MAM sorbed | 6MAM. degraded | $K_d$ | Removal (%) |
| 15 | 213.7 | 14.9 | 12271.4 | 1.6 | 98.3 | 572.5 | 69.8 | 11857.6 | 1.6 | 95.4 |
| 30 | 160.8 | 13.4 | 12325.8 | 1.3 | 98.7 | 616.3 | 62.7 | 11820.9 | 1.3 | 95.1 |
| 45 | 123.8 | 11.1 | 12365.1 | 1.2 | 99.0 | 564.4 | 52.1 | 11883.5 | 1.2 | 95.5 |
| 60 | 101.6 | 9.1 | 12389.3 | 1.2 | 99.2 | 463.4 | 42.6 | 11994.0 | 1.2 | 96.3 |
| 75 | 70.3 | 9.2 | 12420.6 | 2.5 | 99.4 | 373.7 | 50.9 | 12075.4 | 2.5 | 97.0 |
| 90 | 60.1 | 8.4 | 12431.5 | 1.9 | 99.5 | 282.0 | 39.2 | 12178.8 | 1.9 | 97.7 |
| 105 | 59.8 | 10.0 | 12430.2 | 3.3 | 99.5 | 243.2 | 61.3 | 12195.6 | 3.3 | 98.1 |
| 120 | 47.0 | 7.9 | 12445.0 | 3.0 | 99.6 | 214.5 | 49.2 | 12236.3 | 3.0 | 98.3 |
| 135 | 24.8 | 7.6 | 12467.6 | 2.2 | 99.8 | 113.1 | 35.5 | 12351.4 | 2.2 | 99.1 |
| 150 | 18.9 | 7.1 | 12474.0 | 3.1 | 99.8 | 85.2 | 33.1 | 12381.6 | 3.1 | 99.3 |
| 165 | 13.4 | 5.1 | 12481.5 | 5.1 | 99.9 | 61.3 | 23.9 | 12414.8 | 5.1 | 99.5 |
| 180 | 6.2 | 3.3 | 12490.5 | 7.1 | 100.0 | 39.2 | 15.6 | 12445.2 | 7.1 | 99.7 |

**Table 32**: Degradation of Heroin in PS at biotic (19 ± 0.5°C) and abiotic (4 ± 0.5°C) temperature (Data derived from Table 35A & 36A in Appendix 5)

| Time (min) | Conc. of HER (µg in 250 mL of PS) at 19 ± 0.5°C | | | | | Conc. of HER (µg in 250 mL of PS) at 4 ± 0.5°C | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | HER soluble | HER sorbed | HER. degraded | $K_d$ | Removal (%) | HER soluble | HER sorbed | HER. degraded | $K_d$ | Removal (%) |
| 15 | 480.0 | 677.0 | 11343.0 | 18.2 | 96.2 | 1665.4 | 2382.9 | 8451.7 | 18.5 | 86.7 |
| 30 | 414.1 | 652.6 | 11433.3 | 20.4 | 96.7 | 1444.5 | 2293.3 | 8762.3 | 20.5 | 88.4 |
| 45 | 286.3 | 459.4 | 11754.3 | 20.8 | 97.7 | 1030.3 | 1612.4 | 9857.3 | 20.2 | 91.8 |
| 60 | 276.4 | 0.0 | 12223.6 | 0.0 | 97.8 | 1068.9 | 1345.2 | 10085.9 | 16.3 | 91.4 |
| 75 | 204.2 | 0.0 | 12295.8 | 0.0 | 98.4 | 908.5 | 1155.6 | 10435.9 | 16.5 | 92.7 |
| 90 | 167.7 | 0.0 | 12332.3 | 0.0 | 98.7 | 608.6 | 803.3 | 11088.1 | 17.1 | 95.1 |
| 105 | 140.3 | 0.0 | 12359.7 | 0.0 | 98.9 | 476.6 | 530.3 | 11493.1 | 14.4 | 96.2 |
| 120 | 80.4 | 0.0 | 12419.6 | 0.0 | 99.4 | 278.6 | 301.4 | 11920.1 | 14.0 | 97.8 |
| 135 | 61.0 | 0.0 | 12439.0 | 0.0 | 99.5 | 208.0 | 238.7 | 12053.3 | 29.9 | 98.3 |
| 150 | 34.7 | 0.0 | 12465.3 | 0.0 | 99.7 | 154.2 | 186.4 | 12159.4 | 20.2 | 98.8 |
| 165 | 24.0 | 0.0 | 12476.0 | 0.0 | 99.8 | 110.8 | 133.0 | 12256.2 | 15.5 | 99.1 |
| 180 | 14.7 | 0.0 | 12485.3 | 0.0 | 99.9 | 66.4 | 90.0 | 12343.6 | 17.5 | 99.5 |

**Table 33**: Degradation of Diazepam in PS at biotic (19 ± 0.5°C) and abiotic (4 ± 0.5°C) temperature (Data derived from Table 42A & 43 in Appendix 5)

| Time (min) | Conc. of DIAZ (µg in 250 mL of PS) at 19 ± 0.5°C | | | | | Conc. of DIAZ (µg in 250 mL of PS) at 4 ± 0.5°C | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | DIAZ soluble | DIAZ sorbed | DIAZ. degraded | $K_d$ | Removal (%) | DIAZ soluble | DIAZ sorbed | DIAZ. degraded | $K_d$ | Removal (%) |
| 15 | 2015.7 | 4919.3 | 5565.1 | 10.5 | 83.9 | 914.2 | 3866.1 | 7719.6 | 50.6 | 92.7 |
| 30 | 1547.0 | 4504.2 | 6448.8 | 12.6 | 87.6 | 832.3 | 3878.6 | 7789.1 | 40.9 | 93.3 |
| 45 | 1401.2 | 2933.2 | 8165.6 | 10.0 | 88.8 | 741.2 | 3983.4 | 7775.5 | 50.5 | 94.1 |
| 60 | 1093.9 | 3229.7 | 8176.4 | 10.7 | 91.2 | 687.2 | 3677.1 | 8135.7 | 49.3 | 94.5 |
| 75 | 979.9 | 3028.4 | 8491.7 | 13.3 | 92.2 | 294.9 | 3317.1 | 8888.0 | 99.6 | 97.6 |
| 90 | 653.6 | 2690.2 | 9156.2 | 17.6 | 94.8 | 266.3 | 2958.0 | 9275.7 | 97.3 | 97.9 |
| 105 | 597.8 | 2104.9 | 9797.3 | 15.2 | 95.2 | 229.7 | 2603.8 | 9666.5 | 75.7 | 98.2 |
| 120 | 439.4 | 1887.4 | 10173.2 | 18.6 | 96.5 | 193.0 | 2203.2 | 10103.8 | 85.6 | 98.5 |
| 135 | 307.7 | 1506.1 | 10686.2 | 21.2 | 97.5 | 149.8 | 1860.0 | 10490.1 | 93.0 | 98.8 |
| 150 | 190.8 | 1301.3 | 11008.0 | 29.5 | 98.5 | 120.6 | 1446.7 | 10932.7 | 73.2 | 99.0 |
| 165 | 118.8 | 883.8 | 11497.4 | 32.2 | 99.0 | 77.6 | 1065.5 | 11356.8 | 91.8 | 99.4 |
| 180 | 37.0 | 508.1 | 11954.9 | 66.7 | 99.7 | 41.5 | 643.5 | 11815.0 | 91.8 | 99.7 |

*$K_d$ is solid-water partition coefficient.

Figure 29 further demonstrates the results presented in Table 26 – 31, showing how microbial inactivity has slowed down metabolic processes with obvious relative slower changes in concentrations observed indicating the degradation of compounds were both biological and chemical with an increase degradation products and greater partitioning in solid phases.

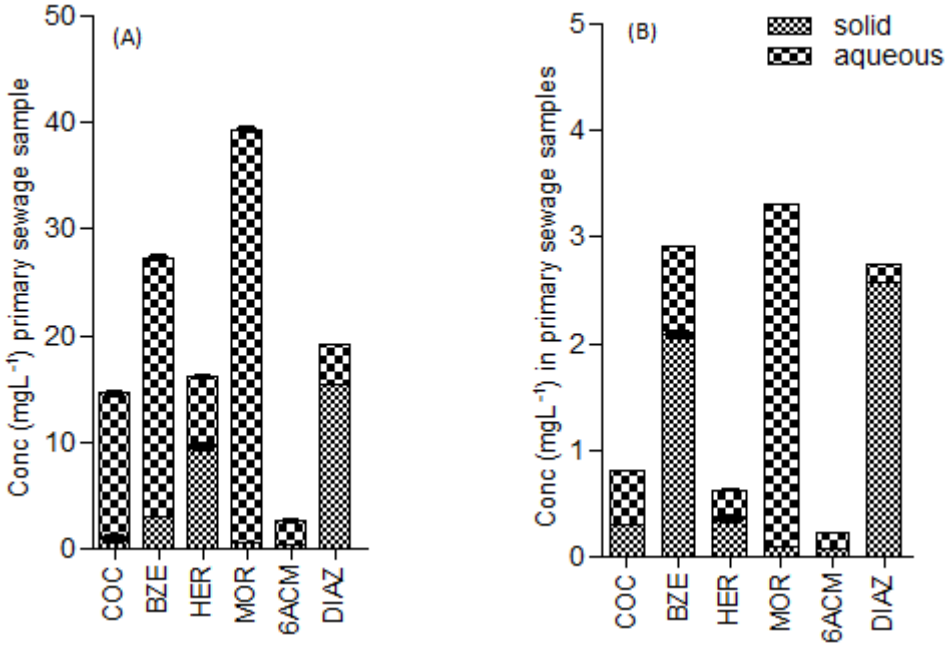



**Figure 29:** Degradation of compounds in primary sludge at abiotic conditions (4± 0.5°C) demonstrating the change in distribution/partitioning at time (A) 15 min and (B) 3h. (Data derived from Table 25 - 30)

Figure 30 below further confirmed the degradations were temperature independent as chemical hydrolysis did occur for all compounds at low temperature.

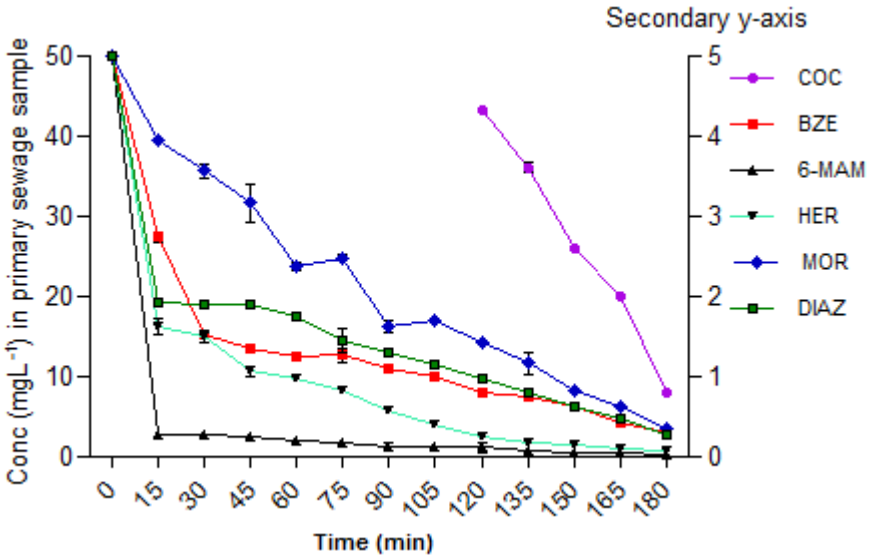



**Figure 30:** Plot showing combined concentrations of compounds in aqueous and solid phases in abiotic conditions (4± 0.5°C). (Data derived from Table 25 - 30)

Inhibition of the biological activities in the samples was expected at 4 ± 0.5° C to slow the metabolic processes. Observed changes in the concentration indicating that the degradation was chemical with possible biological processes may be involved. No changes in the concentrations would have been observed if degradation were only biological, but that was not the case here as metabolic processes though were slowed down compared to degradation at 19± 0.5°C. The changes suggest chemical degradation of compounds indicating that the degradation was both biological and chemical. The temperature change affects the rate of removal of compounds with the $K_d$ values of diazepam, heroin, morphine and benzoylecgonine showing greater sorption at 19± 0.5°C while 6 monoacetylmorphine and cocaine were better sorped at 4± 0.5°C.

### 4.2.5 Evaluation of suspended solids on the removal of compounds

High removals of organic compounds during primary treatment have largely depended on water-solid partition coefficient ($K_d$) values which normally determine the degree of partitioning of compounds between aqueous and solid phases [290]. One of the major removal mechanisms of compounds is the association with the suspended and settleable solids which are removed in sludge after sedimentation in the primary sludge. In the secondary treatment processes, transformation or accumulation of organic pollutant onto the sewage sludge matrix depends on factors like:

i. Sorption onto the surfaces of the biological solids or association with fats and oils
ii. Chemical degradation such as hydrolysis
iii. Biodegradation

iv. Volatilisation

In the current work, the effect of suspended solids on the removal of compounds was done by evaluating the levels of compounds adsorbed onto the solids in primary sludge at 19±0.5$^{o}$C.

The results in Table 34 indicate that in PS, 0.08% of cocaine was sorbed onto solids, further showing adsorption as one of the removal mechanisms with significant removal of diazepam in both primary and secondary sewage samples, compared to cocaine, benzoylecgonine and 6-monoacetylmorphine removals in secondary sludge. Heroin and morphine exhibited negligible associations with the suspended solids and therefore have minimal removals in both primary and secondary sludge samples.

**Table 34:** Adsorption of compounds onto sludge of different suspended solids concentration (%) in 3h.

| | Suspended solids (mg $L^{-1}$) | | | |
|---|---|---|---|---|
| Compounds | 77386.5 (PS) | 70793.5 (SAF-1) | 8465.0 (MSAF) | 3563.5 (HS) |
| Cocaine | 0.08 | 1.83 | 0.17 | 0.09 |
| Benzoylecgonine | 0.51 | 1.69 | 0.59 | 0.73 |
| Heroin | 0.0 | 0.03 | 0.01 | 0.38 |
| Morphine | 0.59 | 0.12 | 0.31 | 0.02 |
| 6-monoacetylmorphine | 0.03 | 1.19 | 0.02 | 0.0 |
| Diazepam | 4.06 | 0.26 | 3.07 | 0.15 |

Further comparison of the effects of adsorption on the removal of compounds at abiotic (4±0.5$^{o}$C) and biotic (19±0.5$^{o}$C) temperatures exhibited similar adsorption pattern (Fig. 31). However, heroin, cocaine and benzoylecgonine showed greater adsorption at chilling temperature whereas morphine and diazepam exhibited better adsorption at biotic temperature. The sorption by organic compounds has therefore be reported with log $K_{ow}$ < 2.5 (low sorption potential), log $K_{ow}$ > 2.5 and < 4.0 (medium sorption potential) and log $K_{ow}$ > 4.0 (high sorption potential) for chemicals that partition to organic phases to estimate a clear relationship with the degree of partitioning of contaminants during treatment [111].

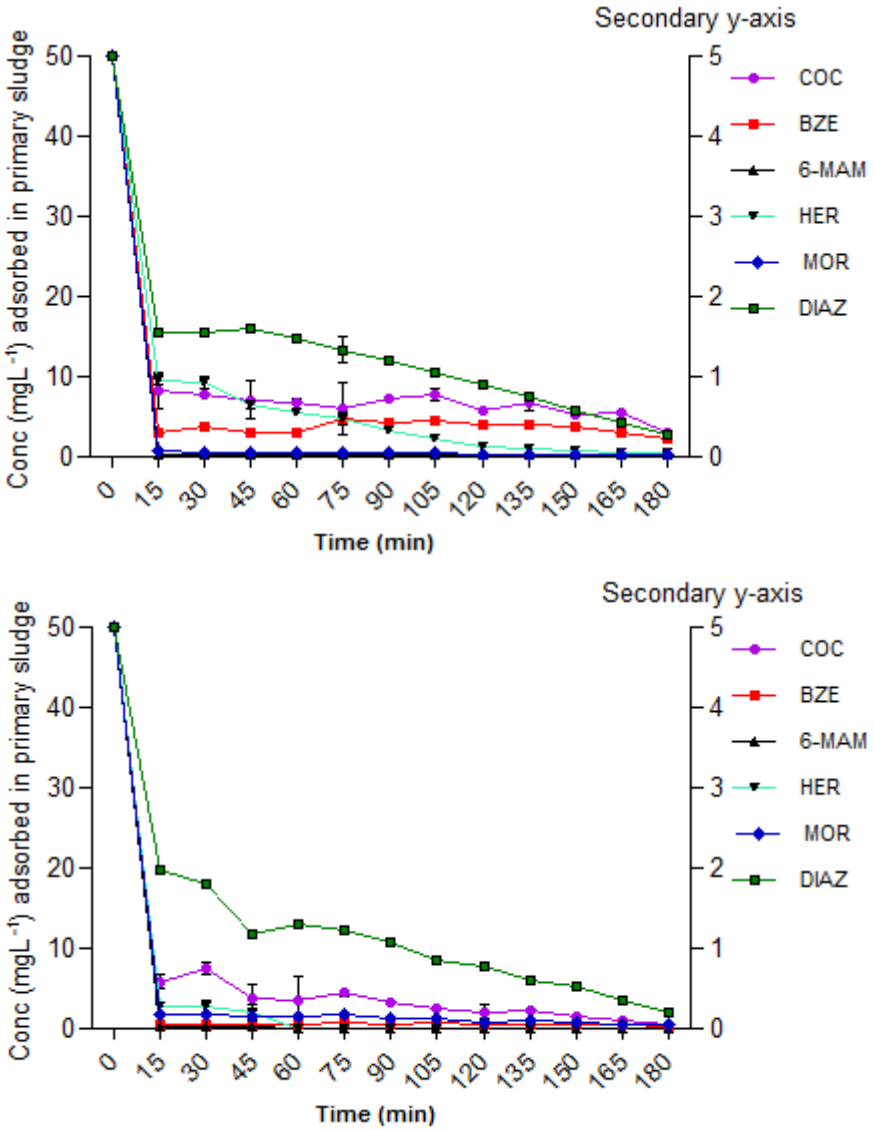



**Figure 31:** Effect of adsorption on the removal of compounds in primary sludge at (A) 4±0.5°C (B) 19±0.5°C. (Data derived from Table 31 - 36)

The solid-water partition coefficients ($K_d$) in the current study were in the range for cocaine (0.2 – 0.9), benzoylecgonine (0.2 – 2.5), morphine (0.2 – 1.3), 6-monoacetylmorphine (0.1 – 0.5), heroin (0.7 -1.7) and diazepam (2.1–13.7). The association of diazepam with biological solids may have exhibited adsorption as an important removal mechanism during primary and secondary wastewater treatment while other drugs have no significant adsorption. Also, removal efficiencies above 75 % in primary sludge and biological secondary sludge for all the compounds were observed. The $K_d$ values of compounds studied in this work showed positive correlation with their removal efficiencies as reported for ten quinolone and fluoroquinolone antibiotics in sludge samples [290].

### 4.2.6. Effect of nature of sludge on degradation.

Table 35 – 40 below compares the effects of degradation of compounds with respect to the nature of sludge sample. Therein are results obtained from the primary sludge samples collected at Molesworth and Stoke Bardolph Nottingham STWs.

**Table 35:** Degradation of cocaine in primary sludge from Molesworth and Stoke Bardolph Nottingham STWs (19 ± 0.5°C) (Data derived from Table 2A & 13A in Appendix 5)

| Time (min) | PS (Molesworth STW) | | | PS (Nottingham) | | |
|---|---|---|---|---|---|---|
| | COC. soluble | COC. degraded | Removal (%) | COC. soluble | COC. degraded | Removal (%) |
| 15 | 731.0 | 11769.0 | 95.3 | 468.7 | 12031.3 | 96.3 |
| 30 | 619.5 | 11880.5 | 96.5 | 394.8 | 12105.2 | 96.8 |
| 45 | 529.5 | 11970.5 | 96.5 | 321.9 | 12178.1 | 97.4 |
| 60 | 513.7 | 11986.3 | 96.6 | 268.7 | 12231.3 | 97.9 |
| 75 | 482.2 | 12017.8 | 97.0 | 215.5 | 12284.5 | 98.3 |
| 90 | 306.1 | 12193.9 | 98.2 | 172.8 | 12327.2 | 98.6 |
| 105 | 284.5 | 12215.5 | 98.2 | 146.5 | 12353.5 | 98.8 |
| 120 | 246.2 | 12253.8 | 98.4 | 121.9 | 12378.1 | 99.0 |
| 135 | 211.4 | 12288.6 | 98.7 | 99.4 | 12400.6 | 99.2 |
| 150 | 160.1 | 12339.9 | 99.0 | 76.0 | 12424.0 | 99.4 |
| 165 | 105.4 | 12394.6 | 99.3 | 54.3 | 12445.7 | 99.6 |
| 180 | 55.5 | 12444.5 | 99.6 | 31.6 | 12468.4 | 99.7 |

**Table 36**: Degradation of BZE in primary sludge from Molesworth and Stoke Bardolph Nottingham STWs (19 ± 0.5°C) (Data derived from Table 15 & 20 in Appendix 5)

| Time (min) | PS (Molesworth STW) | | | PS (Nottingham) | | |
|---|---|---|---|---|---|---|
| | BZE soluble | BZE. degraded | Removal (%) | BZE soluble | BZE degraded | Removal (%) |
| 15 | 496.1 | 12003.9 | 96.7 | 1660.2 | 10839.8 | 86.7 |
| 30 | 465.5 | 12034.5 | 97.2 | 1291.2 | 11208.8 | 89.7 |
| 45 | 407.8 | 12092.2 | 97.4 | 1131.7 | 11368.3 | 90.9 |
| 60 | 377.5 | 12122.5 | 97.7 | 1164.2 | 11335.8 | 90.7 |
| 75 | 382.7 | 12117.3 | 98.1 | 1053.4 | 11446.6 | 91.6 |
| 90 | 332.8 | 12167.2 | 98.4 | 831.9 | 11668.1 | 93.3 |
| 105 | 302.8 | 12197.2 | 98.7 | 731.5 | 11768.5 | 94.1 |
| 120 | 238.7 | 12261.3 | 99.0 | 596.6 | 11903.4 | 95.2 |
| 135 | 225.1 | 12274.9 | 99.2 | 539.7 | 11960.3 | 95.7 |
| 150 | 190.2 | 12309.8 | 99.4 | 414.1 | 12085.9 | 96.7 |
| 165 | 129.4 | 12370.6 | 99.7 | 287.7 | 12212.3 | 97.7 |
| 180 | 88.9 | 12411.1 | 99.8 | 164.2 | 12335.8 | 98.7 |

**Table 37**: Degradation of Morphine in primary sludge from Molesworth and Stoke Bardolph Nottingham STWs (19 ± 0.5°C) (Data derived from Table 22 & 27 in Appendix 5)

| Time (min) | PS (Molesworth STW) | | | PS (Nottingham) | | |
|---|---|---|---|---|---|---|
| | MOR soluble | MOR. degraded | Removal (%) | MOR soluble | MOR. degraded | Removal (%) |
| 15 | 2331.1 | 10168.9 | 84.8 | 9897.3 | 2602.7 | 20.8 |
| 30 | 2108.9 | 10391.1 | 86.6 | 8915.8 | 3584.2 | 28.7 |
| 45 | 1796.5 | 10703.5 | 88.5 | 6472.2 | 6027.8 | 48.2 |
| 60 | 1534.2 | 10965.8 | 90.6 | 6126.5 | 6373.5 | 51.0 |
| 75 | 1427.4 | 11072.6 | 91.8 | 5237.1 | 7262.9 | 58.1 |
| 90 | 1147.8 | 11352.2 | 93.3 | 4599.3 | 7900.7 | 63.2 |
| 105 | 924.8 | 11575.2 | 94.9 | 3377.3 | 9122.7 | 73.0 |
| 120 | 679.1 | 11820.9 | 96.1 | 1376.2 | 11123.8 | 89.0 |
| 135 | 607.2 | 11892.8 | 96.8 | 1127.8 | 11372.2 | 91.0 |
| 150 | 433.6 | 12066.4 | 97.7 | 875.9 | 11624.1 | 93.0 |
| 165 | 289.0 | 12211.0 | 98.7 | 598.9 | 11901.1 | 95.2 |
| 180 | 132.1 | 12367.9 | 99.5 | 362.8 | 12137.2 | 97.1 |

**Table 38**: Degradation of 6-MAM in primary sludge from Molesworth and Stoke Bardolph Nottingham STWs (19 ± 0.5°C) (Data derived from Table 29 & 34 in Appendix 5)

| Time (min) | PS (Molesworth STW) | | | PS (Nottingham) | | |
|---|---|---|---|---|---|---|
| | 6MAM soluble | 6MAM. degraded | Removal (%) | 6MAM soluble | 6MAM. degraded | Removal (%) |
| 15 | 228.6 | 12271.4 | 98.3 | 133.7 | 12366.3 | 98.9 |
| 30 | 174.2 | 12325.8 | 98.7 | 129.8 | 12370.2 | 99.0 |
| 45 | 134.9 | 12365.1 | 99.0 | 108.5 | 12391.5 | 99.1 |
| 60 | 110.7 | 12389.3 | 99.2 | 88.5 | 12411.5 | 99.3 |
| 75 | 79.4 | 12420.6 | 99.4 | 74.1 | 12425.9 | 99.4 |
| 90 | 68.5 | 12431.5 | 99.5 | 45.8 | 12454.2 | 99.6 |
| 105 | 69.8 | 12430.2 | 99.5 | 36.0 | 12464.0 | 99.7 |
| 120 | 55.0 | 12445.0 | 99.6 | 24.4 | 12475.6 | 99.8 |
| 135 | 32.4 | 12467.6 | 99.8 | 18.1 | 12481.9 | 99.9 |
| 150 | 26.0 | 12474.0 | 99.8 | 12.2 | 12487.8 | 99.9 |
| 165 | 18.5 | 12481.5 | 99.9 | 9.2 | 12490.8 | 99.9 |
| 180 | 9.5 | 12490.5 | 100.0 | 5.2 | 12494.8 | 100.0 |

**Table 39**: Degradation of Heroin in primary sludge from Molesworth and Stoke Bardolph Nottingham STWs (19 ± 0.5°C) (Data derived from Table 36A & 41A in Appendix 5)

| Time (min) | PS (Molesworth STW) | | | PS (Nottingham) | | |
|---|---|---|---|---|---|---|
| | HER soluble | HER. degraded | Removal (%) | HER soluble | HER. degraded | Removal (%) |
| 15 | 1157.0 | 11343.0 | 96.2 | 1836.0 | 10664.0 | 85.3 |
| 30 | 1066.7 | 11433.3 | 96.7 | 1657.5 | 10842.5 | 86.7 |
| 45 | 745.7 | 11754.3 | 97.7 | 1261.4 | 11238.6 | 89.9 |
| 60 | 276.4 | 12223.6 | 97.8 | 943.2 | 11556.8 | 92.5 |
| 75 | 204.2 | 12295.8 | 98.4 | 840.6 | 11659.4 | 93.3 |
| 90 | 167.7 | 12332.3 | 98.7 | 767.8 | 11732.2 | 93.9 |
| 105 | 140.3 | 12359.7 | 98.9 | 597.3 | 11902.7 | 95.2 |
| 120 | 80.4 | 12419.6 | 99.4 | 506.5 | 11993.5 | 95.9 |
| 135 | 61.0 | 12439.0 | 99.5 | 377.8 | 12122.2 | 97.0 |
| 150 | 34.7 | 12465.3 | 99.7 | 271.6 | 12228.4 | 97.8 |
| 165 | 24.0 | 12476.0 | 99.8 | 175.8 | 12324.2 | 98.6 |
| 180 | 14.7 | 12485.3 | 99.9 | 97.8 | 12402.2 | 99.2 |

**Table 40**: Degradation of Diazepam in primary sludge from Molesworth and Stoke Bardolph Nottingham STWs (19 ± 0.5°C) (Data derived from Table 43 & 48 in Appendix 5)

| Time (min) | PS (Molesworth STW) | | | PS (Nottingham) | | |
|---|---|---|---|---|---|---|
| | DIAZ soluble | DIAZ. degraded | Removal (%) | DIAZ soluble | DIAZ. degraded | Removal (%) |
| 15 | 6934.9 | 5565.1 | 83.9 | 11970.5 | 529.5 | 4.2 |
| 30 | 6051.2 | 6448.8 | 87.6 | 9533.0 | 2967.0 | 23.7 |
| 45 | 4334.4 | 8165.6 | 88.8 | 8060.3 | 4439.7 | 35.5 |
| 60 | 4323.6 | 8176.4 | 91.2 | 7131.4 | 5368.6 | 42.9 |
| 75 | 4008.3 | 8491.7 | 92.2 | 6317.9 | 6182.1 | 49.5 |
| 90 | 3343.8 | 9156.2 | 94.8 | 5433.0 | 7067.0 | 56.5 |
| 105 | 2702.7 | 9797.3 | 95.2 | 4709.3 | 7790.7 | 62.3 |
| 120 | 2326.8 | 10173.2 | 96.5 | 4024.3 | 8475.7 | 67.8 |
| 135 | 1813.8 | 10686.2 | 97.5 | 3313.0 | 9187.0 | 73.5 |
| 150 | 1492.0 | 11008.0 | 98.5 | 2589.0 | 9911.0 | 79.3 |
| 165 | 1002.6 | 11497.4 | 99.0 | 1774.2 | 10725.8 | 85.8 |
| 180 | 545.1 | 11954.9 | 99.7 | 1043.4 | 11456.6 | 91.7 |

The ability of sewage treatment works to design removal processes that enable interactions with natural solid particles (sediments, microorganisms, clay) and added materials (coagulants, active

carbon) in facilitating the physical-chemical removals of compounds by flotation, settling or by biodegradation are disimilar. Even difference in bacteria population and diversity could exist in different municipal treatment works giving rise to different degradation rates. Municipal treatment works vary in design capacity and location and this has almost certainly resulted in variations in degree of degradation (Figure 32).

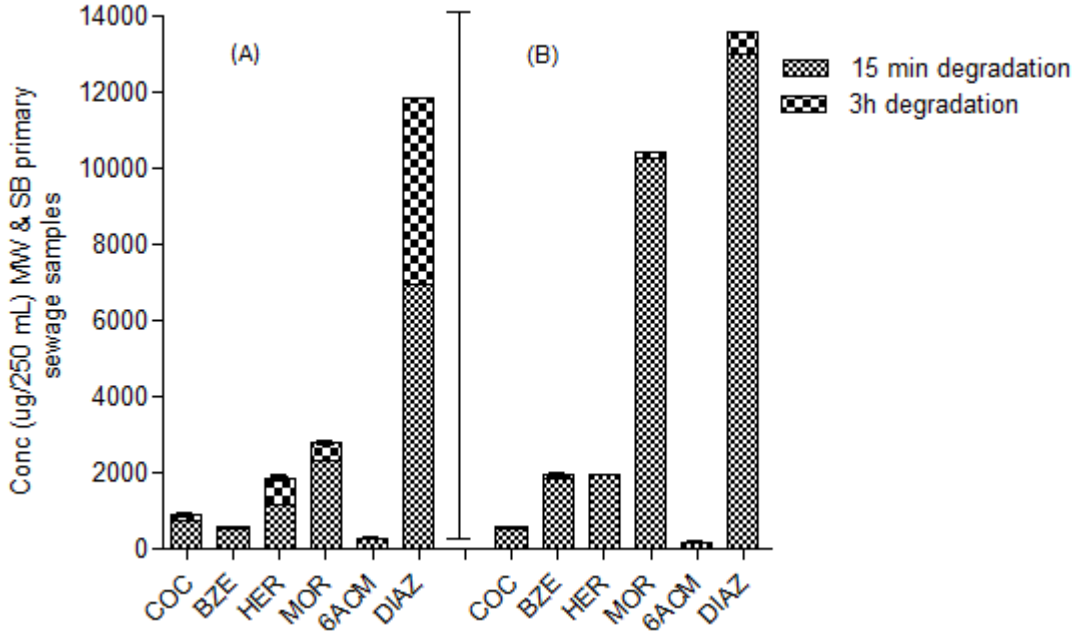



**Figure 32:** Degradation of cocaine in (a) Molesworth and (b) Nottingham STWs, comparing reductions in parent drug over 3h as drugs degrade at 19± 0.5°. (Data derived from Table 38 - 43)

The similarity in degradation and removal profiles as shown from the two STWs where samples were collected surprisingly showed no significant effects of nature of primary sludge samples on degradation, the only difference is the morphine data. Both have high values after 15 mins, and low values after 3hours. Variability in the values of TSS and COD as shown in the characterization (Table 16) is based on Stoke Bardolph STW Nottingahm and Molesworth STWs suggesting many treatment processes exist with different requirements based on peculiar inflow variability to solve problematic situation by adjusting operating conditions.

The degradation products (metabolites) found for cocaine were benzoylecgonine, ecgonine methyl ester and cocaethene and heroin produced morphine and 6-monoacetylmorphine. Attempt to quantify these products were difficult due to their diffuse nature as these compounds were futher being degraded simultaneously as they were been generated from initial corresponding drugs. While other polar degradation products could also not be detected and accurately measured possibly due to the method of analysis or incomplete derivatisation, duplicate measurement of the concentration levels of drugs dissolved in aqueous phase and those sorbed to solid phase were removed from initial concentration to obtain degradation levels.

Finally, benzoylecgonine, morphine and diazepam showed relatively higher degradations in sludge-samples from Molesworth compared to Stoke Bardolph STW Nottingham samples. But as part of the operational challenges encountered as mentioned above, inability to obtain extensive samples from different processing units of the Stoke Bardolph STW, Nottingham limited the empirical comparison between the STWs to only TSS and COD contents. The primary wastewater effluent from primary sedimentation tank was the only access we had due to site restrictions.

Developments on different aspects of drug's transformations in the environment that appeared recently in the chemical literature includes occurrence and fate, treatability by conventional and non-conventional processes, and several miscellaneous others [317-33, 335-339]. There was no information to our knowledge in the literature on the laboratory studies of fate and behaviour of illcit drug using different sewage types. Also not very clear was the use of mass balance calculations to assess degradation and removal patterns of organic compounds from the removal rate data obtained from batch experiments. Therefore, using the current work to provide this important information from the batch studies as presented in Chapter 4 in order to generate mass balance calculations and to estimate influent concentrations of compounds was the main objective duly completed.

# 5. Mass Balance of Compounds in a Sewage Treatment Work

## 5.1 Introduction

There appears to be limited published work on the mass balances for illicit drugs. However, a review of many reported approaches of heavy metals in full-scale sewage treatment has been undertaken to demonstrate mass balance calculation and highlight assumptions inherent in these [264, 344-348]. Herein are some approaches to highlight the merits and demerits often associated with the mass balances of this nature and then offer alternative methods based on the present batch studies.

To estimate the levels of cocaine in wastewaters, Zuccato *et al* [124] first related the concentration of benzoylecgonine (BZE), a main metabolite of cocaine to estimate the loads of parent cocaine in effluent wastewater and this was later applied by other researchers [131, 134]. In their approach, certain assumptions were made: (i) a total of 45% of ingested cocaine dose is excreted as BZE, (ii) no loss or leakage of wastewater along the sewage system, (iii) no accidental discharge or 'dumping' of large quantities of BZE into the sewage system and (iv) the main source of cocaine and metabolites comes from the human urination and the metabolite used in back calculation is the major product.

Also they proposed some parameters relevant to the calculations including (i) the concentration of the main metabolite be ng $L^{-1}$, (ii) the ratio of the molecular masses of the parent drug and metabolites (e.g. BZE/COC), (iii) the influent flow rate ($m^3$ $sec^{-1}$), (iv) the population size that are served by the STW, (v) the percentages of drug dose excreted as major metabolites, (iv) correction factor and (v) proven stability of the main metabolite (BZE) with respect to pH and temperature. In calculating the load (g $day^{-1}$); the concentration of BZE (ng $L^{-1}$), flow rate and molar fraction of cocaine and BZE as well as their molar mass ratio were estimated. Zuccato used the approach to estimate the community consumption of cannabinoids, opiates and cocaine per day/1000 people but the percentage of drug dose excreted as drug target residue (DTR) and correction factor must be known. Bones *et al* [265] used 10% as a percentage of parent cocaine excreted to estimate the level of cocaine consumed with the assumption that cocaine was more stable in aqueous media and found in greater quantity than its main metabolite, benzoylecgonine. It appears the literature differs on the actual percentage of excreted cocaine and BZE from cocaine dose and more information is therefore required to address discrepancies in the literature.

In 2007, mass balances of pharmaceutical products were undertaken at the Soseigawa Municipal STW, Japan. Grab samples were taken 11 times from the effluents from grit chamber, the effluent from secondary sedimentation basin and the effluents from the two pilots scale-submerged MBRs of 175 L effective volume installed at the STW, operated at the same membrane flux and HRT of 6.7 h as this study and the result are presented in Table 41. Simple mass balances were estimated by

**Table 41:** Average concentrations and mass balances of pharmaceuticals in the WWTP and MBRs as found in Soseigawa Municipal STW, Japan [240]

| | WWTP | MBR-A | MBR-B |
|---|---|---|---|
| Water flow ($m^3$/day) | 125 000 | 0.624 | 0.624 |
| Excess sludge (kg/day) | 7865 | 0.035 | 0.033 |
| **Influent concentration[b] ($ngL^{-1}$, n=11)** | | | |
| Clofibric acid | 28 ± 8 | | |
| Diclofenac | 251 ± 100 | | |
| Ketoprofen | 979 ± 237 | | |
| Ibuprofen | 1966 ± 662 | | |
| Mafanamic acid | 221 ± 62 | | |
| naproxen | 276 ± 115 | | |
| **Effluent concentration[b] ($ngL^{-1}$, n=11)** | | | |
| Clofibric acid | 14 ± 4 | 14 ± 5 | 5 ± 4 |
| Diclofenac | 145 ± 32 | 124 ± 29 | 46 ± 17 |
| Ketoprofen | 445 ± 121 | 171 ± 60 | <20[c] |
| Ibuprofen | 40 ± 32 | 106 ± 68 | 35 ± 32 |
| Mafanamic acid | 62 ± 23 | 51 ± 1 | 15 ± 6 |
| naproxen | 99 ± 18 | 11 ± 12 | <10[c] |
| **Amount of pharmaceuticals adsorbed on sludge[b] (ng/g of TSS[d], n=4)** | | | |
| Clofibric acid | <4[c] | <4[c] | <4[c] |
| Diclofenac | 35 ± 7 | 135 ± 200 | 31 ± 7 |
| Ketoprofen | <40[c] | <40 | <40[c] |
| Ibuprofen | 51 ± 8 | 26 ± 8 | 18 ± 6 |
| Mafanamic acid | 130 ± 71 | 111 ± 27 | 92 ± 29 |
| naproxen | <20[c] | <20[c] | <20[c] |
| **Total elimination during wastewater treatment [g/day (WWTP) or ug/day (MBR)]** | | | |
| Clofibric acid | 1.75 | 8.74 | 14.4 |
| Diclofenac | 13.3 | 79.2 | 128 |
| Ketoprofen | 66.8 | 504 | 598 |
| Ibuprofen | 241 | 1160 | 1200 |
| Mafanamic acid | 19.9 | 106 | 129 |
| naproxen | 22.1 | 165 | >166 |
| **Elimination due to sorption [g/day (WWTP) or ug/day (MBR)]** | | | |
| Clofibric acid | <0.031 | <0.14 | <0.13 |
| Diclofenac | 0.28 | 4.7 | 1.0 |
| Ketoprofen | <0.31 | <1.4 | <1.3 |
| Ibuprofen | 0.40 | 0.91 | 0.59 |
| Mafanamic acid | 1.0 | 3.9 | 3.0 |
| naproxen | <0.16 | <0.70 | <0.66 |
| **Sorption vs Elimination (%)** | | | |
| Clofibric acid | <2 | <2 | <0.9 |
| Diclofenac | 2 | 6 | 0.8 |
| Ketoprofen | <0.5 | <0.3 | <0.3 |
| Ibuprofen | 0.2 | 0.1 | 0.05 |
| Mafanamic acid | 5 | 4 | 2 |
| naproxen | <0.7 | <0.4 | <0.4 |

[a]Amount of sludge extracted from each process to maintain a target concentration of biomass, [b] Data are shown with standard deviations. [c]Concentrations were always <LOQ. [d] Dry weight is represented by grams of TSS

inflow concentration minus outflow concentration. However, the problems of representative samplings and effect of rainfall were not taken into account as sampling was in summer at low rainfall, this meant the dilution of the effluent was minimal and for this reason the errors often encountered as a result of rain dilution have been eliminated. Also, the presence of conjugates were

overlooked but it has been reported that pharmaceuticals that enter the STWs are significantly underestimated in studies as dilution of influent wastewater in STW would have occurred and result is an overestimate of performance.

A detailed study of the occurrence and removal of selected pharmacuticals compounds in a sewage treatment works utilising activated sludge treatment was undertaken by Jones *et al* [78] in England, UK. The mass balance was completed using municipal sewage samples collected over four days sampling periods for the study. The example of the simple mass balance of the flow through the works was consistent with the large amount of data as presented in Table 42. To calculate a simple mass balance of the flow of pharmaceuticals along the sewage works processes, Jones *et al* used the formula: $M_{rem} = m_{in} - m_{out}$ [eqn. 9]

Where, $M_{rem}$= mass removed by the activated sludge, $m_{in}$ = mass of compound from settled sewage and $m_{out}$= total mass leaving the works in the final effluent. The main error were from the returned activated sludge, RAS which was mixed with the settled sewage before entering the

**Table 42:** Mass balance (g/d) of pharmaceuticals over 4 day sampling period in England, UK [78] (Note the contrasting approaches to mass balance calculation as compared to Table 44).

| **Compounds** | **Day** | | | | **Removal rate (%)** |
|---|---|---|---|---|---|
| | | $M_{in}$ | $M_{out}$ | ($M_{in}$-$M_{out}$) | |
| Ibuprofen | 1 | 4.82 | 8.38 | 46.44 | 84.71 |
| | 2 | 40.89 | 8.06 | 32.84 | 80.29 |
| | 3 | 47.58 | 5.67 | 41.91 | 88.08 |
| | 4 | 45.54 | 4.27 | 41.27 | 90.62 |
| | Mean | 47.21 | 6.60 | 40.61 | 86.03 |
| Paracetamol | 1 | 28.37 | 3.77 | 24.60 | 86.71 |
| | 2 | 27.72 | 2.10 | 25.62 | 92.42 |
| | 3 | 24.92 | 1.23 | 23.70 | 95.08 |
| | 4 | 24.17 | 1.57 | 22.60 | 93.51 |
| | Mean | 26.30 | 2.17 | 24.13 | 91.93 |
| Salbutamol | 1 | 35.93 | 2.94 | 32.99 | 9183 |
| | 2 | 32.23 | 3.09 | 29.13 | 90.40 |
| | 3 | 44.13 | 2.11 | 42.02 | 95.21 |
| | 4 | 53.81 | 0.82 | 52.98 | 98.47 |
| | Mean | 41.52 | 2.24 | 39.28 | 94.60 |
| Melfenamic acid | 1 | 51.72 | 4.83 | 46.89 | 90.66 |
| | 2 | 60.36 | 5.40 | 54.96 | 91.05 |
| | 3 | 42.87 | 5.62 | 37.25 | 86.89 |
| | 4 | 47.79 | 1.31 | 46.48 | 97.25 |
| | Mean | 50.69 | 4.26 | 46.40 | 91.54 |

activated sludge unit and this was assumed to be 'locked in the system' and removal of selected compounds during primary sedimentation ought to have been carried out in primary tanks prior to the

experiment to completely eliminate bias, but this may be insignificant as the study was limited to activated sludge.As a useful tool in allowing the fate of drugs to be accounted for, as well as assessing analytical quality mass balance calculation is important wherever the data is available. It appears the problems due to sampling logistics and desludging process often introduce high errors in the mass balances of heavy metals through sewage works and these make methods and methodology of reporting mass balances vary considerably [264]. Therefore more work is required to address the discrepancies in the literature, which the current study hopes to provide.

### 5.2 Mass balance calculation from batch studies data with Molesworth sewage samples

The site flow data for the last 24 months in RAF Molesworth shown in Figures 33 and 34 gives indication of the average flows and the range. A meter measures and records the flow to the primary tank. The data is logged and recorded by site operatives. For performance assessment the maximum flow has been taken as the consented 360 $m^3$/d (0.10 Mgal/d), while the average volume treated by the works is approximately 78.4 $m^3$/d (0.02 Mgal/d). The hydraulic retention time (HRT) and average time the flow spends in each treatment unit is a function of the average flow as calculated in equation 11. The process calculation of each process unit including the volume is presented in Appendix D [292]:

Hydraulic Loading Rate (m/h) = flow to tanks ($m^3$/h) / total surface area ($m^2$) [eqn 10]

Hydraulic Retention Time (h) = flow to tanks ($m^3$/h) / total volume of tanks ($m^3$) [eqn 11]

Therefore, HRT = 360 $m^3$/d (15 $m^3$/h) / 78.4 x 3 $m^3$/d (9.81 $m^3$/h)

= ~ 1.5 hr

For mass balances, duplicate samples were collected every 15 minutes over 3h to provide composite batch samples for each process stage. The samples were taken through the process at the following four locations: (1) primary effluent (2) submerged aerated filter-1, (3) mixed submerged aerated filterand & (4) humus tanks effluent. RAF Molesworth currently operates no base housing, so the wastewater composition does not conform to typical ranges and conditions for wastewater. However, the actual TSS and COD were easy to assess as the primary effluent did not include the recirculation flow which would have diluted the concentration by the recirculation flow. The plastic media filter has been replaced by the SAF (a COPA CB750, which has a process volume of 40 cubic meters) hence; this recirculation pumping system has not been used. It was installed to ensure the required wetting rate of the plastic media filter was maintained. In a SAF, the filter is entirely submerged and mechanically aeration is provided.

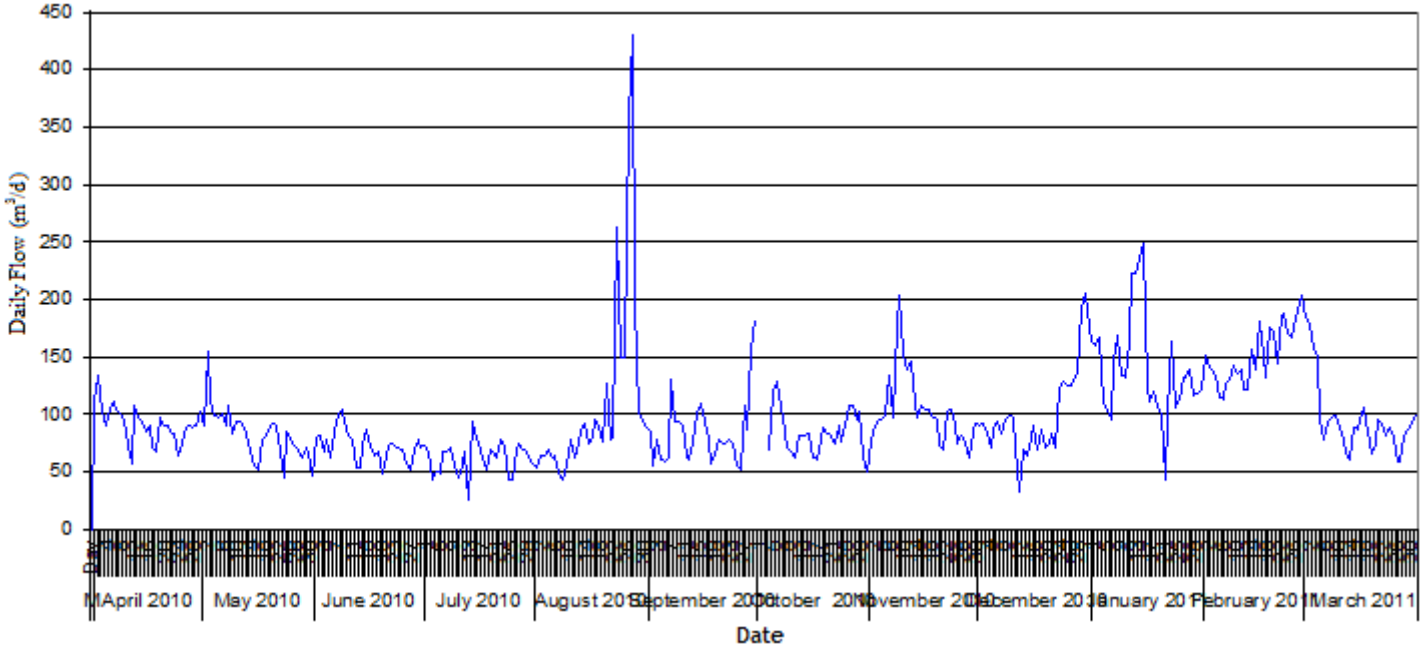



**Figure 33:** RAF Molesworth - Waste Water Flow Data for Year 10-11

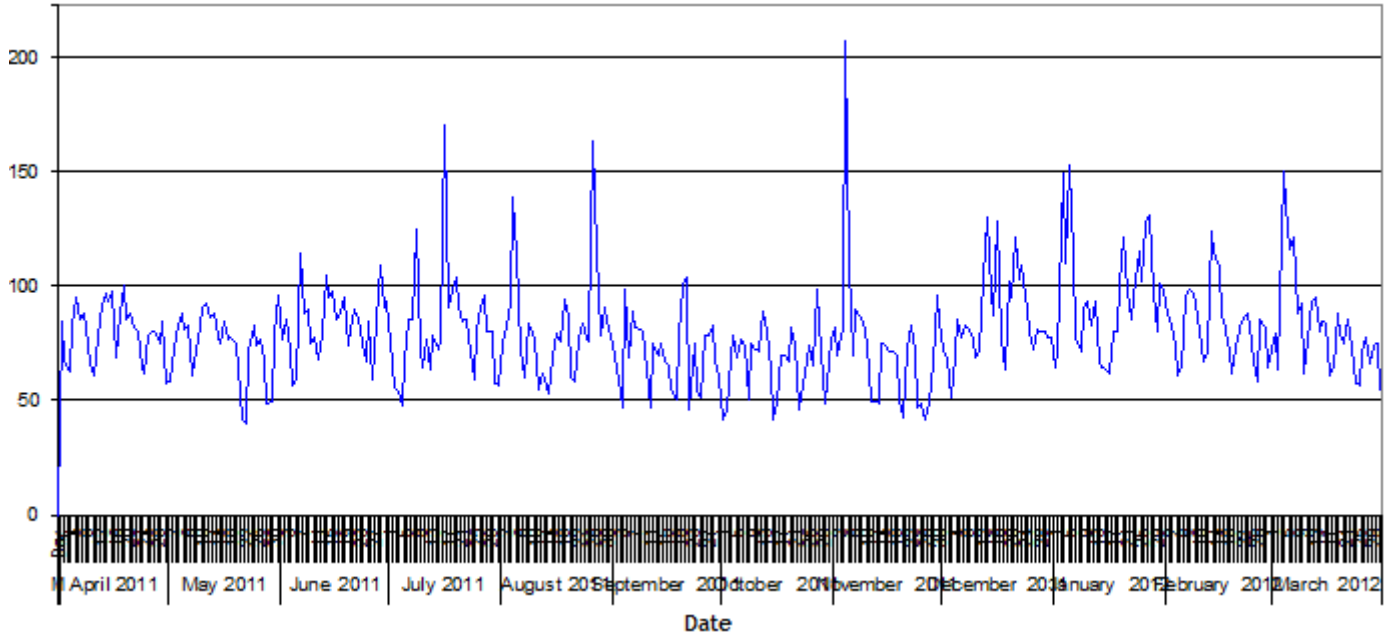



**Figure 34:** RAF Molesworth - Waste Water Flow Data for Year 11-12

### 5.2.1. Procedures used in mass balance calculation:

1. In these batch studies, the removal of compounds was through adsorption to solids and degradation and data table of different sludge experiments are presented in the Appendix 4 as listed below.

