

# **The Fate and Effects of Human Pharmaceuticals in the Aquatic Environment**

**Michael Williams**

In fulfilment of the requirements for the degree of

**Doctor of Philosophy**

A thesis submitted to

**Soil and Land Systems**

**School of Earth and Environmental Sciences**

**The University of Adelaide**

**Australia**

June 2007

# Table of Contents

---

Table of Contents .....	II
List of Figures.....	VI
List of Tables .....	IX
Abstract.....	XI
Abbreviations .....	XIII
Acknowledgements .....	XV
Declaration.....	XVII
Chapter 1. General introduction .....	19
Chapter 2. Literature Review .....	22
<b>2.1 Pharmaceuticals in the aquatic environment.....</b>	<b>22</b>
2.1.1 Entry into the aquatic environment.....	23
2.1.2 Use and classification of human medicines .....	25
<b>2.2 Strategies for the risk assessment of human pharmaceuticals in the aquatic environment.....</b>	<b>29</b>
2.2.1 Ecological risk assessment.....	29
2.2.2 Environmental surveys .....	39
2.2.3 Ecotoxicity Testing .....	42
2.2.3.1 Acute ecotoxicity testing .....	43
2.2.3.2 Chronic ecotoxicity testing .....	45
2.2.4 Use of pharmacological data.....	47
<b>2.3 The fate of human pharmaceuticals in aquatic systems.....</b>	<b>49</b>
2.3.1 The role of sorption in environmental fate and effects .....	50
<b>2.4 Conclusions and summary .....</b>	<b>53</b>
Chapter 3. Analytical method development .....	56
<b>3.1 Introduction.....</b>	<b>56</b>
<b>3.2 Test pharmaceuticals .....</b>	<b>57</b>
<b>3.3 High pressure liquid chromatography (HPLC).....</b>	<b>62</b>
3.3.1 Multi-residue analysis.....	63
3.3.1.1 Stationary phase .....	63
3.3.1.2 Mobile phase .....	64
3.3.1.3 Wavelength of analysis.....	66
3.3.2 HPLC for carbamazepine (CBZ) analysis .....	67
3.3.2.1 Stationary phase .....	68
3.3.2.2 Mobile phase .....	69
3.3.2.3 Wavelength of analysis.....	70
3.3.3 Quality assurance / quality control (QA/QC) .....	70

3.3.3.1 Calibration procedures .....	70
3.3.3.2 Limit of detection / limit of quantification (LOD/LOQ).....	72
3.3.3.3 Matrix effects .....	73
3.3.3.4 Sample analysis .....	76
3.3.4 Clean-up procedures .....	76
3.3.4.1 Filtration .....	77
3.3.4.2 Solid phase extraction (SPE).....	78
<b>3.4 Conclusions.....</b>	<b>83</b>
<b>Chapter 4. Sorption and desorption of human pharmaceuticals .....</b>	<b>84</b>
<b>4.1 Introduction.....</b>	<b>84</b>
<b>4.2 Materials and methods .....</b>	<b>88</b>
4.2.1 Test pharmaceuticals .....	88
4.2.2 Test sediment .....	88
4.2.3 Sorption validation experiments .....	89
4.2.4 Isotherm sorption test procedure.....	91
<b>4.2.5 Desorption isotherm test procedure.....</b>	<b>93</b>
4.2.6 Quality assurance / quality control (QA/QC) .....	94
<b>4.3 Results and discussion .....</b>	<b>95</b>
4.3.1 Sediment characterisation .....	95
4.3.2 Sorption protocol validation .....	96
4.3.3 Sorption isotherms .....	100
4.3.3.1 Sorption isotherm parameters.....	100
4.3.3.2 Sorption of pharmaceuticals – comparisons with other studies.....	105
4.3.4 Desorption isotherms .....	109
4.3.4.1 Desorption of test pharmaceuticals .....	109
4.3.4.2 Desorption hysteresis .....	110
4.3.5 Limitations and implications of batch sorption .....	113
<b>4.4 Conclusions.....</b>	<b>115</b>
<b>Chapter 5. The influence of water quality parameters (pH and Ca<sup>2+</sup> ionic strength) on the sorption of pharmaceuticals.....</b>	<b>116</b>
<b>5.1 Introduction.....</b>	<b>116</b>
<b>5.2 Methods and materials .....</b>	<b>118</b>
5.2.1 Test pharmaceuticals .....	118
5.2.2 Batch sorption .....	118
5.2.3 Adjustment of pH and ionic strength.....	119
5.2.4 Test procedure .....	121
5.2.5 QA/QC.....	121
<b>5.3 Results and discussion .....</b>	<b>122</b>
5.3.1 Variation of sorption as a function of pH .....	122
5.3.1.1 Neutral and acidic pharmaceuticals.....	123
5.3.1.2 Basic pharmaceuticals .....	126
5.3.2 pH-dependent species contribution to sorption.....	126
<b>5.3.3 Variation of sorption as a function of [Ca<sup>2+</sup>].....</b>	<b>130</b>
5.3.3.1 Acidic pharmaceuticals .....	130
5.3.3.2 Basic pharmaceuticals .....	132
5.3.3.3 Neutral pharmaceuticals .....	133
5.3.3.4 Sorption to colloids – implications for K <sub>d</sub> .....	133

