Cranfield University

School of Industrial and Manufacturing Science
Department of Water Sciences

Thesis submitted for the degree of Doctor of Philosophy
Academic year 2005-2006

Andrew Thompson

The Fate and Removal of Pharmaceuticals during Sewage Treatment

Supervisors: Dr Elise Cartmell and Dr Richard Stuetz

Date of presentation: October 2005

This thesis is submitted in partial fulfilment of the requirements for the degree of Doctor of Philosophy

Abstract

Pharmaceuticals, personal care products and their metabolites are continuously entering the environment through many routes, especially from the effluent of sewage treatment plants. The aim of this work was to examine the fate and removal of pharmaceuticals during sewage treatment, and establish ways in which current sewage treatment technologies could be optimised to improve removal. Based on an analysis of pharmaceutical usage and environmental effects, four compounds were selected for further study (triclosan, tetracycline, carbamazepine, and caffeine). Reliable analytical methods were developed, using HPLC-UV, to detect these compounds in sewage samples.

The amounts of removal of the four compounds were quantified using laboratory sorption and biodegradation tests. Both tetracycline and triclosan were shown to be readily biodegradable, and to sorb strongly to biomass, although sorption occurred at different rates. Caffeine degraded rapidly, but did not sorb to biomass, whilst carbamazepine did not sorb or biodegrade. Grab samples were taken before and after every major process unit at four sewage treatment plants (STPs). Although tetracycline was not detected in any samples, triclosan was measured at concentrations up to 5115 ng l^{-1}, caffeine was measured at concentrations up to 82,300 ng l^{-1}, and carbamazepine was measured at concentrations up to 1461 ng l^{-1}. This is the first time carbamazepine and caffeine concentrations have been reported in UK sewage. The grab samples showed that a wide range of pharmaceutical effluent concentrations can be expected. The concentrations of the pharmaceuticals detected in this research were not high enough to cause immediate harm (i.e. death) to aquatic organisms. However, there is insufficient information to determine whether exposure to these low concentrations, typically around PNEC levels, may have an effect over a long period of time.

Further composite sampling conducted at one STP generated data, modelled using Toxchem+, which demonstrated how variations in a wide range of parameters were correlated with the removal of pharmaceuticals. These showed that whilst sludge age may be the most important parameter, pH, temperature, hydraulic retention time, and chemical oxygen demand could have a critical effect on the removal of pharmaceuticals. Several ways of optimising sewage treatment plants have been proposed, including pH adjustments and longer HRTs to enhance sorption, as well as a novel adaptation to activated sludge tanks incorporating two IFAS type bioreactors to enhance biodegradation.

The effects of plant operating events, such as aeration failures, were also investigated. These showed that a typical length of aeration loss (four hours) could result in reduced pharmaceutical removal (through decreases in both sorption and biodegradation) for up to twelve hours.

Overall, this work has shown that it may be possible to adapt current sewage treatment technology to improve removal of pharmaceuticals which sorb or biodegrade readily. With further research, these adaptations could become a viable alternative to tertiary treatment technologies such as ozonation, granular activated carbon, or chlorine dioxide.

Table of Contents

Chapter 1: Introduction 2
1.1 Aims and Objectives 4
1.2 Thesis plan 4

Chapter 2: Literature Review 7
2.1 Usage of PPCPs 7
2.1.1 Usage of PPCPs in the UK 8
2.1.2 Usage of PPCPs in Europe 17
2.1.3 Summary of usage data 20
2.2 Occurrence of PPCPs in the environment 21
Sewage treatment plant effluent 22
Sewage sludge 24
Landfill leachate 26
Streams and rivers 27
Drinking water 28
Summary of pharmaceutical detection data 29
2.2.1 Routes into the environment 30
Reduction of usage and pipe leakage 31
Reducing straight-piping and sewer overflow events 32
Changing the disposal methods for surplus drugs 33
Improving efficiency of sewage treatment plants (STPs) 35
2.3 Environmental significance 36
2.3.1 Risk to humans 36
2.3.2 Risk to aquatic organisms 38
Risk assessment in the US 39
Risk assessments in the EU (including UK) pre-2006 40
Risk assessments in the EU (including UK) post-2006 42
2.3.3 Chemicals with PEC>PNEC or MEC/PNEC in the UK 44
2.3.4 Mixtures of compounds 46
2.4 Degradation products and metabolites 48
2.5 The role played by sewage treatment plants 51
2.5.1 Current Technologies 51
Preliminary treatment 51
Primary sedimentation 51
Secondary treatment 53
Suspended growth systems 53
Fixed film systems 53
2.5.2 Removal mechanisms 55
Photolysis 56
Sorption 57
Biodegradation 60
Hydrolysis 61
2.5.3 Fate of pharmaceuticals during sewage treatment 61
2.5.4 Parameters affecting removal efficiency 64
2.5.5 Tertiary treatments 66
2.6 Summary 67

Chapter 3: Choice of pharmaceuticals for study 70
3.1 Prioritisation 70
3.1.1 Prioritisation by measured environmental concentration 70
3.1.2 Prioritisation by PEC to PNEC and/or MEC to PNEC ratio 72
3.1.3 Prioritisation by bioaccumulation 73
3.1.4 Prioritisation by removal in sewage treatment plants and additional weightings . 74
3.1.5 Scoring 75
3.1.6 Overall selection, modulated by analytical methodology 76
3.2 Details on compounds selected for study 77
3.2.1 Caffeine 77
3.2.2 Carbamazepine 78
3.2.3 Tetracycline 79
3.2.4 Triclosan 83
3.3 Summary 86

Chapter 4: Analytical development 87
4.1 Development of HPLC methods 87
Procurement of reference standards 87
Measurement of standard concentrations 89
Selection and development of the chromatographic method 89
Column and instrument performance 90
4.1.1 Triclosan 93
4.1.2 Tetracycline 97
4.1.3 Caffeine 101
4.1.4 Carbamazepine 105
4.2 Development of Solid Phase Extraction (SPE) 109

Chapter 5: Materials and Methods 114
5.1 Source of Materials 114
5.2 Description of STPs 114
5.2.1 Northend STP 115
5.2.2 Frankton STP 115
5.2.3 Southam STP 116
5.2.4 Finham STP 117
5.3 Sample Collection and Storage 118
5.3.1 Grab sampling 118
5.3.2 Composite sampling 119
5.4 Description and Operation of rigs 119
5.4.1 Rolling Tubes 119
5.4.2 Rotating biological contactor (RBC) 120
5.4.3 Porous Pots 121
5.5 Sorption and desorption tests 123
5.5.1 Preparation of biomass 123
5.5.2 Test procedure 123
5.5.3 Data analysis 125
5.6 Biodegradation tests 126
5.6.1 Preparation of biomass 126
5.6.2 Test procedure 126
5.6.3 Data analysis 127

5.7 Dynamic reactor studies 127
5.7.1 Aeration failure 128
5.7.2 Rainfall event 128
5.8 Wastewater Characterisation 129
5.8.1 Preparation of solids free fraction 129
5.8.2 pH, temperature, and redox potential 129
5.8.3 Chemical oxygen demand (COD) 129
5.8.4 Nitrate, Nitrite, Phosphorus, and Sulphate 129
5.8.5 Ammonia 130
5.8.6 Soluble protein 130
5.8.7 Soluble carbohydrate 130
5.9 Biomass Characterization 130
5.9.1 Preparation of Extracellular Polymeric Substances (EPS) 131
5.9.2 Total carbon, inorganic carbon, and organic carbon 131
5.9.3 Suspended solids 131
5.9.4 Volatile suspended solids 131
5.9.5 Lipid content 131
5.9.6 Extracellular Protein 132
5.9.7 Extracellular carbohydrate 132
5.9.8 Particle size 132
5.10 Summary of HPLC and Solid Phase Extraction (SPE) conditions 133

Chapter 6: Fate of pharmaceuticals in laboratory tests 135
6.1 Sorption and desorption tests 135
6.1.1 Triclosan 135
6.1.2 Tetracycline 139
6.1.3 Carbamazepine 143
6.1.4 Caffeine 143
6.1.5 Comparing sorption tests with reality 146
6.2 Biodegradation 147
6.2.1 Triclosan 147
6.2.2 Tetracycline 148
6.2.3 Carbamazepine 149
6.2.4 Caffeine 150
6.3 Summary 151

Chapter 7: Grab sampling of STPs 153
7.1 Triclosan 153
7.1.1 Northend STP 154
7.1.2 Frankton STP 155
7.1.3 Southam STP 156
7.1.4 Finham STP 158
7.1.5 Fate of Triclosan in STPs 160
7.2 Tetracycline 162
7.3 Carbamazepine 162
7.3.1 Northend STP 162
7.3.2 Frankton STP 163
7.3.3 Southam STP 164
7.3.4 Finham STP 165

7.3.5 Fate of carbamazepine 166
7.4 Caffeine 167
7.4.1 Northend STP 167
7.4.2 Frankton STP 169
7.4.3 Southam STP 169
7.4.4 Fate of caffeine 171
7.5 Summary of findings 171

Chapter 8: Composite Sampling of Southam STP 173
8.1 Pharmaceutical concentrations 173
8.1.1 Triclosan concentrations 173
8.1.2 Tetracycline concentrations 177
8.1.3 Carbamazepine concentrations 177
8.1.4 Caffeine concentrations 179
8.2 Wastewater and biomass parameters - implications for pharmaceutical removal 182
8.2.1 Suspended solids 183
8.2.2 pH 186
8.2.3 Chemical oxygen demand 190
8.2.4 Volatile suspended solids 194
8.2.5 Carbon content (total, organic, and inorganic) 196
8.2.6 Soluble Protein and carbohydrate 199
8.2.7 Fats, oil, and grease (FOG) content 201
8.2.8 Extracellular Protein and Carbohydrate 202
8.3 Summary 204

Chapter 9: Modelling and implications for improving pharmaceutical removal in STPs 205
9.1 Modelling the effect of wastewater and biomass parameters on pharmaceutical removal207
9.1.1 Flow rate 207
9.1.2 Inlet temperature 208
9.1.3 MLSS 210
9.1.4 MLVSS. 211
9.1.5 Summary of Toxchem+ modelling 212
9.2 Adapting sewage treatment technology to improve pharmaceutical removal 212
9.2.1 Sludge retention time 212
9.2.2 Hydraulic retention time 212
9.2.3 pH 213
9.2.4 Temperature 214
9.2.5 Chemical oxygen demand 214
9.3 Summary of proposed modifications to existing sewage treatment technologies 218

Chapter 10: Plant operating events 219
10.1 Aeration Failures – one hour loss test 219
10.2 Aeration Failures – four hour loss test 219
10.2.1 Carbamazepine 221
10.2.2 Tetracycline 221
10.2.3 Caffeine 223
10.2.4 Triclosan 224
10.2.5 Summary of aeration loss tests 224
10.3 Storm Flows 225

Chapter 11: Conclusions 226
11.1 Optimisation of STPs 227
11.2 Costs versus benefits - implications for environmental protection and human health229
11.3 Further work 230

References 233

Appendix A 259
13.1 Pharmaceuticals detected in sewage effluents 259
13.2 Pharmaceuticals detected in streams or rivers 266
13.3 Pharmaceuticals detected in drinking water 271
13.4 Pharmaceuticals detected in landfill leachate 273
13.5 Pharmaceuticals detected in sewage sludges 274

Appendix B 275

Appendix C 277

List of Figures

Figure 2.1: Possible sources and pathways for the occurrence of pharmaceutical residues in the aquatic environment. (Heberer 2002) 30
Figure 2.2: Concentration profile of pharmaceuticals below a "*leaky*" sewer (Ellis 2006) 32
Figure 2.3: Calculation of PEC according to EMEA guideline 4447 42
Figure 2.4: Phase I and Phase II metabolic reactions (Daughton and Ternes 1999) 48
Figure 2.5: The degradation pathway of caffeine (1,3,7-trimethylxanthine) (Georga *et al.* 2001) 49
Figure 2.6: Combinations of existing preliminary, primary, secondary, and tertiary wastewater treatment processes. (a) trickling filters, (b) activated sludge process, (c) rotating biological contactors, (d) enhanced biological nutrient removal, and (e) membrane bioreactors. (Metcalf and Eddy 2003) 52
Figure 2.7: Activated sludge STP diagram (Metcalf and Eddy 2003) 53
Figure 2.8: Percentage sorption as a function of K_d and sludge production, according to Equation 5 59
Figure 2.9: Biological degradation rates for 35 pharmaceuticals (Joss *et al.* 2005) 61
Figure 2.10 Pharmaceutical removal efficiencies during sewage treatment (Ternes 1998) 62

Figure 3.1: Structures of tetracycline and its biological degradation products (Halling-Sorensen *et al.* 2002) 81
Figure 3.2: Photodegradation products of tetracycline (Liang *et al.* 1998) 82
Figure 3.3: Structure of triclosan in phenolic and phenolate forms (Lindstrom *et al.* 2002) 84

Figure 4.1: Calculation of column efficiency and peak asymmetry (Phenomenex 2005/06) 91
Figure 4.2: Factors affecting peak resolution (Phenomenex 2005/06) 92
Figure 4.3: Triclosan chromatograms: (a) 10mg l^{-1} triclosan in methanol, (b) 200 μg l^{-1} in crude sewage, (c) 100 μg l^{-1} in crude sewage, (d) crude sewage (no triclosan), and (e) final effluent (no triclosan) 94
Figure 4.4: Chromatogram of tetracycline Group II compounds at 20 mg l^{-1} in methanol. t = 2.5 epi-tetracycline, t = 2.8 tetracycline, t = 7.0 epi-anhydrotetracycline, t = 9.0 anhydrotetracycline 98
Figure 4.5: Tetracycline chromatograms: (a) 10mg l^{-1} tetracycline in methanol, (b) 400 □ g l^{-1} in crude sewage, (c) 400 □ g l^{-1} in final effluent, (d) crude sewage (no tetracycline), and (e) final effluent (no tetracycline) 99
Figure 4.6: Chromatogram of caffeine and degradation products. t = 19.2 caffeine, t = 12.6 theophylline, t = 11.7 paraxanthine, t = 9.6 1,7-dimethyluric acid, t = 7.1 theobromine 102
Figure 4.7: Caffeine chromatograms: (a) 10mg l^{-1} caffeine in methanol, (b) 61 □ g l^{-1} in crude sewage, (c) 114 ng l^{-1} in final effluent, and (d) crude sewage (no caffeine) 103
Figure 4.8: Chromatogram of carbamazepine (t = 10.7) and 10,11-dihydrocarbamazepine (t = 11.3) 105
Figure 4.9: Carbamazepine chromatograms: (a) 10mg l^{-1} carbamazepine in methanol, (b) 200 □ g l^{-1} in crude sewage, (c) 200 □ g l^{-1} in final effluent, and (d) crude sewage (no carbamazepine). 107
Figure 4.10: Diagram of SPE process (Phenomenex 2005/06) 109
Figure 4.11: Chromatograms of 1 μg l^{-1} triclosan in crude sewage: (a) before SPE, and (b) after SPE. 111
Figure 4.12: Chromatograms of 1 μg l^{-1} carbamazepine in crude sewage: (a) before SPE, and (b) after SPE. 111

Figure 5.1: Flow diagram of Northend STP, showing sampling points 115
Figure 5.2: Flow diagram of Frankton STP, showing sampling points 116
Figure 5.3: Flow diagram of Southam STP, showing sampling points 117
Figure 5.4: Flow diagram of Finham STP, showing sampling points 117
Figure 5.5: Picture of rolling tubes rig 120
Figure 5.6: Picture of the rotating biological contactor rig 121
Figure 5.7: Picture of porous pots rig 122

Figure 6.1: Freundlich sorption isotherms for triclosan with fixed film and suspended growth biomass 136
Figure 6.2: Desorption Freundlich isotherms for triclosan with fixed film and suspended growth biomass 137
Figure 6.3: Profile of triclosan sorption, from an initial concentration of 10 mg l^{-1} 138
Figure 6.4: Freundlich sorption isotherms for tetracycline with fixed film and suspended growth biomass 139
Figure 6.5: Freundlich sorption isotherms for tetracycline with two different samples of suspended growth biomass 140
Figure 6.6: Freundlich desorption isotherms for tetracycline with two different samples of suspended growth biomass 141
Figure 6.7: Profile of tetracycline sorption, from an initial concentration of 10 mg l^{-1} 142
Figure 6.8: Chromatogram of caffeine desorption sample after 22 hours. A=Paraxanthine, B=Theophylline, C=7-Methylxanthine, D=Unknown 144
Figure 6.9: Desorption of caffeine degradation products 145
Figure 6.10: Degradation of triclosan with suspended growth biomass 147
Figure 6.11: Degradation of tetracycline with fixed film biomass 148

Figure 7.1: Concentration of triclosan in samples of crude sewage, settled sewage, RBC effluent, and final effluent from Northend STP (fixed film) 155
Figure 7.2: Concentration of triclosan in samples of crude sewage, settled sewage, humus effluent, and final effluent from Frankton STP (fixed film) 156
Figure 7.3: Concentration of triclosan in samples of crude sewage, oxidation ditch effluent, and final effluent taken from Southam STP (suspended growth) 157
Figure 7.4: Concentration of triclosan in samples of crude sewage, settled sewage, aeration outlet, return activated sludge (RAS), and final effluent at Finham STP (suspended growth). nd = not detected 159
Figure 7.5: The relationship between hydraulic retention time and percentage removal of triclosan. (Range of retention times taken from Metcalf and Eddy (2003) and Severn Trent Design Manual (Green 2004)). 161
Figure 7.6: Concentration of carbamazepine in samples of crude sewage, settled sewage, RBC effluent, and final effluent from Northend STP (fixed film) 163
Figure 7.7: Concentration of carbamazepine in samples of crude sewage, settled sewage, humus effluent, and final effluent from Frankton STP (fixed film) 164
Figure 7.8: Concentration of carbamazepine in samples of crude sewage, oxidation ditch effluent, and final effluent taken from Southam STP (suspended growth) 165
Figure 7.9: Concentration of carbamazepine in samples of crude sewage, settled sewage, aeration outlet, return activated sludge (RAS), and final effluent at Finham STP (suspended growth) 166

Figure 7.10: Concentration of caffeine and degradation products in samples of crude sewage, settled sewage, RBC effluent, and final effluent from Northend STP (fixed film) 168
Figure 7.11: Concentration of caffeine and degradation products in samples of crude sewage, settled sewage, humus effluent, and final effluent from Frankton STP (fixed film) ... 169
Figure 7.12: Concentration of caffeine and degradation products in samples of crude sewage, oxidation ditch effluent, and final effluent taken from Southam STP (suspended growth) 170

Figure 8.1: Concentrations of triclosan in Southam STP. 174
Figure 8.2: Influent sewage flow at Southam STP 175
Figure 8.3: Daily variation of triclosan mass flow 176
Figure 8.4: Concentrations of carbamazepine in Southam STP 177
Figure 8.5 Daily variation of carbamazepine mass flow 178
Figure 8.6: Concentrations of caffeine in Southam STP 180
Figure 8.7: Daily variation of caffeine mass flow 181
Figure 8.8 Daily variation of suspended solids 183
Figure 8.9: Modelling of the effect of SRT on biological degradation rate, with T_{crit} = 10 days185
Figure 8.10: Daily variation of pH 186
Figure 8.11: Correlation between pH and triclosan concentration in Southam STP 187
Figure 8.12: Percentage of triclosan as phenolate form in Southam STP 188
Figure 8.13: Correlation between pH and caffeine concentration in Southam STP 189
Figure 8.14: Daily variation of chemical oxygen demand 190
Figure 8.15: Correlation between chemical oxygen demand and triclosan concentration 191
Figure 8.16: Relationship between influent COD concentration and percentage removal of triclosan and COD, with average COD influent concentration marked in red 191
Figure 8.17 Daily variation of volatile suspended solids 194
Figure 8.18: Correlation between biomass yield and triclosan removal 195
Figure 8.19 Daily variation of total carbon 196
Figure 8.20: Daily variation of inorganic carbon 197
Figure 8.21: Daily variation of total organic carbon 197
Figure 8.22: Correlation between total carbon and triclosan concentration 198
Figure 8.23: Daily variation of soluble protein 199
Figure 8.24: Daily variation of soluble carbohydrate 200
Figure 8.25: Correlation between elimination of protein and elimination of triclosan in biological treatment 201
Figure 8.26: Daily variation of extracellular protein 202
Figure 8.27: Daily variation of extracellular carbohydrate 203

Figure 9.1: The effect of flow rate on the removal of caffeine, carbamazepine, tetracycline, and triclosan, as modelled by Toxchem+ 208
Figure 9.2: The effect of temperature on the removal of caffeine, carbamazepine, tetracycline, and triclosan, as modelled by Toxchem+ 209
Figure 9.3: The effect of MLSS on the removal of caffeine, carbamazepine, tetracycline, and triclosan, as modelled by Toxchem+ 210
Figure 9.4: The effect of MLVSS on the removal of caffeine, carbamazepine, tetracycline, and triclosan, as modelled by Toxchem+ 211
Figure 9.5: Typical arrangement of two IFAS type biofilters as reproduced from patent WO02079103 (Darton and Johnson 2002) 216

Figure 9.6: Proposed modifications (in red) to existing activated sludge sewage treatment technologies 218

Figure 10.1: Dissolved oxygen profile during the four hour aeration loss test 220
Figure 10.2: Ammonia removal profile during aeration loss test 220
Figure 10.3: Removal of tetracycline during the four hour aeration loss test 222
Figure 10.4: Percentage removal of caffeine during aeration loss test 223

Figure 14.1: Screenshot of Toxchem+ model 275

List of Tables

Table 2.1: Top Prescribed drugs for cardiovascular disease (NHS 2005)....8
Table 2.2: Top prescribed antibacterial drugs (NHS 2005)....9
Table 2.3: Top prescribed NSAIDs and analgesics (NHS 2005)....9
Table 2.4: Top prescribed pharmaceuticals for mental health (NHS 2005)....10
Table 2.5: Top prescribed pharmaceuticals for asthma and COPD (NHS 2006)....10
Table 2.6: Top prescribed pharmaceuticals for dyspepsia (NHS 2005).....11
Table 2.7: Top prescribed pharmaceuticals for diabetes (NHS 2006)....11
Table 2.8: Comparison of top 10 prescribed drugs in the UK in 1997 and 2005.....12
Table 2.9: Top thirty prescribed pharmaceuticals in the UK in 1995, 2000 and 2005.....14
Table 2.10: Main use of pharmaceuticals annually (kg) in Germany in 1999 and 2000 (Huschek *et al.* 2004)....16
Table 2.11: Extrapolation of actual usage of seven pharmaceuticals in the UK in 2005....17
Table 2.12: Consumption of prescribed pharmaceuticals in Germany 1993-1995 (tonnes/year)18
Table 2.13: Top 25 pharmaceuticals based on use in Denmark in 1997 (Stuer-Lauridsen *et al.* 2000)....19
Table 2.14: Top ten detected pharmaceuticals in effluent of STPs worldwide....23
Table 2.15: Top ten detected pharmaceuticals in effluent of STPs in UK....24
Table 2.16: Concentrations of pharmaceuticals in sewage sludges ($\mu g\ kg^{-1}$)....25
Table 2.17 Concentrations of pharmaceuticals in landfill leachate (Schneider *et al.* , 2004)....26
Table 2.18: Top ten detected pharmaceuticals worldwide in streams and rivers....27
Table 2.19: Top ten detected pharmaceuticals in the UK streams and rivers....28
Table 2.20: Top 10 highest reported concentrations of pharmaceuticals in drinking water worldwide....29
Table 2.21: Median concentration ranges of pharmaceuticals in different compartments of the aquatic environment (Kiwa 2004; Ternes *et al.* 2004).....30
Table 2.22: Potential daily intake of trimethroprim....37
Table 2.23: Safety factors used in the calculation of predicted no-effects concentrations (EU Directive 67/548/EEC)....41
Table 2.24: Comparison of pre- and post-2006 formulae for calculation of PEC using Germany 2001 consumption data (Huschek *et al.* 2004)....43
Table 2.25: Chemicals detected in UK, where usage data is available (Webb 2000)....44
Table 2.26 Chemicals with PEC:PNEC > 1, with MEC:PNEC where data are available....45
Table 2.27: Estimated human excretion rates of selected drug glucuronide-conjugations (Ternes 1998)....48
Table 2.28: Comparison of hydraulic retention times and effluent qualities of activated sludge, trickling filter, and RBC sewage treatment plants (Metcalf and Eddy 2003)....54
Table 2.29: Literature values of triclosan removal through various types of STP....55
Table 2.30: Distribution coefficient data for pharmaceuticals (Gobel *et al.* 2005; Jones *et al.* 2002; Ternes *et al.* 2004; Urase and Kikuta 2005).....58
Table 2.31: Costs of activated sludge and ozonation treatment processes....66

Table 3.1: Compounds detected in the aquatic environment at concentrations above 200 ng l^{-1}71
Table 3.2: Compounds with PEC greater than PNEC....73
Table 3.3: Compounds with MEC greater than PNEC....73
Table 3.4: Pharmaceuticals with log K_d above 1.5 (Jones *et al.* 2002)....73
Table 3.5: Pharmaceuticals with log K_{ow} above 4.....74

Table 3.6: Compounds with a selection score of three or greater 75
Table 3.7: Properties of caffeine 78
Table 3.8: Properties of carbamazepine 79
Table 3.9: Properties of tetracycline 80
Table 3.10: Properties of triclosan 85

Table 4.1: Purity and source of selected pharmaceuticals 88
Table 4.2: Factors affecting chromatographic performance 90
Table 4.3: HPLC conditions for triclosan and methyl-triclosan analysis 93
Table 4.4: Values of control parameters for triclosan 95
Table 4.5: HPLC conditions for tetracycline family analysis 97
Table 4.6: Values of control parameters for tetracycline 100
Table 4.7: HPLC conditions for caffeine and degradation product analysis 101
Table 4.8: Values of control parameters for caffeine 104
Table 4.9: HPLC conditions for carbamazepine and 10,11-dihydrocarbamazepine analysis 105
Table 4.10: Values of control parameters for carbamazepine 108
Table 4.11: Recoveries (%) of pharmaceuticals during solid phase extraction using Strata-X and Oasis HLB cartridges 112
Table 4.12: Quantification and detection limits in crude sewage after solid phase extraction. 112
Table 4.13: Quantification and detection limits in final effluent after solid phase extraction. 113

Table 5.1: Summary of study STPs 114
Table 5.2: Soil to solution ratios 124
Table 5.3: Summary of HPLC and SPE conditions 134

Table 6.1: Estimate of quantities of caffeine degradation products in biomass 145
Table 6.2: Amounts of triclosan in the liquid solid phases of three samples of activated sludge from Southam STP. 146
Table 6.3: Summary of sorption and biodegradation rates 151

Table 7.1: Numbers of Severn Trent STPs by population equivalent 153
Table 7.2: Literature values of triclosan removal through various types of STP 160

Table 9.1: Removal of pharmaceuticals according to Toxchem+ and literature fate data, compared to literature removals and removal established in this work 205
Table 9.2: Removal of pharmaceuticals according to Toxchem+ with updated sorption and biodegradation rates as demonstrated in this work 206

Acknowledgements

The work outlined herein was sponsored by the UK Engineering and Physical Sciences Research Council (EPSRC) and Severn Trent Water. I would especially like to thank Paul Griffin and the staff of the four Severn Trent sewage treatment plants used in this work, without whose help and cooperation much of the sampling work could not have been completed.

I would like to thank Dr Elise Cartmell and Dr. Richard Stuetz for all their support, enthusiasm and encouragement. I would also like to thank Gudrun Winkler, Jeff Chittim, and Sibel Basakcilardan for their help during their placements.

I would like to thank my parents for their continued love and support.

Finally, and most of all, I would like to thank Heather for all her love, time, warmth and understanding throughout the course of this research, and her patience during the writing of this thesis.

Andrew Thompson

November 2006

Abbreviations and Notation

APEs	Alkylphenol polyethoxylates	ng l^{-1}
AS	Activated sludge	
ASP	Activated sludge plant	
BET	Brunauer, Emmett, and Teller	
BOD	Biological oxygen demand	mg l^{-1}
CAFF	Caffeine	ng l^{-1}
CBZ	Carbamazepine	ng l^{-1}
CDER	Centre for drug evaluation and research	
COD	Chemical oxygen demand	mg l^{-1}
DI	De-ionised	
DO	Dissolved oxygen	mg l^{-1}
DOC	Dissolved organic carbon	
DOM	Dissolved organic matter	mg l^{-1}
DUMP	Disposal of unwanted medicines and poisons	
E1	Estrone	ng l^{-1}
E2	Estradiol	ng l^{-1}
EE2	Ethinylestradiol	ng l^{-1}
EIC	Expected introductory concentration	mg l^{-1}
EPA	Environmental protection agency	
EPS	Extracellular polymeric substances	mg l^{-1}
FDA	Food and drug administration	
GAC	Granular activated carbon	
GCMS	Gas chromatography mass spectrometry	
HLB	Hydrophilic-lipophilic balance	
HPLC	High performance liquid chromatography	
HRT	Hydraulic retention time	hrs
IC	Inorganic carbon	mg l^{-1}
LOD	Limit of detection	ng l^{-1}
LOQ	Limit of quantification	ng l^{-1}
MBBR	Moved bed biofilm reactor	
MBR	Membrane biological reactor	
MCA	Medicines control agency	
MEC	Measured environmental concentration	mg l^{-1}

NOEC	No observed effects concentration	$mg\ l^{-1}$
NP	Nonylphenol	$ng\ l^{-1}$
NSAF	Nitrifying submerged aerated filter	
OECD		
OP	Octylphenol	
PE	Population equivalent	
PEC	Predicted environmental concentration	$mg\ l^{-1}$
PNEC	Predicted no-effects concentration	$mg\ l^{-1}$
PPCPs	Pharmaceuticals and personal care products	
QSAR	Quantitative structure-activity relationship	
RAS	Return activated sludge	
RBC	Rotating biological contactor	
SP	Specific sludge production	
SPE	Solid phase extraction	
SRT	Solids retention time	hrs
SS	Suspended solids	$mg\ l^{-1}$
STP	Sewage treatment plant	
TC	Total carbon	$mg\ l^{-1}$
TET	Tetracycline	$ng\ l^{-1}$
TOC	Total organic carbon	$mg\ l^{-1}$
TRC	Triclosan	$ng\ l^{-1}$
UV	Ultraviolet	
VSS	Volatile suspended solids	$mg\ l^{-1}$

Chapter 1: Introduction

The occurrence and fate of pharmaceutically active substances (PhACs) in the aquatic environment has, over recent years, become recognised as a major issue in environmental chemistry (Daughton and Jones-Lepp 2001; Daughton and Ternes 1999; Halling-Sorensen *et al.* 1998; Kummerer 2001; Stan and Heberer 1997; Verstraeten *et al.* 2001). A pharmaceutical may be described as any chemical used for the diagnosis, treatment (cure/mitigation), alteration, or prevention of disease, health condition, or structure/function of the body (Buser *et al.* 1999; Jones *et al.* 2005). There are currently over 3000 pharmaceutically active substances licensed for use in the UK (Ayscough *et al.* 2000; NHS 2006), along with a much greater number of personal care products (Giger *et al.* 2003). Pharmaceuticals, along with personal care products (such as shampoos, deodorants and soaps) have been consumed with the users giving little attention to what happens to them after they have been used (Bound *et al.* 2006). These chemicals and their metabolites are continuously entering the environment either from direct disposal or from the effluent of sewage treatment plants (STPs) (Bound *et al.* 2006; Heberer 2002; Hirsch *et al.* 1999; Jjemba 2006; Roberts and Thomas 2006; Ternes 1998; Ternes *et al.* 1999).

The effects of most pharmaceuticals have been well studied in humans and some other mammals due to the required approval and registration procedures (EMEA 2006; FDA 1995). However, beyond basic acute toxicological tests, full life cycle toxicological data on these compounds is generally not required and the environmental relevance of these compounds is largely unknown (Ternes 1999). Although these compounds have been evaluated as safe for human or veterinary use, this is not adequate to ensure the protection of the many ecosystems that may be exposed to these compounds and their metabolites (Bound *et al.* 2006; Huschek *et al.* 2004). The interaction of these compounds with the many species with which they may come into contact in the wider environment is unknown (Sumpter and Johnson 2005). Many lower animals have similar receptor systems to those of humans and it is therefore reasonable to assume that there is the potential for other animals to be affected by these compounds (Ternes 1999). For these reasons, it is important that the fate of pharmaceuticals is considered more thoroughly, especially to understand if wastewater treatment processes can be changed to improve removal. The potential for pharmaceuticals

to have an adverse effect on the aquatic environment, or indeed on human health, must be considered and processes changed or introduced, so that the chemicals that have the greatest effect, both on the environment and humans, are removed.

Traditionally, sewage treatment technology has been designed to combat the problems of nutrient enrichment and microbial contamination, while more recent advancements have concentrated on nitrogen and phosphorus removal (Jones *et al.* 1998; Randall and Sen 1996; Rogalla *et al.* 2006; Sriwiriyarat and Randall 2005; Sriwiriyarat and Randall 2005). However, there are many natural and synthetic compounds, such as pharmaceuticals, which may not be removed by these systems, leading to their discharge into the aquatic environment. (Giger *et al.* 2003; Hirsch *et al.* 1999; Sacher *et al.* 2001).

Over recent years, both water companies and regulators have become increasingly concerned about reports of concentrations of pharmaceuticals in sewage effluents, as well as in the wider aquatic environment, such as streams, rivers, groundwater, and drinking water (Ayscough *et al.* 2000; Hilton *et al.* 2004; Kanda *et al.* 2003; Stokes and Churchley 2006; Thompson *et al.* 2005). With increasing analytical development meaning that more compounds can be detected at ever lower concentrations, regulatory drivers such as the Habitats Directive (EC 2000) and the Water Framework Directive (EC 2000) are driving the water companies to look seriously at this issue (Cartwright 2006). Whilst additional tertiary wastewater treatments, such as ozonation or granular activated carbon (GAC) and chlorine dioxide treatment are available, they are often expensive to install and operate. The Environment Agency, in conjunction with Thames Water and Severn Trent are running (2005-2010) an $85 million research programme studying the effectiveness of these three technologies (EA 2006), as part of a wider research programme on endocrine disrupting compounds.

The optimisation of current treatment technologies to maximize removal of pharmaceuticals, if possible, could reduce the need for tertiary treatments. The sewage treatment processes with the most potential for optimisation are the secondary biological processes, since this is where the majority of pharmaceutical removal is seen to occur (Boyd *et al.* 2005; Carballa *et al.* 2005; Jones *et al.* 2005; Miao *et al.* 2005; Nakada *et al.* 2006; Perez *et al.* 2005; Ternes *et al.* 2004; Verenitch *et al.* 2006) This is discussed in detail in section 2.5 of the literature review.

There are two main types of secondary biological treatment systems: suspended growth systems (such as activated sludge (AS)), and fixed film systems (such as rotating biological contactors (RBCs) or biological filters). The biomass in these two systems is of very different makeup and consistency, and it is therefore likely that it interacts with pharmaceuticals in different ways, particularly with regard to sorption and biodegradation. Quantification of the removal of pharmaceuticals by each of these mechanisms for each type of biomass, and investigation of the reasons for any differences, could lead to ways of maximising pharmaceutical removal using current sewage treatment technologies.

1.1 Aims and Objectives

The overall aim of this work was to examine the fate and removal of pharmaceuticals during sewage treatment, and establish ways in which current sewage treatment technologies could be optimised to improve removal. Initially, the work looked at the evaluation of concentrations of pharmaceuticals in final effluents and after individual process units of sewage treatment plants. Once this had been achieved, the work focused on:

(1) Investigation and quantification of the mechanisms responsible for pharmaceutical removal
(2) Investigation of the removal efficiency of activated sludge and fixed film secondary treatment processes for pharmaceuticals
(3) Understanding the factors involved in controlling the removal of pharmaceuticals in secondary treatment processes
(4) Evaluation of the effect of plant operational events on pharmaceutical removal
(5) Assessment of how pharmaceutical removal could be maximized in secondary treatment processes

1.2 Thesis plan

In order to meet the above objectives, a review of available literature was conducted regarding the presence and behaviour of pharmaceuticals in sewage (Chapter 2). Four pharmaceuticals were selected for study, based on measured and predicted environmental concentrations, risk to the environment, potential for bioaccumulation, and known removal

in treatment processes (Chapter 3). The behaviour of these four pharmaceuticals was predicted using current knowledge with the Toxchem+ modelling package.

Robust and precise analytical methods for the detection of these compounds in wastewater, using high performance liquid chromatography with ultraviolet detection, were developed and validated (Chapter 4). Materials and methods for all experiments are detailed in Chapter 5.

Four sewage treatment plans (including a mixture of fixed film and suspended growth processes) were then sampled by grab samples, to establish the fate of the pharmaceuticals (Chapter 6). Laboratory tests were conducted to establish and quantify the sorption and degradation parameters of the selected pharmaceuticals, with both fixed film and suspended growth biomass (Chapter 7).

A further set of composite samples was taken from a suspended growth STP. These samples were tested not only for their pharmaceutical concentration, but also for various wastewater and biomass parameters to establish any relationship between them. These parameters included pH, chemical oxygen demand, soluble protein, soluble carbohydrate, suspended solids, volatile suspended solids, lipid content, extracellular protein, and extracellular carbohydrate (Chapter 8).

In Chapter 9 the results from Chapter 6-8 allowed a refinement of the Toxchem+ model, and the results were compared to those observed at full-scale treatment plants. The model was then used to predict the effect of parameters such as temperature, suspended solids, volatile suspended solids, on the relative amounts of pharmaceutical removal by sorption and biodegradation. These results, combined with results from literature, and the correlations and mechanisms discussed in Chapter 8, were combined to outline how certain treatment processes could be optimised to improve removal of pharmaceuticals

Finally, brief consideration was given to the effect of certain operational events on the removal of pharmaceuticals (Chapter 10). These events included high rainfall, and aeration losses. This data allowed for an assessment of the reliability of treatment processes, which

would be essential information for plant operators, if discharge consents were ever to be imposed for pharmaceuticals.

Chapter 2: Literature Review

Before beginning this research, a comprehensive review of the current literature regarding pharmaceuticals in sewage and the aquatic environment was undertaken. The first section details the usage quantities of pharmaceuticals, and their occurrence in the aquatic environment, and the routes by which pharmaceuticals can enter the environment. The second section covers the environmental significance of pharmaceuticals and the risk they may pose to humans and the wider environment. The processes for risk assessments are discussed, and the results compared with occurrence data. The final section looks at the role of sewage treatment plants, covering the mechanisms for pharmaceutical removal and factors that may influence these mechanisms. Potential modifications for sewage treatment are discussed, including tertiary treatments and optimisation of current treatment technologies.

2.1 Usage of PPCPs

Whilst there are approximately 3000 pharmaceutical substances available for use in the UK (Ayscough *et al.* 2000), information on the quantities of pharmaceuticals used for human treatment in the UK and in the EU is not readily available (Sebastine and Wakeman 2003). In order to prioritise those pharmaceuticals most likely to be present at measurable quantities in the environment, it is necessary to establish which drugs are used to the greatest extent. The most extensive sets of data are available in terms of numbers of prescriptions issued (NHS 2005; NHS 2005; NHS 2005; NHS 2005; NHS 2005; NHS 2006; NHS 2006; NHS 2006). This prescription data does not include hospital or retail usage, although Huschek *et al.* (2004) presented data on these other usage sources for a few compounds (see Table 2.10). Whilst numbers of prescriptions are useful, highly prescribed items may not necessarily represent a high weight of active ingredient. Many authors (Hirsch *et al.* 1999; Jones *et al.* 2002; Khan and Ongerth 2002; Slack *et al.* 2005; Stan and Heberer 1997; Stuer-Lauridsen *et al.* 2000; Ternes 1998) have worked around this problem multiplying the prescription data by the defined daily dose (DDD) for each pharmaceutical, available from the WHO (WHO 2006). The DDD *"is the assumed average maintenance dose per day for a drug used for its main indication in adults"* (WHO 2006). Doses for individual patients and patient groups will often differ from the DDD, and it should be noted that this could lead to a significant source of error where many different therapeutic doses exists for a particular compound.

2.1.1 Usage of PPCPs in the UK

Over the last few years (2004-06), the NHS prescription pricing authority (PPA) has begun publishing data for the years 2000-06 on the number of prescriptions given by therapeutic areas. The areas covered by reports to date include: osteoporosis, asthma and COPD (Chronic obstructive pulmonary disease), mental health, diabetes, cardiovascular disease, NSAIDs and analgesics, antibacterials, and dyspepsia.

Of these sectors, the most highly used were pharmaceuticals for the prevention of Cardiovascular disease (NHS 2005), accounting for 50 million prescriptions per quarter by the end of 2004. This had risen from about 28 million per quarter at the end of 1999 (an increase of 76%) with the largest rises seen in lipid regulators, renin-angiotensin system drugs, and antiplatelets. A wide variety of compounds are used in this sector, with the most highly used drugs for each type of treatment shown in Table 2.1 below.

Table 2.1: Top Prescribed drugs for cardiovascular disease (NHS 2005)

Compound	Therapeutic Group	Million prescriptions/quarter	DDD (mg)	Tonnes /year
Bendroflumethiazide	Diuretic	4.9	2.5	0.049
Atenolol	Beta-blocker	4.5	75	1.35
Simvastatin	Lipid regulator	3.6	15	0.216
Atorvastatin	Lipid regulator	3.1	10	0.124
Furosemide	Diuretic	2.6	40	0.416
Ramipril	ACE inhibitor	2.4	2.5	0.024
Bisoprolol	Beta-blocker	0.7	10	0.028
Losartan	Angiotensin antagonist	0.7	50	0.14
Clopidogrel	Antiplatelet	0.7	75	0.21

Antibacterials are the second highest used sector of drugs, although usage has dropped from 45 million items/quarter in 1994 to around 33 million items in 2004 (NHS 2005). The reasons behind this decrease have not been established, but it could be due either to a decrease in respiratory tract infections, or that GPs are following prescribing guidance for infections more closely (NHS 2005). It should be noted that the tonnage of antibiotics used far exceed the tonnage of cardiovascular drugs used, even though more prescriptions for cardiovascular drugs were issued, since the DDD are considerably higher for antibiotics (1000 – 2000 mg, rather than 2.5 - 75 mg for cardiovascular drugs). The most highly used compounds are shown in Table 2.2 below.

Table 2.2: Top prescribed antibacterial drugs (NHS 2005)

Compound	Therapeutic Group	Million prescriptions/quarter	DDD (mg)	Tonnes /year
Amoxicillin	Penicillin	10.4	1000	41.6
Flucloxacillin	Penicillin	3.2	2000	25.6
Erythromycin	Macrolide	3.2	1000	12.8
Trimethoprim	Trimethroprim	2.8	400	4.48
Phenoxymethylpenicillin	Penicillin	2.3	2000	18.4
Cefalexin	Cephalosporin	2.0	2000	16
Oxytetracycline	Tetracycline	1.0	1000	4
Ciprofloxacin	Quinolone	0.9	1000	3.6
Doxycycline	Tetracycline	0.8	100	0.32
Tetracycline	Tetracycline	0.5	1000	2

Non-steroidal anti-inflammatory drugs (NSAIDs) and analgesics are widely prescribed, mainly for osteoarthritis and back pain (NHS 2005). NSAIDs have shown a slow but steady increase in usage to just over 5 million items per quarter by the end of 2004, dominated by two compounds: diclofenac (1.8 million) and ibuprofen (1.1 million). Worthy of note are two compounds, rofecoxib and celecoxib (Cox-II selective inhibitors), which were introduced in 1999, and together now account for 1.7 million items per quarter. Analgesic prescriptions totalled 8.4 million items per quarter by September 2004, and had shown little change over the previous five years. Of these, 8.1 million items were for paracetamol, or paracetamol combination products (e.g. in combination with dextropropoxyphene, dihydrocodeine, or codeine). Annual usage is shown in Table 2.3 below.

Table 2.3: Top prescribed NSAIDs and analgesics (NHS 2005)

Compound	Therapeutic Group	Million prescriptions/quarter	DDD (mg)	Tonnes /year
Diclofenac	NSAID	1.8	100	0.72
Ibuprofen	NSAID	1.1	1200	5.28
Paracetamol	Analgesic	8.1	3000	97.2
Rofecoxib	NSAID	0.85	25	0.085
Celecoxib	NSAID	0.85	200	0.68

Around 300 out of ever 1000 people in Britain are affected by mental health problems each year. Usage of pharmaceuticals for mental health problems has shown a slow but steady increase over the last five years, from 10 to 12 million items per quarter by the middle of 2005 (NHS 2005). In this sector, the largest therapeutic group is the antidepressants, accounting for 7.3 million items, with the main drugs being fluoxetine (1.0 million), citalopram (1.2), tricyclic acid (2.5), and venlafaxine (0.7).

Other therapeutic groups include the anxiolytics, e.g. diazepam (1.1), and the hypnotics, e.g. zopiclone (1.0). An emerging trend appears to be the increased usage of atypical antipsychotics, which has tripled over the last five years to 0.83 million items per quarter e.g. olanzapine (0.33), risperidone (0.25). As can be seen from Table 2.4, the DDD of these pharmaceuticals are low, mostly below 20 mg, resulting in lower tonnage values than for other groups of compounds.

Table 2.4: Top prescribed pharmaceuticals for mental health (NHS 2005)

Compound	Therapeutic Group	Million prescriptions/quarter	DDD (mg)	Tonnes /year
Fluoxetine	Antidepressant	1.0	20	0.08
Citalopram	Antidepressant	1.2	20	0.096
Tricyclic acid	Antidepressant	2.5	-	-
Venlafaxine	Antidepressant	0.7	100	0.28
Diazepam	Anxiolytics	1.1	10	0.044
Zopiclone	Hypnotics	1.0	7.5	0.03
Olanzapine	Antipsychotics	0.33	10	0.013
Risperidone	Antipsychotics	0.25	5	0.005

Asthma and COPD was ranked as the fifth highest pharmaceutical using sector, with a usage of around nine million items per quarter (NHS 2006). Usage has increased only very slightly over the last five years, from around 8.5 million items per quarter. There are currently 5.2 million people with asthma in the UK, including one in ten children, whilst COPD mainly affects the middle aged and elderly (0.7 million patients in the UK). The pharmaceutical usage is split into two main therapeutic groups: short-acting beta agonists such as salbutamol (4.0 million items per quarter), and corticosteroids such as beclometasone (1.7 million items per quarter) and fluticasone (1.0 million items per quarter). The annual tonnage use is shown in Table 2.5 below.

Table 2.5: Top prescribed pharmaceuticals for asthma and COPD (NHS 2006).

Compound	Therapeutic Group	Million prescriptions/quarter	DDD (mg)	Tonnes /year
Salbutamol	Beta agonist	4.0	12	0.192
Beclometasone	Corticosteroids	1.7	1.5	0.01
Fluticasone	Corticosteroids	1.0	1.5	0.006

The next highest sector was drugs for Dyspepsia (NHS 2005). It is thought that around 40% of adults suffer from this condition, although only 5% consult their GP each year. Usage has increased

from around 5.5 million items per quarter in 1999 to 7 million items per quarter in 2004. In this period there has also been a significant change in the usage of different therapeutic groups to treat the condition. There has been a large increase in proton pump inhibitors, such as lansoprazole (2.4 million items per quarter) and omeprazole (1.2 million items per quarter), whereas the usage of H2-receptor antagoniosts (H2RAs), such as ranitidine (0.9 million items per quarter) has fallen. The annual tonnage use is shown Table 2.6 in below.

Table 2.6: Top prescribed pharmaceuticals for dyspepsia (NHS 2005).

Compound	Therapeutic Group	Million prescriptions/quarter	DDD (mg)	Tonnes /year
Lansoprazole	Proton pump inhibitor	2.4	30	0.288
Omeprazole	Proton pump inhibitor	1.2	20	0.096
Ranitidine	H2RA	0.9	300	1.08

One of the lowest prescription sectors is drugs used for diabetes (NHS 2006), which has shown a strong increase from 2.5 to 4.5 million items per quarter by March 2005. The main drug in this sector is metformin (1.9), whose usage has increased by 138% over the last five years. Usage of sulphonylureas has also increased, such as gliclazide (1.0). Insulins account for around 0.5 million items per quarter. The annual tonnage use is shown Table 2.7 in below.

Table 2.7: Top prescribed pharmaceuticals for diabetes (NHS 2006)

Compound	Therapeutic Group	Million prescriptions/quarter	DDD (mg)	Tonnes /year
Metformin	Biguanide	1.9	2000	15.2
Gliclazide	Sulphonylurea	1.0	160	0.64
Insulin	Insulin	0.5	0.04	0.00008

The smallest sector is drugs used for osteoporosis (NHS 2006). The National Osteoporosis Society estimates that osteoporosis affects 3 million postmenopausal women in the UK (2006). Prescriptions for osteoporosis drugs have tripled over the period 2000-2005, from around 0.8 to 2.9 million items/quarter. Of these drugs (2005), 64% of prescriptions were for alendronic acid (0.8 million). The DDD of 10 mg, gives a usage of 0.032 tonnes per year. Vitamin D is also widely prescribed either on its own, or in combination with alendronic acid.

In 2000, the Environment Agency published a list of the consumption of prescribed drugs in the UK for 1997 (Ayscough *et al.* 2000), which had been obtained from the Department of Health. The top ten for the 2005 prescription data were compared with the levels reported for 1997, as shown in Table 2.8 below.

Table 2.8: Comparison of top 10 prescribed drugs in the UK in 1997 and 2005.

Pharmaceutical	Rank 2005	Rank 1997	Prescriptions 2005 (millions)	Prescriptions 1997 (millions)	Change
Amoxicillin	1	1	41.6	16.6	+ 150%
Paracetamol	2	6	32.4	9.6	+ 237%
Bendroflumethiazide	3	8	19.6	7.6	+ 158%
Atenolol	4	7	18.0	8.1	+122%
Salbutamol	5	2	16.0	16.1	- 1%
Simvastatin	6	47	14.4	2.4	+ 500%
Erythromycin	7=	33	12.8	3.3	+ 288%
Flucoxacillin	7=	49	12.8	2.2	+ 482%
Atorvastatin	9	-	12.4	-	New
Furosemide	10	14	10.4	6.2	+ 68%

This table shows that of the top five compounds in 2005, all were found in the top ten in 1997. This suggests that these are popular compounds that can be expected to continue to be used in large quantities. Salbutamol had dropped three places to fifth highest used pharmaceutical in 2005, with no real change in its usage. This was in line with the whole of the asthma and COPD sector, which has shown no noticeable growth in usage over 2000-2005 (NHS 2006). It should be of note that aspirin was not mentioned in the data presented for 2000-2005 although it had appeared as the third highest used compound in 1997 and is known to be widely prescribed for a variety of conditions.

Of the five new entries into the top ten in 2005, one (atorvastatin) was a new drug that had been introduced, whilst simvastatin was only introduced into use in the mid 1990s, and thus showed a large increase in usage between 1997 and 2005. This meant that the 2005 top ten included five drugs used for the treatment of cardiovascular disease, along with three antibacterials.

A large increase in the usage of flucoxacillin (482%) can be seen between 1997-2005. This is surprising, since the NHS reported only a 32% increase in 2000-2005, which may suggest errors in the data used by Ayscough *et al.* for the usage of flucoxacillin in 1997.

Whilst numbers of prescriptions are useful, highly prescribed items may not necessarily represent a high weight of active ingredient. Unfortunately, there is only a very small amount of publicly available data on the quantities of pharmaceuticals that are used annually, particularly in the UK. Similarly, many compounds are used at concentrations that do not need a prescription and can therefore be sold "over the counter", such as ibuprofen in anti-inflammatory gels, and paracetamol in headache tablets.

There are only two data sets of UK pharmaceutical usage by weight with which the NHS data presented above can be compared: Webb (2000; 2001) and Jones *et al.* (2002). The Webb data sets covers the top 67 compounds based entirely on prescription data for 1995, whereas the Jones *et al.* data covers only the top 25 compounds but includes some "over the counter" usage as well as prescription usage for 2000. It would therefore be expected that in most cases the values quoted by Jones *et al.* would exceed those quoted by Webb, due to better data coverage and increase in usage over time. Whilst the Webb data was obtained directly, the Jones *et al.* data is calculated from prescription usage and DDD information. A summary of this data (top thirty) is given in Table 2.9, and compared to the tonnage information derived above from the NHS data on the number of prescription for the years 2000-2005.

Table 2.9: Top thirty prescribed pharmaceuticals in the UK in 1995, 2000 and 2005.

Pharmaceutical	**Amount prescribed (tonnes)**	**Amount used (tonnes)**	**Amount prescribed (tonnes)**
Data years:	**1995** Webb (2000)	**2000** Jones *et al.* (2002)	**2005** NHS (2000-2006)
Paracetamol	2000	391	97.2
Amoxicillin		71.5	41.6
Flucloxacillin		23.4	25.6
Phenoxymethylpenicillin		22.2	18.4
Cefalexin			16
Metformin	106.1	205	15.2
Erythromycin	67.7	26.4	12.8
Ibuprofen		162	5.28
Trimethroprim			4.48
Oxytetracycline	33.7	27.2	4
Ciprofloxacin			3.6
Tetracycline	4.7		2
Atenolol		28.9	1.35
Ranitidine	69	36.3	1.08
Diclofenac		26.1	0.72
Gliclazide		18.8	0.64
Furosemide			0.416
Lansoprazole	0.434		0.288
Venlafaxine			0.28
Simvastatin			0.216
Salbutamol			0.192
Atorvastatin			0.124
Aspirin	770		
Cimetidine	72	35.6	
Naproxen	60.6	35	
Sulphasalazine		46.4	
Dextropropoxyphene	42.5		
Mesalazine		40.4	
Carbamazepine		40.3	
Quinine	29.7		

All three data sets agree that paracetamol has continued to be the most highly used compounds throughout the last decade, there is little overlap on other compounds both in terms of order and quantities. However, paracetamol, metformin, erythormycin and oxytetracycline do appear on all three lists.

Whilst prescription data is useful to predict usage quantities, it should be remembered that this method will always produce an underestimate. This is because many compounds are used not only as a prescription drug, but also in products at concentrations which do not require a prescription and can therefore be sold "over the counter". For example, whilst ibuprofen is sometime used as a prescribed drug, its major use is in analgesic creams and painkillers (at sub-prescription levels) sold over the counter at any chemist or supermarket. Similarly, triclosan (an antibacterial) has only a small prescription usage (4 tonnes in 2000) (Ayscough *et al.* 2000), but is widely used in washing up liquids, toothpastes, mouthwashes, soaps, and other household products. The problems with these data sets are highlighted by the usage data published by Huschek *et al.* (2004), who gave not only prescription data, but also hospital and retail use for Germany in 1999 and 2000, as shown in Table 2.10. For most of the pharmaceuticals on the list, prescription usage is outweighed by hospital and retail use.

Table 2.10: Main use of pharmaceuticals annually (kg) in Germany in 1999 and 2000 (Huschek *et al.* 2004)

Active Substance	Hospital[1] 1999	Prescription[2] 1999	Retail[1] 1999	Hospital[1] 2000	Prescription[2] 2000	Retail[1] 2000
Acetylsalicylic acid	14,945	64,499	822,829	13,996	73,211	775,398
Paracetamol	24,823	134,659	494,937	24,320	116,113	501,423
Povidone-iodine	476,279	6,642	25,773	452,998	6,131	26,055
Metformin	4,044	322,990	40,973	4,744	377,537	51,177
Ibuprofen	6,337	143,377	110,132	7,112	151,960	141,012
Metamizole sodium	47,130	49,447	66,881	54,928	53,391	74,884
Theophylline	6,074	133,173	7,185	5,877	126,713	11,003
Piracetam	7,496	110,028	16,525	7,335	103,968	15,180
Allopurinol	2,638	113,798	15,386	2,588	113,911	21,580
Amoxicillin	13,052	63,883	30,709	12,908	67,934	32,339
Pentoxifylline	2,575	82,076	7,934	2,401	71,360	7,907
Salicylic acid	1,099	3,568	85,034	2,320	3,451	71,213
Carbamazepine	4,348	77,688	4,887	4,306	75,508	7,896
Ranitidine	6,108	57,401	21,900	5,619	57,296	26,377
Diclofenac	4,682	50,451	26,654	4,692	48,189	29,321
Verapamil	1,652	58,624	12,123	1,590	56,124	13,681
Metoprolol	2,259	56,716	8,687	1,642	68,082	9,430
Bezafibrate	320	32,874	3,392	305	30,525	4,187
Propyphenazone	594	2,876	31,103	514	2,209	28,594
Furosemide	2,529	25,334	4,327	2,601	26,098	4,329
Captopril	593	27,938	3,034	590	28,580	3,898
Phenazone	10	418	29,205	11	327	26,285
Sotalol	542	22,926	3,672	544	22,302	4,706
Hydrochlorothiazide	361	26,286	75	416	26,209	2,782
Erythromycin	1,452	16,894	5,711	1,491	15,742	4,585
Tramadol	3,649	18,251	1,830	3,786	19,674	3,492
Lactitol	8,610	10,861	3,493	6,812	6,577	4,591
Isosorbide dinitrate	712	20,138	1,702	660	18,936	1,924
Naftidrofuryl	289	19,285	1,221	232	15,443	1,818
Isosorbide mononitrate	455	15,019	1,834	420	13,891	1,766
Triamterene	268	14,896	1,303	271	14,128	1,895
Ambroxol	968	9,140	4,985	1,045	7,901	6,183
Dimethyl sulfoxide	505	7,170	7,230	81	5,246	7,312
Diltiazem	273	12,461	1,665	269	11,334	2,163
Irbesartan	78	11,177	3,113	107	14,650	4,091

Original data sources: 1 (IMS Health 2002), 2 (Schwabe and Paffrath 2000; Schwabe and Paffrath 2001)

For compounds that appear in both the NHS data and the Huschek *et al.* (2004) data, the information was extrapolated to give an impression of the actual load potentially entering the environment, as shown in Table 2.11 below. From this data it can be seen that seen that pharmaceutical usage accounts for around 60 to 70% of total usage. In certain cases, such as paracetamol, prescription usage could be as low as 20% of the total.

Table 2.11: Extrapolation of actual usage of seven pharmaceuticals in the UK in 2005

Pharmaceutical	UK Prescription Usage in 2005 (NHS) (tonnes / year)	Prescription usage as a percentage of total usage (Huschek *et al.* 2004)	Extrapolated usage (tonnes / year)
Paracetamol	97.2	20.6	472
Amoxicillin	71.5	59.3	121
Metformin	15.2	87.8	17.3
Erythromycin	12.8	70.2	18.2
Ibuprofen	5.28	55.2	9.6
Ranitidine	1.08	67.2	1.6
Diclofenac	0.72	61.7	1.2

It is also important to consider the role of veterinary medicines. Many compounds, particularly antibiotics (e.g. oxytetracycline, amoxicillin, trimethroprim), used for human health are also used as veterinary medicines, adding to the potential load entering the aquatic environment.

Looking at pharmaceutical usage from another angle, it is necessary to consider that the average age of the European population is increasing. Indeed, the 2001 UK census showed that there are more people over the age of 60 than under the age of 16 in the country, for the first time. Since older people use more pharmaceuticals than younger people (Department of Health, 2000), it can be seen that the general level of pharmaceutical usage is likely to keep increasing steadily as the number of elderly people increases. The figures for 2000 show that elderly people (those aged 60 or over) used 26.5 prescription items per person per year, compared with 4.1 for those under 15 years old, and 6.4 for those aged between 16 and 59 (Department of Health, 2000). From this perspective, it would be worth considering which pharmaceuticals older people are more likely to use. Some diseases such as arthritis become more prevalent in older people, for which anti-inflammatory drugs and analgesics can be used. However, this approach does not significantly help to ascertain which individual chemicals are likely to be the most widely used in future years for a variety of reasons. These will include the replacement of old drugs as new drugs are introduced, and the influence of drug cost on the prescribing habits of general practitioners.

2.1.2 Usage of PPCPs in Europe

Whilst the data on pharmaceutical usage in the UK is limited to the few sources discussed above, there is similar data available for some other European countries, particularly Denmark and Germany. Prescription data from Germany for 1993-1995 is shown in Table 2.12 below. Similar to

Jones *et al.* (2002) for the UK data, these consumption values are based of the daily dose and number of prescriptions, leading to possible inaccuracies as discussed previously. "Over the counter" usage of these drugs, unlike Jones *et al.*, is not taken into account. Insufficient data are available to be able to suggest any trends in the change of usage of compounds.

Table 2.12: Consumption of prescribed pharmaceuticals in Germany 1993-1995 (tonnes/year)

Compound	**1993**	**1994**	**1995**	**1995**
	(Stan and Heberer 1997)	(Stan and Heberer 1997)	(Ternes 1998)	(Hirsch *et al.* 1999)
Ibuprofen	48-96		105	
Carbamazepine			80	
Diclofenac	48-72		75	
Metoprolol		30-60	50	
Bezafibrate	38-57		30	
Clofibric acid	15-21		16	
Fenofibric acid	11-15		15	
Indomethacine		3-10	6	
Gemfibrozil	14		6	
Propanoprolol		2.5-5	3	
Acetylsalicylic acid	23-116			
Ketoprofen		0.7		
Bisoprolol		0.6-1.1		
Fenoterol		0.5		
Clenbuterol		0.001		
Antibiotics				
Amoxicillin				25.5-127.5
Ampicillin				1.8-3.6
Penicillin V				140
Penicillin G				1.8-3.6
Sulfamethoxazole				16.6-76
Trimethoprim				3.3-15
Erythromycin				3.9-19.8
Roxithromycin				3.1-6.2
Clarithromycin				1.3-2.6
Minocycline				0.8-1.6
Doxycycline				8-16

Immediately, similarities can be seen with the UK usage data, with seven compounds appearing in both lists: amoxicillin, aspirin (acetylsalicylic acid), carbamazepine, diclofenac, doxycycline,

erythromycin, and ibuprofen. Again, amoxicillin can be seen as the most used antibiotic. This was also reported by Hartmann *et al.* (1998) who studied the usage of drugs in a Swedish hospital.

For Denmark, Stuer-Laurisen *et al.* (2000) calculated a list of the top 25 pharmaceuticals by weight, again from prescription usage. The most used compound by weight was paracetamol (248.25 tonnes). This list, shown in Table 2.13, again shows similarities with the UK list, with paracetamol, ibuprofen, frusemide, and ibuprofen all near the top. Unlike all other countries discussed, amoxicillin does not appear on the list, although this may be due to an incomplete data set. It is worth noting that frusemide was the most prescribed pharmaceutical for Denmark, whereas for the UK, it was only fourth within the cardiovascular drugs. This may be due to the time difference (eight years) between the two datasets, or may suggest differences in prescribing practices in the two countries.

Table 2.13: Top 25 pharmaceuticals based on use in Denmark in 1997 (Stuer-Lauridsen *et al.* 2000)

Pharmaceutical	Number of prescriptions (million)	Weight used (tonnes)
Paracetamol	82.75	248.25
Acetylsalicylic acid	70.93	212.79
Potassium Chloride	26.7	80.1
Ibuprofen	28.16	33.8
Frusemide	93.6	3.74
Hydrogen Peroxide	15.3	0.916
Terbutaline	23.73	0.47
Enalapril	20.8	0.416
Citalopram	18.4	0.368
Diazepam	20.7	0.207
Bendroflumethiazide	66.83	0.17
Salbutamol	17	0.17
Zopiclone	19.2	0.144
Amlodipine	26.44	0.13
Oestradiol	23.7	0.119
Nitrazepam	23.2	0.116
Gestroden & oestrogen	37.14	0.045
Budesonide	25.82	0.04
Xylometazolin	16.7	0.013
Desogestrel & oestrogen	45.4	0.004
Digoxin	16.5	0.004
Lactic acid	27.4	-
Hydrochlorthiazide	16.0	-
Ketoconazole	14.7	-

2.1.3 Summary of usage data

Across the European countries where usage data of pharmaceuticals has been available, several compounds can be seen to be heavily used over at least the last decade, such as (quoted values, where available, are tonnes used in the UK in 2005): paracetamol (472), amoxicillin (121), aspirin, carbamazepine, diclofenac (1.2), doxycycline, erythromycin, and ibuprofen (5.28). Over the last decade, only two new drugs, simvastatin (0.216 tonnes) and atorvastatin (0.124 tonnes), have made it into the top ten highest used compounds in the UK, in terms of number of prescriptions. Evaluating actual tonnage usage values is somewhat problematic, with different authors producing vastly different estimates. For example, Webb (2000) estimated 2000 tonnes per year usage of paracetamol in the UK, whilst Jones *et al.* (2002) estimated 391 tonnes, and this thesis has estimated 472 tonnes a year. Similarly, this work has shown only about 15 pharmaceuticals used at amounts above one tonne per year, as shown in Table 2.9, whilst Webb (2000) showed over 80 compounds above this usage level.

In the UK, eight out of the top ten most used pharmaceuticals were either cardiovascular or antimicrobial drugs. Antimicrobials (antibiotics) were also the most used therapeutic group in Denmark according to 1995 data (Halling-Sorensen *et al.* 1998). Antimicrobial pharmaceuticals have the highest defined daily dose, typically 2000mg, which is at least one order of magnitude greater than all other groups. This would suggest that antimicrobials are the group of compounds entering the environment in the greatest amounts. Indeed, of the 15 compounds estimated to have annual usage over one tonne in this work, seven are antimicrobials: amoxicillin (41.6), flucoxacillin (25.6), phenoxymethylpenicillin (18.4), erythromycin (12.8), trimethoprim (4.48), oxytetracycline (4.0), and ciprofloxacin (3.6).

Whilst the number of prescriptions used in combination with defined daily dose information is useful in that it gives a good idea of which compounds are most heavily used, it can be misleading since this can significantly underestimate total usage due to the compounds being used in hospitals and as "over the counter" products. This underestimate can be as little as 10% (e.g. metformin), or as much as 80% (e.g. paracetamol) (Huschek *et al.* 2004).

2.2 Occurrence of PPCPs in the environment

Clofibric acid, the main metabolite of the blood lipid regulators clofibrate, etofibrate, and theofibrate, was the first pharmaceutical residue to be detected in the aquatic environment (Garrison *et al.* 1976). Since then, clofibric acid has been detected in many surface waters, and even in the North Sea, near to the mouth of the River Elbe (Germany) (Heberer 2002; Heberer 2002; Stan and Heberer 1997; Stan *et al.* 1999). Clofibric acid was also the first pharmaceutical residue to be detected in drinking water (Stan and Heberer 1997). It was demonstrated that there was a link between concentrations of clofibric acid in drinking water, and the usage of bank filtration and groundwater enrichment in drinking water treatment (Heberer *et al.* 1997; Webb *et al.* 2003). Several studies showed the impact of sewage discharges on surface water quality, and on drinking water contamination (Heberer 2002; Heberer *et al.* 2002).

Since the discovery of clofibric acid, a large number of papers have reported detection of pharmaceuticals in different compartments of the aquatic environment. This includes sewage treatment plant (STP) influents and effluents, sewage sludges, landfill leachate, streams and rivers, and drinking water. These papers include, but are not limited to, the following papers: (Aguera *et al.* 2003; Aherne and Briggs 1989; Ahrer *et al.* 2001; Andreadakis *et al.* 1997; Ayscough *et al.* 2000; Belfroid *et al.* 1999; Bendz *et al.* 2005; Berger *et al.* 1986; Bergh and Breytenbach 1990; Bester 2003; Bester 2005; Boreen *et al.* 2003; Boyd *et al.* 2004; Boyd *et al.* 2003; Buser *et al.* 1998; Buser *et al.* 1999; Buyuksonmez and Sekeroglu 2005; Carballa *et al.* 2005; Carballa *et al.* 2005; Carballa *et al.* 2004; Carballa *et al.* 2003; Castiglioni *et al.* 2006; Davis *et al.* 1999; Desbrow *et al.* 1998; Diaz-Cruz *et al.* 2003; Drewes *et al.* 2001; Ellis 2006; Federle *et al.* 2002; Fielding *et al.* 1981; Giger *et al.* 2003; Gobel *et al.* 2005; Gobel *et al.* 2005; Golet *et al.* 2002; Golet *et al.* 2001; Gomez *et al.* 2006; Halden and Paull 2005; Halling-Sorensen *et al.* 1998; Hammond *et al.* 2005; Hannah *et al.* 1988; Hannah *et al.* 1986; Heberer 2002; Heberer 2002; Heberer *et al.* 1995; Heberer *et al.* 1997; Heberer and Feldmann 2005; Heberer *et al.* 2002; Heberer *et al.* 1998; Heberer and Stan 1996; Heberer and Stan 1997; Hignite and Azarnoff 1977; Hilton *et al.* 2003; Hirsch *et al.* 1996; Hirsch *et al.* 1999; Hirsch *et al.* 2000; Hirsch *et al.* 1998; James *et al.* 1997; Johnson *et al.* 2005; Johnson and Sumpter 2001; Jones *et al.* 2005; Jones *et al.* 2001; Jones *et al.* 2005; Joss *et al.* 2004; Kosjek *et al.* 2005; Koutsouba *et al.* 2003; Kummerer 2001; Kummerer *et al.* 1997; Lee *et al.* 2005; Lin *et al.* 2005; Lindqvist *et al.* 2005; Lindstrom *et al.* 2002; Loraine and Pettigrove 2006; McArdell *et al.* 2003; McAvoy *et al.* 2002; Miao *et al.* 2004; Quintana *et al.* 2005; Rathner and Sonneborn 1979; Reddersen *et al.* 2002; Richardson and Bowron 1985; Roberts

and Thomas 2006; Rodriguez *et al.* 2003; Rogers 1996; Sabaliunas *et al.* 2003; Sacher *et al.* 2001; Siegrist *et al.* 2003; Singer *et al.* 2002; Snyder *et al.* 2003; Soulet *et al.* 2002; Stackelberg *et al.* 2004; Stan and Heberer 1997; Stan *et al.* 1994; Stan *et al.* 1999; Stelzer *et al.* 1985; Stokes and Churchley 2006; Strenn *et al.* 2004; Stumpf *et al.* 1996; Stumpf *et al.* 1999; Tauber 2003; Ternes 1999; Ternes 2000; Ternes 2001),(Ternes 1998; Ternes *et al.* 2005; Ternes and Hirsch 2000; Ternes *et al.* 2004; Ternes *et al.* 2002; Ternes *et al.* 1999; Thompson *et al.* 2005; van der Ven *et al.* 2004; Verenitch *et al.* 2006; Verstraeten *et al.* 2001; Vieno *et al.* 2005; Waggott 1981; Waltman *et al.* 2006; Water 2002; Wilson *et al.* 1996; Yang *et al.* 2006; Zuccato *et al.* 2000; Zuhlke *et al.* 2004; Zwiener and Frimmel 2000). As part of this study, a database of these data was created, which detailed the compound detected, quantities, location, toxicity data, and literature references. This database can be found in Appendix A. This large collection of data is summarised below. It should be noted that whilst papers continue to be published on a regular basis containing reports of pharmaceuticals in the aquatic environment, the actual number of different pharmaceuticals being detected in rising relatively slowly. For example, in 1999 a review by Heberer *et al.* reported just over 80 different compounds, whilst a review by Asycough *et al.* in 2000 reported only 68 different compounds. This work has shown that currently (2006), around 120 different compounds have been reported in the aquatic environment.

Sewage treatment plant effluent

In the literature, there have been reports of 112 different pharmaceuticals worldwide detected in effluent from municipal sewage treatment plants with primary and secondary treatment. The most reported compounds were ibuprofen (21 reports), diclofenac (20), naproxen (14), clofibric acid (12), and ketoprofen (11). Table 2.14 shows a summary of the ten pharmaceuticals that have been detected at the highest levels worldwide.

Table 2.14: Top ten detected pharmaceuticals in effluent of STPs worldwide

Chemical Name	Maximum ng l^{-1}	Median ng l^{-1}	Minimum ng l^{-1}	Country	Information Source
Caffeine	292000	-	-	Canada	Rogers *et al.* (1996)
Ibuprofen	151000	19770	1500	Spain	Gomez *et al.* (2006)
Atenolol	122000	3400	100	Spain	Gomez *et al.* (2006)
Ketorolac	59500	4200	200	Spain	Gomez *et al.* (2006)
Ibuprofen	27256	3086	< 20	UK	Ashton *et al.* (2004)
Iopamidol	15000	660	< 50	Germany	Ternes and Hirsch (2000)
Iopromide	11000	750	< 50	Germany	Ternes and Hirsch (2000)
Clofibric acid	9740	-	-	US	Hignite and Azarnoff (1977)
Metronidazol	9400	5900	1800	Spain	Gomez *et al.* (2006)
Ibuprofen	8800	6500	-	Spain	Santos *et al.* (2005)

Of the top ten compounds detected, caffeine was the pharmaceutical detected at the highest concentration. This is unsurprising since its major use is not as a pharmaceutical, but in beverages. Ibuprofen was the second highest detected, and actually accounted for three of the highest ten concentrations detected. Since ibuprofen was the most used compound in Germany (Ternes 1998), and 8th in the UK list, this is again unsurprising. Similarly, atenolol in third place, was the 13th most used UK compound. Iopamidol and iopromide are both X-ray contrast media and therefore not included in the prescription usage data. The remaining three compounds in the top ten did not appear in the compounds which were highly used. This may suggest that they are poorly removed during sewage treatment. Since many of the compounds which are heavily used did not appear in this list, it may be suggested that they are well removed during sewage treatment, or well degraded within the human body.

In the UK, 42 pharmaceuticals have been reported in the effluent of STPs, including erythromycin (4), ibuprofen (4), and diclofenac (4). Table 2.15 shows the ten highest concentrations reported in UK sewage effluent.

Table 2.15: Top ten detected pharmaceuticals in effluent of STPs in UK

Chemical Name	Maximum ng l^{-1}	Median ng l^{-1}	Minimum ng l^{-1}	Information Source
Ibuprofen	27256	3086	< 20	Ashton *et al.* (2004)
Musk galaxolide	19200	-	7800	Kanda *et al.* (2003)
Musk tonalide	6500	-	2200	Kanda *et al.* (2003)
Ibuprofen	4500	-	< 50	Kanda *et al.* (2003)
Ibuprofen	4239	3063	1979	Roberts and Thomas (2006)
Ibuprofen	3780	-	< 10	Hilton *et al.* (2003)
Diclofenac	2349	424	< 20	Ashton *et al.* (2004)
Erythromycin	1842	< 10	< 10	Ashton *et al.* (2004)
Mefenamic acid	1440	133	< 50	Ashton *et al.* (2004)
Trimethoprim	1288	70	< 10	Ashton *et al.* (2004)

As reported for the worldwide data, ibuprofen features prominently in the list of compounds detected at high concentrations in the UK. Two musk compounds (used in perfumes) also feature, which were not included in the pharmaceutical usage data. All the remaining compounds on this list did appear within the top thirty most used compounds in the UK, suggesting a direct link between consumption and environmental concentrations.

The numbers of pharmaceuticals reported in effluent are small in comparison to the approximately 3000 pharmaceuticals approved for human and veterinary use. The remaining pharmaceuticals have not been detected in effluent, not because they are not there (although this may be true), but rather that no-one has attempted to detect them. However, the data that are available are significant as they generally represent the most prescribed compounds (Ternes 1998).

Sewage sludge

During sewage treatment, many pharmaceuticals partition onto solids producing concentrations in the sewage sludges that can be several orders of magnitude higher than those in the influent (Hannah *et al.* 1988; Hannah *et al.* 1986; Petrasek *et al.* 1983). This may often be the main method through which pharmaceuticals are 'removed' during treatment i.e. they are not found in the liquid effluent stream. The sewage solids are collected in the settling tanks to form the sewage sludges, which eventually need to be disposed of. Ayscough *et al.* (2000) noted that no quantitative data had been presented in literature on concentrations of pharmaceuticals in sludges. Principal reasons for

the lack of this data are the inherent difficulties associated with the laboratory extraction and the analysis of sludge samples. Since 2000, a few papers have been published on sludge data, concerning a total of fourteen pharmaceutical compounds, as shown in Table 2.16 below.

Table 2.16: Concentrations of pharmaceuticals in sewage sludges (μg kg^{-1})

Chemical Name	Concentration mg kg^{-1}	Country	Information Source
Salicylic acid	13748	Australia	Khan and Ongerth (2002)
Paracetamol	4535	Australia	Khan and Ongerth (2002)
Ibuprofen	3988	Australia	Khan and Ongerth (2002)
Carbamazepine	1731	Australia	Khan and Ongerth (2002)
Gemfibrozil	1192	Australia	Khan and Ongerth (2002)
Naproxen	1022	Australia	Khan and Ongerth (2002)
Diclofenac	450	Germany	Ternes *et al.* (2005)
Sulfapyridine	197	Germany	Gobel *et al.* (2005)
Azithromycin	158	Germany	Gobel *et al.* (2005)
Trimethoprim	133	Germany	Gobel *et al.* (2005)
Roxithromycin	131	Germany	Gobel *et al.* (2005)
Sulfamethazine	113	Germany	Gobel *et al.* (2005)
Clarithromycin	41	Germany	Gobel *et al.* (2005)
Galaxolide	0	USA	Buyuksonmez and Sekeroglu (2005)
Ibuprofen	0	USA	Buyuksonmez and Sekeroglu (2005)

The compound detected at the highest concentration, salicylic acid, is a primary degradation product of aspirin (acetylsalicylic acid), which along with paracetamol and ibuprofen appeared towards the top of the lists of the most used compounds. Many members of the heavily used antimicrobial group are also represented. However, there are some surprises on this list, such as gemfibrozil (a lipid regulator) which has not been mentioned previously. Carbamazpeine also appears on this list, contrary to much research (see Chapter 3 – Choice of compounds for study), which had suggested that it does not partition to sludge.

Pharmaceuticals, as well as other organic contaminants, in the sludge could cause environmental effects when sludge is applied to agricultural land. The Urban Wastewater Directive is attempting to promote re-use of sewage sludges, rather than disposal by landfill or incineration. The UK government considers that the spreading of sludge on agricultural land is the best practicable environmental option (Ayscough *et al.* 2000). This is a potential route for pharmaceuticals to be transferred to the terrestrial environment, and from there to humans via crops and grazing livestock

(Wilson *et al.* 1996). Boxall *et al.* (2006) measured the uptake of veterinary pharmaceuticals (such as amoxicillin, oxytetracycline, and trimethoprim) by plants used for human consumption (lettuce and carrot). They measured concentrations up to 10% of the acceptable daily intake (ADI) values for the compounds, concluding that this showed little evidence of an appreciable risk to human health. The authors stated that measurable residues of their veterinary medicines were only likely to occur in soils for up to five months after application of manure. It should be noted that according to the Safe Sludge Matrix (ADAS 2001), crops of these types for human consumption should not be grown for at least 12 months after applications of sludge.

Landfill leachate

As well as disposal directly to wastewater, many unwanted pharmaceuticals are disposed of in domestic waste and find their way to landfill sites (Bound and Voulvoulis 2005). These sites may also be used as disposal routes for the waste products from pharmaceutical production facilities (Ahel *et al.* 2004). Landfill leachate goes to wastewater treatment plants to be treated, before discharge to rivers (Schneider *et al.* 2004). Only a few papers have reported pharmaceutical compounds that have been detected in landfill leachate. These include propyphenazone (concentration not reported) (Ahel *et al.* 1998), various compounds associated with the production of Vitamin C (Ahel *et al.* 2004), as well as ibuprofen, propylphenazone, phenazone, and clofibric acid (concentrations not reported) (Slack *et al.* 2005). Only one reference has been found that quoted actual concentrations, as shown in Table 2.17 below (Schneider *et al.* 2004).

Table 2.17 Concentrations of pharmaceuticals in landfill leachate (Schneider *et al.* , 2004)

Chemical Name	Concentration ng l^{-1}
Ibuprofen	9362
Propylphenazone	9173
Phenazone	5507
Primidone	5011
Diclofenac	3190
Iopamidol	2944
Clofibric acid	2879
Bezafibrate	2773
Carbamazepine	1415
Piroxicam	931
Ketoprofen	697
Diazepam	453

Chemical Name	Concentration ng l^{-1}
Naproxen	445
Iopromide	236
Valproic acid	205
Cyclophosphamide	192
Indometacin	141
Dihydrocodeine	101
Iomeprol	92
Atenolol	44
Ifosfamide	42
Metoprolol	31
Propanolol	10

No data have been found on the amounts of pharmaceuticals found in landfill leachate entering sewage works.

Unfortunately, due to the nature of the materials used in their construction, landfill sites have a tendency to leak (Giardino and Guglielmetti 1997; Mota *et al.* 2004). Leachate from these landfill sites can also find its way directly into groundwater and from there into streams and rivers, completely bypassing sewage treatment systems. This means that landfill sites may be a major source of pharmaceuticals entering the river system (Slack *et al.* 2005).

Streams and rivers

Data have been found on 110 pharmaceuticals that have been detected in streams and rivers worldwide. Ibuprofen (12 reports), diclofenac (10), naproxen (8), and clofibric acid (8) were the most commonly detected pharmaceuticals. The ten highest concentrations detected are shown in Table 2.18.

Table 2.18: Top ten detected pharmaceuticals worldwide in streams and rivers

Chemical Name	Maximum ng l^{-1}	Median ng l^{-1}	Minimum ng l^{-1}	Country	Information source
Paracetamol	10000	110	-	US	Kolpin *et al.* (2002)
Clofibric acid	7300	-	-	Germany	Heberer *et al.* (1997)
Caffeine	6000	81	-	US	Kolpin *et al.* (2002)
Ibuprofen	5044	826	< 20	UK	Ashton *et al.* (2004)
Ibuprofen	5040	-	< 20	UK	Hilton *et al.* (2003)
Salicylic acid	4100	25	< 10	Germany	Ternes (1998)
Clofibric acid	4000	-	-	Germany	Heberer and Stan (1997)
Bezafibrate	3100	350	< 25	Germany	Ternes (1998)
Clofibric acid derivative	2900	-	-	Germany	Heberer *et al.* (1997)
Bisoprolol	2900	-	-	Germany	Hirsch *et al.* (1996)

In the UK, sixteen pharmaceuticals have been reported in streams and rivers, with dextropropoxyphene (4), ibuprofen (3), erythromycin (3), and trimethroprim (3) being the most frequently reported. The highest concentrations detected in UK rivers and streams are shown in Table 2.19 below.

Table 2.19: Top ten detected pharmaceuticals in the UK streams and rivers

Chemical Name	Maximum ng l^{-1}	Median ng l^{-1}	Minimum ng l^{-1}	Information Source
Ibuprofen	5044	826	< 20	Ashton *et al.* (2004)
Ibuprofen	5044	826	< 20	Hilton *et al.* (2003)
Ibuprofen	2370	-	144	Roberts and Thomas (2006)
Erythromycin	1022	< 10	< 10	Ashton *et al.* (2004)
Dextropropoxyphene	1000	-	-	Richardson and Bowron (1985)
Dextropropoxyphene	682	58	< 20	Hilton *et al.* (2003)
Dextropropoxyphene	682	58	< 20	Ashton *et al.* (2004)
Diclofenac	568	0	< 20	Hilton *et al.* (2003)
Diclofenac	568	0	< 20	Ashton *et al.* (2004)
Mefenamic acid	366	62	< 50	Ashton *et al.* (2004)

Perhaps unsurprisingly, the list of pharmaceuticals detected at the highest concentrations in UK streams and rivers is virtually identical to the list of pharmaceuticals detected at the highest concentrations in the effluent of STPs (see Table 2.15). In fact the only compound that was recorded at the highest concentrations in effluents of STPs and is not detected at the highest concentrations in streams and rivers was musk galaxolide. This was because the study that detected the compound in the STP effluent did not extend to study the river concentrations as well (Kanda *et al.* 2003). It can be surmised that musk galaxolide would have been detected in the river system.

Drinking water

Since many drinking water treatment plants abstract their source water from rivers downstream from the effluent of sewage treatment plants, there is the potential for a wide range of pharmaceuticals to enter drinking water. From literature, 104 pharmaceuticals have been tested for in drinking water, of which only 23 have been detected. The most commonly detected compounds were clofibric acid (7 reports), ibuprofen (5), diazepam (4), and phenazone (4). The highest concentrations that have been recorded worldwide are summarised in Table 2.20.

Table 2.20: Top 10 highest reported concentrations of pharmaceuticals in drinking water worldwide

Chemical Name	Maximum ng l^{-1}	Median ng l^{-1}	Minimum ng l^{-1}	Country	Information Source
Phenazone	400	-	-	Germany	Reddersen *et al.* (2002)
Clofibric acid	270	-	-	Germany	Heberer and Stan (1996)
Carbamazepine	258	-	-	US	Stackelberg *et al.* (2004)
Phenazone	250	-	-	Germany	Zuhlke *et al.* (2004)
Clofibric acid	170	-	-	Germany	Heberer and Stan (1996)
Clofibric acid	165	-	-	Germany	Stan *et al.* (1994)
Propylphenazone	120	-	-	Germany	Reddersen *et al.* (2002)
Diatrizoic acid	100	-	-	UK	Seitz *et al.* (2006)
Iopromide	86	-	-	Germany	Ternes (2001)
Diatrizoate	85	-	-	Germany	Ternes (2001)

Of particular note in the compounds found in drinking water is that none of the pharmaceuticals had been previously mentioned as occurring in high concentrations in other areas of the aquatic system. This would suggest that the compounds listed above are the ones most resistant to removal by current treatment technologies (both sewage treatment and drinking water technologies).

Summary of pharmaceutical detection data

From the available data, there does not appear to be any particular therapeutic group that has either been detected more frequently than any other group in sewage effluent, streams and rivers, or drinking water. There are however, specific compounds, such as ibuprofen, diazepam, diclofenac, clofibric acid, carbamazepine, and phenazone, which have been regularly detected in all sections of the aquatic environment. The compounds that have been detected at the highest concentrations are also those that are used in the greatest quantities (ibuprofen, erythromycin, dextropropoxyphene, diclofenac, mefenamic acid). The trend is particularly strong in the UK, whilst weaker for German data.

Concentrations reduce rapidly as the compounds pass through the water system. In sewage effluent the maximum concentration detected was 292,000 ng l^{-1} (caffeine) (Rogers 1996), but this was reduced by a factor of 30, to a maximum level of 10,000 ng l^{-1} (paracetamol) (Kolpin *et al.* 2002) in streams and rivers. Maximum concentrations in drinking water are a further factor of 25 lower, at a maximum of 400 ng l^{-1} (phenazone) (Reddersen *et al.* 2002). Median

concentrations are usually somewhat lower than these maximums, as summarised in Table 2.21 below (Kiwa 2004; Ternes *et al.* 2004).

Table 2.21: Median concentration ranges of pharmaceuticals in different compartments of the aquatic environment (Kiwa 2004; Ternes *et al.* 2004).

Compartment	Concentration range ng l^{-1}
Wastewater effluents	1 – 10,000
Surface water	1 – 1000
Drinking water	1- 100

2.2.1 Routes into the environment

As has been discussed above, many pharmaceuticals have been discovered in all areas of the aquatic environment. In general, the compounds that have been detected most regularly and at the highest concentrations, such as ibuprofen, erythromycin, dextropropoxyphene, diclofenac, and mefenamic acid are those that are the most highly used (by weight, rather than by prescription). The primary pathway of pharmaceuticals into the environment is the use and disposal of medicines (Bound and Voulvoulis 2005). This can occur through many routes, with the major ones summarised in Figure 2.1 below, as described by Heberer (2002).