A. partition/degradation of cocaine in all the four sewage types– Tables: 2A, 5A, 8A, 11A
B. partition/degradation of benzoylecgonine in the four sewage types – Tables: 15, 17, 18, 19
C. partition/degradation of morphine in the four sewage types – Tables: 22, 24, 25, 26
D. partition/degradation of 6-ACM in the four sewage types - Tables: 29, 31, 32, 33
E. partition/degradation of heroin in the four sewage types – Tables: 36A, 38A, 39A, 40A
F. partition/degradation of diazepam in the four sewage types – Tables: 43, 45, 46, 47

2. The percent removal rate data used are taken from Table 21 – 40 using equation 8:

$$\% \text{ removal} = \frac{100\,([\text{initial drug}] - [\text{final drug in aqueous phase}])}{[\text{Initial drug}]}$$

obtained from different sludge of STW units are summarised below in the following order:

Table 43 (cocaine)

Table 44 (benzoylecgonine)

Table 45 (morphine)

Table 46 (6-monoacetylmorphine)

Table 47 (heroin) and

Table 48 (diazepam)

3. The STW process at Molesworth goes through terminal pumping station, screens (no grit trap), primary tank, submerged aerated filter (SAF), humus tank, reed bed, and finally to the outfall chamber. Each dissolved compound in effluent of an STW unit is passed on to the next unit where the compound would again partition into aqueous-solid layers and the new partitioning concentration calculated as presented in Table 43 – 48. The successive partitioning of compound along through STW indicate the measure of mass balance of individual drug through different STW units using equation 10 below:

$$\text{Conc. of drug in effluent of each unit} = (\%\text{ removal rate}) \times [\text{dissolved drug}] \qquad \text{[eqn. 10]}$$

4. The final effluent (µg in 250 ml) was then multiplied by 4 and converted to final concentration expressed in $\mu g L^{-1}$

5. Back-calculation assessment is to cross-check and account for all the masses through the STW bringing the total mass-flow to original batch concentration (12.5 mg in 250 mL).

$$\text{Conc. of drug in effluent of each unit} \div (\%\text{ removal rate}) = [\text{dissolved drug}] \qquad \text{[eqn. 11]}$$

**Mass balance modelling calculation:**

**Note: The percent removal rates data of compounds from all sewage types were used in the mass balance modelling work as presented below in Table 43 – 48.**

For example, mass balance in the first row of Table 43 after 15 min degradation goes thus:

Removal rate of cocaine by sorption & degradation from 12500 µg of cocaine in 250 mL flask:

First PS row = 12500 µg **x** 95.3% (0.953) = 11912.5 µg (removed by sorption/degradation)
= 12500 µg **x** 4.7% (0.047) = 587.5 µg (dissolved in aqueous phase) →

Second SAF row = 587.5 µg (dissolved in aqueous) **x** 63% (0.63) = 370.13 µg (removed/sorbed)
= 587.5 µg **x** 37% (0.370) = 217.4 µg (dissolved in aqueous phase) →

Third MSAF row = 217.4 µg (dissolved in aqueous) **x** 94.9 % (0.949) = 206.31 µg (removed)
= 217.4 µg **x** 5.1% (0.051) = 11.09 µg (dissolved in aqueous phase) →

Fourth HS row = 11.09 µg (dissolved in aqueous phase) **x** 81.0% (0.810) = 8.98 µg
= 11.09 µg **x** 19.0% (0.019) = 2.11 µg (dissolved in aqueous phase) → **to effluent**

Total removed by degradation & sorption = 11912.5 + 370.13 + 206.31 + 8.98

= **12497.89** µg

Final effluent (L) = 2.11 in 250 mL **x** 4 = **8.43** (µg $L^{-1}$)

(**Note**: Above calculation applies to all compounds passing the columns from 15 min to 180 min)

In Table 43**,** the concentration of cocaine from the batch studies experiment using primary (PS), submerged aerated filter-1 (SAF-1), mixed submerged aerated filter (MSAF) and humus sludge are shown. Data derived from Table 2A, 5A, 8A & 11A (Appendix 4) were used to calculate the removal rates of drugs as illustrated above (Section 5.2.1). The removal rates are in **RED** (sorbed & degraded) and amount dissolved in aqueous phase are in **BLACK** for every 15 min timescale. In a STW with a HRT of 2 – 3 hours, for a mass balance the initial concentration goes through the STW, using the corresponding removal rates and the percent dissolved in the aqueous phase to estimate the concentration of drugs that moves through the processing units of STW, and then measured the effluent.

The final effluent concentrations (µg $L^{-1}$) were calculated in the last column by multiplying the final measured drugs by 4 (concentration in µg$L^{-1}$) since the initial batch concentration was 12500 µg in 250 mL. We then used the data to estimate the mass balance calculation and this similarly applies to

benzoylecgonine, morphine, 6 – monoacetylmorphine, heroin and diazepam in Table 43 -48, respectively.

**Table 43**: Concentration and mass balances of cocaine from the batch studies experiment using primary (PS), submerged aerated filter-1 (SAF-1), mixed submerged aerated filter (MSAF) and humus sludge and showing the removal rate of drugs in RED (sorbed & degraded) and amount dissolved in aqueous phase in BLACK. (Data derived from Table 2A, 5A, 8A & 11A in Appendix 4).

| Time (min) | Removal rate (%) from each STW units | | | | Cocaine partition in aqueous and solid phases (µg in 250 mL) | | | | | Mass Balance | Final Effluent (µg $L^{-1}$) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | PS | SAF | MSAF | HS | PS | SAF | MSAF | HS | Total degraded & sorbed | | |
| 15 | 4.7 | 37.0 | 5.1 | 19.0 | 587.50 | 217.38 | 11.09 | 2.11 | | 12500 | 8.43 |
| | 95.3 | 63.0 | 94.9 | 81.0 | 11912.50 | 370.13 | 206.29 | 8.98 | 12497.89 | | |
| 30 | 3.5 | 33.6 | 4.1 | 16.5 | 437.50 | 147.00 | 6.03 | 0.99 | | 12500 | 3.98 |
| | 96.5 | 66.4 | 95.9 | 83.5 | 12062.50 | 290.50 | 140.97 | 5.03 | 12499.01 | | |
| 45 | 3.5 | 26.9 | 3.6 | 14.8 | 437.50 | 117.69 | 4.24 | 0.63 | | 12500 | 2.51 |
| | 96.5 | 73.1 | 96.4 | 85.2 | 12062.50 | 319.81 | 113.45 | 3.61 | 12499.37 | | |
| **60** | 3.4 | 27.2 | 1.8 | 16.8 | 425.00 | 115.60 | 2.08 | 0.35 | | 12500 | 1.40 |
| | 96.6 | 72.8 | 98.2 | 83.2 | 12075.00 | 309.40 | 113.52 | 1.73 | 12499.65 | | |
| 75 | 3.0 | 24.0 | 1.4 | 15.1 | 375.00 | 90.00 | 1.26 | 0.19 | | 12500 | 0.76 |
| | 97.0 | 76.0 | 98.6 | 84.9 | 12125.00 | 285.00 | 88.74 | 1.07 | 12499.81 | | |
| 90 | 1.8 | 20.4 | 1.3 | 12.5 | 225.00 | 45.90 | 0.60 | 0.07 | | 12500 | 0.30 |
| | 98.2 | 79.2 | 98.7 | 87.5 | 12275.00 | 179.10 | 45.30 | 0.52 | 12499.93 | | |
| 105 | 1.8 | 13.8 | 1.2 | 10.7 | 225.00 | 31.05 | 0.37 | 0.04 | | 12500 | 0.16 |
| | 98.2 | 86.2 | 98.8 | 89.3 | 12275.00 | 193.95 | 30.68 | 0.33 | 12499.96 | | |
| **120** | 1.6 | 11.3 | 1.3 | 9.0 | 200.00 | 22.60 | 0.29 | 0.03 | | 12500 | 0.11 |
| | 98.4 | 88.7 | 98.7 | 91.0 | 12300.00 | 177.40 | 22.31 | 0.27 | 12499.97 | | |
| 135 | 1.3 | 9.2 | 0.9 | 7.9 | 162.50 | 14.95 | 0.13 | 0.01 | | 12500 | 0.04 |
| | 98.7 | 90.8 | 99.1 | 92.1 | 12337.50 | 147.55 | 14.82 | 0.12 | 12499.99 | | |
| 150 | 1.0 | 5.3 | 0.6 | 5.9 | 125.00 | 6.63 | 0.04 | 0.00 | | 12500 | 0.01 |
| | 99.0 | 94.7 | 99.4 | 94.1 | 12375.00 | 118.38 | 6.59 | 0.04 | 12500.00 | | |
| 165 | 0.7 | 4.0 | 0.4 | 2.7 | 87.50 | 3.50 | 0.01 | 0.00 | | 12500 | 0.00 |
| | 99.3 | 96.0 | 99.6 | 97.3 | 12412.50 | 84.00 | 3.49 | 0.01 | 12500.00 | | |
| **180** | 0.4 | 2.4 | 0.2 | 1.4 | 50.00 | 1.20 | 0.00 | 0.00 | | 12500 | 0.00 |
| | 99.6 | 97.6 | 99.8 | 98.6 | 12450.00 | 48.80 | 1.20 | 0.00 | 12500.00 | | |

*Black figure = dissolved (aqueous phase); Red figure = sorbed & degraded

**Table 44:** Concentration and mass balance of benzoylecgonine from the batch studies experiment using primary (PS), submerged aerated filter-1 (SAF-1), mixed submerged aerated filter (MSAF) and humus sludge and showing the removal rate of drugs in RED (sorbed & degraded) and amount dissolved in aqueous phase in BLACK. (Data derived from Table 15, 17, 18 & 19 in Appendix 4)

| Time (min) | Removal rate (%) from each STW units | | | | Benzoylecgonine partition in aqueous and solid phases (µg in 250 mL) | | | | | Mass Balance | Final Effluent (µg $L^{-1}$) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | PS | SAF | MSAF | HS | PS | SAF | MSAF | HS | Total degraded & sorbed | | |
| 15 | 3.3 | 48.9 | 51.9 | 30.9 | 412.50 | 201.71 | 104.69 | 32.35 | | 12500 | 129.40 |
| | 96.7 | 51.1 | 48.1 | 69.1 | 12087.50 | 210.79 | 97.02 | 72.34 | 12467.65 | | |
| 30 | 2.8 | 31.4 | 49.0 | 19.6 | 350.00 | 109.90 | 53.85 | 10.55 | | 12500 | 42.22 |
| | 97.2 | 68.6 | 51.0 | 80.4 | 12150.00 | 240.10 | 56.05 | 43.30 | 12489.45 | | |
| 45 | 2.6 | 24.9 | 38.9 | 17.4 | 325.00 | 80.93 | 31.48 | 5.48 | | 12500 | 21.91 |
| | 97.4 | 75.1 | 61.1 | 82.6 | 12175.00 | 244.08 | 49.45 | 26.00 | 12494.52 | | |
| **60** | 2.3 | 20.9 | 38.9 | 16.2 | 287.50 | 60.09 | 23.37 | 3.79 | | 12500 | 15.15 |
| | 97.7 | 79.1 | 61.1 | 83.8 | 12212.50 | 227.41 | 36.71 | 19.59 | 12496.21 | | |
| 75 | 1.9 | 20.0 | 32.8 | 15.8 | 237.50 | 47.50 | 15.58 | 2.46 | | 12500 | 9.85 |
| | 98.1 | 80.0 | 67.2 | 84.2 | 12262.50 | 190.00 | 31.92 | 13.12 | 12497.54 | | |
| 90 | 1.6 | 16.6 | 26.6 | 12.9 | 200.00 | 33.20 | 8.83 | 1.14 | | 12500 | 4.56 |
| | 98.4 | 83.4 | 73.4 | 87.1 | 12300.00 | 166.80 | 24.37 | 7.69 | 12498.86 | | |
| 105 | 1.3 | 14.0 | 18.6 | 11.3 | 162.50 | 22.75 | 4.23 | 0.48 | | 12500 | 1.91 |
| | 98.7 | 86.0 | 81.4 | 88.7 | 12337.50 | 139.75 | 18.52 | 3.75 | 12499.52 | | |
| **120** | 1.0 | 12.0 | 12.2 | 9.4 | 125.00 | 15.00 | 1.83 | 0.17 | | 12500 | 0.69 |
| | 99.0 | 88.0 | 87.8 | 90.6 | 12375.00 | 110.00 | 13.17 | 1.66 | 12499.83 | | |
| 135 | 0.8 | 10.0 | 10.8 | 7.0 | 100.00 | 10.00 | 1.08 | 0.08 | | 12500 | 0.30 |
| | 99.2 | 90.0 | 89.2 | 93.0 | 12400.00 | 90.00 | 8.92 | 1.00 | 12499.92 | | |
| 150 | 0.6 | 7.3 | 8.2 | 5.4 | 75.00 | 5.48 | 0.45 | 0.02 | | 12500 | 0.10 |
| | 99.4 | 92.7 | 91.8 | 94.6 | 12425.00 | 69.53 | 5.03 | 0.42 | 12499.98 | | |
| 165 | 0.3 | 4.8 | 6.6 | 3.5 | 37.50 | 1.80 | 0.12 | 0.00 | | 12500 | 0.02 |
| | 99.7 | 95.2 | 93.4 | 96.5 | 12462.50 | 35.70 | 1.68 | 0.11 | 12500.00 | | |
| **180** | 0.2 | 2.9 | 4.3 | 2.1 | 25.00 | 0.73 | 0.03 | 0.00 | | 12500 | 0.00 |
| | 99.8 | 97.1 | 95.7 | 97.9 | 12475.00 | 24.28 | 0.69 | 0.03 | 12500.00 | | |

*Black figure = dissolved (aqueous phase); Red figure = sorbed & degraded

**Table 45:** Concentration and mass balance of morphine from the batch studies experiment using primary (PS), submerged aerated filter-1 (SAF-1), mixed submerged aerated filter (MSAF) and humus sludge and showing the removal rate of drugs in RED (sorbed & degraded) and amount dissolved in aqueous phase in BLACK. (Data derived from Table 22, 24, 25 & 26 in Appendix 4)

| Time (min) | Removal rate (%) from each STW units | | | | Morphine partition in aqueous and solid phases (µg in 250 mL) | | | | | Mass Balance | Final Effluent (µg $L^{-1}$) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | PS | SAF | MSAF | HS | PS | SAF | MSAF | HS | Total degraded & sorbed | | |
| 15 | 15.2 | 5.6 | 35.3 | 1.0 | 1900.00 | 106.40 | 37.56 | 0.38 | | 12500 | 1.50 |
| | 84.8 | 94.4 | 64.7 | 99.0 | 10600.00 | 1793.60 | 68.84 | 37.18 | 12499.62 | | |
| 30 | 13.4 | 3.3 | 24.0 | 0.7 | 1675.00 | 55.28 | 13.27 | 0.09 | | 12500 | 0.37 |
| | 86.6 | 96.7 | 76.0 | 99.3 | 10825.00 | 1619.73 | 42.01 | 13.17 | 12499.91 | | |
| 45 | 11.5 | 2.9 | 22.0 | 0.6 | 1437.50 | 41.69 | 9.17 | 0.06 | | 12500 | 0.22 |
| | 88.5 | 97.1 | 78.0 | 99.4 | 11062.50 | 1395.81 | 32.52 | 9.12 | 12499.95 | | |
| **60** | 9.4 | 2.6 | 18.6 | 0.5 | 1175.00 | 30.55 | 5.68 | 0.03 | | 12500 | 0.11 |
| | 90.6 | 97.4 | 81.4 | 99.5 | 11325.00 | 1144.45 | 24.87 | 5.65 | 12499.97 | | |
| 75 | 8.2 | 2.0 | 14.8 | 0.5 | 1025.00 | 20.50 | 3.03 | 0.02 | | 12500 | 0.06 |
| | 91.8 | 98.0 | 85.2 | 99.5 | 11475.00 | 1004.50 | 17.47 | 3.02 | 12499.98 | | |
| 90 | 6.7 | 1.7 | 12.5 | 0.3 | 837.50 | 14.24 | 1.78 | 0.01 | | 12500 | 0.02 |
| | 93.3 | 98.3 | 87.5 | 99.7 | 11662.50 | 823.26 | 12.46 | 1.77 | 12499.99 | | |
| 105 | 5.1 | 1.4 | 10.9 | 0.3 | 637.50 | 8.93 | 0.97 | 0.00 | | 12500 | 0.01 |
| | 94.9 | 98.6 | 89.1 | 99.7 | 11862.50 | 628.58 | 7.95 | 0.97 | 12500.00 | | |
| **120** | 3.9 | 1.2 | 9.1 | 0.3 | 487.50 | 5.85 | 0.53 | 0.00 | | 12500 | 0.01 |
| | 96.1 | 98.8 | 90.9 | 99.7 | 12012.50 | 481.65 | 5.32 | 0.53 | 12500.00 | | |
| 135 | 3.2 | 0.7 | 7.6 | 0.3 | 400.00 | 2.80 | 0.21 | 0.00 | | 12500 | 0.00 |
| | 96.8 | 99.3 | 92.4 | 99.7 | 12100.00 | 397.20 | 2.59 | 0.21 | 12500.00 | | |
| 150 | 2.3 | 0.3 | 5.7 | 0.2 | 287.50 | 0.86 | 0.05 | 0.00 | | 12500 | 0.00 |
| | 97.7 | 99.7 | 94.3 | 99.8 | 12212.50 | 286.64 | 0.81 | 0.05 | 12500.00 | | |
| 165 | 1.3 | 0.2 | 4.0 | 0.2 | 162.50 | 0.33 | 0.01 | 0.00 | | 12500 | 0.00 |
| | 98.7 | 99.8 | 96.0 | 99.8 | 12337.50 | 162.18 | 0.31 | 0.01 | 12500.00 | | |
| **180** | 0.5 | 0.1 | 2.4 | 0.1 | 62.50 | 0.06 | 0.00 | 0.00 | | 12500 | 0.00 |
| | 99.5 | 99.9 | 97.6 | 99.9 | 12437.50 | 62.44 | 0.06 | 0.00 | 12500.00 | | |

*Black figure = dissolved (aqueous phase); Red figure = sorbed & degraded

**Table 46:** Concentration and mass balances of 6-monoacetylmorphine from the batch studies experiment using primary (PS), submerged aerated filter-1 (SAF-1), mixed submerged aerated filter (MSAF) and humus sludge and showing the removal rate of drugs in RED (sorbed & degraded) and amount dissolved in aqueous phase in BLACK.. (Data derived from Table 29, 31, 32 & 33 in Appendix 4)

| Time (min) | Removal rate (%) from each STW units | | | | 6MAM partition in aqueous and solid phases (µg in 250 mL) | | | | | Mass Balance | Final Effluent (µg $L^{-1}$) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | PS | SAF | MSAF | HS | PS | SAF | MSAF | HS | Total degraded & sorbed | | |
| 15 | 1.7 | 81.1 | 13.3 | 33.6 | 212.50 | 172.34 | 22.92 | 7.70 | | 12500 | 30.81 |
| | 98.3 | 18.9 | 86.7 | 66.4 | 12287.50 | 40.16 | 149.42 | 15.22 | 12492.30 | | |
| 30 | 1.3 | 56.7 | 11.6 | 29.1 | 162.50 | 92.14 | 10.69 | 3.11 | | 12500 | 12.44 |
| | 98.7 | 43.3 | 88.4 | 70.9 | 12337.50 | 70.36 | 81.45 | 7.58 | 12496.89 | | |
| 45 | 1.0 | 48.2 | 10.1 | 23.3 | 125.00 | 60.25 | 6.09 | 1.42 | | 12500 | 5.67 |
| | 99.0 | 51.8 | 89.9 | 76.7 | 12375.00 | 64.75 | 54.16 | 4.67 | 12498.58 | | |
| **60** | 0.8 | 49.6 | 9.0 | 14.2 | 100.00 | 49.60 | 4.46 | 0.63 | | 12500 | 2.54 |
| | 99.2 | 50.4 | 91.0 | 85.8 | 12400.00 | 50.40 | 45.14 | 3.83 | 12499.37 | | |
| 75 | 0.6 | 41.9 | 7.5 | 10.9 | 75.00 | 31.43 | 2.36 | 0.26 | | 12500 | 1.03 |
| | 99.4 | 58.1 | 92.5 | 89.1 | 12425.00 | 43.58 | 29.07 | 2.10 | 12499.74 | | |
| 90 | 0.5 | 29.8 | 6.1 | 9.9 | 62.50 | 18.63 | 1.14 | 0.11 | | 12500 | 0.45 |
| | 99.5 | 70.2 | 93.9 | 90.1 | 12437.50 | 43.88 | 17.49 | 1.02 | 12499.89 | | |
| 105 | 0.5 | 18.4 | 5.2 | 8.2 | 62.50 | 11.50 | 0.60 | 0.05 | | 12500 | 0.20 |
| | 99.5 | 81.6 | 94.8 | 91.8 | 12437.50 | 51.00 | 10.90 | 0.55 | 12499.95 | | |
| **120** | 0.4 | 14.0 | 4.4 | 6.7 | 50.00 | 7.00 | 0.31 | 0.02 | | 12500 | 0.08 |
| | 99.6 | 86.0 | 95.6 | 93.3 | 12450.00 | 43.00 | 6.69 | 0.29 | 12499.98 | | |
| 135 | 0.2 | 10.9 | 3.6 | 5.0 | 25.00 | 2.73 | 0.10 | 0.00 | | 12500 | 0.02 |
| | 99.8 | 89.1 | 96.4 | 95.0 | 12475.00 | 22.28 | 2.63 | 0.09 | 12500.00 | | |
| 150 | 0.2 | 8.3 | 2.9 | 3.4 | 25.00 | 2.08 | 0.06 | 0.00 | | 12500 | 0.01 |
| | 99.8 | 91.7 | 97.1 | 96.6 | 12475.00 | 22.93 | 2.01 | 0.06 | 12500.00 | | |
| 165 | 0.1 | 6.4 | 2.0 | 2.4 | 12.50 | 0.80 | 0.02 | 0.00 | | 12500 | 0.00 |
| | 99.9 | 93.6 | 98.0 | 97.6 | 12487.50 | 11.70 | 0.78 | 0.02 | 12500.00 | | |
| **180** | 0.0 | 3.2 | 1.1 | 1.4 | 0.00 | 0.00 | 0.00 | 0.00 | | 12500 | 0.00 |
| | 100.0 | 96.8 | 98.9 | 98.6 | 12500.00 | 0.00 | 0.00 | 0.00 | 12500.00 | | |

*Black figure = dissolved (aqueous phase); Red figure = sorbed & degraded

**Table 47:** Concentration and mass balances of heroin from the batch studies experiment using primary (PS), submerged aerated filter-1 (SAF-1), mixed submerged aerated filter (MSAF) and humus sludge and showing the removal rate of drugs in RED (sorbed & degraded) and amount dissolved in aqueous phase in BLACK.. (Data derived from Table 36A, 38A, 39A & 40A in Appendix 4)

| Time (min) | Removal rate (%) from each STW units | | | | Heroin partition in aqueous and solid phases (µg in 250 mL) | | | | | Mass Balance | Final Effluent (µg $L^{-1}$) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | PS | SAF | MSAF | HS | PS | SAF | MSAF | HS | Total degraded & sorbed | | |
| 15 | 3.8 | 0.4 | 0.4 | 12.3 | 475.00 | 1.90 | 0.0076 | 0.0009 | | 12500 | 0.0037 |
| | 96.2 | 99.6 | 99.6 | 87.7 | 12025.00 | 473.10 | 1.8924 | 0.0067 | 12500 | | |
| 30 | 3.3 | 0.4 | 0.4 | 8.9 | 412.50 | 1.65 | 0.0066 | 0.0006 | | 12500 | 0.0023 |
| | 96.7 | 99.6 | 99.6 | 91.1 | 12087.50 | 410.85 | 1.6434 | 0.006 | 12500 | | |
| 45 | 2.3 | 0.4 | 0.3 | 7.3 | 287.50 | 1.15 | 0.0035 | 0.0003 | | 12500 | 0.0010 |
| | 97.7 | 99.6 | 99.7 | 92.7 | 12212.50 | 286.35 | 1.1465 | 0.0032 | 12500 | | |
| **60** | 2.2 | 0.3 | 0.3 | 6.3 | 275.00 | 0.83 | 0.0025 | 0.0002 | | 12500 | 0.0006 |
| | 97.8 | 99.7 | 99.7 | 93.7 | 12225.00 | 274.18 | 0.8225 | 0.0023 | 12500 | | |
| 75 | 1.6 | 0.3 | 0.2 | 4.5 | 200.00 | 0.60 | 0.0012 | 0.0001 | | 12500 | 0.0002 |
| | 98.4 | 99.7 | 99.8 | 95.5 | 12300.00 | 199.40 | 0.5988 | 0.0011 | 12500 | | |
| 90 | 1.3 | 0.2 | 0.2 | 3.0 | 162.50 | 0.33 | 0.0007 | 0.0 | | 12500 | 0.0001 |
| | 98.7 | 99.8 | 99.8 | 97.0 | 12337.50 | 162.18 | 0.3243 | 0.0007 | 12500 | | |
| 105 | 1.1 | 0.2 | 0.2 | 2.5 | 137.50 | 0.28 | 0.0006 | 0.0 | | 12500 | 0.0001 |
| | 98.9 | 99.8 | 99.8 | 97.5 | 12362.50 | 137.23 | 0.2744 | 0.0006 | 12500 | | |
| **120** | 0.6 | 0.1 | 0.1 | 2.1 | 75.00 | 0.08 | 0.0001 | 0.0 | | 12500 | 0.0000 |
| | 99.4 | 99.9 | 99.9 | 97.9 | 12425.00 | 74.93 | 0.0749 | 0.0001 | 12500 | | |
| 135 | 0.5 | 0.1 | 0.1 | 1.1 | 62.50 | 0.06 | 0.0001 | 0.0 | | 12500 | 0.0000 |
| | 99.5 | 99.9 | 99.9 | 98.9 | 12437.50 | 62.44 | 0.0624 | 0.0001 | 12500 | | |
| 150 | 0.3 | 0.1 | 0.1 | 0.9 | 37.50 | 0.04 | 0.0 | 0.0 | | 12500 | 0.0000 |
| | 99.7 | 99.9 | 99.9 | 99.1 | 12462.50 | 37.46 | 0.0375 | 0.0 | 12500 | | |
| 165 | 0.2 | 0.1 | 0.1 | 0.9 | 25.00 | 0.03 | 0.0 | 0.0 | | 12500 | 0.0000 |
| | 99.8 | 99.9 | 99.9 | 99.1 | 12475.00 | 24.98 | 0.0250 | 0.0 | 12500 | | |
| **180** | 0.1 | 0.0 | 0.0 | 0.5 | 12.50 | 0.00 | 0.0 | 0.0 | | 12500 | 0.0000 |
| | 99.9 | 100.0 | 100.0 | 99.5 | 12487.50 | 12.50 | 0.0 | 0.0 | 12500 | | |

*Black figure = dissolved (aqueous phase); Red figure = sorbed & degraded

**Table 48:** Concentration and mass balances of diazepam. from the batch studies experiment using primary (PS), submerged aerated filter-1 (SAF-1), mixed submerged aerated filter (MSAF) and humus sludge and showing the removal rate of drugs in RED (sorbed & degraded) and amount dissolved in aqueous phase in BLACK. (Data derived from Table 43, 45, 46 & 47 in Appendix 4)

| Time (min) | Removal rate (%) from each STW units | | | | Diazepam partition in aqueous and solid phases (µg in 250 mL) | | | | | Mass Balance | Final Effluent (µg $L^{-1}$) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | PS | SAF | MSAF | HS | PS | SAF | MSAF | HS | Total degraded & sorbed | | |
| 15 | 16.1 | 1.0 | 14.5 | 0.5 | 2012.50 | 20.13 | 2.9181 | 0.0146 | | 12500 | 0.0584 |
| | 83.9 | 99.0 | 85.5 | 99.5 | 10487.50 | 1992.38 | 17.2069 | 2.9035 | 12499.99 | | |
| 30 | 12.4 | 1.0 | 11.1 | 0.5 | 1550.00 | 15.50 | 1.7205 | 0.0086 | | 12500 | 0.0344 |
| | 87.6 | 99.0 | 88.9 | 99.5 | 10950.00 | 1534.50 | 13.7795 | 1.7119 | 12499.99 | | |
| 45 | 11.2 | 0.9 | 9.9 | 0.5 | 1400.00 | 12.60 | 1.2474 | 0.0062 | | 12500 | 0.0249 |
| | 88.8 | 99.1 | 90.1 | 99.5 | 11100.00 | 1387.40 | 11.3526 | 1.2412 | 12499.99 | | |
| **60** | 8.8 | 0.8 | 8.6 | 0.4 | 1100.00 | 8.80 | 0.7568 | 0.0030 | | 12500 | 0.0121 |
| | 91.2 | 99.2 | 91.4 | 99.6 | 11400.00 | 1091.20 | 8.0432 | 0.7538 | 12500.00 | | |
| 75 | 7.8 | 0.7 | 6.5 | 0.4 | 975.00 | 6.83 | 0.4436 | 0.0018 | | 12500 | 0.0071 |
| | 92.2 | 99.3 | 93.5 | 99.6 | 11525.00 | 968.18 | 6.3814 | 0.4418 | 12500.00 | | |
| 90 | 5.2 | 0.6 | 4.4 | 0.3 | 650.00 | 3.90 | 0.1716 | 0.0005 | | 12500 | 0.0021 |
| | 94.8 | 99.4 | 95.6 | 99.7 | 11850.00 | 646.10 | 3.7284 | 0.1711 | 12500.00 | | |
| 105 | 4.8 | 0.5 | 4.2 | 0.3 | 600.00 | 3.00 | 0.1260 | 0.0004 | | 12500 | 0.0015 |
| | 95.2 | 99.5 | 95.8 | 99.7 | 11900.00 | 597.00 | 2.8740 | 0.1256 | 12500.00 | | |
| **120** | 3.5 | 0.4 | 3.5 | 0.3 | 437.50 | 1.75 | 0.0613 | 0.0002 | | 12500 | 0.0007 |
| | 96.5 | 99.6 | 96.5 | 99.7 | 12062.50 | 435.75 | 1.6887 | 0.0611 | 12500.00 | | |
| 135 | 2.5 | 0.3 | 2.0 | 0.3 | 312.50 | 0.94 | 0.0187 | 0.0001 | | 12500 | 0.0002 |
| | 97.5 | 99.7 | 98.0 | 99.7 | 12187.50 | 311.56 | 0.9188 | 0.0186 | 12500.00 | | |
| 150 | 1.5 | 0.2 | 1.2 | 0.2 | 187.50 | 0.38 | 0.0045 | 0.0000 | | 12500 | 0.0000 |
| | 98.5 | 99.8 | 98.8 | 99.8 | 12312.50 | 187.13 | 0.3705 | 0.0045 | 12500.00 | | |
| 165 | 1.0 | 0.1 | 0.8 | 0.2 | 125.00 | 0.13 | 0.0010 | 0.0000 | | 12500 | 0.0000 |
| | 99.0 | 99.9 | 99.2 | 99.8 | 12375.00 | 124.88 | 0.1240 | 0.0010 | 12500.00 | | |
| **180** | 0.3 | 0.1 | 0.6 | 0.2 | 37.50 | 0.04 | 0.0002 | 0.0000 | | 12500 | 0.0000 |
| | 99.7 | 99.9 | 99.4 | 99.8 | 12462.50 | 37.46 | 0.0373 | 0.0002 | 12500.00 | | |

*Black figure = dissolved (aqueous phase); Red figure = sorbed & degraded

## 5.3 Back-calculation assessment of compounds.

In Table 49, the result of back-calculation to estimate initial concentration of analytes in raw influent wastewaters using their respective removal rates in reversed order from Table 43 as shown below and arriving back at initial influent concentration of 12500 µg $L^{-1}$. This confirms or checks the mass balance calculation operation has been done correctly.

**Table 49:** Mass Balance back-calculation in reversed order in humus sludge (HS), mixed submerged aerated filter (MSAF), submerged aerated filter-1 (SAF-1) and primary sludge (PS), showing the removal rate of drugs in RED (sorbed & degraded) and amount dissolved in aqueous phase in BLACK. (Data from Table 44)

| | **Removal rate (%) from each STW units** | | | | **Cocaine partition in aqueous and solid phases (µg in 250 mL)** | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Time (min)** | HS | MSAF | SAF | PS | HS | MSAF | SAF | PS | Total degraded & sorbed | **Batch Conc. (µg in 250ml)** |
| 15 | 19.0 | 5.1 | 37.0 | 4.7 | 2.11 | 11.09 | 217.38 | 587.50 | | 12500 |
| | 81.0 | 94.1 | 63.0 | 95.3 | 8.98 | 206.29 | 370.13 | 11912.50 | 12497.89 | |
| 30 | 16.5 | 4.1 | 33.6 | 3.5 | 0.99 | 6.03 | 147.00 | 437.50 | | 12500 |
| | 83.5 | 95.9 | 66.4 | 96.5 | 5.03 | 140.97 | 290.50 | 12062.50 | 12499.01 | |
| 45 | 14.8 | 3.6 | 26.9 | 3.5 | 0.63 | 4.24 | 117.69 | 437.50 | | 12500 |
| | 85.2 | 96.4 | 73.1 | 96.5 | 3.61 | 113.45 | 319.81 | 12062.50 | 12499.37 | |
| **60** | 16.8 | 1.8 | 27.2 | 3.4 | 0.35 | 2.08 | 115.60 | 425.00 | | 12500 |
| | 83.2 | 98.2 | 72.8 | 96.6 | 1.73 | 113.52 | 309.40 | 12075.00 | 12499.65 | |
| 75 | 15.1 | 1.4 | 24.0 | 3.0 | 0.19 | 1.26 | 90.00 | 375.00 | | 12500 |
| | 84.9 | 98.6 | 76.0 | 97.0 | 1.07 | 88.74 | 285.00 | 12125.00 | 12499.81 | |
| 90 | 12.5 | 1.3 | 20.4 | 1.8 | 0.07 | 0.60 | 45.90 | 225.00 | | 12500 |
| | 87.5 | 98.7 | 79.2 | 98.2 | 0.52 | 45.30 | 179.10 | 12275.00 | 12499.93 | |
| 105 | 10.7 | 1.2 | 13.8 | 1.8 | 0.04 | 0.37 | 31.05 | 225.00 | | 12500 |
| | 89.3 | 98.8 | 86.2 | 98.2 | 0.33 | 30.68 | 193.95 | 12275.00 | 12499.96 | |
| **120** | 9.0 | 1.3 | 11.3 | 1.6 | 0.03 | 0.29 | 25.64 | 226.90 | | 12500 |
| | 91.0 | 98.7 | 88.7 | 98.4 | 0.27 | 25.34 | 201.26 | 12273.08 | 12499.92 | |
| 135 | 7.9 | 0.9 | 9.2 | 1.3 | 0.01 | 0.13 | 14.95 | 162.50 | | 12500 |
| | 92.1 | 99.1 | 90.8 | 98.7 | 0.12 | 14.82 | 147.55 | 12337.50 | 12499.99 | |
| 150 | 5.9 | 0.6 | 5.3 | 1.0 | 0.00 | 0.04 | 6.63 | 125.00 | | 12500 |
| | 94.1 | 99.4 | 94.7 | 99.0 | 0.04 | 6.59 | 118.38 | 12375.00 | 12500.00 | |
| 165 | 2.7 | 0.4 | 4.0 | 0.7 | 0.00 | 0.01 | 3.50 | 87.50 | | 12500 |
| | 97.3 | 99.6 | 96.0 | 99.3 | 0.01 | 3.49 | 84.00 | 12412.50 | 12500.00 | |
| **180** | 1.4 | 0.2 | 2.4 | 0.4 | 0.00 | 0.00 | 1.20 | 50.00 | | 12500 |
| | 98.6 | 99.8 | 97.6 | 99.6 | 0.00 | 1.20 | 48.80 | 12450.00 | 12500.00 | |

## 5.4 Application of mass balance to calculate influent concentration of analytes from Nottingham STW effluent.

In this section (Table 50), we applied back-calculation for the selected analytes from Stoke Bardolph STW Nottingham effluent to estimate influent concentrations. In using the removal rates (%) obtained from batch studies data using sewage samples collected from Molesworth STW and applying it to the Stoke Bardolph STW Nottingham to estimate influent (ng $L^{-1}$) in back calculation. The following assumptions that may influence the removal rate (%) and its application in the back-calculation were made:

1. Operational design and treatment policy for Stoke Bardolph Nottingham and Molesworth STWs are assumed to be similar. There was no recirculation section in Molesworth STW and no submerged aerated filter (SAF) processing unit at Stoke Bardolph STW Nottingham.
2. The nature of sewage and its characteristics were assumed to be the representative of most municipal sewage types.
3. The STWs hydraulic retention times were assumed to be the same (though a total of 8 HRT in Molesworth and 16 HRT including recycling process in Stoke Bardolph STW Nottingham).
4. The batch experiments data obtained with the real sewage samples collected from each processing units of the Molesworth STW were assumed to be representative of real-time STW runs, the real time pilot run in the STW was not possible due to site restrictions.

**Table 50:** Estimation of analytes from effluents concentration at 2 HRT using the percent removal rates for Cocaine (Table 43) and Benzoylecgonine (Table 44) in back calculation to estimate influent concentration. (Note the reverse order of sewage sludges: HS →MSAF→SAF-1→PS)

| Analyte | **Analytes (%) in aqueous phase of each STW units.** | | | | **Concentration of analytes (ng $L^{-1}$) in STW units.** | | | | **Estimated influent (µg $L^{-1}$)** |
|---|---|---|---|---|---|---|---|---|---|
| | HS | MSAF | SAF-1 | PS | HS | MSAF | SAF-1 | PS | |
| COC | 9.0<br>91.0 | 1.3<br>98.7 | 11.3<br>88.7 | 1.6<br>98.4 | 1.9<br>19.2 | 21.1<br>1602.8 | 1623.9<br>12747.2 | 14371.1 | 14.3 |
| BZE | 9.4<br>90.6 | 12.2<br>87.8 | 12.0<br>88.0 | 1.0<br>99.0 | 32.9<br>317.1 | 350.0<br>2518.9 | 2868.9<br>21038.2 | 23907.1 | 23.9 |

*Black = dissolved (aqueous phase); Red = sorbed & degraded.

Using the concentration of drugs found from Stoke Bardolph STW Nottingham effluent in Table 18 and applying the percent removal rates of drugs (cocaine, benzoylecgonine and morphine) at 2h timescale for back- calculation as shown in Table 50:

The First HS row: = 1.9 ng $L^{-1}$ of COC (effluent)
Dissolved rate = 1.9 ÷ 9.0% (0.09) = 21.1 ng (dissolved in aqueous phase that goes to MSAF) →
Removal rate = 91.0% = 19.2 ng (removed by sorption/degradation)

Second MSAF row:
Dissolved rate = 21.1ng ÷ 1.3% (0.013) = 1623.9 ng (dissolved in aqueous that goes to SAF-1) →
Removal rate = 98.7% = 1602.8 ng (removed by sorption/degradation)

Third SAF row:
Dissolved rate = 1623.9 ÷ 0.113 ng = 14371.1 ng (dissolved in aqueous phase that goes to PS) →
Removal rate = 88.7% = 12747.2 ng (removed by sorption/degradation)

Fourth PS row:
Dissolved rate = 14371.1 ng (influent)
Removal rate = 98.4% = 883882.7 ng (removed by sorption/degradation)

Table 55 below, makes it easy to compare data of the effluents concentations of cocaine and benzoylecgonine obtained from Stoke Bardolph STW Nottingham with the back calculated influent concentrations for cocaine (14371.1 ng $L^{-1}$) and benzoylecgonine 23907.1 (ng $L^{-1}$).

**Table 51:** Comparing literature influent measurements of drugs

| **Influent measurements of cocaine and benzoylecgonine quoted from other places (Table 4 in Chapter 1)** | | | **Calculated influent based on back calculations from effluent using batch study removal rates** |
|---|---|---|---|
| **Analytes** | **Matrix** | **Measured Influent (ng $L^{-1}$)** | **(µg $L^{-1}$)** |
| **Cocaine** | 5 STPs, Spain | 225.0 | 14.3 |
| | 5 STPs, Belgium | 22 -678 | |
| | 37 STPs, Belgium | 32 – 753 | |
| | 5 STPs, Ireland, UK | 489 ± 117 | |
| | Eastern Spain | 370 – 1000.24 | |
| | 30 STPs, Belgium | 9 – 683 | |
| | 2 STPs, Italy | 218.4 – 421.4 | |
| | 42 STPs, NE Spain | 4 – 4700 | |
| | Barcelona, Spain | 2.40 | |
| **Benzoylecgonine** | 5 STPs, Spain | 2307.0 | 23.9 |
| | 5 STPs, Belgium | 82 – 1898 | |
| | 37 STPs, Belgium | 46 –2258 | |
| | 5 STPs, Ireland UK | 290 ± 11 | |
| | Eastern Spain | 150 – 1000.5 | |
| | 30 STPs, Belgium | 37 – 1550 | |
| | 2 STPs, Italy | 547.4 -197.2 | |
| | 42 STPs, NE Spain | 9 – 7500 | |
| | Barcelona, Spain | 5.24 | |
| | 12 STPs, Germany | 65 ± 5 | |

Our calculated values though seem very high results – (1000s ng $L^{-1}$) may possibly be due to uncertainty in primary removal in our batch studies as no recovery values from the solid phase were used [354], compared with those quoted in other places (Table 4 in Chapter 1). However, the capabilities of the current experimental batch data in generating removal rates

as used in our current mass balance approach have improved on the complications associated with assumptions of Zuccato *et al* [124] in their use of 45% of total ingested cocaine dose to calculate the concentration of excreted as BZE and Bones *et al* [265] used 10%. The literature apparent differences on the actual percentage of excreted cocaine and BZE from cocaine dose coupled with the problems due to sampling logistics and desludging as experienced by prior studies have made methodologies of reporting mass balances varied considerably and this is what the present new approach has addressed.

The removal rates of COC (91.0%), BZE (90.6%), HER (97.9%), (MOR (99.7%), 6MAM (93.3%) and DIAZ (99.7%) in total of 8 HRT compare to the removal efficiencies of some pharmaceuticals like ciprofloxacin (37-86%), ofloxacin (33-66%), norfloxacin (58-87%) and Iomefloxacin (21-72%) are presented, respectively [290].

Clear and simple steps in mass balance calculation of compounds in STWs has been presented in the current work and with some refinement, the conceptual approach may be useful.

# CHAPTER 6: Conclusions

In the current work, six representative compounds of classes of illicit drugs (cocaine, benzoylecgonine, heroin, 6 monoacetyl morphine, morphine and diazepam (pharmaceutical) were studied and these drugs exhibited comparable removals when in contact with both primary and biological secondary treatment sludge during the batch studies of 3h exposure time. The compounds were comparably dissolved, degraded, adsorbed and distributed between both solid and aqueous phases in a pattern that reflects their hydrophobic nature as degradation progressed. Observed rapid removals were exhibited in the first few minutes of contact with both primary and secondary sewage samples with increase in the degradation products as compounds exposed to microbial and chemical hydrolysis but the rate slows down as availability of nutrient source reduces. Elements of this work reaffirm existing knowledge and data derived from batch studies, removal rates and their application to Molesworth and Stoke Bardolph STWs are the novel aspects of this thesis. The main conclusions for the novel work are presented in 8 bullet points:

- Methods were developed for the determination of a range of drugs in wastewaters and sludge samples, which were applied to samples from two sewage treatment works of RAF Molesworth and Stoke Bardolph, Nottingham, UK. This stage of my work confirmed analytical methodologies had necessary capabilities for the work undertaken.
- The present study concentrations of analytes found were between 1.9 and 3147 ng $L^{-1}$ in effluents of Stoke Bardolph STW with the percentage recoveries ranged from 74.5 – 109.6%, with the instrumental limits of detection (LODs) ranges of 0.2 – 12.7 ng $L^{-1}$, and relative standard deviation (RSD) values of 0.6 – 4.7% for all the compounds were achieved. Procaine, bromacil, codeine, lidocaine, ibruprofen, caffeine, nicotine and diazepam were the most abundant compounds with concentrations of 99.2, 1806.8, 33.5, 71.8, 3147, 213.4, 252.5 and 105.2 ng $L^{-1}$, respectively, and the results were in line with values reported by other workers for such analyses.
- A number of degradation batch studies of cocaine, benzoylecgonine, heroin, morphine, 6-monoacetylmorphine and diazepam on sludge samples at two different temperatures showed consistent duplicates results with the degradation of compounds at 19 ± 0.5$^{o}$ C relatively greater but still occurred slowly at 4 ± 0.5$^{o}$ C, demonstrating that degradation was both biotic and abiotic but the degradation of compounds at 19 ± 0.5$^{o}$ C was faster than at 4 ± 0.5$^{o}$ C, by between 5 and 10%. Both biological and chemical degradations affects the removal of compounds in different rates at 4 ± 0.5 $^{o}$C, the degradation/partitioning is therefore temperature dependent.
- Optimal experimental strategy with refinement can be developed by incorporating good temperature process variables with knowledge of individual's compound degradation and

possible transport occurring in the STWs to achieve complete removals as demonstrated in the batch studies.

- Compounds removal by biodegradation, chemical degradation and sorption were the dominant removal processes (volatilization is unlikely) with removal rates of cocaine (91.0%), benzoylecgonine (90.6%), heroin (97.9%), morphine (99.7%), 6 monoacetylmorphine (93.3%) and diazepam (99.7%) having corresponding partitioning coefficients ($K_d$) ranged from 1.2 – 68.1 Kg $L^{-1}$for the same 8 HRT. Diazepam has the highest sorption removal in both primary and secondary sludge treatments and its exhibited tendencies of accumulating in sediments with identical $K_d$ values in all sludge types could possibly provide empirical relationship between drug removal and HRT.
- The characterization of the sewage sludge of RAF Moleswoth and Stoke Bardolph STW, Nottingham demonstrated strong variation in concentration strengths of TSS, CODs and occasional pH changes. In the RAF Molesworth STW, the TSS was 36243.0 mg $L^{-1}$ (influent) and 5.5 mg $L^{-1}$ (effluent), while Stoke Bardolph had 797.9 mg $L^{-1}$ (influent) and 8.6 mg $L^{-1}$ (effluent). The COD at RAF Molesworth was 216.8 $mgdm^{-3}O_2$ (influent) and 1.8 $mgdm^{-3}O_2$ (effluent), while Stoke Bardolph had 36.0 $mgdm^{-3}O_2$ (influent) and 3.6 $mgdm^{-3}O_2$ (effluent). The pH ranges of 7.8 and 8.4 were found between the influent and effluent wastewaters of the two STWs. Also, the average ash and organic carbon contents of the sewage samples were 0.94 g/g and 0.06 g/g, respectively.
- The measurements made from batch studies allowed for the development of a mass balance which indicates that 110.0 ng $L^{-1}$ (cocaine), 690.0 ng $L^{-1}$ (benzoylecgonine), 10.0 ng $L^{-1}$ (morphine), 80.0 ng $L^{-1}$ (6-monoacetylmorphine), 0.0 ng $L^{-1}$ (heroin) and 0.7 ng $L^{-1}$ (diazepam) remained in humus sludge that are passed into effluent in total of 8 HRT from an initial influent concentration of 12500 ng $L^{-1}$. Projected influent concentrations of cocaine (14, 471 ng $L^{-1}$) and benzoylecgonine (23, 907.1 ng $L^{-1}$) at Stoke Bardolph were derived from back-calculating measued final effluent concentrations using this same mass balance approach.
- Influent concentrations of cocaine (14. 4 µg $L^{-1}$) and benzoylecgonine (23.9 µg $L^{-1}$) were obtained from effluent concentrations in back-calculation of removal rates to demonstrate the application of mass balance approach in simple and clear steps. However, the uncertainties as highlighted in section 4.2.1 may be an important impacting factor on our eventual removal rate calculations.

Work encompassed directly measures illicit drug removal rates in laboratory studies for the first time and improvement over prior study where assumptions on removal rates were made. With refinement, the capabilities of the current experimental batch data in generating removal rates of drugs have

however been clearly demonstrated and applied, the conceptual approach developed may be very useful to obtain influent information of any organic compounds in real life (STWs) situations.

## Future **Research**

1. More work is required for batch experiments of other classes of illicit drugs using many STWs at different location. Complete transformation processes of the compounds should be further studied to provide additional exposure data for environmental scientists.

2. Mass balance calculations should be further applied using other STWs and composite sludge samples from all the major processing units be obtained to eliminate erroneous calculation of drug levels based on analytes found in surface water.

