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Prioritising anticancer drugs for environmental monitoring and risk assessment purposes

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KEYWORDS

Pharmaceuticals; wastewater; fate; receiving water.

ABSTRACT

Anticancer drugs routinely used in chemotherapy enter wastewater through the excretion of the non-metabolised drug following administration to patients. This study considers the consumption and subsequent behaviour and occurrence of these chemicals in aquatic systems, with the aim of prioritising a selection of these drugs which are likely to persist in the environment and hence be considered for environmental screening programmes. Accurate consumption data were compiled from a hospital survey in NW England and combined with urinary excretion rates derived from clinical studies. Physical-chemical property data were compiled along with likely chemical fate and persistence during and after wastewater treatment. A shortlist of 12 chemicals (from 65) was prioritised based on their consumption, persistency and likelihood of occurrence in receiving waters and supported by observational studies. The ecological impact of these 'prioritised' chemicals is uncertain however, as the measured concentrations in surface waters generally fall below standard toxicity thresholds.

CAPSULE

Anticancer drugs are a broad group of pharmaceuticals with wide use. Here we shortlist a useful subgroup of 12 drugs that are likely to be of environmental concern.

INTRODUCTION

There is growing concern about the presence of pharmaceuticals in the wider aquatic environment. Common 'over the counter' and prescription medicines as well as veterinary medicines are increasingly reported in waste and surface waters in the scientific literature [1]. Anticancer drugs, however, used in chemotherapy, have received less attention but have high pharmacological potency and possess fetotoxic, genotoxic and teratogenic properties and can induce subtle genetic and cell cycle changes in aquatic fauna and flora under chronic exposure [2, 3]. Due to development of analytical methods some of these chemicals have been reported in hospital waste effluents, influents/effluents in sewage treatment plants (STPs) and river water, in a small but growing number of studies [4-11]. The concern over these substances is their occurrence in freshwater systems which are then abstracted as a potable water supply, hence presenting a risk of human exposure, as well as their wider risk to freshwater and estuarine habitats [2].

Anticancer drugs are classified under antineoplastic and immunomodulating agents using the anatomical therapeutic classification system (ATC); class L. Based on the chemical structure and therapeutic properties they are further subcategorized into five groups; L01A: alkylating agents; L01B: antimetabolites; L01C: plant alkaloids & other natural products; L01D: cytotoxic antibiotics & related substances, and L01X: other antineoplastic agents which relate to their mode of action. Chemotherapy is correctly described as cytotoxic therapy, and refers to the use of drugs to kill or inhibit the growth of cancer cells. Most chemotherapy drugs act as cytotoxic agents by causing damage to deoxyribonucleic acid (DNA) or prevent chromosomal replication by disrupting critical cell processes, which leads to cell death (apoptosis) [12]. There are other

40 treatments that do not kill cancer cells and work by stopping cancer cell replication/division.
41 Targeted anticancer therapy uses advances in cancer biology to specifically target selected
42 effects in cancer cells such as the monoclonal antibodies that target selected effects (tumour cell
43 antigens, growth factors and their receptors).

44 There are a high number of anticancer drugs in use and, in general, many of these compounds are
45 polar, water soluble and non-volatile. The principle sources to wastewater include point release
46 through hospital effluents as well as diffusive release from domestic dwellings from cancer
47 patients (non-hospital bound or 'outpatients') undergoing chemotherapy medication. Therefore,
48 STP discharges are considered as the main source of anticancer drugs to the aquatic environment
49 [2]. Some of these drugs are not fully metabolised and are poorly biodegradable and therefore
50 can resist biological as well as physical removal processes during wastewater treatment in STPs
51 [3]. Even if their concentration in the environment is low, some of these chemicals could be
52 considered to be semi-persistent with ongoing release into the environment [1]. Mixtures of these
53 drugs, that possess a similar pharmacology have the potential to act additively once in the
54 environment, possibly enhancing their overall cytotoxicity and increasing the risk to aquatic
55 organisms [13].

56 Currently, there are over fifty anticancer drugs being routinely used in chemotherapy in the UK,
57 with the estimated consumption of 1.0 and 1.7 tonnes/yr for 5-fluorouracil and capecitabine
58 respectively in 2003 [3]. However, there is a paucity of environmental data on levels of
59 anticancer drugs in aquatic systems, although there are a small number of chemicals including
60 cyclophosphamide, ifosfamide, 5-fluorouracil, which consistently appear in the handful of
61 studies that have targeted anticancer drugs in surface water monitoring [4, 5, 7, 14].

Concentrations in river water (receiving treated effluents) have been predicted for 5-fluorouracil where the input of sewage effluent resulted in concentrations of 5-fluorouracil ranging from 5-50 ng/L during low flow conditions using the GREAT-ER model in the Aire and Calder catchment of North Yorkshire UK. This study assumed no removal or loss of the chemical in the STPs or the river water [3].

The purpose of this current study was to generate a shortlist of anticancer drugs (from the many drugs in use) that are likely to have relevance with regards to their actual occurrence and impact on the wider environment. We therefore examined the specific use and consumption of anticancer drugs in general hospitals operating non-specialist oncology units and attempted to shortlist those chemicals which are likely to be prevalent in receiving water, based on their use/consumption, physical chemical properties and persistence, in order to aid environmental screening programmes. This dataset of 65 pharmaceuticals was then compared to the limited environmental data for these compounds to confirm our assessments regarding the behaviour and likely occurrence in surface waters. While a large suite of anticancer drugs and metabolites might be present in hospital wastewater [4], their numbers and concentrations in STP effluent are likely to diminish, due to biological, chemical and physical removal processes during water treatment and subsequent dilution. A shortlist of target chemicals is useful for screening programmes and also forms the basis for targeted risk assessments. Figure 1 provides a schematic representation of the methodology used in this study.

1. HOSPITAL CONSUMPTION

Consumption data was obtained for hospitals within the North West (NW) of England and used to generate an accurate annual usage dataset for the 65 anticancer drugs listed by these hospitals.

84 The National Health Service (NHS) trusts within the region are listed in Table 1 with their
85 corresponding annual consumption (g/yr) and calculated per capita consumption (µg/cap/d). The
86 survey lists the collective consumption of each chemical administered at all hospital and
87 outpatient sites contained within the NHS trust with the exception of the Greater Manchester &
88 Cheshire cancer network and the Clatterbridge Centre of Oncology. The Greater Manchester and
89 Cheshire cancer network (Table 1) contains 11 trusts; five trusts (Trafford Healthcare NHS trust,
90 Royal Bolton Hospital NHS foundation trust, Salford Royal NHS foundation trust, Mid Cheshire
91 Hospitals NHS foundation trust and Wrightington, Wigan and Leigh NHS foundation trust) have
92 been combined to give the consumption for this region. The highest consumed anticancer drugs
93 from Table 1 are capecitabine > cyclophosphamide > hydroxyurea > 5-fluorouracil > imatinib >
94 gemcitabine. These data complement a French study conducted in the city of Lyon [15]. An
95 unpublished study at the University Hospital in Aachen (Germany) also highlighted dacarbazine,
96 methotrexate and cytarabine as highly consumed chemicals (i.e. > 178 g/yr) [16]. An additional
97 drug with high consumption is Trastuzumab (common name: Herceptin) which is an example of
98 a cytostatic agent i.e. a compound that arrests cells in a specific phase of their cycle. Once
99 cancerous cells are arrested and synchronized they can be targeted with a cytotoxic agent. In this
100 case Trastuzumab is a monoclonal antibody to the HER-2 receptor which is overexpressed in
101 30% of women with metastatic breast cancer. [12]. Anticancer drugs are sometimes applied in
102 combination, for example the cisplatin-etoposide (PE) regime is an example of combinational
103 chemotherapy used to treat different types of lung-cancer where typically cisplatin ($75mg/m^2$)
104 and etoposide ($300mg/m^2$) are given in a 4:1 ratio over three consecutive days [17]. This
105 relationship can be seen in Table 1, where consumption data from the University Hospital of

106 Morecombe Bay show the consumption of cisplatin is proportional to etoposide in this given
107 ratio.

108 Alkylating agents (non-phase specific) are effective against a wide range of malignancies and
109 display a dose-response relationship where usually the threshold dose is limited by the toxicity to
110 normal tissues. The alkylating agents are chemically reactive drugs that interact with DNA to
111 form covalent bonds/adducts causing DNA damage (single or double strand breaks) at any point
112 in the cell cycle and therefore prolonged exposure is not necessary. Whereas antimetabolites (S-
113 phase specific) structurally resemble naturally occurring purines, pyrimidines and nucleic acids
114 and act by inhibiting key enzymes involved in DNA synthesis or by incorporation into DNA and
115 RNA resulting in DNA strand breaks and premature DNA chain termination. One example of an
116 antimetabolite is 5-fluorouracil, (5-FU). 5-FU is one of the most commonly used chemotherapy
117 agents and issued to treat a range of cancers including breast, head and neck, colorectal, stomach,
118 and some skin cancers. It is important that cancer cells are exposed to 5-FU during the synthesis-
119 phase (S-phase) of the cell cycle. For that reason administration is usually a prolonged infusion
120 over days or taken as a daily tablet over weeks, maximizing the chance of exposure to tumour
121 cells during S-phase. Anticancer drugs are dosed according to the patients' body surface area
122 (g/m^2) and the route of administration largely depends on the pharmacological properties of the
123 drug. Since many anticancer drugs exhibit poor oral bioavailability, intravenous injection or
124 catheter is the most common method of drug delivery. Chemotherapy drugs are administered in
125 cycles of one or more days followed by a recovery period for normal tissue; since normal cells
126 have a greater capacity for repair than tumour cells the repeated cycles decrease the tumour
127 population with time [12]. The implications for this type of dosing to cancer patients is that

128 ‘emissions’ of the unmetabolised or partially metabolized drug is continuous and not restricted
129 both spatially or temporally, ensuring continuous input to wastewater.

130 ## 2. DRUG METABOLISM AND EXCRETION

131 Since there are a wide range of anticancer drugs under consideration and a limit of scientific
132 knowledge on both ecotoxicological and occurrence data, we consider that it is best to primarily
133 focus on chemicals for which there is a higher probability for detection in the environment. The
134 combined excretion of pharmaceutical ingredients via urine and faeces is considered the primary
135 route by which the active pharmaceutical compound enters the environment. Table 2 shows the
136 average % urinary excretion of the parent pharmaceuticals for the 65 anticancer drugs surveyed
137 in NW hospitals and calculated from clinical studies. There is considerable variability in the
138 pharmacokinetics of an administered drug and the urinary excretion rates show strong inter-
139 patient variation [16]. Nonetheless, the average percentage urinary excretion of the unchanged
140 parent drug ranged from negligible to >75%. From Table 2, methotrexate and pemetrexed show
141 the lowest metabolism with the highest average urinary excretion rates. Building a preferential
142 list based on the consumption and the corresponding urinary excretion rate permits an evaluation
143 of their likelihood to be present in the wider environment. The following drugs were therefore
144 removed for further consideration, and included chlorambucil, busulfan, lomustine, tioguanine,
145 cladribine, vinblastine, vincristine, trabectedin, idarubicin, mitoxantrone, gefitinib, dasatinib,
146 temsirolimus and everolimus. The monoclonal antibodies L01XC (rituximab, trastuzumab,
147 alemtuzumab, cetuximab, bevacizumab) were also not included in the next stage of assessment
148 due to the lack of information on their physical-chemical properties and possibly low excretion
149 rates, although they may require future evaluation due to their high consumption. For example,

150 trastuzumab, rituximab and cetuximab, have respective consumption rates of 3.04, 0.77 and 0.45
151 kg/yr in the hospitals surveyed in this study.

152 ## 3. BUILDING A FATE PROFILE BASED ON PHYSICAL-CHEMICAL PROPERTIES

153 Table 3 presents physical-chemical properties of a wide range of anticancer drugs and their
154 predicted loss from wastewater during the sewage treatment process. Building a physicochemical
155 profile for each drug allows their partitioning and fate within aquatic systems to be predicted to
156 some extent. The fate of the chemical entering a STP depends on both the nature of the chemical
157 and the treatment process; anticancer drugs may sorb to sludge, undergo volatilisation or remain
158 in the dissolved phase in the aqueous effluent. The latter is most important for chemical
159 breakthrough and occurrence in receiving waters. Sorption to suspended particulate matter and
160 deposition with sludge would be an important route of removal for those compounds with high
161 K_{ow} /$K_{oc.}$ The physical-chemical properties were compiled based on information in the literature
162 as well as predicted data (K_{ow}, K_{oc}) through the use of property estimation models, including
163 SPARC (SPARC performs automated reasoning in chemistry, http://archemcalc.com/sparc/); and
164 EPI-Suite (US-EPA, http://www.epa.gov/oppt/exposure/pubs/episuite.htm) with additional
165 information e.g. SMILES notation, gleaned from ChemSpider [18].