5.3.4 Variation of sorption as a function of solution salts and pH; the case of moderately hard water (MHW) .....	136
5.3.5 Implications for sorption in the aquatic environment .....	138
<b>5.4 Conclusions .....</b>	<b>140</b>
<b>Chapter 6. Can the aquatic distribution of human pharmaceuticals be related to pharmacological data?.....</b>	<b>142</b>
<b>6.1 Statement of contributions .....</b>	<b>142</b>
<b>6.2 Introduction.....</b>	<b>143</b>
6.2.1 Pharmacological principles.....	144
6.2.3 Comparison between volume of distribution ( $V_D$ ) and the partition coefficient ( $K_d$ ) .....	146
<b>6.3 Materials and methods .....</b>	<b>149</b>
6.3.1 Test pharmaceuticals .....	149
6.3.2 Test procedure .....	150
6.3.3 Chemical analysis .....	152
<b>6.4 Results and discussion .....</b>	<b>153</b>
6.4.1 Functional relationship between $K_d$ and $V_D$ .....	153
6.4.2 Comparisons with other studies .....	154
6.4.3 Influence of system parameters on $K_d$ values .....	157
6.4.4 Considerations for ecological risk assessment of pharmaceuticals .....	158
<b>6.5 Conclusions.....</b>	<b>159</b>
<b>Chapter 7: Isotopic dilution of carbamazepine as a measure of its exchangeable fraction.....</b>	<b>160</b>
<b>7.1 Introduction.....</b>	<b>160</b>
7.1.1 Exchangeable (E) value of contaminants.....	161
<b>7.2 Methods and materials .....</b>	<b>164</b>
7.2.1 Principles for determining the E-value .....	164
7.2.2 Test pharmaceutical .....	166
7.2.3 Batch sorption procedure .....	166
7.2.4 Concentration dependence of E-value .....	167
7.2.5 Time dependence of E-value .....	168
7.2.6 Sediment amendment with char.....	169
7.2.7 Analytical methods .....	169
7.2.7.1 Quench curve.....	170
7.2.8 QA/QC considerations.....	171
<b>7.3 Results and discussion .....</b>	<b>171</b>
7.3.1 Concentration dependence of E-value .....	171
7.3.2 Time dependence of E-value .....	174
7.3.4 Use of the E-value and implications for sorption of carbamazepine (CBZ).....	179
7.3.4.1 Comparison with other desorption methods.....	179
7.3.4.2 Presence of char in sediment .....	180
7.3.4.3 Implications for the exchangeability of CBZ .....	182
<b>7.4 Conclusions.....</b>	<b>183</b>
<b>Chapter 8. Ecotoxicology of carbamazepine in sediments; the case of the freshwater midge, <i>Chironomus tepperi</i>.....</b>	<b>185</b>

