8012-17

Occurrence and Distribution of Pharmaceutical Residuals in Bay Sewage and Sewage Treatment

Prepared for

Bay Area Clean Water Agencies

by

Stuart Khan and Jerry Ongerth
University of New South Wales
School of Civil and Environmental Engineering

June 26, 2002
Final Aug. 29, 2005

Chapter 1

1.1 Introduction

Organic compounds are used for an almost infinite variety of purposes in today's society. It is becoming clear that their residues of can be found in surface and groundwater throughout the globe. Increasing numbers of reports documenting the presence of such residues with increasing frequency and in increasingly widespread locations raises questions about their origin, factors controlling their distribution in the environment, how they can be controlled, and ultimately their significance to human health and the environment. Among the organic contaminants identified in work reported over the last 10 to 15 years are residues of pharmaceuticals, or more generally pharmaceutically active compounds (PhAC's). These are compounds that are administered to humans for therapeutic purposes. Included are prescription drugs, non prescription or "over-the-counter" medications, and illicit compounds.

Residuals of pharmaceuticals are of interest and potential concern for a variety of reasons. The are produced specifically for their ability to elicit physiological effects. Their control by the prescription system is based on the likelihood of their potentially serious adverse effects on unintended consumers. Also, the number of drugs in use is large. An internet website maintains a list of the 200 most widely used pharmaceuticals in the USA, http://www.rxlist.com/top200.htm. The quantity of drug use per capita is surprisingly high, estimated to be more than 50 g per capita per year or 135 mg per person per day for the entire population.

Reports identifying pharmaceutical residuals, often along with contaminants in other categories such as endocrine disrupters and personal care products, have resulted typically from application of refined analytical schemes to samples collected from a variety of sources of interest to the individual investigator. The important feature is the capability of the analytical scheme. All analytical schemes are specific to compounds having a limited range of chemical characteristics that permit them to be 'collected' by the concentration procedure and subsequent processing, typically chromatography, perhaps aided by chemical derivatization, with subsequent detection and identification of individual compounds by mass spectrometry. Application of such schemes typically can detect organic residuals of compounds that are relatively hydrophobic and reasonably stable at temperatures used in chromatography. Limits of detection for reasonably skilful and well-controlled application are typically on the order of 0.01 to 0.1 μg/L or in the range of 10 to 100 parts per trillion.

A reasonable characterization of this approach would be an ad hoc survey. It would reveal the presence of compounds identifiable by the analytical procedure and present in the detectable range. Examination of the physical/chemical characteristics of pharmaceutical compounds as a class (for example) would show that a large proportion would not be readily detectable by many of the relatively conventional analytical schemes raising the question of what might be found, or might actually be present, if a truly comprehensive analytical scheme or combination of schemes might be available.

On examination of the topic, pharmaceutical residuals would appear to be a class of compounds that should lend itself to relatively straightforward comprehensive description. Their source should be almost exclusively human excretion as a product of their intended use and human physiology and metabolism. Excretion of human waste is collected efficiently in sewage. Sewage generation has well-defined and reasonably predictable characteristics. Sewage is conveyed efficiently to treatment facilities that also have well-defined characteristics. In addition, the framework for describing the behavior, fate, and distribution of organic compounds in wastewater treatment, and indeed in the environment, is well-established (Clark and McKay, 1998). This line of reasoning led to the umbrella project for which the two California sewage treatment plants, in addition to those in Australia and Germany, were sampled and analyzed for the concentration of selected organic pharmaceutical residuals.

1.2 Purpose and Scope

The purpose of the umbrella project was to examine the predictability of pharmaceutical residual occurrence in municipal sewage and of the fate and distribution of residuals in sewage treatment. The scope included development of a model to predict the concentration of individual pharmaceutical compounds that should appear in raw sewage based on use statistics and their pharmaco/physiological and physical/chemical characteristics in combination with local wastewater generation characteristics. The model was also included components designed to predict the distribution of individual compounds through biological secondary sewage treatment. The model was applied to predict the appearance and distribution of all prescription compounds in use sufficiently high to generate measurable concentrations in raw sewage. For Australia, the location the project, this corresponded to the 50 compounds in highest use on a mass basis. The capability of the predictive model was tested by analysing samples from eight sewage treatment plants located in Australia, Germany, and the USA.

The specific purpose of this project was to determine the profile of pharmaceutical residuals measurable in two large (ca. 100 MGD) California municipal sewage treatment plants for general comparison to more extensively developed Australian data and to accompanying German data. The comparisons were viewed as valuable to evaluation of the predictive model and to providing broader understanding of the general (generalizable) characteristics of pharmaceutical residuals in municipal sewage.

1.3 Objectives and Approach

The objectives of this project were to collect samples of sewage and solids from the subject sewage treatment plants to permit completing a mass balance on residuals of individual pharmaceutical compounds. Concentration measurements for selected compounds provided a basis for evaluating the model and for comparison between local and remote locations having differing compound use characteristics.

Samples were collected by the operating agency staffs and delivered to a central lab for processing to a stable residual. The residuals were then transported by hand to the University of New South Wales where analytical procedures were completed for both liquid and solid residues. Information on the physical and operational characteristics of the individual treatment plants pertaining to completing mass balances was obtained from the operating agencies. All data were incorporated into the Doctoral Dissertation of Stuart Khan (Khan, 2002).

Chapter 2--Predicting PhAC Fate in an Example STP

2.1.1 Predicting PhAC origin and distribution in sewage and sewage treatment.

A theoretical model was developed to predict the concentration of PhAC's in raw sewage and their fate and distribution through sewage treatment and discharge to surface water, Figure 2.1. The model uses pharmaceutical use statistics, physiological metabolism and excretion information, physical and chemical compound characteristics, fugacity relationships and mass balances to predict concentrations generated in a typical population and their resulting partitioning and distribution into the environment. Statistics on pharmaceutical use for the 50 highest use compounds in Australia were used to drive the model, Table 2.1.

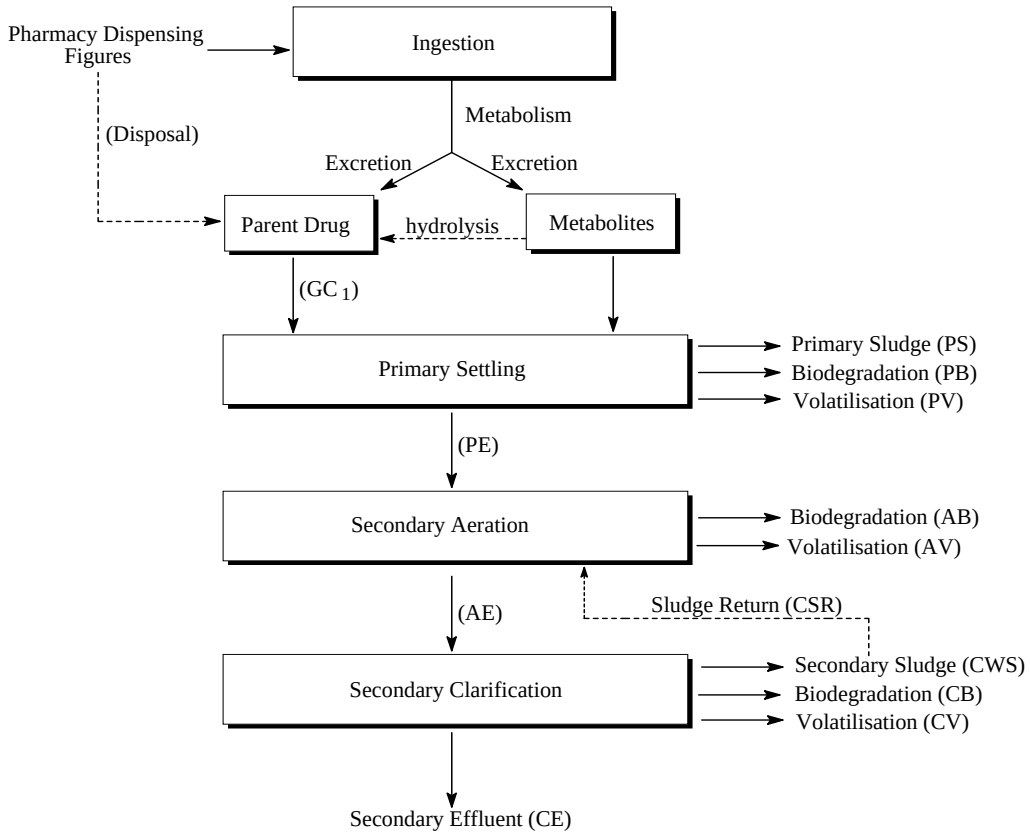


Figure 2.1. Schematic diagram of pharmaceutical residual mass balance

The model was based on mass balances and use of the fugacity concept to predict equilibrium distribution between phases. Mass balance data were obtained from primary sources including: the Australian government, Department of Health & Aged Care (Ref. 3); published characteristics of the individual pharmaceuticals, Table 2.1; and data describing process design and operating features of

Table 2.1. Australian Top 50* Prescription Pharmaceuticals By Mass: Dispensed mass, excretion, residual excretion, and physical/chemical properties.

No.	Name	Annual dispensed mass (kg)	Excreted Biliary / Fecal / Renal unchanged	Excreted Renal hydrolysable conjugates	Molecular Mass (disp.) (g/mol)	Molecular Mass (aqueous) (g/mol)	Henry's Law Const. (Unitless)	Log P	Acid base or neut.	pKa	Aerobic Biodeg. halflife in 2000mg/L MLSS (h)
1	PARACETAMOL	295882	0.03	0.93	151.16	151.16	6E-13	0.46	n	9.5	13
2	METFORMIN (hydrochloride)	90878	1.00	0.00	165.63	129.17	8E-16	-2.6	b	2.8	22
3	LACTULOSE	88099	0.03	0.00	342.3	342.3	8E-16	-4.7	n	n	4
4	AMOXYCILLIN	46204	0.68	0.00	419.45	365.41	2E-21	0.87	a	2.4	9
5	RANITIDINE (hydrochloride)	33724	0.30	0.00	350.87	314.41	3E-15	0.27	a	8.2	64
6	CEPHALEXIN	25408	0.90	0.00	365.41	347.4	3E-17	0.65	a	2.5	6
7	NAPROXEN	22850	0.10	0.60	230.26	230.26	3E-10	3.18	a	4.2	12
8	VALPROATE (sodium)	20889	0.05	0.20	166.2	144.21	3E-06	2.75	a	5	9
9	ASPIRIN	20389	0.00	0.00	180.16	180.16	1E-09	-1.1	a	3.5	15
9b	SALICYLIC ACID (ex-aspirin)	20389	0.08	0.85	180.16	138.12	1E-08	2.26	a	3	20
10	GEMFIBROZIL	20042	0.76	0.00	250.34	250.34	1E-08	4.77	a	4.8	22
11	ALLOPURINOL	19168	0.30	0.00	136.11	136.11	2E-14	-0.6	n	9.4	20
11b	OXIPURINOL (ex-allopurinol)	19168	0.70	0.00	136.11	152.11	2E-18	-0.3	n	n	20
12	SULPHASALAZINE	17998	0.00	0.00	398.39	398.39	2E-18	3.81	a	0.6	56
13	IBUPROFEN	14196	0.10	0.05	206.28	206.28	2E-07	3.97	a	4.4	16
14	CHLOROTHIAZIDE	12181	1.00	0.00	295.72	295.72	4E-12	-1.9	a	6.7	55
15	QUININE (sulphate)	11670	0.20	0.00	782.9	324.43	9E-16	3.44	b	8.8	50
16	ERYTHROMYCIN	10971	0.04	0.00	733.94	733.94	5E-29	3.06	a	8.9	259
17	CEFACLOR	10463	0.73	0.00	385.83	367.81	1E-17	0.35	a	2.4	9
18	CARBAMAZEPINE	9975	0.03	0.00	236.27	236.27	1E-10	2.45	n	n	31
19	VERAPAMIL (hydrochloride)	9786	0.16	0.04	491.07	454.61	9E-15	3.79	b	8.9	119
20	MOCLOBEMIDE	9457	0.01	0.00	268.75	268.75	2E-14	1.16	b	6.2	63
21	PHENOXYMETHYLPENICILLIN	9209	0.30	0.00	350.4	350.4	4E-15	2.09	a	2.7	9
22	DILTIAZEM (hydrochloride)	8669	0.03	0.00	450.98	414.53	9E-17	2.7	b	7.7	34
23	SULPHAMETHOXAZOLE	7322	0.30	0.00	253.28	253.28	1E-12	0.89	a	5.6	49
24	GLICLAZIDE	6842	0.01	0.80	323.42	323.42	8E-13	2.12	b	5.8	49
25	METHYLDOPA	6596	0.68	0.17	238.24	238.24	2E-18	-1.8	a	2.2	15
26	METOPROLOL (tartrate)	6176	0.07	0.00	684.82	267.37	1E-13	1.88	b	9.7	23
27	FRUSEMIDE	5350	0.75	0.14	330.74	330.74	4E-16	2.03	a	3.9	79
28	ATENOLOL	5189	0.93	0.00	266.34	266.34	1E-18	0.23	b	9.6	14
29	FLUCLOXACILLIN	4844	0.58	0.00	453.87	453.87	2E-17	2.58	a	2.7	24
30	CIMETIDINE	4788	0.48	0.00	252.34	252.34	1E-15	0.4	b	6.8	38
31	KETOPROFEN	4439	0.10	0.70	254.28	254.28	2E-11	0	a	4.6	17
32	PHENYTOIN	4431	0.00	0.00	252.27	252.27	1E-11	2.47	a	8	46
33	DICLOFENAC	4389	0.02	0.15	318.13	296.16	5E-12	4.51	a	4.5	50
34	CODEINE (phosphate)	4256	0.10	0.00	397.36	299.37	8E-14	0.6	b	8.2	65
35	CLAVULANIC ACID	3790	0.33	0.00	199.16	199.16	2E-16	-2	a	2.7	5
36	ROXITHROMYCIN	3745	0.74	0.00	837.07	837.07	5E-31	2.75	n	n	362
37	IRBESARTAN	3638	0.80	0.06	428.54	428.54	7E-15	5.31	n	n	28
38	SERTRALINE	3151	0.00	0.00	306.24	306.24	5E-08	5.29	b	8.9	89
39	DICLOXACILLIN	3147	0.60	0.00	470.33	470.33	1E-07	2.91	a	2.8	39
40	METRONIDAZOLE	3147	0.25	0.00	171.16	171.16	2E-11	-0.1	n	2.5	28

41	CAPTOPRIL	2944	0.45	0.00	217.29	217.29	4E-13	0.34	a	3.7	7
42	TRIMETHOPRIM	2694	0.43	0.00	290.32	290.32	2E-14	0.91	b	7.2	42
43	ISOSORBIDE MONONITRATE	2611	0.00	0.00	191.14	191.14	6E-14	-0.4	n	n	21
44	NIZATIDINE	2547	0.60	0.00	331.45	331.45	2E-18	-0.4	b	6.8	68
45	TIAPROFENIC ACID	2413	0.54	0.00	260.31	260.31	1E-11	2.82	a	3	17
46	DOTHIEPIN (hydrochloride)	2286	0.05	0.10	331.9	295.45	1E-09	2.8	n	n	74
47	SIMVASTATIN	2253	0.73	0.00	418.57	418.57	3E-10	4.68	n	n	21
48	HYDROCHLOROTHIAZIDE	2251	1.00	0.00	297.73	297.73	4E-12	-0.1	a	7	72
49	SOTALOL (hydrochloride)	2095	0.75	0.00	308.82	272.4	3E-14	0.24	b	8.3	22
50	DOXYCYCLINE	1773	0.35	0.00	462.46	462.46	5E-24	-0.2	a	7.7	52

*After the elimination of some compounds as described in the text.

sewage treatment plants in Australia, Germany, and the USA. The final model was used to predict the concentration of PhAC's in raw sewage and their distribution through sewage treatment and discharge to surface water. The model takes into account the total mass of each compound prescribed on an annual basis per capita, physiological metabolism and excretion information, physical and chemical compound characteristics (fugacity). Sewage samples were collected from each of the treatment plants as 24 hour composites. Samples were refrigerated as collected and delivered to the lab for processing within 24 hours. Analyses were performed by filtration, extraction, derivatization, and GC/MS analysis as described in detail elsewhere (3)

2.1.2 Predicted concentrations in sewage

Application of the model to each of the top 50 compounds resulted in prediction that 30 of the pharmaceuticals and the two tested metabolites should be present in raw sewage at concentrations of 1 μg/L or greater (Table 2.2, Column 3). The nine highest compounds predicted, each with concentrations greater than 5 μg/L, were: paracetamol (76 μg/L), metformin (37 μg/L), amoxicillin (14 μg/L), cephalexin (11 μg/L), gemfibrozil and oxipurinol (each 8 μg/L), chlorothiazide (6 μg/L), naproxen and ranitidine (each 5 μg/L). The next 18 compounds with concentrations predicted to be between 1 and 5 μg/L were: lactulose, valproate, salicylic acid, allopurinol, quinine, cefaclor, phenoxymethylpenicillin, sulphamethoxazole, gliclazide, methyldopa, frusemide, atenolol, flucloxacillin, cimetidine, ketoprofen, roxithromycin, irbesartin, dicloxacillin, and hydrochlorothiazide.

Application of the model to effluent from the example activated sludge-treated secondary STP predicted that 14 of the compounds would be expected at a concentration of 1 μg/L or greater. Those compounds were paracetamol (35 μg/L), metformin (22 μg/L), amoxycillin and chlorothiazide, (each 5 μg/L), ranitidine, gemfibrozil and oxipurinol (each 4 μg/L), naproxen (3 μg/L), salicylic acid,

allopurinol and frusemide (each 2 μg/L), cefaclor, gliclazide, methyldopa, atenolol, roxithromycin and hydrochlorothiazide (each 1 μg/L) (Table 2.2, Column 6). Among the remainder of the 52 compounds, 23 are predicted to appear in secondary effluent at concentrations between 0.1 and 1 μg/L and 6 more between 0.01 and 0.1 μg/L.

2.1.3 Removal Mechanisms

The percent removals presented in Table 2.2 (Columns 7, 8, 9), refer to the amounts removed by the aggregate of biodegradation and partitioning to sludges on the basis of the original amount present in

Table 2.2. Australian Top50* Prescription Pharmaceuticals: Predicted concentrations in primary clarifier, aeration tank and secondary clarifier.

No.	Name	Influent (μg/l)	Primary Effluent (μg/l)	Aeration Tank Effluent (μg/l)	Clarifier Effluent (μg/l)	Removal to sludge (%)	Biodegrad. (%)	removal total (%)
1	PARACETAMOL	76	74	38	35	3	53	56
2	METFORMIN (hydrochloride)	37	36	23	22	4	40	44
3	LACTULOSE	1	1	3.E-01	3.E-01	1	80	81
4	AMOXYCILLIN	14	14	6	5	2	64	66
5	RANITIDINE (hydrochloride)	5	5	4	4	5	18	23
6	CEPHALEXIN	11	11	4	3	2	72	74
7	NAPROXEN	5	5	2	2	3	55	58
8	VALPROATE (sodium)	1	1	6.E-01	5.E-01	2	62	65
9	ASPIRIN	1.E-03	1.E-03	6.E-04	5.E-04	3	50	53
9b	SALICYLIC ACID (ex-aspirin)	4	4	2	2	4	42	46
10	GEMFIBROZIL	8	8	7	4	8	47	55
11	ALLOPURINOL	3	3	2	2	4	42	46
11b	OXIPURINOL (ex-allopurinol)	8	8	5	4	4	43	46
12	SULPHASALAZINE	9.E-04	9.E-04	8.E-04	7.E-04	5	20	25
13	IBUPROFEN	9.E-01	9.E-01	5.E-01	5.E-01	4	49	52
14	CHLOROTHIAZIDE	6	6	5	5	5	20	25
15	QUININE (sulphate)	1	5.E-01	4.E-01	4.E-01	5	22	27
16	ERYTHROMYCIN	2.E-01	2.E-01	3.E-01	2.E-01	13	7	20
17	CEFACLOR	4	4	1	1	2	64	67
18	CARBAMAZEPINE	2.E-01	2.E-01	1.E-01	1.E-01	6	33	39
19	VERAPAMIL (hydrochloride)	8.E-01	8.E-01	8.E-01	8.E-01	6	11	16
20	MOCLOBEMIDE	5.E-02	5.E-02	4.E-02	4.E-02	5	18	24
21	PHENOXYMETHYLPENICILLIN	1	1	6.E-01	5.E-01	2	65	67
22	DILTIAZEM (hydrochloride)	1.E-01	1.E-01	9.E-02	9.E-02	4	30	35
23	SULPHAMETHOXAZOLE	1	1	9.E-01	9.E-01	5	22	27
24	GLICLAZIDE	1	1	1	1	5	23	29
25	METHYLDOPA	3	3	1	1	3	50	53
26	METOPROLOL (tartrate)	9.E-02	9.E-02	6.E-02	5.E-02	4	39	42
27	FRUSEMIDE	2	2	2	2	5	15	20
28	ATENOLOL	3	2	1	1	3	52	55
29	FLUCLOXACILLIN	1	1	9.E-01	9.E-01	4	38	41

30	CIMETIDINE	1	1	9.E-01	9.E-01	4	27	32
31	KETOPROFEN	1	1	6.E-01	5.E-01	3	47	51
32	PHENYTOIN	2.E-04	2.E-04	2.E-04	2.E-04	6	25	32
33	DICLOFENAC	2.E-01	2.E-01	2.E-01	1.E-01	7	24	30
34	CODEINE (phosphate)	2.E-01	2.E-01	1.E-01	1.E-01	5	18	23
35	CLAVULANIC ACID	6.E-01	6.E-01	2.E-01	2.E-01	2	76	78
36	ROXITHROMYCIN	1	1	2	1	10	4	14
37	IRBESARTAN	2	8.E-01	2	2.E-02	50	49	99
38	SERTRALINE	3.E-03	3.E-03	4.E-03	3.E-03	10	16	26
39	DICLOXACILLIN	1	1	7.E-01	7.E-01	5	27	32
40	METRONIDAZOLE	4.E-01	4.E-01	3.E-01	3.E-01	4	34	38
41	CAPTOPRIL	7.E-01	7.E-01	2.E-01	2.E-01	2	68	70
42	TRIMETHOPRIM	6.E-01	6.E-01	5.E-01	4.E-01	5	25	30
43	ISOSORBIDE MONONITRATE	1.E-04	1.E-04	8.E-05	8.E-05	4	41	45
44	NIZATIDINE	8.E-01	8.E-01	7.E-01	7.E-01	5	17	22
45	TIAPROFENIC ACID	7.E-01	7.E-01	4.E-01	4.E-01	3	47	50
46	DOTHIEPIN (hydrochloride)	1.E-01	1.E-01	1.E-01	8.E-02	9	19	27
47	SIMVASTATIN	9.E-01	5.E-01	9.E-01	3.E-02	37	59	97
48	HYDROCHLOROTHIAZIDE	1	1	1	1	5	16	22
49	SOTALOL (hydrochloride)	7.E-01	7.E-01	5.E-01	4.E-01	4	39	43
50	DOXYCYCLINE	3.E-01	3.E-01	3.E-01	3.E-01	5	22	26

*After the elimination of some compounds as described in the text.

raw sewage. Overall compound removals are predicted to range from a minimum of 14% to as much as 99%. Significant removal should occur in both the aeration tank and the secondary clarifier, with individual variations dependant on the respective compound characteristics. It can be shown that some compounds were predicted to have a greater total concentration in aeration tank effluent (prior to secondary clarification) than in primary tank effluent. These were compounds that are not quickly biodegraded and are highly lipophilic, thus being accumulated in the high biomass concentration of the aeration tank. For these compounds, a significant fraction will be carried into waste secondary sludges.

