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4 5 6 **The Removal of Pharmaceuticals During Wastewater Treatment: Can it be** 7 **Predicted Accurately?**

8
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15 16 17 **Abstract**

18 The presence of active pharmaceutical ingredients (APIs) in the environment is of growing concern and
19 effluents from wastewater treatment works (WwTWs) are one of the major sources. This research
20 combines the outputs of a multimillion pound UK programme of work to evaluate the fate of APIs in
21 the wastewater treatment process. A combination of analysis of measured data and modelling has been
22 applied to 18 APIs, representing a wide range of medicinal application and physico-chemical
23 characteristics. Some isomers (for atorvastatin) and metabolites (for sertraline, carbamazepine and
24 erythromycin) were also included. High variability was observed between removal rates for individual
25 APIs between WwTW, which after statistical analysis could not be explained by the nominal WwTW
26 process (e.g. activated sludge or trickling filter). Nor was there a clear relationship between API removal
27 and physico-chemical parameters such as pKa, charge or log Kow. A publically available sewage
28 process model, SimpleTreat 4.0 which has been rigorously validated and is now being used for exposure
29 assessment with REACH legislation for organic chemicals and within the Biocidal Products Regulation
30 by the European Medicines Agency for APIs, was used to estimate removal rates with which to compare
31 with measured data. SimpleTreat provided estimates of removal rates within +/- 30% of observed values
32 for the majority of the APIs measured, with the use of readily available WwTW specific parameters
33 such as flow, total suspended solids and BOD data. The data and correlations provided in this study
34 provide support for any future considerations regarding the management of API discharge to the aquatic
35 environment.

36

37 **Key words:** pharmaceuticals; modelling; removal efficiency; wastewater treatment; activated sludge;
38 trickling filter

39

40 **1. Introduction**

41

42 The use of active pharmaceutical ingredients (APIs) is increasing throughout the world owing to the
43 widening array of treatments offered, increasing affordability and availability (particularly over the
44 counter sales) combined with a growing population, of which a greater proportion are increasing in age
45 (Jelic et al., 2011). The main source of occurrence of APIs in the environment is considered to be from
46 human use of pharmaceuticals, the majority of which are used, excreted and discharged into the
47 wastewater system (Gardner et al., 2012; Melvin et al., 2016). Owing to the complexity and cost of
48 monitoring micropollutants in environmental matrices and in some cases, the lack of legislation to drive
49 regulation, the availability of fate data can be limited within the public domain. Consequently, there is
50 increasing scrutiny on the levels of APIs entering and being discharged from WwTW (Comber et al.,
51 2018).

52

53 Furthermore, the extent to which of APIs are removed during wastewater treatment can be limited. API
54 removal rates are dependent on concentrations entering the works, the API's chemical structure,
55 solubility, charge, potentially toxicity and the existence of viable bacteria with the requisite
56 catabolic/biodegradative capabilities. It should be noted, however, that specific mechanisms of removal
57 are highly complex and in many cases the contribution of individual factors are poorly understood.
58 Previous studies have demonstrated that API removal efficiency can vary between WwTW treatment
59 technologies and even within a given works. Consequently, the quality of WwTW effluent is currently
60 of interest to the pharmaceutical industry seeking better risk assessments, regulators considering
61 legislation and the water industry in terms of the risks associated with their effluents entering the aquatic
62 environment (Gardner et al., 2013).

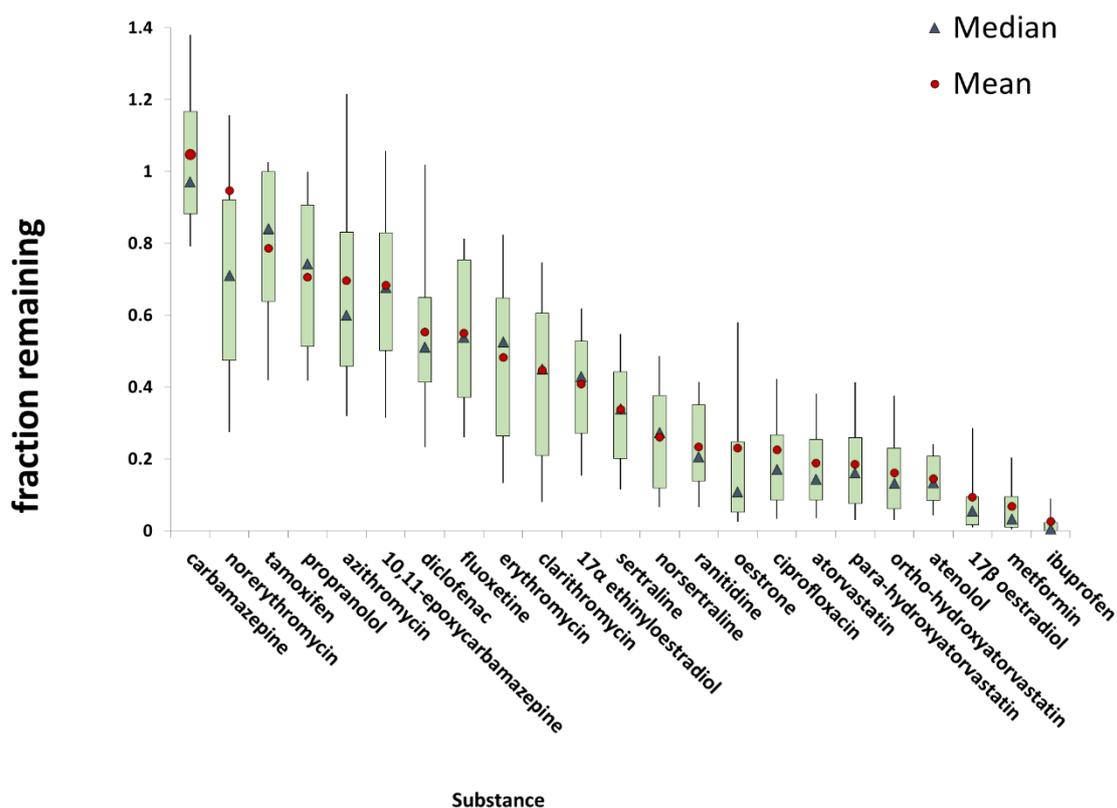
63

64 The range of concentrations found for pharmaceuticals studied in the UK is similar to that observed in
65 continental Europe as well as in the USA (Ashton et al., 2004 and Hope et al., 2012). Most often
66 published data in the literature shows API concentration of less than 100 ng/l in the surface and
67 groundwater, and below 50 ng/l in treated drinking water (WHO, 2011). This is considerably below the
68 human therapeutic dose and any acute toxic limit values for the vast majority of APIs. There is,
69 however, concern regarding potential toxicity and impacts on antimicrobial resistance to the
70 environment when exposed to mixture of APIs and other chemicals and non-chemical stressors (Bound
71 et al., 2006). Many countries have initiated various monitoring programs to investigate the exposure of
72 APIs and to get a better understanding of the pathways and emission sources (Falås, 2012). The

