Targeted Monitoring Programme for Pharmaceuticals in the Aquatic Environment

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Statement of Use
This document reports the targeted monitoring of twelve selected pharmaceutical compounds and metabolites in sewage treatment works (STWs) effluents and surface waters. It is to be used for information by Agency staff in developing policy and future actions.

Keywords
Pharmaceutical compounds, targeted monitoring, trimethoprim, diclofenac, sulfamethoxazole, sulfamethoxazole acetate, paracetamol, mefenamic acid, ibuprofen, erythromycin, dextropropoxyphene, lofepramine, tamoxifen, propranolol.

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EXECUTIVE SUMMARY

The occurrence and potential adverse effect of pharmaceutical compounds in the aquatic environment is a subject of scientific interest and public awareness. To investigate the potential risk posed to the aquatic environment in England and Wales by pharmaceutical substances, the Environment Agency initially commissioned a review of the information in the literature on the occurrence, fate and effects of human pharmaceuticals in the environment (Ayscough *et al*. 2000). The agency has subsequently adopted a screening approach based on the EU Technical Guidance document on risk assessment (1996). Pharmaceutical substances have been ranked on their relative risk, to identify those substances that pose the greatest potential risk to the aquatic environment. A number of substances were identified for further research and these were the candidates for a targeted monitoring programme. The Agency commissioned the Centre for Environment, Fisheries and Aquaculture Science (CEFAS) to conduct a targeted monitoring study of twelve of the selected pharmaceutical compounds and pharmaceutical compound metabolites at UK sewage treatment works (STW). The occurrence data generated by this targeted monitoring programme will be used to verify the Predicted Environmental Concentrations (PECs) derived during the screening process, reduce the uncertainty associated with the screening process and provide actual data to enable the Environment Agency to better determine potential risk.

Analytical methods were developed and validated to determine ng L^{-1} concentrations of the pharmaceutical compounds trimethoprim, diclofenac, sulfamethoxazole, acetyl-sulfamethoxazole, paracetamol, mefenamic acid, ibuprofen, erythromycin, dextropropoxyphene, lofepramine, tamoxifen and propranolol in STW effluents and receiving water samples collected over a three month period during 2002. STW final effluent and receiving water samples were collected from Corby, Great Billing, East Hyde, Harpenden and Ryemeads STWs and analysed for the targeted pharmaceuticals.

Ibuprofen was detected at the highest concentrations in both the effluents (~27 µg L^{-1}) and receiving waters (5 µg L^{-1}). The mean concentration of ibuprofen in STW effluents was 4.2 µg L^{-1} at a frequency of 84 % of the effluent samples collected, whilst a mean ibuprofen concentration of 1.1 µg L^{-1} was determined in receiving waters at a frequency of 70 %. Diclofenac was detected at a mean concentration of 0.6 µg L^{-1} in effluents at a frequency of ~90 % and 0.15 µg L^{-1} in receiving waters at a lower frequency. Propranolol was detected in all of the effluent samples collected at a mean concentration of 0.1 µg L^{-1} and in receiving waters at a mean concentration of 0.04 µg L^{-1}. Mefenamic acid and dextropropoxyphene were detected in ~75% of the effluent samples collected at mean concentrations of between 0.2 and 0.3 µg L^{-1}. Lower concentrations of 0.15 µg L^{-1} for dextropropoxyphene and 0.01 µg L^{-1} for mefenamic acid were determined in receiving waters. Erythromycin, trimethoprim and acetyl-sulfamethoxazole were determined in approximately a third of the effluent samples collected at mean concentrations of between 0.1 and 0.2 µg L^{-1}, whilst lower mean concentrations were determined in receiving waters. Sulfamethoxazole was detected in only 9 % of the effluent samples collected and in none of the receiving water samples. Paracetamol was not detected in any of the samples collected.

The environmental input of each targeted pharmaceutical is also reported using the occurrence data generated by this study and flow data obtained from each STW at the time of sampling.

GLOSSARY

CAS	Chemical abstracts service: A unique number assigned to a chemical compound.
CRM	Consecutive reaction monitoring
DCM	Dichloromethane
ESI	Electrospray ionisation
GFC	Glass fibre filter
HPLC	High performance liquid chromatography
LOD	Limit of detection
MSMS	Tandem mass spectrometry
PE	Population equivalents
PEC	Predicted environmental concentration
RSD	Relative standard deviation
SIM	Selected ion monitoring
SPE	Solid phase extraction
STW	Sewage treatment works

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1 INTRODUCTION

1.1 Background

The presence and potential adverse effect of pharmaceuticals in the aquatic environment has begun to receive increasing interest in the popular and scientific press. This is largely a result of a growing number of scientific papers published in the 1990s which have reported trace levels of pharmaceuticals detected in environmental samples, including sewage effluent, surface water, groundwater and drinking water, most of these data being collected in Germany.

To investigate the potential risk posed to the aquatic environment in England and Wales by pharmaceutical substances, the Environment Agency initially commissioned a review of the information in the literature on the occurrence, fate and effects of human pharmaceuticals in the environment (Ayscough *et al*., 2000).

The Agency has subsequently adopted a screening approach based on the EU Technical Guidance document on risk assessment (1996). Their aim was to rank substances on their relative risk, to enable those substances with the greatest potential to pose a risk to the aquatic environment to be identified. Eleven substances were identified for further research and these were the candidates for a targeted monitoring programme (Table 1.4). This monitoring programme will enable verification of the Predicted Environmental Concentrations (PECs) derived during the screening process, reduce the uncertainty associated with the screening process and provide actual data to enable the Environment Agency to better determine potential risk. This report presents the results of the said targeted monitoring programme.

1.2 Objectives

The overall objective of the study was to conduct a targeted monitoring programme in a number of UK sewage treatment works (STWs) and rivers to determine the concentration (if any) of a selection of specified pharmaceutical substances. The purpose of this monitoring is to help the Environment Agency determine whether these pharmaceuticals are present at concentrations that may be of potential concern in the aquatic environment.

The specific aims were:

1. To devise a suitable field-monitoring regime for a three month sampling programme.

2. To develop analytical methods for three substances: lofepramine, dextropropoxyphene and tamoxifen.

3. To analyse water samples for pharmaceutical substances of interest (Table 1.4).

1.3 Pharmaceutical metabolites

At the inception of this project the Environment Agency requested that pharmaceutical metabolites were also monitored. Unfortunately this was not possible within this study since the available resources were directed towards obtaining robust methods for the analysis of

parent pharmaceuticals. The development of robust methods was required since difficulties were encountered with replicating the performance data of reported methods.

1.4 The selection of substances for targeted monitoring

The aim of this prioritisation was to rank substances on their relative risk, enabling those substances with the greatest potential to pose a risk to the aquatic environment to be identified. The assumptions used in this process are highly conservative and therefore this process is only suitable for relative risks to be identified and conclusions should not be drawn about the potential risk of individual substances.

1.4.1 Risk Characterisation

The potential risk of a substance to the environment is often characterised by comparing the Predicted Environmental Concentration (PEC) value with the Predicted No Effect Concentration (PNEC) (Equation 1), producing a Risk Characterisation Ratio. This approach was used as a starting point in this prioritisation exercise.

$$Risk\ Characterisation\ Ratio = \frac{PEC}{PNEC} \quad \text{(Equation 1)}$$

Therefore, in order to obtain values for the Risk Characterisation Ratio of the pharmaceutical substances, values for PEC and PNEC needed to be obtained.

Prediction of Environmental Concentrations (PEC)

Predicted Environmental Concentrations (PECs) were derived using the approach detailed in the EU technical guidance on risk assessment (1996) (Equation 2). The calculation uses a simple equation based on usage, population and wastewater production to generate the PEC in surface water ($PEC_{w)}$ and provides a likely 'worst case' concentration for the pharmaceuticals in surface waters. It was assumed that there was no removal during sewage treatment and that the effluent is diluted by a factor of 10 in receiving waters.

$$PEC_w = \frac{A \times (100 - R)}{365 \times P \times V \times D \times 100} \quad \text{(Equation 2)}$$

Where:

PEC_w is the predicted concentration in surface water;
A is the amount of substance used per year (mg yr^{-1});
R is the removal rate in sewage treatment (set to 0);
P is the population under consideration (i.e. UK);
V is the volume of wastewater produced per capita per day (assumed to be 150 L)
D is the dilution factor in the environment (default of 10)

Since R, P, V and D were ascribed constant values for all substances, in order to calculate PEC_w, data on the amount of each pharmaceutical used in the UK was required. Tonnage data for the year 1999 for the top 500 substances was obtained from Intercontinental Medical Statistics Health (IMS). These data were taken from the British Pharmaceutical Industry -

audit of purchases by retail pharmacies and dispensing doctors of registered pharmaceutical products. Sales to hospitals and sales of over-the-counter (OTC) products in to outlets such as supermarkets, garages etc. are excluded. The data covered the whole of the UK, including Northern Ireland. PEC_w values were therefore calculated for these top 500 substances.

Prediction of No-Effect Concentrations (PNEC)

Data were collected on properties and effects of the substances on the list. Due to the lack of experimental data in the public domain on the ecotoxicity and environmental behaviour of substances, it was not possible to prioritise the substances based on experimental data alone. Two approaches were therefore used to predict the no effect concentrations.

1) A therapeutic dose approach.
2) An approach using experimental ecotoxicology data and Quantitative Structure-Activity Relationships (QSAR) predictions.

The therapeutic dose approach was used to provide an indication of relative potency of individual substances. This method used a simple equation using the maximum therapeutic dose/1000 to produce a $PNEC_{D.}$

QSARs can be used in the absence of experimental data to provide information on the toxicological effects of a substance from knowledge of its chemical structure and the effects of similar substances. In this case they were used to generate a single acute ecotoxicity value for each compound to which a safety factor of 100 was applied to produce a $PNEC_{T.}$

To enable the substances to be prioritised a single concentration value was chosen to represent acute toxicity (very little chronic toxicity data was publicly available so for consistency acute toxicity data were used). The value was selected from either the predicted or experimental acute toxicity data, with the lowest value for either fish, daphnids or green algae used in the prioritisation.

Results

Risk Characterisation ratios were obtained for each type of PNEC ($PNEC_D$ and $PNEC_T$). The resulting ratios were then used to produce 2 priority lists, one based on dose, and the other based on experimental data and/or QSAR predictions for ecotoxicity (Table 1.1). The ten substances with the highest risk characterisation ratios using both approaches are shown.

Table 1.1 The top ten substances identified by the prioritisation approaches following further screening

Therapeutic Dose Approach ($PEC_w/PNEC_D$)	QSAR/Experimental Approach ($PEC_w/PNEC_T$)
Aminophylline	Lofepramine
Beclametasone	Dextropropoxyphene
Theophylline	Procyclidine
Paracetamol	Tramadol
Norethisterone	Paracetamol
Codeine	Clotrimazole
Furosemide	Thiridazine
Atenolol	Mebeverine
Bendroflumethiazide	Terbinafine
Chlorphenamine	Tamoxifen

Substances with a risk characterisation ratio greater than one are of potential concern (EU, 1996). Using the $PNEC_D$ approach no substances fall into this bracket whilst for the $PNEC_T$ approach three substances had a ratio greater than one, (lofepramine, dextropropoxyphene and procyclidine) indicating that they could potentially be of concern in the aquatic environment. The EU guidance document (1996) indicates that those substances with a ratio greater than one should be further investigated.

The two approaches used rank the compounds on different criteria, and the relationship between therapeutic dose and environmental toxicity is unknown. It would be expected that the QSAR based approach should provide more accurate estimates of toxicity to aquatic life than the human therapeutic dose approach, though the accuracy of many of the QSAR predictions are unknown. However the human therapeutic dose approach enables identification of pharmaceuticals which are designed to be highly potent. Therefore a single ranking list combining both of the PNEC approaches was produced so that all factors (toxicity, usage and dose) were taken into consideration. This list was drawn from the top 100 substances derived by the two methods. The final ranking was based on the risk characterisation ratio of the substances; the higher of the two values produced for each substance ($PNEC_T$ and $PNEC_D$) was used in the final ranking. The top ten compounds from this ranking are shown in Table 1.2.

1.4.2 Persistence, bioaccumulation and toxicity

This approach ignores other factors that are important when assessing environmental risk, including the potential persistence, bioaccumulation and toxicity of a substance (PBT criteria). These effects were examined by applying the OSPAR Dynamic Selection and Prioritisation Mechanism for Hazardous Substances (DYNAMEC) criteria to the substances near the top of the priority list. These criteria are:

Persistency (P): ..... Half-life ($T_{½}$) of 50 days
Liability to Bioaccumulate (B): ..... log K_{ow}>=4 or BCF>=500
Toxicity (T)..... T_{aq}: acute $L(E)C_{50}$=<1 mg/l, long-term NOEC=<0,1 mg/l
or ..... $T_{mammalian}$: CMR or chronic toxicity

None of the substances examined fulfilled all three criteria, however several were found to be either toxic or persistent or both (Tables 1.2 & 1.3). None were found to bioaccumulate. Table 1.2 outlines the relevant information available on the top ten compounds, including whether they have been detected previously in either sewage effluent or surface waters, as reported in the literature and the relevant OSPAR DYNAMEC criteria.

Table 1.2 Relevant information available for the top 10 compounds

Substance	Therapeutic class	Detected?	Method?	OSPAR	
				Persistent	Toxic
Lofepramine	Anti-depressant		Could be developed		
Dextropropoxy-phene	Analgesic		Could be developed		✓
Procyclidine	Anti-depressant		No data		
Tramadol	Analgesic		Not easy to develop		
Paracetamol	Analgesic	✓	✓		
Clotrimazole	Antifungal		No data		
Thioridazine	Anti-depressant		Not easy to develop	✓	✓
Mebeverine	Gastrointestinal		Could be developed		
Aminophylline	Respiratory		No data		
Tamoxifen	Anticancer		Could be developed		

The OSPAR Dynamic criteria for PBT highlighted a number of additional substances that could be of potential concern (Table 1.3) that were not in the top 10 compounds. None of these substances were bioaccumulative according to the OSPAR DYNAMEC criteria.

Table 1.3 Available information on substances highlighted by the OSPAR DYNAMEC criteria

Substance	Therapeutic class	Detected?	Method?	OSPAR	
				Persistent	Toxic
Fluoxetine	Anti-depressant		Not easy to develop	✓	✓
Trimethoprim	Antiinfective	✓	✓		✓
Sulphamethoxazole	Antibiotic	✓	✓		✓
Fenofibrate	Metabolism	✓	✓		✓
Diclofenac	Anti-inflammatory	✓	✓		✓

1.4.3 Deriving a list for targeted monitoring

There are a number of uncertainties associated with the risk characterisation process used. For the PNEC values used, the relationship between therapeutic dose and environmental toxicity is unknown. The accuracy of many of the QSAR predictions is also unknown and chronic effects have not been sufficiently taken into account. Additionally, the assumption involved in the calculation of the PEC_w that all compounds will be unaffected by the sewage treatment

processes is a false one, and could lead to miss ranking of the substances of interest. These uncertainties indicate that a targeted monitoring programme would be justified to determine whether these substances are being released into the aquatic environment.

In deriving a list of compounds to be included in the monitoring programme a number of factors were considered:

- Near the top of the priority list.
- Was the substance highlighted by OSPAR DYNAMEC (PBT Criteria) (Tables 1.2 & 1.3)?
- Has the substance been detected previously in either surface waters or sewage effluent (Tables 1.2 & 1.3)?
- Should substances across a range of different therapeutic classes be selected?
- Is there a reliable analytical method available for the substance?

Analytical Methods and Developing a List for further Investigation

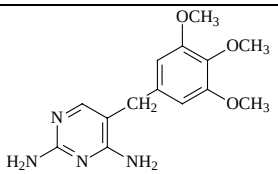
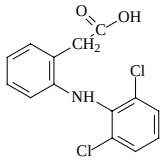
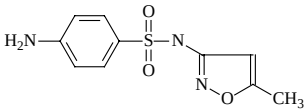
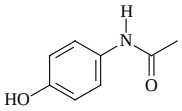
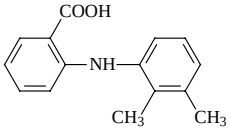
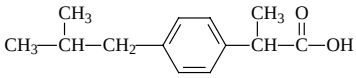
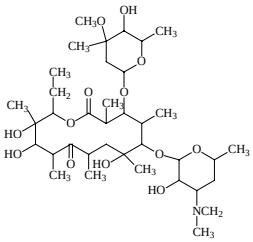
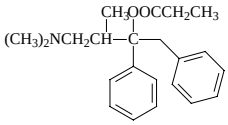
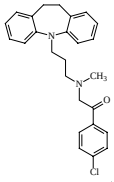
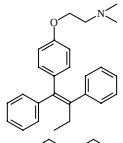
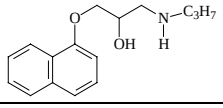
The Agency commissioned a review of the availability of analytical methods with suitable detection limits for a range of the substances on the ranked list. In many cases methods are available, but they would require modification to enable surface water and/or sewage effluent samples to be analysed effectively, which could be costly and time consuming. Few of the top 10 compounds (Table 1.2) have reliable analytical methods available that could be used in a monitoring programme. Despite these substances being potential priorities for a monitoring programme (greatest potential to pose a risk to the aquatic environment) the lack of available methods reduces the feasibility of their inclusion on a final list.

The list below (Table 1.4) represents the best compromise that could be achieved with minimum expenditure on analytical method development, and provides a good initial list for a targeted monitoring programme. All of the substances were ranked close to the top of the prioritisation list produced during the screening process. It includes substances from a range of different therapeutic classes; where the top ranked substance from a particular class was unsuitable (no method), the next ranked substance was chosen. With the exception of tamoxifen, dextropropoxyphene and lofepramine all the substances have analytical methods and have previously been detected in either sewage effluent or surface waters.

The availability of reliable analytical methods restricts the selection of substances for any monitoring programme. None of the anti-depressant therapeutic class substances near the top of the ranking have available analytical methods. These substances are used in high quantities within the UK, indicating they could pose a potential risk to the aquatic environment. As part of this monitoring programme analytical methods were developed for lofepramine, the top ranked anti-depressant, tamoxifen and dextropropoxyphene. Analytical method development was thought to be feasible for all three and both dextropropoxyphene and lofepramine are at the top of the priority list, whilst tamoxifen is also in the top ten.

Ibuprofen, diclofenac, paracetamol and propranolol have all been reported to either biodegrade or to be removed during sewage treatment, though the percentage removal varies. All four substances have been reported in monitoring studies, justifying their inclusion on the proposed list (Table 1.4).

Table 1.4 Pharmaceutical compounds selected for targeted monitoring

Pharmaceutical	Therapeutic class	Chemical abstracts service (CAS) No.	Structure
Trimethoprim	Antibiotic	738-70-5	
Diclofenac	Anti-inflammatory	15307-86-5	
Sulfamethoxazole	Antibiotic	723-46-6	
Paracetamol	Analgesic	103-90-2	
Mefenamic acid	Anti-inflammatory	61-68-7	
Ibuprofen	Analgesic	15687-27-1	
Erythromycin	Antibiotic	114-07-8	
Dextropropoxyphene	Analgesic	469-62-5	
Lofepramine	Anti-depressant	23047-25-8	
Tamoxifen	Anti-cancer	10540-29-1	
Propranolol	Antihypertensive	525-66-6	

2 MATERIALS AND METHODS

2.1 Monitoring sites

Five sewage treatment works (STW) were chosen for the targeted monitoring programme by the Environment Agency (Table 2.1). It was essential that, in order ensure that degradation of the pharmaceutical substances within the water samples was kept to a minimum, the samples were collected and returned to the laboratory within the same day. The laboratory conducting the analysis is based in Essex. It would therefore not have been possible to select sites outside the Southeast of England. The selection of sites for analysis was therefore focussed on the Southeast of England. Those sites chosen for the analysis have also been used previously in similar monitoring studies for trace organic compounds and support was again forthcoming from the relevant local water companies (Anglian Water and Thames Water). Finally, the majority of the STWs selected had a predominantly domestic input. Such sites provide information on the levels of pharmaceuticals via general use in the population rather than from industrial point sources.