In this study, examination of the factors that accounted for removal in the secondary treatment process, in combination with the parameter ranges reported or estimated for the compounds, showed that removal was generally predicted to be dominated by biodegradation. The exceptions occurred where the compound had an exceedingly high Log D_{pH} value combined with a high or medium-range biodegradation half-life.

The influence of differences in partitioning coefficients and aerobic biodegradation rates are illustrated in Figure 2.2. Four compounds were selected to illustrate the range of typical compound distribution and fate. Ketoprofen is an example of a compound with low Log $D_{6.5}$ (-1.91) and low

biodegradation half-life (17 h). It is effectively removed by aeration treatment, but not significantly by partitioning. Roxithromycin has a mid-range Log $D_{6.5}$ (2.75), though it has a very high aerobic biodegradation half-life (362 h) and was removed much less significantly in the aeration tank. In fact the aeration tank effluent was predicted to carry a higher concentration of roxithromycin than the primary effluent due to the elevated biosolids concentration. Irbesartan has a particularly high Log $D_{6.5}$ (5.31) and a medium-low biodegradation half-life (28 h). It was predicted to be significantly removed by both primary and secondary settling. It was further predicted to exhibit an analogous concentration increase to that of roxithromycin in the aeration tank effluent.

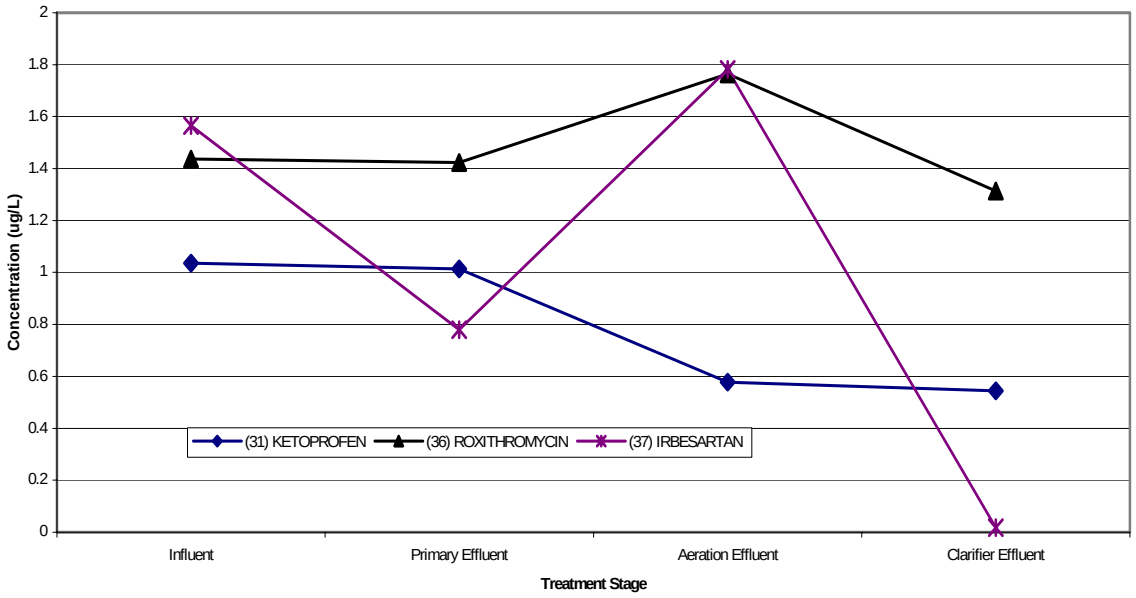


Figure 2.2 Comparison of partitioning and biodegradation rates: Concentrations of Ketoprofen (#31), Irbesartan (#37) and Roxithromycin (#36) in various treatment effluents

2.1.4 Explanation of anomalies

The concentrations of a small number of compounds, such as paracetamol and aspirin, are expected to be significantly underestimated due to the fact that these drugs may be purchased from non-pharmacy sources such as supermarkets (and hence, go uncounted in this study). Other sources of these compounds not considered (and of unknown significance) include international visitors and internet sales.

In comparison to data reported from Europe, an obvious omission from the “top 50” pharmaceuticals of this study is clofibrate and its principal metabolite clofibric acid. This has been shown to be present in most investigations of pharmaceutical residuals elsewhere in the world (Daughton and

Ternes, 1999). The prescription rate of clofibrate has been gradually decreased in Australia with published statistics showing dramatic preference for the alternate drug, gemfibrozil (Australian Commonwealth Department of Health and Aged Care (CDHFS), 1999). As a result, concentrations of clofibric acid in STPs are likely to be very low. However, clofibric acid's demonstrated environmental persistence (Buser *et al.*, 1998a) may result in a continuing measurable concentration in the Australian aquatic environment.

2.1.5 Predicted Concentrations in Sludge

A recent review published by the UK Environment Agency noted that no quantitative data were found on concentrations of pharmaceuticals in sludge, although this is a potential route for lipophilic substances to the terrestrial environment (Ayscough *et al.*, 2000). Principal reasons for the lack of such data are likely to be inherent difficulties associated with the laboratory extraction and the analysis of sludge samples.

Fugacity-based modelling provides a promising method for estimating concentrations of likely target compounds in sludges.

The concentrations of the compounds predicted to occur on both a wet and dry sludge basis were estimated by extending the fugacity model further to calculate the concentrations associated with both the aqueous and the biomass components of the sludge streams. In doing so, I have considered the "sludge stream" to be the pipeline between the primary tank and any later sludge tanks rather than the sludge tanks themselves. The transport and transformation mechanisms, normally considered with the fugacity approach to chemical fates and distributions in a STP, include the various exiting streams along with biodegradation and volatilisation. Each of these is assigned a fugacity rate parameter (D) as described elsewhere (Khan & Ongerth, 2004). The fugacity of the primary sludge stream (f_{PS}) is then:

$$f_{PS} = \frac{f_P \times D_{PS-IN}}{D_{PSB} + D_{PSV} + D_{PS-OUT}}$$

where f_P is the fugacity in the primary tank, $D_{PS\text{-}IN}$ is the flow rate into the primary sludge stream pipeline, D_{PSB} is the loss due to biodegradation, D_{PSV} is the loss due to volatilisation from the pipeline and $D_{PS\text{-}OUT}$ is the flow rate out of the pipeline.

Given that the residence time in the pipeline is short, I have assumed that D_{PSB} and D_{PSV} are negligible. It was then reasonable to further assume $D_{PS\text{-}IN} = D_{PS\text{-}OUT.}$ The fugacity relationship then simplifies to $f_{PS} = f_{P.}$

The chemical concentrations in the aqueous and biomass components of the primary sludge stream are then given by:

$$C_{aqueous} = f_P \times Z_W$$

$$C_{biomass} = f_P \times Z_B$$

where Z_W and Z_B are the fugacity capacities of the chemical in water and biomass respectively. These relationships provide a useful prediction of the chemical concentrations in the filtrate and in the solids of a sludge sample that have been separated from the liquid phase by filtration. The chemical concentration in the wet sludge was calculated as the weighted average of these concentrations according to the sludge biomass concentration. The same relationships can be used for the secondary sludge tank and corresponding pipeline.

Application of the model to each of the top 50 compounds using chemical and physical properties (Khan and Ongerth, 2004) and system characteristics resulted in the predicted concentrations shown in Table 2.3.

Table 2.3. Pharmaceutical residuals in raw influent, primary and secondary sludge; and percentages of residual removal to primary, secondary and total sludge.

		Influent	Concentration in primary sludge			Concentration in secondary sludge		
No.	Name	Aqueous (µg/l)	Aqueous (µg/l)	Dry (µg/kg)	Wet[a] (µg/kg)	Aqueous (µg/l)	Dry (µg/kg)	Wet[a] (µg/kg)
1	PARACETAMOL	76	74	102	77	35	49	36
2	METFORMIN (hydrochloride)	37	36	29	37	22	18	22
3	LACTULOSE	1	1	1	1	3.E-01	2.E-01	3.E-01
4	AMOXYCILLIN	14	14	11	14	5	4	5
5	RANITIDINE (hydrochloride)	5	5	5	5	4	4	4
6	CEPHALEXIN	11	11	9	11	3	3	3
7	NAPROXEN	5	5	11	5	2	5	2
8	VALPROATE (sodium)	1	1	6	2	5.E-01	2	5.E-01
9	ASPIRIN	1.E-03	1.E-03	8.E-04	1.E-03	5.E-04	4.E-04	5.E-04
9b	SALICYLIC ACID (ex-aspirin)	4	4	3	4	2	2	2
10	GEMFIBROZIL	8	7	1725	59	4	873	9
11	ALLOPURINOL	3	3	3	3	2	1	2
11b	OXIPURINOL (ex-allopurinol)	8	8	7	8	4	4	4
12	SULPHASALAZINE	9.E-04	9.E-04	7.E-04	9.E-04	7.E-04	6.E-04	7.E-04
13	IBUPROFEN	9.E-01	9.E-01	14	1	5.E-01	7	5.E-01

14	CHLOROTHIAZIDE	6	6	5	6	5	4	5
15	QUININE (sulphate)	5.E-01	5.E-01	2	6.E-01	4.E-01	1	4.E-01
16	ERYTHROMYCIN	2.E-01	2.E-01	51	2	2.E-01	45	4.E-01
17	CEFACLOR	4	4	3	4	1	1	1
18	CARBAMAZEPINE	2.E-01	2.E-01	9	4.E-01	1.E-01	6	1.E-01
19	VERAPAMIL (hydrochloride)	8.E-01	8.E-01	5	1	8.E-01	4	8.E-01
20	MOCLOBEMIDE	5.E-02	5.E-02	1.E-01	5.E-02	4.E-02	1.E-01	4.E-02
21	PHENOXYMETHYLPENICILLIN	1	1	1	1	5.E-01	4.E-01	5.E-01
22	DILTIAZEM (hydrochloride)	1.E-01	1.E-01	1	1.E-01	9.E-02	6.E-01	9.E-02
23	SULPHAMETHOXAZOLE	1	1	1	1	9.E-01	9.E-01	9.E-01
24	GLICLAZIDE	1	1	33	2	1	25	1
25	METHYLDOPA	3	3	2	3	1	1	1
26	METOPROLOL (tartrate)	9.E-02	9.E-02	7.E-02	9.E-02	5.E-02	4.E-02	5.E-02
27	FRUSEMIDE	2	2	2	2	2	2	2
28	ATENOLOL	3	2	2	2	1	1	1
29	FLUCLOXACILLIN	1	1	1	1	9.E-01	7.E-01	9.E-01
30	CIMETIDINE	1	1	1	1	9.E-01	8.E-01	9.E-01
31	KETOPROFEN	1	1	8.E-01	1	5.E-01	4.E-01	5.E-01
32	PHENYTOIN	2.E-04	2.E-04	1.E-02	6.E-04	2.E-04	1.E-02	2.E-04
33	DICLOFENAC	2.E-01	2.E-01	13	6.E-01	1.E-01	10	2.E-01
34	CODEINE (phosphate)	2.E-01	2.E-01	1.E-01	2.E-01	1.E-01	1.E-01	1.E-01
35	CLAVULANIC ACID	6.E-01	6.E-01	5.E-01	6.E-01	2.E-01	1.E-01	2.E-01
36	ROXITHROMYCIN	1	1	160	6	1	149	2
37	IRBESARTAN	2	2.E-01	7821	235	1.E-02	576	3
38	SERTRALINE	3.E-03	3.E-03	5.E-01	2.E-02	3.E-03	4.E-01	5.E-03
39	DICLOXACILLIN	1	1	8.E-01	1	7.E-01	6.E-01	7.E-01
40	METRONIDAZOLE	4.E-01	4.E-01	4.E-01	4.E-01	3.E-01	3.E-01	3.E-01
41	CAPTOPRIL	7.E-01	7.E-01	5.E-01	7.E-01	2.E-01	2.E-01	2.E-01
42	TRIMETHOPRIM	6.E-01	6.E-01	6.E-01	6.E-01	4.E-01	5.E-01	5.E-01
43	ISOSORBIDE MONONITRATE	1.E-04	1.E-04	1.E-04	1.E-04	8.E-05	7.E-05	8.E-05
44	NIZATIDINE	8.E-01	8.E-01	7.E-01	8.E-01	7.E-01	5.E-01	7.E-01
45	TIAPROFENIC ACID	7.E-01	7.E-01	6.E-01	7.E-01	4.E-01	3.E-01	4.E-01
46	DOTHIEPIN (hydrochloride)	1.E-01	1.E-01	13	5.E-01	8.E-02	10	1.E-01
47	SIMVASTATIN	9.E-01	3.E-01	2938	88	3.E-02	288	2
48	HYDROCHLOROTHIAZIDE	1	1	1	1	1	9.E-01	1
49	SOTALOL (hydrochloride)	7.E-01	7.E-01	6.E-01	7.E-01	4.E-01	3.E-01	4.E-01
50	DOXYCYCLINE	3.E-01	3.E-01	3.E-01	3.E-01	3.E-01	2.E-01	3.E-01

[a] "Wet" sludge is as withdrawn from the primary or secondary clarifier, or digester.

All of the compounds investigated were predicted to be present in wet primary sludge at concentrations at least as high as the concentrations of raw sewage. The more lipophilic compounds, characterised by a Log D_{pH} value greater than 2 are predicted in wet primary sludge at significantly higher concentrations than in raw influent. For example, irbesartan, with a Log $D_{6.5}$ of 5.31, was predicted to be amplified by a factor greater than 100 to a concentration of 235 μg/kg in wet primary sludge.

The relative amplifications of pharmaceutical concentrations in (fresh) wet primary and secondary sludge is shown for the top-20 dispensed pharmaceuticals and two metabolites (Figure 2.3).

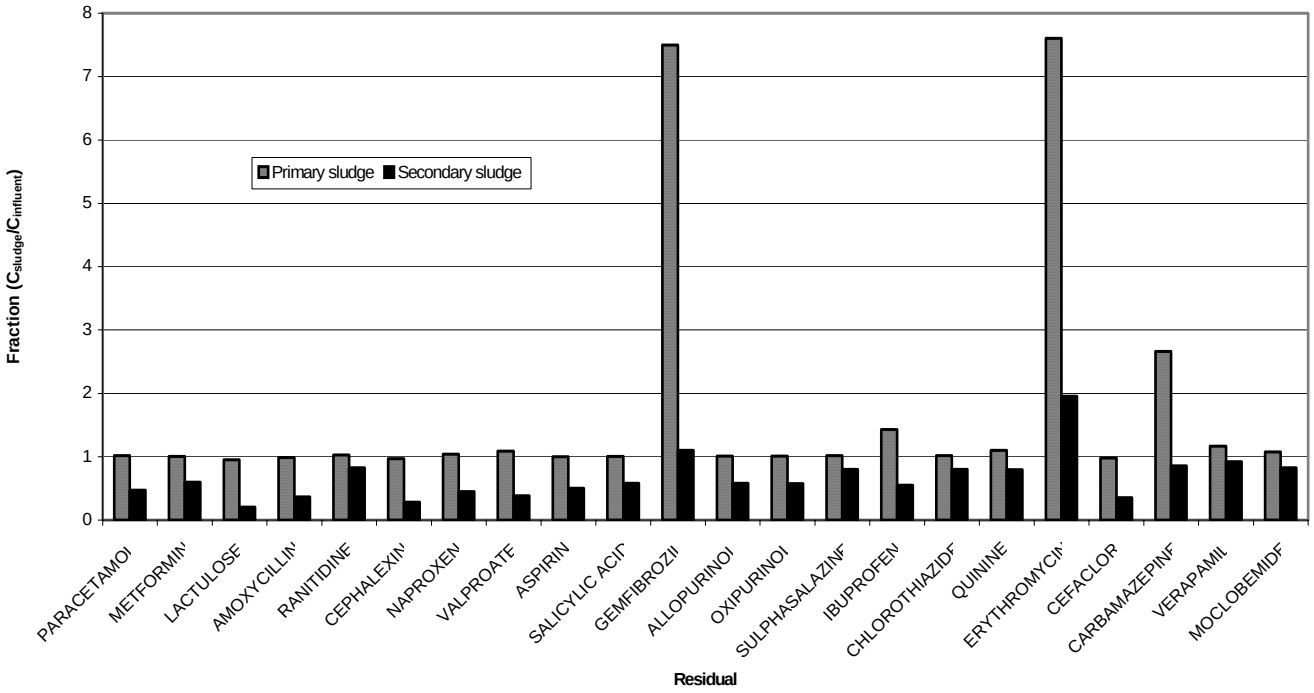


Figure 2.3. Concentrations in primary and secondary sludge as a fraction of raw influent concentration (pH 6.5).

The predicted amplification of the lipophilic chemicals is even more pronounced in the dry sludge solids with irbesartan predicted at 7.8 mg/kg of dry primary sludge. Many of the more hydrophilic compounds are predicted at lower *w/w* concentrations in dried sludge than in wet sludge. In practice, this effect should only be observed in dewatered sludges (eg. by filtration) rather than air dried ones. Predicted concentrations in secondary sludge were consistently lower than were primary sludge concentrations. One of the most notable reductions was predicted for gemfibrozil due to the combination of a large Log $D_{6.5}$ (3.06) resulting in significant loss to primary sludge and a short biodegradation half-life (16 h) resulting in significant degradation in the aeration tank.

2.1.6 Variable adsorption to sluge

A study (Stuer-Lauridsen *et al.*, 2000) estimated the concentrations of furosemide, ibuprofen, oxytetracycline and ciprofloxacin in sewage sludge by Equation 1.

$$C_{sludge} = \frac{M_{act}}{V_w / K_d + M_{sludge}} \qquad \textbf{Equation 1}$$

where M_{act} is the annual consumption of active compound, V_w is the total annual wastewater volume, M_{sludge} is the total annual sludge production and K_d is the sludge-water partition coefficient. Three sets of K_d data were used: 1) K_d as estimated by a QSPR program, 2) K_d as estimated by a QSPR program and corrected for pK_a and 3) an experimentally observed K_d. The predicted concentrations are outlined in Table 2.4. The authors noted that the uncorrected K_d values more closely resembled the experimental values than did the corrected K_ds. They suggested that this may indicate that sorption of the furosemide and ibuprofen ions may be more significant than the hydrophobic sorption of the non-ionised species. They concluded that the assumption that only the hydrophobic sorption is significant in the environment might not be justified indiscriminately for pharmaceuticals.

Table 2.4. Predicted concentrations (mg/kg) of pharmaceuticals in sewage sludge based on estimated and experimental partition coefficients (Stuer-Lauridsen *et al.*, 2000).

	Predicted and measured dry sludge concentrations (mg/kg).		
	K_d (QSPR)	K_d (QSPR corrected for pK_a)	K_d (experimental)
Furosemide	150	0.012	1470
Ibuprofen	34210	180	20330
Oxytetracycline	0.026	-	1990
Ciprofloxacin	0.00095	-	130

Four general mechanisms have been proposed to explain the observed, but under-predicted, sorption of ionisable compounds to nonaqueous phases (Westall *et al.*, 1985):

1. transfer of the neutral organic species from the bulk of the aqueous phase to the bulk of the nonaqueous phase
2. transfer of the ionic organic species with inorganic counterions to the bulk of the nonaqueous phase
3. transfer of the ionic organic species to the aqueous-nonaqueous interface with inorganic counterions in the aqueous phase
4. association of the organic species with specific functional groups of the nonaqueous phase.

Future improvements may be made to the model, by gaining a closer insight to the nature and significance of these mechanisms.

2.2 Conclusion

The calculations presented in this chapter, and the subsequently derived predicted concentrations, will serve as highly valuable tools in priority setting for further research work. The predicted concentrations are not intended to be precise, but should prove indicative of where analytical effort

should be invested. Once biodegradation rates of these compounds in sewage treatment are better understood and characterized, the predictive value of such a model will increase significantly.

Chapter 3--Measured Pharmaceutical Residuals in STP's

3.1 Overview

Sampling and analysis of sewage and sludge was conducted at seven sewage treatment plants in Australia, Germany and the USA to test the predictions generated in Chapter 2. Some of the sampling was conducted to determine the correspondence of PhAC behaviour in sewage to that long-established for the general organic fraction of municipal sewage. The primary data acquired from each of these studies are presented in this chapter.

All extractions, derivatisations and GC-MS methods were as described previously (Khan & Ongerth, 2002c). Only the specific details or any analytical variations are presented with results in this chapter.

3.2 General scan of sewage & sludge: Bondi STP

Non-target screening of sewage extracts was responsible for the earliest reported cases of pharmaceutical residues in effluents (Garrison *et al.*, 1976; Hignite & Azarnoff, 1977; Rogers *et al.*, 1986). Experimentally acquired mass spectra can now be easily and quickly compared to "standard" mass spectra in extensive electronic databases, for rapid and accurate identification.

Many suitable mass spectral databases are commercially available for this task including general databases such as those published by Wiley and the United States National Institute of Standards and Technology (NIST). More specialised databases are also available.

The Bondi STP is one of the three largest STPs in Sydney, serving a population of 480,000 people. It provides high-rate primary treatment and discharges 130 ML/day via a deep-water ocean outfall.

3.2.1 Analytical details

A single sewage grab sample (2L) was collected from the influent stream of the Bondi STP on Wednesday 26/05/99 after a period of 24 hr of dry weather. The sample was transported to the laboratory and processed on the same day. The sample was filtered and determined to have an initial pH of 6.6. Two extraction solutions (500 ml) were prepared from the filtered sample. One was adjusted to pH 2 and the other to pH 7. Both samples were extracted by C18 SPE and eluted with methanol.

A primary sludge sample was collected at a much later date (Tuesday 7/08/01) by staff at the Bondi STP. It was collected at approximately midnight, also under dry weather conditions. The sample was kept refrigerated until 9am the next morning, and then transported to the laboratory for immediate filtration followed by Soxhlet extraction.

Extracts were analysed by GC-MS on full scan mode. All other conditions were as described previously, (Khan & Ongerth, 2004). After carefully adjusting the integration parameters, the chromatograms were thoroughly searched for matches with the Wiley/NIST Registry of Mass Spectral Data (6th Edition). Only matches with an agreement of 90% or greater are reported.