73 Chemical Investigation Program (CIP) in the UK is a large ongoing monitoring programme for priority
74 chemicals, including emerging contaminants such as APIs in WwTW influent, intermediate processes
75 and effluent as well as their impacts on concentrations in receiving waters (Gardner et al., 2013). The
76 first phase of the CIP (known as CIP1) was an extensive project that ran from 2012-2015 with the
77 primary aim to investigate the fate of trace substances in influent, effluent and within the WwTW
78 process. The result from this extensive investigation has been reported previously (Gardner et al., 2012;
79 Gardner et al., 2013; Jones et al., 2013, Comber et al., 2014 and Comber et al., 2018). With respect to
80 process data, removal of 11 commonly detected APIs at 25 WwTWs (on 26 occasions) were reported
81 for influent, primary, secondary and where present, tertiary treatment effluents (Comber et al., 2018).
82 The £140 million investment in second phase of the CIP (known as CIP2) builds on the outputs from
83 CIP1 by extending the range to include the monitoring of a larger number of analytes, and by including
84 river sampling upstream/downstream of WwTW discharges to measure impact on receiving waters. In
85 total, over 60,000 samples have been taken, resulting in over 3 million determinations. CIP2 includes
86 data for 23 APIs (including some metabolites and isomers) for influent and effluent at 44 WwTW,
87 sampled on 20 occasions (Figure 1; Comber et al., 2018). Furthermore, CIP1 and CIP2 include sanitary
88 parameters (total suspended solids (TSS), biochemical oxygen demand (BOD), chemical oxygen
89 demand (COD), pH, dissolved and total organic carbon (DC, TOC), nitrate and phosphate (Gardner et
90 al., 2013).

91
92 Household wastewater quality will vary depending on such things as behaviour and lifestyle, with many
93 sewerage systems also containing stormwater which may also contain APIs (Munro et al., 2019). The
94 sanitary determinands are measured routinely as they are often listed on permits to discharge effluents
95 to receiving waters. The concentrations of these 'sanitary' parameters of BOD, COD, TSS, ammonia
96 define the character of the effluent and provide an indication of works performance based on
97 concentrations (lower concentrations suggest higher works efficiency). The presence of APIs is not
98 measured on a routine basis for most WwTWs owing to cost and lack of legislative drivers.
99 Furthermore, modern risk assessments and chemical management are increasingly reliant on models to
100 predict the fate of chemicals through pathways and fate in the environment. Models often provide
101 predictions of treatment efficiency and effluent concentrations which may then be used in tiered risk
102 assessments and environmental regulation. There are a number of software tools available which to
103 various degrees can model the removal of chemicals through the wastewater treatment processes. Over
104 20 computer programs developed by academia, environmental agencies and commercial sources have
105 been recognised for predicting fate in WwTW (Crechem et al., 2006).

106



107
108

109 **Figure 1: Summary from the CIP 2 program for API median fraction remaining from 44 WwTW**
110 **sampled on 20 occasions (Comber, 2018). Note the abbreviations used here are used**
111 **throughout this paper.**

112
113

114 SimpleTreat is a fundamental tool used on an official EU level for predicting exposure in the
115 environmental risk assessment. Among others, it is the formally recommended model for the essential
116 assessment for chemical covered in the EU directive of Registration, Evaluation, Authorisation and
117 restriction of Chemicals (REACH), as well as for the market authorisation of new pharmaceuticals
118 regulated by European Medicines Agency (EMA) (Franco et al., 2013; EMA et al., 2006). The tool is
119 straightforward to use and requires the input of a limited number of chemical properties parameters:
120 molecular weight (MW), vapour pressure, water solubility, n-Octanol/Water Partition Coefficient (K_{ow})
121 as well as the results from biodegradability assessments, as defined by the Organisation for Economic
122 Co-operation and Development (OECD) guidelines (RIVM, 2013). For basic and acidic compounds,
123 the acid dissociation constant, pK_a is also required to take account the state of ionization of polar
124 molecules in the wastewater (Franco et al., 2013). However, it should be noted that many APIs have
125 more than 1 pK_a value (although rarely do both occur within expected environmental pH conditions)
126 which cannot be accommodated within the current model and that for ionisable substances such as APIs

127 logD incorporating the ionization potential of the chemical within the partitioning calculations would
128 be potentially an improvement. However, previous studies have suggested that SimpleTreat predicts
129 total removal to an accuracy of $\pm 5\%$ compared with the measured values for the majority of routine
130 wastewater determinands which included non-polar persistent organic pollutants but also ionisable
131 compounds such as triclosan ($pK_a=8$) (Crechem et al., 2006).

132

133 Data from CIP therefore offers the opportunity for a detailed examination of the variability of API
134 removal efficiency in light of works type and performance. Specifically, this study utilizes CIP2
135 program outputs, reporting the presence of 23 APIs (including five metabolites of parent APIs) in
136 influent and effluents, combined with CIP1 data on efficiency of 11 API removal from WwTW
137 secondary process, split into Activated Sludge Plants (ASP) and Trickling Filter works (TF) processes.
138 These data, combined with the use of SimpleTreat modelling, has made possible a critical evaluation of
139 removal efficiency at WwTWs, as well as a comparison of monitoring data with default biodegradation
140 constants provided in the literature and the accuracy of modelling using the accepted risk assessment
141 models. By gaining a better understanding of the key factors controlling the removal of APIs during
142 wastewater treatment combined with an assessment of the effectiveness of modelling will inform future,
143 focused investments as well as more accurate and prioritized targeted risk assessments (Gardner et al.,
144 2013).

145

146 **2. Materials and methods**

147

148 **2.1 API selection**

149 The selection of chemicals for CIP1 (Gardner et al., 2012) and CIP2 (Comber et al., 2018) are discussed
150 in detail elsewhere. Briefly, APIs were selected based on a risk assessment approach by comparing the
151 estimated environmental concentrations of nearly 150 pharmaceuticals (screened on usage and
152 perceived hazard from a list of approximately thousand candidate substances) with data for their
153 respective effect concentrations on a variety of receptor organisms in the aquatic environment (UKWIR,
154 2014). For the purposes of CIP2, the list was further refined by selection of substances that were likely
155 to occur in effluents after treatment and that were considered to have the greatest potential as candidates
156 for inclusion on the WFD priority substance list (EU, 2011). This resulted in the list of substances
157 ($n=13$) tabulated in Table A1 of the Electronic Supplementary Information (ESI).