166 Many of the chemicals listed in Table 3 are highly polar with high aqueous solubility (i.e. ~10^3-
167 10^4 mg/L, although a wide range is evident ($1.07x10^{-4}$ – $8.66x10^4$ mg/L). The presence of
168 ionisable functional moieties indicates that a large number of these compounds are likely to be
169 ionised at environmentally relevant pHs. pKa values are given in Table 3 (largely gleaned from
170 pharmaceutical data/reports) for behaviour in blood (pH 7.4). The presence of two or more
171 carboxylic acid groups on methotrexate, for example, results in successive pKa values of 3.8, 4.8

172 and 5.6. The corresponding log K_{ow} and log D_{ow} are also presented in Table 3. K_{ow} values, in
173 general, are low (i.e. < log 2) indicating the propensity of these drugs to remain in the dissolved
174 phase, although there are exceptions to this. For example, lapatinib, mitotane, nilotinib,
175 paclitaxel, vinorelbine, pazopanib, sorafenib and irinotecan, possess log K_{ow}/D_{ow} values that
176 exceed 3, with correspondingly high K_{oc} values indicating a potential for bioconcentration. Since
177 EPI-Suite models were used to derive K_{ow} values then these can only be considered as estimates
178 at best and are unlikely to be accurate for polar molecules containing multiple functional groups.
179 For example doxorubicin (DOX), an anthracycline antibiotic, sorbs to sludge despite its
180 relatively low K_{oc} predicted by EPI-Suite. Between 48 -74% of DOX was removed from the
181 dissolved phase of a model STP when incubated with activated sludge, with 20-40% of this
182 recovered in the sludge suggesting loss through sorption to particulate matter [19] . Tetracycline
183 (TET) is structurally similar to DOX and shows similarly high rates of sorption to organic rich
184 particulate material [20].

185 Cyclophosphamide (CP) is one of the most widely used cytotoxic drugs and is frequently
186 detected in receiving waters. It is therefore appropriate to relate other drugs to CP to give a
187 relative indication of their likely environmental behaviour. For example, CP has a water
188 solubility of 40,000 mg/L and has been detected in hospital effluent at a maximum concentration
189 of 4.5 mg/L [21]. Where data are available, the high aqueous solubilities, low vapour pressures
190 and hence very low Henry's Law constants indicate that it is unlikely that these compounds will
191 volatilise at ambient temperatures from surface waters. The vapour pressures for the drugs range
192 from $<10^{-7}$ to 10^{-2} Pa and the Henry's Law constants range from $<10^{-10}$ to 10^{-5} Pa.m^3/mole. For
193 CP and similar compounds possessing water solubilities in the g/L range, combined with low log

194 K_{ow} values indicate that the chemicals will remain in the dissolved phase and will not be lost
195 from wastewater streams either through volatilisation or through particle settling.

196 3.1.Dissociation and partitioning in aquatic systems

197 Many of the compounds listed in Table 3 possess functional groups with the ability to ionise
198 depending on the ambient pH. At environmentally relevant pHs (e.g. 5-9), fifteen of the listed
199 anticancer drugs will be partially or fully ionised. Figure 2 illustrates the relative proportions of
200 acid-base species as a function of pH for some of the anticancer drugs exhibiting more than one
201 acid or base moiety. More complex sorption related modelling in representative aqueous systems
202 appears necessary to whether the drug is removed from the dissolved phase, with the ambient pH
203 influencing the degree of dissociation for ionisable drugs and hence the D_{ow} and D_{oc}. For
204 example, the particle-water distribution ratio K_d for chemicals containing an amine functional
205 group (hence basic properties) can be affected by changes in pH, although changes over the
206 environmental range of pHs appear to have little effect on K_d and hence sorption [22].

207 Figure 3 illustrates the range of log K_{ow} values for many of the anticancer drugs. Where relevant,
208 D_{ow} values were then calculated from K_{ow} according to for acidic
209 compounds and for basic compounds and reported in Table 3.
210 Typically, chemicals with a log K_{ow} over 2.5 and a log D_{ow} greater than 3 can be considered to
211 partition significantly to organic rich particulate matter and hence be removed from the water
212 column particularly during primary sewage treatment. . Values of K_{ow} and corresponding K_{oc}
213 should be interpreted with caution as Yamamoto, et al. (2005) demonstrated the lack of
214 correlation when plotting the Karickhoff empirical formula ($\log K_{oc} = \log K_{ow} - 0.21$) for
215 pharmaceuticals with experimentally derived K_{oc} values. Furthermore, pharmaceuticals with

ionisable functional groups (e.g. carboxylic acids, or amino groups) will undergo a range of sorption mechanisms with the existence of electron donating and/or accepting functional groups both in the selected pharmaceuticals and soil/sediment matrices affecting electrochemical affinity [23].

In addition to sorption to suspended particulate matter, some anticancer drugs may enter STPs as conjugated/metabolised entities e.g. glucuronide conjugates (or partitioned onto biological macromolecules). The parent molecules may then be 'liberated' presumably through biochemical 'cleavage' during sewage treatment, thus increasing the concentration of the parent molecules in the final effluent. This has been suggested for other pharmaceuticals such as carbamazepine which binds extensively to blood plasma proteins [24]. Anticancer drugs with high protein binding capabilities include sorafenib, oxaliplatin, imatinib, etoposide, paclitaxel, epirubicin, vinorelbine, doxorubicin and cyclophosphamide (fraction sorbed in the range from 0.6-0.99) [25-32], raising the potential for concentrations of the parent molecule to be higher in the final effluent than the raw influent water of a sewage treatment works.

Using the K_{ow}/K_{oc} values reported in Table 3 (and illustrated in Figure 3) (D_{ow} if dissociated at pH 7) then those anticancer drugs likely to show strong tendency for sorption are: Lapatinib > mitotane > nilotinib > paclitaxel > vinorelbine > cisplatin > pazopanib, and possess K_{oc} values >5000. Compounds with K_{oc} values greater than 2000 are sorafenib > irinotecan > erlotinib with potential to sorb to sewage sludge. Bendamustine and carboplatin also have relatively high K_{oc} values (approx 900) [33]. Sorption to particulate matter is therefore likely for these chemicals, with a significant fraction likely to be retained in sewage sludge following primary treatment. Depending on their persistence and resistance to biodegradation these chemicals may eventually

find their way to soil if the sludge is subsequently applied to agricultural land. The remaining anticancer drugs have K_{oc} values <500 and are expected to display limited partitioning to suspended particulate matter in STP influent.

4. DEGRADATION AND ENVIRONMENTAL HALF-LIVES

Those anticancer drugs that exhibit high urinary excretion rates without human metabolic transformation may also resist environmental biotransformation. As with metabolism the chemical structure can be changed by biotransformation, biodegradation and abiotic transformation; the latter including aqueous photolysis and hydrolysis reactions. It is assumed that metabolism and other transformation processes may lead to decreased toxicity, however, in some cases metabolism leads to the activation of some compounds (i.e. metabolism of the pro-drugs capecitabine & tegafur result in 5-fluorouracil).

The present study gives insight into the degradation anticancer drugs and the influence of external factors on these processes. Using literature sources, including specific laboratory degradation tests, as well as the STPWIN model of EPI-SUITE we attempt to assess semi-quantitatively transformation/loss of anticancer drugs in a STP and the wider aquatic environment. STPWIN is based on fugacity principles and attempts to predict the fate of an organic chemical in a conventional STP that uses activated sludge. The model estimates the fate of a chemical as it becomes subject to removal by evaporation, biodegradation or other degradation processes, as well as sorption to sludge and loss in the final effluent.

4.1 Biodegradability

After release with wastewater streams biological degradation by micro-organisms (aerobic/anaerobic), particularly in the secondary stage of sewage treatment, may be a significant loss process for some anticancer drugs. Degradation will be dictated by both the physical-chemical properties of the drug and the characteristics of the treatment plant. Biodegradability tests such as the closed bottle test (CBT) or Zahn-Wellens test (ZWT) are usually carried out with the initial quantity of the test chemical in the order of mM, with the drug serving as the carbon source. Results from these tests should therefore be viewed with caution as levels of anticancer drugs in wastewater are likely to be much lower. Cyclophosphamide [7, 21, 34-36], ifosfamide [7, 35-37], vinblastine [38], vincristine [38] and mitoxantrone [39] have been shown to exhibit poor biodegradability, and a summary of these tests are presented in Table 4. For example, the incubation of CP in activated sludge resulted in no observed degradation after 24 hours. After prolonged incubation for 39 days, only 17% of CP was removed, indicating the stability of this drug [21] and CP showed no degradation for environmentally relevant concentrations (ng/L) over a 24 hour period [7]. Another study investigated the removal of trace levels of CP from sewage waters by using nanofiltration and reverse osmosis, however, CP was not sufficiently removed in these tests and a suggestion of using combined systems was thought to be a promising method for eliminating CP [40].

Other compounds such as treosulfan [39], methotrexate [34], pemetrexed [41], gemcitabine [35, 42], capecitabine [43], cytarabine [34, 42], doxorubicin [19] and epirubicin [19] have been shown to be biodegradable to some extent (Table 4). For example, methotrexate biodegradation increased continuously during the first seven days and reached a constant removal of 95% after 8 days, with little dependence on the initial concentration, however, only 10% of methotrexate was degraded in the first four days of this study [34]. In another study methotrexate biodegradation

281 was tested in accordance to OECD guidelines over a 28 day test period and biodegradation was
282 shown to be negligible [44]. Results for other drugs are presented in Table 4. In some cases
283 biodegradation results are conflicting. For example, for 5-fluorouracil several studies indicate
284 that biodegradation of this drug is negligible [42, 43] yet others show significant biodegradation,
285 with 94% biodegradation over 14 days in a laboratory scale activated sludge plant [19, 34, 43].
286 However, only 15% of 5-FU was removed in the first 24 hours, followed by a steep increase to
287 almost complete degradation between 24 to 48 hours. This is possibly reflected by the low
288 recovery of 5-fluorouracil in sewage sludge (2-5%) after 24 hours [19, 34].

289 Microorganisms in STPs will not have an adaptation period of several days in order to achieve
290 the high degradation efficiencies observed in the above tests and therefore environmental
291 degradation rates are likely to be low and reflect those measured in the first few days of these
292 experiments [34]. Since there is a paucity of empirical biodegradation studies, other anticancer
293 drugs listed in Table 4 were assessed based on modeled biodegradation using STPWIN (v. 4.1.)
294 with consideration to other anticancer drugs in the same ATC classification or with a similar
295 structure. Examples include azacitidine and tegafur which can be compared with the other
296 pyrimidine analogues such as cytarabine, 5-fluorouracil, gemcitabine and capecitabine,
297 suggesting that azacitidine and tegafur will biodegrade to some extent during activated sludge
298 treatment. In general, most anticancer drugs have low biodegradability and it can be assumed
299 that biological degradation in receiving waters will also be negligible [45]. The modeled
300 biodegradation rates are estimates and likely to be an underestimation of degradation occurring
301 in the STP. Modeled biodegradation rates derived using STPWIN are provide in Table 4 and also
302 highlighted with an asterisk in Table 6 (see later section). Biotransformation products may also
303 need consideration with regards to their occurrence in surface waters and possible risk. For

304 example, methotrexate has been shown to degrade into the active metabolite, 7-
305 hydroxymethotrexate, which acts via the same cytotoxic mechanism as methotrexate but with a
306 lower potency. 7-hydroxymethotrexate does not appear to undergo further biodegradation and
307 therefore may persist for longer periods than the parent chemical [34].

308 4.2 Abiotic degradation: photodecay and hydrolysis

309 Photolysis and hydrolysis are potentially major abiotic degradation routes for pharmaceuticals
310 like anticancer drugs in the aquatic environment. While photochemical processes maybe a
311 relevant loss mechanism, they are obviously dependent on the geographic location, the season
312 and the presence of other constituents in the water which may sensitise or shield chemicals from
313 photochemical decay (particle matter, DOC, nitrate etc) [46, 47]. Anticancer drugs that exhibit
314 significant light absorption above 290nm of solar irradiation may be subject to direct photolysis
315 in surface waters, resulting in transformation products of the parent chemical. For example
316 cisplatin and methotrexate have UV maxima absorbance values of 318nm and 298nm (within the
317 solar UV-B range) respectively, and are likely to undergo some direct photolysis [34].

