Tampere University of Applied Sciences
Degree Programme in Environmental Engineering

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Final Thesis

Biodegradability analysis of pharmaceuticals used in developing countries; screening with OxiTop ® - C 110

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Commissioned by TUT - Tampere University of Technology, Department of Chemistry and Environmental Engineering, Finland, Prof. Tuula Tuhkanen

Tampere, 30.10.2010
Tampere University of Applied Sciences, Environmental Engineering, Finland

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Pages	73
Graduation time	2011
Thesis supervisor	Senior Lecturer Eeva-Liisa Viskari, Ph.D.
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ABSTRACT

Recently, a significant number of studies regarding the environmental occurrence and fate of pharmaceuticals and personal care products (PPCPs) used in developed countries has been published. In the developing world, different therapeutic groups are frequently used without the knowledge of their behavior in the aquatic and terrestrial ecosystems. Great number of pharmaceuticals detected from the natural ecosystems around the world and the reported impact on non-target organisms suggest that PPCPs are potent and persistent environmental pollutants.

The herein presented study, conducted with the support of pharmaceutical company in Nairobi; Kenya, analyzed the biodegradation potential of seventeen selected pharmaceuticals typical for the developing world. The test method was based on the Organization for Economic Co-operation and Development (OECD) 301 guidelines and OxiTop®-C 110 system was employed in the measurement. The principle of the measurement was manometric respirometry in a closed bottle system measuring the microbial activity by carbon dioxide evolution. Dextrose monohydrate and carbamazepine were selected to test the functionality of the system and to connect to the literature data.

Only two drugs from seventeen were readily biodegradable. All tested antiviral drugs, antimalarials, antiparasitics, antifungals, antituberculotics, antibacterials, and antiulceratives were found non-degradable, potentially persistent in the environment or affecting the efficiency of wastewater treatment plant (WWTP) processes.

KEYWORDS

Biodegradation, BOD, removal efficiency, developing countries, pharmaceuticals, antiretrovirals, antimalarials, antibacterials, anthelminthics,

FOREWORD

This thesis has been made in co-operation of Tampere University of Technology (TUT) and Tampere University of Applied Sciences (TAMK). The work has been supported by the Universal Corporation Ltd. (UCL), Nairobi, Kenya.

I would like to express my gratitude to Prof. Tuula Tuhkanen and Eeva-Liisa Viskari, Ph.D., for their support and encouragement I received throughout the whole process. Their knowledge, experience and advice have indeed helped me to accomplish the work. I would also like to thank to Marjukka Dyer, Tech.Lic., Mika Nieminen, M.Sc., Seija Haapamäki, Sanna Eljaala and the rest of the TAMK staff for valuable help, advice and overall working atmosphere.

Finally I would like to thank my family and friends for supporting me during the work and everything else that stroke that year. At last but not least I thank my fiancé Mathias von Essen for everything he has done for me, his tolerance and ever-present humor, motivation and optimism.

Tampere, November 2010

Magdaléna Vaňková

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LIST OF ABBREVIATIONS AND SYMBOLS

AO	Acute oral
ATB	Antibiotics
ATU	N-Allylthiourea
BOD	Biological Oxygen Demand
BOD av.	Average Biological Oxygen demand
BOD corr.	Biological Oxygen Demand corrected to Endogenous value
$CaCl_2$	Calcium chloride
CAS #	Chemical Abstract Services register number
CBS	Closed Bottle System
CBT	Closed Bottle Test
CO_2	Carbon dioxide
DNA	Deoxyribonucleic acid
DOC	Dissolved Organic Carbon
EC_{50}	Effective concentration
EMPA	Swiss Federal laboratories for Material Testing and Research
$FeCl_3 \cdot 6H_2O$	Iron (III) chloride hexahydrate
GIT	Gastrointestinal tract
H_2O	Water
HCl	Hydrochloric acid
HIV	Human immunodeficiency syndrome
IPR	Intraperitoneal
K_2HPO_4	Potassium hydrogen phosphate
KH_2PO_4	Potassium dihydrogen phosphate
K_{ow}	Octanol - water partitioning coefficient
LD_{50}	Lethal Dose
M	Mouse
$MgSO_4 \cdot 7H2O$	Magnesium sulfate heptahydrate
Mr	Relative Molecular weight
NA	Nucleic Acid

Na	not available
$Na_2HPO_4 \cdot 2H_2O$	Sodium hydrogen phosphate dihydrate
NaOH	Sodium hydroxide
NH_4Cl	Ammonium chloride
OECD	Organization for Economic Co-operation and Development
OUR	Oxygen Uptake Rate
pKa	Dissociation constant
PPCP	Pharmaceuticals and Personal Care Products
R	Rat
RNA	Ribonucleic acid
SCAS	Semi – Continuous Activated Sludge
TAMK	Tampere University of Applied Sciences
ThOD	Theoretical Oxygen Demand
TUT	Tampere University of technology
UCL	United Corporation Limited
WWTP	Wastewater treatment plant

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1 Introduction

The occurrence and fate of anthropogenic chemical species in our environment has been given great attention for several decades already. The main areas of research were however limited to solvents, detergents and agricultural chemicals. Only during the last two decades, the attention has shifted towards therapeutics (Halling-Sørensen, 1998, Kümmerer, 2004). Therapeutic or pharmaceutical chemicals are biologically active ingredients that are initially planned to increase or moderate the quality of human health. Recent studies however suggest that pharmaceuticals and their residues are refractory compounds persisting in the environment and affecting non-target species in aquatic and terrestrial ecosystems (Boxall, 2006, Heberer, 2002, Vieno, 2007, Quinn, 2008, Wollenberger, 2000).

Pharmaceuticals and Personal Care Products (PPCPs) have been recognized as a potential environmental problem (Kümmerer, 2004). The research on the occurrence and on the fate of PPCPs in the environment has been active particularly in Europe and USA (Carballa Arcos, 2005, Lishman, 2006).

Recent studies provide knowledge on the PPCPs frequently used in developed countries (Kasprzyk-Horden, 2009, Gros, 2009, Ternes, 1998). However the use, occurrence, fate, biotic and abiotic degradation of therapeutics commonly used in developing countries has not been widely studied. In other words, the ecotoxicological knowledge on PPCPs such as antimalarials, antiretrovirals, antituberculotics and antiparasitics is incomplete.

Furthermore, the pressure to develop innovative methods to improve the removal efficiencies of the anthropogenic pollutants from the hydrologic cycle is increasing (Sangave, 2007, Rosal, 2010, Badawy, 2009). Efficient removal of refractory compounds would make the recycling of industrial wastewater possible for irrigation (Badawy, 2009). Closing water loop is one of the options to sustain and minimize the water resource exploitation. Limited quantity of unpolluted water, especially for agricultural purposes in the future, is one of the major challenges of the world (Ternes, 2007). Mainly in sub-Saharan Africa, over-exploration of limited water sources is resulting in decreasing river

flows, falling groundwater levels, increasing water pollution and wetland deterioration (Descheemaeker, 2010).

This thesis has been a part of co-operation of Tampere University of Technology (TUT), Tampere, Finland, and Tampere University of Applied Science (TAMK), Tampere, Finland. The project has been supported by pharmaceutical industry Universal Corporation Limited (UCL), Nairobi, Kenya.

1.1 Objectives of the study

The target of the herein presented work is to determine biodegradability of selected PPCPs. Biodegradation screening is included as a part of the work. Results of the screening are consecutively used to identify non-biodegradable compounds. These chemicals may include e.g. refractory compounds persistent in the environment, therapeutics affecting bacterial species in their natural ecosystems, or toxic and inhibitory chemicals reducing efficiency of biological processes in wastewater treatment plant.

1.2 Scope

From a large portfolio of pharmaceutical therapeutic groups used frequently in developing world, 19 chemicals were selected for the biodegradability analysis. The main selection parameters were the therapeutic use, water solubility and the lacking knowledge of the occurrence, fate and behavior in the environment.

1.3 Outline

The structure of the thesis is as follows. Chapter 2 provides a review of reported occurrence, fate and effects of pharmaceuticals in the environment. Chapter 3 introduces the methods available for biodegradability measurements. Chapter 4 concentrates on the background information of the pharmaceuticals selected for the study. Chapter 5 describes the experimental setup of the biodegradability screening. Chapter 6 presents the results and observations on the analysis, and Chapter 7 concludes and provides proposals for future work.

2 Pharmaceuticals in the environment

During last two decades the interest in the occurrence and fate of pharmaceuticals and personal care products (PPCPs) in the environment has risen dramatically. The main reason of the concern has been rapidly increasing consumption of medical preparations as well as fast development of semi-synthetic and synthetic derivates. Today there are over 100,000 chemical individuals on the market prescribed all over the world (Merck, 2006, Sweetman, 2007, Hardman, 2001).

Another reason for the growing attention to medical substances is the intention of performing a biological effect. Chemical derivates of naturally occurring compounds are designed to be potent, species selective or with a broad spectrum of target organisms. The concern of the development is the change in physico-chemical properties for better membrane penetration, increased toxicity to either target or non-target organism, and possible persistence causing accumulation in the terrestrial and aquatic ecosystems. The constant exposure might cause development of resistant strains of bacteria, affect plant growth or animal and human health.

2.1 Pathways of exposure

Figure 1 presents the pathways possibly undertaken by human and veterinary pharmaceuticals in the environment starting from the sources, through the exposure routes to the receiving ecosystems. Parameters affecting the speed of the passage and severity of the impact in the receiving ecosystem can be found in the same figure. Such important parameters are physico-chemical properties, quantities prescribed, produced or excreted, and the spectrum of animal, plant and micro-organic species affected. The final severity of the PPCPs impact is given by the persistence in the environment influenced by the pharmaceutical degradation rate.

For safe sustainable development, fresh water needs to be free of those persistent non-degradable PPCPs. So that closing water loop would not continue the pharmaceutical circulation in the environment causing constant re-exposure to xenobiotic chemicals.

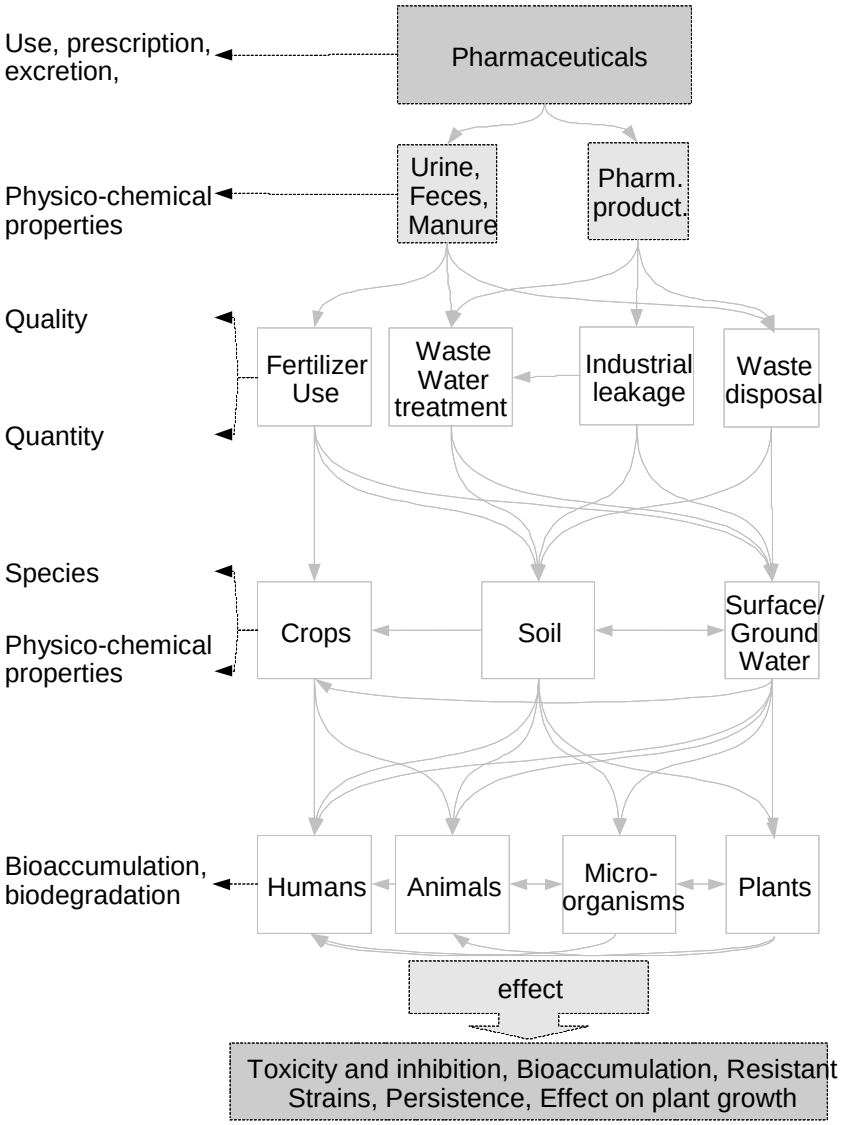


Figure 1 Exposure pathways of PPCPs in the environment

2.2 Reported fate of PPCPs in the environment

Most pharmaceuticals undergo metabolic changes after being digested and absorbed in the target organism. Many medical compounds are metabolized to Phase I or Phase II metabolites (Halling-Sørensen, 1998, Kümmerer, 2004). Phase I reactions consist of reduction, oxidation, hydrolysis, alkylation or dealkylation giving often more reactive or more toxic compounds compared to the parent pharmaceutical. Phase II metabolites (e.g. hydroxylated compounds) are Phase I metabolites modified by glucuronation or sulfatation.

Phase II compounds are often inactive but more water soluble conjugates for enhanced excretion.

The active compound after it is excreted by the recipient via urine or feces can appear in the sewage system as a parent active compound or in the form of more water soluble metabolites (Heberer, 2002). The disposal of PPCPs and their residuals via municipal sewage systems seems of minor interest; however the metabolites can be transformed by sludge bacteria back into the potent parent compound. Furthermore, large number of the therapeutics is excreted unchanged conjugated to polar molecules, glucuronides. The conjugates can be during the wastewater treatment cleaved into the original parent compound and enter the natural aquatic and terrestrial ecosystems. Pharmaceuticals that are disposed incorrectly to the sewage system or dumpsite, or appear in the wastewater through the industrial production are present in active forms, dissolved or as suspended solids.

Fate of individual pharmaceuticals in the environment can differ dramatically. The main fate processes in the environment is sorption and (bio)degradation, photodegradation and hydrolysis can be also significant (Kümmerer, 2004). Depending on the toxicity of the compound towards microorganisms, stability in the presence of air or light, PPCPs can persist for years in our surroundings. For example pentobarbital has been detected in 21 years old anaerobic ground water plume (Eckel, 1993); oxytetracycline, oxolinic acid, flumequine and sarafloxacine have been detected in sediments of fish farms several months after application to the water (Halling-Sørensen, 1998).

Most current studies on the fate of PPCPs are performed on the WWTP influent and effluent concentrations. The detection from municipal sewage and comparison of WWTP influent and effluent concentrations gives the quantity of therapeutics entering natural ecosystems. Furthermore the efficiency of the WWTP and the possible removal of the pharmaceuticals can be drawn to evaluate pharmaceutical persistence. For example chloramphenicol removal has been measured (Kasprzyk-Horden, 2009) from 95 to 100%. 50% of erythromycin-H_2O and 70% of sulphamethoxazole have been removed during wastewater treatment in Wales, UK. Carbamazepine, tramadol, and β-blockers (atenolol) however have not been removed during the same treatment process. Ibuprofene, paracetamol, salbutamol and mefenamic acid (Jones, 2007) have been studied on the

removal in southern England. Even though all chemicals were removed by more than 90% compared to the influent concentration, residues of all compounds were found in the effluent.

Large number of research studies on the fate of pharmaceuticals in the developed world uses the available detail knowledge of consumption patterns and amounts of PPCPs consumed (Winker, 2008, Lindqvist, 2005). The comparison of quantity consumed (and excreted via urine or feces to sewage system) to the quantity detected in the WWTP influent/effluent gives needed knowledge on the removal efficiency during the WWTP processes. A study conducted in Finland furthermore compares removal efficiencies of different WWTP that employ different sets of wastewater treatment processes (Vieno, 2007). Correct selection of physical, biological and chemical treatments could increase the removal efficiency rapidly (Kreuzinger, 2004). In the Finnish study, the removal of atenolol ranged from 37% to 77% in different WWTP.

The amount of pharmaceutical residues, that passes the WWTP barrier and enter the natural environment, and the possibility to develop or improve the WWTP processes to increase the removal are another great positives of the detailed knowledge on the prescription. The same methods of monitoring can be hardly applied to the research in developing countries. Main reason is unknown or inaccurate quantities of PPCPs consumed and insufficient sanitation or centralized sewage system.

2.3 Reported occurrence of PPCPs in the environment

The occurrence of the therapeutics in the environment has been already intensively investigated for more than 15 years. However research studies available provide knowledge mainly on pharmaceuticals and personal care products (PPCPs) used and detected in developed countries (Austria, Canada, USA, Germany, Denmark, Finland, Sweden, Norway, Spain and Netherlands).

More than 80 PPCPs and several metabolites have been detected in μg/l in the sewage effluent and in surface waters downstream from the WWTP (Heberer, 2002). PPCPs have been detected also from groundwater contaminated by landfill leachates and manufacturing residues. The most studied substances and their reported occurrence in the natural

environment can be divided into the following therapeutic groups (Halling-Sørensen, 1998, Heberer, 2002, Kasprzyk-Horden, 2009, Gros, 2009, Jones, 2007, Lindqvist, 2005):

- Analgetics and Anti-inflammatory drugs (Paracetamol, Aspirin, Diclophenac, Ibuprofen)
- Antiepileptic drugs (Carbamazepine)
- Stimulants (Caffeine)
- β-blockers (Atenolol, Propranolol)
- Antibacterials –ATB (Erythromycin, Tetracycline, Oxytetracycline, Sulphamethoxazole, Trimethoprim, Chloramphenicol)
- Hormones (Oral contraceptives, Testosterone, Estrogen, Estradiol, Estrone, 17α-Ethinylestradiol)
- Antineoplastics (Ifosfamide, Cyclophosphamide)
- Lipid lowering agents (Clofibric acid, Bezafibrate)

Substances have been detected (Halling-Sørensen, 1998) from ground water (sulfonamides, antineoplastics), river water (ATBs and hormones), ocean water (pharmaceutical dump-sites), sediments (ATBs) and soil (ATBs, growth promoters and hormones). Research shows that the problem is worldwide, similar results on the occurrence in the environment have been published in Finland, Sweden, Denmark, Germany, UK, China or USA.