158

159

160 **2.2 Sampling strategy**

161 A set number of WwTW were selected for the CIP1 and 2 programs with the justification for which are
162 described elsewhere (Comber et al., 2018), being based on a combination of low dilution in the receiving
163 water, representative types of works (roughly evenly split between ASP and TF), geographic location

164 (covering England, Scotland and Wales), and size (serving populations between 2,000 and 1.6 million).
165 Owing to the varying hydraulic retention times (HRT) for individual works, which are often not
166 accurately known and can be measured in days (Ejhed et al., 2016) meant it was not practical to try and
167 match collection of influent and effluent related to the HRT of the selected WwTW. However, given
168 the mixing that occurs within a given WwTW, combined with sludge returns, inputs from storm tanks
169 and combined sewers it was decided that sample replication based on numerous sampling occasions
170 would derive statistically robust conclusions regarding WwTW performance.

171

172 Data used for this research were (Table A2 in the ESI):

- 173 • **CIP1 program:** 25 WwTW data for influent, after primary settlement and final effluent after
174 secondary and if available tertiary process for 11 APIs. Two samples of each process (spaced
175 more than 4h apart to provide a degree of replication) were taken on between 10 and 15
176 occasions between 2011 and 2013. In this part of the programme two samples.
- 177 • **CIP2 program:** Single samples for 18 APIs and 5 metabolites were spot sampled on 20
178 occasions at 44 WwTWs in the influent and effluent (not intermediate process stages, unlike
179 CIP1) over a two-year period between 2015 and 2017.

180

181 A summary of the CIP sampling strategies is provided in Table A3. Grab samples at various time
182 intervals were used for the collection of aqueous samples. To assess variability within the day, in the
183 CIP1 program, at least one duplicate sample was taking during the same day with a minimum of four-
184 hour period between the sampling. Composite samples were not considered owing to concerns
185 regarding sample stability. A minimum of 15% of the samples were taken at non-working hours
186 (evenings and weekends). The sampling schedule was conducted according to stratified random
187 strategy, indicating that the sampling events are spaced approximately evenly during the year at monthly
188 intervals, but are randomly placed at each interval in the month.

189

190 **2.2 Laboratory analysis**

191 Samples were collected in stainless steel samplers, stored in glass container and transported at 4° C to
192 the analysis laboratories. The samples were stored a maximum of 5 days prior to analysis. The samples
193 for measuring the endocrine disrupting chemicals were preserved by adding 30% hydrochloric acid and
194 copper nitrate (Gardner et al., 2012). The quality assurance/quality control procedures were conducted
195 for experiment preparation, sample collection, sample pre-treatment and analysis for both laboratory
196 tests and field sampling. All the samples were analysed by any of four approved laboratories with
197 ISO17025 accreditation and showed to be able to achieve the analytical performance and quality
198 assurance laid down in the specification (see A1 of ESI). The pharmaceuticals were analysed by LC-
199 MS or GS-MS. The analytical error of all the pharmaceutical measured were considered to be $\pm 50\%$

200 (25% random error and 25% systematic error) or the Limit of Detection (LOD) if this value was larger
201 (Table A4). In accordance to EU regulations, if analysed concentrations were below LOD then the value
202 for LOD was halved to generate a result (EC, 2009). There were no major inter-laboratory error and
203 inter-regional variation, which would otherwise indicate if there was a bias in the procedure of sample
204 handling and analysis method. Further details of the proficiency testing can be found in the supporting
205 information (A1).

206
207

208 **2.3 Data handling and analysis**

209 The data handling and the statistical analysis were conducted with either Microsoft Excel (2016) or
210 IBM SPSS Statistics software (version 20). This study also made use of the tool SimpleTreat (version
211 4.0) for modeling fate in WwTW, application developed by the National Institute for Public Health and
212 the Environment (RIVM). EPI Suite (version 4.11) was used for retrieving some of the non-published
213 physico-chemical data, available from the US EPA (US EPA, 2016).

214

215 In the data handling, the replicates were averaged, and this value was then used for further statistical
216 calculations. Mean, maximum, minimum and percentiles were calculated from the daily average.
217 Fraction remain was calculated from the influent concentration as a fraction of the various stages of the
218 process. The removal was calculated as percentage from the concentration based on the effluent
219 concentration subtracted from the influent then divided by the influent, expressed as a percentage.

220

221

222 **2.4 SimpleTreat 4.0 (RIVM) emission model**

223 The model SimpleTreat 4.0 (RIVM, 2013) was used for estimating the percentage removal in the
224 WwTW for a number of the APIs in the CIP program. SimpleTreat is an established, readily available
225 free to download model often used within regulatory risk assessment frameworks to estimate predicted
226 environmental concentrations for ASP only (not TF WwTW). Input parameters include noting if the
227 chemical is potentially ionisable. Given APIs are often charged, the model accommodates by
228 calculating the proportion of the APIs that is neutral at pH 7.0 and this determines the equation used to
229 calculate the default organic carbon:water partition coefficient (K_{oc}). Molecular weight, K_{ow} , vapour
230 pressure and solubility are other required input variables to the model. Henry's Law Coefficient (H),
231 Organic carbon partition coefficient (K_{oc}), Organic carbon partition coefficient for raw and settled
232 sewage as well as for activated sludge (K_p) can be added as an adjustable input or the model creates a
233 default value.

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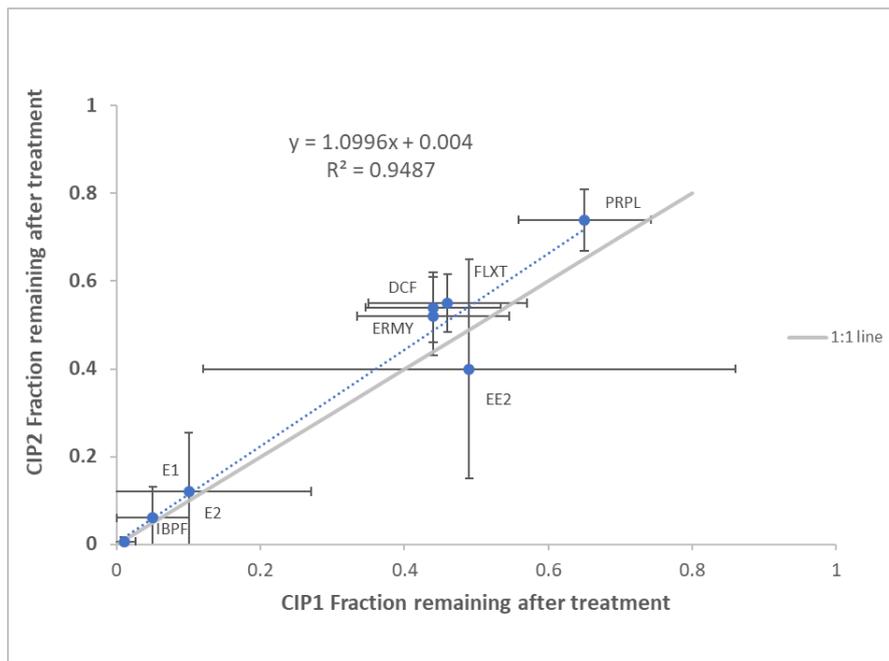
237 3. Results and discussion