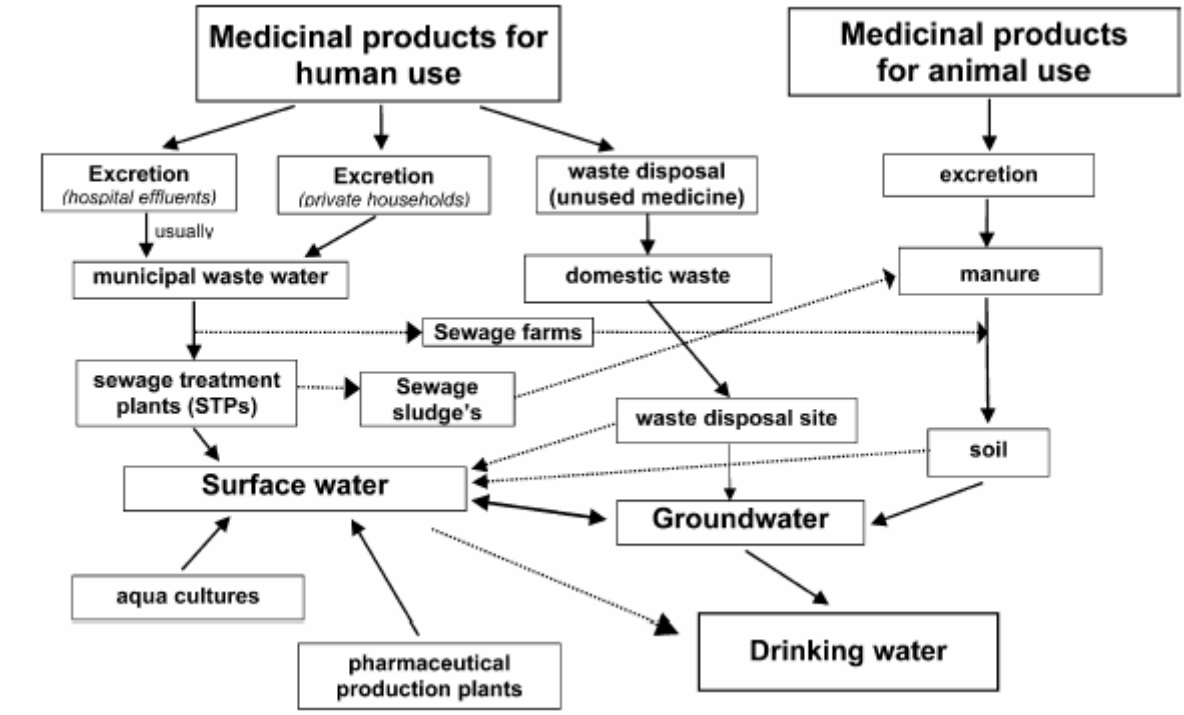


Figure 2.1: Possible sources and pathways for the occurrence of pharmaceutical residues in the aquatic environment. (Heberer 2002)

After normal application, many pharmaceuticals and/or their metabolites are eliminated from the body mainly through the renal system (urine), biliary system (faeces), or a combination of

both depending on the nature of the compound. Thus these compounds, contained in the urine and manure in runoff from agricultural fields and in sludges generated from sewer systems, can enter the environment (Kuhne *et al.* 2000; Kummerer *et al.* 1997). Furthermore, during sewage treatment, most of these compounds are not quantitatively removed and remain in the effluents that get into the surface and groundwater (Doll and Frimmel 2003; Mohle *et al.* 1999; Ternes 1998).

As can be seen from Figure 2.1, the potential for preventing pharmaceuticals and their metabolites from entering the aquatic environment is limited. Some possible solutions include:

- Reducing usage
- Reducing pipe leakage
- Reducing straight-piping and sewer overflow events
- Changing the disposal methods for surplus drugs
- Improving the efficiency of sewage treatment plants

These options are discussed in the sections below.

Reduction of usage and pipe leakage

These two options can be largely ignored in this work, since water companies are continuously working to reduce pipe leakage, and as discussed in Section 2.1.1 , the total usage of pharmaceuticals is unlikely to be reduced, although some individual drugs will be discontinued and replaced by others.

To actually quantify the reduction in pharmaceutical load entering the environment by reducing pipe leakage is somewhat hard, since water companies are reluctant to publish their sewerage leakage rates (unlike drinking water leakage rates, which are a regulatory requirement in the UK). Several authors have attempted to quantify exfiltration from sewage with tracer techniques using rhodamine dye or NaCl tracers, with typical results around 2% to 15% of dry weather flow (DWF) (de Benedittis and Bertrand-Krajewski 2005), and 2.7% of DWF (Rieckermann *et al.* 2005). Cardoso *et al.* (2006) estimated that in worst cases up to 10% DWF may be lost by exfiltration. Fenz *et al.* (2005; 2005) used carbamazepine as a tracer, since it is discharged to the environment only via domestic wastewater, resulting in an estimate of exfiltration losses of 1% DWF. Ellis (2006) studied concentrations of

pharmaceuticals below "*leaky*" sewers, with resulting concentration profiles as shown in Figure 2.2 below. These concentrations were close to detection limits, predominantly due to low exfiltration rates (10^{-5} – 10^{-6} m s^{-1} per metre of sewer (Blackwood *et al.* 2005; Wolf *et al.* 2004)) being diluted with groundwater.

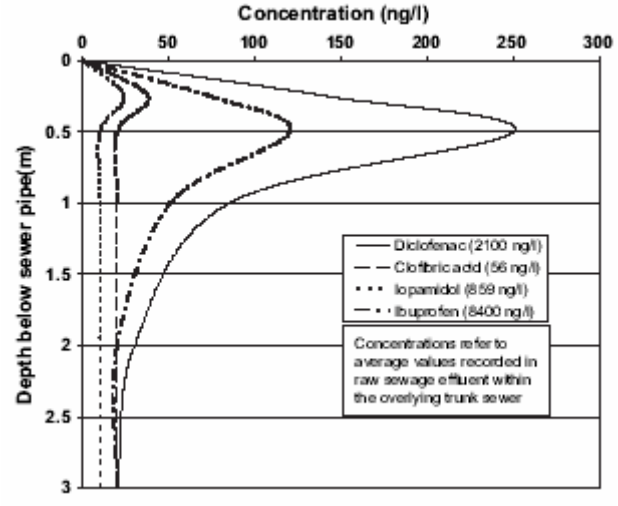


Figure 2.2: Concentration profile of pharmaceuticals below a "*leaky*" sewer (Ellis 2006)

The loss of only 1% DWF can be considered to be insignificant when considering the entire load of pharmaceuticals entering the environment. In any case, fixing this level of leaks would not be considered economical either by water companies or the water regulator.

Reducing straight-piping and sewer overflow events

Straight-piping is the direct hydraulic connection of septic tank leachate to groundwater, particularly common in rural areas (Gray and Booker 2003; Hayes *et al.* 1990). This is a major problem in the US, where about one third of all households use septic tanks (USEPA 1998). Sewer overflow events can be caused by excessive amounts of storm-water, blockages, or structural, mechanical, or electrical failures. The US Environmental Protection Agency (EPA) has estimated that 40,000 overflows occur each year (Metcalf and Eddy 2003). These events can cause a threat to public health and the environment, through the discharge of untreated sewage, which may also contain high concentrations of pharmaceuticals. Similarly to pipe leaks, the water companies (under pressure from the Environment Agency) are seeking to reduce the number of these events that occur each year, and as such these will not be covered by this work. It is not known what proportion of the total amount of pharmaceuticals

entering the aquatic environment each year is due to these types of events, but it is expected to be relatively small (Daughton and Ternes 1999). Considering that most sewere overflow events are caused by storm flows, when pharmaceutical concentrations will be minimal due to being heavily diluted by rainwater, the potential for reducing the amount of pharmaceuticals entering the environment by this route is very low.

Changing the disposal methods for surplus drugs

One potential method of reducing the amount of pharmaceuticals entering the aquatic environment is to change the disposal methods that are employed to get rid of unused or unwanted pharmaceuticals (Bound and Voulvoulis 2005; Slack *et al.* 2005). In the past, it has been common practice simply to either dispose of these medicines in household waste, or to flush them down the toilet (Daughton 2003). These two disposal routes accounted for the disposal of 89.4% of unwanted medicines according to one US survey (Kuspis and Krenzelok 1996) and 74.7% in a UK survey (Bound *et al.* 2006). Over the last few years several countries, including the UK, Italy, and France, have started schemes to collect these unwanted medicines and either recycle them where possible, or else incinerate them. Examples of these schemes include Cyclamed (France), Associazione Indennizzo Resi (Italy), and DUMP (Disposal of Unwanted Medicines and Poisons) (UK).

The Italian scheme, running since 1980, operates on a six-monthly cycle. According to Macarthur (2000), every January and July, wholesalers and pharmacies clear their shelves of out-of-date or damaged stock and draw up an inventory on a special form. The goods are transported by a licensed carrier to the organisation's offices in Rome, where the packaging materials are separated, sealed in bags, quarantined for six months and then shredded. The medicines are incinerated.

In the French Cyclamed scheme (running since 1995), households are encouraged to return all unused medicines and packaging, and even empty packs to pharmacies (Cyclamed http://www.unpf.org/cyclamed/index.htm). Partially used, expired, damaged or other clearly non-reusable stock is placed by the pharmacist in a "destroy" box; any products that could possibly be used by medical charities, in France or overseas, are stored in a second box. When full, the "destroy" boxes are picked up by a wholesaler on his normal rounds and put into an isolated, closed container at the warehouse. Unlike in Italy, where only certified waste

disposal contractors can be used for transport, French wholesalers can be involved as it is labelled non-hazardous "special waste". Wholesalers also supply the communication materials and arrange for a waste contractor to collect containers when full for incineration. Charities involved in Cyclamed collect the supplies initially considered reusable from pharmacies. A secondary selection is made and waste returned to the system. A minimum of twelve months of remaining shelf life is required for the medicines to be used in Asia or Africa and six months for central and Eastern Europe. Any in-date stock can at least be considered for France's homeless people. The adoption of this scheme in other countries could significantly reduce the quantity of human pharmaceuticals entering the environment. For example, in 1998 the French scheme recovered about 10,000 tonnes of pharmaceutical products for reuse, out of an estimated 70,000 tonnes of domestic drug-related waste (Macarthur 2000). It is estimated that the scheme collects as much as 80% of all unused medicines in France (Aumonier 2003).

Data on the operations of the DUMP scheme in the UK are limited, since there is no regular data collection. Surveys in Arran, Ayrshire, Grampian, and Lancashire showed that between 0.5 and 0.8% of drugs were returned unused (Cromarty and Downie 2001), while a similar survey in Devon in 1995 showed that 1.8% were returned unused (Cromarty and Downie 2001). A survey in Scotland in 1999 showed that a total of 47,000 kg of unwanted medicines were returned for disposal, with diclofenac recorded as being the most returned pharmaceutical compound (Craig 2001). Based on this UK data, these schemes have the potential to reduce the amount of pharmaceuticals reaching the aquatic environment by up to 2% (Cromarty and Downie 2001) (assuming no degradation in the original disposal route).

According to the above data at least 98% of pharmaceuticals are consumed as prescribed. A significant amount will pass through the human body unchanged, and along with the metabolites that are formed, pass into the sewerage system, and then into sewage treatment plants, before being discharged into the environment. Therefore, the option that must be investigated most thoroughly is improving the efficiency of sewage treatment plants to remove pharmaceuticals, whether achieved by optimisation of current processes or the addition of new ones.

Improving efficiency of sewage treatment plants (STPs)

Pharmaceuticals are not completely destroyed during sewage treatment (see Section 2.5.3), leading to their discharge both in final effluents into streams and rivers, and also in sewage sludges which may then be applied to land. The improvement of removal of pharmaceuticals during sewage treatment may be achieved by the enhancement of current removal mechanisms in existing technologies. These include sorption to biomass, and biodegradation. Alternatively, tertiary treatments (such as ozonation or granular activated carbon (GAC)) could be added to existing treatment plants for further removal. These approaches are discussed in detail in Section 2.5 .

A recent review of endocrine disrupting chemicals (Johnson and Sumpter 2001) concluded that "*the future must lie in a reassessment of the activated sludge process and an exploration of the existing potential to enhance its biodegradative and sorptive capacity*" due to the high costs of other processes. This approach will need to be followed not just for endocrine disrupters, but also for all micro-organic contaminants such as pharmaceuticals. For example, doubling the hydraulic retention time (HRT), could allow more complete biodegradation to occur, but this would require much larger sewage works.

Of course, there are approaches other than activated sludge methods that may be more effective at removing pharmaceuticals. These could include other suspended growth processes, such as membrane biological reactors (MBRs) or fixed film processes, such as trickling filters, rotating biological contactors (RBCs), or moving bed biofilm reactors (MBBRs). The last method, when used as an adaptation to existing activated sludge plants, looks potentially interesting, as it increases the available biomass, and hence biodegradation capacity, without significantly increasing the amount of sludge produced. As well as these methods, there are secondary treatments, such as ozonation, but these tend to be very costly to build and run. Generally, solutions will need to be able to remove the contaminants without a significant increase to either the current size or the running costs of the plant.

A full discussion of current STP treatment technologies and optimisation option can be found in Section 2.5 .

2.3 Environmental significance

Pharmaceuticals are designed to be biologically active compounds. Once released into the environment, they may come in to contact with a large range of non-target organisms, producing the potential for a wide range of physiological outcomes. A well known example of this is the feminisation of male fish by natural and synthetic hormones, such as estrone (E1), estradiol (E2), and ethinyl-estradiol (EE2) (Johnson and Sumpter 2001). There is also growing evidence for effects of these endocrine disrupting compounds on many other species, such as pseudohermaphroditic offspring produced by polar bears (Wiig *et al.* 1998), and seals in contaminated water that have an excess of uterine fibroids (McLachlan *et al.* 2006). Another example was the use of diclofenac as a painkiller for cattle in India and Pakistan. When vultures ate treated cattle carcasses, the diclofenac induced visceral gout, leading to their death (Oaks *et al.* 2004). This led to up to a 97% decrease in vulture populations in the area and the Indian government to announce its intention to ban the use of diclofenac by September 2005 (Swan *et al.* 2006).

To assess the environmental significance of the concentrations of pharmaceuticals found in the aquatic environment, risk assessments have been conducted for both humans and aquatic organisms. These environmental risk assessments (ERAs) of various compounds are discussed in the sections below.

2.3.1 Risk to humans

For humans, some authors have attempted to make risk assessments based on the usage data from general practitioners (Richardson and Bowron 1985). From this data, a predicted environmental concentration (PEC – see section 2.3.2) was calculated, along with the amount that would be consumed over a typical seventy years lifespan (I_{70}) via drinking water. These consumptions were below the standard daily dose, and so it was concluded that there was no risk to human health. Recent experimental data has confirmed this view. Based on monitoring data of pharmaceuticals from Germany (Ternes 2001), Webb *et al.* (2003) conducted a risk assessment along the same lines as Richardson and Bowron. They calculated that only four compounds had I_{70} values exceeding a standard daily dose: Clenbuterol, Salbutamol, Terbutalin, and EE2. In the worst case, Clenbuterol, a lifetime consumption of drinking water equated to 25.5 daily doses. They concluded this presented no risk to human

health, which was a view shared by Schab *et al.* (2005) who conducted a similar study for 26 pharmaceuticals in US surface waters.

The consumption of pharmaceuticals via drinking water should also be considered in comparison with consumption via other sources. Since pharmaceuticals can be in sewage sludges applied to agricultural land, there is the potential for the uptake of pharmaceuticals by crops. Boxall *et al.* (2006) showed that a range of veterinary medicines could be taken up by crops. In the worst cases, this could account for up to 10% (levamisole, trimethroprim) of the acceptable daily intake (ADI). The Joint FAO/WHO Expert Committee on Food Additives (JECFA) has established acceptable daily intake (ADI) values for a large number of veterinary drugs. The ADI values are expressed on a body weight basis and correspond to the amount that can be ingested daily over a lifetime without appreciable risk (assuming 60kg human body weight). These values tend to be based on no observed effects levels (NOEL) from chronic animal studies, along with a safety factor of 100. For example, trimethroprim has an ADI of 252 ug/person, based on toxicity testing of *Lactobacillus* from the human gut (EMEA 1997). Typical sources of trimethroprim are shown in Table 2.22.

Table 2.22: Potential daily intake of trimethroprim

Source	Concentration	Typical consumption of source product	Total consumption of pharmaceutical	Data Source
Plants (above ground)	82 μg kg^{-1}	500g	41 μg	(Boxall *et al.* 2006)
Plants (below ground)	80 μg kg^{-1}	333g	27 μg	(Boxall *et al.* 2006)
Milk	50 μg kg^{-1}	1500ml	150 μg	(EMEA 1997)
Bovine meat	50 μg kg^{-1}	300g	15 μg	(EMEA 1997)
Water	<10ng l^{-1}	2 litres	<20 ng	(Ternes 2001)

From this table it is clear to see that the consumption of pharmaceuticals through other sources outweighs the consumption through water by a factor of over 10,000. Indeed, according to this data, the greatest risk to human health could be by the transport of pharmaceuticals to land via sewage sludges, rather than by consumption of drinking water.

This consideration of the effects of pharmaceuticals may not, however, give the full picture of risks to human health. This approach looks at individual chemicals, and does not consider the effect that the entire mixture may have. This approach also fails to take into account the

effect that this mixture may have on babies and young children whose bodies are less able to deal with pharmaceuticals. For example, it has recently been shown that there is a link between concentration of phthalates and genital abnormalities in newborn males (Swan *et al.* 2005). Finally, this approach fails to look at the effect these drugs may be having on the flora and fauna of the aquatic environment, which in turn may have a direct effect on human health. An example of this is bacterial resistance, possibly caused by bacteria being exposed to pharmaceuticals in the sewerage system and aquatic environment. Antibiotic residues in the environment are suspected to induce resistance in bacterial strains causing a serious threat for public health as an increasing number of infections can no longer be treated with the presently known antidotes (Hirsch *et al.* 1999). Epidemic diseases in hospitals are often caused by resistant Klebsiellae strains. Stelzer *et al.* (1985) investigated Klebsiellae isolated from a sewage treatment plant in which 90% exhibited insensitivity against ampicillin and 6% showed multiple resistances. Several papers have reported bacteria in sediments that are resistant to various antibiotics used as feed additives in fish farms (Nygaard *et al.* 1992; Sandaa *et al.* 1992).

Finally, a possible failing of the I_{70} method of assessing risk to humans is that it does not cover the effects of continual exposure to low concentrations. For example, in the case of ethinylestradiol, spot concentrations in the milligram or microgram range are necessary to have a detrimental effect on organisms. However, continual exposure to a concentration of a few nanograms per litre can also induce the same extreme effects (Sumpter and Johnson 2005). Therefore, it is possible that the continual exposure to low concentrations of pharmaceuticals, even though they are significantly below the standard daily dose, could still have adverse effects.

Whilst it is not possible to draw a conclusive answer from this data as to whether or not pharmaceuticals pose a risk to human health or the wider environment, it is possible to see that there may be a small risk. Therefore, the precautionary principle warrants further investigation into the effects that pharmaceutical concentrations in the environment may have.

2.3.2 Risk to aquatic organisms

Any new pharmaceutical product must have its quality, safety and effectiveness demonstrated to the regulatory authorities before it can be sold. The risk to aquatic organisms is calculated as the ratio between the predicted environmental concentration (PEC), and the predicted no-effects concentration (PNEC). The regulatory authorities in each county define the method of calculating the values of the PEC and PNEC, meaning that different approaches are taken in different countries. The process used in the US and the EU are discussed and compared. It should be noted that during the course of this research, the formula for the EU calculation was changed. The selection of compounds for study (see Chapter 3:) was based on the original calculation method. For the UK, the regulatory authority is the Medicines and Healthcare products Regulatory Agency (MHRA); for the EU, it is the European Medicines Evaluation Authority (EMEA); and for the US it is the Food and Drug Administration (FDA).

Risk assessment in the US

The environmental risk assessment for the US is well established, having being developed by the FDA in the 1980s. However, in 1995, the FDA Centre for Drug Evaluation and Research (CDER) reduced the requirements after showing that human pharmaceuticals generally had a minimal environmental impact (FDA 1995). The assessment procedure consists of two steps. First, the manufacture must first estimate the Expected Introductory Concentration (EIC), based on an estimate of fifth year production. The standard EIC calculation (FDA 1995) for the aquatic environment is:

$$EIC\ (mg\ l^{-1}) = A \times B \times C \times D$$

Equation 1

Where:

A = kg / year production

B = 1 / litre per day entering publicly owned treatment works (1.115×10^{11})

C = 1 / 365 year/days (conversion factor)

D = 10^6 mg kg^{-1} (conversion factor)

Information available on metabolism to inactive compounds, or environmental mechanisms such as adsorption, biodegradation and hydrolysis can also be taken into account when calculating the EIC (FDA 1995). For the second step, if the EIC of a drug or its metabolites is shown to be less than 1 $\mu g\ l^{-1}$ at its point of entry into the environment (sewage effluent),

no further risk assessment is needed. However, if the EIC is higher than this level, a full environmental assessment has to be conducted. This requires data on environmental fate and a tiered set of ecotoxicity tests. The base set includes effects on microbial respiration and acute toxicity to at least one algal, invertebrate, or fish species.

Risk assessments in the EU (including UK) pre-2006

Until the advent of advent of EMEA guideline 4447 (EMEA 2006), due to come into effect in December 2006, the European procedure was less developed than that for the US, but nonetheless was stricter (Ayscough *et al.* 2000). The EU draft guidance document (CEC/III/5504/94) published in 1995, which specified the documentation required for an environmental risk assessment. The first step is to estimate the predicted environmental concentration (PEC) via equation Equation 2):

$$\text{PEC } (\text{g l}^{-1}) = \frac{A.(100 - R)}{365.P.V.D.100}$$

Equation 2

Where:

A = predicted amount (kg) used per year in the EU country.

R = removal rate (%) due to loss by adsorption, hydrolysis, biodegradation or other naturally occurring process.

P = number of inhabitants of the country

V = volume (m^3) of waste per capita per day (generally 0.15 to 0.30 m^3)

D = dilution factor of waste water by surface water

The estimate should be conducted for the EU country with the maximum A/P ratio, and assuming worst case conditions i.e. no losses (R=0) and no dilution (D=1). Similar to the American procedure, if the PEC is less than 0.01 $\mu g\ l^{-1}$ then no further action is required. It should be noted that this trigger value is a factor of 100 lower than in the American system. If the PEC is greater than this value, then the ratio of the PEC to the predicted no-effects concentration (PNEC) should be calculated. If this ratio is less than unity it can be concluded that there is unlikely to be a risk to the environment and so no further action is required.

For aquatic organisms, it is necessary to be able to predict the concentration at which no effect will be observed in a particular organism. The PNEC is calculated by dividing the effects concentration from acute toxicity tests by a suitable safety factor. These safety factors are defined in EU directive 67/548/EEC Annex V, as shown in Table 2.23. The values used are ideally the no observed effects concentration (NOEC), but more usually are based on acute toxicity tests (e.g. LC_{50}). However, until recently, very little aquatic toxicity data have been available. For example, a review by the Environment Agency in 2000 (Ayscough *et al.* 2000) discovered aquatic toxicity data for only seventeen human pharmaceuticals, and chronic toxicity data for only one compound (ethinylestradiol). The current literature about ecotoxicological effects of human pharmaceutical deals mainly with the acute toxicity in standardized tests and it is generally focused on aquatic organisms. The influence of environmental parameters such as pH on toxicity has only rarely, or not yet been investigated (Fent *et al.* 2006). Such studies would be of importance for instance for acidic pharmaceuticals that may induce different toxicities depending on speciation at different ambient pH. Moreover, effects of drug metabolites have rarely been investigated. Phototransformation products of naproxen, for instance, showed higher toxicities than the parent compound (Isidori *et al.* 2005). At contaminated sites, aquatic life is exposed over the entire life cycle to these compounds. Chronic effects are less investigated and often even related to relative short-term exposures.

Table 2.23: Safety factors used in the calculation of predicted no-effects concentrations (EU Directive 67/548/EEC)

Test type (lowest obtained value to be used)	**Safety factor**
At least one acute EC_{50} from each of the three trophic levels of the base-set.	1000
One chronic NOEC (either fish or Daphnia or a representative organism for saline waters).	100
Two chronic NOECs from species representing two trophic levels (fish and/or Daphnia or a representative organism for saline waters and/or algae).	50
Chronic NOECs from at least three species (normally fish, Daphnia or a representative organism for saline waters and algae) representing three trophic levels.	10
Other cases, including field data or model ecosystems, which allow more precise safety factors to be calculated and applied.	Case by case assessment

Where neither NOEC nor acute toxicity values are available, many researchers have used quantitative structure–activity relationship (QSARs) to predict toxicity. The most extensively validated and used QSAR is the USEPA EPIWIN suit with ECOSAR (Sanderson *et al.* 2003). ECOSAR has previously been successfully (low false negative rates) applied to screening pharmaceuticals (Jones *et al.* 2001) and other complex compounds such as fragrance materials (Salvito *et al.* 2002).

If the PEC / PNEC ratio is greater than one then a more detailed assessment is required. This includes information on degradability or persistence of the pharmaceutical and its relevant metabolites. Full details of this can be found in the EMEA publication EMEA/CVMP/055/96.

Risk assessments in the EU (including UK) post-2006

Coming into force from December 2006, EMEA Guideline 4447 alters the way in which the PEC is calculated (EMEA 2006). In addition to the previously specified tests, compounds suspected to be potential endocrine disruptors or to be highly lipophilic are required to have Phase II screening, irrespective of whether or not they meet the trigger PEC value for further testing. This had been a major criticism of the previous guidelines, since known endocrine disrupting compounds, such as EE2, were not required to complete further testing. The new formula for calculating the PEC is shown in Figure 2.3 below. For pharmaceuticals with a PEC above 0.01, a Phase II analysis is required, as per the pre-2006 regulations.

$$PEC_{SURFACEWATER} = \frac{DOSE_{ai} * F_{pen}}{WASTEW_{inhab} * DILUTION}$$

Parameter	Symbol	Value	Unit
Input			
• Maximum daily dose consumed per inhabitant	DOSEai		[mg·inh^{-1}·d^{-1}]
• Percentage of market penetration	Fpen	0.01(*)	[--]
• Amount of wastewater per inhabitant per day	*WASTEWinhab*	200	[L·inh^{-1}·d^{-1}]
• Dilution factor	*DILUTION*	10	[--]
Output	$PEC_{SURFACEWATER}$		[mg·L^{-1}]
• Local surface water concentration			

Figure 2.3: Calculation of PEC according to EMEA guideline 4447

This new formula allows for an easier calculation of PEC than the previous version, since data on the maximum daily dose per inhabitant is more readily available than the total sales of a compound. The refinement of the PEC in phase 2 of the post-2006 method leads to the same PEC as calculated by the pre-2006 method (Liebig *et al.* 2006).

Huschek *et al.* (2004) calculated the PEC values of the top twenty most used pharmaceuticals in Germany (see Table 2.10) according to both formulae. The same calculations were also conducted by Liebig *et al.* (2006). These data are shown in Table 2.24, and compared to the measured environmental concentration (MEC), where possible. For measured environmental concentrations (MEC), maximum reported values are shown rather than medians, since the PEC calculation should provide the worst-case scenario (i.e. no dilution, or removal by sorption or degradation).

Table 2.24: Comparison of pre- and post-2006 formulae for calculation of PEC using Germany 2001 consumption data (Huschek *et al.* 2004)

Pharmaceutical	PEC post-2006 (μg l^{-1})	PEC pre-2006 (μg l^{-1})	MEC in Germany	MEC data source
Acetylsalicylic acid	15	13.95	1.51	Stumpf *et al.* (1996)
Paracetamol	15	10.38	6.0	Ternes (1998)
Povidone	1	8.07	-	
Metformin	10	8.63	-	
Ibuprofen	6	5.76	3.4	Ternes (1998)
Metanizole	15	3.57	-	
Theophylline	2	2.29	-	
Piracetam	12	2.03	-	
Allopurinol	2	2.38	-	
Amoxicillin	5	1.93	-	
Pentoxifylline	5	1.25	-	
Salicylic acid	7	1.20	0.14	Ternes (1998)
Carbamazepine	5	1.46	6.30	Ternes (1998)
Penicillin V	9.8	1.38	-	
Ranitidine	1.5	1.43	-	
Diclofenac	0.5	1.43	2.10	Ternes (1998)
Verapamil	1.2	1.14	-	
Metoporolol	0.75	1.55	2.20	Hirsch *et al.* (1996)
Iopromide	3.8	1.07	11.0	Ternes and Hirsch (2000)
Sulfamethoxazole	10	0.9	2.00	Hirsch *et al.* (1999)

Whilst predicting environmental concentrations may be essential for conducting an environmental risk assessment, it is of no use if it underestimates the concentration entering the environment. Similarly, it would also be of little use if it significantly overestimated the environmental concentration, potentially limiting the use of a pharmaceutical. For the ten compounds where data was available for Germany, the PEC overestimated for six compounds, and underestimated for four compounds. The pre-2006 formula underestimated for five compounds, but generally estimated closer to the MEC.

Table 2.25 shows a similar comparison for the UK, using PEC data calculated by Webb (2000), using 2000 usage data and the pre-2006 formula. In this case, only one compound was underestimated (diazepam). All other estimates were in good agreement with the MEC, apart from aspirin which was overestimated by a factor of 10000. This may be because paracetamol is readily biodegradable (Ternes 2000)

Table 2.25: Chemicals detected in UK, where usage data is available (Webb 2000)

Chemical Name	PEC (mg l^{-1})	MEC (mg l^{-1})	MEC data source
Ibuprofen	29.8	27.3	Ashton *et al.* (2004)
Diclofenac	4.79	2.3	Ashton *et al.* (2004)
Erythromycin	4.87	1.8	Ashton *et al.* (2004)
Mefenamic acid	2.66	1.4	Ashton *et al.* (2004)
Diazepam	0.18	1	Waggott (1981)
Triclosan	0.73	0.71	Severn Trent (2002)
Dextropropoxyphene	7.81	0.59	Ashton *et al.*(2004)
Propanolol	2.17	0.37	Roberts and Thomas (2006)
Aspirin (acetylsalicylic acid)	141.41	0.01	Severn Trent (2002)
17a-Ethinylestradiol	0.01	0.007	Desbrow *et al.* (1998)

2.3.3 Chemicals with PEC>PNEC or MEC/PNEC in the UK

As discussed in Section 2.3.2 , any compound with a predicted or measured environmental concentration greater than the predicted no-effects concentration could pose a risk to environment. Based on the EMEA approach, Table 2.26 shows a list of compounds in the UK with a PEC/PNEC and/or MEC/PEC ratio greater than 1. PEC data was calculated from UK usage data presented by Webb (2000). The MEC data used was for concentrations measured in sewage effluents in the UK. Where experimental data are available (from literature and ECOTOX database – see Appendix C), the PNEC for each chemical has been calculated by the method outlined in Table 2.23. Where these data are not available, the

chemical toxicity has been predicted by the use of quantitative structure-activity relationships (QSARs). For these compounds, information on the measured environmental concentration (MEC) to PNEC ratio has been included where possible.

Table 2.26 Chemicals with PEC:PNEC > 1, with MEC:PNEC where data are available

Chemical Name	PEC (μg l^{-1})	MEC (μg l^{-1})*	PNEC (ng l^{-1})	PEC/PNEC	MEC/PNEC
Dextropropoxyphene	7.81	0.585	40	195	14
Diltiazem	3.99		60	66	
Triclosan	0.73	0.710	14	52	50
Mebeverine	2.84		70	40	
Tetracycline	0.86		25.1	34	
Gliclazide	3.45		110	31	
Mesalazine	7.40		500	14	
Paracetamol	71.8		6100	11	
Mefenamic acid	2.66	1.44	320	8.3	4.5
Thioridazine	0.70		104	6.7	
Quinine	5.45		1000	5.4	
Ibuprofen	29.8	27.3	7100	4.2	3.8
Verapamil	1.82		450	4.0	
Chloramphenicol	0.07		20	3.5	
Clofibrate	0.28		100	2.8	
Cisapride	0.08		30	2.6	
Aspirin	141.41	0.01	61000	2.3	0.0002
Sulfasalazine	8.50		4900	1.7	
Erythromycin	4.87	1.84	3900	1.2	0.4
Diclofenac	4.79	2.35	4240	1.1	0.5
Fluoxetine	0.37		350	1.0	
Cimetidine	6.55		6500	1.0	
Carbamazepine	7.38		8100	0.9	
Tamoxifen		0.37	120		3.08
Sulfamethoxazole		0.29	130		2.2
Benzydamine		1.00	490		2.0

*** For data sources, please see Table 2.25**

Whilst data is limited, both on predicted and measured concentrations, the above table shows a range of compounds that could be, or are, having an adverse effect on the environment. Several compounds are particularly worthy of note. At the top of the list, dextropropoxyphene has a very high PEC/PNEC ratio. Its MEC/PNEC ratio is an order of magnitude lower, although this is still significantly above unity. Triclosan has very similar PEC and MEC values. This is somewhat surprising since triclosan is readily biodegradable

(Bester 2003; Federle *et al.* 2002; Hundt *et al.* 2000). The similarity in values may be because triclosan is heavily used in non-prescription items (Bester 2003), which is not included in the usage values used for the PEC, leading to a severe underestimate. The same is also true for ibuprofen (Buser *et al.* 1999).

Aspirin has a MEC which is four order of magnitude smaller than its PEC. This may be because it is readily biodegradable (Cleuvers 2004).

2.3.4 Mixtures of compounds

Whilst the methods outlined above give a clear indication of the effects of a single pharmaceutical, sewage effluents contain a wide variety of these compounds. This mixture could contain mixtures of compounds that perform similar effects or have the same mode of action. Cleuvers *et al.* (2003), (2004) evaluated a mixture of NSAIDs (diclofenac, ibuprofen, naproxen, acetylsalicylic acid) using acute *Daphnia* and algal tests. Toxicity of the mixture was found at concentrations at which the single compound showed no or only little effects. The mixture toxicity followed the concept of concentration addition, which means that the concentrations of each compound behaved in an additive fashion. This implies that compounds occurring at concentrations below their individual NOEC can nevertheless contribute to the total effect of the mixture.

Flaherty and Dodson (2005) and Richards *et al.* (2004) conducted experiments on the effects of a mixture of up to five pharmaceuticals on the survival, growth and reproduction of Daphnia. They showed that whilst a chronic fluoxetine exposure (36 $\mu g\ l^{-1}$) significantly increased Daphnia fecundity, and acute clofibric acid exposure (10 $\mu g\ l^{-1}$) significantly increased sex ratio, the same concentrations of fluoxetine and clofibric acid mixed together resulted in significant deformities, including malformed carapaces and swimming setae. It should be noted that whilst concentrations of up to 10 $\mu g\ l^{-1}$ of clofibric acid have been detected in sewage effluent (Hignite and Azarnoff 1977), the highest reported concentration of fluoxetine is only 10 $ng\ l^{-1}$ (Kolpin *et al.* 2002) in river water. Mixtures of three to five antibiotics (total antibiotic concentration 30-500 $\mu g\ l^{-1}$) elicited changes in Daphnia sex ratio. They concluded that individual and mixtures of pharmaceuticals affect normal development and reproduction of Daphnia; and that aquatic toxicity of pharmaceutical mixtures can be unpredictable and complex compared to individual pharmaceutical effects.

From these works it would appear that whilst the safety factor of up to 1000 used in the PNEC calculation may be sufficient for single compounds, it does not accurately reflect the risk posed to the environment caused by a mixture of pharmaceuticals such as can be found in a sewage effluent.

2.4 Degradation products and metabolites

Once administered, pharmaceuticals can be degraded in the body by Phase I and Phase II metabolic reactions, as shown in Figure 2.4.

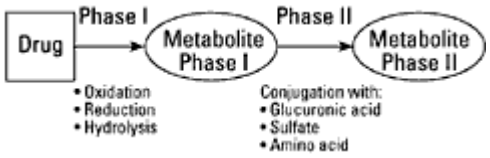


Figure 2.4: Phase I and Phase II metabolic reactions (Daughton and Ternes 1999)

Phase I reactions add reactive functional groups to the pharmaceutical molecule by oxidation (using monooxygenases enzymes), reduction (reductases), and hydrolysis (hydrolases). Phase II reactions make the molecule hydrophilic and more excretable, by covalent conjugation (glucuronidation) (Daughton and Ternes 1999). Phase I products are often more reactive and toxic than the parent compounds, while Phase II products normally result in inactive compounds (Halling-Sorensen *et al.* 1998). Often, 50-90% of administered pharmaceuticals can leave the body unchanged, in the original or biologically active form (Hirsch *et al.* 1999; Ternes 1998). The less toxic conjugates can later be transformed back into the parent compound by certain treatment processes in sewage works (Berger *et al.* 1986; Halling-Sorensen *et al.* 1998). Hence, conjugates can act as storage reservoirs from which the parent drug can be later released into the environment (Daughton and Ternes 1999). Conjugates, such as glucuronides can account for a large proportion of the excreted drug, as estimated by Ternes (1998) in Table 2.27.

Table 2.27: Estimated human excretion rates of selected drug glucuronide-conjugations (Ternes 1998)

Pharmaceutical	% Unchanged	% Glucuronide
Bezafibrate	50	22
Clofibric acid	6	> 90
Gemfibrozil	-	50
Diclofenac	15	< 1
Ibuprofen	1-8	14
Indomethacine	10-20	80

The degradation products and metabolites of pharmaceuticals are often overlooked in research. This is often because degradation route studies are complex, costly, and it is difficult to identify minor degradates. Whilst there are now a multitude of papers recording the concentrations and removal of various pharmaceuticals in the aquatic environment, there are

very few which deal with degradates, such as Halling-Sorensen *et al.* (2002) which looks at the degradation pathways of several tetracyclines.

Boxall *et al.* (2004) discussed the degradation of pesticides, showing that in certain circumstances pesticide degradates are more often found in the environment than the parent compound. They also reported that whilst most pesticide degradates are as toxic or less toxic to the environment than their parents, in some instances degradates are more toxic to the environment than the parent compound (41% less toxic, 39% as toxic, 20% >3x as toxic, 9% >10x more toxic).

An example of the importance of considering degradation products is caffeine (1,3,7-trimethylxanthine), whose degradation pathway is shown in Figure 2.5 below. The PNEC of caffeine has been measured at 87,000 ng l^{-1} (fish - *Leuciscus idus*) (OECD 2001), whilst the PNEC of one of its primary degradation product, theophylline (7% of caffeine degrades to theophylline (Georga *et al.* 2001)), has been measured at 100,000 ng l^{-1} (also fish – *Leuciscus idus*) (OECD 2001).

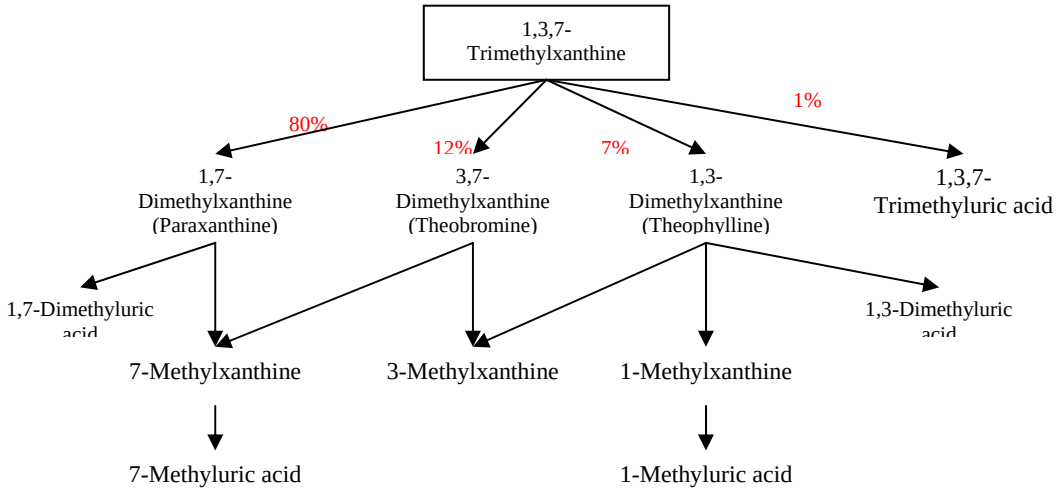


Figure 2.5: The degradation pathway of caffeine (1,3,7-trimethylxanthine) (Georga *et al.* 2001)

Therapeutically, caffeine is commonly used as part of headache preparations, and for its ability to improve the effectiveness of analgesics. It is also used in the treatment of apnea in newborn babies, in some cancer therapies, and electroconvulsive therapy (RxList 2006). Nonetheless, its major usage worldwide is non-medicinal, in beverages, with an average consumption of 100 mg per day (Chambaz *et al.* 2001), equating to a PEC of 50µg l^{-1}.

Theophylline is used as a respiratory muscle relaxant, particularly as a bronchiole dilator for asthma (RxList 2006). In Germany, the PEC of theophylline was calculated at 2290 ng l^{-1} (Huschek *et al.* 2004). Considering that 7% of caffeine degrades to theophylline, this gives an additional load of 3500 ng l^{-1} into the environment, more than doubling the total load. Hence, considering either caffeine or theophylline alone, without information about their degradation pathways, could potentially lead to a misleading environmental risk assessment (ERA). Whilst an ERA for theophylline would require consideration of its degradation products, consideration of the production of theophylline from caffeine degradation would not be required. In this case however, the total PEC of 5790 ng l^{-1} is still well below the PNEC value for theophylline.

This demonstrates that in some cases, not only does the parent compound need to be monitored and removed during sewage treatment, but in some cases so do the primary degradation products. However, in the case of most pharmaceuticals, the primary degradation products are not known, and hence monitoring them is not possible.

In the future, with improved analytical techniques, it should be possible to detect and identify the primary degradation products of pharmaceuticals. There may also be a case for regulators to require identification of primary degradation products at the time environmental risk assessments are carried out for the parent compound, and complete risk assessments for the degradates too.

2.5 The role played by sewage treatment plants

It has been established that some pharmaceuticals have the potential to harm the environment, as described by PEC and/or MEC values greater than the PNEC (see Table 2.26). It has also been shown that a major route for pharmaceuticals to enter the environment is in the effluent from STPs (see Section 2.2.1). As also discussed in section 2.2.1 , the option that can most significantly reduce the total amount of pharmaceuticals entering the environment is that of improving the amount of removal that occurs within sewage treatment plants. This could be achieved either by improving current processes, or the addition of new unit processes, particularly tertiary treatments.

2.5.1 Current Technologies

Conventional sewage treatment plants consist of a three stage process consisting of preliminary treatment, primary sedimentation, and secondary treatment (Metcalf and Eddy 2003). Typical arrangements of process units can be seen in Figure 2.6 (Metcalf and Eddy 2003), arranged in order of total cost of construction and operation per unit volume treated wastewater.

Preliminary treatment

The initial screening of the raw sewage occurs at the preliminary treatment at the head of the works where large floating objects are removed by screens (typically 6mm in two dimensions). Only a very small amount of organic material is removed at the screens. Grit and dense inorganic solids are then removed by the means of settlement in tanks or constant velocity channels – the lighter organics remain in suspension. Little or no removal of pharmaceuticals has been observed during preliminary treatment (Carballa *et al.* 2005).

Primary sedimentation

Raw sewage then enters the primary sedimentation tanks where a significant amount of removal of pharmaceuticals may occur, by adsorption on to solids. Under the influence of gravity, these solids settle to form primary sludge. Typical removal through primary treatment include 35-60% of musks, 40-50% of diazepam, 20-45% of diclofenac, 10-25% of ibuprofen, and 10-30% of naproxen (Carballa *et al.* 2005). Removals of pharmaceuticals have been seen to be higher in wastewaters with a high fat content (around 150 mg l^{-1})

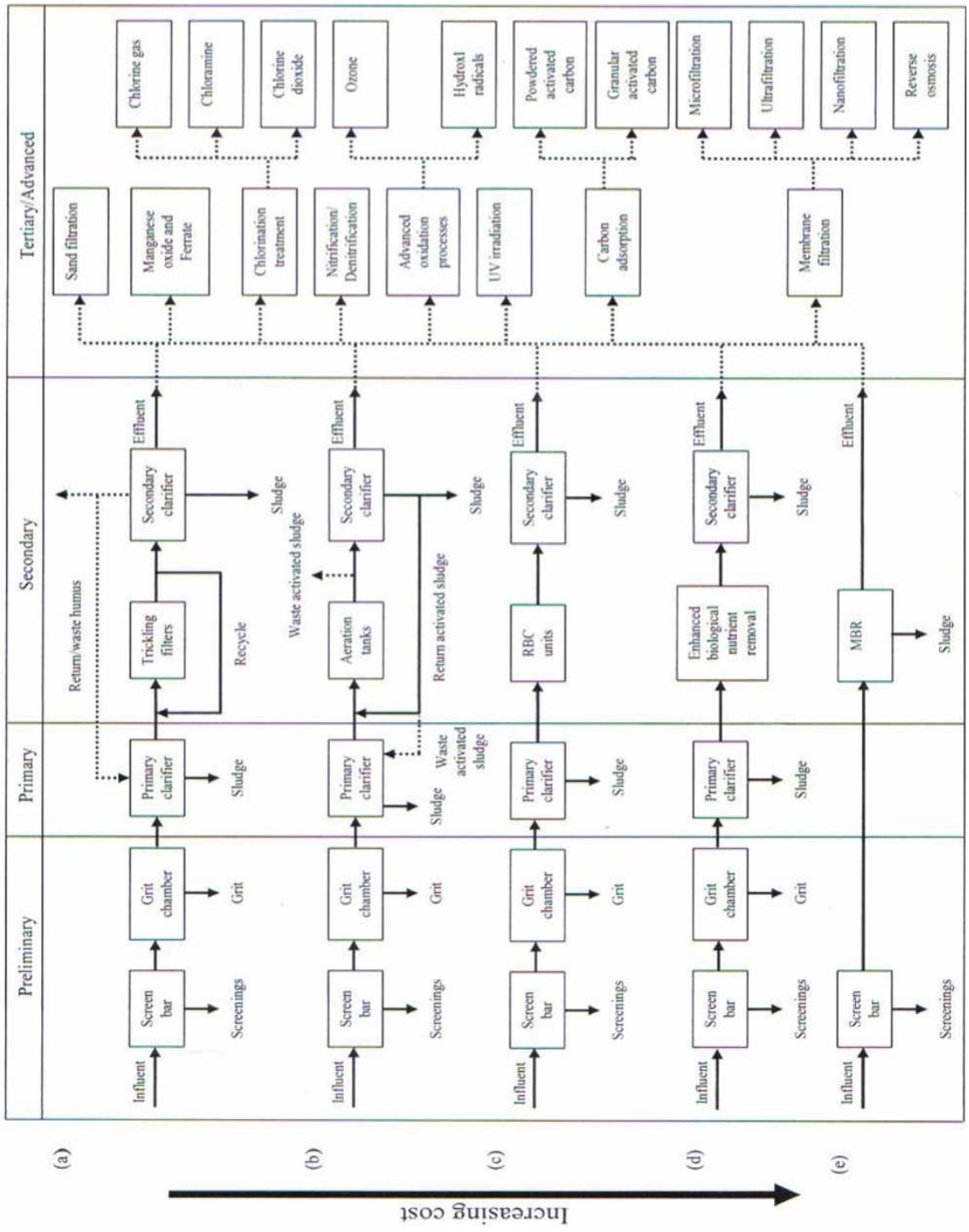


Figure 2.6: Combinations of existing preliminary, primary, secondary, and tertiary wastewater treatment processes. (a) trickling filters, (b) activated sludge process, (c) rotating biological contactors, (d) enhanced biological nutrient removal, and (e) membrane bioreactors. (Metcalf and Eddy 2003)

Secondary treatment

Current secondary treatment technologies can be divided into two distinct types: fixed film, and suspended growth systems (Metcalf and Eddy 2003). Examples of fixed film systems include rotating biological contactors (<2000 population equivalent (PE)) and trickling filters (2,000-50,000 PE) (Green 2004). It should be noted that trickling filters are no longer considered as an option for new builds, with oxidation ditches being the preferred solution due to better effluent quality (Green 2004). Examples of suspended growth systems are oxidation ditches (2000-10,000 PE), and the activated sludge process, which is usually found at the larger sewage treatment plants (>10,000 PE) (Green 2004).

Suspended growth systems

The most common secondary treatment in STPs is the activated sludge (AS) process, used for biological treatment of both municipal and industrial wastewater (Metcalf and Eddy 2003). A basic AS system contains three parts: a reactor in which the micro-organisms for treatment are kept in suspension and aerated (aeration tank); liquid-solid separation (primary and final settling tanks); and a recycle system for returning solids from the final separation tank back into the aeration tank (Metcalf and Eddy 2003). Preceding the AS plant, there are usually inlet screens, a grit chamber, and a primary clarifier, which will remove 50 to 70% of the suspended solids and 25 to 40% of the biological oxygen demand (BOD) (Metcalf and Eddy 2003). A diagram of a typical AS system is shown in Figure 2.7. There have been many modifications to this basic process, mainly aimed at removal of nitrogen and phosphorus (Metcalf and Eddy 2003).

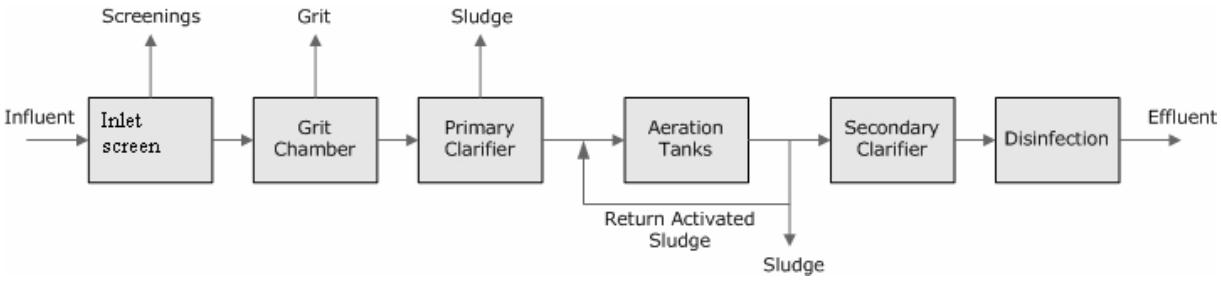


Figure 2.7: Activated sludge STP diagram (Metcalf and Eddy 2003)

Fixed film systems

Fixed film systems, in a trickling filter configuration, have been used for sewage treatment since the late 1890s, with RBCs being introduced in the 1960s (Metcalf and Eddy 2003). In trickling filters, wastewater is distributed continuously over a rock or plastic packing on

which biomass grows. In RBCs, the plastic packing is rotated in the wastewater treatment tank. Both processes provide a large surface area on which biomass can grow. These processes have advantages over suspended growth systems in that:

- less energy is required
- simpler to operate
- no problems with sludge bulking in the secondary clarifiers
- Better sludge thickening
- Less equipment maintenance
- Better recovery from toxic loads

However, fixed film systems tend to have a poorer effluent quality (as defined by biological oxygen demand (BOD) and suspended solids (SS) concentrations) than suspended growth systems, and tend to have problems with low temperatures, odour production, and sloughing events (Metcalf and Eddy 2003). Table 2.28 shows typical hydraulic retention times (HRT) and effluent qualities (BOD and ammonia) for the fixed film and suspended growth systems discussed.

Table 2.28: Comparison of hydraulic retention times and effluent qualities of activated sludge, trickling filter, and RBC sewage treatment plants (Metcalf and Eddy 2003)

	HRT (hours)	Effluent BOD (mg l^{-1})	Effluent ammonia (mg l^{-1})
Activated sludge	15 – 30	2 – 4	< 1
Trickling filter	1 – 8	15 – 30	0.5 – 3
RBC	0.7 – 4	15 – 30	1 – 2

The differences between fixed film and suspended growth technologies appear to have an effect on the pharmaceutical removal during sewage treatment. For example, the removal of triclosan, a common antimicrobial, during sewage treatment has been widely studied. Surveys of various STPs have shown variations in removal of triclosan during sewage treatment, as summarised in Table 2.29.

Table 2.29: Literature values of triclosan removal through various types of STP

Type of works	Influent ($\mu g\ l^{-1}$)	Effluent ($\mu g\ l^{-1}$)	Percentage removal	Author
Activated Sludge	21.9	1.1	95.0	Sabaliunas *et al.* (2003)
Activated Sludge	1.2	0.051	95.8	Bester (2003)
Activated Sludge	5.21	0.24	95.4	McAvoy *et al.* (2002)
Activated Sludge	10.70	0.41	96.2	McAvoy *et al.* (2002)
Activated Sludge	0.67	0.032	95.2	Kanda *et al.* (2003)
Activated Sludge	1.1	0.027	97.5	Kanda *et al.* (2003)
Activated Sludge	0.38	0.16	58.0	Bendz *et al.* (2005)
Trickling filter	7.5	0.34	95.5	Sabaliunas *et al.* (2003)
Trickling filter	3.83	1.61	58.0	McAvoy *et al.* (2002)
Trickling filter	16.6	2.10	86.1	McAvoy *et al.* (2002)
Trickling filter	15.4	2.70	82.5	McAvoy *et al.* (2002)
Trickling filter	2.5	0.14	94.4	Kanda *et al.* (2003)
Trickling filter	3.7	0.13	96.5	Kanda *et al.* (2003)

Generally, STPs with long hydraulic retention times (>15 hours), such as activated sludge, have shown high removal of triclosan (>95%). STPs with shorter retention times, such as trickling filters (1-4 hours), have shown lower and more varied removal (83-96%). However, for each type of process there has been a report of much poorer removal (58%) (Bendz *et al.* 2005; McAvoy *et al.* 2002). The possible reasons for this are unclear, since insufficient information is available in the papers to assess the plant operation. In particular, potentially essential parameters such as sludge ages, hydraulic retention times, and loadings have not been included in these papers.

2.5.2 Removal mechanisms

There are four mechanisms that have the potential to remove substances from the sewage influent stream, preventing their discharge in the liquid final effluent. These are volatilisation, photolysis, sorption, and biodegradation, and are discussed in detail below. Generally, removal of pharmaceuticals during sewage treatment only occurs by the later two mechanisms (Daughton and Ternes 1999; Paxeus 2004; Richardson and Bowron 1985; Ternes *et al.* 2004). A fifth mechanism, hydrolysis, is also discussed, since this can significantly affect detectable concentrations of pharmaceuticals.

Volatilisation.

The volatilisation potential can be estimated using the octanol-water partition coefficient (K_{ow}) and Henry's law constant (H_c) according to the following regime (Rogers 1996):

- If H_c is greater than 10^{-4} and H_c / K_{ow} is greater than 10^{-9}, the compound has a high volatilization potential.
- If H_c is less than 10^{-4} and H_c / K_{ow} is less than 10^{-9} the compound has a low volatilization potential.

In most cases, pharmaceuticals have a low volatilization potential since they have a small Henry's law constant (generally in the range 10^{-4} to 10^{-6}) (Ternes *et al.* 2004). Notable exceptions to this are the musk fragrances, such as tonalide and galaxolide (Ternes *et al.* 2004)., which have Henry's law constants in the range 5 x 10^{-3} and H_c / K_{ow} around 5 x 10^{-9} . These compounds can be readily volatilized during sewage treatment, particularly in STPs that employ surface aeration as opposed to fine bubble aeration, due to the higher amount of air in contact with the wastewater (Ternes *et al.* 2004).

Photolysis

Whilst an important loss mechanism for pharmaceuticals in surface waters (Boreen *et al.* 2003), the amount of photolysis that can occur depends on the configuration of the STP. In particular large aeration tanks or polishing lagoons allow for some photolysis to occur (Metcalf and Eddy 2003). Two pharmaceuticals whose loss due to photolysis has been studied are triclosan and diclofenac (Buser *et al.* 1998; Singer *et al.* 2002; Tixier *et al.* 2002). For both of these, photolysis has been shown to be the major removal mechanism in surface waters during summer months. However, for triclosan, removal by photolysis is only a significant removal mechanism above pH 8 (Tixier *et al.* 2002). Most STPs operate in pH range 6.5 to 8.5 (Metcalf and Eddy 2003), in which most Triclosan (pKa 8.1) will be in its photostable form. Therefore, losses of this compound due to photolysis can be expected to be negligible. Whilst potential losses due to photolysis need to be investigated separately for each compound, they can in general be expected to be negligible during sewage treatment for most pharmaceuticals.

Sorption

Sorption to filterable solids, which are later transferred to the sewage sludge, is one of the two most important mechanisms for preventing pharmaceuticals reaching the effluent of sewage treatment plants. Rogers (1996) suggested that the sorption potential could be estimated using the octanol-water partition coefficient (K_{ow}) as follows:

If log K_{ow} is less than 2.5, the compound has a low sorption potential
If log K_{ow} is between 2.5 and 4, the compound has a medium sorption potential
If log K_{ow} is greater than 4, the compound has a high sorption potential

However, a review of sorption of veterinary pharmaceuticals by Tolls (2001) showed that below a value of around 3, log K_{ow} gave a poor prediction of sorption. This suggested that a number of hydrophobicity-independent mechanisms such as cation exchange, cation bridging at clay surfaces, surface complexation, and hydrogen bonding appear to be involved. These processes are not accounted for by K_{ow}, which suggests that this data treatment is conceptually inappropriate and fails to describe the sorption (Tolls 2001). A better prediction was given by the use of the distribution coefficient K_d, defined as the ratio of the concentrations of a compound in the solid phase (C_s) and the aqueous phase (C_{aq}), as shown in Equation 3:

$$K_d = \frac{C_s}{C_{aq}}$$ **Equation 3**

The K_d can be calculated from batch sorption tests, according to OECD guideline 106 (OECD 2000). For comparison of the sorption to different sewage sludges, the K_d data can be normalised to the organic carbon content (f_{oc}), according to Equation 4, yielding the organic-normalized sorption coefficient K_{oc}.

$$K_{oc} = \frac{K_d}{f_{oc}}$$ Equation 4

However, the normalisation to organic carbon does not take into account a number of hydrophobicity independent mechanisms, such as cation exchange, hydrogen bonding, and surface complexation (Tolls 2001). For compounds where these mechanisms are important (e.g. tetracyclines from complexes with metal ions (Halling-Sorensen *et al.* 2002)), then K_d data should be used for sorption, rather than K_{oc}.

Ternes *et al.* (2004) calculated K_d values for 12 pharmaceuticals with both primary and secondary sludges. Values ranged from <1 to 2500 L kg^{-1} SS. Similar ranges were calculated

by Gobel *et al.* (2005), Jones *et al.* (2002) and Urase and Kikuta (2005). Values for selected pharmaceuticals are shown in Table 2.30 below.

Table 2.30: Distribution coefficient data for pharmaceuticals (Gobel *et al.* 2005; Jones *et al.* 2002; Ternes *et al.* 2004; Urase and Kikuta 2005).

Pharmaceutical	Log K_d (Primary sludge) L kg^{-1} SS	Log K_d (Secondary Sludge) L kg^{-1} SS	Reference
Azithromycin		2.6	Gobel *et al.* (2005)
Caffeine		1.5	Urase and Kikuta (2005)
Carbamazepine	-	0.09	Ternes *et al.* (2004)
Carbamazepine		1.8	Urase and Kikuta (2005)
Carbamazepine		1.4	Jones *et al.* (2002)
Clarithromycin		2.4	Gobel *et al.* (2005)
Clofibric acid	-	0.68	Ternes *et al.* (2004)
Cyclophosphamide	1.7	0.39	Ternes *et al.* (2004)
Diazepam	1.6	1.3	Ternes *et al.* (2004)
Diclofenac	2.7	1.2	Ternes *et al.* (2004)
Diclofenac		1.5	Urase and Kikuta (2005)
Ethinylestradiol	2.4	2.5	Ternes *et al.* (2004)
Galaxolide	3.7	3.3	Ternes *et al.* (2004)
Ibuprofen	-	0.85	Ternes *et al.* (2004)
Ibuprofen		1.9	Urase and Kikuta (2005)
Ibuprofen		2.7	Jones *et al.* (2002)
Iopromide	-	1.0	Ternes *et al.* (2004)
Naproxen		1.4	Urase and Kikuta (2005)
Naproxen		2.33	Jones *et al.* (2002)
Tonalide	3.7	3.4	Ternes *et al.* (2004)
Trimethroprim		2.3	Gobel *et al.* (2005)

From the data in Table 2.30 it can be seen that there is a difference between sorption to primary and secondary sludges. In particular, compounds that adsorb well to primary sludges absorb less well to secondary sludges (e.g. cyclophosphamide, diazepam, galaxolide). Similarly, those compounds that do not adsorb well to primary sludges do adsorb to secondary sludges (e.g. clofibric acid, iopromide), although not to any great extent (Log K_d generally below 1.0). This would suggest that there are significant differences between the makeup of primary and secondary sludges that affect sorption.

It should be noted that where there is data on the same compound from different researchers (carbamazepine, diclofenac, ibuprofen, and naproxen), the values of K_d vary by up to a factor of 100 (carbamazepine). This would suggest that there is variability between different secondary sludges, which affects sorption. Various parameters within sewage sludges have been suggested for this variability, such as fat content (Carballa *et al.* 2005), colloidal protein and polysaccharide concentrations (Holbrook *et al.* 2004; Kreuzinger *et al.* 2004), and organic carbon concentration (Drewes *et al.* 2001) – these are discussed in Section 2.5.4

For estimating the removal by sorption in secondary treatment, the quantity of sludge produced (rather than the suspended solids concentration) is the relevant factor, since return activated sludge (RAS) can be assumed to be in equilibrium with the aqueous phase (Ternes *et al.* 2004). The proportion of sorbed quantities can be obtained via Equation 5 (Ternes *et al.* 2004).

$$\frac{C_{sorbed}}{C_{aqueous} + C_{sorbed}} = \frac{K_d SS}{1 + K_d SS}$$

Equation 5

The effect of the variation of sludge production is shown in Figure 2.8 below, for a range of sludge production of 100 to 500 gSS m^{-3}. Sludge production values are typically 200 to 400 gSS m^{-3} in activated sludge plants (Ternes *et al.* 2004).

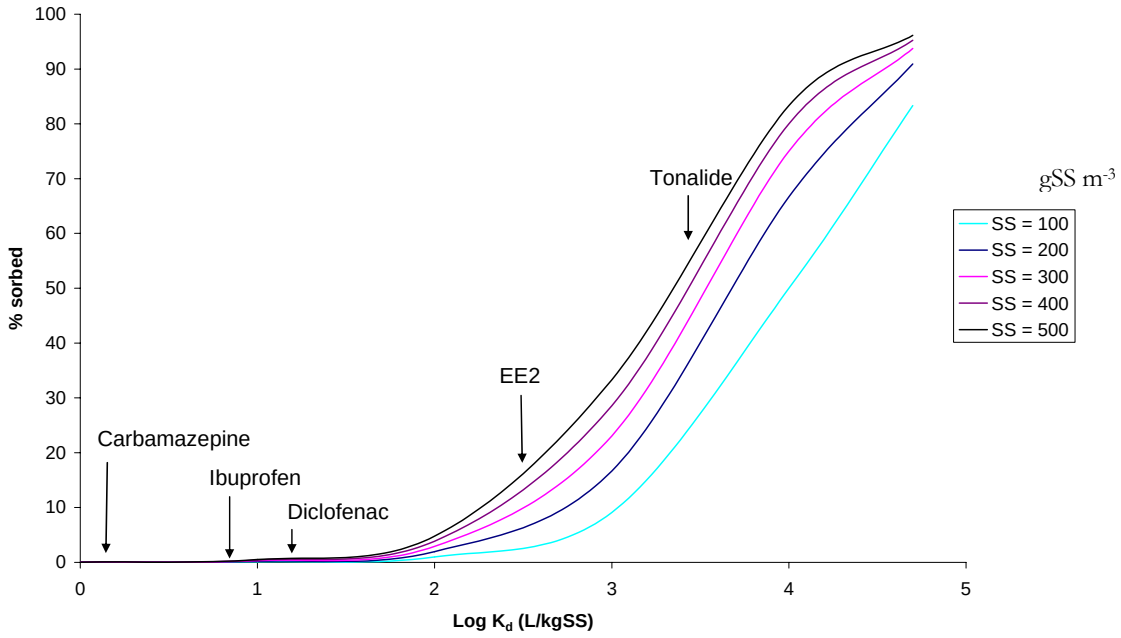


Figure 2.8: Percentage sorption as a function of K_d and sludge production, according to Equation 5

From this figure it can be seen that removal by sorption will be negligible for compounds with a K_d of less than about 500 l kg^{-1} SS (log K_d of below about 1.5).

Biodegradation

The biodegradation of pharmaceuticals to lower molecular weight products, which can sometimes lead to complete mineralization (to CO_2 and H_20), has been shown to vary widely between compounds. For example, paracetamol degrades rapidly during sewage treatment (Ternes *et al.* 2004), whilst carbamazepine does not degrade (Ternes *et al.* 2004). Biological degradation can be described by first order kinetics according to Equation 6 (Schwarzenbach *et al.* 2003).

$$\frac{dC}{dt} = K_{biol} C_0 SS$$ **Equation 6**

The biological degradation rate (K_{biol}) of 35 pharmaceuticals were calculated by Joss *et al.* (2006). Of these compounds, they showed that only 4 compounds (ibuprofen, paracetamol, 17β-estradiol and estrone) would be degraded by more than 90% (K_{biol}>10 L g_{SS}^{-1} d^{-1}). Sixteen compounds were shown to be partially removed (0.1>K_{biol}>10), whereas no biological transformation was shown for 17 compounds (K_{biol}<1). The ranges for each compound are shown in Figure 2.8 below.

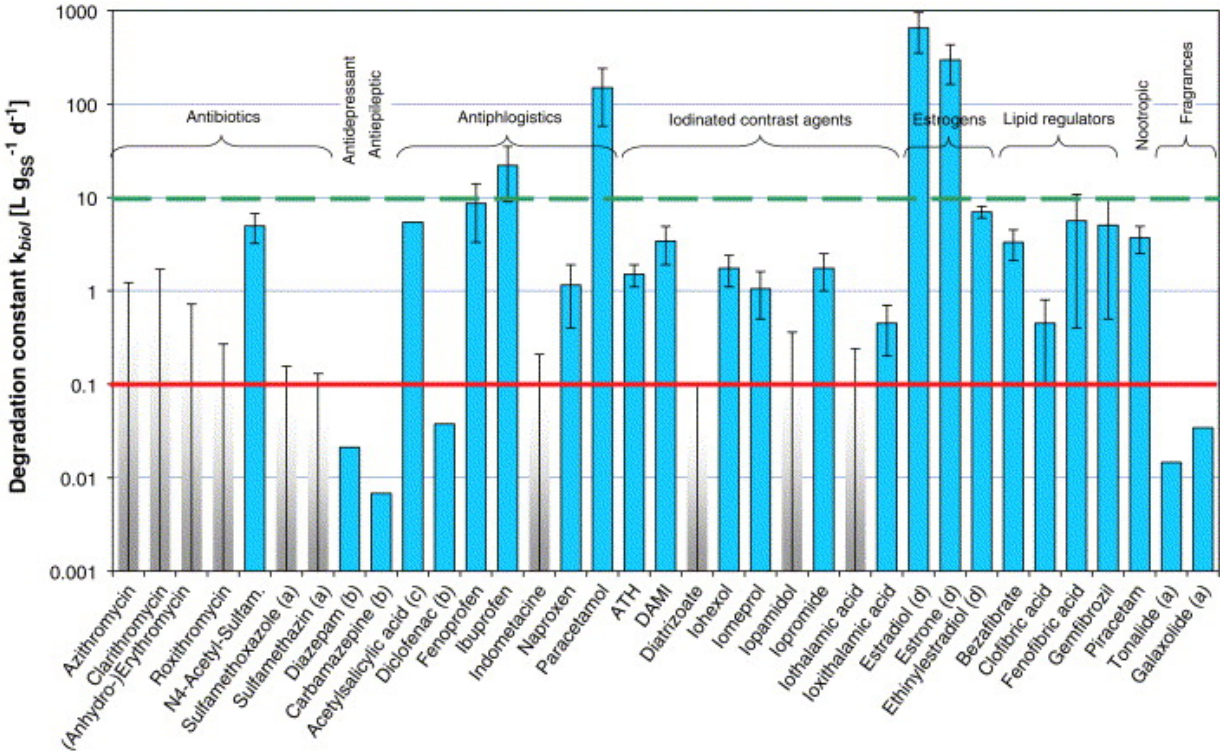


Figure 2.9: Biological degradation rates for 35 pharmaceuticals (Joss *et al.* 2005)

The amount of degradation that occurs may depend on a large range of factors, including sludge floc size (Joss *et al.* 2004), fraction of active biomass within the suspended solids (Joss *et al.* 2005), and diversity of the microbial community within the biomass (Clara *et al.* 2005; Ternes *et al.* 2004). Sludge age has been shown to be the most important parameter affecting degradation (Ternes *et al.* 2004). These factors are discussed in Section 2.5.4

Hydrolysis

Compounds excreted from the human body in the form of glucuronides can later be converted back to their bioactive form via enzymatic or chemical hydrolysis (Daughton and Ternes 1999). As was shown in Table 2.27, up to 90% of certain pharmaceuticals may be excreted in the form of glucuronides (Daughton and Ternes 1999; Ternes 1998). These conjugated pharmaceuticals can then act as storage reservoirs from which the bioactive parent compound can be released. This reformation of the parent compound can account for why some compounds have been measured at higher concentrations in effluents of sewage treatment plants than in the influent (Quintana *et al.* 2004).

Enzymatic hydrolysis reactions can be the first step in the biological degradation pathway of many pharmaceuticals e.g. bezafibrate and acetylsalicylic acid (Quintana *et al.* 2005).

2.5.3 Fate of pharmaceuticals during sewage treatment

As discussed previously, there are three main possible fates for pharmaceuticals once they have entered the sewage treatment plant. Firstly, they can pass straight through the system and end up in the liquid effluent, which is highly undesirable. Secondly, the pharmaceuticals can be partitioned into the sewage sludges (sorption), from which they pass through several further treatment processes, and then in many cases onto agricultural land through sludge disposal. Thirdly, and most desirable, the pharmaceuticals can undergo microbial degradation to *lower molecular weight products*. It is important to emphasise the lower molecular weight, since some transformation processes can form compounds with higher molecular weights such as glucuronides. As mentioned above, in later treatment processes these glucuronide groups can undergo hydrolysis reactions, reforming the original compound.

The removal efficiencies of STPs for pharmaceuticals can vary wildly from plant to plant, and from compound to compound. Where removal efficiencies have been determined, generally

only the disappearance of the parent compound has been tracked, ignoring the issue of whether sorption or biodegradation has occurred (Daughton and Ternes 1999) and the role of metabolites. Many papers have quoted removal efficiencies for a range of pharmaceuticals, such as Clara *et al.* (2005), Ternes (1998), Ternes *et al.* (1999), Kanda *et al.* (2003), Zweiner *et al.* (2000), and others too numerous to list here. Examples of typical removal efficiencies for selected pharmaceuticals in a municipal activated sludge STP are summarised in Figure 2.10 (Ternes 1998).

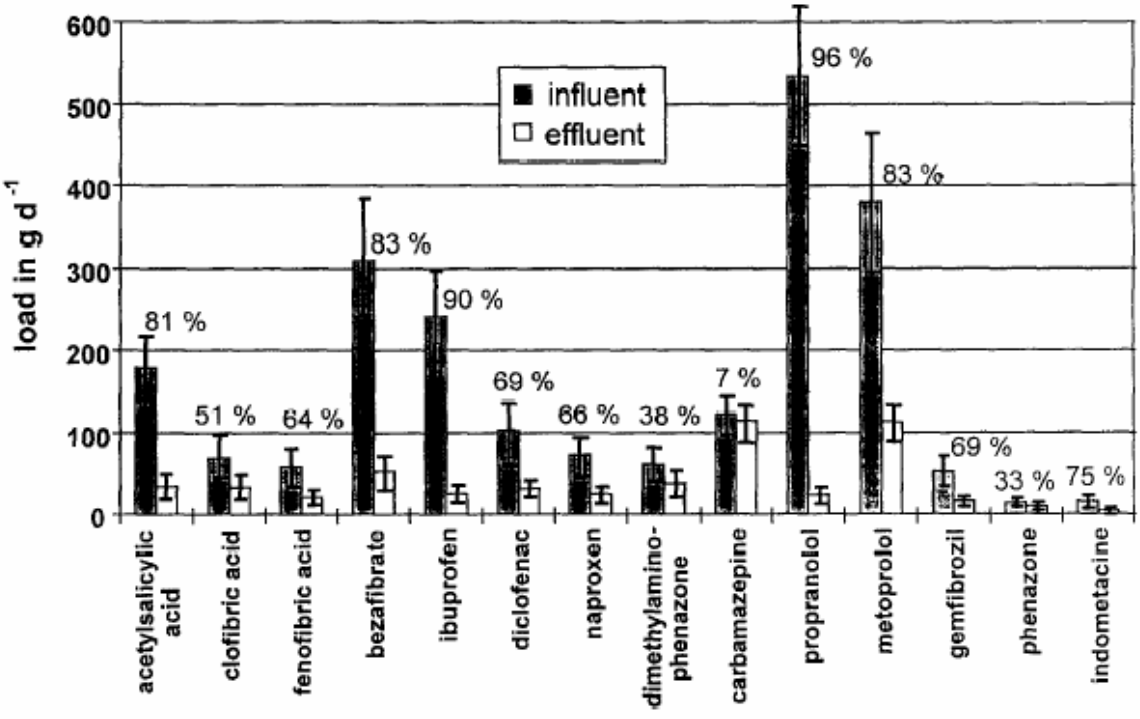


Figure 2.10 Pharmaceutical removal efficiencies during sewage treatment (Ternes 1998)

From this figure it can be seen that there is a wide spectrum in the removal efficiencies of different compounds, ranging from only 7% for carbamazepine to 96% for propranolol and greater than 99.9% for caffeine (data not shown). Clara *et al.* (2005) also noted different behaviours for the different investigated compounds. Some compounds such as the antiepileptic drug carbamazepine were not removed in any of the sampled treatment facilities. Other compounds such as the analgesic ibuprofen or the lipid regulator bezafibrate were nearly completely removed. They also noted that the operation of STPs with SRTs suitable for nitrogen removal (SRT>10 days at 10 °C) increased the removal potential regarding selected pharmaceuticals.

Information on the efficiency of pharmaceutical removal at each stage of the sewage treatment processes is critical to help improve the current treatment methods by showing how and where pharmaceutical removal is currently taking place. Whilst a widespread investigation of distinct types of sewage treatment plants and individual treatment techniques is still lacking (Daughton and Ternes 1999), some papers have studied the effect of particular treatment processes. For example, Carballa *et al.* (2005) studied a range of pharmaceuticals through a sewage treatment plant in Spain, including musk fragrances (galaxolide and tonalide), two analgesics (ibuprofen and naproxen), an antibiotic (sulfamethoxazole) and the X-ray contrast media (Iopromide). In the primary treatment, only the fragrances were partly removed, with efficiencies of 20-50% for galaxolide and tonalide. However, the aerobic treatment caused an important reduction in all compounds detected, between 35 and 75%, with the exception of iopromide. The overall removal efficiency of the STP ranged between 70 and 90% for the fragrances, 45 and 70% for the acidic compounds, and 57% for the antibiotic sulfamethoxazole.

Not only is there a difference between removal efficiencies for different compounds, but also there is a difference between removal efficiencies for the same compound at different STPs. For example, Ternes (1998) and Stumpf *et al.* (1999) sampled ibuprofen concentrations in activated sludge works in Germany and Brazil respectively. Removal efficiencies were recorded at 90% for the German STP, but ranged between 22 and 75% for the Brazilian STPs. Similarly, Ternes (1998) reported only 7% removal for carbamazepine, but Heberer and Feldmann (2005) reported between 3% and 40% removal. For diclofenac, Paxeus (2004) reported removals ranging from <10% to 88% for five STPs, whilst Heberer *et al.* (2002) reported 17% and Ternes (1998) reported 69%. However, critical details that would be necessary for detailed comparison of the performances of the various STPs are missing from most papers, particularly details such as details of unit operations, temperature, HRT, sludge age, and loadings data.

As might be expected, differences in removal efficiencies exist between different types of treatment process. For example, the removal of triclosan was much lower through treatment plants using trickling filter (83-96%) (Sabaliunas *et al.* (2003), McAvoy *et al.* (2002), Bester (2003), Kanda *et al.* (2003)), compared to plants using activated sludge (>95%) (Sabaliunas *et al.* (2003), Bester (2003), McAvoy *et al.* (2002), Kanda *et al.* (2003)), as has been summarised

in Table 2.29. Again, a lack of data on plant design and operating conditions means that a meaningful comparison is not possible.

From these data it is clear to see that compounds are removed to differing amounts at different sewage treatment plants, even when the major unit processes are of the same type. Similarly, there are clear differences in the amounts of removal occurring within different treatment processes, indicating that there may be certain design or operational parameters within the treatment processes that are affecting the removal. Identification of these parameters, and then control of them, could lead to simple changes being made in the sewage treatment processes which lead to improvements in overall removal of pharmaceuticals. Some potential parameters and their effects are discussed in section 2.5.4

2.5.4 Parameters affecting removal efficiency

As has already been discussed, Clara *et al.* (2005) showed that for many pharmaceuticals, there exists a critical sludge age (T_{crit}) of about 10 days, below which little or no degradation occurs. This critical sludge age has been shown to be different for various pharmaceuticals. For example, the critical sludge age for ibuprofen was around 1 day, whilst for estrone it was around 8 days (Ternes *et al.* 2004). However, this is not necessarily true for all pharmaceuticals. A study with triclosan observed no relationship between the solids retention time (SRT) and the level of removal (Federle *et al.* 2002). Nevertheless, variations in removal were observed, suggesting that other parameters may be important in the removal of pharmaceuticals during sewage treatment.

Several studies have noted that the removal of pharmaceuticals in sewage treatment is worse during winter than in summer. Vieno *et al.* (2005) noted concentrations in recipient waters that were five times higher in winter than in summer. Similarly, they noted that high flow events led to an increase in pharmaceutical concentration downstream from the STP. A similar study by Castiglioni *et al.* (2006) also showed that many compounds were removed more in summer than in winter (amoxicillin, atenolol, bezafibrate, enalapril, furosemide, ibuprofen, ranitidine, and sulfamethoxazole). The reasons for this lower removal could be related to several parameters. This could include temperature, a lower temperature in winter leading to a lower rate of biodegradation. Greater rainfall in winter reduces the hydraulic retention time (HRT), which was shown to reduce removal, along with a reduction in specific

sludge production (Kreuzinger *et al.* 2004). Also, many STPs are run at much lower mixed liquor suspended solids (MLSS) concentrations in winter (typically 1500 mg l^{-1} compared to 3000-3300 in summer) due to lower feed concentrations. Therefore, MLSS concentration and F/M ratio may be factors contributing to changes in pharmaceutical removal. Indeed, Strenn *et al.* (2004) observed only slight removal of pharmaceuticals in STPs operated with high F/M ratios.

The Castiglioni *et al.* (2006) study as mentioned above also reported that there was no difference in removals for two compounds (ciprofloxacin and ofloxacin) in summer and winter conditions. Considering the potential removal mechanisms, biodegradation is likely to be temperature related, whilst sorption is not. Therefore, it could be suggested from this data that the floxacin compounds were removed entirely by sorption. However, K_d values are not available to confirm this as the removal mechanism, nor were relevant MLSS and sludge production rates quoted in the original study.

Several authors (Kim *et al.* 2005; Strenn *et al.* 2004; Ternes *et al.* 2004) have observed reduced removal of pharmaceuticals during rainfall events, suggesting that HRT may be an important factor. However, due to the experimental set-up, several other variables also changed in these studies including the MLSS concentration and the F/M ratio. The reduction in MLSS concentration in particular would reduce the capacity for removal by sorption (Kim *et al.* 2005).

Urase *et al.* (2005) demonstrated that pH could affect removal of pharmaceuticals. In particular acidic pharmaceuticals, such as ibuprofen, ketoprofen, caffeine, gemfibrozil, ketoprofen, naproxen, diclofenac, and indomethacine showed higher removal at acidic pH.

Some work has been conducted to attempt to find correlations between pharmaceutical removal and biomass parameters. For example, Holbrook *et al.* (2004) and Kreuzinger *et al.* (2004) noted that sorption (and hence total removal) of 17b-estradiol and 17a-ethinylestradiol was weakly correlated with colloidal protein and polysaccharide concentrations ($r^2 \sim 0.4$). Drewes *et al.* (2001) suggested that organic carbon concentrations were a significant factor in the removal of iodinated X-ray contrast media. Indeed, Stokes and Churchley (2006) found a direct correlation ($r^2 = 0.9994$) between total organic carbon concentrations and estradiol concentrations, implying that removal mechanisms for both are similar.

Whilst most of the above parameters above refer to removal in activated sludge plants, previous discussion had noted differences in removal between suspended growth systems such as activated sludge, and fixed film systems such as biological filters and RBCs. Little work has been conducted to assess the reasons for these differences (such as quantities of available biomass, biomass age, retention times, oxygen availability etc.).

In combination, all these findings suggest that there are many parameters which, when changed, alter the removal of pharmaceuticals. Greater understanding of the effect of each of these parameters, and the magnitude to which they affect removal of pharmaceuticals, may suggest ways that the design of sewage treatment plants could be altered to improve the pharmaceutical removal potential of current treatment technologies.

2.5.5 Tertiary treatments

An alternative to improving current treatment technologies is the addition of further treatment processes. Tertiary treatments, such as the oxidation with ozone or OH radicals have shown high potential for the elimination of pharmaceuticals, ranging from 25% to 90% removal (Huber *et al.* 2005; Huber *et al.* 2005; Ternes *et al.* 2004). For example, the process has been shown to be very effective at removing the three principal endocrine disruptors (EE2, E2, E1), and also at the removal of antibiotics. The removal of antibiotics could lead to a reduction in the potential for the formation of resistant bacteria. However, compared to activated sludge, these oxidation processes are expensive to operate. A cost comparison per cubic metre of treated wastewater is shown in Table 2.31.

Table 2.31: Costs of activated sludge and ozonation treatment processes

	Activated Sludge	**Ozonation**	**Percentage increase**
Capital Cost (Euros)	0.5	0.04	8%
Energy resources (kWh)	0.3	0.3	100%

Due to this cost, ozonation is likely only to be a viable option at large STPs, or in areas where receiving waters contain a high amount of treated wastewater.

Other technologies have not shown such good performance. For example, coagulation and flocculation processes have shown removal between 45% (Ternes *et al.* 2004) and lower than

25% (Snyder *et al.* 2003). Removal of pharmaceuticals by disinfection processes such as chlorine and chlorine dioxide range from 70% to less than 20% (Snyder *et al.* 2003).

The use of granular activated carbon (GAC) has been shown to be effective at removing some pharmaceuticals. Ternes *et al.* (2004) tested the removal of four pharmaceuticals (bezafibrate, clofibric acid, diclofenac, and carbamazepine) by GAC, and found all to be efficiently removed from deionised water. However, as the amount of dissolved organic carbon (DOC) was increased (to 2 mg l^{-1} – equivalent to groundwater), the amount of the pharmaceuticals that was removed by the process was reduced, leading to the breakthrough of clofibric acid, due to competition for sorption sites. It is notable that of the compounds tested, all can be classified as having a medium or high sorption potential (see Section 2.5.2 for definition). This would suggest that pharmaceuticals with a lower sorption potential might be less well removed by the GAC process. Vidic and Suidan (1991) suggested that adsorption to GAC could be improved by adding aeration into the system, although the mechanisms for this improvement were not understood.

Whilst both ozonation and GAC show good potential for the removal of pharmaceuticals, they both have problems that could preclude their use in wastewater treatment. In particular, the cost of ozonation is a problem, whilst GAC shows potential poor removal of pharmaceuticals with a low sorption potential, particularly at DOC concentrations that would be found in a typical wastewater treatment plant. For these reasons, other options need to be investigated for wastewater treatment plants, and in particular the smaller treatment plants where capital cost of treatment processes would be a significant issue.

2.6 Summary

This review has shown which pharmaceuticals are most used, particularly in Europe and the UK. In terms of number of prescriptions used in the UK, cardiovascular drugs were the most used group, accounting for over fifty million prescriptions per quarter in 2004. Amoxcillin (41.6 million) and paracetamol (32 million) were the most prescribed compounds. Over the last decade, only two new drugs, simvastatin and atorvastatin, have made it into the top ten highest used compounds in the UK (in terms of number of prescriptions).

Little data was directly available on the actual weights of pharmaceuticals used. By means of the defined daily dose, these weights were calculated. Antimicrobial pharmaceuticals have the

highest defined daily dose, typically 2000mg, which is at least one order of magnitude greater than all other groups. Combined with prescription number, this would suggest that antimicrobials are the group of compounds entering the environment in the greatest amounts. Indeed, of the 15 compounds estimated to have annual usage over one tonne in this work, seven are antimicrobials: amoxicillin (41.6 tonnes), flucoxacillin (25.6), phenoxymethylpenicillin (18.4), erythromycin (12.8), trimethoprim (4.48), oxytetracycline (4.0), and ciprofloxacin (3.6).

The potential routes into the environment have been studied (see Figure 2.1). Whilst reducing overall usage of pharmaceuticals must be encouraged, the effluents of sewage treatment plants (both through liquid and sludge) have been shown to produce the greatest controllable discharge to the environment.

Removal mechanisms for pharmaceuticals during sewage treatment have been evaluated (sorption, biodegradation, volatilisation, photolysis, and hydrolysis). Sorption and biodegradation are considered to be the mechanisms by which the majority of removal of most pharmaceuticals occur, although the relative amounts by each mechanism vary from compounds to compound. The rates and limits of sorption and biodegradation have been published for a range of pharmaceuticals, predominantly in suspended growth (activated sludge) systems. Little data has been published on the fate of pharmaceuticals in fixed film systems (such as biological filters and rotating biological contactors). The published removal rates vary widely from report to report. For example, removal of ibuprofen has varied from 22% to 90% in various activated sludge plants. Little explanation has been offered for these variations, with critical data often omitted on the design and operation of the sewage treatment plants that have been studied.

Where authors have attempted to quantify the reasons for variation in removal, many parameters have been identified that have the potential to affect the removal of pharmaceuticals during sewage treatment, whether by affecting sorption, biodegradation, or both. These include, but are probably not limited to, sludge retention time, sludge age, hydraulic retention time, sludge production rates, colloidal protein and polysaccharide concentrations, organic carbon content, and pH. With the exception of sludge age, little appears to be understood about the relationship between these parameters and the amounts of pharmaceutical removal that can be expected. Through optimising these parameters, it

may be possible to improve the removal of pharmaceuticals with current treatment technologies. Alternatively, some new tertiary treatments do exist which can readily remove most pharmaceuticals. However, these can be expensive to build and operate.

Chapter 3: Choice of pharmaceuticals for study

In order to understand how STPs remove pharmaceuticals and what factors may be important, it was necessary to select key pharmaceutical compounds to study. Pharmaceuticals can be ranked in many ways, such as their predicted aquatic toxicity, predicted environmental concentrations, reported environmental concentrations, the PEC or MEC to PNEC ratio, reported removal in sewage treatment, and potential to bioaccumulate. A combination of these factors was taken into account when selecting compounds for this study. These factors were a measured environmental concentration greater than 200 ng l^{-1}, predicted environmental concentration greater than predicted no effects concentration, measured environmental concentration greater than predicted no effects concentration, high potential for bioaccumulation (defined as log K_d greater than 1.5, or log K_{ow} greater than 4), and removal in STPs (either high (> 90%) or poor (<10%)). In addition to this, compounds where knowledge of the degradation pathway already existed were given greater weighting, since monitoring of the degradation products could also be conducted. This would help in quantifying losses by degradation, as opposed to sorption, through the treatment processes.

3.1 Prioritisation

3.1.1 Prioritisation by measured environmental concentration

It was decided that chemicals chosen for use in this research should be ones that had already been detected in the aquatic environment. This was to limit the risk of spending a large amount of time developing analytical methods for a particular compound and then being unable to detect it in the environment. Similarly, due to the limitations of the available HPLC-UV equipment (see further discussion in Section 3.1.6), it was decided that only compounds that had been detected in the environment at a concentration greater than 200 ng l^{-1} should be included in the investigation. This figure was chosen since fixed wavelength UV detectors have a sensitivity of about 1 x 10^{-8}g per ml (10 μg l^{-1}) at a signal to noise ratio of two (Scott 2002). This can be combined with solid phase extraction (see Chapter 4.2) to give a concentration factor of 500, which gives a limit of detection of 20 ng l^{-1}. A safety factor of 10 was then included to ensure some variation in detected levels would be possible. This gave a possible 55 compounds to study, as shown in Table 3.1.