318 Cyclophosphamide and ifosfamide show negligible absorption in the solar wavelength range [7].
319 Direct photolysis studies to date generally use a lamp with appropriate filters to simulate sunlight
320 with light irradiance approximately equivalent to midsummer sunlight in California, and the
321 studies do not account for light attenuation in the water column and other site-specific factors
322 [47]. The quantum yield of photo disappearance was calculated to be effectively zero for
323 cytarabine. UV irradiation experiments showed that only 10% of cytarabine was degraded after
324 two hours from which, only 3% of the total energy emitted by the lamp corresponded to the
325 wavelength of cytarabine (271nm) [48]. UV lights fitted as tertiary treatment in some STPs may

result in some loss of cytotoxic drugs. Indirect photolysis involving the transformation of the drug through a reaction with photochemically derived species like the aqueous hydroxyl radical (OH*) is perhaps more relevant for some anticancer drugs present in surface waters. The photolysis rates in river water are generally faster than those in MilliQ laboratory water, with the accelerated rates attributed to photosensitization by dissolved organic carbon (DOM) [47]. Cyclophosphamide and ifosfamide were degraded at faster rates in irradiated lake water, with increasing OH* concentrations enhancing photodegradation rates [7]. However, depending on the structural properties, DOM can retard the reaction by competing for photo-radicals and acting as an optical filter or quencher. The latter may explain slower rates of photodegradation rates in river water compared to MilliQ [47]. UV/H_2O_2 systems were adequate to degrade cytarabine although toxicity assays showed that the photodegradation products were more toxic than the parent chemical [48].

Hydrolysis: the direct nucleophilic substitution reaction with water usually catalysed by hydrogen or hydroxyl ions may also provide a transformation route for cytotoxic drugs. Hydrolysis rates are expressed in terms of the acid- neutral- and base- catalysed hydrolysis rate constants and drugs containing ester and amide functional groups are particularly susceptible to degradation via hydrolysis. To investigate this process, the rate constants for the base driven hydrolysis for 11 relevant anticancer drugs (cyclophosphamide, ifosfamide, vinblastine, vincristine, vinorelbine, etoposide, paclitaxel, docetaxel, cisplatin, topotecan and irinotecan) were generated by SPARC (version 4.5) and reported in Table 4. The SPARC base hydrolysis rate shows that at pH 7, the percent loss of parent compound is negligible in all cases, however, at pH 8.1 (the pH of seawater, relevant for coastal waters), vinblastine, vincristine, vinorelbine, paclitaxel and irinotecan undergo significant hydrolysis with losses ranging over ~ 54-77% (for a

5 day period). This range is an estimate at best but does highlight the relative susceptibility of these drugs to be lost by this process, particularly at higher pHs.

Since the hydrolysis of most anticancer drugs at pH 7 appears to be negligible, biodegradation and photolysis are likely to be the primary degradation routes for the drugs listed in Table 4. In addition to hydrolysis and photolysis, the calculated rate constants for the reaction between dissolved ozone (used in water treatment processes) and cyclophosphamide suggests that ozone reactions will play a minor role in the degradation of this chemical. For other chemicals containing amino groups, such as methotrexate, then reaction with ozone can be rapid [49]. Furthermore, sorption to sediments generally reduces the rates of hydrolysis for acid- or base - catalysed reactions but neutral reactions appear to be unaffected by sorption, so particle-sorbed compounds may undergo some transformation by this process.

5. ECOTOXICITY AND GENOTOXICITY (IMPACT ON THE ENVIRONMENT)

The effects of anticancer drugs on aquatic biota is not well understood, but attempts have been made to assess human exposure risk based on consumption of drinking water containing anticancer drugs in the low ng/L range, i.e. the concentrations observed in surface waters (see next section) [2, 3]. The EU-Directives 93/67/ECC (EC 1996) classifies chemicals according to their EC_{50} values (whereby EC_{50} < 1mg/L is deemed 'very toxic to aquatic organisms'; 1-10mg/L are 'toxic to aquatic organisms' and 10-100mg/L are classified as 'harmful to aquatic organisms' and chemicals with an EC_{50} above 100mg/L are not classified). Predicting the effects of chemicals on biota can be attempted using Quantitative Structure Activity Relationships (QSAR), or measured using a range of acute and chronic ecotoxicity tests, that have been conducted for algae, daphnia and fish, i.e. different trophic levels of freshwater systems [3].

371 However, in general, only limited data exists on the ecotoxicity of both cytotoxic and cytostatic
372 drugs. Where ecotoxicity data does exist, 5-fluorouracil has been found to be of most concern,
373 with EC_{50} values being <<1mg/L for a number of ecotoxicity tests. For example, the highest
374 toxicity was found for bacteria, particularly *Pseudomonas putida* (gram negative bacteria) with
375 EC_{50} values of 0.027mg/L and 0.044mg/L for respective studies [50, 51] and *Vibrio fisheri* (gram
376 positive bacteria) with an EC_{50} value of 0.122mg/L [52]. 5-fluorouracil was also found to have a
377 relatively low EC_{50} value of 0.11mg/L for *Pseudokirchneriella subcapitata,* a species of algae
378 [50], although a markedly higher EC_{50} value of 48mg/L was reported for another strain (*D.*
379 *Subspicatus)* [51]..

380 QSAR derived EC_{50} values categorize cyclophosphamide and ifosfamide as harmful for algae
381 with a corresponding EC_{50} values of 11 mg/L for each. The QSAR data appear to be consistent
382 with experimental data for cyclophosphamide where ecotoxicity tests for daphnia result in EC_{50}
383 values above 100mg/L. QSAR data for ifosfamide with Zebrafish *(Brachydanio rerio)* also
384 predict an EC_{50} value >100mg/L and therefore was considered as low risk for this organism [53].
385 For other anticancer drugs such as cisplatin, paclitaxel and gemcitabine then ecotoxicity tests
386 with the water flea (*D. magna*) have derived relatively low EC_{50} values of 0.64mg/L, >0.74mg/L
387 and 1.0mg/L, respectively [50, 51]. In addition, a relatively low EC_{50} value of 0.015mg/L was
388 observed for methotrexate for the African clawed frog *(Xenopus lawvis)* [54] with similarly low
389 EC_{50}s for fish *(Brachydanio rerio)* and ciliates (protozoa) *(Tetrahymena pyriformis)* [55].
390 Several experimental studies have now investigated the genotoxicity of hospital effluents as an
391 indirect marker of cytotoxic/cytostatic contamination [7, 50, 51, 56, 57]. For example, cytarabine
392 and gemcitabine showed genotoxicity in the umuC biological assay (based on the ability of
393 inducing the expression of the umu operon) in both variants (with and without metabolic

activation), however, the effects were observed at relatively high exposure concentrations (>100mg/L) [51]. Cyclophosphamide and ifosfamide are also known to be present in hospital wastewater, but no independent umuC tests have been carried out to our knowledge. Genotoxicity was shown in about 13% of the hospital wastewater samples but hospital wastewater is generally diluted by at least 100-fold before entering municipal STPs [56]. No significant genotoxicity was observed for the influent to a STP either due to dilution or degradation [56]. In general, the umuC-test for genotoxicity appears to be less sensitive to the studied drugs than other acute and chronic ecotoxicity assays. Results from another study showed that the hospital wastewater from the oncology ward caused DNA damage in single cell gel electrophoresis (SCGE) assays in primary rat hepatocytes [57]. Cisplatin, carboplatin and 5-fluorouracil also contributed to the genotoxicity of the assay, however, they do not account for all the effects seen within the water samples [57].

The effective toxic concentrations of anticancer drugs are generally higher than their expected and/or observed environmental concentrations. However, a maximum concentration of 124µg/L for 5-fluorouracil was reported in hospital effluent in Austria [19] and is close to some of the reported EC_{50} values reported above. These standardized chronic assays only represent exposure to one generation of organisms and prolonged multi-generational exposure might result in a number of unexpected effects, especially for compounds acting on DNA [51]. Other issues that will require further research are the impact of mixtures of anti-cancer drugs as well as the biological effects arising due to exposure to metabolites/transformation products. Several of the chemicals which are likely to be present in receiving waters based on observations, their use data and lack of removal in STPs also demonstrate toxicity. For example, 5-FU, gemcitabine and methotrexate have EC_{50} values <<1mg/L for a number of different organisms that typically show

417 less sensitivity to cyclophosphamide. Although measurements are sparse, concentrations of these
418 chemicals in river and estuarine waters are likely to be in sub-low ng/L range (see Table 6) and
419 therefore the risk of harm due to exposure at this concentration is likely to be minimal. Subtle
420 effects associated with low dose exposure are an issue which requires further attention,
421 particularly for anti-cancer drugs with high cytotoxic potency. Biochemical alterations in MCF-7
422 cells were observed at low-dose exposures (10^{-12} to 10^{-6} M) to cyclophosphamide and
423 representative of concentrations observed in surface waters [58].

424 ## 6. MEASURED ANTICANCER DRUGS IN WASTEWATER AND RECEIVING
425 WATERS

426 Occurrence of anticancer drugs in hospital effluents has been reported in a growing number of
427 studies, with several studies examining cytotoxic/cytostatic agents in the influent and effluent of
428 STPs and a few studies measuring anticancer drugs in surface and receiving waters (Table 6).
429 Most studies focus on hospital wastewaters where no treatment to the sewage is carried out [4-6,
430 19, 21, 36, 37, 59-61]. Other matrices such as effluent wastewater [7, 9-11, 21, 37, 62, 63] and
431 surface waters [7, 10, 11, 63-65] have perhaps received less attention. Based on use patterns for
432 hospitals, capecitabine, 5-fluorouracil and hydroxyurea are expected to be the most abundant
433 chemicals present in hospital wastewater; however to date, there are only a handful of studies
434 that have detected 5-fluorouracil in the environment and it has only been screened for in hospital
435 wastewater [5, 19, 61]. To our knowledge, capecitabine and hydroxyurea have not been screened
436 at all, although capecitabine may contribute to the overall abundance of 5-fluorouracil detected
437 in hospital effluent.

438 The two most studied cytotoxics in the environment are cyclophosphamide and ifosfamide, both
439 of which have high consumption rates and have been found in receiving waters. Based on
440 hospital consumption, gemcitabine is expected to be an abundant chemical in the environment,
441 however, only two studies have reported this chemical with a concentration range of <0.9-
442 38ng/L, similar to etoposide 3.4-380ng/L. Methotrexate, epirubicin and doxorubicin have
443 reported maximum concentrations in the hospital effluent of 4689, 1400 and 1350ng/L
444 respectively, however, they have not been detected in receiving waters to our knowledge.

445 PRIORITY DRUGS FOR ENVIRONMETAL SCREENING PROGRAMMES

446 Table 6 reports the predicted environmental concentrations of the anticancer drugs in STP
447 effluents and their receiving waters based on the hospital usage data (from NW England),
448 populations they serve, mean excretion rate and their potential for (bio)degradation during the
449 sewage treatment processes. Based on this assessment, combined with their physical-chemical
450 properties (e.g. dictating partitioning behavior) then the following anticancer drugs are
451 considered as priority contaminants with respect to their occurrence in the wider environment
452 and could be included in future screening programmes.

453 Cyclophosphamide (CP) (a pro drug) is considered to be a priority chemical and is used alone or
454 in combination with other medications to treat Hodgkin's lymphoma, non-Hodgkin's lymphoma,
455 cutaneous T-cell lymphoma, multiple myeloma, retinoblastoma (cancer in the eye),
456 neuroblastoma, ovarian cancer, breast cancer and certain types of leukaemia.. In the surveyed
457 hospitals in NW England, the consumption of CP (active ingredient) was 78kg/yr. Blackpool
458 Victoria hospital confirmed that CP consumption was by either oral ingestion or injection and
459 treatments are more likely to commence on a Monday to Friday basis during an outpatients'

460 clinic, however, most CP will be taken within the patient's own home resulting in continuous
461 discharge with wastewater throughout the week. The chemical fate profile in this study indicates
462 that CP is not expected to sorb to suspended solids and sediments (log K_{ow} = 0.23-0.97). CP has
463 been shown to be non-biodegradable using laboratory scale sewage treatment studies at
464 concentrations from 90ng/L and shows negligible loss for base-driven hydrolysis and other
465 abiotic processes, hence confirming the persistence of CP in the environment. CP has been
466 detected by a number of studies in hospital effluents, STP wastewaters and receiving waters.