238

239 3.1 Comparison of API data between CIP1 and CIP2

240 Previous data analysis has shown that the CIP data for APIs in WwTW effluents corresponded well
 241 with those reported elsewhere for UK effluents (Comber et al., 2018). To investigate the quality of the
 242 data, and to examine if there had been any systematic shifts in API effluent concentrations between the
 243 CIP1 the median fraction remaining from both the CIP1 and CIP2 were compared for APIs that were
 244 studied in both programs (E1, E2, EE2, IBPF, DCF, FLXT, PRPL and ERM). Taking account of the
 245 significant variability of removal efficiencies for individual APIs, good agreement was obtained
 246 between the fraction remaining in effluent of those APIs common to both CIP1 and CIP2 (Figure 2).
 247 These results provided confidence in the analytical data obtained between the two separate programmes
 248 (using different analytical laboratories in some cases) and that there were no gross changes or variations
 249 between the WwTWs selected for sampling or impacts on removal rates associated with the sampling
 250 periods (e.g. seasonality) or methodologies used.

251



252

253 **Figure 2: Median fraction remaining after treatment comparing CIP1 (25 WwTW) and CIP2**
 254 **(44 WwTW) programs. Solid line is the 1:1 line and the dotted line is fitted linear trend**
 255 **line and error bars are 95% confidence intervals. P value = 4.3×10^{-5} .**
 256

257

258 3.2 Physico-chemical characteristics potentially impacting the API removal in WwTW

259 APIs can be characterised broadly in terms of their charge and their ability to accept or donate protons;
 260 with carboxylic acid APIs acting as acids and amine groups acting as bases under environmentally
 261 relevant pH conditions. The degree of dissociation (reported as pKa) is crucial when the ambient pH of

262 the WwTW effluent is close to the value of the pKa of the API. In some cases, where there are carboxylic
263 acid and amine groups present on the same compound, depending on the ambient pH, the molecule may
264 be rendered charge neutral depending on the size of the molecule and spacing between ionisable sites.
265 The pH of sewage effluent is circumneutral and so for assumption of charge and calculation of LogD,
266 a pH of 7 was assumed (Gardner et al., 2012)

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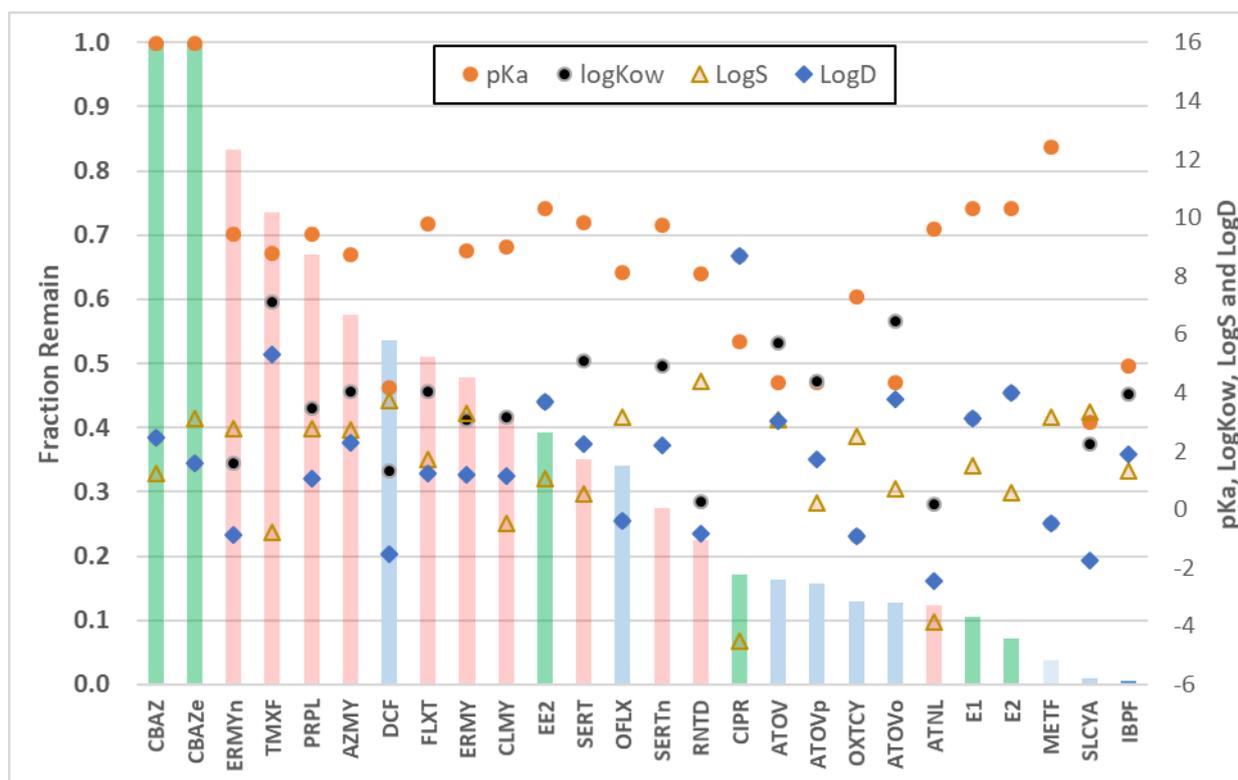
268 This is a particularly important physico-chemical characteristic as the charge on the molecule will in
269 some degree impact on its affinity for particulate matter, complexation/association with organic matter
270 and other counter-ions and affect solubility and partitioning and hence bioavailability to microorganisms
271 (Greenhagen et al., 2014; Tappin et al., 2016). These are all crucial parameters in determining the
272 removal rate during wastewater treatment. As a general rule, biological uptake is mostly associated with
273 neutral molecules, particularly if they are also hydrophobic (Haitzer et al., 1999). Positively charged
274 compounds will show a tendency to sorb strongly to clay minerals and solids which have a
275 predominantly negative charge. Negatively charged compounds therefore generally have lower affinity
276 for sorption and uptake, although for complex molecules with multi-protic sites this is somewhat of an
277 over simplification (Bendz et al., 2005; Katsoyiannis et al., 2007).