The works chosen for the study were:

Corby STW, Northamptonshire.
Great Billing STW, Northamptonshire.
East Hyde STW, Bedfordshire
Harpenden STW, Hertfordshire
Ryemeads STW, Hertfordshire

The locations of these STWs and the associated sampling stations used are shown in Figure 2.1. Details of the sites are shown in Table 2.1.

2.2 Sample collection

Sampling was conducted monthly during the months of May, June and July 2002 at all five sites. Three discrete final effluent samples were taken at hourly intervals on each visit to be analysed independently of each other. One sample was also taken upstream and downstream of the discharge point at each site during the course of each sampling trip. Discharge flow rates were obtained at the time of sampling in order to calculate the overall load of pharmaceutical discharging input from each STW. The pH of each individual sample was also measured, whist the temperature, and salinity of each effluent/water sample was also measured and assumed to be constant.

Samples were taken in the months of May, June and July 2002 when surface water flows were predicted to be low due to low precipitation. Periods of low rainfall were targeted since the dilution of effluent through the STWs would be low and river flows would be correspondingly low. Sampling was performed around the time of peak flow from the STW (information provided by the STW operators). It was anticipated therefore that the concentrations of any compounds detected would represent a ‘worst case scenario’ as dilution would be kept to a minimum. However, heavy rain during July made it unavoidable to collect samples during dry periods (particularly for Corby and Billing). This reflected in the STW effluent discharge flow rates (Appendix A).

Two methods were used to collect effluent and water samples (2.7 L). The first used direct sampling into a silanised, clean amber glass winchester, using a stainless steel water sampler. The second used a stainless steel bucket to collect the water sample, which was then transferred into a silanised, clean amber glass winchester. The method used was dependent on the effluent sampling points available at each STW. No bias was expected from either method.

Table 2.1 Details of the STWs chosen as sites for this study

STW	Treatment process	Population equivalent (PE)	Trade PE
Corby	Inlets works: 6mm screens, 4 mm drum screens, Kaldnes (Moving Fixed Film biological treatment process), intermediate settlement tanks, oxidation ditch (suspended biological treatment process), final settlement tanks and tertiary treatment (sand filter). [Ferric chloride is dosed in the ISTs and the oxidation ditch for phosphate removal. All return liquors are returned post screening]	150,000	105,000
Gt. Billing	Combination of filter and activated sludge plant with 40 % of flows receiving biological P removal, flows are settled in conventional humus tanks, before being discharged to river. Secondary treatment only.	296,100	67,400
East Hyde	Oxidation ditch, final tanks then tertiary sand filters.	143,801	35,478
Harpenden	Primary sedimentation tanks then half of the plant is double biological filtration and the other half is alternate double filtration. Both followed by tertiary sand filters	31,905	324
Ryemeads	Conventional aeration, final tanks then tertiary lagoons	365,071	44,377

2.3 Analysis

Analysis of all compounds was performed using liquid chromatography coupled to electrospray mass spectrometry or tandem mass spectrometry. Analysis followed extraction and pre-concentration of the samples by solid phase extraction (SPE).

Prior to use all glassware was silanised by rinsing with 10 % v/v dimethyldichlorosilane in DCM, followed by DCM (x 2), and methanol (x 2). This was done to minimise the loss of the analytes through adsorption onto the surface of the sampling vessels.

2.3.1 Extraction

Analytes were extracted by SPE following the addition of a known amount of an internal standard in methanol (^{13}C-phenacetin). Each water sample (1 L) was passed through a glass fibre filter (GFC, 0.45 μm) and the pH adjusted to 3 by the addition of concentrated hydrochloric acid. The sample was then passed through a StrataX 6 ml SPE column (200 mg; Phenomenex, UK) at a flow rate of approximately 10 ml min.$^{-1}$. The columns had previously been solvated with 3 x 2 ml methanol, 3 x 2 ml water (normal pH) and 3 x 2 ml water at pH 3. The SPE columns were then dried by vacuum aspiration for 30 min. and frozen at –30^{o}C until elution. Once defrosted, the analytes were eluted with 3 x 2 ml methanol, at a rate of 5 ml min.$^{-1}$ after soaking for 5 min., and reduced in volume to ~100 μl. Each sample was then transferred into an analysis vial, made up to 1 ml with 50:50 methanol: ammonium acetate (40 mM; pH 5.5) and stored at -20°C until analysed.

2.3.2 High performance liquid chromatography

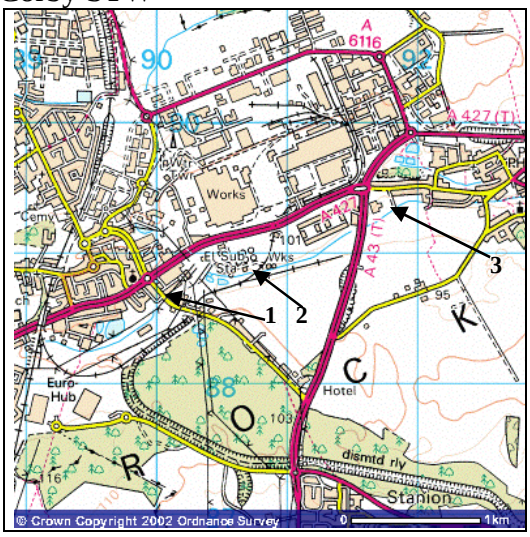
High performance liquid chromatography (HPLC) was carried out on a Thermo-Finnigan Surveyor system (Thermo-Finnigan, Hemel Hempstead, UK). The analytes were separated on a 250 x 2 mm Luna C18 (2) 5 μm column (Phenomenex, Macclesfield, UK) using a water, methanol and 40 mM ammonium acetate in water (adjusted to pH 5.5 by the addition of formic acid) mobile phase. Four solvent gradients were used (Tables 2.2 - 2.5) at a flow rate of 200 μl min^{-1}. The first gradient was used for the separation of the antibiotics erythromycin, sulfamethoxazole, acetyl-sulfamethoxazole, and trimethoprim. The second gradient for mefenamic acid, lofepramine, propranolol, dextropropoxyphene, diclofenac and tamoxifen. The third gradient was used for ibuprofen and the fourth for paracetamol. The injection volume was set to 20 μl and a post-run equilibrium time of 3 min. was used. The HPLC column eluent was split 1:1 and 100 μl min^{-1} was introduced into the MS interface.

2.3.3 Mass Spectrometry

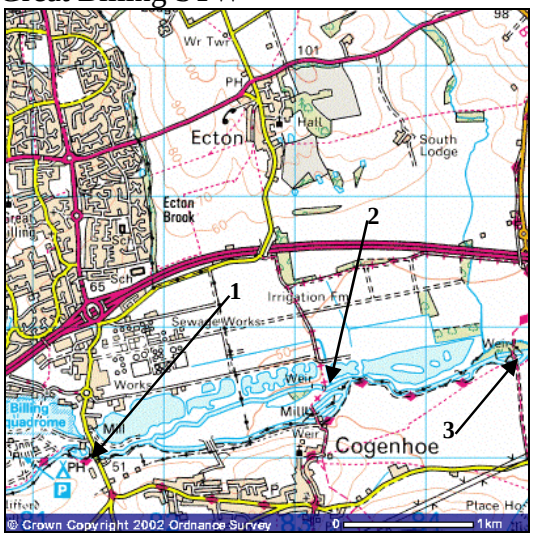
Mass spectra were obtained on a Thermo-Finnigan LCQ Advantage mass spectrometer using electrospray ionisation (ESI). For all the methods used the sheath gas flow was set to 10 (arbitrary units), auxiliary flow was set to 4 (arbitrary units) and the capillary temperature was set at 220^{o}C. Other variable parameters are shown in Table 2.6. The precursor and product ions shown in Table 2.7 were monitored. Approximate retention times are also shown.

Four different methods were set-up following an initial investigation to establish which of the available ionisation modes was the most sensitive for each analyte: the first method was for general pharmaceuticals in positive ionisation mode using tandem mass spectrometry (MSMS) in the Consecutive Reaction Monitoring (CRM) mode (mefenamic acid, diclofenac, propranolol, dextropropoxyphene, lofepramine and tamoxifen); the second, for ibuprofen, used negative ionisation single ion monitoring (SIM), the third method used SIM in the positive ion mode for paracetamol and a fourth method to analyse antibiotics (erythromycin, sulfamethoxazole, acetyl-sulfamethoxazole and trimethoprim) used MSMS in the positive ionisation mode with CRM. The precursor/SIM mass, product ion mass and retention data for the selected pharmaceuticals analysed is shown in Table 2.7. In each case the product ion 1 mass was used for the analysis of the compound in MS/MS mode. In the cases that no identifying fragment could be obtained (paracetamol and ibuprofen) quantification of that

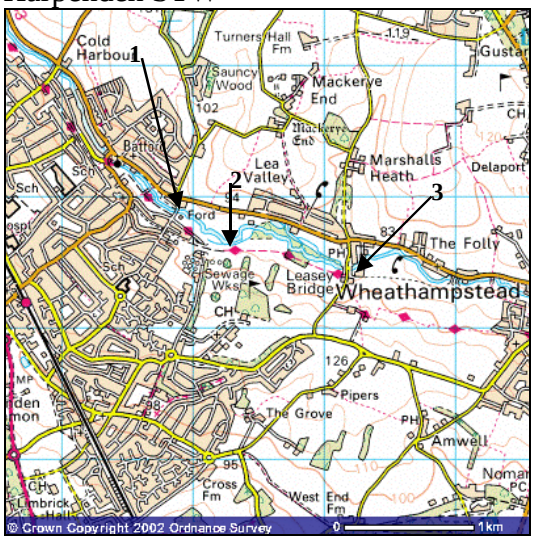
A. Corby STW



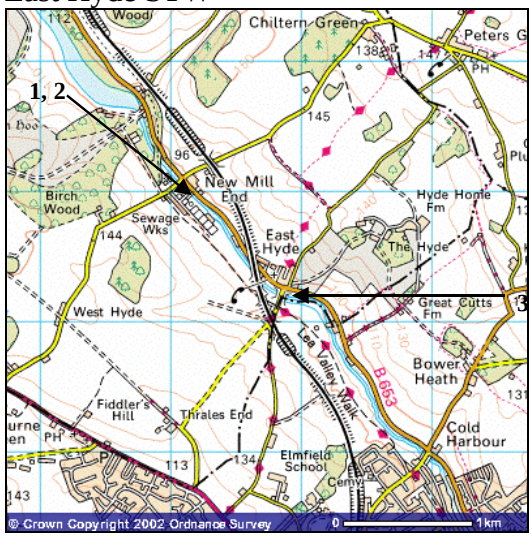
B. Great Billing STW



C. Harpenden STW



D. East Hyde STW



E. Rye Meads STW

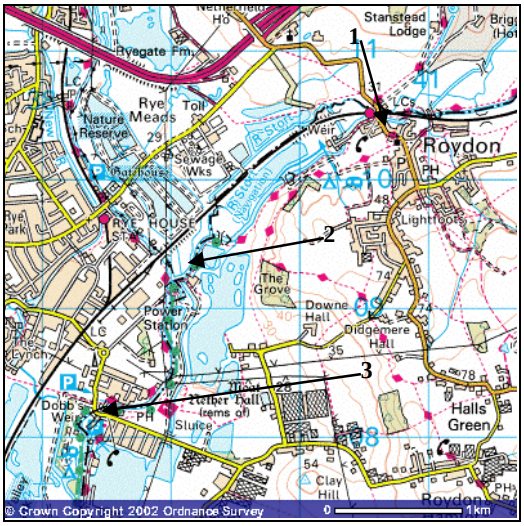


Figure 2.1 Location of sampling points at selected STWs. 1 = upstream, 2 = effluent discharge, 3 = downstream.

Table 2.2 HPLC solvent gradient for the separation of erythromycin, sulfamethoxazole, acetyl-sulfamethoxazole, and trimethoprim

Time	Solvent A	Solvent B	Solvent C
0	10	15	75
2	10	15	75
15	10	90	0
20	10	90	0
25	10	15	75

Solvent A: 40 mM ammonium acetate adjusted to pH 5.5 with formic acid, Solvent B: Methanol, Solvent C: Water.

Table 2.3 HPLC solvent gradient for the separation of mefenamic acid, lofepramine, propranolol, dextropropoxyphene, diclofenac and tamoxifen

Time	Solvent A	Solvent B	Solvent C
0	10	15	75
3	10	15	75
10	10	90	0
20	0	100	0
25	0	100	0
26	10	15	75
30	10	15	75

Solvent A: 40 mM ammonium acetate adjusted to pH 5.5 with formic acid, Solvent B: Methanol, Solvent C: Water.

Table 2.4 HPLC solvent gradient for the separation of ibuprofen

Time	Solvent A	Solvent B	Solvent C
0	0	15	85
3	0	15	85
10	0	100	0
20	0	100	0
25	0	100	0
30	0	15	85

Solvent A: 40 mM ammonium acetate adjusted to pH 5.5 with formic acid, Solvent B: Methanol, Solvent C: Water.

Table 2.5 HPLC solvent gradient for the separation of paracetamol

Time	Solvent A	Solvent B	Solvent C
0	0	10	90
5	0	10	90
25	0	100	0
30	0	10	90

Solvent A: 40 mM ammonium acetate adjusted to pH 5.5 with formic acid, Solvent B: Methanol, Solvent C: Water

compound was performed using the precursor mass and retention time (from HPLC) only. For each method tuning was performed on the ion displaying the weakest signal within that particular method file.

Ion suppression regions were identified in each method used by infusing a dilute solution of analyte at a constant rate into the effluent flowing from the HPLC system to the mass spectrometer to create an elevated constant baseline. A blank sample was then injected into the system. A drop in the baseline identified the regions where eluted material suppresses ionisation in the mass spectrometer.

Table 2.6 Mass spectrometer parameters used

Method	Current (μA)	Voltage (kV)	Capillary Voltage (V)
General positive ionisation MSMS (CRM)	2.0	5.0	31.0
Negative Ionisation SIM/MSMS (CRM)	6.3	4.2	-32.7
Antibiotics (MSMS)	2.0	5.0	2.8
Paracetamol (SIM)	2.0	5.0	31.0

2.3.4 Quality Assurance

All methods were validated by external calibration over a range of 10-5000 ng L^{-1} in order to determine limits of detection for each compound and to establish the linearity of the MS detector. In addition, a ^{13}C- labelled internal standard (^{13}C-phenacetin) was added to each sample to monitor analyte recovery. Calibration standards were made up at concentrations of 0.01, 0.05, 0.1, 0.45, 1.0, 5.0, 10.0 and 45 μg ml^{-1} in a 50:50 mix of methanol: ammonium acetate (pH 5.5).

Table 2.7 Precursor/SIM mass, product ion mass and retention data for the selected pharmaceuticals analysed

Compound	Precursor mass $[M+H]^+$ (SIM)	Product ion 1 (MS/MS)	Retention Time (min)
Paracetamol	152.2	-	14.8
Ibuprofen	205.2*	-	15.5
Sulfamethoxazole	254.2	188.1	4.0
Sulfamethoxazole-acetate	296.1	236.2	4.3
Trimethoprim	292.6	230.2 $[M-2CH_3O]^+$	14.5
Erythromycin	734.7	576.3	19.4
Mefenamic acid	242.2	224.2	17.8
Diclofenac	296.1	277.9	16.6
Propranolol	260.2	183.1	15.3
Dextropropoxyphene	340.1	266.2	16.4
Lofepramine	419.2	224.1	23.8
Tamoxifen	372.3	327.1	20.5

*negative ionisation, $[M-H]^-$

3. RESULTS

3.1 Recoveries and Limits of Detection

Table 3.1 shows the performance data for each compound analysed as part of this programme.

Table 3.1 Performance data for the methods developed for the targeted pharmaceuticals

Compound	% Recoveries[†] (RSD)	LOD[‡] (ng L^{-1})
Sulfamethoxazole	120 (16)	50
Acetyl-sulfamethoxazole	56(5.4)	50
Trimethoprim	123 (2.5)	10
Erythromycin	73 (30)	10
Paracetamol	75 (6.9)	50
Ibuprofen	117 (22)	20
Mefenamic acid	24 (7.9)	50
Diclofenac	62 (20)	20
Propranolol	45 (5.6)	10
Dextropropoxyphene	63 (3.9)	20
Lofepramine	4.2 (35)	10
Tamoxifen	42 (40)	10

[†] Calculated using: Recovery = $100(X_S - X_U)/ K$, where X_S= concentration measured in spiked sample, X_U= concentration measured in unspiked sample and K= known value of the spike in the sample. n=3. 100 ng spiked into each sample. [‡] Limit of detection (LOD) calculated using a signal to noise ratio of 10.

3.2 Targeted pharmaceutical concentrations in STW effluents and receiving waters

A summary of the occurrence data generated by this study is presented in Tables 3.2 to 3.6. A complete set of data is presented in Appendix B.

3.2.1 Corby STW

Propranolol and dextropropoxyphene were detected in all three of the STW effluent samples collected from Corby STW in May (Figure 3.1; Table 3.2). Along with mefenamic acid (detected in #1 and 2) these compounds were present in the discharge samples at a ratio that provides an almost characteristic fingerprint. These three compounds were found downstream of the STW discharge point at a similar concentration ratio to that seen in the STW effluent samples (though dextropropoxyphene is at a higher concentration). Only ibuprofen was detected upstream of the site, at a concentration of 830 ng L^{-1}. However, it is also present in the downstream sample at a concentration of over 5,000 ng L^{-1}. Ibuprofen was only detected in one of the effluent samples collected.

One confounding aspect of these data is that the concentration of mefenamic acid, dextropropoxyphene and ibuprofen determined downstream of the STW discharge was higher than that measured in the STW effluent. One possible explanation is that the capacity of the SPE column to retain compounds was exceeded and that breakthrough may have occurred when analysing the STW effluent. The StrataX SPE sorbent material used in this study was chosen since it has a high surface area and therefore a high capacity to extract compounds from water. If breakthrough did occur then it is likely to have been caused by the presence of

large amounts of co-extracted compounds present in the STW effluent sample collected. It is also difficult to explain the presence of diclofenac and acetyl-sulfamethoxazole in the sample collected downstream of the discharge point.