3. With approval from the relevant environmental agency, real-life pilot runs through some STWs to monitor degradation processes and assess removal efficiencies would be advantageous.

4. Chemical screening of phytotoxicity potential of these drugs and their active metabolites in the environment may be insufficient until the analysis of sediments and fruits grown in sewage – amended soils are investigated. It might give further information about possible transports of these residues to humans through organic foodstuffs. Understanding the adverse effects of other compounds would be a right step towards the development of safer sewage management practices.

---

- **Supplementary data for calibration, wastewater sampling analysis, batch studies and RAF process unit calculation are provided in the appendices.**

# References


[1] Rhind, S.M. **2002**, "Endocrine disrupting compounds and farm animals: their properties, actions and of exposure", *Domestic Animal Endocrinology,* vol. 23 (Special Issue), pp. 179-187.

[2] Rhind, S. M. **2005**, "Are endocrine disrupting compounds a threat to farm animal health, welfare and productivity", *Reproduction in Domestic Animals*. vol.40, pp. 282-290.

[3] Wilkinson, J. M., Hill, J. & Livesey, C.T. **2001**, "Accumulation of potentially toxic elements by sheep grazed on grassland given repeated applications of sewage sludge", *Animal Science*, vol. 72, pp. 179-190.

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

[5] Sharman, M., Read, W.A., Castle, L. & Gilbert, J. **1994**, " Levels of di-(2-ethylhexyl)phthalate and total phthalate esters in milk, cream, butter and cheese" *Food Addit Contam*ination, vol.11, no. 3, pp. 375-385.

[6] Koppe, J. G., Bartonova, A., Bolte, G., Bistrup, M. L., Busby, C., Butter, M., Dorfman, P., Fucic, A., Gee, D., van den Hazel, P. et al. **2006**, "Exposure to multiple environmental agents and their effect", *Acta Paediatr Suppl.*vol.95, pp 106–113.

[7] Heindel, J. J., Gulati, D. K., Mounce, R. C., Russell, S. R. & Lamb, J. C. **1989**, " Reproductive toxicity of three phtalic acid esters in a continuous breeding protocol", *Fundam Appl Toxicology*, vol.12, pp 508-518.

[8] Lottrup, G., Andersson, A. M., Leffers, H., Mortensen, G. K., Toppari, J., Skakkebaeek, N. E. & Main, K. M. **2006**, "Possible impact of phthalates on infant reproductive health. *International Journal of Andrology*", vol. 29, pp. 172–180.

[9] Akingbemi, B.T., Sottas, C. M., Koulova, A.I., Klinefelter, G. R. & Hardy, M.P. **2004**, " 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*, vol.145, pp. 592–603.

[10] Duty, S. M., Singh, N. P., Silva, M. J., Barr, D. B., Brock, J. W, Ryan, L., Herrick, R. F., Christiani, D. C. & Hauser, R. **2003**, " The relationship between environmental exposure to phthalates and DNA damage in human sperm", *Environ Health Perspec*tive, vol.111, pp. 1164–1169.

[11] Davis, B. J., Maronpot, R. R. & Heindel, J. J. **1994**, "Di-(2-ethylhexyl) phthalate suppresses estradiol and ovulation in cycling rats", *Toxicology and Applied Pharmacology,* vol. 128, pp 216–223.

[12] Mylchreest, E., Wallace, D. G., Cattley, R. C. & Foster, P. M. D. **2000**, "Dose-dependent alterations in androgen-regulated male reproductive development in rats exposed to di(n-butyl) phthalate during late gestation", *Toxicology Science.* vol.55, pp. 143-151.

[13] Li, L. H., Jester, W. F., Laslett, A. L. & Orth, J. M. **2000**, "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*, vol.166, no.3, pp. 222-229.

[14] Gray, L. E., Jr., Ostby, J., Monosson, E. & Kelce, W. R. **1999**, "Environmental antiandrogens: low doses of the fungicide vinclozolin alter sexual differentiation of the male rat", Toxicology Industrial. Health, vol. 15, pp. 48–64.

[15] A. Garcia-Gutierrez, A.E. McIntyre & J.N. Lester. **1982**, "Analysis of aldrin and endrin in sewage sludges", *Environ. Technol. Letter,* vol.3, no.12, pp. 541-544.

[16] A.E. McIntyre & J.N. Lester. **1984**, "Occurrence and distribution of persistent organochlorine compounds in UK sewage sludges", *Water Air Soil Pollution,* vol.23, no.4, pp. 397-415.

[17] D.W. Hill and P.L. McCarty. **1967**, "Anaerobic degradation of selected chlorinated hydrocarbon pesticides", *Journal of Water Pollution Cont. Fed.,* vol.39, no.8, pp. 1259-1277.

[18] N.P. Hill, A.E. McIntyre, R. Perry & J.N. Lester. **1983**,"Development of a method for the analysis of chlorophenoxy herbicides in waste waters amd waste water sludges. *International Journal of Environment. Anal. Chem*., vol.15, no.2, pp. 107-130.

[19] J. Folke. **1984**, "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*., vol.7, no.l, pp. 25-32

[20] A.E. McIntyre, R. Perry & J.N. Lester. **1981**, "Analysis and incidence of organophosphorus compounds in sewage sludges", *Bull. Environ. Contam. Toxicol.,* vol.26, pp. 116-123.

[21] R.O. Mumma, D.C. Raupach, J.P. Waldman, S.S.C. Tong, M.L. Jacobs, J.G. Babish, J.H. Hotchkiss, P.C. Wszolek, W.H. Gutenman, CA. Bathe & D.J. Lisk. **1984**, "National survey of elements and other constituents in municipal sewage sludges", *Arch. Environ. Contam. Toxicology,* vol.13, no. l, pp. 75-83.

[22] W.S. Brewer, A.C. Draper & S.S. Wey. **1980,** "The detection of dimethylnitrosamine and diethylnitrosamine in municipal sewage sludge applied to agricultural soils", *Environmental. Pollution* (Ser. B), vol. 1, pp. 37-43.
.
[23] H. Britcher and A. Waggott, 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] D.L. Liu. **1978**, "Measuring petroleum hydrocarbons in digested sewage sludges", *Water Sewage Works,* vol.125, no.3, pp. 40-44.

[25] W. Giger, P.H. Brunner & C. Schaffner. **1984**, "4 Nonylphenol in sewage sludge: accumulation of toxicmetabolites from non-ionic surfactants", *Science,* vol.225, no.4662, pp. 623-625.

[26] M. Tschui, P.H. Brunner & W. Giger. **1985**, "Accumulation of Refractory Cnonylphenol During Mesophilic Anaerobic Sludge Stabilisation", COST 68 Working Party No. 1 Workshop, Utrecht, 22 April.

[27] D.E. Stott &M.A. Tabatabai. 1985, "Identification of phospholipids in soils and sewage sludges by high performance liquid chromatography", *Journal of Environmental Quality,* vol.14, no. l , pp. 107-110.

[28] L. Brown, K.C.C. Bancroft & M.M. **1980**, "Rhead, Laboratory studies on the adsorption of acrylamide monomer by sludge, sediments, clays, peat and synthetic resins", *Water Research,* vol.14, pp. 779.

[29] O'Grady, D.P., Howard, P.H. & Werner, A.F. **1985**, "Activated sludge biodegradation of 12 commercial phthalate esters", *Appl. Environ. Microbialogy,* vol. 49, no.2, pp. 443-445.

[30] Laughlin, R.B. & Linden, 0. **1985**, "Fate & effects of organotin compounds", *Ambio,* vol. 14, no. 2, pp. 88-94.

[31] Vrijhof, H. **1985**, "Organotin compounds and international treaties on the pollution of water by dangerous substances: black or grey list substances", *Science of Total Environ*., vol. 43, no. 3, pp. 221-233.

[32] Craig, P.J., Moreton, P.A. & Rapsonanikis, S. **1983**, "Methylation of mercury, tin and lead in aqueous and sediment environments, in Heavy Metals in the Environment", International Conference Proceedings, Heidelberg.

[33] McEvoy, J. & Giger, W. **1985**, "Accumulation of linear alkylbenzene sulphonate surfactants in sewage sludges", *Naturwissenschaften*, vol.72, pp. S429.

[34] Motschi, H. & McEvoy, J. **1985**, "Influence of metaliadsorbate interactions on the adsorption of linear alkylbenzene sulphonates to hydrous surfaces", *Naturwissenschaften*, vol.72, pp. S.654.

[35] A.J. Sweetman, H.R. Rogers & B. Crathome. **1990**, "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, Commission of the European Communities.

[36] H. Takada & R. Ishiwatari. **1985**, "Quantitation of longchain alkylbenzenes in environmental samples by silica gel column chromatography and high resolution gas chromatography", *Journal of Chromatography*, vol. 346, pp. 28 l-290.

[37] H. Takada & R. Ishiwatari. **1987**, "Linear alkylbenzenes in urban riverine environments in Tokyo: distribution, source and behaviour", *Environ. Sci. Technology,* vol. 21, no.9, pp 875-883.

[38] H. Takada & R. Ishiwatari. **1990**, "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,* vol. 24, pp. 86-91.

[39] K. Verschueren, "Handbook of Environmental Data on Organic Chemicals", 2nd edn., Van Nostrand Reinhold, New York, **1983**.

[40] M.D. Muller. **1982**, "Hexachlorobenzol in der Schweiz- Aussmass und Hintergrunde der Umweltkontamination ". *Chimia,* vol. 11, pp. 437-435.

[41] P.E. Gaffney. **1976**, "Carpets and rugs industry 1: water and wastewater treatment plant operation", *Journal of Water Polluion. Cont. Fed*., vol. 48, pp. 2590.

[42] L.W. Jacobs, & M.J. Zabik. **1983**, "Importance of sludgeborne organic chemicals for land application programs", 6th Annual Madison Conf. on Applic. Res. Pratt. Munic. and Indust. Waste, Univ. Wisconsin, pp. 418-426.

[43] B. Topping. **1987**, "The biodegradability of paradichlorobenzene and its behaviour in model activated sludge plants", *Water Research*, vol. 21, pp. 295-300.

[44] J.C. Spain, & S.F. Nishino. **1987**, "Degradation of 1,4- dichlorobenzene by a Pseudomonas sp", Appl. Environ. *Microbiol.*, vol. 53, pp. 1010-1019.

[45] G. Schraa, Anaerobic degradation, processes and test methods, in G. Angeletti and A. Bjorseth (Ed@, Organic Micropollutants in the Aquatic Environment, Kluwer, Dordrecht.

[46] P.W.W. Kirk, H.R. Rogers, & J.N. Lester. **1989**, "The fate of chlorobenzenes and permethrins during anaerobic sewage sludge digestion", *Chemosphere,* vol. 18, no. (9/10), pp. 1771-1784.

[47] N.C.A. Weerasinghe, M.L. Gross, & D.J. Lisk. **1985,** "Polychlorinated dibenzodioxins and polychlorinated dibenzofurans in sewage sludges", *Chemosphere,* vol. 14, no. 5, pp. 557-564.

[48] H. Hagenmaier, H. Brunner, R. Haig, & A. Berchtoid. **1986**, "PCDDs and PCDFs in sewage sludge, river and lake sediments from South West Germany", *Chemosphere,* vol. 15, pp. 1421-1428.

[49] H. Hagenmaier & B. Brunner. **1987**, "Isomer specific analysis of pentachlorophenol and sodium pentachlorophenate for 2,3,7,8-substituted PCDD and PCDF at sub ppb levels"
*Chemosphere*, vol. 16, pp. 1759-1764.

[50] L.G. Oberg, R. Andersson and C. Rappe. **1992**, "De-novo formation of hepta- and octachlorodibenzo-p-dioxins from pentachlorophenol in municipal sewage sludge", in Proceedings of 'DIOXIN '92', Tampere, Finland..

[51] C. Rappe & R. Andersson. **1992**, "Analyses of PCDDs and PCDFs in wastewater from dishwashers and washing machines", 'DIOXIN '92', Tampere, Finland, Extended
Abstracts. *Organohalogen Compounds*, vol. 9, pp. 191-194.

[52] C. Rappe and R. Andersson. **1992**, "Levels of PCDDs and PCDFs in human faeces", 'DIOXIN '92' Tampere, Finland, Extended Abstracts. *Organohalogen Compounds*, vol. 9, pp. 195-198.

[53] D. Broman, C. Naf, C. Rolff and Y. Zebuhr. **1990,** "Analysis of polychlorinated dibenzo-p-dioxins (PCDD) and polychlorinated dibenzofurans (PCDF) in soil and digested sewage sludge from Stockholm, Sweden", *Chemosphere*, vol. 21, pp. 1213-1220.

[54] H.R. Rogers, P. Ambridge & I. Stenhouse. **1989**, "The Occurrence of PCDDs and PCDFs in Some UK Sewage Sludges", - A Preliminary Investigation, WRc report for the Department of the Environment, DOE 2093-M.

[55] J.M. Neff. **1979**, "Polycyclic Aromatic Hydrocarbons in the Aquatic Environment - Sources, Fates and Biological Effects", Applied Science Publishers, London.

[56] A.E. McIntyre, R. Perry and J.N. Lester. **1981**, "Analysis of polynuclear aromatic hydrocarbons in sewage sludges", *Analytical Letter*, vol. 14, pp. 291-309.

[57] P.C. Wszolek & T. Wachs. **1982**, "Occurrence of polycyclic aromatic hydrocarbons in municipal sewage sludge ashes", *Arch. Environmental. Contamination Toxicology*, vol.11, pp. 69-72.

[58] K. Aichberger & D. Reifenauer. **1983**, "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, Brighton, Reidel, Dordrecht.

[59] P. Nicholls, R. Perry and J.N. Lester. **1979,** "The influence of heat treatment on the metallic and PAH content of sewage sludge", *Science Total Enviroment*, vol. 12, pp. 137-150.

[60] H. Hagenmaier, H. Kaut and P. Krauss, 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] T.R. Bridle and M.D. Webber. **1982**, "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", WRc report 2-S.

[62] Gray, L. E., Ostby, J., Furr, J., Wolf, C. J., Lambright, C., Parks, L., Veeramachaneni, D. N., Wilson, V., Price, M., Hotchkiss, A., Orlando, E. and Guillette, L. (**2001**). Effects of environmental antiandrogens on reproductive development in experimental animals. Human Reproduction Update (7) 248-264.

[63] Ema, M. & Miyawaki, E. **2001**, "Adverse effects on development of the reproductive system", *Reprod Toxicol*ogy, vol.1, no. 5, pp. 189-194.

[64] Couse, J.F. and Korach, K.S. **2004**, "Estrogen receptor-alpha mediates the detrimental effects of neonatal diethylstilbestrol (DES) exposure in the murine reproductive tract", *Toxicology*, vol. 205, pp. 55–63.

[65] Johnston, H., Baker, P. J., Abel, M., Charlton, H. M., Jackson, G., Fleming, L., Kumar, T. R. & O-Shaughnessy, P. J. **2004**, "Regulation of Sertoli cell number and activity by follicle-stimulating hormone and androgen during postnatal development in the mouse", *Endocrinology*, vol. 145, pp. 318-29.

[66] Jobling, S., Reynolds, T., White, R., Parker, M.G. and Sumpter, J.P. **1995**, "A variety of environmentally persistent chemicals, including some phthalate plasticizers, are weakly estrogenic", *Environ Health Perspect*. Vol. 103, pp. 582-587.

[67] Soto, A.M., Sonnenschein, C., Chung, K. L., Fernandez, M. F., Olea, N. and Olea Serrano, F. **1995**, "The e-screen assay as a tool to identify estrogens: an update on estrogenic environmental pollutants", *Environ Health Perspect*. vol.103 (Suppl3), pp.113-122.

[68] Rudel, R. A., Seryak, L. M. and Brody, J. G. **2008**, "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*. Vol. 7, pp. 2.

[69] Safe, S. H. **1990**, "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,* vol. 21, pp. 51-88.

[70] Brouwer, A., Reijnders, P. J. H. and Koeman, J. H. **1989**, "Polychlorinated biphenyl (PCB)-contaminated fish induce vitamin A and thyroid hormone deficiency in the Common Seal", (*Phoca vitulina*). *Aquatic Toxicology*, vol. 15, pp. 99–106.

[71] Simms and Ross, **2001**; Brouwer, A., Reijnders, J. H., Koeman, J. H. **1989**, "Polychlorinated biphenyls (PCB)-contaminated fish induces vitamin A and thyroid deficiency in the common seal Phoca vitulina", *Aquatic Toxicology*, vol. 15, pp. 99-106.

[72] Venners, S.A., Korrick, S., Xu, X., Chen, C., Guang, W. and Huang, A. *et al.*, **2005**, "Preconception serum DDT and pregnancy loss: a prospective study using a biomarker of pregnancy", *America Journal of Epidemiol*ogy, vol. 162, pp. 709–716.

[73] Zimmer, K., Gutleb, A. C., Lyche, J. L., Dahl, E., Oskam, I., Krogenas, A., Skaare, J. U. and Ropstad, E. **2008**, "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 (272) 164–172.

[74] Krishman, V. and Safe, S. **1993**, "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* , vol. 120) 55-61.

[75] Whyatt, R.M., Rauh, V., Barr, D.B., Camann, D.E., Andrews, H.F. and Garfinkel, R. *et al.*, **2004**, "Prenatal insecticide exposures and birth weight and length among an urban minority cohort", *Environ Health Perspect*ive, vol. 112, pp. 1125–1132.

[76] Lilienthal, H. and Winneke, G. **1991**, "Sensitive periods for behavioural toxicity of polychlorinated biphenyls: determination by cross fostering in rats. Fundam Appl Toxicology, vol. 17, pp. 368-375.

[77] Korach, K.S., Sarver, P., Chae, K., McLachlan, J.A. and McKinney, J.D. **1988**, "Estrogen receptor-binding activity of polychlorinated hydrobiphenyls conformationally restricted structural probes. Mol Pharmacology", vol. 33, pp. 130-126

[78] Jones, O.A.H., Voulvoulis, N. & Lester, J.N. **2007**, "The occurrence and removal of selected pharmaceutical compounds in a sewage treatment works utilising activated sludge treatment", *Environmental* Pollution, vol. 145, no. 3, pp. 738-744

[79] Loffler, D., Rombke, J., Meller, M. & Ternes, T.A. **2005**, "Environmental fate of pharmaceuticals in water/sediment systems", *Environmental science & technology*, vol. 39, no. 14, pp. 5209-5218**.**

[80] Ternes, T.A., Stumpf, M., Mueller, J., Haberer, K., Wilken, R.D. & Servos, M. **1999**, "Behaviour and occurrence of estrogens in municipal sewage treatment plants--I. Investigations in Germany, Canada and Brazil",*The Science of the total environment,* vol. 225, no. 1-2, pp. 81-90.

[81] Ternes, T.A. **1998**, "Occurrence of drugs in German sewage treatment plants and rivers", *Water research*, vol. 32, no. 11, pp. 3245-3260.

[82] Jones, O.A.H., Voulvoulis, N. & Lester, J.N. **2007**, "The occurrence and removal of selected pharmaceutical compounds in a sewage treatment works utilising activated sludge treatment", *Environmental Pollution*, vol. 145, no. 3, pp. 738-744.

[83] Daughton, C.G. & Ternes, T.A. **1999**, "Pharmaceuticals and personal care products in the environment: agents of subtle change?", *Environmental health perspectives*, vol. 107 Suppl 6, pp. 907-938.

[84] Zhang, Z.L. & Zhou, J.L. **2007**, "Simultaneous determination of various pharmaceutical compounds in water by solid-phase extraction–liquid chromatography–tandem mass spectrometry", *Journal of Chromatography A*, vol. 1154, no. 1–2, pp. 205-213.

[85] Richardson, S.D. & Ternes, T.A. **2005**, "Water analysis: emerging contaminants and current issues", *Analytical Chemistry*, vol. 77, no. 12, pp. 3807-3838.

[86] Ashton, D., Hilton, M. & Thomas, K.V. **2004**, "Investigating the environmental transport of human pharmaceuticals to streams in the United Kingdom", *Science of The Total Environment*, vol. 333, no. 1–3, pp. 167-184.

[87] Tixier, C., Singer, H.P., Oellers, S. & Muller, S.R. **2003**, "Occurrence and fate of carbamazepine, clofibric acid, diclofenac, ibuprofen, ketoprofen, and naproxen in surface waters", *Environmental science & technology*, vol. 37, no. 6, pp. 1061-1068.

[88] Zuccato, E., Calamari, D., Natangelo, M. & Fanelli, R. **2000**, "Presence of therapeutic drugs in the environment", *Lancet*, vol. 355, no. 9217, pp. 1789-1790.

[89] Ternes, T.A. **2001**, "Analytical methods for the determination of pharmaceuticals in aqueous environmental samples", *TrAC Trends in Analytical Chemistry*, vol. 20, no. 8, pp. 419-434.

[90] Castiglioni, S., Bagnati, R., Calamari, D., Fanelli, R. & Zuccato, E. **2005**, "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*, vol. 1092, no. 2, pp. 206-215

[91] Hernando, M.D., Gómez, M.J., Agüera, A. & Fernández-Alba, A.R. **2007**, "LC-MS analysis of basic pharmaceuticals (beta-blockers and anti-ulcer agents) in wastewater and surface water", *TrAC Trends in Analytical Chemistry*, vol. 26, no. 6, pp. 581-594.

[92] Pérez, S. & Barceló, D. **2007**, "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*, vol. 26, no. 6, pp. 494-514.

[93] M.L.Richardson and J.M. Bowron. **1985** "The fate of pharmaceutical chemicals in the aquatic environment". *J. Pharm. Pharmacol*, vol. 37, pp l-12.

[94] Ternes, T.A., Kreckel, P. & Mueller, J. **1999**, "Behaviour and occurrence of estrogens in municipal sewage treatment plants--II. Aerobic batch experiments with activated sludge", *The Science of the total environment*, vol. 225, no. 1-2, pp. 91-99

[95] Buchberger, W.W. **2007,** "Novel analytical procedures for screening of drug residues in water, waste water, sediment and sludge", *Analytica Chimica Acta*, vol. 593, no. 2, pp. 129-139.

[96] Petrovic, M., Petrovic, M. & Barceló, D. **2007**, "LC-MS for identifying photodegradation products of pharmaceuticals in the environment", *TrAC Trends in Analytical Chemistry*, vol. 26, no. 6, pp. 486-493.

[97] Fatta, D., Achilleos, A., Nikolaou, A. & Meriç, S. **2007**, "Analytical methods for tracing pharmaceutical residues in water and wastewater", *TrAC Trends in Analytical Chemistry*, vol. 26, no. 6, pp. 515-533.

[98] Rahman, F., Langford, K.H., Scrimshaw, M.D. & Lester, J.N. **2001**, "Polybrominated diphenyl ether (PBDE) flame retardants", *The Science of the total environment,* vol. 275, no. 1-3, pp. 1-17.

[99] O'Connor, S. & Aga, D.S. **2007**, "Analysis of tetracycline antibiotics in soil: Advances in extraction, clean-up, and quantification", *TrAC Trends in Analytical Chemistry,* vol. 26, no. 6, pp. 456-465.

[100] Bajad, S. & Shulaev, V. **2007**, "Highly-parallel metabolomics approaches using LC-MS2 for pharmaceutical and environmental analysis", *TrAC Trends in Analytical Chemistry,* vol. 26, no. 6, pp. 625-636.

[101] Daughton, C.G. **2004**, "Non-regulated water contaminants: emerging research", *Environmental Impact Assessment Review,* vol. 24, no. 7-8, pp. 711-732.

[102] CD. Watts, B. Crathome, M. Fielding and C.P. Steel. **1983** "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, Reidel, Dordrecht.

[103] Golet, E.M., Alder, A.C., Hartmann, A., Ternes, T.A. & Giger, W. **2001**, "Trace determination of fluoroquinolone antibacterial agents in urban wastewater by solid-phase extraction and liquid chromatography with fluorescence detection", *Analytical Chemistry,* vol. 73, no. 15, pp. 3632-3638.

[104] Calamari D, Zuccatto, E., Castiglion S.,, Bagnat , R., & Fanalli R. **2003**, "Strategic Survey of Therapeutic Drugs in the Rivers Po and Lambro in Northern Italy", *Environ. Sci. Technol,* vol.37, pp.1241-1248

[105] Hirsch, R., Ternes, T., Haberer, K. & Kratz, K. **1999**, "Occurrence of antibiotics in the aquatic environment", *The Science of the total environment,* vol. 225, no. 1-2, pp. 109-118.

[106] Miao, X., Koenig, B.G. & Metcalfe, C.D. **2002**, "Analysis of acidic drugs in the effluents of sewage treatment plants using liquid chromatography–electrospray ionization tandem mass spectrometry", *Journal of Chromatography A,* vol. 952, no. 1-2, pp. 139-147.

[107] Ternes, T., Bonerz, M. & Schmidt, T. **2001**, "Determination of neutral pharmaceuticals in wastewater and rivers by liquid chromatography–electrospray tandem mass spectrometry", *Journal of Chromatography A,* vol. 938, no. 1–2, pp. 175-185.

[108] Vieno, N.M., Tuhkanen, T. & Kronberg, L. **2006**, "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,* vol. 1134, no. 1–2, pp. 101-111.

[109] Barceló, D. **2007**, "Pharmaceutical-residue analysis", *TrAC Trends in Analytical Chemistry,* vol. 26, no. 6, pp. 454-455.

[110] Beausse, J. **2004**, "Selected drugs in solid matrices: a review of environmental determination, occurrence and properties of principal substances", *TrAC Trends in Analytical Chemistry,* vol. 23, no. 10-11, pp. 753-761.

[111] Rogers, H.R. **1996**, "Sources, behaviour and fate of organic contaminants during sewage treatment and in sewage sludges", *Science of The Total Environment,* vol. 185, no. 1-3, pp. 3-26.

[112] Petrovic, M., Gonzalez, S. & Barceló, D. **2003**, "Analysis and removal of emerging contaminants in wastewater and drinking water", *TrAC Trends in Analytical Chemistry,* vol. 22, no. 10, pp. 685-696.

[113] Hirsch, R., Ternes, T.A., Haberer, K., Mehlich, A., Ballwanz, F. & Kratz, K.L. **1998**, "Determination of antibiotics in different water compartments via liquid chromatography-electrospray tandem mass spectrometry", *Journal of chromatography.A,* vol. 815, no. 2, pp. 213-223.

[114] Alder, A, C., McArdell, C.S., Gole, E.M., Ibric, S., Molnar, E., Nipales, N.S., 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. **2000,** "Occurrence of triiodinated X-ray contrast agents in the aquatic environment", *The Science of the total environment*, vol. 255, no. 1-3, pp. 129-134.

[116] Wick, A., Fink, G., Joss, A., Siegrist, H. & Ternes, T.A. **2009**, "Fate of beta blockers and psycho-active drugs in conventional wastewater treatment", *Water research*, vol. 43, no. 4, pp. 1060-1074.

[117] Ternes, T.A., & Hirsch, R. **2000**, "Occurrence and Behavior of X-ray Contrast Media in Sewage Facilities and the Aquatic Environment. Environ. Sci. Technol. 34, 2741-2748

[118] Ternes, T. A. **2001**, "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, in press.

[119] Halling-Sørensen, B., Nors Nielsen, S., Lanzky, P.F., Ingerslev, F., Holten Lützhøft, H.C. & Jørgensen, S.E. **1998**, "Occurrence, fate and effects of pharmaceutical substances in the environment- A review", *Chemosphere*, vol. 36, no. 2, pp. 357-393.

[120] Jørgensen, S.E. & Halling-Sørensen, B. **2000,** "Drugs in the environment", *Chemosphere*, vol. 40, no. 7, pp. 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. & Fanelli, R. **2005**, "Cocaine in surface waters: a new evidence-based tool to monitor community drug abuse", *Environmental health: a global access science source*, vol. 4, pp. 14.

[125] Hilton, M.J. & Thomas, K.V. **2003**, "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*, vol. 1015, no. 1-2, pp. 129-141.

[126] Berg, T., Lundanes, E., Christophersen, A.S. & Strand, D.H. **2009**, "Determination of opiates and cocaine in urine by high pH mobile phase reversed phase UPLC–MS/MS", *Journal of Chromatography B*, vol. 877, no. 4, pp. 421-432.

[127] Pizzolato, T.M., de Alda, M.J.L. & Barceló, D. **2007**, "LC-based analysis of drugs of abuse and their metabolites in urine", *TrAC Trends in Analytical Chemistry*, vol. 26, no. 6, pp. 609-624.

[128] Dickson, S., Park, A., Nolan, S., Kenworthy, S., Nicholson, C., Midgley, J., Pinfold, R. & Hampton, S. **2007**, "The recovery of illicit drugs from oral fluid sampling devices", *Forensic science international*, vol. 165, no. 1, pp. 78-84.

[129] Beyer, J., Drummer, O.H. & Maurer, H.H. **2009**, "Analysis of toxic alkaloids in body samples", *Forensic science international*, vol. 185, no. 1-3, pp. 1-9.

[130] 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

[131] van Nuijs, A.L.N., Pecceu, B., Theunis, L., Dubois, N., Charlier, C., Jorens, P.G., Bervoets, L., Blust, R., Neels, H. & Covaci, A. **2009**, "Cocaine and metabolites in waste and surface water across Belgium", *Environmental Pollution*, vol. 157, no. 1, pp. 123-129

[132] Gheorghe, A., van Nuijs, A., Pecceu, B., Bervoets, L., Jorens, P.G., Blust, R., Neels, H. & Covaci, A. **2008**, "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*, vol. 391, no. 4, pp. 1309-1319.

[133] Zuccato, E., Castiglioni, S., Bagnati, R., Chiabrando, C., Grassi, P. & Fanelli, R. **2008**, "Illicit drugs, a novel group of environmental contaminants", *Water research*, vol. 42, no. 4-5, pp. 961-968.

[134] Bones, J., Thomas, K.V. & Paull, B. **2007**, "Using environmental analytical data to estimate levels of community consumption of illicit drugs and abused pharmaceuticals", *Journal of environmental monitoring : JEM*, vol. 9, no. 7, pp. 701-707.

[135] Bijlsma, L., Sancho, J.V., Pitarch, E., Ibanez, M. & Hernandez, F. **2009**, "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*, vol. 1216, no. 15, pp. 3078-3089.

[136] van Nuijs, A.L.N., Pecceu, B., Theunis, L., Dubois, N., Charlier, C., Jorens, P.G., Bervoets, L., Blust, R., Neels, H. & Covaci, A. **2009**, "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*, vol. 43, no. 5, pp. 1341-1349.

[137] Castiglioni, S., Zuccato, E., Crisci, E., Chiabrando, C., Fanelli, R. & Bagnati, R. **2006**, "Identification and measurement of illicit drugs and their metabolites in urban wastewater by liquid chromatography-tandem mass spectrometry", *Analytical Chemistry*, vol. 78, no. 24, pp. 8421-8429.

[138] Huerta-Fontela, M., Galceran, M.T., Martin-Alonso, J. & Ventura, F. **2008**, "Occurrence of psychoactive stimulatory drugs in wastewaters in north-eastern Spain", *Science of The Total Environment*, vol. 397, no. 1-3, pp. 31-40.

[139] Postigo, C., de Alda, M.J. & Barcelo, D. **2008**, "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*, vol. 80, no. 9, pp. 3123-3134.

[140] Hummel, D., Loffler, D., Fink, G. & Ternes, T.A. **2006**, "Simultaneous determination of psychoactive drugs and their metabolites in aqueous matrices by liquid chromatography mass Spectrometry", *Environmental science & technology*, vol. 40, no. 23, pp. 7321-7328.

[141] Bartelt-Hunt, S.L., Snow, D.D., Damon, T., Shockley, J. & Hoagland, K. **2009**, "The occurrence of illicit and therapeutic pharmaceuticals in wastewater effluent and surface waters in Nebraska", *Environmental Pollution*, vol. 157, no. 3, pp. 786-791.

[141] Van Bocxlaer, J.F., Clauwaert, K.M., Lambert, W.E., Deforce, D.L., Van den Eeckhout, E.G. & De Leenheer, A.P. **2000**, "Liquid chromatography-mass spectrometry in forensic toxicology", *Mass spectrometry reviews*, vol. 19, no. 4, pp. 165-214.

[142] Hearn, W.L., Flynn, D.D., Hime, G.W., Rose, S., Cofino, J.C., Mantero-Atienza, E., Wetli, C.V. & Mash, D.C. **1991**, "Cocaethylene: a unique cocaine metabolite displays high affinity for the dopamine transporter", *Journal of neurochemistry*, vol. 56, no. 2, pp. 698-701.

[142] Boleda, M.R., Galceran, M.T. & Ventura, F. **2007**, "Trace determination of cannabinoids and opiates in wastewater and surface waters by ultra-performance liquid chromatography–tandem mass spectrometry", *Journal of Chromatography A*, vol. 1175, no. 1, pp. 38-48.

[143] Hummel, D., Loffler, D., Fink, G. & Ternes, T.A. **2006**, "Simultaneous determination of psychoactive drugs and their metabolites in aqueous matrices by liquid chromatography mass Spectrometry", *Environmental science & technology*, vol. 40, no. 23, pp. 7321-7328

[144] Harkey, M.R., Henderson, G.L. & Zhou, C. **1991**, "Simultaneous quantitation of cocaine and its major metabolites in human hair by gas chromatography/chemical ionization mass spectrometry", *Journal of analytical toxicology*, vol. 15, no. 5, pp. 260-265

[145] European Monitoring Centre for Drugs and Drug Addition (EMCDDA), Insight series 9, **2008**. Assessing illicit drug in wastewater ISBN 978-92-9168-317-8.

[146] Ellenhorn M.J., ed. and Barceloux D. G., **1988.** New York Elsevier Sc Publishing Company, Inc, 644-661.

[147] Van Viet, H., Chevalier, P., Sereni, C., Bornet, P., Bautier, P., Degos, C.F. & Rulliere, R. **1990**, "Neurologic complications of cocaine abuse", *Presse medicale (Paris, France : 1983)*, vol. 19, no. 22, pp. 1045-1049.

[148] Kolbrich, E.A., Barnes, A.J., Gorelick, D.A., Boyd, S.J., Cone, E.J. & Huestis, M.A. **2006**, "Major and minor metabolites of cocaine in human plasma following controlled subcutaneous cocaine administration", *Journal of analytical toxicology*, vol. 30, no. 8, pp. 501-510.

[149] Matsubara, K., Kagawa, M. & Fukui, Y. **1984**, "In vivo and in vitro studies on cocaine metabolism: Ecgonine methyl ester as a major metabolite of cocaine", *Forensic science international*, vol. 26, no. 3, pp. 169-180.

[150] Cone, E.J. **1995**, "Pharmacokinetics and pharmacodynamics of cocaine", *Journal of analytical toxicology*, vol. 19, no. 6, pp. 459-478.

[151] Stewart, D.J., Inaba, T., Lucassen, M. & Kalow, W. **1979,** "Cocaine metabolism: cocaine and norcocaine hydrolysis by liver and serum esterases", *Clinical pharmacology and therapeutics*, vol. 25, no. 4, pp. 464-468.

[152] Saxon, A.J., Calsyn, D.A., Haver, V.M. & Delaney, C.J. **1988**, "Clinical evaluation and use of urine screening for drug abuse", *The Western journal of medicine*, vol. 149, no. 3, pp. 296-303.

[153] Ambre, J. **1985**, "The urinary excretion of cocaine and metabolites in humans: a kinetic analysis of published data", *Journal of analytical toxicology*, vol. 9, no. 6, pp. 241-245.

[154] Kloss, M.W., Rosen, G.M. & Rauckman, E.J. **1984,** "Cocaine-mediated hepatotoxicity. A critical review", *Biochemical pharmacology*, vol. 33, no. 2, pp. 169-173.

[155] Kloss, M.W., Rosen, G.M. & Rauckman, E.J. **1984**, "Biotransformation of norcocaine to norcocaine nitroxide by rat brain microsomes", *Psychopharmacology*, vol. 84, no. 2, pp. 221-224.

[156] Hearn, W.L., Flynn, D.D., Hime, G.W., Rose, S., Cofino, J.C., Mantero-Atienza, E., Wetli, C.V. & Mash, D.C. **1991**, "Cocaethylene: a unique cocaine metabolite displays high affinity for the dopamine transporter", *Journal of neurochemistry*, vol. 56, no. 2, pp. 698-701.

[157] Scheidweiler, K.B., Shojaie, J., Plessinger, M.A., Wood, R.W. & Kwong, T.C. **2000**, "Stability of methylecgonidine and ecgonidine in sheep plasma in vitro", *Clinical chemistry*, vol. 46, no. 11, pp. 1787-1795.

[158] Maurer, H.H., Sauer, C. & Theobald, D.S. **2006**, "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*, vol. 28, no. 3, pp. 447-453.

[159] G. Schraa, Anaerobic degradation, processes and test methods, in G. Angeletti and A. Bjorseth (Ed@, Organic Micropollutants in the Aquatic Environment, Kluwer, Dordrecht.

[160] 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).

[161] Ensslin, H.K., Kovar, K. & Maurer, H.H. **1996**, "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*, vol. 683, no. 2, pp. 189-197.

[162] Dollery, C.T. **1991**, "Clinical pharmacology of calcium antagonists", *American journal of hypertension*, vol. 4, no. 2 Pt 2, pp. 88S-95S

[163] Andrew, R., Tasker, R. & Nakatsu, K. **1984**, "Evaluation of 3,6-dibutanoylmorphine as an analgesic : Comparison with morphine and 3,6-diacetylmorphine", *Life Sciences*, vol. 34, no. 17, pp. 1659-1667.

[164] Knodell, R.G., Farleigh, R.M., Steele, N.M. & Bond, J.H. **1982**, "Effects of liver congestion on hepatic drug metabolism in the rat", *The Journal of pharmacology and experimental therapeutics*, vol. 221, no. 1, pp. 52-57.

[165] Hand, C.W., Moore, R.A., McQuay, H.J., Allen, M.C. & Sear, J.W. **1987**, "Analysis of morphine and its major metabolites by differential radioimmunoassay", *Annals of Clinical Biochemistry*, vol. 24 ( Pt 2), no. Pt 2, pp. 153-160.

[166] Canezin, J., Cailleux, A., Turcant, A., Le Bouil, A., Harry, P. & Allain, P. **2001**, "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*, vol. 765, no. 1, pp. 15-27.

[167] Reuschel, S.A., Eades, D. & Foltz, R.L. **1999**, "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*, vol. 733, no. 1-2, pp. 145-159.

[168] Pizzolato, T.M., de Alda, M.J.L. & Barceló, D. **2007**, "LC-based analysis of drugs of abuse and their metabolites in urine", *TrAC Trends in Analytical Chemistry*, vol. 26, no. 6, pp. 609-624.

[169] J.M. Neff. **1979**, "Polycyclic Aromatic Hydrocarbons in the Aquatic Environment - Sources, Fates and Biological Effects", Applied Science Publishers, London.

[170] Postigo, C., de Alda, M.J. & Barcelo, D. **2008**, "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*, vol. 80, no. 9, pp. 3123

[171] Nelson C. C., Foltz R.L in: R. C. Baselt (ed). **1989**. Advances in analytical Toxicology, Vol. II, Year book Medical Publishers, Chicago, IL, p. 140.

[172] Lambert, D.M. & Fowler, C.J. **2005**, "The endocannabinoid system: drug targets, lead compounds, and potential therapeutic applications", *Journal of medicinal chemistry*, vol. 48, no. 16, pp. 5059-5087.

[173] Burns, T.L. & Ineck, J.R. **2006**, "Cannabinoid analgesia as a potential new therapeutic option in the treatment of chronic pain", *The Annals of Pharmacotherapy*, vol. 40, no. 2, pp. 251-260.

[174] ElSohly, M.A. & Slade, D. **2005**, "Chemical constituents of marijuana: The complex mixture of natural cannabinoids", *Life Sciences*, vol. 78, no. 5, pp. 539-548.

[175] A.C. Petrasek, I.J. Kugelman, M. Austem, T.A. Pressley, L.A. Winslow & R.H. Wise. **1983,** "Fate of toxic organic compounds in wastewater treatment plants", *Journal Water Pollut*ion Cont. Fed., vol.55, no. 10, pp. 1286-1296.

[176] S.A. Hannah, B.M. Austem, A.E. Eralp & R.H. Wise. **1986**, "Comparative removal of toxic pollutants by six wastewater treatment processes", *Journal of Water Pollution* Cont. Fed., vol. 58, pp. 27-34.

[177] S.A. Hannah, B.M. Austem, A.E. Eralp and R.A. Dobbs. **1988**, "Removal of organic toxic pollutants by trickling filter and activated sludge", J. Water Pollut. Cont. Fed.. vol.60 , pp. 1281-1283.

[178] G.W. Strachan, D.W. Nelson & L.E. Sonuners, **1983**,"Sewage sludge components extractable with nonaqueous solvents", J. Environ. Qual., vol. 12. No. l, pp. 69-74.

[179] A.C. Petrasek, I.J. Kugelman, B.M. Austem, T.A. Pressley, L.A. Winslow and R.H. Wise. **1983**, "Fate of toxic organic compounds in wastewater treatment plants". *J. Water Pollut.* Cont. Fed., vol. 55, no. 10, pp. 1286-1296.

[180] B. Hultmann. **1982**, "Elimination of organic micropollutants", *Water Sci. Technol.,* vol.14, pp. 73.

[181] J.F.T. Van Rensberg, A. Hassett, S. Theron and S.G. Wiechers. **1980**, "The fate of organic micropollutants through an integrated wastewater treatment/water reclamation system", *Water Technol.,* vol.12, pp. 537-552.

[182] H.H. Tabak, S.A. Quave, C.I. Mashi and E.F. Barth. **1981**, "Biodegradability studies with organic priority pollutant compounds", *J. Water Pollut*. Cont. Fed., 5vol. 3, pp. 1503.

[183] W.B. Horning, E.L. Robinson and A.C. Petrasek. **1984**, "Reduction in toxicity of organic priority pollutants by pilot-scale conventional wastewater treatment process", *Arch. Environ. Contam. Toxicol.,* vol. 13, no. 2, pp. 191-196.

[184] 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

[185] L. Wang, R. Govind & R.A. Dobbs. **1993**, "Sorption of toxic organic compounds on wastewater solids: mechanism and modelling". *Environ. Sci. Technol.,* vol. 27, pp. 1.

[186] L.M. Naylor and R.C. Loehr. **1982**, "Priority pollutants in municipal sewage sludge", Part I. *Biocycle,* vol. 23, no. 4, pp. 18-22.

[187] L.M. Naylor and R.C. Loehr. **1982**, "Priority pollutants in municipal sewage sludge", Part II. *Biocycle*, vol. 23, no. 6, pp. 37-42.

[188] J.N. Lester. **1983,** "Presence of organic micropollutants in sewage sludges", in Processing and Use of Sewage Sludge, Third International Symposium. Brighton, pp.27-30 September 1983.

[189] D. Taylor. **1984**, "Managing organ& in sludge reuse programs", *Biocycle,* vol. 25, no. 6, pp. 20-22.

[190] R.D. Davis, K. Howell, R.J. Oake and P. Wilcox. **1984**, "Significance of organic contaminants in sewage sludges used on agricultural land", in International Conference on Environmental Contamination, London, July, EP, Edinburgh.

[191] P.K. Hopke, M.J. Plewa, P.L. Stapleton and D.L. Weaver. **1984**, "Comparison of the mutagenicity of sewage sludges", *Environ. Sci. Technol.,* vol. 18, pp. 909-916.

[l92] J.G. Babish, B. Johnson, B.O. Brooks and D.J. Lisk. **1982**, "Acute toxicity of organic extracts of municipal sewage sludge in mice", Bull. *Environ. Contam. Toxicol., vol.* 29, pp. 379-384.

[193] T.E. Clevenger, D.D. Hemphill, K. Roberts and W.A. Mullins. **2007**, Chemical composition and possible mutagenicity of municipal sludges. J. Water Pollut. Cont. Fed., vol. 55, no. 12, pp. 1470-1475.

[194] Lind, P. M., Öberg, D., Larsson, S., Kyle, C. E., Örberg, J. and Rhind, S. M. **2009,** "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*, vol. 408, no. 11, pp. 2340-2346.

[195] Sahlstrom, L., De Jones, B. and Aspan, A. **2006**, "Salmonella isolated in sewage sludge trace back to human cases of Salmonellosis. Letters in applied microbiology. vol. 43, pp. 46-52.

[196] Gale, P. **2005**, "Land application of treated sewage sludge: quantifying pathogen risks from consumption of crops", *J. Appl Microbiol*. vol. 98, pp. 380-96

[197] Swanson, R. L., Bortman, M. L., O`Connor, T. P. and Stanford, H. M. **2004**, "Science, policy and the management of sewage materials", The New York City experience. *Marine Pollution Bulletin*", vol. 49, pp. 679–87.

[198] [140] Rhind, S.M**.. 2009,** "Anthropogenic pollutants – a threat to ecosystem sustainability? *Philosophical Transactions of the Royal Society, B., 364, 3391-3401.*

[199] Evers, J. H. L. **2002**, "Female subfertility", *Lancet*, vol. 360, pp. 151-159.

[200] Hallikainen, A. & Vartiainen, T. **1997**, "Food control surveys of polychlorinated dibenzo-*p*-dioxins and dibenzofurans and intake estimates", *Food Addit Contam.* Vol. 14, pp. 355-366.

[201] AmaralMendes, J. J. **2002**", "The endocrine disrupters: a major medical challenge," *Food and Chemical Toxicology*, vol.40, no. 6, pp. 781–788.

[202] Panzica, G. C.,Viglietti-Panzica, C., Mura, E., Quinn Jr, M. J., Lavoie, E., Palanza, P. & Ottinger, M. A. **2007**, "Effects of xenoestrogens on the differentiation of behaviorally-relevant neural circuits", *Frontiers in Neuroendocrinology*. Vol. 28, pp. 179–200.

[203] Ekelund, R., Bergman, A., Granmo, A. and Berggren, M. **1990**, "Bioaccumulation of 4-nonylphenol in marine animals—a re-evaluation", *Environ Pollut*. Vol. 64, pp. 107–20.

[204] Ahel, M., McEvoy J. and Giger, W. **1993**, "Bioaccumulation of the lipophilic metabolites of nonionic surfactants in freshwater organisms", *Environ. Pollut*, vol. 79, pp. 243–248.

[205] Bigbsy, R.M., Caperell-Grant, A. and Madhukar, B.V. **1997**, "Xenobiotics released from fat during fasting produce estrogenic effects in ovariectomized mice" *Cancer Res*, vol. 57, pp. 865–869.

[206] Southworth, G.R. & Keller, J.L. **1984**, "Mobilization of azaarenes from wastewater treatment plant biosludge", *Bulletin of environmental contamination and toxicology,* vol. 32, no. 4, pp. 445-452.

[207] Shima, N., Tsutsumi, H., Kamata, T., Nishikawa, M., Katagi, M., Miki, A. & Tsuchihashi, H. **2006,** "Direct determination of glucuronide and sulfate of p-hydroxymethamphetamine in methamphetamine users' urine", *Journal of Chromatography B,* vol. 830, no. 1, pp. 64-70.

[208] Zhang, J.Y. & Foltz, R.L. **1990**, "Cocaine metabolism in man: identification of four previously unreported cocaine metabolites in human urine", *Journal of analytical toxicology*, vol. 14, no. 4, pp. 201-205.

[209] Castiglioni, S., Zuccato, E., Crisci, E., Chiabrando, C., Fanelli, R. & Bagnati, R. **2006**, "Identification and measurement of illicit drugs and their metabolites in urban wastewater by liquid chromatography-tandem mass spectrometry", *Analytical Chemistry*, vol. 78, no. 24, pp. 8421-8429

[210] Kasprzyk-Hordern, B., Dinsdale, R.M. & Guwy, A.J. **2008**, "The occurrence of pharmaceuticals, personal care products, endocrine disruptors and illicit drugs in surface water in South Wales, UK", *Water research,* vol. 42, no. 13, pp. 3498-3518.

[211] Berg, T., Lundanes, E., Christophersen, A.S. & Strand, D.H. **2009**, "Determination of opiates and cocaine in urine by high pH mobile phase reversed phase UPLC–MS/MS", *Journal of Chromatography B,* vol. 877, no. 4, pp. 421-432.

[212] Marais, A.A.S. & Laurens, J.B. **2009**, "Rapid GC–MS confirmation of amphetamines in urine by extractive acylation", *Forensic science international,* vol. 183, no. 1-3, pp. 78-86.

[213] Ambre, J., Fischman, M. & Ruo, T.I. **1984**, "Urinary excretion of ecgonine methyl ester, a major metabolite of cocaine in humans", *Journal of analytical toxicology,* vol. 8, no. 1, pp. 23-25.

[214] Wylie, F.M., Torrance, H., Seymour, A., Buttress, S. & Oliver, J.S. **2005**a, "Drugs in oral fluid: Part II. Investigation of drugs in drivers", *Forensic science international,* vol. 150, no. 2-3, pp. 199-204.

[215] Wylie, F.M., Torrance, H., Anderson, R.A. & Oliver, J.S. **2005**b, "Drugs in oral fluid Part I. Validation of an analytical procedure for licit and illicit drugs in oral fluid", *Forensic science international,* vol. 150, no. 2-3, pp. 191-198.

[216] Jones, A.W., Holmgren, A. & Kugelberg, F.C. **2008**, "Concentrations of cocaine and its major metabolite benzoylecgonine in blood samples from apprehended drivers in Sweden", *Forensic science international,* vol. 177, no. 2-3, pp. 133-139.

[217] Mueller, C.A., Weinmann, W., Dresen, S., Schreiber, A. & Gergov, M. **2005**, "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,* vol. 19, no. 10, pp. 1332-1338.

[218] Dams, R., Murphy, C.M., Lambert, W.E. & Huestis, M.A. **2003**, "Urine drug testing for opioids, cocaine, and metabolites by direct injection liquid chromatography/tandem mass spectrometry", *Rapid communications in mass spectrometry : RCM,* vol. 17, no. 14, pp. 1665-1670.

[219] Johansen, S.S. & Jensen, J.L. **2005**, "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,* vol. 825, no. 1, pp. 21-28.

[220] Horn, C.K., Klette, K.L. & Stout, P.R. **2003**, "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,* vol. 27, no. 7, pp. 459-463.