Table 3.1: Compounds detected in the aquatic environment at concentrations above 200 ng l^{-1}

Chemical Name	Maximum Concentration (ng l^{-1})	Information Source
Aspirin	3100	Stumpf *et al.* (1999)
Atenolol	122000	Gomez *et al.* (2006)
Benzydamine	1000	Richardson and Bowron (1985)
Bezafibrate	4800	Clara *et al.*(2005)
Biphenylol	2600	Stumpf *et al.* (1999)
Bisoprolol	370	Ternes (1998)
Caffeine	292000	Rogers *et al.* (1986)
Carbamazepine	6300	Ternes (1998)
Chloramphenicol	560	Hirsch *et al.* (1996)
Chlorophene	710	Ternes *et al.* (1998)
Ciprofloxacin	970	Batt *et al.*(2005)
Clarithromycin	240	Hirsch *et al.* (1996)
Clindamycin	1000	Batt *et al.*(2005)
Clofibric acid	9740	Hignite and Azanoff (1977)
Codeine	5700	Gomez *et al.* (2006)
Dextropropoxyphene	585	Ashton *et al.*(2004)
Diatrizoate	8700	Ternes and Hirsch (2000)
Diazepam	1000	Halling-Sorensen *et al.* (1998)
Diclofenac	2349	Ashton *et al.*(2004)
Dimethylaminophenazone	1000	Ternes (1998)
Erythromycin	6000	Hirsch *et al.* (1999)
Fenofibric acid	1200	Ternes (1998)
Gemfibrozil	2090	Lee *et al.*(2005)
Gentisic acid	590	Ternes (1998)
Ibuprofen	151000	Gomez *et al.* (2006)
Ifosfamide	2900	Ternes (1998)
Indomethacine	1000	Stumpf *et al.* (1999)
Iopamidol	15000	Ternes and Hirsch (2000)
Iopromide	11000	Ternes and Hirsch (2000)
Ketoprofen	1760	Santos *et al.*(2005)
Ketorolac	59500	Gomez *et al.* (2006)
Mefenamic acid	1440	Ashton *et al.*(2004)
Methotrexate	1000	Aherne and English (1985)
Metoprolol	2200	Hirsch *et al.* (1996)
Metronidazol	9400	Gomez *et al.* (2006)
Musk galaxolide	5300	Severn Trent (2002)
Musk ketone	410	Yamagishi *et al.* (1981, 1983)
Musk tonalide	1400	Severn Trent (2002)
Nadolol	290	Hirsch *et al.* (1996)
Naproxen	7098	Verenitch *et al.*(2006)
Norflaxacin	210	Costanzo *et al.*(2004)

Chemical Name	Maximum Concentration (ng l^{-1})	Information Source
Paracetamol (acetaminophen)	6000	Ternes (1998)
Phenazone	410	Ternes (1998)
Propanolol	6500	Gomez *et al.* (2006)
Propylphenazone	350	Heberer *et al.* (1998)
Ranitidine	1700	Gomez *et al.* (2006)
Roxithromycin	1000	Hirsch *et al.* (1999)
Salicylic acid	2178	Verenitch *et al.*(2006)
Sulfamethoxazole	6000	Batt *et al.*(2005)
Tamoxifen	369	Roberts and Thomas (2006)
Tetracycline	977	Metcalfe *et al.* (2005)
Tolfenamic acid	1600	Stumpf *et al.* (1999)
Triclosan	710	Severn Trent (2002)
Trimethoprim	1288	Ashton *et al.* (2004)
Tylosin	1041	Yang *et al.* (2006)

3.1.2 Prioritisation by PEC to PNEC and/or MEC to PNEC ratio

As discussed in Section 1.2.2, any compound with a predicted or measured environmental concentration greater than the predicted no-effects concentration could pose a risk to environment. Based on the EMEA approach, Table 3.2 shows a list of compounds with a PEC/PNEC greater than one, and Table 3.3 shows a list of compounds with MEC/PEC ratio greater than one. PEC data was calculated from UK usage data presented by Webb (2000). The MEC data used was for concentrations measured in sewage effluents in the UK. Where experimental data are available, the PNEC for each chemical has been calculated by the method outlined in Table 1.16. Where these data are not available, the chemical toxicity has been predicted by the use of quantitative structure-activity relationships.

Seven compounds appear in both lists – triclosan, chloramphenicol, tetracycline, ibuprofen, dextropropxyphene, mefenamic acid, and paracetamol.

Table 3.2: Compounds with PEC greater than PNEC

Chemical Name	PEC/PNEC
Dextropropoxyphene	**195**
Diltiazem	66.5
Triclosan	**52.1**
Mebeverine	40.6
Tetracycline	**34.3**
Gliclazide	31.4
Mesalazine	14.8
Paracetamol	**11.8**
Mefenamic acid	**8.31**
Thioridazine	6.73
Quinine	5.45
Ibuprofen	**4.20**
Verapamil	4.04
Chloramphenicol	**3.50**
Clofibrate	2.80
Cisapride	2.67
Aspirin (acetylsalicylic acid)	2.32
Sulfasalazine	1.74
Erythromycin	1.25
Diclofenac	1.13
Fluoxetine	1.06
Cimetidine	1.01
Carbamazepine	0.91

Table 3.3: Compounds with MEC greater than PNEC

Chemical Name	MEC/PNEC
Codeine	95.0
Triclosan	**50.7**
Sulfamethoxazole	46.2
Tetracycline	**38.9**
Chloramphenicol	**28.0**
Ibuprofen	**21.3**
Dextropropoxyphene	**14.6**
Ciprofloxacin	12.1
Mefenamic acid	**4.50**
Caffeine	3.36
Tamoxifen	3.08
Gemfibrozil	2.25
Benzydamine	2.04
Chlorophene	1.78
Erythromycin	1.54
Atenolol	1.12
Paracetamol	**0.98**

3.1.3 Prioritisation by bioaccumulation

The best prediction of compounds likely to bioaccumulate is given by the distribution coefficient K_d (Tolls 2001). A value for log K_d greater than 1.5 would suggest a compound is likely to bioaccumulate (Ternes *et al.* 2004). However, values for only 25 compounds were available in 2002, as calculated by Jones *et al.* (2002). Of these, 9 had log K_d values of around 1.5 or higher, as shown in Table 3.4 below.

Table 3.4: Pharmaceuticals with log K_d above 1.5 (Jones *et al.* 2002)

Compound	Log K_d
Quinine	4.56
Mefenamic acid	4.28
Mebeverine	2.98
Sulphasalazine	2.97
Ibuprofen	2.66
Naproxen	2.34
Erythromycin	2.22
Diltiazem	1.86
Carbamazepine	1.41

Whilst not as accurate in predicting the potential for bioaccumulation, octanol-water partition coefficient data are readily available for virtually all pharmaceuticals. Whilst Tolls (2001) noted that log K_{ow} became inaccurate below three, Rogers (1996) suggested that bioaccumulation would be likely for compounds with a log K_{ow} above four. Therefore, all compounds with a log K_{ow} above four are shown in Table 3.5 below.

Table 3.5: Pharmaceuticals with log K_{ow} above 4.

Chemical Name	Log K_{ow}
Terfenadine	7.62
Permethrin	7.43
Lofepramine	7.26
Fusidic acid	6.75
Fosinopril	6.61
Thioridazine	6.45
Tamoxifen	6.3
Clotrimazole	6.26
Miconazole	6.25
Itraconazole	6.16
Dicycloverine	6.05
Alverine	5.91
Tolnaftate	5.81
Terbinafine	5.81
Loratadine	5.66
Clomipramine	5.65
Cinnarizine	5.44
Ticonazole	5.43
Trimipramine	5.43
Naftidrofuryl	5.37
Sertraline	5.29
Mefenamic acid	5.28
Dextropropoxyphene	5.27
Chlorpromazine	5.2
Fenofibrate	5.19
Simvastatin	5.19
Mebeverine	5.12
Ursodeoxycholic acid	5.06

Chemical Name	Log K_{ow}
Imipramine	5.01
Flavoxate	4.95
Chlorhexidine	4.85
Verapamil	4.8
Prochlorperazine	4.79
Glibenclamide	4.79
Procyclidine	4.78
Gemfibrozil	4.77
Paroxetine	4.74
Triclosan	4.66
Fluoxetine	4.65
Olsalazine	4.61
Promazine	4.56
Dosulepin	4.51
Chloroquine	4.5
Promethazine	4.49
Ketoconazole	4.45
Difunisal	4.41
Sulindac	4.28
Rifampicin	4.24
Indometacin	4.23
Benzydamine	4.21
Danazol	4.21
Methadone	4.17
Acemetacin	4.13
Diclofenac	4.02

3.1.4 Prioritisation by removal in sewage treatment plants and additional weightings

Whilst all compounds will be removed to some extents during sewage treatment, those that are removed very well (>90%) or very poorly (<10%) were of interest to this research. By identifying why these compounds are well or poorly removed could help in the assessment of ways to improve current sewage treatment technologies. Three compounds were identified as

being well removed: caffeine 99.9% (Heberer *et al.* 2002), propranolol 96% (Ternes 1998), and ibuprofen 90% (Ternes 1998). One compound was regularly noted as having removal below 10% - carbamazepine. Removals were reported at 8% (Heberer *et al.* 2002) and 7% (Ternes 1998).

Additional weighting was given to caffeine, since the degradation pathway was very well known (see Figure 1.4 (Georga *et al.* 2001)). This includes 4 primary degradation products, 5 secondary degradation products, and 2 tertiary degradation products. All these compounds can be analysed simultaneously by HPLC-UV (Georga *et al.* 2001).

3.1.5 Scoring

From these five criteria (MEC > 200ng l^{-1}, PEC>PNEC, MEC>PNEC, likelihood of bioaccumulation, removal in STPs) scores were awarded on the basis of one point per occurrence of a compound in each category. Therefore, a compound could obtain a maximum score of five. Overall, only one compound scored five points (ibuprofen), five compounds scored four points (caffeine, carbamazepine, dextropropxyphene, erythromycin, and mefenamic acid), nine compounds scored three points, 16 compounds scored two points, and 94 compounds scored one point. Compounds with a score of three or greater are shown in Table 3.6 below.

Table 3.6: Compounds with a selection score of three or greater

Chemical Name	CAS Number	NHS Usage Class	Score	HPLC-UV method available
Ibuprofen	15687-27-1	Analgesic	5	No
Caffeine	58-08-2	Stimulant	4	Yes
Carbamazepine	298-46-4	Mental health	4	Yes
Dextropropoxyphene	469-62-5	Analgesic	4	No
Erythromycin	114-07-8	Antibacterial	4	No
Mefenamic acid	61-68-7	Analgesic	4	No
Triclosan	3380-34-5	Antibacterial	4	Yes
Benzydamine	642-72-8	Analgesic	3	No
Chloramphenicol	56-75-7	Antibacterial	3	Yes
Diclofenac	15307-79-6	Analgesic	3	No
Gemfibrozil	25812-30-0	Cardiovascular disease	3	Yes
Gentisic acid	490-79-9	Aspirin Metabolite	3	No
Paracetamol (acetaminophen)	103-90-2	Analgesic	3	No

Chemical Name	CAS Number	NHS Usage Class	Score	HPLC-UV method available
Tamoxifen	10540-29-1	Antiestrogen	3	No
Tetracycline	60-54-8	Antibacterial	3	Yes

3.1.6 Overall selection, modulated by analytical methodology

The compounds selected in the above sections were checked against known analytical methods for the available detection equipment. For this research, high performance liquid chromatography with ultraviolet detection (HPLC-UV) was the only analytical equipment available. UV detectors function on the capacity of many compounds to absorb light in the wavelength range 180 to 350 nm. The relationship between the intensity of UV light transmitted through the detector cell and the solute concentration, is given by Beers' Law:

$$I_T = I_o e^{-kLc}$$

where $\mathbf{I_o}$ is the intensity of the light entering the cell, $\mathbf{I_T}$ is the intensity of the transmitted light, **L** is the path length of the cell, **c** is the concentration of the solute, and **k** is the molar extinction coefficient of the solute for the specific wavelength of the UV light.

The output from the detector, usually in millivolts, is passed to a scaling amplifier that converts the signal to a voltage that is acceptable to the analog to digital (A/D) converter The A/D converter changes the voltage output to a binary number which is temporarily stored in a register. This process is continuously repeated at a defined rate, called the 'sampling rate'. The current binary number, stored in the register is regularly sampled by the computer and stored. At its most sensitive, this technique has a detection limit of 1×10^{-8}g per ml (10 $\mu g\ l^{-1}$) at a signal to noise ratio of two (Scott 2002).

As can be seen in Table 3.6, in 2002 only six of the fifteen priority compounds were known to have published methods already available for analysis by HPLC-UV, although several others did have published HPLC-MS methods. From these six compounds, it was decided to select an initial group of four compounds, whilst the remaining two could be studied at a later point if time permitted. Therefore, the three compounds that had scored four points (carbamazepine, caffeine, and triclosan) were selected. The fourth was chosen as tetracycline,

as more data was available on the analysis of this compound compared with the other two options. Further details of these compounds are given below.

3.2 Details on compounds selected for study

The sections below give a description of what each selected compound is used for, quantities used, quantities detected in the environment, and a summary of the physical properties of the compound where data are available (e.g. molecular weight, solubility, K_{ow}, K_d, biodegradation rates, toxicity). Where known, degradation products are also discussed. A separate chapter looks at the detection of these compounds and their degradation products. These properties have been used to create a model of the expected fate of the compounds through a simulated STP, using the modelling package Toxchem+. The results of this modelling are compared with known fate in sewage treatment. The design of the model, based on a standard ASP design (Green 2004), used for this modelling can be found in Appendix B.

3.2.1 Caffeine

As a pharmaceutical, caffeine is used in the treatment of apnea in newborn babies, in some cancer therapies, and electroconvulsive therapy (RxList 2006). It is also commonly used as part of headache preparations, for its ability to improve the effectiveness of analgesics. However, its major use is not as pharmaceutical, but as a naturally occurring stimulant in tea and coffee, or as an additive in many beverages. As such, caffeine is ubiquitous in the human diet (Georga *et al.* 2001). As was shown in Figure 1.4, the degradation pathway of caffeine is well-known and most of the degradation products are also detectable using HPLC-UV (Georga *et al.* 2001). Two of the primary degradation products of caffeine, theophylline and theobromine, are also used as pharmaceuticals. Theophylline acts as a powerful bronchodilator, whilst theobromine acts as a stimulant to the central nervous system, to gastric acid secretion, and to cardiac muscle (Georga *et al.* 2001).

Caffeine has been detected in sewage plant influents at concentrations up to 640,000 ng l^{-1} (median 320,000 ng l^{-1}) (Heberer *et al.* 2002). Detections in the effluent of sewage treatment plants have ranged from as low as 220 ng l^{-1} (Bendz *et al.* 2005), to as high as 292,000 ng l^{-1} (Rogers 1996). Typical concentrations appear to be in the order of one to ten micrograms per litre – 2263 ng l^{-1} (Verenitch *et al.* 2006), 4520 ng l^{-1} (Santos *et al.* 2005), 9900 ng l^{-1} (Batt *et al.* 2006). Generally, sewage treatment plants remove about 99.9% of caffeine (Heberer *et al.*

2002), which is sufficient to reduce concentrations below the PNEC level of 87000 ng l^{-1} in most cases. Even though this removal is extremely high, caffeine has still regularly been detected in streams and rivers at concentrations from 265 ng l^{-1} (Heberer *et al.* 2002) to 6000 ng l^{-1} (Kolpin *et al.* 2002). The physical properties of caffeine are summarised in Table 3.7 below.

Table 3.7: Properties of caffeine

Property	Value	Units	Reference
CAS Number	58-08-2	-	ChemIDplus
Molecular Weight	194.19	g mol^{-1}	ChemIDplus
Solubility	21600	mg l^{-1}	ChemIDplus
Henry's law constant	1.9 x 10^{-19}	atm m^3 mo l^{-1}	ChemIDplus
K_d (suspended growth)	-		
K_d (fixed film)	-		
log K_{ow}	-0.07	-	ChemIDplus
Biodegradation rate (suspended growth)	173	L ng^{-1} hr^{-1}	Gutierrez-Sanchez *et al.* (2004)
Biodegradation rate (fixed film)	-		
Toxicity (daphnia)	>100	mg l^{-1} (EC50)	Toxline
Toxicity (algae)	182	mg l^{-1} (EC50)	Toxline
Toxicity (fish)	87	mg l^{-1} (LC50)	Toxline
PNEC	87	μg l^{-1}	(calculated)

From this table it can be seen that no data are available on specific fixed film systems, although a biodegradation rate for suspended growth systems was derived from data in Gutierrez-Sanchez *et al.* (2004). Since K_d data are unavailable, K_{ow} data was used as predictor of sorption. From these data, caffeine was expected to be removed by biodegradation during sewage treatment, with very little removal by sorption. The Toxchem+ model agreed with this, showing 98.9% of caffeine removed by biodegradation, 0.01% removed by sorption, leaving a remaining 1.1% discharged in the final effluent. There was no release to air. This agrees with the observed removal in sewage treatment plants.

3.2.2 Carbamazepine

Carbamazepine is an antiepileptic which is very poorly removed during all types of sewage treatment – only around 7 or 8% (Heberer *et al.* 2002; Ternes 1998). It is highly used, with over 40 tonnes in the UK in 2000 (Jones *et al.* 2002). It also has a PEC to PNEC ratio of around unity (test organisms – algae).

Carbamazepine has been detected in sewage influents at concentrations of up to 3800 ng l^{-1}, with a median concentration of 1780 ng l^{-1} (Heberer 2002). Concentrations in sewage effluent have ranged from 70 ng l^{-1} (Gomez *et al.* 2006) to 6300 ng l^{-1} (Ternes 1998), with an average figure around 1500 ng l^{-1} (Bendz *et al.* 2005; Clara *et al.* 2005). Concentrations in streams and rivers are typically 1000 ng l^{-1} (Bendz *et al.* 2005; Sacher *et al.* 2001; Ternes 1998). The physical properties of carbamazepine are summarised in Table 3.8 below.

Table 3.8: Properties of carbamazepine

Property	Value	Units	Reference
CAS Number	298-46-4	-	ChemIDplus
Molecular Weight	236.27	g mo l^{-1}	ChemIDplus
Solubility	17.7	mg l^{-1}	ChemIDplus
Henry's law constant	1.08 x 10^{-10}	atm m^3 mol^{-1}	ChemIDplus
K_d (suspended growth)	0.02552	l g^{-1}	Jones *et al.* (2002)
K_d (fixed film)	-		
log K_{ow}	2.45	-	ChemIDplus
Biodegradation rate (suspended growth)	0.333	l ng^{-1} hr^{-1}	Ternes *et al.* (2004)
Biodegradation rate (fixed film)	-		
Toxicity (daphnia)	>100	mg l^{-1} (EC50)	Cleuvers (2003)
Toxicity (algae)	25.5	mg l^{-1} (EC50)	Cleuvers (2003)
Toxicity (fish)	-		
Toxicity (QSAR)	8.1	mg l^{-1}	(calculated)
PNEC	8.1	μg l^{-1}	(calculated)

Based on these properties, very little degradation or sorption is expected to occur. The Toxchem+ model, however, showed only 98.2% of carbamazepine emitted in the effluent, with 0.7% removed by biodegradation, and the remaining 1.1% sorbed to solids. This is a slight underestimate of removal compared with observed behaviour.

3.2.3 Tetracycline

Tetracycline is an antibacterial pharmaceutical, or more specifically a broad-spectrum antibiotic, effective against strains of streptococci, gram-negative bacilli, rickettsia (typhoid fever), and spirochaetes (syphilis). It is also used to treat acne, pelvic inflammatory disease, urinary tract infections, bronchitis, and Lyme disease. Bacterial resistance is known to develop readily, and the use of tetracycline is becoming somewhat limited because of this. There were 4.7 tonnes of tetracycline prescribed for human use in the UK in 2000 (Webb

2000). However, this figure does not include agricultural applications, which are the major use of tetracycline (Halling-Sorensen *et al.* 2001).

Tetracycline has been detected in sewage effluent at maximum concentrations of 560 ng l^{-1} (Batt *et al.* 2006) and 977 ng l^{-1} (Miao *et al.* 2004), with a median concentration of 151 ng l^{-1} (Miao *et al.* 2004). Tetracycline has also been reported in streams and rivers at a concentration of 110 ng l^{-1} (Kolpin *et al.* 2002). All these concentrations are above the predicted no-effects concentration. The physical properties of tetracycline are summarised in Table 3.9 below.

Table 3.9: Properties of tetracycline

Property	Value	Units	Reference
CAS Number	60-54-8	-	ChemIDplus
Molecular Weight	444.44	g mol^{-1}	ChemIDplus
Solubility	231	mg l^{-1}	ChemIDplus
Henry's law constant	4.66 x 10^{-24}	atm m^3 mol^{-1}	ChemIDplus
K_d (suspended growth)	1.620	l g^{-1}	Sithole and Guy (1987)
K_d (fixed film)	-		
log K_{ow}	-1.3	-	ChemIDplus
Biodegradation rate (suspended growth)	0.4079	l ng^{-1} hr^{-1}	Kuhne *et al.* (2000)
Biodegradation rate (fixed film)	-		
Toxicity (daphnia)	44.8	mg l^{-1} (EC50)	Wollenberger *et al.* (2000)
Toxicity (algae)	0.09	mg l^{-1} (EC50)	Halling-Sorensen (2000)
Toxicity (fish)	-		
Toxicity (daphnia)	340	mg l^{-1} (NOEC)	Wollenberger *et al.* (2000)
PNEC	90	ng l^{-1}	(calculated)

From this table it can be seen that little biodegradation could be expected, although the rates is slightly higher than those for carbamazepine. Similarly, a prediction of sorption based solely on K_{ow} values would suggest that little sorption could be expected. However, tetracycline is known to forms complexes with metal ions (Halling-Sorensen *et al.* 2002), which will not be accounted for by the K_{ow} model. Using the experimentally derived K_d gives a much increased rate of sorption.

The Toxchem+ model predicted that only 5.22% of the influent tetracycline would be emitted in the liquid effluent, with 4.47% biodegraded, and the remaining 90.31% sorbed to

sludge. This could not be compared to actual removal, since no papers have been found which have recorded the concentrations of tetracycline at both the influent and effluent of a sewage treatment plant.

Tetracycline has six known bioloical degradation products: 4-epi-tetracyline (ETC), anhydro tetracycline (ATC), 4-epi-anhydrotetracyline (EATC), iso-tetracyline, 4-epi-iso-tetracycline, N-desmethyl-iso-tetracycline, and 4-epi-N-desmethyl-iso-tetracycline (Halling-Sorensen *et al.* 2002). The structures of these compounds are shown in Figure 3.1 below.

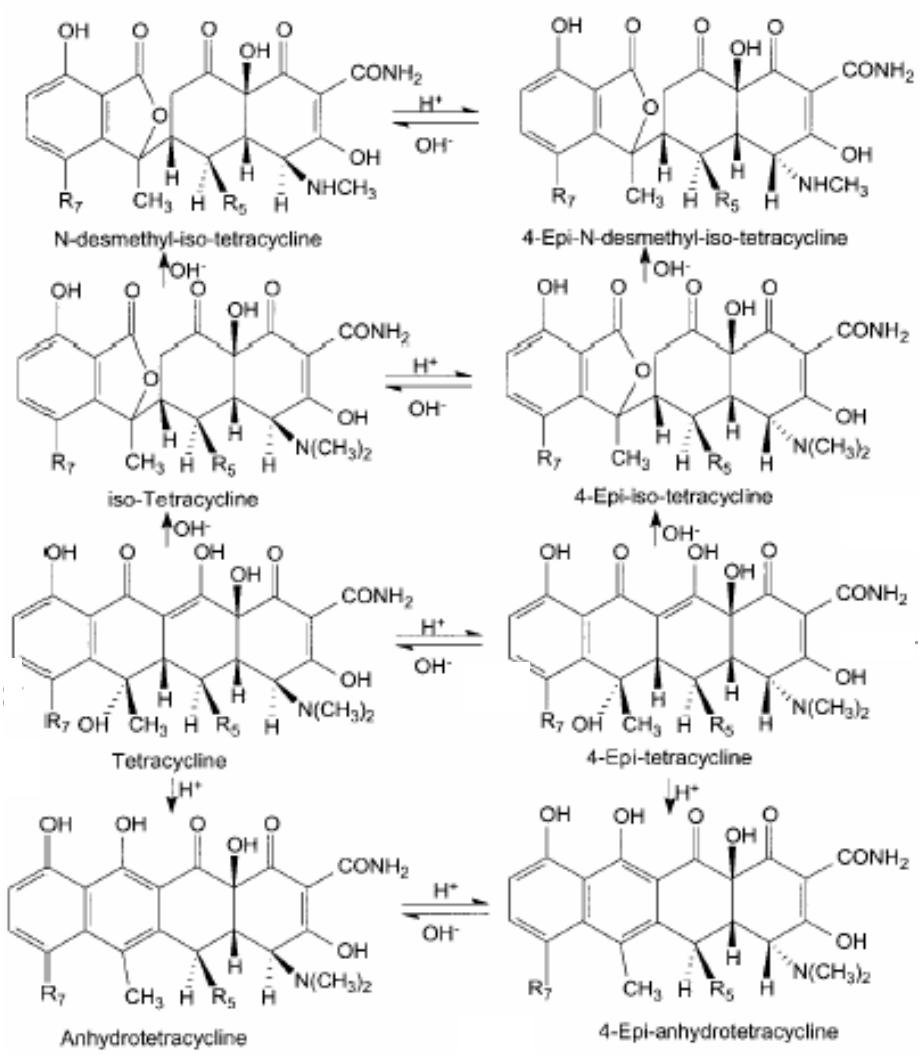


Figure 3.1: Structures of tetracycline and its biological degradation products (Halling-Sorensen *et al.* 2002)

The degradation products most commonly found in the environment are ETC (Halling-Sorensen *et al.* 2002) and N-desmethyl-iso-tetracycline (Zurhelle *et al.* 2000). The route of degradation depends primarily on pH - in alkali conditions, a hydroxyl group is readily

cleaved, leading to formation of iso-tetracycline, with further degradation to N-desmethyl-iso-tetracycline if oxygen is present (Halling-Sorensen *et al.* 2002). At neutral conditions and in the pH range 2-6, ETC is the major degradation product, whilst below pH 2, ATC is the major product (Halling-Sorensen *et al.* 2002). The formations of ETC and ATC are readily reversible (Halling-Sorensen *et al.* 2002; Liang *et al.* 1998). In sewage treatment it is most likely that ETC will be the preferential degradation pathway, since most STPs operate in the relatively neutral pH range 6.5 to 8.5 (Metcalf and Eddy 2003).

Tetracyclines can also undergo direct photolysis reactions. Liang *et al.* (1998) described eight photodegradation products, as shown in Figure 3.2 below. It should be noted that none of the biological degradation products mentioned above were formed.

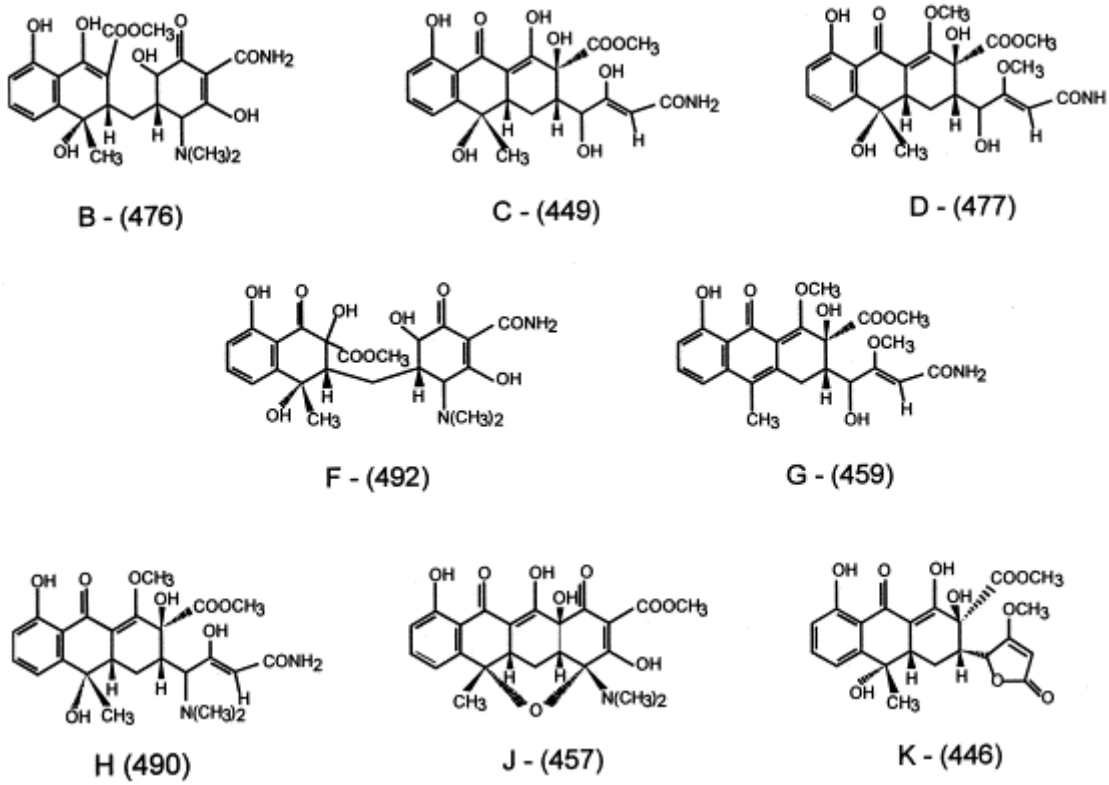


Figure 3.2: Photodegradation products of tetracycline (Liang *et al.* 1998)

A final property of tetracyclines that should not be overlooked is the chelation with divalent and trivalent metal ions, such as Mg^{2+}, Ca^{2+}, Fe^{3+}, and Al^{3+} (Halling-Sorensen *et al.* 2002). This mechanism will cause increased removal from the aqueous phase, compared to that predicted by the K_{ow} method. This removal mechanism will be particularly relevant for STPs that use ferrous, ferric, or alum dosing for P removal.

3.2.4 Triclosan

Triclosan is a highly used non-prescription antibacterial, included in anti-gum-disease toothpaste, deodorant soaps, deodorants, antiperspirants, body washes, detergents, dish-washing liquids, cosmetics and anti-microbial creams, lotions, and hand soaps. It is also used as an additive in plastics, polymers, and textiles to give these materials antibacterial properties. Triclosan is also a pre-dioxin, meaning that trace quantities of dioxins can be found in the products that it is used in, and that dioxins may be formed during its degradation. Prescription usage of triclosan in 2000 was around 4 tonnes (Webb 2000), but this is likely to be a severe underestimate of the total usage considering the wide range of non-prescription applications of triclosan.

Kanda *et al.* (2003) detected triclosan in sewage influent at a median of 3700 ng l^{-1}. They also reported removal ranging from 100% to no removal, although typical removals were 95 to 98%. McAvoy *et al.* (2002) reported triclosan concentrations in influents ranging from 3830 to 15400 ng l^{-1}, and in effluents ranging from 240 to 2700 ng l^{-1}. This equated to removals ranging from 58.0% to 96.2%. They noted that the higher removal rates (95.4%, 96.2%) occurred in activated sludge plants, whilst the lower removal rates (58.0%, 86.1%) occurred in trickling filter plants. Similarly, Thomas and Foster (2005) reported removals of 97.6%, 98.5%, and 99.2% through three activated sludge plants, with an average effluent concentration of 49 ng l^{-1}. Halden and Paull (2005) reported greater than 99% removal of triclosan through a US activated sludge works, with an effluent concentration of 35 ng l^{-1}. All these concentrations remain in excess of the PNEC for triclosan of 14 ng l^{-1} (see Table 3.10). Bester (2003) reported concentrations of triclosan in sewage sludges ranging from 400 to 8800 ng g^{-1}, from a study of 20 sewage plants of various types. The paper made no attempt to relate concentrations to type of treatment. Bester (2003) also reported 96% removal of triclosan through an activated sludge plant, with approximately 30% being sorbed to sludges, and 65% being removed by biodegradation. Bester later reported (Bester 2005) 87% removal in a trickling filter plant. Federle *et al.* (2002) showed in a laboratory activated sludge study that more than 80% of the removal of triclosan was attributed to biodegradation.

Federle *et al.* (2002) also studied a variation in the removal of triclosan during their trials, between 93.9 and 99.3%. This was not linked to SRT, as might have been expected according to Ternes *et al.* (2004). Furthermore, percentage removal was almost constant as influent

levels increased, which is a deviation from pure Monod kinetics. This was explained by triclosan serving as a secondary, rather than primary substrate. Federle *et al.* (2002) also noted a correlation (r^2 = 0.87) between COD removal and triclosan removal. They stated that this indicated that the removal of triclosan was directly related to the efficiency of the activated sludge system.

Singer *et al.* (2002) reported triclosan concentrations in sewage effluents ranging from 42 to 213 ng l^{-1}. Using grab samples, they calculated the K_d of triclosan to be about 16 L g^{-1}. (This is roughly ten times as much as tetracycline). However, this was dependant on pH, since triclosan changes from its phenolic form to its phenolate form at pHs above its pKa (8.1) (Lindstrom *et al.* 2002), as shown in Figure 3.3.

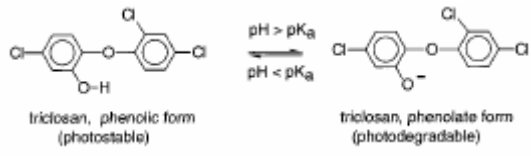


Figure 3.3: Structure of triclosan in phenolic and phenolate forms (Lindstrom *et al.* 2002)

Singer *et al.* (2002) calculated the effect of pH on K_d according to Equation 7 below. Orvos *et al.* (2002) also calculated the K_d of triclosan, at 19.8 and 21.6 l g^{-1}, but made no account of pH in their calculations.

$$K_d = \frac{C_s}{C_{aq}}(1+10^{(pH-pKa)}) \qquad \text{Equation 7}$$

Due to this transformation, photolysis of triclosan is expected to be a significant removal mechanism in surfaces waters (Tixier *et al.* 2002), since the phenolate form of triclosan is photodegradable. However, most STPs operate in pH range 6.5 to 8.5 (Metcalf and Eddy 2003), in which most Triclosan (pKa 8.1) will be in its photostable form. The physical properties of triclosan are summarised in Table 3.10 below.

Table 3.10: Properties of triclosan

Property	Value	Units	Reference
CAS Number	3380-34-5	-	ChemIDplus
Molecular Weight	289.53	g mol^{-1}	ChemIDplus
Solubility	10	mg l^{-1}	ChemIDplus
Henry's law constant	4.99 x 10^{-9}	atm m^3 mol^{-1}	ChemIDplus
pKa	8.1		Lindstrom *et al.* (2002)
K_d (suspended growth)	16 -22	l g^{-1}	Singer *et al.* (2002), Orvos *et al.* (2002)
K_d (fixed film)	-		
log K_{ow}	4.8	-	ChemIDplus
Biodegradation rate (suspended growth)	0.135	l ng^{-1} hr^{-1}	Tixier *et al.* (2002)
Biodegradation rate (fixed film)	-		
Toxicity (daphnia)	390	µg l^{-1} (EC50)	Orvos *et al.* (2002)
Toxicity (algae)	-	µg l^{-1} (EC50)	
Toxicity (fish)	260	µg l^{-1} (EC50)	Orvos *et al.* (2002)
Toxicity (fish)	34.1	µg l^{-1} (NOEC)	Orvos *et al.* (2002)
Toxicity (algae)	700	ng l^{-1} (NOEC)	Ciba Speciality Chemicals (2001)
PNEC	14	ng l^{-1}	(calculated)

Based on these parameters, triclosan is expected both to biodegrade readily and adsorb to sewage solids, producing a high removal. Depending on the pH, photolysis of triclosan could be a significant removal mechanism, especially above pH 8. Using these parameters, the Toxchem+ model predicted 0.26% of the influent triclosan would be found in the liquid effluent, with 33.72% in the sludge, and the remaining 66.02% being removed by biodegradation. It should be noted that loses by photolysis cannot be accounted for in Toxchem+ model.

.

3.3 Summary

The pharmaceuticals discussed above have all been detected at a wide range of concentrations in the influents and effluents of sewage treatment plants, as well as in streams and rivers. In most cases, these concentrations have exceeded the PNEC of those compounds, suggesting that they may pose a threat to the environment.

Four pharmaceuticals (caffeine, carbamazepine, tetracycline, and triclosan) were selected for further study according to a range of factors. These factors were a measured environmental concentration greater than 200 ng l^{-1}, predicted environmental concentration greater than predicted no effects concentration, measured environmental concentration greater than predicted no effects concentration, high potential for bioaccumulation (defined as log K_d greater than 1.5, or log K_{ow} greater than 4), and removal in STPs (either high (> 90%) or poor (<10%)).

The fate of the pharmaceuticals in activated sludge plants has been modelled with Toxchem+, using currently available data. This has shown that whilst the model is broadly accurate for two compounds (triclosan, and caffeine) in terms of overall removal, the quantities removed by sorption and biodegradation are inaccurate compared to experimental values. For tetracycline, removal cannot be compared due to a lack of experimental data. For carbamazepine, the model widely overestimates removal. No modelling has been attempted with trickling filter plants, due to a complete lack of data on the sorption and biodegradation rates with fixed film biomass.

To ensure that the model accurately portrays reality, accurate sorption and biodegradation rates are required for both fixed film and suspended growth biomasses. Once this has been completed for these compounds, it may be possible to see how small changes in the operation and make-up of the treatment plants can affect removal. This can be achieved both by the use of the Toxchem+ model, and by measurement of parameters at full scale treatment works.

Chapter 4: Analytical development

Analytical development is, perhaps, the most important part of any scheme of work involving micropollutants, such as pharmaceuticals. Without robust, reliable, and repeatable analytical methods, any data produced would be completely unreliable.

The analytical development work is split into two sections in this chapter, covering high performance liquid chromatography (HPLC) method development, and the development of solid phase extraction (SPE) techniques. The latter was required as a concentration step, since the concentrations expected in sewage (especially the final effluents) were lower than could be detected directly with HPLC.

4.1 Development of HPLC methods

Analysis was performed using a Shimadzu LC 10AD high pressure liquid chromatograph with UV detector (Shimadzu, Milton Keynes, UK). The column, detection wavelength, mobile phase, and flow rates used were varied depending on the pharmaceuticals being analysed for, and are detailed in the method development. All methods used a 10 μl injection volume. Crude sewage and final effluent samples used in the develpoment of these methods were taken from the Cranfield sewage treatment plant. Four main steps were undertaken in the development of the analytical methods, which are detailed below.

Procurement of reference standards

Before any selection or development of an analytical method can occur, it must be possible to obtain analytical standards of the compounds that are to be analysed. For triclosan, and its primary degradation product methyl-triclosan, it was possible to purchase ready-made analytical standards from Greyhound Chromatography (Birkenhead, UK). These were made up in nonane, at a concentration of 50 mg l^{-1}.

No ready-made standards of the other selected compounds were available. Therefore, the compounds were purchased in powder form, and high concentration stock solutions (1 g l^{-1}) were made up in HPLC grade methanol. These stock solutions were stored at -25 ^{o}C, and

diluted to make analytical standards immediately prior to use. The stock solutions were replaced every six months. Analytical standards were made up from the stock solution to concentrations of 20, 10, 5, 2, 1, and 0.1 mg l^{-1}. It was noted that the powder form of these compounds were not pure, as shown in Table 4.1 below. These purities were taken into account when making up the 1 g l^{-1} stock solutions. In a similar fashion, any degradation products or compounds that were similar to the compound of interest were purchased, where possible. For example, in the analysis of tetracycline, it was important to ensure that the related compounds oxytetracycline, and chlorotetracycline did not interfer with the analysis of tetracycline.

Table 4.1: Purity and source of selected pharmaceuticals

Compound	**Associated compounds**	**Purity (%)**	**Source**
Tetracycline		99.0	Sigma-Aldrich, UK
	Epitetracycline	97.9	
	Anhydrotetracycline	98.5	
	Epianhydrotetracycline	97.4	
	Oxytetracycline	96.2	
	Chlorotetracycline	84.4	
Triclosan		100	Greyhound Chromatography
	Methyl-triclosan	100	
Carbamazepine		99.0	Sigma-Aldrich, UK
	10,11-dihydrocarbamazepine	98.2	
Caffeine		99.0	Sigma-Aldrich, UK
	Theophylline	96.4	
	Paraxanthine	97.8	
	Theobromine	93.1	
	1,7-Dimethyluric acid	98.1	
	7-Methyl xanthine	92.8	

Measurement of standard concentrations

As described previously, standards were prepared at six concentrations. These were chosen so that one was well above the expected detection levels, whilst the others corresponded to the range of concentrations expected in real samples. All six standards were measured at the start and end of each sample run, whilst the highest concentration standard (20 mg l^{-1}) was measured every five samples to ensure correct mode of operation. On occasion, the measurement of the analytical standard showed that other compounds were being retained on the column from previous samples, due to the dirty matrix. This occurred primarily when analysing influent samples. When this occurred, analysis was stopped, and the column backwashed with various concentrations of methanol and acetonitrile as per the manufacturer's instructions. Affected samples were reanalysed to ensure concentrations were being recorded. This process was also conducted for any significant changes to peak shape, peak area, or retention time on the analytical standards.

Selection and development of the chromatographic method

Three options were available for chromatographic methods. These were: use of methods supplied by equipment suppliers (Phenomenex, and Waters), use of methods described in published papers, or complete development of new chromatographic methods.

The first attempts at tetracycline analysis were with a method supplied by Phenomenex (Macclesfield, UK), using their Luna C8 HPLC column. However, when tetracycline standards were run using this method and column, it was found that a pure sample of tetracycline produced two distinct peaks, rather than the single peak expected. Therefore, further chromatographic methods from peer-reviewed journals were considered (Cinquina *et al.* 2001; Oka *et al.* 2000; Oka *et al.* 1984). It was possible to find and utilize published HPLC methods for tetracycline, carbamazepine, and caffeine, whilst it was necessary to develop a method for triclosan, since only methods using gas chromatography with mass spectrometry (GCMS) were found. The HPLC methods used are discussed in the sections on each compound below.

Column and instrument performance

The instrument performance parameters shown in Table 4.2 were calculated from chromatograms of the pharmaceutical standards, and methods adjusted where necessary. The relevant formulas are shown in Figure 4.1 and Figure 4.2. Keeping these parameters within the limits defined within Table 4.2 ensured the reliability of the analytical methods (Phenomenex 2005/06).

Table 4.2: Factors affecting chromatographic performance

Factor	**Units**	**Requirement**
Column efficiency (N)	-	> 2000
Run time (t)	Minutes	2>t>40
Peak asymmetry (A_s)	-	$0.5 \leq A_s \leq 2$
Precision	-	r^2 >0.98 over 10 injections of a standard
Resolution (R)	-	≥ 2 between adjacent peaks
Linearity	-	$r^2 > 0.999$ Over 5 standard concentrations
Limits of detection (LOD) and quantification (LOQ)	ng l^{-1}	< expected environmental concentrations

The formulae for the calculation of column efficiency and peak asymmetry are shown in Figure 4.1. The requirements given for these limits are based on typical concentrations detected in the environment.

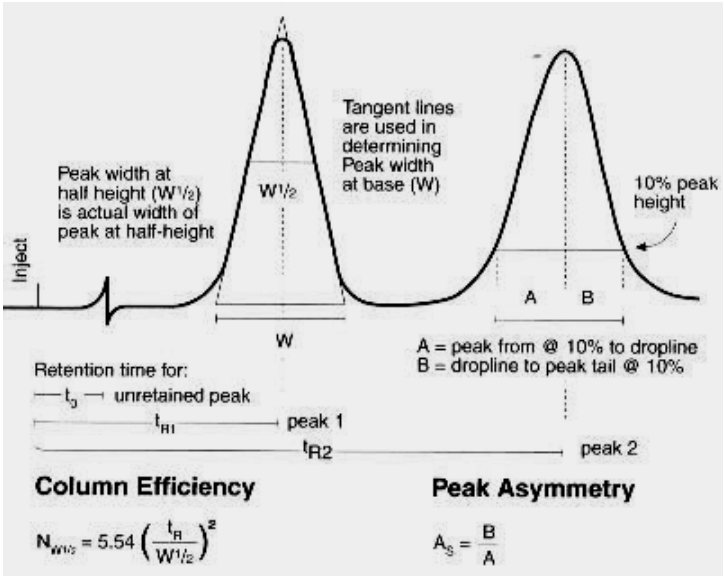


Figure 4.1: Calculation of column efficiency and peak asymmetry (Phenomenex 2005/06)

The resolution of a peak (R) is affected by three controllable factors: selectivity, column efficiency, and the retention factor, as shown in Figure 4.2.

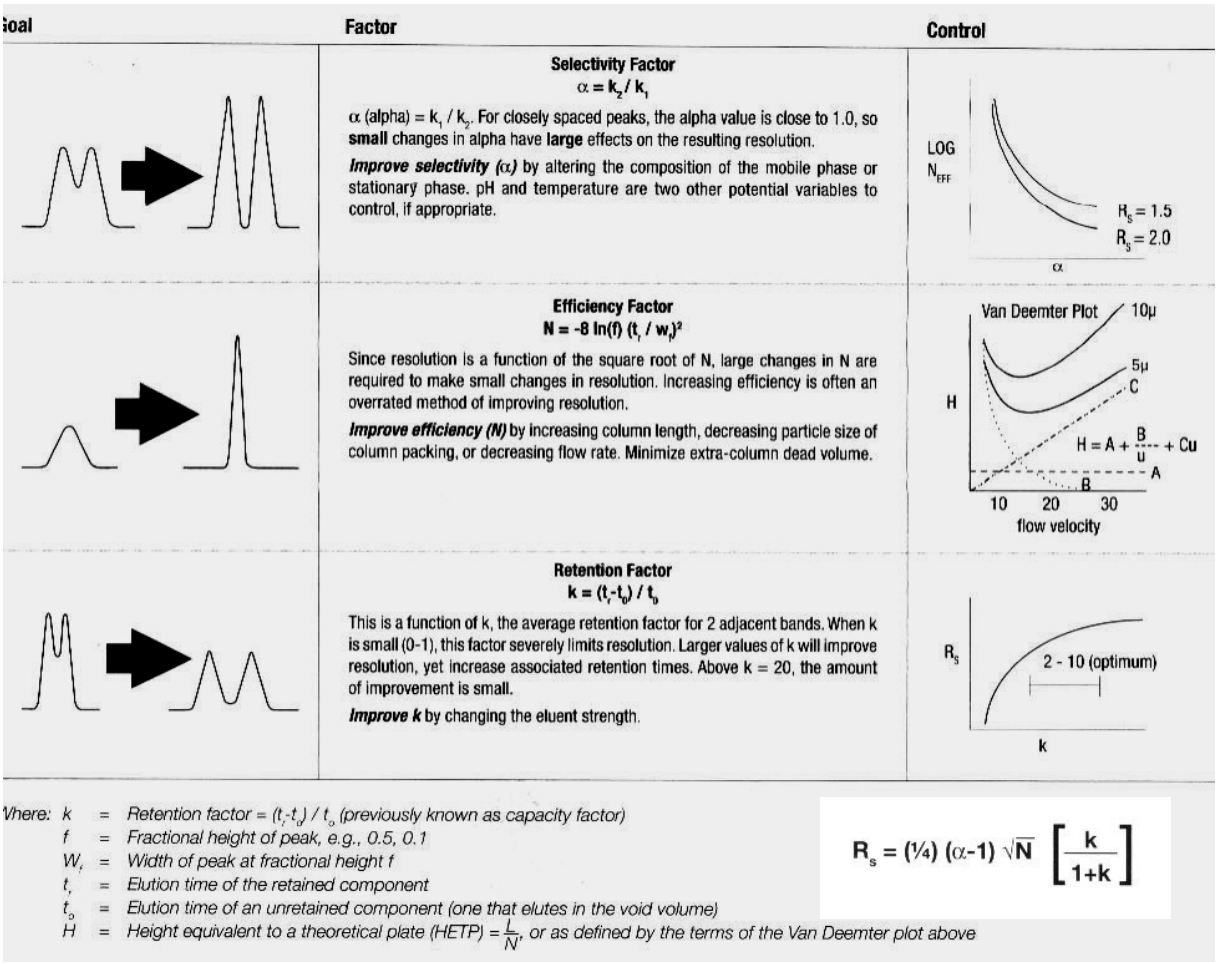


Figure 4.2: Factors affecting peak resolution (Phenomenex 2005/06)

The limit of detection (LOD) is the injected amount that results in a peak with a height at least twice as high as the baseline noise level (EURACHEM 1993). Similarly, according to the EURACHEM the limit of quantification (LOQ) is defined as the injected amount that results in a peak with a height at least ten times as high as the baseline noise level (ie LOQ = LOD x 5). Different matrices produce different amounts of baseline noise, ranging from the dirtiest (noisiest) being influent samples, to the cleanest in methanol samples. Therefore, three LODs are given for all compounds - for influent, effluent, and in methanol solution.

4.1.1 Triclosan

After a thorough review of the literature, no HPLC method was found for the analysis of triclosan. Therefore, a basic method was trialled using various concentrations of acetonitrile and water as a mobile phase, ranging from 100% water to 100% acetonitrile. Initially, the ratio was changed by 5% until the complete range was tested, and then in 0.5% steps around the most favourable ratio. The optimum column efficiency and retention time were found to be at a ratio of acetonitrile and water of 1:1.1, and a flow rate of one millilitre per minute. Both C8 and C18 HPLC columns were trialled, but the C8 showed very little retention of triclosan, so the C18 was chosen. The same conditions were found to be suitable for methyl-triclosan, although the retention time was very long (~64 minutes). Therefore, the flow rate was increased to two millilitres per minute. The HPLC conditions are shown in Table 4.3.

Table 4.3: HPLC conditions for triclosan and methyl-triclosan analysis

Column	Gemini 5µm C18 150x4.6 mm ID (Phenomenex, UK)	
Mobile Phase[1]	47.5% Acetonitrile 52.5% Water	
UV wavelength (nm)	235	
Flow rate (ml min^{-1})	2	
Retention times (min)	Triclosan	12.2
	Methyl-triclosan	31.6

Figure 4.3 shows chromatograms of various triclosan concentrations. These include (a) 10mg l^{-1} triclosan in methanol, (b) 200 µg l^{-1} in crude sewage, (c) 100 µg l^{-1} in crude sewage, (d) crude sewage (no triclosan), and (e) final effluent (no triclosan). For all samples involving sewage, chromatograms were only recorded after five minutes, to reduce the influence of unretained compounds, particularly in crude sewage samples.

[1] For sources and grades of solvents used, please see Materials and Methods

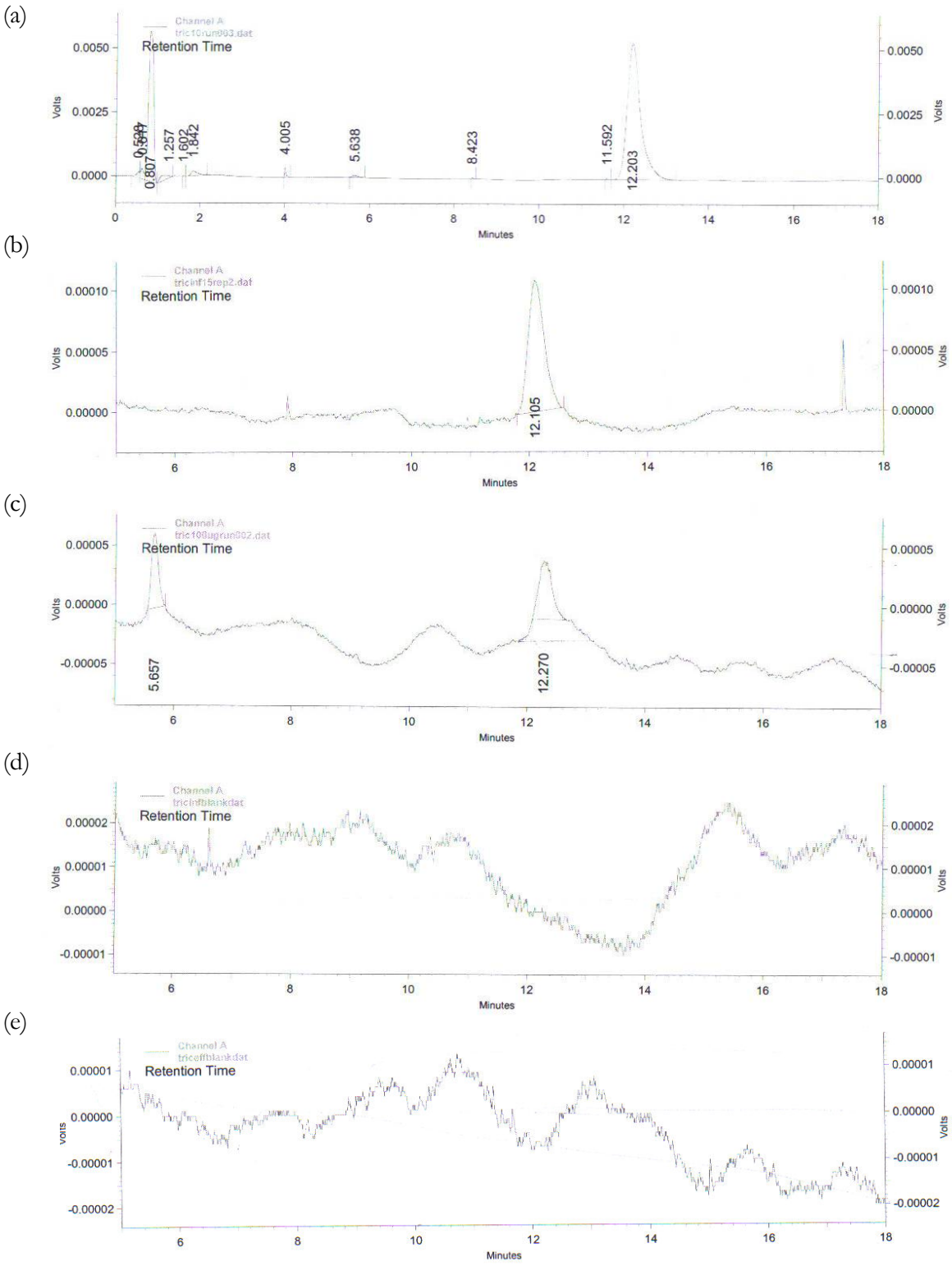


Figure 4.3: Triclosan chromatograms: (a) 10mg l^{-1} triclosan in methanol, (b) 200 µg l^{-1} in crude sewage, (c) 100 µg l^{-1} in crude sewage, (d) crude sewage (no triclosan), and (e) final effluent (no triclosan)

The values for the control parameters are shown in Table 4.4, where column efficiency, resolution, and peak asymmetry were calculated from chromatogram "a" above. The baseline noise for crude and final effluents was measured from chromatograms "d" and "e" above, as 0.000010 and 0.000007 volts respectively. This means that the LOD and LOQ for triclosan in crude sewage were equivalent to peak heights of 0.00002 and 0.00010 respectively, with 0.000014 volts and 0.00007 volts for final effluent. This equated to concentrations in crude sewage: LOQ = 170 $\mu g\ l^{-1}$, LOD = 43 $\mu g\ l^{-1}$; and in final effluent LOQ = 100 $\mu g\ l^{-1}$, LOD = 39 $\mu g\ l^{-1}$. Unsurprisingly, the LOQ of triclosan is much lower in final effluent than in crude sewage, although there is not much difference between the LODs. These results showed that the limits of quantification and detection are not sufficient to detect the expected environmental concentrations. For this reason, the samples needed to be concentrated before analysis, using a solid phase extraction technique.

To illustrate the LOQ and LOD, two chromatograms with crude sewage and triclosan were included in the above figure. Chromatogram "b" at 200 $\mu g\ l^{-1}$ is slightly above the LOQ, whilst chromatogram "c" at 100 $\mu g\ l^{-1}$ is below the LOQ and above the LOD. In the latter chromatogram it is clear to see that the peak, although visible, is affected by the baseline noise and therefore its area cannot be measured accurately.

Table 4.4: Values of control parameters for triclosan

Factor	Requirement	Value
Column efficiency (N)	> 2000	2270
Run time (t)	2 > t > 40	34
Peak asymmetry (A_s)	$0.5 \leq A_s \leq 2$	1.15
Precision	$r^2 > 0.98$	0.993
Resolution (R)	≥ 2	22.6
Linearity	$r^2 > 0.999$	1.000
Limits of quantification	< 710 ng l^{-1}	170 $\mu g\ l^{-1}$ (crude), 100 $\mu g\ l^{-1}$ (final effluent)
Limits of detection		43 $\mu g\ l^{-1}$ (crude), 39 $\mu g\ l^{-1}$ (final effluent)

Since this was a new method, it was suggested that samples should be sent to other laboratories for confirmation of concentration measurements. Six samples were sent to two laboratories (CEH Wallingford – Neville Llewellyn; Severn Trent Laboratories – Anne Brown) running gas chromatography mass spectrometry (GCMS) analysis of triclosan. These samples included two each of crude sewage, mixed liquor, and final effluents, containing triclosan. Details of the chromatography conditions used by these laboratories were not available. The values obtained using the method described here were within 1% of the values obtained by the two GCMS methods, thereby confirming the accuracy of this method.

4.1.2 Tetracycline

For the reliable analysis of tetracycline, it was necessary to split the set of related compounds into two groups, due to the UV response of the various compounds. Firstly, the group (I) of parent compounds (tetracycline, oxytetracycline, and chlortetracycline) were analysed to ensure that their peaks did not interfere with one another. Secondly, the group (II) of transformation products (epi-tetracycline, anhydrotetracycline, and epi-anhydrotetracycline) along with tetracycline were analysed. The analysis of these two groups were based on the methods of (Oka *et al.* 1984), with the conditions shown in Table 4.5. This method was used because it was the only method found in literature that analysed all the tetracycline family and tetracycline degradation product compounds.

Table 4.5: HPLC conditions for tetracycline family analysis

	Group I		Group II	
Column	Luna 5µm C8 150x4.6 mm ID (Phenomenex, UK)		Gemini 5µm C18 150 x 4.6 mm ID (Phenomenex, UK)	
Mobile Phase	13% Methanol 20% Acetonitrile 67% 0.01M Oxalic acid pH 2.0		18% Methanol 18% Acetonitrile 64% 0.2M Oxalic acid pH 2.0	
UV wavelength (nm)	360		400	
Flow rate (ml min^{-1})	1		1	
Retention times (mins)	Oxytetracycline	2.1	Epi-tetracycline	2.5
	Tetracycline	2.2	Tetracycline	2.8
	Chlortetracycline	2.8	Epi-anhydrotetracycline	7.0
			Anhydrotetracycline	9.0

These HPLC condition produced a typical chromatogram for the Group II compounds as shown in Figure 4.4.

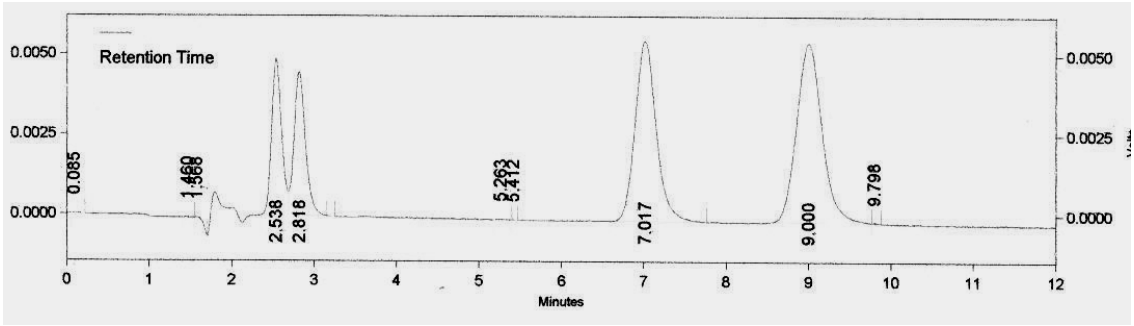


Figure 4.4: Chromatogram of tetracycline Group II compounds at 20 mg l^{-1} in methanol. t = 2.5 epi-tetracycline, t = 2.8 tetracycline, t = 7.0 epi-anhydrotetracycline, t = 9.0 anhydrotetracycline

As can be seen from this chromatogram, the selectivity between the epi-tetracycline and tetracycline is not good, with the bases of the two peaks overlapping slightly. This is due to the nature of the compounds involved. Both compounds have exactly the same chemical structure – the only difference is the position of two functional groups. This similarity means that they behave in a very similar fashion to each other during chromatography. However, the values for the control parameters (based on the tetracycline peak), which are shown in Table 4.6, showed that this amount of separation is sufficient.

Figure 4.5 shows chromatograms of various tetracycline concentrations. These include (a) 10 mg l^{-1} tetracycline in methanol, (b) 400 μg l^{-1} in crude sewage, (c) 400 μg l^{-1} in final effluent, (d) crude sewage (no tetracycline), and (e) final effluent (no tetracycline).

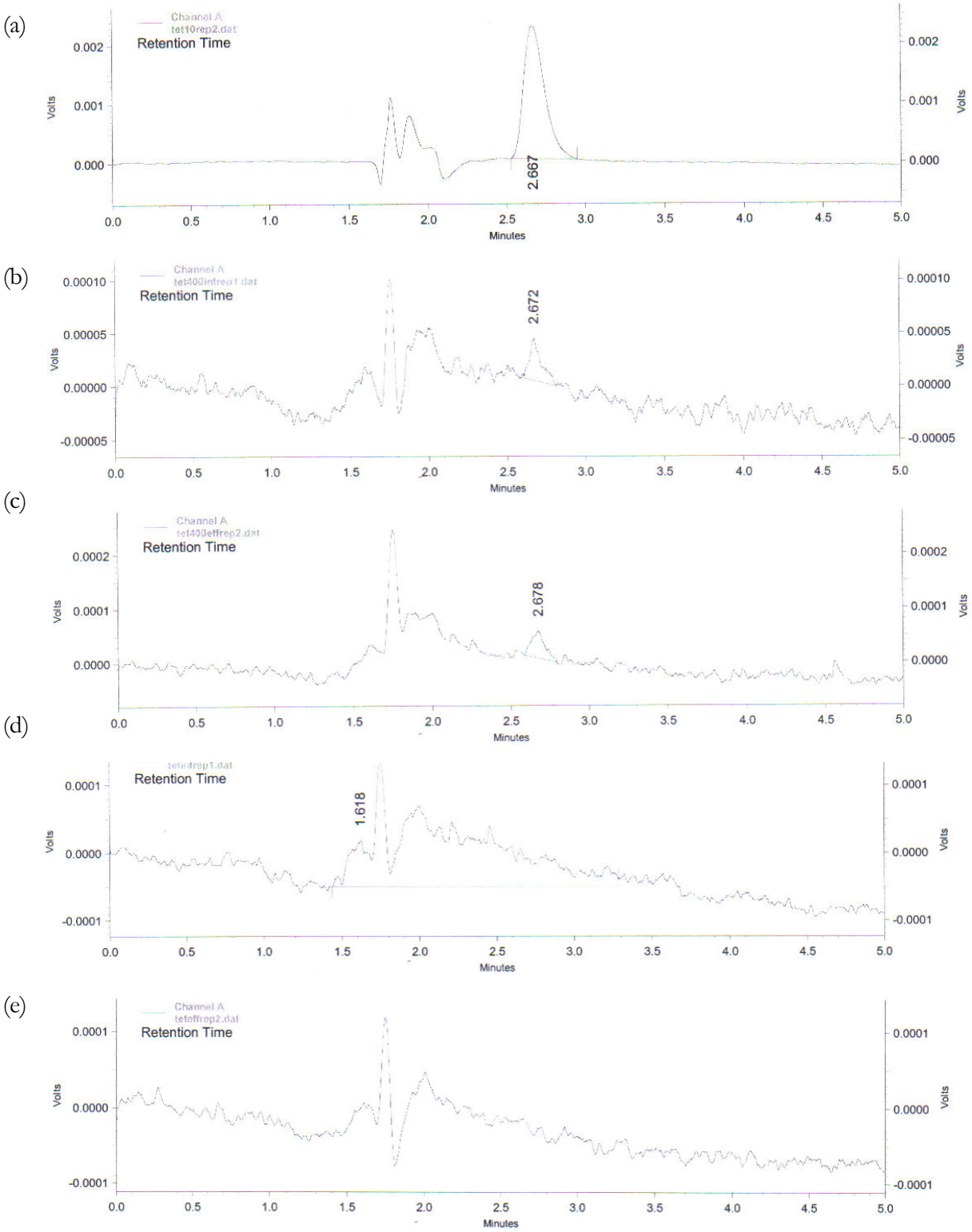


Figure 4.5: Tetracycline chromatograms: (a) 10mg l^{-1} tetracycline in methanol, (b) 400 □g l^{-1} in crude sewage, (c) 400 □g l^{-1} in final effluent, (d) crude sewage (no tetracycline), and (e) final effluent (no tetracycline)

The baseline noise for crude and final effluents was measured from chromatograms "d" and "e" above, as 0.00002 and 0.000005 volts respectively. This means that the LOD and LOQ for tetracycline in crude sewage were equivalent to peak heights of 0.00004 and 0.0002 respectively, with 0.00001 volts and 0.00005 volts for final effluent. This equated to concentrations in crude sewage: LOQ = 499 μg l^{-1}, LOD = 397 μg l^{-1}; and in final effluent LOQ = 403 μg l^{-1}, LOD = 380 μg l^{-1}. These results showed that the limits of quantification and detection are not sufficient to detect the expected environmental concentrations. For this reason, the samples needed to be concentrated before analysis, using a solid phase extraction technique.

Table 4.6: Values of control parameters for tetracycline

Factor	**Requirement**	**Value**
Column efficiency (N)	> 2000	222100
Run time (t)	2 > t > 40	12
Peak asymmetry (A_s)	$0.5 \leq A_s \leq 2$	0.75
Precision	r^2 >0.98	0.988
Resolution (R)	≥ 2	16.2
Linearity	r^2 >0.999	0.9991
Limits of quantification	< 150 ng l^{-1}	499 μg l^{-1} (crude), 403 μg l^{-1} (final effluent)
Limits of detection		397 μg l^{-1} (crude), 380 μg l^{-1} (final effluent)

4.1.3 Caffeine

Caffeine analysis was based on the method of Georga *et al.* (2001), which allowed for the analysis of caffeine and 11 degradation products (4 primary, 4 secondary, and 3 others). This method was chosen since it was the published HPLC method that covered the most caffeine degradation products. According to Georga *et al.* (2001), the dominant degradation pathways in humans were paraxanthine (80%), theobromine (12%), and theophylline (7%). Based on these percentages, it was deemed highly unlikely that any degradation products other than these three compounds and possibly the two primary degradation products of paraxanthine (1,7-dimethyluric acid, 7-methylxanthine) would exist in detectable concentrations in sewage. As a result, only these 5 of the 11 degradation products were selected for the analytical regime. The HPLC conditions are shown in Table 4.7.

Table 4.7: HPLC conditions for caffeine and degradation product analysis

Column	Kromasil 5µm C4 250 x 4.0 mm ID (Phenomenex, UK)	
Mobile Phase	3% Methanol 97% Acetate Buffer (pH 3.5) Changing to 20:80 in 20 minutes	
UV wavelength (nm)	275	
Flow rate (ml min^{-1})	1	
Retention times (mins)	Caffeine	19.2
	Theophylline	12.6
	Paraxanthine	11.7
	1,7-Dimethyluric acid	9.6
	Theobromine	7.1
	7-Methylxanthine	4.8

These HPLC conditions produced a typical chromatogram for caffeine and its degradation products as shown in Figure 4.6.

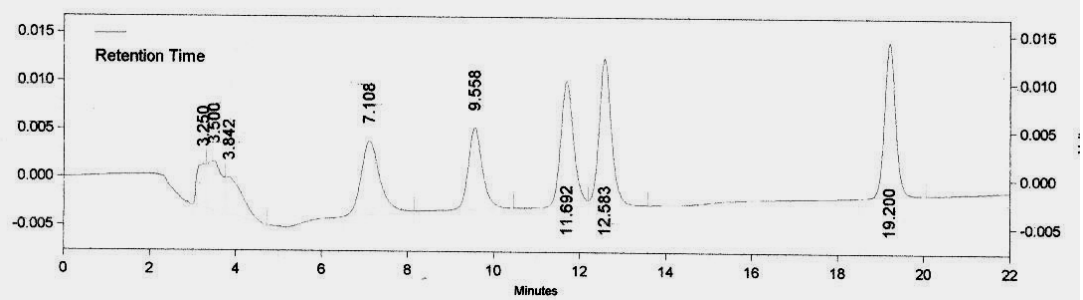


Figure 4.6: Chromatogram of caffeine and degradation products. t = 19.2 caffeine, t = 12.6 theophylline, t = 11.7 paraxanthine, t = 9.6 1,7-dimethyluric acid, t = 7.1 theobromine

As can be seen from this chromatogram, there is very good selectivity between most of the peaks, although the theophylline and paraxanthine peaks are a little close. The values for the control parameters (based on the caffeine peak) are shown in Table 4.8.

Figure 4.7 shows chromatograms of various caffeine concentrations. These include (a) 10mg l^{-1} caffeine in methanol, (b) 61 μg l^{-1} in crude sewage, (c) 114 ng l^{-1} in final effluent, and (d) crude sewage (no caffeine).

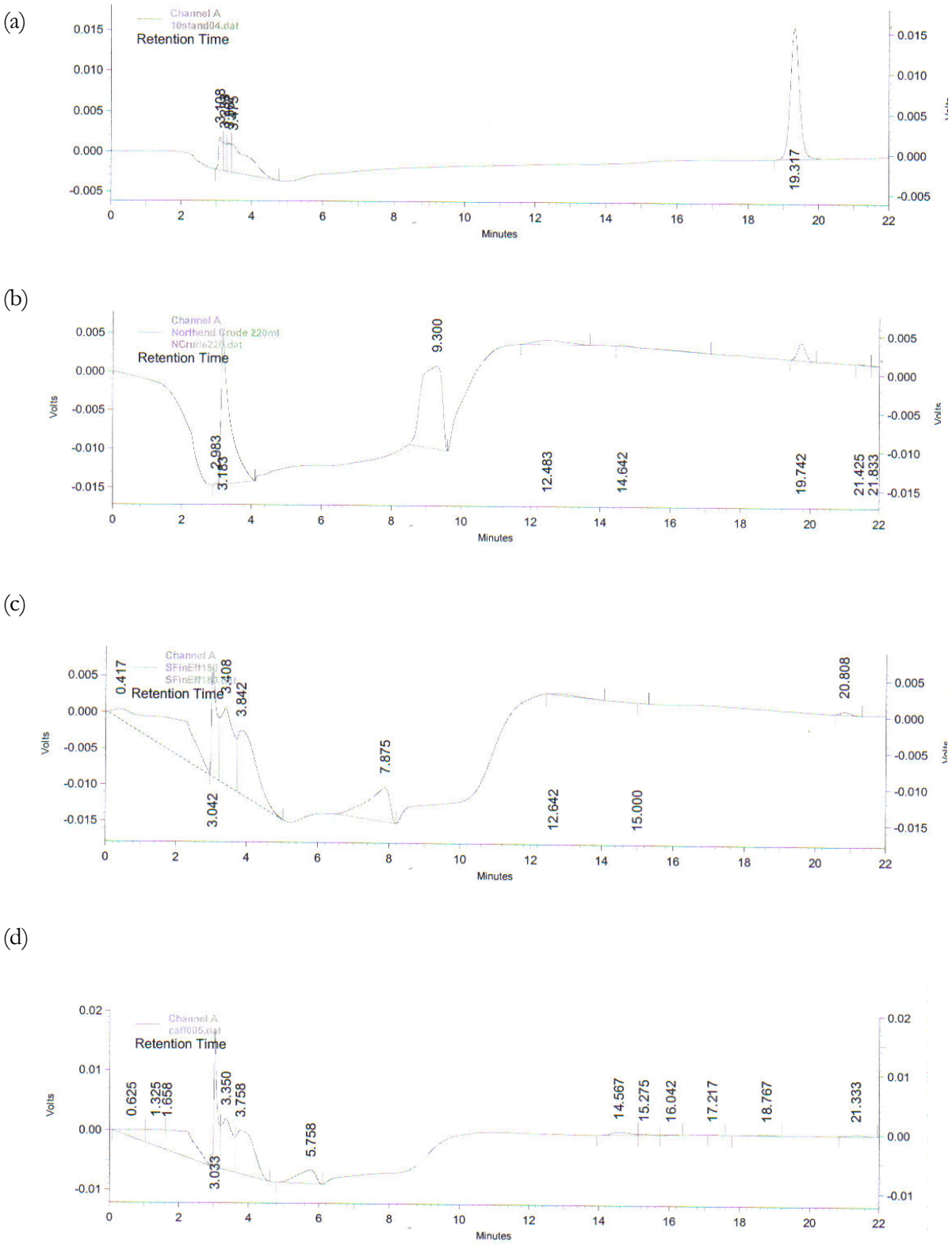


Figure 4.7: Caffeine chromatograms: (a) 10mg l^{-1} caffeine in methanol, (b) 61 □g l^{-1} in crude sewage, (c) 114 ng l^{-1} in final effluent, and (d) crude sewage (no caffeine)

The baseline noise for crude was measured from chromatogram "d" above as 0.000187, and 0.000131 volts for final effluent (chromatogram not shown). This means that the LOD and LOQ for caffeine in crude sewage were equivalent to peak heights of 0.000374 and 0.00187 respectively, with 0.000262 volts and 0.00131 volts for final effluent. This equated to concentrations in crude sewage: LOQ = 18.2 $\mu g\ l^{-1}$, LOD = 0.113 $\mu g\ l^{-1}$; and in final effluent LOQ = 2.73 $\mu g\ l^{-1}$, LOD = 0.078 $\mu g\ l^{-1}$. These showed that the limits of quantification and detection are not sufficient to detect the expected environmental concentrations. Therefore, the samples needed to be concentrated before analysis, using a solid phase extraction technique.

Table 4.8: Values of control parameters for caffeine

Factor	**Requirement**	**Value**
Column efficiency (N)	> 2000	28650
Run time (t)	2 > t > 40	22
Peak asymmetry (A_s)	$0.5 \leq A_s \leq 2$	1
Precision	r^2 >0.98	0.992
Resolution (R)	≥ 2	24.6
Linearity	r^2 >0.999	1.000
Limit of quantification	< 180 ng l^{-1}	18.2 $\mu g\ l^{-1}$ (crude), 2.73 $\mu g\ l^{-1}$ (final effluent)
Limit of detection		0.113 $\mu g\ l^{-1}$ (crude), 0.078 $\mu g\ l^{-1}$ (final effluent)

The limit of quantification in this work was similar to the level quoted in the literature for caffeine in crude sewage of 20 $\mu g\ l^{-1}$ (Georga *et al.* 2001).

4.1.4 Carbamazepine

Although no methods were available in literature were available for the HPLC-UV analysis of carbamazepine in sewage, Levert *et al.* (2002) analysed carbamazepine and epoxy-carbamazepine in serum, and Reith and Cannell (1999) analysed carbamazepine phase I metabolites and their glucuronides in urine. Since the Reith and Cannell method analysed many compounds that could not be purchased for this work, the Levert *et al.* method was used as the basis for this analysis. The HPLC conditions are shown in Table 4.9.

Table 4.9: HPLC conditions for carbamazepine and 10,11-dihydrocarbamazepine analysis

Column	Gemini 5µm C18 150x4.6 mm ID (Phenomenex, UK)	
Mobile Phase	22% Acetonitrile 78% 7mM Sodium acetate (pH 5.4)	
UV wavelength (nm)	240	
Flow rate (ml min^{-1})	1	
Retention times (mins)	Carbamazepine	10.7
	10,11-dihydrocarbamazepine	11.3

These HPLC conditions produced a typical chromatogram for carbamazepine and 10,11-dihydrocarbamazepine as shown in Figure 4.8.

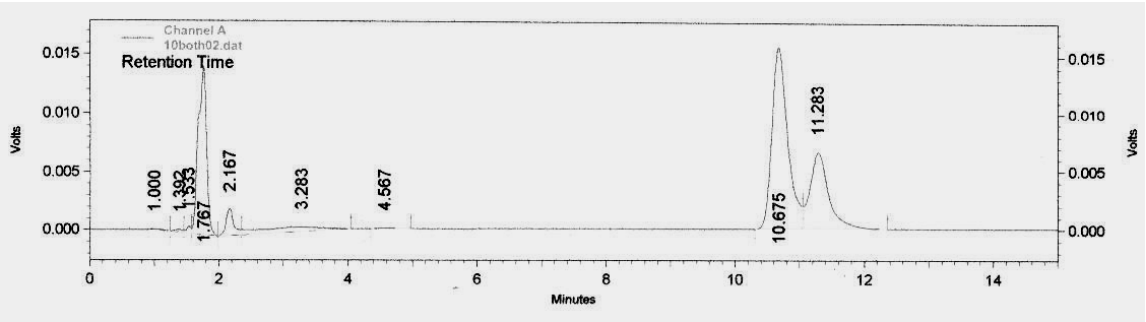


Figure 4.8: Chromatogram of carbamazepine (t = 10.7) and 10,11-dihydrocarbamazepine (t = 11.3)

As can be seen from this chromatogram, there is not good selectivity between the two peaks, although this was just sufficient to satisfy the criterion for peak resolution, as shown in Table 4.10. Separation of carbamazepine and 10,11-dihydrocarbamazepine could not be improved by changes in the content of the mobile phase.

Figure 4.7 shows chromatograms of various carbamazepine concentrations. These include (a) 10mg l^{-1} carbamazepine in methanol, (b) 200 μg l^{-1} in crude sewage, (c) 200 μg l^{-1} in final effluent, and (d) crude sewage (no carbamazepine).

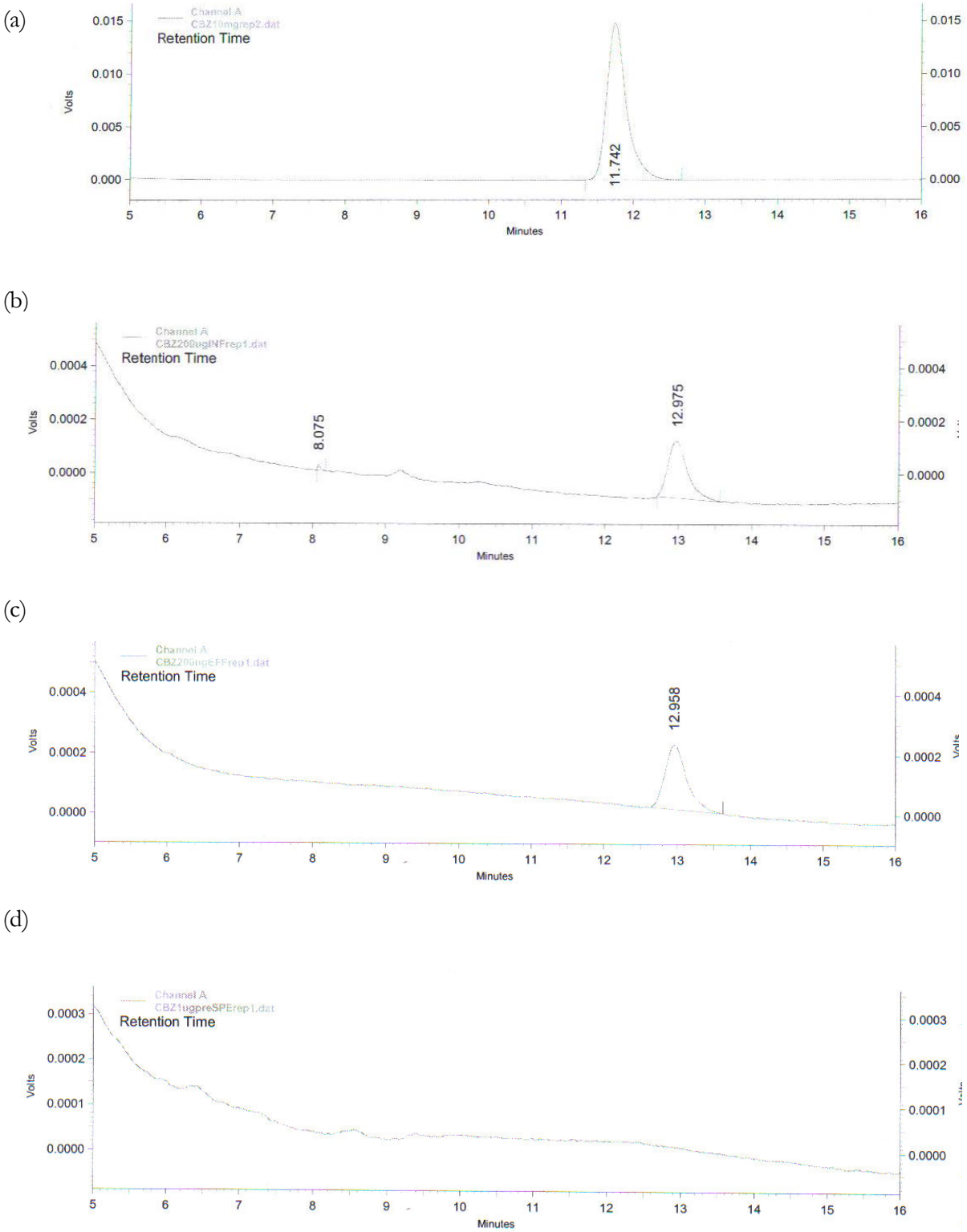


Figure 4.9: Carbamazepine chromatograms: (a) 10mg l^{-1} carbamazepine in methanol, (b) 200 □ g l^{-1} in crude sewage, (c) 200 □ g l^{-1} in final effluent, and (d) crude sewage (no carbamazepine).

The baseline noise for crude was measured from chromatogram "d" above as 0.000015, and 0.000010 volts for final effluent (chromatogram not shown). This means that the LOD and LOQ for caffeine in crude sewage were equivalent to peak heights of 0.00003 and 0.00015 respectively, with 0.00002 volts and 0.00010 volts for final effluent. This equated to concentrations in crude sewage: LOQ = 195 μg l^{-1}, LOD = 188 μg l^{-1}; and in final effluent LOQ = 190 μg l^{-1}, LOD = 186 μg l^{-1}. These showed that the limits of quantification and detection are not sufficient to detect the expected environmental concentrations. Therefore, the samples needed to be concentrated before analysis, using a solid phase extraction technique.

Table 4.10: Values of control parameters for carbamazepine

Factor	**Requirement**	**Value**
Column efficiency (N)	> 2000	20380
Run time (t)	2 > t > 40	15
Peak asymmetry (A_s)	$0.5 \leq A_s \leq 2$	0.8
Precision	r^2 >0.98	0.986
Resolution (R)	≥ 2	2.03
Linearity	r^2 >0.999	0.9993
Limits of quantification	< 2100 ng l^{-1}	195 μg l^{-1} (crude), 190 μg l^{-1} (final effluent)
Limits of detection		188 μg l^{-1} (crude), 186 μg l^{-1} (final effluent)

The limit of detection in this method was significantly worse than the quoted literature value of 66 μg l^{-1} (Levert *et al.* 2002). However, the quoted value was for detection in serum. Crude sewage and final effluent can be considered to be much dirtier matrices than serum, and hence it is not surprising that worse detection limits were encountered.

4.2 Development of Solid Phase Extraction (SPE)

As shown in the sections above, the limits of quantisation were not sufficient to allow for direct analysis of the pharmaceuticals content of samples. Therefore, it was necessary to concentrate the samples prior to analysis. Two processes were considered: liquid-liquid extractions, and solid phase extractions. The former was rejected since few reliable methods were found in a review of the literature, whereas SPE was commonly used for the concentration of pharmaceutical samples (Benito-Pena *et al.* 2006; Castiglioni *et al.* 2005; Gomez *et al.* 2006; Kosjek *et al.* 2005; Lin *et al.* 2005; Loos *et al.* 2003; Reddersen and Heberer 2003). SPE involves passing a large volume sample (up to one litre) through a cartridge that contains a sorbent material, to which the pharmaceuticals attach. The pharmaceutical compounds are then eluted with a small volume of solvent (eg 2ml of methanol).

An initial investigation was conducted according to the procedure suggested by Phenomenex in their equipment catalogue (Phenomenex 2005/06). The process, consisting of five steps is shown in Figure 4.10.

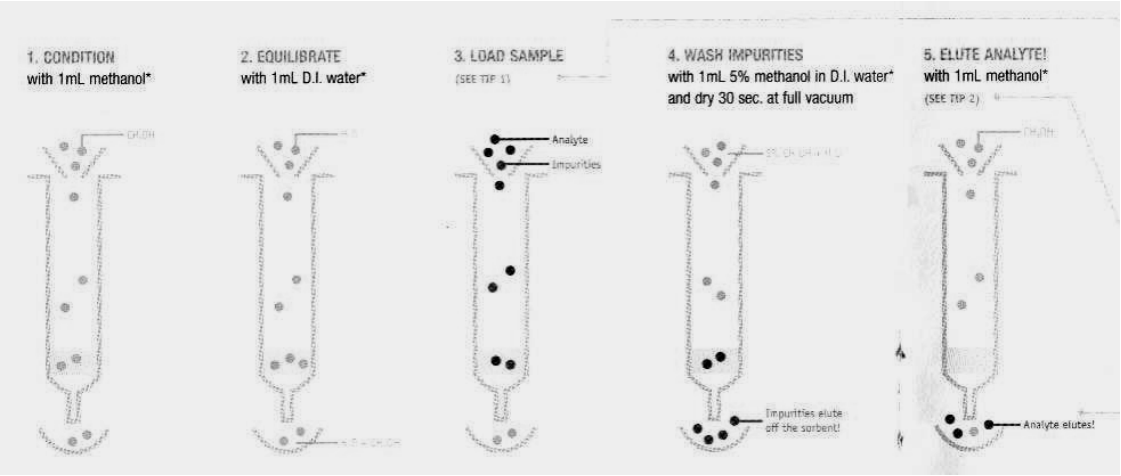


Figure 4.10: Diagram of SPE process (Phenomenex 2005/06)

In the first step, 1 ml of methanol was added to the cartridge to wet the sorbent material, and in the second step 1 ml of deionised (DI) water was added to the cartridge to ensure all the methanol was washed out. Any residual methanol in the cartridge would cause the pharmaceuticals to pass through the cartridge rather than attach to the sorbent material. A sample of up to 1000ml was then passed through the cartridge at a flow rate of approximately

1ml min^{-1}. In the fourth step, 1ml of 5% methanol in DI water was passed through the cartridge, designed to wash off any loosely bound impurities from the sorbent material. Finally, the pharmaceuticals were eluted off the cartridge using two 1 ml volumes of methanol. This was found to elute the pharmaceuticals more completely than using just a 1ml methanol wash as suggested in the diagram.

Many different sorbent materials are available for SPE. In this work, two different common sorbent materials were tested. The first was the Strata-X (Phenomenex, UK), a silica-based sorbent recommended for the extraction of pharmaceuticals and polar metabolites (Phenomenex 2005/06). The second were the Oasis hydrophilic-lipophilic balance (HLB) polymer solid-phase extraction cartridges (Waters, Watford, UK). These had previously been used by Blackwell *et al.* (2004) for the extraction of oxytetracycline from surface and ground water, with recoveries of over 99%.