2.4 Reported effects of PPCPs on non-target species

A review by Halling-Sørensen et.al (1998) gives an overview of reported effects of PPCPs in the environment on non-target organisms. As an example, anti-inflammatory drug ibuprofen has a potential antimicrobial activity against *Staphylococcus aureus* inhibiting the growth at concentrations as low as 150μg/ml. ATB streptomycin is affecting the growth of several blue-green algae species; furazolidone is proven to be toxic to *crustaceans* and to mosquito larvae *Culex pipiens*.

It is generally accepted that the excessive use of ATB in human and veterinary medicine causes the development of resistant bacterial strains. Growing resistant bacterial colonies have been observed on the medium containing ATB neomycin. Resistant strains of micro-

organisms are known also for other therapeutic groups, antimalarials or antiretrovirals. (Hardman, 2001, Sweetman, 2007).

Plant species and PPCPs are yet another large unknown area. Persistent compounds and pharmaceutical residues possessing biological activity present in irrigation water or in organic fertilizer might be accumulated and stored in the agricultural soils. Plants and crops could up-take these chemicals unchanged. This could cause adverse effects on the plant growth and development as well as causing animal and human re-exposure to these xenobiotics. Kumar et.al (2005) tested the plant uptake of chlortetracycline by green onion, cabbage and corn. All three plants fertilized by affected manure contained chlortetracycline. (Kumar, 2005). Oxytetracycline was found inhibitory for the growth and development of roots and shoots of alfalfa (*Medicago sativa* L.) (Kong, 2007). Fluoxetine HCl known better under trade name Prozac® has been detected in all parts of cauliflower (*Brassicaceae*), however no statistically valuable negative effects were recognized (Radshaw, 2008). Decline in plant growth was observed by Boxall et. al (2006) for phenylbutazone, oxytetracycline and enrofloxacin when lettuce and carrot were exposed. Florfenicol and trimethoprim were uptaken by both plants and detected in leaves and roots, levamisol was present in the lettuce leaves, diazinon and enrofloxacin were found in the carrot root (Boxall, 2006). It has to be pointed out that the pharmaceutical concentration tested in mentioned studies was significantly higher than the concentration detected in the natural water bodies and soils (Kümmerer, 2004).

3 Methods of biodegradability analysis

Estimation of degradation rates is rather challenging process, especially for biodegradability since biological processes and living organisms are involved (Struijs, 1995). Degradation can take place through abiotic processes, such as photochemical reactions and hydrolysis, or through biotic processes, i.e. biological degradation. Biodegradation is an important process in the WWTP for removal of the substances and potential pollutants from the sewage (Kümmerer, 2000). Current knowledge on the WWTP removal suggests that many PPCPs (caffeine, ibuprofen or iodinated contrast media) are eliminated mainly by biodegradation (Ternes, 2007). Therefore biodegradability was given attention in this study.

Biodegradability refers to the transformation of organic molecules by bacteria, fungi or yeast to simple molecules through biological activity. In other words, biodegradability expresses the potential of micro-organisms to degrade (mineralize) organic matter or organic pollutants. Water and carbon dioxide are the final products of the degradation pathways (Murphy, 2002). If all parts of parent compound are reduced to basic inorganic elements with or without the presence of oxygen, the parent compound is degradable. Biodegradation is often monitored as a degree of mineralization by means of oxygen uptake rate (OUR), removal of dissolved organic carbon (DOC) or carbon dioxide evolution.

With the growing attention to the fate and persistence of organic pollutants in our environment during last 40 years, also methods to study biodegradability developed. Large number of those methods concentrates on the behavior of organic molecules in the aquatic ecosystems (Reuschenbach, 2003).

3.1 Ready Biodegradability: OECD 301

Following six methods mentioned in the paragraphs below are permit for screening chemicals on their ready biodegradability in aerobic aqueous medium. Mentioned test can be also combined; CO_2/DOC combination is one example.

Tests are performed in the dark at constant temperature. At least 70% removal of DOC and 60% removal of ThOD within 28 days is the pass level of the test for a chemical individual.

If pass level is reached, compound is readily biodegradable. If the desired percentage is not reached or time period is longer, compound is not readily biodegradable. Ready biodegradability tests are often used for quick selection of degradable compounds (Struijs, 1995).

All tests mentioned in this paragraph have virtually the same principle and can be performed on a wide range of physico-chemical and biological properties (Struijs, 1995). Even more, the test mineral medium used in all tests is of the same composition: KH_2PO_4 (0,625mM), K_2HPO_4 (1,249mM), $Na_2HPO_4 \cdot 2H_2O$ (1,877mM), NH_4Cl (0,093mM), $CaCl_2$ (0,248mM), $MgSO_4 \cdot 7H_2O$ (0,0913mM) and $FeCl_3 \cdot 6H_2O$ (0,0009mM). (OECD(a), 1992) (Reuschenbach, 2003).

The volatility, solubility, vapor pressure, adsorption and test compound concentration are vital values for further selection of appropriate method (OECD(a), 1992).

3.1.1 DOC die-away test (ISO 7827, OECD 301 A)

Non-volatile compounds with solubility greater than 100mg/l can be tested by DOC die-away test. Higher microbial cell concentrations are allowed in the test compared to MITI (301 C) mentioned in paragraph 3.1.3. (OECD(a), 1992).

Known concentration (DOC) of a known compound is measured in inoculated medium for 28 days without light at 22 ± 2°C. The biodegradation is calculated as a percentage from DOC removed compared to original DOC. Another possibility is supplemental chemical analysis on the parent compound in the beginning and in the end of the measurement. Samples should be taken daily to ensure sufficient amount of data.

3.1.2 CO_2 evolution test (ISO 9439, OECD 310 B) - Modified Sturm Test

Biodegradability of non-volatile compounds, where the formula, carbon content and purity are known can be tested by CO_2 evolution test. Test is suitable also for poorly soluble and adsorbing compounds. Test substance in inoculated medium is the sole source of carbon in the system. CO_2 produced by the microbial activity is trapped by NaOH and determined by titration of the residual hydroxide. Biodegradability is presented as a percentage of $ThCO_2$. Sampling should take place every second day during the first 10 days and every fifth day for the rest of the measuring period (OECD(a), 1992).

3.1.3 MITI (OECD 301 C)

Formula and purity of the tested substance should be known in order to calculate theoretical oxygen demand (ThOD) and perform the measurement. MITI test can be used also for insoluble and volatile compounds. The principle of the MITI test is automatic measurement of the oxygen uptake in an enclosed respirometer. Values of the oxygen uptake expressed in mg/l are compared to the original ThOD. The result of the comparison is the percentage of biodegradation (OECD(a), 1992).

3.1.4 Closed bottle test (ISO 10707, OECD 301 D)

As in the previous test also closed bottle test CBT can be used for insoluble or volatile compounds. Small number of microorganism from a mixed population is added to the inoculated media with the tested substance. The mixture is kept in full closed containers for desired period of time. Biodegradability is expressed by comparison of consumed oxygen during the degradation to the original ThOD (OECD(a), 1992).

3.1.5 Modified OECD screening (OECD 301 E)

Substances with solubility over 100mg/l and non-volatile substances can be tested by modified OECD screening method. The method is similar to DOC die-away test, however compared to 301 A lower concentration of the microorganisms is allowed (OECD(a), 1992).

3.1.6 Manometric respirometry test (ISO 9408, OECD 301 F)

By manometric respirometry test, insoluble or volatile compounds can be assessed. Higher substance concentrations (100mg/l) compared to the previous methods can be tested by manometric respirometry. Biodegradability is determined by the oxygen consumption or by CO_2 evolution. Oxygen consumption is determined by measuring the amount of oxygen that has to be produced and added to the system to maintain the same pressure or constant gas volume. CO_2 is trapped in hydroxide sorbent and determined by titration (OECD(a), 1992).

The principle of manometric respirometry is used in the automated systems for Biological Oxygen Demand determination (BOD), such as OxiTop® or Sampromat® mentioned later in this chapter.

3.1.7 Combined CO_2/DOC test

The combined CO_2 and DOC test is a reliable test for aerobic biodegradability determination in aquatic ecosystems (Strotmann, 1995). The new test combines the study of two parameters for biodegradability analysis, the removal of dissolved oxygen (DOC) and carbon dioxide production. The test provides sufficient reliability of the obtained results and larger amount of data compared to CO_2 evolution test, DOC die-away test or respiratory test performed alone.

3.2 Inherent Biodegradability: OECD 302

The original test method was adopted in 1981 as OECD 302 B method for testing inherent biodegradability (OECD(b), 1992). The original method was merged with tests developed by EMPA and final change was implemented in 1992 concerning the mineral medium composition. The new composition is identical with the OECD 301 guideline.

3.2.1 Semi-continuous activated sludge test (SCAS): OECD 302 A

Semi-continuous activated sludge test (ISO 9887, OECD 302 A) uses activated sludge as inoculum as following Zahn-Wellens test. (Pagga, 1997) Both methods have high degradability potential and can be used to determine the potential inherent biodegradability.

3.2.2 Zahn-Wellens/EMPA: OECD 302 B

The method is similar to ISO 9888 standard to measure inherent biodegradability. The mixture of the mineral medium, test substance and rather large amount of activated sludge is aerated at 20 - 25ºC in the dark for 28 days. The biodegradation is monitored by DOC determination in the filtered samples on daily bases.

The solubility, vapor pressure and structure of the test substance are necessary properties to be known. Substances that are non-volatile, do not adsorb significantly, and are not lost by foaming can be tested in the inherent biodegradability test. Zahn-Wellens test is usually applied after one of the tests on ready biodegradability (OECD 301), the inhibition and physical properties are therefore in many cases already known. The biodegradation must reach 70% of DOC removal within 14 days.

3.2.3 Modified Zahn-Wellens test

Modified Zahn-Wellens test enables continuous and parallel determination of oxygen consumption and CO_2 production (Norr, 2001). The modification is using inexpensive laboratory equipment designed as a closed system apparatus. Oxygen consumption is measured by the pressure change measurement and the CO_2 production is determined by the conductivity measurement. The system allows the inherent biodegradability measurement for poorly soluble compounds and for adsorbing or volatile substances. The measuring signals are transmitted to PC and stored automatically, which allows the continuous and parallel measurement of the parameters for biodegradability determination. The setting parameters of the measurement (test concentration, temperature, inoculums concentration, test medium composition, pH and duration) are as the parameters described for Zahn-Wellens test.

3.3 Automated determination of biodegradability

With the development of PC technology, commercially available automated systems to measure biodegradability appeared. Both presented systems, Sapromat and OxiTop-C are based on the manometric respirometry measuring principle in a Closed Bottle System (CBS) where Biological Oxygen Demand (BOD) is determined.

The value of BOD expressed in mg/l shows the amount of oxygen required to decompose the organic matter present in water sample by aerobic digestion. BOD can be used as a parameter for expressing the water quality in the wastewater management and assessing the behavior of the treatment systems (Mara, 2003). BOD can be also used to describe the organic material strength of influents and effluents and to monitor environmental pollutants. (Roppola, 2009)

For the assessment of pharmaceutical behavior in the WWTP, BOD can be used to evaluate the efficiency of substance removal from wastewater during the biological treatment operations. Furthermore BOD measurement can be used to study the effects on microorganisms, the substance toxicity or inhibition.

Biodegradation is an important part of the WWTP removal operations. While great number of pharmaceuticals is proven to poses toxic or inhibitory effects on the biotic populations of

WWTP the PPCPs can cause decrease in removal efficiency of PPCPs as well as the removal of other organic matter from the wastewater and causing consequently secondary organic pollution. (Roppola, 2009)

BOD tests for water quality determination are performed under strictly defined conditions for the time period of 5 or 7 days. BOD measurements applied to the ready biodegradability analysis of pharmaceuticals have a prolonged time period of 28 days (Mara, 2003, OECD(a), 1992).

3.3.1 OxiTop ® – C

OxiTop-C is a BOD self-measuring mercury free system applicable also for respiration or biogas determination tests. The principle is based on the pressure measurement in a closed bottle system. CO_2 produced by microbial activity is absorbed by NaOH creating vacuum. The pressure difference can be read by the operating unit and is presented as mg/l BOD.

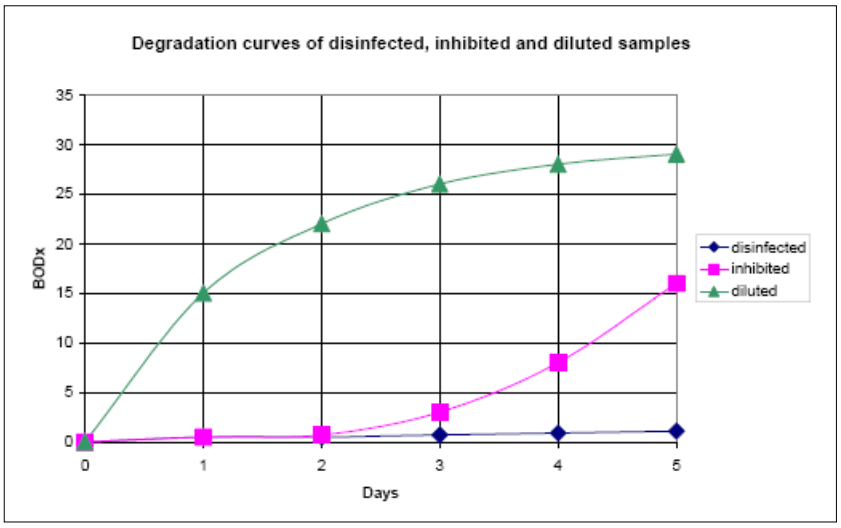


Figure 2 Data presentation by OxiTop measuring system, (WTW, 2009)

Figure 2 shows the example of data presentation by OxiTop measuring system. The curve of diluted sample shows the ideal biodegradation curve of a readily biodegradable substance. Inhibited curve is typical for substances inhibiting the metabolism of microorganisms and result in slow increase of oxygen consumption. Disinfected sample curve does not show any BOD value and is typical for toxic substances; microorganisms are killed shortly after the exposure to the chemical hence no oxygen is consumed.

OxiTop - C measurement simulates biological processes of WWTP. Any disturbance in the simulated process or low BOD value points on possible disturbance in the WWTP processes, inhibition of bacteria, toxic effects and potentially persistant compounds.

3.3.2 Sapromat ® E

Sapromat BOD measuring system consists of 12 independent measuring vessels (500ml) with CO_2 traps containing soda lime. Each vessel is placed into a water bath at constant temperature and connected to manometer and an electrochemical oxygen production unit, see Figure 3. During the biodegradation, oxygen from the system is consumed creating sub-pressure which induces the manometer and the electrochemical unit producing oxygen until the pressure is even. The oxygen consumption (BOD) values are controlled continuously, transferred to a computer and used for the biodegradability calculation as a comparison of the ThOD and BOD (Reuschenbach, 2003, H+P, 2010).

Figure 3 Sapromat ® E measuring system (H+P, 2010) with the water bath and PC measuring unit

3.4 Other available methods

Activated sludge simulation test (ISO 11733, OECD 303) for wastewater plants and Low test concentrations test (ISO 14592) may be used to establish kinetic biodegradability parameters, which explain far more details about the biodegradation processes (Pagga,

1997). Test at low test concentrations is often used to simulate biodegradation in natural lakes and ponds. Anaerobic biodegradability test (ISO 11734) simulates the environment in digesters of WWTP. Marine degradation test (OECD 306) is a special aquatic test for marine environments. Aerobic composting test (ISO 14855) is a method simulating composting facility and is used to study biodegradability of polymers (Pagga, 2001).

Only one internationally harmonized method is available for degradation in soils or in sediments. Soil test (OECD 304) requires the ^{14}C-labelled material and measures the ^{14}C-labelled carbon dioxide produced (Pagga, 1997).

3.5 Comparison of the test methods

For the ready biodegradation studies most commonly used methods are the DOC die-away test, the CO_2 evolution test and manometric respirometry. The limiting parameters for the tests application are the water solubility, volatility of the compound and the toxicity to the used inoculum. In OECD 301 tests, the test substance has to be the sole source of carbon. Unknown impurities cannot be calculated to the original ThOD. If unknown impurities are present, final comparison of ThOD/BOD will not return true biodegradability value.

Since it is not possible to distinguish between real biodegradation and abiotic degradation processes, all tests for ready and inherent biodegradability based on DOC measurements are limited. (Pagga, 1997). It is also difficult to distinguish between the oxidized part of the substance and the part incorporated to the biomass. Furthermore, only bacteria are used in the aquatic settings. Apart from bacteria, fungi and yeast contribute considerably to the degradation process in compost or in soils; however their growth is limited in the aquatic environment (Pagga, 2001). All tests performed in the aquatic environment have therefore lower degradation values compared to aerobic composting test. A great advantage of the classical biodegradation methods is the inexpensive apparatus using conventional laboratory glass.

Sapromat has a low maintenance, and large mode of application for the BOD measurements. Sapromat measuring system provides large amount of data accessible on PC. With the control unit, measuring system can be left for several days for independent

operation. (H+P, 2010) The main advantage of the Sapromat system is the possibility to extend nearly infinitely the measurement period. (Reuschenbach, 2003)

The advantages of OxiTop-C system are simple operation and compact design, controllability and large measuring range, up to 400 000 mg/l BOD. The system enables simultaneous and grouped start, management, storage and tracking of 100 measuring heads. The reliable, accurate data is presented graphically and can be easily transported to PC. OxiTop-C system can operate individually for the whole period of measurement. The data can be called up at any point of the measurement for test control (WTW, 2009).