Table 3.2 Summary of the targeted pharmaceutical concentrations in Corby STW samples

Sample type	Pharmaceutical	Mean	Median	Max.	Min.	Detected		
			(ng L^{-1})			May	June	July
Discharge	Paracetamol	<50	<50	<50	<50			
	Mefenamic acid	271	128	1103	<50	✓	✓	✓
	Diclofenac	605	309	2246	<20		✓	✓
	Propranolol	48	57	63	22	✓	✓	✓
	Dextropropoxyphene	185	189	350	<20	✓	✓	✓
	Lofepramine	<10	<10	<10	<10			
	Tamoxifen	<10	<10	23	<10			✓
	Ibuprofen	1745	2274	3297	<20	✓	✓	✓
	Erythromycin	<10	<10	20	<10		✓	
	Sulfamethoxazole	<50	<50	112	<50	✓		
	Trimethoprim	<10	<10	<10	<10			
	Acetyl-sulfamethoxazole	158	<50	871	<50			✓
Upstream	Paracetamol	<50	<50	<50	<50			
	Mefenamic acid	<50	<50	<50	<50			
	Diclofenac	<20	<20	<20	<20			
	Propranolol	<10	<10	<10	<10			
	Dextropropoxyphene	<20	<20	<20	<20			
	Lofepramine	<10	<10	<10	<10			
	Tamoxifen	<10	<10	<10	<10			
	Ibuprofen	308	90	834	<20	✓	✓	
	Erythromycin	<10	<10	<10	<10			
	Sulfamethoxazole	<50	<50	<50	<50			
	Trimethoprim	<10	<10	<10	<10			
	Acetyl-sulfamethoxazole	<50	<50	<50	<50			
Downstream	Paracetamol	<50	<50	<50	<50			
	Mefenamic acid	96	105	182	<50	✓	✓	
	Diclofenac	375	556	568	<20	✓	✓	
	Propranolol	17	18	35	<10	✓	✓	
	Dextropropoxyphene	223	<20	670	<20	✓		
	Lofepramine	<10	<10	<10	<10			
	Tamoxifen	<10	<10	<10	<10			
	Ibuprofen	2011	988	5044	<20	✓	✓	
	Erythromycin	29	<10	88	<10		✓	
	Sulfamethoxazole	<50	<50	<50	<50			
	Trimethoprim	<10	<10	<10	<10			
	Acetyl-sulfamethoxazole	136	198	210	<50	✓		✓

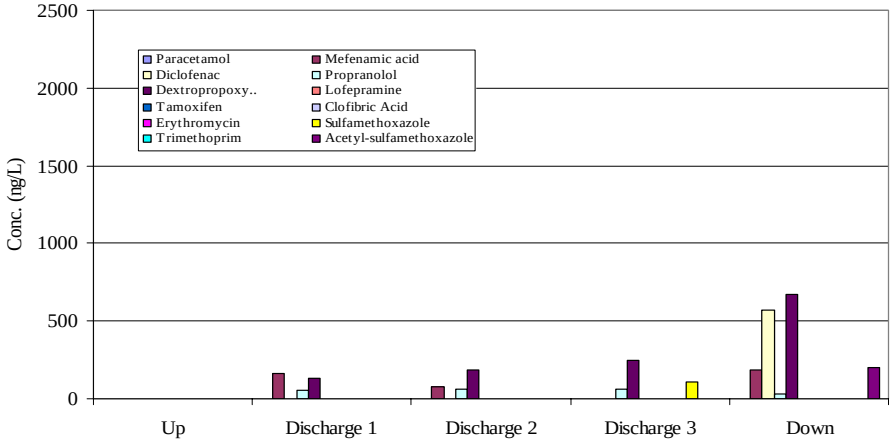


Figure 3.1 Concentration of selected pharmaceuticals (excluding ibuprofen) in samples collected from Corby STW during May 2002

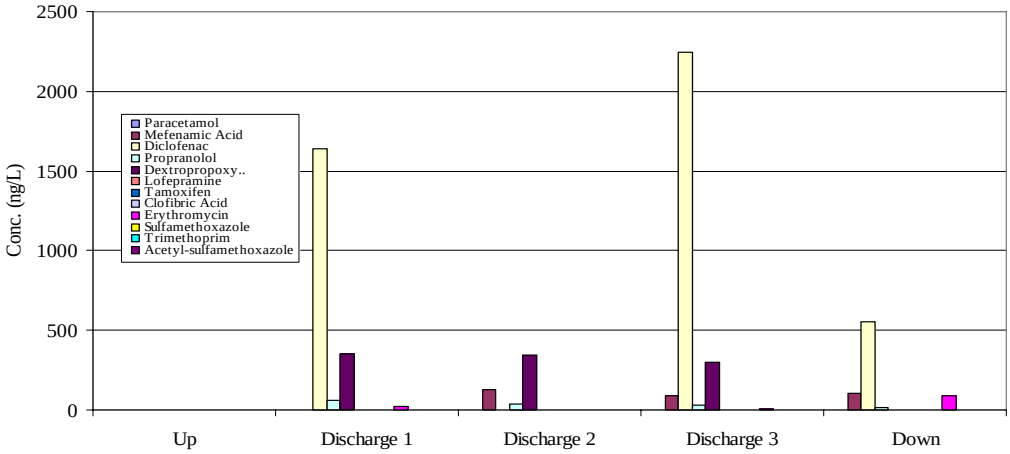


Figure 3.2 Concentration of selected pharmaceuticals (excluding ibuprofen) in samples collected from Corby STW during June 2002

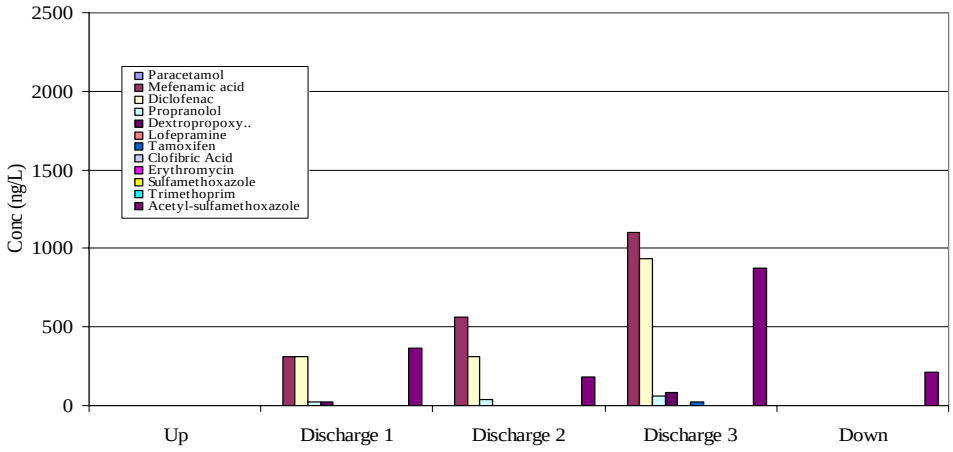


Figure 3.3 Concentration of selected pharmaceuticals (excluding ibuprofen) in samples collected from Corby STW during July 2002

In June 2002, ibuprofen, mefenamic acid, propranolol, dextropropoxyphene, diclofenac and erythromycin were all detected in the final effluent of Corby STW (Figure 3.2). Mefenamic acid, diclofenac and propranolol were also measured, at lower concentrations than those of the final effluent, in the sample collected downstream. Dextropropoxyphene was not detected downstream, whilst ibuprofen was detected in both the upstream and downstream samples. Diclofenac and erythromycin were detected in two of the three effluent samples collected (#1 and 3), whist mefenamic acid was detected in effluent samples #2 and 3. Propranolol, ibuprofen and dextropropoxyphene were detected in all discharge samples in June. Diclofenac was determined to be present at concentrations of around 2,000 ng L^{-1} in the STW effluent, whilst erythromycin was determined to be present at concentrations near the LOD of the method (10 ng L^{-1}), though it was also detected at 88 ng L^{-1} downstream.

In July 2002, ibuprofen, mefenamic acid, propranolol, dextropropoxyphene, diclofenac, tamoxifen and acetyl-sulfamethoxazole were all detected in the final effluent of Corby STW (Figure 3.3). Only acetyl-sulfamethoxazole was detected downstream of the discharge point.

3.2.2. Great Billing STW

A full data set is only available for the effluent samples collected from Gt. Billing STW in May 2002; an incomplete data set is available from the up- or downstream samples, due to the poor internal standard recovery. Ibuprofen, mefenamic acid, diclofenac, propranolol, dextropropoxyphene, erythromycin and trimethoprim were all detected in the three discharge samples collected (Table 3.3). Acetyl-sulfamethoxazole was also detected in the first effluent sample collected. Ibuprofen was detected at the highest concentration. Mefenamic acid and propranolol were also detected in the sample collected downstream of the works (Table 3.3). None of the targeted pharmaceutical compounds were determined upstream of the effluent discharge point.

Ibuprofen, mefenamic acid, diclofenac, propranolol, dextropropoxyphene, erythromycin and trimethoprim were also detected in the effluent samples collected from Gt. Billing STW in June and July 2002 (Figure 3.4 and 3.5). Acetyl-sulfamethoxazole was also detected in the effluent samples collected in July 2002. In June 2002, ibuprofen, propranolol, dextropropoxyphene, erythromycin and trimethoprim were determined in the sample collected downstream, whilst in July 2002 mefenamic acid, diclofenac, propranolol, erythromycin, trimethoprim and acetyl-sulfamethoxazole were all detected downstream. The concentration of certain pharmaceuticals in the final effluent from Gt. Billing STW were consistently higher than those measured in the effluents of other STWs visited as part of this study.

Table 3.3 Summary of the targeted pharmaceutical concentrations in Great Billing STW samples

Sample type	Pharmaceutical	Mean	Median	Max.	Min.	Detected		
			(ng L^{-1})			May	June	July
Discharge	Paracetamol	<50	<50	<50	<50			
	Mefenamic acid	614	720	1440	74	✓	✓	✓
	Diclofenac	715	411	2349	230	✓	✓	✓
	Propranolol	152	152	264	73	✓	✓	✓
	Dextropropoxyphene	220	248	368	78	✓	✓	✓
	Lofepramine	<10	<10	<10	<10			
	Tamoxifen	<10	<10	<10	<10			
	Ibuprofen	5823	3781	27256	<20	✓	✓	✓
	Erythromycin	107	123	176	16	✓	✓	✓
	Sulfamethoxazole	<50	<50	<50	<50			
	Trimethoprim	365	174	1288	83	✓	✓	✓
	Acetyl-sulfamethoxazole	487	<50	2235	<50	✓		✓
Upstream	Paracetamol	<50	<50	<50	<50			
	Mefenamic acid	<50	<50	<50	<50			
	Diclofenac	<20	<20	<20	<20			
	Propranolol	<10	<10	<10	<10			
	Dextropropoxyphene	<20	<20	<20	<20			
	Lofepramine	<10	<10	<10	<10			
	Tamoxifen	<10	<10	<10	<10			
	Ibuprofen	<20	<20	<20	<20			
	Erythromycin	28	28	57	<10			✓
	Sulfamethoxazole	<50	<50	<50	<50			
	Trimethoprim	<10	<10	<10	<10			
	Acetyl-sulfamethoxazole	<50	<50	<50	<50			
Downstream	Paracetamol	<50	<50	<50	<50			
	Mefenamic acid	143	65	366	<50	✓		✓
	Diclofenac	30	<20	91	<20			✓
	Propranolol	93	37	215	25	✓	✓	✓
	Dextropropoxyphene	52	<20	155	<20		✓	
	Lofepramine	<10	<10	<10	<10			
	Tamoxifen	<10	<10	<10	<10			
	Ibuprofen	414	414	828	<20		✓	
	Erythromycin	677	677	1022	331		✓	✓
	Sulfamethoxazole	<50	<50	<50	<50			
	Trimethoprim	41	41	42	39		✓	✓
	Acetyl-sulfamethoxazole	119	119	239	<50			✓

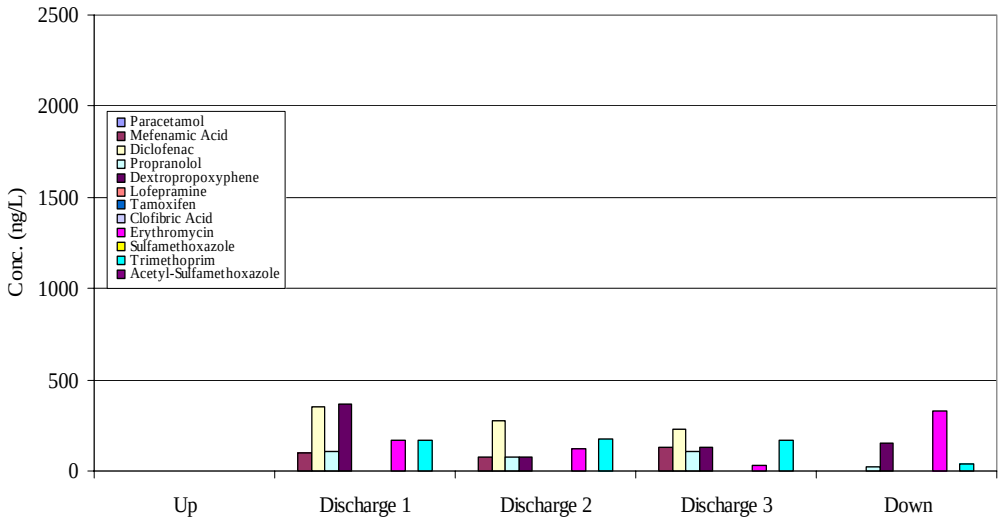


Figure 3.4 Concentration of selected pharmaceuticals (excluding ibuprofen) in samples collected from Great Billing STW during June 2002

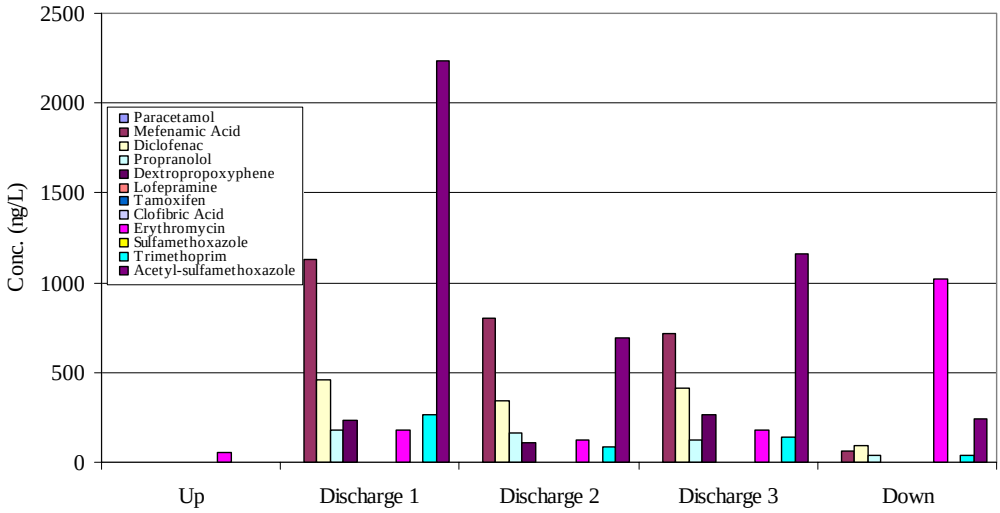


Figure 3.5 Concentration of selected pharmaceuticals (excluding ibuprofen) in samples collected from Great Billing STW during July 2002

3.2.3 Rye Meads STW

In May 2002, ibuprofen, mefenamic acid, diclofenac, propranolol, dextropropoxyphene, erythromycin, trimethoprim and acetyl-sulfamethoxazole were determined in the STW final effluent samples collected (Figure 3.6; Table 3.4). Low concentrations of erythromycin and trimethoprim were detected in samples collected upstream of the works, whilst ibuprofen, mefenamic acid, propranolol, erythromycin, trimethoprim and acetyl-sulfamethoxazole were detected in the sample collected downstream of the STW.

In June 2002, ibuprofen, mefenamic acid, diclofenac, propranolol, dextropropoxyphene and trimethoprim were determined in the STW final effluent samples collected. Only dextropropoxyphene and ibuprofen were detected in the downstream sample (Figure 3.7). Erythromycin was determined at high concentrations in the final effluent samples collected in July, whilst the receiving waters also contained mefenamic acid, diclofenac, propranolol, dextropropoxyphene, erythromycin and trimethoprim (Figure 3.8).

Table 3.4 Summary of the targeted pharmaceutical concentrations in Rye Meads STW samples

Sample type	Pharmaceutical	Mean (ng L^{-1})	Median	Max.	Min.	Detected		
						May	June	July
Discharge	Paracetamol	<50	<50	<50	<50			
	Mefenamic acid	240	142	874	51	✓	✓	✓
	Diclofenac	515	215	2098	<20	✓	✓	✓
	Propranolol	78	77	156	16	✓	✓	✓
	Dextropropoxyphene	231	201	585	<20	✓	✓	✓
	Lofepramine	<10	<10	<10	<10			
	Tamoxifen	<10	<10	<10	<10			
	Ibuprofen	3663	3628	8039	<20	✓	✓	✓
	Erythromycin	402	21	1842	<10	✓		✓
	Sulfamethoxazole	<50	<50	<50	<50			
	Trimethoprim	121	86	326	28	✓	✓	✓
	Acetyl-sulfamethoxazole	<50	<50	218	<50	✓		
Upstream	Paracetamol	<50	<50	<50	<50			
	Mefenamic acid	<50	<50	<50	<50			
	Diclofenac	<20	<20	<20	<20			
	Propranolol	<10	<10	<10	<10			
	Dextropropoxyphene	<20	<20	<20	<20			
	Lofepramine	<10	<10	<10	<10			
	Tamoxifen	<10	<10	<10	<10			
	Ibuprofen	722	610	1555	<20		✓	✓
	Erythromycin	<10	<10	23	<10	✓		
	Sulfamethoxazole	<50	<50	<50	<50			
	Trimethoprim	18	17	36	<10	✓		✓
	Acetyl-sulfamethoxazole	<50	<50	<50	<50			
Downstream	Paracetamol	<50	<50	<50	<50			
	Mefenamic acid	97	62	228	<50	✓		✓
	Diclofenac	61	<20	182	<20			✓
	Propranolol	24	27	46	<10	✓		✓
	Dextropropoxyphene	46	58	81	<20		✓	✓
	Lofepramine	<10	<10	<10	<10			
	Tamoxifen	<10	<10	<10	<10			
	Ibuprofen	1012	1046	1989	<20	✓	✓	
	Erythromycin	208	112	511	<10	✓		✓
	Sulfamethoxazole	<50	<50	<50	<50			
	Trimethoprim	19	16	40	<10	✓		✓
	Acetyl-sulfamethoxazole	55	<50	164	<50	✓		

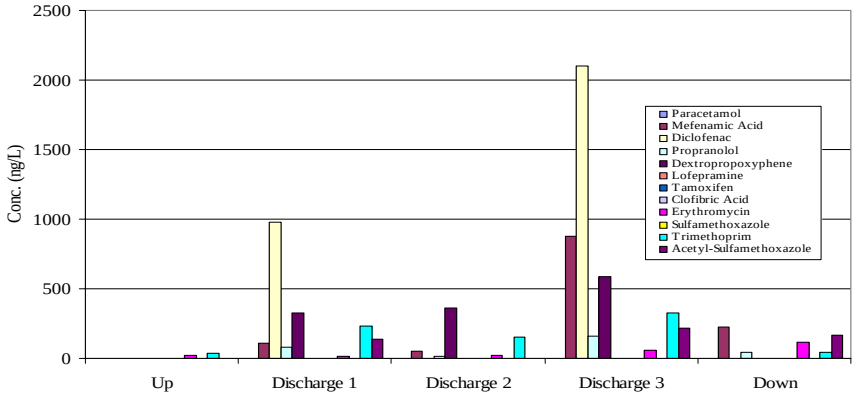


Figure 3.6 Concentration of selected pharmaceuticals (excluding ibuprofen) in samples collected from Rye Meads STW during May 2002