[221] Stout, P.R., Horn, C.K. & Klette, K.L. **2002**, "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,* vol. 26, no. 5, pp. 253-261.

[222] Dams, R., Benijts, T., Lambert, W.E. & De Leenheer, A.P. **2002**, "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,* vol. 773, no. 1, pp. 53-61.

[223] Shima, N., Tsutsumi, H., Kamata, T., Nishikawa, M., Katagi, M., Miki, A. & Tsuchihashi, H. **2006**, "Direct determination of glucuronide and sulfate of p-hydroxymethamphetamine in methamphetamine users' urine", *Journal of Chromatography B,* vol. 830, no. 1, pp. 64-70.

[224] Weinmann, W., Vogt, S., Goerke, R., Müller, C. & Bromberger, A. **2000**, "Simultaneous determination of THC-COOH and THC-COOH-glucuronide in urine samples by LC/MS/MS", *Forensic science international*, vol. 113, no. 1-3, pp. 381-387.

[225] Weinmann, W., Goerner, M., Vogt, S., Goerke, R. & Pollak, S. **2001**, "Fast confirmation of 11-nor-9-carboxy-Δ9-tetrahydrocannabinol (THC□COOH) in urine by LC/MS/MS using negative atmospheric-pressure chemical ionisation (APCI)", *Forensic science international*, vol. 121, no. 1-2, pp. 103-107.

[226] Maralikova, B. & Weinmann, W. **2004**, "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*, vol. 811, no. 1, pp. 21-30.

[227] http://www.drugabuse.gov/researchreports/cocaine/cocaine2.html. **2012**

[228] Needham, S.R., Jeanville, P.M., Brown, P.R. & Estape, E.S. **2000**, "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*, vol. 748, no. 1, pp. 77-87.

[229] Jeanville, P.M., Estape, E.S., Needham, S.R. & Cole, M.J. **2000**, "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*, vol. 11, no. 3, pp. 257-263.

[230] Cone, E.J., Tsadik, A., Oyler, J. & Darwin, W.D. **1998,** "Cocaine metabolism and urinary excretion after different routes of administration", *Therapeutic drug monitoring*, vol. 20, no. 5, pp. 556-560.

[231] Canezin, J., Cailleux, A., Turcant, A., Le Bouil, A., Harry, P. & Allain, P. **2001**, "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*, vol. 765, no. 1, pp. 15-27.

[232] Dams, R., Huestis, M.A., Lambert, W.E. & Murphy, C.M. **2003**, "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, vol. 14, no. 11, pp. 1290-1294.

[233] Ternes, T.A., Joss, A. & Siegrist, H. **2004,** "Scrutinizing pharmaceuticals and personal care products in wastewater treatment", *Environmental science & technology*, vol. 38, no. 20, pp. 392A-399A

[234] Zwiener, C., Seeger, S., Glauner, T. & Frimmel, F.H. **2002**, "Metabolites from the biodegradation of pharmaceutical residues of ibuprofen in biofilm reactors and batch experiments", *Analytical and bioanalytical chemistry*, vol. 372, no. 4, pp. 569-575.

[235] Stumpf, M., Ternes, T.A., Wilken, R.D., Rodrigues, S.V. & Baumann, W. **1999**, "Polar drug residues in sewage and natural waters in the state of Rio de Janeiro, Brazil", *The Science of the total environment*, vol. 225, no. 1-2, pp. 135-141.

[236] Langford, K.H., Lester, J.N., **2002**. "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. CRC Press, Florida, USA.

[237] Hunter, M., Stephenson, T., Kirk, P.W.W., Perry, R., Lester, J.N., **1986**, "Effects of salinity gradients and heterotrophic microbial activity on biodegradation of nitrolotriacetic acid in laboratory simulations of the estuarine environment", *Appl. Environ, Microbiol*. vol. 51, pp.919-925.

[238] Langford, K.H., Scrimshaw, M. D., Birkett, J.W. & Lester, J.N. **2005**, "Degradation of nonylphenolic surfactants in activated sludge batch tests", *Water Research*, vol. 39, pp. 870-876.

[239] Quintana, J.B., Weiss, S. & Reemtsma, T. **2005**, "Pathways and metabolites of microbial degradation of selected acidic pharmaceutical and their occurrence in municipal wastewater treated by a membrane bioreactor", *Water research*, vol. 39, no. 12, pp. 2654-2664.

[240] Kimura, K., Hara, H., Watanabe, Y. **2007** "Elimination of selected acidic pharmaceuticals from municipal wastewater by an activated sludge system and membrane bioreactors" *Environ. Sci .Technol*. 41, pp 3708-3714

[241] Heberer, T. & Feldmann, D. **2005**, "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*, vol. 122, no. 3, pp. 211-218.

[242] Postigo, C., Lopez de Alda, M.J. & Barceló, D. **2008**, "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*, vol. 27, no. 11, pp. 1053-1069.

[243] Venners, S.A., Korrick, S., Xu, X., Chen, C., Guang, W. and Huang, A. *et al.*, **2005**, "Preconception serum DDT and pregnancy loss: a prospective study using a biomarker of pregnancy", *America Journal of Epidemiol*ogy, vol. 162, pp. 709–716.

[244] Zimmer, K., Gutleb, A. C., Lyche, J. L., Dahl, E., Oskam, I., Krogenas, A., Skaare, J. U. and Ropstad, E. **2008**, "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 (272) 164–172.

[245] Krishman, V. and Safe, S. **1993**, "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*, vol. 120) 55-61.

[246] Whyatt, R.M., Rauh, V., Barr, D.B., Camann, D.E., Andrews, H.F. and Garfinkel, R. *et al.*, **2004,** "Prenatal insecticide exposures and birth weight and length among an urban minority cohort", *Environ Health Perspect*ive, vol. 112, pp. 1125–1132.

[247] Lilienthal, H. and Winneke, G. **1991**, "Sensitive periods for behavioural toxicity of polychlorinated biphenyls: determination by cross fostering in rats. Fundam Appl Toxicology, vol. 17, pp. 368-375.

[248] Korach, K.S., Sarver, P., Chae, K., McLachlan, J.A. and McKinney, J.D. **1988**, " Estrogen receptor-binding activity of polychlorinated hydrobiphenyls conformationally restricted structural probes. Mol Pharmacology", vol. 33, pp. 130-126

[249] Carballa, M., Omil, F., Lema, J.M., Llompart, M., García-Jares, C., Rodríguez, I., Gómez, M. & Ternes, T. **2004,** "Behavior of pharmaceuticals, cosmetics and hormones in a sewage treatment plant", *Water research*, vol. 38, no. 12, pp. 2918-2926.

[250] Quintana, J.B., Weiss, S. & Reemtsma T. **2005**, "Pathways and metabolites of microbial degradation of selected acidic pharmaceutical and their occurrence in municipal wastewater treated by a membrane bioreactor", *Water Research*, vol. 39, pp.2654-2664.

[251] Ternes, T.A., Stueber, J., Herrmann, N., McDowell, D., Ried, A., Kampman, T. M., Teiser, B. **2003**, "Ozonation: a tool for the removal of pharmaceuticals, contrast media and musk frangrances from wastewater", *Water Research*, vol. 37, pp. 1976-1982.

[252] Huber, M.M., Canonical, S., Park, G.Y., von Gunten, U., **2003**. "Oxidation of pharmaceuticals during ozonation and advanced oxidation processes", *Environ. Sci. Technol*. vol. 37, pp. 1016-1024.

[253] Kloepler, A., Gnirss, R., Jekel, M., Reemtsma, T., **2004**. "Occurrence of benzothiazoles in municipal wastewater and their fate in biological treatment", *Water Sci. Technol*. vol. 50 (5), pp. 203-208.

[254] Zuehlke, S., Duennbier, U., Lesjean, B., Gniss, R., Buisson, H., **2003**. "Longtime comparison of trace organics removal performance between convectional and membrane activated sludge processes". Poceedings of the WEFTEC 2003 Conference, Los Angeles, October 2003.

[255] Metcalf and Eddy. **1991**, "Wastewater Engineering: Treatment Disposal Reuse", McGraw-Hill, Inc.

[256] Chen, H.C., Wang, P.L., Ding, W.H. **2008**" Using liquid chromatography-ion trap mass spectrometry to determine pharmaceutical residues in Taiwanese rivers and wastewaters" *Chemosphere*, 72, pp 863-869.

[257] Perez, S., Eichhorn, P., Celiz, M.D. & Aga, D.S. **2006**, "Structural characterization of metabolites of the X-ray contrast agent iopromide in activated sludge using ion trap mass spectrometry", *Analytical Chemistry*, vol. 78, no. 6, pp. 1866-1874.

[258] Steger-Hartmann, T., Lange, R., Schweinfurth, H., Tschampel, M. & Rehmann, I. **2002**, "Investigations into the environmental fate and effects of iopromide (ultravist), a widely used iodinated X-ray contrast medium", *Water research*, vol. 36, no. 1, pp. 266-274.

[259] Eichhorn, P., Ferguson, P.L., Perez, S. & Aga, D.S. **2005**, "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*, vol. 77, no. 13, pp. 4176-4184.

[260] Schreiber, F. & Szewzyk, U. **2008**, "Environmentally relevant concentrations of pharmaceuticals influence the initial adhesion of bacteria", *Aquatic Toxicology*, vol. 87, no. 4, pp. 227-233.

[261] Kasprzyk-Hordern, B., Dinsdale, R.M and Guwy, A.J. 2009, " The removal of pharmaceuticals, personal care products, endocrine disruptors and illicit drugs during wastewater treatmen and its impact on the quality of receiving waters.

[262] European Community, European Communities List of 129 Priority Substances, Direcive 76/464/EEC, Commission of the European Community.

[263] Carballa, M., Francisco, O., Juan, M.L., Maria, L., Carmen, G-J., Isaac, R., Mariano, G., Ternes, T., **2004**, "Behaviour of pharmaceuticals, cosmetics and hormones in a sewage plant", *Water Research*, vol. 38, pp. 2918-2926.

[264] Goldstone, M.E and Lester, J.N., **1991**, "The balance of heavy metals through sewage treatment works", *The Science of the Total Environment*, vol.105, pp. 259-266.

[265] Radtke, T. M., and Gist, G. L. **1989**, "Wastewater Sludge Disposal: Antibiotic Resistant Bacteria May Pose Health Hazard." *Journal of Environmental Health*, vol. 52, no. 2), pp. 102-5.

[266] Kasprzyk-Hordern, B., Dinsdale, R.M. & Guwy, A.J. **2007**, "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*, vol. 1161, no. 1-2, pp. 132-145.

[267] Wood, M., Laloup, M., Fernandez, M.d.M.R., Jenkins, K.M., Young, M.S., Ramaekers, J.G., Boeck, G.D. & Samyn, N. **2005**, "Quantitative analysis of multiple illicit drugs in preserved oral fluid by solid-phase extraction and liquid chromatography–tandem mass spectrometry", *Forensic science international*, vol. 150, no. 2-3, pp. 227-238.

[268] Huerta-Fontela, M., Galceran, M.T., Martin-Alonso, J. & Ventura, F. **2008**a, "Occurrence of psychoactive stimulatory drugs in wastewaters in north-eastern Spain", *Science of The Total Environment,* vol. 397, no. 1-3, pp. 31-40.

[269] Zuccato, E., Chiabrando, C., Castiglioni, S., Bagnati, R. & Fanelli, R. **2008a**, "Estimating Community Drug Abuse by Wastewater Analysis", *Environmental health perspectives,* vol. 116, no. 8, pp. 1027-1032.

[270] van Nuijs, A.L.N., Pecceu, B., Theunis, L., Dubois, N., Charlier, C., Jorens, P.G., Bervoets, L., Blust, R., Neels, H. & Covaci, A. **2009**b, "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,* vol. 43, no. 5, pp. 1341-1349.

[271] Phillips, D.L., Tebbett, I.R. & Bertholf, R.L. **1996**, "Comparison of HPLC and GC-MS for measurement cocaine and metabolites in human urine", *Journal of analytical toxicology,* vol. 20, no. 5, pp. 305-308.

[272] Drummer, O.H., Gerostamoulos, D., Chu, M., Swann, P., Boorman, M. & Cairns, I. **2007**, "Drugs in oral fluid in randomly selected drivers", *Forensic science international,* vol. 170, no. 2-3, pp. 105-110.

[273] Concheiro, M., de Castro, A., Quintela, O., López-Rivadulla, M. & Cruz, A. **2005**, "Determination of MDMA, MDA, MDEA and MBDB in ora)l fluid using high performance liquid chromatography with native fluorescence detection", *Forensic science international,* vol. 150, no. 2-3, pp. 221-226.

[274] Pujadas, M., Pichini, S., Civit, E., Santamariña, E., Perez, K. & de la Torre, R**. 2007**, "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,* vol. 44, no. 2, pp. 594-601.

[275] Dignac, M.A., Ginestet, P., Rybacki, D., Bruchet, A., Urbain, V & Scribe, P. **2000**, "Fate of wastewater organic pollution during activated sludge treatment: Nature of residual orgnic matter", *Water Research*, vol. 34, no. 17, pp. 4185-4194.

[276] Waumans D., Steven P., Braneel N and Tytgat J. Lab. of Toxicology, Campu,Gasthuisberg,O&N2,Leuven Belgium (jan.tytgat@pharm.kuleuven.be.

[277] Van Bocxlaer, J.F., Clauwaert, K.M., Lambert, W.E., Deforce, D.L., Van den Eeckhout, E.G. & De Leenheer, A.P. **2000**, "Liquid chromatography-mass spectrometry in forensic toxicology", *Mass spectrometry reviews,* vol. 19, no. 4, pp. 165-214.

[278] Akre, C., Fedeniuk, R. & MacNeil, J.D. **2004**, "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,* vol. 129, no. 2, pp. 145-149.

[279] Sacher, F., Lange, F.T., Brauch, H. & Blankenhorn, I. **2001**, "Pharmaceuticals in groundwaters: Analytical methods and results of a monitoring program in Baden-Württemberg, Germany", *Journal of Chromatography A,* vol. 938, no. 1-2, pp. 199-210.

[280] Steller H. **1995**, "Mechanisms and genes of cellular suicide. *Science,* vol. 267, pp. 1445–1449.

[281] Meakins, N.C., Bubb, J.M. & Lester, J.N. **1994**, "The behaviour of the s-triazine herbicides, atrazine and simazine, during primary and secondary biological waste water treatment", *Chemosphere,* vol. 28, no. 9, pp. 1611-1622.

[282] Standing Committee of Analysts, **1980**. Suspended, settleable and dissolved solids in waters and effluents, HMSO, London.

[283] Eaton, A.D., Clesceri, L.S and Greenberg, A.E., **1995**, "Standard Methods for Examination of Water and Wastewater, 19th ed. APHA, AWWA, WPCF.

[284] Lester, J.N and Birkett, J.W. "Microbiology and chemistry for Environmental Scientist and Engineers 2nd Edition Chapter 14 & 20. Publishers F & N Spon. ISBN 0-414-22680-X

[285] Wilson, P.C. and Boman, B.J., **2011**, "Characterization of selected organo-nitrogen herbicides in South Florida canals: Exposure and risk assessment", *Sci. of Total Environment*, vol. 412-413, pp. 119-26

[286] Stumpf, M., Ternes, T.A., Haberer, K., Seel, P., Baumann, W., Nachweis von Arzneimittelruckstanden in Klaranlagen und Fliebgewassern, **1996**, "Determination of drugs in sewage treatment plants and river water", *Vom Wasser*. Vol. 87, pp. 291-303.

[287] Zakkour, P.D., Gaterell., M.R., Griffin, P., Gochin, R. J., and Lester, J. N. , **2001**. "Anaerobic treatment of domestic wastewater in temperate climates: Treatment plant modelling with economic considerations*", Water. Res*. Vol. 35, No. 17, pp. 4137–4149

[288] www.stwater.co.uk/upload/pdf/Stoke_Bardolph.pdf, **2012.**

[289] http://www.lenntech.com/wwtp/wwtp-overview.htm#ixzz1ynZrW2x2, **2012.**

[290] Ai Jia, Yi Wan, Yang Xiao and Jianying Hu., **2012**, "Occurrence and fate of quinolone and fluoroquinolone antibiotics in a municipal sewage treatment plant", *Water Research*, vol. 46, pp. 387 -394

[291] Ze-hua Liu**.,** Yoshinori Kanjo., Satoshi Mizutani, **2009**. "Removal mechanisms for endocrine disrupting compounds (EDCs) in wastewater treatment — physical means, biodegradation, and chemical advance oxidation: A review", *Science of The Total Environment*, vol. 407, Issue 2, pp. 731–748

[292] USAFE OMTAP – RAF Molesworth:Phase I Diagnostic Report. MHW Project number: 41513965 and Document Number: MDH06/RO541. March **2007** and UK Departrment for Environment, Food and Rural Affairs (http://www.defra.gov.uk/environment/)

[293] American Public Health Association, Standard Methods for the Examination of Water and Wastewater, 18th Edition, 1992, various pages.

[294] Sophonsiri, C. & Morgenroth, E. **2004**, "Chemical composition associated with different particle size fractions in municipal, industrial, and agricultural wastewaters", *Chemosphere*, vol. 55, no. 5, pp. 691-703.

[295] Pierre Servais., Martin Seidl., Jean-Marie Mouchel, **1999**. "Comparison of parameters characterizing organic matter in a combined sewer durig rainfall events and dry matter", *Water Environment Research*, vol. 71, 4, pp. 408-417

[296] Guellil, A., Thomas, F., Block, J.C., Bersillon, J.L. & Ginestet, P. **2001**, "Transfer of organic matter between wastewater and activated sludge flocs", *Water research*, vol. 35, no. 1, pp. 143-150.

[297] Lester, J.N. **2002** "Presence of organic micropollutants in sewage sludges. First Int. Symposium on Processing and Use of Sewage sludge". Department of the Environment/Commission of the European Communities, Brighton, UK. Pp. 150-157.

[298] Baker, D.R. & Kasprzyk-Hordern, B. **2011**, "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*, vol. 1218, no. 44, pp. 8036-8059.

[299] Baker, D.R. & Kasprzyk-Hordern, B. **2011**, "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,* vol. 1218, no. 12, pp. 1620-1631.

[300] Harman, C., Reid, M. & Thomas, K.V. **2011**, "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,* vol. 45, no. 13, pp. 5676-5682.

[301] Hedgespeth, M.L., Sapozhnikova, Y., Pennington, P., Clum, A., Fairey, A. & Wirth, E. 2012, "Pharmaceuticals and personal care products (PPCPs) in treated wastewater discharges into Charleston Harbor, South Carolina", *Science of The Total Environment,* vol. 437, no. 0, pp. 1-9.

[302] Karolak, S., Nefau, T., Bailly, E., Solgadi, A. & Levi, Y. **2010**, "Estimation of illicit drugs consumption by wastewater analysis in Paris area (France)", *Forensic science international,* vol. 200, no. 1–3, pp. 153-160.

[303] Kasprzyk-Hordern, B. & Baker, D.R. **2012**, "Enantiomeric profiling of chiral drugs in wastewater and receiving waters", *Environmental science & technology,* vol. 46, no. 3, pp. 1681-1691.

[304] Kasprzyk-Hordern, B. & Baker, D.R. **2012**, "Estimation of community-wide drugs use via stereoselective profiling of sewage", *Science of The Total Environment,* vol. 423, no. 0, pp. 142-150.

[305] Kasprzyk-Hordern, B., Dinsdale, R.M. & Guwy, A.J. **2009**, "Illicit drugs and pharmaceuticals in the environment – Forensic applications of environmental data. Part 1: Estimation of the usage of drugs in local communities", *Environmental Pollution,* vol. 157, no. 6, pp. 1773-1777.

[306] Kasprzyk-Hordern, B., Kondakal, V.V.R. & Baker, D.R. **2010**, "Enantiomeric analysis of drugs of abuse in wastewater by chiral liquid chromatography coupled with tandem mass spectrometry", *Journal of Chromatography A,* vol. 1217, no. 27, pp. 4575-4586.

[307] Lai, F.Y., Ort, C., Gartner, C., Carter, S., Prichard, J., Kirkbride, P., Bruno, R., Hall, W., Eaglesham, G. & Mueller, J.F. **2011**, "Refining the estimation of illicit drug consumptions from wastewater analysis: Co-analysis of prescription pharmaceuticals and uncertainty assessment", *Water research,* vol. 45, no. 15, pp. 4437-4448.

[308] Lai, K.M., Scrimshaw, M.D. & Lester, J.N. **2002**, "Prediction of the bioaccumulation factors and body burden of natural and synthetic estrogens in aquatic organisms in the river systems", *Science of The Total Environment,* vol. 289, no. 1–3, pp. 159-168.

[309] Metcalfe, C., Tindale, K., Li, H., Rodayan, A. & Yargeau, V. **2010**, "Illicit drugs in Canadian municipal wastewater and estimates of community drug use", *Environmental Pollution,* vol. 158, no. 10, pp. 3179-3185.

[310] Postigo, C., de Alda, M.L. & Barceló, D. **2011**, "Evaluation of drugs of abuse use and trends in a prison through wastewater analysis", *Environment international,* vol. 37, no. 1, pp. 49-55.

[311] Postigo, C., López de Alda, M.J. & Barceló, D. **2010**, "Drugs of abuse and their metabolites in the Ebro River basin: Occurrence in sewage and surface water, sewage treatment plants removal efficiency, and collective drug usage estimation", *Environment international,* vol. 36, no. 1, pp. 75-84.

[312] Putschew, A., Wischnack, S. & Jekel, M. **2000,** "Occurrence of triiodinated X-ray contrast agents in the aquatic environment", *The Science of the total environment,* vol. 255, no. 1-3, pp. 129-134.

[313] ter Laak, T.L., van der Aa, M., Houtman, C.J., Stoks, P.G. & van Wezel, A.P. **2010**, "Relating environmental concentrations of pharmaceuticals to consumption: A mass balance approach for the river Rhine", *Environment international,* vol. 36, no. 5, pp. 403-409.

[314] van Nuijs, A.L.N., Mougel, J., Tarcomnicu, I., Bervoets, L., Blust, R., Jorens, P.G., Neels, H. & Covaci, A. **2011**, "Sewage epidemiology — A real-time approach to estimate the consumption of illicit drugs in Brussels, Belgium", *Environment international,* vol. 37, no. 3, pp. 612-621.

[315] Brausch, J.M., Connors, K.A., Brooks, B.W. & Rand, G.M. **2012**, "Human pharmaceuticals in the aquatic environment: a review of recent toxicological studies and considerations for toxicity testing", *Reviews of environmental contamination and toxicology,* vol. 218, pp. 1-99.

[316] Brooks, G.T. **1980**, "Perspectives of the chemical fate and toxicity of pesticides", *Journal of environmental science and health.Part.B, Pesticides, food contaminants, and agricultural wastes,* vol. 15, no. 6, pp. 755-793.

[317] Chojnacka, K. **2010**, "Biosorption and bioaccumulation--the prospects for practical applications", *Environment international,* vol. 36, no. 3, pp. 299-307.

[318] Daughton, C.G. **2011**, "Illicit drugs: contaminants in the environment and utility in forensic epidemiology", *Reviews of environmental contamination and toxicology,* vol. 210, pp. 59-110.

[319] Escher, B.I. & Fenner, K. **2011**, "Recent advances in environmental risk assessment of transformation products", *Environmental science & technology,* vol. 45, no. 9, pp. 3835-3847.

[320] Fatta-Kassinos, D., Meric, S. & Nikolaou, A. **2011**, "Pharmaceutical residues in environmental waters and wastewater: current state of knowledge and future research", *Analytical and bioanalytical chemistry,* vol. 399, no. 1, pp. 251-275.

[321] Guellil, A., Thomas, F., Block, J.C., Bersillon, J.L. & Ginestet, P. **2001**, "Transfer of organic matter between wastewater and activated sludge flocs", *Water research,* vol. 35, no. 1, pp. 143-150.

[322] Hintelmann, H. **2010,** "Organomercurials. Their formation and pathways in the environment", *Metal ions in life sciences,* vol. 7, pp. 365-401.

[323] Kasprzyk-Hordern, B. **2010**, "Pharmacologically active compounds in the environment and their chirality", *Chemical Society Reviews,* vol. 39, no. 11, pp. 4466-4503.

[324] Katagi, T. **2010**, "Bioconcentration, bioaccumulation, and metabolism of pesticides in aquatic organisms", *Reviews of environmental contamination and toxicology,* vol. 204, pp. 1-132.

[325] Kern, S., Baumgartner, R., Helbling, D.E., Hollender, J., Singer, H., Loos, M.J., Schwarzenbach, R.P. & Fenner, K. **2010**, "A tiered procedure for assessing the formation of biotransformation products of pharmaceuticals and biocides during activated sludge treatment", *Journal of environmental monitoring : JEM,* vol. 12, no. 11, pp. 2100-2111.

[326] Lin, A.Y., Panchangam, S.C. & Chen, H.Y. **2010**, "Implications of human pharmaceutical occurrence in the Sindian river of Taiwan: a strategic study of risk assessment", *Journal of environmental monitoring : JEM,* vol. 12, no. 1, pp. 261-270.

[327] Perelo, L.W. 2010, "Review: In situ and bioremediation of organic pollutants in aquatic sediments", *Journal of hazardous materials,* vol. 177, no. 1-3, pp. 81-89.

[328] Puig, S., van Loosdrecht, M.C., Colprim, J. & Meijer, S.C. **2008**, "Data evaluation of full-scale wastewater treatment plants by mass balance", *Water research,* vol. 42, no. 18, pp. 4645-4655.

[329] Ra, J.S., Lee, S.H., Lee, J., Kim, H.Y., Lim, B.J., Kim, S.H. & Kim, S.D. **2011**, "Occurrence of estrogenic chemicals in South Korean surface waters and municipal wastewaters", *Journal of environmental monitoring : JEM,* vol. 13, no. 1, pp. 101-109.

[330] Ra, J.S., Lee, S.H., Lee, J., Kim, H.Y., Lim, B.J., Kim, S.H. & Kim, S.D. **2011**, "Occurrence of estrogenic chemicals in South Korean surface waters and municipal wastewaters", *Journal of environmental monitoring : JEM,* vol. 13, no. 1, pp. 101-109.

[331] Rujiralai, T., Bull, I.D., Llewellyn, N. & Evershed, R.P. **2011,** "In situ polar organic chemical integrative sampling (POCIS) of steroidal estrogens in sewage treatment works discharge and river water", *Journal of environmental monitoring : JEM,* vol. 13, no. 5, pp. 1427-1434.

[332] Schilling, K. & Zessner, M. **2011**, "Foam in the aquatic environment", *Water research,* vol. 45, no. 15, pp. 4355-4366.

[333] Sophonsiri, C. & Morgenroth, E. **2004**, "Chemical composition associated with different particle size fractions in municipal, industrial, and agricultural wastewaters", *Chemosphere,* vol. 55, no. 5, pp. 691-703.

[334] Thomann, R.V. 1**995**, "Modeling organic chemical fate in aquatic systems: significance of bioaccumulation and relevant time-space scales", *Environmental health perspectives,* vol. 103 Suppl 5, pp. 53-57.

[335] Ulrich, E.M., Morrison, C.N., Goldsmith, M.R. & Foreman, W.T. **2012**, "Chiral pesticides: identification, description, and environmental implications", *Reviews of environmental contamination and toxicology,* vol. 217, pp. 1-74.

[336] Westgate, P.J. & Park, C. **2010**, "Evaluation of proteins and organic nitrogen in wastewater treatment effluents", *Environmental science & technology,* vol. 44, no. 14, pp. 5352-5357.

[337] Yu, Y., Huang, Q., Wang, Z., Zhang, K., Tang, C., Cui, J., Feng, J. & Peng, X. **2011**, "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,* vol. 13, no. 4, pp. 871-878.

[338] Ziylan, A. & Ince, N.H. **2011,** "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,* vol. 187, no. 1-3, pp. 24-36.

[339] Eiceman, G.A. (**2000**). Gas Chromatography. In R.A. Meyers (Ed.), *Encyclopedia of Analytical Chemistry: Applications, Theory, and Instrumentation*, pp. 10627. Chichester: Wiley. ISBN 0-471-97670-9

[340] McLafferty, F. W.; Hertel, R. H. and Villwock, R. D. **1974** "Probability based matching of mass spectra. Rapid identification of specific compounds in mixtures". *Organic Mass Spectrometry* 9 (7): 690–702. doi:10.1002/oms.1210090710.

[341] Stein, SE; Scott DR **1994**. "Optimization and testing of mass spectral library search algorithms for compound identification". *J Am Soc Mass Spectrom* 5 (9): 859–866. doi:10.1016/1044-0305(94)87009-8.

[342] Stalling DL, Gehrke CW, Zumwalt RW. A new silylation reagent for amino acids bis(trimethylsilyl)trifluoroacetamide (BSTFA). *Biochemical and Biophysical Research Communications*. **1968** May 23;31(4):616-22. PMID 5656249.

[343] Prichard and Barwick Quality assurance in analytical chemistry. Originally: Horwitz (**1990**) *Pure Appl. Chem.* 62, 1193-1208.

[344] I P. Roberts, H.R. Heigi, A. Webber and H.R. Kr/idhenb/ihl. (**1977**), "Metals in municipal wastewater and their elimination in sewage treatment". *Prog. Water Technol.,* 8, 301-306

[345] J.N. Lester, R.M Harrison and R. Perry. (1979), "The balance of heavy metals through a sewage treatment works. I. Lead, cadmium and copper". *Sci Total Environ.,* 12, 13-23.

[346] S. Stoveland, M. Astruc, J.N. Lester and R. Perry. (**1979**), "The balance of heavy metals through a sewage treatment works, ll. Chromium, nickel and zinc". *Sci. Total Environ.,* 12, 25-34.

[347] A.C. Petrasek and l.J. Kugelman. (**1983),** "Metals removals and partitioning m conventtonal wastewater treatment plants". J. Water Pollut. Control Fed., 55, 1183-1189.

[348] W. Firk. (**1986**), "Heavy metals in sewage and occurring sludges -- the bahmces of three sewage treatment plants", in J.N. Lester, R. Perry and R.M. Sterritt (Eds), Int. Conf. Chemicals in the Environment, Lisbon, I-3 July, Selper Ltd, pp. 432-442.

[349] Kadam, A.; Ozaa, G.; Nemadea, P.; Duttaa, S.; Shankar, H. (**2008**). "Municipal wastewater treatment using novel constructed soil filter system". *Chemosphere* (Elsevier) **71** (5): 975–981

[350] Water and Environmental Health at London and Loughborough (**1999**). "Waste water Treatment Options."Technical brief no. 64. London School of Hygiene & Tropical Medicine and Loughborough University.

[351] M.J. Martı´nez , Bueno , S. Ucle´s , M.D. Hernando , E. Da´voli , A.R. Ferna´ndez-Alba. **2011**, "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,* vol. 4 5, pp 2 3 3 1 - 2 3 4 1

[352] M.J. Gomez, A. Aguera , M. Mezcua , J. Hurtado , F. Mochol´I, A.R. Fernandez-Alba. **2007**, "Simultaneous analysis of neutral and acidic pharmaceuticals as well as related compounds by gas chromatography–tandem mass spectrometry in wastewater" *Talanta*, vol. 73, pp. 314–320

[353] Suarez, S; Carballa, M and Omil, F. (**2008**), "How are pharmaceutical and personal care products (PPCPs) removed from urban wastewaters". *Rev. Environ Sci Biotechnol*, vol. 7, pp. 125-138

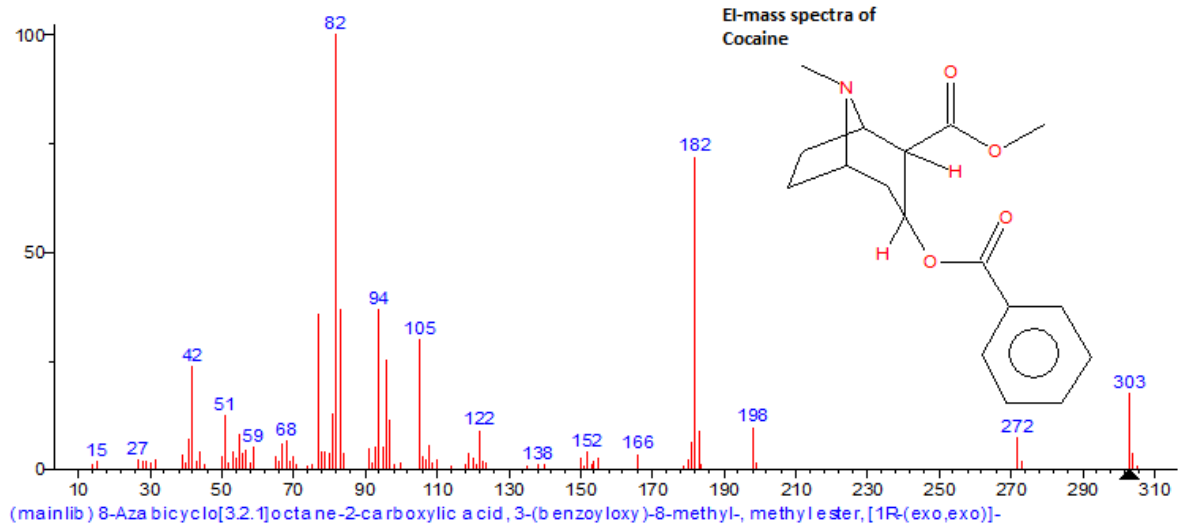
[354] Oulton, R. L., Kohn. T and Cwiertny, D.M. (**2010**), "Pharmaceuticals and personal care products in effluents matrices: A survey of transformation and removal during wastewater treatment and implications for wastewater management". J. of Environ. Monitor, vol. 12, pp. 1956 – 1978.

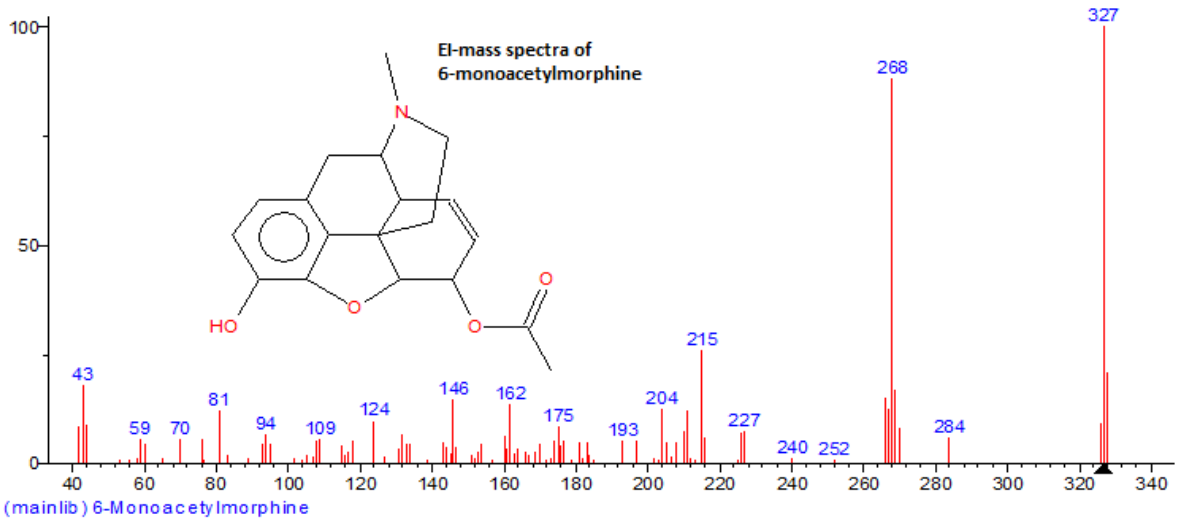
---

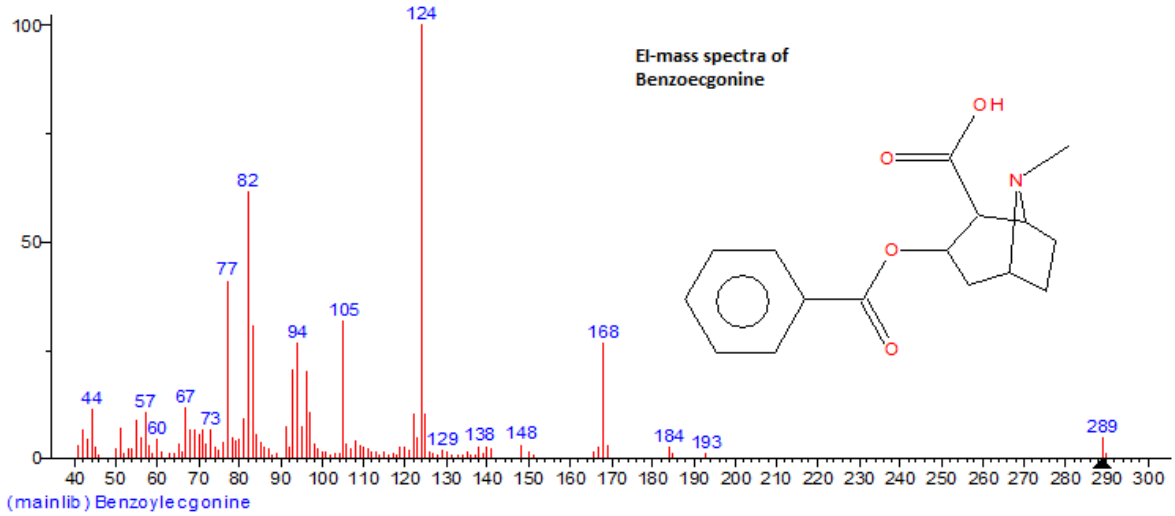
**Total number of words = 52, 748**

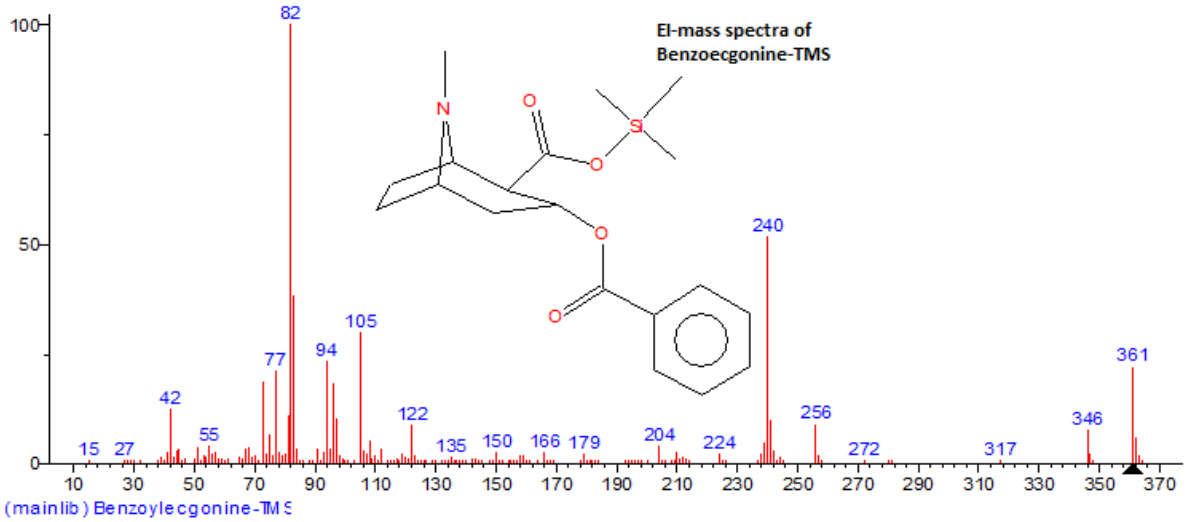
# APPENDIX SECTION

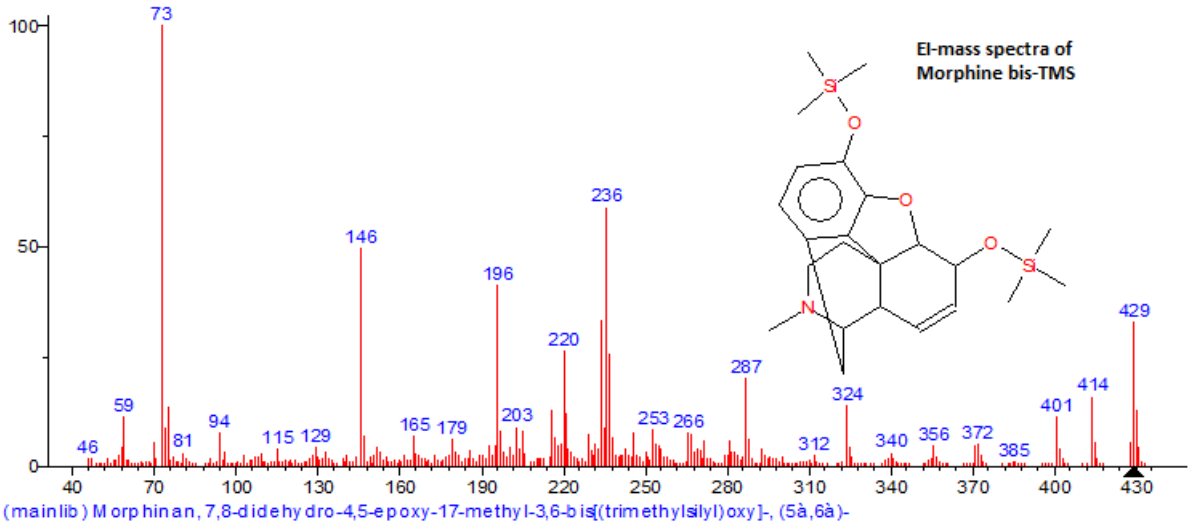
# Appendix 1: Reference Mass Spectra of Selected Compounds from GC NIST Library

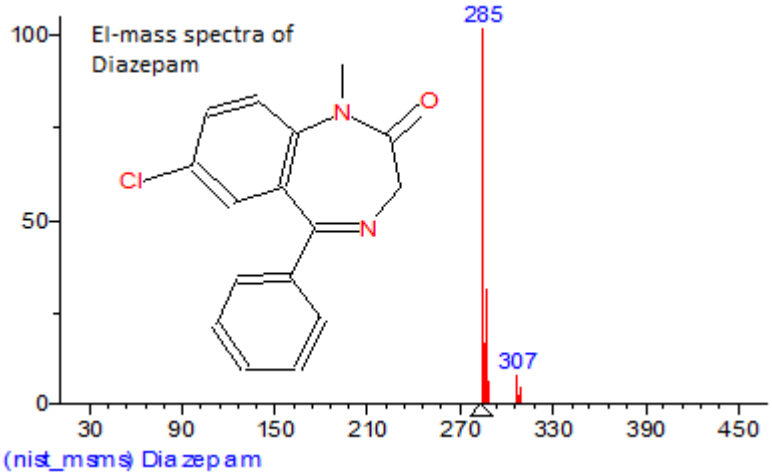


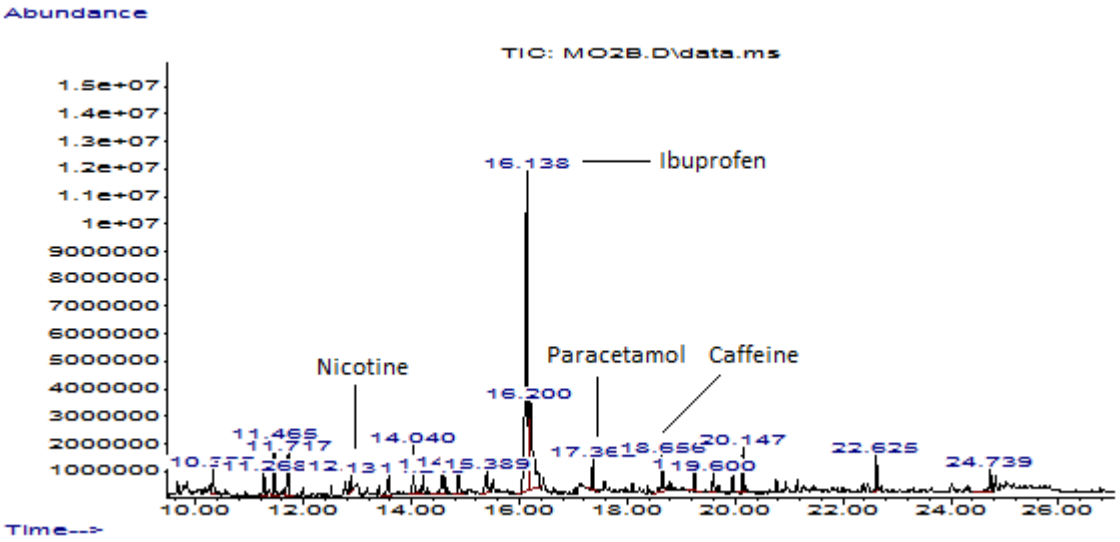

EI-mass spectra of
Benzoecgonine
OH
O
N
O
O
100
50
0
124
82
77
105
94
168
44
57
60
67
73
129
138
148
184
193
289
40 50 60 70 80 90 100 110 120 130 140 150 160 170 180 190 200 210 220 230 240 250 260 270 280 290 300
(mainlib) Benzoylecgonine

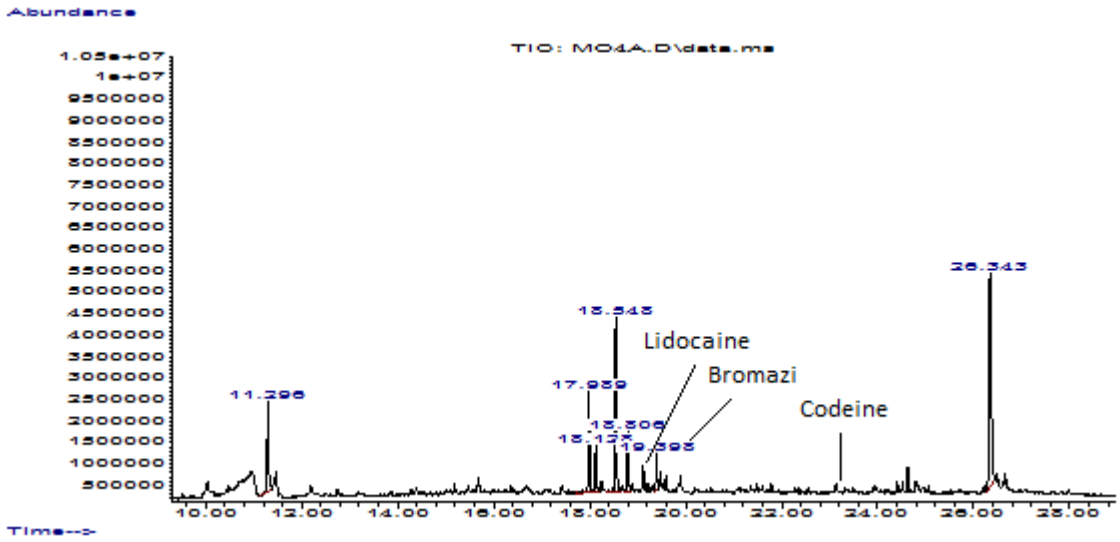

EI-mass spectra of
Benzoecgonine-TMS
N
O
O
Si
O
O
100
50
0
82
240
105
77
94
361
42
122
256
346
15
27
55
135
150
166
179
204
224
272
317
10 30 50 70 90 110 130 150 170 190 210 230 250 270 290 310 330 350 370
(mainlib) Benzoylecgonine-TMS


EI-mass spectra of
Morphine bis-TMS
73
146
196
236
220
287
324
429
414
401
59
94
46
81
115
129
165
179
203
253
266
312
340
356
372
385
100
50
0
40
70
100
130
160
190
220
250
280
310
340
370
400
430
(mainlib) Morphinan, 7,8-didehydro-4,5-epoxy-17-methyl-3,6-bis[(trimethylsilyl)oxy]-, (5à,6à)-


EI-mass spectra of
Diazepam
285
307
100
50
0
30
90
150
210
270
330
390
450
(nist_msms) Diazepam

## 2. Examples of GC TIC traces of analytes from wastewater samples:

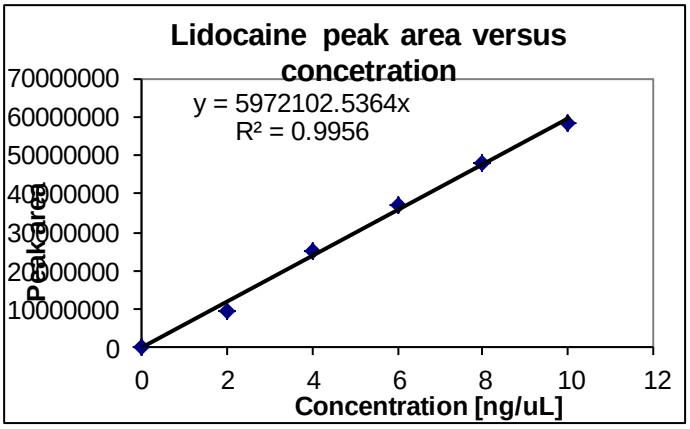


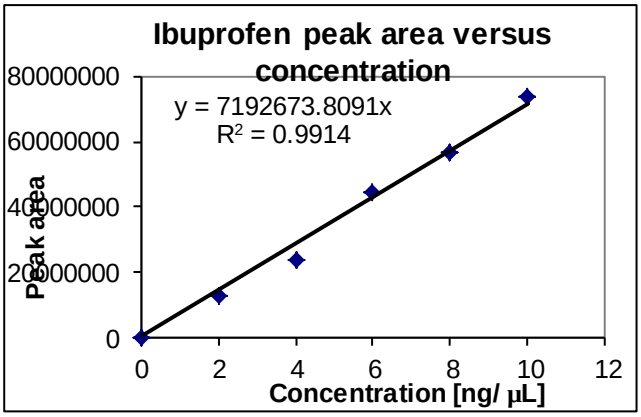



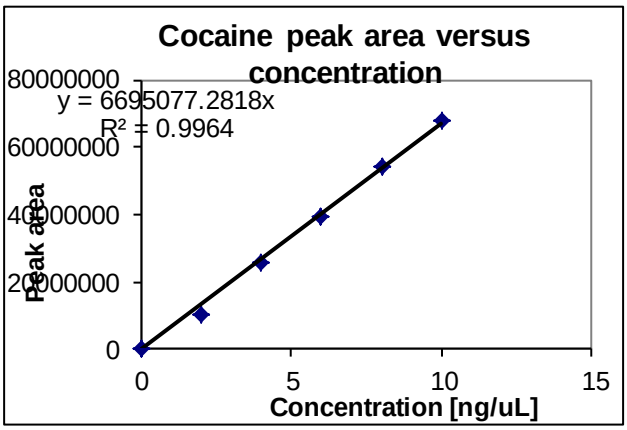
# Appendix 2A- Linear Calibration Data and Graphs for Analysed Wastewaters:

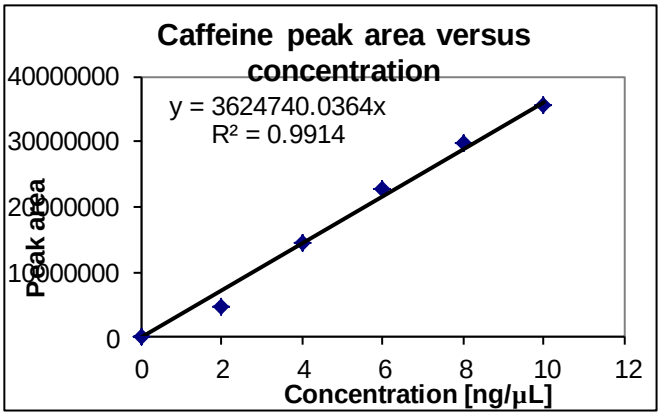
| Compounds | Calibration equations | Coefficient of variations |
|---|---|---|
| Ibuprofen | **y** = 7192673.8091**x** | $R^2$ = 0.9914 |
| Benzocaine | **y** = 12643449.0909**x** | $R^2$ = 0.9898 |
| Caffeine | **y** = 3624740.0364**x** | $R^2$ = 0.9914 |
| Lidocaine | **y** = 5972102.5364**x** | $R^2$ = 0.9956 |
| Cocaine | **y** = 6695077.2818**x** | $R^2$ = 0.9964 |
| Codeine | **y** = 26382267.9909**x** | $R^2$ = 0.9914 |
| Amphetamine | **y** = 2603437.4545**x** | $R^2$ = 0.9719 |
| Metamphetamine | **y** = 3936488.1455**x** | $R^2$ = 0.9534 |
| Ecgonine methyl ester | **y** = 9712404.1364**x** | $R^2$ = 0.9850 |
| Methadone | **y** = 8283520.2818**x** | $R^2$ = 0.9978 |
| 6- acetylcodeine | **y** = 363398.0455**x** | $R^2$ = 0.9950 |
| 6- acetylmorphine | **y** = 968887.8455**x** | $R^2$ = 0.9956 |
| Diacetylmorphine | **y** = 4861518.1909**x** | $R^2$ = 0.9671 |
| Diazepam | **y** = 7186720.3364**x** | $R^2$ = 0.9768 |
| Procaine | **y** = 1562839.1455**x** | $R^2$ = 0.9852 |
| Aspirin | **y** = 3317023.0818**x** | $R^2$ = 0.9647 |
| Bromacil | **y** = 1351922.4355**x** | $R^2$ = 0.9947 |
| Benzoylecgonine | **y**= 113780**x** | $R^2$ = 0.9989 |
| Morphine | **y**= 2380012.11**x** | $R^2$ = 1.00 |
| Ephedrine | **y**= 1335354.68**x** | $R^2$ = 0.9902 |

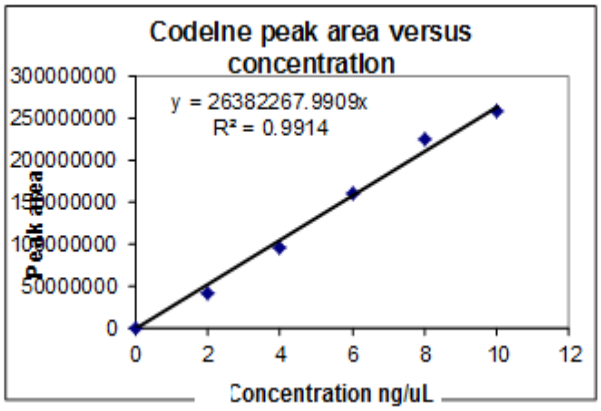
## Individual calibration graphs

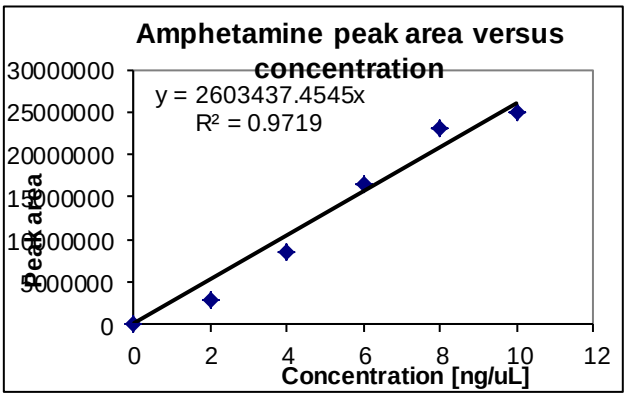


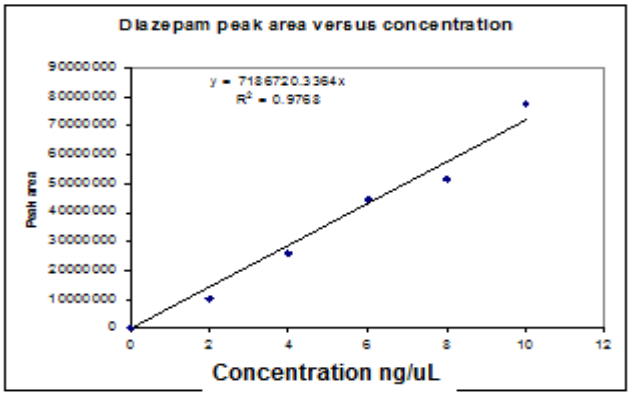



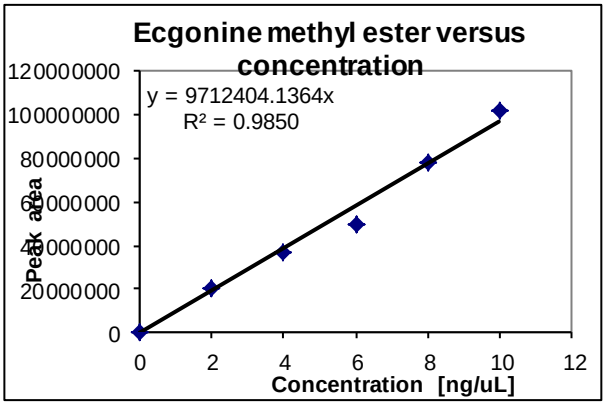



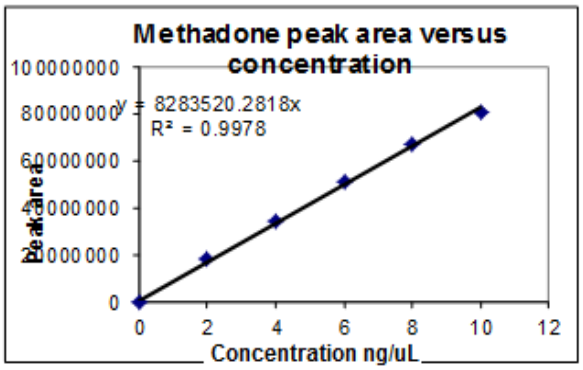



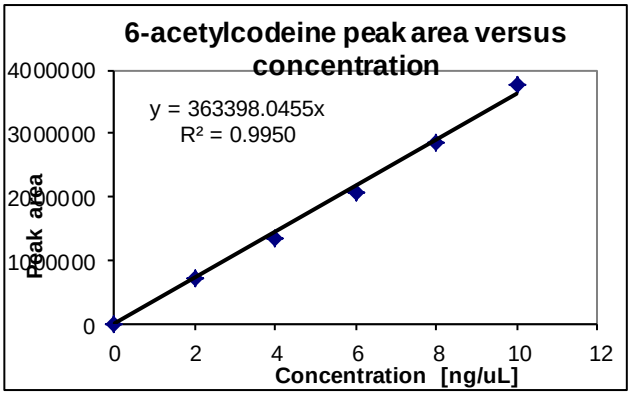

Codeine peak area versus concentration
y = 26382267.9909x
R² = 0.9914
Peak area
Concentration ng/uL

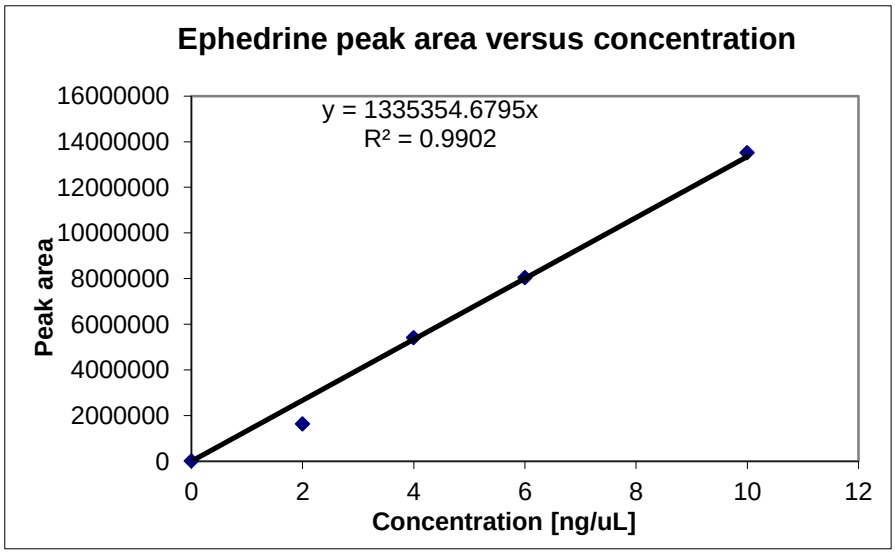

Amphetamine peak area versus concentration
y = 2603437.4545x
R² = 0.9719
Peak area
Concentration [ng/uL]

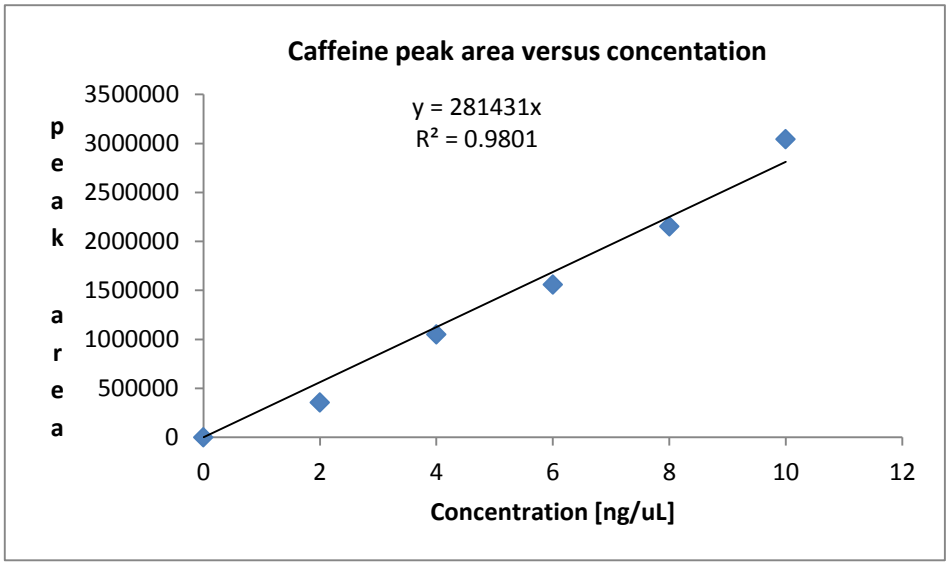

Diazepam peak area versus concentration
y = 7186720.3364x
R² = 0.9768
Peak area
Concentration ng/uL

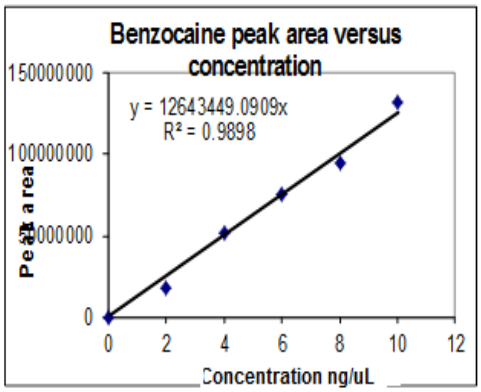

Ecgonine methyl ester versus concentration
y = 9712404.1364x
R² = 0.9850
Peak area
Concentration [ng/uL]

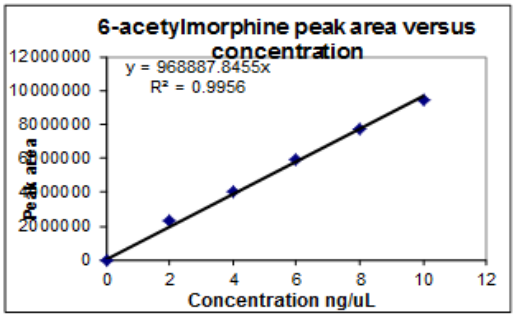

Methadone peak area versus concentration
y = 8283520.2818x
R² = 0.9978
Peak area
Concentration ng/uL

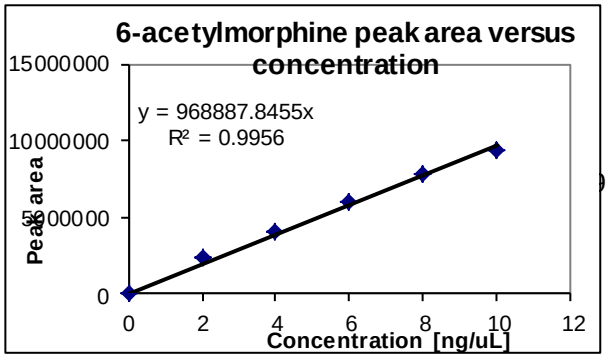

6-acetylcodeine peak area versus concentration
y = 363398.0455x
R² = 0.9950
Peak area
Concentration [ng/uL]

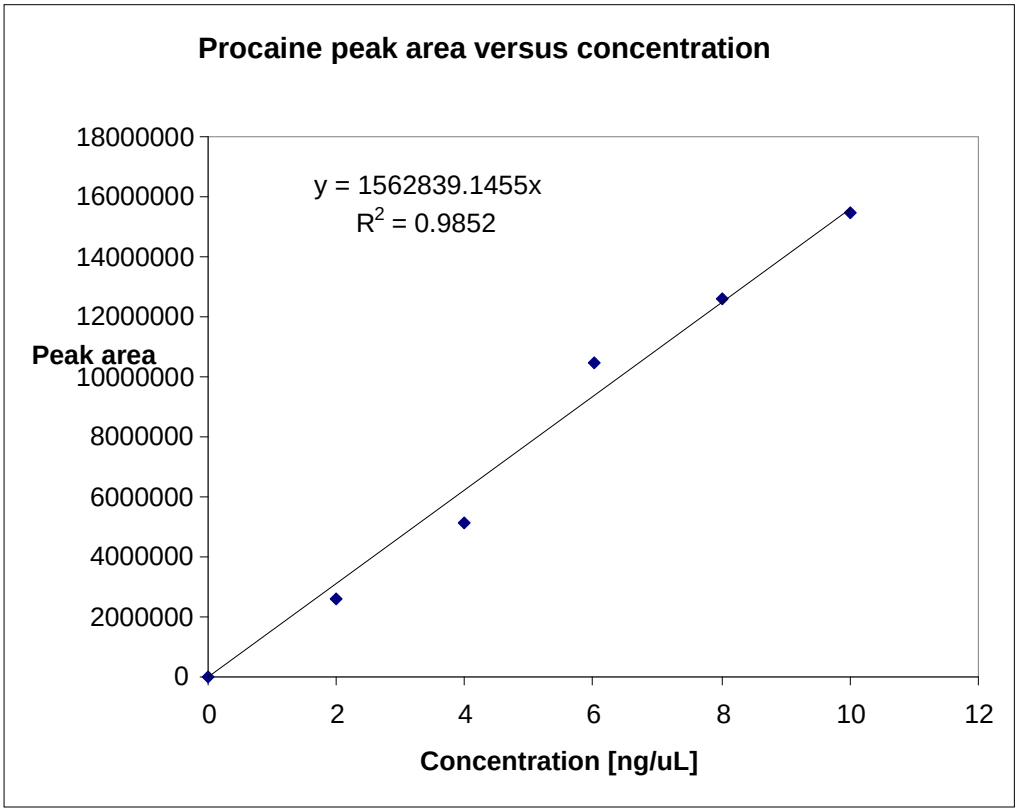

Ephedrine peak area versus concentration
y = 1335354.6795x
R² = 0.9902
Peak area
Concentration [ng/uL]

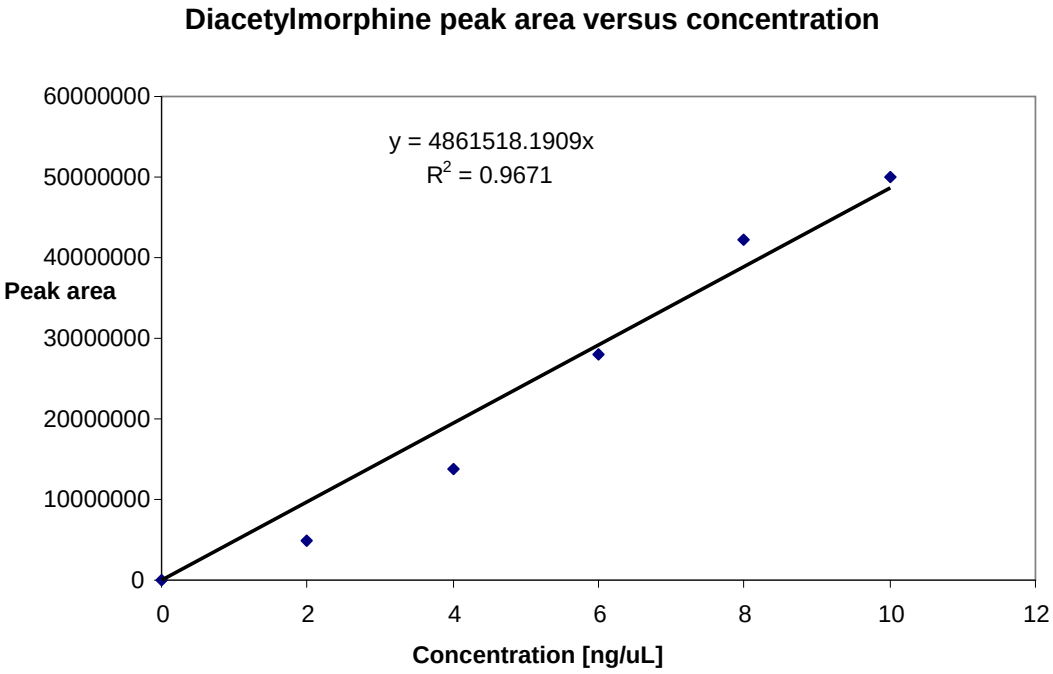

Caffeine peak area versus concentation
y = 281431x
R² = 0.9801
peak area
Concentration [ng/uL]

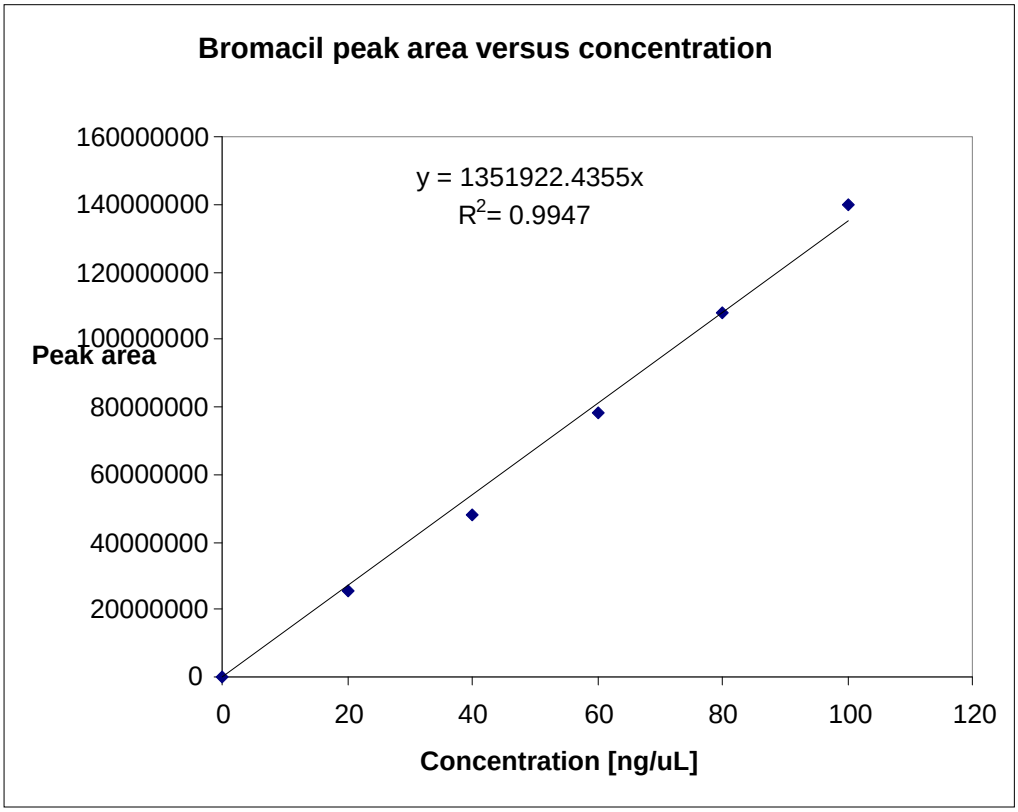

Benzocaine peak area versus concentration
y = 12643449.0909x
R² = 0.9898
Peak area
Concentration ng/uL

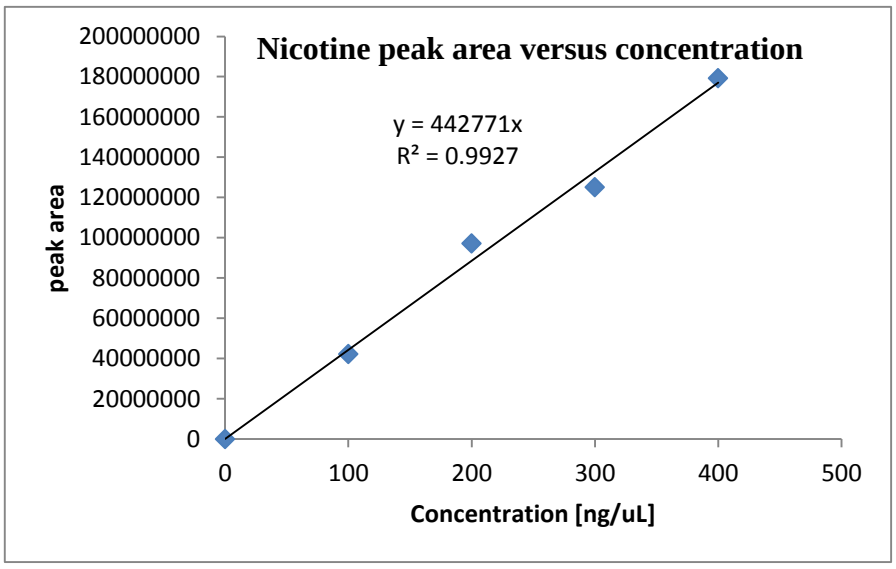

6-acetylmorphine peak area versus concentration
y = 968887.8455x
R² = 0.9956
Peak area
Concentration ng/uL

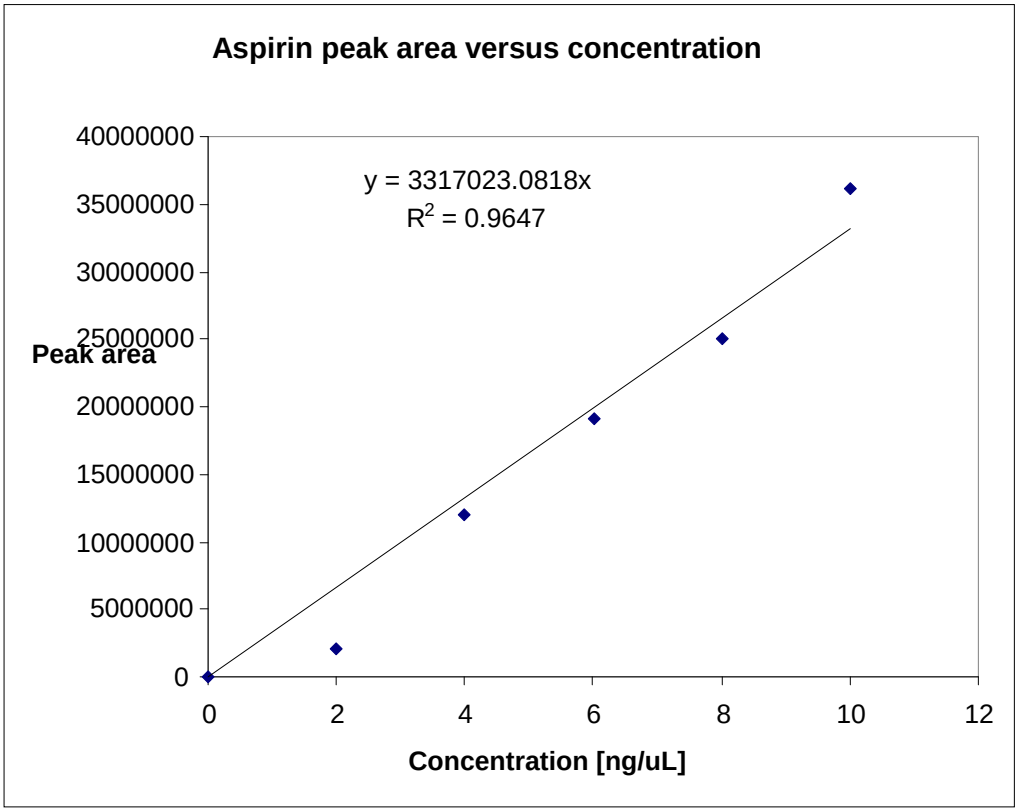

6-acetylmorphine peak area versus concentration
y = 968887.8455x
R² = 0.9956
Peak area
Concentration [ng/uL]

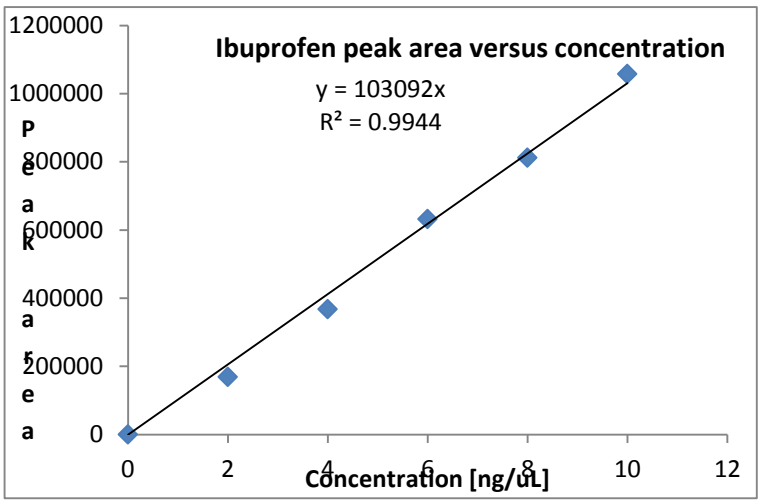

Procaine peak area versus concentration
y = 1562839.1455x
R2 = 0.9852
Peak area
18000000
16000000
14000000
12000000
10000000
8000000
6000000
4000000
2000000
0
0
2
4
6
8
10
12
Concentration [ng/uL]


Diacetylmorphine peak area versus concentration
y = 4861518.1909x
R2 = 0.9671
Peak area
60000000
50000000
40000000
30000000
20000000
10000000
0
0
2
4
6
8
10
12
Concentration [ng/uL]


Bromacil peak area versus concentration
y = 1351922.4355x
R2= 0.9947
Peak area
160000000
140000000
120000000
100000000
80000000
60000000
40000000
20000000
0
0
20
40
60
80
100
120
Concentration [ng/uL]


Nicotine peak area versus concentration
y = 442771x
R² = 0.9927
peak area
200000000
180000000
160000000
140000000
120000000
100000000
80000000
60000000
40000000
20000000
0
0
100
200
300
400
500
Concentration [ng/uL]


Aspirin peak area versus concentration
y = 3317023.0818x
R2 = 0.9647
Peak area
40000000
35000000
30000000
25000000
20000000
15000000
10000000
5000000
0
0
2
4
6
8
10
12
Concentration [ng/uL]


Ibuprofen peak area versus concentration
y = 103092x
R² = 0.9944
1200000
1000000
800000
600000
400000
200000
0
0
2
4
6
8
10
12
Concentration [ng/uL]

# Appendix 2B– Linear Calibration Data and Graphs for Batch Studies:

Cocaine: **(y)** =13223636.07**x** $\mathbf{R^2}$ = 1.00

Ecgoninemethylester :**(y)** =101000**x** $\mathbf{R^2}$ = 0.9877

Cocaethene: **(y)** =103540**x** $\mathbf{R^2}$ = 0.9947.

Benzoylecgonine:**(y)** =113780**x** $\mathbf{R^2}$ = 0.9989

Morphine:**(y)** =2380012.11**x** $\mathbf{R^2}$ = 1.00

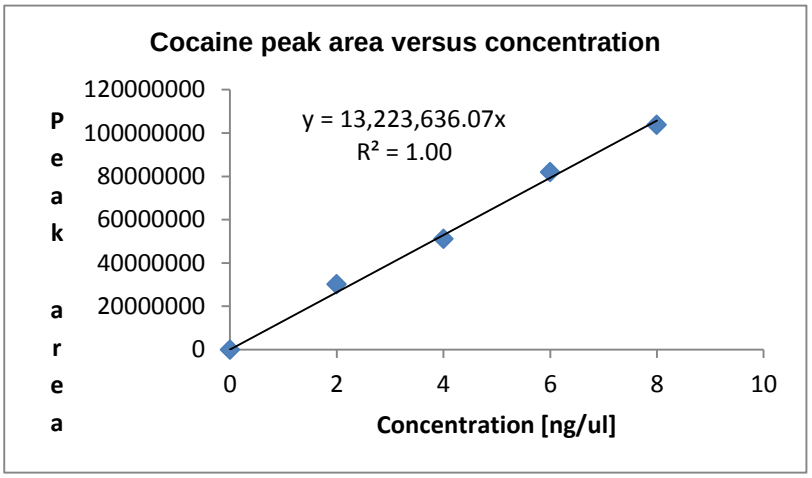
6-monoacetylmorphine: **(y)** =878750**x** $\mathbf{R^2}$ = 0.9940

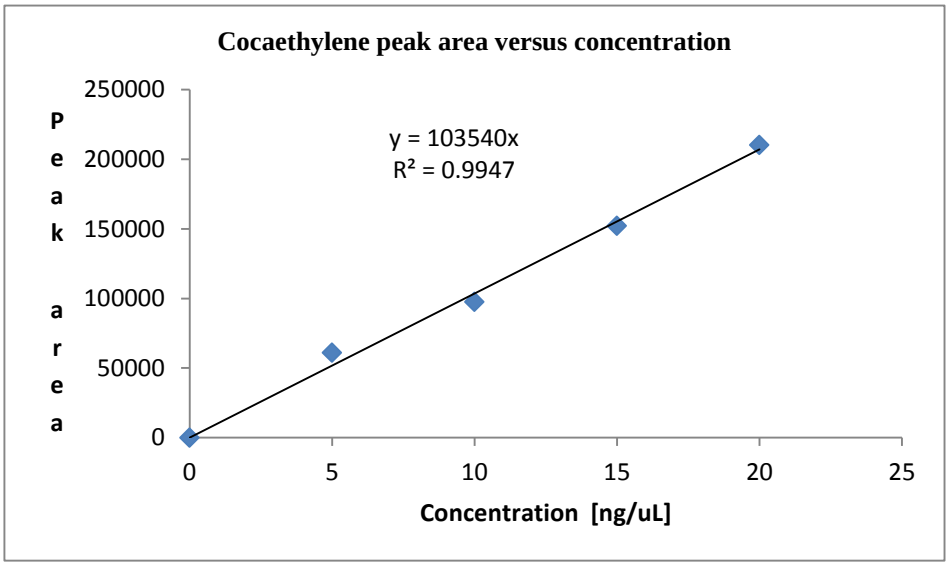
Heroin: **(y)** =106632**x** $\mathbf{R^2}$ = 0.998

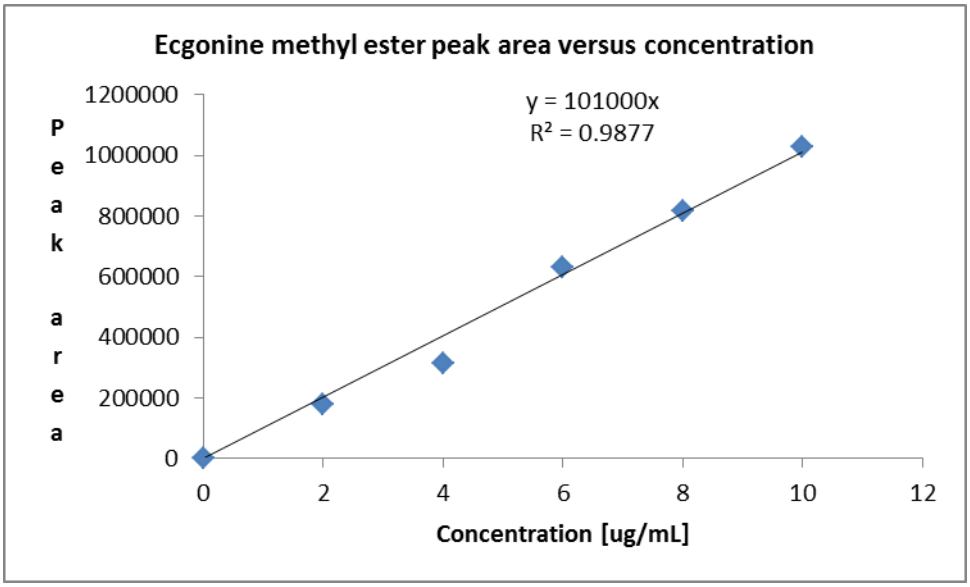
Diazepam: **(y)** =78682**x** $\mathbf{R^2}$ = 0.9989

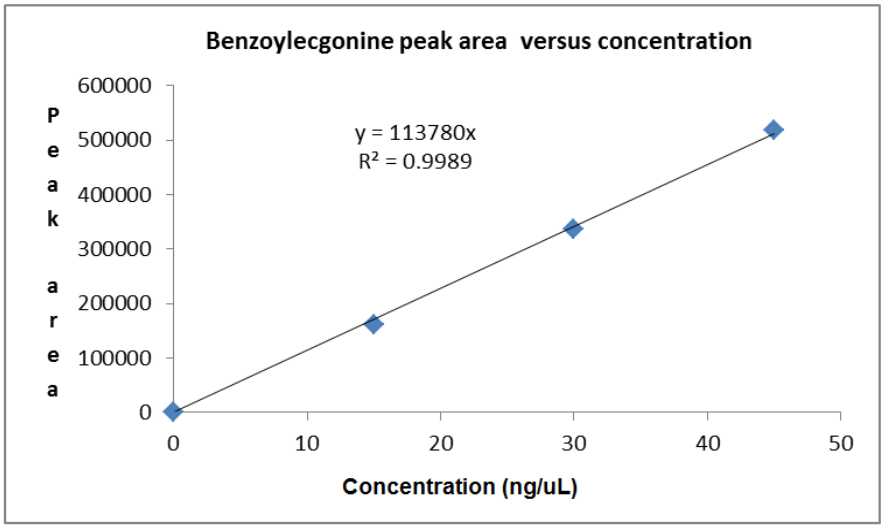
Nordazepam: **(y)** =348405**x** $\mathbf{R^2}$ = 0.999

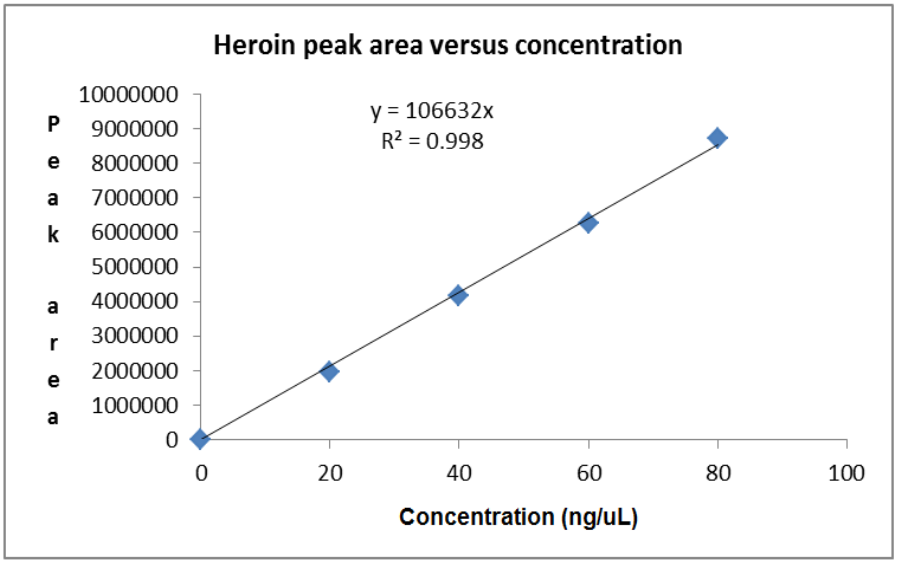
---

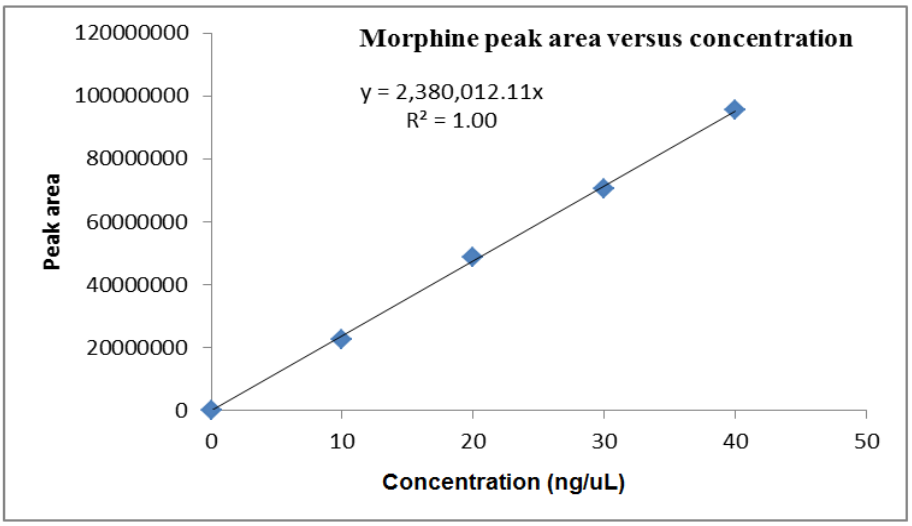


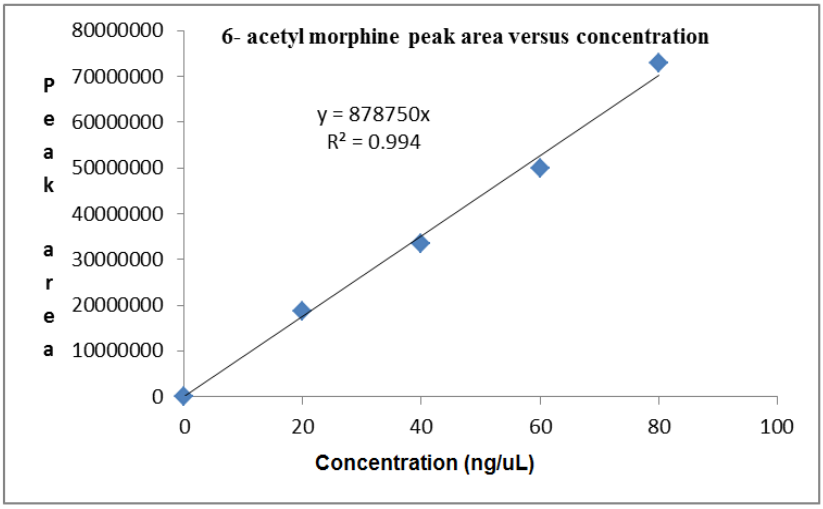

Cocaethylene peak area versus concentration
Peak area
250000
200000
150000
100000
50000
0
y = 103540x
R² = 0.9947
0
5
10
15
20
25
Concentration [ng/uL]

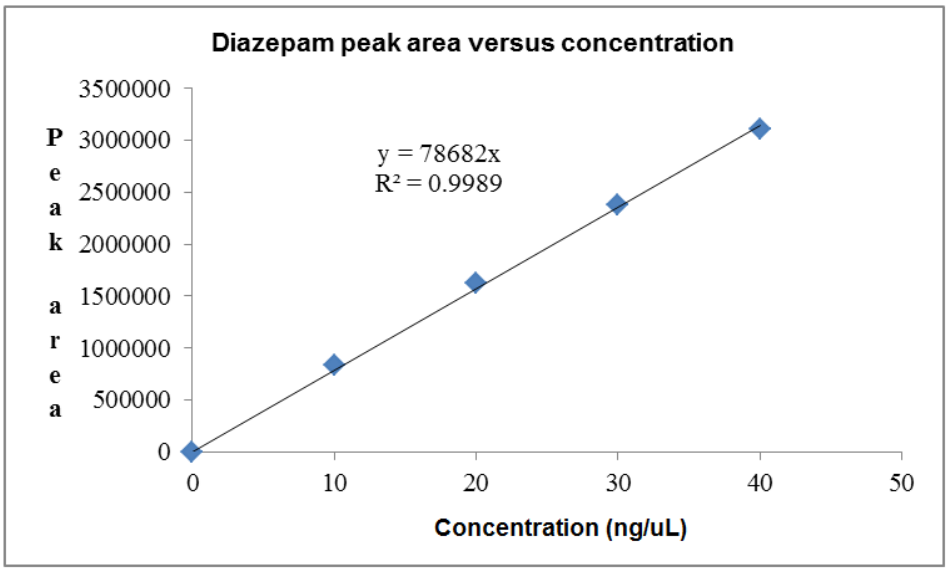

Ecgonine methyl ester peak area versus concentration
Peak area
1200000
1000000
800000
600000
400000
200000
0
y = 101000x
R² = 0.9877
0
2
4
6
8
10
12
Concentration [ug/mL]

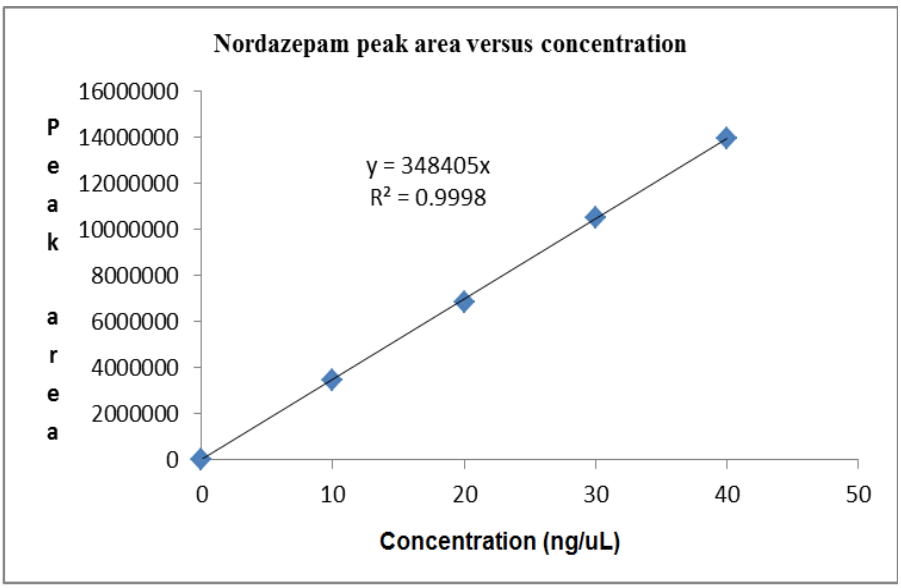

Benzoylecgonine peak area versus concentration
y = 113780x
R² = 0.9989
Peak area
600000
500000
400000
300000
200000
100000
0
0
10
20
30
40
50
Concentration (ng/uL)


Heroin peak area versus concentration
y = 106632x
R² = 0.998
Peak area
10000000
9000000
8000000
7000000
6000000
5000000
4000000
3000000
2000000
1000000
0
0
20
40
60
80
100
Concentration (ng/uL)


Morphine peak area versus concentration
y = 2,380,012.11x
R² = 1.00
Peak area
120000000
100000000
80000000
60000000
40000000
20000000
0
0
10
20
30
40
50
Concentration (ng/uL)


6- acetyl morphine peak area versus concentration
y = 878750x
R² = 0.994
Peak area
80000000
70000000
60000000
50000000
40000000
30000000
20000000
10000000
0
0
20
40
60
80
100
Concentration (ng/uL)


Diazepam peak area versus concentration
Peak area
3500000
3000000
2500000
2000000
1500000
1000000
500000
0
y = 78682x
R² = 0.9989
0
10
20
30
40
50
Concentration (ng/uL)


Nordazepam peak area versus concentration
Peak area
16000000
14000000
12000000
10000000
8000000
6000000
4000000
2000000
0
y = 348405x
R² = 0.9998
0
10
20
30
40
50
Concentration (ng/uL)

# Appendix 3: GC TIC data for analysis of Stoke Bardolph, Nottingham STW wastewaters

| **Compounds** | **Water Sampling dates and TIC area** | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | **Mon; 22/02/10** | **Thur; 25/02/10** | **Thur; 04/03/10** | **Thur; 11/03/10** | **Mon; 15/03/10** | **Thur; 18/03/10** | **Fri; 23/04/10** | **Sat; 24/04/10** |
| Cocaine | 129537<br>131538 | | | | | | | |
| Benzoylecgonine-*O*-TMS | | | | 125648<br>124070 | 905197<br>964038<br>930387 | 456995<br>474036<br>453764 | | |
| Codeine-*O*-TMS | 2843877<br>2828066 | 1265427<br>1203968 | 857768<br>859669 | 7750259<br>7588535 | 8116051<br>8958348<br>8716846 | 6694918<br>6590851<br>4740367 | 1980035 | 4649455<br>4603701<br>4458251 |
| Diazepam | 288823<br>284285 | | | 3090946<br>2901362 | 4193148<br>4342158<br>4167055 | 7525654<br>7593518 | | |
| Morphine-bis- *O*-TMS | | | | 380541<br>393180 | | | | |
| Ephedrine- *O*-TMS | | | 70455 | | | | | |
| Dihydrocodeine-*O*-TMS | | | | 1223462<br>4230932 | 2433785<br>6829090 | 2741835 | | |
| Dihydromorphine-*O*-TMS | | | | 1843927 | | 8602830 | | |
| Lidocaine | 2236362<br>2224158 | 4554012<br>4581522 | | 1355039 | 1289848<br>1276897<br>1246592 | 938076<br>947060 | 4264405<br>4157302 | 2586923<br>2604099 |
| Diacetylmorphine (Heroin) | | | | 2385847<br>2388462 | | | | |
| Ibruprofen--*O*-TMS | | | | 8317097<br>8857220 | | | | |
| Bromacil | 4989517<br>5285145 | | 634139<br>636344 | | 1421622<br>1024792 | 861113<br>872471 | 5655181<br>5525176 | 8167878<br>8116136 |
| Procaine | 2583272<br>2478914 | | | | 1564681<br>1534684 | | | |
| Amphetamine | | | | | | | 100296 | |
| Ecgonine methyl ester | | | | | | | 785137 | |

# Appendix 4 – Concentration Data of Sewage Batch Studies

## Batch Studies Data Table

Table 1a. Partition/Degradation of cocaine in primary sludge batch tests (4± 0.5°C)

| Time (min) | aqueous phase (ug) | | | solid phase (ug) | | | Total cocaine | % COC | % removal | EME, CE & Residual products |
|---|---|---|---|---|---|---|---|---|---|---|
| | Sample 1 | Sample 2 | Mean | Sample 1 | Sample 2 | Mean | | | | |
| 15 | 3420.3 | 3440.2 | 3430.2 | 201.4 | 210.4 | 205.9 | 3636.1 | 29.1 | 72.6 | 8863.9 |
| 30 | 3114.1 | 3113.8 | 3114.0 | 193.3 | 193.3 | 193.3 | 3307.3 | 26.5 | 75.1 | 9192.7 |
| 45 | 2923.2 | 2926.1 | 2924.7 | 170.4 | 180.0 | 175.2 | 3099.9 | 24.8 | 76.6 | 9400.1 |
| **60** | 2234.1 | 2236.7 | 2235.4 | 166.3 | 163.8 | 165.0 | 2400.4 | 19.2 | 82.1 | 10099.6 |
| 75 | 1690.0 | 1689.0 | 1689.5 | 141.6 | 154.5 | 148.0 | 1837.6 | 14.7 | 86.5 | 10662.4 |
| 90 | 1446.1 | 1439.5 | 1442.8 | 177.5 | 177.4 | 177.5 | 1620.3 | 13.0 | 88.5 | 10879.7 |
| 105 | 1210.0 | 1185.9 | 1197.9 | 192.7 | 190.2 | 191.4 | 1389.4 | 11.1 | 90.4 | 11110.6 |
| **120** | 940.3 | 941.8 | 941.1 | 140.5 | 140.7 | 140.6 | 1081.7 | 8.7 | 92.5 | 11418.3 |
| 135 | 732.9 | 733.8 | 733.4 | 166.7 | 163.3 | 165.0 | 898.4 | 7.2 | 94.1 | 11601.6 |
| 150 | 518.3 | 519.1 | 518.7 | 126.9 | 125.5 | 126.23 | 644.9 | 5.2 | 95.9 | 11885.1 |
| 165 | 365.6 | 365.4 | 365.5 | 133.0 | 132.3 | 132.7 | 498.2 | 4.0 | 97.1 | 12001.8 |
| **180** | 125.0 | 125.3 | 125.2 | 74.3 | 74.2 | 74.2 | 199.4 | 1.6 | 99.0 | 12300.6 |