When comparing OxiTop-C and Sapromat systems, the difference in the degradation value is negligible (bellow 10%), lag phases are quite consistent and the degradation rate is not consistently greater or smaller for one system (Reuschenbach, 2003). A common disadvantage for both automated systems is expensive apparatus.

Reuschenbach et.al. presented a study comparing the classical biodegradability tests with the new automated systems using aniline as a reference substance. Test methods were Zahn-Wellens test for inherent biodegradability, DOC die-away test, CO_2 evolution test, OxiTop-C and Sapromat both systems being based on the method of manometric respirometry. Degradation rate was again comparable for both respirometry systems, Sapromat and OxiTop. DOC die-away test had the highest degradation rate, at the same time CO_2 evolution test the lowest. The lag periods were the longest for Sapromat, but comparable for OxiTop, DOC die-away and CO_2 evolution test. Last three mentioned tests also showed greater final degradation percentage compared to Sapromat system (Reuschenbach, 2003).

4 Pharmaceuticals

In the following paragraphs the pharmaceuticals selected for the biodegradability analysis are introduced. Separate paragraphs are organized based on the therapeutic group 4.1, toxicity 4.2, physico-chemical properties 4.3, and on the biodegradability and wastewater removal information 4.4.

4.1 Therapeutic group

Chemical species concerned in the study were divided into groups according to their therapeutic application. The therapeutic application is in several cases connected with the possible target organism; virus, bacteria, protozoa etc. The main reason for this decision was the following experimental section of the work, where the effect on microorganisms has a crucial role on the BOD value and the final biodegradation rate.

The mentioned groups are antiviral chemical species affecting viruses 4.1.1, antimicrobial pharmaceuticals affecting prokaryotic cells such as bacteria – antibiotics (ATB) 4.1.2, and antimicrobial pharmaceuticals affecting eukaryotic cells such as protozoal organisms 4.1.3, helminths 4.1.4 and fungi 4.1.6.

Following groups include pharmaceuticals affecting functions and systems of higher organisms, for example anti-inflammatory drugs 4.1.5, antiulceratives and antiepileptics 0. (Tortora, 2007)

4.1.1 Antiviral pharmaceuticals

Viruses are intracellular parasites that are using metabolism of the hosting cell and posing a difficult problem for the choice of efficient chemotherapy. Selective toxicity for the virus is harder to achieve compared to higher organisms. (Bennett, 2003)

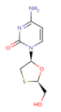
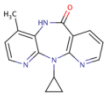
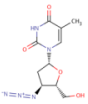
The main mechanisms of action are the inhibition of the adsorption to the hosting cell, repression of the viral nucleic acid (NA) and the interference with the transcription and translation of the viral NA. (Bednář, 1999)

The most common diseases treated by the antiviral pharmaceuticals are chickenpox (*Varicella-zoster*), Herpes simplex virus, HIV and Hepatitis B, C, or D. (Bennett, 2003)

Because of the location and the lacking information, three antiretroviral pharmaceuticals for treating HIV infections were selected, see Table 1.

HIV is the causative agent for acquired immunodeficiency syndrome (AIDS). (Sweetman, 2007) Two subtypes of the virus have been recognized, HIV-1 occurring worldwide and HIV-2 typical for West Africa. HIV uses the CD4 receptors to enter T-lymphocytes and monocytes/macrophages where the viral RNA is translated to DNA by reverse transcriptase enzymatic catalysis. Transcribed viral DNA is then inserted to the genome of the host cell. Viral replication results in constant depletion of T-lymphocytes and reduction in the immune response of the host organism.

Table 1 Antiretroviral pharmaceuticals

Chemical	**CAS #**	**Formula**	**Mr (g/mol)**	**Therapeutic use**
Lamivudine	134678-17-4	$C_8H_{11}N_3O_3S$ 	229,256	Antiviral (HIV, Hepatitis B)
Nevirapine (viramune)	129618-40-2	$C_{15}H_{14}N_4O$ 	266,298	Antiviral (HIV)
Zidovudine	30516-87-1	$C_{10}H_{13}N_5O_4$ 	267,241	Antiviral (HIV)

Reference: (DrugBank. Wishart, 2010, Merck, 2006, Sweetman, 2007, ChemBlink, 2007).

Lamivudine is a white powder soluble in water exhibiting polymorphism. Lamivudine is in the cells converted to active antiviral triphosphate (Sweetman, 2007). Triphosphate stops DNA synthesis of retroviruses through competitive inhibition of reverse transcriptase and the incorporation to the viral DNA. Lamivudine is rapidly absorbed after oral dose. The bioavailability is between 80 and 87%. Lamivudine is metabolized intracellularly, hepatic metabolism is low and clearance takes place mainly by renal excretion of the unchanged compound. Lamivudine is a potent inhibitor of HIV-1 and HIV-2, including strains resistant to zidovudine. Lamivudine is also one of the promising antivirals used in the

treatment of hepatitis B infections. However, resistance against lamivudine has been reported for HIV and hepatitis B virus.

Nevirapine is white powder practically insoluble in water and sparingly soluble in dichlormethane and methyl alcohol. Nevirapine is metabolized by cytochrome P450 isoenzymes and readily absorbed after oral doses. The bioavailability of nevirapine is greater than 90%. Nevirapine is mainly excreted in the urine as glucuronide conjugates of hydroxylated metabolites (Sweetman, 2007).

Zidovudine is white or brownish powder soluble in water and dehydrated alcohol, which should be protected from light; photodegradation of the drug is expected. After absorption by organism, zidovudine is metabolized to triphosphate which inhibits the synthesis of retroviral DNA. Zidovudine is rapidly absorbed by gastrointestinal tract (GIT) and undergoes first-pass hepatic transformation (bioavailability 60-70%). After intercellular metabolic change to triphosphate it is metabolized by liver to inactive glucuronide. Zidovudine is excreted via urine as metabolite and parent compound (Sweetman, 2007). It has been proven zidovudine possesses activity against Epstein-Barr virus and Gram-negative bacteria. Long-term monotherapy causes formation of zidovudine-resistive strains of HIV and therefore it is used in combination with other pharmaceuticals. Zidovudine shows haematologic toxicity and is reported to be carcinogenous in rodents.

4.1.2 Antibacterial pharmaceuticals

This paragraph is dedicated to the pharmaceutical species treating bacterial infections, i.e. infections caused by simple organisms with a prokaryotic cell structure. These chemical species are often referred to as antibacterials or antibiotics (Sweetman, 2007). Bacteriostatics and disinfectants are often mentioned in the same therapeutic group even though the mechanism of action on the target organism is different.

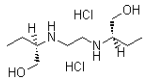
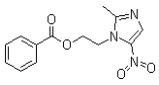
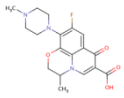
The very diverse group of antibacterial pharmaceuticals is often divided into subgroups according to their chemical structure, their mode of activity or according to the spectrum of antimicrobial activity. Mentioned objectives are often interconnected and similarity in the chemical structure results in the similarity of microbial activity. Most commonly referred groups are aminoglycosides, antimycobacterials, cefalosporines and β-lactams,

chloramphenicols, glycopeptides, macrolides, quinolones, sulfonamides and tetracyclines (Sweetman, 2007).

The mechanism of effect can be classified as (Bennett, 2003):

- Inhibition of cell wall synthesis (β-lactams – penicillin and cephalosporin)
- Inhibition of the protein synthesis (aminoglycosides, tetracycline, macrolides)
- Inhibition of the NA synthesis (sulphonamides, quinolones)

Table 2 Antibacterial pharmaceuticals

Chemical	**CAS #**	**Formula**	**Mr (g/mol)**	**Therapeutic use**
Ethambutol HCl (antimycobacterials)	1070-11-7	$C_{10}H_{24}N_2O_2$. 2HCL 	277,230	Antibacterial *(Mycobacterium tuberculosis)*
Metronidazole Benzoate (imidazols)	13182-89-3	$C_{13}H_{13}N_3O_4$ 	275,300	Antibacterial, antiprotozoal (*Giardia lamblia, Trichomonas)*
Ofloxacin (quinolones)	82419-36-1	$C_{18}H_{20}FN_3O_4$ 	361,368	Antibacterial *(Chlamydia, M. tuberculosis, M. leprae)*
Isoniazid	54-85-3	$C_6H_7N_3O$ 	137,1393	Antibacterial Antitubercular

Reference: (DrugBank. Wishart, 2010, Merck, 2006, Sweetman, 2007, ChemBlink, 2007).

For the purpose of the study, four antibacterial pharmaceuticals were chosen ethambutol, ofloxacin, metronidazole benzoate and isoniazid. Details about the antibacterial group, chemical formula and structure, the molecular weight and the therapeutic group can be found from Table 2.

Ethambutol hydrochloride is a white crystalline powder freely soluble in water and alcohol. 2% solution is slightly acidic with pH 3,7 – 4,0 and should be stored in air-tight containers, short half-life and fast degradation is expected. Ethambutol is absorbed by GIT by 80% of

the oral dose. Ethambutol is partially metabolized in the liver forming aldehyde and dicarboxylic acid derivates. These metabolites are inactive and excreted in the urine, most of the drug appears as a parent compound in the urine within 24 hours (8 – 15% is present as inactive metabolites) and 20% of the dose is excreted in feces (Sweetman, 2007). Ethambutol is used to treat *Mycobacterium* infections, however when used alone resistant strains of *M.tuberculosis* are produced.

Metronidazole benzoate is a white crystalline powder or flakes insoluble in water, slightly soluble in alcohol and soluble in acetone. Photodegradation is expected, metronidazole b. should be therefore protected from light. Metronidazole is almost completely absorbed after oral application. Accumulation in the organism may occur after multiple doses. Metronidazole benzoate is hydrolyzed to metronidazole in GIT in order to be absorbed. Metronidazole is metabolized in the liver by side-chain oxidation and glucuronide formation. The majority of metronidazole is excreted in urine mainly as metabolites (1-(2-hydroxyethyl)-2-hydroxymethyl-5-nitroimidazole and 2-methyl-5-nitroimidazole-1-acetic acid), small amount appears in feces (Sweetman, 2007). Metronidazole is carcinogenic in bacterial assays and even though it has been classified as carcinogenic for animals, the carcinogenity for humans is ambiguous. Metronidazole is prescribed against several protozoal microorganisms (*Blastocystis hominis*, *Giardia intestinalis* and *G.lamblia*, *Trichomonas vaginalis*), and is also active antibacterial (*Bacteroides*, *Clostridium* spp., *Helicobacter pylori*). Resistance to metronidazole and to other imidazole antibacterials has been reported.

Ofloxacin is a yellow crystalline powder, soluble in water and in methyl alcohol, soluble in dichloromethane and in glacial acetic acid. Ofloxacin should be stored in air-tight containers and protected from light. Ofloxacin is rapidly absorbed from GIT (~100%), up to 80% of the dose is excreted by tubular secretion and glomerular filtration in kidneys resulting in high urinary concentrations (less than 5% is excreted as metabolites). 4-8% may be excreted in feces (Sweetman, 2007). Ofloxacin belonging to the fluoroquinolone group is more active compared to ciprofloxacin (*Chlamydia trachomatis*, *Mycobacterium leprae* and *M.tuberculosis*). S-(-) isomer of ofloxacin, levofloxacin has twice the activity of ofloxacin. Resistance has been reported for strains of *Neisseria gonorrhoeae*.

Isoniazid is a white powder soluble in water and sparingly soluble in alcohol unstable when exposed to air and light. 5% solution has pH between 6,0 to 8,0. Primary metabolic route is the acetylation to acetylisoniazid in liver and small intestine. Acetylisoniazid is hydrolyzed to isonicotinic acid and monoacetylhydrazine. Isonicotinic acid is conjugated with glycine to isonicotinuric acid, monoacetylhydrazine is acetylated do diacetylhydrazine. Un-metabolized isoniazid is hydrolyzed to hydrazones. Metabolites do not poses tuberculostatic activity and are less toxic compared to the parent compound. Over 75% of the dose is excreted via urine mainly as metabolites. Isoniazid is highly active against *M. tuberculosis* and may be also used to treat other mycobacterial infections (*M. kansasii*). (Sweetman, 2007) Resistance against isoniazid develops rapidly when used alone in the clinical infection treatment.

4.1.3 Antimalarial pharmaceuticals

Pharmaceuticals described in this paragraph and Table 3 are chemical species affecting parasitic infections caused by *Plasmodium* protozoa. *Plasmodium* species cause malaria, a serious and potentially fatal disease.

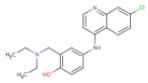
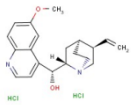
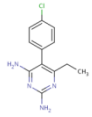
Amodiaquine is odorless powder practically insoluble in water and slightly soluble in alcohol which should be stored in air tight containers. Amodiaquine hydrochloride is a crystalline powder soluble in water and alcohol, and slightly soluble in chloroform. Amodiaquine is readily absorbed from GIT and metabolized in the liver to the active compound leaving only very small amount of amodiaquine unchanged in the excreted urine. The half life of amodiaquine in the blood plasma is long and the parent compound with the active metabolite can be detected in urine several months after the administration.

Quinine sulfate is a water soluble white powder that should be protected from light. Quinine is rapidly and almost completely absorbed from GIT. After extensive hepatic metabolic transformation, quinine is excreted mainly in urine. (Sweetman, 2007)

Pyrimethamine is white crystalline powder practically insoluble in water, sparingly soluble in alcohol and unstable exposed to light. Pyrimethamine is after absorption from GIT concentrated in liver, kidneys, lungs and spleen. Pyrimethamine is excreted by kidney, metabolites are detected in urine. Pyrimethamine is used in the treatment of malaria and

toxoplasmosis. The antimalarial activity is through the inhibition of the NA synthesis of the malaria parasite. Main activity is detected against *Plasmodium falciparum* and *P.vivax*. Because of the resistant strains development, pyrimethamine is currently recommended mainly for the treatment of toxoplasmosis.

Table 3 Antimalarial pharmaceuticals

Chemical	**CAS #**	**Formula and structure**	**Mr (g/mol)**	**Therapeutic use**
Amodiaquine HCl	69-44-3	$C_{20}H_{22}ClN_3O$ (2HCl) 	464,800	Antimalarial, Amebicide
Quinine Sulfate	804-63-7	$2(C_{20}H_{24}N_2O_2)\cdot H_2SO_4$ 	746,910	Antimalarial, Analgetic
Pyrimethamine	58-14-0	$C_{12}H_{13}ClN_4$ 	248,711	Antimalarial (toxoplasmosis)

Reference: (DrugBank. Wishart, 2010, Merck, 2006, Sweetman, 2007, ChemBlink, 2007).

4.1.4 Anthelmintic pharmaceuticals

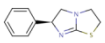
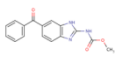
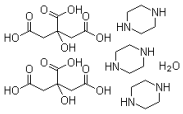
Helminth infections are one of the most common human infections around the world, large proportion of the population is however affected in the tropical regions. In developing countries, helminth infections pose a large threat to the public health and contribute to other problems and diseases (malnutrition, pneumonia and anemia). The eradication of helminth infection does not depend only on the use and availability of chemotherapeutics, but also on the hygiene, proper food preparation and satisfactory sanitation systems with uncontaminated water supply (Sweetman, 2007).

Helminths are a very diverse group of eukaryotic parasites differing with the life cycle, structure, development and physiology, location within the host and their sensitivity to

chemotherapeutics (Brunton, 2006). Worms pathogenic for humans are *nematodes* (roundworms), *trematodes* (flatworms) and *cestodes* (tapeworms).

Anthelmintics (Table 4) are pharmaceuticals treating infections caused by parasitic worms either by expelling the worms from the GIT or systematically by eradicating the adult worms and the developmental forms (Bennett, 2003).

Table 4 Anthelmintic pharmaceuticals

Chemical	**CAS #**	**Formula and structure**	**Mr (g/mol)**	**Therapeutic use**
Levamisole HCL	16595-80-5	 $C_{11}H_{12}N_2S.HCl$	240,750	Anthelmintic (Nematodes), Immuno-modulans
Mebendazole (benzimidazol carbamate derivate)	31431-39-7	 $C_{16}H_{13}N_3O_3$	295,293	Anthelmintic (Nematodes)
Piperazine Citrate	144-29-6	 $2(C_6H_8O_7)\cdot 3(C_4H_{10}N_2)\cdot H_2O$	642,700	Anthelmintic

Reference: (DrugBank. Wishart, 2010, Merck, 2006, Sweetman, 2007, ChemBlink, 2007).

Levamisole hydrochloride is a white crystalline powder freely soluble in water unstable exposed to light. 5% water solution has pH from 3,0 to 4,5. Levamisole is active against intestinal nematodes in particular ascariasis and hookworm infections. The sensitive worms are paralyzed and excreted from the intestines (Sweetman, 2007).

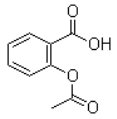
Mebendazole is a white powder showing polymorphism, practically insoluble in water, alcohol or dichlormethane, unstable exposed to light. Mebendazole is active against most nematodes, against some larval stages and ova. It destroys the cytoplasmic microtubules in the worm cells causing the death within several days (Sweetman, 2007).

Piperazine citrate is white granular powder, soluble in water and practically insoluble in alcohol. Piperazine is absorbed from GIT and excreted partly as metabolites via urine. Prescription prevails against intestinal nematodes *Ascaris* and *Enterobius* (Sweetman, 2007).

4.1.5 Anti-inflammatory pharmaceuticals

Anti-inflammatory pharmaceuticals are used in relief of inflammation, pain or fever. They are often grouped as salicylates (aspirin), anti-rheumatic drugs, gold compounds, non-steroidal anti-inflammatory drugs (diclofenac), opioid analgesics and para-aminophenols (paracetamol). Aspirin has been chosen for the study because of wide world use.