<b>8.1 Introduction.....</b>	<b>185</b>
8.1.1 Ecotoxicology of pharmaceuticals.....	185
8.1.2 Ecotoxicology of carbamazepine (CBZ) .....	188
8.1.3 The use of <i>Chironomus tepperi</i> (freshwater midge) for ecotoxicity testing .....	189
<b>8.2 Methods and materials .....</b>	<b>190</b>
8.2.1 Test chemicals .....	190
8.2.2 Test sediment and solutions .....	190
8.2.3 <i>Chironomus tepperi</i> culturing.....	191
8.2.4 Degradation of CBZ within solution .....	192
8.2.5 Na <sup>+</sup> /K <sup>+</sup> -ATPase assay .....	193
8.2.6 <i>In vitro</i> assay .....	196
8.2.7 Aqueous exposure.....	197
8.2.8 Sediment exposure.....	197
8.2.9 Statistical analysis.....	199
<b>8.3 Results and Discussion.....</b>	<b>200</b>
8.3.1 Aqueous degradation of pharmaceuticals .....	200
8.3.2 Na <sup>+</sup> /K <sup>+</sup> -ATPase assay validation .....	202
8.3.3 <i>In vitro</i> exposure .....	203
8.3.4 Aqueous exposure.....	204
8.3.5 Sediment exposure.....	209
8.3.6 Effect of exposure period.....	215
8.3.7 The use of biomarkers for ecotoxicological screening .....	216
<b>8.4 Conclusions.....</b>	<b>219</b>
<b>Chapter 9. Conclusions and recommendations.....</b>	<b>221</b>
<b>9.1 Sorption of human pharmaceuticals .....</b>	<b>221</b>
<b>9.2 The use of pharmacological data to estimate the potential sorption of     pharmaceuticals .....</b>	<b>222</b>
<b>9.3 Exchangeability of carbamazepine (CBZ) within a water / sediment system     .....</b>	<b>223</b>
<b>9.4 Ecotoxicological impacts of carbamazepine (CBZ) on <i>Chironomus tepperi</i>     .....</b>	<b>225</b>
<b>9.5 Application of results to the regulatory approach to ecological risk     assessment (ERA).....</b>	<b>227</b>
<b>9.6 Summary.....</b>	<b>229</b>
<b>References.....</b>	<b>231</b>
<b>Appendices.....</b>	<b>251</b>

## List of Figures

---

- Figure 2.1: Conceptual overview of the entry of human pharmaceuticals into the aquatic environment showing partitioning into different compartments within an aquatic system. *Italics indicate likely processes that can remove pharmaceuticals from system.* .....24
- Figure 2.2: Scheme of decision process for risk assessment of a new drug for the European Medicines Agency (EMA) and Food and Drug Administration (FDA) (FDA-CDER 1998; Bound and Voulvoulis 2004; EMA-CPMP 2005). .....34
- Figure 3.1: Chromatogram of the mixture of pharmaceuticals at 280 nm using HPLC-PDA.....67
- Figure 3.2: UV spectrum of (A) CBZ and (B) FLX showing relative absorbance intensity at various wavelengths of light .....69
- Figure 3.3: Representative calibration curve, depicting a typical response of CBZ for a concentration range from 10-1000  $\mu\text{gL}^{-1}$ . Note that x-axis units were automatically generated by the software. ....71
- Figure 3.4: The percent change in peak area of pharmaceuticals spiked into 10 mM  $\text{CaCl}_2$  (■) and 10 mM  $\text{CaCl}_2$  mixed with sediment (□), relative to pharmaceuticals spiked in 18.2  $\text{M}\Omega\text{cm}^{-1}$  water.....75
- Figure 3.5: Recovery efficiency of pharmaceuticals for (A) HLB, (B) MCX/ $\text{C}_{18}$  and (C)  $\text{C}_{18}$  solid phase extraction matrices at pH 3 adjusted with  $\text{H}_3\text{PO}_4$  (■), pH adjusted with  $\text{NaH}_2\text{PO}_4/\text{H}_3\text{PO}_4$  (□) and pH 7 adjusted with  $\text{NaHPO}_4/\text{H}_3\text{PO}_4$  (□).....83
- Figure 4.1: Replicate NMR spectra of sediment used for batch sorption experiments. NMR spectra A-D represent 4 sediment replicates.....97
- Figure 4.2: Percentage of spiking concentration of test pharmaceuticals in solution, compared with initial spiking concentration ( $C_0$ ), after various batch sorption shaking times for A: CIM (□), DCF (△), DIL (◇), DMI (◇), FLX (△), and IMI (□); B: ATL (◇), CAF (◇), CBZ (□), IBU (△) and PAC (△). Error bars are the standard error of triplicate samples. In all cases,  $\%C/C_0$  is 100 at  $t = 0$  h. ....98
- Figure 4.3: Effect of spiking pharmaceuticals as a mixture (■) in batch sorption system, compared with spiking each drug individually (□) into the batch sorption system. Error bars are the standard error of triplicate samples..... 103
- Figure 4.4: The effect of sterilisation (■), particle size (0.425 mm (□) and 2 mm (■)) and sediment : solution ratio (1 : 10 (■)), compared with 1 : 100 for all other treatments) on distribution coefficient ( $K_d$ ) of test pharmaceuticals. For the sterile and 1/10 sediment : solution treatments DCF, EE2 and FLX were not detected. DCF was

also not detected for the 2 mm sediment treatment. Error bars are the standard error of triplicate samples. .... 103