In order to determine the concentration step achievable by using SPE, crude and final effluent samples were spiked with the test pharmaceuticals. The SPE procedure was trialled with sample volumes of 500, 1000, and 1500ml of the two types of sewage. Pharmaceuticals were spiked into each volume of at a five concentrations, from 10 mg l^{-1}, down to a factor of 500 below the limit of detection. The samples were immediately filtered to remove the majority of suspended solids, extracted into 2 ml methanol as described above, and then measured via the HPLC methods as previously described.

A sample volume of 1000ml was found to be the maximum practicable sample volume, since the cartridges tended to block if much more sample was added, particularly for crude samples. This lead to either a very long time being required to pass the sample through the SPE cartridge (in excess of eight hours), or complete blockage of the cartridge. Using a sample volume of 1000ml achieved a 500x concentration step.

Figure 4.11 and Figure 4.12 show crude sewage spiked with 1 μg l^{-1} of triclosan and carbamazepine respectively, before and after SPE. Whilst these concentrations are below the limit of detection without SPE, they can not only be detected but also quantified when SPE is used.

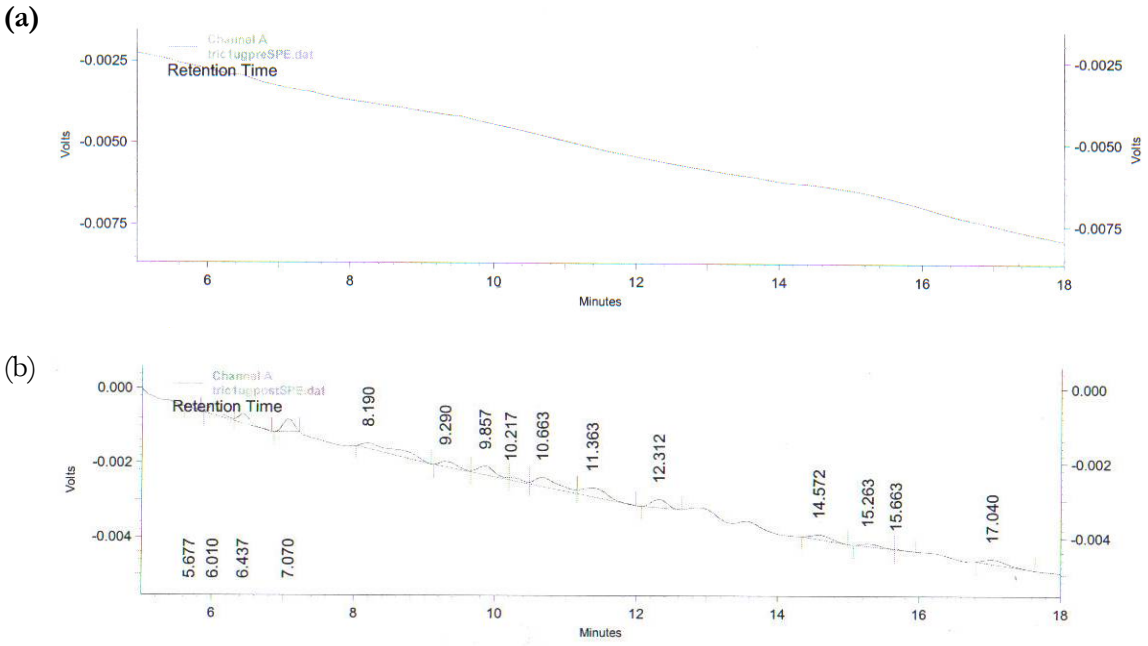


Figure 4.11: Chromatograms of 1 µg l^{-1} triclosan in crude sewage: (a) before SPE, and (b) after SPE.

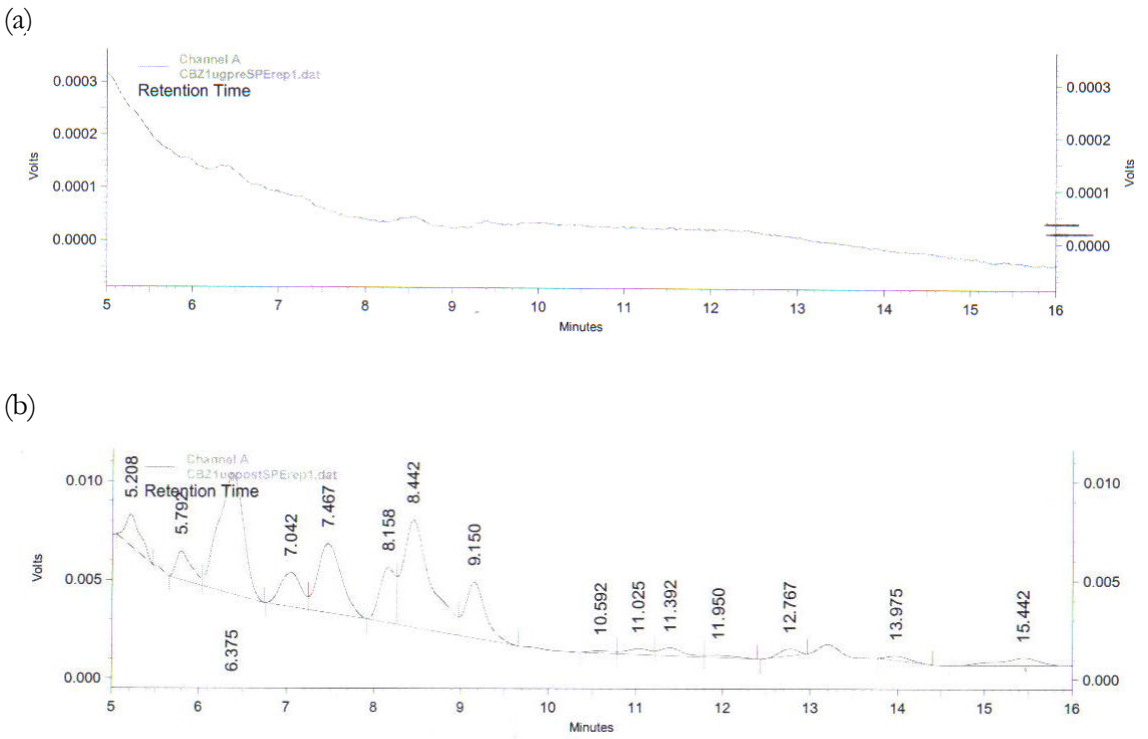


Figure 4.12: Chromatograms of 1 µg l^{-1} carbamazepine in crude sewage: (a) before SPE, and (b) after SPE.

Recoveries of the four pharmaceuticals with the two different sorbent materials are shown in Table 4.11. As can be seen from this table, neither sorbent material was superior to the other for all four compounds. As a result, Strata-X was chosen for the extraction of caffeine and carbamazepine, whilst Oasis HLB was chosen for the extraction of tetracycline and triclosan. When only a single environmental sample was available from which all four pharmaceuticals had to be extracted, Oasis HLB cartridges were used, because on average they had better recoveries than Strata-X, and also showed less tendency to block up during extractions.

Table 4.11: Recoveries (%) of pharmaceuticals during solid phase extraction using Strata-X and Oasis HLB cartridges

	Strata-X	**Oasis HLB**
Caffeine	**95.4 ± 3.2**	92.6 ± 4.4
Carbamazepine	**101 ± 1.3**	102 ± 3.4
Tetracycline	96.9 ± 4.9	**100.3 ± 3.2**
Triclosan	89.7± 2.6	**94.0 ± 1.0**

Using solid phase extraction prior to HPLC analysis significantly reduced the detection limits by a factor of 500, as shown in Table 4.12 and Table 4.13 for crude sewage and final effluent respectively.

Table 4.12: Quantification and detection limits in crude sewage after solid phase extraction

	Required detection	**Limit of quantification with SPE**	**Limit of detection with SPE**
Caffeine	< 180 ng l^{-1}	36 ng l^{-1}	1 ng l^{-1}
Carbamazepine	< 2100 ng l^{-1}	390 ng l^{-1}	376 ng l^{-1}
Tetracycline	< 150 ng l^{-1}	1000 ng l^{-1}	795 ng l^{-1}
Triclosan	< 710 ng l^{-1}	340 ng l^{-1}	86 ng l^{-1}

Table 4.13: Quantification and detection limits in final effluent after solid phase extraction

	Required detection	Limit of quantification with SPE	Limit of detection with SPE
Caffeine	< 180 ng l^{-1}	5 ng l^{-1}	1 ng l^{-1}
Carbamazepine	< 2100 ng l^{-1}	380 ng l^{-1}	372 ng l^{-1}
Tetracycline	< 150 ng l^{-1}	805 ng l^{-1}	760 ng l^{-1}
Triclosan	< 710 ng l^{-1}	200 ng l^{-1}	78 ng l^{-1}

Even with SPE, the quantification limits for tetracycline are above the levels likely to be detected in the environment. The other three pharmaceuticals should be detectable in the environment using SPE before HPLC analysis.

Chapter 5: Materials and Methods

5.1 Source of Materials

All pharmaceutical compounds were purchased from Sigma-Aldrich (Gillingham, United Kingdom). Analytical standards of triclosan and methyl-triclosan were obtained from Greyhound Chromatography (Swansea, UK). Methanol and acetonitrile were of high-performance liquid chromatography (HPLC) grade and were purchased, along with all other common chemicals, from Fisher Scientific (Loughborough, United Kingdom).

5.2 Description of STPs

Four STPs were sampled as part of this work: Northend, Frankton, Southam and Finham. A summary of these STPs is given in Table 5.1, whilst full details can be found in the sections below.

Table 5.1: Summary of study STPs

STP	Primary Treatment	Secondary Treatment	Tertiary Treatment	Average flow (m^3 d^{-1})	Population equivalent
Northend	Septic tank	Rotating biological contactor	Reed beds	103	449
Frankton	Primary sedimentation tank	Biological filters	Polishing lagoon	1134	2,309
Southam	-	Oxidation ditches	-	7145	14,275
Finham	Primary sedimentation tank with iron dosing	Activated sludge plant	-	125,000	494,387

5.2.1 Northend STP

This small STP, serving a population of 449, consisted of two identical rotating biological contactors (RBCs) followed by reed beds. The RBCs had a total surface area of 9249 m^2. The STP served a small village and surrounding area, which contained no known industrial effluent. Samples were obtained from the crude inlet, the settled wastewater tank entering RBC 2, effluent from the RBCs, and final effluent. A flow diagram is shown in Figure 5.1.

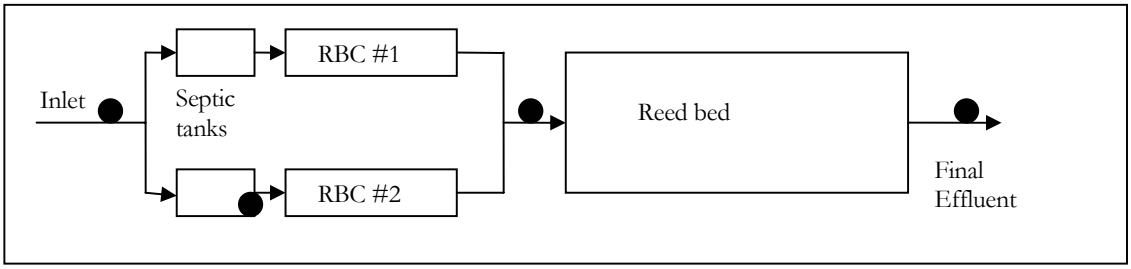


Figure 5.1: Flow diagram of Northend STP, showing sampling points

Sample points are marked on the above figure. The inlet sample was taken from the flow over the inlet v-notch weir. The settled sewage sample was taken from the weir between the septic tank and RBC chamber. The RBC effluent sample was taken in the outlet channel, after the humus tank. Due to the design of the RBC, it was not possible to obtain a sample before the humus tank. The final effluent sample was taken from the Environment Agency sample point.

5.2.2 Frankton STP

This rural STP, serving a population of 2309, consisted of a settling tank followed by two biological filters. These filters had a total volume of 2,256 m^3, containing media with a specific surface area of 100 m^2/m^3. Following this were two humus tanks and a polishing lagoon. The STP served an entirely domestic catchment. Samples were taken from the crude inlet, the settled wastewater tank, humus tank effluent, and final effluent. No sample point was available between the biofilters and the humus tanks (underground pipework). A flow diagram is shown in

Figure 5.2.

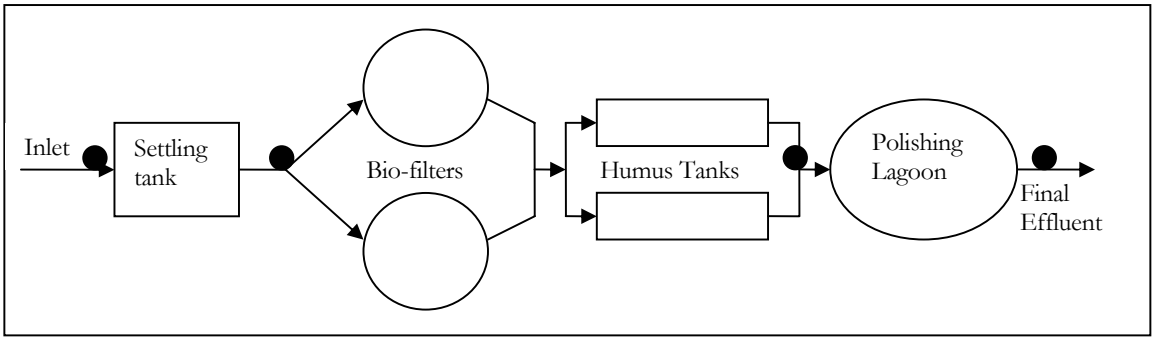


Figure 5.2: Flow diagram of Frankton STP, showing sampling points.

Sample points are marked on the above figure. The inlet sample was taken from the (covered) channel between the screens and the primary settling tanks, with the settled sewage sample taken from the outlet weir of the tanks. The humus tank effluent sample was taken from the combined outlet pipe, and the final effluent sample was taken from the Environment Agency sample point.

5.2.3 Southam STP

This STP treated the waste from three catchments. These catchments were mainly domestic, with a little light industry, coving a total population equivalent of 14,275. The effluent from industry accounted for approximately 2% of the biological oxygen demand (BOD) load. Treatment consisted of screens and grit removal, followed by two oxidation ditches with the flow split equally between them, designed to have a nominal hydraulic retention time (HRT) of 30 hours. After the ditches, the flows recombined and were then split into two final settlement tanks, before a final effluent discharge to a local river. Samples were obtained from the crude inlet (before the screens), the effluent of the oxidation ditch, and the final effluent. A flow diagram is shown in Figure 5.3.

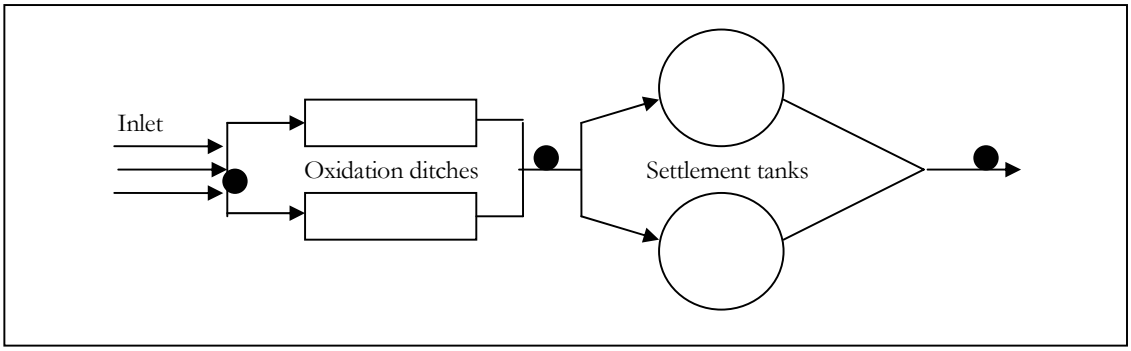


Figure 5.3: Flow diagram of Southam STP, showing sampling points

Sample points are marked on the above figure. The crude sewage sample was taken in the combined inlet channel before the inlet screens. The oxidation ditch effluent sample was taken at the outlet weir, and the final effluent sample was taken from the Environment Agency sample point.

5.2.4 Finham STP

Finham STP was the largest site sampled, serving two catchments with a total population equivalent of 494,387. The STP contains three activated sludge plants (ASPs), each consisting of four aeration lanes with a nominal HRT of 11.8 hours, and each having an initial anoxic zone. Iron is added at the inflow to the activated sludge plants to enhance phosphorus removal. Samples were taken from both influents (Sowe inlet and Sharebourne inlet), the primary settlement tank, the end of the aeration zone, the inflow of the returned activated sludge (RAS), and the final effluent channel. A flow diagram with sampling points is shown in Figure 5.4.

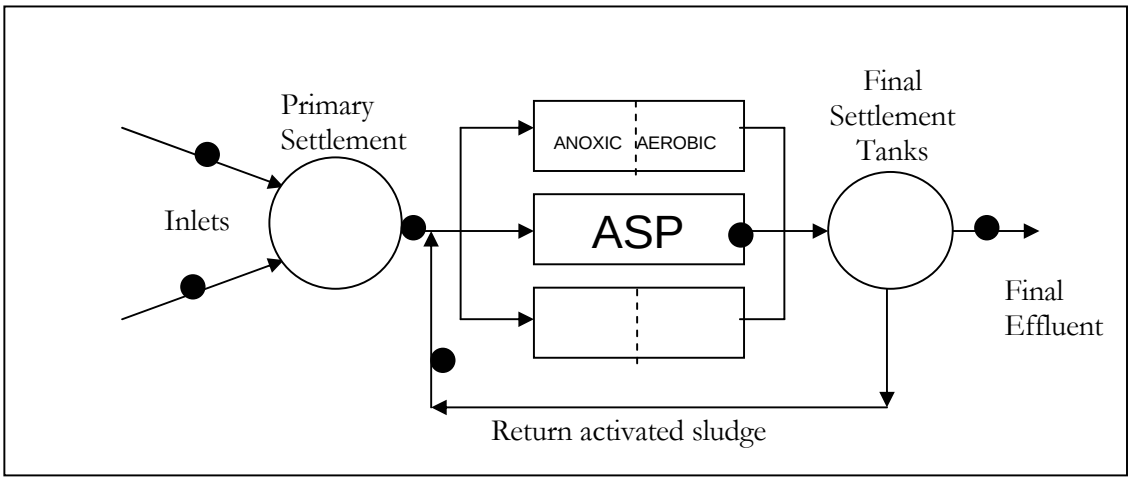


Figure 5.4: Flow diagram of Finham STP, showing sampling points

Sample points are marked on the above figure. The inlet samples were taken in the flow channels immediately after the inlet screens. Settled sewage and RAS samples were taken from the weirs at the chamber where the two flows combined. The ASP effluent was taken from the final zone of lane 4 of ASP 2. The final effluent sample was taken from the Environment Agency sample point.

5.3 Sample Collection and Storage

Two different types of sampling regimes were undertaken: grab sampling, and composite sampling. The grab samples were used to give an indication of the typical pharmaceutical concentrations that were to be found at each STP. The composite samples were used to give a more in depth view of how small changes affected the operation of the STPs.

5.3.1 Grab sampling

Grab samples were taken on seven occasions from Northend, six from Southam and Frankton, and on three occasions from Finham, between February and July 2004. Sampling visits were arranged on an ad-hoc basis, when sampling equipment, laboratory equipment, and Severn Trent staff were available. Samples were taken at approximately the same time of day from each works - Northend: 10-11am, Southam: 11am-12, Frankton: 12-1pm, and Finham: 1 – 2pm. On each sampling occasion, one litre grab samples were collected in an aluminium sample can and transferred into amber glass bottles. On each occasion, single samples were collected from all marked sample points within each STP. All the samples were kept in cool boxes during the sampling campaigns (4-6 ^{o}C) and the samples were delivered to the analytical laboratory within four hours of collection. Samples were immediately passed through glass fibre filters (Scheicher and Schuell) and stored in a refrigerator below 4 ^{o}C prior to extraction, which occurred within 48 hours. Samples were analysed immediately after extraction.

Grab samples were taken to provide comparable effluent quality data as would be collected by regulatory authorities. Due to practical constraints all samples were collected within a one hour period and therefore samples did not necessarily have the same input concentrations since the retention times within each works were about twenty-four hours.

5.3.2 Composite sampling

Composite sampling was undertaken only at Southam STP, between 13th and 15th September 2005. Since equipment and time restrictions only allowed for the sampling of one STP, this site was chosen for two reasons. Firstly, Severn Trent indicated that they were more interested in suspended growth systems, and saw this as the future of sewage treatment. Secondly, of the two suspended growth systems already sampled (Southam and Finham STPs), only Southam STP had flow meters installed, so that mass balances and removals could be calculated.

Samples were collected hourly, for a total of 60 hours from the influent, oxidation ditch, and final effluent of the STP (as previously described) using auto-sampler machines (ISCO model 3700 autosampler). These were set up to take 250ml samples every 15 minutes, producing a one litre composite samples every hour. Every twenty-four hours these samples were transferred to amber glass bottles and returned, on ice, to the laboratory. The samples were then stored in a cold room, at 2 ^{o}C, until they could be processed (within forty-eight hours).

5.4 Description and Operation of rigs

Three pilot scale rigs were operated at the pilot hall at the Cranfield University sewage works. These were used initially to produce biomass for sorption, desorption, and biodegradation studies, as well as for plant operating event tests (porous pots rig only). Biomass was grown using a settled sewage feed from the Cranfield STP.

5.4.1 Rolling Tubes

Rolling tubes are designed to imitate biological filters, such as those found at Frankton STP. As shown in Figure 5.5, the rolling tubes consisted of six hollow acrylic tubes, rotated by a motor at a speed of 20 rpm (as suggested by OECD guideline 303B (OECD 2001)). Each tube was 30.5 cm long and 5 cm internal diameter, supported on rubber wheels within a metal supporting frame. Each tube had an outside lip, 5mm deep, to retain it on the wheels, and a 5mm internal lip to retain the feed liquid. The tubes were supplied with a constant feed of 250 ml hr^{-1} settled sewage. The tubes were inclined to produce a residence time of 125 seconds for the feed in a clean tube. The rig was obtained from previous work at Cranfield University – no

manufacturer details were available. These rates, speeds and times were sufficient to achieve 80% removal of chemical oxygen demand (COD) after two weeks of biomass growth.

Figure 5.5: Picture of rolling tubes rig

The fixed film biomass was allowed to mature for a period of at least two weeks before being collected for use and had reached a steady-state, defined as achieving a level of COD removal greater than 75% for a period of at least seven days. The exact length of time to reach this level depended on the time of year and ambient conditions. This time included at least one complete slough cycle. Biomass was collected by manually scraping off the inside of the rolling tubes, then prepared for use as detailed in sections 5.5.1 and 5.6.1

5.4.2 Rotating biological contactor (RBC)

A pilot scale rotating biological contactor (loaned from Severn Trent Water – manufacturer details were not available), as shown in Figure 5.6, was fed with settled sewage at a flow rate of 120ml per minute, producing an HRT of 30 hours. The rig consisted of 14 plastic discs, with a total surface area of approximately 31 m^2. The discs were rotated at a fixed speed of 2 rpm (not adjustable), and were approximately 40% immersed in the sewage. The unit was manually desludged once per week, by means of a valve underneath the unit (not visible in figure).

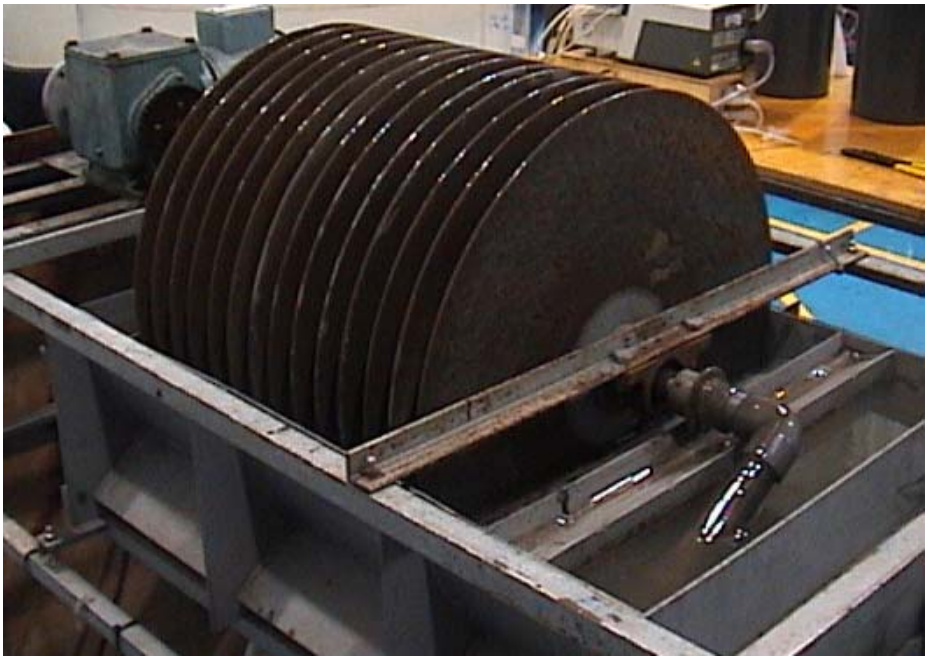


Figure 5.6: Picture of the rotating biological contactor rig

The fixed film biomass was allowed to mature for a period of at least four weeks before being collected for use and had reached a steady-state, defined as achieving a level of ammonia removal greater than 95% for a period of at least seven days. The exact length of time to reach this level depended on the time of year and ambient conditions. This time included at least one complete slough cycle. Biomass was collected by manually scraping off the discs, and then prepared for use as detailed in sections 5.5.1 and 5.6.1 .

5.4.3 Porous Pots

Six porous pots units, as shown in Figure 5.7, were operated with a mean hydraulic retention time of six hours (as specified in OECD guideline number 303 (OECD 2001)). The rig was obtained from previous work at Cranfield University – no manufacturer details were available. The porous pot system consisted of an inner porous polyethylene cylinder with a conical bottom held in a slightly larger vessel of the same shape, made of impervious plastic. Effluents collect in the annular space, and overflow to a drain. No settlement occurred. Aeration was kept constant at a flow rate of one litre per minute. The pots were inoculated with 1 litre of activated sludge from Finham STP to enhance the bacterial communities within the pots.

Figure 5.7: Picture of porous pots rig

In the initial stages after inoculation, the pots had a tendency to become blocked. When this occurred, the contents of the pot were poured into a clean liner. Blocked liners were rinsed and then soaked in dilute sodium hypochlorite solution until next required. The liners were rinsed before use to remove any remaining hypochlorite solution.

The insides of the pot liners were scraped twice daily to minimise the growth of fixed film biomass. Biomass was only collected after the rigs has reached a steady-state, defined as achieving a level of ammonia removal greater than 95% for a period of at least seven days. The biomass was collected for use in sorption and biodegradation tests, and prepared as described in sections 5.5.1 and 5.6.1 .

5.5 Sorption and desorption tests

Sorption and desorption tests with fixed film and suspended growth biomasses was conducted according to OECD guideline number 106 (OECD 2000) as described in the sections below, to assess the amount of each pharmaceutical that partitioned into the biomass.

5.5.1 Preparation of biomass

Biomass samples for the tests were collected immediately prior to use from the three pilot scale rigs. Biomass from the RBC and rolling tubes, simulating fixed film biomass, was scraped off the plastic media into glass containers. Biomass from the porous pots, simulating suspended growth biomass, was decanted into bottles and centrifuged (10g, 5 minutes) to remove excess liquid. Both types of biomass were lyophilised for forty-eight hours, and then desiccated for an hour at 105 °C. This process was selected not only to remove all water from the samples, but also to prevent any biodegradation during the sorption tests. Finally, samples were ground to a fine uniform powder before use, to reduce any effect that variation in particle size might have on sorption.

5.5.2 Test procedure

200ml of the test pharmaceutical, at an initial concentration of 20 mg l^{-1} in 0.01 M $CaCl_2$, were added to biomass soils of known dry weight. This volume was chosen since it was the maximum capacity of the centrifuge. The initial pharmaceutical concentration was chosen based on the analysable range (without SPE), which was as low as 1 μg l^{-1} in the worst case (tetracycline). Since the OECD guideline aimed to achieve 90-95% sorption for a reliable test, the final concentration needed to be detectable. Assuming 90% sorption, a safety factor of 10, and a final aqueous concentration of 1 μg l^{-1}, gave a lowest starting concentration of 0.1 mg l^{-1}.

The OECD guideline selects soil to solution ratios for sorption testing according to the octanol-water partition coefficient (K_{ow}). However, the expected distribution coefficient (K_d) of the pharmaceutical is generally thought to be a better descriptor of sorption than K_{ow}. Therefore, where K_d was not known (caffeine), the soil to solution ratio was chosen based on the K_{ow} value, according to the OECD guidelines. For the remaining three compounds, the

soil to solution ratios were chosen according to K_d values, as shown in Table 5.2. Due to the practicalities of measurement and container size, it was not possible to use a soil to solution ratio of below 0.1 mg cm^{-3}. Similarly, due to the amount of available biomass, it was not practical to use a soil to solution ratio above 20 mg cm^{-3}.

Table 5.2: Soil to solution ratios

Pharmaceutical	Soil to solution ratio (mg cm^{-3})	Log K_{ow}	K_d	Reference
Caffeine	20	-0.07	-	ChemIDplus
Carbamazepine	0.5	2.45	0.02552	Jones *et al.* (2002)
Tetracycline	0.25	-1.3	1.620	Sithole and Guy (1987)
Triclosan	0.1	4.8	16 22	Singer *et al.*(2002) Orvos *et al.* (2002)

The mixtures of biomass and pharmaceutical solution were agitated, with magnetic stirring bars, for 48 hours. Light was excluded to avoid the potential for degradation by photolysis. After 1, 2, 4, 8, 12, 24, and 48 hours, the test vessels were centrifuged (10.3g, 5 minutes) and 1ml samples of the supernatant were taken. These samples were then measured for their pharmaceutical content by HPLC. All tests were run in duplicate, at a range of pharmaceutical concentrations, typically 0.1, 1, 2, 5, 10, and 20 mg l^{-1}. In addition two control samples were run. The first contained the test pharmaceutical, but no biomass. Any loss of pharmaceutical in this sample would suggest sorption to the container. A second control contained biomass in deionised water, and no pharmaceutical. Any pharmaceutical concentrations detected in this sample could be assumed to have desorbed from the biomass, and hence interfere with the sorption test. This was thought to be likely for caffeine and triclosan.

Immediately at the end of the sorption test, the test vessels were centrifuged (10.3g, 15 minutes), and all liquid removed. This was replaced by 200ml of 0.01 M $CaCl_2$ (no pharmaceutical content), for the desorption test. The mixtures were agitated, and samples taken in an identical manner to the sorption tests. However, whenever a sample was taken, an identical volume (1ml) of pure $CaCl_2$ was added, to ensure the total volume remained constant throughout the test.

5.5.3 Data analysis

From these results, the sorption and desorption isotherms were plotted, and attempts were made to fit the data to both the Freundlich and Langmuir models, although as discussed later, correlations were only made using the Freundlich model. The Freundlich adsorption equation is shown in Equation 8 below.

Equation 8

$$C_s^{ads} = K_F^{ads} (C_{aq}^{ads})^{\frac{1}{n}}$$

where:

C_s^{ads} is the concentration of pharmaceutical adsorbed to the biomass ($\mu g\ g^{-1}$)

C_{aq}^{ads} is the concentration of pharmaceutical remaining in the aqueous phase ($\mu g\ cm^{-3}$)

K_F^{ads} is the Freundlich adsorption coefficient

n is the regression constant; 1/n generally ranges between 0.7-1.0 indicating nonlinearity

When n=1, the Freundlich adsorption coefficient is equal to the distribution coefficient, K_d. The results of the tests for each pharmaceutical with each biomass were plotted on a linearized plot, and the correlation coefficient r^2 of the log equation was calculated.

To compare the effects of different biomasses, first the correlation coefficients for each set of pharmaceutical and biomass was calculated. Then, the datasets were combined, and the correlation coefficients were recalculated. If the coefficient for the combined set was higher than or equal to the individual datasets, then it could be concluded that the different biomasses had no effect on sorption. However, if the correlation coefficient for the combined set was lower than for the individual sets, it could be concluded that the different biomasses did have an effect on sorption. Previous researchers comparing sorption isotherms have merely compared individual Freundlich adsorption coefficients to infer difference (Celis *et al.* 1998; Mesquita *et al.* 2002; Williams *et al.* 2006; Yu *et al.* 2004), without any statistical test. No statistical tests are suggested in the OECD guidelines for these tests (OECD 2000).

5.6 Biodegradation tests

Biodegradation tests were conducted, according to OECD guideline 301D - closed bottle test (OECD 1992), to assess the amount of each pharmaceutical that is removed by degradation during sewage treatment.

5.6.1 Preparation of biomass

Biomass was collected immediately prior to use in the tests. Fixed film biomass was scrapped off the discs of the RBC rig then centrifuged (10.3g, 5 minutes) and all supernatant removed. Suspended growth biomass was collected by emptying the entire contents of one of the porous pot units into a container. This was allowed to settle for ten minutes and excess water was then removed. Finally, the biomass was centrifuged (10.3g, 5 minutes) and all supernatant removed.

5.6.2 Test procedure

Test pharmaceutical were diluted in a mineral medium (as specified in the guidelines) to produce an initial concentration of 5 mg l^{-1}, as required by the guidelines, and aerated until oxygen saturation was reached (about 20 minutes). Biomass was added to produce a solids concentration of 5 g SS l^{-1}. The tests were conducted in darkness at a constant temperature of 20 ^{o}C.

It should be noted that the concentration of 5 mg l^{-1} used in these tests is higher than would be expected in the environment for all test compounds. Whilst this was not expected to cause a problem for caffeine and carbamazepine, there was the potential for triclosan and tetracycline to inhibit biodegradation since their primary use is as antibacterial and antibiotic respectively. Voets *et al.* (1976) had previously conducted the same tests for triclosan at concentrations between 1 and 5 mg l^{-1} and noted no degradation. Federle *et al.* (2002) suggested this result may have been due to the antimicrobial nature of triclosan, but then went on to show over 98% removal of an influent concentration of 2 mg l^{-1}. To confirm biological activity was not inhibited, oxygen concentrations were measured at each sample point, and compared for all four pharmaceuticals. If a difference between the oxygen usages were to be found, it could be suspected that some inhibition had occurred.

Tests were conducted in 250ml BOD bottles, with duplicate bottles prepared for each sample point: 7, 14, 21, 28, 56, 84 and 112 days. At each sample point, the dissolved oxygen concentration was measured (with a Hanna HI 8424 DO meter (Hanna Instruments, Leighton Buzzard, UK)) and a liquid sample taken for HPLC analysis. For substances containing nitrogen (tetracycline, caffeine), nitrate and nitrite concentrations were also measured to account for oxygen usage. For nitrate and nitrite detection method, please see section 5.8.4 . The tests were abandoned if the dissolved oxygen level fell below 0.5 mg l^{-1}, as this could have limited the biological activity.

5.6.3 Data analysis

From the results of the sorption tests, the amount of pharmaceutical sorbed to the biomass could be established from the Freundlich isotherms. Therefore, if the amount of pharmaceutical remaining in the aqueous phase was lower than that predicted by the sorption tests, it could be concluded that degradation had occurred. The amount of degradation was plotted against time, and the rate of degradation per unit biomass was calculated according to Equation 9 (Schwarzenbach *et al.* 2003).

$$\frac{dC}{dt} = K_{biol} C_0 SS$$ **Equation 9**

5.7 Dynamic reactor studies

Dynamic reactor studies were conducted to establish the fate of the four selected pharmaceuticals (caffeine, carbamazepine, tetracycline, and triclosan) when certain common events occurred. These events were aeration failure, and heavy rainfall. In both cases, the simulations were conducted using the porous pot units, to simulate the effect in activated sludge plants.

The six pots were split in to three pairs, all fed with real sewage. The first pair was used as a process control to detect the diurnal variation caused by changes in the feed makeup. The remaining two pairs had a high concentration (low volume) feed of pharmaceutical added. One of these pairs was used as a pharmaceutical control, to see how diurnal variations affected the fate of the pharmaceuticals, whilst the final pair was used as for the tests. The pharmaceutical was introduced to the pots at a concentration of 3000 μg l^{-1} in methanol at a flow rate of 0.01

ml min^{-1}. This was combined with a sewage feed of 6 ml min^{-1}, to produce an overall pharmaceutical concentration of 5000 ng l^{-1}. Assuming a best-case scenario of up to 99% removal of triclosan and caffeine, and up to 90% of carbamazepine and tetracycline, this influent concentration would ensure that there was sufficient pharmaceutical remaining in the effluent to be above the limit of detection.

5.7.1 Aeration failure

Two aeration loss tests were conducted, for lengths of one and four hours. These were considered to be typical periods for which aeration may be lost at STPs, depending on their staffing levels and back-up systems (e.g. electrical generators).

100ml samples were taken every 30 minutes for one hour preceding the test, every 30 minutes throughout the aeration loss, and every 30 minutes for four hours after the test, from the influent and effluent of all six porous pots. Temperature, pH, DO, redox potential, COD, ammonia, and pharmaceutical concentrations and degradates (where possible) were measured. For the methods used for each of these parameters, please see section 5.8 . The results from each pair of pots were averaged for each parameter.

5.7.2 Rainfall event

A rainfall event was conducted by adding a water feed to the pair of test pots, at a flow rate of 6 ml min^{-1} for a period of four hours. This simulated a doubling of the flows. This increased the overall dilution of the feed, but also decreased the hydraulic retention time. Samples were taken in the same manner as for the aeration failure test.

Apart from dilution, storm flows have other properties that could affect the removal of pharmaceuticals. These include an initial high ammonia spike, followed by an increase in suspended solids (Lawler *et al.* 2006). These properties were not simulated in these tests, due to a lack of time and resources.

5.8 Wastewater Characterisation

The wastewater samples that were collected were characterised to allow determination of the differences in wastewater composition that could affect sorption and biodegradation of the test pharmaceuticals.

5.8.1 Preparation of solids free fraction

For determination of COD, and soluble proteins and carbohydrate, a preparation of biomass fraction free of suspended solids is required. The solid free fractions were prepared by centrifuging samples for 20 minutes at 10.3g in a Rotanta 96 R centrifuge (Hettich Zentrifugen, Germany). The supernatant was decanted and filtered through glass fibre filter paper (Patterson Scientific, Bedfordshire, UK) to remove any residual suspended particles.

5.8.2 pH, temperature, and redox potential

Temperature, redox potential, and pH were measured using a HANNA HI8424 pH meter (Hanna Instruments, Leighton Buzzard, UK). The meter was calibrated before use at pH 4, 7, and 10 using standard buffer solutions (VWR International Ltd).

5.8.3 Chemical oxygen demand (COD)

The COD was determined using Merck Spectroquant COD cell test (range 25-1500 mg l^{-1} COD). A 2 ml sample of solid free supernatant was added to the cell test vial, shaken, and then heated at 150°C for 2 hours. After cooling to room temperature the COD value (mg l^{-1}) was measured in a Spectroquant Nova 60 Spectrophometer. Where necessary, samples were diluted to ensure the measured value was within the range of the test kit.

5.8.4 Nitrate, Nitrite, Phosphorus, and Sulphate

Nitrate, nitrite, phosphorus, and sulphate were measured using a Dionex Ion Chromatograph (Dionex, UK). The column was an Ion-Pac AS9-HC, with an eluent of 9 mM sodium carbonate at a flow rate of 1 ml per minute. The ions were detected with suppressed conductivity using an ATLAS suppresser operated at 58 mA.

5.8.5 Ammonia

Ammonia concentration was determined using Merck Spectroquant ammonia cell test (range 0.2-8.0 or 4.0-80 mg l^{-1}). A sample (0.1 ml or 1 ml respectively) of solid free supernatant was added to the cell test vial with 1 dose of reagent. Colour was left to develop for 15 minutes, and the ammonia concentrations (mg l^{-1}) were measured in a Spectroquant Nova 60 Spectrophometer. Where necessary, samples were diluted to ensure the measured value was within the range of the test kit.

5.8.6 Soluble protein

Soluble protein concentrations were determined by using the Bradford method (Bradford 1976). A 1 ml sample of suspended solid free supernatants is added to 1 ml of Bradford reagent (Sigma – Aldrich, Gillingham, UK). After 20 minutes, the sample was diluted with 2 ml deionised water. The absorbance was measured against a blank at 595 nm in a Jenway 6505 UV / Visible Spectrophotometer and results were calculated from a calibration curve obtained from protein standard bovine serum albumin.

5.8.7 Soluble carbohydrate

Soluble carbohydrate concentrations were determined by the phenol – sulphuric acid method (Dubois *et al.* 1956). A 0.4 ml sample of suspended solid – free supernatant was added to 0.4 ml of 5% (w/w) phenol solution (Sigma – Aldrich, Gillingham UK) and then mixed with 2 ml concentrated sulphuric acid (98% / 1.84 Specific Gravity (Fisher Chemicals, Loughborough, UK)). Colour was allowed to develop for 10 minutes before transfer to cuvette and absorbance measurement against a blank at 480 nm (Jenway 6505 UV / Visible Spectrophotometer). Carbohydrate concentration (mg l^{-1}) was calculated from a calibration curve constructed using a glucose standard.

5.9 Biomass Characterization

The biomass samples that were collected were characterised to allow determination of the differences in biomass composition that could affect sorption and biodegradation of the test pharmaceuticals.

5.9.1 Preparation of Extracellular Polymeric Substances (EPS)

Extracellular substances were extracted based on the heating method of (Zhang *et al.* 1999). 50 ml of each oxidation ditch sample were centrifuged at 4.6 g for 5 minutes and the supernatant decanted. 50 ml DI water was added to the sludge pellet and then hand shaken and placed into an oven (60 min at 105°C). The bottles were then centrifuged at 7500 rpm for 5 min at room temperature. Finally, the supernatant was filtrated through glass fibre filter paper (Patterson Scientific, Bedfordshire, UK). The obtained supernatants are used for determination of extracellular protein and extracellular carbohydrate.

5.9.2 Total carbon, inorganic carbon, and organic carbon

Total carbon and inorganic carbon were measured using a Shimadzu TOC-5000A analyser (Shimadzu, Milton Keynes, UK). Total organic carbon was then calculated from those values.

5.9.3 Suspended solids

Suspended solids (SS) content was determined by standard method 2540D (APHA 1998). Glass fibre filter papers (Patterson Scientific, Bedfordshire, UK) were ignited in a furnace at 550 ^{o}C for one hour and cooled in a desiccator until needed, then weighed immediately prior to use. Well-mixed samples were filtered under vacuum through the filter papers, using a volume chosen to yield a dried residue between 2.5 and 200 mg. The filter papers were then dried overnight in an oven at 105 ^{o}C, cooled in a desiccator and reweighed, and the suspended solids concentration calculated.

5.9.4 Volatile suspended solids

Volatile suspended solids (VSS) content was determined by standard method 2540E (APHA 1998). The dried filter papers obtained from the measurement of the suspended solids were ignited at 550 ^{o}C for two hours in a furnace and then cooled in a desiccator. The filter paper was re-weighed and the volatile suspended solids concentration calculated.

5.9.5 Lipid content

The lipid content of sludges were determined according to standard method 5220E (APHA 1998). Twenty grams of centrifuged sludge (10.3g, 5 minutes) were mixed with 25 g of magnesium sulphate monohydrate and left for thirty minutes until solidified. The mixture was

then ground into a fine powder and added to an extraction thimble in a soxhlet extraction apparatus. The fat was extracted for four hours at a rate of 20 cycles per hour, using 100 ml of solvent (80% n-hexane, 20% MTBE). After four hours, the solvent was recovered, and the amount of lipids extracted was calculated.

5.9.6 Extracellular Protein

Extracellular protein was determined using a protein diagnostic kit (Sigma – Aldrich, Gillingham, UK). 0.2 ml of sample was mixed with 2.2 ml Biuret reagent and kept for 10 min at room temperature. Then 0.1 ml of Folin and Ciocalteu's Phenol reagent was added and colour allowed to develop for 30 minutes at room temperature. The samples are transferred to cuvettes and measured against a blank at 595 nm (Jenway 6505 UV/VIS). The concentration was calculated from a calibration curve obtained from protein standard.

5.9.7 Extracellular carbohydrate

Extracellular carbohydrate concentrations were determined by the phenol – sulphuric acid method (Dubois *et al.* 1956). A 0.4 ml sample of suspended solid – free supernatant was added to 0.4 ml of 5% (w/w) phenol solution (Sigma – Aldrich, Gillingham UK) and then mixed with 2 ml concentrated sulphuric acid (98% / 1.84 Specific Gravity (Fisher Chemicals, Loughborough, UK)). Colour was allowed to develop for 10 minutes before transfer to cuvette and absorbance measurement against a blank at 480 nm (Jenway 6505 UV / Visible Spectrophotometer). Carbohydrate concentration (mg l^{-1}) was calculated from a calibration curve constructed using a glucose standard.

5.9.8 Particle size

Sludge particle sizes were measured using the Malvern Mastersizer 2000 particle analyser (Malvern Instruments Ltd, Worcestershire, UK). The mastersizer uses an optical unit to detect the light scattering pattern of sludge particles dispersed in deionised water. Diluted sludge suspensions are circulated through a measurement cell where the particle fields are exposed to a laser beam. The pattern of light scatter in analysed using Mie theory to calculate the particle sizes. Sludge samples were added to the deionised water tank supplying the particle suspension to the measurement cell until the laser obscuration was between 10 and 20%. The stirrer was

set at 350 rpm. The following parameters were recorded: mass median diameter, volume mean diameter, surface area mean diameter, and specific surface area.

5.10 Summary of HPLC and Solid Phase Extraction (SPE) conditions

The steps taken in the development of the HPLC and solid phase extraction methods has been discussed in the previous chapter. Table 5.3 shows a summary of these conditions (limit of detection refers to parent compound only).

Table 5.3: Summary of HPLC and SPE conditions

	Caffeine	**Carbamazepine**	**Tetracycline**	**Triclosan**
Liquid Chromatograph	Shimadzu LC 10AD	Shimadzu LC 10AD	Shimadzu LC 10AD	Shimadzu LC 10AD
SPE cartridges [recovery %]	Strata-X [95%] (Phenomenex, UK)	Strata-X [101%] (Phenomenex, UK)	Oasis HLB [100%] (Waters, UK)	Oasis HLB [94%] (Waters, UK)
Column	Kromasil 5µm C4 250 x 4.0 mm ID (Phenomenex, UK)	Gemini 5µm C18 150x4.6 mm ID (Phenomenex, UK)	Gemini 5µm C18 150x4.6 mm ID (Phenomenex, UK)	Gemini 5µm C18 150x4.6 mm ID (Phenomenex, UK)
Injection volume	10 µl	10 µl	10 µl	10 µl
Mobile Phase	Acetate buffer (pH 3.5), methanol at 97:3 v/v changing to 80:20 in 20 minutes	22% Acetonitrile 78% 7mM Sodium acetate (pH 5.4)	64% 0.2M Oxalic acid 18% Acetonitrile 18% Methanol	52.5% Water 47.5% Acetonitrile
Flow rate	1 ml min^{-1}	2 ml min^{-1}	1 ml min^{-1}	2 ml min^{-1}
UV detection	275 nm	240 nm	400 nm	235 nm
Elution times (minutes)	Caffeine: 19.2 Theophylline: 12.6 Paraxanthine: 11.7 1,7-DMU[1]: 9.6 Theobromine: 7.1 7-Methylxanthine: 4.8	Carbamazepine: 10.7 10,11-DHCBZ[2]: 11.3	Epi-tetracycline: 2.5 Tetracycline: 2.8 Epi-anhydrotetracycline: 7.0 Anhydrotetracycline: 9.0	Triclosan: 16.2 Methyl-triclosan: 31.6
Limit of quantification (Crude) after SPE	36 ng l^{-1}	390 ng l^{-1}	1000 ng l^{-1}	340 ng l^{-1}
Limit of detection (Crude) after SPE	1 ng l^{-1}	376 ng l^{-1}	795 ng l^{-1}	86 ng l^{-1}
Limit of quantification (Final effluent) after SPE	5 ng l^{-1}	380 ng l^{-1}	805 ng l^{-1}	200 ng l^{-1}
Limit of detection (Final effluent) after SPE	1 ng l^{-1}	372 ng l^{-1}	760 ng l^{-1}	78 ng l^{-1}

1: 1,7-DMU = 1,7 Dimethyluric acid
2: 10,11-DHCBZ = 10,11-Dihydrocarbamazepine

Chapter 6: Fate of pharmaceuticals in laboratory tests

As discussed in the literature review, there are four main mechanisms that remove substances from the sewage influent stream, preventing their discharge in the liquid final effluent. Of these, only two are generally relevant for the removal of pharmaceuticals: sorption and biodegradation (Ternes *et al.* 2004). These two mechanisms were investigated for each of the four pharmaceuticals, by use of laboratory sorption and biodegradation tests. The results of these tests were used to improve the parameterisation of the Toxchem+ fate model.

6.1 Sorption and desorption tests

As described in the materials and methods chapter, sorption and desorption tests were conducted according to OECD guideline number 106 (OECD 2000), to assess the amount of each pharmaceutical that partitioned into the biomass, both fixed film and suspended growth. These are important to be able to determine optimum mixed liquor suspended solids (MLSS) concentrations, and hence the maximum removal achievable. The difference between the sorption and desorption isotherms will allow an indication of what will happen to pharmaceuticals under cases of variable influent concentrations (e.g. diurnal variations, or storm events). Significantly, the rate at which sorption occurs can be determined from the sorption tests, which will allow for an assessment of the minimum HRT required for removal by sorption.

6.1.1 Triclosan

Sorption tests were conducted with triclosan with samples of fixed film (one test each with biomass from the RBC and rolling tubes rigs) and suspended growth biomass (two tests with biomass from the porous pots) at a soil to solution ratio of 0.1 mg cm^{-3}. The Freundlich sorption isotherms obtained from the combined results are shown in Figure 6.1.

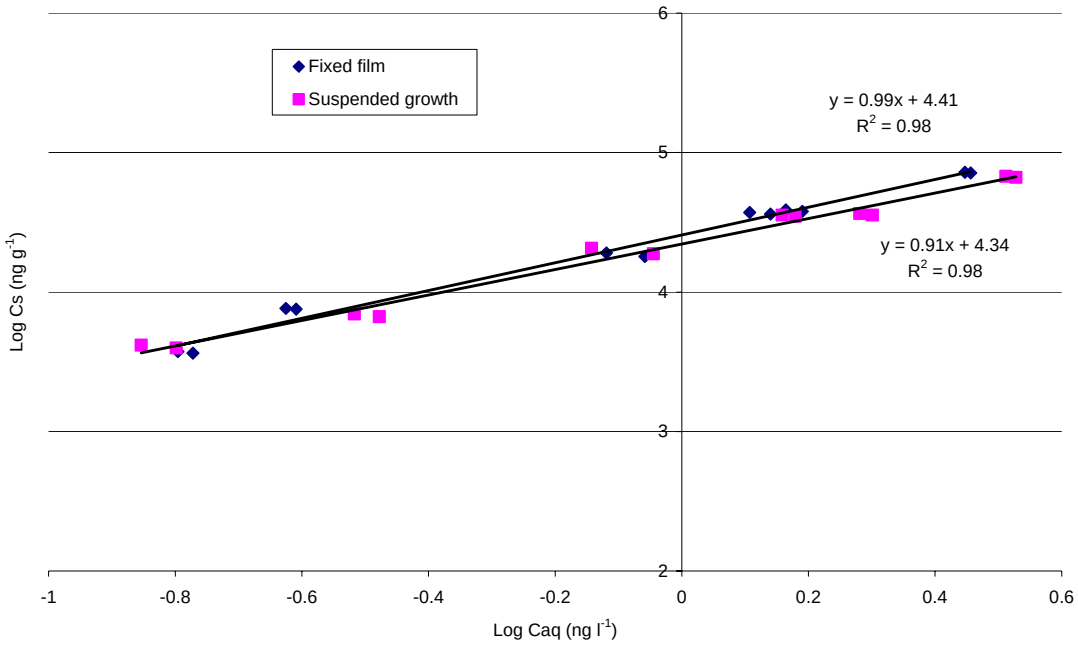


Figure 6.1: Freundlich sorption isotherms for triclosan with fixed film and suspended growth biomass

Triclosan was rapidly and strongly sorbed by the biomass, reaching sorption equilibrium in around four hours. Since triclosan has a high octanol-water partition coefficient (log K_{ow} = 4.8), it was expected that it would partition very strongly into the biomass, which these sorption isotherms show, with around 70% of the triclosan sorbing to the biomass. The regression coefficient is close to unity for both types of biomass, suggesting that triclosan is absorbed into the bulk of the biomass, meaning that desorption will be limited. Individually, the two biomasses both had correlation coefficients (r^2) of about 0.98. Combining the results for the biomasses produced a group with a correlation coefficient which was also 0.98. This suggested that sorption of biomass is independent of variation in the biomass.

The distribution coefficient (K_d) and organic carbon partition coefficient (K_{oc}) values were in the ranges 21483 to 24071, and 55084 to 61720 respectively. These K_d values are broadly consistent with the value of 21592 previously published by Orvos *et al.* (2002) for an activated sludge sample. This small difference between the K_d values for the two types of biomass suggests that the effect of differences in biomass do not affect the sorption of triclosan.

The results of desorption tests for triclosan with both suspended and fixed film biomass are shown in Figure 6.2.

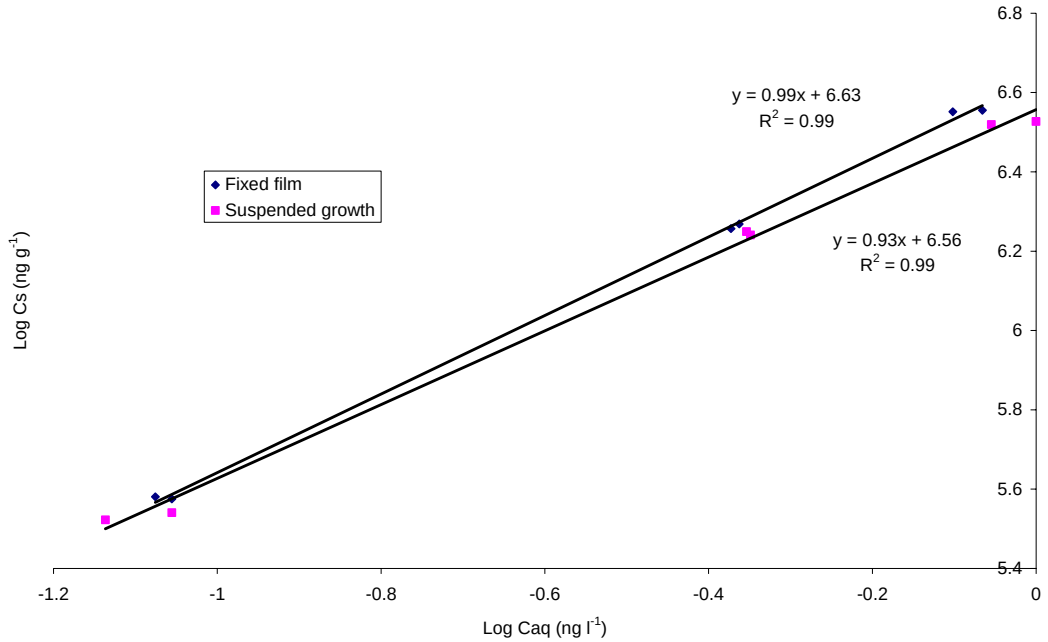


Figure 6.2: Desorption Freundlich isotherms for triclosan with fixed film and suspended growth biomass

As for the sorption tests, the Freundlich desorption isotherms are very similar for both types of biomass, with the correlation coefficient for the combined data set of 0.99. This suggested that desorption of triclosan was independent of biomass makeup. Since the Freundlich desorption coefficients (log K_f^{des} = 6.63) are two orders of magnitude greater than the Freundlich sorption coefficients (log K_f^{ads} = 4.41), little desorption is expected to occur. For example, if an STP were to suddenly switch from an influent condition of 1000 ng l^{-1} of triclosan, to an influent condition of no triclosan (which could be due to diurnal variation or a heavy storm flow), and keeping a steady MLSS of 3000 mg l^{-1}, then only around 0.01% of the sorbed triclosan (or around 0.1 ng) would be desorbed back into the liquid phase.

Figure 6.3 shows the profile of triclosan sorption in a standard sorption test starting at 10 mg l^{-1}.

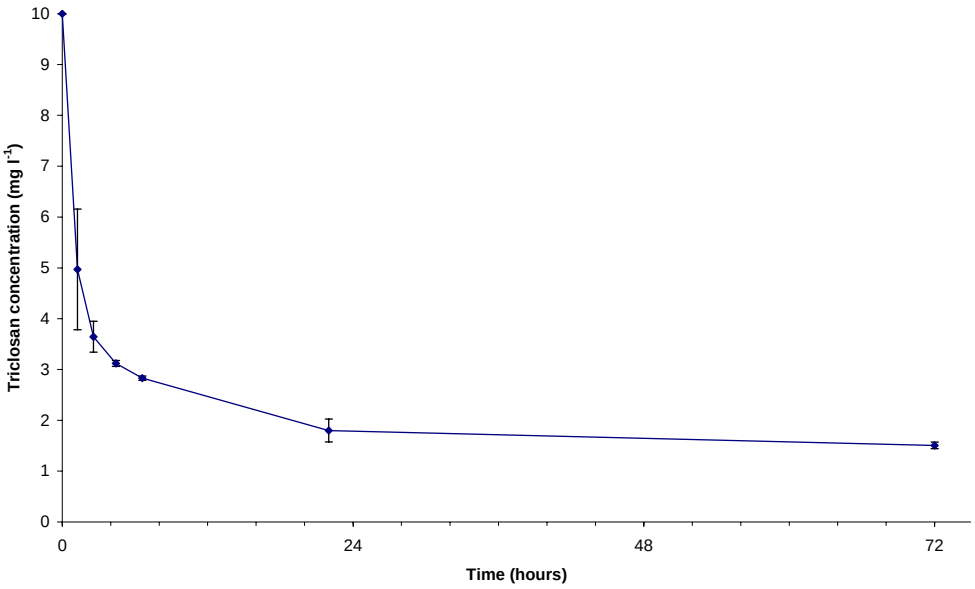


Figure 6.3: Profile of triclosan sorption, from an initial concentration of 10 mg l^{-1}

From this figure it can be seen that over 80% of the total sorption occurs within the first four hours of the test, and around 95% within 20 hours. There was no measurable difference in the rates of sorption to the different types of biomass. Depending on the effluent concentrations required, and the amount of biodegradation that may occur, then this information can be used to determine the optimum HRT. In the worst-case scenario, where no degradation occurs, and a tight discharge consent were to be imposed, the maximum possible amount of sorption would be required. From this data, it can be suggested that an HRT of around 24 hours would be suitable for this condition.

These results suggest that sorption is a very important removal mechanism for triclosan (log K_d 4.33 - 4.41), with sorption occurring rapidly (>80% in four hours). If triclosan does not biodegrade, then it is likely to remain contained in the sewage sludges, with the potential for transportation into the environment via sludge reuse. However, triclosan is unlikely to be washed off the sludge by rainfall, although the potential for uptake by crops could be an issue.

6.1.2 Tetracycline

Few pharmaceuticals have such a high octanol-water partition coefficient as triclosan, and it was therefore expected that a greater difference between fixed film and suspended growth biomasses would be seen with a compound with a lower K_{ow}, such as tetracycline (log K_{ow} = 1.33). Figure 6.4 shows the Freundlich sorption isotherms obtained for tetracycline with samples of the two types of biomass (three suspended growth tests, four fixed film tests (two RBC biomass, and two rolling tubes biomass)).

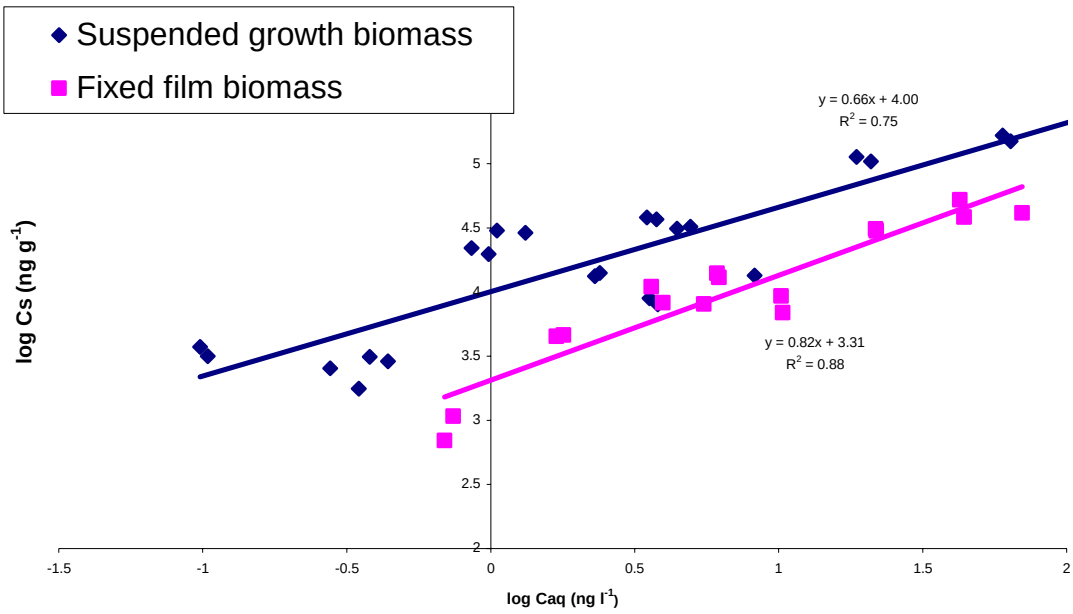


Figure 6.4: Freundlich sorption isotherms for tetracycline with fixed film and suspended growth biomass

Several differences are immediately obvious between the results for tetracycline when compared to the previous results for triclosan. There is a much greater difference between the results for the two biomasses, and the regression constants are much lower (0.66 – 0.82), suggesting that adsorption (to the surface of the biomass rather than absorption into the bulk of the biomass) is the prevalent mechanism. This is an important result for two reasons. First, it suggests that tetracycline could be desorbed easily from biomass. Possibly the more important implication, however, is that as tetracycline concentrations increase within a system with a fixed amount of biomass, the total percentage of tetracycline removed by sorption will decrease. For example (at a suspended growth biomass concentration of 3000 mg l^{-1}), at an influent concentration of 1000 ng l^{-1}, 77.4 % of tetracycline would become sorbed to the biomass, but at 10,000 ng l^{-1} only 21.4 % would be removed by sorption. This type of information could be critical for controlling STPs where

large fluctuations are seen in the load of pharmaceuticals entering the plant. Failure to take this non-linearity into account, as assumed when used distribution coefficients (K_d) can lead to gross overestimates of removal. For example, using the same conditions above, setting $K_d = K_f$ and n=1, would suggest 94.4% removal of tetracycline at an influent concentration of 1,000 ng l^{-1}, and 60.0% at an influent concentration of 10,000 ng l^{-1}. It should be noted that there is no facility to include this non-linearity in modelling packages such as Toxchem+.

There is also quite a spread of data away from the regression lines, producing lower correlation coefficients (r^2 = 0.75 for suspended growth biomass, and 0.88 for fixed film biomass) than were seen for triclosan. However, the correlation coefficient for the combined dataset was much lower, at 0.57, suggesting that there is a significant difference between the two biomasses. This difference is further exposed when the results for each type of biomass are separated out. For example, Figure 6.5 shows the Freundlich sorption isotherms for tetracycline with two different suspended growth biomasses. The first was from a standard activated sludge system (Southam STP), whilst the second sample was taken from an activated sludge system that has iron added to it for phosphorus removal (Finham STP).

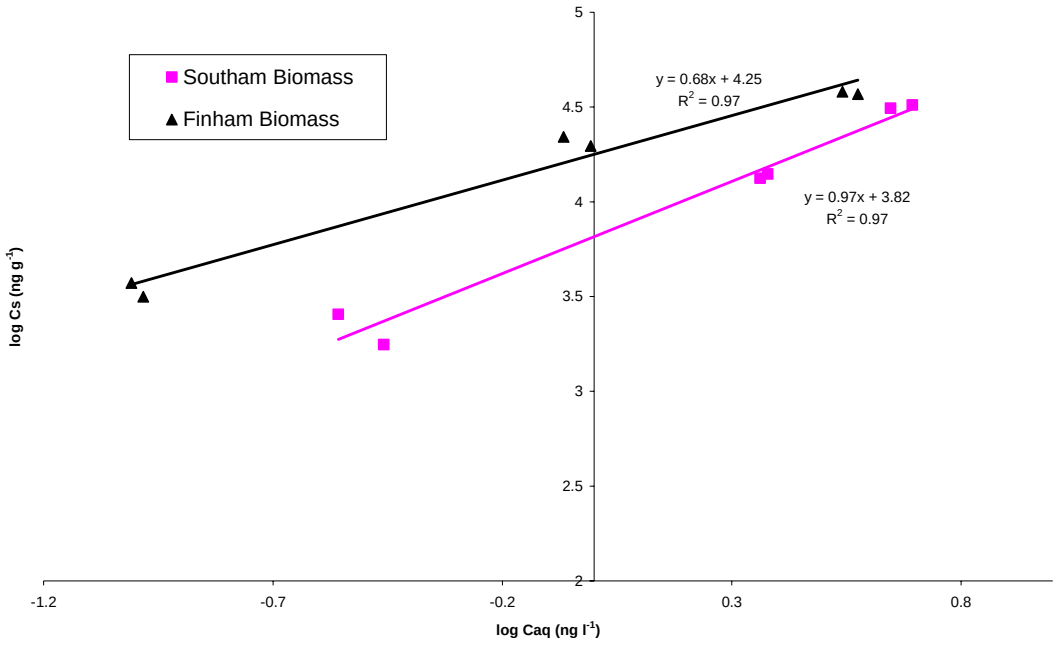


Figure 6.5: Freundlich sorption isotherms for tetracycline with two different samples of suspended growth biomass

Since the correlation coefficients are both datasets are greater in this figure (r^2 = 0.97) than in the previous figure with the combined dataset (r^2 = 0.75), this suggests that there are significant differences between the two different samples of suspended growth biomass.

Since it is well known that tetracyclines form complexes with metal ions, such as the iron added to the Finham STP biomass, it should not be surprising that greater sorption was seen with this biomass. However, this does demonstrate that differences in the biomass makeup can cause a significant change in the amount of sorption of tetracycline that can occur. For example, at a biomass concentration of 3000 mg l^{-1} and a tetracycline influent concentration of 1000 ng l^{-1}, the best suspended growth biomass tested here (Southam STP) would remove 87.5%, whilst the average fixed film biomass would remove only 65.7%.

A typical desorption isotherms for tetracycline are shown in Figure 6.6 below.

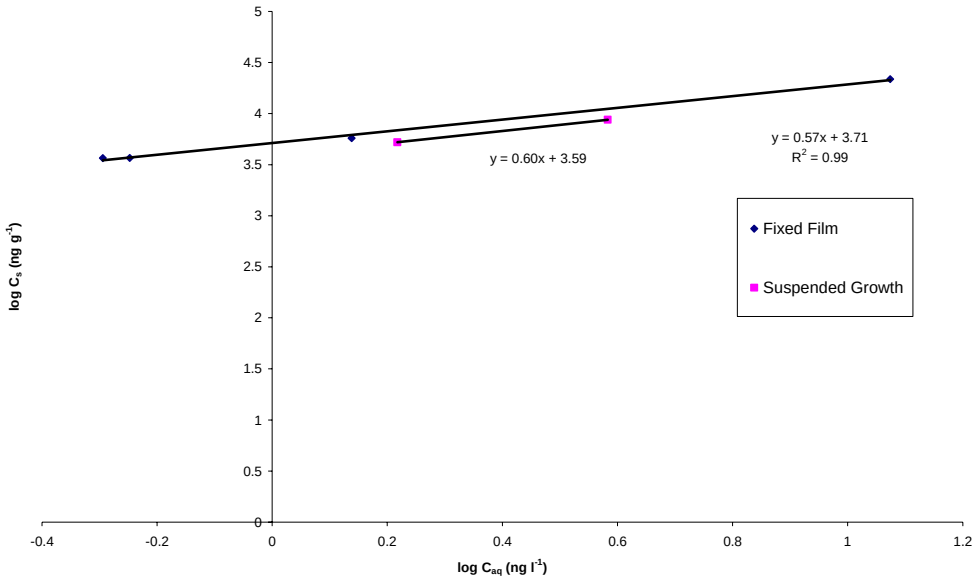


Figure 6.6: Freundlich desorption isotherms for tetracycline with two different samples of suspended growth biomass

As can be seen from the above figure, desorption tests for tetracycline with both suspended growth and fixed film biomass gave virtually identical Freundlich desorption isotherms with constants (log K_F) of 3.71 and 3.59 respectively. Due to difficulties in performing the tests, insufficient data was collected on the desorption of tetracycline from

suspended growth biomass to determine categorically that the two biomasses behave in the same way.

The difference between the sorption (log K_f = 3.31) and desorption (log K_f = 3.71) isotherms for tetracycline are much closer together than for triclosan, suggesting that there will be more potential for tetracycline to be desorbed from biomass. Following the same conditions as for the example with triclosan above (3000 mg l^{-1} MLSS, 1000ng l^{-1} normal tetracycline influent concentration), if sorbed quantities of tetracycline associated with the MLSS were suddenly subjected to an influent without any tetracycline, then around 20% of the sorbed tetracycline could be expected to desorb, producing an effluent with around 200 ng l^{-1} of tetracycline.

Figure 6.7 below shows the profile of tetracycline sorption in a standard sorption test starting at 10 mg l^{-1}.

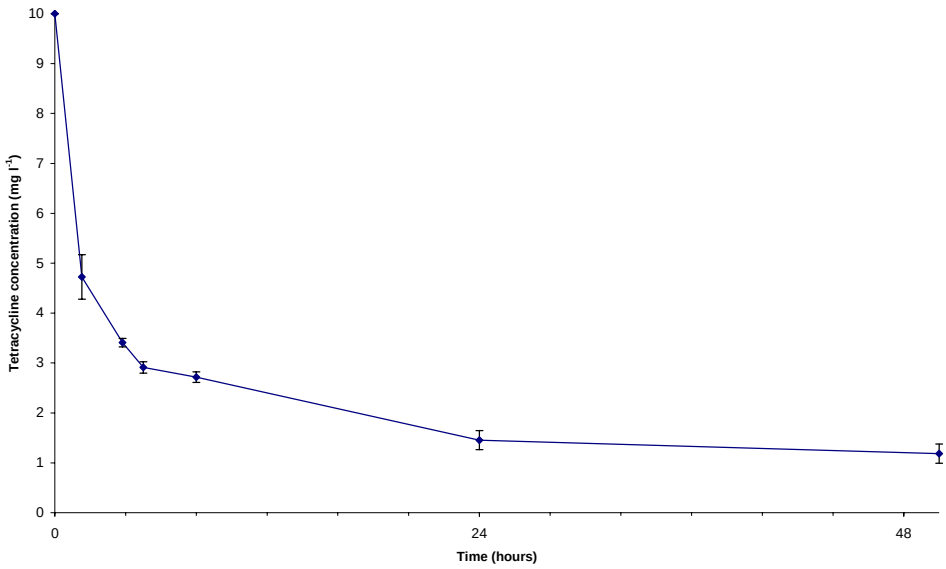


Figure 6.7: Profile of tetracycline sorption, from an initial concentration of 10 mg l^{-1}

As can be seen from the above figure, the rate of sorption for tetracycline is somewhat slower than for triclosan, with around 70% of sorption having occurred in four hours, and about 95% in 24 hours. Again, following the same argument as for triclosan above, this data would suggest that an HRT of around 30 hours would be suitable for maximising tetracycline removal. Similarly, Kim *et al.* (2005) suggested sorption of tetracycline to soils reached equilibrium after around 24 hours, as did Sithole and Guy (1987) and Rabolle and Spliid (2000).This is approximately double the current guideline levels for HRT for Severn Trent (Green 2004).

6.1.3 Carbamazepine

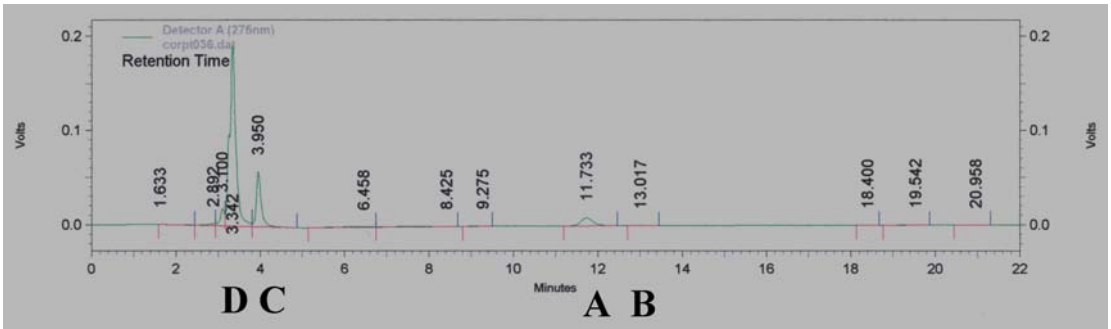
Sorption tests were conducted for carbamazepine. Based on its octanol-water partition coefficient (log K_{ow} =2.25), sorption could have been expected at a level somewhere between that observed for triclosan and tetracycline. However, no sorption of carbamazepine was observed to either type of biomass.

The tests had been conducted at a soil to solution ratio of 0.5 mg cm^{-3}, as suggested by the OECD guidelines (OECD 2000) for a compound with this K_{ow} value. Since no sorption had been observed, the soil to solution ratio was increased to 20 mg cm^{-3}. This was the maximum practicable ratio for these tests, due to the amount of biomass required, and the stirring mechanisms available. Again, no sorption was observed with either type of biomass. Since no sorption occurred, it was not possible to conduct desorption tests.

6.1.4 Caffeine

Sorption tests were conducted for caffeine, at the maximum soil to solution ratio of 20 mg cm^{-3}. Similar to carbamazepine above, no sorption was observed to either type of biomass. This was expected, since caffeine has a very low octanol-water partition coefficient (log K_{ow} = 0.16), which suggested that it would remain in the aqueous phase.

Whilst no sorption was observed, the control samples did produce an interesting result. When HPLC analysis was conducted on the control containing biomass and no caffeine, several caffeine degradation products were detected. A chromatogram of a sample taken after 22 hours is shown in Figure 6.8, which shows four peaks, labelled A to D.



Detector A (275nm)

Pk #	Retention Time	Area	Area %	Height	Height %
1	1.633	13572	0.469	54	0.019
2	2.892	33690	1.163	2384	0.843
3	3.100	127673	4.408	18123	6.405
4	3.342	1991347	68.752	194420	68.713
5	3.950	475647	16.422	57984	20.493
6	6.458	35246	1.217	605	0.214
7	8.425	25663	0.886	159	0.056
8	9.275	3548	0.122	133	0.047
9	11.733	176935	6.109	8511	3.008
10	13.017	5465	0.189	302	0.107
11	18.400	956	0.033	59	0.021
12	19.542	3989	0.138	96	0.034
13	20.958	2675	0.092	116	0.041

Figure 6.8: Chromatogram of caffeine desorption sample after 22 hours. A=Paraxanthine, B=Theophylline, C=7-Methylxanthine, D=Unknown

Based on retention times, the first fours peaks were identified as paraxanthine (A), theophylline (B), and 7-methylxanthine (C). The compound responsible for Peak D was not conclusively identified but was suspected to be 7-methyluric acid. Figure 6.9 shows the quantities and rate of desorption of the three identified compounds. Since it was not possible to obtain many data points, lines are fitted to give a rough idea of the rate of desorption.

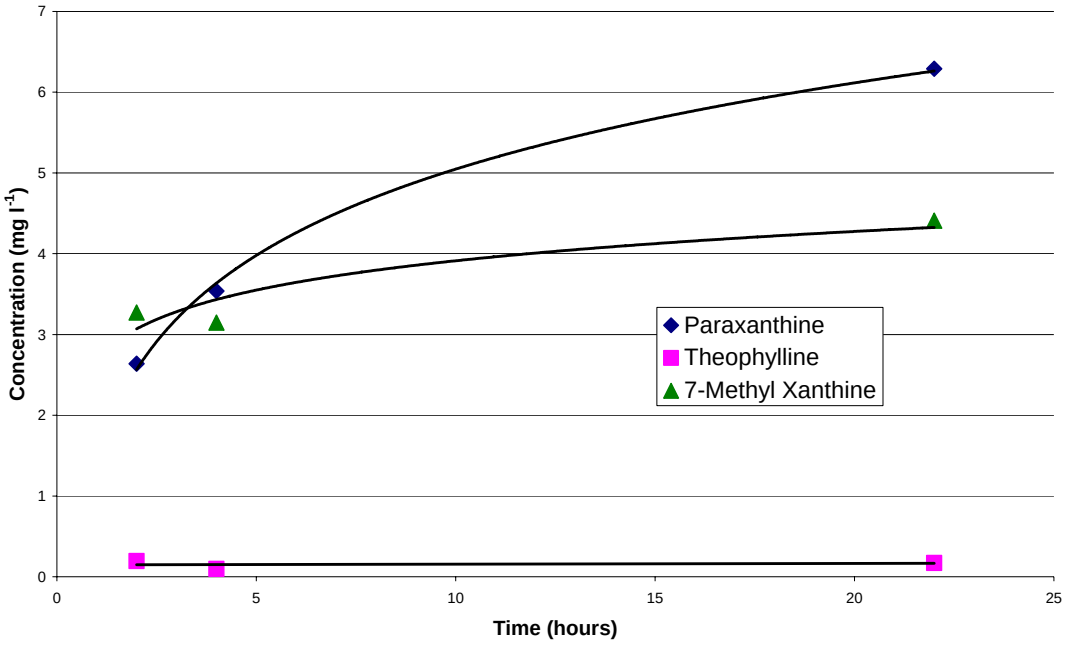


Figure 6.9: Desorption of caffeine degradation products

These desorption tests were conducted with 2 g biomass in 100 ml water. Therefore, an estimate of the total amounts of the caffeine and its degradation products within the biomass can be made. This is summarised in Table 6.1.

Table 6.1: Estimate of quantities of caffeine degradation products in biomass

Compound	Quantity (mg per g biomass)
Caffeine	Not detected
Paraxanthine	0.325
Theophylline	0.01
7-Methylxanthine	0.225
7-Methyluric acid	Not yet quantified

Although the quantities of caffeine and its degradation products were not measured in the aqueous phase from which these biomass samples were taken, the particular degradation products found are consistent with those seen in the sampling of Northend, Frankton, and Southam STPs. In all these cases caffeine, paraxanthine and 7-methylxanthine were detected. In addition to these three compounds, trace amounts of paraxanthine, and larger amounts of 7-methyluric acid were detected. This would suggest that these two compounds are indeed present in STPs, but mainly in the solid rather than aqueous phase.

This would suggest that although sorption is not a relevant removal mechanism for caffeine, it is for many of degradation products. This is an important finding, since for

many pharmaceuticals (caffeine included) the primary degradation product is often more polar and more toxic to the environment than the parent compound.

6.1.5 Comparing sorption tests with reality

Whilst sorption tests produce a relatively easy method for estimating the amount of a compound that has adsorbed to biomass, there is no guarantee that it does indeed represent what is happening in reality, particularly since there may have already been some of the test compound sorbed to the biomass used in the sorption test. This was demonstrated by the desorption of several caffeine degradation products. The most likely candidate for this would be triclosan, since it adsorbs most strongly, and the minimal amount of desorption that may have occurred in the control tests would have been far too small to detect (around 0.1 ng l^{-1}).

From the sorption testing, sorption of triclosan to biomass appeared to be independent of the type of biomass. To confirm that the sorption test results could be used to estimate sorption to biomass in real sewage treatment works, a method was developed to extract triclosan from biomass.

Three samples of activated sludge were taken from Southam STP. The amount of triclosan in the liquid phase was measured, and then the triclosan in the solid phase was extracted, yielding the results shown in Table 6.2.

Table 6.2: Amounts of triclosan in the liquid solid phases of three samples of activated sludge from Southam STP.

Triclosan in liquid phase	174 ng l^{-1}
Triclosan extracted from biomass	589 ng g^{-1}
Suspended solids	2476 mg l^{-1}

An estimate of the amount of triclosan in the solid phase, based on the Freundlich isotherm for triclosan with suspended solids (log K_f = 4.34, 1/n= 0.91), gave a total of 594 ng g^{-1}. The difference between these values is only 0.85%, which is well within the limits of experimental error. Therefore, it can be said that sorption tests are an accurate method of measuring the amounts of triclosan adsorbed to the solid phase of sewage samples.

6.2 Biodegradation

Biodegradation tests were conducted to assess how much each compound degraded, the rate of degradation, and to see whether any degradation products could be identified.

6.2.1 Triclosan

Biodegradation tests with biomass at a concentration of 3 g l^{-1} showed that triclosan was readily biodegradable (at a temperature of 20 °C). Figure 6.10 below shows the degradation of triclosan over a period of 63 days, plotting both the observed aqueous triclosan concentration, and the total amount of triclosan (aqueous and solid phases). The solid phase concentration was extrapolated from the measured aqueous concentration by use of the Freundlich isotherms. No degradation was observed in control tests.

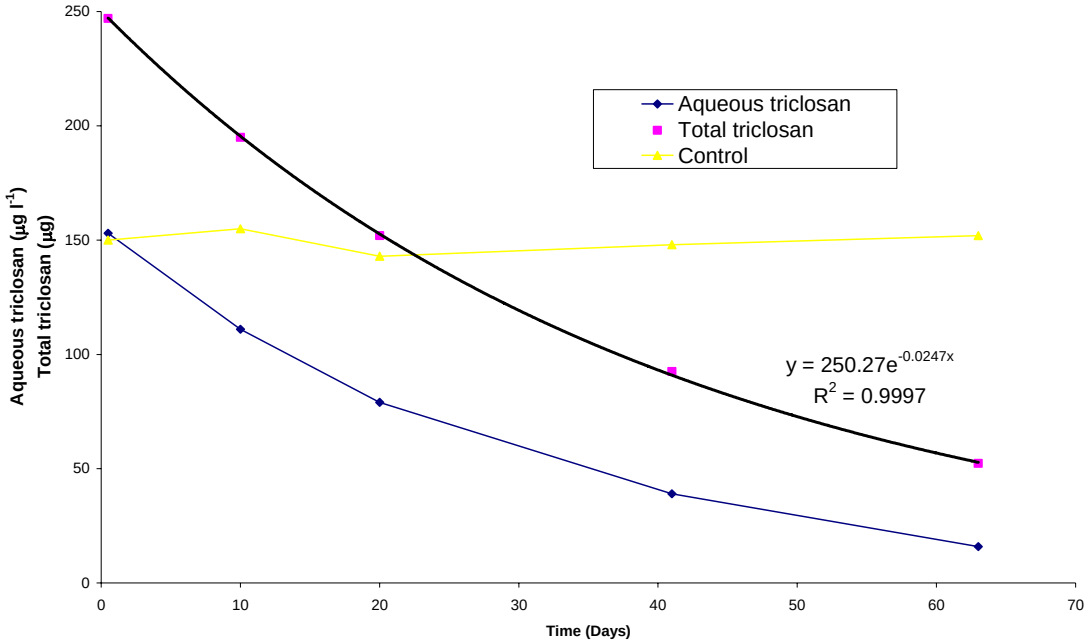


Figure 6.10: Degradation of triclosan with suspended growth biomass

The biodegradation rate was calculated according to Equation 10 below, assuming first order kinetics (Schwarzenbach *et al.* 2003).

$$\frac{dC_i}{dt} = k_{i,biol} \cdot SS \cdot C_i \qquad \textbf{Equation 10}$$

The biodegradation rate was the same for both types of biomass at k = 8.23 l gSS^{-1} d^{-1}. No literature data was available for comparison of degradation rates. No degradation products were observed. This was as expected, as the only known triclosan degradation product

(methyl triclosan) is even more polar than triclosan and so would be expected to be found exclusively sorbed to the biomass.

The combination of the results from these sorption and degradation experiments have two immediate implications for STP design. First, the SRT must be sufficient to allow the community of degrading bacteria to establish, in order to produce the rate of degradation established above. This has not been covered by these experiments, but as was noted in the literature review it has been investigated by other authors and is discussed further in Chapter 7. Second, the HRT must be sufficient for sorption and/or degradation to occur. For triclosan, sorption is rapid, with around 80% occurring in four hours, and 95% in twenty hours. However, for an influent flow of 1000 ng l^{-1} of triclosan and 3000 mg l^{-1} MLSS, then an HRT of only 0.28 days (6.7 hours) is required to achieve an effluent concentration of 1 ng l^{-1}. Therefore, an HRT of around 8 hours should be sufficient to achieve >99.9% removal of triclosan.

6.2.2 Tetracycline

Tetracycline biodegradation tests, with biomass at a concentration of 3 g l^{-1}, did show a decrease of tetracycline concentrations, as shown in Figure 6.11. In addition to this there was an increase in the concentration of epi-tetracycline, and a second unknown degradation product.

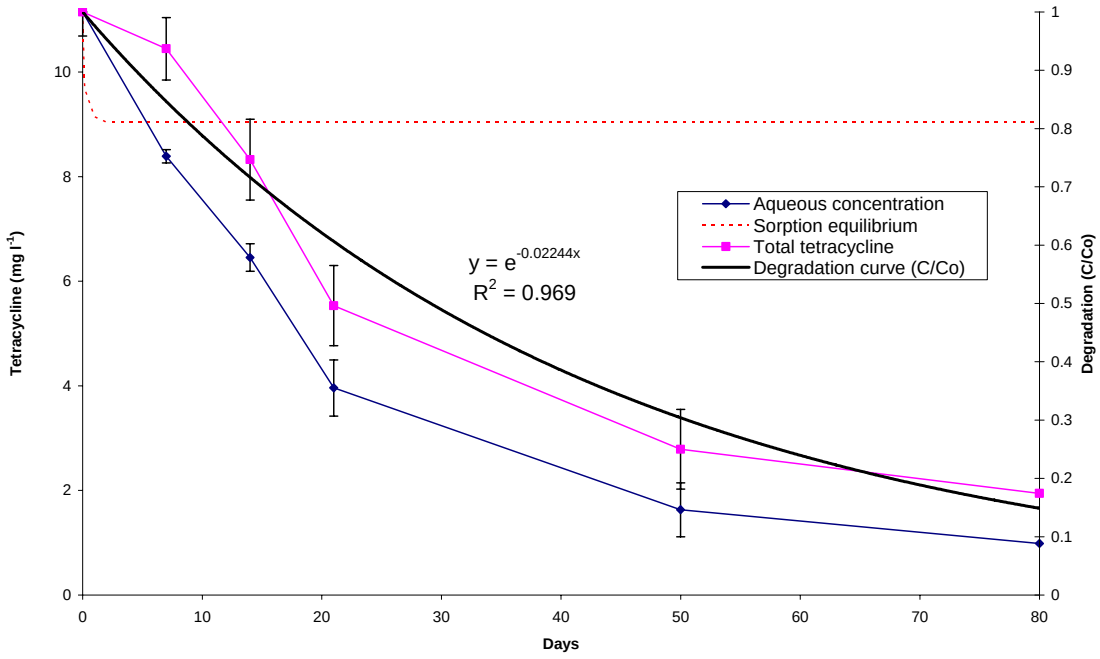


Figure 6.11: Degradation of tetracycline with fixed film biomass

The above figure shows the measured decrease in aqueous tetracycline concentration (blue line). From this the amount of tetracycline adsorbed to the solid phase was calculated and the total amount of tetracycline remaining was plotted as a function of the initial amount of tetracycline (pink line). An exponential regression line was plotted to this data.