Table 5 Anti-inflammatory pharmaceuticals

Chemical	CAS #	Formula	Mr (g/mol)	Therapeutic use
Aspirin (Acetylsalicylic acid) (Salicylate NSAID)	50-78-2	 $C_9H_8O_4$	180,157	Anti-inflammatory, Anticoagulant

Reference: (DrugBank. Wishart, 2010, Merck, 2006, Sweetman, 2007, ChemBlink, 2007).

Aspirin (Table 5) is a white crystalline powder, slightly soluble in water and freely soluble in alcohol. Aspirin is readily absorbed in non- ionized form from GIT, stomach and intestine. Aspirin acts as inhibitor of cyclo-oxygenase which directly inhibits the production of prostaglandins and tromboxanes. Elimination is by hepatic metabolism to salicyluric acid, salicyl phenolic glucuronide, salicylic acyl glucuronide, gentisic and gentisuric acid. With the increasing therapeutic dose and increasing alkalinity of the urine also unchanged parent compound is excreted.

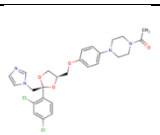
4.1.6 Antifungal pharmaceuticals

Antifungal pharmaceuticals are preparations used in the treatment and prophylaxis of fungal infections. Most fungi pathogenic in humans can be classified as yeasts and moulds and are saprophytic in nature (Sweetman, 2007). They cause infections when airborne spores reach lungs, skin or eye. Example of pathogenic fungi is *Aspergillus*, *Candida* or *Pneumocystis*.

Ketoconazole (Table 6) is a white powder, practically insoluble in water, sparingly soluble in alcohol and freely soluble in dichlormethane, unstable exposed to light. Ketoconazole is an imidazole derivate that inhibits ergosterol synthesis and alters the permeability of the fungal cell membrane. It is metabolized by liver and excreted as inactive metabolites in

feces. Ketoconazole has wide spectrum of antifungal activity and resistance to this chemotherapeutic is rare. Ketoconazole is proven to be hepatotoxic.

Table 6 Antifungal pharmaceuticals

Chemical	CAS #	Formula	Mr (g/mol)	Therapeutic use
Ketoconazole (Imidazole derivates)	65277-42-1	 $C_{26}H_{28}Cl_2N_4O_4$	531,431	Antifungal for immune-suppresed patients

Reference: (DrugBank. Wishart, 2010, Merck, 2006, Sweetman, 2007, ChemBlink, 2007).

4.1.7 Other therapeutic use and PCPs

In this paragraph, pharmaceuticals with effect on higher organism and its separate systems are discussed. The groups of pharmaceuticals are very large and diverse, therefore only the mechanism of action of the chosen chemical species will be described in more detail, see Table 7.

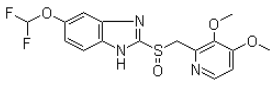
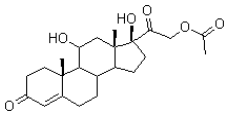
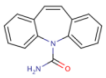
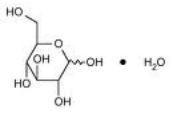
Pantoprazole is a proton pump inhibitor which can be prescribed in gastro-oesophageal reflux disease, peptic ulcer diseases and pathological hypersecretory states. Pantoprazole is metabolized in liver by cytochrome P450 isoenzyme to desmethylpantoprazole and excreted in urine.

Hydrocortisone acetate is a white crystalline powder practically insoluble in water, unstable exposed to light. Hydrocortisone is absorbed by GIT and metabolized in the liver to hydrogenated and degraded forms (tetrahydrocortisone and tetrahydrocortisole). Metabolites are excreted via urine conjugated to glucuronides (Sweetman, 2007).

Carbamazepine is a white crystalline powder that exhibits polymorphism and is unstable exposed to air. It is slightly soluble in water, sparingly in alcohol and acetone, freely soluble in dichlormethane. Carbamazepine is a dibenzazepine derivate with antiepileptic and psychotropic properties. It is used to treat epilepsy, trigeminal neuralgia and in prophylaxis of bipolar disorder. Carbamazepine is irregularly and slowly absorbed from GIT and extensively metabolized by liver by cytochrome P450 isoenzymes CYP3A4 and CYP2C8. Carbamazepine is excreted mainly in urine in the form of metabolites

(carbamazepine-10,11-epoxide), which keeps the original activity of the parent compound (Sweetman, 2007).

Table 7 Antiulcerative, antiepileptic and glucocorticoid pharmaceuticals

Chemical	CAS #	Formula	Mr (g/mol)	Therapeutic use
Pantoprazole	102625-70-7	 $C_{16}H_{15}F_2N_3O_4S$	383,370	Antiulcerative
Hydrocortisone Acetate	50-03-3	 $C_{23}H_{32}O_6$	404,500	Glucocorticoid
Carbamazepine	298-46-4	 $C_{15}H_{12}N_2O$	236,2686	Antiepileptic, analgesic
Dextrose Monohydrate	5996-10-1	 $C_6H_{12}O_6.H_20$	198,170	Additive

Reference: (DrugBank. Wishart, 2010, Merck, 2006, Sweetman, 2007, ChemBlink, 2007).

Dextrose monohydrate and Carbamazepine were chosen as compounds of comparison with the source literature, dextrose as a readily biodegradable, non-toxic non-inhibitory compound while Carbamazepine as a non-biodegradable persistent compound according to source information (Kasprzyk-Horden, 2009, Joss, 2005, Zhang, 2008, Ternes, 1998).

4.2 Toxicity

In the following the toxicity values were collected. Oral Acute dose LD_{50} for rat (mouse) is the most common available value for all pharmaceutical species, Table 8 (p.41).

Effective concentration EC_{50} then expresses the toxicity to *Daphnia magna*, algae or sludge bacteria. EC_{50} value used to express the environmental toxicity is another example next to biodegradability of incomplete available information. Existing studies concentrate on the research of the medical treatment and effects on target organisms while the knowledge on

the effects of individual pharmaceuticals on the non-target organisms is missing. As an example, amodiaquine is largely studied on the toxicity effects towards *Plasmodium falciparum* (Brasseur, 1995), environmental toxicity is not available.

The EU directive 93/67/EEC classifies chemicals according to their EC_{50} value to following classes: very toxic to aquatic organisms (<1mg/l), toxic to aquatic organisms (1-10mg/l), and harmful to aquatic organisms (10-100mg/l). Chemicals with EC_{50} value over 100mg/l are not classified. (CEC, 1996)

4.3 Physico-chemical properties

Table 9 (p.42) presents the collected values describing the physico-chemical properties. pK_a value describes the tendency of molecules or ions to dissociate in water. Octanol-water partitioning coefficient (K_{ow}) is a ratio of chemical concentration in octanol and in water at defined conditions. K_{ow} is used to determine the fate of chemicals in the environment and possible bioaccumulation or adsorption to solids (Morganwalp, 2010).

4.4 Removal of pharmaceuticals and Biodegradability

Table 10 (p.43) presents current available information on the removal efficiency (%) of selected pharmaceuticals during WWTP processes and the biodegradability (%). As mentioned before, the information on the selected pharmaceuticals is scarce. Bioavailability (%) is a value describing the availability of therapeutics to the receiving organism. Half life value mentioned in the Table 10, is the half life of the therapeutics in the blood plasma.

Table 8 Toxicity of pharmaceuticals: Acute Oral dose LD_{50} to rat (r) or a mouse (m) and effective concentration EC_{50} to *Daphnids*, algae and activated sludge bacteria

Toxicity of pharmaceuticals: Acute Oral LD50 for rat (r) or mouse (m), Effective concentration for Daphnids and Algae							
Chemical	**AO LD_{50} (mg/kg)**	**note**	**Reference**	**EC_{50} (mg/l)**			**Reference**
				Daphnids (mg/l)	**Algae (mg/l)**	**Sludge b. (mg/l)**	
Amodiaquine HCl	550	m	a			102,82	(Basnyat, 2010)
Aspirin (Acetylsalicylic acid)	200	r	d	8858,00	61,00	50,43	(Sanderson, 2003) (Basnyat, 2010)
Carbamazepine	4025	r	(Merck, 2001)	76,30	85,00		(Huschek, 2004)
Dextrose Monohydrate	25800	r	d				
Ethambutol HCl	6800	r	d				
Hydrocortisone Acetate	2300	IPR m	d				
Isoniazid	160	r	d	23,00	25,00		(Sanderson, 2009)
Ketoconazole	86	r	a				
Lamivudine	>2000	r	d	>1000			(Sanderson, 2009)
Levamisole HCL	180	r	d			15,02	(Basnyat, 2010)
Mebendazole	620	m	a				
Metronidazole Benzoate	330	r	d		12,50		(Sanderson, 2009)
Nevirapine (viramune)	400	r	d				
Ofloxacin USP	3590	r	d	31,75 17,41	1,44		(Isidori, 2005), (Sanderson, 2009)
Pantoprazole	1000	m	d				
Piperazine Citrate	11200	r	d				
Pyrimethamine	440	r	d	4,80	20,00		(Sanderson, 2009)
Quinine Sulfate B.P	620	r	d	NA	NA	-19,00	(Sanderson, 2009) (Basnyat, 2010)
Zidovudine	3084	r	d	>100		886,80	(Sanderson, 2009) (Basnyat, 2010)

Reference: a - DrugBank. Wishart, 2010, d - MSDS

Table 9 Physico-chemical properties of pharmaceuticals

Physico-chemical properties of selected pharmaceuticals					
Chemical	**pK_a**	**logP (hydrofobicity)**	**log K_{ow}**	**Reference**	**Photodegradability**
Amodiaquine HCl	na	3,70 a			No b
Aspirin (Acetylsalicylic acid)	3,49 a	1,40 a	1,13	(Sanderson, 2003)	No b
Carbamazepine	Neutral	2,3 a	2,25	(Sanderson, 2003)	No b
Dextrose Monohydrate	na	na	na		Na
Ethambutol HCl	na	-0.3 a			No b
Hydrocortisone Acetate	na	0,5 a			Yes b
Isoniazid	1,82 a	-0,8 a	0,97 -0,81	(Sanderson, 2003), (Sanderson, 2009)	Yes b
Ketoconazole	na	4,0 a			Yes b
Lamivudine	na	-1,40 a	na		Yes b
Levamisole HCL	na	2,30 a			Yes b
Mebendazole	na	2,80 a			Yes b
Metronidazole Benzoate	2,40 a	-0,1 a	-0,1	(Sanderson, 2009)	Yes b
Nevirapine (viramune)	na	2,50 a			No b
Ofloxacin USP	7,90 a	2,10 a	0	(Sanderson, 2009)	Yes b
Pantoprazole	3,92 a	0,50 a			No b
Piperazine Citrate	9,73 a	-0,80 a			No b
Pyrimethamine	7,34 a	2,7 a	2,41	(Sanderson, 2009)	Yes b
Quinine Sulfate B.P	na	2,6 a	3,43	(Sanderson, 2009)	Yes b
Zidovudine	9,85c	0,05 a	0,23	(Sanderson, 2009)	Yes b

Reference: a - DrugBank. Wishart, 2010, b - Sweetman, 2007, c - Jjemba, 2006

Table 10 Biodegradability and removal of pharmaceuticals: background information

Bioavailability, half life in organism, removal% in WWTP and Biodegradability						
Chemical	**Bioavailability %**	**Half life**	**Removal %**	**Reference**	**Biodegradability %**	**Reference**
Amodiaquine HCl	Readily av. b	1-10d+ b				
Aspirin (Acetylsalicylic acid)	na	0,25-9h a	81	(Fent, 2005)		
Carbamazepine	na	25-65h a	0-10 7-8	(Zhang, 2008) (Joss, 2005) (Ternes, 1998)	0	(Ternes, 2007)
Dextrose Monohydrate	na	na				
Ethambutol HCl	80 a	3-4h a				
Hydrocortisone Acetate	Readily av. b	6-8h a				
Isoniazid	Readily av. b	1-6h b				
Ketoconazole	Moderate a, b	2-8h b				
Lamivudine	87 a	5-7h a				
Levamisole HCL	na	4,4-5,6h a				
Mebendazole	5-10 a	2,5-5,5h a				
Metronidazole Benzoate	20-100 b	8h a			5	(Kümmerer, 2000)
Nevirapine (viramune)	90 a	45h a				
Ofloxacin USP	98 a	9h a	57 56 75 - 88	(Gros, 2009) (Zorita, 2009) (Vieno, 2007)	0	(Kümmerer, 2000)
Pantoprazole	77 a,b	1h a				
Piperazine Citrate	100 a	na				
Pyrimethamine	100 b	96h a				
Quinine Sulfate B.P	76-88 a	18h a				
Zidovudine	65 a	0,5-3h a				

Reference: a - DrugBank. Wishart, 2010, b - Sweetman, 2007

5 Experimental: Biodegradability analysis by OxiTop ® - C 110

Base information on the biodegradability or removal from wastewater is incomplete for the chosen therapeutics. Therefore ready biodegradability was tested to obtain a starting value on the biological degradation potential.

Based on the physico-chemical properties of the selected chemical species, manometric respirometry in a closed bottle system (CBS/CBT) was chosen as an appropriate test method. The main parameters for this decision were the solubility of tested substances and the test concentration. The water solubility ranged from freely soluble to practically insoluble substances. For manometric respirometry the test concentration is higher compared to other test methods, which was important parameter in order to maintain high accuracy of the solution preparation.

In order to test large amount of pharmaceuticals and get needed accurate data, the automated OxiTop-C 110 system was chosen for the biodegradability analysis, see Figure 4.

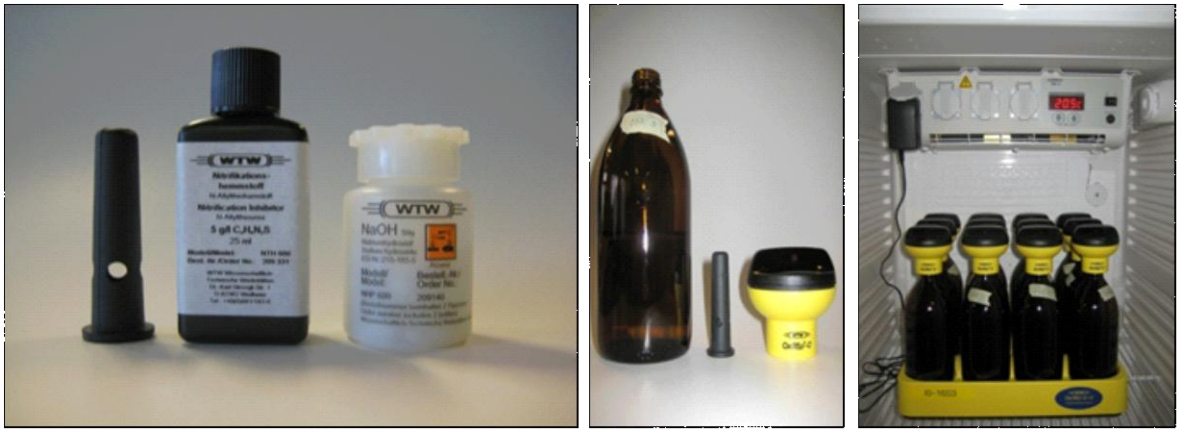


Figure 4 OxiTop measuring system - (left) rubber sleeve as a CO_2 trap, ATU and NaOH pellets, (middle) measuring vessel, rubber sleeve and measuring head, (right) Incubator and magnetic stirrer

5.1 Principle

The principle of the test was the measurement of CO_2 produced by inoculated bacteria in a closed bottle system when pharmaceutical was added. Increasing concentration of CO_2

trapped and measured by the head of OxiTop-C 110 system showed the progressing degradation process.

The CO_2 evolution is an indirect measurement method that can be easily presented as oxygen consumption, i.e. biological oxygen demand (BOD). As each pharmaceutical was tested individually as a sole source of carbon the biodegradation was presented as a ratio of theoretical oxygen demand (ThOD) and BOD (WTW, 2009, manual, 2000).

5.2 Objective of the analysis

The main target of the experimental part is the biodegradability analysis of the pharmaceuticals used extensively in developing countries. Based on biodegradation results, pharmaceuticals likely to enter and persist in the natural environments are identified.

5.3 Theoretical Oxygen demand

Theoretical oxygen demand (ThOD), used as a reference value in the final biodegradation evaluation, was calculated individually for selected substances using following equation (1) and formula (2) (Techobanoglous, 1987).

$$C_cH_hO_oN_nP_pS_s + (c + 0{,}25h - 0{,}5o + 1{,}25n + 1{,}25p + 1{,}5s)\ O_2 \rightarrow cCO_2 + (0{,}5h - 0{,}5n - 1{,}5p - s)\ H_2O + nNO_3^- + pPO_4^{-3} + sSO_4^{-2} + (n + 3p + 2s)\ H^+ \quad (1)$$

where small letters represent the amount of moles (c) carbon, (h) hydrogen, (o) oxygen, (n) nitrogen, (p) phosphorus and (s) sulfur.

Equation (1) was used to calculate moles of oxygen $n(O_2)$ needed for complete oxidation of the organic compound. Value of $n(O_2)$ was then substituted to formula (2) to calculate the ThOD.

$$\frac{\qquad\qquad}{\qquad\qquad} \quad (2)$$

where $n(O_2)$ is amount of moles of oxygen (mol), $Mr(O_2)$ is relative molecular weight of oxygen (g/mol), c(pharmaceutical) is the actual concentration used in the measurement (g/l), and Mr(pharmaceutical) is the relative molecular weight of pharmaceutical (g/mol).

To perform the BOD test the total oxygen in the CBS cannot become a limiting factor during the whole time of the experiment. Stating correct filling volume (the wastewater level in the bottle and amount of air above the water level) is a crucial moment in the experiment setting. ThOD was therefore used as an ideal reachable BOD value to set the correct filling volume and appropriate range of the measurement (WTW, 2009).

5.4 Methods

In this paragraph the methods and the setting of the analysis are described. Paragraph 5.4.1 shows the composition and preparation of the mineral medium (the base solution of artificial wastewater), 5.4.2 describes the pharmaceutical solution preparation, inoculum and test conditions are described in 5.4.3 and 5.4.4 respectively. Endogenous BOD measurement is mentioned in 5.4.5. And finally the system test compounds are to be found from 5.4.6.