467 Capecitabine (CAP) is the pro drug of 5-fluorouracil, developed with the goal of improving
468 tolerability through tumour specific conversion into the active drug. The antimetabolite has
469 activity against numerous types of neoplasms. Despite the very low excretion rates of
470 capecitabine, the 357kg/yr consumption in surveyed hospitals creates a high environmental load
471 placing capecitabine second on the list for preferential chemicals. Correspondence with the
472 hospitals confirmed the consumption of CAP is by oral chemotherapy, where patients consume a
473 tablet once or twice daily within their own homes. CAP has a low Kow (log K_{ow} = 0.77 -1.17)
474 and does not sorb appreciably to particulate matter To date no biodegradation studies are
475 available to the authors' knowledge but if CAP was to degrade in a similar manner to 5-
476 fluorouracil, then a high biodegradation rate might be expected, although at present this is
477 highly uncertain.. Furthermore, as CAP serves as a pro-drug to 5FU then it may also contribute
478 to the overall load of 5-fluorouracil.

479 5-fluorouracil (5-FU) principle uses are in colorectal cancer and pancreatic cancer and
480 sometimes used in the treatment of inflammatory breast cancer. 5-FU can also be administered
481 topically (as a cream) for treating some types of cell carcinomas of the skin. As a pyrimidine

analogue it induces cell cycle arrest by mimicking Uracil (natural component of normal cells). The consumption of 5-FU in the surveyed hospitals was 23kg/yr with an 18% excretion rate of the active ingredient. Similarly to CAP the chemical fate of 5-FU is driven by its low K_{ow} (log K_{ow} = -2.1 – 0.91) indicating its preference to stay in the dissolved phase. Biodegradation will account for some loss during sewage treatment. No evidence of abiotic degradation is apparent and furthermore results from EPI suite imply a residence time of ~20 days if released from effluent wastewaters. To date 5-FU has only been detected in hospital effluents.

Hydroxyurea (HU) is used to treat skin cancer, chronic myelocytic leukemia and metastatic cancers and is by far the most consumed anticancer drug without consideration to its other applications (treating adult patients with sickle cell anemia). The antimetabolite interferes with DNA synthesis during the S-phase of cell division. In the surveyed hospitals 64kg/yr of HU was consumed with some 58% excreted from the patients in a non-metabolised form possibly resulting in a high environmental load. Besse et al. (2012) showed a 14% increase in the national consumption of HU in France from 5757kg/yr to 6839kg/yr in 2004 and 2008 respectively. The chemical fate of HU suggests that it is unlikely to sorb significantly to particulate matter during sewage treatment (i.e. K_{oc} = 3) (log K_{ow} = -1.83 to -1.27). To our knowledge no biodegradation data is available for HU and model predictions possibly underestimate its loss, particularly when incubated with activated sludge. Urease catalyses the hydrolysis of urea into ammonia and carbamate [66], the enzyme also has affinity for HU where it catalyses the hydrolysis of HU into hydroxylamide and ultimately acetohydroxamic acid [66, 67]. HU also undergoes hydrolysis so the environmental persistence of this chemical is likely to be low relative to other drugs prioritized here [66]. Assuming a biodegradation rate of 95%, then HU is perhaps less of a priority chemical for environmental screening, although even with a biodegradation rate of 99%

505 it still ranks above ifosfamide (a chemical detected in STP effluents). To our knowledge HU has
506 not yet been screened in wastewaters.

507 Imatinib (IM) is now licensed to treat 10 different types of cancer and is designed to specifically
508 inhibit a tyrosine kinase receptor (trait of certain cancer cells). IM has an annual consumption of
509 20kg/yr in the NW hospitals surveyed here and approximately 10% of the active ingredient is
510 excreted in urine unchanged. Besse et al. (2012) reported a 50% increase in consumption of IM
511 from 2004 to 2008 (584kg/yr to 874kg/yr, respectively). The chemical fate indicates that IM will
512 undergo some retardation in sludge due to IM being partially charged at pH 6 (K_{oc} = 16 & log
513 D_{ow} = 0.19). The loss of IM from biodegradation has been reported at 9-12% over a 28 day
514 period. Since sorption to sludge is to be expected and fecal excretion of the unchanged drug is
515 63%, this drug is of concern for contamination of soils (if sludge is dispersed onto fields). To our
516 knowledge no studies have reported IM in aquatic systems or other environmental
517 compartments.

518 Methotrexate (MET) is from the same family as pemetrexed and is consumed (1.3kg/yr) and
519 excreted (~80%) to a similar extent. Blackpool hospital confirmed that MET is primarily used to
520 treat inpatients and administered 7days/week, however, consumption is likely to increase on
521 outpatient clinic days as this drug is also used to treat rheumatoid arthritis. Besse et al. (2012)
522 estimated that consumption is likely to be an underestimated due to medical uses of MET under
523 another ATC class (L04). The chemical fate profile of MET shows a very low Kow (log K_{ow} = -
524 2.59 to -0.24) and correspondingly low Koc(K_{oc} = 20) demonstrating its preference to remain in
525 the dissolved r phase with potential to pass through STPs and enter receiving water MET did not
526 make the preferential list in previous studies [15] due to a removal rate of 95% when incubated

with activated sludge. Biodegradation data for MET shows that 98% is removed from incubation with activated sludge, however, only 10% was removed in the first four days, akin to the incubation time at STPs. The combined physical-chemical properties and literature is in agreement with the confirmed detection of MET in sewage effluent at 12.6ng/L [9].

Carboplatin (CARBO) is commonly used to treat ovarian and lung cancers and 5.2kg/yr was consumed in the NW hospitals. The chemical has a high urinary excretion rate (~55%) resulting in a significant environmental load. The environmental fate profile of this platinum-based anticancer drug suggests that retardation in sludge would be a dominant removal process for this chemical from the wastewater stream (K_{oc} = 891). Effluents of wastewater treatment plants may contain CARBO and also diaqua-cisplatin (the major degradation product) and analysis has demonstrated that CARBO was present in the influent and effluent of a STP [68] possibly due to its low log K_{ow} (-2.3 to 1.06). To our knowledge no other biodegradation studies are available for CARBO and this assessment is based on the results from a pilot membrane bioreactor system conducted at a hospital in Vienna, Austria [68].

Gemcitabine (GEM) is a pyrimidine analogue with its main uses including non-small lung cancer, pancreatic cancer and bladder cancer. In the surveyed hospitals from NW England, the consumption of GEM (active ingredient) was 13kg/yr, with approximately 8% of the active ingredient being excreted in patients' urine. Like the other priority chemicals GEM's fate profile indicates high mobility with a lack of partitioning to particulate matter (K_{oc} = 1) and preference for the aqueous phase (log K_{ow} = -2.01 to 0.14). A biodegradation rate of ~30% highlights GEM as a priority chemical. The persistence of GEM in the environment is confirmed from studies detecting GEM in hospital effluents, STP wastewaters and receiving waters.

549 Etoposide (ET) is a plant alkaloid mitotic inhibitor derived from the root of the May apple
550 (*Podophyllum peltatum)*. Its main uses are to treat germ cell tumours, lung cancer, lymphomas,
551 leukemia and ovarian cancer. Consumption within the NW hospitals was only 1.2kg/yr, however,
552 ET has an average urinary excretion rate of 40%. The chemical fate profile indicates that ET will
553 have preference for the aqueous phase with negligible partitioning to particulate matter (log K_{ow}
554 = 0.04 to 1.97). To our knowledge no biodegradation data exists for ET, however, its
555 environmental persistence is confirmed by studies measuring its presence in hospital wastewaters
556 and STP wastewaters. Currently there are no studies measuring ET in receiving water, however,
557 Yin et al. (2010) measured a concentration of 3.4ng/L in wastewater effluent within China.

558 Ifosfamide (IF) is usually used to treat sarcoma, testicular cancer and some types of lymphomas.
559 IF is administered via injection or IV drip to both inpatients and outpatients although
560 consumption is likely to show a 'spike' on outpatient clinic days. IF is a structural analogue to
561 CP and its mechanism of action is presumed to be the same. In the surveyed hospitals 1.3kg/yr of
562 IF was consumed, of which ~26% is excreted as the parent drug in the patient's urine. The
563 chemical fate profile is similar to CP (K_{oc} = 51 & log K_{ow} = 0.1 to 0.97) and so it is also likely to
564 stay in the aqueous phase of the STP and be released to effluent waters. IF has also been shown
565 to be non-biodegradable using a STP simulation test and shows negligible loss for base-driven
566 hydrolysis, hence confirming the persistence of IF in the aquatic environment. IF has been
567 detected by a number of studies in hospital effluents, STP wastewaters and receiving waters.

568 Darcarbazine (DAC) is used in the treatment of metastatic malignant melanoma, Hodgkin's
569 disease, soft tissue sarcomas and neuroblastoma. DAC is a cell cycle non specific drug and
570 metabolic activation is required for antitumour activity. 0.75kg/yr DAC was consumed in the

571 NW hospitals, 36% of which is released unchanged in patients urine. In France, DAC
572 consumption increased by 58% from 2004 to 2008 (9kg/yr to 29kg/yr, respectively) [15]. The
573 chemical fate profile for DAC indicates its potential to resist sorption to sewage sludge during
574 treatment (K_{oc} = 15) and remain in the dissolved phase (log K_{ow} = -1.9 to 0.12). To our
575 knowledge no biodegradation data is available for this chemical and therefore we suggest further
576 assessment of this drug.

577 Treosulfan (TREO) is usually given to treat ovarian cancer and as a high dose treatment for
578 leukemia. In the NW hospitals reported in this study, 1.56kg/yr of TREO was consumed, of
579 which 22% is excreted unchanged. TREO is in its neutral form at environmentally relevant pHs
580 and is very highly mobile in soil (K_{oc} = 1) and has a low log K_{ow} ranging from -3.4 to -0.8, it is
581 unlikely that TREO will be lost during sewage treatment. Only a 10% biodegradation rate was
582 observed in activated sludge over 5 days confirming the persistence. Since TREO is often
583 administered in high doses (1 – 5g) it is expected to be periodically present in sewage effluents
584 and possibly detected in areas where local hospitals utilise this specialist chemotherapy.

585 7.1 Other anticancer drugs of potential concern

586 Of the 12 priority chemicals identified in this study, some of them had already been selected by
587 other prioritisation methods [15]. Other compounds such as mitotane, vinorelobine, paclitaxel,
588 nilotinib and lapatinib, while persistent, are not expected to be present in the dissolved phase of
589 the effluent, instead we predict that they may bioaccumulate or be retained in soil following
590 dispersion of sewage sludge onto farmland.

591 Mitotane is used to treat inoperable or advanced adrenocortical cancer, with a usual dose of 2-
592 10g/day in three or four divided doses. In the NW hospitals, mitotane consumption was 4.5kg/yr;
593 however, this value is from one hospital trust and may be the result of specialist chemotherapy
594 for this rare cancer. Mitotane is perhaps better known as *o,p'*-DDD, a constituent of the
595 insecticide DDT, withdrawn from the market due to its ability to bioaccumulate (log K_{oc} > 5) and
596 associated toxicity (e.g. endocrine disruption, mutagenic and carcinogenic effects) [69]. Its
597 endocrine effects have been demonstrated on certain fish species [69]. This chemical is a
598 persistent organic pollutant.

599 Paclitaxel (PAC) was consumed in the NW hospital at 0.86kg/yr with a urinary excretion rate of
600 6.5% for the un-metabolized drug. However, 61% of paclitaxel is seen in fecal excretion. This is
601 to be expected due to its high log K_{ow} value (over 5) and hence high K_{oc} (>5000) and this
602 hydrophobic compound is predicted to sorb to sludge and be effectively removed from the water
603 column by particle settling. It has been reported to have a 42% increase in consumption (Besse
604 et.al).

605 Lapatinib was consumed at relatively high amounts (4.6kg/yr) and has a low urinary excretion
606 rate for the unmetabolised drug, but similar to PAC, lapatinib has a high fecal excretion rate
607 (92%). Lapatinib is expected to be present in the sludge of STPs due to the high log D_{ow} of its
608 weak base, and hence high D_{oc} (>5000). Consumption of this drug has shown an increase from
609 2004 by some 116% from >1kg/yr (2004) to 116kg/yr (2008) [15]. Besse et al., (2012) reported
610 lapatinib as a priority drug likely to be present in the aquatic system. However, like other
611 anticancer drugs that are moderately persistent and posses' high K_{ow}/D_{ow} values, this compound
612 is likely to be present in the sludge following primary treatment.