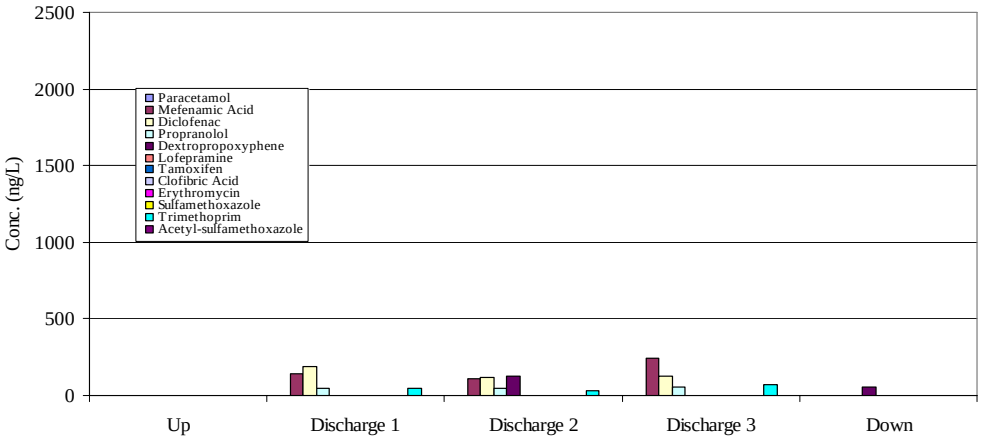


Figure 3.7 Concentration of selected pharmaceuticals (excluding ibuprofen) in samples collected from Rye Meads STW during June 2002

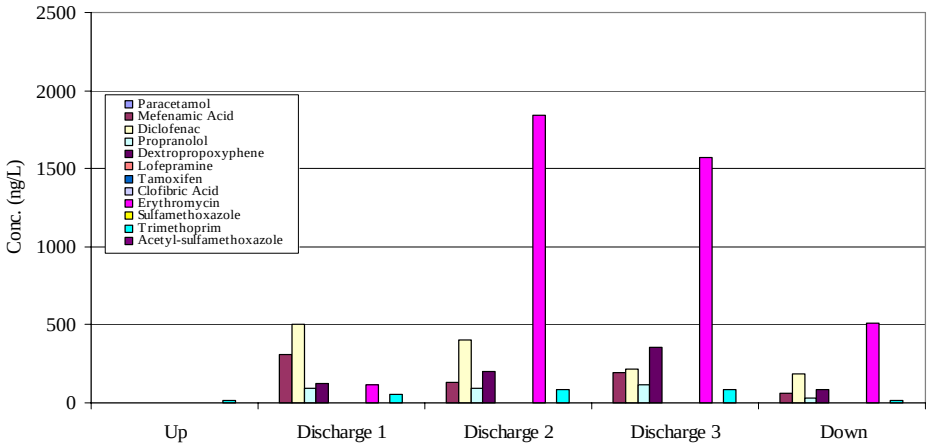


Figure 3.8 Concentration of selected pharmaceuticals (excluding ibuprofen) in samples collected from Rye Meads STW during July 2002

3.2.4 East Hyde STW

The May data for East Hyde should be viewed with caution since it was not possible to analyse for mefenamic acid, diclofenac, propranolol, lofepramine, dextropropoxyphene and tamoxifen in effluent sample #1 due to the poor recovery of the internal standard ^{13}C-phenacetin. Ibuprofen, diclofenac, propranolol, dextropropoxyphene, trimethoprim and acetyl-sulfamethoxazole were all determined in other effluent samples collected in May 2002 (Figure 3.9). Ibuprofen was the only targeted pharmaceutical detected upstream, whilst ibuprofen, mefenamic acid, diclofenac, propranolol and dextropropoxyphene were detected in the sample collected downstream (Table 3.5; Figure 3.9).

In June 2002, ibuprofen, diclofenac, propranolol, and sulfamethoxazole were detected in the first two effluent samples collected, whilst ibuprofen, mefenamic acid, propranolol, dextropropoxyphene and sulfamethoxazole were detected in effluent sample #3 (Figure 3.10). Ibuprofen, diclofenac and propranolol were detected in the downstream sample, whilst only ibuprofen was detected upstream. Ibuprofen, mefenamic acid, diclofenac, propranolol and dextropropoxyphene were also detected in the effluent sample in July 2002, along with erythromycin (Figure 3.11). Ibuprofen and trimethoprim were detected upstream, whilst ibuprofen, mefenamic acid, propranolol, dextropropoxyphene, and trimethoprim were all determined in the sample collected downstream (Table 3.5; Figure 3.11).

3.2.5 Harpenden STW

In May 2002, ibuprofen, mefenamic acid, diclofenac, trimethoprim and acetyl-sulfamethoxazole were detected in the final effluent of Harpenden STW (Figure 3.12). Ibuprofen, propranolol and trimethoprim were detected in the sample collected upstream, whilst ibuprofen, mefenamic acid, diclofenac, propranolol and dextropropoxyphene were detected in the sample collected downstream. The same compounds were also detected in June and July 2002 (Figures 3.13 and 3.14).

3.3 Environmental load

The environmental input of each targeted pharmaceutical was calculated for each STW effluent sampling event using effluent flow rates supplied by the STW operators (Figures 3.15, 3.16, and 3.17). The data is expressed as gram of pharmaceutical compound per hour. The pharmaceutical input and flow data are provided in Appendix C.

Table 3.5 Summary of the targeted pharmaceutical concentrations in East Hyde STW samples

Sample type	Pharmaceutical	Mean	Median	Max.	Min.	Detected		
			(ng L^{-1})			May	June	July
Discharge	Paracetamol	<50	<50	<50	<50			
	Mefenamic acid	53	<50	201	<50		✓	✓
	Diclofenac	350	447	565	<20	✓	✓	✓
	Propranolol	72	69	123	33	✓	✓	✓
	Dextropropoxyphene	267	232	523	<20	✓	✓	✓
	Lofepramine	<10	<10	<10	<10			
	Tamoxifen	<10	<10	42	<10			✓
	Ibuprofen	3358	3248	6847	980	✓	✓	✓
	Erythromycin	<10	<10	38	<10			✓
	Sulfamethoxazole	<50	<50	132	<50		✓	
	Trimethoprim	<10	<10	39	<10	✓		
	Acetyl-sulfamethoxazole	<50	<50	188	<50	✓		
Upstream	Paracetamol	<50	<50	<50	<50			
	Mefenamic acid	<50	<50	<50	<50			
	Diclofenac	<20	<20	<20	<20			
	Propranolol	<10	<10	<10	<10			
	Dextropropoxyphene	<20	<20	<20	<20			
	Lofepramine	<10	<10	<10	<10			
	Tamoxifen	<10	<10	<10	<10			
	Ibuprofen	761	569	1441	272	✓	✓	✓
	Erythromycin	<10	<10	<10	<10			
	Sulfamethoxazole	<50	<50	<50	<50			
	Trimethoprim	<10	<10	10	<10			✓
	Acetyl-sulfamethoxazole	<50	<50	<50	<50			
Downstream	Paracetamol	<50	<50	<50	<50			
	Mefenamic acid	70	59	150	<50	✓		✓
	Diclofenac	133	176	222	<20	✓	✓	
	Propranolol	26	29	34	16	✓	✓	✓
	Dextropropoxyphene	99	111	187	<20	✓		✓
	Lofepramine	<10	<10	<10	<10			
	Tamoxifen	<10	<10	<10	<10			
	Ibuprofen	1217	818	2116	716	✓	✓	✓
	Erythromycin	<10	<10	<10	<10			
	Sulfamethoxazole	<50	<50	<50	<50			
	Trimethoprim	<10	<10	20	<10			✓
	Acetyl-sulfamethoxazole	<50	<50	<50	<50			

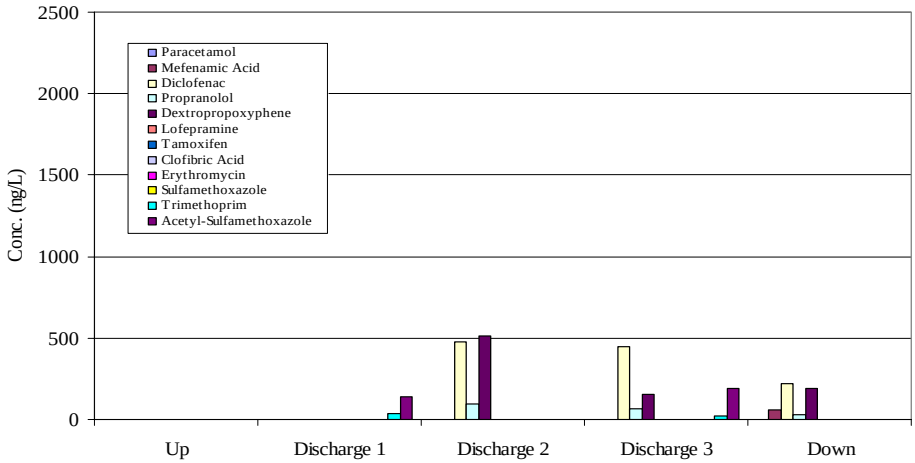


Figure 3.9 Concentration of selected pharmaceuticals (excluding ibuprofen) in samples collected from East Hyde STW during May 2002

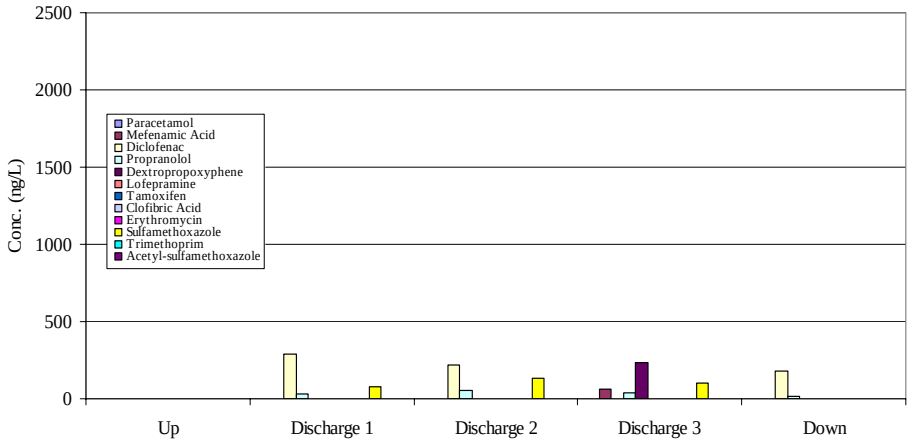


Figure 3.10 Concentration of selected pharmaceuticals (excluding ibuprofen) in samples collected from East Hyde STW during June 2002

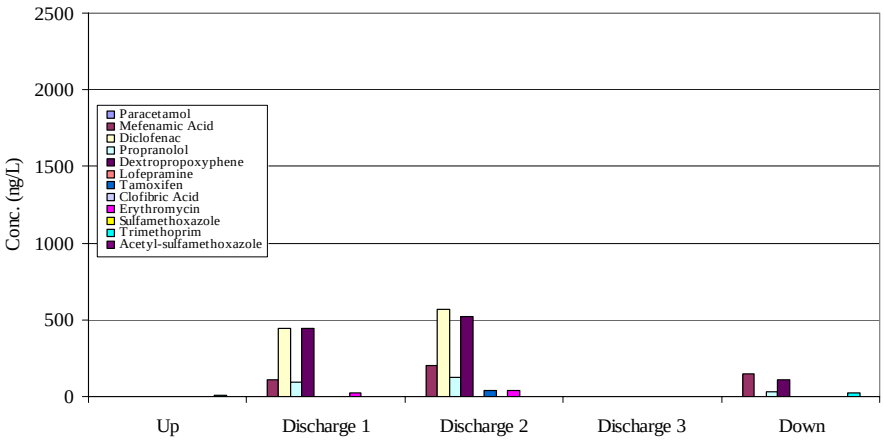


Figure 3.11 Concentration of selected pharmaceuticals (excluding ibuprofen) in samples collected from East Hyde STW during July 2002

Table 3.6 Summary of the targeted pharmaceutical concentrations in Harpenden STW sample

Sample type	Pharmaceutical	Mean	Median	Max.	Min.	Detected		
			(ng L^{-1})			May	June	July
Discharge	Paracetamol	<50	<50	<50	<50			
	Mefenamic acid	121	131	254	<50	✓	✓	✓
	Diclofenac	774	712	1457	328	✓	✓	✓
	Propranolol	114	88	284	28	✓	✓	✓
	Dextropropoxyphene	97	<20	453	<20	✓		✓
	Lofepramine	<10	<10	<10	<10			
	Tamoxifen	<10	<10	<10	<10			
	Ibuprofen	6587	3002	24444	<20	✓	✓	✓
	Erythromycin	<10	<10	<10	<10			
	Sulfamethoxazole	<50	<50	<50	<50			
	Trimethoprim	134	126	227	51	✓	✓	✓
	Acetyl-sulfamethoxazole	57	<50	282	<50	✓		✓
Upstream	Paracetamol	<50	<50	<50	<50			
	Mefenamic acid	<50	<50	<50	<50			
	Diclofenac	<20	<20	<20	<20			
	Propranolol	45	21	115	<10	✓		✓
	Dextropropoxyphene	<20	<20	<20	<20			
	Lofepramine	<10	<10	<10	<10			
	Tamoxifen	<10	<10	<10	<10			
	Ibuprofen	227	<20	681	<20	✓		
	Erythromycin	<10	<10	<10	<10			
	Sulfamethoxazole	<50	<50	<50	<50			
	Trimethoprim	13	12	27	<10	✓		✓
	Acetyl-sulfamethoxazole	<50	<50	<50	<50			
Downstream	Paracetamol	<50	<50	<50	<50			
	Mefenamic acid	<50	<50	68	<50			✓
	Diclofenac	174	<20	522	<20	✓		
	Propranolol	43	39	64	26	✓	✓	✓
	Dextropropoxyphene	314	260	682	<20	✓		✓
	Lofepramine	<10	<10	<10	<10			
	Tamoxifen	<10	<10	<10	<10			
	Ibuprofen	413	413	826	<20	✓		
	Erythromycin	<10	<10	<10	<10			
	Sulfamethoxazole	<50	<50	<50	<50			
	Trimethoprim	<10	<10	<10	<10			
	Acetyl-sulfamethoxazole	51	51	102	<50	✓		

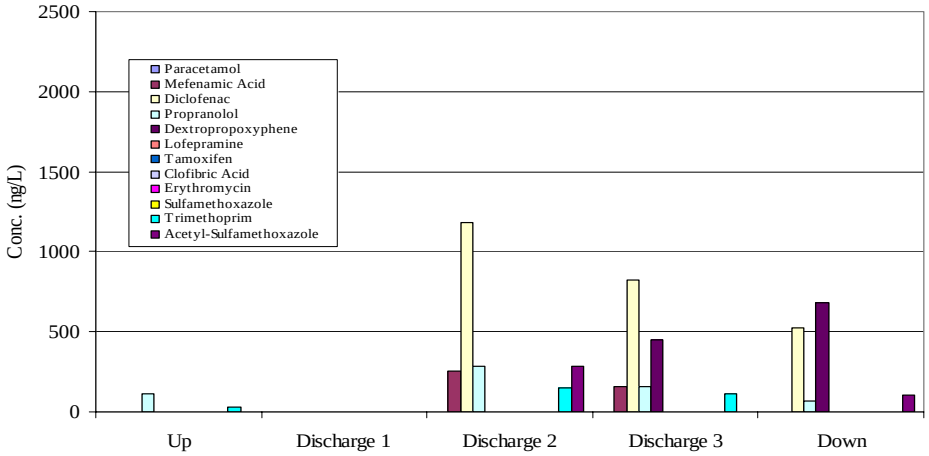


Figure 3.12 Concentration of selected pharmaceuticals (excluding ibuprofen) in samples collected from Harpenden STW during May 2002

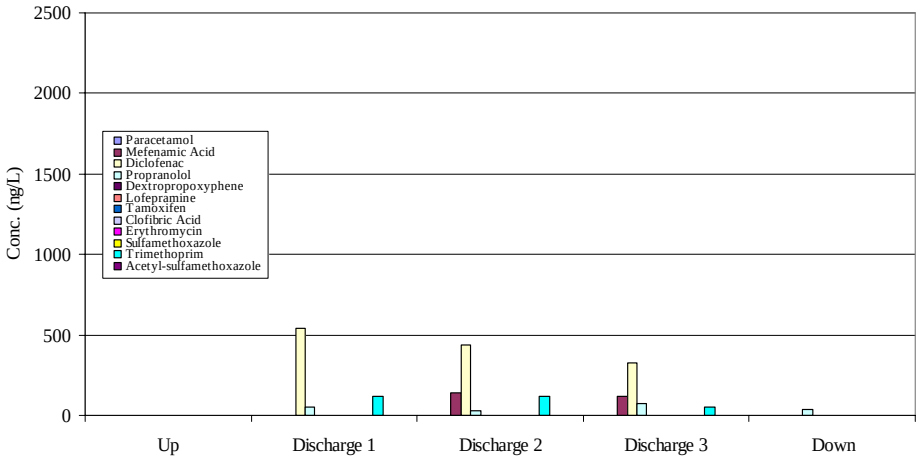


Figure 3.13 Concentration of selected pharmaceuticals (excluding ibuprofen) in samples collected from Harpenden STW during June 2002

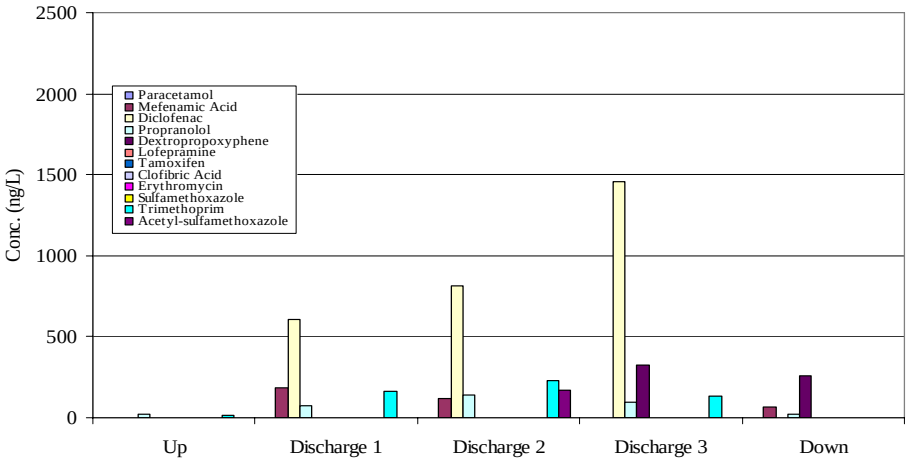


Figure 3.14 Concentration of selected pharmaceuticals (excluding ibuprofen) in samples collected from Harpenden STW during July 2002