5.4.1 Mineral medium

In the biodegradability analysis, the tested compound has to be the sole source of carbon. To fulfill this condition a mineral medium of known composition resembling wastewater was prepared. The mineral medium composition can be found in OECD guidelines for testing chemicals on biodegradability (OECD(a), 1992), see Table 11. Chemicals used in the preparation were of pro-analysis purity purchased from Merck.

Table 11 Mineral medium preparation according to OECD 301

		Stock solution		Mineral solution	
sol.	**Chemical**	**mass (g)**	**V (ml)**	**V (ml)**	**Total V(ml)**
a	KH_2PO_4	8,50	1000	10	1000
	K_2HPO_4	21,75			
	$Na_2HPO_4.2H_2O$	33,40			
	NH_4Cl	0,50			
b	$CaCl_2.2H_2O$	36,40	1000	1	
c	$MgSO_4.7H_2O$	22,50	1000	1	
d	$FeCl_3.6H_2O$	0,25	1000	1	

Reference: (OECD(a), 1992)

The final mineral solution was prepared by diluting 10ml of stock solution **a** in 800ml of distilled water, 1ml of each stock solution **b**, **c**, and **d** were added and filled to final volume of 1000ml.

5.4.2 Test compound

Each pharmaceutical was tested individually on the biodegradation. Test substances were received from UCL, Nairobi, Kenya as powders.

Test concentration was 50mg/l. Rather high concentration was decided to maintain desired accuracy and because wastewater from pharmaceutical industry was in question. At this concentration in several cases toxic and inhibitory effects were expected based on available EC_{50} values. However the preference was given to the possibility of final comparison of the effects at the same concentration.

Solutions of the test substances were prepared according to the water solubility based on given OECD 301 guidelines. Compounds with solubility over 1g/l were prepared into solutions with distilled water (1g/l) and adequate volume was transferred from the pharmaceutical stock solution to the test bottle. Solutions of sparingly (0,1g/l – 1g/l) soluble compounds were prepared from stock solution **a**. Adequate weight of poorly soluble and insoluble compounds was added to the ready mineral medium from a weighing vessel. The reason for described action was the water volume reduction in the system. Excess water could reduce the buffering capacity of prepared mineral medium and disturb the measurement; bacterial inoculum is often sensitive to pH change.

5.4.3 Inoculum

Activated sludge from Viinikanlahti wastewater treatment plant, Tampere, Finland was used as the bacterial inoculum. Fresh samples were taken each day of the measurement in the time period from May to September 2010. Inoculum was aerated and filtrated before the measurement. Each closed bottle system was added 0,5ml of inoculum.

5.4.4 Test conditions

Two different period lengths of biodegradation measurements were performed, 7 and 28 days. The solutions were kept in dark and incubated at constant temperature 20±1°C. 20 drops/l of N-Allylthiourea ($C_4H_8N_2S$) ATU were used as a nitrification inhibitor. Three

replicates of each substance were measured. Each measurement round was accompanied with three blanks to measure endogenous BOD for final biodegradability correction.

5.4.5 Endogenous BOD

Endogenous BOD is the oxygen consumption from the basic respiration activity of starved bacteria without any added degradable substance. That means, blank system to measure endogenous BOD contained mineral medium, 0,5ml of inoculum and 20 drops ATU/l. The blank values were used for correction of the test values (Reuschenbach, 2003).

According to the literature, the blank BOD value should range between 20mg/l - 60mg/l. If the value drops bellow 20mg/l, change of the procedure or system check-up is needed. (OECD(a), 1992, Reuschenbach, 2003)

5.4.6 System test compounds

System test compounds, dextrose monohydrate and carbamazepine were chosen to test the set conditions and the functionality of the test. Dextrose monohydrate was chosen as a ready biodegradable compound in order to gain a target degradation curve. Carbamazepine is a non-degradable compound with low percentage of WWTP removal.

5.5 Results

System test compounds, dextrose monohydrate was readily biodegradable, while carbamazepine was non-biodegradable.

Out of following 17 compounds, only two PPCPs were readily biodegradable (aspirin and hydrocortisone acetate) and two compounds were low-degradable (metronidazole benzoate and piperazine citrate).

13 compounds (excluding already mentioned carbamazepine) were non-biodegradable, toxic or inhibitory to activated sludge bacteria and potentially refractory in the environment. Therapeutics belonging to the non-degradable group were antiretrovirals, antimalarials, anthelminthics (excluding piperazine citrate), antibacterials (excluding metronidazole benzoate), antifungals, antituberculotics and antiulceratives. More detailed information on the observations can be found from the following paragraphs.

5.5.1 Endogenous BOD

Endogenous BOD was measured every round with test compounds. The final endogenous BOD values ranged between 2,5 – 14,1mg/l, 7,0mg/l being the most common endogenous BOD value.

Degradation curves for endogenous BOD_7 and endogenous BOD_{28} can be seen from Figure 5. No significant difference between the final values of the two measurement periods was observed.

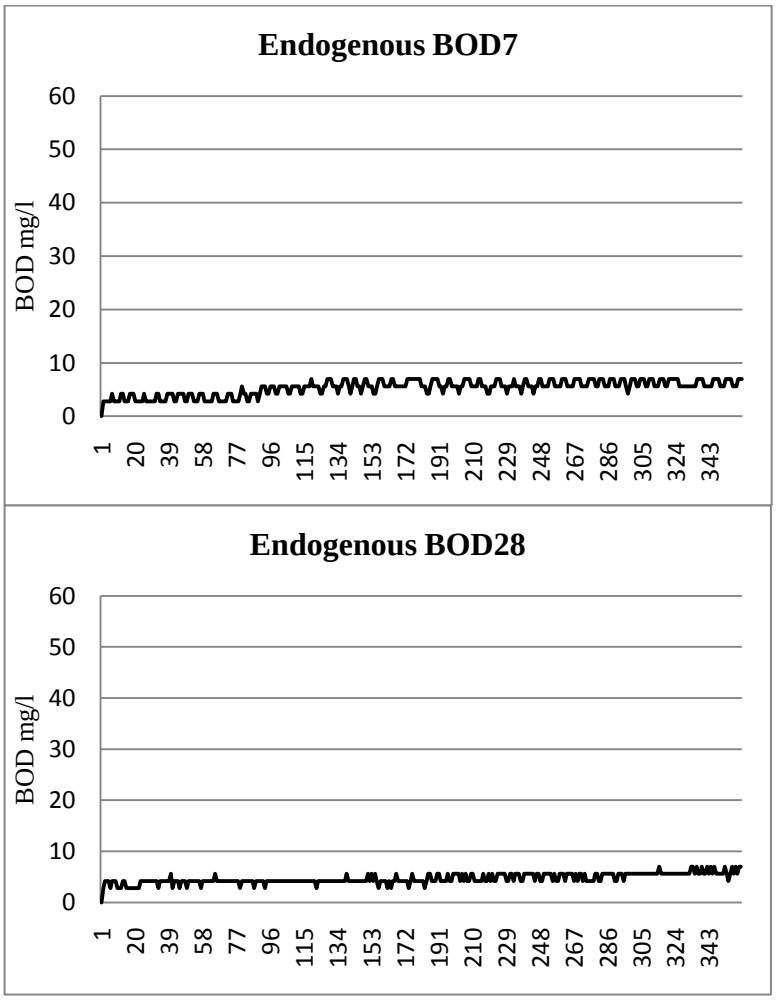


Figure 5 Endogenous BOD_7 and endogenous BOD_{28} degradation curves.

The functionality of the test was measured on degradability of system test compound, i.e. dextrose monohydrate, because the endogenous BOD was not in the recommended range 20 – 60mg/l. Degradation curve of dextrose can be seen from Figure 6.

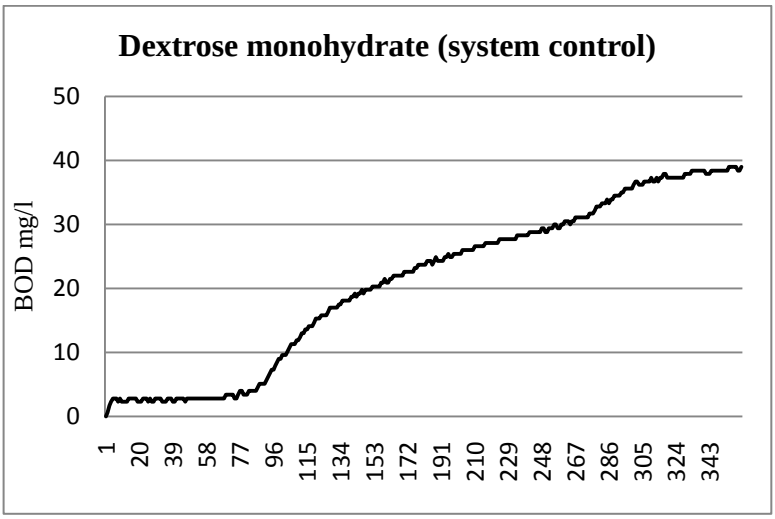


Figure 6 Degradation curve of dextrose monohydrate. Dextrose was tested as a system control compound on the degradation potential. Values in the chart are not corrected with endogenous BOD.

Biodegradation of dextrose reached 73% in 7 days. The system setup was considered functional and no change was introduced. The low endogenous BOD was probably caused by the filtration of the suspended solids from the inoculum causing the decrease in the organic matter concentration. The initial lag phase, which can be seen from Figure 6, was caused most probably by temperature shock.

5.5.2 BOD_7

Antiretrovirals (nevirapine and zidovudine), antibacterials (ethambutol, ofloxacin and metronidazole benzoate), antimalarials (amodiaquine and quinine sulfate) and anthelminthics (levamisole and mebendazole) were tested for BOD_7.

Except of metronidazole benzoate (18%), all tested chemicals degraded by less than 5% during 7 days, see Table 12. BOD_7 value of amodiaquine was lower than the endogenous value of the corresponding measurement, which resulted in negative biodegradability percentage.

All biodegradation curves of pharmaceuticals tested on BOD_7 can be found from Appendix A Biodegradation curves: BOD7

Table 12 BOD_7 of selected pharmaceuticals. ThOD - theoretical oxygen demand, BOD av. is average Biological oxygen demand calculated from 3 replicates, BOD corr. is the corrected BOD value with endogenous BOD of corresponding measurement.

Chemical	ThOD (mg/l)	BOD av. (mg/l)	BOD corr.(mg/l)	Biodegradability%
Amodiaquine Base	98,967	7,1	-0,7	**-0,7%**
Ethambutol HCl	100,999	7,0	4,23	**4%**
Levamisole HCL	121,288	7,0	0	**0%**
Mebendazole	116,495	6,1	1,9	**2%**
Metronidazole Benzoate	104,613	25,8	18,8	**18%**
Nevirapine (viramune)	138,191	7,5	4,7	**3%**
Ofloxacin USP	109,584	7,5	0,5	**0,5%**
Quinine Sulfate B.P	112,463	5,1	0,9	**0,8%**
Zidovudine	104,774	7,5	4,7	**4%**

5.5.3 BOD_{28}

Anti-inflammatory aspirin, glucocorticoid hydrocortisone acetate, antiulcerative pantoprazole, antiretroviral lamivudine, antiepileptic carbamazepine (system test compound), antibacterial isoniazid, anthelmintic piperazine citrate, antifungal ketoconazole and antimalarial pyrimethamine were tested for BOD_{28}.

Table 13 BOD_{28} of selected pharmaceuticals. ThOD - theoretical oxygen demand, BOD av. is average Biological oxygen demand calculated from 3 replicates, BOD corr. is the corrected BOD value with endogenous BOD of corresponding measurement.

Chemical	ThOD (mg/l)	BOD av. (mg/l)	BOD corr. (mg/l)	Biodegradability%
Aspirin (Acetylsalicylic acid)	79,930	52,6	46,07	**58%**
Carbamazepine	135,439	8,9	-2,8	**-2%**
Hydrocortisone Acetate	110,754	78,5	71,9	**65%**
Isoniazid	128,337	9,9	-1,87	**-1%**
Ketoconazole	129,462	4,2	1,9	**1%**
Lamivudine	101,197	8,5	-3,3	**-3%**
Pantoprazole	95,991	12,7	6,17	**6%**
Piperazine Citrate	89,622	36,1	31,9	**36%**
Pyrimethamine	130,272	4,2	1,9	**1%**

Anti-inflammatory Aspirin (58%) and glucocorticoid hydrocortisone acetate (65%) were considered readily biodegradable compounds based on the observed BOD value and on the shape of the degradation curve. Anthelmintic piperazine citrate was considered degradable (36%).

Based on the shape of the biodegradation curve with a mild and slow increase of the BOD value (Appendix B), antiulcerative pantoprazole was identified as a compound with inhibitory effects to activated sludge.

Carbamazepine, isoniazid, ketoconazole, lamivudine and pyrimethamine pose toxic effects towards activated sludge bacteria.

Charts of all PPCPs mentioned in this paragraph can be found from Appendix B Biodegradation curves: BOD28.

5.5.4 Observation of OxiTop-C 110

OxiTop-C 110 system is a very easy and fast screening method for ready biodegradability analysis. The system does not require controlling and tested compounds are individually sampled in regular intervals which would under different circumstances require time. The length of intervals depends on the whole measuring time, the sampling covers the chosen testing period completely with 360 samples.

What was observed was rather high fluctuation of the BOD value in several cases, for example mebendazole benzoate. Even more, especially for non-biodegradable compounds where the BOD value was low, certain similarity in the reached values was observed. It seams that the system is able to record the values in steps, discrete values. As an example a short row of values reached during the whole screening are shown: 0; 1,4; 2,8; 4,2; 5,6; 7,0. Values in between were not reached, which in the cases of low BOD might cause accuracy problem. This phenomenon can explain the fluctuating values visible from presented charts.

6 Conclusion

During last two decades, research studies on the occurrence and fate of PPCPs in the environment commonly used in developed world have been published. PPCPs have been recently detected from soils, sediments, surface or groundwater around the world. Furthermore, the behavior of PPCPs in the WWTP and the removal from the wastewater has become an important question.

This study provided information on the biodegradability of PPCPs frequently used in developing world that are lacking knowledge on their WWTP removal, behavior in the environment and occurrence and fate in the aquatic and terrestrial ecosystems.

Ready biodegradability test according to OECD 301 guidelines was performed on selected PPCPs. Screening with OxiTop-C 110 based on the manometric respirometry was performed. Chosen method allowed collection of large amount of data on the biodegradability process and enabled clear presentation of the results. A question appeared following the values reached during individual measurements. The system seems to reach only discrete values of BOD. This becomes only visible when low-degradable and non-degradable compounds are tested and raises a question of accuracy in these measurements.

Therapeutic groups preferred in the experiment were antiretrovirals, antibacterials, antimalarials, anthelmintics, antituberculotics, antifungals, antiulceratives and glucocorticoids.

Because of limited knowledge available on the selected chemical species, biodegradable dextrose monohydrate and non-biodegradable antiepileptic carbamazepine were chosen as system test compounds (Heberer, 2002). Both drugs behaved as was expected based on the background information. Performed test results were therefore considered valid for all tested compounds.

From the tested pharmaceuticals only anti-inflammatory aspirin and glucocorticoid hydrocortisone acetate were found readily biodegradable. The results for aspirin comply with the literature data (Fent, 2005).

All tested antiretrovirals (anti-HIV drugs), zidovudine, lamivudine and nevirapine were found non-biodegradable during the simulated WWTP biological treatment in a CBS. There are no reference data available on the therapeutic group, however the results were considered valid due to consistency with results of the test compounds. All anti-HIV drugs are potential environmental pollutants. Nevirapine should be high lightened in this group because of its long half life, high toxicity to higher organisms (rat) and stability when exposed to light.

From the group of antibacterials, ethambutol HCl and ofloxacin did not degrade during the same test. The biodegradability of ofloxacin was 0,5% which is consistent with the literature data (0%) mentioned in paragraph 4.4 (Kümmerer, 2000). Higher percentage of removal during WWTP shows on possible other processes taking place next to biodegradation (Vieno, 2007, Fent, 2005). The biodegradation value of metronidazole benzoate was measured 18%, which is higher than the background information (5%) (Kümmerer, 2000).

All tested antimalarials, amodiaquine, quinine sulfate and pyrimethamine were found non-biodegradable. Similarly to antiretrovirals, for the therapeutic group of antimalarials there are no available literature data on their biodegradability. The observed biodegradation values ranged from -0,7% to 1%. Therefore antimalarials were considered toxic to activated sludge bacteria.

In the group of anthelminthics, piperazine citrate degraded by 36%. Mebendazole and levamisole were toxic to activated sludge bacteria based on the BOD value and on the biodegradation curve. This statement is supported by the EC_{50} value tested on activated sludge bacteria for levamisole, see paragraph 4.2. The low EC_{50} value is indicating the toxicity to sludge bacteria (Basnyat, 2010).

Antitubercular isoniazid was found non-biodegradable. The negative biodegradability percentage (Table 13), low AO LD_{50} dose and low EC_{50} (Sanderson, 2009) values for *Daphnids* and for algae are suggesting that isoniazid might be toxic to sludge bacteria.

Antifungal ketoconazole and antiulcerative pantoprazole were found non-biodegradable. Based on the observed values and biodegradation curves ketoconazole was considered toxic to sludge bacteria and pantoprazole inhibitory.

Based on the observations, all non-biodegradable and low- biodegradable compounds studied in this thesis should be excluded from the wastewater. These chemicals can potentially pass WWTP processes and enter the natural environment causing water and soil pollution. Secondly, the constant exposure to these xenobiotics can cause the development of resistant species of viruses, bacteria or protozoa. Resistant species would consequently increase the demand for stronger therapeutics increasing also the negative effect on the environment. And finally, toxic and inhibitory compounds could decrease the efficiency of WWTP biological processes by decreasing the bacterial population and cause secondary pollution.