Table 1b. Partition/Degradation of **ecgonine methyl ester** in primary sludge batch tests (4± 0.5°C)

| Time (min) | aqueous phase (ug) | | | solid phase (ug) | | | Total EME | CE & other Residual products |
|---|---|---|---|---|---|---|---|---|
| | Sample 1 | Sample 2 | Mean | Sample 1 | Sample 2 | Mean | | |
| 15 | 1800 | 1800 | 1800 | - | - | - | 1800 | 7063.9 |
| 30 | 1702 | 1725 | 1725 | - | - | - | 1725 | 7467.7 |
| 45 | 1449 | 1470 | 1470 | - | - | - | 1470 | 7930.1 |
| **60** | 1121 | 1121 | 1121 | - | - | - | 1121 | 8978.6 |
| 75 | 1241 | 1224 | 1241 | - | - | - | 1241 | 9421.4 |
| 90 | 1140 | 1155 | 1140 | - | - | - | 1140 | 9739.7 |
| 105 | 962 | 949 | 962 | - | - | - | 962 | 10148.6 |
| **120** | 792 | 803 | 803 | - | - | - | 803 | 10615.3 |
| 135 | 666 | 657 | 684 | - | - | - | 684 | 10917.6 |
| 150 | 588 | 581 | 588 | - | - | - | 588 | 11297.1 |
| 165 | 425 | 430 | 425 | - | - | - | 425 | 11576.8 |
| **180** | 267 | 270 | 267 | - | - | - | 267 | 12033.6 |

Table 1c. Partition/Degradation of **cocaethylene** in primary sludge batch tests (4± 0.5°C)

| Time (min) | aqueous phase (ug) | | | solid phase (ug) | | | Total CE | CE & other Residual products |
|---|---|---|---|---|---|---|---|---|
| | Sample 1 | Sample 2 | Mean | Sample 1 | Sample 2 | Mean | | |
| 15 | 650 | 625 | 650 | - | - | - | 650 | 6413.9 |
| 30 | 644 | 621 | 644 | - | - | - | 644 | 6823.7 |
| 45 | 525 | 546 | 546 | - | - | - | 546 | 7384.1 |
| **60** | 475 | 418 | 456 | - | - | - | 456 | 8522.6 |
| 75 | 442 | 442 | 442 | - | - | - | 442 | 8979.4 |
| 90 | 450 | 450 | 450 | - | - | - | 450 | 9289.7 |
| 105 | 312 | 312 | 312 | - | - | - | 312 | 9836.6 |
| **120** | 264 | 264 | 264 | - | - | - | 264 | 10351.3 |
| 135 | 216 | 216 | 216 | - | - | - | 216 | 10701.6 |
| 150 | 189 | 189 | 189 | - | - | - | 189 | 11108.1 |
| 165 | 115 | 115 | 115 | - | - | - | 115 | 11461.8 |
| **180** | 96 | 96 | 96 | - | - | - | 96 | 11937.6 |

Table 2a. Partition/Degradation of cocaine in primary sludge batch tests (19± 0.5°C)

| Time (min) | aqueous phase (ug) | | | solid phase (ug) | | | Total cocaine | % COC | % removal | EME, CE & Residual products |
|---|---|---|---|---|---|---|---|---|---|---|
| | Sample 1 | Sample 2 | Mean | Sample 1 | Sample 2 | Mean | | | | |
| 15 | 583.7 | 589.3 | 586.5 | 142.8 | 146.2 | 144.5 | 731.0 | 5.8 | 95.3 | 11769.0 |
| 30 | 430.3 | 436.3 | 433.3 | 184.6 | 187.8 | 186.2 | 619.5 | 5.0 | 96.5 | 11880.5 |
| 45 | 435.0 | 435.0 | 435.0 | 91.1 | 97.9 | 94.5 | 529.5 | 4.2 | 96.5 | 11970.5 |
| **60** | 424.4 | 427.3 | 425.8 | 82.0 | 93.7 | 87.9 | 513.7 | 4.1 | 96.6 | 11986.3 |
| 75 | 372.0 | 371.2 | 371.6 | 109.6 | 111.5 | 110.6 | 482.2 | 3.9 | 97.0 | 12017.8 |
| 90 | 225.0 | 225.5 | 225.3 | 81.7 | 80.0 | 80.8 | 306.1 | 2.4 | 98.2 | 12193.9 |
| 105 | 224.2 | 218.3 | 221.3 | 63.4 | 63.1 | 63.3 | 284.5 | 2.3 | 98.2 | 12215.5 |
| **120** | 196.4 | 195.2 | 195.8 | 48.8 | 51.9 | 50.3 | 246.2 | 2.0 | 98.4 | 12253.8 |
| 135 | 157.6 | 158.6 | 158.1 | 52.5 | 54.0 | 53.2 | 211.4 | 1.7 | 98.7 | 12288.6 |
| 150 | 121.0 | 123.1 | 122.1 | 38.0 | 38.0 | 38.0 | 160.1 | 1.3 | 99.0 | 12339.9 |
| 165 | 82.5 | 82.7 | 82.6 | 22.3 | 23.3 | 22.8 | 105.4 | 0.8 | 99.3 | 12394.6 |
| **180** | 46.2 | 45.9 | 46.0 | 9.4 | 9.6 | 9.5 | 55.5 | 0.4 | 99.6 | 12444.5 |

Table 2b. Partition/Degradation of **ecgonine methyl ester** in primary sludge batch tests (19± 0.5$^{o}$C)

| Time (min) | aqueous phase (ug in10 mL) | | | solid phase (ug in10 mL) | | | Total EME | Other residuals |
|---|---|---|---|---|---|---|---|---|
| | Sample 1 | Sample 2 | Mean | Sample 1 | Sample 2 | Mean | | |
| 15 | 25 | 25 | 25 | - | - | - | 25 | 11744 |
| 30 | 23 | 23 | 23 | - | - | - | 23 | 11857.5 |
| 45 | 84 | 63 | 74 | - | - | - | 74 | 11886.5 |
| **60** | 76 | 76 | 76 | - | - | - | 76 | 11910.3 |
| 75 | 85 | 85 | 85 | - | - | - | 85 | 11932.8 |
| 90 | 75 | 75 | 75 | - | - | - | 75 | 12118.9 |
| 105 | 65 | 78 | 65 | - | - | - | 65 | 12150.5 |
| **120** | 88 | 88 | 88 | - | - | - | 88 | 12165.8 |
| 135 | 63 | 63 | 63 | - | - | - | 63 | 12225.6 |
| 150 | 49 | 42 | 46 | - | - | - | 46 | 12290.9 |
| 165 | 30 | 25 | 30 | - | - | - | 30 | 12364.6 |
| **180** | 18 | 18 | 18 | - | - | - | 18 | 12426.5 |

Table 2c. Partition/Degradation of **cocaethylene** in primary sludge batch tests (19± 0.5$^{o}$C)

| Time (min) | aqueous phase (ug ) | | | solid phase (ug) | | | Total CE | Other Residual products |
|---|---|---|---|---|---|---|---|---|
| | Sample 1 | Sample 2 | Mean | Sample 1 | Sample 2 | Mean | | |
| 15 | 150 | 150 | 150 | 225 | 200 | 225 | 375 | 11394 |
| 30 | 184 | 184 | 184 | 184 | 184 | 184 | 368 | 11512.5 |
| 45 | 147 | 147 | 147 | 168 | 168 | 168 | 315 | 11655.5 |
| **60** | 152 | 152 | 152 | 114 | 133 | 114 | 266 | 11720.3 |
| 75 | 119 | 136 | 119 | 153 | 119 | 136 | 255 | 11762.8 |
| 90 | 135 | 135 | 135 | 105 | 105 | 105 | 240 | 11953.9 |
| 105 | 91 | 104 | 91 | 91 | 91 | 91 | 182 | 12033.5 |
| **120** | 66 | 77 | 66 | 55 | 55 | 77 | 121 | 12132.8 |
| 135 | 54 | 54 | 54 | 72 | 72 | 72 | 126 | 12162.6 |
| 150 | 63 | 63 | 63 | 28 | 28 | 28 | 98 | 12241.9 |
| 165 | 50 | 50 | 50 | 40 | 40 | 40 | 90 | 12304.6 |
| **180** | 27 | 27 | 27 | 30 | 24 | 27 | 54 | 12390.5 |

Table 3a. Total cocaine degradation in primary sludge (filtered) batch tests (19 ± 0.5°C)

| Time (min) | aqueous phase (ug) | | Total cocaine (ug) | % COC | % Removal | CE & Residual products |
|---|---|---|---|---|---|---|
| | Sample 1 | Sample 2 | | | | |
| 15 | 1393.0 | 1373.1 | 1383.1 | 11.1 | 88.9 | 11116.9 |
| 30 | 1153.8 | 1152.9 | 1153.3 | 9.2 | 90.8 | 11346.7 |
| 45 | 976.2 | 985.5 | 980.8 | 7.8 | 92.2 | 11519.2 |
| **60** | 822.4 | 823.1 | 822.8 | 6.6 | 93.4 | 11677.2 |
| 75 | 803.5 | 805.7 | 804.6 | 6.4 | 93.6 | 11695.4 |
| 90 | 695.0 | 705.3 | 700.1 | 5.6 | 94.4 | 11799.9 |
| 105 | 587.3 | 578.7 | 583.0 | 4.7 | 95.3 | 11917.0 |
| **120** | 485.3 | 482.0 | 483.6 | 3.9 | 96.1 | 12016.4 |
| 135 | 357.5 | 358.0 | 357.7 | 2.9 | 97.1 | 12142.3 |
| 150 | 243.2 | 243.4 | 243.3 | 1.9 | 98.1 | 12256.7 |
| 165 | 114.9 | 115.3 | 115.1 | 0.9 | 99.1 | 12384.9 |
| **180** | 62.5 | 62.7 | 62.6 | 0.5 | 99.5 | 12437.4 |

Table 3b. Total **cocaethylene** degradation in primary sludge (filtered) batch tests (19 ± 0.5°C)

| Time (min) | aqueous phase (ug) | | Total CE (ug) | Residual products |
|---|---|---|---|---|
| | Sample 1 | Sample 2 | | |
| 15 | 275 | 275 | 275 | 10841.9 |
| 30 | 276 | 276 | 276 | 11070.7 |
| 45 | 462 | 441 | 462 | 11057.2 |
| **60** | 437 | 437 | 437 | 11240.2 |
| 75 | 425 | 425 | 425 | 11270.4 |
| 90 | 375 | 375 | 375 | 11424.9 |
| 105 | 351 | 364 | 364 | 11553 |
| **120** | 308 | 297 | 308 | 11708.4 |
| 135 | 270 | 261 | 261 | 11881.3 |
| 150 | 203 | 203 | 203 | 12053.7 |
| 165 | 145 | 150 | 145 | 12239.9 |
| **180** | 114 | 111 | 111 | 12326.4 |

Table 4a. Partition/Degradation of **cocaine** in SAF-1 batch tests (4 ± 0.5°C)

| Time (min) | aqueous phase (ug) | | | solid phase (ug) | | | Total cocaine | % COC | % removal | CE & Residual products |
|---|---|---|---|---|---|---|---|---|---|---|
| | Sample 1 | Sample 2 | Mean | Sample 1 | Sample 2 | Mean | | | | |
| 15 | 3934.1 | 3911.0 | 3922.5 | 4252.4 | 4265.3 | 4258.9 | 8181.4 | 65.5 | 68.6 | 4318.6 |
| 30 | 3438.0 | 3375.6 | 3406.8 | 4038.3 | 4053.3 | 4045.8 | 7452.6 | 59.6 | 72.7 | 5047.4 |
| 45 | 2113.6 | 2080.0 | 2096.8 | 4637.2 | 4691.6 | 4664.4 | 6761.2 | 54.1 | 83.2 | 5738.8 |
| **60** | 1888.0 | 1875.5 | 1881.7 | 4289.6 | 4321.6 | 4305.6 | 6187.4 | 49.5 | 84.9 | 6312.6 |
| 75 | 1600.1 | 1584.0 | 1592.1 | 4078.2 | 4046.2 | 4062.2 | 5654.2 | 45.2 | 87.3 | 6845.8 |
| 90 | 1143.8 | 1135.1 | 1139.4 | 3445.2 | 3453.3 | 3449.3 | 4588.7 | 36.7 | 90.9 | 7911.3 |
| 105 | 888.0 | 880.8 | 884.4 | 2901.1 | 2930.3 | 2915.7 | 3800.1 | 30.4 | 92.9 | 8699.9 |
| **120** | 663.3 | 661.0 | 662.1 | 2505.5 | 2529.7 | 2517.6 | 3179.7 | 25.4 | 94.7 | 9320.3 |
| 135 | 440.0 | 500.0 | 470.0 | 1997.1 | 2008.5 | 2002.8 | 2472.8 | 19.8 | 96.2 | 10027.2 |
| 150 | 388.0 | 369.9 | 378.9 | 1545.2 | 1561.5 | 1553.3 | 1932.2 | 15.5 | 97.0 | 10567.8 |
| 165 | 259.0 | 243.3 | 251.1 | 1092.8 | 1101.7 | 1097.3 | 1348.4 | 10.8 | 98.0 | 11151.6 |
| **180** | 134.7 | 135.8 | 135.2 | 633.2 | 627.0 | 630.1 | 765.4 | 6.1 | 98.9 | 11734.6 |

Table 4b. Partition/Degradation of **cocaethylene** in SAF-1 sludge batch tests (4± 0.5°C)

| Time (min) | aqueous phase (ug) | | | solid phase (ug) | | | Total CE | Other Residual products |
|---|---|---|---|---|---|---|---|---|
| | Sample 1 | Sample 2 | Mean | Sample 1 | Sample 2 | Mean | | |
| 15 | 400 | 400 | 400 | 400 | 400 | 400 | 800 | 3518.6 |
| 30 | 506 | 506 | 506 | 368 | 368 | 368 | 874 | 4173.4 |
| 45 | 420 | 420 | 420 | 294 | 294 | 294 | 714 | 5024.8 |
| **60** | 361 | 361 | 361 | 494 | 475 | 494 | 855 | 5457.6 |
| 75 | 340 | 357 | 357 | 425 | 425 | 425 | 765 | 6080.8 |
| 90 | 300 | 300 | 300 | 360 | 345 | 345 | 660 | 7251.3 |
| 105 | 260 | 260 | 260 | 195 | 195 | 195 | 455 | 8244.9 |
| **120** | 220 | 220 | 220 | 198 | 198 | 198 | 429 | 8891.3 |
| 135 | 180 | 180 | 180 | 234 | 234 | 234 | 414 | 9613.2 |
| 150 | 161 | 154 | 161 | 175 | 175 | 175 | 336 | 10231.8 |
| 165 | 120 | 110 | 115 | 120 | 130 | 125 | 240 | 10911.6 |
| **180** | 93 | 93 | 93 | 66 | 66 | 66 | 159 | 11575.6 |

Table 5a. Partition/Degradation of **cocaine** in SAF-1 sludge batch tests (19 ± 0.5°C)

| Time (min) | aqueous phase (ug) | | | solid phase (ug) | | | Total cocaine | % COC | % removal | EME, CE & Residual |
|---|---|---|---|---|---|---|---|---|---|---|
| | Sample 1 | Sample 2 | Mean | Sample 1 | Sample 2 | Mean | | | | |
| 15 | 4600.1 | 4638.8 | 4619.4 | 3975.0 | 3992.2 | 3983.6 | 8603.0 | 68.8 | 63.0 | 3897.0 |
| 30 | 4227.3 | 4169.9 | 4198.6 | 3665.6 | 3604.4 | 3635.0 | 7833.6 | 62.7 | 66.4 | 4666.4 |
| 45 | 3388.7 | 3345.4 | 3367.1 | 3130.2 | 3173.6 | 3151.9 | 6519.0 | 52.2 | 73.1 | 5981.0 |
| **60** | 3386.7 | 3423.8 | 3405.2 | 2404.5 | 2365.0 | 2384.7 | 5790.0 | 46.3 | 72.8 | 6710.0 |
| 75 | 3025.0 | 2981.3 | 3003.1 | 2123.9 | 2095.3 | 2109.6 | 5112.7 | 40.9 | 76.0 | 7387.3 |
| 90 | 2564.9 | 2542.8 | 2553.9 | 1422.8 | 1360.1 | 1391.4 | 3945.3 | 31.6 | 79.6 | 8554.7 |
| 105 | 1730.5 | 1714.4 | 1722.5 | 862.1 | 845.8 | 853.9 | 2576.4 | 20.6 | 86.2 | 9923.6 |
| **120** | 1403.3 | 1423.0 | 1413.1 | 1011.3 | 1026.2 | 1018.8 | 2431.9 | 19.5 | 88.7 | 10068.1 |
| 135 | 1144.3 | 1166.0 | 1155.2 | 658.2 | 659.9 | 659.1 | 1814.2 | 14.5 | 90.8 | 10685.8 |
| 150 | 666.9 | 649.8 | 658.3 | 574.2 | 584.0 | 579.1 | 1237.4 | 9.9 | 94.7 | 11262.6 |
| 165 | 514.9 | 489.9 | 502.4 | 312.9 | 309.3 | 311.1 | 813.4 | 6.5 | 96.0 | 11686.6 |
| **180** | 306.5 | 305.9 | 306.2 | 230.7 | 227.6 | 229.1 | 535.4 | 4.3 | 97.6 | 11964.6 |

Table 5b. Partition/Degradation of **ecgonine methyl ester** in SAF-1 sludge batch tests (19± 0.5°C)

| Time (min) | aqueous phase (ug) | | | solid phase (ug) | | | Total EME | CE & other residuals |
|---|---|---|---|---|---|---|---|---|
| | Sample 1 | Sample 2 | Mean | Sample 1 | Sample 2 | Mean | | |
| 15 | 275 | 275 | 275 | 125 | 125 | 125 | 400 | 3497 |
| 30 | 207 | 207 | 207 | 184 | 230 | 207 | 414 | 4252.4 |
| 45 | 63 | 63 | 63 | 252 | 252 | 252 | 315 | 5666 |
| **60** | 133 | 133 | 133 | 152 | 152 | 152 | 285 | 6425 |
| 75 | 102 | 102 | 102 | 238 | 255 | 255 | 357 | 7030.3 |
| 90 | 75 | 75 | 75 | 120 | 120 | 120 | 195 | 8359.7 |
| 105 | 52 | 52 | 52 | 234 | 247 | 247 | 208 | 9715.6 |
| **120** | 88 | 88 | 88 | 110 | 110 | 110 | 198 | 9870.1 |
| 135 | 90 | 99 | 99 | 108 | 108 | 108 | 207 | 10478.8 |
| 150 | 28 | 28 | 28 | 77 | 70 | 77 | 105 | 11157.6 |
| 165 | 35 | 35 | 35 | 60 | 60 | 60 | 95 | 11591.6 |
| **180** | 18 | 18 | 18 | 48 | 48 | 48 | 66 | 11898.6 |

Table 5c. Partition/Degradation of **cocaethylene** in SAF-1 batch tests (19 ± 0.5°C)

| Time (min) | aqueous phase (ug) | | | solid phase (ug) | | | Total CE | other residuals |
|---|---|---|---|---|---|---|---|---|
| | Sample 1 | Sample 2 | Mean | Sample 1 | Sample 2 | Mean | | |
| 15 | 350 | 350 | 350 | 150 | 150 | 150 | 500 | 2997 |
| 30 | 322 | 345 | 345 | 138 | 115 | 138 | 460 | 3792.4 |
| 45 | 294 | 294 | 294 | 126 | 126 | 126 | 420 | 5246 |
| **60** | 266 | 266 | 266 | 152 | 152 | 152 | 437 | 5988 |
| 75 | 238 | 221 | 238 | 119 | 119 | 119 | 357 | 6673.3 |
| 90 | 195 | 195 | 195 | 90 | 90 | 90 | 270 | 8089.7 |
| 105 | 169 | 169 | 169 | 117 | 91 | 104 | 260 | 9455.6 |
| **120** | 143 | 132 | 132 | 110 | 121 | 110 | 253 | 9617.1 |
| 135 | 108 | 108 | 108 | 99 | 99 | 99 | 216 | 10262.8 |
| 150 | 84 | 84 | 84 | 70 | 70 | 70 | 154 | 11003.6 |
| 165 | 60 | 60 | 60 | 90 | 85 | 85 | 145 | 11446.6 |
| **180** | 42 | 42 | 42 | 54 | 54 | 54 | 96 | 11802.6 |

Table 6a. Total **cocaine** degradation in (filtered) SAF-1 sludge batch tests (19 ± 0.5°C)

| Time (min) | aqueous phase (ug) | | Total cocaine (ug) | % COC | % Removal | CE & Residual |
|---|---|---|---|---|---|---|
| | Sample 1 | Sample 2 | | | | |
| 15 | 6089.3 | 6101.2 | 6095.2 | 48.8 | 51.2 | 6404.8 |
| 30 | 5435.2 | 5440.0 | 5437.6 | 43.5 | 56.5 | 7062.4 |
| 45 | 4883.2 | 4706.1 | 4794.6 | 38.4 | 61.6 | 7705.4 |
| **60** | 4581.4 | 4599.4 | 4590.4 | 36.7 | 63.3 | 7909.6 |
| 75 | 3791.0 | 3773.4 | 3782.2 | 30.3 | 69.7 | 8717.8 |
| 90 | 3233.4 | 3258.8 | 3246.1 | 26.0 | 74.0 | 9253.9 |
| 105 | 2810.5 | 2774.7 | 2792.6 | 22.3 | 77.7 | 9707.4 |
| **120** | 2241.9 | 2311.6 | 2276.8 | 18.2 | 81.8 | 10223.2 |
| 135 | 1633.9 | 1596.6 | 1615.2 | 12.9 | 87.1 | 10884.8 |
| 150 | 1202.0 | 1208.2 | 1205.1 | 9.6 | 90.4 | 11294.9 |
| 165 | 803.4 | 832.9 | 818.1 | 6.5 | 93.5 | 11681.9 |
| **180** | 429.0 | 428.5 | 428.7 | 3.4 | 96.6 | 12071.3 |

Table 6b. Total **cocaethylene** degradation (filtered) SAF-1 sludge batch tests (19 ± 0.5°C)

| Time (min) | aqueous phase (ug) | | Total CE (ug) | Residuals |
|---|---|---|---|---|
| | Sample 1 | Sample 2 | | |
| 15 | 3125 | 3125 | 3125 | 3279.8 |
| 30 | 2829 | 2852 | 2829 | 4210.4 |
| 45 | 2583 | 2625 | 2604 | 5080.4 |
| **60** | 2451 | 2299 | 2375 | 5610.6 |
| 75 | 2397 | 2397 | 2397 | 6320.8 |
| 90 | 2310 | 2310 | 2310 | 6943.9 |
| 105 | 2002 | 1976 | 1989 | 7731.4 |
| **120** | 1760 | 1760 | 1760 | 8463.2 |
| 135 | 1449 | 1458 | 1449 | 9426.8 |
| 150 | 1106 | 1106 | 1106 | 10188.9 |
| 165 | 810 | 795 | 800 | 10886.9 |
| **180** | 477 | 480 | 480 | 11591.3 |

Table 7a. Partition/Degradation of **cocaine** in MSAF-1 sludge batch tests (4± 0.5°C)

| Time (min) | aqueous phase (ug) | | | solid phase (ug) | | | Total cocaine | % COC | % removal | EME, CE & Residual |
|---|---|---|---|---|---|---|---|---|---|---|
| | Sample 1 | Sample 2 | Mean | Sample 1 | Sample 2 | Mean | | | | |
| 15 | 5488.0 | 5458.8 | 5473.4 | 418.0 | 442.2 | 430.1 | 5903.5 | 47.2 | 56.2 | 6596.5 |
| 30 | 5176.5 | 5182.0 | 5179.3 | 391.0 | 448.2 | 419.6 | 5598.9 | 44.8 | 58.6 | 6901.1 |
| 45 | 4877.7 | 4863.0 | 4870.3 | 355.5 | 374.0 | 364.7 | 5235.0 | 41.9 | 61.0 | 7265.0 |
| **60** | 3832.8 | 4141.0 | 3986.9 | 355.5 | 360.6 | 358.0 | 4344.9 | 34.8 | 68.1 | 8155.1 |
| 75 | 3812.6 | 3784.3 | 3798.5 | 372.1 | 385.8 | 378.9 | 4177.4 | 33.4 | 69.6 | 8322.6 |
| 90 | 3536.8 | 3554.3 | 3545.5 | 276.5 | 288.7 | 282.6 | 3828.2 | 30.6 | 71.6 | 8671.8 |
| 105 | 3046.4 | 3035.5 | 3040.9 | 250.2 | 257.2 | 253.7 | 3294.6 | 26.4 | 75.7 | 9205.4 |
| **120** | 2300.8 | 2301.8 | 2301.3 | 277.4 | 277.2 | 277.3 | 2578.5 | 20.6 | 81.6 | 9921.5 |
| 135 | 1884.6 | 1877.9 | 1881.2 | 167.9 | 167.8 | 167.8 | 2049.1 | 16.4 | 85.0 | 10450.9 |
| 150 | 1409.3 | 1416.9 | 1413.1 | 136.8 | 139.5 | 138.1 | 1551.2 | 12.4 | 88.7 | 10948.8 |
| 165 | 985.3 | 992.8 | 989.1 | 112.1 | 117.4 | 114.7 | 1103.8 | 8.8 | 92.1 | 11396.2 |
| **180** | 608.6 | 607.6 | 608.1 | 67.9 | 67.1 | 67.5 | 675.6 | 5.4 | 95.1 | 11824.4 |

Table 7b. Partition/Degradation of **cocaethylene** in MSAF batch tests (4± 0.5°C)

| Time (min) | aqueous phase (ug) | | | solid phase (ug) | | | Total CE | other residuals |
|---|---|---|---|---|---|---|---|---|
| | Sample 1 | Sample 2 | Mean | Sample 1 | Sample 2 | Mean | | |
| 15 | 2600 | 2575 | 2575 | - | - | - | 2575 | 4021.5 |
| 30 | 2369 | 2415 | 2392 | - | - | - | 2392 | 4509.1 |
| 45 | 2163 | 2205 | 2184 | - | - | - | 2184 | 5081 |
| **60** | 1957 | 1976 | 1976 | - | - | - | 1976 | 6179.1 |
| 75 | 1853 | 1870 | 1853 | - | - | - | 1853 | 6469.6 |
| 90 | 1515 | 1560 | 1545 | - | - | - | 1545 | 7126.8 |
| 105 | 1417 | 1430 | 1430 | - | - | - | 1430 | 7775.4 |
| **120** | 1331 | 1276 | 1298 | - | - | - | 1298 | 8623.5 |
| 135 | 1161 | 1170 | 1170 | - | - | - | 1170 | 9280.9 |
| 150 | 959 | 952 | 959 | - | - | - | 959 | 9989.8 |
| 165 | 765 | 755 | 760 | - | - | - | 760 | 10636.2 |
| **180** | 447 | 444 | 444 | - | - | - | 444 | 11380.4 |

Table 8a. Partition/Degradation of **cocaine** in MSAF batch tests (19± 0.5°C)

| Time (min) | aqueous phase (ug) | | | solid phase (ug) | | | Total cocaine | % COC | % removal | EME, CE & Residual |
|---|---|---|---|---|---|---|---|---|---|---|
| | Sample 1 | Sample 2 | Mean | Sample 1 | Sample 2 | Mean | | | | |
| 15 | 647.7 | 635.9 | 641.8 | 90.7 | 93.1 | 91.9 | 733.7 | 5.9 | 94.9 | 11858.2 |
| 30 | 509.4 | 510.9 | 510.1 | 115.4 | 113.9 | 114.7 | 624.8 | 5.0 | 95.9 | 11989.9 |
| 45 | 446.6 | 456.0 | 451.3 | 101.1 | 99.0 | 100.1 | 551.4 | 4.4 | 96.4 | 12048.7 |
| **60** | 231.9 | 230.0 | 231.0 | 93.5 | 92.3 | 92.9 | 323.9 | 2.6 | 98.2 | 12269.0 |
| 75 | 179.2 | 179.5 | 179.4 | 77.4 | 77.5 | 77.5 | 256.8 | 2.1 | 98.6 | 12320.6 |
| 90 | 162.1 | 163.4 | 162.7 | 68.5 | 68.2 | 68.4 | 231.1 | 1.8 | 98.7 | 12337.3 |
| 105 | 146.4 | 146.4 | 146.4 | 65.3 | 65.1 | 65.2 | 211.6 | 1.7 | 98.8 | 12353.6 |
| **120** | 160.8 | 161.7 | 161.2 | 55.3 | 55.4 | 55.4 | 216.6 | 1.7 | 98.7 | 12338.8 |
| 135 | 114.3 | 115.9 | 115.1 | 43.1 | 42.1 | 42.6 | 157.7 | 1.3 | 99.1 | 12384.9 |
| 150 | 74.5 | 74.8 | 74.7 | 35.9 | 35.1 | 35.5 | 110.2 | 0.9 | 99.4 | 12425.3 |
| 165 | 47.5 | 47.4 | 47.4 | 30.2 | 30.4 | 30.3 | 77.7 | 0.6 | 99.6 | 12452.6 |
| **180** | 28.7 | 29.9 | 29.3 | 21.9 | 21.6 | 21.7 | 51.0 | 0.4 | 99.8 | 12470.7 |

Table 8b. Partition/Degradation of **ecgonine methyl ester** in MSAF-1 sludge batch tests (19± 0.5°C)

| Time (min) | aqueous phase (ug) | | | solid phase (ug) | | | Total EME (ug) | CE & other Metabolites (ug) |
|---|---|---|---|---|---|---|---|---|
| | Sample 1 | Sample 2 | Mean | Sample 1 | Sample 2 | Mean | | |
| 15 | 0 | 0 | 0 | - | - | - | 0 | 11858.2 |
| 30 | 0 | 0 | 0 | - | - | - | 0 | 11989.9 |
| 45 | 0 | 0 | 0 | - | - | - | 0 | 12048.7 |
| **60** | 114 | 114 | 114 | - | - | - | 114 | 12155 |
| 75 | 102 | 102 | 102 | - | - | - | 102 | 12218.6 |
| 90 | 45 | 45 | 45 | - | - | - | 45 | 12292.3 |
| 105 | 39 | 39 | 39 | - | - | - | 39 | 12314.6 |
| **120** | 33 | 33 | 33 | - | - | - | 33 | 12305.8 |
| 135 | 45 | 45 | 45 | - | - | - | 45 | 12339.9 |
| 150 | 21 | 21 | 21 | - | - | - | 21 | 12404.3 |
| 165 | 15 | 15 | 15 | - | - | - | 15 | 12437.6 |
| **180** | 24 | 24 | 24 | - | - | - | 24 | 12446.7 |

Table 8c. Partition/Degradation of **cocaethylene** in MSAF-1 sludge batch tests (19± 0.5°C)

| Time (min) | aqueous phase (ug) | | | solid phase (ug) | | | Total CE (ug) | Other metabolites |
|---|---|---|---|---|---|---|---|---|
| | Sample 1 | Sample 2 | Mean | Sample 1 | Sample 2 | Mean | | |
| 15 | 200 | 225 | 225 | - | - | - | 225 | 11633.2 |
| 30 | 184 | 184 | 184 | - | - | - | 184 | 11805.9 |
| 45 | 147 | 147 | 147 | - | - | - | 147 | 11901.7 |
| **60** | 133 | 133 | 133 | - | - | - | 133 | 12022 |
| 75 | 136 | 119 | 119 | - | - | - | 119 | 12099.6 |
| 90 | 90 | 90 | 90 | - | - | - | 90 | 12202.3 |
| 105 | 78 | 78 | 78 | - | - | - | 78 | 12236.6 |
| **120** | 88 | 88 | 88 | - | - | - | 88 | 12217.8 |
| 135 | 90 | 81 | 81 | - | - | - | 81 | 12258.9 |
| 150 | 91 | 98 | 98 | - | - | - | 98 | 12306.3 |
| 165 | 65 | 65 | 65 | - | - | - | 65 | 12372.6 |
| **180** | 36 | 33 | 33 | - | - | - | 33 | 12413.7 |

Table 9a. Total **cocaine** degradation in MSAF sludge (filtered) batch tests (19± 0.5°C)

| Time (min) | aqueous phase (ug) | | Total cocaine (ug) | % COC | % Removal | CE & Residual |
|---|---|---|---|---|---|---|
| | Sample 1 | Sample 2 | | | | |
| 15 | 995.3 | 1002.2 | 998.7 | 8.0 | 92.0 | 11501.3 |
| 30 | 898.8 | 889.6 | 894.2 | 7.2 | 92.8 | 11605.8 |
| 45 | 838.9 | 841.2 | 840.0 | 6.7 | 93.3 | 11660.0 |
| **60** | 782.0 | 789.5 | 785.8 | 6.3 | 93.7 | 11714.2 |
| 75 | 670.0 | 674.6 | 672.3 | 5.4 | 94.6 | 11827.7 |
| 90 | 552.0 | 550.9 | 551.5 | 4.4 | 95.6 | 11948.5 |
| 105 | 452.2 | 447.8 | 450.0 | 3.6 | 96.4 | 12050.0 |
| **120** | 369.2 | 365.7 | 367.5 | 2.9 | 97.1 | 12132.5 |
| 135 | 264.9 | 262.4 | 263.7 | 2.1 | 97.9 | 12236.3 |
| 150 | 187.9 | 177.8 | 182.9 | 1.5 | 98.5 | 12317.1 |
| 165 | 132.8 | 133.3 | 133.1 | 1.1 | 98.9 | 12366.9 |
| **180** | 73.1 | 71.1 | 72.1 | 0.6 | 99.4 | 12427.9 |

Table 9b. Total **cocaethylene** degradation in MSAF sludge (filtered) batch tests (19 ± 0.5°C)

| Time (min) | aqueous phase (ug) | | Total CE (ug) | Other metabolites (ug ) |
|---|---|---|---|---|
| | Sample 1 | Sample 2 | | |
| 15 | 525 | 525 | 525 | 10976.3 |
| 30 | 506 | 506 | 506 | 11099.8 |
| 45 | 462 | 483 | 483 | 11177 |
| **60** | 418 | 380 | 399 | 11315.2 |
| 75 | 442 | 425 | 425 | 11402.7 |
| 90 | 315 | 315 | 315 | 11633.5 |
| 105 | 299 | 286 | 286 | 11764 |
| **120** | 242 | 253 | 242 | 11890.5 |
| 135 | 198 | 207 | 207 | 12029.3 |
| 150 | 175 | 175 | 175 | 12142.1 |
| 165 | 135 | 140 | 135 | 12231.9 |
| **180** | 78 | 81 | 81 | 12346.9 |

Table 10a. Partition/Degradation of **cocaine** in Humus sludge batch tests (4± 0.5°C)

| Time (min) | aqueous phase (ug) | | | solid phase (ug) | | | Total cocaine | % COC | % removal | EME, CE & Residual |
|---|---|---|---|---|---|---|---|---|---|---|
| | Sample 1 | Sample 2 | Mean | Sample 1 | Sample 2 | Mean | | | | |
| 15 | 5099.7 | 5053.2 | 5076.5 | 2355.7 | 2351.3 | 2353.5 | 7429.9 | 59.4 | 59.4 | 5070.1 |
| 30 | 4686.5 | 4640.7 | 4663.6 | 2065.8 | 2010.5 | 2038.2 | 6701.7 | 53.6 | 62.7 | 5798.3 |
| 45 | 4374.4 | 4221.2 | 4297.8 | 1914.9 | 1883.8 | 1899.4 | 6197.1 | 49.6 | 65.6 | 6302.9 |
| **60** | 3602.3 | 3766.6 | 3684.4 | 1558.3 | 1555.3 | 1556.8 | 5241.2 | 41.9 | 70.5 | 7258.8 |
| 75 | 3596.7 | 3626.4 | 3611.6 | 1463.9 | 1436.9 | 1450.4 | 5062.0 | 40.5 | 71.1 | 7438.0 |
| 90 | 3204.3 | 3154.3 | 3179.3 | 1213.5 | 1173.6 | 1193.5 | 4372.9 | 35.0 | 74.6 | 8127.1 |
| 105 | 2122.8 | 2178.2 | 2150.5 | 1027.7 | 1056.8 | 1042.3 | 3192.7 | 25.5 | 82.8 | 9307.3 |
| **120** | 1939.3 | 1888.9 | 1914.1 | 711.6 | 703.8 | 707.7 | 2621.8 | 21.0 | 84.7 | 9878.2 |
| 135 | 1311.5 | 1335.9 | 1323.7 | 573.5 | 569.1 | 571.3 | 1895.0 | 15.2 | 89.4 | 10605.0 |
| 150 | 1111.0 | 1117.1 | 1114.1 | 438.8 | 439.3 | 439.0 | 1553.1 | 12.4 | 91.1 | 10946.9 |
| 165 | 856.6 | 864.4 | 860.5 | 315.0 | 314.3 | 314.6 | 1175.1 | 9.4 | 93.1 | 11324.9 |
| **180** | 430.1 | 430.9 | 430.5 | 181.8 | 182.0 | 181.9 | 612.4 | 4.9 | 96.6 | 11887.6 |

Table 10b. Partition/Degradation of **cocaethylene** in Humus sludge batch tests (4± 0.5°C)

| Time (min) | aqueous phase (ug) | | | solid phase (ug) | | | Total CE (ug) | BZE & other metabolites |
|---|---|---|---|---|---|---|---|---|
| | Sample 1 | Sample 2 | Mean | Sample 1 | Sample 2 | Mean | | |
| 15 | 400 | 350 | 375 | 450 | 450 | 450 | 825 | 4245.1 |
| 30 | 230 | 230 | 230 | 575 | 368 | 460 | 690 | 5108.3 |
| 45 | 252 | 252 | 252 | 441 | 441 | 441 | 693 | 5609.9 |
| **60** | 323 | 285 | 304 | 342 | 361 | 361 | 665 | 6593.8 |
| 75 | 272 | 272 | 272 | 340 | 340 | 340 | 612 | 6826 |
| 90 | 285 | 270 | 270 | 240 | 255 | 240 | 525 | 7602.1 |
| 105 | 260 | 208 | 234 | 299 | 299 | 299 | 533 | 8774.3 |
| **120** | 176 | 187 | 176 | 253 | 253 | 253 | 429 | 9449.2 |
| 135 | 189 | 207 | 198 | 198 | 198 | 198 | 396 | 10209 |
| 150 | 196 | 196 | 196 | 175 | 175 | 175 | 371 | 10575.9 |
| 165 | 135 | 135 | 135 | 90 | 95 | 95 | 225 | 11099.9 |
| **180** | 72 | 75 | 72 | 60 | 57 | 60 | 132 | 11755.6 |

Table 10c. Partition/Degradation of **benzoylecgonine** in Humus sludge batch tests (4± 0.5°C)

| Time (min) | aqueous phase (ug) | | | solid phase (ug) | | | Total BZE (ug) | Other metabolites |
|---|---|---|---|---|---|---|---|---|
| | Sample 1 | Sample 2 | Mean | Sample 1 | Sample 2 | Mean | | |
| 15 | 300 | 300 | 300 | - | - | - | 300 | 3945.1 |
| 30 | 299 | 299 | 299 | - | - | - | 299 | 4809.3 |
| 45 | 168 | 189 | 168 | - | - | - | 168 | 5441.9 |
| **60** | 171 | 171 | 171 | - | - | - | 171 | 6422.8 |
| 75 | 221 | 221 | 221 | - | - | - | 221 | 6605 |
| 90 | 135 | 135 | 135 | - | - | - | 135 | 7467.1 |
| 105 | 117 | 117 | 117 | - | - | - | 117 | 8657.3 |
| **120** | 77 | 77 | 77 | - | - | - | 77 | 9372.2 |
| 135 | 171 | 180 | 171 | - | - | - | 171 | 10038 |
| 150 | 126 | 140 | 133 | - | - | - | 133 | 10442.9 |
| 165 | 55 | 60 | 55 | - | - | - | 55 | 11044.9 |
| **180** | 72 | 72 | 72 | - | - | - | 72 | 11683.6 |

Table 11a. Partition/Degradation of **cocaine** in Humus sludge batch tests (19± 0.5°C)

| Time (min) | aqueous phase (ug) | | | solid phase (ug) | | | Total cocaine | % COC | % removal | EME, CE & Residual |
|---|---|---|---|---|---|---|---|---|---|---|
| | Sample 1 | Sample 2 | Mean | Sample 1 | Sample 2 | Mean | | | | |
| 15 | 2393.2 | 2365.0 | 2379.1 | 240.1 | 239.9 | 240.0 | 2619.1 | 21.0 | 81.0 | 9880.9 |
| 30 | 2092.9 | 2042.7 | 2067.8 | 207.9 | 202.8 | 205.3 | 2273.1 | 18.2 | 83.5 | 10226.9 |
| 45 | 1856.8 | 1848.3 | 1852.6 | 169.1 | 186.5 | 177.8 | 2030.4 | 16.2 | 85.2 | 10469.6 |
| **60** | 2085.3 | 2107.3 | 2096.3 | 114.2 | 113.2 | 113.7 | 2210.0 | 17.7 | 83.2 | 10290.0 |
| 75 | 1890.2 | 1897.2 | 1893.7 | 108.1 | 108.8 | 108.4 | 2002.1 | 16.0 | 84.9 | 10497.9 |
| 90 | 1570.1 | 1551.9 | 1561.0 | 107.3 | 108.1 | 107.7 | 1668.7 | 13.3 | 87.5 | 10831.3 |
| 105 | 1350.8 | 1333.9 | 1342.4 | 67.9 | 66.4 | 67.1 | 1409.5 | 11.3 | 89.3 | 11090.5 |
| **120** | 1128.5 | 1131.0 | 1129.7 | 65.4 | 65.5 | 65.4 | 1195.2 | 9.6 | 91.0 | 11304.8 |
| 135 | 991.0 | 983.6 | 987.3 | 54.9 | 54.6 | 54.7 | 1042.1 | 8.3 | 92.1 | 11457.9 |
| 150 | 740.7 | 738.2 | 739.4 | 35.1 | 35.8 | 35.4 | 774.9 | 6.2 | 94.1 | 11725.1 |
| 165 | 337.9 | 341.5 | 339.7 | 22.9 | 22.9 | 22.9 | 362.6 | 2.9 | 97.3 | 12137.4 |
| **180** | 170.3 | 168.9 | 169.6 | 10.6 | 10.5 | 10.6 | 180.1 | 1.4 | 98.6 | 12319.9 |

Table 11b. Partition/Degradation of **ecgonine methyl ester** in Humus sludge batch tests (19± 0.5°C)

| Time (min) | aqueous phase (ug) | | | solid phase (ug) | | | Total EME (ug) | CE & other metabolites |
|---|---|---|---|---|---|---|---|---|
| | Sample 1 | Sample 2 | Mean | Sample 1 | Sample 2 | Mean | | |
| 15 | 1350 | 1375 | 1350 | - | - | - | 1350 | 8530.9 |
| 30 | 1058 | 1035 | 1058 | - | - | - | 1058 | 9168.9 |
| 45 | 945 | 945 | 945 | - | - | - | 945 | 9524.6 |
| **60** | 874 | 874 | 874 | - | - | - | 874 | 9416 |
| 75 | 544 | 544 | 544 | - | - | - | 544 | 9953.9 |
| 90 | 600 | 615 | 615 | - | - | - | 615 | 10216.3 |
| 105 | 507 | 494 | 494 | - | - | - | 494 | 10596.5 |
| **120** | 803 | 781 | 792 | - | - | - | 792 | 10512.8 |
| 135 | 630 | 630 | 630 | - | - | - | 630 | 10827.9 |
| 150 | 581 | 574 | 581 | - | - | - | 581 | 11144.1 |
| 165 | 410 | 410 | 410 | - | - | - | 410 | 11727.4 |
| **180** | 297 | 300 | 297 | - | - | - | 297 | 12022.9 |

Table 11c. Partition/Degradation of **cocaethylene** in Humus sludge batch tests (19± 0.5°C)

| Time (min) | aqueous phase (ug) | | | solid phase (ug) | | | Total CE (ug) | Other metabolites |
|---|---|---|---|---|---|---|---|---|
| | Sample 1 | Sample 2 | Mean | Sample 1 | Sample 2 | Mean | | |
| 15 | 1625 | 1625 | 1625 | 1375 | 1375 | 1375 | 3000 | 5530.9 |
| 30 | 1909 | 1909 | 1909 | 897 | 897 | 897 | 2806 | 6362.9 |
| 45 | 1722 | 1680 | 1701 | 924 | 987 | 945 | 2646 | 6878.6 |
| **60** | 1045 | 1026 | 1026 | 665 | 665 | 665 | 1710 | 7706 |
| 75 | 1190 | 1156 | 1173 | 918 | 935 | 935 | 2091 | 7862.9 |
| 90 | 810 | 825 | 810 | 765 | 765 | 765 | 1575 | 8641.3 |
| 105 | 858 | 871 | 858 | 624 | 650 | 637 | 1495 | 9101.5 |
| **120** | 484 | 484 | 484 | 352 | 352 | 352 | 836 | 9676.8 |
| 135 | 900 | 891 | 900 | 279 | 279 | 279 | 1179 | 9648.9 |
| 150 | 693 | 686 | 686 | 210 | 217 | 210 | 903 | 10241.1 |
| 165 | 615 | 605 | 610 | 250 | 245 | 250 | 860 | 10867.4 |
| **180** | 375 | 357 | 366 | 174 | 171 | 171 | 537 | 11485.9 |

Table 12a. Total **cocaine** degradation in (filtered) Humus sludge batch tests (19 ± 0.5°C)

| Time (min) | aqueous phase (ug) | | Total cocaine (ug) | % COC | % Removal | CE & Residual |
|---|---|---|---|---|---|---|
| | Sample 1 | Sample 2 | | | | |
| 15 | 3144.1 | 3066.6 | 3105.4 | 24.8 | 75.2 | 9394.6 |
| 30 | 2769.3 | 2769.2 | 2769.3 | 22.2 | 77.8 | 9730.7 |
| 45 | 2380.1 | 2384.8 | 2382.4 | 19.1 | 80.9 | 10117.6 |
| **60** | 2256.5 | 2256.5 | 2256.5 | 18.1 | 81.9 | 10243.5 |
| 75 | 1995.8 | 2011.6 | 2003.7 | 16.0 | 84.0 | 10496.3 |
| 90 | 1426.1 | 1400.7 | 1413.4 | 11.3 | 88.7 | 11086.6 |
| 105 | 1213.7 | 1228.6 | 1221.1 | 9.8 | 90.2 | 11278.9 |
| **120** | 1000.8 | 1006.2 | 1003.5 | 8.0 | 92.0 | 11496.5 |
| 135 | 841.6 | 832.8 | 837.2 | 6.7 | 93.3 | 11662.8 |
| 150 | 579.1 | 580.8 | 580.0 | 4.6 | 95.4 | 11920.0 |
| 165 | 352.9 | 349.9 | 351.4 | 2.8 | 97.2 | 12148.6 |
| **180** | 150.9 | 154.1 | 152.5 | 1.2 | 98.8 | 12347.5 |

Table 12b. Total cocaethylene degradation in (filtered) Humus sludge batch tests (19 ± 0.5°C)

| Time (min) | aqueous phase (ug) | | Total CE (ug) | Other metabolites (ug) |
|---|---|---|---|---|
| | Sample 1 | Sample 2 | | |
| 15 | 1400 | 1400 | 1400 | 7994.6 |
| 30 | 1472 | 1518 | 1495 | 8235.7 |
| 45 | 1449 | 1407 | 1428 | 8689.6 |
| **60** | 1292 | 1311 | 1292 | 8951.5 |
| 75 | 1326 | 1139 | 1224 | 9272.3 |
| 90 | 960 | 1020 | 990 | 10096.6 |
| 105 | 1014 | 988 | 1001 | 10277.9 |
| **120** | 1397 | 1397 | 1397 | 10099.5 |
| 135 | 1044 | 1134 | 1089 | 10573.8 |
| 150 | 840 | 826 | 833 | 11087 |
| 165 | 595 | 590 | 595 | 11553.6 |
| **180** | 636 | 621 | 630 | 11717.5 |

Table 13a. Total **cocaine** degradation in Stoke primary effluent batch tests (19 ± 0.5°C)

| Time (min) | aqueous phase (ug) | | Total cocaine (ug) | % COC | % Removal | EME, CE & Residual |
|---|---|---|---|---|---|---|
| | Sample 1 | Sample 2 | | | | |
| 15 | 468.0 | 469.5 | 468.7 | 3.7 | 96.3 | 12031.3 |
| 30 | 384.3 | 405.3 | 394.8 | 3.2 | 96.8 | 12105.2 |
| 45 | 320.6 | 323.3 | 321.9 | 2.6 | 97.4 | 12178.1 |
| **60** | 267.6 | 269.8 | 268.7 | 2.1 | 97.9 | 12231.3 |
| 75 | 216.3 | 214.7 | 215.5 | 1.7 | 98.3 | 12284.5 |
| 90 | 173.0 | 172.6 | 172.8 | 1.4 | 98.6 | 12327.2 |
| 105 | 146.8 | 146.1 | 146.5 | 1.2 | 98.8 | 12353.5 |
| **120** | 121.5 | 122.3 | 121.9 | 1.0 | 99.0 | 12378.1 |
| 135 | 99.7 | 99.1 | 99.4 | 0.8 | 99.2 | 12400.6 |
| 150 | 76.0 | 75.9 | 76.0 | 0.6 | 99.4 | 12424.0 |
| 165 | 54.3 | 54.4 | 54.3 | 0.4 | 99.6 | 12445.7 |
| **180** | 31.6 | 31.6 | 31.6 | 0.3 | 99.7 | 12468.4 |