3.2.2 Analytical data

Organic chemicals identified in the non-target analysis of raw sewage and primary sludge are presented in order of GC-MS elution (Table 3.1).

Table 3.1. Non-target raw sewage (26/05/99) & sludge analysis (7/08/01), Bondi STP (>90% match)

Compound	Ph 2	Ph 7	Sludge
2-(2-Butoxy-ethoxy)ethanol		✓	
Capric acid	✓	✓	✓
Methylcyclodecane	✓		
Cyclotetradecane		✓	
1-Pentadecene		✓	
Cyclopropane, nonyl-	✓		
2,6-Di-t-butyl-4-methylene-2,5-cyclohexadiene-1-one	✓		
Methyl laurate	✓		
Lauric acid	✓	✓	✓
Diethyl phthalate	✓	✓	
Ibuprofen	✓	✓	
Cyclopentadecane	✓		
Methylydihydrojasmonate95		✓	
1-Ethyl-2-Methyl cyclododecane	✓		
Methyl isomyristate	✓		
Methylmyristate		✓	
(Z)-4-Vinylcyclooctene		✓	
Cyclododecyne	✓		
(Z)6,(Z)9-Pentadecadien-1-ol	✓	✓	
7R,8S)-cis-anti-cis-7,8-Epoxytricyclo[7.3.0.0(2,6)]dodecane		✓	
Myristic acid	✓	✓	✓
N-Butylbenzenesulfonylamine	✓	✓	
1-Dodecanol, ethoxy-	✓		
Pentadecanoic acid	✓		✓
Tetradecanoic acid, 12-methyl-, (S)-	✓		
Versalide (Musk 36A)			✓
Caffeine	✓	✓	
Pentadecanol	✓		

Compound	Ph 2	Ph 7	Sludge
Isobutyl phthalate		✓	
1-Tetradecene		✓	
Methyl palmitoleate	✓	✓	
Cyclotetradecane	✓		
Methylpalmitate	✓	✓	
9-Hexadecenoic acid		✓	✓
Dibutylphtalate	✓	✓	
Palmitic acid	✓	✓	✓
Margaric acid (Heptadecanoic acid)	✓		
Methyl oleate	✓		
Methyl stearate	✓		
2(1H)-Naphthalenone, octahydro-4a,7,7-trimethyl-, trans-		✓	
2-cis-9-Octadecenyloxy ethanol		✓	
1-Octadecene		✓	
Palmitelaidic acid, TMS			✓
5-Chloro-3'-methyl-1'-phenylspiro(indoline-2,4'-(2)pyrazoline)-5'-one			✓
5-Chloro-2-(2,4-dichlorophenoxy)phenol (Triclosan)			✓
Methyl octadec-8-enoate		✓	
Oleic acid, methyl ester		✓	
Stearic acid, methyl ester		✓	
Linoleic acid			✓
Oleic acid	✓	✓	✓
Stearic acid		✓	✓
Palmitic amide	✓		
(R)-(-)-14-Methyl-8-hexadecyn-1-ol	✓		
14 -Pregnane	✓		
7,15-dimethoxy[2,2](2,6)pyridinophane		✓	
Phenanthren-1-carboxylic acid, 1,2,3,4,4A,9,10,10A-octahydro-7-isopropyl-1,4A-dimethyl		✓	
Butyl benzyl phthalate	✓	✓	
Oleic acid amide	✓		
Dehydroabietic acid	✓		
Dodecenyl succinic anhydride	✓		✓
2-Ethylhexyl diphenyl phosphate	✓	✓	
3α-Hydroxy-5β-androstane-17-one		✓	
Dicyclohexyl phthalate		✓	
Bis(2-ethylhexyl) phthalate	✓	✓	✓
Omega-Pentadecalactone (Muskolactone)	✓		
5α-Androstane-3,17-dione		✓	
Cis-8(10)-p-Menthen-9-ol			✓
Squalene		✓	✓
Cholesta-3,5-diene		✓	
5α-Cholestan-3-one			✓
Dihydrocholesterol		✓	
Cholesterol		✓	

3.3 General scan of extracted sewage components: Castle Hill STP

Non-target screening of raw influent was also undertaken at the Castle Hill STP. A number of significant method differences were incorporated with the intention of recovering a more diverse range of compounds.

Castle Hill Sewage Treatment Plant is a tertiary (additional phosphorous removal) STP with disinfection. It serves a population of 23,000 and operates with an average dry weather discharge of 4.4 ML/day. This effluent is discharged to a small inland creek (Cattai Creek). Since June 2000 UV irradiation, rather than chlorination, has been used for disinfection of effluent from Castle Hill STP.

Figure 3.1. Aerated activated sludge treatment tanks, Castle Hill STP

Figure 3.2. Secondary clarifier, Castle Hill STP

3.3.1 Analytical details

Composite raw sewage samples were collected during dry weather, during four consecutive 24-hour periods, from 21/12/01 – 24/12/01. The samples were filtered and then combined. The initial pH was determined to be 6.5. Two 200ml solutions were prepared for extractions at pH 2 and pH 7. SPE was undertaken with PS-DVB sorbent.

In the previous analysis large sections of the total ion chromatogram were swamped with considerable quantities of common long chain fatty acids. This problem was somewhat resolved by gentle derivatisation with BSTFA at room temperature for 30 minutes before analysis. This had the effect of increasing the volatility of these compounds, resulting in earlier elution and narrower peaks.

Extracts were analysed by GC-MS on full scan mode and the chromatograms were searched for matches with the Wiley/NIST Registry of Mass Spectral Data (6th Edition). This library was most suitable for this task as it features many common TMS derivatives. Only matches with an agreement of 90% or greater are reported.

3.3.2 Analytical data

Species identified in the non-target analysis of raw sewage are presented in order of GC-MS elution (Table 3.2). In cases where TMS derivatives were identified, the number of TMS groups is indicated.

Table 1.2. Non-target screening of raw sewage at Castle Hill STP (>90% match)

Compound	pH 2	pH 7
Phosphoric acid (3TMS)	✓	
Phenylacetic acid (1TMS)	✓	✓
5-ethyl-3,12-dioxatricyclo[4.4.2.0(1,6)]dodecan-4-one	✓	
2-methoxy-10-oxo-5,11a-dimethyl-7,8-dihydrophenanthrene		✓
3-Phenylpropionic acid (1 TMS)	✓	✓
Decanoic acid (1 TMS)	✓	✓
cis-4-hydroxy-cyclohexylcarboxylic acid (2TMS)	✓	
3-Hydroxybenzoic acid (2TMS)	✓	
p-Methoxybenzoic acid (1TMS)		✓
p-hydroxybenzoic acid (2TMS)	✓	
Cinnamic acid (1TMS)		✓
4-Hydroxyphenylacetic acid (2TMS)	✓	
4-Hydroxyphenylethanol (2TMS)		✓
Ibuprofen (1TMS)		✓
1,1,4a,8a.α-tetramethyl-7-oxoperhydrophenanthrene		✓
Vanillyl alcohol (2TMS)		✓
Lauric acid (1TMS)	✓	✓
Propylparaben (1TMS)		✓
m-Hydroxyphenylpropionic acid (2TMS)	✓	✓
Acetaminophen (1 TMS)	✓	✓
4- Hydroxyphenylpropionic acid (2TMS)	✓	
Triethylene glycol (2TMS)		✓
Isovanillic acid (2TMS)	✓	
3-Vanillylpropanol (2TMS)		✓
Homovanillic acid (2TMS)	✓	
Azelaic acid (2TMS)	✓	
Myristic acid (TMS)	✓	✓
Caffeine	✓	✓
m-Hydroxyphenylhydracrylate (3TMS)	✓	

Compound	pH 2	pH 7
Vanillylpropionic acid (2TMS)		✓
(2-Hydroxyphenyl)pentanoic acid (2TMS)		✓
m-Coumaric acid (2TMS)	✓	
Vanillylacetic acid (2TMS)	✓	
p-Coumaric acid (TMS)	✓	
1-Hexadecanol (1TMS)	✓	
Iminostilbene	✓	✓
Palmitic acid (TMS)	✓	✓
Palmitoleic acid (TMS)	✓	✓
Ferulic acid (TMS)	✓	✓
Heptadecanoic acid (TMS)	✓	✓
2,2',3,4,4',5-Hexachlorobiphenyl		✓
Z-9-Octadecen-1-ol (TMS)	✓	✓
Naproxen metabolite (2TMS)		✓
Linolic acid (TMS)		✓
Oleic acid (TMS)	✓	✓
11-cis-Octadecenoic acid (TMS)	✓	✓
Stearic acid (TMS)	✓	✓
Dehydroabietic acid (TMS)	✓	
11-Hydroxycephalotaxine	✓	
5α-Androstan-3α-ol-17-one (TMS) (Androsterone)	✓	✓
11-keto-etiocholanolone (TMS)		✓
Squalene	✓	✓
5α-Cholest-7-ene	✓	✓
Cholesterol (TMS)	✓	✓

A detailed discussion of the analytical results obtained in this study are presented in section 4.3.

3.4 Determination of variation among grab-samples

Sewage is a highly dynamic medium with rapid fluctuation in both flow rate and composition. Accordingly, it was necessary to gain an understanding of the representativeness of a single sewage grab-sample. Some variation was expected in the analysis of a series of samples collected from the same source within a few minutes of each other. This variation may be the result of varying sewage content over this short period. It may also result from variations occurring during the extraction and analysis of samples. The grab-sample variation analysis was undertaken with samples collected at the Castle Hill STP. A description of this STP was given in section 3.3.

3.4.1 Analytical details

Five raw sewage samples (each 1L) were manually extracted from the transfer line between the grit channels and the primary sedimentation tank. Sampling was undertaken on a weekday (Tuesday 11 September 2001) and commenced at 12 noon. All samples were collected within a period of 10 min.

Samples were filtered and extracted by SPE with PS-DVB cartridges at pH 2 and pH 7. They were then derivatised and analysed by GC-MS in SIM mode.

3.4.2 Analytical data

The results of the grab-sample variation analysis are displayed in Table 3.4.

Table 3.4. Short-term variation of pharmaceutical concentration (μg/l) among sewage grab-samples

Pharmaceutical	1	2	3	4	5	Mean & std. Dev.	Coeff. of variation %
Salicylic acid	8.79	8.90	8.83	9.26	8.63	8.88 ± 0.23	3
Ibuprofen	2.24	2.00	2.25	2.43	2.62	2.31 ± 0.23	10
Paracetamol	132.5	176.7	146.6	149.3	135.3	148.1 ± 17.5	12
Metronidazole	0.25	0.58	n.d.	0.45	n.d.	0.43 ± 0.17	40
Methamphetamine	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	
Gemfibrozil	1.58	1.54	1.47	1.45	1.48	1.50 ± 0.06	4
Captopril	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	
Naproxen	4.26	3.42	3.84	3.71	3.97	3.84 ± 0.31	8
Methadone	0.08	n.d.	0.04	0.10	0.03	0.06 ± 0.03	50
Ketoprofen	0.13	0.12	0.12	0.14	0.13	0.13 ± 0.01	8
Phenytoin	0.20	0.12	0.16	0.19	0.23	0.18 ± 0.04	22
Carbamazepine	0.32	0.23	0.23	0.28	0.26	0.26 ± 0.04	15
Morphine	0.48	0.47	0.51	0.42	0.70	0.52 ± 0.11	21

n.d.: not detected.

A detailed discussion of the analytical results obtained in this study are presented in section 4.4.

3.5 Diurnal analysis at Castle Hill STP, Sydney

This study was devised to provide information on the variation of concentration of pharmaceutical residues over a 24-hour cycle. The analysis was undertaken with samples collected from the Castle Hill STP. A description of this STP was given in section 3.3.

3.5.1 Analytical details

All samples were 1L grab samples collected from the STP grit chamber over a 24-hour period (7am, Tuesday 11 Sep 2001 – 7am, Wednesday 12 Sep 2001). The first three samples (7am, 11 am and 3pm, Tuesday) were manually collected and immediately filtered. They were then stored overnight at 4^{O}C. The remaining four samples (7pm and 11pm, Tuesday; and 3am and 7 am, Wednesday) were collected by means of an autosampler. They were stored in the refrigerated autosampler until collection the Wednesday morning and were filtered that afternoon. All samples were processed on the Wednesday. Samples were filtered and extracted by SPE with PS-DVB cartridges at pH 2 and pH 7. They were then derivatised and analysed by GC-MS in SIM mode.

3.5.1.1 Analytical data

Concentrations of pharmaceuticals and total organic carbon (TOC) in raw sewage samples collected every four hours over a 24-hour cycle are shown in Table 3.4.

Table 3.4. Diurnal analyses of pharmaceuticals (μg/l) and TOC (mg/l) at Castle Hill STP

Pharmaceutical	7am	11am	3pm	7pm	11pm	3am	7am
Salicylic acid	13.72	5.71	9.86	9.98	11.66	0.16	4.34
Ibuprofen	3.80	0.80	1.47	2.64	2.97	1.96	1.17
Paracetamol	162.65	86.63	177.82	112.88	142.01	145.77	123.94
Metronidazole	n.d.	n.d.	0.89	n.d.	n.d.	n.d.	n.d.
Methamphetamine	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Gemfibrozil	1.87	1.75	0.80	1.27	0.21	1.85	2.44
Captopril	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Naproxen	11.11	1.35	6.62	4.33	5.32	3.89	1.81
Methadone	0.03	0.01	0.05	n.d.	n.d.	n.d.	0.02
Ketoprofen	0.18	0.36	0.19	0.19	0.83	0.20	0.10
Phenytoin	n.d.	n.d.	n.d.	0.08	n.d.	0.02	0.03
Carbamazepine	n.d.	0.05	0.08	0.11	0.10	0.13	0.08
Morphine	n.d.	0.20	0.43	0.42	0.31	0.31	0.09
TOC (mg/l)	82	95	115	144	149	112	18

n.d.: not detected.

A detailed discussion of the analytical results obtained in this study are presented in section 4.5.

3.6 Seven-day sewage analysis, Castle Hill STP, Sydney

This analysis was undertaken in order to observe any variation that may have been perceptible over a 7-day cycle, as well as to provide averages and standard deviations of concentrations over comparable days. It was undertaken with samples collected from the Castle Hill STP. A description of this STP was given in section 3.3.

3.6.1 Sampling and analysis details

All samples in this analysis were 24-hour composites. The raw samples were collected with an autosampler from the screenings channel. Raw samples were collected over a 7-day period from Tuesday (18/12/01) to Monday (24/12/01). The raw samples from Saturday and Sunday were stored in the refrigerated autosampler ($4^{O}C$) until collected on the Monday morning. The primary and secondary composites were supplied by staff at the STP as surplus to their normal testing requirements. These were only available on weekday mornings; hence no primary and secondary results are available for Saturday and Sunday. Samples were filtered and extracted by SPE with PS-DVB cartridges at pH 2 and pH 7. They were then derivatised and analysed by GC-MS in SIM mode.

3.6.2 Analytical data

Concentrations of pharmaceuticals measured in raw (Table 3.5), primary treated (Table 3.6) and secondary treated (Table 3.7) sewage are shown below.

Table 3.5. Concentration of pharmaceuticals (μg/l) in raw sewage, Castle Hill STP

Pharmaceutical	Tue	Wed	Thu	Fri	Sat	Sun	Mon
Salicylic acid	11.95	11.36	10.19	17.96	18.25	7.22	15.68
Ibuprofen	2.59	2.77	2.20	3.13	2.84	2.16	2.91
Paracetamol	105.64	102.70	106.67	104.76	105.78	102.58	102.62
Metronidazole	n.d.	n.d.	n.d.	n.d.	n.d.	0.55	n.d.
Methamphetamine	0.19	0.07	0.11	0.18	0.44	0.13	0.36
Gemfibrozil	1.34	1.77	1.60	1.38	1.57	1.34	1.38
Captopril	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Naproxen	6.30	6.31	6.87	6.95	6.18	6.28	5.97
Methadone	n.d.	n.d.	n.d.	n.d.	0.07	n.d.	0.02
Ketoprofen	0.86	0.88	1.04	0.86	0.82	0.86	0.89
Phenytoin	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Carbamazepine	n.d.	0.59	n.d.	n.d.	n.d.	n.d.	n.d.
Morphine	0.21	0.21	0.19	0.25	0.46	0.22	0.46

n.d.: not detected.

Table 3.6. Concentration of pharmaceuticals (μg/l) in primary effluent, Castle Hill STP.

Pharmaceutical	Tue	Wed	Thu	Fri	Mon
Salicylic acid	5.21	4.88	5.71	6.41	8.40
Ibuprofen	2.77	2.02	1.93	2.14	2.38
Paracetamol	24.24	32.83	24.72	29.46	27.66
Metronidazole	n.d.	n.d.	n.d.	n.d.	n.d.
Methamphetamine	n.d.	n.d.	n.d.	0.15	0.22
Gemfibrozil	1.22	1.39	1.21	1.22	1.20
Captopril	n.d.	n.d.	n.d.	n.d.	n.d.
Naproxen	5.40	5.30	4.59	5.81	6.59
Methadone	n.d.	n.d.	n.d.	0.01	n.d.
Ketoprofen	1.01	0.88	1.32	1.01	0.87
Phenytoin	n.d.	n.d.	n.d.	n.d.	n.d.
Carbamazepine	n.d.	n.d.	n.d.	n.d.	n.d.
Morphine	0.17	0.19	0.17	0.20	0.20

n.d.: not detected.

Table 3.7. Concentration of pharmaceuticals (μg/l) in secondary effluent, Castle Hill STP.

Pharmaceutical	Tue	Wed	Thu	Fri	Mon
Salicylic acid	0.32	0.43	0.19	0.46	0.50
Ibuprofen	0.12	0.46	0.30	0.09	0.15
Paracetamol	0.64	n.d.	n.d.	0.20	0.33
Metronidazole	n.d.	n.d.	n.d.	n.d.	n.d.
Methamphetamine	n.d.	n.d.	n.d.	n.d.	n.d.
Gemfibrozil	n.d.	0.28	0.25	0.26	0.21
Captopril	n.d.	n.d.	n.d.	n.d.	n.d.
Naproxen	0.30	0.55	0.25	0.31	0.35

Methadone	n.d.	n.d.	n.d.	n.d.	n.d.
Ketoprofen	0.64	0.62	0.55	0.59	0.53
Phenytoin	n.d.	n.d.	n.d.	n.d.	n.d.
Carbamazepine	0.59	n.d.	n.d.	n.d.	n.d.
Morphine	0.02	0.02	0.02	n.d.	n.d.

n.d.: not detected.

A detailed discussion of the analytical results obtained in this study are presented in section 4.6.

3.7 Sewage and sludge analysis for Bondi STP, Sydney

This study was undertaken specifically to examine the degree of removal of compounds to primary sludge and the effect of primary sludge digestion. It was undertaken at the Bondi STP, which was described in Section 3.2.

3.7.1 Analytical details

Requests for samples were made to staff at the Bondi STP. Samples were collected from the raw influent, primary effluent, primary sludge and digested sludge. A duty operator collected the samples at approximately midnight, Tuesday 7/08/01. All samples were 2.5 L grab samples. After collection, samples were refrigerated overnight at the STP then collected early on the following morning. Samples were promptly transported to the laboratory and filtered. Aqueous filtrates were extracted by SPE with PS-DVB cartridges at pH 2 and pH 7. They were then derivatised and analysed by GC-MS in SIM mode. Solid residues of the primary sludge and digested sludge were extracted by Soxhlet extraction.

3.7.2 Analytical data

The concentrations of pharmaceuticals detected in raw influent (aqueous), primary effluent (aqueous), primary sludge (aqueous, dry, combined) and digested sludge (aqueous and dry) are shown in Table 3.8 (Khan & Ongerth, 2002).

Table 3.8 Concentration of pharmaceuticals in raw and primary sewage as well as primary and digested sludge from Bondi STP

Pharmaceutical	Raw Influent	Primary Effluent	Primary Sludge			Digested Sludge	
	aqueous (µg/l)	aqueous (µg/l)	aqueous (µg/l)	dry (µg/kg)	combined (µg/kg)	aqueous (µg/l)	dry (µg/kg)
Paracetamol	291.91	254.25	42.08	4535	178.14	2.17	0.0006
Naproxen	3.01	3.16	1.91	1022	32.58	0.11	0.0010
Salicylic acid	16.08	12.80	11.44	13748	423.87	1.40	0.0022
Gemfibrozil	3.00	1.24	1.53	1192	37.30	n.d.	n.d.

Ibuprofen	3.58	3.13	1.53	3988	121.18	6.45	0.0064
Metronidazole	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.0039
Ketoprofen	0.40	0.47	0.65	495	15.51	0.30	0.0011
Methadone	0.28	0.11	0.02	552	16.57	0.23	0.0016
Phenytoin	0.29	0.21	0.56	n.d.	0.56	0.01	0.0001
Carbamazepine	0.10	0.06	2.51	1731	54.44	5.98	0.0135
Morphine	1.39	0.88	1.28	n.d.	1.28	2.23	n.d.

n.d.: not detected.

A detailed discussion of the analytical results obtained in this study are presented in section 4.7.

3.8 Sewage analysis for three STPs in Berlin, Germany

A considerable quantity of the published data for pharmaceutical residues in sewage originates from Germany. Specifically, much of this data relates to STPs in Berlin and has been reported by Dr. Thomas Heberer and colleagues at The Technical University of Berlin. For the month of October 2001 Stuart Khan worked in the lab of Dr. Heberer at the Technical University of Berlin. During that time, a number of samples were simultaneously extracted and analysed by Dr. Heberer's group and Dr. Khan. The purpose of was to obtain data on PhAC's in Berlin sewage and STP's and to compare data obtained independent analytical methods applied to the same samples. Six major STPs treat the municipal sewage of Berlin. Three of them were tested in this analysis. They were Schönerlinde, Ruhleben and Falkenberg.

3.8.1 Analytical details

Samples were collected as 24-hour composites of raw influent and final effluent from all three of the selected Berlin STPs.

These samples were extracted on PS-DVB sorbent in Berlin. They were then packaged and sent by mail to Sydney, where they were eluted from the SPE tubes and analysed by GC-MS.

3.8.2 Analytical data

Concentrations of pharmaceuticals measured in raw influents and final effluents from Schönerlinde (SCH), Ruhleben (RUH) and Falkenberg (FAL) STPs are shown in Table 3.9.