278

279 There was no clear relationship between removal rates and groups of acid, basic and neutral APIs
280 (Figure 3). Poor DCF removal may be a result of the combination of chemical structure, specifically
281 the presence of halogen functional groups (Verlicchi, 2012) and its hydrophilic nature ($\log K_{ow} 1.5$)
282 reducing bioavailability and increasing persistence. As observed previously (Tappin et al., 2016) the
283 data show that it is not possible to accurately predict removal of the selected APIs during wastewater
284 treatment using charge, Log Kow, solubility (LogS), pKa, or LogD which is Kow corrected for the
285 charge on any given molecule for a specific pH (7.0 assumed in this case). The only conclusion which
286 may be drawn is that the majority of the basic APIs show poorer removal, possibly owing to reduced
287 bioavailability of the positively charge molecule (Yamamoto et al., 2009).

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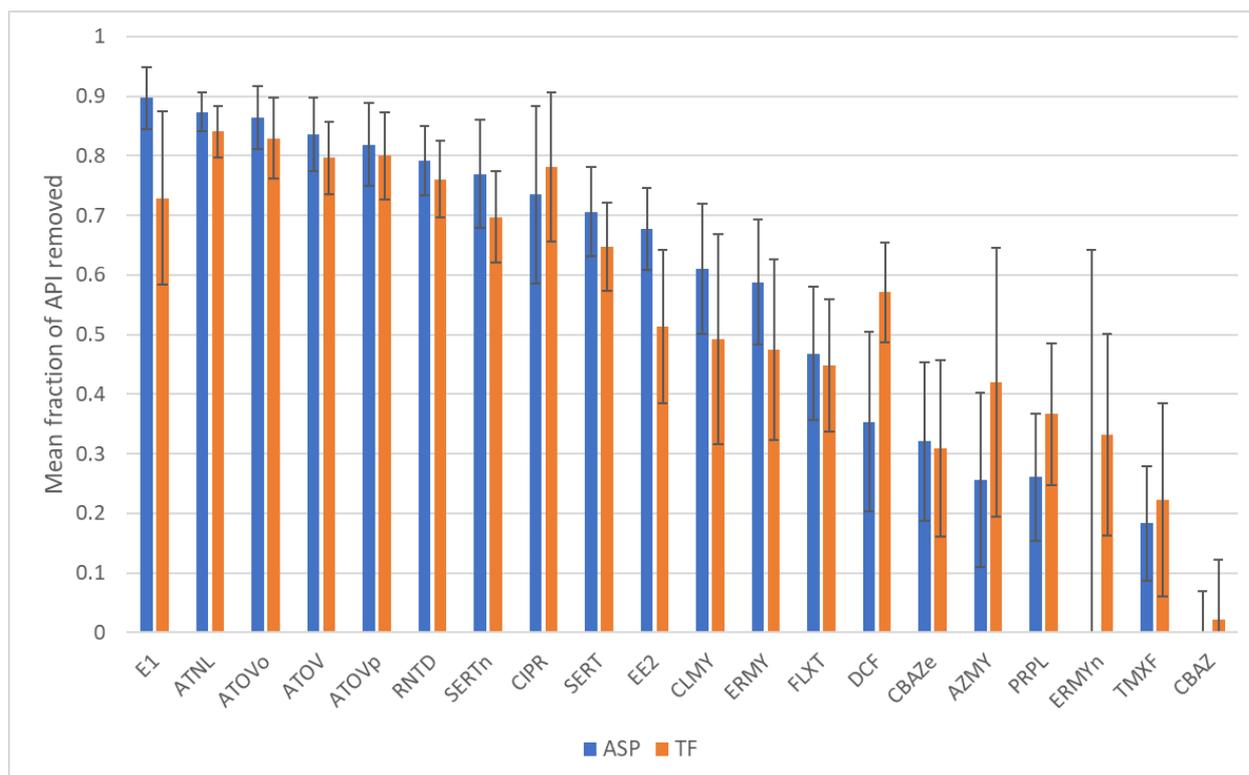


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Figure 3: The order of total fraction remaining (median) for APIs as function of pKa, LogS, LogD and logKow for the CIP2 and CIP1 APIs not covered by CIP2 (blue colour for acidic compounds; red for basic; green for neutral or zwitterions)

298 3.3 Variation in efficiency of API removal by different works technology (ASP vs TF)

299 Major investments have been made across the UK to upgrade WwTWs from TF to ASP as they are
300 generally more efficient and reliable in removing BOD and suspended solids, as required by permits to
301 discharge to receiving waters (Water UK, 2018). The CIP API data were therefore examined to
302 determine if there were any significant differences in treatment efficiency between TF and ASP (Figure
303 4, Table A5). The CIP1 data contained 9 TF and 13 ASP WwTWs and the CIP2 data compared 15 TF
304 and 18 ASP WwTWs APIs percentage removal. For the CIP1 data (Table A5) the secondary process
305 was separated out (i.e. not total percentage removal) to provide a more accurate comparison with the
306 CIP2 data.



307

308 **Figure 4: CIP2 mean fractional removal rates for ASP and TF WwTW with 95%ile error bars.**

309

310 Data from both CIP1 and CIP2 data, indicate that although in many cases the mean performance for
 311 API removal at ASP works is better than that for TF, which has been reported elsewhere for a different
 312 set of chemicals (Falås, 2012) however, for none of the 23 compounds measured was the difference
 313 statistically significant. What is also noteworthy is the fact that for APIs where removal may be
 314 considered good (e.g. greater than 70%) then variance between works (ASP and TF) are generally lower
 315 than where removal rates are poorer. These data therefore indicate that the type of technology is less
 316 critical for the overall removal efficiency of APIs than WwTW specific processes and characteristics
 317 such as hydraulic retention times, sludge retention times, sludge return management and
 318 biodegradability of the API itself. Another potentially complicating factor is API conjugation.
 319 Metabolic transformations include glucuronidation, sulphation, acetylation of the parent API to increase
 320 solubility and aid excretion. Conjugated metabolites can undergo retransformation back to the parent
 321 form following cleavage of the conjugated moiety which has been hypothesised to occur within WwTW
 322 for estrogens, carbamazepine and diclofenac which are included in the CIP suite of determinands.
 323 Although the potential significance of deconjugation during wastewater treatment has been
 324 acknowledged, detailed empirical evidence is still scarce, being limited to estrogens, because of
 325 analytical challenges (Polesel et al., 2016; Brown and Wong, 2018). As a consequence discussion
 326 relating to absolute removal rates have to be viewed in this light, although comparisons between
 327 different processes is more of a relative comparison.

328

329 **3.4 The relationship between sanitary determinands and pharmaceutical removal**

330 The benefit of gathering concentration data regarding the sanitary determinands (AMON, BOD, COD
331 and TSS) in combination with that for APIs, allows the ability to seek correlations between metrics
332 which indicate the overall performance of a WwTW with respect to API removal. If such relationships
333 can be established, then there are multiple benefits:

- 334 • Majority of the WwTW routinely measure the sanitary determinant so this data is already
335 available. The ability to predict a WwTW's potential API removal efficiency based on a cheap
336 and readily available sanitary determinands analysis data, without any issues possibly associated
337 with time delays with the analysis method and sampling strategy for APIs (Roberts, 2006).
- 338 • By extension, the capability of being able to apply the outputs into available models (like for
339 example SimpleTreat) predicting API removal based on input variables associated with TSS,
340 AMON, BOD etc.
- 341 • Ultimately, allow the potential for optimising WwTW operations (through for example,
342 hydraulic retention time, increased biological treatment, use of coagulants etc.) to achieve the
343 desired API removal efficiency without additional expenditure on tertiary treatment.