Figure 4.5: Log-transformed sorption isotherms for (A): DCF (◇), DMI (△), EE2 (□), FLX (◆), IMI (▣) and PRL (△); and (B): ATL (◇), CAF (▣), CBZ (△), CIM (◇), DIL (△) and IBU (□). Data is log transformed for aqueous concentrations ( $C_{aq}$ ) and apparent sediment concentrations ( $C_s$ ). Error bars are standard error of triplicate samples..... 104

Figure 4.6: Log-transformed sorption and desorption isotherms for selected drugs; CAF (sorption (▣) and desorption (□)), CBZ ((△) and (△)), EE2 ((◇) and (◇)) and FLX ((●) and (○)). Regression lines are for sorption isotherm only. Desorption isotherms were derived from dilution method. Error bars are standard error of triplicate samples. .... 111

Figure 5.1: The effect of pH on the extent of ionisation of a drug with a mono-acidic (solid line) or mono-basic (dashed line) functional group. The x-axis shows the difference between the  $pK_a$  value of the drug and the pH of solution it is dissolved in. .... 123

Figure 5.2: Distribution coefficient ( $K_d$ ) plotted against solution pH for drugs that were (A) basic; ATL (◇), CIM (▣), DMI (△), DIL (◇), FLX (□), IMI (△) or (B) neutral and acidic; CAF (◇), CBZ (▣), DCF (△), IBU (□), PAC (◇) within the measured pH range. The  $pK_a$  values for each drug are marked on the x-axis with solid, black lines. Error bars are standard error of the mean  $K_d$  value. .... 125

Figure 5.3: Log distribution coefficient ( $\log K_d$ ) plotted against  $\alpha$  for (A) CIM and (B) IBU. At  $\alpha = 0$ , basic CIM is in its unionised form, while acidic IBU is in its ionised form. The ionisation state then changes until  $\alpha = 1$ , where CIM is ionised and IBU is unionised..... 129

Figure 5.4: The effect of  $Ca^{2+}$  (expressed as molarity) on the  $K_d$  value of pharmaceuticals with (A) basic; ATL (◇), CIM (△), DIL (▣), DMI (◇), FLX (△), IMI (□) and (B) acidic or neutral; CAF (◇), CBZ (▣), IBU (◇), PAC (□) functional groups. Error bars are standard error of triplicate  $K_d$  values. .... 134

Figure 5.5: The  $K_d$  value of DCF based on the solution concentration of  $Ca^{2+}$ , expressed as molarity..... 135

Figure 5.6: The  $K_d$  value of pharmaceuticals in moderately hard water (■), moderately hard water with 1 mM  $CaCl_2$  (▣) and 1 mM  $CaCl_2$  (■). Error bars are standard error of triplicate  $K_d$  values. .... 137

Figure 6.1: The relationship between  $K_d$  and  $V_D$  for (A) the 10 mM  $CaCl_2$ /sediment system, (B) the 10 mM  $CaCl_2$ /soil system and (C) the MHW/sediment system. The values are mean  $\pm$  s.e. for  $K_d$  while error bars are not shown for  $V_D$  values..... 156

Figure 7.1: Labile and non-labile pool of an organic contaminant in a water sediment system. The exchangeable pool is where the radiolabelled isotope added in solution can exchange with the sediment-bound non-labelled analyte. Aqueous concentration

( $C_{aq}$ ) and solids-bound concentration ( $C_s$ ) are used to define the partition coefficient ( $K_d$ ) of the analyte..... 162

Figure 7.2: Effect of concentration of carbamazepine ([CBZ]) on the percentage of originally spiked CBZ remaining within the exchangeable pool. Error bars shown are standard error of triplicate samples..... 172