7 Discussion and future work

The results of this thesis raise several important questions. First of all, I would like to emphasize that the used inoculum was sampled at a WWTP in Finland and therefore the bacteria have possibly never before been exposed to most of the tested chemicals. This could explain the large number of refractory compounds. On the other hand though, such first exposure shows truly the potential to affect the natural populations. It would be interesting to subject the same therapeutics to the same test with inoculum exposed earlier to these xenobiotics. Such experiment could present accommodation ability of the sludge bacteria or the potential for resistant population development.

Another problem that can be identified is the question of the toxic and inhibitory chemicals in the WWTP influent. It is clear that the toxic impact reduction during the biological treatment requires innovative wastewater treatment processes or source separation to reduce the chemical concentration entering sewage system. Intervention at the source could be feasible when industrial production is in questions. The reduction of water used for equipment cleaning, cleaning with compressed air, isolating cleaning water from the rest of grey and black water and introduce separate treatment techniques are only few theoretical ideas of possible actions to undertake.

Isolating the industrial wastewater from the household wastewater would be beneficial also for the recovery of the pharmaceuticals and reduction of the wastewater volume. Ozonation is one of the innovative methods of refractory compound treatments. This method involves ozone generation which is energy demanding and expensive. Reduction of the wastewater volume could reduce the costs and make the method more accessible. Furthermore, it is more than obvious that development of new innovative treatment methods is necessary.

Fourth point is not tightly connected to the content of this thesis. Nevertheless, I would like to mention the problem of PPCPs and their residues that are excreted from receiving organisms via urine or feces. In developed countries with sufficient sanitation systems, these enter the centralized sewage system from household wastewater and travel to municipal WWTP. In the WWTP, these can affect the efficiency of the treatment. In

developing countries the situation is even more complicated due to insufficient sanitation. It is of course not clear, but this route could be the main pathway causing the reported occurrence of PPCPs in the environment. Again, the behavior in black and yellow water, soils, surface and ground water and the potential effect on the organisms should be widely studied and treatment processes developed.

8 Bibliography

Badawy, M.I., Wahaab, R.A., El-Kalliny, A.S. 2009. *Fenton-biological treatment processes for the removal of some pharmaceuticals from industrial wastewater.* Giza, Egypt : Elsevier - Journal of Hazardous Metrials 167, pp. 567-574, 2009. Available at: www.elsevier.com/locate/jhazmat.

Basnyat, P. 2010. *Draft of Master of Science Thesis.* Tampere, Finland : Tampere University of Technology. Dep. of Bio- and Environmental Engineering, 2010.

Bednář, M., Fraňková, V., Schindler, J., Souček, A., Vávra, J. 1999. *Lékařská Mikrobiologie - bakteriologie, virologie, parazitologie.* Praha, Czech Republic : Marvil, 1999.

Bennett, P.N., Brown, M.J. 2003. *Clinical Pharmacology.* 9th ed. Cambridge, UK : Churchill Livingstone, 2003. ISBN 0443064806.

Boxall, A.B.A., Johnson, P., Smith, E.J., Sinclair, C.J., Stutt., E., Levy, L. 2006. *Uptake of Veterinary Medicines from Soils into Plants.* York, UK : Americam Chemical Society - Journal of Agriculture and Food Chemistry 54, pp.2288-2297, 2006.

Brasseur, P. at.al. 1995. *Sensitivity of Plasmodium falciparum to amodiaquine and chloroquine in central Africa: a comparative study in vivo and in vitro.* Rouen, France : Transactions of the Royal Society of Tropacal Medicine and Hygiene 89, pp. 528-530, 1995.

Brunton, L.L., Lazo, J.S, Parker, K.L. 2006. *Goodman and Gilman´s The Pharmacological Basis of Therapeutics.* 11th ed. USA : McGraw-Hill Medical Publishing Division, 2006. ISBN 0-07-142280-3.

Carballa Arcos, M. 2005. *Fate of pharmaceuticals and Personal Care Products (PPCPs) in Sewege Treatment Plants focusing on the anaerobic digestion of sludge.* Santiago de Compostela, Spain : Universidade de Santiago de Compostela, 2005.

CEC, Commission of the european Commuties. 1996. *Technical guidance document in support of commission directive 93/67/EEC on risk assessment for new notified substances and commission regulation (EC) No 1488/94 on risk assessment for existing substances, Part II.* Luxembourg : Office for official publications of the european Communities, 1996.

Descheemaeker, K., Amede, T., Haileslassie, A. 2010. *Improving water productivity in mixed crop-livestock farming systems of sub-Saharan Africa.* Addis Ababa, Ethiopia : Elsevier - Agricultural Water Management 97, pp. 579-586, 2010.

DrugBank. Wishart, D. 2010. *DrugBank - Chemical On-line Database.* Alberta : s.n., 2010. Available at: www.drugbank.ca.

Eckel, W.P., Ross, B., Isensee, R. 1993. *Pentobarbital Found in Ground Water.* s.l. : Ground Water Vol.31, No.5, pp. 801-804, 1993.

Fent, K., Weston, A.A., Caminada, D. 2005. *Ecotoxicology of human pharmaceuticals.* Basel, Switzerland : Elsevier - Aquatic Toxicology 76, pp. 122-159, 2005. Available at: elsevier.com/locate/aquatox.

Gros, M., Petrovic M., Ginebreda, A., Barceló, D. 2009. *Removal of pharmaceuticals during wastewater treatment and environemntal risk assessment using hazard indexes.* Barcelona, Spain : Elsevier - Environement International (Article in Press), No.pp. 12, 2009. Available at: www.elsevier.com/locate/envint.

H+P, Laboratoriotechnik AG. 2010. *Sapromat E - BOD measuring unit.* Oberschleisheim : s.n., 2010.

Halling-Sørensen, B., Nielsen, S.N., Lanzky, P.F., Ingerslev, F., Lützhøft, H.C.H, Jørgensen, S.E. 1998. *Occurence, Fate and Effects of Pharmaceutical Substances in the Environment-A Review.* Copenagen, Denmark : Elsevier - Chemosphere 36, pp. 357-393, 1998.

Hardman, J.G., Limbird, L.E., Gilman, A.G. 2001. *Goodman and Gilman´s The Pharmacological Basis of Therapeuticals.* 10th. Nashvill, Tennessee, USA : McGraw-Hill Medical Publishing Division, 2001. ISBN 0-07-135469-7.

Heberer, T. 2002. *Occurence, fate, and removal of pharmaceutical residues in the aquatic environment: a review of recent research data.* Berlin, Germany : Elsevier - Toxicology Letters 131, pp. 5-17, 2002. Available at: www.elsevier.com/locate/toxlet.

Huschek, G., Hansen, P.D., Maurer, H.H., Krengel, D., Kayser, A. 2004. *Environmental risk assessment of medicinal products for human use according to European Commission recommendations.* s.l. : Elsevier - Environmetal Toxicology 19, pp. 226-240, 2004. Available at: www.elsevier.com/locate/envtox.

ChemBlink. 2007. *ChemBlink Formum - The Community of Chemical Professionals.* 2007. Available at: www.chemblink.com.

Isidori, M., Lavorgna, M., Nardelli, A., Pascarella, A. 2005. *Toxic and genotoxic evaluation of six antibiotics on non-target organisms.* s.l. : Elsevier - Science of the Total Environment 346, pp. 87-98, 2005.

Jjemba, P.K. 2006. *Ecretion and ecotoxicity of pharmaceuticals and personal care products in the environment.* Cincinnati, USA : Elsevier - Ecotoxicology and Environment Safety 63, pp. 113-130, 2006. Available at: www.elsevier.com/locate/ecoenv.

Jones, O.A.H., Voulvoulis, N., Lester, J.N. 2007. *The occurence and removal of selected pharmaceutical compounds in a sewege treatment works utilising activated sludge treatment.* London, UK : Elsevier - Environmental Pollution 145, pp. 738-744, 2007. Available at: www.elsevier.com/locate/envpol.

Joss, A. et al. 2005. *Removal of pharmaceuticals and fragrances in biological wastewater treatment.* Dübendorf, Switzerland : Elsevier - Water Research 39, pp. 3139-3152, 2005. Available at: www.elsevier.com/locate/waters.

Kasprzyk-Horden, B., Dinsdale, R.M., Guwy, A.J. 2009. *The removal of pharmaceuticals, personal care products, endocrine disruptors and illicit drugs during wastewater treatment and its impact on the quality of recieving waters.* Queensgate, Huddersfield, UK : Elsevier - Water Research 43, pp. 363-380, 2009. Available at: www.elsevier.com/locate/waters.

Kong, W.D., Zhu, Y.G., Liang, Y.C., Zhang, J., Smith, F.A., Yang,M. 2007. *Uptake of oxytetracycline and its phytotoxicity to alfalfa (Medicago sativa L.).* Beijing, China : Elsevier- Environmental Pollution 147, pp. 187-193, 2007. Availabel at: www.elsevier.com/locate/envpol.

Kreuzinger, N., Clara, M., Strenn, B., Krolss, H. 2004. *Relevance of the sludge retention time (SRT) as design criteria for wastewater treatment plants for the removal of endocrine disruptors and pharmaceuticals from wastewater.* Vienna, Austria : Water Science and Technology Vol. 50, No.5, pp. 149-156, 2004.

Kumar, K., Gupta, S.C., Baidoo, S.K., Chander, Y., Rosen, C.J. 2005. *Antibiotic Uptake by Plants from soil Fertilized with Animal Manure.* Madison, USA : ASA, CSSA, SSSA - reproduced from Journal of Environmental Quality 34, pp. 2082-2085 , 2005.

Kümmerer, K. (ed.). 2004. *Pharmaceuticals in the Environment. Sources, Fate, Effects and Risks. 2nd ed.* Berlin, Germany : Springer-Verlag Berlin Heidelberg, 2004. ISBN 3-540-21342-2.

Kümmerer, K., Al-Ahmad, A., Mersch-Sundermann, V. 2000. *Biodegradability of some antibiotics, elimination of the genotoxicity and affection of wastewater bacteria in a simple test.* Freiburg, Germany : Pergamon - Chemosphere 40, pp. 701-710, 2000.

Lindqvist, N., Tuhkanen, T., Kronberg, L. 2005. *Occurence of acidic pharmaceuticals in raw and treated sewages and in receiving waters.* Tampere, Turku, Finland : Elsevier - Water Research vol. 39, pp. 2219-2228, 2005. Available at: www.elsevier.com/locate/watres.

Lishman, L., et.al. 2006. *Occurence and reductions of pharmaceuticals and personal care products and etrogens by municipal wastewater treatment plants in Ontario, Canada.* Burlington, Ontario, Canada : Elsevier - Science of Total Environment 367, pp. 544-558, 2006. Available at: www.elsevier.com/locate/scitotenv.

manual, OxiTop. 2000. *Respirometric determination of the BOD5 of sewege polluted with inhibitory or toxic substances using the OxiTop measuring system.* Weilheim, Germany : Wissenschaftlich-Technische Werkstätten GmbH, 2000. Available at: www.WTW.com.

Mara, D., Horan, N., ed. 2003. *The Handbook of Water and Wastewater Microbiology.* London, UK : Academic Press - An Imprint of Elsevier, 2003. ISBN 0-12-470100-0.

Merck. 2006. *Merck Index - An Ancyclopedia of chemical drugs and biologicals.* White station, NJ, USA : Merck research laboratiories, Merck CO., INC, 2006.

—. 2001. *Merck Index - An Encyclopedia of chemical drugs and biologicals.* 13rd ed. White Station, NJ, USA : Merck Research Laboratories, Merck CO., INC, 2001. ISBN 0911910-13-1.

Morganwalp, D.W., Ulrich, J. 2010. *USGS, Science for a changing world. Toxic Substances Hydrology Program.* on-line : USGS - U.S. Geological Survey, 2010. Available at: http://toxics.usgs.gov/definitions/kow.html .

Murphy, B.L., Morrison, R.D. ed. 2002. *Introduction to Environmental Forensics.* London, UK : Elsevier Academic Press, 2002. ISBN 0-12-511355-2.

Norr, C., Meinecke, S., Brackemann, H. 2001. *Modification of the Zahn-Wellens test: determination of the biodegradability of poorly soluble, adsorbing and volatile substances by measurement of oxygen consumption and carbon dioxide production.* Berlin, Germany : Pergamon - Chemosphere 44, pp. 553-559, 2001. Available at: www.elsevier.com/locate/chemosphere.

OECD(a). 1992. *Guideline for testing of chemicals - Ready Biodegradability (OECD 301).* 1992.

OECD(b). 1992. *OECD guideline for testing of chemicals - Zahn-Wellens/EMPA Test, 302.* 1992.

Pagga, U. 1997. *Testing biodegradability with standardized methods.* Ludwigshafen, Germany : Pergamon - Chemosphere, Vol.35, No.12, pp. 2953-2972, 1997.

Pagga, U., Schäfer, A., Müller, R-J., Pantke, M. 2001. *Determination of the aerobic biodegradability of polymeric material in aquatic batch tests.* Ludwigshafen, Germany : Pergamon - Chemosphere 42, pp. 319-331, 2001.

Quinn, B., Gagné, F., Blaise, C. 2008. *An investigation into the acute and chronic toxicity of eleven pharmaceuticals (and their solvents) found in wastewatetr effluent on the cnidarian, Hydra attenuata.* Montréal, Québec, Canada : Elsevier - Science of the Total Environment 389, pp. 306-314, 2008. Available at: www.elsevier.com/locate/scitotenv.

Radshaw, C.H., Wootton, V.G., Rowland, S.J. 2008. *Uptake of pharmaceutical Fluoxetine Hydrochloride from growth medium by Brassicaceae.* Plymouth, Devon, UK : Elsevier - Phytochemistry 69, pp. 2510-2516, 2008. Available at: www.elsevier.com/locate/phytochem.

Reuschenbach, P., Pagga, U., Strotmann, U. 2003. *A Critical corparison of respirometric biodegradation tests based on OECD 301 and related methods.* Ludwigshafen, Germany : Elsevier - Water Research 37, pp. 1571 - 1582, 2003. Available at: www.elsevier.com/locater/waters.

Roppola, K. 2009. *Environmental application of manometric respirometris methods.* Oulu, Finland : Faculty of Science, Department of Chemistry, University of Oulu, P.0.Box 3000, FI-90014, 2009. Acta Univ. Oulu A 527.

Rosal, R. et al. 2010. *Occurrence of emerging pollutants in urban wastewater and their removal through biological treatment followed by ozonation.* Alclalá de Henares, Almería, Spain : Elsevier - Water Research vol. 44, pp. 578-588, 2010. Available at: www.elsevier.com/locate/watres.

Sanderson, H., Johnson, D.J., Wilson, C.J., Brain, R.A., Solomon, K.R. 2003. *Probabilistic hazard assessment of environmentally occurring pharmaceuticals toxicity to fish, daphnids and algae by ECOSAR screening.* Guelph, Ontario, Canada : Elsevier - Toxicology Letters 144, pp. 383-395, 2003. Avalable at: www.elsevier.com/locate/toxlet.

Sanderson, H., Thomsen, M. 2009. *Comparative analysis of pharmaceuticals versus industrial chemicals acute aquatic toxicity classification according to the United Nations classification system for chemicals.* Roskilde, Denmark : Elsevier - Toxicology Letters 187, pp. 84-93, Assessment of the (Q)SAR predictability of pharmaceuticals acute aquatic toxicity and their predominant acute toxic mode-of-action, 2009. Available at: www.elsevier.com/locate/toxlet.

Sangave, P.C., Gogate, P.R., Pandit, A.B. 2007. *Combination of ozonation with conventional aerobic oxidation for distillery wastewater treatment.* Mumbai, India : Elsevier - Chemosphere vol. 68, pp. 32-41, 2007. Available at: www.elsevier.com/locate/chemosphere.

Strotmann, U.J., Schwarz, H., Pagga, U. 1995. *The combined CO2/DOC test- a new method to determine the biodegradability of organic compounds.* Ludwigshafen : Pergamon - Chemosphere, Vol.30, No.3, p.525-538, 1995.

Struijs, J., van den Berg, R. 1995. *Standardized biodegradability tests: extrapolation to aerobic environments.* Bilthoven : Pergamon - Water Research, Vol. 29, No.1, pp.255-262, 1995.

Sweetman, S. C., ed. 2007. *Martindale - The Complete Drug Reference.* 35th ed. London, UK : Pharmaceutical Press, 2007. ISBN-10 0 85369 687 X.

Techobanoglous, G., Sroeder, E.D. 1987. *Water Quality - Characteristics, modeling, modification.* University of California at Davis : Addison-Wesley publishing company, 1987. ISBN 0-201-05433-7.

Ternes, T.A. 1998. *Occurence of drugs in German sewege treatment plants and rivers.* Koblenz, Germany : Elsevier - Water research 32 (11), pp. 3245-3260, 1998.

Ternes, T.A., Bonerz, M., Herrmann, N., Teiser, B., Andersen, H.R. 2007. *Irrigation of treated wastewater in Braunschweig, Germany: An option to remove pharmaceuticals and musk fragrances.* Brauschweig, Germany : Elsevier - Chemosphere 66, pp. 894-904, 2007. Available at: www.elsevier.com/locate/chemosphere.

Tortora, G.J., Funke, B.R., Case, C.L. 2007. *Microbiology.* 9th ed. San Francisco, USA : Pearson Education, Inc., 2007. ISBN 0-321-39602-2.

UCL.co.ke. 2010. *Universal corporation LTD.* Nairobi : ucl, 2010. Available at: http://ucl.co.ke.

Vieno, N., Tuhkanen, T., Kronberg, L. 2007. *Elimination of pharmaceuticals in sewege treatment plants in Finland.* Tampere, Turku, Finland : Elsevier- Water Research vol.41, pp. 1001-1012, 2007. Avalible at: www.sciencedirect.com/locate/watres.

Winker, M., Tettenborn, F., Gulyas, H., Otterpohl, R. 2008. *Comparison of analytical and theoretical pharmaceutical concentrations in human urine in Germany.* Hamburg, Germany : Elsevier - Science Direct, Water Research 42, pp. 3633-3640, 2008. Available at: www,elsevier.com/locate/watres.