344

345 Many UK WwTW receive a combination of both crude sewage from domestic and industrial sources
346 and surface water runoff. Runoff contributes flow but is unlikely to contain APIs or significant BOD.
347 Industrial discharges are often rich in BOD but their flow in most cases is insignificant compared with
348 that from domestic sources. Flows and loads of down the drain chemicals such as APIs to WwTW vary
349 within and between days and seasons; furthermore, the proportion of loads from industrial and domestic
350 flows may also vary. Consequently, WwTW capacities are generally described as population
351 equivalents (PE) which is the normalised unit per capita loading, representing the ratio of the sum of
352 the pollution load produced during 24 hours by industrial facilities and services to the individual
353 pollution load in household sewage produced by one person in the same time. Given that population
354 and consented flow data were available for all WwTW (Table A6), an analysis of normalised data was
355 carried out by multiplying the individual WwTW flow (measured where available, consented otherwise)
356 and then dividing by the PE, thereby taking account of individual WwTW demographics.

357

358 Any observed correlation between API removal and sanitary determinands is likely to reflect a
359 combination of works efficiency and API physico-chemical characteristics (Table A7). For example, a
360 WwTW with high TSS removal suggests efficient settlement and sludge separation and so APIs with a
361 high tendency to sorb to solids (i.e. high log K_{oc}); alternatively, a high BOD and API removal
362 correlation suggests the API is susceptible to biodegradation or co-metabolism. Correlations do not
363 necessarily mean a cause and effect relationship, so there may be other factors influencing the
364 correlation. Furthermore, this would suggest that the process parameters (PE and flow) probably do not

365 account as the only factors for the observed variation in API removal between various plants. PE and
366 measured/consented flow is an indication of the burden of the plant due to for example the population
367 size and the industries present in the area; but these are static values and do not take into account the
368 variability within the year. However, these variations can be seen when looking at the correlation
369 between the measured sanitary determinands and the APIs removal in the WwTW. It was found that
370 with or without normalisation of the data the correlation between sanitary determinands and API analysis
371 concentration was not sufficiently good to allow useably accurate predictions of API concentrations
372 from sanitary determinand surrogate data (Table A6). There were also no differences seen when
373 separating out data from TF and ASP technology processes.

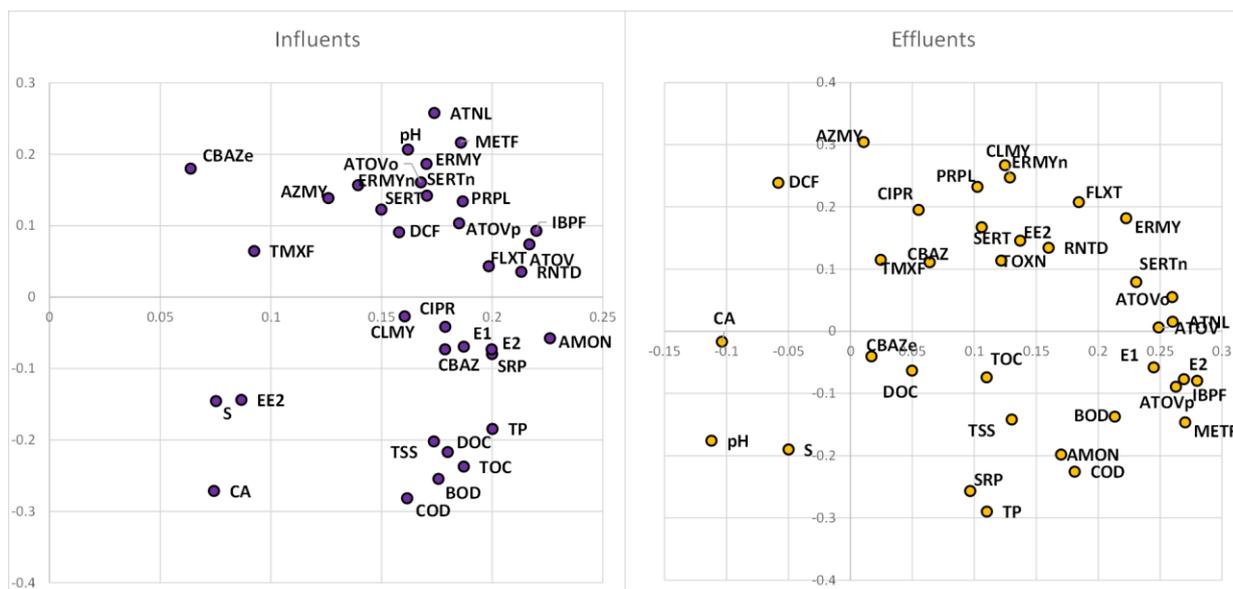
374

375 To move beyond simple correlations a Principle Component Analysis (PCA) was performed on the
376 influent and effluent CIP2 data, where the proximity of determinands on the charts would suggest a
377 degree of relationship/co-variance. (Figure 5). The data presented, however, largely supports that
378 generated from the correlation analysis (Table A7). For the influents it can be seen that the sanitary
379 determinands (BOD, COD, TSS, TP and DOC/TOC) are grouped together showing the expected strong
380 signal from domestic wastewater which would be likely to contain similar ratios owing to a common
381 source. The APIs do not relate to the sanitary determinands, most likely owing to their inputs relating
382 to prescription and/or seasonal use. For the effluents a slightly different pattern is observed. The sanitary
383 determinands are more separated, likely to be a result of varying treatment (i.e. a potential bias for TSS
384 removal during primary treatment and BOD by secondary treatment). The APIs reflect this with certain
385 APIs (e.g. E1, E2, IBPF, ATOV_p, METF) more associated with their biodegradability and so align with
386 BOD. In other words, high performing works reducing BOD to very low levels, are likely to also reduce
387 the concentrations of more easily degradable APIs. Overall, the lack of clear and distinct groupings
388 reflects the complexity of removal mechanisms related to this class of compounds as well as the
389 potential influence of API de-conjugation during the sewage treatment process (Brown and Wong,
390 2018). Overall, it may be concluded that although there appears loose associations for certain physico-
391 chemical parameters for certain classes of APIs, the biodegradation and partitioning processes with
392 sewage treatment are highly complex and likely to include other interactions such as electrostatic,
393 complexation and cation-bridging mechanisms which would be likely to interact with APIs and thus
394 influence their sorption behaviour and bioavailability (Toll, 2001). However, given APIs often exhibit
395 low logK_{ow} (<4.0) and high solubility, their interaction with the particulate phase during primary
396 treatment would be expected to be less significant than potential biodegradation loss mechanisms during
397 secondary treatment (Table A7).