Figure 7.3: Sorption isotherm for carbamazepine from batch sorption experiments for triplicate samples ..... 173

Figure 7.4: Effect of concentration of carbamazepine ([CBZ]) on the distribution coefficient ( $K_d$ ) value of unlabelled carbamazepine ( $^{12}\text{C}$ -CBZ; ■) and radiolabelled carbamazepine ( $^{14}\text{C}$ -CBZ; ■) ..... 173

Figure 7.5: Effect of time of batch sorption experiments on the percentage of originally spiked carbamazepine remaining within the exchangeable pool for unfiltered treatment (■), 0.45  $\mu\text{m}$  filtered treatment (■), 0.2  $\mu\text{m}$  filtered treatment (□) and char-amended sediment treatment (■). Error bars shown are standard error of triplicate samples. .... 175

Figure 7.6: Effect of time of batch sorption experiments on the distribution coefficient ( $K_d$ ) value of (A) unlabelled carbamazepine ( $^{12}\text{C}$ -CBZ) and (B) radiolabelled carbamazepine ( $^{14}\text{C}$ -CBZ) for unfiltered treatment (■), 0.45  $\mu\text{m}$  filtered treatment (■) and 0.2  $\mu\text{m}$  filtered treatment (□). Error bars shown are standard error of triplicate samples..... 177

Figure 8.1: Concentration of carbamazepine in solution (C) compared with initial spiking concentration ( $C_0$ ) following exposure to light and colloidal material; treatments were 18.2  $\text{M}\Omega\text{cm}^{-1}$  water (—◆—), moderately hard water (MHW) mixed with sediment and sterilised (—■—) and MHW mixed with sediment and re-inoculated (—▲—); controls were protected from light for 18.2  $\text{M}\Omega\text{cm}^{-1}$  water (···◆···), MHW mixed with sediment and sterilised (···■···) and MHW mixed with sediment and re-inoculated (···▲···). Error bars are standard errors of the mean of triplicate samples. .... 201

Figure 8.2: Amount of P released during  $\text{Na}^+/\text{K}^+$ -ATPase assay depending on (A) the volume of 25 mM  $\text{Na}_2\text{ATP}$ , (B) the volume of 0.5 mM ouabain (OUB) added to the reaction solution and (C) the length of time the assay was allowed to proceed. Values are means of duplicate samples..... 206

Figure 8.3:  $\text{Na}^+/\text{K}^+$ -ATPase activity in *C. tepperi* cell homogenate exposed in vitro to varying concentrations of carbamazepine (CBZ). Error bars are the standard error of four replicates. The molarity of CBZ is relative to the volume of cell homogenate. 207

Figure 8.4: (A) The  $\text{Na}^+/\text{K}^+$ -ATPase activity of *C. tepperi* cell homogenates, (B) the mean wet weight of larvae and (C) the percentage survival of added *C. tepperi* following a 48 h exposure to aqueous concentrations of carbamazepine (CBZ). \* indicates significantly different values using ANOVA followed by Tukey's multiple comparison test. Error bars are standard errors of the mean of four replicates ..... 208

## List of Tables

---

Table 2.1: Top 50 human medicines used in Australia in 2000 ranked according to mass prescribed.....	25
Table 2.2: The ranking of pharmaceuticals based on prescription data in a number of different countries. Anatomical and therapeutic categories are listed in italics .....	27
Table 2.3: Overview of types of pharmaceuticals found in environmental surveys and their concentrations, grouped by their anatomical therapeutic chemical (ATC) code. Wastewater includes water sampled from wastewater treatment plants (WWTPs), while surface water and ground water were sampled post-WWTP .....	37
Table 2.4: Overview of pharmaceuticals used in ecotoxicological assays .....	38
Table 3.1: Structures and selected physicochemical properties of selected test pharmaceuticals.....	58
Table 3.2: HPLC parameters for selected pharmaceuticals .....	68
Table 4.1: Selected physicochemical properties of the sediment used for sorption experiments. Values shown are mean $\pm$ standard deviation of 4 replicates.....	96
Table 4.2: Integral regions for replicate solid-state $^{13}\text{C}$ NMR analysis for sediment replicates A-D .....	96
Table 4.3: Sorption parameters for sorption isotherm experiment and literature comparisons. Additional information relating to solid phases from literature values are given in Appendix.....	105
Table 4.4: Isotherm parameters for desorption isotherm experiment .....	112
Table 6.1: Comparison of the volume of distribution ( $V_D$ ) and partition coefficient ( $K_d$ ) processes and the system and compound-specific parameters that influence their respective values in the human body or aquatic ecosystem.....	148
Table 6.2: Physicochemical properties of solid phases used for the batch sorption experiments .....	150
Table 6.3: Literature dissociation constants ( $\text{pK}_a$ ), octanol-water partition coefficient ( $K_{OW}$ ) and $V_D$ values of selected pharmaceuticals.....	151
Table 8.1: Summary of the F-values determined for the various assays by ANOVA. The $F_{\text{critical}}$ values are the critical F-values based on the number of degrees of freedom and desired confidence level for the analysis. The null hypothesis ( $H_0$ ) is that there is no difference between the mean values determined at each treatment.....	214