Wollenberger, L., Halling-Sørensen, B., Kusk, K.O. 2000. *Acute and chronic toxicity of veterinary antibiotics to Daphnia magna.* Lyngby, Denmark : Pergamon - Chemosphere 40, pp. 723-730, 2000.

WTW. 2009. *BOD measuring system OxiTop-C 110.* Weilheim : s.n., 2009. (sited on 26.08.2010). Available at: www.wtw.com.

Zhang, Y., Geissen, S-U., Gal, C. 2008. *Carbamazepine and Diclofenac: Removal in wastewater treatment plants and occurence in water bodies.* Berlin, Germany : Elsevier - Chemosphere 73, pp. 1151-1161, 2008. Available at: www.elsevier.com/locate/chemosphere.

Zorita, S., Märtensson, L., Mathiasson, L. 2009. *Occurence and removal of pharmaceuticals in municipal sewege treatment in the south of Sweden.* Lund, Sweden : Elsevier - Science of the Total Environment 407, pp. 2760-2770, 2009. Available at: www.elsevier.com/locate/scitotenv.

Appendix A Biodegradation curves: BOD_7

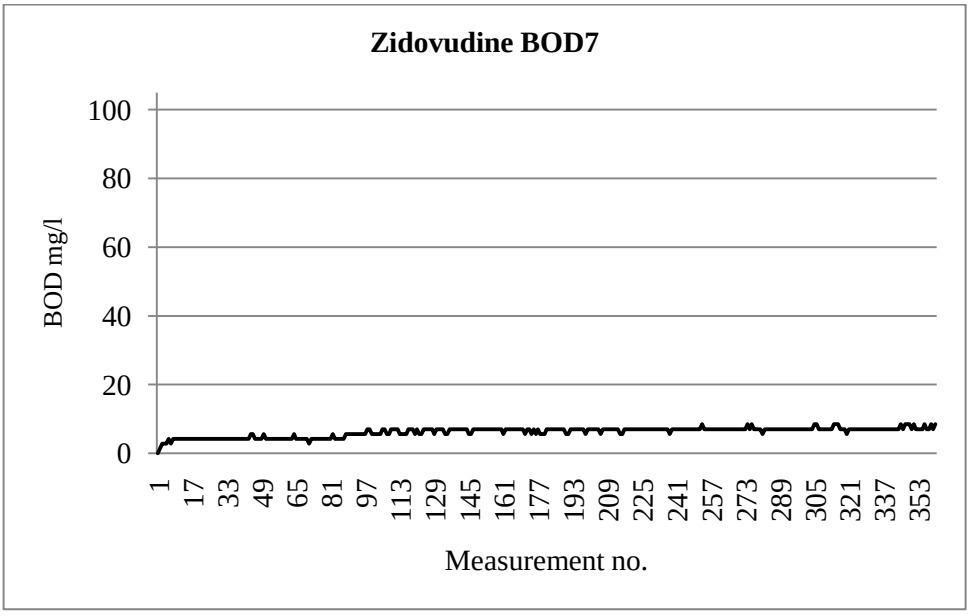


Figure 7 Biodegradation curve: Zidovudine

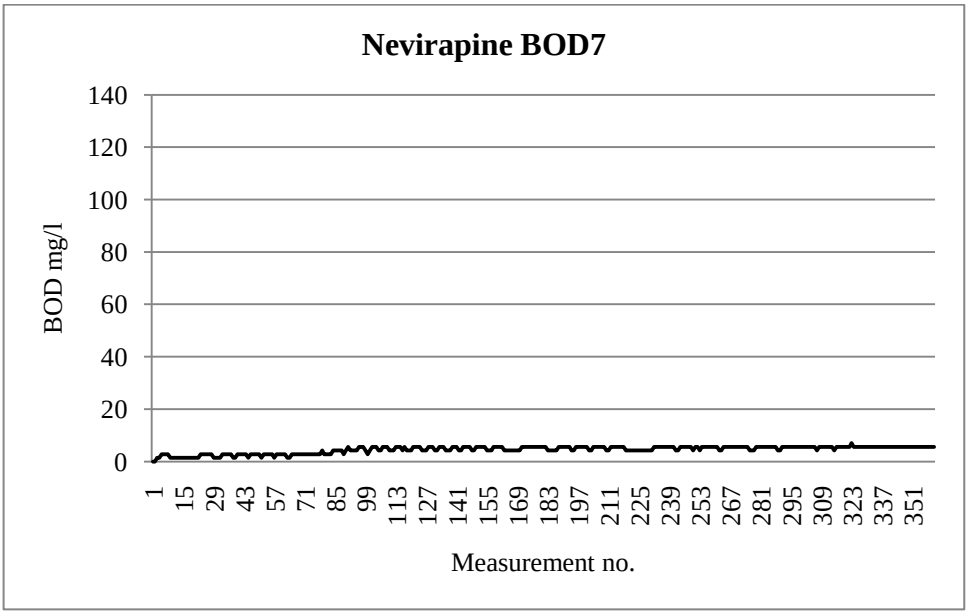


Figure 8 Biodegradation curve: Nevirapine

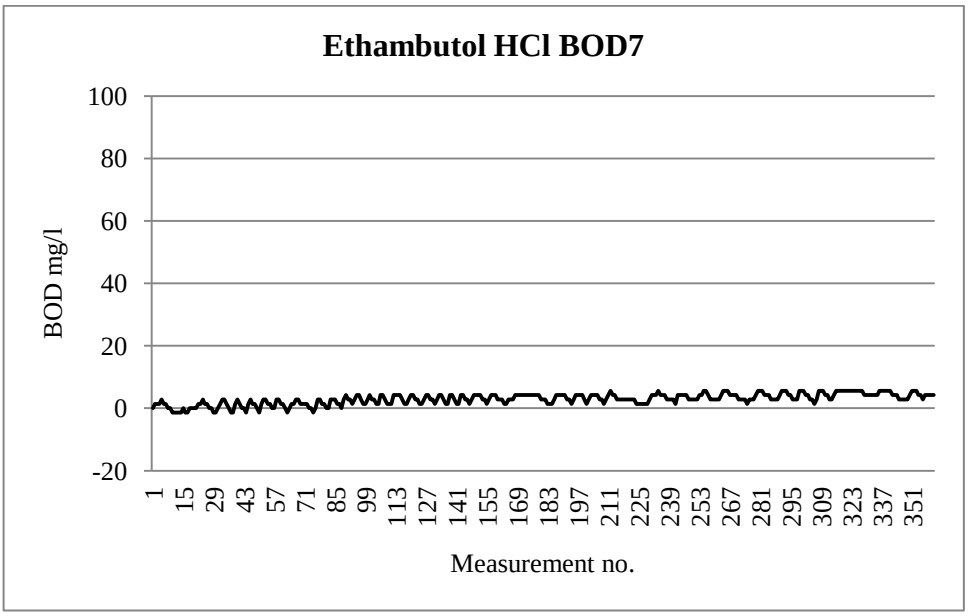


Figure 9 Biodegradation curve: Ethambutol HCl

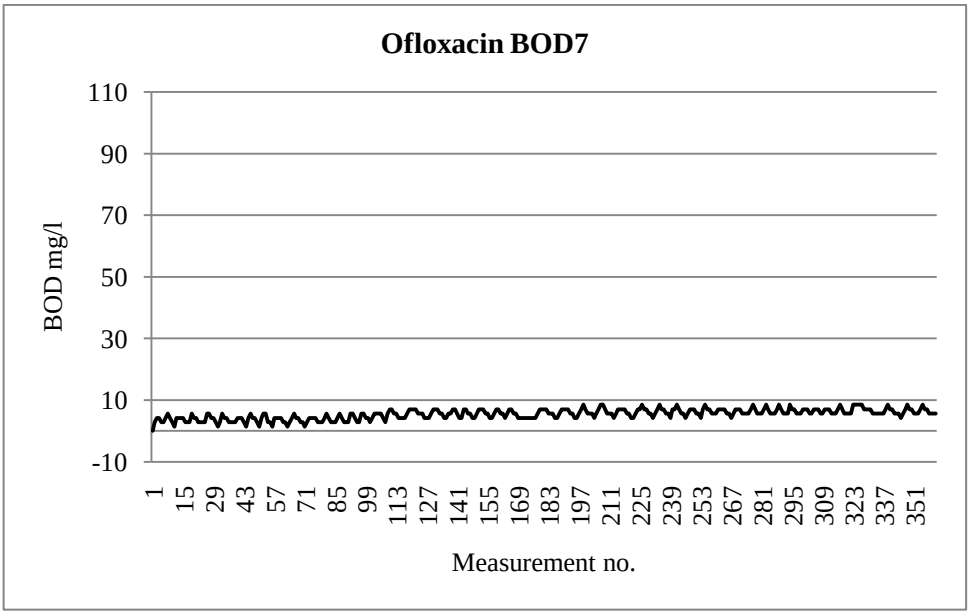


Figure 10 Biodegradation curve: Ofloxacin

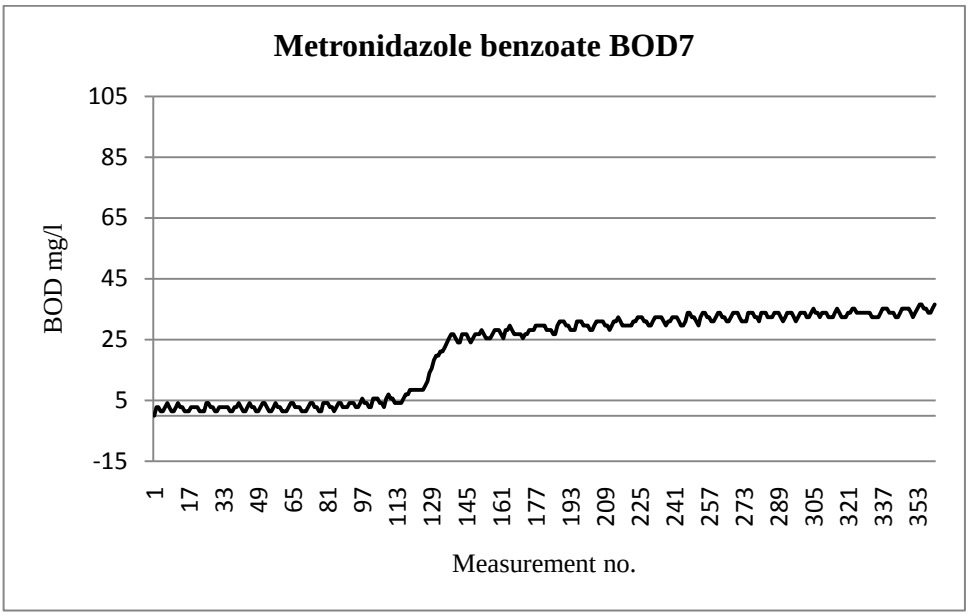