398

399

400



401

402 **Figure 5: Principal component analysis (axes unlabelled as simply pca1 and pca2) of the influents**
 403 **and effluents for CIP2. CA=calcium; S=sulphur; TP=total phosphorus; SRP=soluble**
 404 **reactive phosphorus; TOXN=total oxidisable nitrogen.**

405

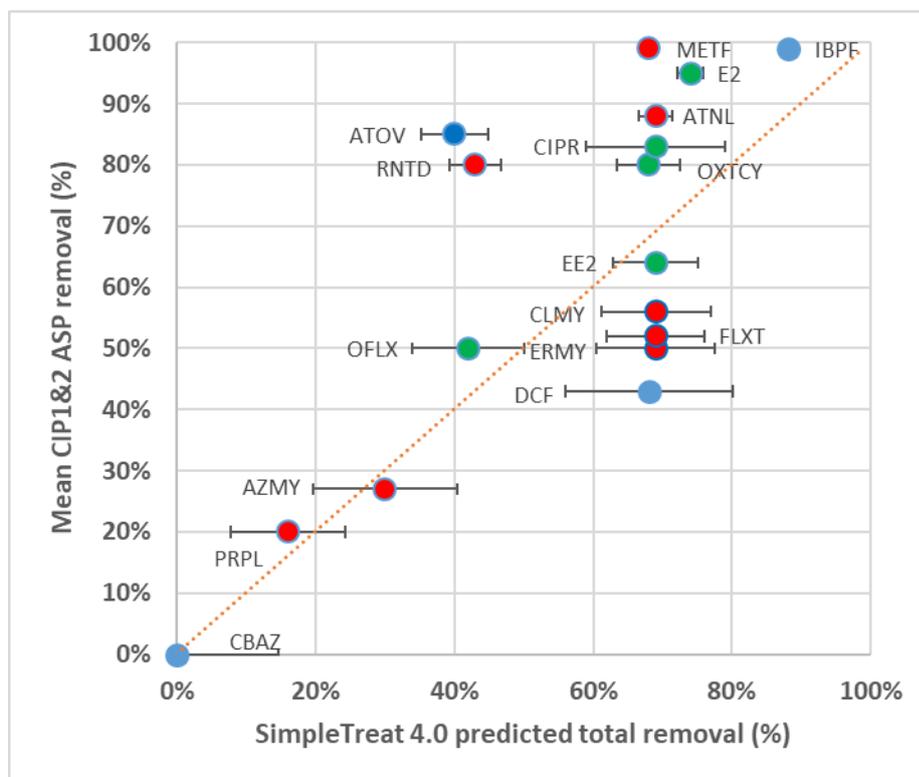
406

407 3.6 SimpleTreat 4.0 (RIVM) emission model

408 The observed variability in estimating API effluent concentrations from sanitary determinands leads
 409 onto the question of whether established models used within the risk assessment process can provide a
 410 better outcome. The freely available model SimpleTreat 4.0 was used for estimating the percentage
 411 removal in the WwTW for a number of the APIs in the CIP program and predictions compared with
 412 observed data from the CIP datasets. The ASP process can be left default or site-specific flow, sewage
 413 solids and BOD can be inputted along with loading rate and pH. Surface aeration (default) or bubble
 414 aeration can be selected as mode of operation. For the purposes of this exercise, given that flows, BOD
 415 and TSS were available for individual WwTW they were input into the model to generate a degree of
 416 WwTW-specific outputs. The key and most sensitive variable however, is the biodegradation rate
 417 employed for the secondary treatment process (hr^{-1}). Data for biodegradation, in particular official
 418 OECD testing data, is not readily available in literature for APIs. A series of defaults are available based
 419 on standard OECD tests which indicate if a compound is readily biodegradable (1 hr^{-1}), readily
 420 biodegradable, failing the 10-day window (0.3 hr^{-1}) and inherently biodegradable fulfilling specific
 421 criteria (0.1 hr^{-1}). Inherently biodegradable, not fulfilling specific criteria or not biodegradable are
 422 assumed to be persistent (0 hr^{-1}). However, for APIs a OECD 301 biodegradability assessment is not
 423 mandated if OECD 308 data are generated, provided the pharmaceutical passes the Phase 1 of the tiered
 424 assessment approach, in other words, it has a $\text{PEC}_{\text{surfacewater}} < 10 \text{ ng/l}$ and $\log K_{ow} > 4.5$ and as well as
 425 certain mode of action (EMEA, 2006). Consequently, not all the APIs in the CIP program could be
 426 estimated in the models (Table A9). When API removal data from both CIP1 and 2 was available, an
 427 average value was used for comparison with SimpleTreat predictions (Figure 6).

428

429



430

431 **Figure 6: SimpleTreat 4.0 predicted removal versus measured data with 95% confidence**
 432 **intervals for the CIP1 and CIP2 data (red dotted line=1:1; blue colour for acidic**
 433 **compounds; red for basic; green for neutral or zwitterions)**

434

435

436 Overall good agreement was obtained between SimpleTreat and the CIP measured data, with 13 of the
 437 APIs predicted to be within 30% of the CIP measured value, with no obvious systematic bias. This is
 438 in agreement with previously reported assessments (Crechem, 2006). In broad terms, there tended to be
 439 better agreement for neutral/zwitterionic APIs than for the charged compounds (at ambient wastewater
 440 pH). In general, it was found that SimpleTreat tended to under estimate the percentage removal for 10
 441 of the APIs, particularly for those more readily degraded, which being conservative (i.e. there is greater
 442 removal in reality than predicted, so less API is being discharged than predicted) meets the
 443 precautionary principle for risk management (UN, 1992). However, this places potential costs on
 444 society that are not warranted, so it needs to be applied as a screen for further validation.

445

446 Furthermore it was possible to reverse engineer biodegradation rate constants for API removal during
 447 secondary treatment using the SimpleTreat 4.0 model. For CIP1 ASP WwTW data were collected for
 448 influent, as well as after both primary and secondary treatment, unlike the CIP2 WwTW where only
 449 influent and effluent concentrations were measured. Consequently the CIP1 dataset allowed the
 450 efficiency of secondary treatment alone to be calculated as a percentage of API removal. For each of

451 the CIP1 WwTW where API concentrations were greater than the limit of detection, API characteristics,
 452 flow, BOD and TSS were input into SimpleTreat and the secondary treatment biodegradation rate
 453 adjusted until the predicted percent removal of the API matched that observed at the WwTW. This
 454 generated a series of rate constants for biodegradation for 9 APIs for between 7 and 13 WwTW
 455 secondary processes. The mean, median and range of these derived rate constants could then be
 456 compared with default constants generated from OECD laboratory tests that are applied in models as
 457 risk assessment to critically assess their efficacy under real-life conditions (Table 1).