Table 8.2: Summary of results from Tukey's multiple comparison test for the effect of time on emergence following sediment (chronic) exposure. Treatments with the same letter denotes there was no significant difference found between the means. ....215

## Abstract

---

There is relatively little known about the fate of human pharmaceuticals once they are released into the aquatic environment and what adverse impacts these compounds have on exposed aquatic organisms. Both of these factors are essential in defining the potential risk pharmaceuticals pose in the aquatic environment.

For this project up to 14 human therapeutic agents were selected as representative compounds to assess both their fate and effects within model aquatic systems. Considering sediments often serve as a repository for aquatic contaminants, the interaction of the selected pharmaceuticals with sediment was assessed. The sorption of the selected pharmaceuticals was found to be highly variable. Furthermore, the solution pH and ionic strength, due to  $\text{Ca}^{2+}$ , were found to exert a large degree of influence on the extent of sorption observed. These solution parameters, among others, may therefore make it difficult to predict the fate of pharmaceuticals, in terms of their association with sediments, using standardised assessment methods alone.

There is an extensive pool of knowledge on pharmaceuticals, in terms of their pharmacological profile, so their distribution within the human body (using the volume of distribution or  $V_D$ ) was compared with their distribution within a sediment / water system (using the partition coefficient or  $K_d$ ). The correlation between the  $V_D$  and  $K_d$  indicated this relationship provided a reasonable basis for estimating the distribution of drugs within the test sediment / water systems. This finding suggests that further exploration of the use of pharmacological data in understanding the potential fate of pharmaceuticals in aquatic systems is warranted.

The extent of the pharmaceuticals respective desorption values was also found to be highly variable within a standard test system. Further analysis on the desorption of carbamazepine, an anti-epileptic drug, was undertaken using an isotopic dilution technique. Observations from the isotopic dilution study indicated that both contact time with sediment and the quality of organic carbon could play an important role in the potential for sediments to irreversibly sorb carbamazepine present in aquatic systems. The desorption hysteresis observed for the other pharmaceuticals also indicates considerable effort is still required to address the issue of whether sediments can be a means of reducing exposure of pharmaceuticals to aquatic organisms (a “sink”) or a means of increasing exposure to sediment-dependent organisms (a “source”).

The necessity for further work on investigating the role that sorption with sediments may play in the fate and effects of human pharmaceuticals was highlighted by a series of ecotoxicological assays in both sediment and solution-only systems. Sediment-dwelling freshwater midges, *Chironomus tepperi*, were exposed to carbamazepine in both short- and long-term assays. Wet weight was found to be significantly reduced during short-term assays, while the development of *C. tepperi* larvae was found to be significantly inhibited when exposed to spiked sediment, over a longer exposure period. For these assays, the aqueous phase may have been a more important route of exposure of carbamazepine for the midges.

This study has indicated that sediments are likely to play an important role in the fate of pharmaceuticals and, subsequently, their effects. However, considerably more effort is required to assess the role sediments have and how this knowledge can be linked with current regulatory ecological risk assessments.