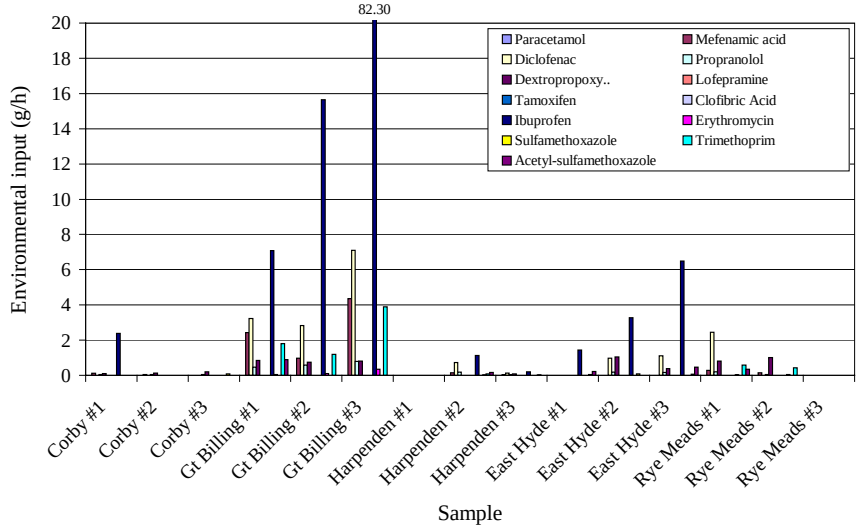


Figure 3.15 Pharmaceutical inputs (g/h) from selected STW final effluent discharges during May 2002

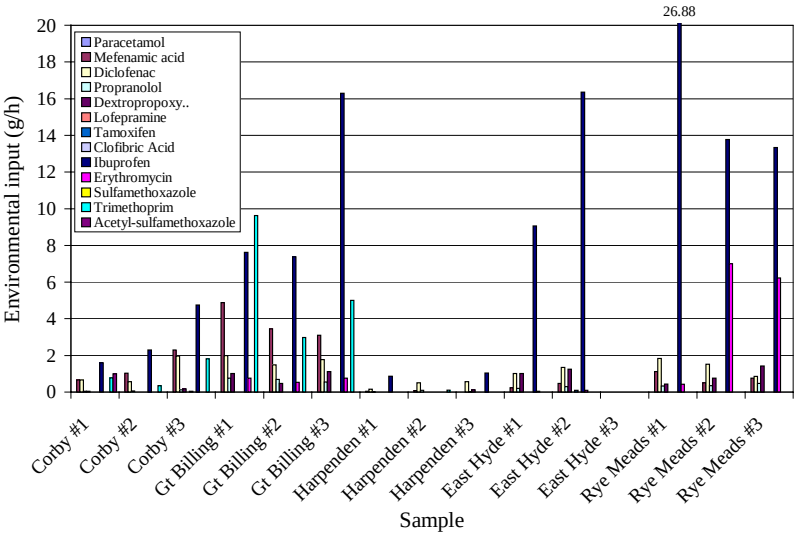


Figure 3.16 Pharmaceutical inputs (g/h) from selected STW final effluent discharges during June 2002

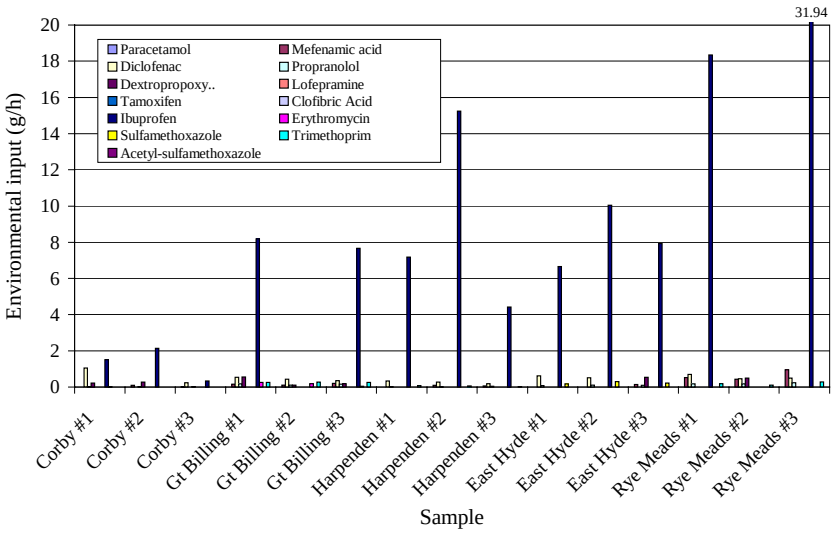


Figure 3.17 Pharmaceutical inputs (g/h) from selected STW final effluent discharges during July 2002

4. DISCUSSION

4.1 Sampling

The pharmaceutical compounds studied as part of this investigation were selected following an assessment of the risk that they pose to the environment. The Environment Agency performed this ranking following a review of human pharmaceuticals in the environment (Ayscough *et al*. 2000), as described in Section 1.4.

The programme of work described within this report focuses on the occurrence of pharmaceutical compounds in STW final effluent discharges. STWs were assessed since alternative sources, such as sites of manufacture, provide a discharge of a very specific nature. STW effluents include waste from hospitals/medical centres as well as domestic sewage that may contain prescription and other 'over the counter' drugs. Additionally the STWs identified for this study were known not to receive trade effluent from the pharmaceutical industry. Therefore the specificity of the discharges monitored was kept to a minimum and the discharges selected are expected to be representative of an average STW. Previous studies have shown that many pharmaceutical compounds are not removed by STWs and therefore STW effluents are potentially a major source of pharmaceuticals in surface waters (Ayscough *et al*. 2000 and references therein). In addition to assessing the concentration of pharmaceuticals in STW effluents, up- and down stream samples were also collected from the receiving water body. The downstream sample was taken in order to establish the concentration of targeted pharmaceutical compounds present in the receiving water downstream of the selected STWs. The upstream sample was taken in order to establish the contribution of pharmaceutical compounds from the STW discharge. In order to establish the pharmaceutical load discharging from each STW, discharge flow rates were also obtained for the time of sampling. The time of sampling was at peak flow of the STWs (section 2.2). Since environmental load is dependent upon flow rate and concentration of the compound within the sample, it is likely that the calculated loads within this study are higher than the average for each STW.

Samples were taken in the months of May, June and July 2002 when surface water flows were predicted to be low due to low precipitation. Periods of low rainfall were targeted since the dilution of effluent through the STWs would be low and river flows would also be low. The concentrations of any compounds detected would therefore represent a 'worst case scenario'. Heavy rain during July made it difficult to collect samples during periods of low flow (particularly when Corby and Billing STWs were sampled). This reflected in the STW effluent discharge flow rates (Appendix A).

4.2 Analysis methods

Robust methods are required to successfully analyse environmental surface water and effluent samples. At the initiation of this study methods had been reported in the peer-reviewed literature for nine of the targeted compounds (Hirsch *et al*. 1998; Ahrer *et al*. 2001). No methods had been reported for dextropropoxyphene, lofepramine, tamoxifen or acetyl-sulfamethoxazole. In addition, the only published method for the analysis of paracetamol in environmental samples reported a recovery of 0 % and is therefore unsuitable for use in this programme. In the early stages of this study attempts were made to reproduce the performance data reported in the literature for the published methods (Hirsch *et al*. 1998;

Ahrer *et al*. 2001), however satisfactory performance data could not be generated following the methods as described. Therefore new methods incorporating an off-line multi-residue solid phase extraction (SPE) procedure followed by liquid chromatography coupled to electrospray tandem mass spectrometry (LC-ESI-MS^2) were developed. A multi-residue SPE step was chosen in order to keep the number of samples collected, analysis times and costs to a minimum, whilst LC-ESI-MS^2 was used in order ensure high sensitivity and specificity.

4.2.1 Analysis method for mefenamic acid, diclofenac, propranolol, lofepramine, dextropropoxyphene and tamoxifen

The method used for the analysis of mefenamic acid, diclofenac, propranolol, dextropropoxyphene, lofepramine and tamoxifen was shown to be good, with good recoveries, LODs and % RSDs (Table 3.1). Comparison with literature data shows mefenamic acid, diclofenac and propranolol to have slightly lower recoveries than those quoted (Ahrer *et al*. 2001; Sacher *et al*. 2001). However, the recovery of mefenamic acid was sufficiently low to be of concern whilst the percentage recovery for lofepramine was too low to be used in this study. The performance data for both dextropropoxyphene and tamoxifen were sufficiently robust for environmental monitoring. None of the analytes eluted in the ion suppression region of the chromatogram.

4.2.2 Analysis method for ibuprofen

Electrospray ionisation operated in the negative ionisation mode was used to measure the concentration of ibuprofen in the collected samples. The method was run under neutral pH conditions in order to assist the negative ionisation of the analyte compounds. The method is constrained by the fact that the internal standard ^{13}C-phenacetin does not ionise well under negative conditions and must therefore be measured in a following run under positive ionisation conditions. When validated, this approach gave good recoveries (117%), good limits of detection and is reproducible (RSD= 22%). Spiked samples were run with each monthly batch and the performance monitored against a series of external calibration standards. Ibuprofen did not elute in the ion suppression region of the chromatogram. The performance of the method is comparable with all previously published methods (Ahrer *et al*. 2001; Sacher *et al*. 2001; La Farre *et al*. 2001; Ollers *et al*. 2001).

4.2.3 Analysis method for erythromycin, trimethoprim, sulfamethoxazole and acetyl-sulfamethoxazole

This method showed good recoveries for all analytes (Table 3.1). For erythromycin, sulfamethoxazole and trimethoprim the performance data comparable to, or better than, those reported in the peer reviewed scientific literature (Hirsch *et al*. 1998). As with the other methods developed, none of the analytes eluted in the ion suppression region of the chromatogram. This is the first reported method for the analysis of acetyl-sulfamethoxazole in environmental samples. All performance data are considered sufficiently robust for environmental monitoring.

4.2.4 Analysis method for paracetamol

Though many methods exist for the analysis of paracetamol in biological media (e.g. blood and urine) no reliable reported method exists for the analysis at the ng L^{-1} concentration in surface waters. Ahrer *et al.* (2001) reports a recovery of 0 % for paracetamol due to the breakthrough of paracetamol during the SPE procedure. The method described in this report, with a recovery of 75 %, a LOD of 50 ng L^{-1} and an RSD of 6.9 %, should be viewed as excellent given the comparatively high recovery and reproducibility.

4.2.5 Summary

The four analysis methods developed in combination with a generic SPE extraction procedure are suitably robust for the analysis of 12 of the 13 compounds in the targeted monitoring programme using the same SPE pre-concentration procedure. Generally the performance of these methods are comparable to, or better than, those reported in the peer-reviewed literature. Where they are inferior they are still adequate for a monitoring programme of this type, with the exception of lofepramine.

4.3 Targeted pharmaceutical concentrations in effluents and receiving waters

A cursory assessment of the pharmaceutical compound concentrations determined from each STW suggests that the secondary treated effluent from Great Billing STW consistently provided the highest concentration of targeted pharmaceutical compounds. Each of the other STW effluents collected during this study had received tertiary treatment with the concentration of selected pharmaceuticals apparently dependent on the dilution received from trade effluent. A summary of all previously reported data referred to in this section is presented in Appendix D (Table A9). A summary of the data recorded for each substance is provided in the following sections. Where available, data on human metabolism of the substance and degradation in STW is provided. Environmental toxicity data for each of the substances was sought to enable the concentrations reported during this study to be put into context with reported effects data. Very few studies reported quantitative results, such as lethal or effect concentrations and the majority of test results were carried out *in vitro* and involved cellular or biochemical endpoints.

Ibuprofen was determined at significantly higher concentrations than any other targeted pharmaceutical compound. During this investigation ibuprofen was regularly determined in STW effluents at a median concentration ~ 3,000 ng L^{-1} and downstream in the receiving waters at concentrations of between <20 and ~5,000 ng L^{-1} (Table 4.1). High concentrations of ibuprofen in STW effluents is not surprising since it has previously been reported at concentrations of up to 3,400 ng L^{-1} in STW effluents (Table A9; Ayscough *et al*. 2000; Ollers *et al*. 2001; La Farre *et al*. 2001; Kolpin *et al*. 2002). This is probably due to the amount of ibuprofen used as a prescription and an 'over-the-counter' anti-inflammatory and painkiller, combined with a low degree of human metabolism. Buser *et al*. (1999) reported that 70-80% of the human therapeutic dose of ibuprofen would be excreted as the parent compound or as metabolites, whilst it has also been reported as relatively persistent in aquatic systems ($t_{½}$ = 50 days; Singer *et al*. 2002).

Ibuprofen has also been previously reported as 'inherently biodegradable' during the STW process (Ayscough *et al*. 2000). However the performance of different sewage treatment

processes varies. Data from a study of the removal of 11 pharmaceutical compounds in a Brazilian sewage treatment works suggested that ibuprofen would be more effectively removed by activated sludge treatment (75% removal) than via biological filtration (22% removal) (Stumpf *et al*. 1999).

La Farre *et al*. (2001) assessed the toxicity of ibuprofen on the bioluminescence of *Vibrio fischeri* using the rapid toxicity assessment kits ToxAlert and Microtox. *V. fischeri* EC50 of 12.1 and 19.1 mg L^{-1} were reported for the two tests respectively. Knoll/BASF (1995) report a 96h EC50 and LC50 for *Skeletonema costatum* of 7.1 and 173 mgL^{-1} respectively, whist a 48h EC50 of 9.06 mgL^{-1} is reported for *D. magna*. In this study the mean concentration of ibuprofen in STW final effluents was 4.2 μgL^{-1} with a maximum concentration of 27.2 μgL^{-1} determined in a sample collected from Great Billing STW in May a mean concentration of 1.1 μgL^{-1} was determined in receiving waters.

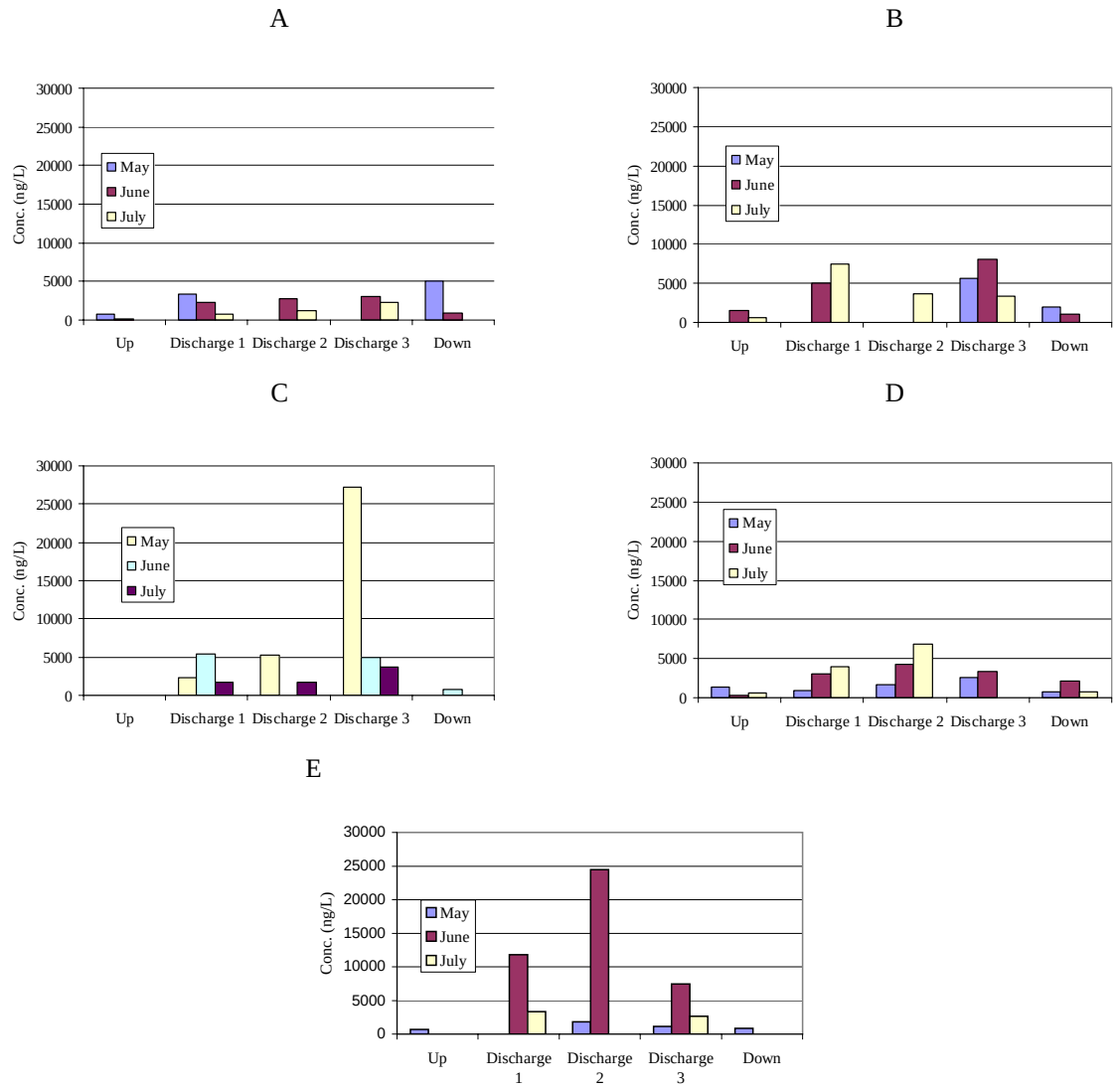


Figure 4.1 Monthly concentrations of ibuprofen in samples collected from A. Corby, B. Rye Meads, C. East Hyde, D. Harpenden and E. Great Billing STWs

Table 4.1 Summary ibuprofen data

Sample type	Mean	Median	Max	Min	Frequency (%)†
		(ng L^{-1})			
Upstream	432	181	1555	<20	57
Final effluent	4201	3086	27256	<20	84
Downstream	1105	826	5044	<20	69

† Percentage of samples analysed where pharmaceutical was detected.

Diclofenac was found to have the second highest mean concentration in the effluents collected (424 ng L^{-1}; Table 4.2). No data on the total UK usage were readily available, however in 1997 there were over a million prescription items issued (Ayscough *et al*. 2000). Diclofenac has been reported at concentrations of up to ~2000 ng L^{-1} in the sewage effluents (Table A9; Ayscough *et al*. 2000; Ollers *et al*. 2000; La Farre *et al*. 2001). Though Diclofenac sodium is known to be eliminated through metabolism and subsequent urinary and biliary excretion of the glucuronide and the sulfate conjugates of the metabolites. Less than 1% of the parent drug is excreted unchanged (eMC, 2002). Diclofenac is reported to have a short half-life of around 8 days (Singer *et al*. 2002) and has also been shown to rapidly photodegrade ($t_{½}$ = 4 h; Ayscough *et al*., 2000), suggesting that once it enters the environment it will rapidly degrade. Additionally Ternes (1998) found a 69% reduction in diclofenac in sewage effluent following primary sedimentation, aeration and phosphate removal treatment.

Diclofenac was detected in approximately half the surface water samples collected downstream of STW effluent discharges (mean concentration 154 ng L^{-1}). Previous studies have also reported the occurrence of diclofenac in surface waters usually at concentrations of up to 1,000 ng L^{-1} (Table A9). La Farre *et al*. (2001) has reported a *V. fischeri* EC50 of 13.5 and 13.7 mg L^{-1}. The results indicate that diclofenac is moderately toxic to *V. fischeri.*

Table 4.2 Summary diclofenac data

Sample type	Mean	Median	Max	Min	Frequency (%)†
		(ng L^{-1})			
Upstream	<20	<20	<20	<20	0
Final effluent	599	424	2349	<20	86
Downstream	154	0	568	<20	47

† Percentage of samples analysed where pharmaceutical was detected.