458

459 **Table 1: Reverse engineered default rate constant generated by SimpleTreat 4.0 using CIP1**
 460 **secondary ASP removal data.**

API	Default Rate constant (hr ⁻¹)	SimpleTreat 4.0 fitted secondary treatment rate constant for CIP 1 ASP (hr ⁻¹)					
		mean	sd	median	n	min	max
DCF	0.3	0.02	0.02	0.003	13	0	0.1
ERMY	0.3	0.22	0.42	0.038	9	0	1.3
FLXT	0.3	1.99	2.5	0.325	8	0.002	5
EE2	0.3	1.77	2.18	0.39	11	0	5
IBPF	1	0.91	0.54	1.1	9	0.15	1.5
OXTCY	0.3	0.67	1.34	0.22	13	0	5
OFLX	0.1	0.84	1.69	0.062	9	0.032	5
PRPL	0.002	1.19	2.01	0.038	7	0.019	5
E2	0.3	2.81	2.15	2.2	11	0.3	5

461

462 By using a combination of the SimpleTreat model and observed CIP1 secondary removal data, it was
 463 possible to fit a biodegradation rate for secondary treatment and compare it with default OECD derived
 464 values (Table 1). Firstly, given the variability in the datasets, fitted first order degradation rates varied
 465 considerably, with maximum and minimum varying by 2 orders of magnitude in some of cases, although
 466 all of the APIs tested, apart from DCF, default degradation rate lay between the observed minimum and
 467 maximum value. As already notes DCF, the steroid estrogens and CBAZ may be susceptible to undergo
 468 de-conjugation during the treatment process and so observed 'removal rates' may not reflect modelled
 469 assumptions or ready test biodegradation data; although the latter would be subject to similar possible
 470 microbiological interactions (Brown and Wong, 2018). The median CIP1 fitted degradation rate was
 471 within a factor of 2 of the default for OFLX, OXTCY, FLXT, IBPF and EE2; within an order of
 472 magnitude for ERMY and E2, but the default rate constant was considerably higher for the anionic DCF
 473 and lower for the cationic PRPL. In regulatory risk assessments it is often assumed that there is zero
 474 WwTW removal and in most cases there are no risks and hence there is little need to refine; hence few
 475 WwTW data are currently generated. However, from a conservative risk assessment point of view, a
 476 default degradation rate being lower than observed is desirable, as it will lead to an over estimate/worst
 477 case for effluent concentration and hence PEC. This was the case for four of the APIs, but given that
 478 another three were within a few % of the fitted values, as well as E2 and PRPL, where PECs could be
 479 generated significantly lower than likely observed concentrations, owing to the over optimistic

480 degradation rates being applied. However, taking account to that the WwTW conditions of BOD, TSS,
481 partitioning to sludge etc, overall removal rates for PRPL are close between observed and predicted,
482 although DCF SimpleTreat removal estimates are significantly higher than observed, owing to the much
483 higher degradation rate applied.

484

485 Overall, the SimpleTreat estimates of API removal are encouraging and the application of easily
486 available WwTW metrics (flow, TSS, BOD) allows accurate predictions to be used which would allow
487 for tentative risk assessments to be undertaken where measured data are not available.

488

489 Finally, it is important to consider the wider impacts of these finding, particularly relating to the risk
490 assessments required for chemicals likely to enter the environment. Provide sufficient data is available
491 then a similar approach should be able to be applied to other substances of concern that occur in
492 wastewater including illicit drugs, pesticides and other classes of APIs such as antiretrovirals (Munro
493 et al., 2019). Furthermore, reverse engineering biodegradation half-lives using monitoring data is quite
494 an expensive way to achieve this and can only be done reliably once an API is in patient use and after
495 WwTWs have adapted to potentially biodegrade the compound. APIs are ‘down the drain’ chemicals
496 and current regulations from the EMA require the determination of LogKoc and LogKow as well as the
497 OECD 301 (ready biodegradability) and 308 (aerobic and anaerobic transformation) tests. Using
498 SimpleTreat to reverse fit secondary treatment biodegradation rates showed that a wide variation in rate
499 constants are generated, reflecting the observed data, with median values which can differ considerably
500 from values generated from OECD ready biodegradation tests. The likely reason for these differences
501 are the artificial conditions used within such tests, in particular, fixed temperatures, elevated API
502 concentrations, low biomass concentrations and variable inoculums (Martin et al.,2018). There is no
503 requirement to conduct a 314B (activated sludge die-away) or 303 (aerobic sewage simulation) tests
504 within the required ERA for EMA. Given the variation in removal observed at WwTW and the need to
505 get a realistic PEC for surface waters, so that those APIs of greatest risk can be prioritised, the EMA
506 guidelines may need to be amended to reflect this. This might include giving greater consideration to
507 WwTW removal in Phase II Tier A and/or B. The draft revision out for consultation (EMA, 2018)
508 allows the OECD 301 test to be waived if the OECD314B test has been completed, which is a positive
509 move and the results presented here do support the need for greater consideration of WwTW within the
510 ERA process. The application of this approach might also help the water industry to prioritise on those
511 drugs with low removal much earlier.

512

513 **4 Conclusions**

514

515 The removal of APIs observed between and within the individual WwTW is shown by CIP monitoring
516 to be highly variable and of greater significance than any variance between overall type of treatment

517 (e.g. ASP versus TF). There was no usable correlation found between concentrations of sanitary
518 determinands such as AMON, BOD, COD and TSS and observed those of APIs. The only conclusion
519 that could be drawn was that high performing WwTWs (with high levels of sanitary determinand
520 removal) lead to the strong likelihood that APIs too, will be more effectively removed. Relatively
521 accurate estimates of removal were achievable using the latest version of the SimpleTreat model for
522 ASP WwTWs, which accounts for the charge present, a significant (but not only) controlling factor in
523 the fate of APIs during wastewater treatment. SimpleTreat was capable of predicting API removal with
524 an uncertainty of +/- 30% for the majority of the APIs tested, based on readily available WwTW specific
525 parameters such as flow, total suspended solids and BOD. This has been achieved without any account
526 of processes such as de-conjugation which is poorly understood at the present time.

527

528 Overall, it may be concluded that SimpleTreat using some easily obtainable WwTW parameters such
529 as TSS and BOD concentrations, offers a relatively refined modelling option for API risk assessment
530 purposes, provided there is confidence in the degradation rate constants used. The data and modelling
531 presented here supports the move towards greater consideration of WwTW within the ERA process for
532 APIs.

533

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539

540

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