## Abbreviations

---

ACR	acute:chronic ratio
ANOVA	analysis of variance
ASM	Australian statistics on medicines
ATC	anatomical therapeutic category
ATL	atenolol
ATP	adenosine triphosphate
BSA	bovine serum albumin
Ca	calcium
C <sub>aq</sub>	aqueous concentration of drug
CAF	caffeine
CBZ	carbamazepine
CEC	cation exchange capacity
CH <sub>3</sub> CN	acetonitrile
CHOOH	formic acid
CIM	cimetidine
C <sub>s</sub>	solid phase concentration of drug
CSIRO	Commonwealth Scientific and Industrial Research Organisation
CV	coefficient of variation
DCF	diclofenac
DDD	defined daily dose
DIL	diltiazem
DMI	desipramine
DPH	diphenhydramine
dpm	disintegrations per minute
E-value	isotopically exchangeable value
EC	electrical conductivity
EC50	concentration where 50% effect observed relative to control
EE2	17 $\alpha$ -ethynylestradiol
EMA	European medicines agency
ERA	ecological risk assessment
FDA	food and drug administration
FLX	fluoxetine
<i>g</i>	gravities
GC	gas chromatography
H	hysteresis index
HPLC	high pressure (or performance) liquid chromatography
I	ionic strength
IBU	ibuprofen
IMI	imipramine
K <sub>d</sub>	partition coefficient
K <sub>f</sub>	Freundlich coefficient
K <sub>OC</sub>	partition coefficient normalised to organic carbon content of sediment
K <sub>OW</sub>	octanol-water partition coefficient
LC50	concentration where 50% lethality observed relative to control
LOD	limit of detection
LOEC	lowest observable effect concentration
LOQ	limit of quantitation

LSC	liquid scintillation counting
MDL	method detection limit
MHW	moderately hard water
Milli-Q	ultrapure water (18.2 MΩcm <sup>-1</sup> )
ML	minimum limit of detection
MS	mass spectrometry
NaCHOO	sodium formate
Na <sup>+</sup> /K <sup>+</sup> -ATPase	sodium/potassium adenosine triphosphatase
NMR	nuclear magnetic resonance
NSAID	non-steroidal antiinflammatory drug
OC	organic carbon
OECD	Organisation for Economic Cooperation and Development
OTC	over-the-counter
OUB	ouabain
PAC	paracetamol
PDA	poly diode array
PEC	predicted environmental concentration
P <sub>i</sub>	inorganic phosphate
pK <sub>a</sub>	acid dissociation constant
PRL	propranolol
PTFE	polytetrafluoroethylene
QA	quality assurance
QC	quality control
SEI	sucrose/ethylenediaminetetraacetic acid/imidazole
SPE	solid phase extraction
SSRI	selective serotonin re-uptake inhibitor
TGA	Therapeutic Goods Administration
UV	ultraviolet
V <sub>D</sub>	volume of distribution
WHO	World Health Organisation
WWTP	wastewater treatment plant

## Acknowledgements

---

Having come to the end of writing this thesis, I cannot remember a longer period of constant introspection, waging a meditative conflict with my egos and their various opinions of interpreting scientific information. I am not sure if my inner critic or narcissist would have emerged the victor but I have no doubt either of these scenarios would have eventuated if not for the guidance and support that I was able to rely on. So, to briefly turn my attention away from my angst-ridden musings, I would like to pay tribute to the following people who were an unfailing social web, who provided the necessary and highly appreciated guidance, advice, support and coping mechanisms, licit or otherwise.

However, I should start by acknowledging two institutions, CSIRO Land and Water and University of Adelaide Earth and Environmental Sciences, which provided me with the necessary infrastructure and support to undertake my experiments.

As for the individuals involved throughout this project, my supervisors were always a source of technical wisdom and scientific inspiration. I will always be grateful to my principal supervisor, Rai Kookana, for his seemingly endless patience and willingness to take an interest in any idea I thought was worth pursuing. His ability for subtle redirection has been the basis for the development of my professional skills and I will always be thankful for the confidence in me he maintained for the duration of my project. Carine Saison came to my project when it had reached the mid-way point, the PhD equivalent of entering the Equatorial doldrums. She immediately put some wind back into my sails and helped me back on course, all under very testing personal circumstances. Her personal strength, enthusiasm for science and passion for obscure literature are assets that I will always try to incorporate into my own life. I would also like to thank Mike McLaughlin for his down-to-earth attitude and accessibility despite his mind-boggling work schedule. His sharp intellect and deep knowledge, depressing for the mere mortal to behold, were always at my disposal when required.