A B

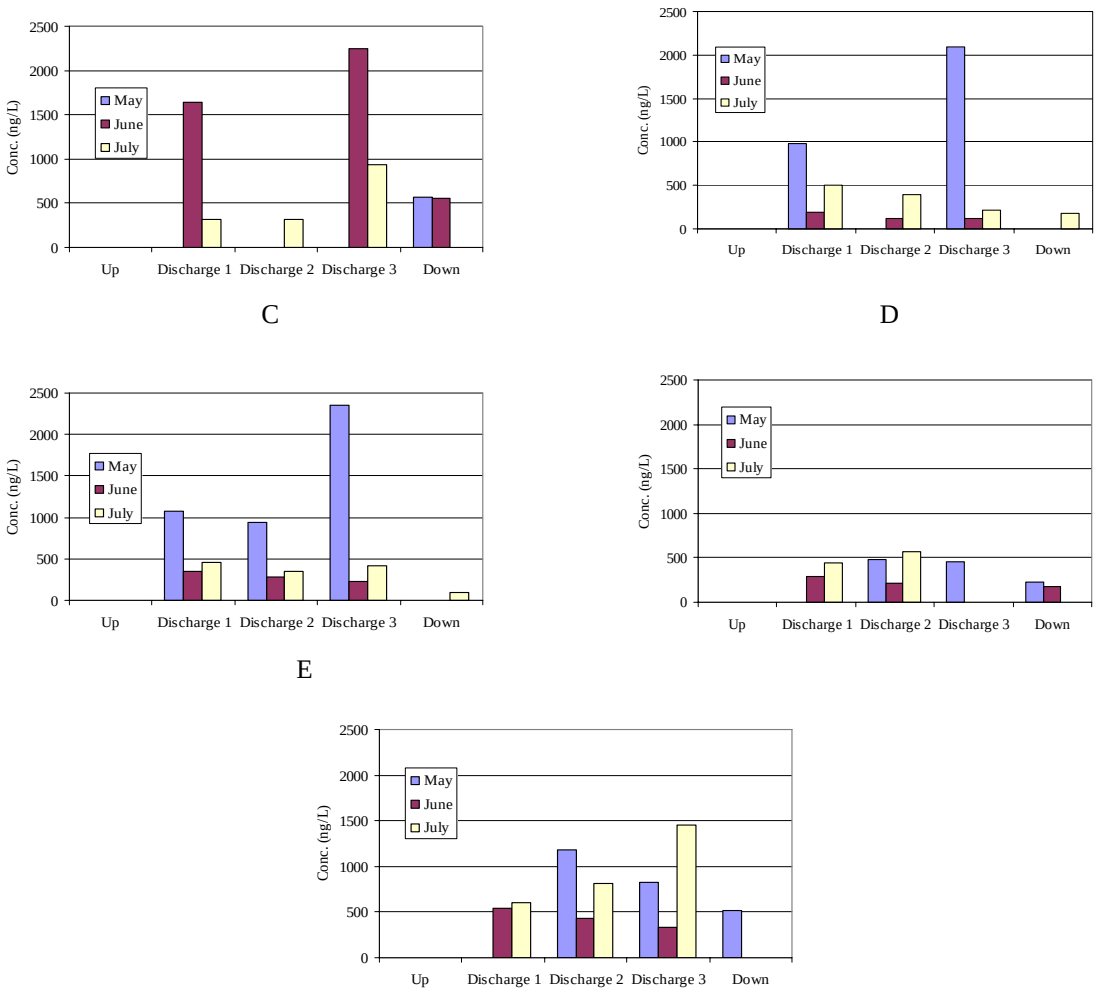


Figure 4.2 Monthly concentrations of diclofenac in samples collected from A. Corby, B. Rye Meads, C. East Hyde, D. Harpenden and E. Great Billing STWs

Mefenamic acid, propranolol and **dextropropoxyphene** were detected in the low to mid ng L^{-1} range (Tables 4.3, 4.4 and 4.5). Propranolol was the only targeted pharmaceutical compound to be detected in 100 % of the final effluent samples. Both propranolol and dextropropoxyphene are used in large quantities in the UK, 11.8 and 42.5 tonnes/annum respectively (Ayscough *et al*. 2000). No usage data could be found for mefenamic acid. All three compounds are thought to undergo significant metabolism within the human body. Propranolol is excreted via the urine largely as metabolites with very little unchanged propranolol, whist dextropropoxyphene is excreted in the urine mainly as metabolites (Martindale, 1993). EMC (2002) reports that 52% of a dose of mefenamic acid is recovered from the urine; 6% as mefenamic acid, 46% as metabolites. Additionally the removal of propranolol in sewage influent during primary settling, aerobic treatment and phosphate removal has been reported to be almost complete (96%) (Ternes, 1998), whist Rogers (1996) reports that dextropropoxyphene is non-biodegradable.

Lilius *et al*. (1994) and Calleja *et al*. (1994) report similar 24 h *D. magna* EC50 for dextropropoxyphene; 14.6 and 19 mg L^{-1}. Calleja *et al*. (1994) also report *Artemia salina*, *Streptocephalus proboscideus* and *Brachionus calyciflorus* 24 h LC50 values of 308, 7.6 and 4.2 mg L^{-1} respectively. The same studies were also performed on propranolol reporting LC50

values of 2.7, 15.87, 407, 1.87 and 2.59 mg L^{-1} . In both cases these data would indicate moderate to high toxicity. No toxicity data for mefenamic acid was obtained.

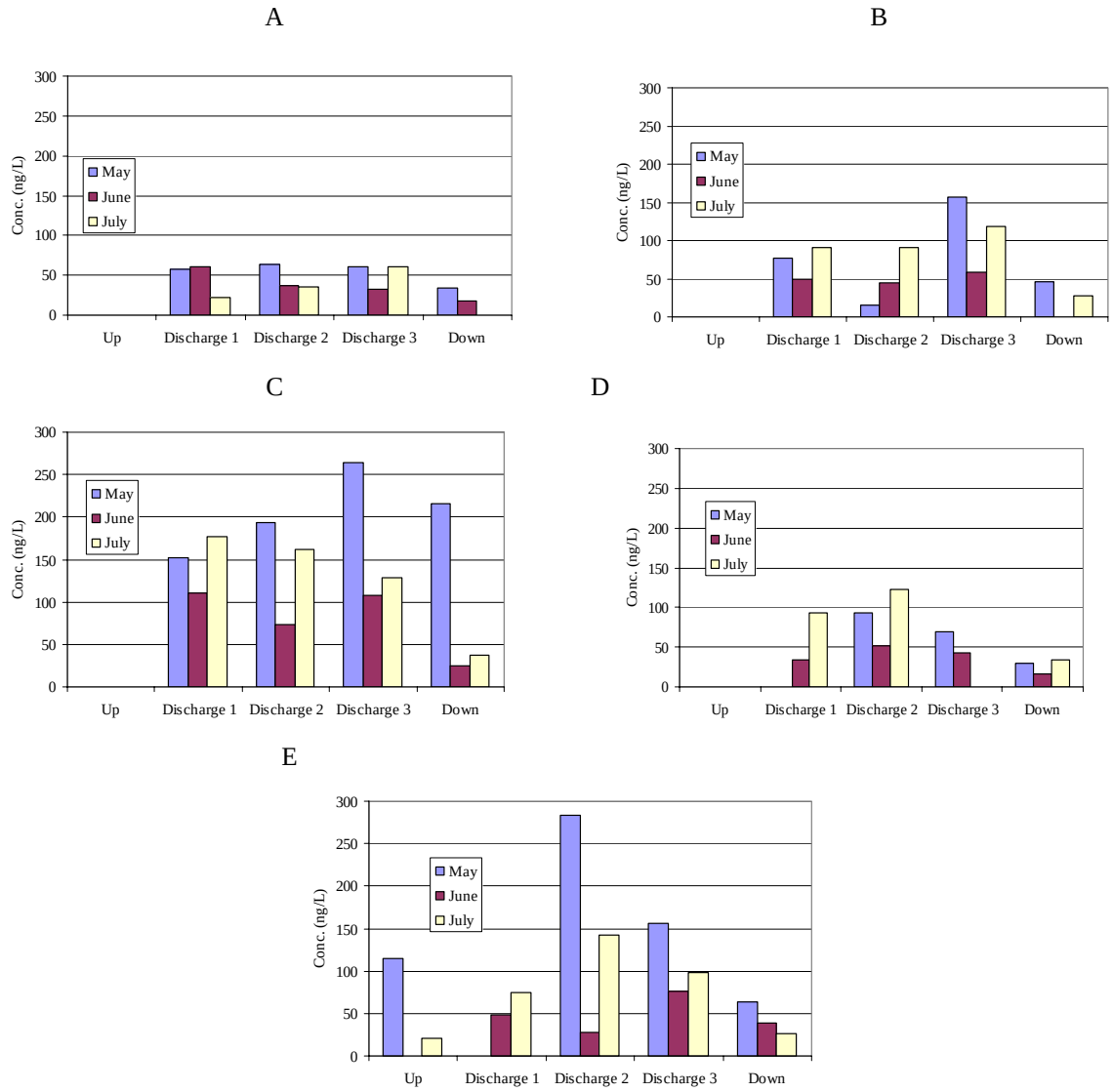


Figure 4.3 Monthly concentrations of propranolol in samples collected from A. Corby, B. Rye Meads, C. East Hyde, D. Harpenden and E. Great Billing STWs.

Table 4.3 Summary propranolol data

Sample type	Mean	Median	Max	Min	Frequency (%)†
		(ng L^{-1})			
Upstream	10	<10	115	<10	14
Final effluent	93	76	284	16	100
Downstream	41	29	215	<10	87

† Percentage of samples analysed where pharmaceutical was detected.

Previous studies on the occurrence of propranolol in Germany found concentrations of propranolol up to 1,300 ng L^{-1} in sewage effluent and up to 590 ng L^{-1} in surface waters (Table A9; Ayscough *et al*. 2000). Propranolol was also the most frequently detected in

surface waters downstream of STW effluent discharges (Table 4.3). No previous data could be found on the presence of mefenamic acid and dextropropoxyphene in sewage effluents or surface waters.

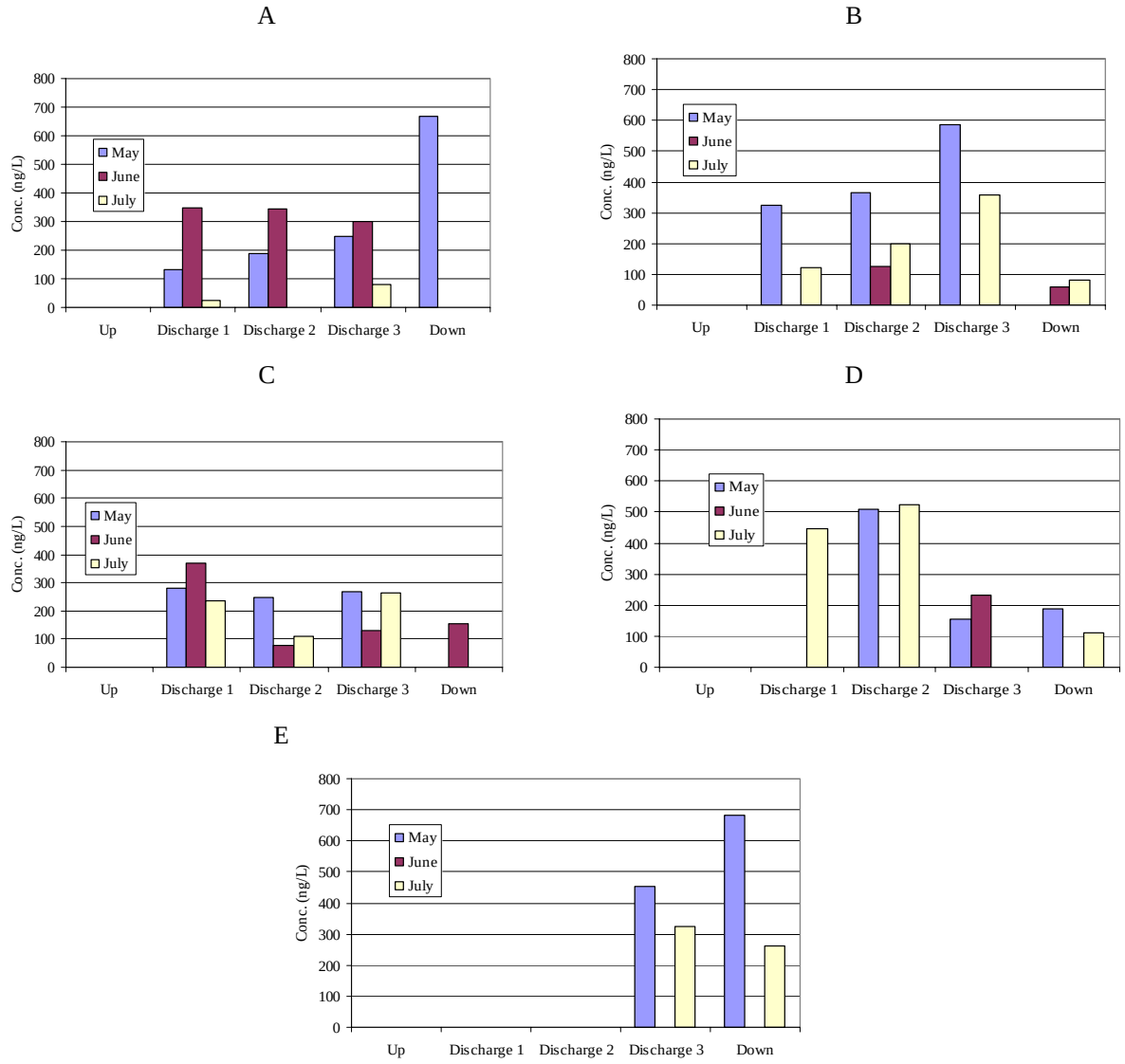


Figure 4.4 Monthly concentrations of dextropropoxyphene in samples collected from A. Corby, B. Rye Meads, C. East Hyde, D. Harpenden and E. Great Billing STWs

Table 4.4 Summary dextropropoxyphene data

Sample type	Mean	Median	Max	Min	Frequency (%)[†]
		(ng L^{-1})			
Upstream	<20	<20	<20	<20	0
Final effluent	199	195	585	<20	74
Downstream	147	58	682	<20	53

[†] Percentage of samples analysed where pharmaceutical was detected.

A B

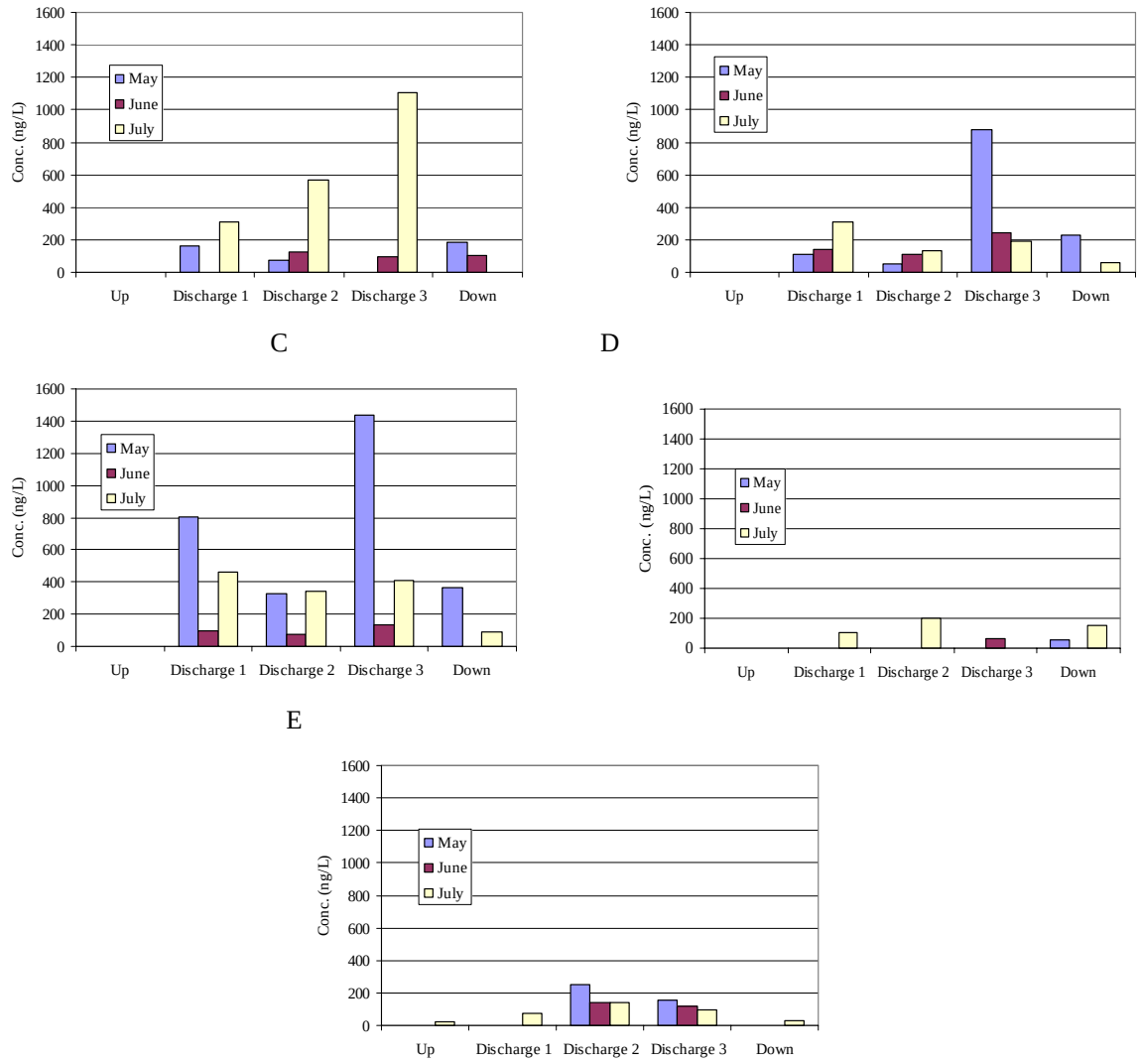


Figure 4.5 Monthly concentrations of mefenamic acid in samples collected from A. Corby, B. Rye Meads, C. East Hyde, D. Harpenden and E. Great Billing STWs

Table 4.5 Summary mefenamic acid data

Sample type	Mean	Median	Max	Min	Frequency (%)[†]
		(ng L^{-1})			
Upstream	<50	<50	<50	<50	0
Final effluent	273	133	1440	<50	81
Downstream	86	62	366	<50	60

[†] Percentage of samples analysed where pharmaceutical was detected.

Of the antibiotics analysed in this study **trimethoprim** was found to be the most frequently occurring (Tables 4.6 and 4.7). **Erythromycin** occurred less frequently in the samples collected but was sometimes found at higher concentrations. UK usage of erythromycin is high at 68 tonnes/annum (Ayscough *et al.* 2000). No total usage data were available for trimethoprim, though in 1997 it was prescribed 2.8 million times compared to erythromycin's 3.3 million

(Ayscough *et al*. 2000). Little metabolism of erythromycin is reported to occur, only 5% being eliminated in the urine (EMC, 2002), whilst trimethoprim is excreted via the urine largely unchanged (Martindale,1993). No data on the degradation of trimethoprim in STW could be located.