Overall, 56% of the loss of tetracycline was shown to be formation of epi-tetracycline. It should be noted that tetracycline can readily be reformed from epi-tetracycline. Without access to techniques such as mass spectrometry, structural identification of the unknown degradation product could not be completed. From the regression line, the overall degradation rate (k) of tetracycline was calculated as 7.48 l gSS^{-1} d^{-1}, which is only slightly slower than for triclosan. However, it should be noted that only a small amount of degradation occurred in the first seven days of the experiment. This may be because the high concentrations of tetracycline used in the experiment forced an adaptation of the microbial species in the biomass, before degradation could occur. If this initial lag period is ignored, the degradation rate was 8.86 l gSS^{-1} d^{-1}, which was slightly higher than for triclosan.

Since tetracycline is only slowly, and weakly, adsorbed by biomass, little advantage could be gained by basing sewage treatment design on this parameter. However, the rapid degradation of tetracycline would suggest that for an influent flow of 1000 ng l^{-1} of tetracycline and 3000 mg l^{-1} MLSS, then an HRT of between 6.2 and 6.7 hours is required to achieve an effluent concentration of 1 ng l^{-1}. This assumes that the biomass is accustomed to degrading the influent concentrations of tetracycline. In the case where a sudden high concentration of tetracycline occurred, then it is unlikely that an increase in the amount of tetracycline degradation would be observed.

6.2.3 Carbamazepine

No degradation of carbamazepine was observed in tests with either fixed film or suspended growth biomass. Ternes *et al.* (2004) had suggested a degradation rate of 0.08 l gSS^{-1} d^{-1}, which would account for the very small amounts of carbamazepine removal observed in some STPs, typically 7 to 8% (Daughton and Ternes 1999; Heberer and Feldmann 2005; Ternes 1998). This would have been equivalent to the loss of around 0.0005 mg in a 60 day test, and as such would have not been detectable by the methods used.

On one sampling occasion in summer 2005, Thomas Heberer noted carbamazepine removal of around 40% (unpublished data). He commented (Heberer, 2005) that this sample had been taken during a period of very low rainfall (and hence long HRT), combined with an extreme high in temperature. This would have allowed for a long time for degradation to occur, possibly combined with an increased degradation rate due to the high temperature. Therefore, these are both factors that should be considered in the design of STPs. However, following the same calculation as for the previous two compounds, to allow the degradation of an influent carbamazepine from 1000 ng l^{-1} in the influent to 1 ng l^{-1} in the effluent with 3000 mg l^{-1} MLSS would require an astounding HRT of 29 days. This is simply not practical due to the large amount of land and tank volume that would be required.

6.2.4 Caffeine

Samples from initial biodegradation tests of caffeine with both types of biomass showed that no detectable amounts of caffeine or its degradation products remained in the test vessels after seven days. The tests were repeated and sampled after one day with the same results.

Published data has shown that caffeine can have a degradation rate up to 33,000,000 l gSS^{-1} hr^{-1} (Gutierrez-Sanchez *et al.* 2004). Calculations at this degradation rate show that the entire amount of caffeine within the test vessels would have been consumed within less than an hour of the start of the test. From the results reported here, all that can be said about the rate of degradation is that it exceeded 1500 l gSS^{-1} hr^{-1} showing that caffeine is readily biodegraded by sewage biomasses.

6.3 Summary

A summary of the sorption and biodegradation rates for the four pharmaceuticals is shown in Table 6.3.

Table 6.3: Summary of sorption and biodegradation rates

	Sorption	**Log K_f**	**Biodegradability**	**Rate** ($l\ gSS^{-1}\ hr^{-1}$)
Caffeine	None	-	Readily	> 1500
Carbamazepine	None	-	Not readily	-
Tetracycline	Medium	3.31 – 4.25	Not readily	7.48 – 8.86
Triclosan	High	4.34 – 4.41	Readily	8.23

The sorption and biodegradation tests have shown that both mechanisms play a part in the removal of pharmaceuticals during sewage treatment. However, the magnitude of removal caused by each mechanism varies from compound to compound. Triclosan is biodegraded rapidly, but also is strongly absorbed by biomass. This means that in conditions where biodegradation fails (e.g. low temperatures), triclosan will still be removed by sorption. Similarly, systems should cope well with fluctuating loads of triclosan, whereas tetracycline would be desorbed back into the aqueous phase. Similarly to triclosan, tetracycline is rapidly biodegraded. No removal of carbamazepine could be detected in either type of test, although other authors have shown that small amounts of biodegradation may occur. Caffeine was rapidly removed by biodegradation.

These tests have shown that the HRT of an STP is important for removal to occur. It must be long enough for sorption to occur and for enough biodegradation to occur. Analogous to this is the SRT, which must be long enough for appropriate bacterial communities to be established. A figure of 10 days has been suggested by other authors. Temperature will, of course, have an important impact on the rate of biodegradation.

A final aspect that should be considered in biodegradation, is the type of bacteria responsible for degrading the pharmaceuticals (i.e. autotrophs or heterotrophs). Autotrophs (usually associated with BOD removal) can cope with fluctuating loads and populations can rapidly increase. Heterotrophs (usually associated with ammonia removal) are much slower growing (requiring longer SRTs) than autotrophs, and are affected more by temperature variations. Studies with estrogens have suggested that it is the heterotrophic bacteria that are responsible for estrogen removal (Schmidt and Schuphan 2002), although it is not the ammonia utilising bacteria within that population that are responsible (Shi *et al.*

2004). The implications of this may be visible in the location of where biodegradation takes place through secondary treatment processes. In particular, when high BOD loads occur, it may be possible that degradation of pharmaceuticals is completely inhibited, in a similar way to that sometimes seen with ammonia removal.

Chapter 7: Grab sampling of STPs

Based on discussion with Severn Trent Water, four STPs were selected to be sampled for their pharmaceutical concentrations, as described in the Materials and Methods chapter. As shown in Table 7.1, Severn Trent Water not only covers large urban STPs, but also has a wide variety of small rural STPs on which there is a paucity of published data for pharmaceutical removal. The four selected STPs covered a wide range of treatment sizes, ranging from Northend STP at a population equivalent (PE) of only 449, to Finham STP at a PE of about 495,000. The four STPs also covered a range of different treatment technologies, including a rotating biological contactor (Northend STP), a biological filter (Frankton STP), an oxidation ditch (Southam STP), and a standard activated sludge plant with additional iron dosing for phosphorus removal (Finham STP). Full details of these STPs can be found in the Materials and Methods chapter.

Table 7.1: Numbers of Severn Trent STPs by population equivalent

Population Equivalent	Number of STPs
> 200,000	9
50,000 to 200,000	25
< 50,000	~ 1000

It should be noted that at the time this section of work was planned and was started, there were only two previous reports of pharmaceuticals and personal care products in the influent and effluent from UK sewage treatment plants. This excludes the reports of the endocrine disrupting compounds, and detections of PhACs in surface waters. The detected compounds included a detection of bleomycin (Aherne *et al.* 1990), as well as aspirin, ibuprofen, clofibric acid, triclosan, galaxolide, tonalide, diatrizoate, cyclophosphamide, and fluvoxamine (Kanda *et al.* 2003). Several other reports of pharmaceuticals in UK sewage influents and effluents, including the EA studies (Hilton *et al.* 2003), were published after this work had begun.

7.1 Triclosan

Samples were obtained on seven occasions at Northend STP, six each at Southam and Frankton STP, and three at Finham STP. Two previous sets of data (Kanda *et al.* 2003) for Northend and Southam STPs have been included for comparison, which were sampled on

consecutive days in December 2002. For clarity, error bars are not shown on the graphs. Errors were around 2.5% of the values shown, based on repeated analysis of the samples.

7.1.1 Northend STP

The concentrations of triclosan measured after each unit process at Northend STP are shown in Figure 7.1. The influent concentrations to Northend STP varied considerably (594 to 4945 ng l^{-1}), but this corresponded with previous measurements at the site by Kanda *et al.* who had recorded 1300 and 2700 ng l^{-1} (Kanda *et al.* 2003). The variation in influent triclosan concentrations was most likely caused by variations in rainfall. However, there was no automated flow metre available at the STP to enable calculation of triclosan mass flows.

Settled sewage triclosan concentrations were in the range < LOQ to 1939 ng l^{-1}. There were no samples taken at this point by Kanda *et al.*. Taking the average of these concentrations would suggest that a removal of 56% had occurred during settlement. This is somewhat strange since the two mechanisms that were expected to be responsible for the removal of triclosan – sorption and biodegradation – are unlikely to be responsible for this removal. Sorption is not likely to be responsible, since it is probable that aqueous and sorbed triclosan are already in equilibrium due to the long contact time in the sewer prior to reaching the works. Biodegradation is unlikely to responsible for removal, since there is no oxygen available. The explanation for this apparent removal is that the grab samples were not timed with the flow through the works. Therefore the concentrations recorded in the settled sewage did not directly correspond to the concentrations in the influent.

Concentrations of triclosan in the effluent of the RBC, after biological treatment and settlement of humus solids, were significantly lower. It should be noted that due to the structural design of RBCs, as well as health and safety considerations, it was not possible to sample between the end of the RBC discs and the start of the humus settlement zones. Concentrations ranged from < LOD to 1106 ng l^{-1}. This was a wider range of concentrations than recorded by Kanda *et al.* (150 and 620 ng l^{-1}).

Concentrations of triclosan in the effluent from the reed bed (final effluent) were similar to those in the effluent from the RBC, ranging from <LOD to 1117 ng l^{-1}. However, Kanda *et al.* had shown a drop in triclosan concentrations (to 81 and 140 ng l^{-1}).

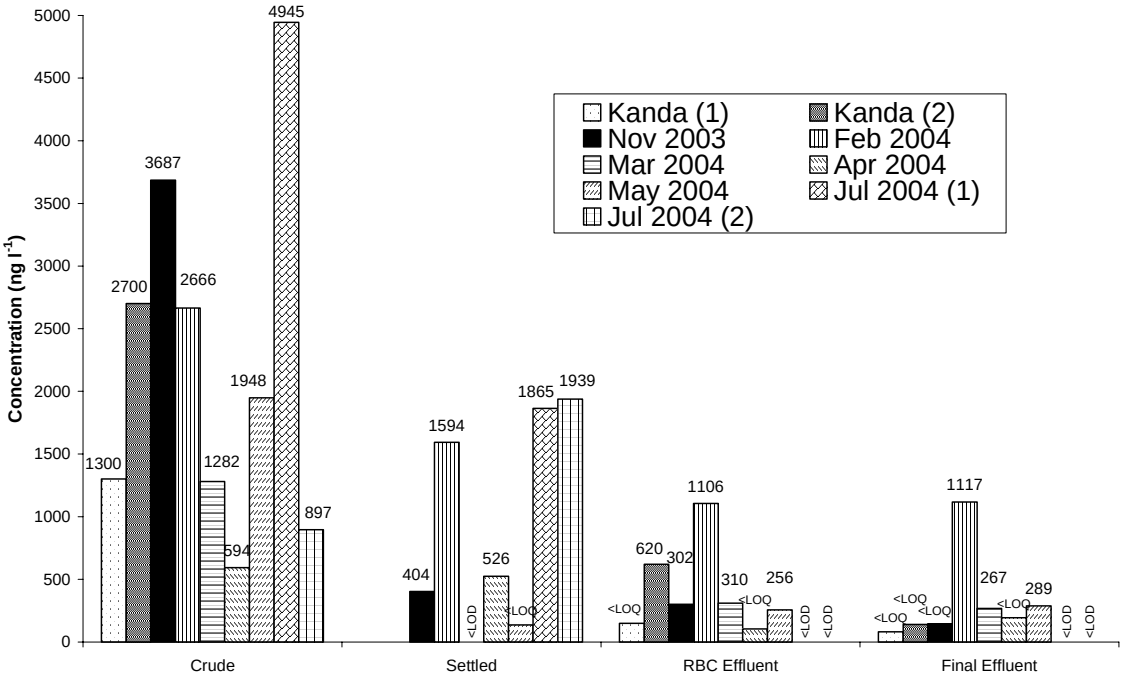


Figure 7.1: Concentration of triclosan in samples of crude sewage, settled sewage, RBC effluent, and final effluent from Northend STP (fixed film)

As noted previously, triclosan has a PNEC and NOEC of 7 and 700 ng l^{-1} respectively. Although all final effluent samples exceeded the PNEC level, only the February 2004 sample exceeded the NOEC level. This would suggest that triclosan is entering the environment at levels that may be of concern.

Of particular interest in this sampling regime was the result from the February 2004 sampling, where very high concentrations were observed in the RBC effluent and final effluent, compared to all other sampling regimes. It was noted that the weather conditions were particularly cold on this day, although no actual measurements were taken. For reference, a layer of ice had to broken in the channel between the effluent of the RBC and the reed bed in order to take a sample (liquid was flowing under the surface ice). This may suggest that the sewage temperature had dropped, possibly leading to an inhibition of biological degradation.

7.1.2 Frankton STP

The concentrations of triclosan measured after each unit process at Frankton STP are shown in Figure 7.2. The influent concentrations were more stable than for Northend STP, ranging from 993 to 2202 ng l^{-1} in this work (although Kanda *et al.* had measured 2500 and 3700 ng l^{-}

[1]). The biological treatment process also gave more consistent effluent concentrations than Northend, ranging from <LOD to 418 ng l^{-1} in the humus effluent. Final effluent concentrations were lower than Northend STP, at <LOD to 290 ng l^{-1}.

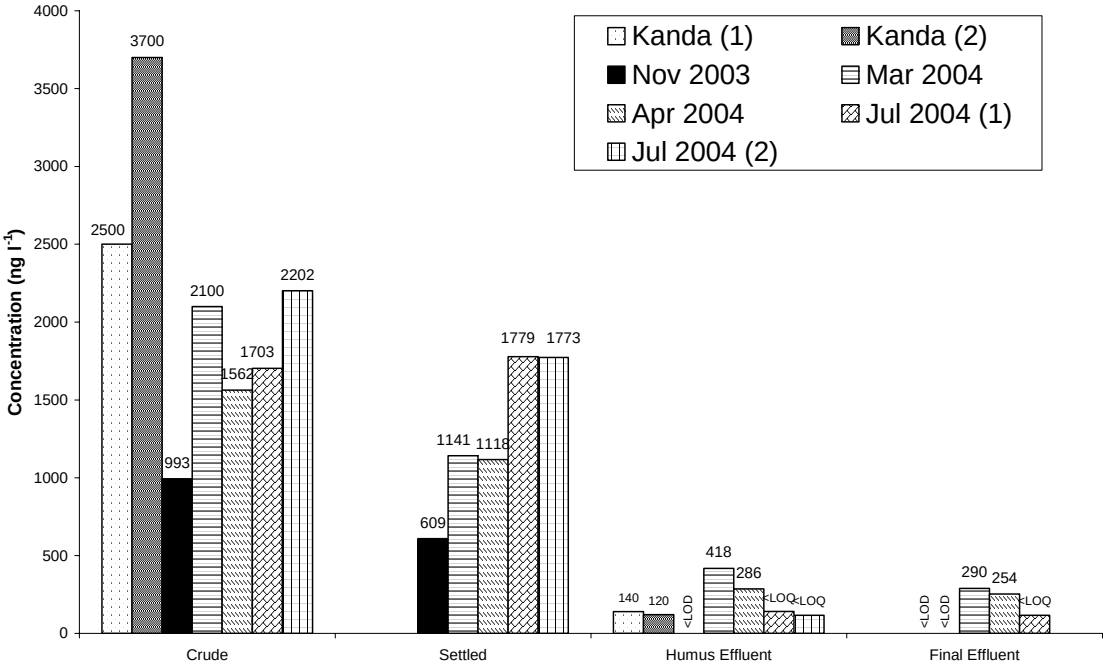


Figure 7.2: Concentration of triclosan in samples of crude sewage, settled sewage, humus effluent, and final effluent from Frankton STP (fixed film)

The influent at Frankton STP showed much less variation (993 to 3700 ng l^{-1})than Northend STP. As for Northend STP, flow meter data did not exist to compare the variation in daily load. Overall, Frankton STP achieved lower concentrations of triclosan in the final effluent. As for Northend STP, most of the samples from the last two sample points (humus effluent and final effluent) were below the theoretical limit of quantification. However, the values obtained may suggest that a small amount of removal is occurring in the polishing lagoon between these two sample points. Although all samples exceeded the PNEC level for triclosan, the final effluent concentrations were below the NOEC level (700ng l^{-1}).

7.1.3 Southam STP

The concentrations of triclosan measured after each unit process at Southam STP are shown in Figure 7.3. The influent concentrations ranged from 1598 to 5115 ng l^{-1} (although Kanda *et al.* had reported lower concentrations at 1100 and 670 ng l^{-1}). Only very small amounts of triclosan were detected in the oxidation ditch effluent (< LOD to < LOQ ng l^{-1}) with only

three samples above the LOD. Similarly all final effluent were below the LOQ, again with only three samples above the LOD.

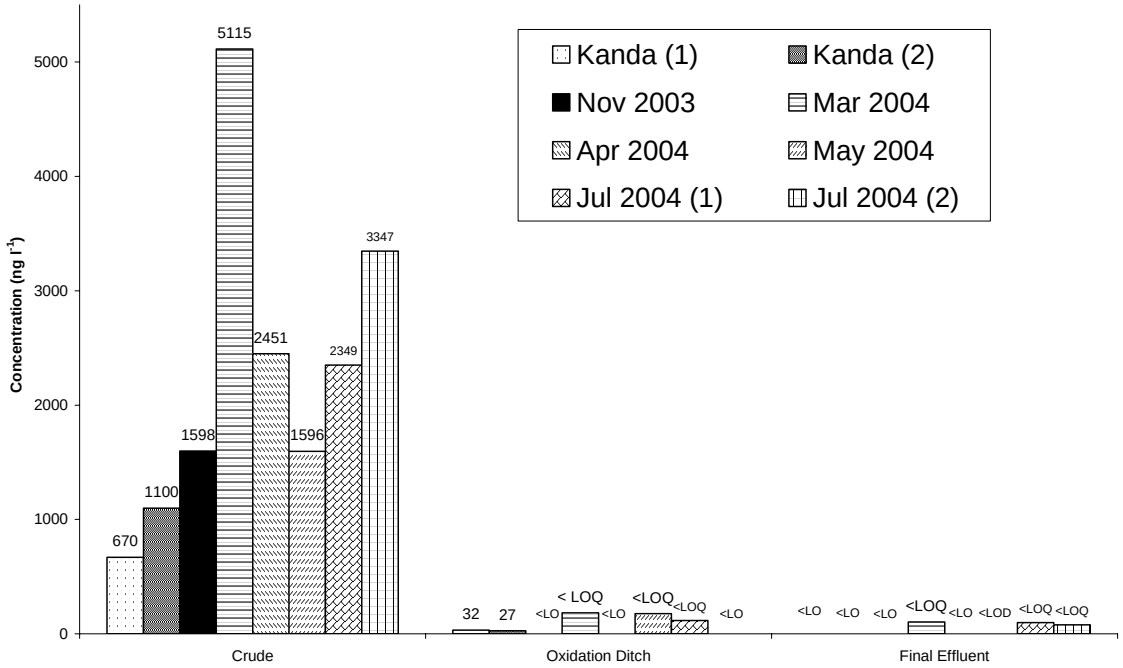


Figure 7.3: Concentration of triclosan in samples of crude sewage, oxidation ditch effluent, and final effluent taken from Southam STP (suspended growth)

Unlike the other STPs sampled, it is possible to estimate the removal efficiencies achieved in this plant. This is because an oxidation ditch performs as a complete-mix reactor, or CSTR. In the CSTR, the tank contents are rapidly mixed, and concentrations throughout the tank can be considered to be uniform. Assuming that the tank is well designed so that no short-circuiting can occur, the concentrations in the tank effluent can be considered to be invariant with time (Metcalf and Eddy 2003).

Overall, the STP removed 97.1% (± 1.0%) of the influent triclosan. This removal was made up of 96.2% (± 0.98%) removal during the biological treatment in the oxidation ditch. This removal could be due to either sorption to biomass, or due to biodegradation. Since triclosan has been shown to be removed rapidly by both processes, it could be suggested that removal was caused by both, although quantification between the two is not possible. A further 1.0% (± 0.45%) removal was observed through the settlement tanks. This amount of removal is small enough that it could be discounted as experimental error. However, if it is removal, then it is probably due to biodegradation, aided by oxygen produced by slight de-nitrification

occurring in the final settlement tanks. It is unlikely to be sorption, since aqueous and sorbed triclosan should already be in equilibrium by this point.

The influent at Southam STP (1596 to 5115 ng l^{-1}) had much higher concentrations than both Northend and Frankton STP. This is probably since Southam serves a much higher population density than the previous two STPs, which would suggest that a lower dilution from rainwater runoff and infiltration may occur. Although Southam does serve a higher population than the previous two STPs, this would not account for the higher concentration, since usage of triclosan per head of population can be assumed to be the same, as can the volume of wastewater (dilution) per head of population.

Similarly to the previous two STPs, most of the samples from the last two sample points (oxidation ditch effluent and final effluent) after the biological step (oxidation ditch) were below the theoretical limit of quantification. The values obtained do suggest that a small amount of removal is occurring in the final settling tanks. Although all samples exceeded the PNEC level for triclosan, the final effluent concentrations were well below the NOEC.

7.1.4 Finham STP

The concentrations of triclosan measured after each unit process at Finham STP are shown in Figure 7.4. It was not possible to determine the overall removal of the STP. This was because there are two separate inlets (from Sowe and Sharebourne respectively). Neither of these inlets had flow meters on them, and so there was no way of telling how much of the total triclosan flow came from each inlet. This was surprising, since without flow meters, the plant operators had no way of telling the flow entering the works, and relating it to the capacity of the works. (This has since been rectified as part of an AMP4 capital scheme). Hence it was not possible to identify the overall removal, since some of the apparent removal could have been accounted for by the high concentrations in the Sharebourne inlet (<LOD to 2219 ng l^{-1}) being diluted by the lower concentrations of the Sowe inlet (<LOQ to 1575 ng l^{-1}). However, it is possible to see that triclosan was strongly removed, since concentrations in the final effluent were all below the level of detection. The majority of the removal appeared to occur during biological treatment in the activated sludge plant.

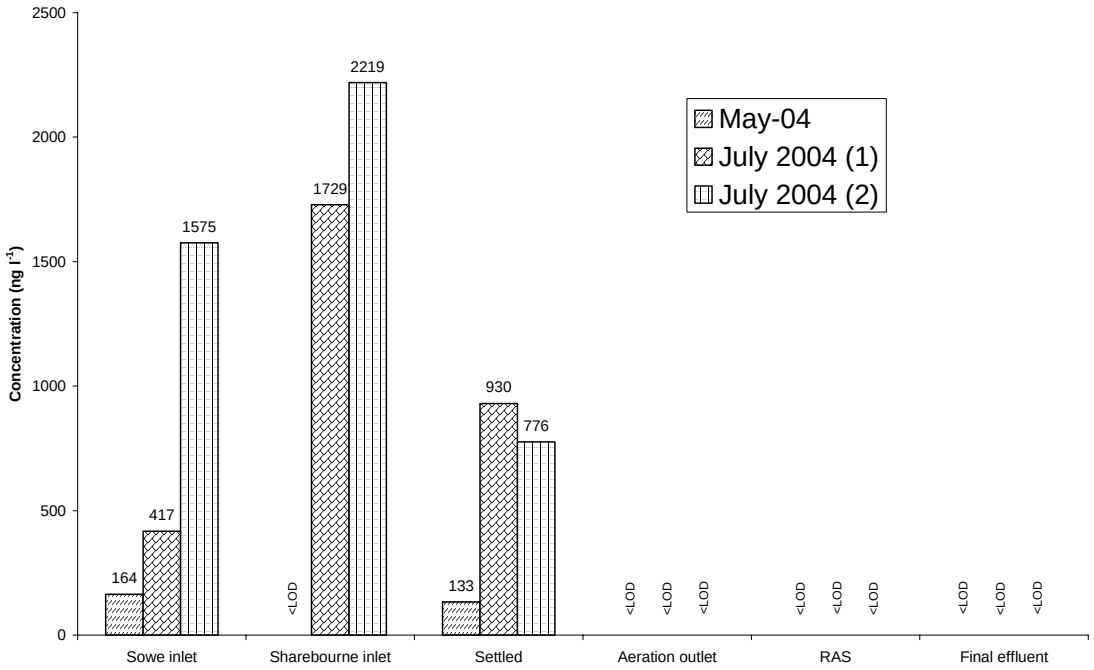


Figure 7.4: Concentration of triclosan in samples of crude sewage, settled sewage, aeration outlet, return activated sludge (RAS), and final effluent at Finham STP (suspended growth). nd = not detected

It is notable that on two of the three sampling occasions, the Sharebourne inlet contained a much higher concentration of triclosan than the Sowe inlet, and further investigation of the catchments may demonstrate the reason for this. Although all samples exceeded the PNEC level for triclosan, the final effluent concentrations were well below the NOEC level (700 ng l^{-1}).

7.1.5 Fate of Triclosan in STPs

Surveys of various STPs have shown variations in the removal of triclosan during sewage treatment, as summarised in Table 7.2. Although, as per the discussion above, there are inherent dangers with comparing the influent and effluent concentrations of grab samples, McAvoy *et al.* (2002) has done this and their results are included in the table, along with other authors who used time-representative grab sampling.

Table 7.2: Literature values of triclosan removal through various types of STP

Type of works	Influent ($\mu g\ l^{-1}$)	Effluent ($\mu g\ l^{-1}$)	Percentage removal	Author
Activated Sludge	21.9	1.1	95.0	Sabaliunas *et al.* (2003)
Activated Sludge	1.2	0.051	95.8	Bester (2003)
Activated Sludge	5.21	0.24	95.4	McAvoy *et al.* (2002)
Activated Sludge	10.70	0.41	96.2	McAvoy *et al.* (2002)
Activated Sludge	0.67	0.032	95.2	Kanda *et al.* (2003)
Activated Sludge	1.1	0.027	97.5	Kanda *et al.* (2003)
Trickling filter	7.5	0.34	95.5	Sabaliunas *et al.* (2003)
Trickling filter	3.83	1.61	58.0	McAvoy *et al.* (2002)
Trickling filter	16.6	2.10	86.1	McAvoy *et al.* (2002)
Trickling filter	15.4	2.70	82.5	McAvoy *et al.* (2002)
Trickling filter	2.5	0.14	94.4	Kanda *et al.* (2003)
Trickling filter	3.7	0.13	96.5	Kanda *et al.* (2003)

Generally, STPs with long hydraulic retention times such as activated sludge (HRTs typically 8 to 14 hours) have shown high removal of triclosan (95 to 97.5%). STPs with shorter retention times, such as trickling filters (6 -12 hours), have shown lower and more varied removal (58-96%). This broadly agreed with the results presented in this work, in which over 97% of triclosan was removed in the oxidation ditch at Southam STP (HRT ~ 24 hours), and through the trickling filters (91%) at Frankton STP. However, the RBCs at Northend STP removed only around 26% of triclosan during biological treatment, which had an HRT of typically 1 to 4 hours. The only other report of triclosan removal through an RBC was by Kanda *et al.* (2003), on the same STP.

The variation in these results, although small, would suggest that there are some parameters that are affecting the ability of the STPs to remove triclosan. From the data produced from the grab samples, it is not possible to determine what these parameters may be, since no specific measurements apart from triclosan concentrations were taken. This aspect was considered further for the composite sampling work, and the tests were designed to cover as

many parameters as possible that could be affecting the sedimentation and biological processes. For example, the differences could be due to the residence times in the biological processes as shown in Figure 7.5.

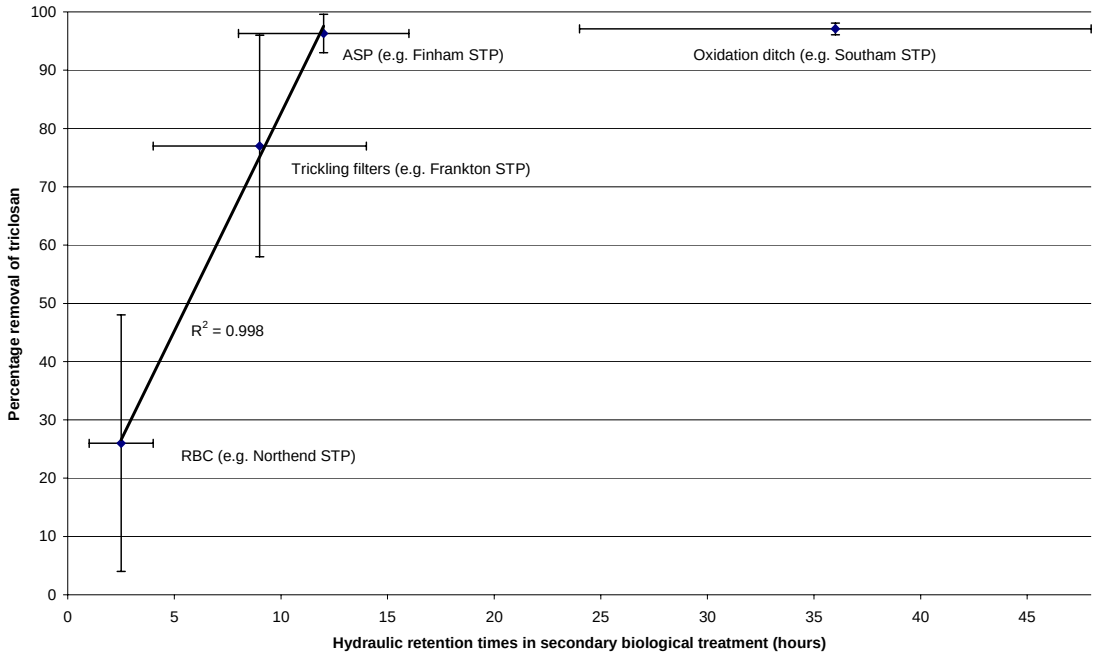


Figure 7.5: The relationship between hydraulic retention time and percentage removal of triclosan. (Range of retention times taken from Metcalf and Eddy (2003) and Severn Trent Design Manual (Green 2004)).

This relationship been retention time and removal has been shown to be true by many authors e.g. (Ternes *et al.* 2004) with an increase in retention time leading to increased amounts of removal. A consequence of sampling at an oxidation ditch, which has a long HRT, has shown that a substantial increase in HRT will not further increase the removal of triclosan, as illustrated above. Indeed, from the above data, a maximum optimal HRT of around 15 hours could be derived. This is similar to the optimal HRTs suggested by the laboratory sorption and biodegradation test (20 hours and 8 hours respectively).

A consequence of this relationship between removal and HRT would be reduced removal during rainfall events (when residence times are reduced because of increased flow). Therefore, reducing rain and other water input into the sewer system would result in an increase in overall removal. In this case, systems which have longer HRT such as oxidation ditches, would see much less variation in their removal. At this stage insufficient information

is available to consider further fate modelling with Toxchem+. This will be considered further with the composite sampling – see Chapter 9.

7.2 Tetracycline

Four sets of samples were taken from each of Northend, Southam, and Frankton sewage treatment plants. A further three sets of samples were taken from Finham sewage treatment plant. In every sample, from all four STPs, the concentrations of tetracycline and its degradation products were below the limit of detection. This was not unexpected, since the limit of detection for tetracycline was 760 ng l^{-1}, whilst tetracycline has been detected in sewage effluent at maximum concentrations of 560 ng l^{-1} (Batt *et al.* 2006) and 977 ng l^{-1} (Miao *et al.* 2004), with a median concentration of 151 ng l^{-1} (Miao *et al.* 2004). Tetracycline has also been reported in streams and rivers at a concentration of 110 ng l^{-1} (Kolpin *et al.* 2002).

7.3 Carbamazepine

One set of carbamazepine samples were obtained from each of the four sewage treatment plants. For clarity, error bars are not shown on the graphs. Errors were around 5% of the values shown, based on repeated analysis of the samples. These results form the first reported detection of carbamazepine in UK sewage treatment plants.

7.3.1 Northend STP

The concentrations of carbamazepine measured after each unit process at Northend STP are shown in Figure 7.6. These ranged from 1368 ng l^{-1} in the influent, to 1149 ng l^{-1} in the final effluent.

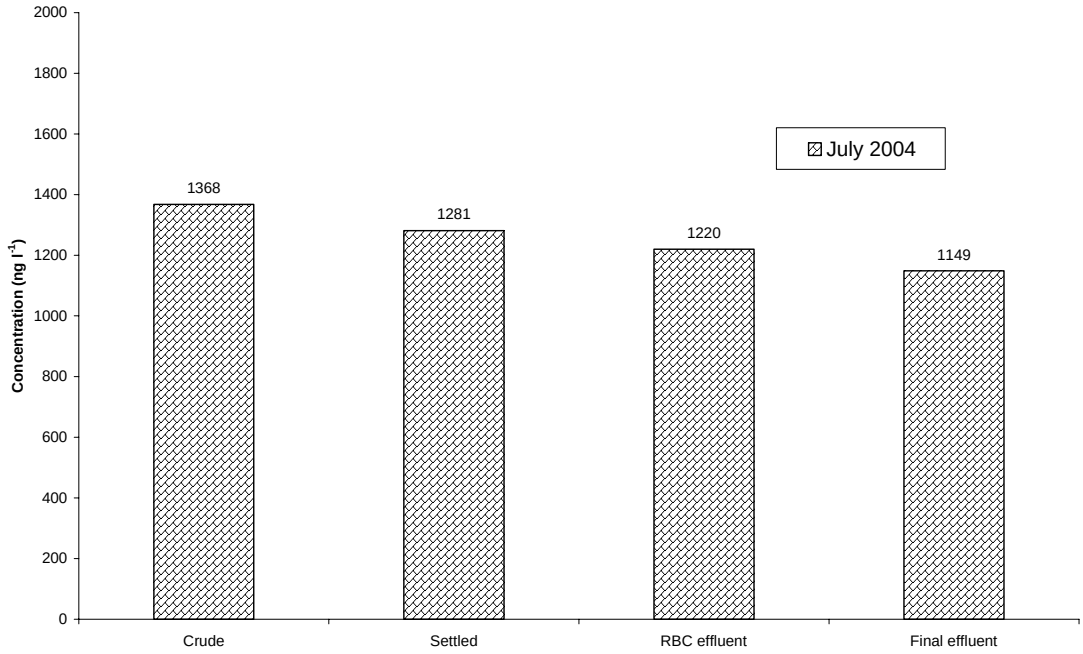


Figure 7.6: Concentration of carbamazepine in samples of crude sewage, settled sewage, RBC effluent, and final effluent from Northend STP (fixed film)

Overall, a 16% reduction was seen in carbamazepine concentrations through the STP, split roughly evenly between the three treatment processes. This is almost double the amount of removal that has been regularly reported in literature. This would suggest that the reduction is due, at least in part, to the final effluent sample being related to a different influent load, due to the long residence times in the plant, particularly through the reed bed.

As noted previously, carbamazepine has a PNEC of 8100 ng l^{-1}. There was no available data for the NOEC. The final effluent sample did not exceed the PNEC level.

7.3.2 Frankton STP

The concentrations of carbamazepine measured after each unit process at Frankton STP are shown in Figure 7.7. These were slightly higher than for Northend, ranging from 1461 ng l^{-1} in the influent, to 1263 ng l^{-1} in the final effluent.

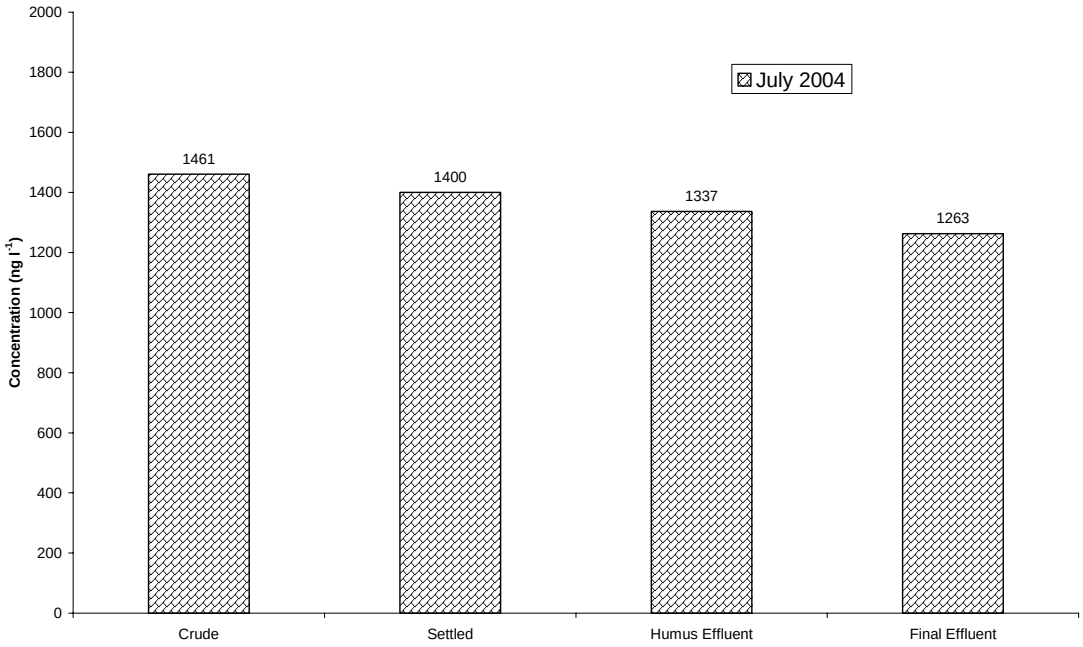


Figure 7.7: Concentration of carbamazepine in samples of crude sewage, settled sewage, humus effluent, and final effluent from Frankton STP (fixed film)

Overall, a fourteen percent reduction was seen in carbamazepine concentrations through the STP, split roughly evenly between the three treatment processes. Similar to Northend STP, this amount of removal is higher than has been regularly reported in literature. This would suggest that the reduction is due to the final effluent sample being related to a different influent load, due to the long residence times in the plant, particularly through the polishing lagoon. The final effluent samples did not exceed the PNEC level.

7.3.3 Southam STP

The concentrations of carbamazepine measured after each unit process at Southam STP are shown in Figure 7.8. These were much lower than for either Northend or Frankton and ranged from 662 ng l^{-1} in the influent to 615 ng l^{-1} in the final effluent.

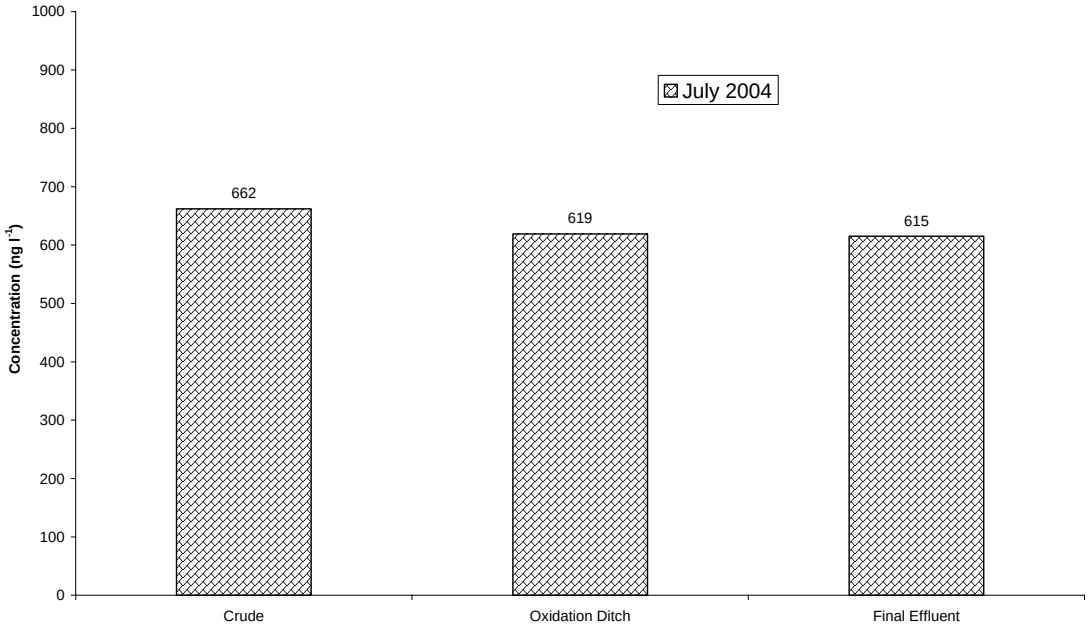


Figure 7.8: Concentration of carbamazepine in samples of crude sewage, oxidation ditch effluent, and final effluent taken from Southam STP (suspended growth)

Overall, the STP removed only 7.1% of the influent carbamazepine. This removal was made up of 6.5% removal during the biological treatment in the oxidation ditch. A further 0.6% removal was observed through the final settlement tanks. As discussed above for triclosan, since oxidation ditches are generally considered to be CSTRs, it is appropriate to quote removal through the plant. It is likely that the small amount of removal seen across the final tanks, may have been due to experimental error, rather than actual removal.

The overall removal of carbamazepine was similar to the removals predominantly quoted in literature. The final effluent samples at Southam STP did not exceed the PNEC level.

7.3.4 Finham STP

The concentrations of carbamazepine measured after each unit process at Finham STP are shown in Figure 7.9. Similarly to the triclosan samples, it was not possible to determine the overall removal of the STP due to the lack of flow meter data, which would have shown the relative amounts entering through the two inlets. Although there appears to be good removal when comparing the concentrations in the Sharebourne inlet (1214 ng l^{-1}) and the final effluent (< LOD), this may simply be due to dilution by the Sowe inlet. However, the difference between the concentration of carbamazepine in the settled sewage (383 ng l^{-1}) and

the outlet of the aeration tank (<LOD) show that there is some removal occurring within the activated sludge plant. No carbamazepine was detected in the return activated sludge (RAS).

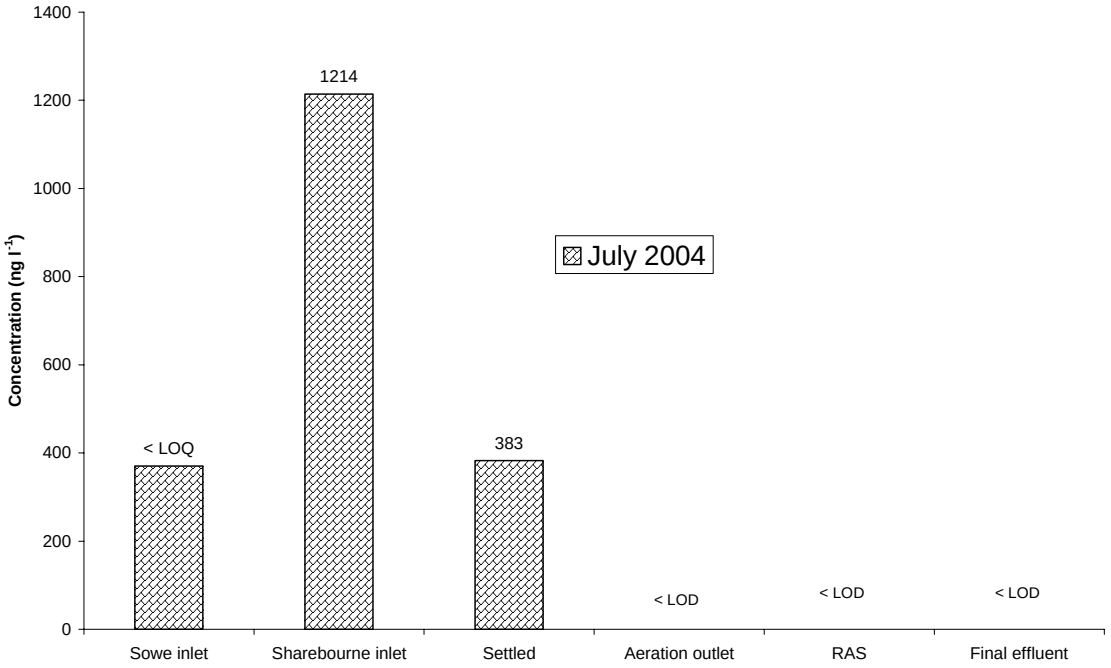


Figure 7.9: Concentration of carbamazepine in samples of crude sewage, settled sewage, aeration outlet, return activated sludge (RAS), and final effluent at Finham STP (suspended growth).

Although it is tempting to report that a significant amount of removal has occurred in the aeration lanes of this STP, this cannot be done since the grab samples were not time paced. Lack of data about the relative flow rates of the two influent compounds the problem. However, similar to the other three STPs, the final effluent samples at Finham STP did not exceed the PNEC level for carbamazepine.

7.3.5 Fate of carbamazepine

Whilst it may be difficult to discern any useful information from the data presented here, due to lack of flow data and flow placed sampling, the actual concentrations detected are of interest. However, it should be noted that these are the first reported detections of carbamazepine in UK sewage influent and effluents. Final effluent concentrations have been in line with those previously published around the world, ranging from 70 ng l^{-1} (Gomez *et al.* 2006) to 6300 ng l^{-1} (Ternes 1998), with an average figure around 1500 ng l^{-1} (Bendz *et al.* 2005; Clara *et al.* 2005). Of important note is that all final effluent samples in this sampling regime were below the predicted no-effects concentration (8100 ng l^{-1}). Similarly, all final effluent samples were below the predicted environmental concentration (7380 ng l^{-1} (Webb

2000)).This is an important results, since of the four pharmaceuticals monitored in this study, carbamazepine is the only one whose usage is accurately known, and therefore the only one for which a reliable estimation of the PEC could be made. It is useful to note that even though the PEC calculation assumed no removal during sewage treatment (EMEA 2006), the MECs are still lower than the PEC. Since carbamazepine showed the least removal of all the four pharmaceuticals in the laboratory tests, this would suggest that the PEC calculation produces an overestimate, thereby producing a factor of safety when used as part of a PEC/PNEC calculation in assessing environmental risk of pharmaceuticals. This has also been concluded by authors studying a wider range of pharmaceuticals (Bound and Voulvoulis 2006; Huschek *et al.* 2004; Liebig *et al.* 2006; Nakamura 2006).

7.4 Caffeine

Caffeine was the last compound for which an analytical method was developed, and hence there was only a single opportunity to collect samples. On this occasion, it was not possible to visit Finham STP due to construction activities on site. The concentrations for the remaining three STPs are presented below. Whilst analysis was conducted for all major caffeine degradation products, only concentrations of paraxanthine and 7-methylxanthine were detected. For clarity, error bars are not shown on the graphs. Errors were around 2% of the values shown, based on repeated analysis of the samples. These results form the first report of caffeine in UK sewage treatment plants.

7.4.1 Northend STP

The concentrations of caffeine and degradation products measured after each unit process at Northend STP are shown in Figure 7.10. Overall, the STP removed 98.7% of the influent caffeine. This removal was made up of 16.0% removal during the primary settlement of sewage solids, 82.7% removal during the biological treatment of the rotating biological contactors (RBCs), and a further 0.03% removal was observed in the reed bed. As previously explained, these removals should only be considered qualitatively.

There was a noticeable increase in both paraxanthine and 7-methylxanthine concentrations through the primary settlement, whilst at the same time there was a drop in caffeine concentrations. Although this could be accounted for by the samples not being time

representative, it may also suggest that degradation of caffeine occurred the primary settlement.

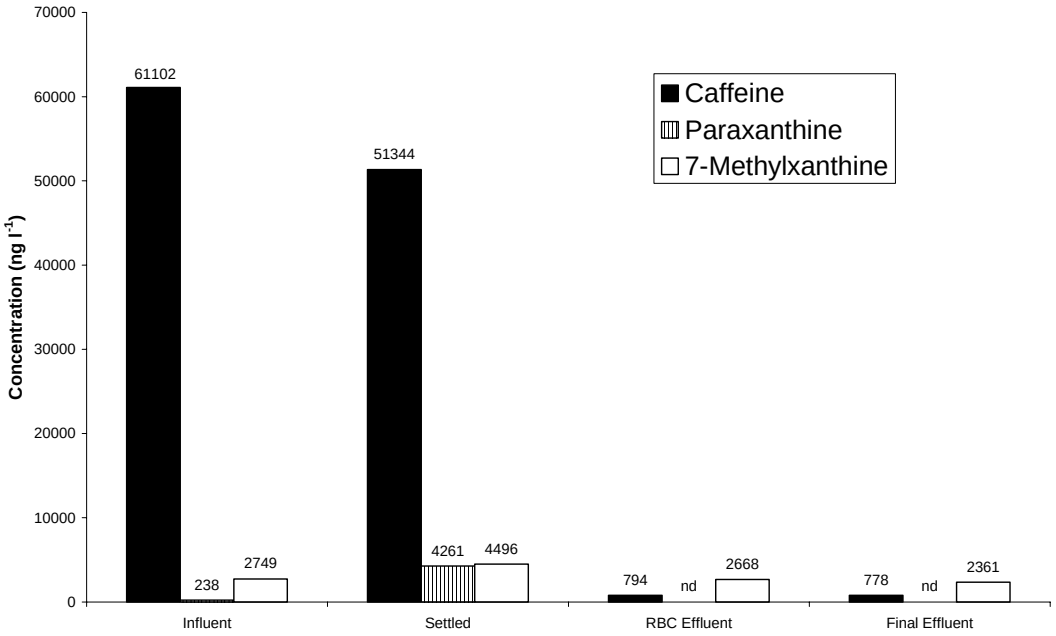


Figure 7.10: Concentration of caffeine and degradation products in samples of crude sewage, settled sewage, RBC effluent, and final effluent from Northend STP (fixed film)

Similarly to triclosan, caffeine concentrations appeared to drop rapidly through this sewage treatment works, with the exception of the reed bed. Where drops in caffeine concentration occurred during the primary settlement, there was a corresponding rise in concentrations of paraxanthine and 7-methylxanthine. Although it is tempting to suggest that this shows that there is biodegradation occurring across the primary settlement tanks and across to a much greater extent across the RBC, it should be remembered that the samples were not timed through the works. Therefore, the settled sewage sample refers to a different influent concentration than the one recorded, and hence no conclusion can be drawn regarding the amount of removal occurring.

As noted previously, caffeine has a PNEC of 87,000 ng l^{-1}. There was no available data for the NOEC. The final effluent samples did not exceed the PNEC level.

7.4.2 Frankton STP

The concentrations of caffeine and degradation products measured after each unit process at Frankton STP are shown in Figure 7.11.

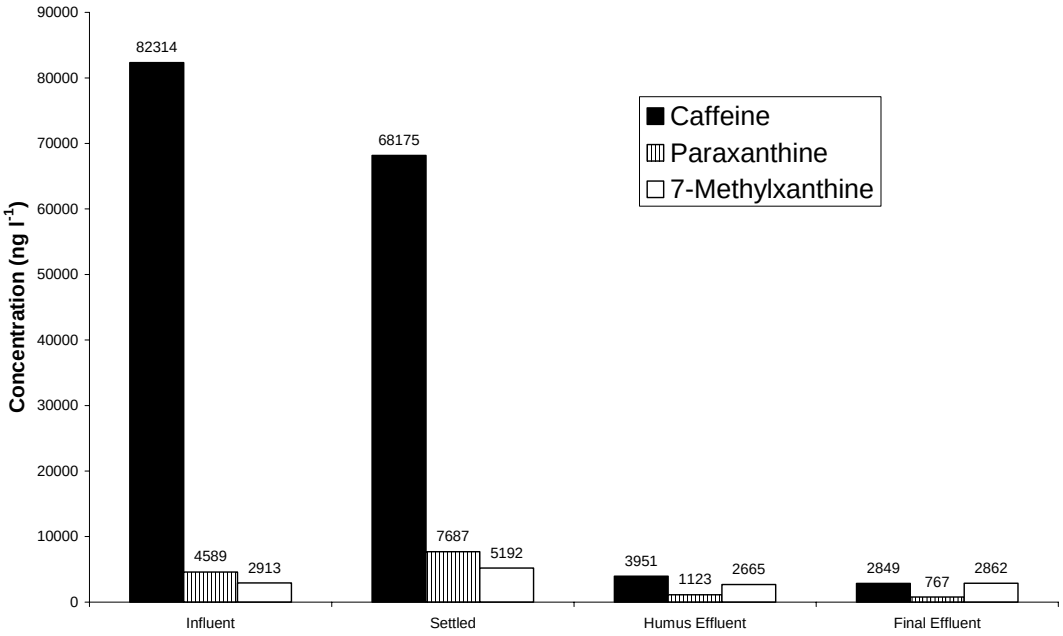


Figure 7.11: Concentration of caffeine and degradation products in samples of crude sewage, settled sewage, humus effluent, and final effluent from Frankton STP (fixed film)

Similar to Northend STP, Frankton STP also appeared to show high removal of caffeine., with both the settlement stage and biological stage appearing to show biological conversion of caffeine to paraxanthine and 7-methylxanthine. Again, it should be remembered that the samples were not timed through the works and that therefore, the settled sewage sample refers to a different influent concentration than the one recorded. Hence no conclusion can be drawn regarding the amount of removal occurring. The final effluent samples did not exceed the PNEC level.

7.4.3 Southam STP

The concentrations of caffeine and degradation products measured after each unit process at Southam STP are shown in Figure 7.12. Overall, the STP removed only 99.3% of the influent caffeine. This removal was made up of 92.4% removal during the biological treatment in the oxidation ditch. A further 6.9% removal was observed through the settlement tanks. As discussed previously, an oxidation ditch performs as a CSTR, meaning that the ditch effluent concentrations should be invariant with time. Therefore, grab samples

can be compared, even though they were not time paced. It should be noted that the concentration of caffeine in the influent of Southam STP was around four times lower than for Northend and Frankton STPs.

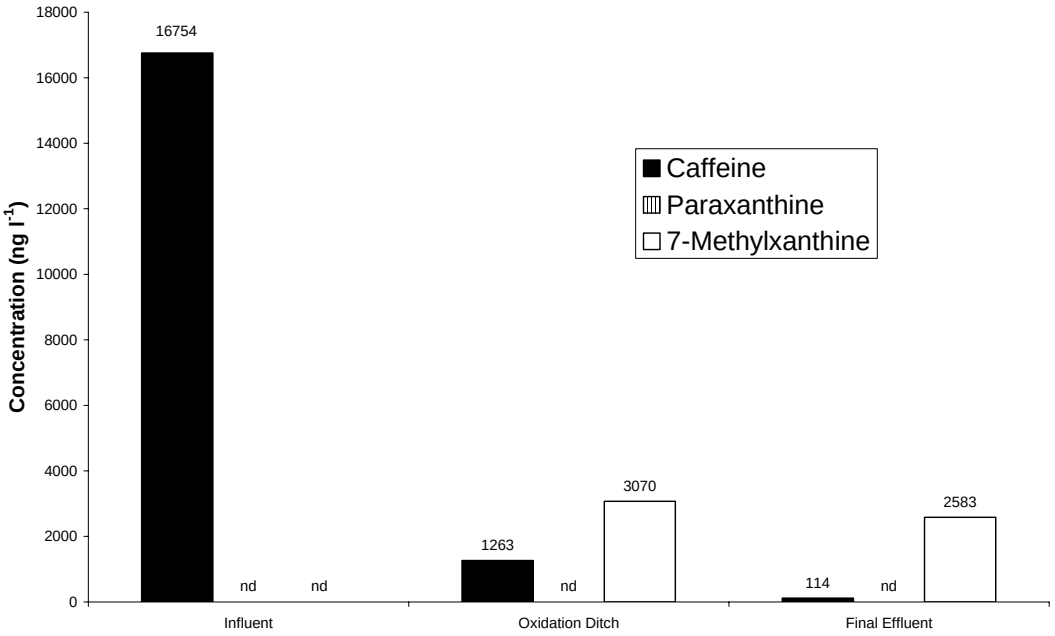


Figure 7.12: Concentration of caffeine and degradation products in samples of crude sewage, oxidation ditch effluent, and final effluent taken from Southam STP (suspended growth)

During the biological stage, where most of the caffeine is removed, it should be noted that there is an increase in 7-methylxanthine concentrations. This would help to confirm that biodegradation is occurring. It is interesting that no paraxanthine was detected, suggesting that it is readily degraded to 7-methylxanthine, once it has been formed from the degradation of caffeine. Further removal of caffeine and 7-methylxanthine was detected across the final sedimentation tanks. This is surprising, since no dissolved oxygen is available within the tank. However, it may be possible that oxygen is available from denitrification, allowing for the degradation.

It is interesting to note that the effluent of the biological stage at Southam STP actually contained more caffeine than at Northend STP, even though there appeared to be a greater concentration of caffeine at Northend STP. This would appear to be contrary to the popular assumption that nitrifying activated sludge processes provide better biological treatment than RBCs. The final effluent sample at Southam STP did not exceed the PNEC level.

7.4.4 Fate of caffeine

These results form the first reported results of caffeine concentrations in UK sewage influent and effluent. As was established in the laboratory tests, caffeine is readily biodegradable, but does not sorb to sludges. No difference had been observed in the degradation rates with suspended growth and fixed film biomass. Therefore, it was surprising to see relatively poor removal in the oxidation ditch at Southam STP, which provided a long hydraulic retention time for biological degradation, compared with the lower concentrations in the fixed film processes at Northend and Frankton STPs which provided relatively short hydraulic retention times. This is the opposite of what was observed for triclosan. In fact this result is probably simply an artefact of the sampling regime. For Southam STP, the CSTR nature of the oxidation ditch meant that the effluent sample of the ditch was comparable (in terms of percentage removal) to the influent sample, even though the samples were not time paced. However, for the plug flow type reactors at Northend and Frankton STPs, the humus effluent concentrations recorded refer to a different influent concentration, which was presumably much lower than that recorded by these samples. This can be confirmed by looking at the "removal" observed across the primary sedimentation tanks at Northend and Frankton STPs. From the laboratory tests, caffeine can only be removed by degradation, and not sorption, and therefore no removal should have been observed across the primary tanks.

7.5 Summary of findings

These results form the first recorded detections of both carbamazepine and caffeine in UK sewage influent and effluent, as well as building on a limited dataset of triclosan detections. These results have shown that three of the four selected pharmaceuticals (triclosan, carbamazepine, and caffeine) exist at detectable concentrations within the selected sewage treatment works. Only tetracycline was not seen at detectable concentrations, possibly due to poor detection limits. The influent and effluent concentrations of these compounds varied not only between each STP, but between sampling occasions at each STP. The wide variation in influent concentrations between STPs was not necessarily expected for compounds such as caffeine and triclosan, where usage could be expected to be uniform in each catchment, but could be explained by different amounts of infiltration and run-off in the different types of catchment. In particular, Northend and Frankton STPs covered rural catchments, whereas Southam STP covered a small town, and Finham STP covered the city of Coventry.

These initial results showed that both triclosan and caffeine were strongly removed during biological treatment. This would suggest that both compounds undergo significant amounts of biodegradation, as was found in the laboratory fate tests. In contrast to these two compounds, carbamazepine appeared to be poorly removed during sewage treatment. This suggested that carbamazepine was not significantly removed by sorption or biodegradation, again confirming the results of the laboratory fate tests.

Some differences were observed in the ability of the various types of treatment process to removal the various compounds. Of particular interest was the higher concentrations of caffeine in the effluent of the oxidation ditch at Southam STP, compared to the RBC at Northend STPor the bacteria beds at Frankton STP. This would suggest that the suspended growth system was performing more poorly than fixed film systems. Conversely, for triclosan, the concentration in the effluent of the oxidation ditch was much lower and more consistent than from the fixed film processes.

Whilst the sampling regime employed in this section of work meant that little can be concluded in terms of percentage removal, it is clear that there is quite a wide variation in the effluent concentration of the pharmaceuticals particularly from the biological stages of each of the STPs, but also in the final effluent. The next chapter looks at these differences in more detail.

Chapter 8: Composite Sampling of Southam STP

This set of experiments involved taking composite samples from a single sewage treatment works (Southam STP). Detailed bi-hourly analysis of the variations in biomass and wastewater composition, combined with variations in pharmaceutical concentrations and removal, were used to examine the behaviour of the selected pharmaceuticals and what factors could affect the removal of pharmaceuticals.

Southam STP was chosen for two reasons. Firstly, future expansion of biological treatment is concentrated on suspended growth type systems. Therefore, the research would be most relevant if it concentrated on a suspended growth system. Secondly, in the choice between Finham and Southam STPs, there was a distinct advantage in having access to flow rate data. This was readily available at Southam STP, where the flow rate data was recorded automatically every fifteen minutes (on the Severn Trent Rtemis system). As noted in the previous chapter, although the total flow through Finham STP was measured by a final effluent flow meter, there was no way of measuring the flow derived from each of the two influents at the STP.

8.1 Pharmaceutical concentrations

The pharmaceutical concentrations were measured hourly for a total of sixty hours at the influent, oxidation ditch effluent, and final effluent of Southam STP. The concentrations detected are presented in the sections on each compound below in terms of a mass flow (mg hr^{-1}). In all cases, only aqueous concentrations of the pharmaceuticals were monitored, since there was insufficient time to conduct extractions from biomass. It had previously been established that it was possible to accurately predict the solid phase concentrations of triclosan from sorption isotherms, whilst based on the laboratory sorption test results there were not expected to be detectable concentrations of tetracycline, carbamazepine or caffeine in the solid phase.

8.1.1 Triclosan concentrations

Triclosan concentrations through Southam STP are shown in Figure 8.1 below. Concentrations in the influent ranged from <LOQ to 6190 ng l^{-1}, with a median of 1800 ng l^{-1}. These maximum and minimum values both exceeded the range previously recorded for

triclosan. The median was towards the lower end of the concentrations recorded during the grab sampling (1596 to 5115 ng l^{-1}), although much higher than reported by Kanda *et al.* (Kanda *et al.* 2003) at the same works (670 and 1100 ng l^{-1}).

From Figure 8.1 it would appear that concentrations of triclosan in the oxidation ditch effluent are approximately constant. This confirms the assumption used during the grab sampling that the output of an ideal CSTR, such as an oxidation ditch, is time invariant. In reality, there was some variation in the concentrations, ranging from below the limit of detection (86 ng l^{-1}) to below the limit of quantification (340 ng l^{-1}), with a very approximate median of 150 ng l^{-1}. It is interesting to note, that whilst there is a wide range in variation of triclosan load entering the works, there is only a very small variation in the oxidation ditch effluent load.

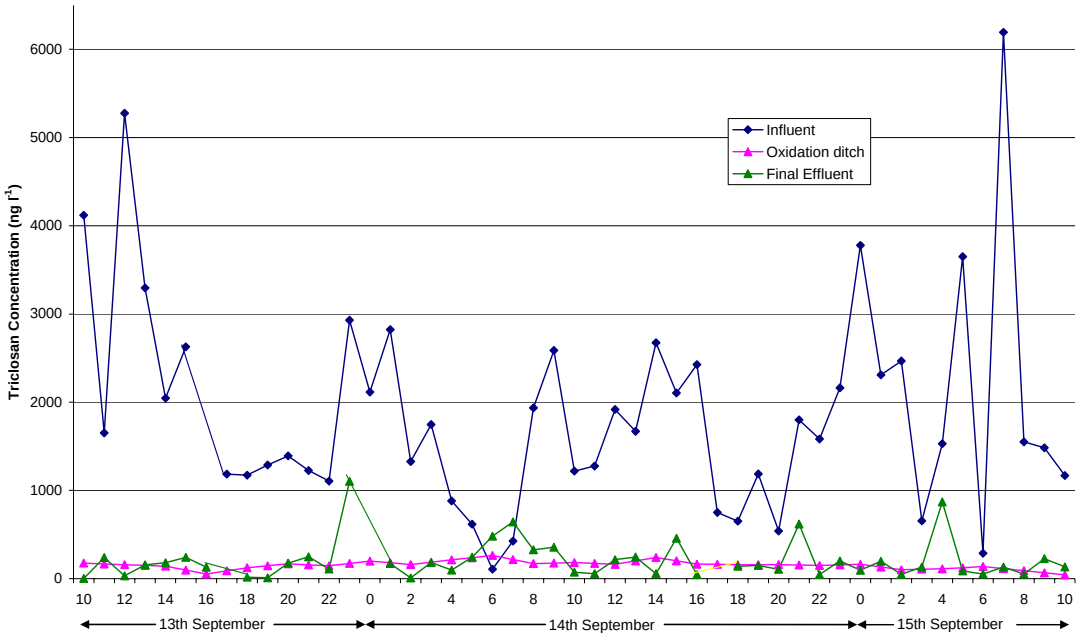


Figure 8.1: Concentrations of triclosan in Southam STP.

The concentrations had approximately the same median value as for the oxidation ditch effluent, at 146 ng l^{-1} (LOQ in final effluent: 200 ng l^{-1}). However, the range of concentrations was much wider, from <LOD to 870 ng l^{-1}. The reasons for this are discussed below in combination with the flow data.

The sampling was conducted during a dry weather period, as can be seen in the influent flow graph shown in Figure 8.2. Flows were recorded as a two hour average. Annual average flow

for this STP was recorded as 296 m^3 hr^{-1}, but the average flow rate recorded during this sampling was much lower at 167 m^3 hr^{-1}. Indeed, at no point did the recorded flow reach the annual average flow.

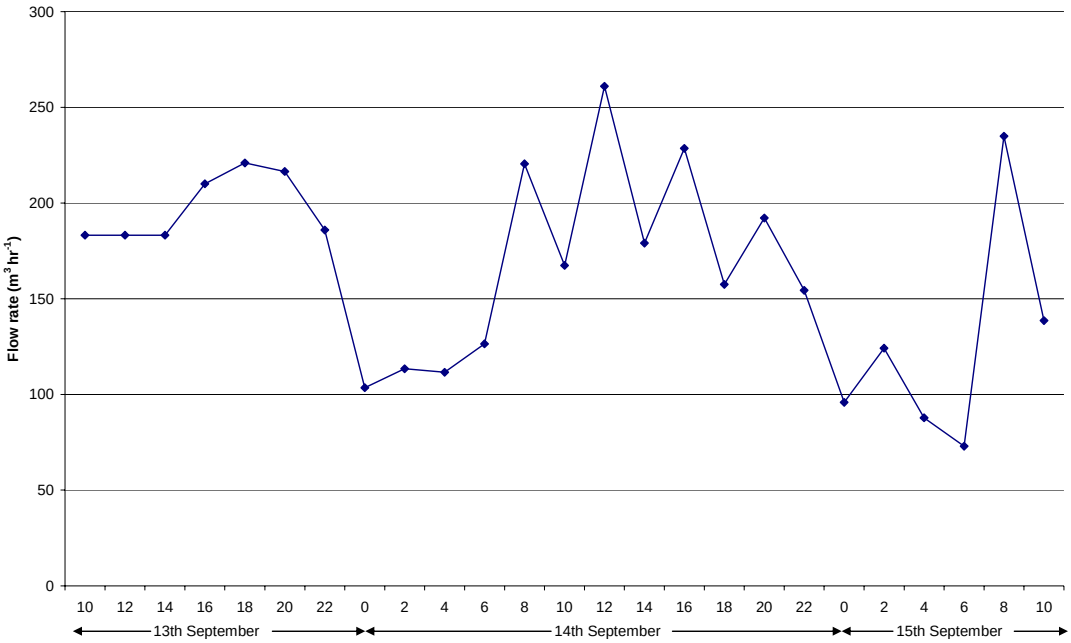


Figure 8.2: Influent sewage flow at Southam STP

Of note from the flow data are that much of the usual diurnal pattern is not visible, particularly during the daytime, although flow does drop during the early morning (0000 to 0600). Further investigation of the sewage inlet showed that the flow to the works came from three distinct sources – gravity flow from the town of Southam, as well as pumped flow from two large villages nearby. These pump stations delivered flow based on levels in their wet wells, thereby masking the normal diurnal pattern. This was of particular concern to the site operators, since during warm periods with low flow, the sewage from the pumping stations could remain in the pipes for a long time before reaching the works, causing septicity and odour problems (see section 8.2.2 on pH).

Combining the triclosan concentrations and flow data provided triclosan mass flow, as can be seen in Figure 8.3 below. The mass flow of triclosan in the influent varied widely over the time period monitored from <LOQ to 970 mg hr^{-1}, with a median of 304 mg hr^{-1}. The concentrations detected in both the oxidation ditch samples (<LOQ to 44 mg hr^{-1}; median 26 mg hr^{1}) and the final effluent samples (<LOD to 76 mg hr^{-1}; average 21 mg hr^{-1}) were significantly lower than the influent and showed much less variability than when considering

the triclosan concentrations alone. There were, however, still several points at which the mass flow in the final effluent exceeded the mass flow in the oxidation ditch effluent, suggesting either experimental error, or an additional input of triclosan after the oxidation ditch. Further investigation of the STP showed that the final effluent BOD monitor had an automatic cleaning system, which discharged downstream of the final settlement tanks. Since the cleaning chemicals contained an (un-named) antibacterial agent to prevent bacterial growth in the BOD monitor, it was presumed that this could be triclosan, leading to the higher effluent values observed here. However, it was not possible to obtain information on the chemicals used, quantities, or times at which automatic cleaning occurred. Additionally, operators were observed emptying buckets into the final effluent channel, which may have accounted for the peak in the final effluent sample at 1200 on 14th September. Again, volumes and chemicals involved could not be identified.

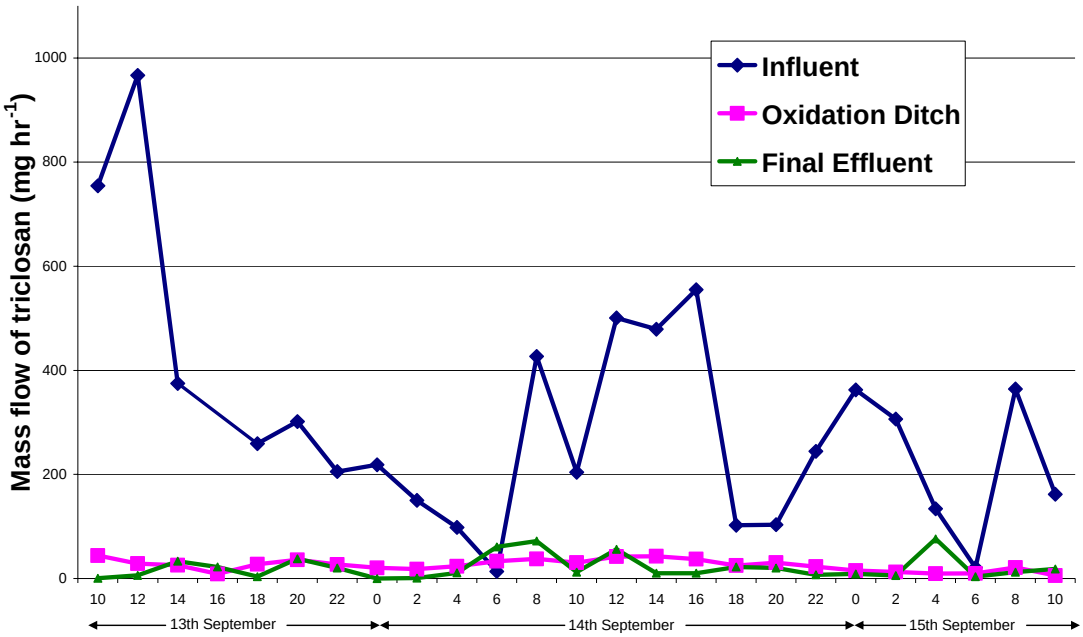


Figure 8.3: Daily variation of triclosan mass flow

Based on these mass flow rates, and the population equivalent of 14275 for the STP, gave a usage of triclosan of 0.51 mg per head per day. This is significantly lower than estimated by previous studies, such as 2.82 mg d^{-1} (Halden and Paull 2005), 3.4-3.8 mg d^{-1} (Sabaliunas *et al.* 2003), 2.8-5.5 mg d^{-1} (McAvoy *et al.* 2002).

Overall, the STP removed around 93.1% of the triclosan during this sampling period, made up of 91.6% through the oxidation ditch and a further 1.5% through the final tanks. In total,

this was a little lower than the removal levels (97.1%) previously reported from the initial grab sampling work, which showed 96.2% and 1.0% removal in the two areas respectively.

8.1.2 Tetracycline concentrations

All samples were analysed by HPLC-UV for tetracycline and degradation products. However, as was found for the grab samples, none of the composite samples contained a concentration above the detection limit. It can therefore be concluded that any emissions of tetracycline in the effluent of Southam STP are below the level of the predicted no-effects concentration (PNEC), and can pose no threat to the aquatic environment.

8.1.3 Carbamazepine concentrations

Carbamazepine concentrations through Southam STP are shown in Figure 8.4 below. Concentrations in the influent ranged from <LOQ to 1365 ng l^{-1}, with a median of 688 ng l^{-1}. This median was virtually identical to the crude concentration measured in the grab sampling (662 ng l^{-1}). Since this is the first report of carbamazepine in UK sewage influent, there are no reports with which to compare it. Reports from Germany have suggested a somewhat higher median concentration of 1780 ng l^{-1} (Heberer 2002).

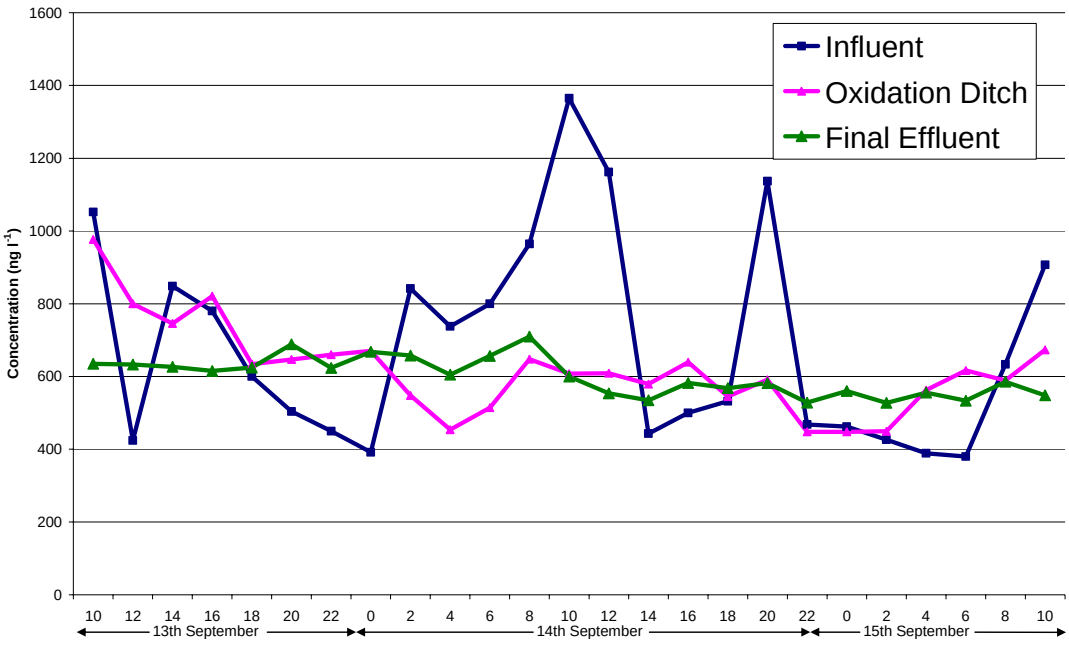


Figure 8.4: Concentrations of carbamazepine in Southam STP

There is more variation in the concentrations of carbamazepine in the oxidation effluent than was observed for triclosan, ranging from 448 to 977 ng l^{-1}, with a median of 619 ng l^{-1}. This

was actually the same concentration as measured in the grab samples. The concentrations of carbamazepine in the final effluent were more stable, ranging from 528 to 710 ng l^{-1}, with a median of 600 ng l^{-1}, slightly lower than measured in the grab samples (615 ng l^{-1}). As for triclosan, the concentrations in the final effluent were often observed to be higher than in the oxidation ditch effluent. The reasons for this are discussed after flow rates have been considered.

Combining the concentrations of carbamazepine in Figure 8.4 with the flows already presented in Figure 8.2, produces the mass flow of carbamazepine, as shown in Figure 8.5 below. Similar to triclosan, the lowest mass flows of carbamazepine were observed between 0000 and 0600. The highest mass flows were more defined than for triclosan, at around 1000 and 2000. Since carbamazepine is a pharmaceutical usually taken orally twice per day, it is likely to be mostly excreted in two peaks corresponding to the morning and evening dose, although with a certain time delay.

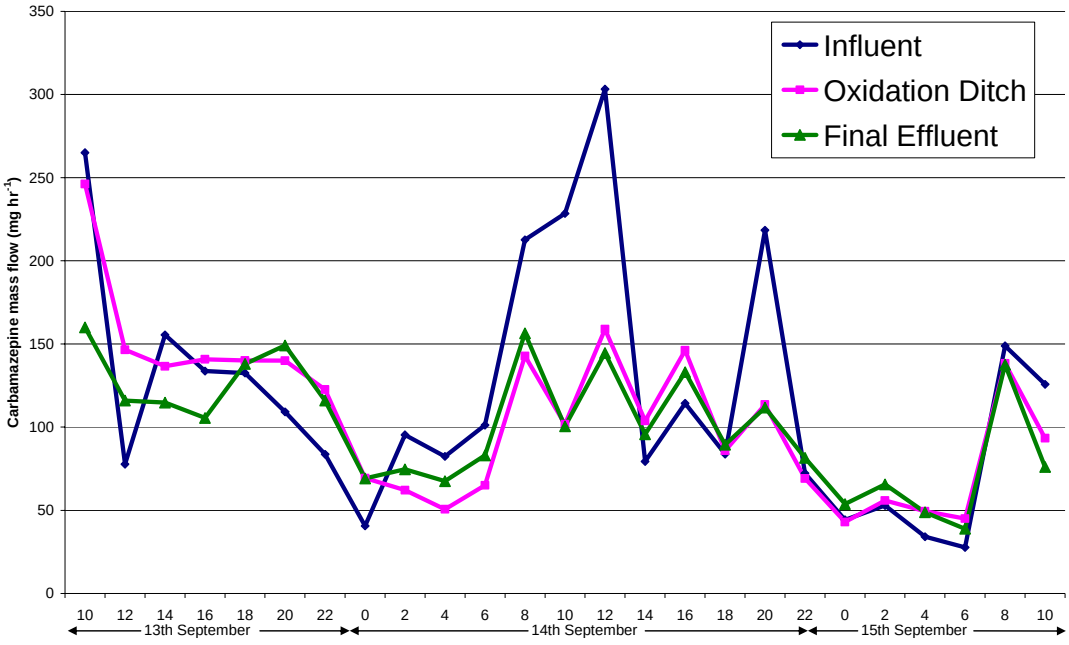


Figure 8.5 Daily variation of carbamazepine mass flow

The influent mass flow of carbamazepine varied from 28 to 303 mg hr^{-1}, with a median of 121 mg hr^{-1}. The concentrations detected in both the oxidation ditch effluent samples (43 to 246 mg hr^{-1}; median 107 mg hr^{-1}) and the final effluent samples (39 to 160 mg hr^{-1}; median 101 mg hr^{-1}) were slightly lower than the influent and were more attenuated. Based on UK

usage of carbamazepine of 40 tonnes in the year 2000 (Jones *et al.* 2002), and a population of 60 million, would give an expected concentration of 1.8 mg per person per day. Of the consumed carbamazepine, approximately 56% can be expected to be excreted in urine as the parent compound (Amore *et al.* 1997), with a further 35% excreted as carbamazepine-10,11-diol (Bourgeois and Wad 1984). Based on the parent compound excretion rate, this yields an expected carbamazepine load of 1.01 mg per person per day. Using the above mass flow rates, and the population equivalent of 14275 for the STP, gave a usage of carbamazepine of 0.20 mg per head per day.

Overall, the STP removed around 12.8% of the carbamazepine during this sampling period, with 10.0% of the removal occurring in the oxidation ditch, and a further 2.8% removal occurring through the final sedimentation tanks. This is higher than both the low removal levels (7.1% overall) previously reported from the initial grab sampling work (which was made up of 6.5% and 0.6% removal in the oxidation ditch and final tanks respectively) and literature values which have typically reported only around 7 or 8% removal (Heberer *et al.* 2002; Ternes 1998).

8.1.4 Caffeine concentrations

Caffeine concentrations through Southam STP are shown in Figure 8.6 below. Concentrations in the influent ranged from 525 to 55000 ng l^{-1}, with a median of 17,750 ng l^{-1}. This median was slightly higher than the crude concentration measured in the grab sampling (16754 ng l^{-1}). Caffeine concentrations were below the limit of detection (1 ng l^{-1}) in all oxidation ditch and final effluent samples, suggesting that essentially complete removal of caffeine occurs within the STP. Although no reports of caffeine concentrations in UK sewage influents exist prior to this measurement, researchers in Germany reported concentrations up to 640,000 ng l^{-1}, with a median of 320,000 ng l^{-1} (Heberer *et al.* 2002). They also reported a median effluent concentration of 220 ng l^{-1}, although maximum effluent concentrations of up to 292,000 ng l^{-1} have been reported (Rogers 1996).

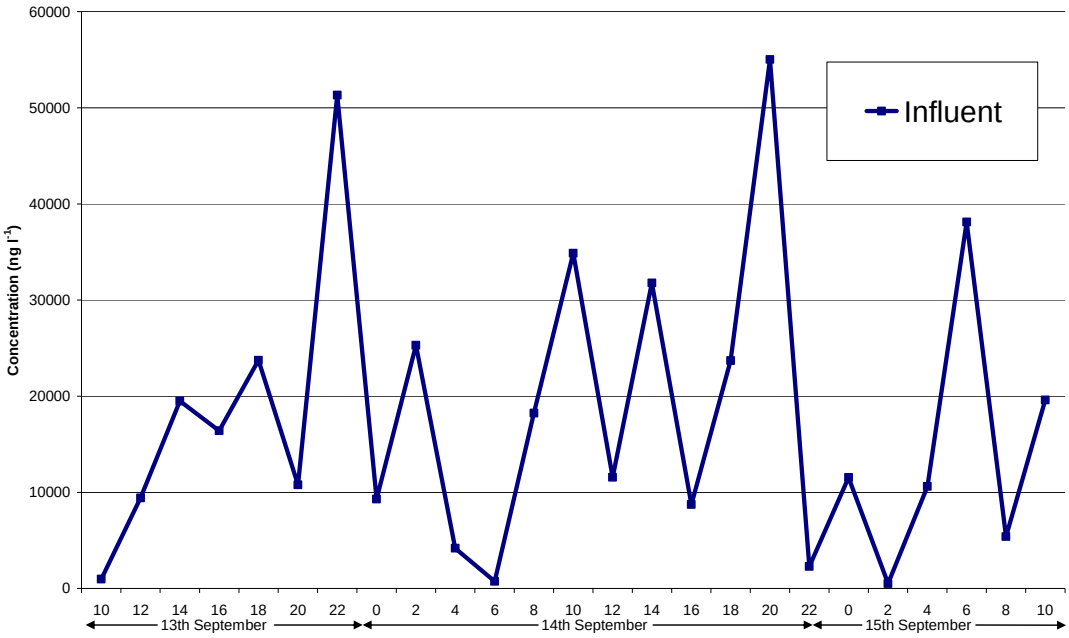


Figure 8.6: Concentrations of caffeine in Southam STP

Concentrations in the influent varied from 65 to 10574 mg hr^{-1}, with a median of 2563 mg hr^{-1} (equivalent to 16000 ng l^{-1}). None of the oxidation ditch effluent or final effluent samples had a concentration of caffeine above the detection limit of 12 ng l^{-1}. Similarly, no degradation products were found in either the final effluent or oxidation ditch effluent. A trace (below quantifiable limits) of paraxanthine and theophylline were found in three (out of sixty) influent samples. The daily variation in the mass flow of caffeine in the influent is shown Figure 8.7.

Combining the concentrations of caffeine in Figure 8.6 with the flows already presented in Figure 8.2, produces the mass flow of caffeine, as shown in Figure 8.7 below. These ranged from 65 to 10574 mg hr^{-1}, with a median of 2960 mg hr^{-1}. Similar to triclosan and carbamazepine, the lowest mass flows of caffeine were observed between 0000 and 0600. The highest mass flows were observed at around 1800 and 2200. Based on normal human caffeine consumption, it may have been expected to see another peak earlier in the day (around 1000-1200). However, the nature of the pumped flow influent to this STP may have masked this.

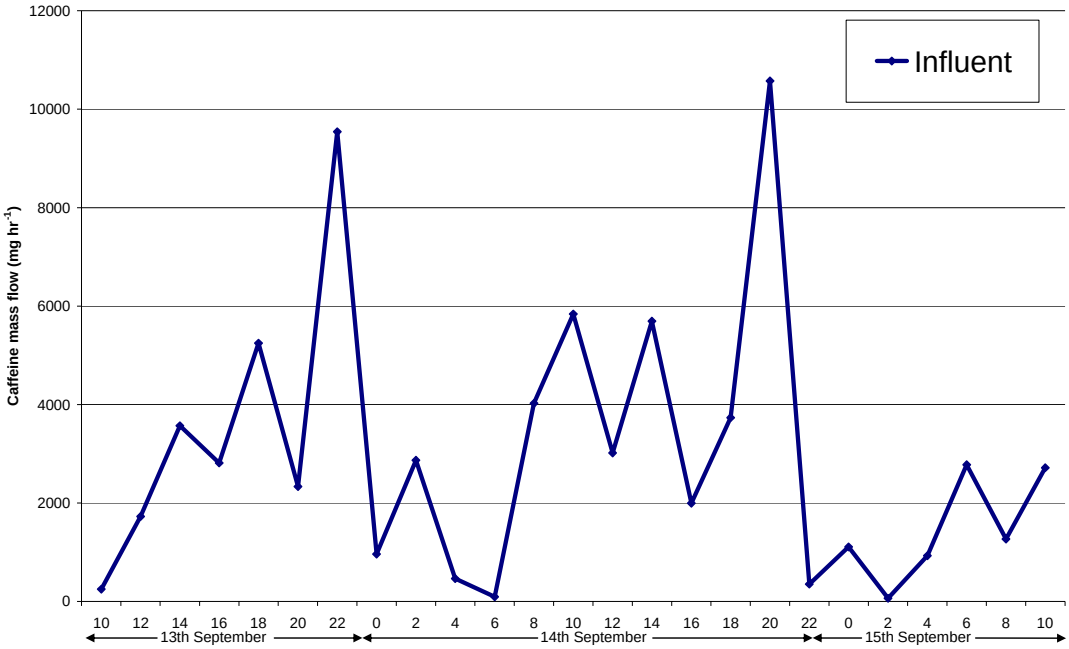


Figure 8.7: Daily variation of caffeine mass flow

Since caffeine was not detected other than in the influent, it was not possible to give an exact figure for total removal, although based on the analytical detection limits, it was possible to say removal was greater than 99.96%. This is similar to the high removal levels (99.3%) previously reported from the initial grab sampling work.

Based on the above figure, a caffeine excretion of 4.98 mg per person per day can be calculated. Data on excreted caffeine concentrations are limited, but Chambaz *et al.* (2001) suggested between 1.2% and 2.8% of consumed caffeine was excreted in urine, whilst Georga *et al.* (2001) suggested less than 2%. This would equate to between 180 and 400 mg per person, or roughly 3 to 6 cups of coffee per person per day - a reasonable figure considering the large potential errors in this calculation due to the excretion rate.

8.2 Wastewater and biomass parameters - implications for pharmaceutical removal

The removal of dissolved and particulate carbonaceous BOD and organic matter found in wastewater, of which pharmaceuticals can be considered to be a subset, is accomplished biologically using a variety of micro-organisms, principally bacteria. For the micro-organisms to effectively oxidise the dissolved and particulate carbonaceous organic matter into simple end products and additional biomass, certain optimal conditions must prevail. Whilst these conditions include the supply of sufficient nutrients (such as oxygen, ammonia, and phosphorus), many other factors can have an effect. For example, temperature should ideally be between 12 and 18 ºC (Metcalf and Eddy 2003), whilst pH should be within the range 6.5 to 7.5 (Metcalf and Eddy 2003). Similarly, sorption can also be affected by the wastewater and biomass conditions, as was demonstrated and discussed in the laboratory sorption tests.