Table 13b. Total **ecgonine methylester** degradation in Stoke primary effluent batch tests (19 ± 0.5°C)

| Time (min) | aqueous phase (ug) | | Total EME (ug) | CE & other metabolites (ug) |
|---|---|---|---|---|
| | Sample 1 | Sample 2 | | |
| 15 | 125 | 125 | 125 | 11781.3 |
| 30 | 115 | 115 | 115 | 11875.2 |
| 45 | 105 | 105 | 105 | 11968.1 |
| **60** | 76 | 76 | 76 | 12079.3 |
| 75 | 85 | 68 | 77 | 12131.5 |
| 90 | 75 | 75 | 75 | 12177.2 |
| 105 | 52 | 52 | 52 | 12249.5 |
| **120** | 55 | 55 | 55 | 12268.1 |
| 135 | 54 | 54 | 54 | 12292.6 |
| 150 | 42 | 42 | 42 | 12340 |
| 165 | 40 | 40 | 40 | 12365.7 |
| **180** | 24 | 24 | 24 | 12420.4 |

Table 13c. Total **cocaethylene** degradation in Stoke primary effluent batch tests (19 ± 0.5°C)

| Time (min) | aqueous phase (ug) | | Total CE (ug in10 mL) | Other metabolites (ug ) |
|---|---|---|---|---|
| | Sample 1 | Sample 2 | | |
| 15 | 250 | 250 | 250 | 11531 |
| 30 | 230 | 230 | 230 | 11645 |
| 45 | 210 | 189 | 200 | 11769 |
| **60** | 152 | 171 | 162 | 11918 |
| 75 | 119 | 119 | 119 | 12013 |
| 90 | 105 | 105 | 105 | 12072 |
| 105 | 78 | 78 | 78 | 12172 |
| **120** | 66 | 66 | 66 | 12202 |
| 135 | 63 | 63 | 63 | 12230 |
| 150 | 42 | 42 | 42 | 12298 |
| 165 | 35 | 35 | 35 | 12331 |
| **180** | 18 | 18 | 18 | 12402 |

Table 14. Partition/Degradation of **BZE** in primary sludge batch tests (4 ± 0.5°C)

| Time (min) | aqueous phase (ug) | | | solid phase (ug) | | | Total BZE | % BZE | % removal | Residual |
|---|---|---|---|---|---|---|---|---|---|---|
| | Sample 1 | Sample 2 | Mean | Sample 1 | Sample 2 | Mean | | | | |
| 15 | 6112.8 | 6090.3 | 6101.6 | 736.5 | 736.8 | 736.7 | 6838.2 | 54.7 | 51.2 | 5661.8 |
| 30 | 2924.3 | 2904.2 | 2914.3 | 898.6 | 906.6 | 902.6 | 3816.8 | 30.5 | 76.7 | 8683.2 |
| 45 | 2624.2 | 2614.0 | 2619.1 | 723.9 | 726.1 | 725.0 | 3344.2 | 26.8 | 79.0 | 9155.8 |
| **60** | 2367.3 | 2367.1 | 2367.2 | 727.4 | 728.9 | 728.2 | 3095.4 | 24.8 | 81.1 | 9404.6 |
| 75 | 1972.0 | 1979.1 | 1975.5 | 1147.2 | 1178.7 | 1162.9 | 3138.4 | 25.1 | 84.2 | 9361.6 |
| 90 | 1688.8 | 1692.1 | 1690.4 | 1043.8 | 1033.5 | 1038.7 | 2729.1 | 21.8 | 86.5 | 9770.9 |
| 105 | 1366.5 | 1361.6 | 1364.0 | 1124.0 | 1113.7 | 1118.8 | 2482.9 | 19.9 | 89.1 | 10017.1 |
| **120** | 979.8 | 978.4 | 979.1 | 983.2 | 973.7 | 978.5 | 1957.6 | 15.7 | 92.2 | 10542.4 |
| 135 | 830.4 | 841.4 | 835.9 | 1010.0 | 1009.8 | 1009.9 | 1845.8 | 14.8 | 93.3 | 10654.2 |
| 150 | 633.7 | 633.3 | 633.5 | 926.3 | 926.6 | 926.5 | 1560.0 | 12.5 | 94.9 | 10940.0 |
| 165 | 356.2 | 353.4 | 354.8 | 704.4 | 707.4 | 705.9 | 1060.7 | 8.5 | 97.2 | 11439.3 |
| **180** | 209.6 | 210.7 | 210.1 | 519.8 | 517.8 | 518.8 | 728.9 | 5.8 | 98.3 | 11771.1 |

Table 15. Partition/Degradation of **BZE** in primary sludge batch tests (19± 0.5°C)

| Time (min) | aqueous phase (ug) | | | solid phase (ug) | | | Total BZE | % BZE | % removal | Residual |
|---|---|---|---|---|---|---|---|---|---|---|
| | Sample 1 | Sample 2 | Mean | Sample 1 | Sample 2 | Mean | | | | |
| 15 | 407.6 | 404.9 | 406.3 | 89.8 | 89.9 | 89.8 | 496.1 | 4.0 | 96.7 | 12003.9 |
| 30 | 356.6 | 354.2 | 355.4 | 109.6 | 110.6 | 110.1 | 465.5 | 3.7 | 97.2 | 12034.5 |
| 45 | 320.0 | 318.8 | 319.4 | 88.3 | 88.6 | 88.4 | 407.8 | 3.3 | 97.4 | 12092.2 |
| **60** | 288.7 | 288.7 | 288.7 | 88.7 | 88.9 | 88.8 | 377.5 | 3.0 | 97.7 | 12122.5 |
| 75 | 240.5 | 241.4 | 240.9 | 139.9 | 143.7 | 141.8 | 382.7 | 3.1 | 98.1 | 12117.3 |
| 90 | 205.9 | 206.4 | 206.2 | 127.3 | 126.0 | 126.7 | 332.8 | 2.7 | 98.4 | 12167.2 |
| 105 | 166.6 | 166.1 | 166.3 | 137.1 | 135.8 | 136.4 | 302.8 | 2.4 | 98.7 | 12197.2 |
| **120** | 119.5 | 119.3 | 119.4 | 119.9 | 118.7 | 119.3 | 238.7 | 1.9 | 99.0 | 12261.3 |
| 135 | 101.3 | 102.6 | 101.9 | 123.2 | 123.1 | 123.2 | 225.1 | 1.8 | 99.2 | 12274.9 |
| 150 | 77.3 | 77.2 | 77.3 | 113.0 | 113.0 | 113.0 | 190.2 | 1.5 | 99.4 | 12309.8 |
| 165 | 43.4 | 43.1 | 43.3 | 85.9 | 86.3 | 86.1 | 129.4 | 1.0 | 99.7 | 12370.6 |
| **180** | 25.6 | 25.7 | 25.6 | 63.4 | 63.1 | 63.3 | 88.9 | 0.7 | 99.8 | 12411.1 |

Table 16. Total degradation of **BZE** in filtered primary sludge batch tests (19 ± 0.5°C)

| Time (min) | aqueous phase (ug) | | Total BZE (ug) | % BZE | % Removal | Residual |
|---|---|---|---|---|---|---|
| | Sample 1 | Sample 2 | | | | |
| 15 | 9363.3 | 9031.9 | 9197.6 | 73.6 | 26.4 | 3302.4 |
| 30 | 8136.2 | 8176.2 | 8156.2 | 65.2 | 34.8 | 4343.8 |
| 45 | 7056.3 | 7082.3 | 7069.3 | 56.6 | 43.4 | 5430.7 |
| **60** | 5896.4 | 5794.4 | 5845.4 | 46.8 | 53.2 | 6654.6 |
| 75 | 5101.4 | 4811.0 | 4956.2 | 39.6 | 60.4 | 7543.8 |
| 90 | 4085.7 | 4174.8 | 4130.3 | 33.0 | 67.0 | 8369.7 |
| 105 | 3235.3 | 3135.6 | 3185.5 | 25.5 | 74.5 | 9314.5 |
| **120** | 2676.0 | 2631.7 | 2653.9 | 21.2 | 78.8 | 9846.1 |
| 135 | 2216.6 | 2134.0 | 2175.3 | 17.4 | 82.6 | 10324.7 |
| 150 | 1650.3 | 1620.0 | 1635.2 | 13.1 | 86.9 | 10864.8 |
| 165 | 1220.5 | 1227.8 | 1224.2 | 9.8 | 90.2 | 11275.8 |
| **180** | 617.7 | 622.4 | 620.0 | 5.0 | 95.0 | 11880.0 |

Table 17. Partition/Degradation of **BZE** in SAF-1 batch tests (19 ± 0.5°C)

| Time (min) | aqueous phase (ug) | | | solid phase (ug) | | | Total BZE | % BZE | % removal | Residual |
|---|---|---|---|---|---|---|---|---|---|---|
| | Sample 1 | Sample 2 | Mean | Sample 1 | Sample 2 | Mean | | | | |
| 15 | 6061.7 | 6173.2 | 6117.5 | 2824.4 | 2824.4 | 2824.4 | 8941.9 | 71.5 | 51.1 | 3558.1 |
| 30 | 3919.4 | 3919.4 | 3919.4 | 1755.7 | 1755.7 | 1755.7 | 5675.1 | 45.4 | 68.6 | 6824.9 |
| 45 | 3123.6 | 3102.3 | 3112.9 | 1175.1 | 1175.1 | 1175.1 | 4288.1 | 34.3 | 75.1 | 8211.9 |
| **60** | 2622.2 | 2608.1 | 2615.1 | 1656.0 | 1656.0 | 1656.0 | 4271.2 | 34.2 | 79.1 | 8228.8 |
| 75 | 2495.2 | 2495.2 | 2495.2 | 1443.9 | 1443.9 | 1443.9 | 3939.0 | 31.5 | 80.0 | 8561.0 |
| 90 | 2076.8 | 2061.7 | 2069.2 | 1183.1 | 1183.1 | 1183.1 | 3252.3 | 26.0 | 83.4 | 9247.7 |
| 105 | 1746.9 | 1746.9 | 1746.9 | 1032.4 | 1035.0 | 1033.7 | 2780.6 | 22.2 | 86.0 | 9719.4 |
| **120** | 1505.9 | 1505.9 | 1505.9 | 857.2 | 856.6 | 856.9 | 2362.8 | 18.9 | 88.0 | 10137.2 |
| 135 | 1256.0 | 1256.0 | 1256.0 | 693.6 | 691.7 | 692.7 | 1948.6 | 15.6 | 90.0 | 10551.4 |
| 150 | 912.6 | 912.6 | 912.6 | 519.0 | 520.9 | 519.9 | 1432.5 | 11.5 | 92.7 | 11067.5 |
| 165 | 600.8 | 600.8 | 600.8 | 368.3 | 368.3 | 368.3 | 969.1 | 7.8 | 95.2 | 11530.9 |
| **180** | 364.8 | 364.8 | 364.8 | 211.3 | 211.3 | 211.3 | 576.2 | 4.6 | 97.1 | 11923.8 |

Table 18. Partition/Degradation of **BZE** in MSAF batch tests (19 ± 0.5°C)

| Time (min) | aqueous phase (ug) | | | solid phase (ug) | | | Total BZE | % BZE | % removal | Residual |
|---|---|---|---|---|---|---|---|---|---|---|
| | Sample 1 | Sample 2 | Mean | Sample 1 | Sample 2 | Mean | | | | |
| 15 | 6478.7 | 6488.7 | 6483.7 | 1064.6 | 1071.9 | 1068.3 | 7551.9 | 60.4 | 48.1 | 4948.1 |
| 30 | 5830.2 | 6296.2 | 6063.2 | 873.3 | 873.0 | 873.1 | 6936.4 | 55.5 | 51 | 5563.6 |
| 45 | 4858.2 | 4858.2 | 4858.2 | 746.8 | 740.1 | 743.4 | 5601.6 | 44.8 | 61.1 | 6898.4 |
| **60** | 4184.7 | 4013.6 | 4099.1 | 663.0 | 667.1 | 665.1 | 4764.2 | 38.1 | 61.1 | 7735.8 |
| 75 | 3503.1 | 3145.4 | 3324.2 | 593.5 | 616.0 | 604.7 | 3929.0 | 31.4 | 67.2 | 8571.0 |
| 90 | 2321.1 | 2321.1 | 2321.1 | 519.7 | 544.2 | 532.0 | 2853.1 | 22.8 | 73.4 | 9646.9 |
| 105 | 1491.2 | 1558.0 | 1524.6 | 465.6 | 458.1 | 461.8 | 1986.5 | 15.9 | 81.4 | 10513.5 |
| **120** | 1354.3 | 1351.7 | 1353.0 | 394.8 | 394.6 | 394.7 | 1747.8 | 14.0 | 87.8 | 10752.2 |
| 135 | 1022.6 | 1022.6 | 1022.6 | 327.0 | 326.4 | 326.7 | 1349.3 | 10.8 | 89.2 | 11150.7 |
| 150 | 803.2 | 843.0 | 823.1 | 251.2 | 250.6 | 250.9 | 1074.0 | 8.6 | 91.8 | 11426.0 |
| 165 | 541.4 | 541.4 | 541.4 | 168.8 | 171.3 | 170.1 | 711.4 | 5.7 | 93.4 | 11788.6 |
| **180** | 321.9 | 321.9 | 321.9 | 73.5 | 73.5 | 73.5 | 395.4 | 3.2 | 95.7 | 12104.6 |

Table 19. Partition/Degradation of **BZE** in HS batch tests (19 ± 0.5°C)

| Time (min) | aqueous phase (ug) | | | solid phase (ug) | | | Total BZE | % BZE | % removal | Residual |
|---|---|---|---|---|---|---|---|---|---|---|
| | Sample 1 | Sample 2 | Mean | Sample 1 | Sample 2 | Mean | | | | |
| 15 | 3844.8 | 3874.0 | 3859.4 | 758.2 | 798.7 | 778.4 | 4637.8 | 37.1 | 69.1 | 7862.2 |
| 30 | 2434.1 | 2453.5 | 2443.8 | 699.1 | 699.1 | 699.1 | 3142.9 | 25.1 | 80.4 | 9357.1 |
| 45 | 2178.4 | 2173.5 | 2175.9 | 650.8 | 674.2 | 662.5 | 2838.4 | 22.7 | 82.6 | 9661.6 |
| **60** | 2024.8 | 2024.8 | 2024.8 | 518.7 | 518.7 | 518.7 | 2543.5 | 20.3 | 83.8 | 9956.5 |
| 75 | 1972.4 | 1978.7 | 1975.5 | 416.7 | 416.7 | 416.7 | 2392.2 | 19.1 | 84.2 | 10107.8 |
| 90 | 1614.9 | 1608.2 | 1611.6 | 372.1 | 367.0 | 369.6 | 1981.1 | 15.8 | 87.1 | 10518.9 |
| 105 | 1413.8 | 1403.1 | 1408.5 | 334.3 | 337.5 | 335.9 | 1744.4 | 14.0 | 88.7 | 10755.6 |
| **120** | 1169.9 | 1167.8 | 1168.8 | 276.8 | 276.8 | 276.8 | 1445.6 | 11.6 | 90.6 | 11054.4 |
| 135 | 881.0 | 876.7 | 878.9 | 208.4 | 208.4 | 208.4 | 1087.2 | 8.7 | 93.0 | 11412.8 |
| 150 | 680.8 | 678.4 | 679.6 | 166.3 | 164.5 | 165.4 | 845.0 | 6.8 | 94.6 | 11655.0 |
| 165 | 435.2 | 448.8 | 442.0 | 136.0 | 136.0 | 136.0 | 578.0 | 4.6 | 96.5 | 11922.0 |
| **180** | 266.3 | 266.3 | 266.3 | 91.1 | 91.1 | 91.1 | 357.4 | 2.9 | 97.9 | 12142.6 |

Table 20. Total degradation of **BZE** in Stoke primary effluent batch tests (19 ± 0.5°C)

| Time (min) | aqueous phase (ug) | | Total BZE (ug) | % BZE | % Removal | Residual |
|---|---|---|---|---|---|---|
| | Sample 1 | Sample 2 | | | | |
| 15 | 1662.5 | 1657.9 | 1660.2 | 13.3 | 86.7 | 10839.8 |
| 30 | 1288.5 | 1293.8 | 1291.2 | 10.3 | 89.7 | 11208.8 |
| 45 | 1129.4 | 1134.0 | 1131.7 | 9.1 | 90.9 | 11368.3 |
| **60** | 1174.0 | 1154.4 | 1164.2 | 9.3 | 90.7 | 11335.8 |
| 75 | 1051.3 | 1055.6 | 1053.4 | 8.4 | 91.6 | 11446.6 |
| 90 | 838.0 | 825.8 | 831.9 | 6.7 | 93.3 | 11668.1 |
| 105 | 730.9 | 732.1 | 731.5 | 5.9 | 94.1 | 11768.5 |
| **120** | 596.5 | 596.8 | 596.6 | 4.8 | 95.2 | 11903.4 |
| 135 | 539.9 | 539.4 | 539.7 | 4.3 | 95.7 | 11960.3 |
| 150 | 414.2 | 414.0 | 414.1 | 3.3 | 96.7 | 12085.9 |
| 165 | 288.3 | 287.2 | 287.7 | 2.3 | 97.7 | 12212.3 |
| **180** | 164.8 | 163.5 | 164.2 | 1.3 | 98.7 | 12335.8 |

Table 21. Partition/Degradation of **Morphine** in Primary Sludge batch tests (4± 0.5°C)

| Time (min) | aqueous phase (ug) | | | solid phase (ug) | | | Total MOR | % MOR | % removal | Residual |
|---|---|---|---|---|---|---|---|---|---|---|
| | Sample 1 | Sample 2 | Mean | Sample 1 | Sample 2 | Mean | | | | |
| 15 | 9713.2 | 9719.6 | 9716.4 | 137.1 | 136.8 | 137.0 | 9853.4 | 78.8 | 22.3 | 2646.6 |
| 30 | 8784.6 | 8745.1 | 8764.9 | 121.3 | 121.9 | 121.6 | 8886.5 | 71.1 | 29.9 | 3613.5 |
| 45 | 7729.0 | 7818.9 | 7773.9 | 120.4 | 120.6 | 120.5 | 7894.4 | 63.2 | 37.8 | 4605.6 |
| **60** | 5789.3 | 5813.2 | 5801.2 | 110.1 | 107.0 | 108.5 | 5909.8 | 47.3 | 53.6 | 6590.2 |
| 75 | 6048.0 | 6066.8 | 6057.4 | 109.0 | 108.7 | 108.9 | 6166.3 | 49.3 | 51.5 | 6333.7 |
| 90 | 3914.4 | 3941.1 | 3927.8 | 89.9 | 92.4 | 91.1 | 4018.9 | 32.2 | 68.6 | 8481.1 |
| 105 | 4165.4 | 4151.5 | 4158.5 | 77.4 | 83.2 | 80.3 | 4238.8 | 33.9 | 66.7 | 8261.2 |
| **120** | 3504.0 | 3504.6 | 3504.3 | 66.8 | 65.2 | 66.0 | 3570.3 | 28.6 | 72.0 | 8929.7 |
| 135 | 2862.9 | 2808.1 | 2835.5 | 61.4 | 62.5 | 62.0 | 2897.5 | 23.2 | 77.3 | 9602.5 |
| 150 | 1979.3 | 1973.7 | 1976.5 | 49.3 | 49.9 | 49.6 | 2026.1 | 16.2 | 84.2 | 10473.9 |
| 165 | 1490.5 | 1480.9 | 1485.7 | 36.2 | 35.5 | 35.8 | 1521.5 | 12.2 | 88.1 | 10978.5 |
| **180** | 804.5 | 804.6 | 804.6 | 23.0 | 23.2 | 23.1 | 827.7 | 6.6 | 93.6 | 11672.3 |

Table 22. Partition/Degradation of **Morphine** in Primary Sludge batch tests (19± 0.5°C)

| Time (min) | aqueous phase (ug) | | | solid phase (ug) | | | Total MOR | % MOR | % removal | Residual |
|---|---|---|---|---|---|---|---|---|---|---|
| | Sample 1 | Sample 2 | Mean | Sample 1 | Sample 2 | Mean | | | | |
| 15 | 1891.9 | 1911.6 | 1901.8 | 431.0 | 427.7 | 429.3 | 2331.1 | 18.6 | 84.8 | 10168.9 |
| 30 | 1692.3 | 1665.4 | 1678.9 | 428.5 | 431.5 | 430.0 | 2108.9 | 16.9 | 86.6 | 10391.1 |
| 45 | 1411.7 | 1457.8 | 1434.8 | 358.2 | 365.1 | 361.7 | 1796.5 | 14.4 | 88.5 | 10703.5 |
| **60** | 1178.2 | 1178.6 | 1178.4 | 351.7 | 359.8 | 355.8 | 1534.2 | 12.3 | 90.6 | 10965.8 |
| 75 | 1065.4 | 993.0 | 1029.2 | 394.6 | 401.8 | 398.2 | 1427.4 | 11.4 | 91.8 | 11072.6 |
| 90 | 840.0 | 847.4 | 843.7 | 303.1 | 305.1 | 304.1 | 1147.8 | 9.2 | 93.3 | 11352.2 |
| 105 | 634.9 | 648.5 | 641.7 | 284.8 | 281.5 | 283.1 | 924.8 | 7.4 | 94.9 | 11575.2 |
| **120** | 495.1 | 478.5 | 486.8 | 194.0 | 190.5 | 192.3 | 679.1 | 5.4 | 96.1 | 11820.9 |
| 135 | 399.2 | 399.4 | 399.3 | 206.0 | 209.8 | 207.9 | 607.2 | 4.9 | 96.8 | 11892.8 |
| 150 | 284.5 | 290.4 | 287.4 | 145.6 | 146.7 | 146.2 | 433.6 | 3.5 | 97.7 | 12066.4 |
| 165 | 162.4 | 165.0 | 163.7 | 126.5 | 124.0 | 125.3 | 289.0 | 2.3 | 98.7 | 12211.0 |
| **180** | 58.1 | 58.5 | 58.3 | 73.8 | 73.7 | 73.8 | 132.1 | 1.1 | 99.5 | 12367.9 |

Table 23. Total degradation of **Morphine** in filtered primary sludge batch tests (19 ± 0.5°C)

| Time (min) | aqueous phase (ug) | | Total MOR (ug) | % MOR | % Removal | Residual |
|---|---|---|---|---|---|---|
| | Sample 1 | Sample 2 | | | | |
| 15 | 841.2 | 831.1 | 836.2 | 6.7 | 93.3 | 11663.8 |
| 30 | 713.0 | 721.5 | 717.3 | 5.7 | 94.3 | 11782.7 |
| 45 | 651.2 | 650.6 | 650.9 | 5.2 | 94.8 | 11849.1 |
| **60** | 593.5 | 600.1 | 596.8 | 4.8 | 95.2 | 11903.2 |
| 75 | 510.9 | 510.8 | 510.9 | 4.1 | 95.9 | 11989.1 |
| 90 | 456.7 | 457.2 | 457.0 | 3.7 | 96.3 | 12043.0 |
| 105 | 353.9 | 353.9 | 353.9 | 2.8 | 97.2 | 12146.1 |
| **120** | 297.6 | 294.8 | 296.2 | 2.4 | 97.6 | 12203.8 |
| 135 | 242.1 | 242.1 | 242.1 | 1.9 | 98.1 | 12257.9 |
| 150 | 179.9 | 179.9 | 179.9 | 1.4 | 98.6 | 12320.1 |
| 165 | 136.6 | 136.4 | 136.5 | 1.1 | 98.9 | 12363.5 |
| **180** | 77.1 | 77.1 | 77.1 | 0.6 | 99.4 | 12422.9 |

Table 24. Partition/Degradation of **Morphine** in SAF-1 sludge batch tests (19± 0.5°C)

| Time (min) | aqueous phase (ug) | | | solid phase (ug) | | | Total MOR | % MOR | % removal | Residual |
|---|---|---|---|---|---|---|---|---|---|---|
| | Sample 1 | Sample 2 | Mean | Sample 1 | Sample 2 | Mean | | | | |
| 15 | 696.5 | 700.8 | 698.7 | 98.5 | 98.7 | 98.6 | 797.3 | 6.4 | 94.4 | 11702.7 |
| 30 | 410.0 | 412.4 | 411.2 | 128.4 | 128.3 | 128.4 | 539.6 | 4.3 | 96.7 | 11960.4 |
| 45 | 360.9 | 365.5 | 363.2 | 115.9 | 116.1 | 116.0 | 479.2 | 3.8 | 97.1 | 12020.8 |
| **60** | 326.4 | 331.6 | 329.0 | 91.5 | 90.6 | 91.0 | 420.0 | 3.4 | 97.4 | 12080.0 |
| 75 | 247.7 | 250.0 | 248.9 | 70.1 | 67.0 | 68.6 | 317.5 | 2.5 | 98.0 | 12182.5 |
| 90 | 208.0 | 212.9 | 210.5 | 58.9 | 65.4 | 62.2 | 272.6 | 2.2 | 98.3 | 12227.4 |
| 105 | 170.8 | 171.5 | 171.2 | 63.6 | 63.1 | 63.3 | 234.5 | 1.9 | 98.6 | 12265.5 |
| **120** | 145.5 | 145.2 | 145.3 | 60.5 | 60.6 | 60.5 | 205.9 | 1.6 | 98.8 | 12294.1 |
| 135 | 89.3 | 86.0 | 87.7 | 40.3 | 41.1 | 40.7 | 128.4 | 1.0 | 99.3 | 12371.6 |
| 150 | 41.7 | 41.7 | 41.7 | 36.2 | 35.9 | 36.1 | 77.8 | 0.6 | 99.7 | 12422.2 |
| 165 | 24.2 | 24.0 | 24.1 | 25.8 | 26.0 | 25.9 | 50.1 | 0.4 | 99.8 | 12449.9 |
| **180** | 13.7 | 13.7 | 13.7 | 15.3 | 15.3 | 15.3 | 29.0 | 0.2 | 99.9 | 12471.0 |

Table 25. Partition/Degradation of **Morphine** in MSAF-1 sludge batch tests (19± 0.5°C)

| Time (min) | aqueous phase (ug) | | | solid phase (ug) | | | Total MOR | % MOR | % removal | Residual |
|---|---|---|---|---|---|---|---|---|---|---|
| | Sample 1 | Sample 2 | Mean | Sample 1 | Sample 2 | Mean | | | | |
| 15 | 4424.8 | 4402.9 | 4413.8 | 221.6 | 215.3 | 218.4 | 4632.3 | 37.1 | 64.7 | 7867.7 |
| 30 | 3044.9 | 2964.4 | 3004.6 | 245.4 | 243.7 | 244.6 | 3249.2 | 26.0 | 76.0 | 9250.8 |
| 45 | 2728.9 | 2770.8 | 2749.8 | 186.1 | 184.6 | 185.3 | 2935.2 | 23.5 | 78.0 | 9564.8 |
| **60** | 2325.9 | 2321.2 | 2323.5 | 223.4 | 226.1 | 224.8 | 2548.3 | 20.4 | 81.4 | 9951.7 |
| 75 | 1846.7 | 1846.7 | 1846.7 | 242.8 | 244.7 | 243.8 | 2090.5 | 16.7 | 85.2 | 10409.5 |
| 90 | 1568.1 | 1557.0 | 1562.6 | 240.2 | 239.2 | 239.7 | 1802.3 | 14.4 | 87.5 | 10697.7 |
| 105 | 1365.9 | 1362.0 | 1364.0 | 191.6 | 192.8 | 192.2 | 1556.1 | 12.4 | 89.1 | 10943.9 |
| **120** | 1145.2 | 1140.2 | 1142.7 | 209.8 | 213.5 | 211.7 | 1354.4 | 10.8 | 90.9 | 11145.6 |
| 135 | 941.4 | 948.7 | 945.1 | 147.4 | 148.1 | 147.7 | 1092.8 | 8.7 | 92.4 | 11407.2 |
| 150 | 720.1 | 711.7 | 715.9 | 102.7 | 105.1 | 103.9 | 819.8 | 6.6 | 94.3 | 11680.2 |
| 165 | 498.1 | 494.9 | 496.5 | 66.9 | 67.1 | 67.0 | 563.5 | 4.5 | 96.0 | 11936.5 |
| **180** | 297.6 | 295.4 | 296.5 | 38.8 | 39.8 | 39.3 | 335.8 | 2.7 | 97.6 | 12164.2 |

Table 26. Partition/Degradation of **Morphine** in Humus sludge batch tests (19± 0.5°C)

| Time (min) | aqueous phase (ug) | | | solid phase (ug) | | | Total MOR | % MOR | % removal | Residual |
|---|---|---|---|---|---|---|---|---|---|---|
| | Sample 1 | Sample 2 | Mean | Sample 1 | Sample 2 | Mean | | | | |
| 15 | 119.8 | 120.8 | 120.3 | 14.2 | 14.8 | 14.5 | 134.8 | 1.1 | 99.0 | 12365.2 |
| 30 | 94.9 | 89.9 | 92.4 | 13.8 | 14.4 | 14.1 | 106.5 | 0.9 | 99.3 | 12393.5 |
| 45 | 78.0 | 76.9 | 77.5 | 12.7 | 13.0 | 12.8 | 90.3 | 0.7 | 99.4 | 12409.7 |
| **60** | 69.0 | 67.8 | 68.4 | 10.7 | 9.7 | 10.2 | 78.6 | 0.6 | 99.5 | 12421.4 |
| 75 | 61.3 | 61.4 | 61.3 | 9.9 | 9.7 | 9.8 | 71.1 | 0.6 | 99.5 | 12428.9 |
| 90 | 42.3 | 43.0 | 42.7 | 10.3 | 10.4 | 10.3 | 53.0 | 0.4 | 99.7 | 12447.0 |
| 105 | 39.6 | 39.0 | 39.3 | 12.3 | 12.0 | 12.1 | 51.4 | 0.4 | 99.7 | 12448.6 |
| **120** | 33.8 | 33.6 | 33.7 | 8.9 | 8.9 | 8.9 | 42.6 | 0.3 | 99.7 | 12457.4 |
| 135 | 42.2 | 42.6 | 42.4 | 6.9 | 7.0 | 7.0 | 49.4 | 0.4 | 99.7 | 12450.6 |
| 150 | 29.8 | 30.0 | 29.9 | 6.5 | 6.5 | 6.5 | 36.5 | 0.3 | 99.8 | 12463.5 |
| 165 | 19.1 | 19.0 | 19.0 | 3.8 | 3.8 | 3.8 | 22.9 | 0.2 | 99.8 | 12477.1 |
| **180** | 11.8 | 11.8 | 11.8 | 2.0 | 2.0 | 2.0 | 13.8 | 0.1 | 99.9 | 12486.2 |

Table 27. Total degradation of **Morphine in** Stoke primary effluent batch tests (19 ± 0.5°C)

| Time (min) | aqueous phase (ug) | | Total MOR (ug) | % MOR | % Removal | Residual |
|---|---|---|---|---|---|---|
| | Sample 1 | Sample 2 | | | | |
| 15 | 9962.7 | 9831.8 | 9897.3 | 79.2 | 20.8 | 2602.7 |
| 30 | 8913.9 | 8917.8 | 8915.8 | 71.3 | 28.7 | 3584.2 |
| 45 | 6415.1 | 6529.2 | 6472.2 | 51.8 | 48.2 | 6027.8 |
| **60** | 6416.2 | 5836.8 | 6126.5 | 49.0 | 51.0 | 6373.5 |
| 75 | 5195.0 | 5279.3 | 5237.1 | 41.9 | 58.1 | 7262.9 |
| 90 | 4617.6 | 4581.0 | 4599.3 | 36.8 | 63.2 | 7900.7 |
| 105 | 3348.1 | 3406.5 | 3377.3 | 27.0 | 73.0 | 9122.7 |
| **120** | 1373.7 | 1378.8 | 1376.2 | 11.0 | 89.0 | 11123.8 |
| 135 | 1131.4 | 1124.3 | 1127.8 | 9.0 | 91.0 | 11372.2 |
| 150 | 875.8 | 876.0 | 875.9 | 7.0 | 93.0 | 11624.1 |
| 165 | 606.6 | 591.3 | 598.9 | 4.8 | 95.2 | 11901.1 |
| **180** | 362.9 | 362.8 | 362.8 | 2.9 | 97.1 | 12137.2 |

Table 28. Partition/Degradation of **6-ACM** in primary sludge batch tests (4± 0.5°C)

| Time (min) | aqueous phase (ug) | | | solid phase (ug) | | | Total 6ACM | % 6ACM | % removal | Residual |
|---|---|---|---|---|---|---|---|---|---|---|
| | Sample 1 | Sample 2 | Mean | Sample 1 | Sample 2 | Mean | | | | |
| 15 | 570.4 | 574.7 | 572.5 | 69.7 | 69.7 | 69.8 | 642.4 | 5.1 | 95.4 | 11857.6 |
| 30 | 610.8 | 621.8 | 616.3 | 63.1 | 63.1 | 62.7 | 679.1 | 5.4 | 95.1 | 11820.9 |
| 45 | 559.5 | 569.3 | 564.4 | 51.8 | 51.8 | 52.1 | 616.5 | 4.9 | 95.5 | 11883.5 |
| **60** | 461.6 | 465.1 | 463.4 | 43.5 | 43.5 | 42.6 | 506.0 | 4.0 | 96.3 | 11994.0 |
| 75 | 376.9 | 370.5 | 373.7 | 50.5 | 50.5 | 50.9 | 424.6 | 3.4 | 97.0 | 12075.4 |
| 90 | 291.3 | 272.7 | 282.0 | 39.2 | 39.2 | 39.2 | 321.2 | 2.6 | 97.7 | 12178.8 |
| 105 | 252.4 | 233.9 | 243.2 | 61.3 | 61.3 | 61.3 | 304.4 | 2.4 | 98.1 | 12195.6 |
| **120** | 201.1 | 227.8 | 214.5 | 49.0 | 49.0 | 49.2 | 263.7 | 2.1 | 98.3 | 12236.3 |
| 135 | 113.8 | 112.3 | 113.1 | 35.7 | 35.7 | 35.5 | 148.6 | 1.2 | 99.1 | 12351.4 |
| 150 | 83.7 | 86.8 | 85.2 | 33.4 | 33.4 | 33.1 | 118.4 | 0.9 | 99.3 | 12381.6 |
| 165 | 60.0 | 62.5 | 61.3 | 24.2 | 24.2 | 23.9 | 85.2 | 0.7 | 99.5 | 12414.8 |
| **180** | 39.5 | 38.9 | 39.2 | 15.6 | 15.6 | 15.6 | 54.8 | 0.4 | 99.7 | 12445.2 |

Table 29. Partition/Degradation of **6-ACM** in primary sludge batch tests (19± 0.5°C)

| Time (min) | aqueous phase (ug) | | | solid phase (ug) | | | Total 6ACM | % 6ACM | % removal | Residual |
|---|---|---|---|---|---|---|---|---|---|---|
| | Sample 1 | Sample 2 | Mean | Sample 1 | Sample 2 | Mean | | | | |
| 15 | 213.3 | 214.2 | 213.7 | 15.3 | 14.5 | 14.9 | 228.6 | 1.8 | 98.3 | 12271.4 |
| 30 | 159.6 | 162.0 | 160.8 | 13.8 | 12.9 | 13.4 | 174.2 | 1.4 | 98.7 | 12325.8 |
| 45 | 122.7 | 124.8 | 123.8 | 11.4 | 10.9 | 11.1 | 134.9 | 1.1 | 99.0 | 12365.1 |
| **60** | 101.2 | 102.0 | 101.6 | 9.5 | 8.7 | 9.1 | 110.7 | 0.9 | 99.2 | 12389.3 |
| 75 | 71.0 | 69.6 | 70.3 | 9.6 | 8.7 | 9.2 | 79.4 | 0.6 | 99.4 | 12420.6 |
| 90 | 63.9 | 56.3 | 60.1 | 8.6 | 8.1 | 8.4 | 68.5 | 0.5 | 99.5 | 12431.5 |
| 105 | 59.7 | 59.9 | 59.8 | 10.3 | 9.7 | 10.0 | 69.8 | 0.6 | 99.5 | 12430.2 |
| **120** | 44.1 | 50.0 | 47.0 | 8.1 | 7.8 | 7.9 | 55.0 | 0.4 | 99.6 | 12445.0 |
| 135 | 25.0 | 24.6 | 24.8 | 7.8 | 7.3 | 7.6 | 32.4 | 0.3 | 99.8 | 12467.6 |
| 150 | 19.0 | 18.9 | 18.9 | 7.3 | 6.8 | 7.1 | 26.0 | 0.2 | 99.8 | 12474.0 |
| 165 | 13.2 | 13.7 | 13.4 | 5.3 | 4.9 | 5.1 | 18.5 | 0.1 | 99.9 | 12481.5 |
| **180** | 6.3 | 6.2 | 6.2 | 3.4 | 3.2 | 3.3 | 9.5 | 0.1 | 100.0 | 12490.5 |

Table 30. Total degradation of **6-ACM** in filtered primary sludge batch tests (19 ± 0.5°C)

| Time (min) | aqueous phase (ug) | | Total cocaine (ug) | % 6ACM | % Removal | Residual |
|---|---|---|---|---|---|---|
| | Sample 1 | Sample 2 | | | | |
| 15 | 55.9 | 56.4 | 56.2 | 0.4 | 99.6 | 12443.8 |
| 30 | 40.4 | 40.1 | 40.2 | 0.3 | 99.7 | 12459.8 |
| 45 | 38.5 | 38.4 | 38.5 | 0.3 | 99.7 | 12461.5 |
| **60** | 33.1 | 33.1 | 33.1 | 0.3 | 99.7 | 12466.9 |
| 75 | 23.8 | 23.7 | 23.8 | 0.2 | 99.8 | 12476.2 |
| 90 | 20.4 | 20.4 | 20.4 | 0.2 | 99.8 | 12479.6 |
| 105 | 17.3 | 17.1 | 17.2 | 0.1 | 99.9 | 12482.8 |
| **120** | 14.1 | 14.0 | 14.1 | 0.1 | 99.9 | 12485.9 |
| 135 | 11.1 | 11.1 | 11.1 | 0.1 | 99.9 | 12488.9 |
| 150 | 8.9 | 8.5 | 8.7 | 0.1 | 99.9 | 12491.3 |
| 165 | 5.6 | 5.6 | 5.6 | 0.0 | 100.0 | 12494.4 |
| **180** | 4.1 | 4.1 | 4.1 | 0.0 | 100.0 | 12495.9 |

Table 31. Partition/Degradation of **6-ACM** in SAF-1 sludge batch tests (19± 0.5°C)

| Time (min) | aqueous phase (ug) | | | solid phase (ug) | | | Total 6ACM | % 6ACM | % removal | Residual |
|---|---|---|---|---|---|---|---|---|---|---|
| | Sample 1 | Sample 2 | Mean | Sample 1 | Sample 2 | Mean | | | | |
| 15 | 10215.7 | 10055.8 | 10135.8 | 1687.0 | 1700.2 | 1693.6 | 11829.4 | 94.6 | 18.9 | 670.6 |
| 30 | 7067.3 | 7116.3 | 7091.8 | 1038.5 | 1051.4 | 1045.0 | 8136.8 | 65.1 | 43.3 | 4363.2 |
| 45 | 6139.1 | 5908.8 | 6023.9 | 1043.5 | 1043.5 | 1043.5 | 7067.4 | 56.5 | 51.8 | 5432.6 |
| **60** | 6119.3 | 6272.2 | 6195.8 | 913.6 | 920.0 | 916.8 | 7112.6 | 56.9 | 50.4 | 5387.4 |
| 75 | 5263.7 | 5204.4 | 5234.1 | 667.0 | 669.2 | 668.1 | 5902.2 | 47.2 | 58.1 | 6597.8 |
| 90 | 3753.7 | 3695.6 | 3724.6 | 676.6 | 651.1 | 663.9 | 4388.5 | 35.1 | 70.2 | 8111.5 |
| 105 | 2302.7 | 2302.7 | 2302.7 | 899.3 | 892.9 | 896.1 | 3198.7 | 25.6 | 81.6 | 9301.3 |
| **120** | 1648.9 | 1851.9 | 1750.4 | 753.3 | 756.4 | 754.9 | 2505.3 | 20.0 | 86.0 | 9994.7 |
| 135 | 1360.5 | 1360.5 | 1360.5 | 601.7 | 612.1 | 606.9 | 1967.4 | 15.7 | 89.1 | 10532.6 |
| 150 | 1025.8 | 1058.7 | 1042.2 | 517.3 | 517.3 | 517.3 | 1559.6 | 12.5 | 91.7 | 10940.4 |
| 165 | 786.4 | 812.0 | 799.2 | 291.4 | 293.4 | 292.4 | 1091.6 | 8.7 | 93.6 | 11408.4 |
| **180** | 410.6 | 378.5 | 394.6 | 148.6 | 149.0 | 148.8 | 543.4 | 4.3 | 96.8 | 11956.6 |

Table 32. Partition/Degradation of **6-ACM** in MSAF sludge batch tests (19± 0.5°C)

| Time (min) | aqueous phase (ug) | | | solid phase (ug) | | | Total 6ACM | % 6ACM | % removal | Residual |
|---|---|---|---|---|---|---|---|---|---|---|
| | Sample 1 | Sample 2 | Mean | Sample 1 | Sample 2 | Mean | | | | |
| 15 | 1660.3 | 1659.4 | 1659.8 | 252.7 | 265.9 | 259.3 | 1919.2 | 15.4 | 86.7 | 10580.8 |
| 30 | 1450.2 | 1444.2 | 1447.2 | 187.3 | 182.7 | 185.0 | 1632.2 | 13.1 | 88.4 | 10867.8 |
| 45 | 1259.8 | 1269.5 | 1264.6 | 195.7 | 196.1 | 195.9 | 1460.5 | 11.7 | 89.9 | 11039.5 |
| **60** | 1115.4 | 1136.4 | 1125.9 | 167.4 | 186.2 | 176.8 | 1302.7 | 10.4 | 91.0 | 11197.3 |
| 75 | 942.9 | 936.8 | 939.8 | 148.0 | 164.4 | 156.2 | 1096.0 | 8.8 | 92.5 | 11404.0 |
| 90 | 746.8 | 770.3 | 758.6 | 131.9 | 134.5 | 133.2 | 891.8 | 7.1 | 93.9 | 11608.2 |
| 105 | 647.1 | 653.2 | 650.1 | 114.0 | 119.4 | 116.7 | 766.8 | 6.1 | 94.8 | 11733.2 |
| **120** | 553.6 | 553.6 | 553.6 | 89.2 | 101.1 | 95.1 | 648.7 | 5.2 | 95.6 | 11851.3 |
| 135 | 452.2 | 448.3 | 450.2 | 28.0 | 30.7 | 29.4 | 479.6 | 3.8 | 96.4 | 12020.4 |
| 150 | 354.3 | 360.6 | 357.4 | 5.9 | 6.7 | 6.3 | 363.7 | 2.9 | 97.1 | 12136.3 |
| 165 | 245.3 | 243.0 | 244.1 | 4.9 | 5.4 | 5.2 | 249.3 | 2.0 | 98.0 | 12250.7 |
| **180** | 135.5 | 137.0 | 136.2 | 2.6 | 3.2 | 2.9 | 139.1 | 1.1 | 98.9 | 12360.9 |

Table 33. Partition/Degradation of **6-ACM** in Humus sludge batch tests (19± 0.5°C)

| Time (min) | aqueous phase (ug) | | | solid phase (ug) | | | Total 6ACM | % 6ACM | % removal | Residual |
|---|---|---|---|---|---|---|---|---|---|---|
| | Sample 1 | Sample 2 | Mean | Sample 1 | Sample 2 | Mean | | | | |
| 15 | 4228.1 | 4166.1 | 4197.1 | 5.0 | 5.0 | 5.0 | 4202.1 | 33.6 | 66.4 | 8297.9 |
| 30 | 3906.6 | 3376.7 | 3641.6 | 4.4 | 4.4 | 4.4 | 3646.1 | 29.2 | 70.9 | 8853.9 |
| 45 | 2915.0 | 2917.2 | 2916.1 | 2.4 | 2.4 | 2.4 | 2918.5 | 23.3 | 76.7 | 9581.5 |
| **60** | 1809.4 | 1737.6 | 1773.5 | 2.9 | 2.8 | 2.8 | 1776.3 | 14.2 | 85.8 | 10723.7 |
| 75 | 1433.8 | 1303.6 | 1368.7 | 3.0 | 2.6 | 2.8 | 1371.5 | 11.0 | 89.1 | 11128.5 |
| 90 | 1267.8 | 1201.0 | 1234.4 | 1.8 | 1.8 | 1.8 | 1236.2 | 9.9 | 90.1 | 11263.8 |
| 105 | 1035.3 | 1010.1 | 1022.7 | 2.2 | 2.2 | 2.2 | 1024.9 | 8.2 | 91.8 | 11475.1 |
| **120** | 831.5 | 831.5 | 831.5 | 1.7 | 1.7 | 1.7 | 833.2 | 6.7 | 93.3 | 11666.8 |
| 135 | 622.3 | 627.5 | 624.9 | 1.4 | 1.4 | 1.4 | 626.3 | 5.0 | 95.0 | 11873.7 |
| 150 | 434.8 | 427.5 | 431.1 | 1.0 | 1.0 | 1.0 | 432.2 | 3.5 | 96.6 | 12067.8 |
| 165 | 302.7 | 302.7 | 302.7 | 0.8 | 0.8 | 0.8 | 303.5 | 2.4 | 97.6 | 12196.5 |
| **180** | 168.8 | 188.0 | 178.4 | 0.5 | 0.5 | 0.5 | 179.0 | 1.4 | 98.6 | 12321.0 |

Table 34. Total degradation of **6-ACM** in Stoke Baldoph effluent batch tests (19 ± 0.5°C)

| Time (min) | aqueous phase (ug) | | Total 6ACM (ug) | % 6ACM | % Removal | Residual |
|---|---|---|---|---|---|---|
| | Sample 1 | Sample 2 | | | | |
| 15 | 134.8 | 132.6 | 133.7 | 1.1 | 98.9 | 12366.3 |
| 30 | 135.6 | 124.1 | 129.8 | 1.0 | 99.0 | 12370.2 |
| 45 | 108.5 | 108.4 | 108.5 | 0.9 | 99.1 | 12391.5 |
| **60** | 88.1 | 88.9 | 88.5 | 0.7 | 99.3 | 12411.5 |
| 75 | 73.0 | 75.2 | 74.1 | 0.6 | 99.4 | 12425.9 |
| 90 | 44.4 | 47.2 | 45.8 | 0.4 | 99.6 | 12454.2 |
| 105 | 36.2 | 35.9 | 36.0 | 0.3 | 99.7 | 12464.0 |
| **120** | 24.3 | 24.5 | 24.4 | 0.2 | 99.8 | 12475.6 |
| 135 | 19.2 | 17.1 | 18.1 | 0.1 | 99.9 | 12481.9 |
| 150 | 12.2 | 12.2 | 12.2 | 0.1 | 99.9 | 12487.8 |
| 165 | 9.2 | 9.2 | 9.2 | 0.1 | 99.9 | 12490.8 |
| **180** | 5.2 | 5.2 | 5.2 | 0.0 | 100.0 | 12494.8 |

Table 35a. Partition/Degradation of **Heroin** in primary sludge batch tests (4± 0.5°C)

| Time (min) | aqueous phase (ug) | | | solid phase (ug) | | | Total HER | % HER | % removal | Residual |
|---|---|---|---|---|---|---|---|---|---|---|
| | Sample 1 | Sample 2 | Mean | Sample 1 | Sample 2 | Mean | | | | |
| 15 | 1660.7 | 1670.1 | 1665.4 | 2369.3 | 2396.6 | 2382.9 | 4048.3 | 32.4 | 86.7 | 8451.7 |
| 30 | 1444.6 | 1444.4 | 1444.5 | 2306.3 | 2280.2 | 2293.3 | 3737.7 | 29.9 | 88.4 | 8762.3 |
| 45 | 1030.3 | 1030.3 | 1030.3 | 1601.9 | 1622.9 | 1612.4 | 2642.7 | 21.1 | 91.8 | 9857.3 |
| **60** | 1068.7 | 1069.2 | 1068.9 | 1336.1 | 1354.3 | 1345.2 | 2414.1 | 19.3 | 91.4 | 10085.9 |
| 75 | 908.9 | 908.2 | 908.5 | 1160.7 | 1150.4 | 1155.6 | 2064.1 | 16.5 | 92.7 | 10435.9 |
| 90 | 615.3 | 601.9 | 608.6 | 797.0 | 809.7 | 803.3 | 1411.9 | 11.3 | 95.1 | 11088.1 |
| 105 | 477.3 | 475.8 | 476.6 | 531.5 | 529.2 | 530.3 | 1006.9 | 8.1 | 96.2 | 11493.1 |
| **120** | 277.7 | 279.4 | 278.6 | 299.1 | 303.6 | 301.4 | 579.9 | 4.6 | 97.8 | 11920.1 |
| 135 | 208.8 | 207.1 | 208.0 | 236.6 | 240.8 | 238.7 | 446.7 | 3.6 | 98.3 | 12053.3 |
| 150 | 154.3 | 154.1 | 154.2 | 186.4 | 186.4 | 186.4 | 340.6 | 2.7 | 98.8 | 12159.4 |
| 165 | 110.8 | 110.8 | 110.8 | 131.2 | 134.7 | 133.0 | 243.8 | 2.0 | 99.1 | 12256.2 |
| **180** | 65.4 | 67.5 | 66.4 | 91.1 | 88.9 | 90.0 | 156.4 | 1.3 | 99.5 | 12343.6 |

Table 35b. Partition/Degradation of **6-ACM** in primary sludge batch tests (4± 0.5°C)

| Time (min) | aqueous phase (ug) | | | solid phase (ug) | | | Total 6ACM (ug) | MOR & other metabolites (ug) |
|---|---|---|---|---|---|---|---|---|
| | Sample 1 | Sample 2 | Mean | Sample 1 | Sample 2 | Mean | | |
| 15 | 625 | 650 | 650 | - | - | - | 650 | 7801.7 |
| 30 | 575 | 575 | 575 | - | - | - | 575 | 8187.3 |
| 45 | 504 | 504 | 504 | - | - | - | 504 | 9353.3 |
| **60** | 437 | 437 | 437 | - | - | - | 437 | 9648.9 |
| 75 | 408 | 408 | 408 | - | - | - | 408 | 10027.9 |
| 90 | 360 | 345 | 360 | - | - | - | 360 | 10728.1 |
| 105 | 312 | 312 | 312 | - | - | - | 312 | 11181.1 |
| **120** | 242 | 242 | 242 | - | - | - | 242 | 11678.1 |
| 135 | 225 | 225 | 225 | - | - | - | 225 | 11828.3 |
| 150 | 154 | 154 | 154 | - | - | - | 154 | 12005.4 |
| 165 | 110 | 110 | 110 | - | - | - | 110 | 12146.2 |
| **180** | 57 | 57 | 57 | - | - | - | 57 | 12286.6 |