I would like to thank other scientists at CSIRO Land and Water and the University of Adelaide for their willingness to down tools and grant me an indulgence in many cumulative hours of discussion, seemingly for the joy of engaging in scientific debate and providing me with a devil's advocate whenever a whim took me into their presence. It would be unfair to single out anyone as every employee apparently suffered my presence; however, Kris Broos, Jason Kirby, Enzo Lombi, Rebecca Hamon, Michael Karkainen and Ron Smernik were particularly remiss in consistently allowing me to enter their offices and accepting manuscripts for their perusal. But by far did Anu Kumar find herself adept at addressing any of my diverse range of concerns I always seemed to present her. She unfailingly provided me access to her deep knowledge on ecotoxicology, her sharp political skills for treading the dizzying heights of workplace relations and, most importantly, an inexhaustible reserve of sympathy. Anu has had an unswerving faith in my scientific ability (even when I didn't) ever since I have known her and I will always consider her to be the Atlas (or female variant thereof!) of my scientific career.

PhD students hold a fairly cynical theory they are merely cheap labour. However, compared with the highly competent technicians who seemed to roam the laboratories

and swoop in to save our experiments from imminent disaster, we could only be classified as merely cheap. Hai Doan, Colin Rivers, Sonia Grocke, Tasha Waller and Lester Smith embodied the labour and support that is necessary to keep a good scientific laboratory humming along. Their work played a critical role in supporting a number of experiments, especially when entering the murky waters of animal husbandry and toxicity assays; they are also a damn nice bunch of people! As for my fellow apprentices, I was infinitely fortunate to share offices and a laboratory with such a diverse, intelligent and funny group of people. To undertake a PhD project can be both highly enlightening and painful but having such a fantastic group of people to share the experience can make it both more enjoyable and less overwhelming. I would especially like to thank Dougal Currie, Therese McBeath, Mark Whatmuff, Dan Smith, Sam Stacey, Warwick Dougherty, Sean Mason and Laurence Jassogne who were often recipients, willing or otherwise, of my need for an outlet of humour, desperate last-minute requests for consumables from their likewise miniscule stockpiles and general banter that ranged from witty to delirious but always removed us from our immediate predicaments. They were, of course, very clever and committed people and I wish them all the best in their future, post-doctoral experiences.

Anyone reading this document will not realise the enormous and silent support that is provided by the people who exist outside of laboratory. My father, Des Williams, is a slight aberration in that he is, in fact, a scientist and did play a very important part in some of the technical aspects of my project, thanks to his profound knowledge in the realm of the pharmaceutical sciences. It is a watershed in one's life when one suddenly realises, after years of denial and rebellion, the fact that they have so many of their parent's characteristics ingrained into their DNA. In this case, I feel a sense of pride that I have inherited a number of his characteristics and the fact that we had the opportunity to work together. Also, for the other half of my DNA (and hot meals and concerned phone messages and other general requirements of a motherly nature) I cannot neglect to thank you so much for, well, being my mum. I would like to also highlight the other profoundly less scientific relationships I had with the rest of my immediate family, my in-laws and close friends who would drag me off camping, surfing, walking and talking to keep my inner geek in check. And most of all, to my beautiful, understanding wife, Chels, who committed herself to me during this time when I was in the most time- and financially-poor state of my life. I could write another thesis just on this unique woman and how she remains the basis of strength, joy and sanity in my life.

There were many other people who, no matter how small, played important roles throughout my project. These scientists were often involved in passing conversations with me at conferences or passing comments on my manuscripts or even hosting me in their laboratories for relatively fleeting moments. Perhaps these people will not read this thesis or even remember our encounters but I was always amazed at how warm and positive the majority of professional scientists have been towards scientists-in-training. It has made me feel very optimistic about my career choice and I look forward to reciprocating this role when I am given the opportunity.

## **Declaration**

---

This work contains no material which has been accepted for the award of any other degree or diploma in a university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text.

I give consent to this copy of my thesis when deposited in the University Library, being made for loan and photocopying, subject to the provisions of the Copyright Act 1968.

Components of the research described in this thesis have been published (as listed below). The author acknowledges that copyright of published material contained within this thesis resides with the copyright holder of these works.

Michael Williams \_\_\_\_\_

### **Publication arising from this thesis**

**Williams M.**, Saison C.L.A., Williams D.B. and Kookana R.S. (2006) Can aquatic distribution of human pharmaceuticals be related to pharmacological data? *Chemosphere* **65** (11) 2253-2259