As discussed in the literature review, other researchers have identified parameters that appear to either affect pharmaceutical removal, or be directly correlated with pharmaceutical removal sharing a controlling mechanism. For example, Holbrook *et al.* (2004) and Kreuzinger *et al.* (2004) noted that sorption (and hence total removal) of 17b-estradiol and 17a-ethinylestradiol was weakly correlated with colloidal protein and polysaccharide concentrations ($r^2 \sim 0.4$). Drewes *et al.* (2001) suggested that organic carbon concentrations were a significant factor in the removal of iodinated X-ray contrast media. Indeed, Stokes and Churchley (2006) found a direct correlation ($r^2 = 0.9994$) between total organic carbon concentrations and estradiol concentrations, implying that removal mechanisms for both are similar. Urase *et al.* (2005) demonstrated that pH could affect removal of pharmaceuticals. In particular acidic pharmaceuticals, such as ibuprofen, ketoprofen, gemfibrozil, ketoprofen, naproxen, diclofenac, and indomethacine showed higher removal at acidic pH.

The sections below show the concentrations of the wastewater and biomass parameters measured during the composite sampling work at Southam STP, along with their implications for pharmaceutical removal. In general, these implications are based solely on the triclosan results, since not enough information was available from the other three compounds to produce meaningful results.

8.2.1 Suspended solids

The concentration of suspended solids in the influent varied from 79 to 359 mg l^{-1} (median 201) during the sampling period. The concentration in the oxidation ditch varied from 2021 to 3061 mg l^{-1}, with a median of 2476 mg l^{-1}. Surprisingly, on two occasions the suspended solids concentration in the final effluent, exceeded the Environment Agency discharge consent level for the EA (20 mg l^{-1}), as shown in Figure 8.8. These two readings may have been due to the sampling method causing biomass to slough off the final effluent pipe work. Overall, the suspended solids in the final effluent ranged from 4 to 44 mg l^{-1}, with an average of 11 mg l^{-1}, equivalent to an overall removal of 99.6%. The STP is designed to have an average SS concentration of 10 mg l^{-1}, in order to meet compliance with the 95 percentile discharge consent. The two results recorded above this level would not result in a consent breach on their own, but may be symptomatic of an underlying solids problem at the STP.

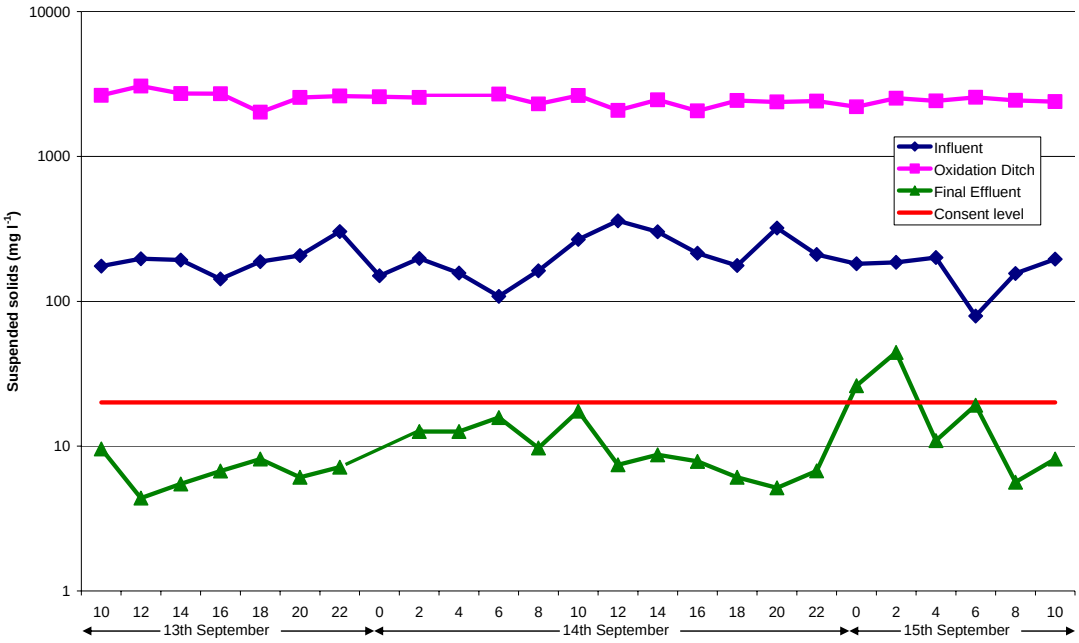


Figure 8.8 Daily variation of suspended solids

Suspended solids concentrations can be expected to affect triclosan removal in two ways. In the influent, a higher amount of suspended solids would produce a higher potential for sorption, according to Equation 11 below. This is the Freundlich sorption equation, as previously discussed, where C_s equals the mass of sorbed pharmaceutical divided by the total suspended solids.

$$C_s^{ads} = K_F^{ads} (C_{aq}^{ads})^{\frac{1}{n}}$$

Equation 11

The same would be true in the oxidation ditch mixed liquor, but a higher suspended solids concentration here would also suggest that a higher amount of active biomass for degradation was also present, as described by Equation 12 below (Schwarzenbach *et al.* 2003; Ternes *et al.* 2004).

$$\frac{dC}{dt} = K_{biol} C_0 SS$$ **Equation 12**

However, the data collected in this research did not show a correlation between either influent suspended solids concentration and triclosan concentration, or between mixed liquor suspended solids concentration and triclosan removal, suggesting other factors may be the cause of the variations seen in removal.

Previous work by Ternes *et al.* (2004) has suggested the most important parameter affecting pharmaceutical removal is sludge age, through three independent processes: increasing biodiversity, share of active biomass within the total suspended solids, and decrease of specific sludge production. Clara *et al.* (2005) showed that for many pharmaceuticals, there exists a critical sludge age (T_{crit}) of about 10 days, below which little or no degradation occurs. This critical sludge age has been shown to be different for various pharmaceuticals. For example, the critical sludge age for ibuprofen was around 1 day, whilst for estrone it was around 8 days (Ternes *et al.* 2004). Some pharmaceuticals, such as bisphenol-A showed a wide range of critical sludge age (Clara *et al.* 2005).

These variations in the critical sludge age demonstrate that the bacteria responsible for degradation can take various lengths of time to adapt in order to degrade the pharmaceutical of interest. It is possible to mathematically model this "biological adaptation rate", as well as the critical sludge age, according to equation 6.2, which generates the graph as shown in Figure 8.9:

$$k_{i,biol} = k_{i,biol}^{\max} \cdot \frac{1}{\pi} \tan^{-1} \left[\frac{\zeta\left(\frac{SRT}{T_{crit}}\right)}{1-\left(\frac{SRT}{T_{crit}}\right)^2} \right]$$ **Equation 13**

$k_{i,biol}^{\max}$ = maximum degradation rate (L gSS^{-1} d^{-1})

SRT = Sludge retention time (d)

T_{crit} = critical sludge age (d)

ζ = "biological adaptation rate"

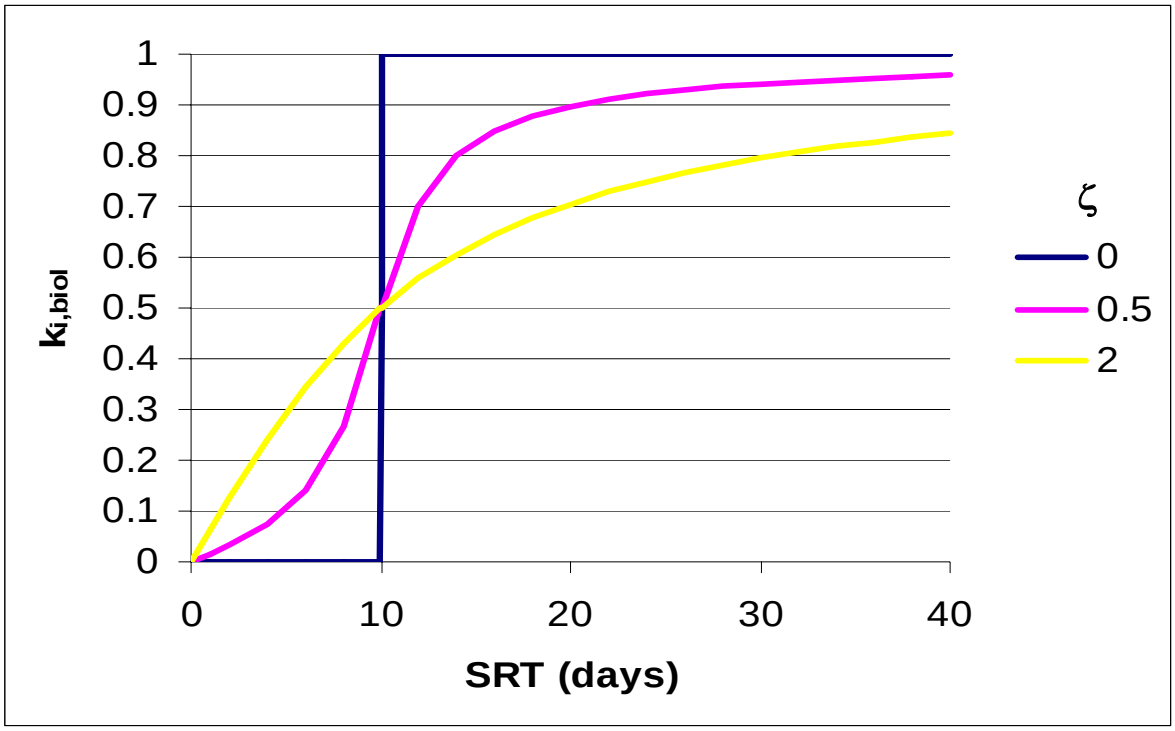


Figure 8.9: Modelling of the effect of SRT on biological degradation rate, with T_{crit} = 10 days

The use of the "biological adaptation rate" term reproduces not only the theoretical situation (ζ=0), in which degradation occurs immediately after a critical sludge age has been reached, but also the situation in which the bacteria take a long time (e.g. ζ=2) to adapt to use the selected pharmaceutical as a substrate. Values for $k_{i,biol}^{max}$ and T_{crit} have been determined by both Ternes *et al.* (2004) and Clara *et al.* (2005), but further work will need to be conducted to produce values for the biological adaptation rate.

In this study, all suspended solids samples taken from Southam STP had an identical sludge age which, according to the Ternes *et al.* (2004) equation, would suggest that similar amounts of removal through the STP would be expected for all samples. However, differing amount of pharmaceutical removal were observed, which is not accounted for by either the original Ternes *et al.* (2004) equation (Equation 12), or the modification of it presented here (Equation 13). Therefore, the reaction rate constant must also depend on additional factors,

other than just the SRT. Indeed, previous studies with triclosan have observed no relationship between SRT and level of observed removal (Federle *et al.* 2002).

8.2.2 pH

The variation in pH during the sampling period is shown in Figure 8.10. Values ranged from 5.8 to 7.6 in the influent (median 7.1), 6.8 to 7.0 in the oxidation ditch (median 6.9), and 7.0 to 7.2 (median 7.1) in the final effluent. The pH levels in the oxidation ditch were slightly more acidic than often associated with a nitrifying STP, with pH of 7.5 to 8.0 being optimal for nitrification, although 7.0 to 7.2 is often more common.

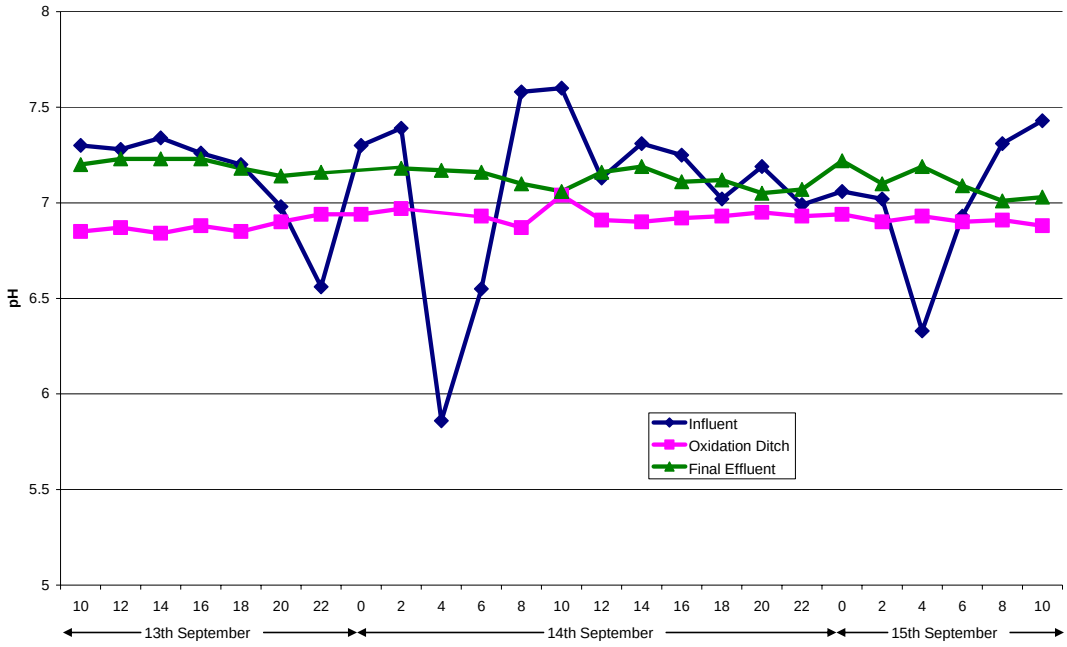


Figure 8.10: Daily variation of pH

As can be seen in the figure, the influent tended to be most acidic (pH 5.8 – 6.5) in the early morning. This may have been due to the pumped flow nature of part of the STP influent. During periods of low flow, such as at night, the warm sewage would be retained in the pipes for a long time, which could lead to septicity.

Figure 8.11 below shows the correlation between pH and triclosan concentration. Although no direct correlation can be observed between the two parameters, it clearly shows the wide variation in pH and triclosan concentration observed in the influent, compared to the closely grouped data points for the oxidation ditch and final effluent. All final effluent samples had a pH between 7.00 and 7.25.

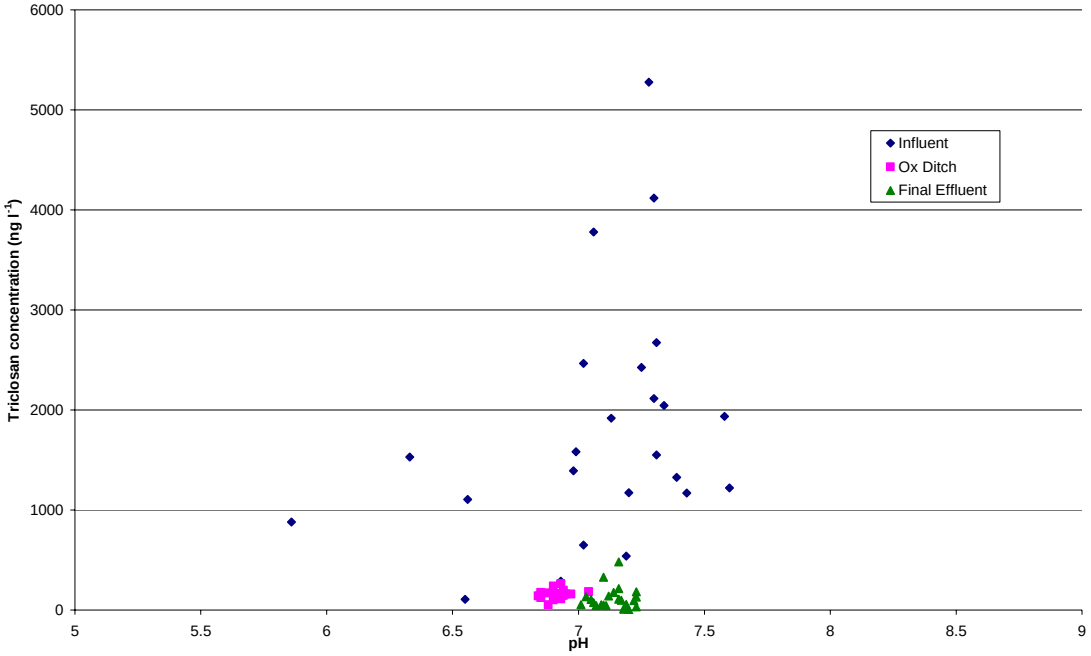


Figure 8.11: Correlation between pH and triclosan concentration in Southam STP

Tixier *et al.* (2002) had suggested that pH could have a significant effect on the removal of triclosan. Above pH 8, photolysis of triclosan (pKa 8.1) becomes a significant removal mechanism. However, in this study, all samples were below pH 7.6, and so triclosan could be expected to be mostly in its photo-stable (phenolic) form (see Figure 8.12 for structures), in which removal due to photolysis can be expected to be negligible. According to Sabaliunas *et al.* (2003), the fraction of triclosan present in its ionic form can be estimated according to Equation 14.

$$C_{ion} = C_{un-ion}[1+10^{(pH-pKa)}] \qquad \textbf{Equation 14}$$

The phenolate form of triclosan, although available for photolysis, does not sorb to biomass (Sabaliunas *et al.* 2003; Thomas and Foster 2005), and therefore cannot be removed via primary sedimentation, resulting in more triclosan passing forward to secondary biological treatment. Using the above equation, the percentage of triclosan in the ionic form was calculated, as shown in Figure 8.12 below.

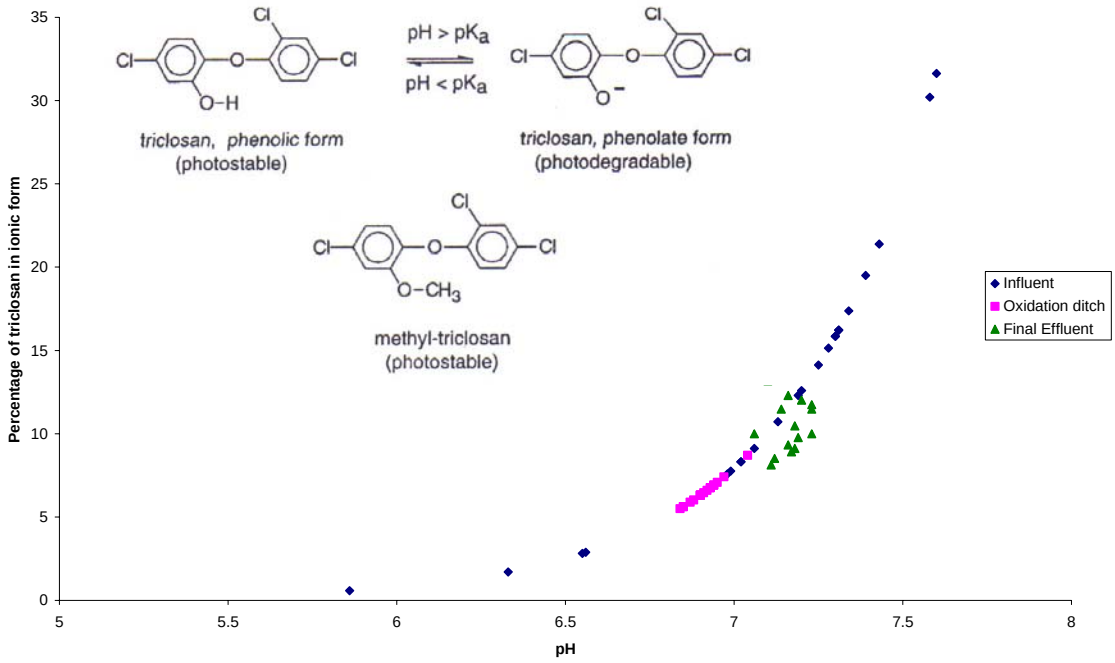


Figure 8.12: Percentage of triclosan as phenolate form in Southam STP

At the highest pHs up to 32% of the triclosan in the Southam influent was in its phenolate, non-bioavailable form. In an STP with primary sedimentation (which Southam STP does not have), this would have resulted in a load of triclosan of up to 800 ng l^{-1} passing forward to the secondary biological treatment. Thomas and Foster (2005) found a similar effect for acidic pharmaceuticals such as ibuprofen, naproxen, ketoprofen, and diclofenac. At the pHs in their study (6.2-7.4) more than 98% of the acidic drugs were expected to be in their ionized form, and hence not sorbed to sludges. A qualitative methanol extraction failed to detect any of the acidic drugs in the sludges.

In the above figure both the influent and oxidation ditch samples show complete agreement with the Sabaliunas equation, whilst the final effluent samples are more dispersed. This is due to most of the final effluent samples being below the LOQ, and hence a large amount of error has been incurred in estimating the concentrations.

Caffeine has pKa values of 0.6 and 14, indicating that it can be both a weak acid and a weak base. Therefore, pH variations in the ranges measured in STPs would not be expected to have any effect on the equilibrium between the ionized and unionized forms. Measured caffeine concentrations are shown against pH in Figure 8.13 below, as expected showing no discernable pattern.

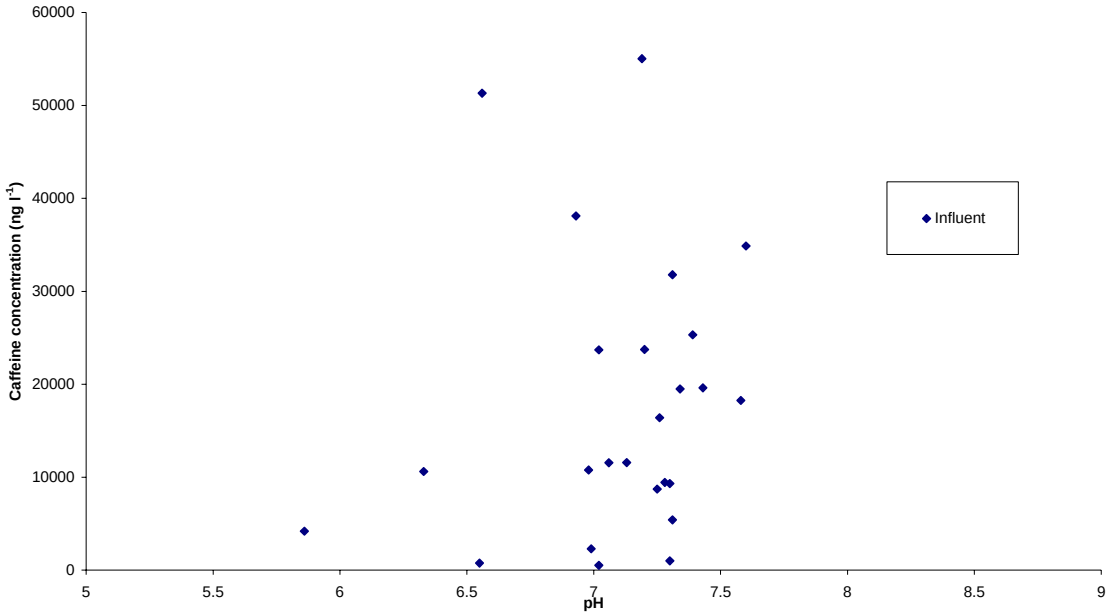


Figure 8.13: Correlation between pH and caffeine concentration in Southam STP

From the results and discussion above it is possible to see that certain pharmaceuticals can be affected by changes in pH. Therefore, it may be possible to manipulate the influent pH by chemical dosing to ensure that the target pharmaceuticals are in their unionised forms by the time they reach the primary sedimentation tanks. This would achieve a reduction in the load of the pharmaceutical moving forward to biological treatment, and help to improve the total removal.

pH adjustment, in terms of line or sodium carbonate addition, is already regularly used to improve denitrification. These results may suggest that if improvement in removal of acidic pharmaceuticals is required, then calcium carbonate should not be added until after the primary sedimentation tanks, rather than into the influent.

For triclosan, however, simply reducing the pH may not be the optimal solution, due to its photolysis reaction. If the STP in question had a large liquid surface area open to sunlight, then photolysis might account for more triclosan than could be achieved by sorption to primary solids. Design of such an STP would have to carefully consider the amounts of removal achievable by each mechanism, and the costs (and availability) of chemical dosing necessary to adjust the pH.

8.2.3 Chemical oxygen demand

The variation in chemical oxygen demand during the sampling period is shown in Figure 8.14. Concentrations ranged from 83 to 263 mg l^{-1} in the influent (median 169 mg l^{-1}), 19 to 51 mg l^{-1} in the oxidation ditch (median 34 mg l^{-1}), and 19 to 34 mg l^{-1} (median 25 mg l^{-1}) in the final effluent.

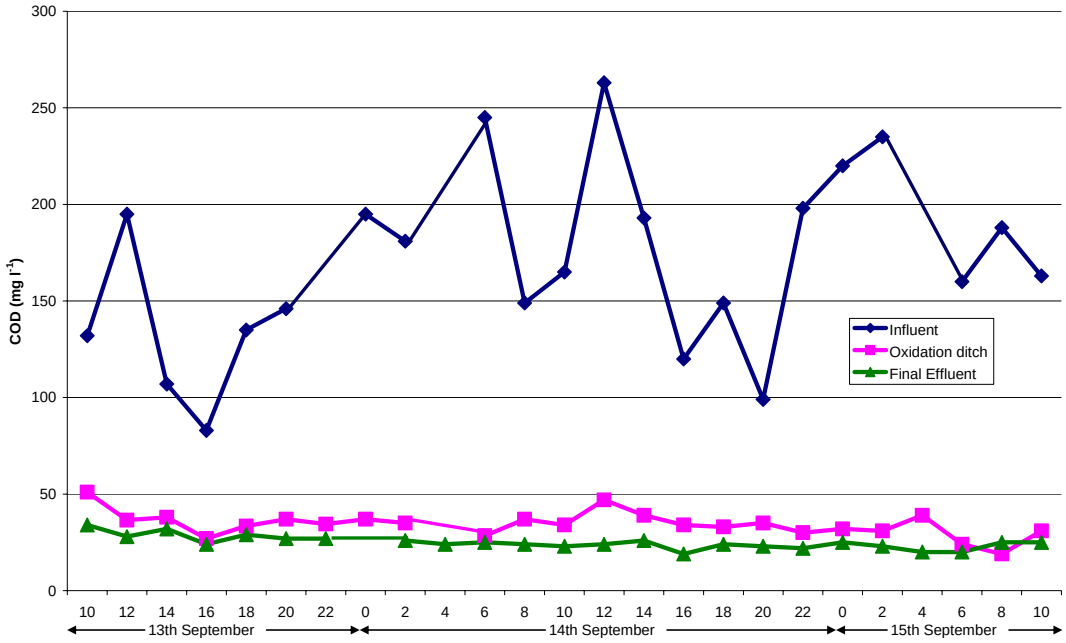


Figure 8.14: Daily variation of chemical oxygen demand

Overall, 85% of the COD was removed by the STP, with the majority (79.9%) occurring in the oxidation ditch, and the remaining 5.1% occurring in the humus tanks.

Chemical oxygen demand removal is usually a good indicator of the biological activity of the sewage treatment works. As such, removal of compounds that are highly biodegradable, such as triclosan, could be expected to show a similar pattern to that of the removal of COD. Triclosan concentrations are plotted against COD concentrations in Figure 8.15 below. The final effluent showed a very compact range of COD concentration (19-32 mg l^{-1}), whilst the concentration of triclosan was spread over a much wider range (83-263 mg l^{-1}). This would suggest that removal of triclosan cannot be directly related to COD removal.

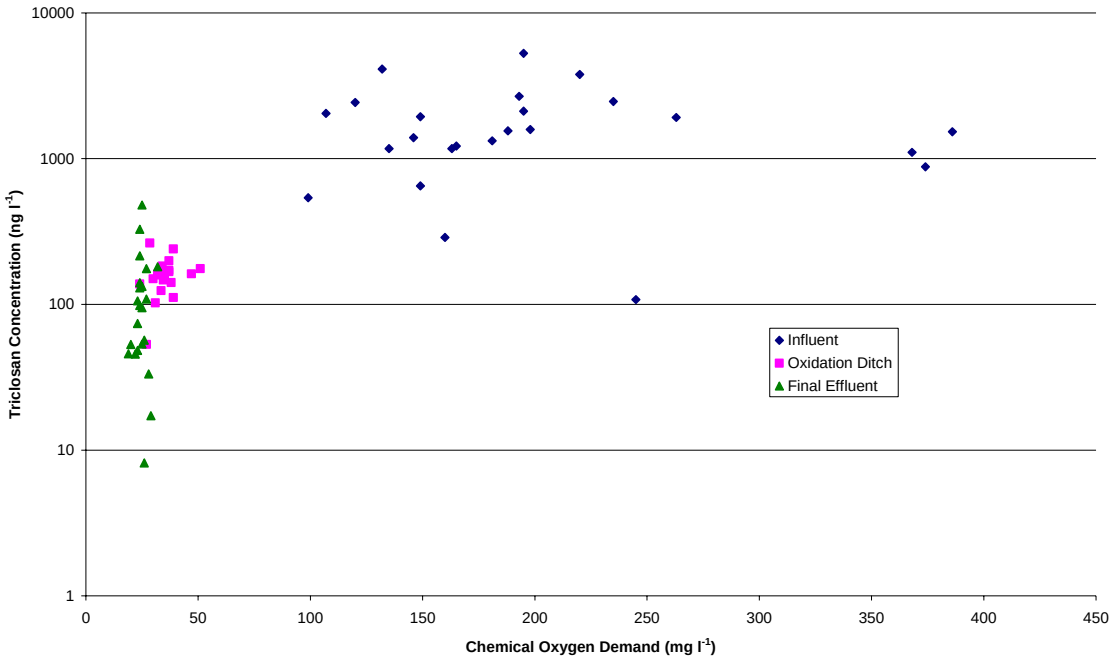


Figure 8.15: Correlation between chemical oxygen demand and triclosan concentration

Plotting the percentage removal of COD and triclosan against influent COD concentration, as shown in Figure 8.16, produces a more interesting relationship. The average influent COD concentration (170 mg l^{-1}) is shown in red.

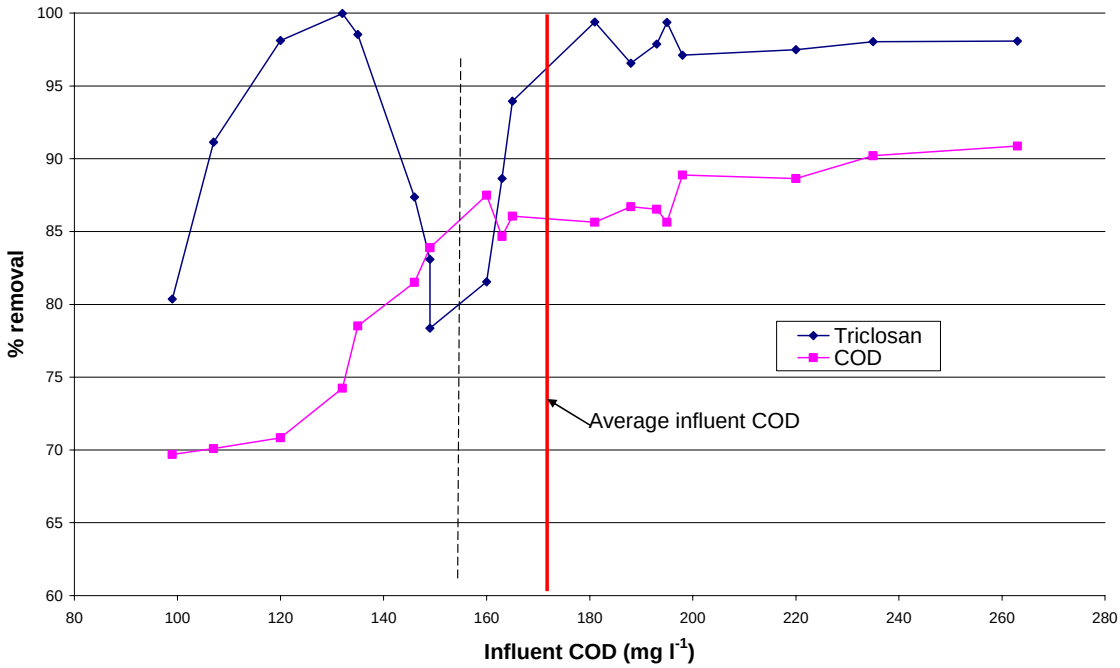


Figure 8.16: Relationship between influent COD concentration and percentage removal of triclosan and COD, with average COD influent concentration marked in red

Figure 8.16 appears to show two distinct relationships. When the influent COD is around or above the average value, triclosan removal appears constant around 97 to 98%, and COD percentage removal gradually increases with COD concentration. However, when the influent COD concentration falls below the average value, percentage COD removal rapidly falls off to around 70%. Percentage triclosan also starts to fall off in a similar manner. However, below a certain value (around 155 mg COD l^{-1} in this case), percentage triclosan removal rapidly increases again, reaching a higher percentage removal (~100%) than is usual. The removal of triclosan then falls again, as COD influent concentration continues to decrease.

Kayombo *et al.* (2003) studied the effect of COD substrate concentration on bacterial growth rates. They observed μ_{max} for heterotrophic bacteria (usually associated with BOD removal) at around 200 mg l^{-1} and remaining at a similar rate for concentrations up to as high as 800 mg l^{-1}. For autotrophic bacteria (usually associated with ammonia), μ_{max} was observed at 110 mg l^{-1}. These values correspond well with the two peaks seen in triclosan removal, at around 190 mg l^{-1} COD (and above) and at around 120 mg l^{-1} COD. This would suggest that triclosan can be removed well by both autotrophic and heterotrophic bacteria, depending on the influent COD conditions.

Little research has been published on the relative abilities of autotrophic and heterotrophic bacteria to remove PhACs, although a study by Shi *et al.* (2004) suggested that degradation of estradiol to estrone was caused by heterotrophic bacteria rather than by autotrophic bacteria. Nonetheless, autotrophic bacteria (in this case *Nitrosomonas europaea*) did degrade estradiol, but without the formation of estrone. The differences in the type of bacteria that perform the degradation of each pharmaceutical may help to explain the differences seen in critical sludge age as detailed previously. For example, since the critical sludge age for ibuprofen was around 1 day (Ternes *et al.* 2004)., which may suggest it is degraded by the rapidly growing heterotrophic bacteria, whilst for estrone it was around 8 days (Ternes *et al.* 2004), which may suggest it is degraded by the slower growing autotrophic bacteria. Some pharmaceuticals, such as bisphenol-A showed a wide range of critical sludge age (Clara *et al.* 2005), which may suggest they are degraded to different extents by both types of bacteria. Indeed, Federle *et al.* (2002) could find no correlation between sludge age and the degradation of triclosan, which

could be explained by triclosan being well degraded by both heterotrophic and autotrophic bacteria, supporting the above results.

In terms of controlling removal of triclosan, and indeed other pharmaceuticals, this produces an interesting scenario. From the above data, it would appear that autotrophic bacteria remove triclosan to the greatest extent (up to 100%), but can only sustain this removal for a narrow range of COD concentration (110 – 130 mg l^{-1}). Heterotrophic bacteria, on the other hand, achieve slightly less removal (about 98%), but can sustain this from 180 mg l^{-1} COD to at least 270 mg l^{-1} COD (and quite possibly up to 800 mg l^{-1} COD according to the Kayombo *et al.* (2003) data). Unfortunately, both these ranges exclude the average COD concentration for this STP (170 mg l^{-1} COD).

There are two possible options to optimise COD removal – either dilute the feed to meet the optimal concentration for the autotrophic bacteria, or add load to meet the optimal concentration for the heterotrophic bacteria. The first option could be achieved easily by returning an amount of the final effluent to the head of the works. The second option could be achieved by either adding various compounds to the feed (potentially expensive), or by the addition of high strength return liquors from sludge dewatering or similar if available. Both options would require the installation of online COD/BOD monitors, and would incur significant pumping costs.

On average, Southam achieved 93.1% removal of triclosan. The option of increasing the COD load into the works could potentially achieve up to 98% removal, whilst the dilution option could potentially achieve up to 100%. However, the cost of either of these options would have to be balanced both against cost and the requirements for removal. In the case of triclosan, the PNEC level is 14 ng l^{-1}. At Southam, the average influent triclosan concentration was 1800 ng l^{-1}, suggesting that around 99.2% removal would be required to reduce effluent concentrations below the PNEC level. This would suggest that the dilution option outlined above to achieve maximum removal by the autotrophic bacteria would be necessary.

8.2.4 Volatile suspended solids

The variation in volatile suspended solids (VSS) during the sampling period is shown in Figure 8.17. Concentrations ranged from 72 to 329 mg l^{-1} in the influent (median 173 mg l^{-1} - equivalent to 86% of the measured suspended solids concentration). This median concentration is typical for a medium strength wastewater (Metcalf and Eddy 2003). VSS in the oxidation ditch effluent ranged from 1575 to 2341 mg l^{-1} (median 1934 mg l^{-1} – equivalent to 78% of the suspended solids), and from 4 to 33 mg l^{-1} (median 9 mg l^{-1} – equivalent to 80% of the suspended solids) in the final effluent.

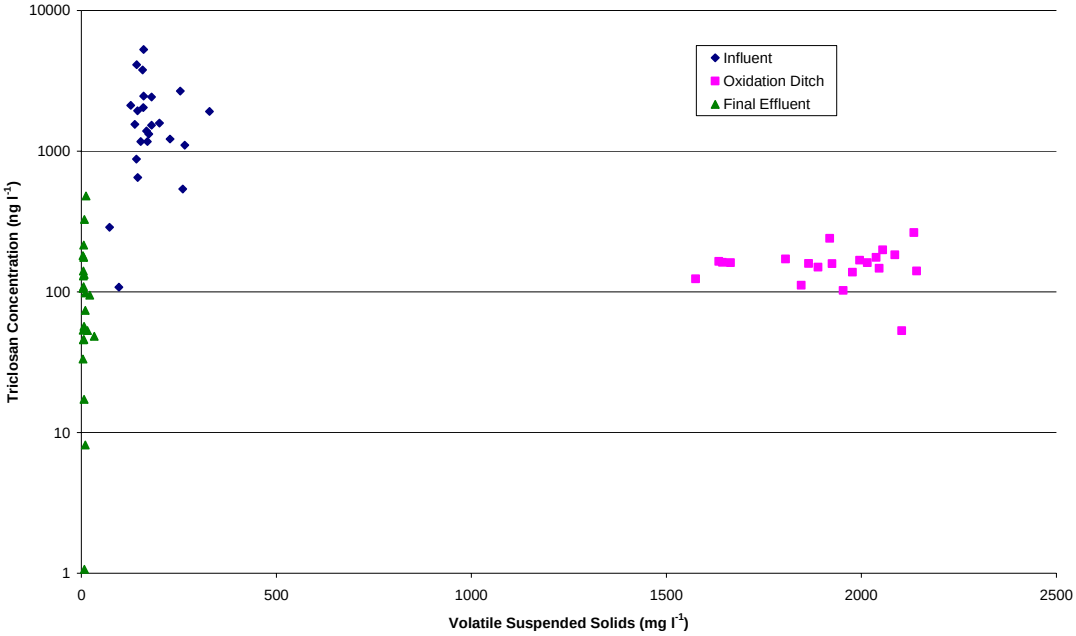


Figure 8.17 Daily variation of volatile suspended solids

No correlation was observed directly between triclosan concentrations and volatile solids concentrations. Indeed, the wide variation of triclosan concentrations in the final effluent (1 to 480 ng l^{-1}) was associated with only a very small range of VSS concentration (4 to 33 mg l^{-1}) suggesting that the two parameters may be completely independent. However, VSS is most generally used as a measure of biomass growth within a biological treatment process, according to Equation 15 below (Metcalf and Eddy 2003). Typical values for the biomass yield (Y) are between 0.30 and 0.50 g VSS per g COD (Metcalf and Eddy 2003).

$$Y = \text{g VSS produced} / \text{g COD utilized} \qquad \textbf{Equation 15}$$

Ternes *et al.* (2004) had suggested that pharmaceutical removal could be related to biomass production (SP) according to Equation 16, where SA is the sludge age, and k_{biol} is the biological degradation rate.

$$\frac{C_{out}}{C_{in}} = e^{-k_{biol}SP.SA}$$ **Equation 16**

In this sampling regime at Southam, the sludge age could be assumed to be constant. Therefore, the above equation implies that the observed removal of triclosan would be directly proportional to the biomass yield. However, as shown in Figure 8.18 below, no such correlation was observed. Indeed, triclosan removal appeared to be independent of biomass yield.

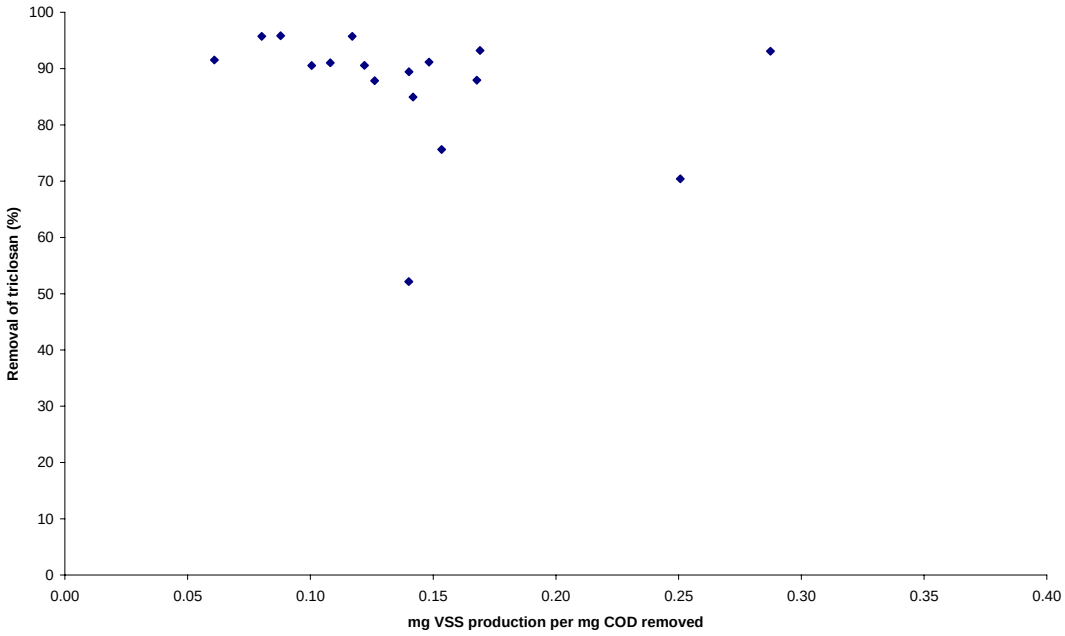


Figure 8.18: Correlation between biomass yield and triclosan removal

It should be noted that the measured yield values (0.08 to 0.35) are somewhat lower than would normally be expected. These low values, as well as the lack of observed correlation in this work, may be due to the fact that only soluble COD (sCOD) values were measured. In reality, VSS production and biomass growth will be from the soluble readily biodegradable COD (rbCOD). The sCOD measurement is a combination of both rbCOD and the non-biodegradable soluble COD fractions. Typically, rbCOD is around 8-25% of total COD, and soluble non-biodegradable COD accounts for a further 4-10%. Therefore, excluding the soluble non-biodegradable COD from the measured COD would have increased the measured yield values, and may have demonstrated more of a correlation between the biomass production and triclosan removal.

8.2.5 Carbon content (total, organic, and inorganic)

The variation in total carbon during the sampling period is shown in Figure 8.19. Concentrations ranged from 78 to 154 mg l^{-1} in the influent (median 113 mg l^{-1}), 72 to 96 mg l^{-1} in the oxidation ditch (median 79 mg l^{-1}), and 48 to 75 mg l^{-1} (median 58 mg l^{-1}) in the final effluent.

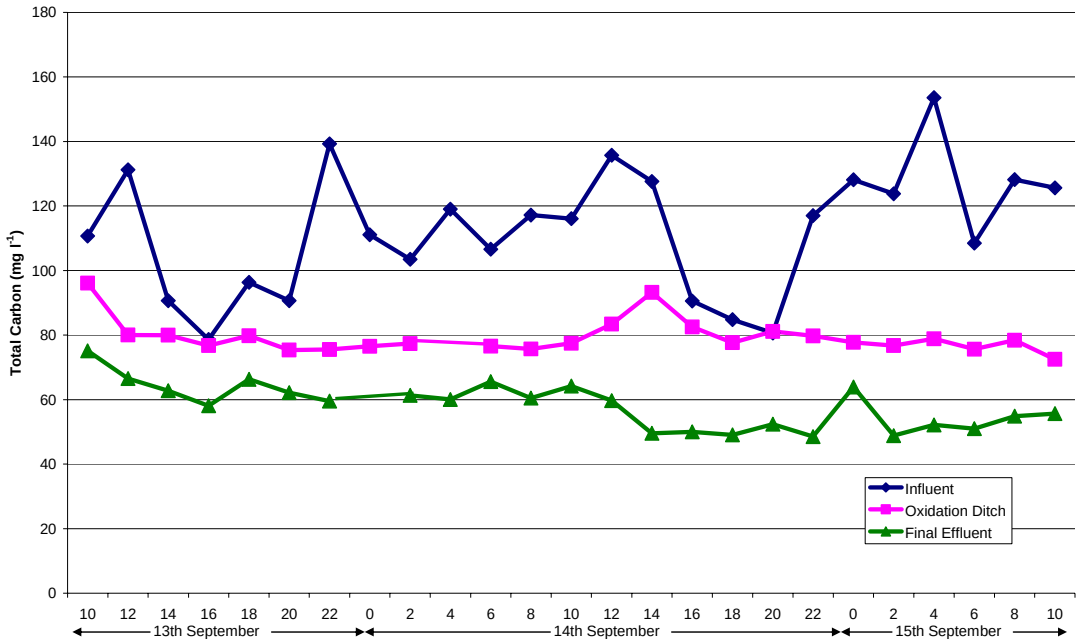


Figure 8.19 Daily variation of total carbon

The variation in inorganic carbon during the sampling period is shown in Figure 8.20. Concentrations ranged from 48 to 87 mg l^{-1} in the influent (median 67 mg l^{-1}), 45 to 50 mg l^{-1} in the oxidation ditch (median 47 mg l^{-1}), and 27 to 43 mg l^{-1} (median 37 mg l^{-1}) in the final effluent.

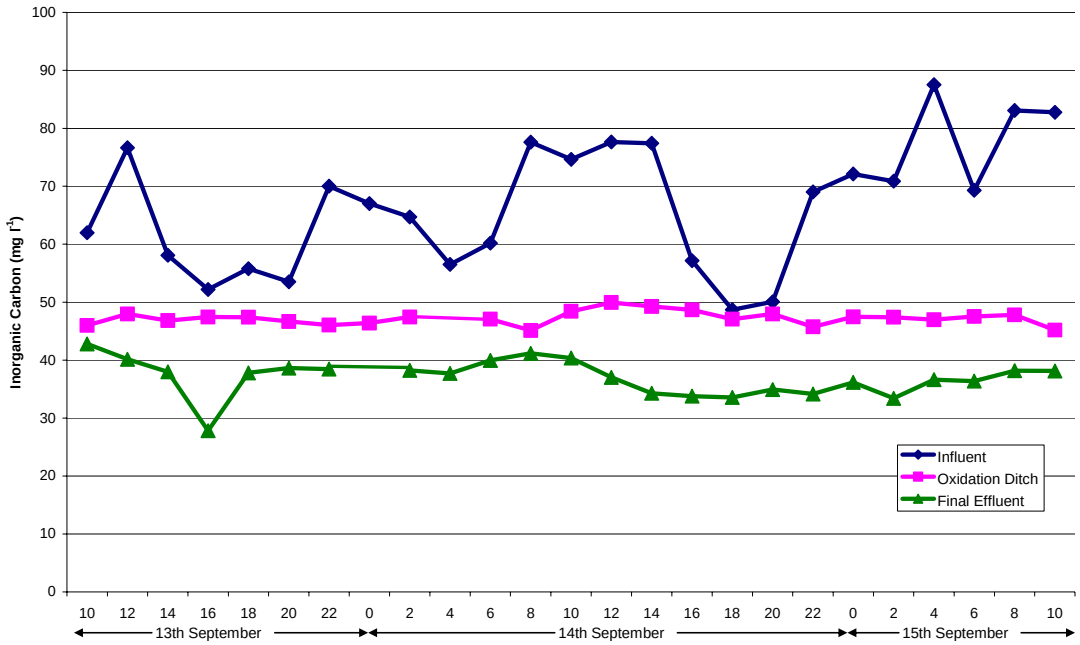


Figure 8.20: Daily variation of inorganic carbon

The variation in total organic carbon during the sampling period is shown in Figure 8.21. Concentrations ranged from 26 to 69 mg l^{-1} in the influent (average 45 mg l^{-1}), 27 to 50 mg l^{-1} in the oxidation ditch (average 32 mg l^{-1}), and 14 to 32 mg l^{-1} (average 21 mg l^{-1}) in the final effluent.

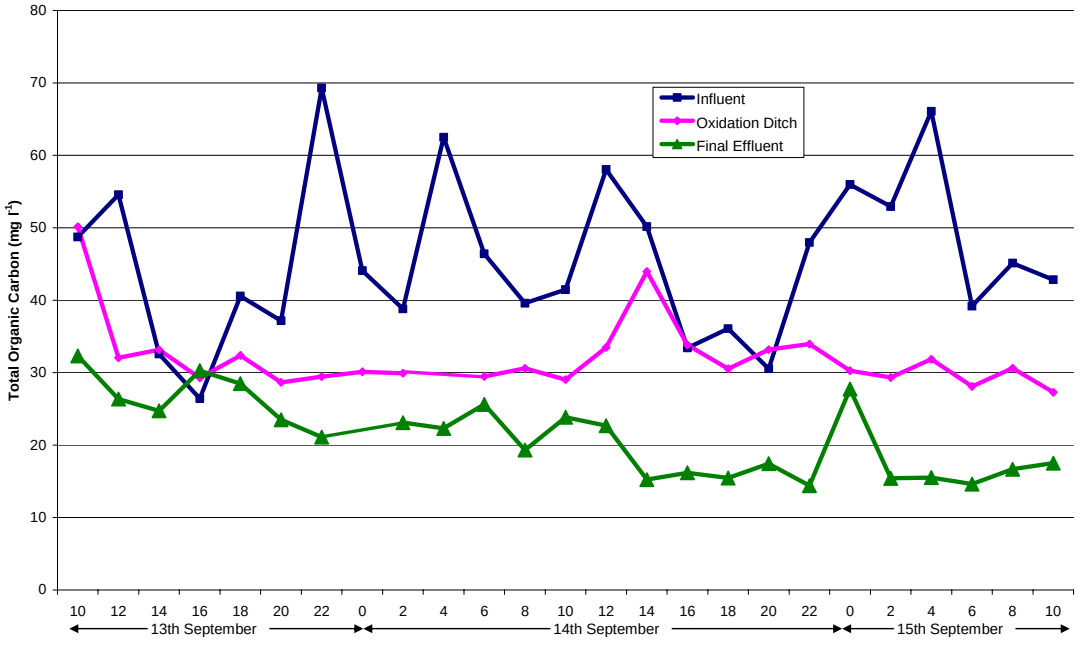


Figure 8.21: Daily variation of total organic carbon

Several authors have suggested that pharmaceutical concentrations may be correlated with carbon concentrations. Drewes *et al.* (2001) suggested that organic carbon concentrations were a significant factor in the removal of iodinated X-ray contrast media, whilst Stokes and Churchley (2006) found a direct correlation (r^2 = 0.9994) between total organic carbon concentrations and estradiol concentrations, implying that removal mechanisms for both are similar.

Due to the sorption properties of triclosan, as discussed earlier, it may have been expected that increased levels of carbon, whether organic or inorganic, may have led to greater removal. However, in this study, triclosan concentrations were very poorly correlated with carbon content. Correlation coefficients ranged from 0.56 for total carbon, as shown in Figure 8.22 below, to 0.44 for total organic carbon, and 0.58 for inorganic carbon.

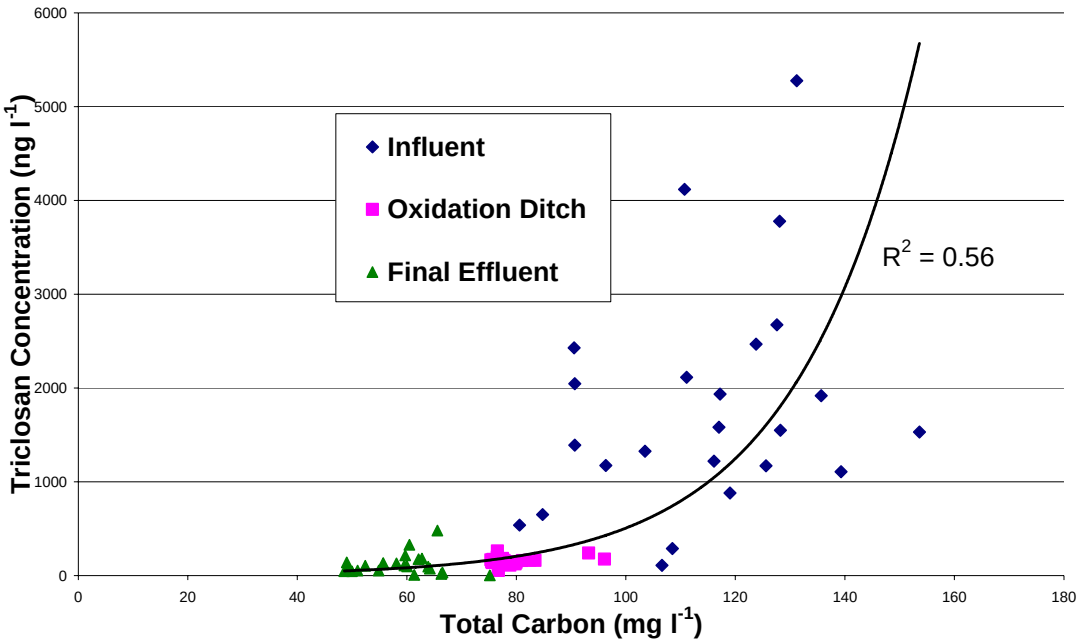


Figure 8.22: Correlation between total carbon and triclosan concentration

Generally, this correlation is too weak to suggest that a relationship exists between triclosan and carbon content, particularly considering the wide spread in the influent data. However, the relationships observed by Drewes *et al.* (Drewes *et al.* 2001) and Stokes and Churchley (Stokes and Churchley 2006) suggest further investigation of this area is required. A relationship between these parameters could be readily exploited, for example by the addition of tertiary sand filters, which can readily reduce the final effluent organic carbon

concentrations, and thereby could reduce the pharmaceutical concentrations entering the aquatic environment.

8.2.6 Soluble Protein and carbohydrate

The variation in soluble protein during the sampling period is shown in Figure 8.23. Concentrations ranged from 12 to 31 mg l^{-1} in the influent (average 23 mg l^{-1}), 4 to 9 mg l^{-1} in the oxidation ditch (average 7 mg l^{-1}), and 4 to 11 mg l^{-1} (average 6 mg l^{-1}) in the final effluent.

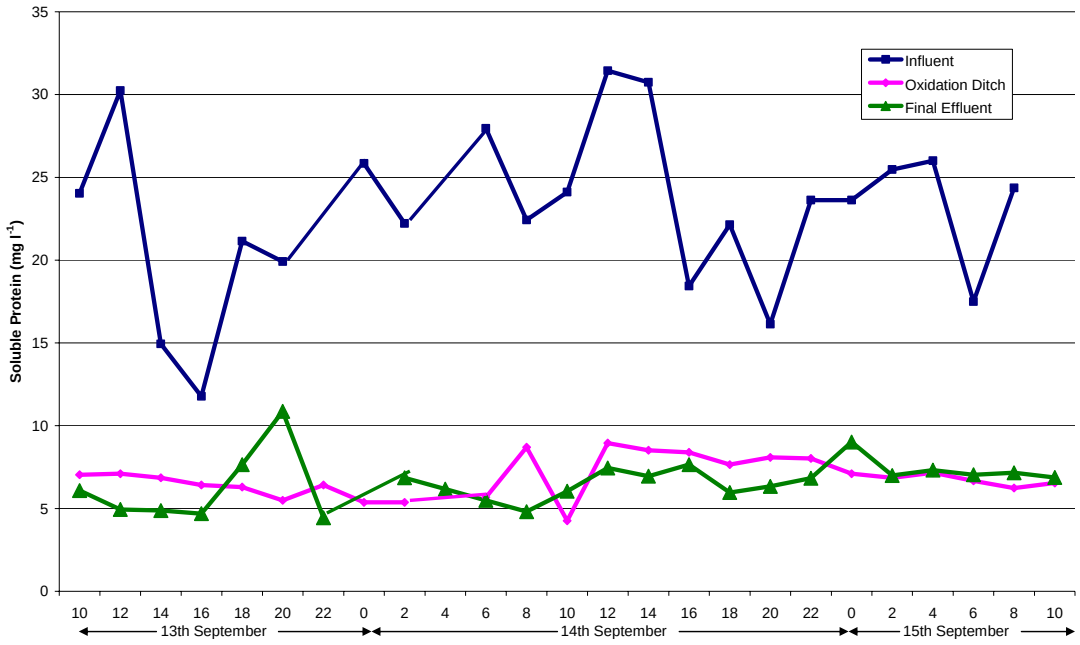


Figure 8.23: Daily variation of soluble protein

Overall, 73.9% of the soluble protein was removed by the STP, with the majority (69.6%) occurring in the oxidation ditch, and the remaining 4.3% occurring in the final tanks.

The variation in soluble carbohydrate during the sampling period is shown in Figure 8.24. Concentrations ranged from 3 to 15 mg l^{-1} in the influent (average 8 mg l^{-1}), 1 to 13 mg l^{-1} in the oxidation ditch (average 6 mg l^{-1}), and 3 to 7 mg l^{-1} (average 5 mg l^{-1}) in the final effluent. As can be seen from Figure 8.24, there is a wide variation in the concentrations measured, with the final effluent concentrations often exceeding the influent. Although concentrations were measured in triplicate, a very high standard deviation was observed, suggesting that the method employed may not have been very reliable. A possible reason for this is that the detection method relies on a phenol-sulphuric acid reaction (5% phenol solution). Phenols

can be expected in wastewater (0.3 mg l^{-1} (Dignac *et al.* 2000)), which may therefore interfere with the photometric determination of carbohydrates.

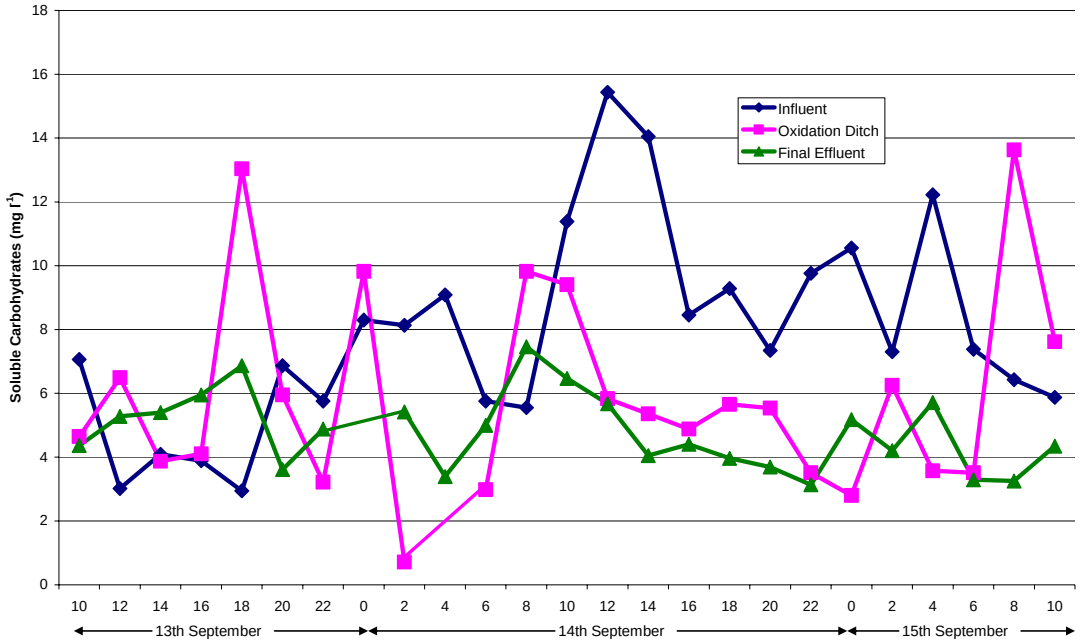


Figure 8.24: Daily variation of soluble carbohydrate

Overall, only 38% of the soluble carbohydrate was removed by the STP, with the majority of that (25%) occurring in the oxidation ditch, whilst the remaining 13% occurring in the humus tanks.

Soluble proteins and carbohydrates play a significant role as carbon sources for heterotrophic bacteria in wastewater (Gremm and Kaplan 1997). They are capable of forming complexes with metals and toxic substances (Jorand *et al.* 1998), and thereby reducing their bioavailaility in the environment. Huber (1999) reported that proteins and carbohydrates formed complexes with endocrine disruptors, suggesting that proteins and carbohydrates may be a sink for pollutants such as pharmaceuticals in wastewater discharges. Huber also noted that up to 25% of the organic carbon in the effluent of STPs consisted of protein and carbohydrate. Figure 8.25 below shows a weak correlation ($r^2 = 0.56$) between the elimination of protein and elimination of triclosan in biological treatment. No correlations were observed for carbohydrate.

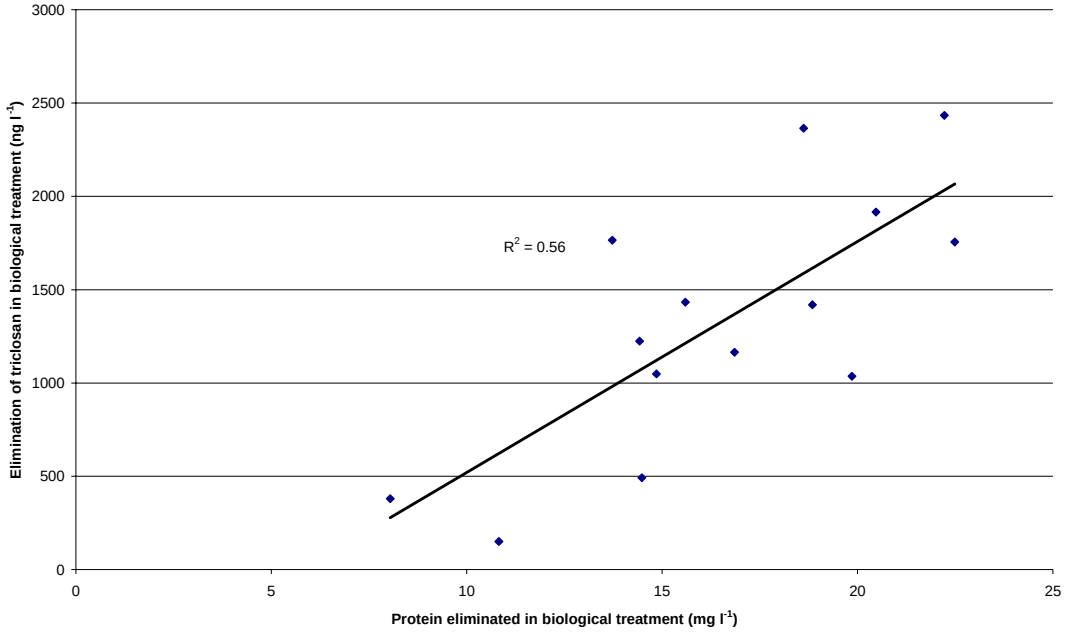


Figure 8.25: Correlation between elimination of protein and elimination of triclosan in biological treatment

As noted above for carbon, this is too weak a correlation to form any firm conclusions. However it would suggest that protein and triclosan share a removal mechanism. Since protein and carbohydrate make up a large proportion of the DOC in the final effluent, it may be important to remove more of them as BOD consents tighten. This could therefore have the added benefit of removing increased amounts of pharmaceuticals.

However, according to Wilen *et al.* (2003), an increase in soluble protein concentrations in activated sludges was correlated with the flocculation ability. Flocculation is an important step in activated sludge treatment that brings about the collisions between destabilised particles needed to form larger particles that can be readily removed by settling or filtration (Metcalf and Eddy 2003). Therefore, soluble protein is a parameter that must be rigorously controlled for optimal sewage treatment efficiency. To improve removal of triclosan during sewage treatment, soluble protein concentrations would need to be reduced as much as possible. However, doing this could significantly reduce the flocculation ability during the activated sludge process.

8.2.7 Fats, oil, and grease (FOG) content

Carballa *et al.* (2004) and Carballa *et al.* (2005) suggested that better pharmaceutical removal occurred in sludges with higher fat content. Antusch (1999) reported that sorption of

phenols on to activated sludge biofilms increased with fat content (and also decreasing pH). Since triclosan is phenolic in character, and has already been shown to have increasing sorption with decreasing pH, it was expected that a correlation would be observed between triclosan removal and the fat content of the biomass. However, there was very little variation observed in the fat content of the oxidation ditch biomass samples, being constantly at about 3.5 mg FOG per gram biomass, equivalent to an average of 8.6 mg l^{-1}. Literature values for fat in raw municipal wastewater are in the range 10 – 200 mg l^{-1} (Metcalf and Eddy 2003)), although the majority of this will be removed during primary treatment. This lack of variation suggested that either no correlation existed, or that there was an error in the experimental method for the measurement of FOG.

8.2.8 Extracellular Protein and Carbohydrate

As shown in Figure 8.26, the extracellular protein in the oxidation ditch varied between 28 and 60 mg l^{-1}, with a median of 43 mg l^{-1}.

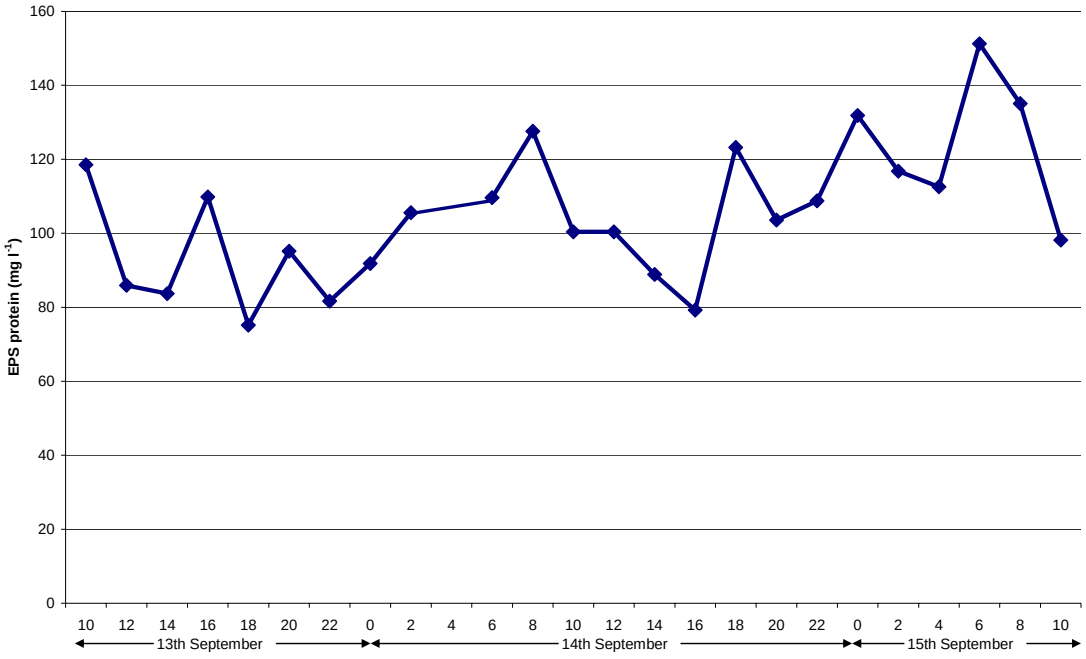


Figure 8.26: Daily variation of extracellular protein

As shown in Figure 8.27, the extracellular carbohydrate in the oxidation ditch varied between 10 and 29 mg l^{-1}, with an average of 17 mg l^{-1}.

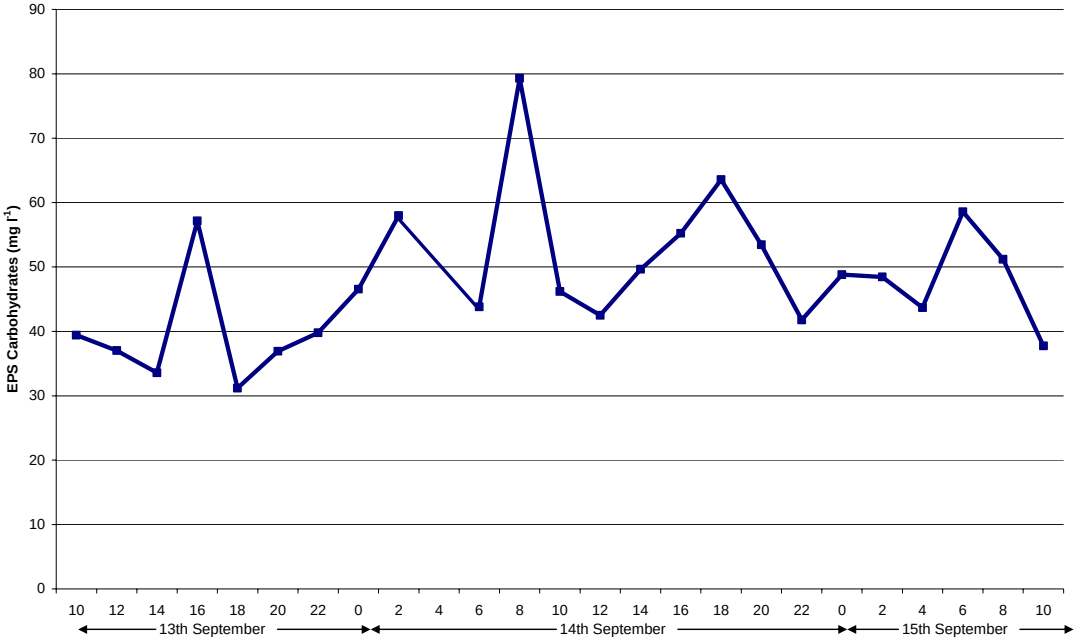


Figure 8.27: Daily variation of extracellular carbohydrate

Biofilms are an accumulation of micro-organisms, extracellular polymeric substances (EPS), multivalent cations, inorganic particles, as well as colloidal and dissolved organic compounds (Wingender *et al.* 1999). EPS act as a cushion layer for micro-organisms within biofilms, and consist of carbohydrates, proteins, DNA, and lipids (Goodwin and Foster 1985). EPS are considered to play an important role in dewatering of activated sludge (increased EPS produces poor settleability and dewatering (Urbain *et al.* 1993))and in the removal of pollutants from wastewater by sorption (Flemming and Wingender 2001; Liu *et al.* 2001; Urbain *et al.* 1993). In general, biofilms with higher EPS have shown better resistance to toxic shocks, possibly due to diffusion barriers within the biofilm matrix (Wuertz *et al.* 1998). Not only can an increase in the EPS fraction affect the overall removal by increasing sorption (Spath *et al.* 1998), but it can also enhance biodegradation by removing inhibitory compounds from solution (Bouwer 1989).

In this study, however, no correlation could be found between triclosan concentrations and extracellular protein or carbohydrate. Although the literature discussed above may suggest that triclosan removal, particularly by sorption, should increase with higher EPS, the earlier sorption test results showed no variation with biomass type. This may suggest that triclosan adsorbs too strongly to biomass to be affected by small variations in EPS, although this is a mechanism that may affect more weakly binding pharmaceuticals.

8.3 Summary

It has been well established by many researchers, mostly as part of the POSEIDON European project, that certain parameters are key in the removal of pharmaceuticals, such as sludge age (including MLSS concentration, inert fraction, and biomass production rate), and hydraulic retention time (wastewater dilution) (Ternes *et al.* 2004). However, other researchers have noted variations in pharmaceutical removal that could not be associated with changes in either sludge age or HRT (Federle *et al.* 2002).

The experimental results detailed in this chapter have shown that certain parameters affect the removal of pharmaceuticals during sewage treatment. These include pH, suspended solids, and chemical oxygen demand. Mechanisms for these relationships have been discussed. Relationships were not found between pharmaceutical removal and biomass yield (Ternes *et al.* 2004), carbon content (Drewes *et al.* 2001; Stokes and Churchley 2006) or FOG content (Carballa *et al.* 2005; Carballa *et al.* 2004) contrary to the results of other researchers. This may be due to the particular pharmaceuticals or the STP used in this research. Experimental results suggested a weak correlation ($r^2 = 0.56$) between pharmaceutical removal and soluble protein removal. Holbrook *et al.* (2004) and Kreuzinger *et al.* (2004) had also reported this as a weak correlation ($r^2 = 0.4$).

The following chapter uses the relationships outlined above, first to model pharmaceutical removal, and then to suggest possible improvements to STPs to improve pharmaceutical removal.

Chapter 9: Modelling and implications for improving pharmaceutical removal in STPs

As mentioned in the earlier chapter on the choice of pharmaceutical compounds for study (see Chapter 3:), computer-modelling packages can be used to assess the fate of compounds during sewage treatment. In this work, Toxchem+ has been used to assess the fate of the pharmaceuticals studied, using the model of a small ASP, as outlined in Appendix B. As discussed in the choice of compounds chapter, using the model to predict the fate of compounds with sorption and biodegradation data available from literature suggested fates as shown in Table 9.1 below, and were compared with literature values. These are based on an influent concentration of 1000 ng l^{-1} for each compound in this model. Removal values for each compound in this work as suggested by the grab sampling (Southam results only) and composite results are shown for comparison.

Table 9.1: Removal of pharmaceuticals according to Toxchem+ and literature fate data, compared to literature removals and removal established in this work

	Toxchem+ modelling (%)			Overall removal (%)			
Pharmaceutical	**Effluent**	**Sludge**	**Biodeg**	**Toxchem+**	**Literature**	**Grab samples**	**Composite samples**
Caffeine	1.1	0.01	98.9	98.9	>99.9	99.3	>99.96
Carbamazepine	98.2	1.1	0.7	1.8	7-8	7.1	12.8
Tetracycline	77.0	6.3	16.7	23.0	-	-	-
Triclosan	1.5	1.0	97.5	98.5	95-97.5	97.1	93.1

For caffeine and triclosan, Toxchem+ appeared to slightly underestimate removal, whilst for carbamazepine, removal appeared to be overestimated. The model was then refined, by adding in the results from the laboratory sorption and biodegradation tests as discussed in Chapter 6: (see Table 6.3 for values). Values for carbamazepine were not updated, since the literature values were more sensitive to the small amounts of biodegradation and sorption of carbamazepine than could be detected in this study. This resulted in Toxchem+ fate predictions, as shown in Figure 9.2 below.

Table 9.2: Removal of pharmaceuticals according to Toxchem+ with updated sorption and biodegradation rates as demonstrated in this work

	Toxchem+ modelling (%)			Overall removal (%)
Pharmaceutical	**Effluent**	**Sludge**	**Biodegradation**	**Toxchem+**
Caffeine	-	-	100	100
Carbamazepine	98.2	1.1	0.7	1.8
Tetracycline	11.5	66.6	21.9	88.5
Triclosan	11.6	67.4	21.0	88.4

For caffeine, the refinement of these parameters increased the removal of caffeine to 100%, entirely by biodegradation. This is very similar to literature values, as previously discussed, and those recorded in this work on overall removal. The results for triclosan with the improved modelling parameters are somewhat lower (88%) than the composite samples removal values (93%) and lower still than typical literature values (95-97.5%), whereas the model had previously overestimated removal. It would appear that the improved sorption parameters (previously relying on log K_{ow}) had increased the amount of sorption, which has had the added effect of reducing the amount of degradation that occurs, even though the new degradation rate was slighter higher than the original literature value. The most significant change with the updated fate parameters is the removal of tetracycline, increased from a prediction of 23% to just over 88%, with a similar split between sorption and biodegradation as for triclosan. The update sorption parameters clearly show that using log K_{ow} values underestimate sorption, as predicted by Tolls (2001). Whilst many papers have reported sewage effluent concentrations of tetracycline, none have reported overall removal through an STP. This may help to explain, combined with poor detection limits, why this pharmaceutical was never detected in environmental samples in this work.

A benefit of using modelling packages like Toxchem+ is that they can show how various parameters may affect pharmaceutical removal. For example, changes in flow rates, temperature, MLSS, and MLVSS can be modelled by Toxchem+. However, the disadvantage of this modelling package is that key wastewater parameters, such as COD, ammonia, organic carbon content, and pH cannot be modelled. Similarly, soluble protein, carbohydrate, and EPS cannot be modelled. These significantly limit the usefulness as the package for wastewater modelling. In the water industry, either GPS-X or Biowin are the modelling packages of choice. However, these packages do not currently have the ability to allow the

user to input contaminants, such as pharmaceuticals, to assess their fate. This problem has been discussed with the company Envirosim, manufacturer of Biowin, and they have shown strong interest in both including this functionality in future versions of their software, as well as further research to elucidate all mechanism and rates involved in the removal of pharmaceuticals during sewage treatment (Dold 2006).

9.1 Modelling the effect of wastewater and biomass parameters on pharmaceutical removal

As noted above, five parameters can be modelled using Toxchem+ that have been shown to affect pharmaceutical removal (from composite sampling and literature). These are: pharmaceutical concentration, flow rate, inlet temperature, MLSS, and MLVSS.

9.1.1 Flow rate

The modelling above was conducted at a flow of 12000 m^3 per day, with an aeration tank volume of 6000 m^3. This gave a hydraulic retention time of 12 hours – Severn Trent specify between 8 and 14 hours in the design of new ASPs (Green 2004). Changes in the influent flow rate will alter the hydraulic retention time of the activated sludge system. Therefore, slower flow rates would be expected to increase sorption and biodegradation, whilst higher flow rates would be expected to have the opposite effect. Figure 9.1 below shows the effect of flow rates ranging from 3,000 m^3 to 72,000 m^3 per day (equivalent to HRT of between 2 hours and 48 hours).

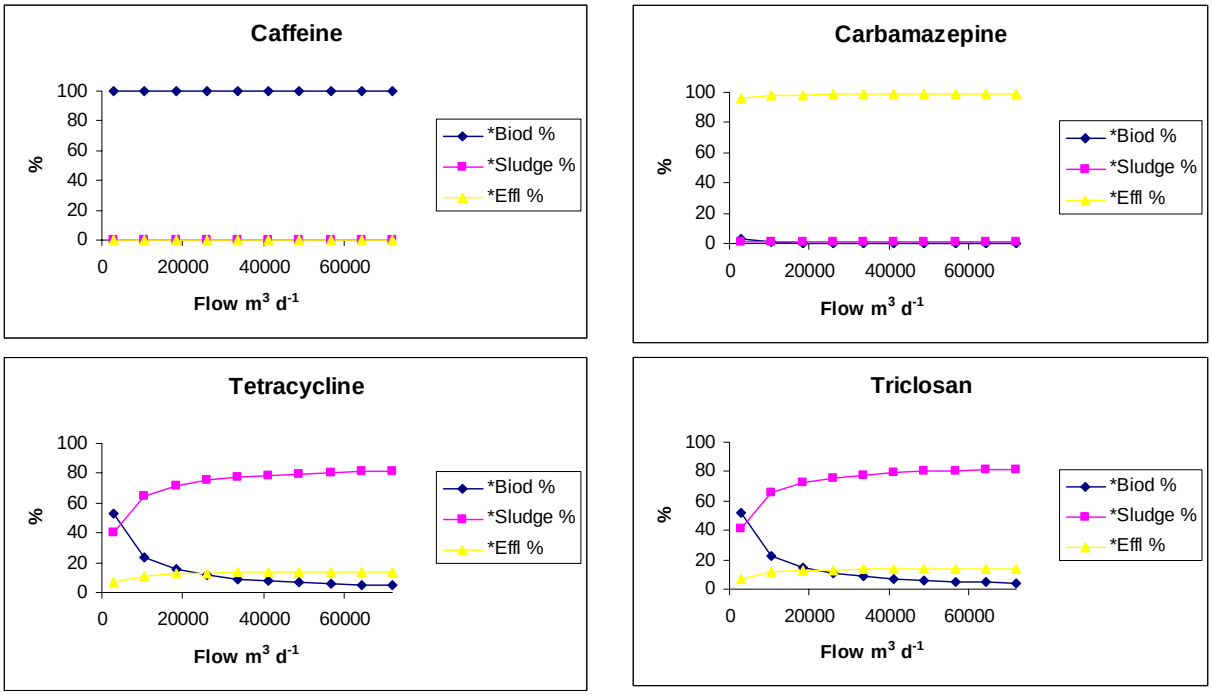


Figure 9.1: The effect of flow rate on the removal of caffeine, carbamazepine, tetracycline, and triclosan, as modelled by Toxchem+

Key: Biod % = percent biodegraded; Sludge % = percent sorbed to sludge; Effl % = percent remaining in final effluent

Variation in flow rate had no effect on caffeine removal. Carbamazepine removal ranged from 1.2% at maximum flow to nearly 4% at minimum flow. Removal of both tetracycline and triclosan ranged from 93% at minimum flow to 86% at maximum flow. Although the amount of removal did not vary greatly, the mechanism responsible for that removal did. In both cases, the amount of biodegradation dropped from around 50% at the low flows, to barely 4% at maximum flows, whilst sorption rose from 41% to 82%.

Noting the above result for tetracycline and triclosan, it immediately becomes apparent that this model does not portray reality, in that sorption increases with flow. Whilst triclosan was shown to sorb rapidly to biomass, reaching equilibrium in only about 4 hours, tetracycline took nearly 24 hours to reach equilibrium. This time to reach sorption equilibrium is not accounted for in this model, and therefore the model severely overestimates sorption for compounds which take longer than the HRT to reach sorption equilibrium.

9.1.2 Inlet temperature

The modelling above was conducted at a sewage inlet temperature of 15 °C. Sewage temperatures in the UK typically range between 8 and 20 °C with an average of 12 °C (Green

2004). Potentially, more extreme temperatures could be experienced in the future, depending on the extent and effects of global warming. The effect of temperature, which will affect biodegradation rates, is shown in Figure 9.2 below.

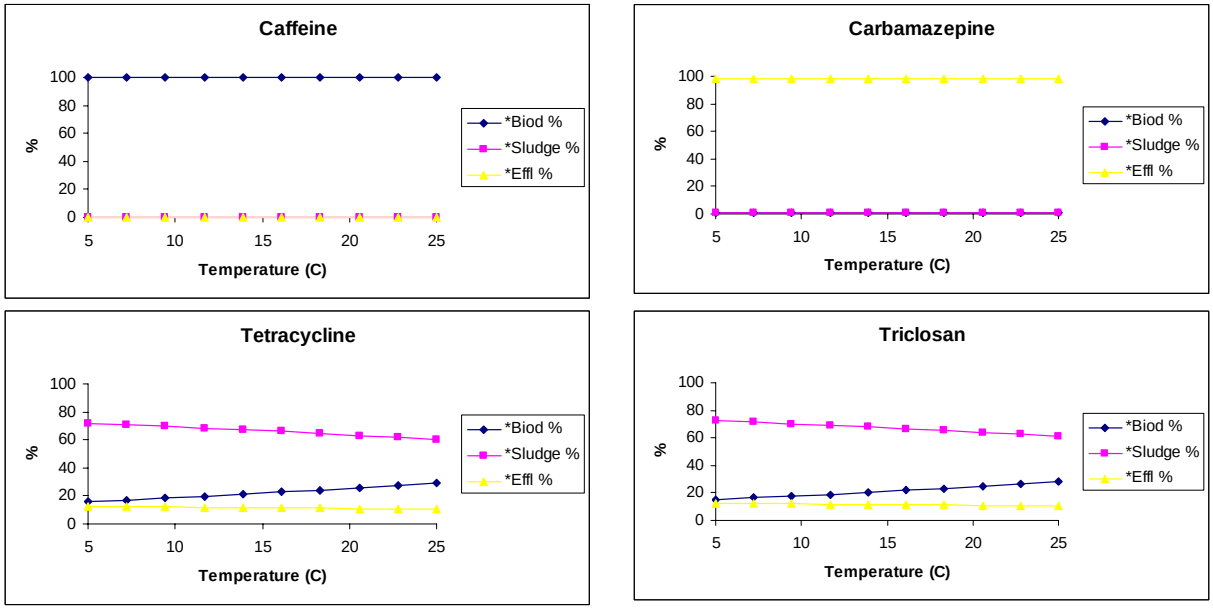


Figure 9.2: The effect of temperature on the removal of caffeine, carbamazepine, tetracycline, and triclosan, as modelled by Toxchem+

Key: Biod % = percent biodegraded; Sludge % = percent sorbed to sludge; Effl % = percent remaining in final effluent

Temperature showed no effect on caffeine removal, and carbamazepine removal varied between 1.6 and only 2.2%. Combined with the information on flow rate above, these parameters would not appear to support the suggestions of Heberer (Heberer 2005) who observed nearly 40% carbamazepine removal on one occasion in very hot and dry conditions, when the HRT of the ASP system he was studying reached about 24 hours.

Removal of both tetracycline and triclosan ranged from 87.6% to 89.6%, suggesting that changes in temperature had little effect on overall removal. Again, this is contrary to the effect observed at Northend STP during the spot sampling, when poor triclosan removal coincided with very low temperatures on one occasion. In both cases, a 10% increase in biodegradation was counterbalanced by a similar decrease in removal by sorption.

It is possible that the Toxchem+ model does not include an accurate kinetic representation of biodegradation at either high or low temperatures. No information was gathered on the variation of degradation due to temperature during this investigation, and these modelling

results combined with the sparse experimental results at temperature extremes would suggest that more research needs to be conducted into this area. Should temperature later be found to be critical in improving pharmaceutical removal, a simple modification can be suggested to sewage treatment plants where CHP schemes exist, in that waste heat (or gas) streams could be passed through the influent sewage to increase its temperature.

9.1.3 MLSS

Typically, activated sludge plants are run at an MLSS between 1500 and 3300 mg l^{-1}, varying for summer and winter conditions (Green 2004). MLSS can be expected to affect sorption, by changing the amount of biomass available to sorb to, as well as biodegradation, by altering the number of bacteria available. The effect of changes in MLSS is shown in Figure 9.3 below.

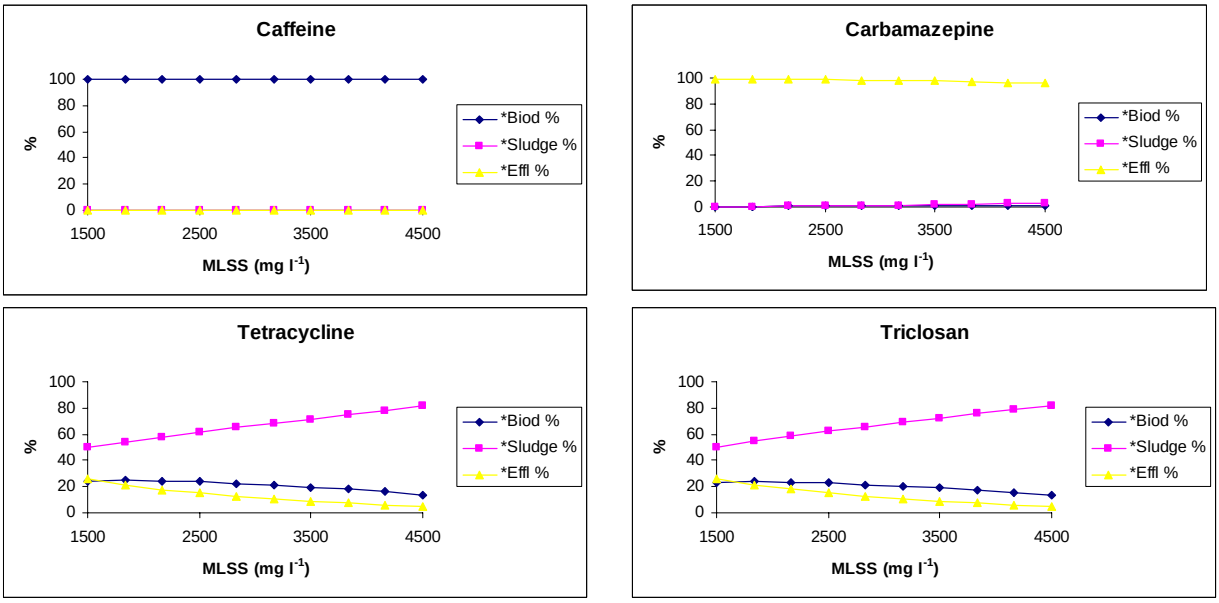


Figure 9.3: The effect of MLSS on the removal of caffeine, carbamazepine, tetracycline, and triclosan, as modelled by Toxchem+

Key: Biod % = percent biodegraded; Sludge % = percent sorbed to sludge; Effl % = percent remaining in final effluent

Once again, caffeine is unaffected by changes in MLSS. Carbamazepine removal ranged between 0.75% at 1500 mg l^{-1}, to 4.3% at 4500 mg l^{-1}, predominantly by sorption. This represented the most important of the modelled parameters on carbamazepine, although flow rate also had a significant effect. Tetracycline and triclosan removal both rose from 74% to 95.3% over the range of MLSS. In both cases, this was caused by a significant (50 – 82%)

increase in removal by sorption, but coupled with a small but nonetheless important fall in biodegradation (24 – 13%). This would suggest, as previously noted by many authors (Carballa *et al.* 2005; Carballa *et al.* 2004; Clara *et al.* 2005; Federle *et al.* 2002; Kreuzinger *et al.* 2004; Ternes *et al.* 2004), that MLSS is the most significant parameter in removing pharmaceuticals (assuming a high sludge age exists within the system).