Table 3.9. Influent and effluent concentrations for three Berlin STPs

Pharmaceutical	SCH		RUH		FAL	
	Raw	Effluent	Raw	Effluent	Raw	Effluent
Salicylic acid	87.24	0.21	108.64	0.33	183.71	0.15
Ibuprofen	12.82	0.21	11.15	0.09	16.69	0.18
Paracetamol	49.16	0.69	37.53	1.15	58.29	0.53

Metronidazole	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Methamphetamine	n.d.	n.d.	0.33	n.d.	n.d.	n.d.
Gemfibrozil	0.57	0.17	0.37	n.d.	0.65	0.15
Captopril	0.53	n.d.	0.55	0.08	0.65	n.d.
Naproxen	0.76	0.52	0.84	0.29	0.72	0.25
Methadone	0.08	0.04	n.d.	0.04	0.09	n.d.
Ketoprofen	n.d.	0.32	0.23	0.14	0.09	0.12
Phenytoin	0.46	0.15	n.d.	0.09	n.d.	0.16
Carbamazepine	6.39	5.26	3.89	3.02	8.39	8.04
Morphine	0.97	0.15	0.74	0.01	1.12	0.08

n.d.: not detected.

These results are compared with those obtained by German colleagues from the Technical University of Berlin in section 4.8.

3.9 Sewage & sludge analysis for the East Bay Municipal Utilities District, USA

Apart from Europe (specifically Germany), the other source of significant available data is the USA. Tests were undertaken at two large STPs in California. The first of these was East Bay Municipal Utilities District (EBMUD).

EBMUD is a large secondary STP. It services an effective population of 600,000 and treats an average dry-weather flow of 300 ML/day (80 MG/day). Treatment steps include pre-chlorination (for odour control), screening, grit removal, primary sedimentation, secondary aeration with high-purity oxygen activated sludge, final clarification, chorine disinfection and dechlorination. The sludge undergoes digestion, dewatering and composting. The effluent is discharged one mile off the East Bay shore through a deep-water outfall into San Francisco Bay.

3.9.1 Analytical details

Samples were collected from the various stages of the primary and secondary treatment processes. These were chosen to define the overall mass-balance of pharmaceutical residues. The sampling points are defined in Figure 3.3 and Table 3.10.

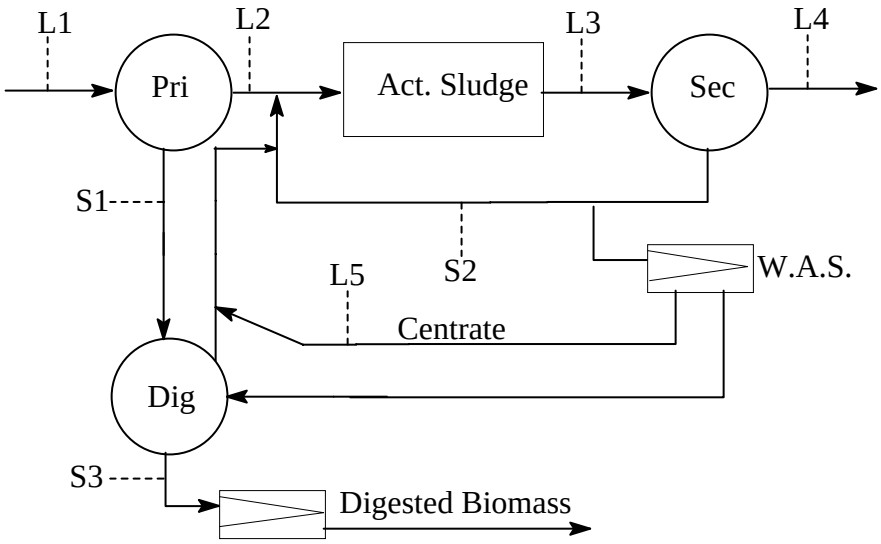


Figure 3.3. EBMUD Location of sampling points

Where possible, 24-hour composites were used. These were supplied from surplus to EBMUD routine analytical needs. Where composites were not available, grab samples were collected as defined in Table 3.10.

Table 3.10. Description of EBMUD samples.

Sample	Source	Type
L1	Primary influent	24 hr composite
L2	Primary effluent	Grab (noon)
L3	Secondary mixed liquor	Grab (noon)
L4	Effluent EPS	24 hr composite
L5	Dewatering centrate	Grab (noon)
S1	Primary sludge (PSLX)	Grab (noon)
S2	Secondary return activated sludge	Grab (noon)
S3	Digested sludge (DSLC)	24 hr composite

After filtration, both aqueous and biomass fractions of various samples were extracted for analysis. Aqueous phases were extracted using a commercial SPE disk extraction manifold, described previously (Khan, 2002). Empore SDB-XC SPE disks were used for this extraction process. Biomass fractions were extracted by accelerated by accelerated solvent extraction apparatus. All samples were evaporated to dryness and returned to Sydney for GC-MS analysis.

3.9.2 Analytical data

The results of this analysis are presented in two sections. First are the aqueous fractions of all samples (both liquid and sludge samples) in section 3.9.2.1. Following are the biomass fractions of the sludge samples in section 3.9.2.2.

3.9.2.1 Aqueous fractions

The concentrations of pharmaceuticals measured in the aqueous fractions of samples from EBMUD are shown in Table 3.11.

Table 3.11. Concentrations of pharmaceutical residues in aqueous fractions of EBMUD sewage samples (μg/l)

Pharmaceutical	L1	L2	L3	L4	L5	S1	S2	S3
Salicylic acid	15.73	21.74	0.79	1.08	0.68	32.05	1.65	8.09
Ibuprofen	3.68	4.73	n.d.	0.21	6.69	2.93	0.33	16.14
Paracetamol	1.48	n.d.	n.d.	1.33	n.d.	20.36	n.d.	n.d.
Metronidazole	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Methamphetamine	n.d.	3.93	n.d.	n.d.	3.19	n.d.	n.d.	n.d.
Gemfibrozil	0.33	0.44	0.79	1.29	3.68	0.09	0.64	5.89
Captopril	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Naproxen	1.10	5.12	n.d.	0.80	n.d.	n.d.	0.05	n.d.
Methadone	n.d.	n.d.	0.24	0.62	n.d.	0.29	n.d.	0.34
Ketoprofen	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Phenytoin	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Carbamazepine	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Morphine	1.09	1.29	1.42	n.d.	2.49	n.d.	2.65	2.39

n.d.: not detected.

3.9.2.2 Biomass fractions

The concentrations of pharmaceuticals measured in the biomass fractions of the EBMUD samples are shown in Table 3.12. The S1 (primary sludge biomass) sample was not able to be properly measured. The reason was that oils associated with the extracted sample separated from the solvent used in the GC-MS analysis. Two separate phases persisted after the derivatisation reaction. An attempted separate analysis of each phase did not yield usable results.

Table 3.12 Concentrations of PhAC residues in EBMUD sludge

Pharmaceutical	S1	S2	S3
Salicylic acid	not measurable	11.43	6.95
Ibuprofen	not measurable	n.d.	19.54
Paracetamol	not measurable	127.39	n.d.
Metronidazole	not measurable	n.d.	n.d.
Methamphetamine	not measurable	n.d.	n.d.
Gemfibrozil	not measurable	213.69	32.02
Captopril	not measurable	n.d.	n.d.

Naproxen	not measurable	17.96	n.d.
Methadone	not measurable	n.d.	n.d.
Ketoprofen	not measurable	n.d.	n.d.
Phenytoin	not measurable	n.d.	n.d.
Carbamazepine	not measurable	n.d.	n.d.
Morphine	not measurable	n.d.	n.d.

(n.d.: not detected. All measurements expressed as μg/kg dried residue)

A detailed discussion of the analytical results obtained in this study are presented in section 4.9.

3.10 Sewage and sludge analysis for San Jose/Santa Clara STP.

San Jose/Santa Clara STP is one of the largest sewage treatment facilities in California, Figure 3.4. It services an effective population of 1.26 million people. It has an average dry weather influent rate of 450 ML/day (120 MG/day). Approximately 65% of the flow originates from residential sources.

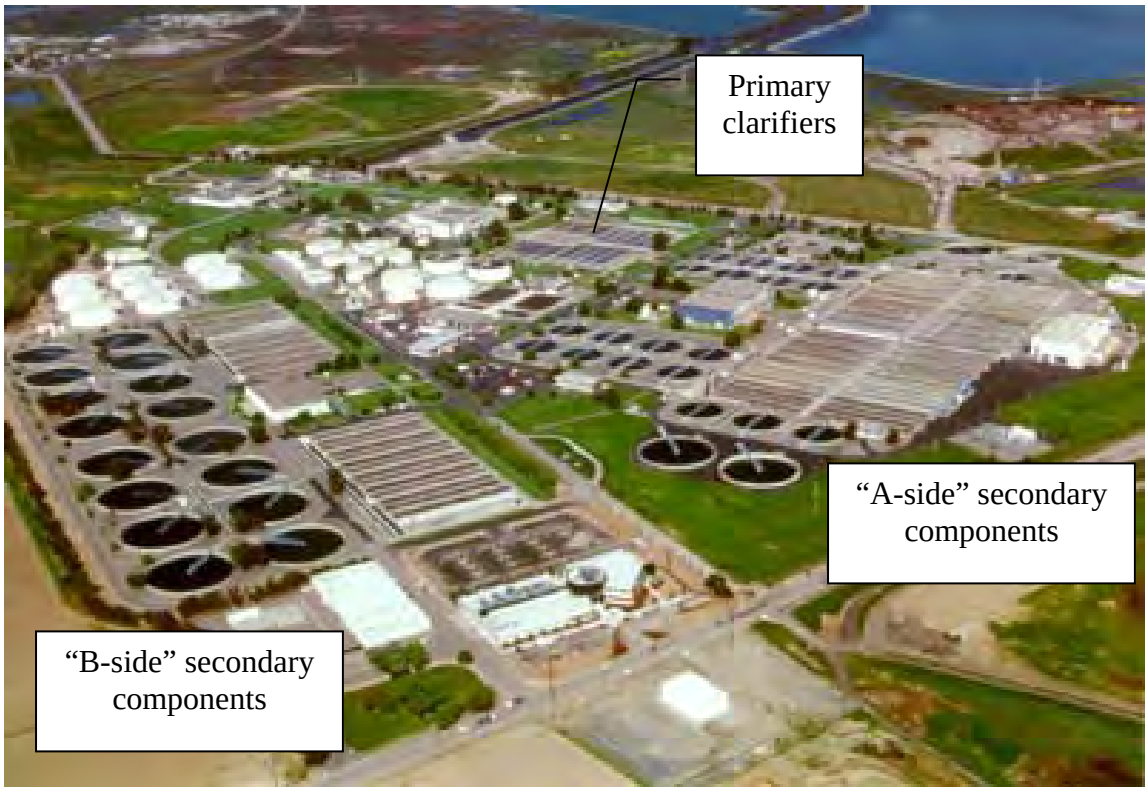


Figure 3.4. Aerial view of the San Jose/Santa Clara STP

The primary settling tanks are arranged in four groups of four. These are designated A, B, C & D. Hydraulically, they are maintained with as close to equal flow as possible and the primary effluent from the four groups is all combined in a single channel before being distributed to the aeration tanks. This keeps the influent to both of the secondary aeration parts (see below) of the STP as similar as

possible. The sludge from the A and B primaries is combined, as is the sludge for the C and D primaries. The A/B primary sludge is piped to digesters separately from the C/D primary sludge.

Two separate secondary aeration parts of the plant are similarly designated “A-Side” and “B-side”. Previously they have operated with different modes of nutrient removal. However, they currently both operate as biological nitrogen removal (BNR) plants and are being operated in parallel. The “A” and “B” labels in the secondary components of the plant do not refer to the alphabetical labels in the primary components. Following activated sludge treatment and secondary clarification, the suspended solids are further removed by filtration through a multi-layered filter bed of stone, sand and anthracite coal. The effluent is disinfected by chlorination before discharge through a slough into the southern-most tip of San Francisco Bay.

3.10.1 Analytical details

Samples of sewage and sludge were collected from numerous locations throughout the STP, Figure 3.5. Sampling sites, Table 3.13, were selected with an aim of providing a comprehensive picture of the presence and behaviour of pharmaceuticals throughout the treatment process.

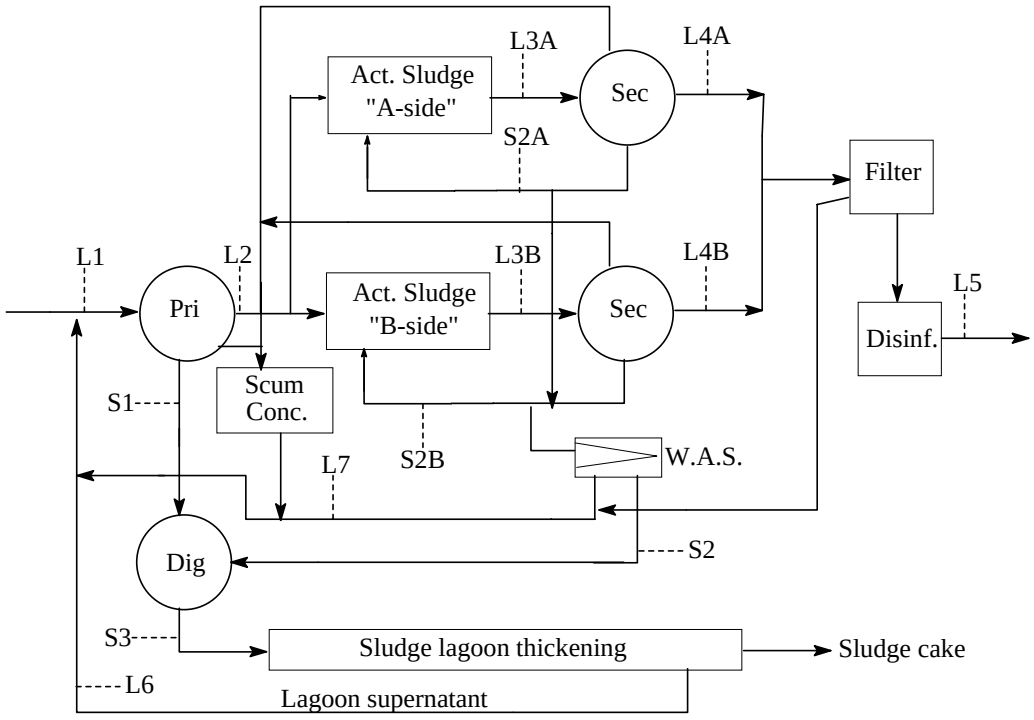


Figure 3.5. San Jose/Santa Clara STP schematic with sampling locations

Where possible, 24-hour composite samples were used in the analysis. Where composites were not available, grab samples were used. All composite samples were collected over 24 h commencing at

noon on Thursday November 1, 2001. All grab samples were 1L and were collected on Friday November 2, 2001.

Table 3.13 Description of samples from San Jose/Santa Clara STP

Sample	Source	Type
L1	Raw sewage	24 hr. composite
L2	Primary effluent	24 hr. composite
L3A	Mixed liquor "A-side"	grab
L3B	Mixed liquor "B-side"	grab
L4A	Secondary clarifier effluent "A-side"	24 hr. composite
L4B	Secondary clarifier effluent "B-side"	24 hr. composite
L5	Final effluent	24 hr. composite
L6	Lagoon supernatant	grab
L7	Concentrated sludge	grab
S1 "A/B"	Primary sludge A/B side	grab
S1 "C/D"	Primary sludge C/D side	grab
S2A	Return activated sludge "A-side"	grab
S2B	Return activated sludge "B-side"	grab
S3	Sludge to lagoon	grab

After filtration, both aqueous and biomass fractions of various samples were extracted for analysis. Aqueous phases were extracted using a commercial SPE disk extraction manifold, described previously (Khan, 2002). Empore SDB-XC SPE disks were used for this extraction process. An Accelerated Solvent Extraction apparatus was used to extract the biomass fractions. All samples were evaporated to dryness and transported to Sydney for GC-MS analysis.

3.10.2 Analytical data

The results of this analysis are presented in three sections according to the type of sample (liquid or sludge) and the fraction (aqueous or biomass) after filtration of sludge samples.

3.10.2.1 Liquid samples (aqueous fractions)

The concentrations of pharmaceuticals measured in the liquid samples from the San Jose/Santa Clara STP are shown in Table 3.14.

Table 3.14. Concentration of pharmaceuticals in liquid samples from San Jose/Santa Clara STP, (μg/l)

Pharmaceutical	L1	L2	L3A	L3B	L4A	L4B	L5	L6	L7
Salicylic acid	3.41	11.50	0.77	0.03	0.19	0.04	0.09	0.13	57.88
Ibuprofen	0.68	3.82	0.11	0.02	1.36	n.d.	n.d.	0.83	7.37
Paracetamol	1.59	1.61	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	22.93
Metronidazole	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

Methamphetamine	4.04	1.28	n.d.	n.d.	n.d.	n.d.	n.d.	0.22	n.d.
Gemfibrozil	0.34	0.31	n.d.	0.20	n.d.	n.d.	n.d.	0.65	0.06
Captopril	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Naproxen	5.06	1.16	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Methadone	0.08	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Ketoprofen	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Phenytoin	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Carbamazepine	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Morphine	n.d.	0.39	n.d.	n.d.	n.d.	n.d.	n.d.	0.50	n.d.

n.d.: not detected.

3.10.2.2 Sludge samples (aqueous fractions)

The concentrations of the pharmaceuticals measured in the aqueous fractions of the filtered sludge samples are shown in Table 3.15.

Table 3.15 Concentrations of pharmaceuticals in aqueous fractions of sludge samples from San Jose/Santa Clara STP (μg/l)

Pharmaceutical	S1 "A/B"	S1 "C/D"	S2A	S2B	S3
Salicylic acid	9.23	51.93	0.03	0.20	3.31
Ibuprofen	0.51	1.92	0.01	0.10	7.20
Paracetamol	n.d.	4.12	n.d.	n.d.	n.d.
Metronidazole	n.d.	n.d.	n.d.	n.d.	n.d.
Methamphetamine	n.d.	3.78	n.d.	n.d.	0.42
Gemfibrozil	0.02	0.20	0.02	n.d.	1.00
Captopril	n.d.	n.d.	n.d.	n.d.	n.d.
Naproxen	0.04	0.06	n.d.	0.11	0.25
Methadone	n.d.	n.d.	n.d.	n.d.	n.d.
Ketoprofen	n.d.	0.67	n.d.	0.26	n.d.
Phenytoin	n.d.	n.d.	n.d.	n.d.	n.d.
Carbamazepine	n.d.	n.d.	n.d.	n.d.	n.d.
Morphine	n.d.	0.64	n.d.	n.d.	1.19

n.d.: not detected.

3.10.2.3 Sludge samples (biomass fractions)

The concentrations of pharmaceuticals measured in the biomass fractions of the filtered sludge samples are shown in Table 3.16.

Table 3.16. Concentrations of pharmaceuticals in biomass fractions of sludge samples from San Jose/Santa Clara STP (μg/kg)

Pharmaceutical	S1 "A/B"	S1 "C/D"	S2A	S2B	S3
Salicylic acid	4.80	n.d.	14.83	14.54	4.51
Ibuprofen	n.d.	5.29	n.d.	n.d.	29.13
Paracetamol	50.85	32.27	125.74	n.d.	n.d.
Metronidazole	n.d.	n.d.	n.d.	n.d.	n.d.
Methamphetamine	n.d.	n.d.	n.d.	n.d.	n.d.
Gemfibrozil	836.58	n.d.	325.81	48.73	43.23

Captopril	n.d.	n.d.	n.d.	n.d.	n.d.
Naproxen	n.d.	n.d.	n.d.	n.d.	n.d.
Methadone	442.03	862.95	n.d.	n.d.	n.d.
Ketoprofen	n.d.	n.d.	n.d.	n.d.	n.d.
Phenytoin	n.d.	n.d.	n.d.	n.d.	n.d.
Carbamazepine	n.d.	n.d.	n.d.	n.d.	n.d.
Morphine	n.d.	n.d.	n.d.	n.d.	n.d.

n.d.: not detected.

A detailed discussion of the analytical results obtained in this study are presented in section 4.10.

3.11 Removal of pharmaceuticals from domestic wastewater by advanced water recycling technology

In 2000, the Queensland Government, as a component of the Queensland Water Recycling Strategy, commissioned the construction of the Advanced Water Recycling Demonstration Plant (AWRDP) (Gibson & Apostolidis, 2001). The plant consists of eight modules, each housing a different water treatment technology. Each module is designed as a relocatable unit so that it can be transported to various sites throughout Queensland. The influent to the AWRDP was taken from treated effluent from the nearby Brendale Wastewater Treatment Plant (Pine Rivers Shire Council). The modules included lime clarification (LC), dissolved air flotation (DAF), dual media filtration (DMF), ozonation (O_3), biological activated carbon filtration (BAC), microfiltration (MF), combined reverse-osmosis/nanofiltration (RO/NF) and UV-disinfection. Individual modules could be placed on-line or bypassed very quickly and simply. The outlay of the AWRDP modules is shown in Figure 3.6.

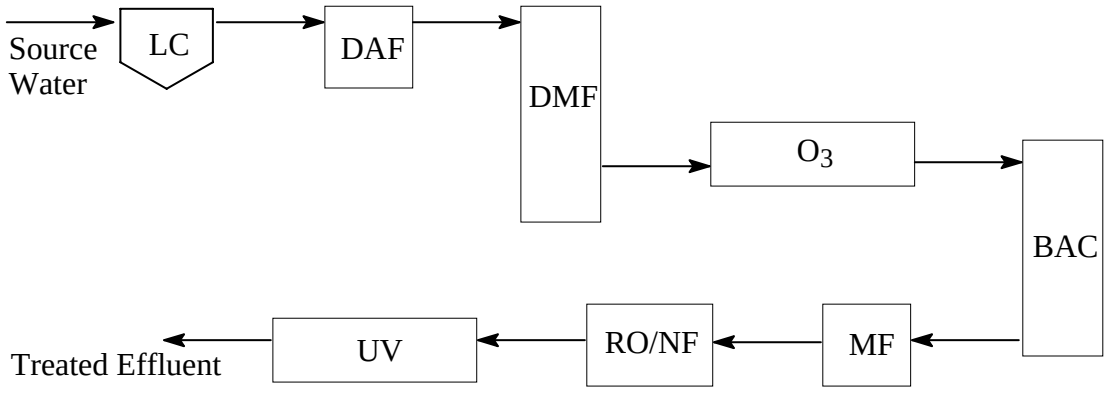


Figure 3.6. Schematic illustration of QLD AWRDP showing individual modules

3.11.1 Analytical details

Initial experiments were undertaken to measure the concentration of seven pharmaceutical residues after treatment by various modules under two different plant configurations.

In addition to investigating the treatability of contaminants that were measurable in the various module influents, the small scale of the AWRDP permitted the undertaking of spiking experiments. By spiking measurable concentrations of the seven pharmaceutical compounds, it was possible to gain significantly more information on the behaviour of these compounds during advanced treatment.

Two pharmaceutical spiking experiments were conducted. The first study incorporated both the microfiltration and RO/NF modules. The pharmaceuticals were spiked into the feed tank of the microfiltration module. Samples for analysis were taken from the microfiltration module (feed and permeate) and the RO/NF module (RO permeate, RO retentate, NF permeate, NF retentate). The second spiking experiment incorporated only the RO/NF module. The feed tank to the RO/NF module was spiked. Samples were then taken from the RO/NF feed, RO permeate, RO retentate, NF permeate, NF retentate.