613 The monoclonal antibodies rituximab, herceptin, cetuximab and bevacizumab may also be
614 present in sewage treatment effluents although these are protein-based antibodies and are likely
615 to be susceptible to biodegradation. However, there is a lack of research on their environmental
616 fate and impact, especially for herceptin which is a widely used drug for breast cancers.

617 CONCLUSIONS

618 There are a wide range of anti-cancer drugs used in chemotherapy with consumption on a
619 regional basis ranging from 10s to <1 kg/year. For those drugs which are only partially
620 metabolized then wastewater and release from STPs are the major route of entry into the wider
621 environment. From the hospitals surveyed in this study many of the anticancer drugs are
622 administered in outpatient clinics ensuring that release of anticancer drugs is diffusive across an
623 urban catchment and not restricted to point-release with waste effluents from individual
624 hospitals. Consumption data combined with rates of human metabolism provide an assessment of
625 the likelihood of occurrence of these drugs in wastewater streams and hence their presence in
626 STPs. Compiling physical-chemical property data, particularly K_{ow} and corresponding K_{oc}
627 values, combined with degradation behaviour allows the prioritization of a small number of
628 chemicals. These may be of environmental concern, in that they are present in final effluent and
629 hence can enter receiving waters or are likely to persist in sewage sludge. Of the 65 anticancer
630 drugs in use, approximately twelve drugs have been identified as sufficiently persistent to
631 warrant inclusion in environmental screening programmes. Concentrations measured in surface
632 waters for these chemicals are generally well below the EC_{50} values reported for a range of
633 aquatic organisms. However, we recommend that further work be conducted in this area,

particularly low dose ‘mixture’ effects, as well as understanding the environmental persistence and fate of key metabolites and transformation products.

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Table 1: Use/consumption data of anticancer drugs in hospitals in NW England as well as selected studies

Drug name	Consumption for NW England hospitals shown in g/year and (µg/capita/d)						Average (range) for NW hospitals (g/yr)	International studies	
	University hospitals of Morecombe Bay[2]	Lancashire teaching hospitals[3]	Blackpool teaching hospitals NHS foundation[4]	East Lancashire teaching hospitals[5]	Greater Manchester & Cheshire cancer network[6]	Clatterbridge centre for oncology[7]		France, 2008 [14] (kg/yr)	University Hospital, Geneva Switzerland [64] (g/yr)1
Cyclophosphamide	1300 (9.8)	610 (4.3)	1900 (15.8)	1300 (7.1)	1400 (2.6)	71000 (84.5)	12918(310-71000)	310	610
Chlorambucil	12 (0.1)	-	10 (0.1)	-	12 (0.0)	-	6 (10-12)	8	-
Melphalan	1 (0.0)	-	20 (0.2)	-	1 (0.0)	-	4 (1-20)	5	-
Ifosfamide	150 (1.1)	-	-	-	120 (0.2)	1000 (1.2)	423 (120-1000)	100	450
Bendamustine	-	-	1 (0.0)	-	91 (0.2)	-	15 (1-91)	-	-
Busulfan	<1	-	30 (0.2)	-	-	-	5 (<1-30)	-	-
Treosulfan	200 (1.5)	860 (6.0)	400 (3.3)	100 (0.5)	-	-	260 (100-860)	-	-
Carmustine	-	-	6 (0.0)	-	-	-	1 (6)	2	-
Lomustine	-	-	6 (0.0)	-	-	42 (0.0)	8 (6-42)	3	-
Temozolomide	-	120 (0.8)	-	-	-	900 (1.1)	170 (120-900)	54	-

Dacarbazine	83 (0.6)	36 (0.3)	64 (0.5)	60 (0.3)	50 (0.1)	460 (0.5)	126 (36-460)	29	-
Methotrexate	12 (0.1)	-	130 (1.1)	7 (0.0)	260 (0.5)	900 (1.1)	218 (7-900)	75	410
Pemetrexed	160 (1.2)	150 (1.1)	130 (1.1)	140 (0.8)	35 (0.1)	870 (1.0)	248 (35-870)	37	-
Mercaptopurine	16 (0.1)	-	26 (0.2)	-	90 (0.2)	-	22 (16-90)	95	-
Tioguanine	-	-	-	-	2 (0.0)	-	<1 (2)	2	-
Cladribine	<1	1 (0.0)	<1	-	<1	-	<1 (<1-1)	-	-
Fludarabine	11 (0.1)	-	9 (0.1)	-	16 (0.0)	-	6 (9-16)	6	6
Cytarabine	8 (0.1)	-	940 (7.8)	64 (0.4)	970 (1.8)	-	330 (8-970)	130	670
5-Fluorouracil	2500 (18.9)	3100 (21.8)	1800 (14.9)	790 (4.3)	1800 (3.4)	13000 (1.5)	3832 (790-13000)	1700	3100
Tegafur	-	58 (0.4)	-	-	-	58 (0.0)	19 (58)	37	-
Gemcitabine	1300 (9.8)	2300 (16.1)	860 (7.1)	1600 (8.8)	110 (0.2)	6800 (0.8)	2162 (110-6800)	380	660
Capecitabine	29000 (218.8)	64000 (449.3)	34000 (282.1)	-	-	230000 (27.4)	59500 (29000-230000)	5100	-
Azacitidine	18 (0.1)	-	11 (0.1)	28 (0.2)	17 (0.0)	-	12 (11-28)	-	-
Vinblastine	2 (0.0)	-	2 (0.0)	<1	1 (0.0)	-	1 (<1-2)	1	-

Vincristine	1 (0.0)	1 (0.0)	1 (0.0)	<1	1 (0.0)	<1	1 (<1-1)	□1	1
Vinorelbine	13 (0.1)	19 (0.1)	45 (0.4)	-	1 (0.0)	220 (0.0)	50 (1-220)	13	-
Etoposide	190 (1.4)	90 (0.6)	210 (1.7)	3 (0.0)	38 (0.1)	700 (0.1)	205 (3-700)	41	110
Paclitaxel	35 (0.3)	200 (1.4)	39 (0.3)	57 (0.3)	140 (0.3)	390 (0.0)	144 (35-390)	39	79
Docetaxel	58 (0.4)	74 (0.5)	44 (0.4)	53 (0.3)	43 (0.1)	260 (0.0)	89 (44-260)	27	43
Trabectedin	-	-	-	-	-	6 (0.0)	1 (6)	-	-
Dactinomycin	-	-	-	-	-	72 (0.0)	12 (72)	-	-
Doxorubicin	26 (0.2)	6 (0.0)	14 (0.1)	27 (0.1)	32 (0.1)	88 (0.0)	32 (6-88)	17	30
Daunorubicin	-	-	15 (0.1)	-	11 (0.0)	-	4 (11-15)	1	-
Epirubicin	87 (0.7)	95 (0.7)	70 (0.6)	96 (0.5)	61 (0.1)	44 (0.0)	76 (44-96)	18	41
Idarubicin	<1	-	1 (0.0)	-	1 (0.0)	-	<1 (<1-1)	<1	-
Mitoxantrone	<1	-	1 (0.0)	-	<1	-	<1 (<1-1)	<1	-
Bleomycin	1 (0.0)	4 (0.0)	2 (0.0)	<1	2 (0.0)	7 (0.0)	3 (<1 -4)	1	-
Mitomycin C	16 (0.1)	<1	11 (0.1)	13 (0.1)	3 (0.0)	<1	8 (<1-16)	3	9
Cisplatin	45 (0.3)	190 (1.3)	39 (0.3)	43 (0.2)	16 (0.0)	370 (0.0)	117 (16-370)	23	67
Carboplatin	320 (2.4)	950 (6.7)	540 (4.5)	600 (3.3)	15 (0.0)	2800 (0.3)	871 (15-2800)	84	330

Oxaliplatin	96 (0.7)	130 (0.9)	110 (0.9)	89 (0.5)	74 (0.1)	510 (0.1)	168 (74-510)	33	64
Procarbazine	24 (0.2)	-	-	-	3 (0.0)	120 (0.0)	25 (3-120)	35	-
Rituximab	-	120 (0.8)	320 (2.7)	-	310 (0.6)	18 (0.0)	128 (18-320)	72	-
Trastuzumab	250 (1.9)	370 (2.6)	160 (1.3)	260 (1.4)	-	2000 (0.2)	507 (160-2000)	56	-
Alemtuzumab	-	-	-	-	30 (0.1)	-	5 (30)	-	-
Cetuximab	17 (0.1)	110 (0.8)	57 (0.5)	-	-	270 (0.0)	76 (17-270)	55	-

"-" = not recorded

[1] Calculated using the average dose (mg) from NW hospital survey

[2] Comprising of Furness general hospital, Royal Lancaster Infirmary, Westmorland general hospital, Queen Victoria hospital, Ulverston community health centre. Population served 363,000

[3] Comprising of Royal Preston hospital (Rosemere cancer foundation) and Chorley and South Ribble hospital. Population served 390,000

[4] Comprising of Blackpool Victoria hospital, Clifton hospital, Fleetwood hospital and three elderly rehabilitation hospitals. Population served 330,000

[5] Comprising of Burnley general hospital, Royal Blackburn hospital and Inpatient rehabilitation services are also provided at Pendle community hospital and the Rakehead unit at Burnley general hospital. Outpatient and diagnostic services are also provided at the Accrington Victoria, Clitheroe hospital, Rossendale and St Peters Primary health care centre's. Population served 500,000

[6] Comprising of NHS Trafford (Trafford general hospital host to the Trafford Macmillan care centre); Bolton NHS Foundation trust (Royal Bolton hospital); Salford Royal NHS Foundation trust (Salford Royal hospital); Mid Cheshire hospitals NHS foundation trust (Leighton hospital, Victoria Infirmary and Elmhurst intermediate care centre); Wrightington, Wigan and Leigh NHS foundation trust (Royal Albert Edward Infirmary, Leigh Infirmary, Wrightington hospital and Thomas Linacre centre (provides the majority of out-patient services for the Trust). Population served 1,463,000

[7] Comprising of outpatient clinics at Linda McCartney centre (Royal Liverpool University hospital), the Countess of Chester, Southport hospital, Halton general hospital, Aintree University hospital, Broadgreen hospital and The Liverpool Woman's. Population served 2,300,000

Table 2: Average urinary excretion rates of the unchanged parent drug

% of administered drug excreted (i.e. not metabolized)					
<5%	5-15%	15-25%	25-45%	45-75%	>75%
Chlorambucil[1, 2, 3, 4] [70] Busulfan[1, 2, 5] [71] Lomustine[1, 2] Capecitabine[1, 2] [43] Trabectedin[1, 2] [72] Idarubicin[2] [73] Gefitinib[1, 2] Sorafenib[1, 2] Dasatinib[1] Laptinib[1] [74] Nilotinib[2, 5] Temsirolimus[1, 2] Everolimus[1, 2] Pazopanib[1, 2] Bortezomib*** Trastuzumab*** Alemtuzumab*** Rituximab*** Cetuximab*** Bevacizumab***	Temozolomide[1, 2] [2, 75] Mercaptopurine[1, 2] Cytarabine[2] Gemcitabine[1] [76] Vinblastine[2] [77] Vincristine[2,] [78] Vinorelbine[1, 2] Paclitaxel[1, 2] Docetaxel[1, 2] Doxorubicin[1, 2, 3] [3] Epirubicin[1, 2] [3] Mitoxantrone[1, 2] Mitomycin[1, 2] Procarbazine[2, 4] Imatinib[1, 2] Erlotinib[1, 2] Mitotane[1, 2, 4, 6] [79] Eribulin[1]	Cyclophosphamide[1, 2, 3] [3, 80-83] Melphalan[1, 2] [84] Bendamustine[1] Treosulfan [85-88] Tioguanine [89, 90] 5-Fluorouracil[1, 2] [3, 43, 76] Tegafur[1] Dactinomycin[1] Sunitinib[1, 2] [91] Irinotecan[1, 2]	Ifosfamide[2, 3] [83, 92, 93] Dacarbazine[1, 2] Cladribine[1, 2] Fludarabine[1, 2] Etoposide[1, 2, 3] Daunorubicin[1, 2] [94] Cisplatin[1] [2] Oxaliplatin[1, 2] [2] Topotecan[1, 2]	Carmustine[1, 2] Azacitidine[2] Bleomycin[1, 2] Carboplatin[1, 2] [2] Hydroxyurea[1, 2] Tretinoin[1, 2]	Methotrexate[2, 3] [95] Pemetrexed[1, 2, 3] [96]