Table 35c. Partition/Degradation of **Morphine** in primary sludge batch tests (4± 0.5°C)

| Time (min) | aqueous phase (ug) | | | solid phase (ug) | | | Total MOR (ug) | Other metabolites (ug) |
|---|---|---|---|---|---|---|---|---|
| | Sample 1 | Sample 2 | Mean | Sample 1 | Sample 2 | Mean | | |
| 15 | 2.25 | 2.25 | 2.25 | - | - | - | 2.25 | 7799.45 |
| 30 | 2.76 | 2.76 | 2.76 | - | - | - | 2.76 | 8184.54 |
| 45 | 2.31 | 2.31 | 2.31 | - | - | - | 2.31 | 9350.99 |
| **60** | 1.71 | 1.71 | 1.71 | - | - | - | 1.71 | 9647.19 |
| 75 | 1.87 | 1.7 | 1.87 | - | - | - | 1.87 | 10026.03 |
| 90 | 1.5 | 1.5 | 1.5 | - | - | - | 1.5 | 10726.6 |
| 105 | 1.69 | 1.69 | 1.69 | - | - | - | 1.69 | 11179.41 |
| **120** | 1.54 | 1.54 | 1.54 | - | - | - | 1.54 | 11676.56 |
| 135 | 1.35 | 1.35 | 1.35 | - | - | - | 1.35 | 11826.95 |
| 150 | 1.12 | 1.12 | 1.12 | - | - | - | 1.12 | 12004.28 |
| 165 | 0.8 | 0.8 | 0.8 | - | - | - | 0.8 | 12145.4 |
| **180** | 0.51 | 0.51 | 0.51 | - | - | - | 0.51 | 12286.09 |

Table 36a. Partition/Degradation of **Heroin** in primary sludge batch tests (19± 0.5°C)

| Time (min) | aqueous phase (ug) | | | solid phase (ug) | | | Total HER | % HER | % removal | Residual |
|---|---|---|---|---|---|---|---|---|---|---|
| | Sample 1 | Sample 2 | Mean | Sample 1 | Sample 2 | Mean | | | | |
| 15 | 479.8 | 480.2 | 480.0 | 672.8 | 681.1 | 677.0 | 1157.0 | 9.3 | 96.2 | 11343.0 |
| 30 | 413.8 | 414.3 | 414.1 | 669.4 | 635.8 | 652.6 | 1066.7 | 8.5 | 96.7 | 11433.3 |
| 45 | 286.3 | 286.3 | 286.3 | 478.7 | 440.0 | 459.4 | 745.7 | 6.0 | 97.7 | 11754.3 |
| **60** | 272.0 | 280.7 | 276.4.0 | 0.0 | 0.0 | 0.0 | 276.4 | 2.2 | 97.8 | 12223.6 |
| 75 | 203.9 | 204.6 | 204.2 | 0.0 | 0.0 | 0.0 | 204.2 | 1.6 | 98.4 | 12295.8 |
| 90 | 168.1 | 167.3 | 167.7 | 0.0 | 0.0 | 0.0 | 167.7 | 1.3 | 98.7 | 12332.3 |
| 105 | 140.6 | 140.1 | 140.3 | 0.0 | 0.0 | 0.0 | 140.3 | 1.1 | 98.9 | 12359.7 |
| **120** | 80.1 | 80.7 | 80.4 | 0.0 | 0.0 | 0.0 | 80.4 | 0.6 | 99.4 | 12419.6 |
| 135 | 61.1 | 60.8 | 61.0 | 0.0 | 0.0 | 0.0 | 61.0 | 0.5 | 99.5 | 12439.0 |
| 150 | 34.8 | 34.6 | 34.7 | 0.0 | 0.0 | 0.0 | 34.7 | 0.3 | 99.7 | 12465.3 |
| 165 | 24.0 | 24.0 | 24.0 | 0.0 | 0.0 | 0.0 | 24.0 | 0.2 | 99.8 | 12476.0 |
| **180** | 14.7 | 14.6 | 14.7 | 0.0 | 0.0 | 0.0 | 14.7 | 0.1 | 99.9 | 12485.3 |

Table 36b. Partition/Degradation of **6-ACM** in primary sludge batch tests (19± 0.5°C)

| Time (min) | aqueous phase (ug) | | | solid phase (ug) | | | Total 6ACM (ug) | MOR & Other metabolites (ug) |
|---|---|---|---|---|---|---|---|---|
| | Sample 1 | Sample 2 | Mean | Sample 1 | Sample 2 | Mean | | |
| 15 | 125 | 125 | 125 | 175 | 175 | 175 | 300 | 11043 |
| 30 | 115 | 115 | 115 | 138 | 138 | 138 | 253 | 11180.3 |
| 45 | 105 | 105 | 105 | 168 | 168 | 168 | 273 | 11481.3 |
| **60** | 95 | 95 | 95 | 152 | 152 | 152 | 247 | 11976.6 |
| 75 | 119 | 119 | 119 | 119 | 119 | 119 | 221 | 12074.8 |
| 90 | 90 | 90 | 90 | 105 | 105 | 105 | 195 | 12137.3 |
| 105 | 78 | 78 | 78 | 91 | 91 | 91 | 169 | 12190.7 |
| **120** | 66 | 66 | 66 | 66 | 55 | 55 | 121 | 12298.6 |
| 135 | 54 | 54 | 54 | 63 | 63 | 63 | 117 | 12322 |
| 150 | 42 | 42 | 42 | 56 | 56 | 56 | 98 | 12367.3 |
| 165 | 30 | 30 | 30 | 40 | 40 | 40 | 70 | 12406 |
| **180** | 30 | 27 | 27 | 21 | 21 | 21 | 51 | 12434.3 |

Table 36c. Partition/Degradation of **Morphine** in primary sludge batch tests (19± 0.5°C)

| Time (min) | aqueous phase (ug) | | | solid phase (ug) | | | Total MOR (ug) | Other metabolites (ug) |
|---|---|---|---|---|---|---|---|---|
| | Sample 1 | Sample 2 | Mean | Sample 1 | Sample 2 | Mean | | |
| 15 | 5 | 5 | 5 | - | - | - | 5 | 11038 |
| 30 | 5.75 | 5.75 | 5.75 | - | - | - | 5.75 | 11174.55 |
| 45 | 4.83 | 4.83 | 4.83 | - | - | - | 4.83 | 11476.47 |
| **60** | 3.04 | 3.04 | 3.04 | - | - | - | 3.04 | 11973.56 |
| 75 | 4.59 | 4.59 | 4.59 | - | - | - | 4.59 | 12070.21 |
| 90 | 3.45 | 3.45 | 3.45 | - | - | - | 3.45 | 12133.85 |
| 105 | 3.51 | 3.51 | 3.51 | - | - | - | 3.51 | 12187.19 |
| **120** | 3.41 | 3.41 | 3.41 | - | - | - | 3.41 | 12295.19 |
| 135 | 3.78 | 3.69 | 3.78 | - | - | - | 3.78 | 12318.22 |
| 150 | 2.52 | 2.52 | 2.52 | - | - | - | 2.52 | 12364.78 |
| 165 | 1.95 | 1.8 | 1.85 | - | - | - | 1.85 | 12404.15 |
| **180** | 1.2 | 1.17 | 1.2 | - | - | - | 1.2 | 12433.1 |

Table 37a. Total degradation of **Heroin** in filtered primary sludge batch tests (19 ± 0.5°C)

| Time (min) | aqueous phase (ug) | | Total HER (ug) | % HER | % Removal | Residual |
|---|---|---|---|---|---|---|
| | Sample 1 | Sample 2 | | | | |
| 15 | 1735.1 | 1725.9 | 1730.5 | 13.8 | 86.2 | 10769.5 |
| 30 | 1499.5 | 1466.6 | 1483.0 | 11.9 | 88.1 | 11017.0 |
| 45 | 1350.2 | 1343.8 | 1347.0 | 10.8 | 89.2 | 11153.0 |
| **60** | 1056.5 | 1064.5 | 1060.5 | 8.5 | 91.5 | 11439.5 |
| 75 | 642.0 | 657.2 | 649.6 | 5.2 | 94.8 | 11850.4 |
| 90 | 592.8 | 599.7 | 596.3 | 4.8 | 95.2 | 11903.7 |
| 105 | 556.8 | 556.8 | 556.8 | 4.5 | 95.5 | 11943.2 |
| **120** | 376.1 | 376.1 | 376.1 | 3.0 | 97.0 | 12123.9 |
| 135 | 267.8 | 265.6 | 266.7 | 2.1 | 97.9 | 12233.3 |
| 150 | 209.8 | 210.3 | 210.1 | 1.7 | 98.3 | 12289.9 |
| 165 | 147.0 | 147.0 | 147.0 | 1.2 | 98.8 | 12353.0 |
| **180** | 92.6 | 89.3 | 91.0 | 0.7 | 99.3 | 12409.0 |

Table 37b. Total degradation of **6-ACM** in filtered primary sludge batch tests (19 ± 0.5°C)

| Time (min) | aqueous phase (ug) | | Total 6ACM (ug) | MOR & other metabolites (ug) |
|---|---|---|---|---|
| | Sample 1 | Sample 2 | | |
| 15 | 950 | 1075 | 1025 | 9744.5 |
| 30 | 1058 | 1127 | 1081 | 9936 |
| 45 | 1071 | 1092 | 1071 | 10082 |
| **60** | 1007 | 1007 | 1007 | 10432.5 |
| 75 | 969 | 986 | 986 | 10864.4 |
| 90 | 765 | 795 | 780 | 11123.7 |
| 105 | 637 | 663 | 650 | 11293.2 |
| **120** | 605 | 638 | 627 | 11496.9 |
| 135 | 531 | 531 | 531 | 11702.3 |
| 150 | 434 | 441 | 441 | 11848.9 |
| 165 | 330 | 330 | 330 | 12023 |
| **180** | 240 | 240 | 240 | 12169 |

Table 37c. Total degradation of **Morphine** in filtered primary sludge batch tests (19 ± 0.5°C)

| Time (min) | aqueous phase (ug) | | Total MOR (ug) | Total metabolites (ug) |
|---|---|---|---|---|
| | Sample 1 | Sample 2 | | |
| 15 | 25 | 25 | 25 | 9719.5 |
| 30 | 46 | 69 | 69 | 9867 |
| 45 | 63 | 63 | 63 | 10019 |
| **60** | 38 | 38 | 38 | 10394.5 |
| 75 | 68 | 68 | 68 | 10796.4 |
| 90 | 60 | 60 | 60 | 11063.7 |
| 105 | 52 | 52 | 52 | 11241.2 |
| **120** | 44 | 44 | 44 | 11452.9 |
| 135 | 45 | 45 | 45 | 11657.3 |
| 150 | 49 | 49 | 49 | 11799.9 |
| 165 | 30 | 30 | 30 | 11993 |
| **180** | 21 | 21 | 21 | 12148 |

Table 38a. Partition/Degradation of **Heroin** in SAF-1 sludge batch tests (19± 0.5°C)

| Time (min) | aqueous phase (ug) | | | solid phase (ug) | | | Total HER | % HER | % removal | Residual |
|---|---|---|---|---|---|---|---|---|---|---|
| | Sample 1 | Sample 2 | Mean | Sample 1 | Sample 2 | Mean | | | | |
| 15 | 55.4 | 55.4 | 55.4 | 80.1 | 80.0 | 80.0 | 135.4 | 1.1 | 99.6 | 12364.6 |
| 30 | 46.0 | 46.0 | 46.0 | 72.7 | 72.3 | 72.5 | 118.6 | 0.9 | 99.6 | 12381.4 |
| 45 | 45.1 | 49.1 | 47.1 | 66.1 | 63.4 | 64.8 | 111.9 | 0.9 | 99.6 | 12388.1 |
| **60** | 37.0 | 35.6 | 36.3 | 62.4 | 62.4 | 62.4 | 98.7 | 0.8 | 99.7 | 12401.3 |
| 75 | 35.9 | 31.0 | 33.4 | 37.6 | 38.3 | 38.0 | 71.4 | 0.6 | 99.7 | 12428.6 |
| 90 | 28.3 | 27.2 | 27.8 | 38.9 | 38.9 | 38.9 | 66.7 | 0.5 | 99.8 | 12433.3 |
| 105 | 21.4 | 21.8 | 21.6 | 29.2 | 27.9 | 28.6 | 50.1 | 0.4 | 99.8 | 12449.9 |
| **120** | 17.7 | 17.8 | 17.7 | 17.1 | 17.5 | 17.3 | 35.0 | 0.3 | 99.9 | 12465.0 |
| 135 | 16.1 | 16.3 | 16.2 | 21.7 | 21.1 | 21.4 | 37.6 | 0.3 | 99.9 | 12462.4 |
| 150 | 9.4 | 9.0 | 9.2 | 16.3 | 15.5 | 15.9 | 25.1 | 0.2 | 99.9 | 12474.9 |
| 165 | 7.5 | 6.5 | 7.0 | 6.3 | 6.3 | 6.3 | 13.3 | 0.1 | 99.9 | 12486.7 |
| **180** | 4.6 | 4.7 | 4.6 | 3.4 | 3.4 | 3.4 | 8.0 | 0.1 | 100.0 | 12492.0 |

Table 38b. Partition/Degradation of **Morphine** in SAF-1 sludge batch tests (19± 0.5°C)

| Time (min) | aqueous phase (ug) | | | solid phase (ug) | | | Total (ug) | Other metabolites (ug) |
|---|---|---|---|---|---|---|---|---|
| | Sample 1 | Sample 2 | Mean | Sample 1 | Sample 2 | Mean | | |
| 15 | 19.0 | 19.0 | 19.0 | 0.8 | 0.8 | 0.8 | 19.8 | 12344.9 |
| 30 | 19.1 | 2.1 | 10.6 | 2.1 | 2.3 | 2.3 | 12.9 | 12368.5 |
| 45 | 11.1 | 11.3 | 11.3 | 2.5 | 2.3 | 2.5 | 13.7 | 12374.5 |
| **60** | 15.8 | 16.7 | 16.2 | 4.2 | 4.2 | 4.2 | 20.3 | 12381.0 |
| 75 | 17.0 | 16.0 | 16.5 | 3.9 | 3.6 | 3.7 | 20.2 | 12408.4 |
| 90 | 13.4 | 13.4 | 13.4 | 3.5 | 3.6 | 3.5 | 16.8 | 12416.5 |
| 105 | 13.4 | 14.2 | 13.8 | 5.1 | 5.2 | 5.2 | 19.0 | 12430.9 |
| **120** | 14.0 | 14.3 | 14.2 | 3.4 | 3.3 | 3.4 | 17.5 | 12447.5 |
| 135 | 11.6 | 11.7 | 11.6 | 4.7 | 4.6 | 4.6 | 16.3 | 12446.1 |
| 150 | 10.0 | 9.9 | 10.0 | 3.3 | 3.3 | 3.3 | 13.3 | 12461.6 |
| 165 | 8.0 | 7.8 | 7.9 | 2.2 | 2.2 | 2.2 | 10.1 | 12476.6 |
| **180** | 5.1 | 5.3 | 5.2 | 0.7 | 0.7 | 0.7 | 5.9 | 12486.1 |

Table 39a. Partition/Degradation of **Heroin** in MSAF-1 sludge batch tests (19± 0.5°C)

| Time (min) | aqueous phase (ug) | | | solid phase (ug) | | | Total HER | % HER | % removal | Residual |
|---|---|---|---|---|---|---|---|---|---|---|
| | Sample 1 | Sample 2 | Mean | Sample 1 | Sample 2 | Mean | | | | |
| 15 | 53.3 | 51.1 | 52.2 | 8.3 | 8.1 | 8.2 | 60.4 | 0.5 | 99.6 | 12439.6 |
| 30 | 45.1 | 45.3 | 45.2 | 9.4 | 9.9 | 9.7 | 54.8 | 0.4 | 99.6 | 12445.2 |
| 45 | 39.1 | 37.3 | 38.2 | 10.8 | 9.4 | 10.1 | 48.3 | 0.4 | 99.7 | 12451.7 |
| **60** | 33.5 | 34.0 | 33.8 | 14.6 | 14.5 | 14.6 | 48.3 | 0.4 | 99.7 | 12451.7 |
| 75 | 28.5 | 28.4 | 28.5 | 11.2 | 11.2 | 11.2 | 39.7 | 0.3 | 99.8 | 12460.3 |
| 90 | 26.8 | 28.0 | 27.4 | 14.2 | 13.9 | 14.1 | 41.5 | 0.3 | 99.8 | 12458.5 |
| 105 | 23.9 | 23.5 | 23.7 | 12.3 | 12.5 | 12.4 | 36.1 | 0.3 | 99.8 | 12463.9 |
| **120** | 18.1 | 17.8 | 17.9 | 8.4 | 8.4 | 8.4 | 26.4 | 0.2 | 99.9 | 12473.6 |
| 135 | 13.0 | 13.8 | 13.4 | 7.2 | 6.6 | 6.9 | 20.3 | 0.2 | 99.9 | 12479.7 |
| 150 | 8.7 | 9.1 | 8.9 | 3.9 | 3.9 | 3.9 | 12.7 | 0.1 | 99.9 | 12487.3 |
| 165 | 6.6 | 6.6 | 6.6 | 2.9 | 2.9 | 2.9 | 9.5 | 0.1 | 99.9 | 12490.5 |
| **180** | 4.4 | 4.4 | 4.4 | 1.7 | 1.7 | 1.7 | 6.1 | 0.0 | 100.0 | 12493.9 |

Table 39b. Partition/Degradation of **Morphine** in MSAF-1 sludge batch tests (19± 0.5°C)

| Time (min) | aqueous phase (ug) | | | solid phase (ug) | | | Total MOR (ug) | Other metabolites (ug) |
|---|---|---|---|---|---|---|---|---|
| | Sample 1 | Sample 2 | Mean | Sample 1 | Sample 2 | Mean | | |
| 15 | 47.3 | 44.8 | 46.0 | 0.3 | 0.3 | 0.3 | 46.0 | 12393.6 |
| 30 | 46.5 | 46.7 | 46.5 | 0.2 | 0.2 | 0.2 | 46.7 | 12398.5 |
| 45 | 34.9 | 34.9 | 34.9 | 0.4 | 0.4 | 0.4 | 35.3 | 12416.4 |
| **60** | 40.1 | 42.6 | 41.4 | 1.0 | 0.8 | 1.0 | 42.2 | 12409.5 |
| 75 | 46.4 | 43.5 | 45.1 | 0.9 | 0.9 | 0.9 | 45.9 | 12414.4 |
| 90 | 30.9 | 31.8 | 31.4 | 2.3 | 2.3 | 2.3 | 33.6 | 12424.9 |
| 105 | 38.2 | 37.7 | 38.0 | 2.0 | 2.0 | 2.0 | 39.9 | 12424.0 |
| **120** | 33.6 | 34.8 | 34.2 | 3.5 | 3.5 | 3.5 | 37.6 | 12436.0 |
| 135 | 28.1 | 28.6 | 28.4 | 4.4 | 4.5 | 4.4 | 32.9 | 12446.9 |
| 150 | 27.4 | 26.4 | 27.0 | 1.3 | 1.3 | 1.3 | 28.3 | 12459.0 |
| 165 | 19.6 | 19.6 | 19.6 | 0.9 | 0.9 | 0.9 | 20.5 | 12470.1 |
| **180** | 13.6 | 13.6 | 13.6 | 0.4 | 0.4 | 0.4 | 14.0 | 12479.9 |

Table 40a. Partition/Degradation of **Heroin** in Humus sludge batch tests (19± 0.5°C)

| Time (min) | aqueous phase (ug) | | | solid phase (ug) | | | Total HER | % HER | % removal | Residual |
|---|---|---|---|---|---|---|---|---|---|---|
| | Sample 1 | Sample 2 | Mean | Sample 1 | Sample 2 | Mean | | | | |
| 15 | 1562.8 | 1514.0 | 1538.4 | 721.9 | 721.9 | 721.9 | 2260.3 | 18.1 | 87.7 | 10239.7 |
| 30 | 1124.7 | 1105.9 | 1115.3 | 554.6 | 629.3 | 591.9 | 1707.2 | 13.7 | 91.1 | 10792.8 |
| 45 | 918.6 | 918.9 | 918.7 | 623.3 | 623.3 | 623.3 | 1542.1 | 12.3 | 92.7 | 10957.9 |
| **60** | 775.6 | 789.2 | 782.4 | 488.8 | 502.9 | 495.9 | 1278.3 | 10.2 | 93.7 | 11221.7 |
| 75 | 562.2 | 566.2 | 564.2 | 319.6 | 308.1 | 313.9 | 878.1 | 7.0 | 95.5 | 11621.9 |
| 90 | 354.3 | 388.6 | 371.5 | 315.2 | 324.5 | 319.8 | 691.3 | 5.5 | 97.0 | 11808.7 |
| 105 | 318.3 | 318.3 | 318.3 | 267.1 | 269.1 | 268.1 | 586.4 | 4.7 | 97.5 | 11913.6 |
| **120** | 258.4 | 275.7 | 267.1 | 201.1 | 201.1 | 201.1 | 468.2 | 3.7 | 97.9 | 12031.8 |
| 135 | 131.8 | 131.8 | 131.8 | 163.1 | 130.7 | 146.9 | 278.7 | 2.2 | 98.9 | 12221.3 |
| 150 | 116.5 | 118.5 | 117.5 | 98.1 | 97.1 | 97.6 | 215.1 | 1.7 | 99.1 | 12284.9 |
| 165 | 107.3 | 107.3 | 107.3 | 80.0 | 80.3 | 80.2 | 187.5 | 1.5 | 99.1 | 12312.5 |
| **180** | 60.7 | 60.7 | 60.7 | 48.7 | 47.4 | 48.0 | 108.8 | 0.9 | 99.5 | 12391.2 |

Table 40b. Partition/Degradation of **6ACM** in Humus sludge batch tests (19± 0.5°C)

| Time (min) | aqueous phase (ug) | | | solid phase (ug) | | | Total (ug) | Other metabolites (ug) |
|---|---|---|---|---|---|---|---|---|
| | Sample 1 | Sample 2 | Mean | Sample 1 | Sample 2 | Mean | | |
| 15 | 197.5 | 175.8 | 186.8 | 5.0 | 4.8 | 4.8 | 191.5 | 10048.2 |
| 30 | 173.7 | 185.4 | 179.6 | 30.8 | 31.3 | 31.1 | 210.7 | 10582.1 |
| 45 | 148.3 | 150.6 | 149.5 | 32.6 | 32.6 | 32.6 | 182.1 | 10775.8 |
| **60** | 134.7 | 134.5 | 134.5 | 49.4 | 50.5 | 50.0 | 184.5 | 11037.2 |
| 75 | 131.8 | 131.8 | 131.8 | 9.2 | 9.5 | 9.4 | 141.1 | 11480.8 |
| 90 | 102.5 | 100.1 | 101.3 | 21.3 | 21.5 | 21.5 | 122.7 | 11686.0 |
| 105 | 95.7 | 94.1 | 94.9 | 18.5 | 19.0 | 18.7 | 113.6 | 11800.0 |
| **120** | 69.7 | 70.2 | 70.0 | 13.3 | 13.3 | 13.3 | 83.3 | 11948.5 |
| 135 | 56.4 | 55.4 | 56.0 | 10.4 | 9.7 | 10.1 | 66.1 | 12155.2 |
| 150 | 45.3 | 39.1 | 38.7 | 7.6 | 7.4 | 7.5 | 46.2 | 12238.7 |
| 165 | 34.7 | 34.7 | 34.7 | 3.7 | 3.7 | 3.7 | 38.4 | 12274.1 |
| **180** | 26.2 | 26.2 | 26.2 | 1.8 | 1.8 | 1.8 | 28.0 | 12363.2 |

Table 40c. Partition/Degradation of **Morphine** in Humus sludge batch tests (19± 0.5°C)

| Time (min) | aqueous phase (ug) | | | solid phase (ug) | | | Total (ug) | Other metabolites (ug) |
|---|---|---|---|---|---|---|---|---|
| | Sample 1 | Sample 2 | Mean | Sample 1 | Sample 2 | Mean | | |
| 15 | 8.0 | 7.8 | 8.0 | 0.0 | 0.0 | 0.0 | 8.0 | 10040.2 |
| 30 | 8.1 | 7.6 | 7.8 | 0.9 | 1.2 | 1.2 | 8.7 | 10573.4 |
| 45 | 7.8 | 8.2 | 8.0 | 1.5 | 1.5 | 1.5 | 9.5 | 10766.4 |
| **60** | 7.0 | 7.0 | 7.0 | 3.4 | 3.8 | 3.6 | 10.6 | 11026.6 |
| 75 | 6.5 | 6.5 | 6.5 | 1.0 | 1.0 | 1.0 | 7.5 | 11473.3 |
| 90 | 3.9 | 3.9 | 3.9 | 3.0 | 3.2 | 3.0 | 6.9 | 11679.1 |
| 105 | 4.0 | 4.9 | 4.6 | 3.5 | 4.2 | 3.8 | 8.3 | 11791.7 |
| **120** | 4.1 | 3.3 | 3.7 | 2.9 | 2.9 | 2.9 | 6.6 | 11941.9 |
| 135 | 3.4 | 3.4 | 3.4 | 3.9 | 3.6 | 3.8 | 7.2 | 12148.0 |
| 150 | 2.8 | 2.8 | 2.8 | 3.4 | 3.4 | 3.4 | 6.2 | 12232.5 |
| 165 | 1.9 | 1.9 | 1.9 | 2.1 | 1.9 | 2.0 | 3.9 | 12270.3 |
| **180** | 1.4 | 1.4 | 1.4 | 1.2 | 1.2 | 1.2 | 2.6 | 12360.6 |

Table 41a. Total degradation of **Heroin** in Stoke Bardoph effluent batch tests (19 ± 0.5°C)

| Time (min) | aqueous phase (ug) | | Total HER (ug ) | % HER | % Removal | Residual |
|---|---|---|---|---|---|---|
| | Sample 1 | Sample 2 | | | | |
| 15 | 1835.3 | 1836.8 | 1836.0 | 14.7 | 85.3 | 10664.0 |
| 30 | 1662.6 | 1652.5 | 1657.5 | 13.3 | 86.7 | 10842.5 |
| 45 | 1274.7 | 1248.1 | 1261.4 | 10.1 | 89.9 | 11238.6 |
| **60** | 939.4 | 946.9 | 943.2 | 7.5 | 92.5 | 11556.8 |
| 75 | 847.6 | 833.7 | 840.6 | 6.7 | 93.3 | 11659.4 |
| 90 | 765.9 | 769.7 | 767.8 | 6.1 | 93.9 | 11732.2 |
| 105 | 603.7 | 590.9 | 597.3 | 4.8 | 95.2 | 11902.7 |
| **120** | 516.7 | 496.3 | 506.5 | 4.1 | 95.9 | 11993.5 |
| 135 | 374.9 | 380.8 | 377.8 | 3.0 | 97.0 | 12122.2 |
| 150 | 257.0 | 286.2 | 271.6 | 2.2 | 97.8 | 12228.4 |
| 165 | 175.8 | 175.8 | 175.8 | 1.4 | 98.6 | 12324.2 |
| **180** | 97.2 | 98.4 | 97.8 | 0.8 | 99.2 | 12402.2 |

Table 41b. Total degradation of **6-ACM** in Stoke Baldoph effluent batch tests (19 ± 0.5°C)

| Time (min) | aqueous phase (ug) | | Total 6ACM (ug) | MOR & other metabolites (ug) |
|---|---|---|---|---|
| | Sample 1 | Sample 2 | | |
| 15 | 975.0 | 950.0 | 962.5 | 9701.5 |
| 30 | 1058.0 | 1127.0 | 1092.5 | 9750 |
| 45 | 966.0 | 966.0 | 966 | 10272.6 |
| **60** | 893.0 | 912.0 | 902.5 | 10654.3 |
| 75 | 901.0 | 884.0 | 892.5 | 10766.9 |
| 90 | 795.0 | 780.0 | 787.5 | 10944.7 |
| 105 | 780.0 | 767.0 | 773.5 | 11129.2 |
| **120** | 671.0 | 649.0 | 660 | 11333.5 |
| 135 | 648.0 | 702.0 | 675 | 11447.2 |
| 150 | 399.0 | 406.0 | 402.5 | 11825.9 |
| 165 | 305.0 | 305.0 | 305 | 12019.2 |
| **180** | 201.0 | 195.0 | 198 | 12204.2 |

Table 41c. Total degradation of **Morphine** in Stoke Baldoph effluent batch tests (19 ± 0.5°C)

| Time (min) | aqueous phase (ug) | | Total MOR (ug) | Total metabolites (ug) |
|---|---|---|---|---|
| | Sample 1 | Sample 2 | | |
| 15 | 50 | 50 | 50 | 9651.5 |
| 30 | 69 | 69 | 69 | 9681 |
| 45 | 63 | 63 | 63 | 10209.6 |
| **60** | 38 | 38 | 38 | 10616.3 |
| 75 | 68 | 85 | 77 | 10689.9 |
| 90 | 60 | 60 | 60 | 10884.7 |
| 105 | 52 | 52 | 52 | 11077.2 |
| **120** | 55 | 55 | 55 | 11278.5 |
| 135 | 45 | 45 | 45 | 11402.2 |
| 150 | 35 | 35 | 35 | 11790.9 |
| 165 | 25 | 25 | 25 | 11994.2 |
| **180** | 18 | 18 | 18 | 12186.2 |

Table 42a. Partition/Degradation of **Diazepam** in primary sludge batch tests (4± 0.5°C)

| Time (min) | aqueous phase (ug) | | | solid phase (ug) | | | Total DIAZ | % DIAZ | % removal | Residual |
|---|---|---|---|---|---|---|---|---|---|---|
| | Sample 1 | Sample 2 | Mean | Sample 1 | Sample 2 | Mean | | | | |
| 15 | 916.6 | 911.9 | 914.2 | 3866.1 | 3866.1 | 3866.1 | 38.2 | 92.7 | 4780.4 | 7719.6 |
| 30 | 835.3 | 829.3 | 832.3 | 3877.8 | 3879.4 | 3878.6 | 37.7 | 93.3 | 4710.9 | 7789.1 |
| 45 | 738.6 | 743.7 | 741.2 | 3989.7 | 3977.0 | 3983.4 | 37.8 | 94.1 | 4724.5 | 7775.5 |
| **60** | 687.2 | 687.2 | 687.2 | 3673.4 | 3680.8 | 3677.1 | 34.9 | 94.5 | 4364.3 | 8135.7 |
| 75 | 294.9 | 294.9 | 294.9 | 3286.7 | 3347.6 | 3317.1 | 28.9 | 97.6 | 3612.0 | 8888.0 |
| 90 | 266.3 | 266.3 | 266.3 | 2958.0 | 2958.0 | 2958.0 | 25.8 | 97.9 | 3224.3 | 9275.7 |
| 105 | 228.6 | 230.8 | 229.7 | 2603.8 | 2603.8 | 2603.8 | 22.7 | 98.2 | 2833.5 | 9666.5 |
| **120** | 192.6 | 193.4 | 193.0 | 2203.2 | 2203.2 | 2203.2 | 19.2 | 98.5 | 2396.2 | 10103.8 |
| 135 | 149.8 | 149.8 | 149.8 | 1860.0 | 1860.0 | 1860.0 | 16.1 | 98.8 | 2009.9 | 10490.1 |
| 150 | 120.5 | 120.6 | 120.6 | 1446.7 | 1446.7 | 1446.7 | 12.5 | 99.0 | 1567.3 | 10932.7 |
| 165 | 76.1 | 79.2 | 77.6 | 1065.5 | 1065.5 | 1065.5 | 9.1 | 99.4 | 1143.2 | 11356.8 |
| **180** | 41.5 | 41.5 | 41.5 | 643.5 | 643.5 | 643.5 | 5.5 | 99.7 | 685.0 | 11815.0 |

Table 42b. Partition/Degradation of **Nor-dazepam** in primary sludge batch tests (4± 0.5°C)

| Time (min) | aqueous phase (ug in10 mL) | | | solid phase (ug in10 mL) | | | Total CE (ug in10 mL) | Other metabolites |
|---|---|---|---|---|---|---|---|---|
| | Sample 1 | Sample 2 | Mean | Sample 1 | Sample 2 | Mean | | |
| 15 | 1 | 1 | 1 | 0 | 0 | 0 | 2 | 227 |
| 30 | 1 | 1 | 1 | 0 | 0 | 0 | 2 | 228 |
| 45 | 1 | 1 | 1 | 0 | 0 | 0 | 2 | 235 |
| **60** | 1 | 1 | 1 | 1 | 1 | 1 | 2 | 234 |
| 75 | 1 | 1 | 1 | 0 | 0 | 0 | 2 | 258 |
| 90 | 1 | 1 | 1 | 0 | 0 | 0 | 1 | 258 |
| 105 | 1 | 1 | 1 | 0 | 0 | 0 | 1 | 261 |
| **120** | 1 | 1 | 1 | 0 | 0 | 0 | 1 | 261 |
| 135 | 1 | 1 | 1 | 0 | 0 | 0 | 1 | 265 |
| 150 | 1 | 1 | 1 | 0 | 0 | 0 | 1 | 267 |
| 165 | 1 | 1 | 1 | 0 | 0 | 0 | 1 | 289 |
| **180** | 1 | 1 | 1 | 0 | 0 | 0 | 1 | 304 |

Table 43. Partition/Degradation of **Diazepam** in primary sludge batch tests (19± 0.5°C)

| Time (min) | aqueous phase (ug) | | | solid phase (ug) | | | Total DIAZ | % DIAZ | % removal | Residual |
|---|---|---|---|---|---|---|---|---|---|---|
| | Sample 1 | Sample 2 | Mean | Sample 1 | Sample 2 | Mean | | | | |
| 15 | 2019.1 | 2012.2 | 2015.7 | 4919.3 | 4919.3 | 4919.3 | 6934.9 | 55.5 | 83.9 | 5565.1 |
| 30 | 1543.6 | 1550.3 | 1547.0 | 4504.2 | 4504.2 | 4504.2 | 6051.2 | 48.4 | 87.6 | 6448.8 |
| 45 | 1400.6 | 1401.7 | 1401.2 | 2933.2 | 2933.2 | 2933.2 | 4334.4 | 34.7 | 88.8 | 8165.6 |
| **60** | 1093.9 | 1093.9 | 1093.9 | 3229.7 | 3229.7 | 3229.7 | 4323.6 | 34.6 | 91.2 | 8176.4 |
| 75 | 979.7 | 980.1 | 979.9 | 3028.4 | 3028.4 | 3028.4 | 4008.3 | 32.1 | 92.2 | 8491.7 |
| 90 | 655.3 | 651.9 | 653.6 | 2690.2 | 2690.2 | 2690.2 | 3343.8 | 26.8 | 94.8 | 9156.2 |
| 105 | 606.9 | 588.7 | 597.8 | 2104.9 | 2104.9 | 2104.9 | 2702.7 | 21.6 | 95.2 | 9797.3 |
| **120** | 439.4 | 439.5 | 439.4 | 1887.4 | 1887.4 | 1887.4 | 2326.8 | 18.6 | 96.5 | 10173.2 |
| 135 | 306.9 | 308.5 | 307.7 | 1506.1 | 1506.1 | 1506.1 | 1813.8 | 14.5 | 97.5 | 10686.2 |
| 150 | 191.1 | 190.4 | 190.8 | 1301.3 | 1301.3 | 1301.3 | 1492.0 | 11.9 | 98.5 | 11008.0 |
| 165 | 118.8 | 118.8 | 118.8 | 883.8 | 883.8 | 883.8 | 1002.6 | 8.0 | 99.0 | 11497.4 |
| **180** | 37.1 | 36.9 | 37.0 | 508.1 | 508.1 | 508.1 | 545.1 | 4.4 | 99.7 | 11954.9 |

Table 44. Total degradation of **Diazepam** in filtered primary sludge batch tests (19 ± 0.5°C)

| Time (min) | aqueous phase (ug) | | Total DIAZ (ug) | % DIAZ | % Removal | Residual |
|---|---|---|---|---|---|---|
| | Sample 1 | Sample 2 | | | | |
| 15 | 11454.1 | 11408.3 | 11431.2 | 91.4 | 8.6 | 1068.8 |
| 30 | 9701.1 | 9701.1 | 9701.1 | 77.6 | 22.4 | 2798.9 |
| 45 | 9808.8 | 9808.8 | 9808.8 | 78.5 | 21.5 | 2691.2 |
| **60** | 7803.9 | 7807.9 | 7805.9 | 62.4 | 37.6 | 4694.1 |
| 75 | 6852.8 | 6852.8 | 6852.8 | 54.8 | 45.2 | 5647.2 |
| 90 | 5620.6 | 5592.6 | 5606.6 | 44.9 | 55.1 | 6893.4 |
| 105 | 4864.4 | 4874.7 | 4869.5 | 39.0 | 61.0 | 7630.5 |
| **120** | 4081.6 | 4081.1 | 4081.3 | 32.7 | 67.3 | 8418.7 |
| 135 | 3252.1 | 3244.4 | 3248.2 | 26.0 | 74.0 | 9251.8 |
| 150 | 2506.8 | 2520.3 | 2513.5 | 20.1 | 79.9 | 9986.5 |
| 165 | 1774.3 | 1775.0 | 1774.7 | 14.2 | 85.8 | 10725.3 |
| **180** | 1039.5 | 1034.3 | 1036.9 | 8.3 | 91.7 | 11463.1 |

Table 45. Partition/Degradation of **Diazepam** in SAF-1sludge batch tests (19± 0.5°C)

| Time (min) | aqueous phase (ug) | | | solid phase (ug) | | | Total DIAZ | % DIAZ | % removal | Residual |
|---|---|---|---|---|---|---|---|---|---|---|
| | Sample 1 | Sample 2 | Mean | Sample 1 | Sample 2 | Mean | | | | |
| 15 | 131.2 | 131.2 | 131.2 | 447.1 | 447.1 | 447.1 | 578.3 | 4.6 | 99.0 | 11921.7 |
| 30 | 120.7 | 120.7 | 120.7 | 400.3 | 400.3 | 400.3 | 521.0 | 4.2 | 99.0 | 11979.0 |
| 45 | 106.7 | 106.4 | 106.5 | 349.3 | 349.3 | 349.3 | 455.9 | 3.6 | 99.1 | 12044.1 |
| **60** | 93.3 | 94.3 | 93.8 | 280.2 | 283.0 | 281.6 | 375.4 | 3.0 | 99.2 | 12124.6 |
| 75 | 83.3 | 83.3 | 83.3 | 254.8 | 254.8 | 254.8 | 338.2 | 2.7 | 99.3 | 12161.8 |
| 90 | 70.8 | 71.2 | 71.0 | 209.5 | 208.0 | 208.8 | 279.8 | 2.2 | 99.4 | 12220.2 |
| 105 | 60.6 | 60.4 | 60.5 | 168.8 | 168.8 | 168.8 | 229.3 | 1.8 | 99.5 | 12270.7 |
| **120** | 50.0 | 51.0 | 50.5 | 143.3 | 141.3 | 142.3 | 192.8 | 1.5 | 99.6 | 12307.2 |
| 135 | 37.2 | 36.3 | 36.8 | 111.9 | 113.7 | 112.8 | 149.6 | 1.2 | 99.7 | 12350.4 |
| 150 | 28.6 | 28.6 | 28.6 | 84.8 | 84.8 | 84.8 | 113.4 | 0.9 | 99.8 | 12386.6 |
| 165 | 11.8 | 11.8 | 11.8 | 58.4 | 58.4 | 58.4 | 70.2 | 0.6 | 99.9 | 12429.8 |
| **180** | 6.9 | 6.9 | 6.9 | 33.1 | 33.1 | 33.1 | 40.0 | 0.3 | 99.9 | 12460.0 |

Table 46. Partition/Degradation of **Diazepam** in MSAF-1sludge batch tests (19± 0.5°C)

| Time (min) | aqueous phase (ug) | | | solid phase (ug) | | | Total DIAZ | % DIAZ | % removal | Residual |
|---|---|---|---|---|---|---|---|---|---|---|
| | Sample 1 | Sample 2 | Mean | Sample 1 | Sample 2 | Mean | | | | |
| 15 | 1811.6 | 1824.8 | 1818.2 | 4640.2 | 4623.0 | 4631.6 | 6449.8 | 51.6 | 85.5 | 6050.2 |
| 30 | 1368.1 | 1409.7 | 1388.9 | 3924.6 | 3924.6 | 3924.6 | 5313.6 | 42.5 | 88.9 | 7186.4 |
| 45 | 1251.6 | 1231.6 | 1241.6 | 3702.0 | 3745.2 | 3723.6 | 4965.2 | 39.7 | 90.1 | 7534.8 |
| **60** | 1080.7 | 1080.7 | 1080.7 | 3010.2 | 2993.3 | 3001.7 | 4082.5 | 32.7 | 91.4 | 8417.5 |
| 75 | 804.2 | 829.6 | 816.9 | 2466.9 | 2474.1 | 2470.5 | 3287.4 | 26.3 | 93.5 | 9212.6 |
| 90 | 536.9 | 553.4 | 545.1 | 2280.3 | 2333.0 | 2306.7 | 2851.8 | 22.8 | 95.6 | 9648.2 |
| 105 | 525.7 | 521.4 | 523.6 | 1817.9 | 1811.5 | 1814.7 | 2338.2 | 18.7 | 95.8 | 10161.8 |
| **120** | 427.7 | 444.8 | 436.2 | 1511.8 | 1511.8 | 1511.8 | 1948.0 | 15.6 | 96.5 | 10552.0 |
| 135 | 247.5 | 247.2 | 247.3 | 1196.0 | 1196.0 | 1196.0 | 1443.3 | 11.5 | 98.0 | 11056.7 |
| 150 | 147.9 | 147.9 | 147.9 | 945.0 | 948.3 | 946.6 | 1094.5 | 8.8 | 98.8 | 11405.5 |
| 165 | 96.5 | 96.9 | 96.7 | 654.1 | 654.5 | 654.3 | 751.0 | 6.0 | 99.2 | 11749.0 |
| **180** | 73.0 | 72.8 | 72.9 | 382.9 | 384.3 | 383.6 | 456.5 | 3.7 | 99.4 | 12043.5 |

Table 47. Partition/Degradation of **Diazepam** in HS- sludge batch tests (19± 0.5°C)

| Time (min) | aqueous phase (ug) | | | solid phase (ug) | | | Total DIAZ | % DIAZ | % removal | Residual |
|---|---|---|---|---|---|---|---|---|---|---|
| | Sample 1 | Sample 2 | Mean | Sample 1 | Sample 2 | Mean | | | | |
| 15 | 66.1 | 66.1 | 66.1 | 21.2 | 21.2 | 21.2 | 87.3 | 0.7 | 99.5 | 12412.7 |
| 30 | 61.9 | 62.0 | 61.9 | 20.3 | 20.3 | 20.3 | 82.3 | 0.7 | 99.5 | 12417.7 |
| 45 | 61.3 | 61.3 | 61.3 | 19.9 | 19.7 | 19.8 | 81.0 | 0.6 | 99.5 | 12419.0 |
| **60** | 54.0 | 54.0 | 54.0 | 19.5 | 19.4 | 19.5 | 73.5 | 0.6 | 99.6 | 12426.5 |
| 75 | 47.4 | 47.2 | 47.3 | 19.3 | 19.3 | 19.3 | 66.6 | 0.5 | 99.6 | 12433.4 |
| 90 | 43.1 | 43.2 | 43.1 | 19.0 | 18.8 | 18.9 | 62.0 | 0.5 | 99.7 | 12438.0 |
| 105 | 41.9 | 41.9 | 41.9 | 18.9 | 18.8 | 18.9 | 60.7 | 0.5 | 99.7 | 12439.3 |
| **120** | 42.3 | 42.3 | 42.3 | 18.9 | 18.7 | 18.8 | 61.1 | 0.5 | 99.7 | 12438.9 |
| 135 | 31.5 | 31.8 | 31.6 | 19.3 | 19.4 | 19.3 | 50.9 | 0.4 | 99.7 | 12449.1 |
| 150 | 22.9 | 22.9 | 22.9 | 19.5 | 19.5 | 19.5 | 42.4 | 0.3 | 99.8 | 12457.6 |
| 165 | 23.2 | 23.2 | 23.2 | 19.4 | 19.5 | 19.5 | 42.6 | 0.3 | 99.8 | 12457.4 |
| **180** | 20.3 | 20.3 | 20.3 | 18.9 | 19.1 | 19.0 | 39.3 | 0.3 | 99.8 | 12460.7 |

Table 48. Total degradation of **Diazepam** in Stoke Bardolph effluent batch tests (19 ± 0.5°C)

| Time (min) | aqueous phase (ug) | | Total DIAZ (ug) | % DIAZ | % Removal | Residual |
|---|---|---|---|---|---|---|
| | Sample 1 | Sample 2 | | | | |
| 15 | 11989.3 | 11951.7 | 11970.5 | 95.8 | 4.2 | 529.5 |
| 30 | 9527.2 | 9538.9 | 9533.0 | 76.3 | 23.7 | 2967 |
| 45 | 8060.3 | 8060.3 | 8060.3 | 64.5 | 35.5 | 4439.7 |
| **60** | 7116.3 | 7146.4 | 7131.4 | 57.1 | 42.9 | 5368.6 |
| 75 | 6321.8 | 6314.1 | 6317.9 | 50.5 | 49.5 | 6182.1 |
| 90 | 5433.1 | 5432.9 | 5433.0 | 43.5 | 56.5 | 7067 |
| 105 | 4707.5 | 4711.2 | 4709.3 | 37.7 | 62.3 | 7790.7 |
| **120** | 4042.8 | 4005.8 | 4024.3 | 32.2 | 67.8 | 8475.7 |
| 135 | 3310.9 | 3315.1 | 3313.0 | 26.5 | 73.5 | 9187 |
| 150 | 2582.6 | 2595.4 | 2589.0 | 20.7 | 79.3 | 9911 |
| 165 | 1772.0 | 1776.4 | 1774.2 | 14.2 | 85.8 | 10725.8 |
| **180** | 1044.5 | 1042.3 | 1043.4 | 8.3 | 91.7 | 11456.6 |

# Appendix 5 – Sewage Treatment Work Process Calculations [292]

## 1.1 Primary Settlement Tank

| | | |
|---|---|---|
| Flow to PST | | |
| DWF | 63 (=average / 1.25) | $m^3/d$ |
| Average | 78 | |
| FFT | 360 | |
| Design recirc. | 250.6 (2.9 l/s) | |
| Temporary Recirc | 276.5 (3.2 l/s) | |
| Dimensions of PST | | |
| No. | 1 | |
| Diameter | 6 | m |
| Side Wall Depth | 1.7 | m |
| Floor Slope | $60^o$ | |
| Cone Depth | = Tan60 x pi/180 x 6/2 = 5.2 | |
| Total SA | $=pi \times (6/2)^2 = 28.3$ | $m^2$ |
| Total Volume | $= pi \times (6/2)^2 \times 1.7 + (pi \times (6/2)^2 \times 5.2/3 \times 0.7) = 82.5$ (30% of cone for sludge) | $m^3$ |

Hydraulic loading rate (m/h) = flow to tanks ($m^3$/h) / total SA ($m^2$) Retention time (h) = flow to tanks ($m^3$/h) / total volume of tanks ($m^3$)

## 1.2 Filter

| | | |
|---|---|---|
| Flow to filters | | |
| DWF | 63 (=average / 1.25) | $m^3/d$ |
| Average | 78 | |
| FFT | 360 | |
| Design recirc. | 250.6 (2.9 l/s) | |
| Temporary Recirc | 276.5 (3.2 l/s) | |
| Average BOD Conc. | 9.8 (based on crude loads, assuming 25% removal in PST) | mg/l |
| Average Ammonia Conc. | 5 (based on crude loads, assuming 50% removal in PST) | mg/l |
| Dimensions | | |
| No. | 1 | |
| Diameter | 11.3 | m |
| Side Wall Depth | 1.7 | m |
| Total SA | $=pi \times (11.3/2)^2 = 100$ | $m^2$ |
| Total Volume | $=pi \times (20/2)^2 \times 1.7 = 170$* | $m^3$ |
| *Neglects centre column | | |

Wetting rate ($m^3/m^2/d$) = flow to tanks ($m^3$/d) / total SA ($m^2$)

Required Volume for plastic media filter calculated using WEF MOP 8 calc for combined filters to meet an effluent quality of 5 mg/l (on a MAC and a 95%ile basis)

## 1.3 Humus Tank

| Flow to Humus Tank | | |
|---|---|---|
| DWF | 63 (=average / 1.25) | $m^3/d$ |
| Average | 78 | |
| FFT | 360 | |
| Design recirc. | 250.6 (2.9 l/s) | |
| Temporary Recirc | 276.5 (3.2 l/s) | |
| Dimensions of Humus Tank | | |
| No. | 1 | |
| Diameter | 5 | m |
| Side Wall Depth | 2 | m |
| Floor Slope | $60^o$ | |
| Cone Depth | = Tan60 x pi/180 x 5/2 = 4.3 | |
| Total SA | $=pi \times (5/2)^2 = 19.6$ | $m^2$ |
| Total Volume | $= pi \times (6/2)^2 \times 2 + (pi \times (6/2)^2 \times 4.3/3 \times 0.7) = 45.8$ (30% of cone for sludge) | $m^3$ |

Hydraulic loading rate (m/h) = flow to tanks ($m^3$/h) / total SA ($m^2$) Retention time (h) = flow to tanks ($m^3$/h) / total volume of tanks ($m^3$)

## 1.4 Reed Beds

| Reed Beds | | |
|---|---|---|
| DWF | 63 (=average / 1.25) | $m^3/d$ |
| Average | 78 | |
| FFT | 360 | |
| Design recirc. | 0 | |
| Temporary Recirc | 0 | |
| Base PE | 1600 non residents | |
| Dimensions of Humus Tank | | |
| No. | 2 | |
| Dimensions | 24m x 12.5m | m |
| Side Wall Depth | 2 | m |
| Total Area | $600m^2$ | $m^2$ |

Required area = 1 $m^2$/PE x PE/3 (only on site for 1/3 time) = 1 x 1600 / 3 = 530 $_{m^2}$