3.11.2 Analytical data

The concentrations of pharmaceuticals were measured in the various stages of the AWRDP. Sampling was undertaken in two plant configurations. The first was with the ozone treatment offline (9 Apr 2002) (Table 3.17), and the second with ozone treatment running (17 Apr 2002) (Table 3.18).

Table 3.17. Concentration of pharmaceuticals (μg/l) in AWRDP with ozone offline

Source	DMF	BAC	MF	RO per	RO ret	NF per
Salicylic Acid	0.33	0.23	0.13	0.16	2.21	0.17
Ibuprofen	n.d.	n.d.	n.d.	n.d.	0.31	n.d.
Metronidazole	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Gemfibrozil	0.18	n.d.	n.d.	n.d.	0.86	n.d.
Naproxen	n.d.	n.d.	n.d.	n.d.	0.70	n.d.
Ketoprofen	n.d.	n.d.	n.d.	n.d.	0.37	n.d.
Carbamazepine	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

n.d.: not detected.

Table 3.18. Concentration of pharmaceuticals (μg/l) in AWRDP with ozone online

Source	DMF	O_3	BAC
Salicylic Acid	0.65	0.26	0.34
Ibuprofen	n.d.	n.d.	n.d.
Metronidazole	n.d.	n.d.	n.d.
Gemfibrozil	n.d.	n.d.	n.d.
Naproxen	n.d.	n.d.	n.d.
Ketoprofen	n.d.	n.d.	n.d.
Carbamazepine	n.d.	n.d.	n.d.

n.d.: not detected.

Concentrations of pharmaceuticals in various stages of the AWRDP during spiking experiments are shown for two plant configurations.

The first of these was conducted with the ozone treatment module offline (11 Apr 2002). Six stock solutions, each containing salicylic acid (3.2 mg), ibuprofen (1.6 mg), gemfibrozil (1.2 mg), naproxen (0.8 mg) and ketoprofen (0.8 mg) in 200 ml of distilled water were prepared. These were dosed into the feed tank of the MF module at 9:00 am, 9:10 am, 9:20 am, 9:30 am, 9:40 am and 9:50 am. The recovered concentrations from the RO/NF module, at three sampling times, are shown in Table 3.19.

Table 3.19. Concentration of pharmaceuticals (μg/l) in AWRDP during spiking experiments with ozone offline

Source	RO/NF feed	NF per	NF ret	RO per	RO/NF feed	NF per	NF ret	RO per	RO/NF feed	NF per	NF ret	RO per
Time	9:30	9:30	9:30	9:30	10:00	10:00	10:00	10:00	10:15	10:15	10:15	10:15
Salicylic Acid	2.33	19.89	11.42	0.25	4.79	3.80	10.38	n.d.	n.d.	4.12	5.82	n.d.
Ibuprofen	0.36	0.47	0.72	n.d.	0.68	0.14	1.95	n.d.	0.36	0.22	1.88	n.d.
Gemfibrozil	0.76	0.43	1.08	n.d.	1.91	n.d.	5.31	n.d.	0.41	0.32	4.30	n.d.
Naproxen	0.31	0.39	1.11	n.d.	0.53	n.d.	3.17	n.d.	n.d.	n.d.	2.02	n.d.
Ketoprofen	0.22	n.d.	1.11	n.d.	n.d.	n.d.	2.30	n.d.	n.d.	n.d.	1.99	n.d.

n.d.: not detected.

The second spiking experiment was conducted with the ozone treatment module online (19 Apr 2002). Six stock solutions, each containing salicylic acid (1.3 mg), ibuprofen (2.2 mg), gemfibrozil (1.2 mg), naproxen (0.8 mg) and ketoprofen (1.1 mg) in 200 ml of distilled water were prepared. These were dosed into the feed tank of the RO/NF module at 9:00 am, 9:10 am, 9:20 am, 9:30 am, 9:40 am and 9:50 am. The recovered concentrations from the RO/NF module, at two sampling times, are shown in Table 3.20.

Table 3.20. Concentration of pharmaceuticals (μg/l) in AWRDP during spiking experiments with ozone online

Source	MF feed	MF	RO ret	RO per	NF ret	NF per	MF feed	MF	RO ret	RO per	NF ret	NF per
Time	9:45	9:45	9:45	9:45	9:45	9:45	10:15	10:15	10:15	10:15	10:15	10:15
Salicylic Acid	6.87	3.07	1.44	n.d.	0.57	n.d.	0.56	n.d.	n.d.	n.d.	n.d.	n.d.
Ibuprofen	1.87	0.79	1.04	n.d.	2.83	0.85	0.50	n.d.	n.d.	n.d.	0.96	n.d.
Gemfibrozil	2.19	1.53	1.95	n.d.	4.65	0.28	0.70	n.d.	0.15	n.d.	3.36	n.d.
Naproxen	1.65	0.63	1.32	n.d.	2.76	0.49	0.38	n.d.	n.d.	n.d.	0.65	n.d.
Ketoprofen	0.86	0.29	0.82	n.d.	3.09	0.32	n.d.	n.d.	n.d.	n.d.	0.04	n.d.

n.d.: not detected.

A detailed discussion of the analytical results obtained in this study are presented in section 4.11.

Chapter 4--Discussion, PhAC Fate in Sewage & Sludge

4.1 Overview

The analytical data that were presented in Chapter 3 are discussed in detail in the current chapter. Factors that influenced or contributed to the observed concentrations of pharmaceutical residuals are considered here. Specific aspects of the discussion include biodegradation and partitioning between aqueous and biomass phases.

Details pertaining to the measurability, as well as to the reproducibility and accuracy of measurements are also included in this chapter. An objective of the project was to provide evidence of the usefulness and shortcomings of the predictive model described in Section 2. Such evidence pertaining to the acquired analytical data is presented here.

A series of discrete analytical studies were undertaken to provide individual parts to the bigger picture of the pharmaceuticals in sewage issue. These are outlined in Table 4.1. Discussions relating to each of these studies are presented in the following sections. The section numbers correspond to the relevant section numbers where the analytical data were presented in Chapter 3.

Table 4.1. Overview of discrete analytical studies

Section	Title	Purpose
4.2	General scan of sewage and sludge: Bondi STP	Investigate major volatile organics component
4.3	General scan of sewage: Castle Hill STP	Investigate derivatised volatile organics component
4.4	Determination of variation among grab-samples	Determine representativeness of single grab-samples
4.5	Diurnal analysis at Castle Hill STP	Explore diurnal variation of pharmaceuticals in sewage
4.6	Seven-day sewage analysis, Castle Hill STP	Explore daily variation of pharmaceuticals in sewage as well as removal efficiency of treatment processes
4.7	Sewage & Sludge analysis for Bondi STP	Explore partitioning to biomass in primary and digested primary sludge
4.8	Sewage Analysis for 3 STPs in Berlin, Germany	Explore geographic variation and compare with results obtained by well established methods
4.9	Sewage & Sludge Analysis East Bay Municipal Utilities District, USA	Further explore geographic variation in pharmaceutical profile
4.10	Sewage & Sludge Analysis San Jose/Santa Clara, USA	Explore geographic variation and mass-balance through a large STP
4.11	Removal by advanced treatment processes	Investigate removal efficiencies of some advanced treatment processes

4.2 General scan of sewage and sludge: Bondi STP

A number of biologically relevant compounds were detected in the general scan of sewage and sludge extracts from Bondi STP (Kahn, 2002). Most significant was ibuprofen, which is a pharmaceutical, identified frequently as most likely to be present in considerable concentrations. Ibuprofen was detected in this analysis in its non-derivatised form. Analysis of Bondi samples provided qualitative data only.

Among other observed biological compounds were the commonly known alkaloid, caffeine, and the antibacterial/anti-microbial agent, triclosan. Caffeine is an ingredient of coffees, teas and numerous carbonated soft drinks. It is certain to be ubiquitous in raw sewage. Triclosan is used in anti-gum-disease toothpaste as well as in deodorants, antiperspirants, detergents, dishwashing liquids, cosmetics and anti-microbial creams, lotions, and hand soaps. Triclosan has been shown to be only partially removed during conventional sewage treatment processes and, hence, it is measurable in surface waters (Lindström *et al.*, 2002).

A number of steroidal compounds were identified. These included cholesterol, dihydrocholesterol, 5α-cholestan-3-one and cholesta-3,5-diene. 14β pregnane is a progestinal steroid hormone and 3α-hydroxy-5β-androstane-17-one is an androgenic steroid hormone. Squalene, which was also found but not a steroid, is an important intermediate in the biochemical synthesis of cholesterol.

Omega-pentadecalactone (Adams *et al.*, 1998) and versalide are musky, synthetic perfumes frequently used in soaps. Methyldihydrojasmonate95 is an artificial peach/apricot flavouring agent.

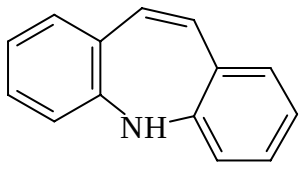
Not surprisingly, many of the most prevalent chemicals are short-chain fatty acids, which are components of soaps, shampoos and detergents. Examples include stearic acid, palmitic acid, lauric acid, capric acid, oleic acid and linoleic acid. Some amides of these acids were also observed as were methyl esters of many of the acids. It is likely that methyl esters were formed by reaction with methanol solvent in the GC-MS inlet.

A number of phthalates were observed: diethyl phthalate, isobutyl phthalate, dibutyl phthalate, butyl benzyl phthalate, dicyclohexyl phthalate and bis(2-ethylhexyl) phthalate. These are industrial compounds used as plasticisers in PVC and some other products including paints. Phthalates are ubiquitous in sewage (Alcock *et al.*, 1999).

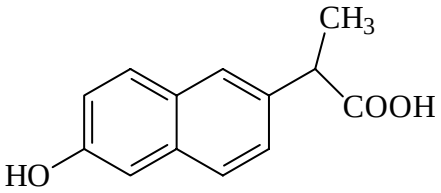
4.3 General scan of extracted sewage samples: Castle Hill STP

A number of significant biological compounds were identified in the general scan of extracted sewage from Castle Hill STP (Khan, 2002). In this analysis, it was possible to detect a wider range of less volatile compounds than those identified from Bondi STP (section 4.2). This was achieved by derivatising the extracted samples with BSTFA to deliver TMS derivatives. The effect was twofold: First, the increased volatility of the abundant long-chain organic acids caused these compounds to be eluted earlier and in narrower peaks, thus obscuring less of the total chromatograph. Second, the increased volatility of some further compounds of interest allowed them to be adequately chromatographed and observed without thermal decomposition.

Ibuprofen was again observed (this time as a TMS derivative). Paracetamol (listed as acetaminophen in the mass-spectral database) was observed as a consequence of the derivatisation. This compound was predicted to be the highest concentration pharmaceutical in raw sewage (Table 2.1). Iminostilbene (Figure 4.1) is both a manufacturing precursor and urinary metabolite of the antiepileptic drug, carbamazepine. A further compound was identified by the Wiley mass-spectral database as "naproxen metabolite". The identity of this compound was confirmed to be the major metabolite 6-*o*-desmethylnaproxen (Figure 4.1), by inspection of its mass spectrum. Both carbamazepine and naproxen were shown, in Table 2.1, to be high use (by mass) drugs. However, diminished concentrations of the parent drugs were predicted because of extensive metabolism. This is very consistent with the results obtained.



Iminostilbene



6-*o*-desmethylnaproxen

Figure 4.1. Structures of identified pharmaceutical metabolites

Also found was 5α-androstan-3α-ol-17-one (androsterone), a male hormone that is biosynthesised from progesterone. It is one of the major urinary metabolites of testosterone. 11-keto-etiocholanolone is also a natural urinary steroid. Many of the other steroids, phthalates and long chain organic acids described in section 4.2, were again observed in this analysis. As with the previous section, only qualitative data were available from this analysis.

4.4 Determination of variation among grab-samples

This analysis was undertaken as a precursor to the diurnal analysis at Castle Hill STP (Section 4.5). In the diurnal analysis, concentration and load variations were to be considered on a time scale of hours. The current tasks were undertaken as a check that the finer-detail variations (i.e. on the order of minutes) were not of the same order as the hourly variations..

The data for this analysis are presented in section 3.4. All five samples were collected from the same location within a period of ten minutes. Reasons reported for variations between samples include:

- short-term flow variations.
- short-term load variations.
- variations in homogeneity of sewerage chamber content.
- variations in sampling, extraction and analysis of samples.

The means and standard deviations were calculated (Table 3.3). Non-detect samples were omitted from the calculation. The coefficients of variation (CV) were calculated from the mean and standard deviation (σ) by the equation:

$$CV = \frac{\sigma}{\text{mean}} \times 100$$

In all cases the coefficients of variation (from 3-5 analyses) were no greater than 50%, indicating adequate reproducibility within data sets of this size. Coefficients of 10% or less were obtained for salicylic acid, ibuprofen, gemfibrozil, naproxen and ketoprofen. The collection, extraction and analysis of these compounds was therefore considered highly reproducible and the short-term concentration variation insignificant at the sampling time that the experiment was performed.

4.5 Diurnal analysis at Castle Hill STP

In an STP, the overall flow-rate exhibits significant diurnal variation. This experiment was undertaken to investigate the diurnal variations in the concentrations of pharmaceutical residues. The data obtained are presented in Table 4.2. To a first approximation, it may have been expected that the concentration of contaminants may have exhibited a reverse diurnal pattern. That is, lower concentrations may have been expected with the increased dilution associated with flow-rate peaks in the cycle. In fact, concentration variations proved to be more complicated.

The actual excretion of pharmaceuticals into a particular sewerage system was also expected to exhibit a highly diurnal nature. This would reflect variations in factors such as typical times of drug ingestion, lapse times for excretion peaks, peak times of elimination to the sewerage system and population shifts into and out of an area during a working-day cycle.

This investigation was undertaken at the Castle Hill STP, which was described in section 3.3. The Castle Hill STP treats predominantly domestic municipal sewage. The rate of flow of influent to such an STP typically exhibits a diurnal nature as shown in Figure 4.2 and Figure 4.3. The diurnal pattern reflects patterns of community water usage. Much of the detail is attributable to factors, generally

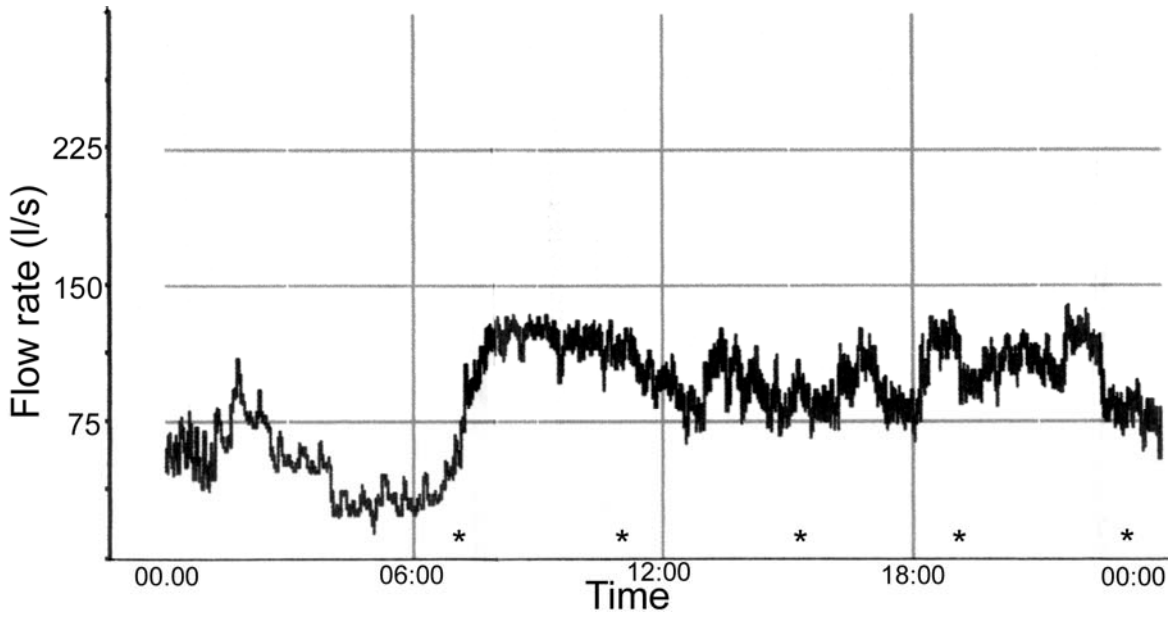


Figure 4.2. Sewage flow at Castle Hill, Tues 11 Sep 2001 (sampling times)**

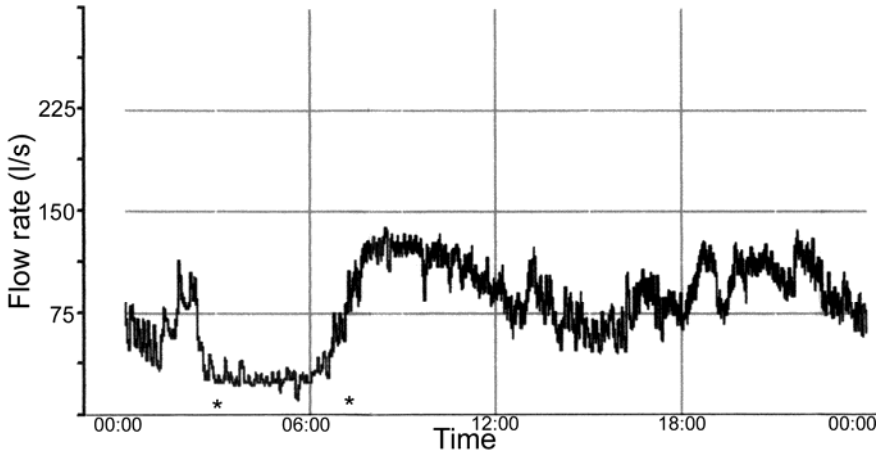


Figure 4.3. Sewage flow at Castle Hill, Wed. 12 Sep 2001 (sampling times)**

categorised as washing (baths, showers, dishes and clothes). The phasing of the diurnal flow curve is influenced by the length of the sewers and, hence, the average delay incurred between wastewater generation and the time it takes for it to reach the STP.

4.5.1 Diurnal concentration variation of pharmaceuticals

An initial assumption may be that times of peak flow might be translated as times of peak dilution and, hence, minimum concentration of organic matter. In fact, the reverse is generally the case. The concentration of organic matter in sewage is commonly measured by its biochemical oxygen demand (BOD) or the total organic carbon (TOC) concentration. BOD, TOC and suspended solids concentrations typically roughly parallel the flow variation during dry weather and in the absence of major industrial discharges. That is, they exhibit the same pattern in phase with Figure 4.2 and Figure 4.3 (Metcalf & Eddy. Inc., 1991). The explanation is that these parameters originate predominantly from the same sources as the flow variations. Much of the measured BOD and TOC is contributed by human excretions, as well as soaps and detergents used in washing.

On the other hand, specific organic compounds originating solely from human excretions (such as pharmaceutical residuals) may not be expected to follow the same concentration variation as the BOD and TOC. It seems likely that the ratio of sewage generated from toilet flushing to that generated by washing may also exhibit peaks and troughs. If this is the case, we might expect to see peak concentrations of pharmaceuticals at times where this “flushing-to-washing” ratio is greatest. Predicting these times is, however, less straightforward.

A few assumptions can be made to help postulate the diurnal pattern of the flushing-to-washing ratio and, hence, presumably the pharmaceutical concentration variation. Peak flushing times will likely

coincide with peak washing times (i.e. morning and evening). Similarly, a trough in the flushing volume may be expected to coincide with a trough in washing volume in the very early hours of the morning (say 2 am – 5 am), however, the flushing trough may be less severe than the washing trough. A decrease in washing activity is assumed to be primarily responsible for the observed afternoon flow-minimum (see Figure 4.3). However, it may be assumed that flushing activity again exhibits a less severe trough than washing.

It follows from these assumptions that while the total flushing volume and the total washing volume may each exhibit a diurnal pattern consistent with Figure 4.2 and Figure 4.3, the flushing-to-washing ratio may be somewhat the inverse. The conclusion is that peak pharmaceutical concentrations may be expected in the very early morning and in the late afternoon. If this were the case, measurement of pharmaceuticals at these concentration peaks would be further facilitated by the minimums in BOD and TOC concentrations, which occur at those times. This hypothesis was tested by analysing grab samples collected every four hours over a 24-hour cycle. The sample times are marked with asterisks in Figure 4.2 and Figure 4.3. The concentration variations observed were, in most cases, greater than the short-term sample variations observed in Sections 4.3 and 4.4, thereby indicating that diurnal concentration variation was indeed observable by this method of sampling. A clear pattern, on the other hand, was less evident.

Paracetamol was the pharmaceutical measured in highest concentrations. Paracetamol concentrations were compared to TOC concentrations over the diurnal cycle (Figure 4.4). A significant concentration peak for this drug was indeed observed in the afternoon sample (3pm). However, there was less evidence of the postulated early morning concentration peak. The only observed TOC concentration peak was in the evening (around 11pm).

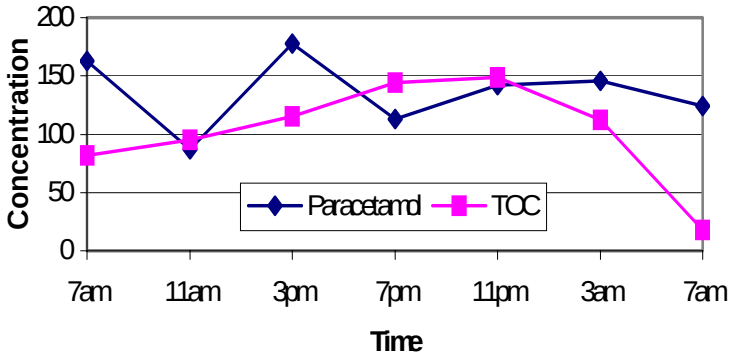


Figure 4.4. Diurnal concentration variation of paracetamol (µg/l) and TOC (mg/l)

The diurnal concentration variations of salicylic acid, ibuprofen, gemfibrozil, naproxen and ketoprofen were compared (Figure 4.5). It was apparent that while some aspects of the overall trends were consistent among the different pharmaceuticals, some considerable variations were also observed.