9.1.4 MLVSS.

MLVSS, in combination with COD, was suggested by the composite sampling as a parameter that may affect pharmaceutical removal. Figure 9.4 below shows the effect of MLVSS on pharmaceutical removal, according to Toxchem+. MLVSS is plotted as a percentage of MLSS.

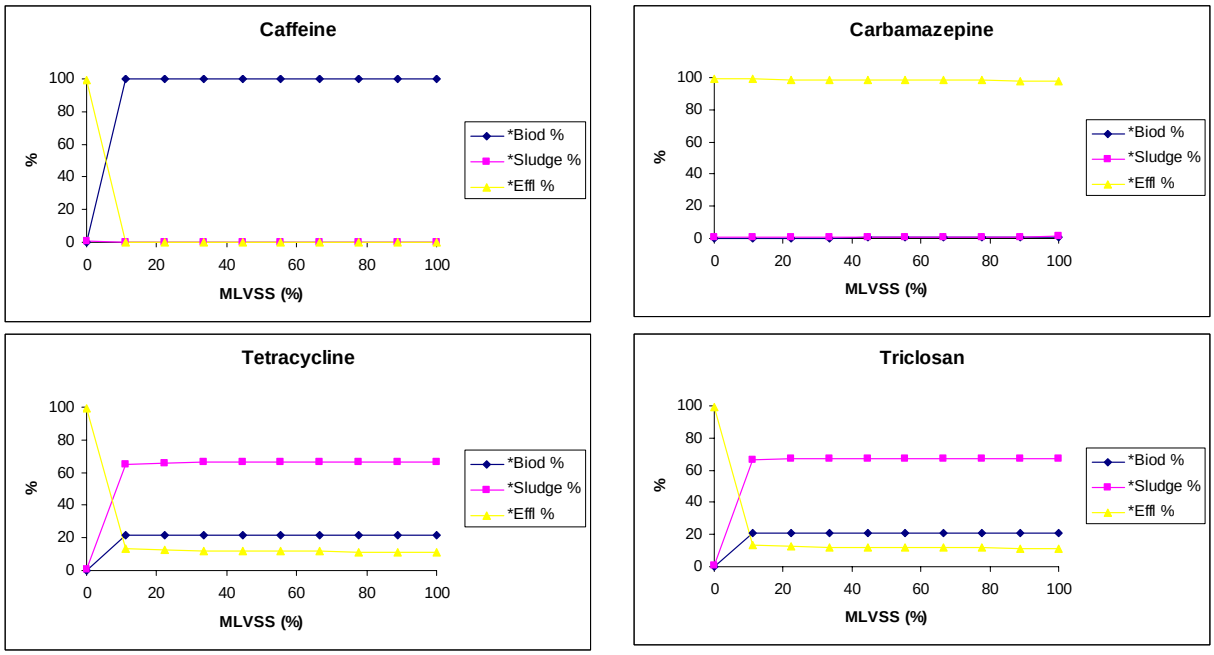


Figure 9.4: The effect of MLVSS on the removal of caffeine, carbamazepine, tetracycline, and triclosan, as modelled by Toxchem+

Key: Biod % = percent biodegraded; Sludge % = percent sorbed to sludge; Effl % = percent remaining in final effluent

At 0% MLVSS, all the pharmaceuticals show no removal. As MLVSS ranges between 10 and 100%, carbamazepine removal increases from 1.1 to 2.1%, whilst triclosan and tetracycline removal increase from 86.6% to 88.5%. This suggests that MLVSS does have a small effect on pharmaceutical removal, but the effectiveness of Toxchem+ to show the true effects is probably masked by the model's inability to model COD removal.

9.1.5 Summary of Toxchem+ modelling

It has been demonstrated that although Toxchem+ may be a useful tool for assessing the fate of certain organic compounds, its predictions for the removal of pharmaceuticals appear to be inaccurate. Of the four compounds modelled, only the prediction for the highly biodegradable caffeine matched environmental sample data. The model underestimated triclosan removal (by 10%) and carbamazepine removal (by 5%). It is likely that the removal of tetracycline was overestimated, since the model did not take into account the slow rate of sorption.

In general, this model lacks the ability to model the effect of many potentially key parameters that may affect the removal of pharmaceuticals, such as COD and ammonia concentrations, and biomass yield. Whilst other models, such as GPS-X and Biowin do model these parameters, it is not possible to use them for pharmaceutical fate modelling, since they do not allow the user to input chemical data. The software manufacturers have identified this as an area for future development.

9.2 Adapting sewage treatment technology to improve pharmaceutical removal

Combining parameters identified in literature, with those identified by the laboratory fate tests, spot sampling, composite sampling, and modelling in this work has led to the possibility of improving current sewage treatment technology.

9.2.1 Sludge retention time

Clearly, from literature, sludge retention time is the key parameter, requiring a sludge age in excess of ten days. However, increasing the sludge age beyond above 15 days gives diminishing returns (Ternes *et al.* 2004), in that the number of compounds being degraded increases, but the increase is less prominent. Therefore a recommendation of this work is that sludge ages of activated sludge work should be 15 days. Currently, Severn Trent design on a sludge age of 12 days (Green 2004).

9.2.2 Hydraulic retention time

From the laboratory fate data in this work, it would appear that hydraulic retention time is the next key parameter. In the results and conclusions of the POSEIDON report sorption onto sludge was "*assumed to be fast compared to biologic degradation or hydraulic retention time*". The laboratory fate work has shown that even for a compound with a high log K_{ow} such as

triclosan (log K_{ow} = 4.8), which could be assumed to be one of the faster sorbing compounds on this basis, sorption still takes at least 4 hours to get near to equilibrium. This is not fast compared to a typical average hydraulic retention time of only 8 – 12 hours (Green 2004). This assumption is clearly ludicrous for a compound such as tetracycline, which took nearly 24 hours to reach sorption equilibrium. Therefore, a major recommendation of this work is to ensure that new ASP treatment works have the highest hydraulic retention time possible - ideally 24 hours as can be achieved with oxidation ditches. Toxchem+ modeling showed that increases in retention time can improve the removal of even the pharmaceuticals most resistant to treatment. For example, at the lowest flow rates (and hence the longest HRT), carbamazepine removal trebled compared to normal values predicted by the model. Although the values of removal may be open to question due to the reliability of the model, it does indicate that significant improvements can be made. The problem with this modification is that it would increase civil construction costs, and there may not be the necessary land available on the treatment works. Equally, excessive HRTs may lead to settlement problems due to an increased potential for denitrification towards the end of the ASP lanes.

9.2.3 pH

pH has the potential to be another critical factor for the removal of some pharmaceuticals. Altering the pH, relative to the pKa of the target compound, will change the amount of the compound in its unionised form – the form in which it can sorb to solids and biodegrade. Although costs of chemical addition to alter pH may be high, as demonstrated with triclosan this could achieve up to 30% additional removal through sorption to solids removed in primary settlement. Recent drinking water research with coagulation using aluminium (pH 6) and ferric sulphate (pH 4.5) by Vieno *et al.* (2006) has shown that removals of 77% of diclofenac, 50% of ibuprofen, and 36% of bezafibrate could be achieved in the presence of high organic matter content. Since the chemicals used to alter pH are predominantly based on iron salts or aluminium (Fe^{2+}, Fe^{3+} or Al^{3+}) if flocculation is required, additional removal of tetracycline through complexation could also be achieved. Alternatively, a strong acid could be used, or sewage could be stored and allowed to go septic (although this could lead to odour problems). The ability of pH adjustment to increase removal pharmaceuticals will be limited by the pKa of the target compounds, and also the length of time available for sorption between the pH dosing point and the effluent of the primary sedimentation. This

may require enlarged primary tanks, leading to similar construction cost and land problems as detailed for the extended HRT option above. Although cost (and availability) of chemical may be a problem, these are the same chemicals as used for chemical phosphorus removal. Therefore, any site using iron dosing for chemical phosphorus removal may already be reducing its pharmaceutical discharge, particularly of the acidic pharmaceuticals. A final disadvantage of using iron or aluminium dosing is that tertiary solids removal (such as sandfilters) will be required to ensure compliance with the inevitable iron discharge consent. It should be noted that pH adjustment will not affect all pharmaceuticals. For example, sorption of norfloxacin and ciprofloxacin to sludge was found to be independent of pH (Lindberg *et al.* 2006), whereas greater removal of sulfachloropyridazine, tylosin, and oxytetracycline was found to occur in acidic conditions (ter Laak *et al.* 2006).

9.2.4 Temperature

As observed qualitatively during the spot sampling, temperature could play an important role in the biodegradation of pharmaceuticals. Although the above Toxchem+ modelling did show an increase in biodegradation with an increase in temperature, this was counterbalanced by a decrease in sorption. As noted in the literature review, biodegradation is a more preferable removal mechanism than sorption, since it implies complete destruction of the compound, rather than transfer of the compound to the sludge stream. Carballa *et al.* (2005) found that although better removal of pharmaceuticals generally occurred better at 25 ^{o}C, they noted that for some compounds (ibuprofen and naproxen) the removal was no better than at 12 ^{o}C. Therefore, an ideal modification to sewage treatment works would be to ensure that influent sewage was always maintained as close to 25 ^{o}C as possible, or at least at 12 ^{o}C. As mentioned previously, this could potentially be achieved by utilisation of waste heat from onsite CHP systems. Although heat losses from an ASP system would be large, especially in winter conditions, insulating a conventional system would be impractical. For covered ASPs, which are becoming increasingly more common for odour control, heat loss may not be as much of an issue.

9.2.5 Chemical oxygen demand

Whilst sludge age, MLSS, and HRT are key parameters for pharmaceutical removal, most research prior to this work has tended to overlook the chemical oxygen demand of the sewage. This parameter is key for the bacteria, since it is an effective measure of their available food, and will control the bacterial population growth rates. As demonstrated by

Kayombo *et al.* (2003), autotrophic and heterotrophic bacteria appear to have maximum growth rates at approximately 110 mg COD l^{-1} and > 200 mg COD l^{-1} respectively. In a conventional ASP, the highest growth rates of heterotrophic bacteria occur in the first third of the ASP (in the high COD concentrations), whilst ammonia removal and maximum autotrophic bacterial growth occurs in the final two thirds of ASPs. Whilst it is important to maximise the number of bacteria in an ASP system to maximise the potential for biodegradation, there is an operational limit to the MLSS concentration in a suspended growth system before settlement failures occur in the final settlement tanks. For this reason, MLSS concentration are usually limited to 3300 mg l^{-1} (Green 2004). Some modifications to the activated sludge system have attempted to get around this problem, such as the IFAS (Integrated fixed-film activated sludge) system. This system involves the addition of small plastic media to the activated sludge tank. Biomass grows on the plastic media, increasing the available biomass, but without a significant addition of load to the final settlement tanks. The media are re-circulated around the activated sludge, creating a fixed film biomass on the media that has a similar bacterial community to the activated sludge (Sriwiriyarat and Randall 2005). This system is currently being evaluated by many water companies and researchers for enhance biological phosphorus and total nitrogen removal (Jones *et al.* 1998; Randall and Sen 1996; Rogalla *et al.* 2006; Sriwiriyarat and Randall 2005; Sriwiriyarat and Randall 2005). A major advantage of this system is that coarse bubble aeration systems can be used, which uses significantly less electricity than current fine bubble diffused aeration systems.

In 2002, Johnson *et al.* produced a patented modification (patent number WO02079103) to the IFAS system aimed at enhancing removal of steroid estrogens (Darton and Johnson 2002). The patent states "*An activated sludge tank for treating waste water, e.g. municipal sewage, has a biofilter located in the first 50 % of the length of the tank, spaced from the inlet end by a first activated sludge zone, and occupying less than 40 % of the length of the tank. All the water passes through the biofilter. A second activated sludge zone, which is aerated, is located on the outlet end side of the biofilter. In the tank the activated sludge has typically an average concentration in the range of 1 to 6 g/L, and the biofilter has a biomass concentration of at least 10 g/L. The presence of the biofilter increases the removal of micro-organic components such as steroid oestrogens*." The authors noted that, typically, solids concentrations of 15 to 40 g l^{-1} were established in the proposed biofilter. They also noted that a second biofilter further enhanced endocrine compound removal. Overall, final effluent concentrations of 10 ng l^{-1} E2 and 0.1 ng l^{-1} EE2 were claimed, which are the same as the PNECs for those compounds

(Johnson and Sumpter 2001). A drawing of a typical arrangement is reproduced from the patent in Figure 9.5 below. It shows a hybrid aeration tank comprising activated sludge and two localised biofilters which each comprise moveable biofilm carriers between two mesh screens.

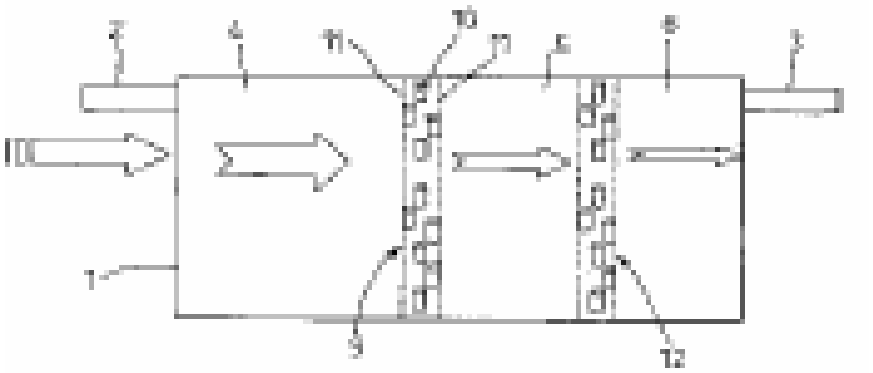


Figure 9.5: Typical arrangement of two IFAS type biofilters as reproduced from patent WO02079103 (Darton and Johnson 2002)

Whilst it was implicitly claimed that two separate biofilters produced superior removal to a single biofilter with volume equal the total of the pair, no reason or mechanism was proposed to explain this. The correlations of pharmaceutical removal with COD concentrations in the composite sampling of this work, and the correspondence of this with the maximum growth rates for the heterotrophic and autotrophic bacteria, it would suggest that a more formal arrangement of the above proposed system can be made. Rather than forming a bacterial community on the media that is the same as the rest of the activated sludge tank, the two biofilters may be forming two very specialised communities. On the first biofilter, which will receive a high COD concentration, a community of heterotrophic bacteria can be expected to occur. Similarly, on the second biofilter, which will receive a much lower COD concentration, autotrophic bacteria can be expected to dominate. Therefore, the two biofilters should be placed within the activated sludge tank so that they receive COD concentrations equivalent to that at which their maximum growth rate occurs, as calculated by Kayombo *et al.* (2003). As a result, the positioning of the two filters will depend on the influent COD concentrations at each STP, and individual systems will need to be designed accordingly. Although this system would require further research to prove its abilities and robustness in a full-scale system, it provides a simple alternative to proposed energy intensive tertiary treatment technologies. This modification also has the advantage that it could actually reduce current OPEX due to lower aeration costs. Potential

disadvantages of this system include rags blocking of the screens used to contain the media. Performance against removal of pharmaceuticals that are resistant to both biodegradation and sorption, such as carbamazepine, remains to be proven.

9.3 Summary of proposed modifications to existing sewage treatment technologies

Figure 9.6 below shows an arrangement of the proposed modifications (in red) to existing sewage treatment technologies.

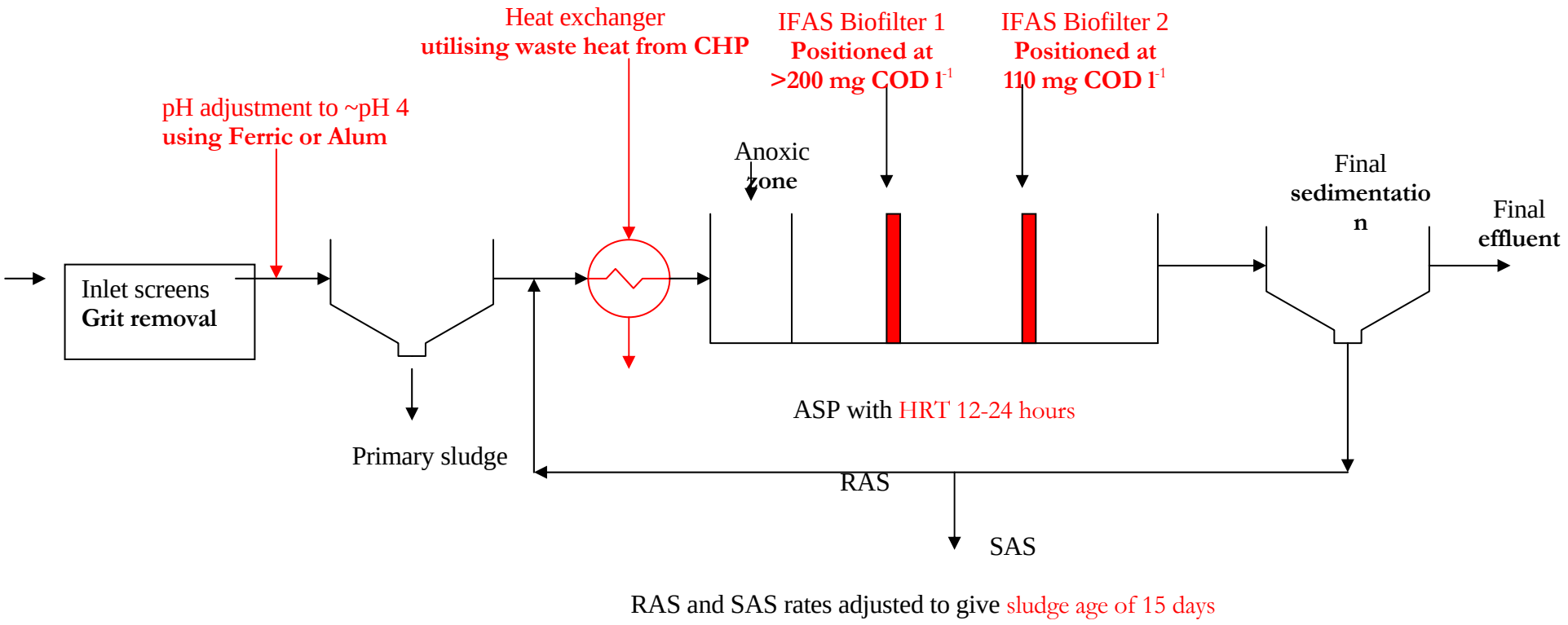


Figure 9.6: Proposed modifications (in red) to existing activated sludge sewage treatment technologies

Chapter 10: Plant operating events

Whilst the results from the previous sets of experiments have shown how sewage treatment plants could be altered to improve the removal of pharmaceuticals, there are uncontrollable daily events within sewage treatment operations that could affect the removal of pharmaceuticals. These include occurrences such as rainfall events, or loss of aeration (which could be caused by blockages, or blower or electricity failure). The last set of experiments involved a series of pilot-scale tests to see what effect these type of events would have on the removal of pharmaceuticals. These tests were run on the porous pots units, simulating an activated sludge plant. As described in the Materials and Methods (see Chapter 5:), pots 5 and 6 were used as a process control (without any pharmaceutical feed). Pots 3 and 4 were dosed with pharmaceuticals, but were otherwise used as controls. Pots 1 and 2 were dosed with pharmaceuticals, and used as the test pots subject to variations in flow and aeration.

10.1 Aeration Failures – one hour loss test

In the first aeration failure test, run for a duration of one hour, no change was observed in the values of any of the monitored parameters or the amount of removal of the pharmaceuticals in the test pots, compared to the control pots.

10.2 Aeration Failures – four hour loss test

A second aeration failure test was run for four hours. Similar to the one hour test, there was no variation in pH, redox potential, and effluent chemical oxygen demand. However, there was an observable change in dissolved oxygen and ammonia removal, as well as pharmaceutical removal. The profile of dissolved oxygen in the porous pot units through the test is shown in Figure 10.1. In this figure, the period for which the aeration to pots 1 and 2 was turned off can clearly be seen (09:30 – 13:30). After this period when aeration was restored, the dissolved oxygen levels within these two pots rapidly returned to normal levels.

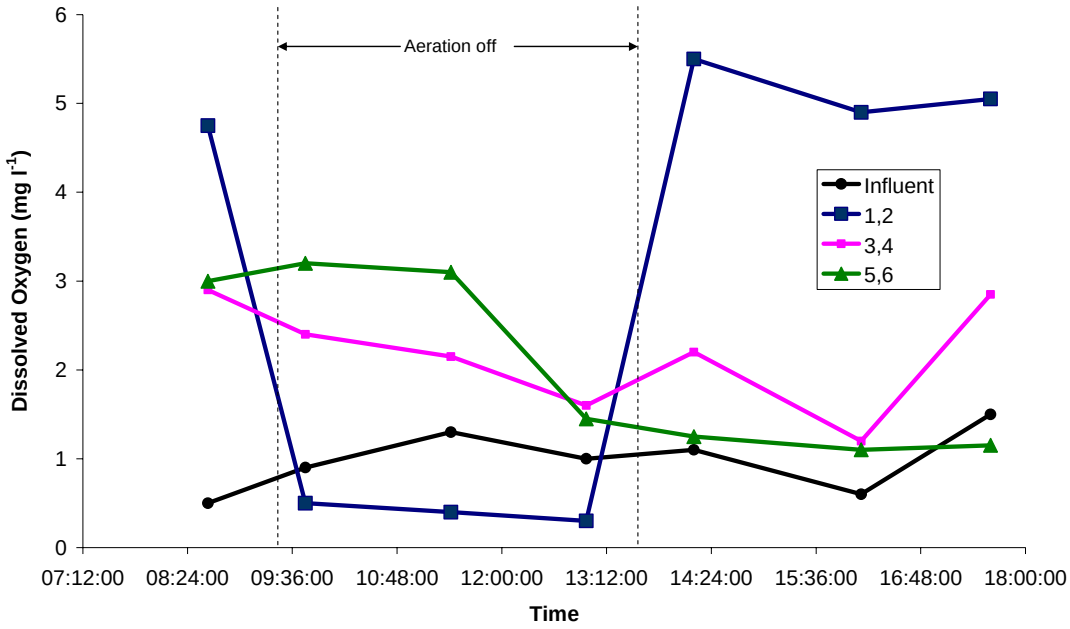


Figure 10.1: Dissolved oxygen profile during the four hour aeration loss test

For the ammonia removal, as shown in Figure 10.2, ammonia removal in pots 1 and 2 dropped off after the aeration was turned off. This decrease in removal continued for about another two to three hours after the aeration was returned, before it began to recover.

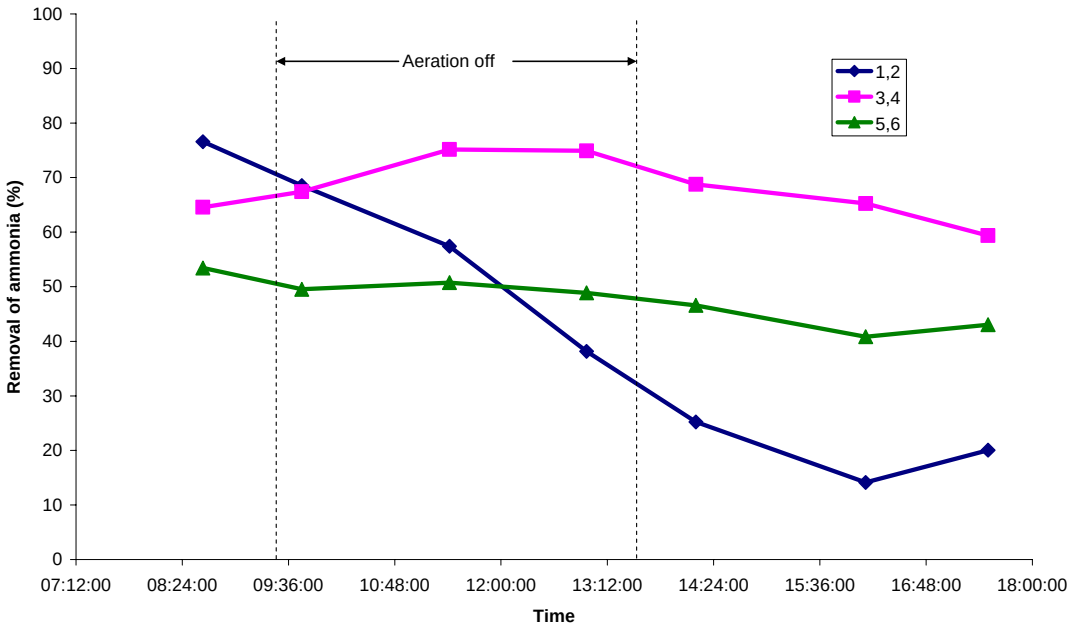


Figure 10.2: Ammonia removal profile during aeration loss test

The effect of the aeration loss on the removal of the pharmaceuticals is discussed in the sections below.

10.2.1 Carbamazepine

Removal of carbamazepine was consistent at 18.4% before and throughout the tests. No variation in removal was observed during the aeration loss. This lack of variation in carbamazepine removal was not surprising, since very little removal of carbamazepine has been observed in full scale sewage treatment plants, typically 6 to 8% (Heberer *et al.* 2002; Ternes 1998) and 12.8% (composite samples from Southam STP). However, the levels of removal in these tests were higher (18.4%) than was expected based on both the above reports and the laboratory fate tests, which had shown no sorption, nor biodegradation. None of the parameters that, when at extremes, have been shown to slightly alter carbamazepine removal, such as HRT or temperature, could be explanations for this higher removal. Potentially, this may have been due to a biomass within the system that had become particularly adapted to degrading the relatively high (compared to environmental conditions) concentrations of carbamazepine entering the system. However, if biodegradation were responsible, then a reduction in removal would have been expected during the un-aerated period. Since this did not occur, it could be suggested that a high level of sorption was responsible for the high removal. However, since no evidence can be found to support either of these hypotheses, then no conclusion can be drawn for the high removal observed in this test.

10.2.2 Tetracycline

In the control pots, tetracycline removal was high, at 92.7%. When the aeration was turned off, tetracycline removal dropped off rapidly, and continued to do so for two hours after aeration was turned back on, in a similar fashion to the ammonia removal. At worst, tetracycline removal dropped to 19.4%. Removal of tetracycline was strongly correlated with the removal of ammonia ($r^2 = 0.97$), suggesting that the reduction in tetracycline removal was due to a loss of biodegradation.

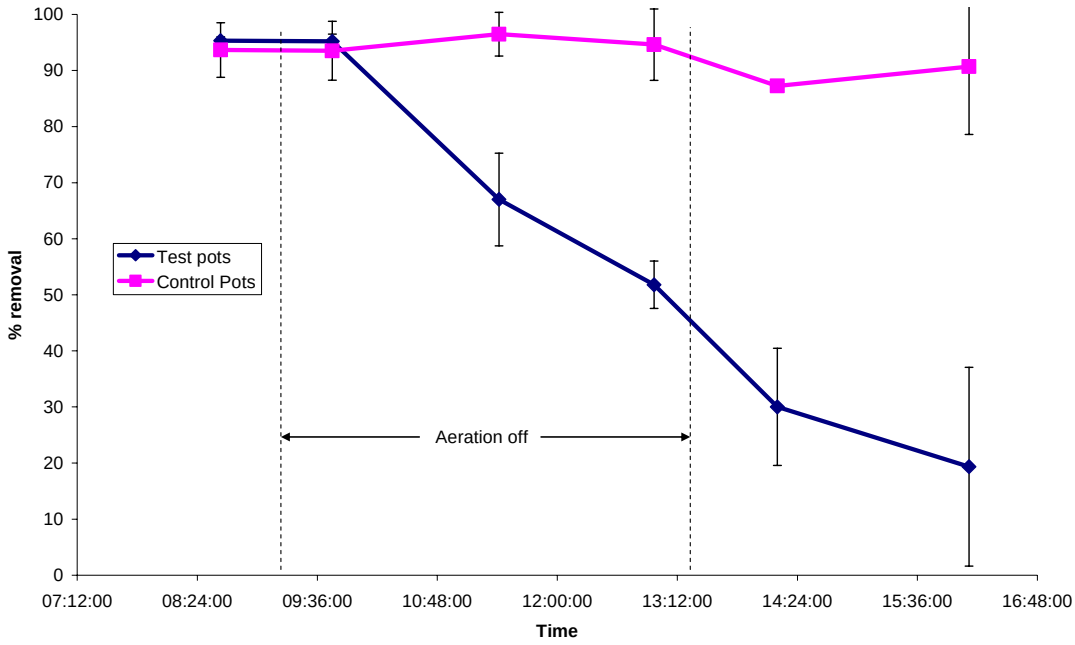


Figure 10.3: Removal of tetracycline during the four hour aeration loss test

The laboratory fate tests, as discussed earlier, showed that tetracycline was both readily biodegradable, and slowly adsorbed to biomass. When aeration was turned off, the amount of oxygen available for biodegradation was rapidly used up, meaning that the only remaining removal mechanism was sorption to biomass. Since the sorption of tetracycline (equilibrium of about 24 hours) is slow compared to the HRT of this system (8.33 hours), incomplete sorption could be expected, leading to the low removal observed in this test system.

It is interesting to note that two hours of aeration was required before any improvement in tetracycline removal was observed. This would have significant implications for the operation of an STP that had to keep tetracycline effluent concentrations beneath a discharge consent level. Two options could be considered. The first being to have standby blowers or generators (as is common practice) to minimize any down time – no drop off in removal was observed with a one hour loss of aeration. However, if alterative air supplies are not possible, an alternative would be to attempt to maximize the HRT of the system to maximize sorption. This could be achieved by temporary use of storm tanks to hold excess flow, although this use of storm tanks would require EA approval.

10.2.3 Caffeine

Removal of caffeine in the control pots was consistent at about 85.8%. No degradation products were detected. In the pots where the aeration was turned off, removal dropped off in a similar manner to tetracycline, reaching a minimum of 18.7%, as shown in Figure 10.4. As for tetracycline, the removal of caffeine was correlated with the removal of ammonia, although the correlation ($r^2 = 0.91$) was not quite as strong as for tetracycline ($r^2 = 0.97$). This again suggests that the reduction in removal is due to a loss of biodegradation.

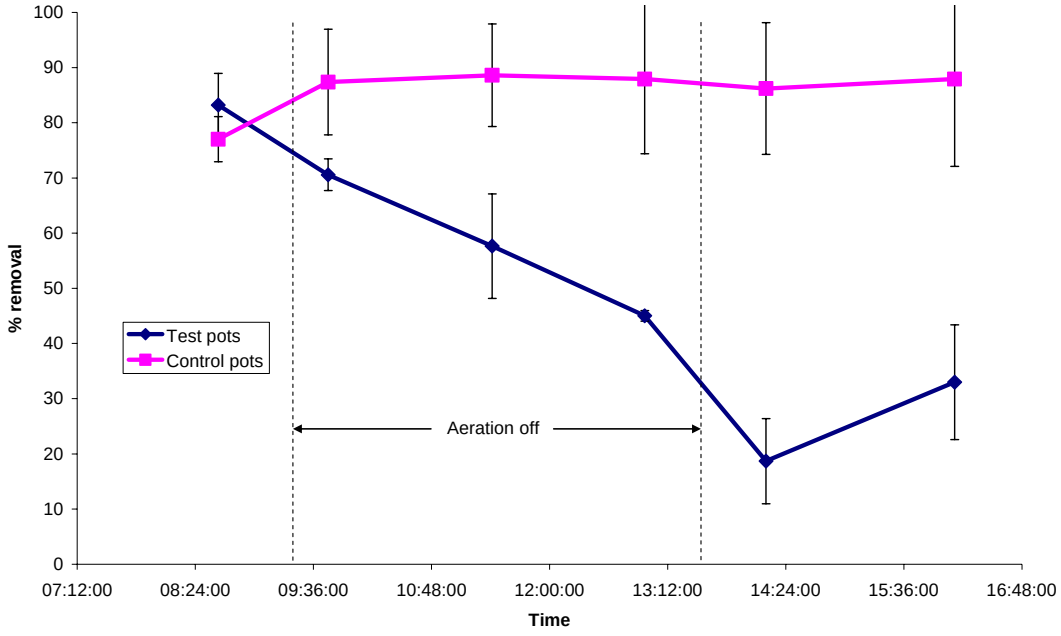


Figure 10.4: Percentage removal of caffeine during aeration loss test

The rate at which the removal of caffeine is reduced in the system after the loss of aeration is higher than that of tetracycline. After four hours only around 35% of caffeine is removed, compared with nearly 50% of tetracycline. Since caffeine is known not to sorb to biomass (see laboratory fate data as discussed earlier), the higher removal of tetracycline after four hours must be due to sorption. It is interesting to note that although there is no measurable dissolved oxygen in the system after four hours, some biodegradation of caffeine is still occurring. This may be due to oxygen entrained within the biomass, or due to denitrification. No data are available on the anoxic (or anaerobic) degradation rates of caffeine.

The removal of caffeine started to improve about one hour after aeration was restored. This presumably related to the length of time it took for oxygen to diffuse into the biomass.

10.2.4 Triclosan

Triclosan removal was constant at around 98.0% through the control pots. Whilst the aeration was turned off the removal of triclosan did appear to reduce very slightly. However, this was not possible to quantify, since triclosan was so well removed that all samples were below the theoretical quantification limit.

Based on the laboratory fate tests, it could have been expected that the loss of aeration would lead to a reduction in the amount of biodegradation, and hence to a reduction in the amount of removal of triclosan. Conversely, the Toxchem+ modelling, as discussed in section 9.2.4 , showed that a reduction in biodegradation (as modelled by temperature decrease) was balanced by an increase in removal due to sorption. Since the rate of sorption of triclosan was faster (about four hours) than the hydraulic retention time of the system (8.33 hours), sorption equilibrium could have been reached. Therefore, in this case it is likely that although the amount of biodegradation may have reduced due to lack of dissolved oxygen, the overall removal of triclosan was preserved by an increase in the amount of sorption. To prove this, concentrations of triclosan degradation products could be monitored, but this was not possible in this study due to the limitations of the analytical equipment available.

10.2.5 Summary of aeration loss tests

No results of the effect of loss of aeration on the removal of pharmaceuticals during sewage treatment have previously been published in the literature. The tests in this work showed that loss of aeration reduced removal of two of the pharmaceuticals (tetracycline and caffeine), whilst two others (triclosan and carbamazepine) were mostly unaffected. The removal of tetracycline and caffeine were strongly correlated with the removal of ammonia, having correlation coefficients of 0.97 and 0.91 respectively, suggesting that the reduction in removal was due to a reduction in biodegradation. The removal of caffeine was reduced more severely than that of tetracycline, since some of the tetracycline was removed by sorption instead.

Loss of aeration has the potential to be a significant, but not insurmountable problem for the removal of pharmaceuticals. For example, no loss of removal was observed when aeration was stopped for only one hour. This is significant, since many oxidation ditch STPs (within Severn Trent) use anoxic periods of around one hour for denitrification (Green 2004). Longer periods without aeration would start to reduce pharmaceutical removal in some cases,

although pharmaceuticals that sorb strongly and quickly to biomass may not be affected (e triclosan). For pharmaceuticals that sorb more slowly, such as tetracycline, increasing the HRT to increase the amount of sorption may mitigate the reduction in biodegradation.

10.3 Storm Flows

A four hour storm flow test was conducted. However, baseline monitoring indicated that shortly after the start of the test, there was a dramatic failure in the nitrification in all six porous pot units. This resulted in all measurements taken being rendered meaningless, since any changes caused by the test were smaller than that causing the nitrification failure. Due to a lack of further time and resources, it was not possible to repeat the storm flow tests.

Strenn *et al.* (2004) observed reduced removal of diclofenac and ibuprofen during rainfall events, which was attributed to a decreased the HRT of the system, although another paper by the same authors noted that the reduced removal could be caused by the rainfall changing the F/M ratio of the ASP (Kreuzinger *et al.* 2004). A decrease in removal during storm events was also noted by Ternes *et al.* (2004). Conversely, Kim *et al.* (2005) found no reduction in tetracycline removal when HRT was reduced from 24 hours to 7.4 hours.

Kolpin *et al.* (2004) noted that in a survey of 105 pharmaceuticals in rivers (of which only 61 were ever detected), that whilst 51 were regularly detected in low flow conditions and 28 were detected in normal flow conditions, only 24 were detected in high flow conditions. This would suggest that whatever the effect of storm flows on the removal of pharmaceuticals is within sewage treatment processes, the overall effect within the aquatic system is to dilute the pharmaceutical concentrations to below normal levels. Therefore, storm events would not pose any direct threat to the compliance with discharge consents for pharmaceutical removal.

Chapter 11: Conclusions

The occurrence and fate of pharmaceutically active substances (PhACs) in the aquatic environment has, over recent years, become recognised as a major issue in environmental chemistry. These substances have been reported in all sections of the aquatic environment, including sewage, rivers, lakes, groundwater, and drinking water. Only 120 out of over 3000 pharmaceuticals licensed for use in the UK have been detected in the environment, although the number of different pharmaceuticals that have been tested for is likely to be perhaps no more than about twice this. The actual number is hard to predict since non-detections are rarely reported in the literature. The effluent of sewage treatment plants was established as a key source of contamination of the aquatic environment.

Understanding of the fate of pharmaceuticals during sewage treatment could lead to new methods and processes to enhance their removal. The overall aim of this research was to further this understanding. To this end, four pharmaceuticals were selected for study. Reliable analytical methods were developed for the detection of these pharmaceuticals in wastewater samples, using a combination of high performance liquid chromatography and solid phase extraction. This combination allowed for detection of the target pharmaceuticals at what was expected to be environmentally relevant concentrations. Three of the four selected pharmaceuticals (triclosan, carbamazepine, and caffeine) were detected in samples taken from sewage treatment plants. The fourth pharmaceutical, tetracycline, was not detected in any environmental sample. These results represented the first reported detections of carbamazepine and caffeine in UK STPs.

Grab sampling was conducted at four STPs. From this, it was established that not only was there a difference in the way that differing pharmaceuticals were removed through each type of unit process, but also that there were differences in the performance of the same type of unit processes at different STPs. Laboratory based sorption and biodegradation tests were conducted that allowed the removal of the pharmaceuticals by each mechanism to be quantified. This began to show where optimisation of STPs could be undertaken to improve removal of pharmaceuticals.

Further sampling at Southam STP, this time taking composite samples, allowed for data to be collected on a wide range of parameters that were suspected to have some influence on the removal of pharmaceuticals. The design of this work, using multiple samples from a single STP, meant that the sludge age was the same for all the samples. This was important since other researchers had shown sludge age to the most significant factor for pharmaceutical removal. Eliminating the variation of this parameter allowed the influence of other parameters to be identified more clearly. Finally, the effects of operational events were investigated.

The concentrations of the pharmaceuticals detected in this research were not high enough to cause immediate harm (i.e. death) to aquatic organisms. However, there is insufficient information to determine whether exposure to these low concentrations, typically around PNEC levels, may have an effect over a long period of time.

From all these results, it was possible to draw a number of conclusions as to ways in which pharmaceutical removal could be optimised during sewage treatment. These are detailed and discussed in the sections below.

11.1 Optimisation of STPs

The major conclusion of this work is that it is indeed possible to optimise the current technology within sewage treatment plants to improve the removal of pharmaceuticals. This could be achieved in a variety of ways, which will result in different amounts of improvement of removal of different pharmaceuticals.

As has been recorded by many authors, but not directly as a part of the experimental work in this research, sludge age needs to be sufficient to produce a diverse biomass. Historically, a minimum of four years has been stated as a minimum sludge age in order to achieve nitrification (Metcalf and Eddy 2003), although ten or twelve days is often used in practice (Green 2004). Increasing the sludge age further will allow for a greater diversification of the bacteria within the biomass, producing greater potential for degradation of pharmaceuticals. However, increasing the sludge age beyond above 15 days gives diminishing returns (Ternes *et al.* 2004), in that the number of compounds being degraded increases, but the increase is

less prominent. Therefore it is recommended that the sludge age of ASPs should be increased to 15 days.

When modelling pharmaceutical removal by sorption, many authors have made the assumption that the sorption process is fast compared to the hydraulic retention time of the system. The results of this work have shown that this assumption is not valid, even for fast sorbing compounds such as triclosan. Much progress has been made over recent years in assessing the distribution coefficients, and hence the maximum sorption potential of pharmaceuticals e.g. Ternes *et al.* (2004). However, to achieve the maximum possible sorption, hydraulic retention times need to be made longer. The sorption tests with tetracycline suggest that as long as twenty-four hours could be necessary. In storm conditions, where hydraulic retention times can be drastically reduced, dilution appears to outweigh any negative effects of reduced sorption.

Increasing the total amount of biomass in an ASP would be another way of increasing removal of pharmaceuticals. However, simply increasing the MLSS concentration would alter the F/M ratio, and potentially overload the final settlement tanks. An alternative to this, as proposed by Darton and Johnson (2002) is to include two or more "biofilters" into the activated sludge tank. The biofilters, typically only two meters in width comprise of small buoyant plastic media on which biomass grows, enclosed by screens. This can support biomass populations from 15 to 40 g l^{-1}. Although this system has been shown to effective remove estrogens, the reasoning behind its success has been somewhat limited. Potentially, simply the amount of biomass or the long sldge age provided by these biofilters could be the answer, but results seem to exceed this. The results of this work have shown that triclosan was removed to different extents depending on the COD concentrations. These concentrations have been suggested, by Kayombo *et al.* (2003), to relate to the maximum growth rates of autotrophic and heterotrophic bacteria, at approximately 110 mg COD l^{-1} and > 200 mg COD l^{-1} respectively. It is therefore recommended that this system be trialled further, with the biofilters positioned where these COD concentrations occur most frequently. This system also has the advantage that the air supply to the biofilters can be via coarse bubble aeration, which is substantially cheaper than a diffused air system.

Maintaining the oxygen flow into biomass will ensure optimal conditions for pharmaceutical removal. Whilst activated sludge plants generally already have an excess of dissolved oxygen,

a failure of the aeration system, for whatever reason, could be severely detrimental to pharmaceutical removal. Although aeration failures of around an hour showed no noticeable deterioration in pharmaceutical removal, a four hour failure lead to up to an 80% drop in removal. Once aeration was restored, pharmaceutical removal took up to two hours to show any improvement in removal. This could have significant implications for the management of aeration systems, should pharmaceutical discharge consents ever be implemented.

Finally, altering the influent pH may help reduce the pharmaceutical loads reaching the biological treatment stages. This is achieved by reducing the pH until the target pharmaceutical has been transformed to its unionised form – the form in which it can sorb to solids and biodegrade. The pH drop required would depend on the pKa of the pharmaceuticals that are targeted for removal. This would increase the amount of pharmaceuticals adsorbed to primary sludges. However, this pH adjustment would also strip alkalinity. Therefore, an alkalinity dosing point would be required after the primary settlement tanks.

11.2 Costs versus benefits - implications for environmental protection and human health

From this research it has become clear that there are many different parameters affecting the removal of pharmaceuticals. However, not all pharmaceuticals are affected in the same way. With over 3000 pharmaceuticals licensed for use in the UK, which have a wide range of functional groups and biodegradation pathways, it highly unlikely that there is any single way of optimising sewage treatment that will improve removal of all pharmaceuticals. Indeed, even the combination of all the optimisation proposed in this work may not substantially improve the removal of the pharmaceuticals most resistant to sorption and biodegradation, such as carbamazepine.

Therefore, the important question that remains is how much removal, of which pharmaceuticals, is required to ensure the protection of the environment and human health? If the answer to that question is that complete removal of *all* pharmaceuticals to below detection limits is required, then it is almost certainly necessary to look at tertiary treatments such as ozonation, chlorine dioxide, or GAC. These tend to be costly and energy intensive processes. Indeed, it could be argued that on large STPs, the pollution caused from power and chemical generation for these processes could cause more environmental damage than

the compounds that the processes are trying to remove. Therefore, are tertiary treatments for pharmaceutical removal the best practicable environmental option? This is an argument currently being tested with the Environment Agency for STPs requiring large amounts of chemical dosing for phosphorus removal. Equally, would the consumer really want a solution that removes all pharmaceuticals but substantially increases water bills?

The practical solution to this dilemma would be to decide on specific limits for specific pharmaceuticals. Whilst these limits may always be open for debate, especially considering the relatively unknown effects of mixtures of compounds, and the practically unpredictable effects of a lifetime's consumption a sub-therapeutic levels, it must be considered that water may only be a very minor part of a person's non-therapeutic consumption. Indeed, as demonstrated for trimethroprim, water could account for less than 0.01% of the total daily intake. For the wider aquatic environment, existing regulations giving PNECs would seem like a practical concentration to aim for in sewage treatment effluents.

11.3 Further work

The key to optimisation of sewage treatment processes for pharmaceutical removal lies, not in new processes, nor in changes to existing ones, but rather in establishing how much removal of which pharmaceuticals is required. In order to do this, much further research is required to elucidate the long term effect of small concentrations of pharmaceuticals both to human health and the aquatic environment. Once this has been established, a more objective discussion on the optimisation of sewage treatment processes can ensue, with a view to determination of the best practicable environmental option.

In order to establish which pharmaceuticals need to be removed more completely during sewage treatment, it is necessary to know what the concentrations currently are in effluents and streams and rivers. Of the 3000 pharmaceuticals licensed for use in the UK, there are reliable analytical methods for possibly less than 10% of these compounds in sewage. The degradation products of pharmaceuticals should also be considered in more detail. Although this work did set out to study degradation products where known, few were detected in environmental samples. However, the few primary degradation products that were detected had been shown to be more toxic to the environment than the parent compound. There is a danger that whilst the amount of degradation of parent compounds could be increased, this

may only lead to an increase in the quantities of primary degradation products present. Potentially, this could lead to an overall increase in the toxicity of the final effluents. Therefore, it is essential that more degradation products are identified, and monitored in the environment.

A major piece of future work will be to test the proposals made in this work for the optimisation of sewage treatment plants for the removal of pharmaceuticals. In particular, establishing how much load can be removed by pH adjustment, compared to the costs of chemicals will be essential. A detailed design for the proposed biofilters will also be required, which will not only need to take account of process issues, but also consider the practicalities of operation and maintenance. For example, how can the screens needed to contain the media be kept free from rags and hence not create a hydraulic restriction.

Once the properties of target pharmaceuticals have been established, through laboratory sorption and biodegradation tests as conducted in this research, modelling of pharmaceutical removal could become a viable proposition. With the cooperation of a major modelling firm, such as Envirosim (Biowin) or Hydromantis (GPS-X and ToxChem+), a useful tool could be created which could not only be used to predict effluent concentrations, but also to show how operational changes to sewage treatment processes could affect pharmaceutical removal. If proven accurate, modelling could significantly reduce the amount of sampling of pharmaceuticals that is required, and hence reduce costs.

Finally, there is one aspect of sewage treatment that has been almost completely ignored in this research, which is what happens to pharmaceuticals once they have become sorbed to sludges. Sewage sludges undergo many treatment processes whilst on the STP, such as dewatering, anaerobic digestion, and liming. Outside of the STP, sludges may be applied to farmland, incinerated, or occasionally disposed of in landfill. If applied to farmland, there is the potential for uptake by crops, which could eventually lead back to the human food chain. Further research is required to establish the fate of pharmaceuticals through the sludge treatment route. In particular, it would be interesting to establish the fate of pharmaceuticals in sludges treated according to the Safe Sludge Matrix (ADAS 2001), designed to promote the highest possible standard of food safety and give the food industry confidence that sludge reuse on agricultural land is safe. If it were to be established that high levels of

pharmaceuticals were ending up in the human food chain due to sludge application on farmland, it could significantly threaten this sludge disposal route.

Chapter 12: References

ADAS (2001). The Safe Sludge Matrix - Guidelines for the Application of Sewage Sludge to Agricultural Land. Wolverhampton, UK, ADAS.

Aguera, A., Fernadez-Alba, A. R., Piedra, L., Mezcua, M. and M., G. (2003). "Evaluation of Triclosan and Bipheylol in Marine Sediments and Urban Wastewaters by Presurized Liquid Extraction and Solipd Phase Extraction Followed by Gcms and Lcms." Analytica Chimica Acta **480**: 193-205.

Ahel, M., Mikac, N., Cosovic, B., Prohic, E. and Soukup, V. (1998). "The Impact of Contamination from a Municipal Solid Waste Landfill (Zagreb, Croatia) on Underlying Soil." Water Science and Technology **37**(8): 203-210.

Ahel, T., Mijatovic, I., Matosic, M. and Ahel, M. (2004). "Nanofiltration of a Landfill Leachate Containing Pharmaceutical Intermediates from Vitamin C Production." Food Technology and Biotechnology **42**(2): 99-104.

Aherne, G. W. and Briggs, R. (1989). "The Relevance of the Presence of Certain Synthetic Steroids in the Aquatic Environment." Journal of Pharmacy and Pharmacology **41**: 735-736.

Aherne, G. W., Hardcastle, A. and Nield, A. H. (1990). "Cytotoxic Drugs and the Aquatic Environment; Estimation of Bleomycin in River and Water Samples." Journal of Pharmacy and Pharmacology **42**: 741-42.

Ahrer, W., Scherwenk, E. and Buchberger, W. (2001). "Determination of Drug Residues in Water by the Combination of Liquid Chromatography or Capillary Electroporesis with Electrospray Mass Spectrometry." Journal of Chromatography A **910**: 69-78.

Amore, B. M., Kalhorn, T. F., Skiles, G. L., Hunter, A. P., Bennett, G. D., Finnell, R. H., Nelson, S. D. and Slattery, J. T. (1997). "Characterization of Carbamazepine Metabolism in a Mouse Model of Carbamazepine Teratogenicity." Drug Metabolism and Disposition **25**(8): 953-962.

Andreadakis, A. D., C.M., K., N., K. and D., A. (1997). "Determination of the Impact of Toxic Inflows on the Performance of Activated Sludge by Wastewater Characterization." Water Science and Technology **36**(2-3): 45-52.

Antusch, E. (1999). Lokalisierung Organischer Schadstoffemissionen in Kommunale Abwasserkanale Durch Sielhautuntersuchungen. Karlsruhe, Universitat Fridericiana zu Karlsruhe.

APHA (1998). "Alpha Standard Methods for Examination of Water and Wastewater." American Public Health Association.

Ashton, D., Hilton, M. and Thomas, K. V. (2004). "Investigating the Environmental Transport of Human Pharmaceuticals to Streams in the United Kingdom." Science of the Total Environment **333**(1-3): 167-184.

Aumonier, J. (2003). "Risk Management of Pharmaceuticals in the Environment: The Human Pharmaceutical Industry Viewpoint." Proceedings of Envirpharma European conference on human and vetinary pharmaceuticals in the environment.

Ayscough, N. J., Fawell, J., Franklin, G. and Young, W. (2000). R&D Technical Report P390: Review of Human Pharmaceuticals in the Environment. Bristol, UK Environment Agency.

Batt, A. L., Bruce, I. B. and Aga, D. S. (2006). "Evaluating the Vulnerability of Surface Waters to Antibiotic Contamination from Varying Wastewater Treatment Plant Discharges." Environmental Pollution **142**(2): 295-302.

Belfroid, A. C., Van der Horst, A., Vethaak, A. D., Schafer, A. J., Rijs, G. B. J., Wegener, J. and Cofino, W. P. (1999). "Analysis and Occurence of Estrogenic Hormones and Their Glucuronides in Surface Water and Waste Water in the Netherlands." Science of the Total Environment **225**: 101-108.

Bendz, D., Paxeus, N. A., Ginn, T. R. and Loge, F. J. (2005). "Occurrence and Fate of Pharmaceutically Active Compounds in the Environment, a Case Study: Hoje River in Sweden." Journal of Hazardous Materials **122**(3): 195-204.

Benito-Pena, E., Partal-Rodera, A. I., Leon-Gonzalez, M. E. and Moreno-Bondi, M. C. (2006). "Evaluation of Mixed Mode Solid Phase Extraction Cartridges for the Preconcentration of Beta-Lactam Antibiotics in Wastewater Using Liquid Chromatography with Uv-Dad Detection." Analytica Chimica Acta **556**(2): 415-422.

Berger, K., Petersen, B. and Buening-Pfaue, H. (1986). "Persistence of Drugs Occuring in Liquid Manure in the Food Chain." Archiv fuer Lebensmittelhygeine **37**(4): 99-102.

Bergh, J. J. and Breytenbach, J. (1990). "High-Performance Liquid Chromatographic Analysis of Trimethroprim in the Presence of Its Degradation Products." Journal of Chromatography **513**: 392-396.

Bester, K. (2003). "Triclosan in a Sewage Treatment Process - Balances and Monitoring Data." Water Research **37**: 3891-3896.

Bester, K. (2005). "Fate of Triclosan and Triclosan-Methyl in Sewage Treatment Plants and Surface Waters." Archives of Environmental Contamination and Toxicology **49**(1): 9-17.

Blackwell, P. A., Lutzhoft, H. C. H., Ma, H.-P., Halling-Sorensen, B., Boxall, A. B. A. and Kay, P. (2004). "Fast and Robust Simultaneous Determination of Three Vetinary Antibiotics in Groundwater and Surface Water Using a Tandem Solid-Phase Extraction

with High-Performance Liquid Chromatography-Cv Detection." Journal of Chromatography A **1045**: 111-117.

Blackwood, D. J., Ellis, J. B., Revitt, D. M. and Gilmour, D. J. (2005). "Factors Influencing Exfiltration Processes in Sewers." Water Science and Technology **51**(2): 147-154.

Boreen, A. L., Arnold, W. A. and McNeill, K. (2003). "Photodegradation of Pharmaceuticals in the Aquatic Environment: A Review." Aquatic Sciences **65**(4): 320-341.

Bound, J. P., Kitsou, K. and Voulvoulis, N. (2006). "Household Disposal of Pharmaceuticals and Perception of Risk to the Environment." Environmental Toxicology and Pharmacology **21**(3): 301-307.

Bound, J. P. and Voulvoulis, N. (2005). "Household Disposal of Pharmaceuticals as a Pathway for Aquatic Contamination in the United Kingdom." Environmental Health Perspectives **113**(12): 1705-1711.

Bound, J. P. and Voulvoulis, N. (2006). "Predicted and Measured Concentrations for Selected Pharmaceuticals in Uk Rivers: Implications for Risk Assessment." Water Research **40**(15): 2885-2892.

Bourgeois, B. F. and Wad, N. (1984). "Carbamazepine-10,11-Diol Steady-State Serum Levels and Renal Excretion During Carbamazepine Therapy in Adults and Children." Theraputic drug monitoring **6**(3): 259-265.

Bouwer, E. (1989). Transformation of Xenobiotics in Biofilms. Structure and Function of Biofilms. Characklis, W. and Wilderer, P. New York, John Wiley and Sons**:** 251-267.

Boxall, A. B. A., Johnson, P., Smith, E. J., Sinclair, C. J., Stutt, E. and Levy, L. S. (2006). "Uptake of Veterinary Medicines from Soils into Plants." Journal of Agricultural and Food Chemistry **54**(6): 2288-2297.

Boxall, A. B. A., Sinclair, C. J., Fenner, K., Kolpin, D. W. and Maund, S. J. (2004). "When Synthetic Chemicals Degrade in the Environment." Environmental Science and Technology **38**: 369A-375A.

Boyd, G. R., Palmeri, J. M., Zhang, S. Y. and Grimm, D. A. (2004). "Pharmaceuticals and Personal Care Products (Ppcps) and Endocrine Disrupting Chemicals (Edcs) in Stormwater Canals and Bayou St. John in New Orleans, Louisiana, USA." Science of the Total Environment **333**(1-3): 137-148.

Boyd, G. R., Reemtsma, H., Grimm, D. A. and Mitra, S. (2003). "Pharmaceuticals and Personal Care Products (Ppcps) in Surface and Treated Waters of Louisiana, USA and Ontario, Canada." Science of the Total Environment **311**(1-3): 135-149.

Boyd, G. R., Zhang, S. Y. and Grimm, D. A. (2005). "Naproxen Removal from Water by Chlorination and Biofilm Processes." Water Research **39**(4): 668-676.

Bradford, M. (1976). "A Rapid and Sensitive Method for the Quantitation of Microgram Quantities of Protein Utilizing the Principle of Protein-Dye Binding." Analytical Biochemistry **72**: 248-254.

Buser, H.-R., Poiger, T. and Muller, M. D. (1998). "Occurence and Fate of the Pharmaceutical Drug Diclofenac in Surface Waters: Rapid Photodegradation in a Lake." Environmental Science and Technology **32**(22): 3449-3456.

Buser, H.-R., Poiger, T. and Muller, M. D. (1999). "Occurence and Environmental Behaviour of the Pharmaceutical Drug Ibuprofen in Surface Waters and in Wastewater." Environmental Science and Technology **33**: 2529-2535.

Buyuksonmez, F. and Sekeroglu, S. (2005). "Presence of Pharmaceuticals and Personal Care Products (Ppcps) in Biosolids and Their Degradation During Composting." Journal of Residuals Science & Technology **2**(1): 31-40.

Carballa, M., Omil, F. and Lema, J. M. (2005). "Removal of Cosmetic Ingredients and Pharmaceuticals in Sewage Primary Treatment." Water Research **39**(19): 4790-4796.

Carballa, M., Omil, F., Lema, J. M., Llompart, M., Garcia, C., Rodriguez, I., Gomez, M. and Ternes, T. (2005). "Behaviour of Pharmaceuticals and Personal Care Products in a Sewage Treatment Plant of Northwest Spain." Water Science and Technology **52**(8): 29-35.

Carballa, M., Omil, F., Lema, J. M., Llompart, M., Garcia-Jares, C., Rodriguez, I., Gomez, M. and Ternes, T. (2004). "Behaviour of Pharmaceuticals, Cosmetics and Hormones in a Sewage Treatment Plant." Water Research **38**: 2918-2926.

Carballa, M., Omil, F., Lema, J. M. and Ternes, T. (2003). "Fate of Ppcps During Anaerobic Treatment of Sewage Sludge." POSEIDON Symposium - Presentation of Project Results.

Cardoso, A., Prigiobbe, V., Giulianelli, M., Baer, E., de Benedittis, J. and Coelho, S. T. (2006). "Assessing the Impact of Infiltration and Exfiltration in Sewer Systems Using Performance Indicators: Case Studies of the Apuss Project." Water Practice & Technology **1**(1).

Cartwright, N. (2006). Edcs - the Problem and Way Forward. Endocrine disrupting chemicals in the water environment, Nottingham, UK.

Castiglioni, S., Bagnati, R., Calamari, D., Fanelli, R. and Zuccato, E. (2005). "A Multiresidue Analytical Method Using Solid-Phase Extraction and High-Pressure Liquid Chromatography Tandem Mass Spectrometry to Measure Pharmaceuticals of Different Therapeutic Classes in Urban Wastewaters." Journal of Chromatography A **1092**(2): 206-215.

Castiglioni, S., Bagnati, R., Fanelli, R., Pomati, F., Calamari, D. and Zuccato, E. (2006). "Removal of Pharmaceuticals in Sewage Treatment Plants in Italy." Environmental Science & Technology **40**(1): 357-363.

Celis, R., Barriuso, E. and Houot, S. (1998). "Effect of Liquid Sewage Sludge Addition on Atrazine Sorption and Desorption by Soil." Chemosphere **37**(6): 1091-1107.

Chambaz, A., Meirim, I. and Decombaz, J. (2001). "Urinary Caffeine after Coffee Consumption and Heat Dehydration." International Journal of Sports Medicine **22**(5): 366-372.

Cinquina, A., Longo, F., Anastasi, G., Gianetti, L. and Cozzani, R. (2001). "Validation of a High-Performance Liquid Chromatography Method for the Determination of Oxytetracycline, Tetracycline, Chlortetracycline and Doxycycline in Bovine Milk and Muscle." Journal of Chromatography A.

Clara, M., Kreuzinger, N., Strenn, B., Gans, O. and Kroiss, H. (2005). "The Solids Retention Time - a Suitable Design Parameter to Evaluate the Capacity of Wastewater Treatment Plants to Remove Micropollutants." Water Research **39**(1): 97-106.

Clara, M., Kreuzinger, N., Strenn, B., Gans, O. and Kroiss, H. (2005). "The Solids Retention Time - a Suitable Design Parameter to Evaluate the Capacity of Wastwater Treatment Plants to Remove Micropollutants." Water Research.

Clara, M., Strenn, B., Gans, O., Martinez, E., Kreuzinger, N. and Kroiss, H. (2005). "Removal of Selected Pharmaceuticals, Fragrances and Endocrine Disrupting Compounds in a Membrane Bioreactor and Conventional Wastewater Treatment Plants." Water Research **39**(19): 4797-4807.

Cleuvers, M. (2003). "Aquatic Ecotoxicity of Pharmaceuticals Including the Assessment of Combination Effects." Toxicology Letters **142**(3): 185-194.

Cleuvers, M. (2004). "Mixture Toxicity of the Anti-Inflammatory Drugs Diclofenac, Ibuprofen, Naproxen, and Acetylsalicylic Acid." Ecotoxicology and Environmental Safety **59**(3): 309-315.

Craig, M. (2001). "Disposal of Unwanted Medicianl Products (Dump) Campaign and Audit - February 2001." Records of Argyll and Clyde NHS Board Lomand and Argyll Primary Care NHS Trust.

Cromarty, E. and Downie (2001). "Drug Wastage - What Is Acceptable?" The Pharmaceutical Journal **267**(7167): 424.

Cyclamed Www.Unpf.Org/Cyclamed/Index.Htm.

Darton, R. C. and Johnson, A. C. (2002). Treatment of Waste Water - Patent Number Wo02079103. UK.

Daughton, C. G. (2003). "Cradle-to-Cradle Stewardship of Drugs for Minimizing Their Environmental Disposition While Promoting Human Health. Ii. Drug Disposal, Waste Reduction, and Future Directions." Environmental Health Perspectives **111**(5): 775-785.

Daughton, C. G. and Jones-Lepp, T. (2001). Pharmaceuticals and Personal Care Products in the Environment: Scientific and Regulatory Isses. Washington DC.

Daughton, C. G. and Ternes, T. A. (1999). "Pharmaceuticals and Personal Care Products in the Environment: Agents of Subtle Change?" Environmental Health Perspectives **107**(Supplement 6): 907-938.

Davis, M., Cleveland, C. and Sharar, M. (1999). "Endocrine Disruptors in Wastwater: Is There Cause for Concern?" Water Environment Federation, WEFTEC.

de Benedittis, J. and Bertrand-Krajewski, J. L. (2005). "Measurement of Exfiltration in Urban Sewer Systems Using Nacl as an Artificial Tracer." Houille Blanche-Revue Internationale De L Eau(5): 26-34.

Desbrow, C., Routledge, E. J., Brighty, G. C., Sumpter, J. P. and Waldock, M. (1998). "Identification of Estrogenic Chemicals in Stw Effluent. 1. Chemical Fractionation and *in Vitro* Biological Screening." Environmental Science and Technology **32**: 1549-1558.

Diaz-Cruz, M. S., de Alda, M. J. L. and Barcelo, D. (2003). "Environmental Behavior and Analysis of Veterinary and Human Drugs in Soils, Sediments and Sludge." Trac-Trends in Analytical Chemistry **22**(6): 340-351.

Dignac, M. F., Ginestet, P., Rybacki, D., Bruchet, A., Urbain, V. and Scribe, P. (2000). "Fate of Wastewater Organic Pollution During Activated Sludge Treatment: Nature of Residual Organic Matter." Water Research **34**(17): 4185-4194.

Dold, P. (2006). Modelling of Pharmaceutical Fate. Comms., P.

Doll, T. E. and Frimmel, F. H. (2003). "Fate of Pharmaceuticals-Photodegradation by Simulated Solar Uv-Light." Chemosphere **52**(10): 1757-1769.

Drewes, J. E., Fox, P. and Jekel, M. (2001). "Occurrence of Iodinated X-Ray Contrast Media in Domestic Effluents and Their Fate During Indirect Potable Reuse." Journal of Environmental Science and Health Part a-Toxic/Hazardous Substances & Environmental Engineering **36**(9): 1633-1645.

Dubois, M., Gilles, K. A., Hamilton, J. K., Rebers, P. A. and Smith, F. (1956). "Colorimetric Method for Determination of Sugars and Related Substances." Analytical Chemistry **28**(3): 350-356.

EA (2006). Www.Environment-Agency.Gov.Uk.

EC (2000). Directive 2000/60/Ec of the European Parliment and of the Council of 23rd October 2000 Establishing a Framework for Community Action in the Field of Water Policy. 2000/60/EC. Brussels, European Commission.

EC (2000). European Commission Directive 92/43/Eec on the Conservation of Natural Habitats and of Wild Fauna and Flora. 92/643/EEC, Brussels.

Ellis, J. B. (2006). "Pharmaceutical and Personal Care Products (Ppcps) in Urban Receiving Waters." Environmental Pollution **144**: 184-189.

EMEA (1997). Trimethroprim Summary Report Emea/Mrl/255/97-Final. products, C. f. v. m.

EMEA (2006). Guideline on the Environmental Risk Assessment of Medicinal Products for Human Use Emea.Chmp/Swp/4447/00. London, UK.

EURACHEM (1993). Eurachem Guidance Document No. 1/Welac Guidance Document No. Wgd 2: Accreditation for Chemical Laboratories: Guidance on the Interpretation of the En 45000 Series of Standards and Iso/Iec Guide 25.

FDA (1995). Guiudeance for Industry for the Submission of an Environmental Assessment in Human Drug Applications and Supplements.

Federle, T. W., Kaiser, S. K. and Nuck, B. A. (2002). "Fate and Effects of Triclosan in Activated Sludge." Environmental Toxicology and Chemistry **21**(7): 1330-1337.

Fent, K., Weston, A. A. and Caminada, D. (2006). "Ecotoxicology of Human Pharmaceuticals." Aquatic Toxicology **76**(2): 122-159.

Fenz, R., Blaschke, A. P., Clara, M., Kroiss, H., Mascher, D. and Zessner, M. (2005). "Monitoring of Carbamazepine Concentrations in Wastewater and Groundwater to Quantify Sewer Leakage." Water Science and Technology **52**(5): 205-213.

Fenz, R., Blaschke, A. P., Clara, M., Kroiss, H., Mascher, D. and Zessner, M. (2005). "Quantification of Sewer Exfiltration Using the Anti-Epileptic Drug Carbamazepine as Marker Species for Wastewater." Water Science and Technology **52**(9): 209-217.

Fielding, M., Gibson, T. M., James, H. A., McLoughlin, K. and Steel, C. P. (1981). Organic Micropollutants in Drinking Water.

Flaherty, C. M. and Dodson, S. I. (2005). "Effects of Pharmaceuticals on Daphnia Survival, Growth, and Reproduction." Chemosphere **61**(2): 200-207.

Flemming, H. C. and Wingender, J. (2001). "Relevance of Microbial Extracellular Polymeric Substances (Epss) - Part Ii: Technical Aspects." Water Science and Technology **43**(6): 9-16.

Garrison, A. W., Pope, J. D. and Allen, F. R. (1976). Identification and Analysis of Organic Pollutants in Water. Ann Arbor, Ann Arbor Science Publishers Inc.

Georga, K. A., Samanidou, V. F. and Papadoyannis, I. N. (2001). "Use of Novel Solid-Phase Extraction Sorbent Materials for High-Performance Liquid Chromatography Quantification of Caffeine Metabolism Products Methylxanthines and Methyluric Acids in Samples of Biological Origin." Journal of Chromatography B **759**: 209-218.

Giardino, V. G. and Guglielmetti, J. L. (1997). "Long-Term Performance of a Hazardous Waste Landfill." Geotextiles and Geomembranes **15**(4-6): 255-267.

Giger, W., Alder, A. C., Golet, E. M., Kohler, H. P. E., McArdell, C. S., Molnar, E., Siegrist, H. and Suter, M. J.-F. (2003). "Occurrence and Fate of Antibiotics as Trace Contaminants in Wastewaters, Sewage Sludges, and Surface Waters." Chimia **57**(9): 485-491.

Gobel, A., Thomsen, A., McArdell, C. S., Alder, A. C., Giger, W., Theiss, N., Loffler, D. and Ternes, T. A. (2005). "Extraction and Determination of Sulfonamides, Macrolides, and Trimethoprim in Sewage Sludge." Journal of Chromatography A **1085**(2): 179-189.

Gobel, A., Thomsen, A., McArdell, C. S., Joss, A. and Giger, W. (2005). "Occurrence and Sorption Behavior of Sulfonamides, Macrolides, and Trimethoprim in Activated Sludge Treatment." Environmental Science & Technology **39**(11): 3981-3989.

Golet, E. M., Alder, A. C. and Giger, W. (2002). "Environmental Exposure and Risk Assessment of Fluoroquinolone Antibacterial Agents in Wastewater and River Water of the Glatt Valley Watershed, Switzerland." Environmental Science & Technology **36**(17): 3645-3651.

Golet, E. M., Alder, A. C., Hartmann, A., Ternes, T. A. and Giger, W. (2001). "Trace Determination of Fluoroquinolone Antibacterial Agents in Solid-Phase Extraction Urban Wastewater by and Liquid Chromatography with Fluorescence Detection." Analytical Chemistry **73**(15): 3632-3638.

Gomez, M. J., Petrovic, M., Fernandez-Alba, A. R. and Barcelo, D. (2006). "Determination of Pharmaceuticals of Various Therapeutic Classes by Solid-Phase Extraction and Liquid Chromatography-Tandem Mass Spectrometry Analysis in Hospital Effluent Wastewaters." Journal of Chromatography A **1114**(2): 224-233.

Goodwin, J. and Foster, C. (1985). "A Further Examination into the Composition of Activated Sludge Surfaces in Relation to Their Settlement Characteristics." Water Research **19**: 527-533.

Gray, S. and Booker, N. (2003). "Wastewater Services for Small Communities." Water Science and Technology **47**(7-8): 65-71.

Green, P. (2004). Process Selection Criteria for Sewage Treatment & on-Site Pumping Stations. The Severn Trent Water - Design Manual. Rimen, P. Birmingham, Severn Trent Water.

Gremm, T. J. and Kaplan, L. A. (1997). "Dissolved Carbohydrates in Streamwater Determined by Hplc and Pulsed Amperometric Detection." Limnology and Oceanography **42**(2): 385-393.

Gutierrez-Sanchez, G., Roussos, S. and Augur, C. (2004). "Effect of Nitrogen Source on Caffeine Degradation by Aspergillus Tamarii." Letters in Applied Microbiology **38**(1): 50-55.

Halden, R. U. and Paull, D. H. (2005). "Co-Occurrence of Triclocarban and Triclosan in Us Water Resources." Environmental Science & Technology **39**(6): 1420-1426.

Halling-Sorensen, B. (2000). "Algal Toxicity of Antibacterial Agents Used in Intensive Farming." Chemosphere **40**(7): 731-739.

Halling-Sorensen, B., Jensen, J., J., T. and Montforts, M. H. M. M. (2001). Worst-Case Estimations of Prdeicted Environmental Soil Concentrations (Pec) of Selected Vetinary Antibiotics and Residues Used in Danish Agriculture. Pharmaceuticals in the Environment. Kummerer, K. Berlin, Springer-Verlag.

Halling-Sorensen, B., Sengelov, G. and J., T. (2002). "Toxicity of Tetracyclines and Tetracycline Degradation Products to Environmentally Relevant Bacteria, Including Selected Tetracycline-Resistant Bacteria." Environmental Contamination and Toxicology **42**: 263-271.

Halling-Sorensen, S., Nielsen, S. N., Lansky, P. F., Ingerslev, F., Lutzhoft, H. C. H. and Jorgensen, S. E. (1998). "Occurence, Fate and Effects of Pharmaceutical Substances in the Environment - a Review." Chemosphere **36**(2): 357-393.

Hammond, E., Humphrey, A., Nwankpa, C. and LaiHing, K. (2005). "Pharmaceuticals and Personal Care Products in Residential and Industrial Effluent Wastewater." Abstracts of Papers of the American Chemical Society **229**: U508-U508.

Hannah, S. A., Austern, B. M., Eralp, A. E. and Dobbs, R. A. (1988). "Removal of Organic Toxic Pollutants by Trickling Filter and Activated Sludge." Journal of the Water Pollution Control Federation **60**: 1281-1283.

Hannah, S. A., Austern, B. M., Eralp, A. E. and Wise, R. H. (1986). "Comparative Removal of Toxic Pollutants by Six Wastewater Treatment Processes." Journal of the Water Pollution Control Federation **58**: 27-34.

Hartmann, A., Alder, A. C., Koller, T. and Widmer, R. M. (1998). "Identification of Fluoroquinone Antibiotics as the Main Source of Umuc Genotoxicity in Native Hospital Wastewater." Environmental Toxicology and Chemistry **17**(3): 377-382.

Hayes, S., Newland, L., Morgan, K. and Dean, K. (1990). "Septic-Tank and Agricultural Nonpoint Source Pollution within a Rural Watershed." Toxicological and Environmental Chemistry **26**(1-4): 137-155.

Heberer, T. (2002). "Occurence, Fate, and Removal of Pharmaceutical Residues in the Aquatic Environment: A Review of Recent Research Data." Toxicology letters **131**: 5-17.

Heberer, T. (2002). "Tracking of Persistent Pharmaceutical Residues from Municipal Sewage to Drinking Water." Journal of Hydrology **266**(175-189).

Heberer, T. (2005). Carbamazepine Removal - Pers Comms. Thompson, A. S.

Heberer, T., Butz, S. and Stan, H. J. (1995). "Analysis of Phenoxycarboxylic Acids and Other Acidic Compounds in Tap, Ground, Surface and Sewage Water at the Low Ng/L-Level." International Journal of Environmental and Analytical Chemistry **58**: 43-53.

Heberer, T., Dunnbier, U., Reilich, C. H. and Stan, H. J. (1997). "Detection of Drugs and Drug Metabolites in Ground Water Samples of a Drinking Water Treatment Plant." Fresenius Environmental Bulletin **6**: 438-443.

Heberer, T. and Feldmann, D. (2005). "Contribution of Effluents from Hospitals and Private Households to the Total Loads of Diclofenac and Carbazepine in Municipal Sewage Effluents - Modeling Versus Measurements." Journal of Hazardous Materials **122**: 211-218.

Heberer, T., Reddersen, K. and Mechlinski, A. (2002). "From Municipal Sewage to Drinking Water: Fate and Removal of Pharmaceutical Residues in the Aquatic Environment in Urban Areas." Water Science and Technology **46**(3): 81-88.

Heberer, T., Schmidt-Baumler, K. and Stan, H. J. (1998). "Occurence and Distribution of Organic Contaminants in the Aquatic System in Berlin. Part I: Drug Residues and Other Polar Contaminants in Berlin Surface and Groundwater." Acta Hydrochimica et Hydrobiologica **26**: 272-278.

Heberer, T. and Stan, H. J. (1996). "Occurence of Polar Organic Contaminants in Berlin Drinking Water." Vom Wasser **86**: 19-31.

Heberer, T. and Stan, H. J. (1997). "Determination of Clofibric Acid and N-(Phenylsulfonyl)-Sarcosine in Sewage, River and Drinking Water." International Journal of Environmental Analytical Chemistry **67**: 113-124.

Hignite, C. and Azarnoff, D. L. (1977). "Drugs and Drug Metabolites as Environmental Contaminants: Chlorophenoxyisobutyrate and Salicylic Acid in Sewage Water Effluent." Life Sciences **20**(2): 337-341.

Hilton, M., Thomas, K. V. and Ashton, D. (2003). Targeted Monitoring Programme for Pharmaceuticals in the Aquatic Environment. R&D Technical Report P6-012/06/TR. Bristol, Environment Agency**:** 62.

Hilton, M., Thomas, K. V. and Ashton, D. (2004). Targeted Monitoring Programme for Pharmaceuticals in the Aquatic Environment. R&D Technical Report P6-012/06/TR. Bristol, Environment Agency**:** 62.

Hirsch, R., Ternes, T. A., Haberer, K. and Kratz, K. L. (1996). "Determination of Beta-Blockers and B2-Sympatomimetics in the Aquatic Environment." Vom Wasser **87**(263-274).

Hirsch, R., Ternes, T. A., Haberer, K. and Kratz, K. L. (1999). "Occurence of Antibiotics in the Aquatic Environment." Science of the Total Environment **225**: 109-118.

Hirsch, R., Ternes, T. A., Lindart, A., Haberer, K. and R.-D., W. (2000). "A Sensitive Method for the Determination of Iodine Containing Diagnostic Agents in Aqueous Matrices Using Lc-Electrospray-Tandem-Ms Detection." Fresenius Journal of Analytical Chemistry **366**: 835-841.

Hirsch, R., Ternes, T. A., Mehlig, A., Ballwans, F., Kratz, K. L. and Haberer, K. (1998). "Determination of Antibiotics in Different Water Compartments Via Lc-Electrospray-/Ms/Ms." Journal of Chromatography **815**: 213-223.

Holbrook, R. D., Love, N. G. and Novak, J. T. (2004). "Sorption of 17b-Estradiol and 17a-Ethinylestradiol by Colloidal Organic Carbon Derived from Biological Wastewater Treatment Systems." Environmental Science and Technology **38**: 3322-3329.

Huber, M. M., Gobel, A., Joss, A., Hermann, N., Loffler, D., McArdell, C. S., Ried, A., Siegrist, H., Ternes, T. A. and von Gunten, U. (2005). "Oxidation of Pharmaceuticals During Ozonation of Municipal Wastewater Effluents: A Pilot Study." Environmental Science & Technology **39**(11): 4290-4299.

Huber, M. M., S., C., Park, G.-Y. and Von Gunten, U. (2005). "Oxidation of Pharmaceuticals During Ozonation and Advanced Oxidation Processes." Environmental Science and Technology.

Huber, S. (1999). "Einfluss Der Prozessfuhrung Auf Menge Und Zusammensetzung Von Proteinen Und Polysacchariden Im Ablauf Von Sequencing-Batch-Reaktoren." Berichte aus der Wasserggute und Abfallwirtschaft, Technische Universitat Munchen **152**.

Hundt, K., Martin, D., Hammer, E., Jonas, U., Kinermann, M. and Schauer, F. (2000). "Transformation of Triclosan by *Trametes Versicolor* and *Pycnoporus Cinnabarinus*." Applied and Environmental Microbiology: 4157-4160.

Huschek, G., Hansen, P. D., Maurer, H. H., Krengel, D. and Kayser, A. (2004). "Environmental Risk Assessment of Medicinal Products for Human Use According to European Commission Recommendations." Environmental Toxicology **19**(3): 226-240.

IMS Health (2002). "Chemical Profile for Germany 2000-2001."

Isidori, M., Lavorgna, M., Nardelli, A., Parrella, A., Previtera, L. and Rubino, M. (2005). "Ecotoxicity of Naproxen and Its Phototransformation Products." Science of the Total Environment **348**(1-3): 93-101.

James, H. A., Fielding, M., Franklin, O., Williams, D. and Lunt, D. (1997). Steroid Concentrations in Trteated Sewage Effluents and Water Courses-Implication for Water Supplies.

Jjemba, P. K. (2006). "Excretion and Ecotoxicity of Pharmaceutical and Personal Care Products in the Environment." Ecotoxicology and Environmental Safety **63**(1): 113-130.

Johnson, A. C., Aerni, H.-R., Gerritsen, A., Gibert, M., Giger, W., Hylland, K., Jurgens, M., Nakari, T., Pickering, A., Suter, M. J.-F., Svenson, A. and Wettsein, F. E. (2005). "Comparing Steroid Estrogen, and Nonylphenol Content across a Range of European Sewage Plants with Different Treatment and Management Practices." Water Research **39**: 47-58.

Johnson, A. C. and Sumpter, J. P. (2001). "Removal of Endocrine Disrupting Chemicals in Activated Sludge Treatment Works." Environmental Science and Technology **35**(24): 4697-4703.

Jones, O. A., Lester, J. N. and Voulvoulis, N. (2005). "Pharmaceuticals: A Threat to Drinking Water?" Trends in Biotechnology **23**(4): 163-167.

Jones, O. A. H., Voulvoulis, N. and Lester, J. N. (2001). "Human Pharmaceuticals in the Aquatic Environment: A Review." Environmental Technology **22**: 1383-1394.

Jones, O. A. H., Voulvoulis, N. and Lester, J. N. (2002). "Aquatic Environmental Assessment of the Top 25 English Prescription Pharmaceuticals." Water Research **36**(20): 5013-5022.

Jones, O. A. H., Voulvoulis, N. and Lester, J. N. (2005). "Human Pharmaceuticals in Wastewater Treatment Processes." Critical Reviews in Environmental Science and Technology **35**(4): 401-427.

Jones, R. M., Sen, D. and Lambert, R. (1998). "Full Scale Evaluation of Nitrification Performance in an Integrated Fixed Film Activated Sludge Process." Water Science and Technology **38**(1): 71-78.

Jorand, F., Boue-Bigne, F., Block, J. C. and Urbain, V. (1998). "Hydrophobic/Hydrophilic Properties of Activated Sludge Exopolymeric Substances." Water Science and Technology **37**(4-5): 307-315.

Joss, A., Andersen, H., Ternes, T., Richle, P. R. and Siegrist, H. (2004). "Removal of Estrogens in Municipal Wastewater Treatment under Aerobic and Anaerobic Conditions: Consequences for Plant Optimization." Environmental Science & Technology **38**(11): 3047-3055.

Joss, A., Keller, E., Alder, A. C., Gobel, A., McArdell, C. S., Ternes, T. and Siegrist, H. (2005). "Removal of Pharmaceuticals and Fragrances in Biological Wastewater Treatment." Water Research **39**(14): 3139-3152.

Joss, A., Zabczynski, S., Gobel, A., Hoffmann, B., Loffler, D., McArdell, C. S., Ternes, T. A., Thomsen, A. and Siegrist, H. (2006). "Biological Degradation of Pharmaceuticals in Municipal Wastewater Treatment: Proposing a Classification Scheme." Water Research **40**(8): 1686-1696.

Kanda, R., Griffin, P., James, H. and Fothergill, J. (2003). "Pharmaceuticals and Personal Care Products in Sewage Treatment Works." Journal of Environmental Monitoring **5**: 823-830.

Kayombo, S., Mbwette, T. S. A., Katima, J. H. Y. and Jorgensen, S. E. (2003). "Effects of Substrate Concentrations on the Growth of Heterotrophic Bacteria and Algae in Secondary Facultative Ponds." Water Research **37**(12): 2937-2943.

Khan, S. J. and Ongerth, J. E. (2002). "Estimation of Pharmaceutical Residues in Primary and Secondary Sewage Sludge Based on Quantities of Use and Fugacity Modelling." Water Science and Technology **46**(3): 105-113.

Kim, S., Eichhorn, P., Jensen, J. N., Weber, A. S. and Aga, D. S. (2005). "Removal of Antibiotics in Wastewater: Effect of Hydraulic and Solid Retention Times on the Fate of Tetracycline in the Activated Sludge Process." Environmental Science & Technology **39**(15): 5816-5823.

Kiwa, N. V. (2004). Pharmaceuticals and Personal Care Products in the Water. Report 04/TX/03/06, UKWIR.

Kolpin, D. W., Furlong, E. T., Meyer, M. T., Thurman, E. M., Zaugg, S. D., Barber, L. B. and Buxton, H. T. (2002). "Pharmaceuticals, Hormones, and Other Organic Wastewater Contaminants in U.S. Streams, 1999-2000: A National Reconnaissance." Environmental Science and Technology **36**(1202-1211).

Kolpin, D. W., Skopec, M., Meyer, M. T., Furlong, E. T. and Zaugg, S. D. (2004). "Urban Contribution of Pharmaceuticals and Other Organic Wastewater Contaminants to Streams During Differing Flow Conditions." Science of the Total Environment **328**(1-3): 119-130.

Kosjek, T., Heath, E. and Krbavcic, A. (2005). "Determination of Non-Steroidal Anti-Inflammatory Drug (Nsaids) Residues in Water Samples." Environment International **31**(5): 679-685.

Koutsouba, V., Heberer, T., Fuhrmann, B., Schmidt-Baumler, K., Tsipi, D. and Hiskia, A. (2003). "Determination of Polar Pharmaceuticals in Sewage Water of Greece by Gas Chromatography-Mass Spectrometry." Chemosphere **51**(2): 69-75.

Kreuzinger, N., Clara, M., Strenn, B. and Kroiss, 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." Water Science and Technology **50**(5): 149-156.

Kuhne, M., Ihnen, D., Moller, G. and Agthe, O. (2000). "Stability of Tetracycline in Water and Liquid Manure." Journal of Veterinary Medicine Series a-Physiology Pathology Clinical Medicine **47**(6): 379-384.

Kummerer, K. (2001). Pharmaceuticals in the Environment: Sources, Fate, Effects and Risks, Springer.

Kummerer, K., StegerHartmann, T. and Meyer, M. (1997). "Biodegradability of the Anti-Tumour Agent Ifosfamide and Its Occurrence in Hospital Effluents and Communal Sewage." Water Research **31**(11): 2705-2710.

Kuspis, D. A. and Krenzelok, E. P. (1996). "What Happens to Expired Medications? A Survey of Community Medication Disposal." Veterinary and Human Toxicology **38**(1): 48-49.

Lawler, D. M., Petts, G. E., Foster, I. D. L. and Harper, S. (2006). "Turbidity Dynamics During Spring Storm Events in an Urban Headwater River System: The Upper Tame, West Midlands, Uk." Science of the Total Environment **360**(1-3): 109-126.

Lee, H. B., Peart, T. E. and Svoboda, M. L. (2005). "Determination of Endocrine-Disrupting Phenols, Acidic Pharmaceuticals, and Personal-Care Products in Sewage by Solid-Phase Extraction and Gas Chromatography-Mass Spectrometry." Journal of Chromatography A **1094**(1-2): 122-129.

Levert, H., Odou, P. and Robert, H. (2002). "Simultaneous Determination of Four Antiepileptic Drugs in Serum by High-Performance Liquid Chromatography." Biomedical Chromatography **16**: 19-24.

Liang, Y., Denton, B. and Bates, R. (1998). "Stability Studies of Tetracycline in Methanol Solution." Journal of Chromatography A **827**: 45-55.

Liebig, M., Moltmann, J. F. and Knacker, T. (2006). "Evaluation of Measured and Predicted Environmental Concentrations of Selected Human Pharmaceuticals and Personal Care Products." Environmental Science and Pollution Research **13**(2): 110-119.

Lin, W. C., Chen, H. C. and Ding, W. H. (2005). "Determination of Pharmaceutical Residues in Waters by Solid-Phase Extraction and Large-Volume on-Line Derivatization with Gas Chromatography-Mass Spectrometry." Journal of Chromatography A **1065**(2): 279-285.