Figure 11 Biodegradation curve: Metronidazole benzoate

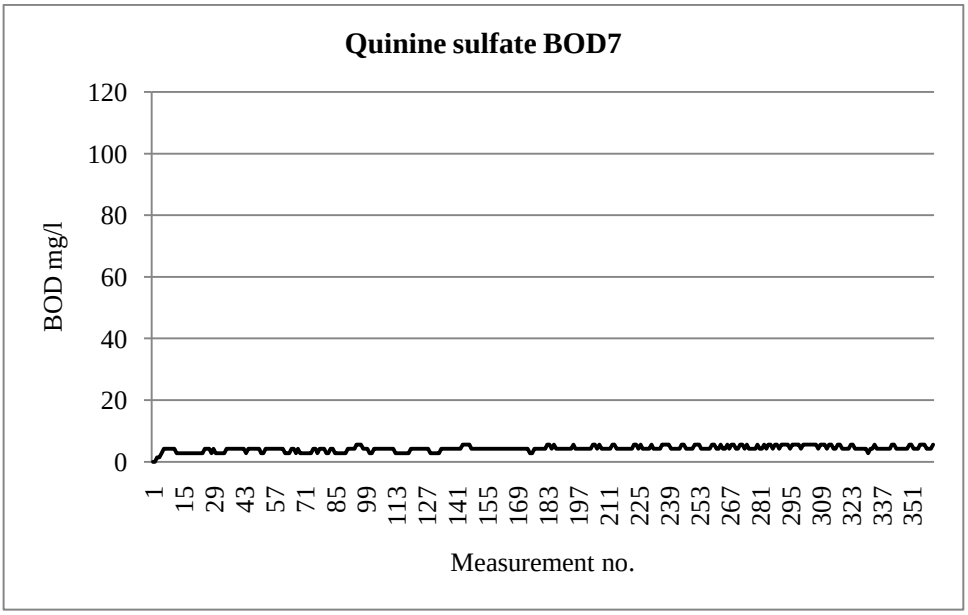


Figure 12 Biodegradation curve: Quinine sulfate

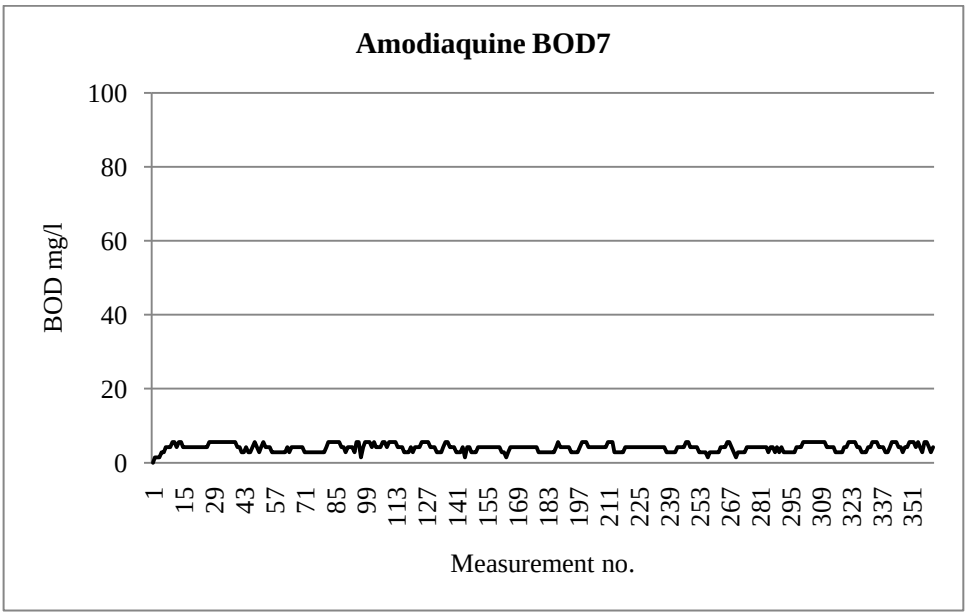


Figure 13 Biodegradation curve: Amodiaquine

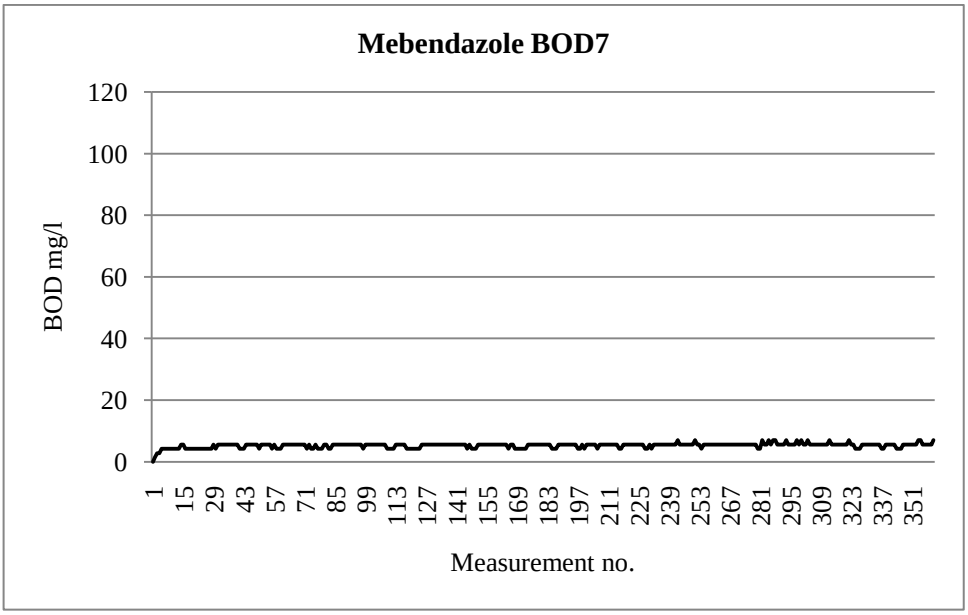


Figure 14 Biodegradation curve: Mebendazole

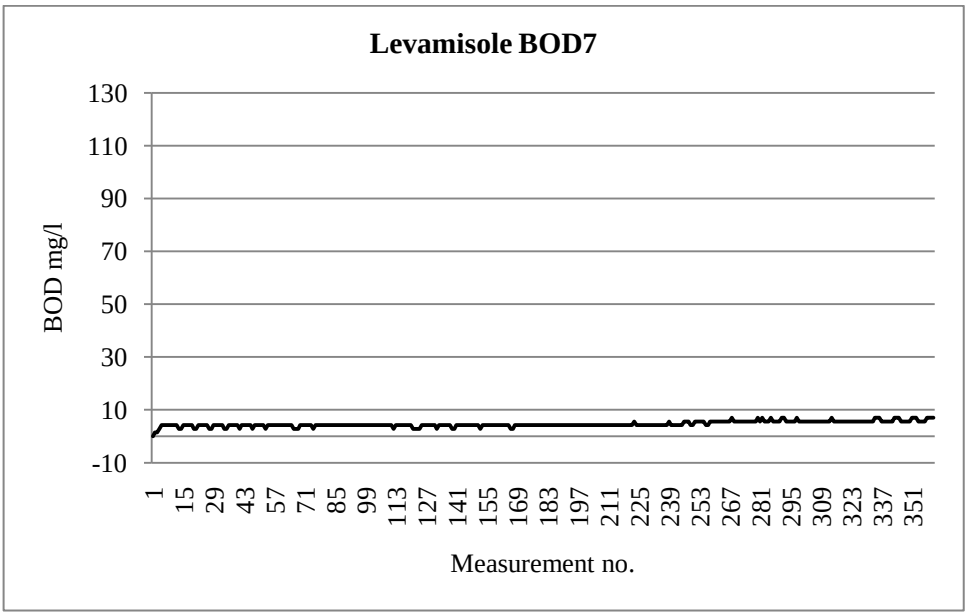


Figure 15 Biodegradation curve: Levamisole

Appendix B Biodegradation curves: BOD_{28}

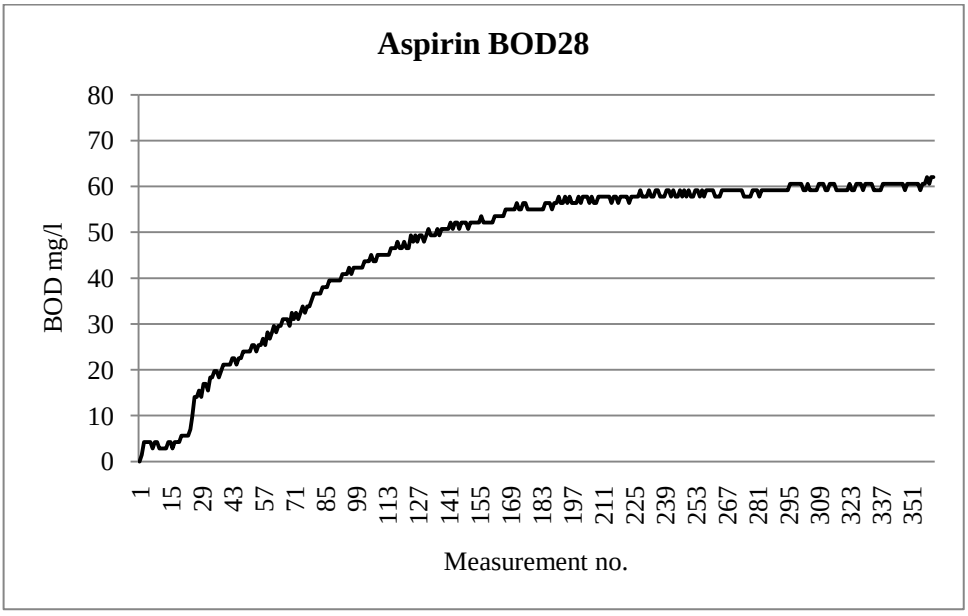


Figure 16 Biodegradation curve: Aspirin

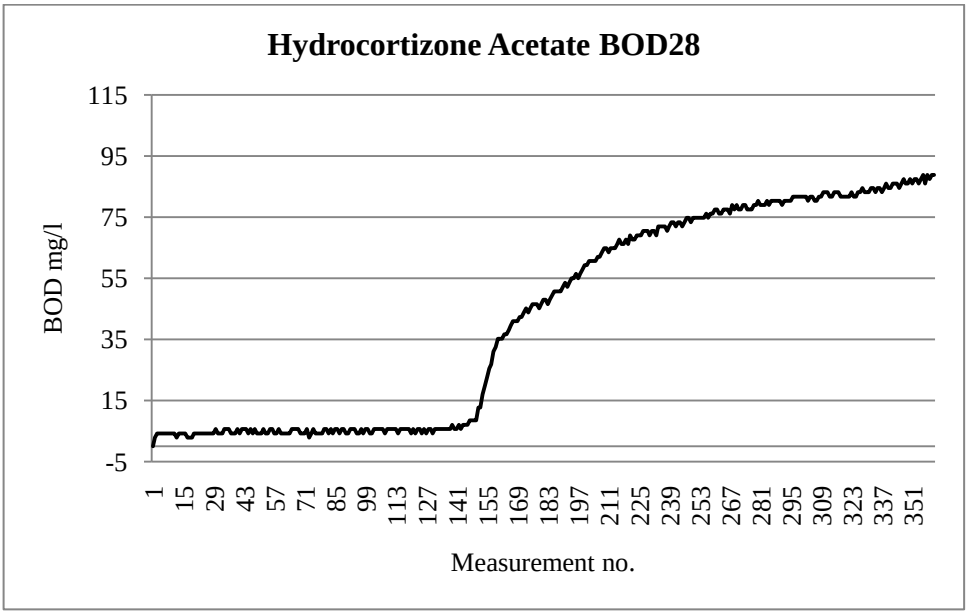


Figure 17 Biodegradation curve: Hydrocortisone acetate

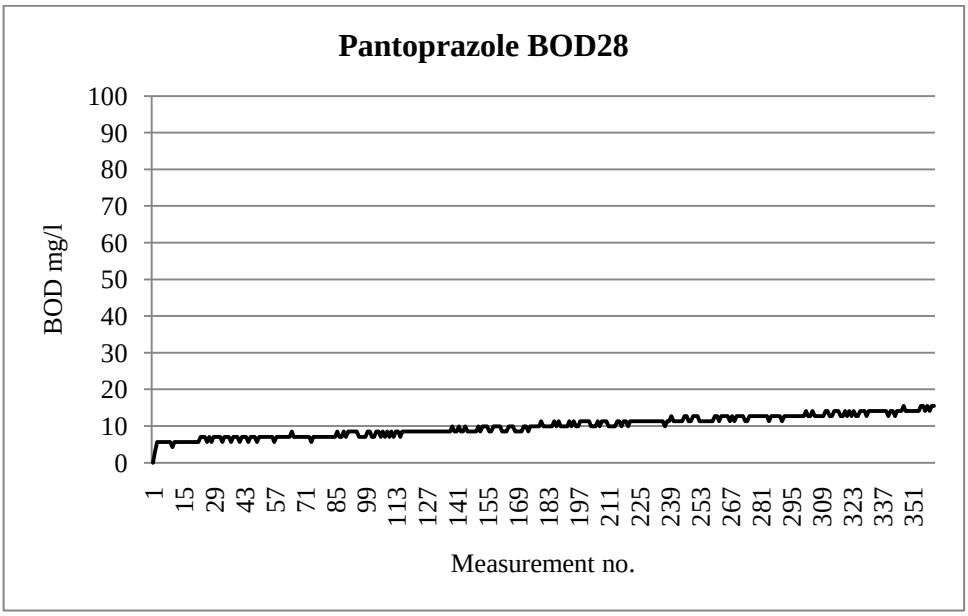


Figure 18 Biodegradation curve: Pantoprazole

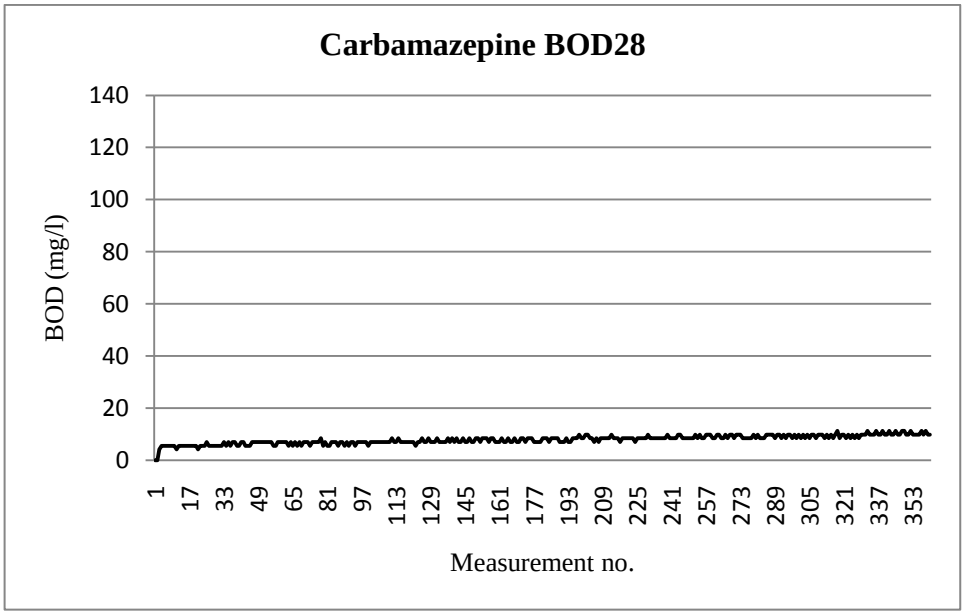


Figure 19 Biodegradation curve: Carbamazepine

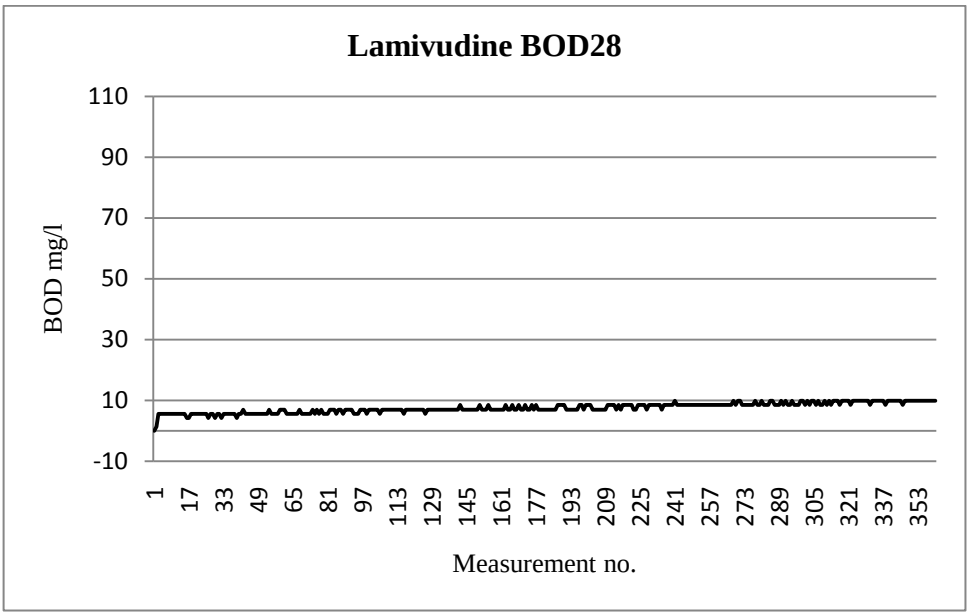


Figure 20 Biodegradation curve: Lamivudine

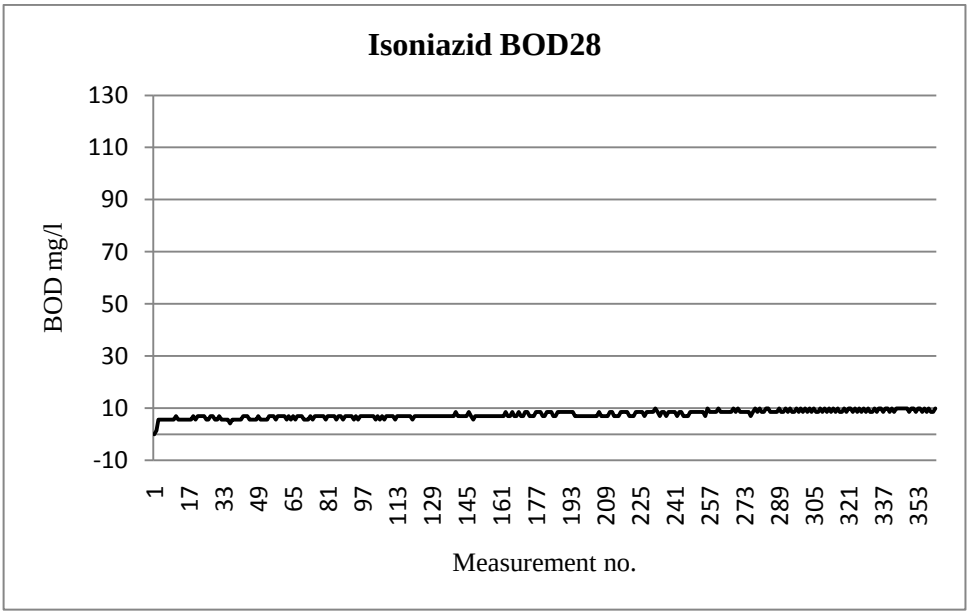


Figure 21 Biodegradation curve: Isoniazid

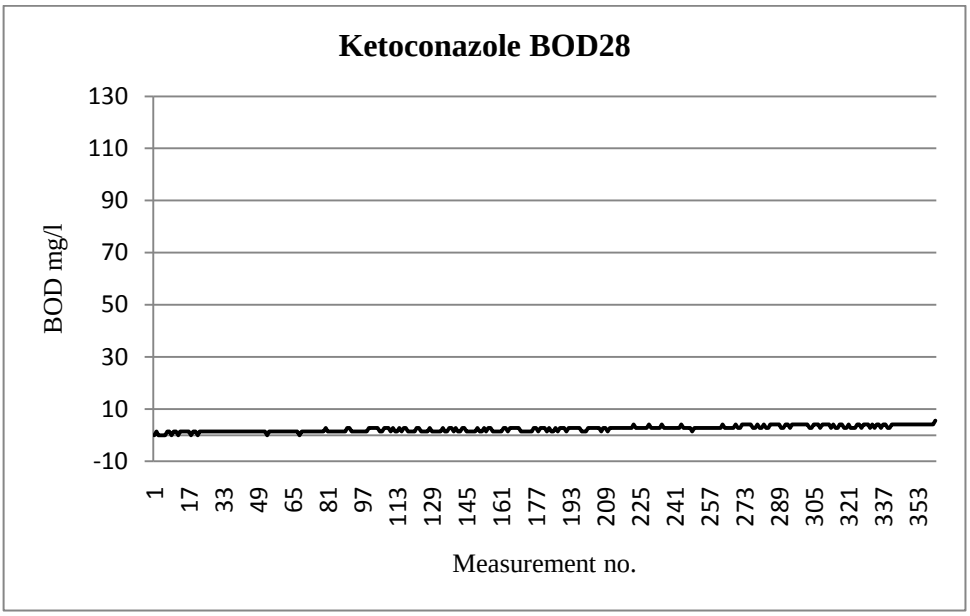


Figure 22 Biodegradation curve: Ketoconazole

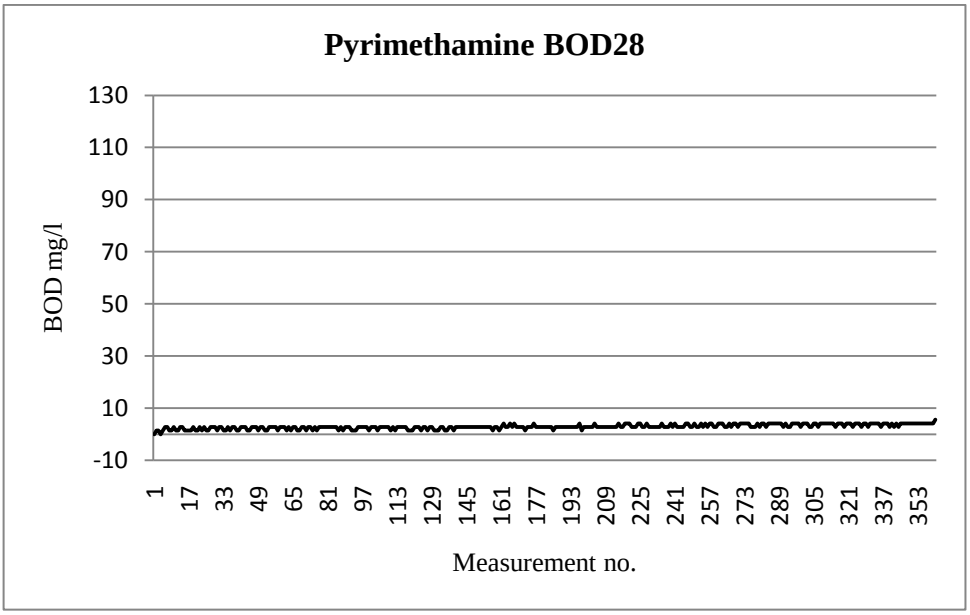


Figure 23 Biodegradation curve: Pyrimethamine

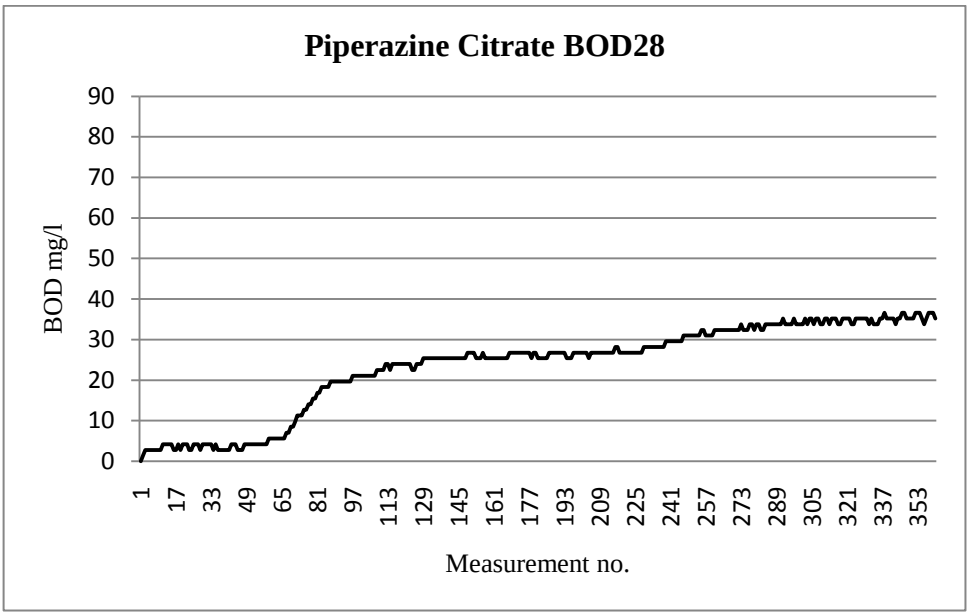


Figure 24 Biodegradation curve: Piperazine Citrate