[1] electronic Medicines Compendium (eMC) (http://www.medicines.org.uk/EMC)
[2] Product monograph (http://www.bccancer.bc.ca/HPI/DrugDatabase/DrugIndexPro/default.htm)
[3] RxList (http://www.rxlist.com/script/main/hp.asp)
[4] Drugs.com (http://www.drugs.com/)
[5] Ema (http://www.ema.europa.eu/ema/)
[6]ToxNet (http://toxnet.nlm.nih.gov/)
*** No available data (Urinary excretion data was not available for six L01X anticancer drugs)

Table 3: Physiochemical properties of the investigated cytotoxic drugs and classification according to the Anatomical Therapeutic Classification (ATC) system

ATC	Drug name	pKa	Charge at pH 7.4	Weak acid/weak base	Log Kow	Log Dow at pH 7.4	Koc[4]	BCF[4]	Solubility in water (mg/L) at 25°C[4]
L01AA01	Cyclophosphamide	2.84, 6.00 [97, 98]	Neutral	Acid [98]	0.63		44	3	4.00E+04
L01AA03	Melphalan	1.83, 9.13 [99]	Neutral	Acid [100]	-0.52		14	3	2.71E+02
L01AA05	Ifosfamide	1.45-4.0 [97, 98]	Neutral	Base [98]	0.86		51	3	3.78E+03
L01AA09	Bendamustine	0.88, 4.17, 6.94[5]	Negative	Zwitterion/Acid		2.84	977	3	2.69E+01
L01AB02	Treosulfan	12.36*	Neutral	Acid	-2.09*		1	3	7.00E+04[8]
L01AD01	Carmustine	12.27*	Neutral	Acid	1.53		89	5	1.83E+03
L01AX03	Temozolomide	N/A [101]	Neutral	Base	1.15		29	3	1.81E+03
L01AX04	Dacarbazine	4.42*	Neutral	Base	-0.24		15	10	4.22E+03
L01BA01	Methotrexate	3.80, 4.8, 5.6 [102]	Negative	Acid		-1.41	20	3	4.98E+03
L01BA04	Pemetrexed	3.6, 4.4 [102]	Negative	Acid		-2.43	60	3	1.84E+02
L01BB02	Mercaptopurine	7.9 [103]	Neutral	Acid [103]	0.67 [103]		40	3	1.98E+04
L01BB05	Fludarabine	3.2, 5.8*	Negative	Acid		-1.22	2	3	1.44E+04
L01BC01	Cytarabine	4.2 [5]	Neutral	Base	-2.15		1	3	8.66E+04
L01BC02	5-Fluorouracil	7.6-8.0, 13.0 [5, 98, 102]	Neutral	Acid [98]	-0.93		4	3	1.11E+04
L01BC03	Tegafur	7.98[1]	Neutral	Acid	-0.27		6	3	3.64E+03
L01BC05	Gemcitabine	3.6 [5]	Neutral	Base	-1.24		1	3	1.53E+04[6]
L01BC06	Capecitabine	8.8 [102]	Neutral	Acid	0.96*		8	3	8.23E+02
L01BC07	Azacitidine	2-3 [104]	Neutral	Base	-2.17		1	3	8.89E+04
L01CA04	Vinorelbine	7.4, 5.4 [105, 106]	Positive	Base	4.72*	4.57	30200	604	9.86E-03
L01CB01	Etoposide	9.8 [107]	Neutral	Acid	0.60		19	3	5.87E+01
L01CD01	Paclitaxel	11.99	Neutral	Zwitterion [98]	5.25		58884	750	1.07E-04
L01CD02	Docetaxel	12.02*	Neutral	Acid	3.64*		27	65	5.17E-03
L01DA01	Dactinomycin	8.06 [108]	Neutral	Acid	1.42*				

L01DB01	Doxorubicin	7.34, 8.3, 9.46 [97, 102, 109]	Positive	Base [98]		-1.93	389	3	5.34E+02
L01DB02	Daunorubicin	8.4 [97, 109]	Positive	Base [98]		-0.14	490	1	1.23E+02
L01DB03	Epirubicin	7.7 [97]	Positive	Base*		-0.30	372	4	6.25E+02
L01DC01	Bleomycin	7.3 [110]	Positive	Base		-0.47			
L01DC03	Mitomycin C	3.2	Neutral	Base	-0.38		76	3	8.11E+03
L01XA01	Cisplatin	6.6, 5.5, 7.3 [111]			-2.40	-2.19	12589 [68]		
L01XA02	Carboplatin	0.24, 3.55*		Base	-1.78	0.01	891 [68]		
L01XA03	Oxaliplatin	7.35, 9.99*		Base	-1.63	-1.42			
L01XB01	Procarbazine	6.8*	Neutral	Base	-0.82*		18	3	8.32E+03
L01XE01	Imatinib	8.07, 3.73, 2.56, 1.52*	Positive	Base		0.19	16	3	6.48E+01
L01XE03	Erlotinib	5.42*	Neutral	Base	2.96*		2188	42	9.97E+00
L01XE04	Sunitinib	8.95*	Positive	Base		0.76	891	29	1.52E+01
L01XE05	Sorafenib	11.55, 2.03[2]*	Neutral	Base/Acid	4.39*		2884	366	2.14E-01
L01XE07	Lapatinib	3.80, 7.20[2]*	Positive	Base		4.72	426580	1127	9.06E-02
L01XE08	Nilotinib	2.1, 5.4[1]	Neutral	Base	3.60		79433	110	3.90E-01
L01XE11	Pazopanib	2.1, 6.4, 10.2[3]	Neutral	Base/Acid	3.38*		12023	79	2.32E+00
L01XX05	Hydroxyurea	10.6*	Neutral	Acid	-1.27		3	3	7.91E+04
L01XX14	Tretinoin	5.0*	Negative	Acid		4.30			
L01XX17	Topotecan	0.60, 6.99, 10.50 [112]	Positive	Base/Acid		-3.13	107	3	3.30E+02
L01XX19	Irinotecan	8.1*	Positive	Base		3.24	2818	355	3.64E-02
L01XX23	Mitotane	N/A	Neutral	N/A	6.11*		154882	4989	1.00E-01
L01XX32	Bortezomib	13.82*	Neutral	Acid	1.47*		766	4	2.13E+02
L01XX41	Eribulin	9.59[2]	Positive	Base		-0.34	1288	14	2.70E+00

[1] www.ema.europa.eu/docs/en_GB/.../WC500034398.pdf
[2] ACD properties calculator (http://www.chemicalize.org/structure)
[3] http://www.medicines.org.au/files/gwpvotri.pdf
[4] BCF predicted with EPI SUITE: linear relationship with kow does not hold for many compounds with high polarity (see text)
[5] http://www.faqs.org/patents/app/20090264488
[6] MSDS gemcitabine (http://ehs.lilly.com/msds/Gemzar.pdf)

[7] MSDS imatinib (http://export.fass.se/pdfprint/servlet/se.itsip.pdfprint.servlets.ConvertServlet?nplId=20031111000058&docTypeId=78&userType=2&paraImported=null&orgNplId=null&showParaLink=null&hasEnvSection=yes&paraInfo=null&orgCompany=null&docId=ID18IILYT1XUZ1XGCS_IDX0000000180&fontSize=standard)

[8] MSDS treosulfan (http://www.medac.de/medac_international/data/SDS/treosulfan_E.pdf)

*From predicted data

** Calculated as an average, with consideration to dastinib a similar compound to basic ionization

Table 4: Degradative loss processes: biodegradation and hydrolysis

Drug Name	Biodegradation				Hydrolysis			% STP total removal (EPI suite)	Ref.
	Test	Incubation (days)	Initial conc.	Results	Log k_{OH} (L/mole.s)[1]	% loss in 5days			
						at pH 7.0	at pH 8.1		
Cyclophosphamide	ZWT[2] (OECD 302B)	28	51.7mg/L	No degradation	-8.458	Neg.	Neg.	1.86	[21]
	OECD confirmatory	10	375, 750mg/L	0±5% degradation					[34]
	CBT[3] (OECD 1992)	40	4.3mg/L	28-66% degradation in 40 days. Chemical structurally related to CP					[35]
	ZWT[2] (OECD 1992)	40	200mg/L	5-72% degradation in 28 days. Chemical structurally related to CP					[35]
	AS incubation	1	90, 900ng/L	No degradation					[7]
Ifosfamide	ZWT[2] (OECD 302B)	42	51.7mg/L	No degradation	-7.397	Neg.	Neg.	1.88	[37]
	STP simulation test (OECD 1992)	42	11.4µg/L	Negligible					[37]
	CBT[3] (OECD 1992)	40	4.3mg/L	28-66% degradation in 40 days. Chemical					[35]

				structurally related to IF					
	ZWT[2] (OECD 1992)	40	200mg/L	5-72% degradation in 28 days. Chemical structurally related to IF					[35]
Treosulfan	CBT[3] (OECD 301D)	40	5mg/L	30% degradation in the first 28 days (40% in 40 days)	N/A	N/A	N/A	1.85	[39]
Methotrexate	OECD confirmatory (AS incubation)	10	10, 20mg/L	98±6% degradation. > 10% in the first four days	N/A	N/A	N/A	1.85	[34]
Pemetrexed	Ready biodegradability	29	N/A	20% was released as CO^2	N/A	<10% (at 50°C)[4]	N/A	1.85	MSDS[4]
	Biodegradation (sludge)	1	N/A	>99% disappearance when incubated with 1.5g/L sludge solids. After 1 hour incubation 90% of pemetrexed had degraded					MSDS[4]
Fludarabine	N/A	N/A	N/A	N/A	-4.097	Neg.	Neg.	1.85	N/A
Cytarabine	OECD confirmatory AS incubation	10	12.5, 25mg/L	60±8% degradation, 2 days = ~10%	N/A	N/A	N/A	1.85	[34]
	CBT[3] (OECD 301D)	40	N/A	50% degradation after 20days					[42]

	ZWT[2] (OECD 302B)	40	N/A	>95% degradation HOW MANY DAYS?					[42]
5-fluorouracil	CBT[3] (OECD 301D)	28 and 40	9.02mg/L	No degradation	N/A	N/A	N/A	1.85	[42]
	ZWT[2] (OECD 302B)	28	854mg/L	2% degradation					[42]
	OECD 303A confirmatory AS incubation	10	5, 10, 20mg/L	100±4% (15% after 1 day and a sharp increase to 100% on day 2) Higher degradation rate with lower concentrations					[34]
	AS incubation	50	50ug/L	<60% removal after 50 days					[113]
	AS incubation	1	5, 500ug/L	Complete degradation					[19]
	ZWT[2] (0ECD 302B)	21	270mg/L	No degradation, using pre-adapted AS					[43]
	Inherent biodegradation test, 4g AS/L and closed test vessels	14	0.2, 11.4mg/L	97.5->100% degradation. > 25% Biodegradation in 1day					[43]
	OECD 303A	3	10mg/L	38-92% biodegradation					[43]
Gemcitabine	CBT[3] (OECD 301D)	40	1660mg/L	45% Degradation	N/A	Neg.[5]	N/A	1.85	[42]
	ZWT[2] (OECD 302B)	40	1660mg/L	50% degradation					[42]
	Aerobic	28		30%					MSDS[5]

	biodegradation			degradation					
Capecitabine	ZWT[2] (OECD 303B)	28	N/A	58% degradation (15% removed in 7 days)	N/A	N/A	N/A	1.85	[43]
	Like OECD 302C	21	30mg/L	41% mineralization, 27% mineralization in 14 days					[43]
	Like OECD 302C	84		55-66% mineralization, 29% mineralization in 28 days					[43]
Vinblastine	CBT[3]	28	N/A	10% Degradation	0.016/-0.835/-1.261	0.63-4.48	7.95-56.40	NOT ON LIST	[38]
	ZWT[2]	40	N/A	18% degradation					[38]
Vincristine	CBT[3]	28	N/A	30% degradation	-0.788/0.149	0.70-6.09	8.86-76.60	NOT ON LIST	[38]
Vinorelbine	N/A	N/A	N/A	N/A	-0.844/0.004/-1.261	0.24-4.36	2.98-54.90	66.90	N/A
Etoposide	N/A	N/A	N/A	N/A	-2.689	0.01	0.11	1.86	N/A
Paclitaxel	N/A	N/A	N/A	N/A	-0.297/-1.669/0.000/-0.433	0.09-4.32	1.17-54.40	84.19	N/A
Docetaxel	N/A	N/A	N/A	N/A	-0.317/-1.689/-0.452/4.667	0.00-2.08	0.00-26.20	16.63	N/A
Doxorubicin	AS incubation	1	2500ug/L	48-74% degradation (20-40% recovered in sludge, 6-	N/A	N/A	N/A	1.85	[19]