Lindberg, R. H., Olofsson, U., Rendahl, P., Johansson, M. I., Tysklind, M. and Andersson, B. A. V. (2006). "Behavior of Fluoroquinolones and Trimethoprim During Mechanical, Chemical, and Active Sludge Treatment of Sewage Water and Digestion of Sludge." Environmental Science & Technology **40**(3): 1042-1048.

Lindqvist, N., Tuhkanen, T. and Kronberg, L. (2005). "Occurrence of Acidic Pharmaceuticals in Raw and Treated Sewages and in Receiving Waters." Water Research **39**(11): 2219-2228.

Lindstrom, A., Buerge, I. J., Poiger, T., Bergqvist, P. A., Muller, M. D. and Buser, H. R. (2002). "Occurrence and Environmental Behavior of the Bactericide Triclosan and Its Methyl Derivative in Surface Waters and in Wastewater." Environmental Science & Technology **36**(11): 2322-2329.

Liu, Y., Lam, M. C. and Fang, H. H. P. (2001). "Adsorption of Heavy Metals by Eps of Activated Sludge." Water Science and Technology **43**(6): 59-66.

Loos, R., Hanke, G. and Eisenreich, S. J. (2003). "Multi-Component Analysis of Polar Water Pollutants Using Sequential Solid-Phase Extraction Followed by Lc-Esi-Ms." Journal of Environmental Monitoring **5**(3): 384-394.

Loraine, G. A. and Pettigrove, M. E. (2006). "Seasonal Variations in Concentrations of Pharmaceuticals and Personal Care Products in Drinking Water and Reclaimed Wastewater in Southern California." Environmental Science & Technology **40**(3): 687-695.

Macarthur, D. (2000). "Any Old Drugs? Two Schemes for the Disposal of Unwanted Medicines in Europe." The Pharmaceutical Journal **264**(7082): 223-4.

McArdell, C. S., Molnar, E., Suter, M. J. F. and Giger, W. (2003). "Occurrence and Fate of Macrolide Antibiotics in Wastewater Treatment Plants and in the Glatt Valley Watershed, Switzerland." Environmental Science & Technology **37**(24): 5479-5486.

McAvoy, D. C., Schatowitz, B., Jacob, M., Hauk, A. and Eckhoff, W. S. (2002). "Measurement of Triclosan in Wastewater Treatment Systems." Environmental Toxicology and Chemistry **21**(7): 1323-1329.

McLachlan, J. A., Simpson, E. and Martin, M. (2006). "Endocrine Disrupters and Female Reproductive Health." Best Practice & Research Clinical Endocrinology & Metabolism **20**(1): 63-75.

Mesquita, M. E., Carranca, C. and Menino, M. R. (2002). "Influence of Ph on Copper - Zinc Competitive Adsorption by a Sandy Soil." Environmental Technology **23**(9): 1043-1050.

Metcalf and Eddy (2003). Wastewater Engineering: Treatment and Reuse. New York, McGraw Hill.

Miao, X. S., Bishay, F., Chen, M. and Metcalfe, C. D. (2004). "Occurrence of Antimicrobials in the Final Effluents of Wastewater Treatment Plants in Canada." Environmental Science & Technology **38**(13): 3533-3541.

Miao, X. S., Yang, J. J. and Metcalfe, C. D. (2005). "Carbamazepine and Its Metabolites in Wastewater and in Biosolids in a Municipal Wastewater Treatment Plant." Environmental Science & Technology **39**(19): 7469-7475.

Mohle, E., Kempter, C., Kern, A. and Metzger, J. W. (1999). "Examination of the Degradation of Drugs in Municipal Sewage Plants Using Liquid Chromatography-Electrospray Mass Spectrometry." Acta Hydrochimica et Hydrobiologica **27**(6): 430-436.

Mota, R., Monteiro Santos, F. A., Mateus, A., Marques, F. O., Goncalves, M. A., Figueiras, J. and Amaral, H. (2004). "Granite Fracturing and Incipient Pollution beneath a Recent Landfill Facility as Detected by Geoelectrical Surveys." Journal of applied geophysics **57**(1): 11-22.

Nakada, N., Tanishima, T., Shinohara, H., Kiri, K. and Takada, H. (2006). "Pharmaceutical Chemicals and Endocrine Disrupters in Municipal Wastewater in Tokyo and Their Removal During Activated Sludge Treatment." Water Research **40**(17): 3297-3303.

Nakamura, K. (2006). "Calculation and Problems of Predicted Evironmental Concentration (Pec) for Aquatic Ecological Risk Assessment." Journal of Pesticide Science **31**(1): 70-72.

NationalOsteoporosisSociety (2006). Osteoporosis Facts and Figures V1.1.

NHS (2005). Nhs Business Services Authority Prescription Pricing Division Prescribing Review - Antibacterial Drugs 1994 - 2004.

NHS (2005). Nhs Business Services Authority Prescription Pricing Division Prescribing Review - Drugs for Dyspepsia 1999-2004.

NHS (2005). Nhs Business Services Authority Prescription Pricing Division Prescribing Review - Mental Health 2000-2005.

NHS (2005). Nhs Business Services Authority Prescription Pricing Division Prescribing Review - Nsaids and Analgesics 1999-2004.

NHS (2005). Nhs Business Services Authority Prescription Pricing Division Prescribing Review - Primary Prevention of Cardiovascular Disease 1999-2004.

NHS (2006). Dictionary of Medicines and Devices.

NHS (2006). Nhs Business Services Authority Presciption Pricing Division Precribing Review - Drugs Used for Osteoporosis 2000-2005.

NHS (2006). Nhs Business Services Authority Presciption Pricing Division Precribing Review - Drugs Used in Asthma and Copd 2000-2005.

NHS (2006). Nhs Business Services Authority Presciption Pricing Division Precribing Review - Drugs Used in Diabetes 2000-2005.

NHS (2006). Nhs Business Services Authority Prescription Pricing Division Prescribing Review - Drugs Used for Osteoporosis 2000-2005.

NHS (2006). Nhs Business Services Authority Prescription Pricing Division Prescribing Review - Drugs Used in Asthma and Copd 2000-2005.

NHS (2006). Nhs Business Services Authority Prescription Pricing Division Prescribing Review - Drugs Used in Diabetes 2000-2005.

Nygaard, K., Lunestad, B. T., Hektonen, H., Berge, J. A. and Hormazabal, V. (1992). "Resistance to Oxytetracycline, Oxolinic Acid and Furazolidone in Bacteria from Marine Sediments." Aquaculture **104**(31-36).

Oaks, L., Gilbert, M., Virani, M. Z., Watson, R. T., Meteyer, C. U., Rideout, B. A., Shivaprasad, H. L., Ahmed, S., Chaudhry, M. J. I., Arshad, M., Mahmood, S., Ali, A. and Khan, A. A. (2004). "Diclofenac Residues as the Cause of Vulture Population Decline in Pakistan." Nature **427**: 630-633.

OECD (1992). "Oecd Guideline for Testing of Chemicals 301. Ready Biodegradability."

OECD (2000). "Oecd Guidline for the Testing of Chemicals 106. Adsorption - Desorption Using a Batch Equilibrium Method."

OECD (2001). Caffeine. SIDS Initial assessment report for SIAM 13. Bern, Switzerland.

OECD (2001). "Oecd Guideline for the Testing of Chemicals 303. Simulation Test - Aerobic Sewage Treatment."

OECD (2001). Theophylline. SIDS Initial assessment report for SIAM 13. Bern, Switzerland.

Oka, H., Ito, Y. and Matsumoto, H. (2000). "Chromatographic Analysis of Tetracycline Antibiotics in Foods." Journal of Chromatography A **882**(1-2): 109-133.

Oka, H., Uno, K., Harada, K., Yasaka, K. and Suzuki, M. (1984). "A Simple Method for the Analysis of Tetracyclines Using Reversed-Phase High-Performance Liquid Chromatography." Journal of Chromatography **298**: 435-443.

Orvos, D. R., Versteeg, D. J., Inauen, J., Capdevielle, M., Rothenstein, A. and Cunningham, V. (2002). "Aquatic Toxicity of Triclosan." Environmental Toxicology and Chemistry **21**(7): 1338-1349.

Paxeus, N. (2004). "Removal of Selected Non-Steroidal Anti-Inflammatory Drugs (Nsaids), Gemfibrozil, Carbamazepine, Beta-Blockers, Trimethoprim and Triclosan in

Conventional Wastewater Treatment Plants in Five Eu Countries and Their Discharge to the Aquatic Environment." Water Science and Technology **50**(5): 253-260.

Perez, S., Eichhorn, P. and Aga, D. S. (2005). "Evaluating the Biodegradability of Sulfamethazine, Sulfamethoxazole, Sulfathiazole, and Trimethoprim at Different Stages of Sewage Treatment." Environmental Toxicology and Chemistry **24**(6): 1361-1367.

Petrasek, A. C., Kugelman, I. J., Austern, B. M., Pressley, T. A., Winslow, L. A. and Wise, R. H. (1983). "Fate of Toxic Organic Compounds in Wastewater Treatment Plants." Journal of the Water Pollution Control Federation **55**: 1286-1296.

Phenomenex (2005/06). Chromatography Columns and Supplies Catalogue.

Quintana, J. B., Carpinteiro, J., Rodriguez, I., Lorenzo, R. A., Carro, A. M. and Cela, R. (2004). "Determination of Natural and Synthetic Estrogens in Water by Gas Chromatography with Mass Spectrometric Detection." Journal of Chromatography A **1024**: 177-185.

Quintana, J. B., Weiss, S. and Reemtsma, T. (2005). "Pathway's and Metabolites of Microbial Degradation of Selected Acidic Pharmaceutical and Their Occurrence in Municipal Wastewater Treated by a Membrane Bioreactor." Water Research **39**(12): 2654-2664.

Rabolle, M. and Spliid, N. H. (2000). "Sorption and Mobility of Metronidazole, Olaquindox, Oxytetracycline, and Tylosin in Soil." Chemosphere **40**: 715-722.

Randall, C. W. and Sen, D. (1996). "Full-Scale Evaluation of an Integrated Fixed-Film Activated Sludge (Ifas) Process for Enhanced Nitrogen Removal." Water Science and Technology **33**(12): 155-162.

Rathner, M. and Sonneborn, M. (1979). "Biologically Active Oestrogens in Potable Water and Effluents." Forum Stadte-Hygiene **30**: 45-49.

Reddersen, K., A and B (2002). "Identification and Significance of Phenazone Drugs and Their Metabolites in Ground and Drinking Water." Chemosphere **49**: 539-544.

Reddersen, K. and Heberer, T. (2003). "Multi-Compound Methods for the Detection of Pharmaceutical Residues in Various Waters Applying Solid Phase Extraction (Spe) and Gas Chromatography with Mass Spectrometric (Gc-Ms) Detection." Journal of Separation Science **26**(15-16): 1443-1450.

Reith, D. M. and Cannell, G. R. (1999). "An Hplc Assay for Carbamazepine Phase I Metabolites and Their Glucuronides in Urine." Journal of Liquid Chromatography & Related Technologies **22**(12): 1907-1918.

Richards, S. M., Wilson, C. J., Johnson, D. J., Castle, D. M., Lam, M., Mabury, S. A., Sibley, P. K. and Solomon, K. R. (2004). "Effects of Pharmaceutical Mixtures in Aquatic Microcosms." Environmental Toxicology and Chemistry **23**(4): 1035-1042.

Richardson, M. L. and Bowron, J. M. (1985). "The Fate of Pharmaceutical Chemicals in the Environment." Journal of Pharmacy and Pharmacology **37**: 1-12.

Rieckermann, J., Borsuk, M., Reichert, P. and Gujer, W. (2005). "A Novel Tracer Method for Estimating Sewer Exfiltration." Water Resources Research **41**(5).

Roberts, P. H. and Thomas, K. V. (2006). "The Occurrence of Selected Pharmaceuticals in Wastewater Effluent and Surface Waters of the Lower Tyne Catchment." Science of the Total Environment **356**(1-3): 143-153.

Rodriguez, I., Quintana, J. B., Carpinteiro, J., Carro, A. M., Lorenzo, R. A. and Cela, R. (2003). "Determination of Acidic Drugs in Sewage Water by Gas Chromatography-Mass Spectrometry as Tert.-Butyldimethylsilyl Derivatives." Journal of Chromatography A **985**(1-2): 265-274.

Rogalla, F., Johnson, T. L. and McQuarrie, J. (2006). "Fixed Film Phosphorus Removal - Flexible Enough?" Water Science and Technology **53**(12): 75-81.

Rogers, H. R. (1996). "Sources, Behaviour and Fate of Organic Contaminants During Sewage Treatment and in Sewage Sludges." Science of the Total Environment **185**: 3-26.

RxList (2006). "The Internet Drug Index." Available via http://www.rxlist.com.

Sabaliunas, D., Webb, S. F., Hauk, A., Jacob, M. and Eckhoff, W. S. (2003). "Environmental Fate of Triclosan in the River Aire Basin, Uk." Water Research **37**: 3145-3154.

Sacher, F., Lange, F. L., H.-J., B. and I., B. (2001). "Pharmaceuticals in Groundwaters. Analytical Methods and Results of a Monitoring Program in Baden-Wurttemberg, Germany." Journal of Chromatography A **938**: 199-210.

Salvito, D. T., Senna, R. J. and Federle, T. W. (2002). "A Framework for Prioritizing Fragrance Materials for Aquatic Risk Assessment." Environmental Toxicology and Chemistry **21**(6): 1301-1308.

Sandaa, R.-A., Torsvik, V. L. and Goksoyr, J. (1992). "Transferable Drug Resistance in Bacteria from Fish-Farm Sediment." Canadian Journal of Microbiology **38**: 1061-1065.

Sanderson, H., Johnson, D. J., Wilson, C. J., Brain, R. A. and Solomon, K. R. (2003). "Probabilistic Hazard Assessment of Environmentally Occurring Pharmaceuticals Toxicity to Fish, Daphnids and Algae by Ecosar Screening." Toxicology Letters **144**(3): 383-395.

Santos, J. L., Aparicio, I., Alonso, E. and Callejon, M. (2005). "Simultaneous Determination of Pharmaceutically Active Compounds in Wastewater Samples by Solid Phase Extraction and High-Performance Liquid Chromatography with Diode Array and Fluorescence Detectors." Analytica Chimica Acta **550**(1-2): 116-122.

Schmidt, B. and Schuphan, I. (2002). "Metabolism of the Environmental Estrogen Bisphenol a by Plant Cell Suspension Cultures." Chemosphere **49**(1): 51-59.

Schneider, C., Kuch, B., Braun, M. and J.W., M. (2004). Pharmaceuticals in Lanfill Leachates and Receiving Wwtp Influents. SETAC.

Schwab, B. W., Hayes, E. P., Fiori, J. M., Mastrocco, F. J., Roden, N. M., Cragin, D., Meyerhoff, R. D., D'Aco, V. J. and Anderson, P. D. (2005). "Human Pharmaceuticals in Us Surface Waters: A Human Health Risk Assessment." Regulatory Toxicology and Pharmacology **42**(3): 296-312.

Schwabe, U. and Paffrath, D., Eds. (2000). Arzneiverordnungsreport 2000. Berlin, Springer Verlag.

Schwabe, U. and Paffrath, D., Eds. (2001). Arzneiverordnungsreport 2001. Berlin, Springer Verlag.

Schwarzenbach, R. P., Gschwend, P. M. and Imboden, D. M. (2003). Environmental Organic Chemistry. Hoboken, NJ, Wiley-Interscience.

Scott, R. P. W. (2002). Liquid Chromatography Detectors, library4science.com.

Sebastine, I. M. and Wakeman, R. J. (2003). "Consumption and Environmental Hazards of Pharmaceuticals Substances in the Uk." Process Safety and Environmental Protection **81**(B4): 229-235.

Seitz, W., Jiang, J. Q., Weber, W. H., Lloyd, B. J., Maier, M. and Maier, D. (2006). "Removal of Iodinated X-Ray Contrast Media During Drinking Water Treatment." Environmental Chemistry **3**(1): 35-39.

Shi, J., Fujisawa, S., Nakai, S. and Hosomi, M. (2004). "Biodegradation of Natural and Synthetic Estrogens by Nitrifying Activated Sludge and Ammonia-Oxidizing Bacterium Nitrosomonas Europaea." Water Research **38**(9): 2323-2330.

Siegrist, H., Joss, A., Alder, A. C., Golet, E. M., Gobel, A., Keller, E., McArdell, C. S., Kreuzinger, N., Clara, M., Ternes, T. A., Hermann, N., K., M., Zabaczynski, S. and Surmacz, J. (2003). "Fate of Pharmaceuticals, Personal Care Products and Hormones in Municipal Wastewater Treatment - a New Challenge for Wastewater Management?" POSEIDON Symposium Presentation of Project Results.

Singer, H., Muller, S., Tixier, C. and Pillonel, L. (2002). "Triclosan: Occurence and Fate of a Widely Used Biocide in the Aquatic Environment: Field Measurements in Wastewater Treatment Plants, Surface Waters and Lake Sediments." Environmental Science and Technology **36**: 4998-5004.

Sithole, B. B. and Guy, R. D. (1987). "Models for Tetracycline in Aquatic Environments .2. Interaction with Humic Substances." Water Air and Soil Pollution **32**(3-4): 315-321.

Slack, R. J., Gronow, J. R. and Voulvoulis, N. (2005). "Household Hazardous Waste in Municipal Landfills: Contaminants in Leachate." Science of the Total Environment **337**(1-3): 119-137.

Slack, R. J., Zerva, P., Gronow, J. R. and Voulvoulis, N. (2005). "Assessing Quantities and Disposal Routes for Household Hazardous Products in the United Kingdom." Environmental Science & Technology **39**(6): 1912-1919.

Snyder, S. A., Westerhoff, P., Yoon, Y. and Sedlak, D. L. (2003). "Pharmaceuticals, Personal Care Products, and Endocrine Disruptors in Water: Implications for the Water Industry." Environmental Engineering Science **20**(5): 449-469.

Soulet, B., Tauxe, A. and Tarradellas, J. (2002). "Analysis of Acidic Drugs in Swiss Wastewaters." International Journal of Environmental Analytical Chemistry **82**(10): 659-667.

Spath, R., Flemming, H. C. and Wuertz, S. (1998). "Sorption Properties of Biofilms." Water Science and Technology **37**(4-5): 207-210.

Sriwiriyarat, T. and Randall, C. W. (2005). "Evaluation of Integrated Fixed Film Activated Sludge Wastewater Treatment Processes at High Mean Cells Residence Time and Low Temperatures." Journal of Environmental Engineering-Asce **131**(11): 1550-1556.

Sriwiriyarat, T. and Randall, C. W. (2005). "Performance of Ifas Wastewater Treatment Processes for Biological Phosphorus Removal." Water Research **39**(16): 3873-3884.

Stackelberg, P. E., A and B (2004). "Persistence of Pharmaceutical Compounds and Other Organic Wastewater Contaminents in a Conventional Drinking-Water-Treatment Plant." Science of the Total Environment **329**: 99-113.

Stan, H. J. and Heberer, T. (1997). Pharmaceuticals in the Aquatic Environment. Dossier Water Analysis. Suter, M. J.-F.**:** M20-M23.

Stan, H. J., Heberer, T. and Linkerhagner, M. (1994). "Occurence of Clofibric Acid in the Aquatic System - Does the Medical Application Cause Contamination of Suirface, Ground and Drinkingwater?" Vom Wasser **83**: 57-68.

Stan, H. J., Heberer, T. and Schmidt-Baumler, K. (1999). "Occurrence and Distribution of Organic Contaminants in the Aquatic System in Berlin. Part Ii: Substituted Phenols in Berlin Surface Water." Acta Hydrochimica et Hydrobiologica **27**(3).

Stelzer, W., Ziegert, E. and Schneider, E. (1985). "The Occurence of Antibiotic-Resistant Klebsiellae in Wastewater." Zentralbl Mikrobiol **140**: 283-291.

Stokes, L. and Churchley, J. (2006). Edc Levels at Ilkeston Stw and How to Zap Them. Endocrine disrupting chemicals in the water environment, Nottingham, UK.

Strenn, B., Clara, M., Gans, O. and Kreuzinger, N. (2004). "Carbamazepine, Diclofenac, Ibuprofen and Bezafibrate - Investigations on the Behaviour of Selected Pharmaceuticals During Wastewater Treatment." Water Science and Technology **50**(5): 269-276.

Stuer-Lauridsen, F., Birkved, M., Hansen, L. P., Lutzhoft, H. C. H. and Halling-Sorensen, B. (2000). "Environmental Risk Assessment of Human Pharmaceuticals in Denmark after Normal Therapeutic Use." Chemosphere **40**(7): 783-793.

Stumpf, M., Ternes, T. A., Haberer, K., Seel, P. and Baumann, W. (1996). "Determination of Drugs in Sewage Treatment Plants and River Water." Vom Wasser **86**: 291-303.

Stumpf, M., Ternes, T. A., Wilken, R.-D., Rodrigues, S. V. and Baumann, W. (1999). "Polar Drug Residues in Sewage and Natural Waters in the State of Rio De Janeiro, Brazil." Science of the Total Environment **225**: 135-141.

Sumpter, J. P. and Johnson, A. C. (2005). "Lessons from Endocrine Disruption and Their Application to Other Issues Concerning Trace Organics in the Aquatic Environment." Environmental Science and Technology **39**(12): 4321-4332.

Swan, G., Naidoo, V., Cuthbert, R., Green, R. E., Pain, D. J., Swarup, D., Prakash, V., Taggart, M., Bekker, L., Das, D., Diekmann, J., Diekmann, M., Killian, E., Meharg, A., Patra, R. C., Saini, M. and Wolter, K. (2006). "Removing the Threat of Diclofenac to Critically Endangered Asian Vultures." Plos Biology **4**(3): 395-402.

Swan, S. H., Main, K. M., Liu, F., Stewart, S. L., Kruse, R. L., Calafat, A. M., Mao, C. S., Redmon, J. B., Ternand, C. L., Sullivan, S. and Teague, J. L. (2005). "Decrease in Anogenital Distance among Male Infants with Prenatal Phthalate Exposure." Environmental Health Perspectives **113**(8): 1056-1061.

Tauber, R. (2003). "Quantitative Analysis of Pharmaceuticals in Drinking Water from Ten Canadian Cities." Enviro-Test Laboratories, Xenos Division, Ontario, Canada.

ter Laak, T. L., Gebbink, W. A. and Tolls, J. (2006). "The Effect of Ph and Ionic Strength on the Sorption of Sulfachloropyridazine, Tylosin, and Oxytetracycline to Soil." Environmental Toxicology and Chemistry **25**(4): 904-911.

Ternes, T. (1998). "Occurence of Drugs in German Sewage Treatment Plants and Rivers." Water Research **32**: 3245-3260.

Ternes, T. (1999). "Drugs and Hormones as Pollutants of the Aquatic Environment: Determination and Ecotoxicological Impacts." Science of the Total Environment **225**(1-2): 1-2.

Ternes, T. (2000). Pharmaceuticals in Rivers, Groundwater and Drinking Water. International Seminar on Pharmaceuticals in the Environment, Brussels.

Ternes, T. (2001). Pharmaceuticals and Metabolites as Contaminants of the Aquatic Environment. Pharmaceuticals and Personal Care Products in the Environment - Scientific and Regulatory Issues. Daughton, C. G. and Jones-Lepp, T. Washington DC, USA, American Chemical Society. **ACS Symposium Series 791:** 39-54.

Ternes, T., Bonerz, M., Herrmann, N., Loffler, D., Keller, E., Lacida, B. B. and Alder, A. C. (2005). "Determination of Pharmaceuticals, Iodinated Contrast Media and Musk Fragrances in Sludge by Lc/Tandem Ms and Gc/Ms." Journal of Chromatography A **1067**(1-2): 213-223.

Ternes, T., Herrmann, N., Bonerz, M., Knacker, T., Siegrist, H. and Joss, A. (2004). "A Rapid Method to Measure the Solid-Water Distribution Coefficient (K-D) for Pharmaceuticals and Musk Fragrances in Sewage Sludge." Water Research **38**(19): 4075-4084.

Ternes, T. and Hirsch, R. (2000). "Occurrence and Behaviour of X-Ray Contrast Media in Sewage Facilities and the Aquatic Environment." Environmental Science and Technology **34**(13): 2741-2748.

Ternes, T., Janex-Habibi, M.-L., Knacker, T., Kreuzinger, N. and Siegrist, H. (2004). Assessment of Technologies for the Removal of Pharmaceuticals and Personal Care Products in Sewage and Drinking Water Facilities to Improve the Indirect Potable Water Reuse. POSEIDON report. Ternes, T.

Ternes, T., Meisenheimer, M., McDowell, D., Sacher, F., Brauch, H.-J., Haist-Gulde, B., Preuss, G., Wilme, U. and Zulei-Seibert, N. (2002). "Removal of Pharmaceuticals During Drinking Water Treatment." Environmental Science and Technology **36**: 3855-3863.

Ternes, T., Stumpf, M., Mueller, J., Haberer, K., Wilken, R.-D. and Servos, M. (1999). "Behaviour and Occurence of Estrogens in Municipal Sewage Treatment Plants - I. Investigations in Germany, Canada and Brazil." Science of the Total Environment **225**: 81-90.

Thomas, P. M. and Foster, G. D. (2005). "Tracking Acidic Pharmaceuticals, Caffeine, and Triclosan through the Wastewater Treatment Process." Environmental Toxicology and Chemistry **24**(1): 25-30.

Thompson, A. S., Griffin, P., Stuetz, R. and Cartmell, E. (2005). "The Fate and Removal of Triclosan During Sewage Treatment." Water Environment Research **77**(1).

Tixier, C., Singer, H., Canonica, S. and Muller, S. R. (2002). "Phototransformation of Triclosan in Surface Waters: A Relevant Elimination Process for This Widely Used Biocide - Laboratory Studies, Field Measurements, and Modeling." Environmental Science and Technology **36**(3482-3489).

Tolls, J. (2001). "Sorption of Veterinary Pharmaceuticals in Soils: A Review." Environmental Science & Technology **35**(17): 3397-3406.

Urase, T., Kagawa, C. and Kikuta, T. (2005). "Factors Affecting Removal of Pharmaceutical Substances and Estrogens in Membrane Separation Bioreactors." Desalination **178**(1-3): 107-113.

Urase, T. and Kikuta, T. (2005). "Seperate Estimation of Adsorption and Degradation of Pharmaceutical Substances and Estrogens in the Activated Sludge Process." Water Research **39**: 1289-1300.

Urbain, V., Block, J. C. and Manem, J. (1993). "Bioflocculation in Activated-Sludge - an Analytic Approach." Water Research **27**(5): 829-838.

USEPA (1998). National Water Quality Inventory: 1998 Report to Congress. EPA 305(b) report. USEPA. Washington DC, USEPA.

van der Ven, K., Van Dongen, W., Maes, B. U. W., Esmans, E. L., Blust, R. and De Coen, W. M. (2004). "Determination of Diazepam in Aquatic Samples by Capillary Liquid Chromatography-Electrospray Tandem Mass Spectrometry." Chemosphere **57**(8): 967-973.

Verenitch, S. S., Lowe, C. J. and Mazumder, A. (2006). "Determination of Acidic Drugs and Caffeine in Municipal Wastewaters and Receiving Waters by Gas Chromatography-Ion Trap Tandem Mass Spectrometry." Journal of Chromatography A **1116**(1-2): 193-203.

Verstraeten, I. M., Heberer, T. and Schyett, T. (2001). Occurrence, Characteristics, and Transport and Fate of Pesticides, Pharmaceutical Active Compounds, and Industrial and Personal Care Products at Bank-Filtration Sites. Bank Filtration for Water Supply. Ray, C. Dordrecht, Kluwer Academic Publishers.

Vidic, R. D. and Suidan, M. T. (1991). "Role of Dissolved Oxygen on the Adsorption Capacity of Activated Carbon for Synthetic and Natural Organic Matter." Environmental Science and Technology **25**: 1612-1618.

Vieno, N., Tuhkanen, T. and Kronberg, L. (2006). "Removal of Pharmaceuticals in Drinking Water Treatment: Effect of Chemical Coagulation." Environmental Technology **27**(2): 183-192.

Vieno, N. M., Tuhkanen, T. and Kronberg, L. (2005). "Seasonal Variation in the Occurrence of Pharmaceuticals in Effluents from a Sewage Treatment Plant and in the Recipient Water." Environmental Science & Technology **39**(21): 8220-8226.

Voets, J. B., Pipyn, P., Van Lanker, P. and Verstraete, W. (1976). "Degradation of Microbicides under Different Environmental Conditions." Journal of Applied Bacteriology **40**: 67-42.

Waggott, A. (1981). Trace Organic Substances in the River Lee (Great Britain). Chemistry in Water Reuse. Cooper, W. J. Ann Arbor, MI, Ann Arbor Science. **2:** 55-59.

Waltman, E. L., Venables, B. J. and Waller, W. Z. (2006). "Triclosan in a North Texas Wastewater Treatment Plant and the Influent and Effluent of an Experimental Constructed Wetland." Environmental Toxicology and Chemistry **25**(2): 367-372.

Water, S. T. (2002). Monitoring Pharmaceuticals and Personal Health Care Products in Sewage Treatment Works.

Webb, S. F. (2000). Risk Assessment for Pharmaceuticals. Proceedings of International Seminar on Pharmaceuticals in the Environment, Brussels Technological Institute.

Webb, S. F. (2001). A Data Based Perspective on the Environmental Risk Assessment of Human Pharmaceuticals Iii - Indirect Human Exposure. Pharmaceuticals in the Environment. Kummerer, K., Springer**:** 221-230.

Webb, S. F., Ternes, T., Gilbert, M. and Olejniczak, K. (2003). "Indirect Human Exposure to Pharmaceuticals Via Drinking Water." Toxicology letters **142**: 157-167.

WHO (2006). Defined Daily Doses Http://Www.Whocc.No/Atcddd/. WHO Collaborating Centre for Drug Statistics Methodology.

Wiig, O., Derocher, A. E., Cronin, N. M. and Skaare, J. U. (1998). "Female Pseudohermaphrodite Polar Bears at Svalbard." Journal of Wildlife Diseases **34**(4): 792-796.

Wilen, B.-M., Jin, B. and Lant, P. (2003). "The Influence of Key Chemical Constituents in Activated Sludge on Surface and Flocculating Properties." Water Research **37**: 2127-2139.

Williams, C. F., Williams, C. E. and Adamsen, E. J. (2006). "Sorption-Desorption of Carbamazepine from Irrigated Soils." Journal of Environmental Quality **35**(5): 1779-1783.

Wilson, S. C., Duarte-Davidson, R. and Jones, K. C. (1996). "Screening the Environmental Fate of Organic Contaminants in Sewage Sludges Applied to Agricultural Soils: 1. The Potential for Downward Movement to Groundwaters." Science of the Total Environment **185**: 45-57.

Wingender, J., Neu, T. and Flemming, H. (1999). What Are Bacterial Extracellular Polymeric Substances. Microbial Extracellular Polymeric Substances - Characterization, Structure and Function. Wingender, J., Neu, T. and Flemming, H. Berlin, Springer.

Wolf, L., Held, I., Eiswirth, M. and Hotzl, H. (2004). "Impact of Leaky Sewers on Groundwater Quality." Acta Hydrochimica et Hydrobiologica **32**(4-5): 361-373.

Wollenberger, L., Halling-Sorensen, B. and Kusk, K. O. (2000). "Acute and Chronic Toxicity of Veterinary Antibiotics to Daphnia Magna." Chemosphere **40**(7): 723-730.

Wuertz, S., Pfleiderer, P., Kriebitzsch, K., Spath, R., Griebe, T., Coello-Oviedo, D., Wilderer, P. A. and Flemming, H. C. (1998). "Extracellular Redox Activity in Activated Sludge." Water Science and Technology **37**(4-5): 379-384.

Yang, S. W., Cha, J. M. and Carlson, K. (2006). "Trace Analysis and Occurrence of Anhydroerythromycin and Tylosin in Influent and Effluent Wastewater by Liquid Chromatography Combined with Electrospray Tandem Mass Spectrometry." Analytical and Bioanalytical Chemistry **385**(3): 623-636.

Yu, Z. Q., Xiao, B. H., Huang, W. L. and Peng, P. (2004). "Sorption of Steroid Estrogens to Soils and Sediments." Environmental Toxicology and Chemistry **23**(3): 531-539.

Zhang, X., Bishop, P. and Kinkle, P. (1999). "Comparison of Extraction Methods for Quantifying Extracellular Polymers in Biofilms." Water Science and Technology **39**(7): 211-218.

Zuccato, E., Calamari, D., Natangelo, M. and Fanelli, R. (2000). "Presence of Theraputic Drugs in the Environment." The Lancet **355**: 1789-1790.

Zuhlke, S., A and B (2004). "Detection and Identification of Phenazone-Type Drugs and Their Microbial Metabolites in Ground and Drinking Water Applying Solid-Phase Extraction and Gas Chromatography with Mass Spectrometric Detection." Journal of Chromatography A **1050**: 201-209.

Zurhelle, G., Petz, M., Mueller-Seitz, E. and Siewert, E. (2000). "Metabolites of Oxytetracycline, Tetracycline, and Chlortetracycline and Their Distribution in Egg White, Egg Yolk, and Hen Plasma." Journal of Agricultural and Food Chemistry **48**(12): 6392-6396.

Zwiener, C. and Frimmel, F. H. (2000). "Oxidative Treatment of Pharmaceuticals in Water." Water Research **34**(6): 1881-1885.

Zwiener, C., Glauner, T. and Frimmel, F. H. (2000). "Biodegradation of Pharmaceutical Residues Investigated by Spe-Gc/Itd-Ms and on-Line Derivatization." High resolution chromatography **23**(7-8): 474-478.

Chapter 13: Appendix A

13.1 Pharmaceuticals detected in sewage effluents

Pharmaceutical	Concentration ng l^{-1} (maximum)	Concentration ng l^{-1} (median)	Country	Information Source
17a-Estradiol	5	3	Netherlands	Belfroid *et al.* (1999)
17a-Ethinylestradiol	7		UK	Aherne and Briggs (1989)
17a-Ethinylestradiol	7		UK	Desbrow *et al.* (1998)
17a-Ethinylestradiol	15	1	GER	Ternes *et al.* (1999a)
17a-Ethinylestradiol	62	17	GER	Stumpf *et al.* (1999b)
17a-Ethinylestradiol	42	9	Canada	Ternes *et al.* (1999a)
17b-Estradiol	48		UK	Desbrow *et al.* (1998)
17b-Estradiol	12	5.5	Netherlands	Belfroid *et al.* (1999)
17b-Estradiol	0		GER	Ternes *et al.* (1999a)
17b-Estradiol	77		US	Tilton *et al.* (2002)
3,4,5,6-Tetrabromo-o-cresol				Ternes *et al.* (1998)
Aspirin (acetylsalicylic acid)	620		US	Hignite and Azanoff (1977)
Aspirin (acetylsalicylic acid)	290		GER	Stan and Heberer (1997)
Aspirin (acetylsalicylic acid)	1500	220	GER	Ternes (1998)
Aspirin (acetylsalicylic acid)	1510		GER	Stumpf *et al.* (1996)
Aspirin (acetylsalicylic acid)	3100	50	BRA	Stumpf *et al.* (1999)
Aspirin (acetylsalicylic acid)	10		UK	Severn Trent (2002)
Atenolol	122000		Spain	Gomez *et al.* (2006)
Atenolol	160		Sweden	Bendz *et al.* (2005)
Atorvastatin	22		Canada	Miao and Metcalfe (2002)
Benzydamine	1000		UK	Richardson and Bowron (1985)
Betaxolol	190		GER	Hirsch *et al.* (1996)
Betaxolol	190	57	GER	Ternes (1998)
Bezafibrate	4600	2200	GER	Ternes (1998)
Bezafibrate	2100	1010	BRA	Stumpf *et al.* (1999)
Bezafibrate	4800		Austria	Clara *et al.* (2005)
Bezafibrate	800		Finland	Lindqvist *et al.* (2005)
Biphenylol	2600		GER	Stumpf *et al.* (1999)
Bisoprolol	370		GER	Hirsch *et al.* (1996)
Bisoprolol	370	57	GER	Ternes (1998)
Bleomycin	19		UK	Aherne *et al.* (1990)
Caffeine	1000		UK	Richardson and Bowron (1985)
Caffeine	292000		Canada	Rogers *et al.* (1996)
Caffeine	9900		US	Batt *et al.* (2005)
Caffeine	3000	180		Heberer (2002)
Caffeine	2263		Canada	Verenitch *et al.* (2006)
Caffeine	4520		Spain	Santos *et al.* (2005)
Caffeine	220		Sweden	Bendz *et al.* (2005)
Carazolol	120	0	GER	Ternes (1998)

Pharmaceutical	Concentration ng l^{-1} (maximum)	Concentration ng l^{-1} (median)	Country	Information Source
Carbamazepine	6300	2100	GER	Ternes (1998)
Carbamazepine		1630		Heberer (2002)
Carbamazepine	70		Spain	Gomez *et al.* (2006)
Carbamazepine	1594		Austria	Clara *et al.* (2005)
Carbamazepine	750		Spain	Santos *et al.* (2005)
Carbamazepine	1180		Sweden	Bendz *et al.* (2005)
Carbamazepine	420		Taiwan	Lin *et al.* (2004)
Cephalexin	78		Australia	Costanzo *et al.* (2004)
Chloramphenicol	560	0	GER	Hirsch *et al.* (1996)
Chlorophene	710			Ternes *et al.* (1998)
Chlorotetracycline	0		GER	Hirsch *et al.* (1996)
Chloroxylenol				Ternes *et al.* (1998)
Ciprofloxacin	132		Australia	Costanzo *et al.* (2004)
Ciprofloxacin	970		US	Batt *et al.* (2005)
Clarithromycin	240	01	GER	Hirsch *et al.* (1996)
Clenbuterol	180		GER	Hirsch *et al.* (1996)
Clenbuterol	80	0	GER	Ternes (1998)
Clindamycin	1000		US	Batt *et al.* (2005)
Clofibrate	0	0	GER	Ternes (1998)
Clofibric acid	9740		US	Hignite and Azanoff (1977)
Clofibric acid	4550		GER	Stan and Heberer (1997)
Clofibric acid	1560		GER	Stumpf *et al.* (1999)
Clofibric acid	680		GER	Heberer *et al.* (1998)
Clofibric acid	1600	360	GER	Ternes (1998)
Clofibric acid	1030	102	BRA	Stumpf *et al.* (1999)
Clofibric acid	2000		US	Garrison *et al.* (1976)
Clofibric acid	10		UK	Severn Trent (2002)
Clofibric acid		480		Heberer (2002)
Clofibric acid	44		UK	Roberts and Thomas (2006)
Clofibric acid	248		Spain	Macia *et al.* (2004)
Clofibric acid	185		Switzerland	Soulet *et al.* (2002)
Clotrimazole	27		UK	Roberts and Thomas (2006)
Cloxacillin	0		GER	Hirsch *et al.* (1996)
Codeine	5700		Spain	Gomez *et al.* (2006)
Cyclophosphamide	20	0	GER	Ternes (1998)
Cyclophosphamide	17			Steger-Hartmann *et al.* (1997)
Cyclophosphamide			UK	Severn Trent (2002)
Dextropropoxyphene	281		UK	Hilton *et al.* (2003)
Dextropropoxyphene	64		UK	Roberts and Thomas (2006)
Dextropropoxyphene	585		UK	Ashton *et al.* (2004)
Diatrizoate	8700		GER	Ternes and Hirsch (2000)
Diatrizoate			UK	Severn Trent (2002)
Diazepam	40	0	GER	Ternes (1998)
Diazepam	1000			Halling-Sorensen *et al.* (1998)

Pharmaceutical	Concentration ng l^{-1} (maximum)	Concentration ng l^{-1} (median)	Country	Information Source
Diazepam	1000		UK	Waggott (1981)
Diazepam	660		Belgium	Ven *et al.* (2004)
Diclofenac	90		GER	Stan and Heberer (1997)
Diclofenac	2000		GER	Stumpf *et al.* (1996)
Diclofenac	960		GER	Heberer *et al.* (1998)
Diclofenac	2100	810	GER	Ternes (1998)
Diclofenac	500		GER	Stumpf *et al.* (1998)
Diclofenac	930	130	BRA	Stumpf *et al.* (1999)
Diclofenac	370		SWI	Buser *et al.* (1998a)
Diclofenac		3020		Heberer (2002)
Diclofenac	1070		UK	Hilton *et al.* (2003)
Diclofenac	448		Canada	Verenitch *et al.* (2006)
Diclofenac	1900		Spain	Gomez *et al.* (2006)
Diclofenac	598		UK	Roberts and Thomas (2006)
Diclofenac	1680		Austria	Clara *et al.* (2005)
Diclofenac	250		Canada	Lee *et al.* (2005)
Diclofenac	300		Finland	Lindqvist *et al.* (2005)
Diclofenac	120		Sweden	Bendz *et al.* (2005)
Diclofenac	2349		UK	Ashton *et al.* (2004)
Diclofenac	365		Greece	Koutsouba *et al.* (2003)
Diclofenac	570		Switzerland	Soulet *et al.* (2002)
Dicloxacillin	0		GER	Hirsch *et al.* (1999)
Dimethylaminophenazone	1000	0	GER	Ternes (1998)
Doxycycline	0		GER	Hirsch *et al.* (1999)
Erythromycin	6000	2500	GER	Hirsch *et al.* (1999)
Erythromycin	116		UK	Hilton *et al.* (2003)
Erythromycin	30		Spain	Gomez *et al.* (2006)
Erythromycin	290		UK	Roberts and Thomas (2006)
Erythromycin	1842		UK	Ashton *et al.* (2004)
Estrone	76		UK	Desbrow *et al.* (1998)
Estrone	47	4.5	Netherlands	Belfroid *et al.* (1999)
Estrone	70	9	GER	Ternes *et al.* (1999a)
Estrone	48	3	Canada	Ternes *et al.* (1999a)
Etofibrate	0	0	GER	Ternes (1998)
Fenofibrate	100		GER	Stan and Heberer (1997)
Fenofibrate	30	0	GER	Ternes (1998)
Fenofibric acid	50		GER	Stan and Heberer (1997)
Fenofibric acid	172		GER	Stumpf *et al.* (1996)
Fenofibric acid	1200	380	GER	Ternes (1998)
Fenofibric acid	750	50	BRA	Stumpf *et al.* (1999)
Fenoprofen	0		GER	Stan and Heberer (1997)
Fenoprofen	0		GER	Stumpf *et al.* (1996)
Fenoprofen	0	0	GER	Ternes (1998)
Fenoterol	8		GER	Hirsch *et al.* (1996)

Pharmaceutical	Concentration ng l^{-1} (maximum)	Concentration ng l^{-1} (median)	Country	Information Source
Fenoterol	60	0	GER	Ternes (1998)
Fluvoxamine			UK	Severn Trent (2002)
Gemfibrozil	120		GER	Stan and Heberer (1997)
Gemfibrozil	190		GER	Stumpf *et al.* (1996)
Gemfibrozil	950	130	BRA	Stumpf *et al.* (1999)
Gemfibrozil	250		GER	Stumpf *et al.* (1998)
Gemfibrozil	1500	400	GER	Ternes (1998)
Gemfibrozil		70		Heberer (2002)
Gemfibrozil	403		Canada	Verenitch *et al.* (2006)
Gemfibrozil	2090		Canada	Lee *et al.* (2005)
Gemfibrozil	180		Sweden	Bendz *et al.* (2005)
Gemfibrozil	250		Australia	Khan and Ongerth (2004)
Gentisic acid	590	0	GER	Ternes (1998)
Gentisic acid	0			Ternes *et al.* (1998)
Ibuprofen	3000	600	BRA	Stumpf *et al.* (1999)
Ibuprofen	81	6	SWI	Buser *et al.* (1999)
Ibuprofen	1300		UK	Severn Trent (2002)
Ibuprofen	140		GER	Stan and Heberer (1997)
Ibuprofen	3350		GER	Stumpf *et al.* (1996)
Ibuprofen	280		GER	Heberer *et al.* (1998)
Ibuprofen	3400	370	GER	Ternes (1998)
Ibuprofen		100		Heberer (2002)
Ibuprofen	2350		UK	Hilton *et al.* (2003)
Ibuprofen	6718		Canada	Verenitch *et al.* (2006)
Ibuprofen	151000		Spain	Gomez *et al.* (2006)
Ibuprofen	4239		UK	Roberts and Thomas (2006)
Ibuprofen	2400		Austria	Clara *et al.* (2005)
Ibuprofen	2170		Canada	Lee *et al.* (2005)
Ibuprofen	8800		Spain	Santos *et al.* (2005)
Ibuprofen	3900		Finland	Lindqvist *et al.* (2005)
Ibuprofen	150		Sweden	Bendz *et al.* (2005)
Ibuprofen	30		Taiwan	Lin *et al.* (2004)
Ibuprofen	27256		UK	Ashton *et al.* (2004)
Ibuprofen	2100		Spain	Rodriguez *et al.* (2002)
Ibuprofen	300		Switzerland	Soulet *et al.* (2002)
Ibuprofen	220		Australia	Khan and Ongerth (2004)
Ifosfamide	2900	0	GER	Ternes (1998)
Indometacin		800		Heberer (2002)
Indometacin	490		Canada	Lee *et al.* (2005)
Indomethacine	50		GER	Stan and Heberer (1997)
Indomethacine	1000	50	GER	Stumpf *et al.* (1999)
Indomethacine	600	270	GER	Ternes (1998)
Iopamidol	15000		GER	Ternes and Hirsch (2000)
Iopromide	11000		GER	Ternes and Hirsch (2000)

Pharmaceutical	Concentration ng l^{-1} (maximum)	Concentration ng l^{-1} (median)	Country	Information Source
Iopromide	5060		Austria	Clara *et al.* (2005)
Ketoprofen	0		GER	Stan and Heberer (1997)
Ketoprofen	0		GER	Stumpf *et al.* (1996)
Ketoprofen	380	200	GER	Ternes (1998)
Ketoprofen	650	170	BRA	Stumpf *et al.* (1999)
Ketoprofen		230		Heberer (2002)
Ketoprofen	268		Canada	Verenitch *et al.* (2006)
Ketoprofen	90		Canada	Lee *et al.* (2005)
Ketoprofen	1760		Spain	Santos *et al.* (2005)
Ketoprofen	1200		Finland	Lindqvist *et al.* (2005)
Ketoprofen	330		Sweden	Bendz *et al.* (2005)
Ketoprofen	190		Switzerland	Soulet *et al.* (2002)
Ketoprofen	590		Australia	Khan and Ongerth (2004)
Ketorolac	59500		Spain	Gomez *et al.* (2006)
Meclofenamic acid	0	0	GER	Ternes (1998)
Meclofenamic acid	79		Canada	Verenitch *et al.* (2006)
Medazepam			GER	Franke *et al.* (1995)
Mefenamic acid	807		UK	Hilton *et al.* (2003)
Mefenamic acid	396		UK	Roberts and Thomas (2006)
Mefenamic acid	1440		UK	Ashton *et al.* (2004)
Mefenamic acid	1000		Switzerland	Soulet *et al.* (2002)
Menstranol	4	0	GER	Ternes *et al.* (1999a)
Metaclofenamic acid	0		GER	Ternes (1998)
Methicillin	0		GER	Hirsch *et al.* (1999)
Methotrexate	1000			Aherne and English (1985)
Metoprolol	2200		GER	Hirsch *et al.* (1996)
Metoprolol	2200	730	GER	Ternes (1998)
Metoprolol	190		Sweden	Bendz *et al.* (2005)
Metoprolol	70		US	Huggett *et al.* (2002)
Metronidazol	9400		Spain	Gomez *et al.* (2006)
Morphine	20		Australia	Khan and Ongerth (2004)
Musk ambrette			UK	Severn Trent (2002)
Musk Cashmeran			UK	Severn Trent (2002)
Musk celestolide	91		UK	Severn Trent (2002)
Musk galaxolide	5300		UK	Severn Trent (2002)
Musk ketone	410		JAP	Yamagishi *et al.* (1981, 1983)
Musk ketone	96		UK	Severn Trent (2002)
Musk moskene			UK	Severn Trent (2002)
Musk Phantolide	48		UK	Severn Trent (2002)
Musk tibetene			UK	Severn Trent (2002)
Musk tonalide	1400		UK	Severn Trent (2002)
Musk Traseolide	95		UK	Severn Trent (2002)
Musk xylene	36		JAP	Yamagishi *et al.* (1981, 1983)
Musk xylene			UK	Severn Trent (2002)

Pharmaceutical	Concentration ng l^{-1} (maximum)	Concentration ng l^{-1} (median)	Country	Information Source
Nadolol	290		GER	Hirsch *et al.* (1996)
Nadolol	60	25	GER	Ternes (1998)
Nadolol	70		US	Huggett *et al.* (2002)
Nafcillin	0		GER	Hirsch *et al.* (1999)
Naproxen	520	300	GER	Ternes (1998)
Naproxen	3000	600	BRA	Stumpf *et al.* (1998)
Naproxen		80		Heberer (2002)
Naproxen	106		US	Boyd (2003)
Naproxen	7098		Canada	Verenitch *et al.* (2006)
Naproxen	2540		Canada	Lee *et al.* (2005)
Naproxen	2440		Spain	Santos *et al.* (2005)
Naproxen	1900		Finland	Lindqvist *et al.* (2005)
Naproxen	250		Sweden	Bendz *et al.* (2005)
Naproxen	170		Taiwan	Lin *et al.* (2004)
Naproxen	232		Spain	Macia *et al.* (2004)
Naproxen	106		US	Boyd *et al.* (2003)
Naproxen	2560		Spain	Rodriguez *et al.* (2002)
Naproxen	350		Australia	Khan and Ongerth (2004)
N-methylphenacetin			GER	Heberer *et al.* (1998)
Norethisterone	20		UK	Aherne and Briggs (1989)
Norflaxacin	210		Australia	Costanzo *et al.* (2004)
o-Hydroxyhippuric acid	0	0	GER	Ternes (1998)
Oxacillin	0		GER	Hirsch *et al.* (1999)
Oxazepam		250		Heberer (2002)
Oxytetracycline	0		GER	Hirsch *et al.* (1999)
Paracetamol (acetaminophen)	6000	0	GER	Ternes (1998)
Paracetamol (acetaminophen)	390		Australia	Khan and Ongerth (2004)
Penicillin G	0		GER	Hirsch *et al.* (1999)
Penicillin V	0		GER	Hirsch *et al.* (1999)
Phenazone			GER	Heberer *et al.* (1998)
Phenazone	410	160	GER	Ternes (1998)
Phenazone		520		Heberer (2002)
Phenobarbitol		30		Heberer (2002)
Primidone		140		Heberer (2002)
Propanolol	98		GER	Hirsch *et al.* (1996)
Propanolol	290	170	GER	Ternes (1998)
Propanolol	6500		Spain	Gomez *et al.* (2006)
Propanolol	373		UK	Roberts and Thomas (2006)
Propanolol	30		Sweden	Bendz *et al.* (2005)
Propanolol	80		US	Huggett *et al.* (2002)
Propranolol	152		UK	Hilton *et al.* (2003)
Propranolol	284		UK	Ashton *et al.* (2004)

Pharmaceutical	Concentration ng l^{-1} (maximum)	Concentration ng l^{-1} (median)	Country	Information Source
Propylphenazone	350		GER	Heberer *et al.* (1998)
Propylphenazone			GER	Franke *et al.* (1995)
Propylphenazone		50		Heberer (2002)
Ranitidine	1700		Spain	Gomez *et al.* (2006)
Roxithromycin	1000	680	GER	Hirsch *et al.* (1999)
Roxithromycin	69		Austria	Clara *et al.* (2005)
Salbutamol	0		GER	Hirsch *et al.* (1996)
Salbutamol	170	0	GER	Ternes (1998)
Salicylic acid	140	0	GER	Ternes (1998)
Salicylic acid	500			Ternes *et al.* (1998)
Salicylic acid		40		Heberer (2002)
Salicylic acid	2178		Canada	Verenitch *et al.* (2006)
Salicylic acid	320		Canada	Lee *et al.* (2005)
Salicylic acid	380		Australia	Khan and Ongerth (2004)
Sulfamethazine	0		GER	Hirsch *et al.* (1999)
Sulfamethoxazole	294		UK	Hilton *et al.* (2003)
Sulfamethoxazole	70		Sweden	Bendz *et al.* (2005)
Sulfamethoxazole	132		UK	Ashton *et al.* (2004)
Sulfamethoxazole	6000		US	Batt *et al.* (2005)
Sulphamethoxazole	2000	400	GER	Hirsch *et al.* (1999)
Sulphamethoxazole	91		Austria	Clara *et al.* (2005)
Tamoxifen	369		UK	Roberts and Thomas (2006)
Tamoxifen	42		UK	Ashton *et al.* (2004)
Terbutaline	9		GER	Hirsch *et al.* (1996)
Terbutaline	120	0	GER	Ternes (1998)
Tetracycline	0		GER	Hirsch *et al.* (1999)
Tetracycline	977	151	Canada	Miao *et al.* (2004)
Tetracycline	560		US	Batt *et al.* (2005)
Timolol	10		GER	Hirsch *et al.* (1998)
Timolol	70	0	GER	Ternes (1998)
Tolfenamic acid	0	0	GER	Ternes (1998)
Tolfenamic acid	1600		BRA	Stumpf *et al.* (1999)
Triclosan	710		UK	Severn Trent (2002)
Triclosan	360		Canada	Lee *et al.* (2005)
Triclosan	160		Sweden	Bendz *et al.* (2005)
Triclosan	21		US	Boyd *et al.* (2003)
Trimethoprim	599		UK	Hilton *et al.* (2003)
Trimethoprim	30		Spain	Gomez *et al.* (2006)
Trimethoprim	322		UK	Roberts and Thomas (2006)
Trimethoprim	40		Sweden	Bendz *et al.* (2005)
Trimethoprim	1288		UK	Ashton *et al.* (2004)
Trimethroprim	660	320	GER	Hirsch *et al.* (1999)
Trimethroprim	530		US	Batt *et al.* (2005)
Tylosin	1041		US	Yang *et al.* (2006)

13.2 Pharmaceuticals detected in streams or rivers

Pharmaceutical	Concentration ng l^{-1} (maximum)	Concentration ng l^{-1} (median)	Country	Information Source
17a-Ethinylestradiol	15		UK	Aherne and Briggs (1989)
17a-Ethinylestradiol	4	0	Netherlands	Belfroid *et al.* (1999)
17a-Ethinylestradiol	0		GER	Ternes *et al.* (1999)
Amoxicillin			Italy	Calamari (2004)
Aspirin (acetylsalicylic acid)	340	0	GER	Ternes (1998)
Atenolol	242		Italy	Zuccato *et al.* (2000)
Atenolol	241		Sweden	Bendz *et al.* (2005)
Betaxolol	28		GER	Hirsch *et al.* (1996)
Betaxolol	28	0	GER	Ternes (1998)
Bezafibrate	3100	350	GER	Ternes (1998)
Bezafibrate	250	100	GER	Stumpf *et al.* (1998)
Bezafibrate	203		Italy	Zuccato *et al.* (2000)
Bezafibrate	20		Austria	Ahrer *et al.* (2001)
Bisoprolol	2900		GER	Hirsch *et al.* (1996)
Bisoprolol	2900	0	GER	Ternes (1998)
Bleomycin	17		UK	Aherne *et al.* (1990)
Caffeine	6000	81	US	Kolpin *et al.* (2002)
Caffeine	310		US	Batt *et al.* (2005)
Caffeine	265		GER	Heberer *et al.* (2002)
Caffeine	1590		Canada	Verenitch *et al.* (2006)
Carazolol	110	0	GER	Hirsch *et al.* (1996)
Carbamazepine	1100	250	GER	Ternes (1998)
Carbamazepine	900		GER	Sacher *et al.* (2001)
Carbamazepine	1100		Sweden	Bendz *et al.* (2005)
Cephalexin	27		Australia	Costanzo *et al.* (2004)
Chloramphenicol	60	0	GER	Hirsch *et al.* (1999)
Chlorotetracycline	690	420	US	Kolpin *et al.* (2002)
Cimetidine	580	74	US	Kolpin *et al.* (2002)
Ciprofloxacin	30	20	US	Kolpin *et al.* (2002)
Ciprofloxacin	41		Australia	Costanzo *et al.* (2004)
Ciprofloxacin	170		US	Batt *et al.* (2005)
Clarithromycin	260	0	GER	Hirsch *et al.* (1999)
Clenbuterol	50	0	GER	Ternes (1998)
Clofibrate	0	0	GER	Ternes (1998)
Clofibrate	40		UK	Richardson and Bowron (1985)
Clofibric acid	7300		GER	Heberer *et al.* (1997)
Clofibric acid	4000		GER	Stan and Heberer (1997)
Clofibric acid	1750		GER	Heberer *et al.* (1995)
Clofibric acid	550	66	GER	Ternes (1998)
Clofibric acid	180			Stumpf *et al.* (1996)

Pharmaceutical	Concentration ng l^{-1} (maximum)	Concentration ng l^{-1} (median)	Country	Information Source
Clofibric acid	90		BRA	Stumpf *et al.* (1999)
Clofibric acid			Italy	Zuccato *et al.* (2000)
Clofibric acid	44		Austria	Ahrer *et al.* (2001)
Clofibric acid derivative	2900		GER	Heberer *et al.* (1997)
Clotrimazole	34		UK	Roberts and Thomas (2006)
Cloxacillin	0		GER	Hirsch *et al.* (1999)
Codeine	19	12	US	Kolpin *et al.* (2002)
Cyclophosphamide	0	0	GER	Ternes (1998)
Cyclophosphamide	10		Italy	Zuccato *et al.* (2000)
Dehydronifedipine	30	12	US	Kolpin *et al.* (2002)
Dextropropoxyphene	1000		UK	Richardson and Bowron (1985)
Dextropropoxyphene	98		UK	Roberts and Thomas (2006)
Dextropropoxyphene	682		UK	Ashton *et al.* (2004)
Dextropropoxyphene	682	260	UK	Hilton *et al.* (2003)
Diatrizoate			GER	Ternes and Hirsch (2000)
Diazepam	0	0	GER	Ternes (1998)
Diazepam			US	Genicola (1999)
Diazepam	10			Halling-Sorensen *et al.* (1998)
Diazepam	10		UK	Waggot (1981)
Diazepam	1		Italy	Zuccato *et al.* (2000)
Diclofenac	1030		GER	Heberer *et al.* (2002)
Diclofenac	489		GER	Stumpf *et al.* (1996)
Diclofenac	500	200	GER	Stumpf *et al.* (1998)
Diclofenac	1200	150	GER	Ternes (1998)
Diclofenac	450	20	BRA	Stumpf *et al.* (1999)
Diclofenac	590		GER	Sacher *et al.* (2001)
Diclofenac	568		UK	Hilton *et al.* (2003)
Diclofenac	1200		Sweden	Bendz *et al.* (2005)
Diclofenac	568		UK	Ashton *et al.* (2004)
Diclofenac	392		Austria	Ahrer *et al.* (2001)
Dicloxacillin	0		GER	Hirsch *et al.* (1999)
Digoxine	0	0	US	Kolpin *et al.* (2002)
Dimethylaminophenazone	340	0	GER	Ternes (1998)
Doxycycline	0	0	GER	Hirsch *et al.* (1999)
Enalapril	1		Italy	Calamari (2004)
Enrofloxacine	0	0	US	Kolpin *et al.* (2002)
Erythromycin	1000			Watts *et al.* (1983)
Erythromycin	1700	150	GER	Hirsch *et al.* (1999)
Erythromycin	17		Italy	Zuccato *et al.* (2000)
Erythromycin	49		GER	Sacher *et al.* (2001)
Erythromycin	112		UK	Hilton *et al.* (2003)
Erythromycin	70		UK	Roberts and Thomas (2006)
Erythromycin	1022		UK	Ashton *et al.* (2004)
Etofibrate	0		GER	Ternes (1998)

Pharmaceutical	Concentration ng l^{-1} (maximum)	Concentration ng l^{-1} (median)	Country	Information Source
Fenofibrate	100		GER	Heberer *et al.* (1997)
Fenofibrate	0	0	GER	Ternes (1998)
Fenofibric acid	280	45	GER	Ternes (1998)
Fenoprofen	0		GER	Stumpf *et al.* (1996)
Fenoprofen	0	0	GER	Ternes (1998)
Fenoterol	61	0	GER	Ternes (1998)
Fluoxetine	12	12	US	Kolpin *et al.* (2002)
Furosemide	88		Italy	Zuccato *et al.* (2000)
Gemfibrozil	790	48	US	Kolpin *et al.* (2002)
Gemfibrozil	510	52	GER	Ternes (1998)
Gemfibrozil	35		GER	Heberer *et al.* (2002)
Gemfibrozil	18		Canada	Verenitch *et al.* (2006)
Gemfibrozil	510		Sweden	Bendz *et al.* (2005)
Gentisic acid	1200	0	GER	Ternes (1998)
Hydrochlorothiazide	256		Italy	Calamari (2004)
Ibuprofen	8			Buser *et al.* (1999)
Ibuprofen	55		GER	Heberer *et al.* (2002)
Ibuprofen	139		GER	Stumpf *et al.* (1996)
Ibuprofen	1000	200	US	Kolpin *et al.* (2002)
Ibuprofen	530	70	GER	Ternes (1998)
Ibuprofen	8	4	SWI	Buser *et al.* (1999)
Ibuprofen	92		Italy	Zuccato *et al.* (2000)
Ibuprofen	5040		UK	Hilton *et al.* (2003)
Ibuprofen	10		Canada	Verenitch *et al.* (2006)
Ibuprofen	2370		UK	Roberts and Thomas (2006)
Ibuprofen	530		Sweden	Bendz *et al.* (2005)
Ibuprofen	5044		UK	Ashton *et al.* (2004)
Ifosfamide	0	0	GER	Ternes (1998)
Indomethacine	26		GER	Stumpf *et al.* (1996)
Indomethacine	200	40	GER	Ternes (1998)
Iopamidol	2400		GER	Ternes and Hirsch (2000)
Iopamidol	300		GER	Sacher *et al.* (2001)
Iopromide	210		GER	Ternes and Hirsch (2000)
Ketoprofen	120	0	GER	Ternes (1998)
Ketoprofen	65		GER	Heberer *et al.* (2002)
Ketoprofen	120		Sweden	Bendz *et al.* (2005)
Lincomycin	730	60	US	Kolpin *et al.* (2002)
Lincomycin	14		Italy	Zuccato *et al.* (2000)
Meclofenamic acid	0	0	GER	Ternes (1998)
Meclofenamic acid	109		Canada	Verenitch *et al.* (2006)
Mefenamic acid	366		UK	Hilton *et al.* (2003)
Mefenamic acid	366		UK	Ashton *et al.* (2004)
Meprobamate			USA	Eckel *et al.* (1993)
Metformin	150	110	US	Kolpin *et al.* (2002)

Pharmaceutical	Concentration ng l^{-1} (maximum)	Concentration ng l^{-1} (median)	Country	Information Source
Methicillin	0		GER	Hirsch *et al.* (1999)
Methotrexate	0			Aherne and English (1985)
Methylbenzylidene			GER	Nagtegaal *et al.* (1997)
Metoprolol	2200	45	GER	Ternes (1998)
Metoprolol	2200		Sweden	Bendz *et al.* (2005)
Musk ambrette	0		SWI	Muller *et al.* (1996)
Musk celestolide	8		GER	Winkler *et al.* (1998)
Musk celestolide	3		SWI	Muller *et al.* (1996)
Musk galaxolide	152		GER	Winkler *et al.* (1998)
Musk galaxolide	136		SWI	Muller *et al.* (1996)
Musk ketone	23		JAP	Yamagishi *et al.* (1981, 1983)
Musk ketone	8		SWI	Muller *et al.* (1996)
Musk moskene	0		SWI	Muller *et al.* (1996)
Musk tibetene	0		SWI	Muller *et al.* (1996)
Musk tonalide	88		GER	Winkler *et al.* (1998)
Musk tonalide	75		SWI	Muller *et al.* (1996)
Musk xylene	23		JAP	Yamagishi *et al.* (1981, 1983)
Musk xylene	10		GER	Winkler *et al.* (1998)
Musk xylene	1		SWI	Muller *et al.* (1996)
Nadolol	0	0	GER	Ternes (1998)
Nafcillin	0		GER	Hirsch *et al.* (1999)
Naproxen	390	70	GER	Ternes (1998)
Naproxen	95		GER	Heberer *et al.* (2002)
Naproxen	107		US	Boyd (2003)
Naproxen	271		Canada	Verenitch *et al.* (2006)
Naproxen	390		Sweden	Bendz *et al.* (2005)
Naproxen	30		Taiwan	Lin *et al.* (2004)
Naproxen	107		US	Boyd *et al.* (2003)
Naproxen	38		Austria	Ahrer *et al.* (2001)
N-methylphenacetin	470		GER	Heberer *et al.* (1997)
Norethisterone	17		UK	Aherne and Briggs (1989)
Norflaxacin	120	120	US	Kolpin *et al.* (2002)
Norflaxacin	80		Australia	Costanzo *et al.* (2004)
o-Hydroxyhippuric acid	0	0	GER	Ternes (1998)
Omeprazole			Italy	Calamari (2004)
Oxacillin	0		GER	Hirsch *et al.* (1999)
Oxytetracycline	0		GER	Hirsch *et al.* (1999)
Oxytetracycline	340	340	US	Kolpin *et al.* (2002)
Paracetamol (acetaminophen)	10000	110	US	Kolpin *et al.* (2002)
Penicillin G	0		GER	Hirsch *et al.* (1999)
Penicillin V	0		GER	Hirsch *et al.* (1999)
Pentobarbitol			USA	Eckel *et al.* (1993)
Phenazone	1250		GER	Heberer *et al.* (1997)
Phenazone	950	24	GER	Ternes (1998)

Pharmaceutical	Concentration ng l^{-1} (maximum)	Concentration ng l^{-1} (median)	Country	Information Source
Phenazone	25		GER	Sacher *et al.* (2001)
Phensuximide			USA	Eckel *et al.* (1993)
Propanolol	590	12	GER	Ternes (1998)
Propanolol	107		UK	Roberts and Thomas (2006)
Propanolol	215		UK	Ashton *et al.* (2004)
Propranolol	215		UK	Hilton *et al.* (2003)
Propranolol	590		Sweden	Bendz *et al.* (2005)
Propylphenazone	1465		GER	Heberer *et al.* (1997)
Ranitidine	10	10	US	Kolpin *et al.* (2002)
Ranitidine	9		Italy	Zuccato *et al.* (2000)
Roxithromycin	180	50	US	Kolpin *et al.* (2002)
Salbutamol	0	0	US	Kolpin *et al.* (2002)
Salbutamol	35	0	GER	Ternes (1998)
Salbutamol	5		Italy	Zuccato *et al.* (2000)
Salicylic acid	4100	25	GER	Ternes (1998)
Salicylic acid	243		Canada	Verenitch *et al.* (2006)
Sarafloxacin	0	0	US	Kolpin *et al.* (2002)
Sotalol	560		GER	Sacher *et al.* (2001)
Sulfamethazine	220	220	US	Kolpin *et al.* (2002)
Sulfamethoxazole	410		GER	Sacher *et al.* (2001)
Sulfamethoxazole	480		Sweden	Bendz *et al.* (2005)
Sulfamethoxazole	370		US	Batt *et al.* (2005)
Sulphadimethoxine	60	60	US	Kolpin *et al.* (2002)
Sulphamethoxazole	480		GER	Hirsch *et al.* (1999)
Sulphamethoxazole	520	66	US	Kolpin *et al.* (2002)
Sulphamethoxazole	198		UK	Hilton *et al.* (2003)
Sulphamethoxazole	102	51	UK	Hilton *et al.* (2003)
Tamoxifen	212		UK	Roberts and Thomas (2006)
Terbutaline	0	0	GER	Ternes (1998)
Tetracycline	110	110	US	Kolpin *et al.* (2002)
Theophylline	1000			Watts *et al.* (1983)
Timolol	10	0	GER	Ternes (1998)
Tolfenamic acid	0	0	GER	Ternes (1998)
Tolfenamic acid	20		GER	Heberer *et al.* (2002)
Triclosan	2300	140	US	Kolpin *et al.* (2002)
Trimethoprim	40		UK	Hilton *et al.* (2003)
Trimethoprim	19		UK	Roberts and Thomas (2006)
Trimethoprim	200		Sweden	Bendz *et al.* (2005)
Trimethoprim	42		UK	Ashton *et al.* (2004)
Trimethoprim	80		US	Batt *et al.* (2005)
Trimethroprim	200		GER	Hirsch *et al.* (1999)
Trimethroprim	300	13	US	Kolpin *et al.* (2002)
Tylosin	2		Italy	Zuccato *et al.* (2000)
Wafarin	0	0	US	Kolpin *et al.* (2002)

13.3 Pharmaceuticals detected in drinking water

Pharmaceutical	Concentration ng l^{-1}	Country	Information Source
17a-Ethinylestradiol	0	UK	Aherne *et al.* (1985)
17a-Ethinylestradiol	4	UK	Aherne and Briggs (1989)
17a-Ethinylestradiol	0	UK	James *et al.* (1997)
17a-Ethinylestradiol	6	GER	Rurainski *et al.* (1977)
17a-Ethinylestradiol	1	Netherlands	Rathner and Sonnenbon (1979)
17a-Ethinylestradiol		Germany	Ternes (2001)
17b-Estradiol	0	UK	James *et al.* (1997)
Acetylsalicylic acid		Germany	Ternes (2001)
Atenolol		Italy	Zuccato *et al.* (2000)
Atenolol		Germany	Ternes (2001)
Benzylpenicillin		Germany	Ternes (2001)
Betaxolol		Germany	Ternes (2001)
Bezafibrate	27	GER	Stumpf *et al.* (1996)
Bezafibrate		Italy	Zuccato *et al.* (2000)
Bezafibrate	27	Germany	Ternes (2001)
Bisoprolol		Germany	Ternes (2001)
Bleomycin	13	UK	Aherne *et al.* (1990)
Carazolol		Germany	Ternes (2001)
Carbamazepine	24	Canada	Tauber (2003)
Carbamazepine	258	US	Stackelberg *et al.* (2004)
Carbamazepine	30	Germany	Ternes (2001)
Celiprolol		Germany	Ternes (2001)
Chloramphenicol		Germany	Ternes (2001)
Chlorotetracycline		Germany	Ternes (2001)
Clarithromycin		Germany	Ternes (2001)
Clenbuterol		Germany	Ternes (2001)
Clofibrate		Germany	Ternes (2001)
Clofibric acid		UK	Fielding *et al.* (1981)
Clofibric acid	70	GER	Stumpf *et al.* (1996)
Clofibric acid	165	GER	Stan *et al.* (1994)
Clofibric acid	270	GER	Heberer and Stan (1998)
Clofibric acid	170	GER	Heberer and Stan (1996)
Clofibric acid	5	Italy	Zuccato *et al.* (2000)
Clofibric acid	70	Germany	Ternes (2001)
Cloxacillin		Germany	Ternes (2001)
Cyclophosphamide		Italy	Zuccato *et al.* (2000)
Cyclophosphamide		Germany	Ternes (2001)
Diatrizoate	85	Germany	Ternes (2001)
Diatrizoic acid	100	UK	Seitz *et al.* (2006)
Diazepam	10		Halling-Sorensen *et al.* (1998)
Diazepam	10	UK	Waggott (1981)
Diazepam	24	Italy	Zuccato *et al.* (2000)

Pharmaceutical	Concentration ng l^{-1}	Country	Information Source
Diazepam		Germany	Ternes (2001)
Diclofenac	6	GER	Stumpf *et al.* (1996)
Diclofenac		Slovenia	Kosjek *et al.* (2005)
Diclofenac	6	Germany	Ternes (2001)
Dicloxacillin		Germany	Ternes (2001)
Dimethylaminophenazone		Germany	Ternes (2001)
Doxycycline		Germany	Ternes (2001)
Erythromycin		Italy	Zuccato *et al.* (2000)
Estrone	0	UK	James *et al.* (1997)
Etofibrate		Germany	Ternes (2001)
Fenofibrate		Germany	Ternes (2001)
Fenofibric acid	42	Germany	Ternes (2001)
Fenoprofen		Germany	Ternes (2001)
Fenoterol		Germany	Ternes (2001)
Fluoxetine		US	Boyd (2003)
Furosemide		Italy	Zuccato *et al.* (2000)
Gemfibrozil	70	Canada	Tauber (2003)
Gemfibrozil		Germany	Ternes (2001)
Ibuprofen	3	GER	Stumpf *et al.* (1996)
Ibuprofen		Italy	Zuccato *et al.* (2000)
Ibuprofen		US	Loraine and Pettigrove (2006)
Ibuprofen		Slovenia	Kosjek *et al.* (2005)
Ibuprofen	3	Germany	Ternes (2001)
Ifosfamide		Germany	Ternes (2001)
Indometacin		Germany	Ternes (2001)
Iohexol		UK	Seitz *et al.* (2006)
iomeprol		UK	Seitz *et al.* (2006)
Iopamidol		UK	Seitz *et al.* (2006)
Iopamidol	79	Germany	Ternes (2001)
Iopromide		UK	Seitz *et al.* (2006)
Iopromide	86	Germany	Ternes (2001)
Iothalmic acid		Germany	Ternes (2001)
Ioxithalmic acid		Germany	Ternes (2001)
Ketoprofen		Slovenia	Kosjek *et al.* (2005)
Ketoprofen		Germany	Ternes (2001)
Lincomycin		Italy	Zuccato *et al.* (2000)
Methicillin		Germany	Ternes (2001)
Methotrexate	0	UK	Aherne *et al.* (1985)
Metropolol		Germany	Ternes (2001)
Nadolol		Germany	Ternes (2001)
Nafcillin		Germany	Ternes (2001)
Naproxen		US	Boyd (2003)
Naproxen		Slovenia	Kosjek *et al.* (2005)
Norethisterone	0	UK	James *et al.* (1985)
Norethisterone	0	UK	Aherne and Briggs (1989)
Oxacillin		Germany	Ternes (2001)

Pharmaceutical	Concentration ng l^{-1}	Country	Information Source
Oxytetracycline		Germany	Ternes (2001)
Paracetamol (acetaminophen)	0	GER	Ternes (1998)
Pentoxifylline		Germany	Ternes (2001)
Phenazone	250	Germany	Zuhlke *et al.* (2004)
Phenazone	400	Germany	Reddersen *et al.* (2002)
Phenazone	50	Germany	Ternes (2001)
Phenazone		Germany	Ternes (2001)
Phenoxymethylpenicillin		Germany	Ternes (2001)
Progesterone	6	UK	Aherne *et al.* (1985)
Propranolol		Germany	Ternes (2001)
Propylphenazone	80	Germany	Zuhlke *et al.* (2004)
Propylphenazone	120	Germany	Reddersen *et al.* (2002)
Ranitidine		Italy	Zuccato *et al.* (2000)
Roxithromycin		Germany	Ternes (2001)
Salbutamol		Italy	Zuccato *et al.* (2000)
Salbutamol		Germany	Ternes (2001)
Salicylic acid		Germany	Ternes (2001)
Sotalol		Germany	Ternes (2001)
Sulfamethazine		Germany	Ternes (2001)
Sulphamethoxazole		Germany	Ternes (2001)
Terbutaline		Germany	Ternes (2001)
Tetracycline		Germany	Ternes (2001)
Timolol		Germany	Ternes (2001)
Triclosan		US	Boyd (2003)
Triclosan		US	Loraine and Pettigrove (2006)
Trimethoprim		Germany	Ternes (2001)
Tylosin	2	Italy	Zuccato *et al.* (2000)

13.4 Pharmaceuticals detected in landfill leachate

Pharmaceutical	Concentration ng l^{-1}	Country	Information Source
Dihydrocodeine	101	Germany	Schneider *et al.* (2004)
Iopamidol	2944	Germany	Schneider *et al.* (2004)
Phenazone	5507	Germany	Schneider *et al.* (2004)
Carbamazepine	1415	Germany	Schneider *et al.* (2004)
Cyclophosphamide	192	Germany	Schneider *et al.* (2004)
Ifosfamide	42	Germany	Schneider *et al.* (2004)
Diclofenac	3190	Germany	Schneider *et al.* (2004)
Clofibric acid	2879	Germany	Schneider *et al.* (2004)
Propylphenazone	9173	Germany	Schneider *et al.* (2004)
Indometacin	141	Germany	Schneider *et al.* (2004)
Atenolol	44	Germany	Schneider *et al.* (2004)
Ibuprofen	9362	Germany	Schneider *et al.* (2004)
Bezafibrate	2773	Germany	Schneider *et al.* (2004)
Piroxicam	931	Germany	Schneider *et al.* (2004)
Primidone	5011	Germany	Schneider *et al.* (2004)

Pharmaceutical	Concentration ng l^{-1}	Country	Information Source
Naproxen	445	Germany	Schneider *et al.* (2004)
Ketoprofen	697	Germany	Schneider *et al.* (2004)
Metoprolol	31	Germany	Schneider *et al.* (2004)
Iopromide	236	Germany	Schneider *et al.* (2004)
Propanolol	10	Germany	Schneider *et al.* (2004)
Diazepam	453	Germany	Schneider *et al.* (2004)
iomeprol	92	Germany	Schneider *et al.* (2004)
Valproic acid	205	Germany	Schneider *et al.* (2004)

13.5 Pharmaceuticals detected in sewage sludges

Pharmaceutical	Concentration µg kg^{-1} (maximum)	Country	Information Source
Azithromycin	158	Germany	Gobel *et al.* (2005)
Carbamazepine	1731	Australia	Khan and Ongerth (2004)
Clarithromycin	41	Germany	Gobel *et al.* (2005)
Diclofenac	450	Germany	Ternes *et al.* (2005)
Galaxolide	0	USA	Buyuksonmez and Sekeroglu (2005)
Gemfibrozil	1192	Australia	Khan and Ongerth (2004)
Ibuprofen	0	USA	Buyuksonmez and Sekeroglu (2005)
Ibuprofen	3988	Australia	Khan and Ongerth (2004)
Naproxen	1022	Australia	Khan and Ongerth (2004)
Paracetamol (acetaminophen)	4535	Australia	Khan and Ongerth (2004)
Roxithromycin	131	Germany	Gobel *et al.* (2005)
Salicylic acid	13748	Australia	Khan and Ongerth (2004)
Sulfamethazine	113	Germany	Gobel *et al.* (2005)
sulfapyridine	197	Germany	Gobel *et al.* (2005)
Trimethoprim	133	Germany	Gobel *et al.* (2005)

Chapter 14: Appendix B

Figure 14.1 below shows a screenshot of the Toxchem+ model of the activated sludge plant used for determination of pharmaceutical fate in this work. This was sized (in terms of volume and flow rates) to be equivalent to Southam STP used in this work. Air requirements were calculated from the WRc equation (Metcalf and Eddy 2003).

The model assumes the influent is either settled sewage, or like Southam, an unscreened crude sewage. Primary tanks were not included primarily since Southam does not have primary tanks, but also because modelling of the tanks is poorly understood.

Similarly, the model does not have an anoxic zone. Again, this emulated Southam STP. Equally, no information has been found in literature or in the course of this research that described the anaerobic degradation of the selected pharmaceuticals.

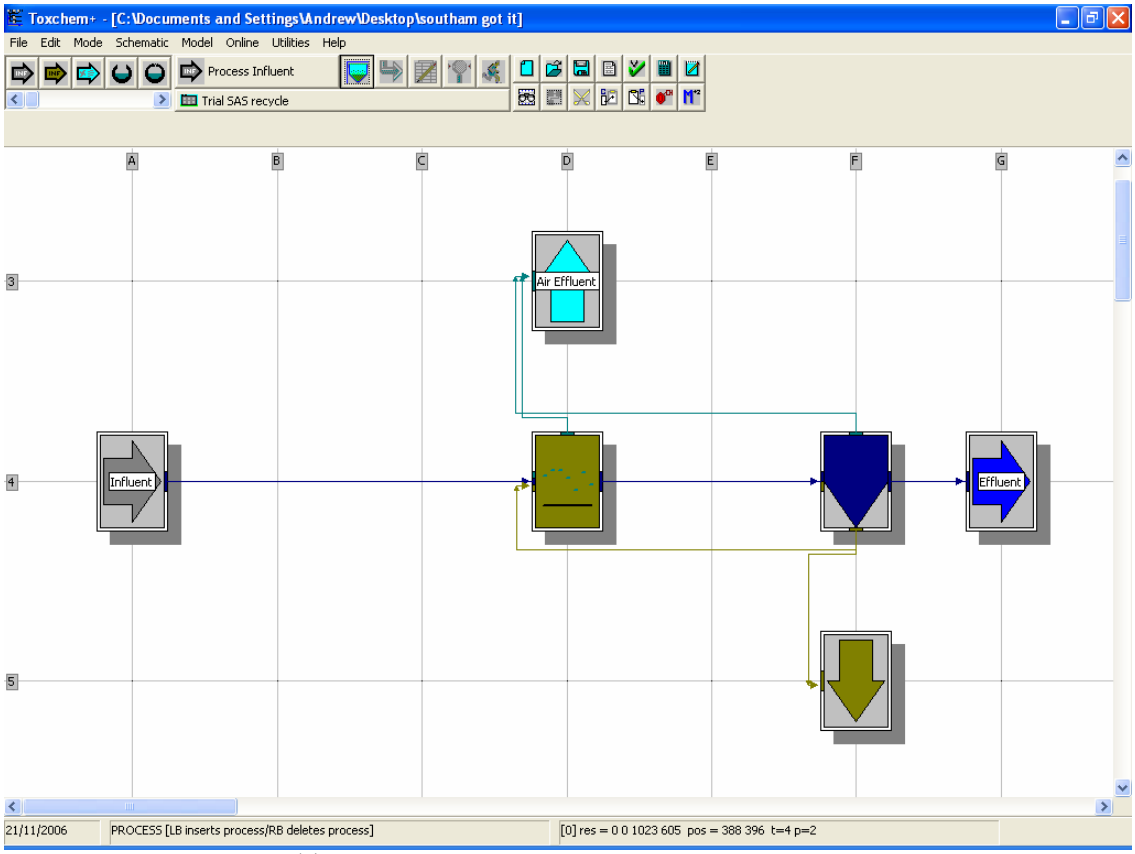


Figure 14.1: Screenshot of Toxchem+ model

The sections below show the required input parameters used for the Toxchem+ model.

Influent:

Flow rate: 12 Ml d^{-1}
SS: 100 mg l^{-1}

VSS ratio: 75%
FOG: 0
Temperature: 15 C

Activated sludge tank

Depth: 5 m
Surface area: 1200 m^2 (HRT = 12 hours)
MLSS: 3000 mg l^{-1}
MLVSS ratio: 75%
DO setpoint: 2 mg l^{-1}
Airflow: 10000 m^3 hr^{-1}

Oxygen transfer efficiency: 25%

Final tanks

Depth: 4m
Surface area: 3000 m^2

Weir length: 32 m
Final effluent SS concentration: 10 mg l^{-1}

SAS: 0.5% dry solids
RAS flow rate: 12000 m^3 d^{-1}

Chapter 15: Appendix C

Chemical Name	CAS Number	L(E)C50 Algae (mg l^{-1})	L(E)C50 Daphnia (mg l^{-1})	L(E)C50 Fish (mg l^{-1})	NOEC Algae (mg l^{-1})	NOEC Daphnia (mg l^{-1})	NOEC Fish (mg l^{-1})	Chronic Toxicity from QSAR	PNEC (ng l^{-1})	PEC (μg l^{-1})
17a-Ethinylestradiol	57-63-6	0.84	0.105	1.61		0.01	0.00000001		0.2	0.01
Acarbose	56180-94-0							1000	1000000	0.17
Allopurinol	315-30-0							79	79000	4.05
Amitiptyline	50-48-6		5						5000	1.01
Amoxicillin	26787-78-0							69	69000	13.1
Aspirin	50-78-2		61						61000	141.41
Atenolol	29122-68-7							10.9	10900	5.31
Azithromycin	83905-01-5							1.6	1600	0.05
Carbamazepine	298-46-4	157						8.1	8100	7.38
Cetirizine	83881-51-0							248	248000	0.05
Chloramphenicol	56-75-7	0.0643	227	0.0413			0.002		20	0.07
Cimetidine	51481-61-9							6.5	6500	6.55
Cisapride	81098-60-4							0.03	30	0.08
Clofibrate	637-07-0	12	0.106			0.01			100	0.28

Chemical Name	CAS Number	L(E)C50 Algae (mg l^{-1})	L(E)C50 Daphnia (mg l^{-1})	L(E)C50 Fish (mg l^{-1})	NOEC Algae (mg l^{-1})	NOEC Daphnia (mg l^{-1})	NOEC Fish (mg l^{-1})	Chronic Toxicity from QSAR	PNEC (ng l^{-1})	PEC (μg l^{-1})
Dextropropoxyphene	469-62-5							0.04	40	7.81
Diazepam	439-14-5		4.3						4300	0.18
Diclofenac	15307-79-6							4.24	4240	4.79
Diethylstilbestrol		10	4	1.09					1090	0.00
Digoxine	20830-75-5		21.2						21200	0.01
Diltiazem	42399-41-7							0.06	60	3.99
Erythromycin	114-07-8							3.9	3900	4.87
Etidronic acid	2809-21-4							23	23000	0.39
Famciclovir	104227-87-4							0.14	140	0.05
Flucloxacillin	5250-39-5							25	25000	4.29
Fluoxetine	54910-89-3							0.35	350	0.37
Gabapentin	60142-96-3							196	196000	0.48
Gliclazide	21187-98-4							0.11	110	3.45
Ibuprofen	15687-27-1	7.1	9.06	173		3	10		7100	29.8
Isoniazid	54-85-3							0.28	280	0.13
Lansoprazole	103577-45-3							0.87	870	0.08
Mebeverine	2753-45-9							0.07	70	2.84
Mefenamic acid	61-68-7							0.32	320	2.66

Chemical Name	CAS Number	L(E)C50 Algae (mg l^{-1})	L(E)C50 Daphnia (mg l^{-1})	L(E)C50 Fish (mg l^{-1})	NOEC Algae (mg l^{-1})	NOEC Daphnia (mg l^{-1})	NOEC Fish (mg l^{-1})	Chronic Toxicity from QSAR	PNEC (ng l^{-1})	PEC (µg l^{-1})
Mesalazine	89-57-6							0.5	500	7.40
Metformin	1115-70-4							1000	1000000	37.78
Metronidazol	443-48-1	64				250			64000	2.85
Naproxen	22204-53-1							23	23000	6.45
Omeprazole	73590-58-6							1	1000	0.72
Orphenadrine	83-98-7							0.65	650	0.20
Oxytetracycline	79-57-2		46.2						46200	4.99
Paracetamol (acetaminophen)	103-90-2	29.6	6.1	19					6100	71.8
Paroxetine	61869-08-7							0.34	340	0.24
Penicillin V	87-08-1							210	210000	4.07
Perindopril	82834-16-0							17.2	17200	0.01
Phenobarbitol	50-06-6			484					484000	0.31
Quinidine	56-54-2							1	1000	0.11
Quinine	130-95-0							1	1000	5.45
Ranitidine	66357-35-5							34	34000	6.67
Sulfasalazine	599-79-1							4.9	4900	8.50
Sumatriptan	103628-46-2							6.1	6100	0.10
Tetracycline	60-54-8	0.0251	44.8			340			25.1	0.86

Chemical Name	CAS Number	L(E)C50 Algae (mg l^{-1})	L(E)C50 Daphnia (mg l^{-1})	L(E)C50 Fish (mg l^{-1})	NOEC Algae (mg l^{-1})	NOEC Daphnia (mg l^{-1})	NOEC Fish (mg l^{-1})	Chronic Toxicity from QSAR	PNEC (ng l^{-1})	PEC (μg l^{-1})
Theophylline	58-55-9							9.7	9700	3.86
Thioridazine	50-52-2							0.104	104	0.70
Tramadol	27203-92-5							1	1000	0.31
Triclosan	3380-34-5	0.0014	0.18	0.18	0.0007		0.034	0.11	14	0.73
Verapamil	52-53-9							0.45	450	1.82