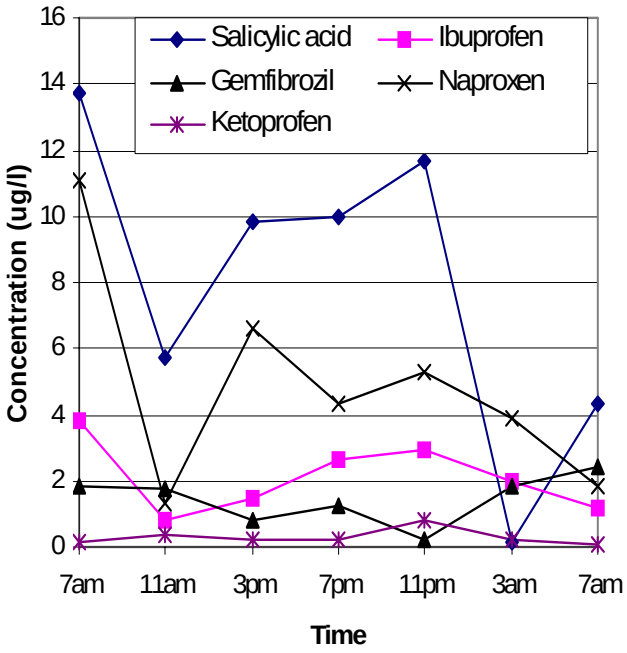


Figure 4.5 Diurnal concentrations of salicylic acid, ibuprofen, gemfibrozil, naproxen and ketoprofen (μg/l)

Furthermore, it was noted that concentrations of specific drugs were, in most cases, significantly different at 7am on the first morning (Tuesday) compared to those at 7am on the second morning (Wednesday). A likely explanation is that at this time, the sewage flow rate is typically changing much more rapidly than when the short-term variation tests were undertaken (12 noon). This rapid rate of change in flow rate at 7am can be seen clearly in Figure 4.2 and Figure 4.3. Hence, short-term variability around this time is expected to be much more significant. Thus, small variations in sampling time, from one day to the next, may have resulted in considerable variations in sample concentration.

A second factor, which may account for some differences between the two mornings, was that the events in the USA of September 11 2001 occurred overnight (Sydney time) during the study. This may have had an unknown effect on people's morning habits, as many may have made adjustments in order to watch television news broadcasts during the late evening and following morning.

4.5.2 Diurnal load variation of pharmaceuticals

The pharmaceutical loads were calculated from the concentration and flow to examine the variations in the total quantities of each of the pharmaceuticals in the STP:

$$\text{Load } (\mu g/s) = \text{Concentration } (\mu g/l) \times \text{Flow rate } (l/s)$$

At each of the given sampling times, the flow was estimated from Figure 4.2 or Figure 4.3. The data are included as "Flow (l/s)" in Table . The load was calculated for each of the eight pharmaceuticals for which there was a full, or near-full, set of diurnal data. The load was also calculated for the total organic carbon (TOC) (Table 4.2).

Table 4.2. Flow rates and loads of pharmaceuticals (μg/s) and TOC (mg/s) at Castle Hill STP.

	7am	11am	3pm	7pm	11pm	3am	7am
Flow (l/s)	53	115	90	102	86	27	75
Salicylic acid	727	657	887	1018	1003	4	326
Ibuprofen	201	92	132	269	255	53	88
Paracetamol	8620	9962	16004	11514	12213	3936	9296
Gemfibrozil	99	201	72	130	18	50	183
Naproxen	589	155	596	442	458	105	136
Ketoprofen	10	41	17	19	71	5	8
Carbamazepine	-	6	7	11	9	4	6
Morphine	-	23	39	43	27	8	7
TOC	4346	10925	10350	14688	12814	3024	1350

The diurnal load variations provide a much clearer description of the quantity of pharmaceutical residues in the system than do the diurnal concentration variations. The limited number of data points did not allow for a complete analysis, however some general observations could be made.

The diurnal paracetamol load variation was compared to the diurnal TOC load variation (Figure 4.6). It was evident that the paracetamol load peaks and TOC load peaks did not coincide. It appears that the paracetamol load peaked in the late afternoon, while the TOC load peaked in the early evening. Moreover, between 3am and 7am, the loads of paracetamol and TOC moved in opposite directions. While the TOC load underwent a sharp increase, the paracetamol load continued to decrease. These observations are consistent with the above speculation regarding variations in sewage generated by flushing and washing.

The diurnal load variations of salicylic acid, ibuprofen, gemfibrozil, naproxen and ketoprofen were compared (Figure). Even with the limited data available, it is clear that the different pharmaceuticals did indeed exhibit different patterns of diurnal load variation. The most extreme

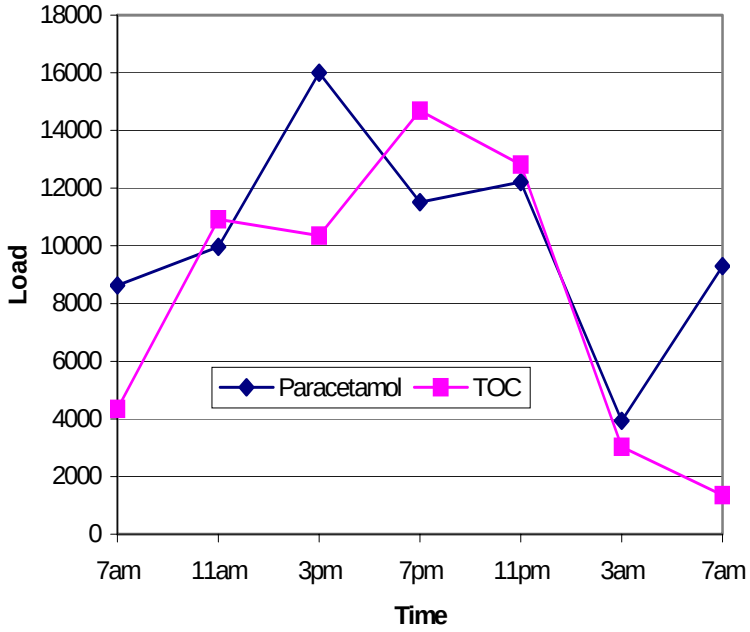


Figure 4.6. Diurnal load of paracetamol (μg/s) and TOC (mg/s).

variation was observed for salicylic acid, which was observed to decrease in load by more than two orders of magnitude between 11pm and 3am.

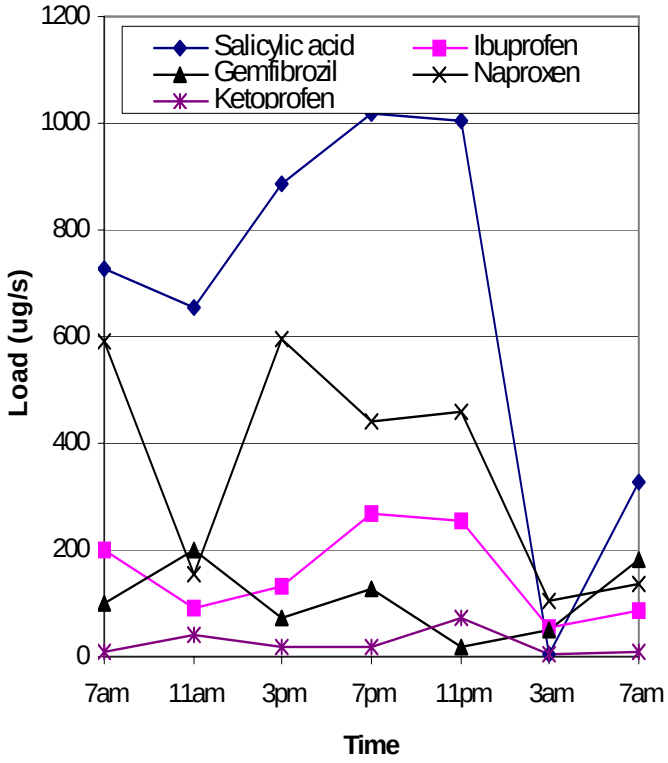


Figure 4.7. Diurnal loads of salicylic acid, ibuprofen, gemfibrozil, naproxen and ketoprofen (μg/s)

4.5.3 Conclusions regarding diurnal variations of pharmaceuticals in sewage

A number of conclusions may be drawn from this study. These include:

1. Diurnal variation of pharmaceutical concentrations and loads in sewage is evident.
2. As a result of the diurnal variability, randomly selected grab-samples are not representative of daily load.
3. Sensitivity of comparing a small number of grab samples is limited somewhat by the short-time variation observed in grab samples.
4. The diurnal pattern of pharmaceutical concentration does not reflect the diurnal variations of the total organic load.
5. Diurnal patterns appear to be variable, even among different pharmaceutical residuals.

The last of these points is, perhaps, the most interesting and warrants further consideration. What factors may be responsible for variations in the diurnal concentrations between different pharmaceuticals?

First, many pharmaceuticals are dispensed with instructions regarding the time of day at which they should be administered. This may vary between different classes of drugs. Examples of instructed administration times include mornings, evenings, before or after meals. Second, pharmaceuticals vary in the time taken from ingestion to excretion. Third, the use of particular drugs varies across age groups. Regularity and times of toilet-use may similarly vary across age groups, resulting in further variation in overall excretions of these drugs. All of these factors may contribute to peak times of excretion, which may vary considerably amongst different drugs. Hence, flushing-generated sewage would be expected to carry variable loads of such drugs over 24-hours.

4.6 Seven-day sewage analysis, Castle Hill STP, Sydney

A seven-day sewage analysis was undertaken at the Castle Hill STP to investigate daily variations in pharmaceutical loads over a typical weekly cycle. The data obtained were also used to compare analytical results with the concentration predictions generated in Section 2.

For seven consecutive days (Tue 18 Dec – Mon 24 December 2001), 24-hour composite raw sewage samples from the Castle Hill STP were collected and analysed. Additionally, 24-hour composite samples of primary and secondary effluents were collected and analysed over five consecutive week-

days (Tue 18, Wed 19, Thu 20, Fri 21, Mon 24 December 2001). The composite periods concluded at 9am each day. The analytical results are presented in section 3.6.

The total flow and rainfall at the STP during the sampling period are shown in Table 4.3 (Rodrigues, 2002). The flow data for each day start and end at midnight. Accordingly, it was not possible to correct the measured concentrations for flow rate, because the flow data were out of phase with the sampling composites.

Table 4.3. Total flow (Ml) and rainfall (mm) for sample period (Rodrigues, 2002)

	Tue	Wed	Thu	Fri	Sat	Sun	Mon
Flow (Ml)	6.25	6.59	6.91	7.04	6.83	6.63	6.92
Rainfall (mm)	3.5	0.0	0.0	4.5	0.0	0.0	0.0

The average flow recorded over the seven days was 6.74 Ml/day with a standard deviation of 0.27 Ml/day. The coefficient of variation was therefore calculated to be 4.0. Since the coefficient of variation was significantly less than 10, the variation in daily flow was not considered to be a significant factor in the overall measured variation in composite pharmaceutical concentrations.

Total sewage flow variations resulting from domestic sources are generally negligible from one day to the next. Typical weekly flow patterns are shown in Figure 4.8 (Metcalf & Eddy. Inc., 1991).

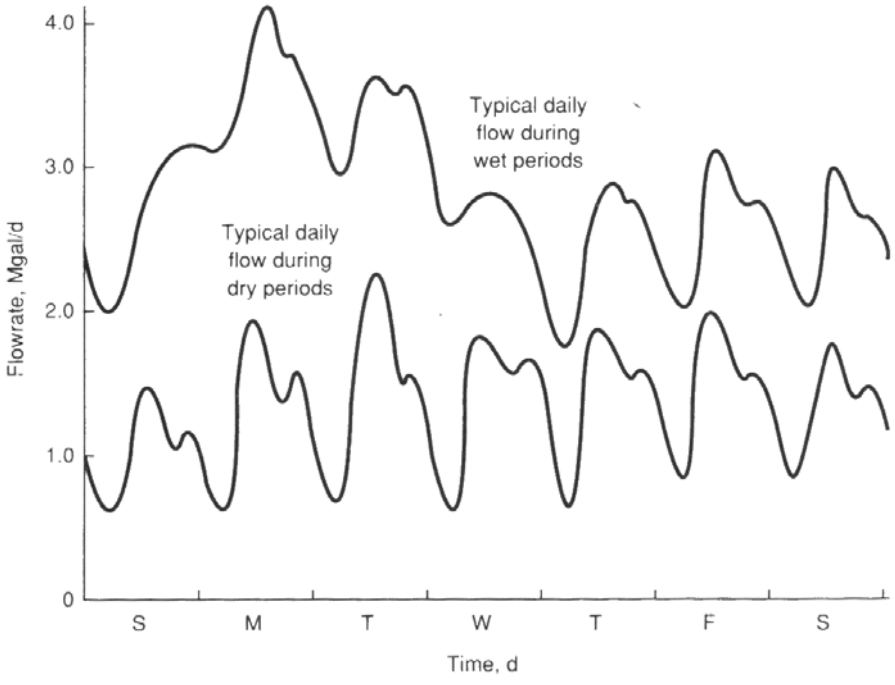


Figure 4.8. Typical weekly flow rate variations during wet and dry periods. Reproduced from (Metcalf & Eddy. Inc., 1991)

Very consistent data were obtained for the 24-hour composites, much more so than for previous single grab samples. Most of the factors contributing to concentration variations in grab samples taken over a 24-hour period (described in section 4.5) were not applicable to 24-hour composites.

The means and standard deviations of the measured pharmaceutical concentrations were calculated for raw influent, primary effluent and secondary effluent (Table 4.4). Samples in which the pharmaceutical was not detected were not used in the calculation. For consistency, only the weekday concentrations were used in the calculation for raw sewage. Only drugs that were detected in at least three of the five samples were included. Failure to detect a compound did not automatically infer a low concentration. Occasionally, significant concentrations of compounds may go undetected due to masking by co-eluting peaks.

Table 4.4. Statistics of pharmaceutical concentrations (μg/l) in Castle Hill STP over five days

Pharmaceutical	Raw influent	Primary effluent	Secondary effluent
Salicylic acid	13.43 ± 3.26	6.12 ± 1.40	0.38 ± 0.13
Ibuprofen	2.72 ± 0.35	2.25 ± 0.34	0.22 ± 0.15
Paracetamol	104.48 ± 1.79	27.78 ± 3.54	0.39 ± 0.23
Metronidazole	-	-	-
Methamphetamine	0.18 ± 0.11	-	-
Gemfibrozil	1.49 ± 0.19	1.25 ± 0.08	0.25 ± 0.03
Captopril	-	-	-
Naproxen	6.48 ± 0.42	5.54 ± 0.73	0.35 ± 0.12
Methadone	-	-	-
Ketoprofen	0.90 ± 0.08	1.02 ± 0.18	0.59 ± 0.05
Phenytoin	-	-	-
Carbamazepine	-	-	-
Morphine	0.26 ± 0.11	0.19 ± 0.02	0.02 ± 0.00

The acquired data were compared to the data predicted in Tables 2.1 and 2.2). The comparisons were made in terms of both absolute concentrations of pharmaceuticals and relative removal rates.

4.6.1 Comparison of absolute concentrations of pharmaceuticals

The concentrations of pharmaceuticals predicted and measured in raw sewage were compared (Figure 4.9). For the six compounds with full sets of analytical data, very close agreement between predicted and measured concentrations generally achieved for raw sewage. Of these six pharmaceuticals, four were under-predicted compared to the analytical results from raw sewage. The remaining two were over-predicted compared to measured results.

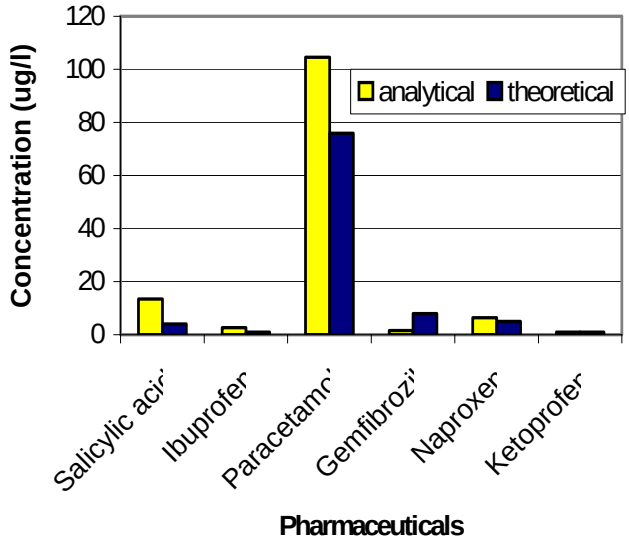


Figure 4.9. Measured vs. predicted pharmaceutical concentrations in raw sewage

The under-predicted compounds were salicylic acid (predicted concentration was 30% of analytical concentration), ibuprofen (33%), paracetamol (73%) and naproxen (77%). The over-predicted compounds were gemfibrozil (537%) and ketoprofen (111%).

Of the under-predicted compounds, purchases from non-pharmacy sources are suspected of being significant for salicylic acid, ibuprofen and paracetamol.

The significantly over-predicted gemfibrozil concentrations warrant close consideration. Gemfibrozil is considerably more lipophilic than the other five compounds. It was therefore predicted to partition towards the sewage biomass to a much greater extent than the others (see Table 2.1). Given that these raw sewage samples were filtered before analysis and that only the aqueous fractions were analysed, it is likely that a considerable amount of gemfibrozil was discarded in the filtered residue (biomass fraction). This may partially account for the significant over-prediction of gemfibrozil in this analysis.

Naproxen and ketoprofen were considered to have been predicted adequately in raw sewage. Concentrations of predicted vs measured compounds in primary effluent were compared (Figure 4.10). In primary effluent, salicylic acid (65%) and ibuprofen (40%) were considered to be under-predicted compared to analytical results. Paracetamol (266%) and gemfibrozil (640%) were considered over-predicted. Naproxen (90%) and ketoprofen (98%) were considered to be satisfactorily predicted. The primary effluent concentrations generally reflect the variations observed for raw sewage, with two notable exceptions. Ratios of predicted-to-measured concentrations for paracetamol and gemfibrozil were dramatically increased compared to that observed for raw sewage.

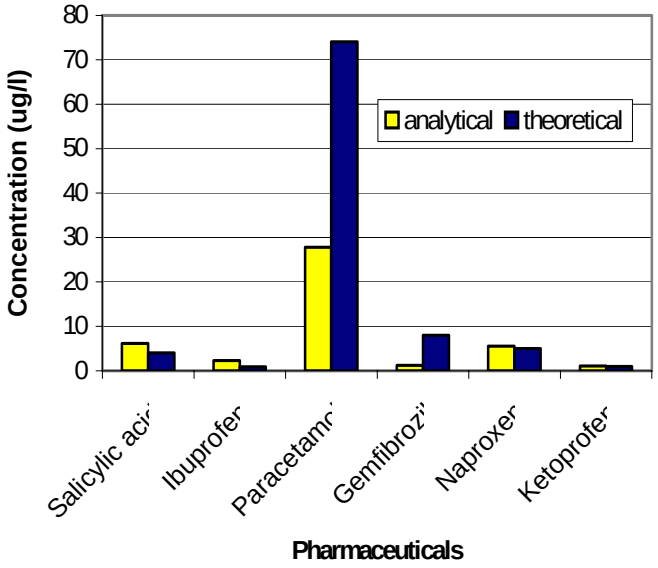


Figure 4.10. Measured vs predicted pharmaceutical concentrations in primary effluent

Not withstanding deficiencies in the chemical analysis, this suggests some combination of three likely possibilities for the predictions regarding these compounds:

1. Predictions underestimated the degree of compound removed to sludge.
2. Predictions underestimated the aerobic biodegradation rate during primary treatment.
3. Predictions suffered from excluding the possible anaerobic degradation in primary treatment.

Predicted vs measured concentrations in secondary effluent were compared (Figure 4.11).

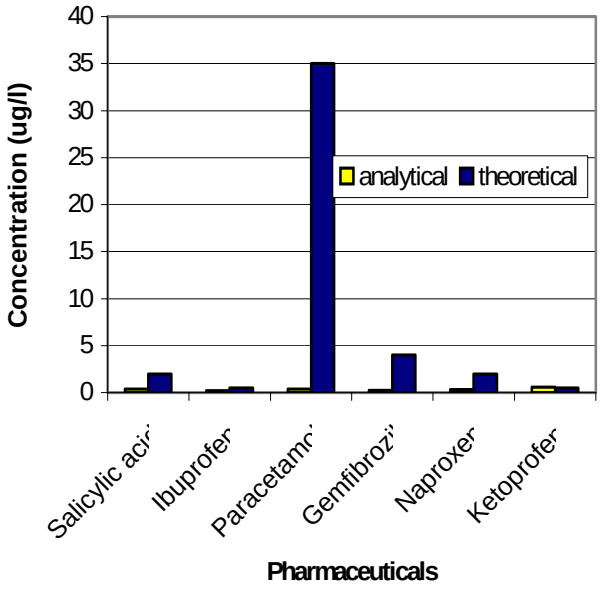


Figure 4.11. Measured vs predicted pharmaceutical concentrations in secondary effluent

As anticipated, secondary effluent concentrations proved considerably more difficult to predict compared to raw sewage and primary effluent concentrations. All compounds, except ketoprofen, were over-predicted in secondary effluent. The predictions, compared with the analytical results, were salicylic acid (526%), ibuprofen (227%), paracetamol (8974%), gemfibrozil (1600%), naproxen (571%) and ketoprofen (85%).

Secondary treatment in this analysis comprised activated sludge aeration followed by secondary clarification. Accordingly, two possible factors are likely to have dominated the under-predicted observed removal rates:

1. under-predicted removal to secondary sludge.
2. under-predicted aerobic biodegradation rates.

In order to identify which of these was the dominant factor, two observations should be considered:

1. Primary effluent concentrations were significantly more successfully predicted than were secondary effluent concentrations.
2. Aerobic biodegradation half-lives were determined to be the most critical and least-well defined parameters in predicting secondary effluent concentrations (Khan, 2002)

These observations strongly suggest that underestimation of aerobic biodegradation half-lives was the major contributing factor to the over-prediction of the pharmaceutical residues in secondary effluent.

4.6.2 Comparison of relative removal rates

The general trend in concentration for the four compounds for which full sets of data are available (salicylic acid, ibuprofen, naproxen and ketoprofen) are shown Figure 4.12. Average concentrations

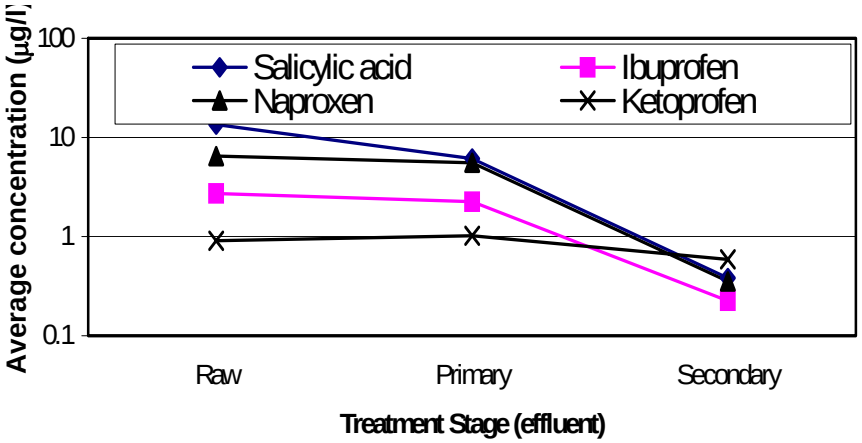


Figure 4.12. Concentration of selected pharmaceuticals in raw influent, primary effluent and secondary effluent (µg/l)

were calculated for weekdays only. Secondary aeration treatment was shown to be significantly more effective than primary treatment for all four of the compounds shown in Figure 4.12.