Hirsch *et al*. (1999) has reported the occurrence of both erythromycin and trimethoprim in German sewage effluents with median values of 2,500 ng L^{-1} and 320 ng L^{-1} respectively. In surface waters erythromycin has been reported to occur at a median concentration of 150 ng L^{-1} and trimethoprim at a maximum concentration of 200 ng L^{-1} (median value below detection limits). A separate study by Kolpin *et al*. (2002) on US streams showed median concentrations in surface waters to be 100 and 150 ng L^{-1} for erythromycin and trimethoprim respectively with a frequency of detection of 21.5 % and 12.5 %. Both compounds would therefore be expected to occur in the effluent discharges of STWs.

An extensive study on the environmental risk of three antibiotic compounds by Halling-Sorensen *et al*. (2000) showed the acute toxicity of trimethoprim to be low; EC50 (growth) values are quoted as 17.8, 110 and 112 mg L^{-1} for bacteria (activated sludge), green algae (*Selenastrum capricornutum*) and cyanobacteria (*Microcystis aeroginosa*) respectively. Additionally a 48 h *D. magna* EC50 (immobilisation) of 123 mg L^{-1} and a zebra fish (*Danio rerio*) NOEC of >100 mg L^{-1} over 72 h are also reported. Kiryu and Moffitt (2002) investigated the toxicity of erythromycin to four salmonid species. LD50 concentrations after 96 h ranged from 350 mg Kg^{-1} to 778 mg Kg^{-1}, indicating low toxicity. However the data is of limited relevance since the fish were injected with erythromycin which does not mimic natural exposure pathways. A study on the 24 h and 48 h LC50 of *D. magna* reported values of 388 and 211 mg L^{-1} (Di delupis *et al*. 1992).

Table 4.6 Summary erythromycin data

Sample type	Mean	Median	Max	Min	Frequency (%)†
		(ng L^{-1})			
Upstream	<10	<10	57	<10	17
Final effluent	109	<10	1842	<10	44
Downstream	159	<10	1022	<10	38

† Percentage of samples analysed where pharmaceutical was detected.

Table 4.7 Summary trimethoprim data

Sample type	Mean	Median	Max	Min	Frequency (%)†
		(ng L^{-1})			
Upstream	<10	<10	36	<10	36
Final effluent	128	70	1288	<10	65
Downstream	12	<10	42	<10	38

† Percentage of samples analysed where pharmaceutical was detected.

Sulfamethoxazole and its metabolite **acetyl-sulfamethoxazole** were irregularly detected in the final effluent samples collected (Table 4.8). Acetyl-sulfamethoxazole occurred at a higher frequency (33%) when compared to sulfamethoxazole (9%). No data could be found on the UK usage of sulfamethoxazole, though it is thought to be non-biodegradable (Ayscough *et al*. 2000). Sulfamethoxazole has been detected in sewage discharge by Hirsch *et al*. (1999) at a median of 400 ng L^{-1} and also in surface waters at 30 ng L^{-1}. While Kolpin *et al*. (2002)

detected sulfamethoxazole in 12.5 % of surface water samples at a median concentration of 150 ng L^{-1}. No previous studies have assessed the occurrence of acetyl-sulfamethoxazole.

Table 4.8 Summary acetyl-sulfamethoxazole data

Sample type	Mean	Median	Max	Min	Frequency (%)[†]
		(ng L^{-1})			
Upstream	0	0	0	0	0
Final effluent	161	0	2235	0	33
Downstream	70	0	239	0	38

[†] Percentage of samples analysed where pharmaceutical was detected.

Of the remaining targeted pharmaceutical compounds, **tamoxifen** was detected twice in sewage discharge samples, while **paracetamol** and **lofepramine** were not detected. No data on the total UK usage of tamoxifen and lofepramine are publicly available, though the number of prescriptions of tamoxifen citrate is low compared to other compounds, being around a million in 1997 (Ayscough *et al*. 2000). Tamoxifen is known to be excreted slowly in the faeces mainly as conjugates, with small amounts excreted in urine, while lofepramine is excreted in urine mainly as metabolites (Martindale, 1993). Additionally no previous data could be found on the occurrence of either tamoxifen or lofepramine in either surface waters or sewage effluents.

In the case of **tamoxifen** the recoveries of the method were acceptable being around 42 %, though for June and July these were 57 and 59 % respectively. It is therefore likely that it is present below the methods 10 ng L^{-1} limit of detection or not at all. However the method for lofepramine was not sufficiently robust and so no assessment can be made on its occurrence in UK STW effluents.

Anderson *et al*. (2001) have conducted a chronic test on the effects of tamoxifen to the development of the marine copepod *A. tonsa*. A 5 day EC10 and EC50 of 8 and 40 μg L^{-1} respectively were reported for inhibition of naupliar development, indicating high acute toxicity. However these values are still 3 orders of magnitude higher than the highest detected concentration in final effluent of 42 ng L^{-1}. All downstream samples were less than 10 ng L^{-1}.

Paracetamol has the highest UK usage of any drug at 2,000 tonnes/annum. This was used by Webb (2000) to calculate a PEC of 367 ng L^{-1} (Ayscough *et al*. 2000). Ternes (1998) reported data from STWs showing a maximum concentration of 6000 ng L^{-1}. However this result was not wholly representative since both the reported median and 90th percentile data were both less than the LOD. Surface water concentrations from the Ternes (1998) study were all lower than the LOD. Paracetamol has been reported to be readily degradable after acclimatisation during sewage treatment, and is known to be mainly excreted as glucuoronides and sulphate conjugates with only 5 % excreted as the parent compound which supports the results of this study (Richardson and Bowron, 1985; Martindale, 1993).

Three studies could be found on the ecotoxicity of paracetamol. Calleja *et al*. (1994), Kuhn *et al*. (1989) and Henschel *et al*. (1997) all studied the effect over 24 h of paracetamol on *D. magna*, reporting EC50 values of 55.5, 13 and 293 mg L^{-1} respectively. Kuhn *et al*. (1989) and Henschel *et al* (1997) also reported *D. magna* 48 h EC50s of 9.2 and 50.0 mg L^{-1}. Henschel *et al*. (1997) reported a 48 h *Brachydanio rerio* embryo EC50 of 378 mg L^{-1} and a 72 h *Scenedesmus subspicatus* EC50 of 134 mg L^{-1}. Calleja *et al*. (1994) performed 24 h LC50 tests on *Artemia salina, Streptocephalus proboscideus* and *Brachionus calyciflorus*

reporting values of 577, 29.6 and 5306 mg L^{-1} respectively. These results indicate that paracetamol has a low toxicity to these species.

4.4 Variability

Significant variability was seen in the occurrence and concentration of the targeted pharmaceutical compounds in the samples collected. Variations were observed between STWs, between the monthly sampling events and between the three samples collected at each STW on a monthly basis. Variation in the concentration of compounds found in STW effluents has been observed in previously reported studies (Environment Agency, 2001). In a study investigating the fate of steroid oestrogens the variable concentration of steroid oestrogens could not be correlated to flow. It was assumed that inputs of natural steroids are related to the (constant) population served by the works and that the variations in steroid concentrations indicate variations in STW plant performance which in turn may be an important factor in determining final effluent concentrations (Environment Agency 2001). Plant performance may control some of the variability observed in the concentration of targeted pharmaceutical compounds, however it is likely that the input of pharmaceutical compounds and their metabolites into STW will be highly variable. The input into a works will be affected by a number of factors including differing use patterns, and the amount used. It is therefore unsurprising that this has been detected in the samples collected for this study.

4.5 Comparison with derived PECs and PNECs

Predicted Environmental Concentrations (PECs) were calculated in the initial screening exercise used to prioritise pharmaceutical substances for targeted monitoring (section 1.4). Table 4.9 compares these values with the mean and maximum concentrations of substances reported downstream during the monitoring programme conducted during this project. In all cases the measured concentrations, expressed as an average for all five sewage treatment works examined, are an order of magnitude lower than the PECs.

Cranfield University determined predicted no-effect concentrations (PNEC) for the initial screening exercise used to prioritise pharmaceutical substances for targeted monitoring (Section 1.4). With the exception of paracetamol and ibuprofen, all the derived PNECs were based on predicted traditional toxicity end-point data (outlined below). These predicted toxicity data should be viewed with caution since the mode of action of these compounds may be significantly different.

- Trimethoprim: no useable experimental data, a predicted algal 96 h EC50 was used.
- Diclofenac: no experimental data, a predicted algal 96 h EC50 was used.
- Sulphamethoxazole: no experimental data, a predicted *D. magna* 48 h LC50 was used.
- Paracetamol: experimental *D. magna* 48 h LC50 was used.
- Mefenamic acid: no experimental data, a predicted fish 96 h LC50 was used.
- Ibuprofen: experimental *D. magna* 48 h LC50 was used.
- Erythromycin: no useable experimental data, a predicted *D. magna* 48 h LC50 was used.

- Dextropropoxyphene: no experimental data, a predicted algal 96 h EC50 was used.
- Lofepramine: no experimental data, a predicted *D. magna* 48 h LC50 was used.
- Tamoxifen: no experimental data, a predicted *D. magna* 48 h LC50 was used.
- Propranolol: no experimental data, a predicted *D. magna* 48 h LC50 was used.

Table 4.9 Comparison of measured environmental concentrations (MEC) and predicted environmental concentrations (PEC)

	PEC (ng L^{-1})	MEC[1] (ng L^{-1})	
		Mean	Maximum
Paracetamol	76400	N/d	N/d
Ibuprofen	10800	1105	5044
Mefenamic acid	677	86	366
Diclofenac	1090	154	568
Propranolol	365	41	215
Dextropropoxyphene	1332	147	682
Lofepramine	140	N/d	N/d
Tamoxifen	63	N/d	N/d
Erythromycin	1594	159	1022
Trimethoprim	289	12	42
Sulphamethoxazole	40	N/d	N/d

[1] Mean data analysed downstream of five STWs. Nd: not detected.

A comparison of PNEC with the mean and maximal measured environmental concentrations (MEC) of pharmaceutical compounds is shown in Table 4.10. For all the pharmaceutical compounds analysed the MEC/PNEC ratios are < 1. This indicates that the pharmaceutical compounds targeted are not being found at levels likely to cause acute toxicity for the range of organisms tested. However, limited ecotoxicological data were available on which to base these conclusions and it has also been suggested that the use of standard acute ecotoxicity data may not be suitable for assessing the risk posed by pharmaceutical compounds given the intended narrow scope of biological activity/effect and potency of pharmaceutical compounds in general. Chronic bioassays conducted over the life-cycle of various organisms from different trophic levels may be more appropriate (Halling-Sørensen *et al*., 1998).

Table 4.10 Comparison of mean and maximal measured environmental concentrations (MEC) with predicted no-effect concentrations (PNEC)

Pharmaceutical	MEC[1] (ng L^{-1})		PNEC (ng L^{-1})	MEC/PNEC ratio
	Mean	Maximum		
Paracetamol	nd	nd	92,000	$< 5.4 \times 10^{-4}$
Ibuprofen	1105	5044	115,000	0.04
Mefenamic acid	86	366	15,000	0.02
Diclofenac	154	568	99,090	5.7×10^{-3}
Propranolol	41	215	23,520	9.1×10^{-3}
Dextropropoxyphene	147	682	800	0.85
Lofepramine	nd	nd	70	< 0.1
Tamoxifen	nd	nd	200	< 0.05
Erythromycin	159	1022	78,000	0.01
Trimethoprim	12	42	26,264	1.6×10^{-3}
Sulphamethoxazole	nd	nd	45,000	1.1×10^{-3}

[1] Mean data analysed downstream of five STWs. Nd: not detected.

4.6 Environmental load

The load data calculated within this report are dependent on the effluent flow rate and concentration of targeted pharmaceutical at the time of sampling. The time of sampling was around the time of peak flow of the STWs (section 2.2). Therefore it is likely that the calculated loads within this study are among the highest for the sites monitored. Within the context of this study, Great Billing had both high final effluent flow rates and high concentrations of targeted pharmaceutical compounds. Therefore the highest loads were calculated to be from Great Billing STW. Similarly, the load data for Rye Meads STW are also high due to its high flow rates. Corby and Harpenden STWs provided the lowest load data; Corby STW due to the combination of low concentration of targeted pharmaceuticals determined in the final effluent and low flow rates. The loads of targeted pharmaceuticals from Harpenden STW were low due to correspondingly low flow rates.

Since the environmental load of each targeted pharmaceutical is dependent on the concentrations presented in Section 3, ibuprofen provides the most significant loads into the receiving waters. These loads are commonly in the low g h^{-1} range, while regularly reaching the order of ten's of g h^{-1}. Within the context of this targeted study, mefenamic acid, diclofenac and to a lesser extent dextropropoxyphene are all regularly providing a significant environmental input. However the concentration of the target compound within the receiving watercourse, and therefore any associated biological effect, is very much dependant on the volume of the receiving waters and the degree of dilution that occurs. Although propranolol was detected in all of the discharge samples collected, an overall mean concentration of ~200 ng L^{-1} suggests that overall input from individual effluent discharges is low in comparison to the other commonly detected target compounds. However, it would appear that propranolol is ubiquitous in the sewage discharges monitored.

5 CONCLUSIONS

- Reliable and robust analytical methods have been developed and validated for the analysis of eleven of the twelve pharmaceutical (or pharmaceutical metabolite) compounds selected by the Environment Agency for targeted monitoring. Difficulties were found in repeating published methods for some of the compounds targeted.

- Environmental occurrence data has been obtained for eleven pharmaceutical (or pharmaceutical metabolite) compounds in samples collected from STW final effluent discharges and receiving waters at five UK STWs.

- Ten pharmaceutical compounds, Ibuprofen, mefenamic acid, diclofenac, propranolol, dextropropoxyphene, erythromycin, trimethoprim, tamoxifen, sulfamethoxazole and acetyl-sulfamethoxazole were detected in STW final effluent samples.

- Eight pharmaceutical compounds, Ibuprofen, mefenamic acid, diclofenac, propranolol, dextropropoxyphene, erythromycin, trimethoprim, and acetyl-sulfamethoxazole were detected in receiving surface water samples.

- Paracetamol and lofepramine were not detected in any of the effluent or receiving water samples collected.

- The anti-inflammatory pharmaceutical ibuprofen was consistently found to be present in the effluent samples collected at the highest median concentration (~3,000 ng L^{-1}).

- Environmental input data showed that significant amounts of the targeted pharmaceutical compounds are entering UK surface waters from STW effluent discharges.

- The results suggest that the screening process used to derive the list of substances to be monitored was a valid approach.

- In all cases the measured downstream concentrations, expressed as an average for all five sewage treatment works examined, are an order of magnitude lower than the derived PECs.

6 RECOMMENDATIONS FOR FURTHER WORK

This report is only one part of a wider investigation by the Agency into assessing the potential risk posed to the aquatic environment in England and Wales by pharmaceutical substances. The recommendations made in this report are only concerned with the findings of this targeted monitoring study. These are primarily aimed at further informing the Agency's risk assessment procedures.

1 Pharmaceutical metabolites

Sulfamethoxazole-acetate, a metabolite analysed as part of this study, was found in some of the effluent and surface water samples collected. The occurrence of this compound suggests that other pharmaceutical metabolites may also be entering the aquatic environment. It is therefore recommended that methods are developed for the analysis of pharmaceutical metabolites and a targeted study performed in order to assess their occurrence in effluents and surface waters.

2 Aquatic toxicity

A fuller assessment of the threat posed to the aquatic environment by the concentration of pharmaceutical compounds determined in this study is recommended.

3 Environmental fate

Certain pharmaceutical compounds appear not to be removed by STW processes. An assessment is required on the fate of pharmaceuticals in sewage treatment and the environment is recommended.

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APPENDIX A STW FINAL EFFLUENT FLOW DATA

Table A1 STW Final effluent flow data and sample times

STW	Date	Day	Sample Time	Flow (l sec^{-1})
Great Billing	20/05/02	Mon	10:00	838
			11:10	838
			12:00	838
	11/06/02	Tue	09:45	425
			11:00	425
			11:45	425
	09/07/02	Tue	10:15	1197
			11:35	1197
			12:15	1197
Corby	20/05/02	Mon	13:10	200
			14:00	196
			15:00	216
	11/06/02	Tue	13:25	180
			14:25	220
			15:20	30
	09/07/02	Tue	13:50	595
			14:40	510
			15:35	580
East Hyde	21/05/02	Tue	08:00	406
			09:00	564
			10:00	684
	13/06/02	Thur	08:30	597
			09:35	648
			10:30	647
	11/07/02	Thur	08:30	630
			09:30	663
			10:30	626
Harpenden	21/05/02	Tue	08:30	165
			09:30	170
			10:30	47
	13/06/02	Thur	09:00	168
			10:00	173
			10:55	164
	11/07/02	Thur	09:00	72
			10:00	174
			11:05	107
Rye Meads	21/05/02	Tue	11:45	696
			12:45	762
			13:50	-
	13/06/02	Thur	12:40	1026
			13:40	1066
			14:55	1103
	11/07/02	Thur	12:20	1009
			13:30	1054
			14:20	1102

Table A2 STW Upstream and Downstream sample times

DATE	DAY	SITE	SAMPLE TIME
20/05/02	Mon	Great Billing-Up	10:30
		Great Billing-Down	10:45
		Corby-Up	13:20
		Corby-Down	13:30
21/05/02	Tue	East Hyde-Up	10:00
		East Hyde-Down	09:15
		Harpenden-Up	08:45
		Harpenden-Down	09:40
		Rye Meads-Up	13:30
		Rye Meads-Down	13:00
11/06/02	Tue	Great Billing-Up	10:05
		Great Billing-Down	10:30
		Corby-Up	13:35
		Corby-Down	13:45
13/06/02	Thur	East Hyde-Up	08:40
		East Hyde-Down	09:45
		Harpenden-Up	10:15
		Harpenden-Down	09:15
		Rye Meads-Up	14:30
		Rye Meads-Down	14:15
09/07/02	Tue	Great Billing-Up	10:35
		Great Billing-Down	11:05
		Corby-Up	14:15
		Corby-Down	14:30
11/07/02	Thur	East Hyde-Up	08:35
		East Hyde-Down	09:45
		Harpenden-Up	09:10
		Harpenden-Down	10:50
		Rye Meads-Up	13:05
		Rye Meads-Down	12:40

APPENDIX B OCCURRENCE DATA FOR TARGETED PHARMACEUTICAL COMPOUNDS

Table A3 Targeted pharmaceutical concentration data for May 2002(ng/l)