				12% recovered in liquid phase). Degraded mainly due to adsorption to sludge					
Epirubicin	CBT[3] (OECD 301D)	N/A	5mg/L	No degradation	N/A	N/A	N/A	1.85	[6]
	ZWT[2] (OECD 302B)	N/A	N/A	Degraded, mainly due to adsorption to sludge					[6]
	ZWT[2] (OECD 302B) CBT[3] (OECD 301D)	N/A	N/A	Eliminated in ZWT but not in CBT					[19]
Mitoxantron	CBT[3] (OECD 301D)	40	5mg/L	No degradation	N/A	N/A	N/A		[39]
Mitomycin	N/A	N/A	N/A	N/A	-2.218	0.03	0.33	1.85	N/A
Cisplatin	OECD screening test	21	0, 0.32, 1.6mg/L	0±2% Degradation	N/A	N/A	N/A	N/A	[34]
Imatinib	Aerobic, 92/69/EC (L383) C.4-C	28	N/A	9-12%; not readily biodegradable	N/A	N/A	N/A	1.85	MSDS[6]
Topotecan	N/A	N/A	N/A	N/A	-0.986	0.45	5.62	1.85	N/A
Irinotecan	N/A	N/A	N/A	N/A	-1.127/0.000	0.32-4.32	4.06-54.40	8.33	N/A

Neg. – negligible
N/A – not available
AS – activated sludge
STP – sewage treatment plant
[1] Second order rate constant estimated using the SPARC model (http://archemcalc.com/sparc/test/login.cfm?CFID=250050&CFTOKEN=79322869)
[2] ZWT - Zahn-Wellens Test (test for inherent biodegradability – OECD302)

[3] CBT – Closed Bottle Test (OECD 301)
[4] MSDS pemetrexed (http://ehs.lilly.com/msds/Alimta.pdf)
[5] MSDS gemcitabine (http://ehs.lilly.com/msds/Gemzar.pdf)
[6] MSDS imatinib (http://export.fass.se/pdfprint/servlet/se.itsip.pdfprint.servlets.ConvertServlet?nplId=20031111000058&docTypeId=78&userType=2&paraImported=null&orgNplId=null&showParaLink=null&hasEnvSection=yes&paraInfo=null&orgCompany=null&docId=ID18IILYT1XUZ1XGCS_IDX0000000180&fontSize=standard)

Table 5: Measured concentrations of anticancer drugs in wastewaters and receiving waters

Drug Name	Concentration ng/L			
	Hospital effluent	STP Influent	STP effluent	Receiving waters
Cyclophosphamide	<2-4500 [4, 21, 36, 60]	<6-143 [7, 8, 21]	<6-<20 [7, 9, 21, 62]	2.2-10.1 [7, 64, 65]
Ifosfamide	<2 -10647 [4, 36, 37, 114]	<0.3-13100 [7, 11, 37, 114, 115]	1.2-2900 [7, 11, 37, 62]	<0.5-41 [7, 64]
Gemcitabine	<0.9 – 38 [5]	9.3 [11]	7.0 [11]	2.4 [11]
Cytarabine		9.2 [11]	14 [11]	13 [11]
Bleomycin			11-19 [10, 63]	<5-17 [10, 63]
Tamoxifen			42-740 [116-118]	25[117]
Methotrexate	<2 – 4689 [4, 59]	59 [8]	12.6 [9]	
Doxorubicin	100-1350 [6, 19]	4.5 [11]		
Vinorelbine		9.1 [11]		
5-fluorouracil	<5 – 124000 [5, 19, 61]			
Epirubicin	100-1400 [6]			

Table 6: Predicted anticancer drugs likely to be present in sewage effluent in England based on 2010-2012 NW consumption values. Predictions assume mean excretion of unchanged drug and minimum a 'best value' to predict sewage treatment plant removal rates.

ATC	Drug	Consumption (kg/year)[1]	Consumption (µg/capita/d)[2]	Excretion of original drug %[3]	Predicted influent load (µg/capita/d)	Predicted % of intact drug after STP biodegradation[4]	Predicted load after STP biodegradation (µg/capita/d)	Predicted effluent conc. (ng/L)[5]	Predicted river water conc. (ng/L)[6]
L01AA01	Cyclophosphamide	77.51	40	21	8.4	98.1*	8.3	41.3	4
L01BC06	Capecitabine	357.00	183	3	5.4	85.0	4.6	23.1	2
L01BC02	Fluorouracil	22.99	12	18	2.1	85.0	1.8	8.9	1
L01XX05	Hydroxyurea	64.00	33	58	18.8	5.0	0.9	4.7	0
L01XE01	Imatinib	20.40	10	9	0.9	98.2*	0.9	4.6	0
L01BA01	Methotrexate	1.31	1	83	0.6	90.0	0.5	2.5	0
L01XA02	Carboplatin	5.23	3	54	1.4	30.0	0.4	2.2	0
L01BC05	Gemcitabine	12.97	7	8	0.5	70.0	0.4	1.8	0
L01CB01	Etoposide	1.23	1	43	0.3	98.1*	0.3	1.3	0
L01XA03	Oxaliplatin	1.01	1	40	0.2	100.0**	0.2	1.0	0
L01AA06	Ifosfamide	1.27	1	26	0.2	98.1*	0.2	0.8	0
L01AX04	Dacarbazine	0.75	0	36	0.1	98.2*	0.1	0.7	0
L01AB02	Treosulfan	1.56	1	22	0.2	70.0	0.1	0.6	0
L01XA01	Cisplatin	0.70	0	33	0.1	98.2*	0.1	0.6	0
L01XE03	Erlotinib	4.14	2	6	0.1	94.6*	0.1	0.6	0
L01BC01	Cytarabine	1.98	1	10	0.1	90.0	0.1	0.5	0
L01BA04	Pemetrexed	1.49	1	80	0.6	10.0	0.1	0.3	0
L01XE04	Sunitinib	0.70	0	16	0.1	98.1*	0.1	0.3	0
L01XX19	Irinotecan	0.56	0	16	0.0	91.7*	0.0	0.2	0
L01AX03	Temozolomide	1.02	1	7	0.0	98.1*	0.0	0.2	0
L01BC07	Azacitidine	0.07	0	68	0.0	98.2*	0.0	0.1	0
L01DB03	Epirubicin	0.45	0	11	0.0	98.2*	0.0	0.1	0
L01CD02	Docetaxel	0.53	0	7	0.0	83.4*	0.0	0.1	0
L01BC03	Tegafur	0.12	0	20	0.0	98.2*	0.0	0.1	0
L01DB01	Doxorubicin	0.19	0	14	0.0	80.0	0.0	0.1	0

L01XX23	Mitotane	4.50	2	6	0.1	7.4*	0.0	0.1	0
L01XE07	Lapatinib	4.60	2	1	0.0	33.1*	0.0	0.1	0
L01AA09	Bendamustine	0.09	0	20	0.0	95.5*	0.0	0.0	0
L01XB01	Procarbazine	0.15	0	11	0.0	98.2*	0.0	0.0	0
L01CA04	Vinorelbine	0.30	0	13	0.0	33.1*	0.0	0.0	0
L01DA01	Dactinomycin	0.07	0	17	0.0	100.0**	0.0	0.0	0
L01BB05	Fludarabine	0.04	0	34	0.0	98.2*	0.0	0.0	0
L01XE08	Nilotinib	0.57	0	2	0.0	84.5*	0.0	0.0	0
L01DC01	Bleomycin	0.02	0	62	0.0	100.0**	0.0	0.0	0
L01XX17	Topotecan	0.03	0	33	0.0	98.2*	0.0	0.0	0
L01CD01	Paclitaxel	0.86	0	7	0.0	15.8*	0.0	0.0	0
L01XX14	Tretinoin	0.02	0	63	0.0	54.5*	0.0	0.0	0
L01DB02	Daunorubicin	0.03	0	25	0.0	98.2*	0.0	0.0	0
L01XX41	Eribulin	0.06	0	9	0.0	98.2*	0.0	0.0	0
L01XE05	Sorafenib	10.28	5	0	0.0	49.9*	0.0	0.0	0
L01XX32	Bortezomib	0.00	0	100	0.0	98.0*	0.0	0.0	0
L01DC03	Mitomycin	0.04	0	10	0.0	98.2*	0.0	0.0	0
L01AA03	Melphalan	0.02	0	20	0.0	98.2*	0.0	0.0	0
L01AD01	Carmustine	0.01	0	57	0.0	98.0*	0.0	0.0	0
L01XE11	Pazopanib	0.05	0	4	0.0	89.4*	0.0	0.0	0
L01BB02	Mitoxantrone	0.00	0	7	0.0	100.0**	0.0	0.0	0

[1] Consumption total of NW survey

[2] Based on NW population of 5346000 from the populations each hospital serves

[3] Mean excretion rate taken from *n* clinical studies

[4] Estimated from EPISUITE biowin model or from biodegradation data table 4. Predictions based on literature values are shown with an asterisk. Where no EPISUITE prediction or literature value could be obtained it was presumed that 100% of the drug remained intact.

[5] 200L/head dilution expected in STP [3]

[6] Further 10-fold dilution in the river [3]

* Predicted from EPISUITE

** No rate available

REFERENCES

1. Jones, O.A., J.N. Lester, and N. Voulvoulis, *Pharmaceuticals: a threat to drinking water?* Trends in Biotechnology, 2005. **23**(4): p. 163-167.
2. Rowney, N.C., A.C. Johnson, and R.J. Williams, *Cytotoxic drugs in drinking water: A prediction and risk assessment exercise for the thames catchment in the United Kingdom.* Environmental Toxicology and Chemistry, 2009. **28**(12): p. 2733-2743.
3. Johnson, A.C., et al., *Do cytotoxic chemotherapy drugs discharged into rivers pose a risk to the environment and human health? An overview and UK case study.* Journal of Hydrology, 2008. **348**(1-2): p. 167-175.
4. Yin, J., et al., *A Preliminary Study on the Occurrence of Cytostatic Drugs in Hospital Effluents in Beijing, China.* Bulletin of Environmental Contamination and Toxicology, 2010. **84**(1): p. 39-45.
5. Kovalova, L., C.S. McArdell, and J. Hollender, *Challenge of high polarity and low concentrations in analysis of cytostatics and metabolites in wastewater by hydrophilic interaction chromatography/tandem mass spectrometry.* Journal of Chromatography A, 2009. **1216**(7): p. 1100-1108.
6. Mahnik, S.N., et al., *Development of an analytical method for the determination of anthracyclines in hospital effluents.* Chemosphere, 2006. **65**(8): p. 1419-1425.
7. Buerge, I.J., et al., *Occurrence and Fate of the Cytostatic Drugs Cyclophosphamide and Ifosfamide in Wastewater and Surface Waters†.* Environmental Science & Technology, 2006. **40**(23): p. 7242-7250.
8. Araceli Garcia-Ac, a.P.A.S., a Christian Gagnonb and S ebastien Sauv e*, *Determination of bezafibrate, methotrexate, cyclophosphamide, orlistat and enalapril in waste and surface waters using on-line solid-phase extraction liquid chromatography coupled to polarity-switching electrospray tandem mass spectrometry.* Journal of Environmental Monitoring, 2009. **11**: p. 830-838.
9. Castiglioni, S., et al., *A multiresidue analytical method using solid-phase extraction and high-pressure liquid chromatography tandem mass spectrometry to measure pharmaceuticals of different therapeutic classes in urban wastewaters.* Journal of Chromatography A, 2005. **1092**(2): p. 206-215.
10. Aherne, G.W., A. Hardcastle, and A.H. Nield, *Cytotoxic drugs and the aquatic environment: estimation of bleomycin in river and water samples.* Journal of Pharmacy and Pharmacology, 1990. **42**(10): p. 741-742.
11. Martín, J., Camacho-Muñoz, D., Santos, J. L., Aparicio, I. and Alonso, E., *Simultaneous determination of a selected group of cytostatic drugs in water using high-performance liquid chromatography–triple-quadrupole mass spectrometry.* J. Sep. Science, 2011. **34**: p. 3166-3177.
12. Caley, A. and R. Jones, *The principles of cancer treatment by chemotherapy.* Surgery (Oxford), 2012. **30**(4): p. 186-190.
13. Lambert, J.C. and J.C. Lipscomb, *Mode of action as a determining factor in additivity models for chemical mixture risk assessment.* Regulatory Toxicology and Pharmacology, 2007. **49**(3): p. 183-194.
14. Llewellyn, N., et al., *Determination of cyclophosphamide and ifosfamide in sewage effluent by stable isotope-dilution liquid chromatography–tandem mass spectrometry.* Journal of Chromatography A, (0).