Analysis of removal during primary and secondary treatment was undertaken by comparing concentrations observed in the effluent of each treatment stage to concentrations observed in the influent of the treatment stage. This was considered for primary settling (Table 4.5) as well as for secondary aeration coupled with secondary clarification (Table 4.6). Only samples for which a compound was detected in both the influent and effluent were used in the calculation of the average and standard deviation.

Table 4.5. Removal rates of pharmaceuticals during primary settling (%)

	Tues	Wed	Thurs	Fri	Mon	Average
Salicylic acid	56	57	44	64	46	54 ± 8
Ibuprofen	-7	27	12	32	18	16 ± 15
Paracetamol	77	68	77	72	73	73 ± 4
Metronidazole	-	-	-	-	-	-
Methamphetamine	-	-	-	17	39	28 ± 16
Gemfibrozil	9	21	24	12	13	16 ± 7
Captopril	-	-	-	-	-	-
Naproxen	14	16	33	16	-10	14 ± 16
Methadone	-	-	-	-	-	-
Ketoprofen	-17	0	-27	-17	2	-12 ± 13
Phenytoin	-	-	-	-	-	-
Carbamazepine	-	-	-	-	-	-
Morphine	19	10	11	20	57	23 ± 19

In most cases, losses of around 15-30% were observed during primary settling. Two exceptions were salicylic acid and paracetamol, observed at much lower concentrations after primary treatment.

The predicted removal rates can likewise be obtained by comparing predicted primary effluent concentrations to predicted influent concentrations (Table 2.2). The predicted removal rates were, in all cases, negligible or insignificant. The model has, therefore, under-predicted the removal rates of most of the analysed pharmaceuticals during primary treatment. With the exception of gemfibrozil, these are not lipophilic compounds. Therefore, it is unlikely that the predominant under-predicted mechanism of the observed loss was by hydrophobic partitioning to sludge. Other, less well-understood mechanisms of sludge partitioning, may have been responsible. Such phenomena were discussed in section 2.1.6.

An alternative explanation is that biodegradation may have been responsible for the observed loss of compound. The significant biochemical oxygen demand (BOD) that is typical of raw sewage can

quickly deplete any available dissolved oxygen. Consequently, primary settling tanks are often highly anaerobic. It is likely, therefore, that anaerobic mechanisms of biodegradation may be responsible for much of the observed removal rates.

A structure-activity relationship (SAR) model was available to help predict the anaerobic biodegradability of some of the compounds (Rorije *et al.*, 1998). A description of the model is given elsewhere (Khan, 2002). The model indicated that salicylic acid, ibuprofen, paracetamol, gemfibrozil, naproxen and ketoprofen were indeed likely to be susceptible to anaerobic biodegradation (Rorije, 2002). SAR tests were not conducted for methamphetamine or morphine. The model does not provide relative degrees or rates of degradation to enable a comparison or ranking between the compounds. The case for an anaerobic rather than aerobic mechanism was strengthened by the higher paracetamol removal observed during primary treatment compared to that observed in secondary aeration treatment.

More often than not, the concentration of ketoprofen was observed to increase after primary treatment. The likely explanation is that ketoprofen was present in raw effluent, partially as its glucuronide conjugate, which is the predominant excreted product. This may then have been somewhat hydrolysed during primary settling, to give further unconjugated ketoprofen.

The observed rates of removal of pharmaceuticals during secondary aeration treatment and clarification are shown in Table 4.6.

Table 4.6. PhAC residual removals during aeration for secondary treatment (%)

	Tues	Wed	Thurs	Fri	Mon	Average
Salicylic acid	84	89	70	86	83	82 ± 7
Ibuprofen	77	96	94	76	84	85 ± 9
Paracetamol	62	-	-	-47	16	10± 55
Metronidazole	-	-	-	-	-	-
Methamphetamine	-	-	-	-	-	-
Gemfibrozil	-	95	95	95	94	95 ± 0
Captopril	-	-	-	-	-	-
Naproxen	82	90	82	81	81	83 ± 4
Methadone	-	-	-	-	-	-
Ketoprofen	98	99	98	98	98	98 ± 0
Phenytoin	-	-	-	-	-	-
Carbamazepine	-	-	-	-	-	-
Morphine	91	90	91	-	-	91 ± 0

Most measured compounds exhibited removal rates of 80-90% during secondary treatment. Removal of paracetamol was highly erratic, however in one case, its concentration was observed to increase. It

is likely that competing processes of aerobic biodegradation and hydrolysis of conjugates may partially explain the observed variability.

The observed removal rates were generally greater than the predicted removal rates shown in Table 2.2. It was not possible to conclude whether the under-predicted parameter was adsorption to biomass, aerobic biodegradation or a combination of the two.

4.7 Sewage and sludge analysis for Bondi STP, Sydney

This study was undertaken at a major primary STP in Sydney. The analytical results are described in section 3.7. The purpose was to determine the degree of correspondence between predicted values (Table 2.1) and measured concentrations of pharmaceuticals in raw influent, primary effluent, primary sludge and digested sludge (Table 4.7). It was assumed that predicted concentrations in primary effluent and primary sludge for Castle Hill STP, would apply reasonably well for Bondi STP. The experiment was designed to provide detail regarding partitioning of the pharmaceuticals between aqueous and biomass phases.

Concentrations of representative compounds measured in the raw sewage influent appear to closely match the predicted concentrations. Relatively higher concentrations observed for paracetamol, salicylic acid, and ibuprofen may be attributed to the relatively high proportion of non-pharmacy purchase of those drugs. Three pharmaceuticals that were not identified in the Castle Hill raw sewage analysis were identified in raw sewage at Bondi. These were methadone, phenytoin and carbamazepine.

Precise analysis of the degree of match between the predicted and analytical results is not given as the model results were derived for a different STP. Furthermore, these results are for grab-samples only and, as such, do not represent the overall diurnal variation in pharmaceutical concentrations. The concentration of pharmaceuticals in sewage and sludge at Bondi STP are presented relative the raw influent concentrations in Table 4.7.

Table 4.7. Concentrations (*w/w*) of pharmaceuticals in sewage and sludge at Bondi STP relative to the concentrations in raw influent (%)

Pharmaceutical	Raw Influent	Primary Effluent	Primary Sludge			Digested Sludge	
	aqueous	aqueous	aqueous	dry	combined	aqueous	dry
Paracetamol	100	87	14	1554	61	1	0
Naproxen	100	105	63	33953	1082	4	0
Salicylic acid	100	80	71	85498	2636	9	0

Gemfibrozil	100	41	51	39733	1243	-	-
Ibuprofen	100	87	43	111397	3385	180	0
Ketoprofen	100	118	163	123750	3878	75	0
Methadone	100	39	7	197143	5918	82	1
Phenytoin	100	72	193	-	193	3	0
Carbamazepine	100	60	2510	1731000	54440	5980	14
Morphine	100	63	92	-	92	160	-

n.d.: not detected.

The removal rates of pharmaceuticals during primary settling at Bondi STP were, in most cases, similar to those observed at Castle Hill STP (section 4.6). The major exceptions were salicylic acid, paracetamol and gemfibrozil. Salicylic acid (20% removal) and paracetamol (13% removal) did not exhibit the same degree of removal (54% and 73% respectively) that had been observed during primary settling at Castle Hill STP. This may be a consequence of the high-rate primary settlement that is used at Bondi compared with the more conventional rate at Castle Hill. Gemfibrozil, on the other hand, was removed much more effectively at Bondi STP (59%) compared with removal during primary settling at Castle Hill STP (16%).

In this experiment, sludge samples were also collected. The sludge samples were filtered and the aqueous and dry fractions were analysed separately.

The concentrations predicted in the aqueous phase of primary sludge (Table 4.7) relative to the predicted influent concentrations were comparable to the measured analytical concentrations relative to the analytical influent concentrations, in most cases. However, the observed relatively high concentration of carbamazepine was difficult to explain.

The relative analytical and theoretical concentrations of pharmaceuticals in aqueous and biomass fractions of primary sludge are shown in Figure 4.13 as a percentage of influent concentration. Concentrations of pharmaceuticals associated with the biomass phase were less successfully predicted by the model. In most cases, significantly higher relative concentrations were measured than those predicted. Possible reasons include unaccounted-for hydrophilic interactions that may be responsible for pharmaceutical adsorption to solids (in addition to lipid partitioning). In addition, errors may have been incurred by the extrapolation of concentration from extraction of a relatively small sample size (<10g dry weight).

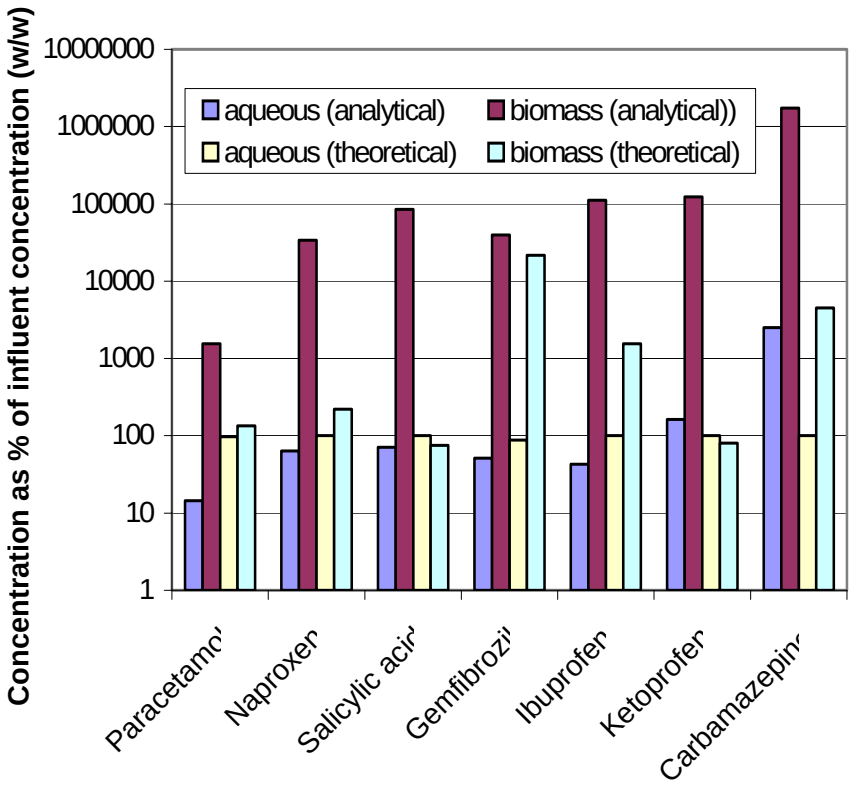


Figure 4.13. Analytical and theoretical concentrations of pharmaceuticals in aqueous and biomass fractions of primary sludge (as % influent concentration)

The high sludge adsorption results are not without precedence. Very similar observations were reported in Denmark (Stuer-Lauridsen *et al.*, 2000). A discussion of potential mechanisms for this under-predicted high sludge adsorption was presented in Section 2.1.6.

Most of the compounds were shown to persist in the aqueous component of digested sludge, exhibiting some resistance to anaerobic degradation. Concentrations in the solids component of digested sludge were extremely low, even when compared to those in the aqueous phase of the same sample. This indicated that once digested, the sludge solids did not retain their lipophilic (or other adsorbing) properties and that all of the investigated compounds had partitioned extensively to the aqueous phase.

4.8 Sewage analysis for three STPs in Berlin, Germany

The raw influents and final effluents of three large STPs in the city of Berlin (Germany) were investigated for pharmaceutical residues (Khan, 2002). The STPs were Schönerlinde (SCH), Ruhleben (RUH) and Falkenberg (FAL).

The raw sewage results show some interesting differences when compared to raw sewage from Castle Hill STP, Australia. Most strikingly, much higher concentrations of salicylic acid, ibuprofen and carbamazepine (see discussion below) were observed in Berlin. On the other hand, considerably higher concentrations of paracetamol and naproxen were observed in Sydney. Paracetamol and salicylic acid are often used to treat similar conditions and may be used rather interchangeably for the treatment of many symptoms. The same is true for ibuprofen and naproxen. It is possible that these concentration trends reflect variable regional preferences of interchangeable drugs.

Another important factor is Berlin's low per capita water use (130 L/person/day) compared with Australia (270 L/person/day) and the USA (about 300 L/person/day).

For the purpose of comparison, the samples described in this section were also analysed by Kirsten Reddersen at the Technical University of Berlin. Reddersen is a Ph.D. student working with Dr. Thomas Heberer, an experienced researcher in the analysis of pharmaceuticals from aqueous samples. Reddersen's analytical method is considerably different to the one described in this thesis. The main differences are:

- SPE sorbent (C18)
- Preextraction volume (500 ml, diluted to 1L)
- Elution solvent (MeOH)
- Derivatisation scheme (PFBBr and TBDMSTFA)
- Target analytes.
- GC-MS temperature profile, inlet liner etc.

Reddersen's results are presented in Table 4.8.

Table 4.8. Reddersen results for compounds common to both methods (Reddersen, 2001)

	SCH		RUH		FAL	
	Raw	Effluent	Raw	Effluent	Raw	Effluent
Gemfibrozil	n.d.	0.06	n.d.	0.02	n.d.	0.06
Ibuprofen	5.9	0.08	6.1	0.04	12.3	0.05
Ketoprofen	n.d.	0.15	n.d.	0.15	n.d.	0.13
Naproxen	0.61	0.45	0.78	0.4	1.15	0.33
Carbamazepine	1.27	2.65	0.66	2.09	2.56	3.7

n.d.: not detected.

Reddersen's analytical methods are well established and known to be reliable. However, they have been optimised for analysis of comparatively clean samples such as environmental surface waters and final sewage effluents. Consequently, some analytical difficulties were caused by interfering matrix compounds in the raw sewage samples. This is the reason for compounds not being detected in the

influents and that influent concentrations of carbamazepine were markedly less than the effluent concentrations.

The level of agreement between the results obtained for the methods presented in this thesis and the results obtained by Reddersen are mixed. The three compounds for which full sets of data are available for comparison are naproxen, ibuprofen and carbamazepine.

The results for naproxen are very similar, both in raw sewage and final effluent. Naproxen is an "analytically agreeable" compound. It is generally well extracted and easily derivatised. It is chemically stable and provides useful high intensity, high *m/z* mass peaks. Poor agreement between methods for this compound would have indicated considerable analytical deficiencies.

The results for ibuprofen are less well matched, however the results obtained by the methods of this thesis appear to be a reasonably consistent factor of two times Reddersen's results. Hence some systematic variation appears to be responsible.

The least-well matched results are for carbamazepine, which vary by factors of about two to four. As noted, Reddersen experienced complications in some of the raw sewage analysis and for this compound returned lower values for raw sewage than for treated effluent. However, this is a compound for which I have also experienced poor reproducibility in terms of GC-MS detection and poor recoveries. So it is not possible, without further testing, to identify where the major analytical deficiencies lie. It is conceivable that variable derivatisation efficiency may be a source of error.

Comparing effluent-only concentrations of gemfibrozil, the methods are in agreement that Schönerlinde (SCH) and Falkenberg (FAL) STPs were approximately equal in concentration and that Ruhleben (RUH) was somewhat lower. Again a systematic difference of a factor of 2-3 has prevailed. Effluent concentrations for ketoprofen are generally in closer agreement.

Removal efficiencies calculated for the pharmaceutical residues in the Berlin STPs are presented in Table 4.9. Some compounds, most notably salicylic acid, ibuprofen, paracetamol and morphine, were easily removed during sewage treatment. Others such as naproxen, ketoprofen, phenytoin and carbamazepine were considerably less-efficiently removed.

Table 4.9. Removal of pharmaceuticals (%) in Berlin STPs

	SCH	RUH	FAL
Salicylic acid	100	100	100
Ibuprofen	98	99	99
Paracetamol	99	97	99
Metronidazole	-	-	-
Methamphetamine	-	-	-
Gemfibrozil	69	100	78
Captopril	-	-	-
Naproxen	31	65	65
Methadone	51	-	100
Ketoprofen	-	38	-29
Phenytoin	67	-	-
Carbamazepine	18	22	4
Morphine	85	99	93

The observed removal rates of pharmaceuticals from the three Berlin STPs were generally comparable to those observed at Castle Hill STP (section 4.6). Removal rates of salicylic acid, ibuprofen and (especially) paracetamol more closely approached completeness in Berlin. Naproxen and ketoprofen appear to have been more effectively removed in the Castle Hill analysis. The negative removal rate of ketoprofen at Falkenberg (FAL) is, as previously, suspected to be due to hydrolysis of ketoprofen conjugates during sewage treatment. The same phenomenon was observed during primary settling at Castle Hill STP (Table 4.5) and Bondi STP (Table 4.7).

In comparing removal efficiencies with Reddersen's results (Table 4.10), calculated removal rates for ibuprofen are the same, confirming the systematic variation mentioned above. Rates for naproxen are also very close, with both methods in agreement that the least efficient removal occurred at Schönerlinde (SCH). Carbamazepine comparisons were hampered as described above, however, both methods agree that the relative removal rates of the compounds was ibuprofen > naproxen > carbamazepine.

Table 4.10. Removal of pharmaceuticals calculated with Reddersen's results

	SCH	RUH	FAL
Gemfibrozil	-	-	-
Ibuprofen	99	99	100
Ketoprofen	-	-	-
Naproxen	26	49	71
Carbamazepine	-109	-217	-45

The conclusion derived from this study was that each method appears to give fairly consistent results, however, systematic differences, between the methods, of a factor of 2-4 are evident for some compounds.

4.9 Sewage & sludge analysis for East Bay Municipal Utilities District, USA

The concentrations of pharmaceutical residues at East Bay Municipal Utilities District (EBMUD) STP were determined as a comparison to the data obtained from Australian and German STPs. The results and analytical details were presented in section 3.9.

It was intended to collect and analyse a series of samples in order to establish a full mass-balance of some pharmaceuticals in the system. However, two factors prevented the mass-balance from being achieved. The first was the difficulties encountered in analysing the biomass phase of the S1 (primary sludge) samples as described in section 3.9.2.2. Previous investigations have shown that this is an important component of the overall mass-balance (see section 4.7). The second factor was that 24-hour composite samples were only available for the raw influent (L1) and final effluent (L4). All other samples were collected by grab-sampling at noon. Previous results have shown that such grab-samples are not comparable to 24-hour composites due to significant diurnal variations (see section 4.5). This incomparability was further apparent by inspection of the analytical results. In all cases, Sample L1 (raw influent, composite) exhibited lower concentrations than Sample L2 (primary effluent, noon grab) and Sample L3 (secondary clarifier influent, noon grab) exhibited lower concentrations than Sample L4 (secondary effluent, composite). These observed anomalies could be explained by diurnal variations, as well as by variable degrees of biodegradation corresponding to variable sample ages (time between collection and extraction) and variable sample biomass concentrations.

4.9.1 Comparison of influent concentrations with Australian data

The variations in influent pharmaceutical concentrations from region to region provide an indication of the relative degrees of drug utilisation between the regions. As previously noted, typical per-capita municipal water use and wastewater generation are not constant between regions, so this variation must also be considered when comparing concentrations.

The influent concentration data from EBMUD were compared to the average pharmaceutical concentrations determined for Castle Hill STP (Table 4.4). The concentrations of 13 pharmaceuticals

from the two STPs are shown in Figure 4.14. The influent pharmaceutical concentrations at Castle Hill STP and EBMUD were generally similar. The clear exception was paracetamol (acetaminophen), which was determined to be 104.48 ± 1.79 μg/l at Castle Hill but was only 1.48 μg/l at EBMUD. Given the relative agreement for the remaining detected pharmaceuticals, this appears to be a significant regional variation in the utilisation of paracetamol. Further testing, however, would be required to adequately preclude other variables including water use, seasonal variations, as well as sampling and analytical variations.

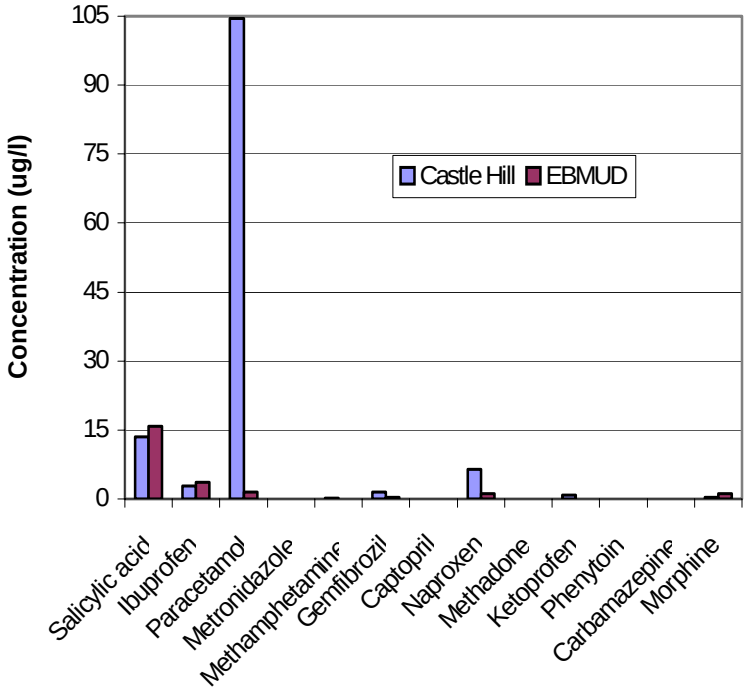


Figure 4.14. Comparison of influent pharmaceutical concentrations (μg/l) for Castle Hill STP and EBMUD

4.9.2 Overall removal rates

As discussed above, only the results for the sewage samples that were collected as 24-hour composites can be reasonably compared. The raw influent (L1) and final effluent (L4) were sampled by this means and, therefore, provide an indication of the overall removal rates of the pharmaceuticals during secondary treatment at EBMUD. The percentage removal rates of pharmaceuticals during treatment were salicylic acid (93%), ibuprofen (94%), paracetamol (10%) and gemfibrozil (-74%). The negative removal rate for gemfibrozil may be the result of accelerated biodegradation in the composite raw sewage sample (high biosolids) compared with the final effluent sample (low biosolids). The removal rate for paracetamol is considerably lower than has been observed in some of the other investigations, however, this compound has previously exhibited

somewhat erratic removal efficiency during secondary aeration treatment (see section 4.6.2). Morphine was effectively removed, being detected in the influent, but not the effluent. Methadone was identified in the effluent, but not in the influent. This is likely to be the result of difficulties endured in the analysis of this particular compound in high-background samples rather than the absence of methadone in the influent.