	Paracetamol	Mefenamic Acid	Diclofenac	Propranolol	Dextroprop-oxyphene	Lofepramine	Tamoxifen	Ibuprofen	Erythromycin	Sulfamethoxazole	Trimethoprim	Acetyl-Sulfamethoxazole
Blank	<50	nsa	nsa	nsa	nsa	nsa	nsa	<20	<10	<50	<10	<50
Corby#1	<50	164	<20	57	132	<10	<10	3300	<10	<50	<10	<50
Corby#2	<50	76	<20	63	189	<10	<10	<20	<10	<50	<10	<50
Corby#3	<50	<50	<20	61	248	<10	<10	<20	<10	112	<10	<50
Corby-Up	<50	<50	<20	<10	<20	<10	<10	834	<10	<50	<10	<50
Corby-Dn	<50	182	568	35	670	<10	<10	5040	<10	<50	<10	198
Gt. Billing#1	<50	807	1070	152	281	<10	<10	2350	16	<50	599	294
Gt. Billing#2	<50	325	936	193	248	<10	<10	5180	34	<50	396	<50
Gt. Billing#3	<50	1440	2350	264	268	<10	<10	27300	116	<50	1290	<50
Gt. Billing-Up	nsa	nsa	nsa	nsa	nsa	nsa	nsa	nsa	nsa	nsa	nsa	nsa
Gt. Billing-Dn	nsa	366	<20	215	<20	<10	<10	nsa	nsa	nsa	nsa	nsa
Harpenden#1	<50	nsa	nsa	nsa	nsa	nsa	nsa	nsa	nsa	nsa	nsa	nsa
Harpenden#2	<50	254	1180	284	<20	<10	<10	1830	<10	<50	147	282
Harpenden#3	<50	155	821	157	453	<10	<10	1150	<10	<50	113	<50
Harpenden-Up	<50	<50	<20	115	<20	<10	<10	681	<10	<50	27	<50
Harpenden-Dn	<50	<50	522	64	682	<10	<10	826	<10	<50	<10	102
East Hyde#1	<50	nsa	nsa	nsa	nsa	nsa	nsa	980	<10	<50	39	143
East Hyde#2	<50	<50	478	93	510	<10	<10	1610	<10	<50	<10	<50
East Hyde#3	<50	<50	449	69	156	<10	<10	2630	<10	<50	25	188
East Hyde-Up	<50	<50	<20	<10	<20	<10	<10	1440	<10	<50	<10	<50
East Hyde-Dn	<50	59	222	29	187	<10	<10	818	<10	<50	<10	<50
Rye Meads#1	<50	110	979	77	323	<10	<10	<20	15	<50	232	136
Rye Meads#2	<50	51	<20	16	365	<10	<10	<20	21	<50	155	<50
Rye Meads#3	<50	874	2100	156	585	<10	<10	5580	55	<50	326	218
Rye Meads-Up	<50	<50	<20	<10	<20	<10	<10	<20	23	<50	36	<50
Rye Meads-Dn	<50	228	<20	46	<20	<10	<10	1990	112	<50	40	164

nsa = not successfully analysed

Table A4 Targeted pharmaceutical concentration data for June 2002 (ng/l)

	Paracetamol	Mefenamic acid	Diclofenac	Propranolol	Dextroprop-oxyphene	Lofepramine	Tamoxifen	Ibuprofen	Erythromycin	Sulfamethoxazole	Trimethoprim	Acetyl-sulfamethoxazole
Blank	<50	<50	<20	<10	<20	<10	<10	342	<10	<50	<10	<50
Corby#1	<50	<50	1640	61	350	<10	<10	2350	20	<50	<10	<50
Corby#2	<50	128	<20	36	346	<10	<10	2710	<10	<50	<10	<50
Corby#3	<50	92	2250	32	298	<10	<10	3090	10	<50	<10	<50
Corby-Up	<50	<50	<20	<10	<20	<10	<10	90	<10	<50	<10	<50
Corby-Dn	<50	105	556	18	<20	<10	<10	988	88	<50	<10	<50
Gt. Billing#1	<50	99	351	110	368	<10	<10	5360	164	<50	169	<50
Gt. Billing#2	<50	74	279	73	78	<10	<10	<20	123	<50	174	<50
Gt. Billing#3	<50	131	230	108	129	<10	<10	5010	31	<50	170	<50
Gt. Billing-Up	<50	<50	<20	<10	<20	<10	<10	<20	<10	<50	<10	<50
Gt. Billing-Dn	<50	<50	<20	25	155	<10	<10	828	331	<50	42	<50
Harpenden#1	<50	<50	543	49	<20	<10	<10	11800	<10	<50	122	<50
Harpenden#2	<50	143	437	28	<20	<10	<10	24400	<10	<50	115	<50
Harpenden#3	<50	119	328	76	<20	<10	<10	7460	<10	<50	51	<50
Harpenden-Up	<50	<50	<20	<10	<20	<10	<10	<20	<10	<50	<10	<50
Harpenden-Dn	<50	<50	<20	39	<20	<10	<10	<20	<10	<50	<10	<50
East Hyde#1	<50	<50	292	33	<20	<10	<10	3090	<10	79	<10	<50
East Hyde#2	<50	<50	219	51	<20	<10	<10	4300	<10	132	<10	<50
East Hyde#3	<50	63	<20	43	232	<10	<10	3400	<10	99	<10	<50
East Hyde-Up	<50	<50	<20	<10	<20	<10	<10	272	<10	<50	<10	<50
East Hyde-Dn	<50	<50	176	16	<20	<10	<10	2120	<10	<50	<10	<50
Rye Meads#1	<50	142	192	49	<20	<10	<10	4960	<10	<50	51	<50
Rye Meads#2	<50	110	119	44	127	<10	<10	<20	<10	<50	28	<50
Rye Meads#3	<50	240	124	59	<20	<10	<10	8040	<10	<50	70	<50
Rye Meads-Up	<50	<50	<20	<10	<20	<10	<10	1560	<10	<50	<10	<50
Rye Meads-Dn	<50	<50	<20	<10	58	<10	<10	1050	<10	<50	<10	<50

nsa = not successfully analysed

Table A5 Targeted pharmaceutical concentration data for July 2002 (ng/l)

	Paracetamol	Mefenamic acid	Diclofenac	Propranolol	Dextroprop-oxyphene	Lofepramine	Tamoxifen	Ibuprofen	Erythromycin	Sulfamethoxazole	Trimethoprim	Acetyl-sulfamethoxazole
Blank	<50	<50	<20	<10	<20	<10	<10	<20	<10	<50	<10	<50
Corby#1	<50	310	309	22	25	<10	<10	746	<10	<50	<10	365
Corby#2	<50	565	315	35	<20	<10	<10	1260	<10	<50	<10	185
Corby#3	<50	1100	938	60	80	<10	23	2270	<10	<50	<10	871
Corby-Up	<50	<50	<20	<10	<20	<10	<10	<20	<10	<50	<10	<50
Corby-Dn	<50	<50	<20	<10	<20	<10	<10	<20	<10	<50	<10	210
Gt. Billing#1	<50	1130	461	177	234	<10	<10	1770	176	<50	267	2240
Gt. Billing#2	<50	801	345	162	110	<10	<10	1710	125	<50	83	690
Gt. Billing#3	<50	720	411	128	262	<10	<10	3780	175	<50	139	1160
Gt. Billing-Up	<50	<50	<20	<10	<20	<10	<10	<20	57	<50	<10	<50
Gt. Billing-Dn	<50	65	91	37	<20	<10	<10	<20	1020	<50	39	239
Harpenden#1	<50	184	609	75	<20	<10	<10	3320	<10	<50	163	<50
Harpenden#2	<50	115	814	143	<20	<10	<10	<20	<10	<50	227	172
Harpenden#3	<50	<50	1460	99	325	<10	<10	2680	<10	<50	130	<50
Harpenden-Up	<50	<50	<20	21	<20	<10	<10	<20	<10	<50	12	<50
Harpenden-Dn	<50	68	<20	26	260	<10	<10	nsa	nsa	nsa	nsa	nsa
East Hyde#1	<50	108	447	93	447	<10	<10	3990	24	<50	<10	<50
East Hyde#2	<50	201	565	123	523	<10	42	6850	38	<50	<10	<50
East Hyde#3	nsa	nsa	nsa	nsa	nsa	nsa	nsa	nsa	nsa	nsa	nsa	nsa
East Hyde-Up	<50	<50	<20	<10	<20	<10	<10	569	<10	<50	10	<50
East Hyde-Dn	<50	150	<20	34	111	<10	<10	716	<10	<50	20	<50
Rye Meads#1	<50	308	505	90	120	<10	<10	7400	120	<50	54	<50
Rye Meads#2	<50	135	401	90	201	<10	<10	3630	1840	<50	86	<50
Rye Meads#3	<50	193	215	118	359	<10	<10	3360	1570	<50	87	<50
Rye Meads-Up	<50	<50	<20	<10	<20	<10	<10	610	<10	<50	17	<50
Rye Meads-Dn	<50	62	182	27	81	<10	<10	<20	511	<50	16	<50

nsa = not successfully analysed

APPENDIX C ENVIRONMENTAL LOAD AND EFFLUENT DISCHARGE FLOW DATA

Table A6 Environmental input data for May 2002 (g/h)

	Paracetamol	Mefenamic acid	Diclofenac	Propranolol	Dextroprop-oxyphene	Lofepramine	Tamoxifen	Ibuprofen	Erythromycin	Sulfamethoxazole	Trimethoprim	Acetyl-sulfamethoxazole
Corby #1	nd	0.12	nd	0.04	0.10	nd	nd	2.38	nd	nd	nd	nd
Corby #2	nd	0.05	nd	0.04	0.13	nd	nd	nd	nd	nd	nd	nd
Corby #3	nd	nd	nd	0.05	0.19	nd	nd	nd	nd	0.09	nd	nd
Gt Billing #1	nd	2.44	3.23	0.46	0.85	nd	nd	7.09	0.05	nd	1.81	0.89
Gt Billing #2	nd	0.98	2.83	0.58	0.75	nd	nd	15.70	0.10	nd	1.20	nd
Gt Billing #3	nd	4.35	7.09	0.80	0.81	nd	nd	82.30	0.35	nd	3.89	nd
Harpenden #1	nd	nsa	nsa	nsa	nsa	nsa	nsa	nsa	nsa	nsa	nsa	nsa
Harpenden #2	nd	0.16	0.72	0.17	nd	nd	nd	1.12	nd	0.02	0.09	0.17
Harpenden #3	nd	0.03	0.14	0.03	0.08	nd	nd	0.19	nd	nd	0.02	nd
East Hyde #1	nd	nsa	nsa	nsa	nsa	nsa	nsa	1.43	nd	nd	0.06	0.21
East Hyde #2	nd	nd	0.97	0.19	1.04	nd	nd	3.27	nd	0.08	nd	nd
East Hyde #3	nd	nd	1.11	0.17	0.38	nd	nd	6.49	nd	nd	0.06	0.46
Rye Meads #1	nd	0.28	2.46	0.19	0.81	nd	nd	nd	0.04	nd	0.58	0.34
Rye Meads #2	nd	0.14	nd	0.04	1.00	nd	nd	nd	0.06	nd	0.42	nd
Rye Meads #3	nsa	nsa	nsa	nsa	nsa	nsa	nsa	nsa	nsa	nsa	nsa	nsa

nsa=not successfully analysed
nd=not detected

Table A7 Environmental input data for June 2002 (g/h)

	Paracetamol	Mefenamic acid	Diclofenac	Propranolol	Dextroprop-oxyphene	Lofepramine	Tamoxifen	Ibuprofen	Erythromycin	Sulfamethoxazole	Trimethoprim	Acetyl-sulfamethoxazole
Corby #1	nd	nd	1.06	0.04	0.23	nd	Nd	1.52	0.01	nd	nd	nd
Corby #2	nd	0.10	nd	0.03	0.27	nd	Nd	2.14	nd	nd	nd	nd
Corby #3	nd	0.01	0.24	nd	0.03	nd	Nd	0.33	nd	nd	nd	nd
Gt Billing #1	nd	0.15	0.54	0.17	0.56	nd	Nd	8.19	0.25	nd	0.26	nd
Gt Billing #2	nd	0.11	0.43	0.11	0.12	nd	Nd	nd	0.19	nd	0.27	nd
Gt Billing #3	nd	0.20	0.35	0.16	0.20	nd	Nd	7.66	0.05	nd	0.26	nd
Harpenden #1	nd	nd	0.33	0.03	nd	nd	Nd	7.19	nd	nd	0.07	nd
Harpenden #2	nd	0.09	0.27	0.02	nd	nd	Nd	15.30	nd	nd	0.07	nd
Harpenden #3	nd	0.07	0.19	0.05	nd	nd	Nd	4.42	nd	nd	0.03	nd
East Hyde #1	nd	nd	0.63	0.07	nd	nd	Nd	6.65	nd	0.17	nd	nd
East Hyde #2	nd	nd	0.51	0.12	nd	nd	Nd	10.00	nd	0.31	nd	nd
East Hyde #3	nd	0.15	nd	0.10	0.54	nd	Nd	7.93	nd	0.23	nd	nd
Rye Meads #1	nd	0.52	0.71	0.18	nd	nd	Nd	18.30	nd	nd	0.19	nd
Rye Meads #2	nd	0.42	0.46	0.17	0.49	nd	Nd	nd	nd	nd	0.11	nd
Rye Meads #3	nd	0.95	0.49	0.23	nd	nd	Nd	31.90	nd	nd	0.28	nd

Table A8 Environmental input data for July 2002 (g/h)

	Paracetamol	Mefenamic acid	Diclofenac	Propranolol	Dextroprop-oxyphene	Lofepramine	Tamoxifen	Ibuprofen	Erythromycin	Sulfamethoxazole	Trimethoprim	Acetyl-sulfamethoxazole
Corby #1	nd	0.66	0.66	0.05	0.05	nd	Nd	1.60	nd	nd	nd	0.78
Corby #2	nd	1.04	0.58	0.06	nd	nd	Nd	2.30	nd	nd	nd	0.34
Corby #3	nd	2.30	1.96	0.13	0.17	nd	0.05	4.75	nd	nd	nd	1.82
Gt Billing #1	nd	4.87	1.99	0.76	1.01	nd	Nd	7.61	0.76	nd	1.15	9.63
Gt Billing #2	nd	3.45	1.49	0.70	0.48	nd	Nd	7.39	0.54	nd	0.36	2.97
Gt Billing #3	nd	3.10	1.77	0.55	1.13	nd	Nd	16.30	0.76	nd	0.60	5.00
Harpenden #1	nd	0.05	0.16	0.02	nd	nd	Nd	0.87	nd	nd	0.04	nd
Harpenden #2	nd	0.07	0.51	0.09	nd	nd	Nd	nd	nd	nd	0.14	0.11
Harpenden #3	nd	nd	0.57	0.04	0.13	nd	Nd	1.04	nd	nd	0.05	nd
East Hyde #1	nd	0.24	1.01	0.21	1.02	nd	Nd	9.06	0.05	nd	nd	nd
East Hyde #2	nd	0.48	1.35	0.29	1.25	nd	0.10	16.40	0.09	nd	nd	nd
East Hyde #3	nsa	nsa	nsa	nsa	nsa	nsa	Nsa	nsa	nsa	nsa	nsa	nsa
Rye Meads #1	nd	1.12	1.84	0.33	0.44	nd	Nd	26.90	0.43	nd	0.20	nd
Rye Meads #2	nd	0.51	1.52	0.34	0.76	nd	Nd	13.80	6.99	nd	0.32	nd
Rye Meads #3	nd	0.77	0.85	0.47	1.43	nd	Nd	13.30	6.22	nd	0.35	nd

APPENDIX D PREVIOUSLY REPORTED OCCURRENCE DATA FOR TARGETED PHARMACEUTICALS

Table A9 Previously reported occurrence data for targeted pharmaceuticals

Pharmaceutical	Concentration detected (ng L^{-1})	Value	Waters	LOD (ng L^{-1})	Reference
Paracetamol	nd, nd, 6000	Median, 90percentile,max	Sewage effluent	500	Ternes (1998)
	nd		Surface waters	150	Ternes (1998)
	nd			-	Ahrer *et al.* (2001)
Mefenamic acid	nd, 10, 11, 14		Surface waters	0.2	Ahrer *et al.* (2001)
Diclofenac	1000		Sewage effluent	-	Stan and Heberer (1997)
	nd-1590		Sewage effluent	50	Stumpf *et al.* (1996)
	135,760		Sewage effluent	-	Heberer *et al.* (1998)
	810, 1600, 2100	median, 90percentile,max	Sewage effluent	50	Ternes (1998)
	310-930		Sewage effluent	1	Buser *et al.* (1998a)
	130, 930	median, max	Sewage effluent	50	Stumpf *et al.* (1999)
	381		Sewage effluent	5	La Farre *et al.* (2001)
	100-700		Sewage effluent	0.9-3.6	Ollers *et al.* (2001)
	90		Surface waters	-	Stan and Heberer (1997)
	nd-489		Surface waters	5	Stumpf *et al.* (1996)
	nd-960		Surface waters	1	Heberer *et al.* (1998)
	150, 800, 1200	median, 90percentile,max	Surface waters	10	Ternes (1998)
	200, 500	median, max	Surface waters	-	Stumpf *et al.* (1998)
	20, 450	median, max	Surface waters	10	Stumpf *et al.* (1999)
	nd-370		Surface waters	1	Buser *et al.* (1998a)
	16, 20, 20, 36		Surface waters	0.3	Ahrer *et al.* (2001)
	51, 56, 147, 484		Surface waters	5	Farre *et al.* (2001)
	nd-150		Surface waters	0.9-3.6	Ollers *et al.* (2001)
Propranolol	nd-290		Sewage effluent	25	Hirsch *et al.* (1996)
	730, 1300, 290	median, 90percentile,max	Sewage effluent	25	Ternes (1998)
	nd-98		Surface waters	3	Hirsch *et al.* (1996)
	12, 440, 590	median, 90percentile,max	Surface waters	5	Ternes (1998)

Pharmaceutical	Concentration detected (ng L^{-1})	Value	Waters	LOD (ng L^{-1})	Reference
Erythromycin	2500, 5100, 6000	median, 90percentile, max	Sewage effluent	20	Hirsch *et al.* (1999)
	150, 630, 1700	median, 90percentile, max	Surface waters	20	Hirsch *et al.* (1999)
	100, 170	median, max	Surface waters	50	Kolpin *et al.* (2002)
Sulfamethoxazole	400, 900, 2000	median, 90percentile, max	Sewage effluent	20	Hirsch *et al.* (1999)
	30,140, 480	median, 90percentile, max	Surface waters	20	Hirsch *et al.* (1999)
	~1000		Surface waters	-	Watts *et al.* (1983)
	150, 1900	median, max	Surface waters	50	Kolpin *et al.* (2002)
	66, 520	median, max	Surface waters	23	Kolpin *et al.* (2002)
Trimethoprim	320, 620, 660	median, 90percentile, max	Sewage effluent	20	Hirsch *et al.* (1999)
	nd, 90, 200	median, 90percentile, max	Surface waters	20	Hirsch *et al.* (1999)
	150, 710	median, max	Surface waters	30	Kolpin *et al.* (2002)
	13, 300	median, max	Surface waters	14	Kolpin *et al.* (2002)
Ibuprofen	3350		Sewage effluent	-	Stan and Heberer (1997)
	nd-3350		Sewage effluent	50	Stumpf *et al.* (1996)
	10		Sewage effluent	-	Heberer *et al.* (1998)
	370, 1200, 3400	median, 90percentile, max	Sewage effluent	50	Ternes (1998)
	600, 3000	median, max	Sewage effluent	50	Stumpf *et al.* (1999)
	+		Sewage effluent	-	Rogers *et al.* (1986)
	868		Sewage effluent	43	La Farre *et al.* (2001)
	5-1500		Sewage effluent	0.9-3.6	Ollers *et al.* (2001)
	140		Surface waters	-	Stan and Heberer (1997)
	nd-139		Surface waters	5	Stumpf *et al.* (1996)
	nd-280		Surface waters	5	Heberer *et al.* (1998)
	70, 280, 530	median, 90percentile, max	Surface waters	10	Ternes (1998)
	190	max	Surface waters	10	Stumpf *et al.* (1999)
	nd		Surface waters	0.6	Ahrer *et al.* (2001)
	nd, 130, 468, 1500		Surface waters	43	La Farre *et al.* (2001)
	nd-80		Surface waters	0.9-3.6	Ollers *et al.* (2001)
	200, 1000		Surface waters	18	Kolpin *et al.* (2002)