4.9.3 Partitioning to and from biomass in sludge samples

As discussed above, the biomass fraction of primary sludge sample (S1) extracted in this investigation did not prove suitable for GC-MS analysis. However, the secondary sludge sample (S2) was filtered and the aqueous and biomass fractions were successfully analysed. Four of the analytes were identified to be associated with the filtered biomass from secondary sludge. These were salicylic acid (11.43 μg/kg), paracetamol (127.39 μg/kg), gemfibrozil (213.69 μg/kg) and naproxen (17.96 μg/kg). The concentrations of these compounds in the aqueous fraction of the same sludge sample were determined to be salicylic acid (1.65 μg/l), paracetamol (n.d.) gemfibrozil (0.64μg/l) and naproxen (0.05 μg/l). Residuals of ibuprofen (0.33 μg/l) and morphine (2.65 μg/l) were also identified in the aqueous phase, though not in the biomass phase. These results indicate that paracetamol, gemfibrozil and naproxen were considerably associated with the biomass phase; salicylic acid was moderately associated with the biomass phase. Ibuprofen and morphine were associated predominantly with the aqueous phase in secondary sludge.

After centrifugation of the waste activated sludge, some of the pharmaceuticals were measurable in the centrate sample (L5). These were salicylic acid (0.68 μg/l), ibuprofen (6.69 μg/l), methamphetamine (3.19 μg/l), gemfibrozil (3.68 μg/l) and morphine (2.49 μg/l). The detection of methamphetamine in the centrate suggests that the detectability of this compound was enhanced by the concentrating effect of centrifugation.

A number of the pharmaceuticals persisted in measurable concentrations in the digested sludge sample (S3). Three of these were determined to be associated with the digested biomass phase. These were salicylic acid (6.95 μg/kg), ibuprofen (19.54 μg/kg) and gemfibrozil (32.02 μg/kg). The aqueous phase of the digested sludge was determined to consist of salicylic acid (8.09 μg/l), ibuprofen (16.14 μg/l), gemfibrozil (5.89 μg/l), methadone (0.34 μg/l) and morphine (2.39 μg/l). The feed to the sludge digestion tanks consists mainly of primary sludge rather than secondary sludge. Therefore, these results are not considered to be comparable with the secondary sludge results, above.

4.10 Sewage & sludge analysis for San Jose/Santa Clara STP

The data from the sewage and sludge analysis for San Jose/Santa Clara STP are shown in section 3.10.2. Various flow and composition details were required to undertake a mass-balance analysis of selected pharmaceuticals in the STP. These are presented in Table 4.11.

Table 4.11. Mass balance parameters for SJ/SC STP

Mass balance parameter	Fraction
PRIMARY SETTLING	
Fraction of influent that flows to primary effluent	0.9956
Fraction of influent that flows to primary sludge	0.0044
Fraction of flow through tanks A/B	0.50
Fraction of flow through tanks C/D	0.50
Solids concentration in sludge from tanks A/B	0.0034
Solids concentration in sludge from tanks C/D	0.0036
ACTIVATED SLUDGE (AS) TREATMENT	
Fraction of primary effluent that flows to “A-side” AS tanks	0.684
Fraction of primary effluent that flows to “B-side” AS tanks	0.316
SECONDARY CLARIFICATION	
Fraction of “A-side” clarifier influent recycled back to AS tank	0.65
Fraction of “B-side” clarifier influent recycled back to AS tank	0.70
Fraction of “A-side” clarifier influent removed as waste AS	0.02
Fraction of “B-side” clarifier influent removed as waste AS	0.02
Solids concentration in “A-side” clarifier sludge	0.0081
Solids concentration in “B-side” clarifier sludge	0.0076

The sampling for this analysis was undertaken in dry weather and the plant-recorded flow rate for the sampling day was 479 Ml (126.3 MG). As stated in section 3.10, the flow through each primary tank was approximately equal. For mass balance reasons this was only of interest with respect to the sludge streams. The quantity of flow responsible for generating the A/B sludge should have been about equal to that generating the C/D sludge. About twice as much primary effluent flows to the “A-side” compared to the “B-side” activated sludge systems.

The mass-balance for the pharmaceutical residuals was calculated by considering the flows and concentrations during the various stages of sewage treatment as a proportion of the total amount of salicylic acid in the raw influent (per unit time). The calculation was given by the following equation:

$$\text{Mass (\%)} = \frac{\text{Concentration}}{\text{Influent concentration}} \times \frac{\text{Flow rate}}{\text{Influent flow rate}}$$

Salicylic acid was the only compound with measurable loads in each of the important compartments contributing to the overall mass-balance of the system. Accordingly, salicylic acid was selected as the

best example with which to illustrate the mass balance calculations. Data for other measured compounds are included where applicable.

The mass-balance figures are given for salicylic acid in Table 4.12. The data are presented as overall loads of compound in aqueous and biomass phases of samples representing the various stages of the sewage treatment process. The figures are given, normalised to the raw sewage (influent) load of salicylic acid.

Table 4.12. Mass balance for salicylic acid at SJ/SC STP (as % of influent load)

Source	Aqueous (%)	Biomass (%)	Total (%)
Raw sewage	100.00	-	100.00
Primary effluent	335.76	-	335.76
Mixed liquor "A-side"	15.38	-	15.38
Mixed liquor "B-side"	0.28	-	0.28
Secondary clarifier effluent "A-side"	3.72	-	3.72
Secondary clarifier effluent "B-side"	0.36	-	0.36
Final effluent	2.58	-	2.58
Primary sludge A/B side	0.59	0.00	0.59
Primary sludge C/D side	3.34	0.00	3.33
Return activated sludge "A-side"	0.39	1.56	0.40
Return activated sludge "B-side"	1.28	0.71	1.28
Waste activated sludge "A-side"	0.01	0.05	0.01
Waste activated sludge "B-side"	0.04	0.02	0.04

The mass-balance results indicate that salicylic acid was almost entirely associated with the aqueous phases of the samples for which both aqueous and biomass phases were tested. Biomass salicylic acid loads had only very minor bearing on the total loads. This was also the case for ibuprofen, methamphetamine and naproxen. However gemfibrozil, methadone and, to a lesser extent, paracetamol had significant proportions of the total loads associated with biomass.

The raw influent of the STP was split evenly between the "A/B" settling tanks and the "C/D" settling tanks. It is unclear, therefore why such considerable salicylic acid load differences were measured in the primary sludges. This phenomenon was observed for all of the compounds that were measurable in the primary sludge. These were ibuprofen, paracetamol, methamphetamine, gemfibrozil and methadone. It is possible that some post-sampling biodegradation had occurred in the sludge samples and therefore, variations of a few hours in sample age may have had a significant effect. Regardless, the sludge loads were relatively minor components of the total post-primary treatment load in all cases. The total salicylic acid load after primary treatment, as a percentage of the load prior to primary treatment was given by:

	Primary effluent	+	Primary sludge “A/B”	+	Primary sludge “C/D”
=	335.76	+	0.59	+	3.33
=	339.68 %				

Aspirin is excreted predominantly as (potentially hydrolysable) conjugates of salicylic acid (Moffat, 1986). The observed increase in free salicylic acid concentration after primary settling was presumed to be the result of hydrolysis of such conjugates. The “removal” rate for salicylic acid during primary settling was then –240%. Negative removal rates were also observed for ibuprofen (-460%) and paracetamol (-1%), both of which are also excreted largely as conjugates. Other calculated removal rates were for methamphetamine (68%), gemfibrozil (9%), naproxen (77%) and methadone (100%). Given that removal to sludge was included in the mass balance calculation and that these are not believed to be volatile compounds (see individual tables), the removal rates were assumed to be attributable to biodegradation during primary settling.

During secondary treatment, twice as much primary effluent is treated by the “A-side” compared to the “B-side” of the plant (see Table 4.11). The load of salicylic acid after activated sludge treatment on the “A-side” of the plant, as a percentage of load delivered by the combination of primary effluent and return activated sludge (RAS) was given by:

$$\frac{\text{Mixed liquor “A”}}{(\text{Fraction “A”} \times \text{Primary effluent}) + \text{RAS “A”}} \times 100$$

$$= \frac{15.38}{(0.684 \times 335.76) + 0.40} \times 100$$

$$= 6.68\%$$

Accordingly, the removal rate for salicylic acid during activated sludge treatment on the “A-side” of the plant was 93.3%. Similarly, ibuprofen was removed 97.1%. Other compounds were shown to be efficiently removed by virtue of the fact that they were observed in the primary effluent, but were not detected in the mixed liquor of the “A-side” activated sludge tank. These were paracetamol, methamphetamine, gemfibrozil and naproxen.

The salicylic acid load after activated sludge treatment on the “B-side” of the plant, as a percentage of load delivered by the combination of primary effluent and return activated sludge (RAS) was given by:

$$\frac{\text{Mixed liquor “B”}}{(\text{Fraction “B”} \times \text{Primary effluent}) + \text{RAS “B”}} \times 100$$

$$= \frac{0.28}{(0.316 \times 335.76) + 1.28} \times 100$$

$$= 0.26\%$$

The removal rate for salicylic acid during activated sludge treatment on the “B-side” of the plant was 99.7%. Ibuprofen was removed 99.5% and gemfibrozil was removed 35.%. Compounds observed in the primary effluent, but not detected in the mixed liquor of the “A-side” activated sludge tank were paracetamol, methamphetamine and naproxen. Again, it was assumed that the loss of compounds during activated sludge treatment was due to biodegradation.

The load of salicylic acid after secondary clarification on the “A-side” of the plant, as a percentage of the load in mixed liquor was given by:

$$\frac{\text{Clarifier “A”}}{\text{Mixed liquor “A”}} \times 100$$

$$= \frac{3.72}{15.38} \times 100$$

$$= 24.18\%$$

The determined removal rates during clarification on the “A-side” were for salicylic acid (75.8%) and ibuprofen (-1112%). The reason for the observed increase in ibuprofen during clarification is unknown. However, it is likely that inaccuracies may be partially attributable to the fact that the mixed liquor samples were only available as “grab-samples” and may not have been representative of the overall daily load. The clarifier effluent samples were collected as 24-hour composites.

The load of salicylic acid after secondary clarification on the “B-side” of the plant, as a percentage of the load in mixed liquor was given by:

$$\frac{\text{Clarifier “B”}}{\text{Mixed liquor “B”}} \times 100$$

$$= \frac{0.36}{0.28} \times 100$$

$$= 130.66\%$$

The removal rate for salicylic acid during clarification on the "B-side" of the plant was –30.7%. In this case, ibuprofen and gemfibrozil were observed in the mixed liquor, but were not detected after clarification. Again, the reason for the elevated salicylic acid concentration is unknown and non-representative grab-sampling of mixed liquor samples was presumed to be a contributing factor.

The salicylic acid load after filtration and disinfection, as a percentage of the load carried by the secondary clarifier effluent, is given by:

$$\frac{\text{Final effluent}}{\text{Clarifier "A"} + \text{Clarifier "B"}} \times 100$$

$$= \frac{2.58}{3.72 + 0.36} \times 100$$

$$= 63.11\%$$

Residual salicylic acid was observed to be removed 36.9% during filtration and disinfection. Ibuprofen, which was measurable in the "A-side" clarifier effluent was not measurable after filtration and disinfection. Other compounds were not detected in the clarifier effluents nor the final effluent.

The overall results of the mass-balance investigation have indicated that the total measurable loads of all investigated compounds were largely eliminated by biodegradation during sewage treatment. Since an unknown quantity of hydrolysable conjugates of some compounds appeared to have been present in the influent of the plant, it was not possible to determine the degree of biodegradation during primary settling. However, the results indicate that biodegradation during activated sludge treatment was an effective means for the removal for all of the detected compounds and that further removal of the few remaining residuals occurred during filtration and disinfection.

4.11 Removal of pharmaceuticals from domestic wastewater by advanced water recycling technology.

This analysis was undertaken to determine the effectiveness of a range of advanced water recycling technologies for the removal of pharmaceuticals from treated sewage effluent. The results are presented in section 3.11. The technologies investigated included ozonation, microfiltration, nanofiltration and reverse-osmosis.

Ozonation is a chemical oxidation process used for the degradation of organic contaminants in water (Chiron *et al.*, 2000). The ozone is generated by an electric discharge method in the presence of air or

oxygen. One of the disadvantages of ozonation is that the process does not actually remove the contaminants from the water stream, but degrades them. Consequently, degradation products remain of concern in ozone-treated water. Ozonation was only briefly examined in this study. The influent to the ozonation module was the effluent from the dual-media filtration module. By comparing the concentrations of pharmaceuticals in the ozonation influent to the ozonation effluent it was intended to determine the effectiveness of ozonation in the removal of these compounds. Since the influent is already quite highly treated wastewater, only salicylic acid was measurable (Table 3.18). The result obtained indicates that salicylic acid was partially degraded from the influent (0.65 μg/l) to the effluent (0.26 μg/l) during ozonation.

Microfiltration is a physical membrane filtration process that is used to remove particles, above a certain size, from water (Schäfer, 1999). Microfiltration pores are relatively large (typically 0.1-1 μm). The removal of organic substances is possible if they are associated with particulates in the water. Initial testing of the wastewater indicated that salicylic acid was partially removed during membrane filtration. The previous module (biological activated carbon) exhibited an effluent concentration of 0.23 μg/l, while the microfiltration effluent was reduced to 0.13 μg/l (Table 3.17). None of the other analytes were observed in either effluent.

Spiking experiments were undertaken to further investigate the pharmaceutical removal efficiency of microfiltration (Table 3.20). During the spiking period, all of the analytes were observed in both the feed (influent) to the microfiltration unit and the effluent, however a partial reduction in concentration was evident for all pharmaceuticals (Figure 4.15). Most of the pharmaceuticals were not measurable in samples collected from the microfiltration unit 25 minutes after the cessation of spiking (Table 3.20).

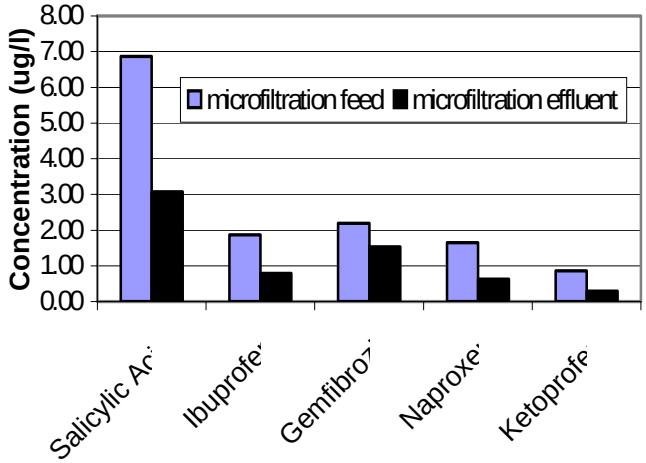


Figure 4.15. Removal of pharmaceuticals by microfiltration during spiking experiment.

The observed concentration reductions could be attributable to adsorption on the membrane rather than removal by size exclusion. This possibility could potentially be confirmed by the examination of removal over extended periods of operation, in which time the adsorptive capacity of the membrane for a particular compound may reach saturation. Another possible explanation is that compounds may have been removed by adsorption to larger particles which were retained by the membrane filtration.

Nanofiltration is a membrane filtration process in which organic solutes and multivalent ions are removed by a combination of size exclusion and charge interactions (Schäfer, 1999). Nanofiltration membranes feature so-called nano-pores, which are charged channels. Mass transfer is not only controlled by solution-diffusion but also by charge effects and convection through these nano-pores. For many trace contaminants with a molecular mass greater than 200 amu, nanofiltration is a useful option allowing higher water fluxes and hence a better economic feasibility than reverse osmosis.

Spiking tests undertaken for the nanofiltration units gave somewhat erratic results. In some cases, the membrane permeate concentrations were greater then the measured concentration in the feed to the module (Table 3.20). This was likely to be partially the result of variations caused by the stepwise (non-continuos) dosing regime and the time delay between sample collections. However, the membrane permeate and membrane retentate concentrations were generally similar and often featured permeate concentrations greater than the retentate concentrations. This indicated that the compounds were generally not being effectively removed by nanofiltration and concentrated in the retentate. In a later experiment where the nanofiltration permeates and retentates were monitored after spiking of the microfiltration module, there was some evidence of pharmaceutical concentration in the retentate (Table 3.20). However, the overall removal rates from the microfiltration effluent to the nanofiltration permeate were generally not significant.

Previous studies have indicated that organics may be adsorbed, on initial spiking, to the nanofiltration membrane. Once the influent concentration drops, the compounds then desorbs from the membrane (Chang *et al.*, 2002). This process may have contributed to the observations described here.

Reverse-osmosis is a membrane filtration method similar to nanofiltration. However, reverse osmosis membranes are much denser polymer matrices where permeation can only occur via a solution-diffusion process. In terms of organics rejection, reverse osmosis membranes generally display higher performances and are less dependant on pH-variations. The use of reverse osmosis in potable reuse of wastewater has been recently discussed (Ramirez *et al.*, 2002).

The results of the spiking tests undertaken in this study indicate that the reverse osmosis process was highly effective in the removal of all of the tested pharmaceutical compounds (Table 3.19 and Table 3.20). Only in one case was any of the pharmaceuticals identified in the reverse osmosis permeate (salicylic acid, 0.25 μg/l) whereas they were regularly observed in the feed (Table 3.19) and the retentates (Table 3.20) at significant concentrations.

Of the advanced wastewater recycling technologies considered in this study, only reverse-osmosis was able to demonstrate significant and consistent removal of the investigated pharmaceutical compounds. As such, reverse osmosis shows promising potential for the effective routine removal of a wider range of pharmaceutical residues from sewage.

REFERENCES

1. Adams, T. B., Greer, D. B., Doull, J., Munro, I. C., Newberne, P., Portoghese, P. S., Smith, R. L., Wagner, B. M., Weil, C. S., Woods, L. A. and Ford, R. A. (1998) The FEMA GRAS assessment of lactones used as flavour ingredients. *Food Chem. Toxicol.,* **36**(4), 249-278.

2. Alcock, R. E., Sweetman, A. and Jones, K. C. (1999) Assessment of organic contaminant fate in waste water treatment plants. I: Selected compounds and physicochemical properties. *Chemosphere*, **38**(10), 2247-2262

3. Australian Commonwealth Department of Health and Aged Care (1999a) Australian Statistics on Medicines 1998. Pharmaceutical Benefits Advisory Committee, Commonweath of Australia, Canberra Buser et al., 1999

4. Ayscough, N. J., Fawell, J., Franklin, G. and Young, W. (2000) Review of human pharmaceuticals in the environment, R&D Technical Report P390, UK Environment Agency, Bristol

5. Buser, H.-R., Muller, M. D. and Theobald, N. (1998) Occurrence of the pharmaceutical drug clofibric acid and the herbicide mecoprop in various Swiss lakes and in the North Sea. *Environ. Sci. Technol.,* **32**, 188-192

6. Chang, S., Waite, T. D., Schäfer, A. I. and Fane, A. G. (2002) Binding of estrone to hollow fibre membranes in microfiltration of solutions containing trace estrone. In: Proceedings of the 3rd International Water Association World Water Congress Melbourne

7. Chiron, S., Fernandez-Alba, A., Rodriguez, A. and Garcia-Calvo, E. (2000) Pesticide chemical oxidation: state-of-the-art. *Water Res.,* **34**(2), 366-377.

8. Clark, B., Henry, J. G. and Mackay, D. (1995) Fugacity analysis and model of organic chemical fate in a sewage treatment plant. *Environ. Sci. Technol.,* **29**, 1488-1494.

9. Daughton, C. G. and Ternes, T. A. (1999) Pharmaceuticals and personal care products in the environment: Agents of subtle change? *Environ. Health Persp.,* **107**(Sup 6), 907-938

10. Garrison, A. W., Pope, J. D. and Allen, F. R. (1976) GC/MS analysis of organic compounds in domestic wastewaters. In: Identification and Analysis of Organic Pollutants in Water (Ed, Keith, L. H.) Ann Arbor Science Publishers Inc, Ann Arbor, 517-556

11. Hignite, C. and Azarnoff, D. L. (1977) Drugs and drug metabolites as environmental contaminants: Chlorophenoxyisobutyrate and salicylic acid in sewage water effluent. *Life Sci.*, **20**, 337-342

12. Khan, Stewart, 2002. Occurrence, Behaviour, and Fate of Pharmaceutical Residues in Sewage Treatment. Doctoral Thesis, University of New South Wales, Sydney, Australia.

13. Khan, S, and JE Ongerth*, 2002. Estimation of pharmaceutical residues in primary and secondary sewage sludge based on quantities of use and fugacity modelling. Wat Sci Technol, 46(3):105-113, August, 2002.

14. Kahn, S, and JE Ongerth, 2004. Modelling of pharmaceutical residues in Australian sewage by quantities of use and fugacity calculations. Chemosphere 54: 355 -367

15. Lindström, A., Buerge, I. J., Poiger, T., Bergqvist, P. A., Müller, 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. *Environ. Sci. Technol.*, **36**(11), 2322-2329

16. Metcalf & Eddy. Inc. (1991) Wastewater Engineering. Treatment, Disposal and Reuse. 3rd Ed., McGraw-Hill, Inc., New York

17. Ramirez, J. A. L., Alonso, J. M. Q., Marquez, D. S. and Asano, T. (2002) Indirect potable reuse and reverse osmosis: challenging the course to 'new water'. *Water 21*, (June), 56-59

18. Reddersen, K. (2001) Technical University of Berlin, Berlin. *Personal communication*

19. Rodrigues, E. (2002), Castle Hill Sewage Treatment Plant. *Personal communication*

20. Rogers, I. H., Birtwell, I. K. and Kruzynski, G. M. (1986) Organic extractables in municipal wastewater, Vancouver, British Columbia. *Water Poll. Res. J. Canada.*, **21**(2), 187-204.

21. Rorije, E., Eriksson, L., Verboom, H., Verhaar, H. J. M., Hermens, J. L. M. and Peijnenburg, W. J. G. M. (1997) Predicting reductive transformation rates of halogenated aliphatic compounds using different QSAR approaches. *Environ. Sci. Pollut. Res.*, **4**, 47-54.

22. Schäfer, A. I. (1999) Natural Organics Removal Using Membranes, A thesis presented to Chemical Engineering and Industrial Chemistry, University of New South Wales, Sydney

23. Stuer-Lauridsen, F., Birkved, M., Hansen, L. P., Lutzhoft, H. C. and Halling-Sorensen, B. (2000) Environmental risk assessment of human pharmaceuticals in Denmark after normal therapeutic use. *Chemosphere*, **40**(7), 783-93

24. Westall, J. C., Leuenberger, C. and Schwarzenbach, R. P. (1985) Influence of pH and ionic strength on the aqueous-nonaqueous distribution of chlorinated phenols. *Environ. Sci. Technol.*, **19**(2), 193-8.