15. Besse, J.-P., J.-F. Latour, and J. Garric, *Anticancer drugs in surface waters: What can we say about the occurrence and environmental significance of cytotoxic, cytostatic and endocrine therapy drugs?* Environment International, 2012. **39**(1): p. 73-86.
16. Kovalova, L., *Cytostatics in the aquatic environment: analysis, occurence, and possibilities for removal*, 2009, Aachen University.
17. Ardizzoni, A., et al., *The combination of etoposide and cisplatin in non-small-cell lung cancer (NSCLC).* Annals of Oncology, 1999. **10**(suppl 5): p. S13-S17.
18. *Chemspider*. Available from: http://www.chemspider.com.
19. Mahnik, S.N., et al., *Fate of 5-fluorouracil, doxorubicin, epirubicin, and daunorubicin in hospital wastewater and their elimination by activated sludge and treatment in a membrane-bio-reactor system.* Chemosphere, 2007. **66**(1): p. 30-37.
20. Leung, H.W., et al., *Distribution, fate and risk assessment of antibiotics in sewage treatment plants in Hong Kong, South China.* Environment International, 2012. **42**(0): p. 1-9.
21. Steger-Hartmann, T., K. Kümmerer, and A. Hartmann, *Biological Degradation of Cyclophosphamide and Its Occurrence in Sewage Water.* Ecotoxicology and Environmental Safety, 1997. **36**(2): p. 174-179.
22. Hörsing, M., et al., *Determination of sorption of seventy-five pharmaceuticals in sewage sludge.* Water Research, 2011. **45**(15): p. 4470-4482.
23. Yamamoto, H., et al., *Persistence and partitioning of eight selected pharmaceuticals in the aquatic environment: Laboratory photolysis, biodegradation, and sorption experiments.* Water Research, 2009. **43**(2): p. 351-362.
24. Radjenovic, J., M. Petrovic, and D. Barceló, *Analysis of pharmaceuticals in wastewater and removal using a membrane bioreactor.* Analytical and bioanalytical chemistry, 2007. **387**(4): p. 1365-1377.
25. Graham, M.A., et al., *Clinical Pharmacokinetics of Oxaliplatin: A Critical Review.* Clinical Cancer Research, 2000. **6**(4): p. 1205-1218.
26. de Kogel, C.E. and J.H.M. Schellens, *Imatinib.* The Oncologist, 2007. **12**(12): p. 1390-1394.
27. Fleming, R.A., et al., *Factors affecting in vitro protein binding of etoposide in humans.* Journal of Pharmaceutical Sciences, 1992. **81**(3): p. 259-264.
28. Pernkopf, I., et al., *[Binding of epirubicin to human plasma protein and erythrocytes: interaction with the cytoprotective amifostine]*. Pharmazie, 1996. **51**(11): p. 897-901.
29. Urien, S., et al., *Vinorelbine high-affinity binding to human platelets and lymphocytes: distribution in human blood.* Cancer Chemotherapy and Pharmacology, 1993. **32**(3): p. 231-234.
30. Chassany, O., et al., *Comparative serum protein binding of anthracycline derivatives.* Cancer Chemotherapy and Pharmacology, 1996. **38**(6): p. 571-573.
31. Bagley, C.M., F.W. Bostick, and V.T. DeVita, *Clinical Pharmacology of Cyclophosphamide.* Cancer Research, 1973. **33**(2): p. 226-233.
32. Villarroel, M., et al., *Plasma protein binding of sorafenib, a multi kinase inhibitor: in vitro and in cancer patients.* Investigational New Drugs, 2012. **30**(6): p. 2096-2102.
33. McCall, P.J., et al., *Estimation of chemical mobility in soil from liquid chromatographic retention times.* Bulletin of Environmental Contamination and Toxicology, 1980. **24**(1): p. 190-195.

34. Kiffmeyer, T., et al., *Trace enrichment, chromatographic separation and biodegradation of cytostatic compounds in surface water.* Fresenius' Journal of Analytical Chemistry, 1998. **361**(2): p. 185-191.
35. Kümmerer, K., et al., *Biodegradability of antineoplastic compounds in screening tests: influence of glucosidation and of stereochemistry.* Chemosphere, 2000. **40**(7): p. 767-773.
36. StegerHartmann, T., K. Kummerer, and J. Schecker, *Trace analysis of the antineoplastics ifosfamide and cyclophosphamide in sewage water by two-step solid-phase extraction and gas chromatography mass spectrometry.* Journal of Chromatography A, 1996. **726**(1-2): p. 179-184.
37. Kümmerer, K., T. Steger-Hartmann, and M. Meyer, *Biodegradability of the anti-tumour agent ifosfamide and its occurrence in hospital effluents and communal sewage.* Water Research, 1997. **31**(11): p. 2705-2710.
38. Al-Ahmad, A. and K. Kummerer, *Biodegradation of the antineoplastics vindesine, vincristine, and vinblastine and their toxicity against bacteria in the aquatic environment.* Cancer Detection and Prevention, 2001. **25**(1): p. 102-107.
39. AlAhmad, A., K. Kummerer, and G. Schon, *Biodegradation and toxicity of the antineoplastics mitoxantron hydrochloride and treosulfane in the closed bottle test (OECD 301 D).* Bulletin of Environmental Contamination and Toxicology, 1997. **58**(5): p. 704-711.
40. Wang, L., et al., *Cyclophosphamide removal from water by nanofiltration and reverse osmosis membrane.* Water Research, 2009. **43**(17): p. 4115-4122.
41. EMA. *Alimta : EPAR - Scientific discussion*. 2006 [cited 2011 02/06/2011]; Available from: http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Scientific_Discussion/human/000564/WC500025606.pdf.
42. Kummerer, K. and A. Al-Ahmad, *Biodegradability of the anti-tumour agents 5-fluorouracil, cytarabine, and gemcitabine: Impact of the chemical structure and synergistic toxicity with hospital effluent.* Acta hydrochimica et hydrobiologica, 1997. **25**(4): p. 166-172.
43. Straub, J.O., *Combined environmental risk assessment for 5-fluorouracil and capecitabine in Europe.* Integrated Environmental Assessment and Management, 2010. **6**(S1): p. 540-566.
44. K. -P. Henschela, A.W., M. Diedricha and A. Fliednerb, *Environmental Hazard Assessment of Pharmaceuticals.* Regulatory Toxicology and Pharmacology, 1997. **25**(3): p. 220-225.
45. Kosjek, T. and E. Heath, *Occurrence, fate and determination of cytostatic pharmaceuticals in the environment.* TrAC Trends in Analytical Chemistry, 2011. **30**(7): p. 1065-1087.
46. *Methods for assessing the effects of mixtures of chemicals / edited by Velimir B. Vouk ... [et al.] ; prepared by Scientific Group on Methodologies for the Safety Evaluation of Chemicals (SGOMSEC)*. IPCS joint symposia ; 6, ed. V.B. Vouk, et al.1987, Chichester [West Sussex] ; New York :: Wiley.
47. Lin, A.Y.-C. and M. Reinhard, *Photodegradation of common environmental pharmaceuticals and estrogens in river water.* Environmental Toxicology and Chemistry, 2005. **24**(6): p. 1303-1309.

48. Ocampo-Pérez, R., et al., *Degradation of antineoplastic cytarabine in aqueous phase by advanced oxidation processes based on ultraviolet radiation.* Chemical Engineering Journal, 2010. **165**(2): p. 581-588.
49. Garcia-Ac, A., et al., *Oxidation kinetics of cyclophosphamide and methotrexate by ozone in drinking water.* Chemosphere, 2010. **79**(11): p. 1056-1063.
50. Radka Zounková, P.O., Lenka Doležalová, Klára Hilscherová, Blahoslav Maršálek, Luděk Bláha, *Ecotoxicity and genotoxicity assessment of cytostatic pharmaceuticals.* Environmental Toxicology and Chemistry, 2009. **26**(10): p. 2208-2214.
51. Zounkova, R., et al., *Ecotoxicity and genotoxicity assessment of cytotoxic antineoplastic drugs and their metabolites.* Chemosphere, 2010. **81**(2): p. 253-260.
52. Backhaus, T. and L.H. Grimme, *The toxicity of antibiotic agents to the luminescent bacterium Vibrio fischeri.* Chemosphere, 1999. **38**(14): p. 3291-3301.
53. Sanderson, H., et al., *Probabilistic hazard assessment of environmentally occurring pharmaceuticals toxicity to fish, daphnids and algae by ECOSAR screening.* Toxicology Letters, 2003. **144**(3): p. 383-395.
54. Bantle JA, F.R., Burton DT, Fort DJ, Dawson DA, Linder G, Rayburn JR, Hull M, Kumsher-King M, Gaudet-Hull AM, Turley SD., *FETAX interlaboratory validation study: phase III--Part 1 testing.* Journal of applied toxicology, 1996. **16**(6): p. 517-28.
55. Henschel, K.P., et al., *Environmental Hazard Assessment of Pharmaceuticals.* Regulatory Toxicology and Pharmacology, 1997. **25**(3): p. 220-225.
56. Giuliani, F., et al., *Detection of genotoxic activity in native hospital waste water by the umuC test.* Mutation Research/Genetic Toxicology, 1996. **368**(1): p. 49-57.
57. Ferk, F., et al., *Genotoxic effects of wastewater from an oncological ward.* Mutation Research/Genetic Toxicology and Environmental Mutagenesis, 2009. **672**(2): p. 69-75.
58. Strong, R.H., CJ; Jones, K. C.; Martin, F.L., *Environmentally relevant levels of the genotoxin, cyclophosphamide induce alterations in MCF-7 cells detectable by biospectroscopy approaches.* Mutagenesis, 2012. **27**: p. 814.
59. Aherne, G.W., J. English, and V. Marks, *The role of immunoassay in the analysis of microcontaminants in water samples.* Ecotoxicology and Environmental Safety, 1985. **9**(1): p. 79-83.
60. Moldovan, Z., *Occurrences of pharmaceutical and personal care products as micropollutants in rivers from Romania.* Chemosphere, 2006. **64**(11): p. 1808-1817.
61. Mahnik, S.N., et al., *Determination of 5-fluorouracil in hospital effluents.* Analytical and Bioanalytical Chemistry, 2004. **380**(1): p. 31-35.
62. Ternes, T.A., *Occurrence of drugs in German sewage treatment plants and rivers.* Water Research, 1998. **32**(11): p. 3245-3260.
63. Halling-Sørensen, B., et al., *Occurrence, fate and effects of pharmaceutical substances in the environment- A review.* Chemosphere, 1998. **36**(2): p. 357-393.
64. Valcárcel, Y., et al., *Detection of pharmaceutically active compounds in the rivers and tap water of the Madrid Region (Spain) and potential ecotoxicological risk.* Chemosphere. **In Press, Corrected Proof**.
65. Zuccato, E., et al., *Presence of therapeutic drugs in the environment.* The Lancet, 2000. **355**(9217): p. 1789-1790.
66. Marlier, J.F., et al., *A Kinetic and Isotope Effect Investigation of the Urease-Catalyzed Hydrolysis of Hydroxyurea.* Biochemistry, 2010. **49**(37): p. 8213-8219.

67. Gale, G.R., *Inhibition of urease by hydroxyurea.* Biochemical Pharmacology, 1965. **14**(5): p. 693-698.
68. Lenz, K., et al., *Fate of cancerostatic platinum compounds in biological wastewater treatment of hospital effluents.* Chemosphere, 2007. **69**(11): p. 1765-1774.
69. Gerić, M., et al., *Cytogenetic status of human lymphocytes after exposure to low concentrations of p,p'-DDT, and its metabolites (p,p'-DDE, and p,p'-DDD) in vitro.* Chemosphere, 2012. **87**(11): p. 1288-1294.
70. Alberts, D.S., et al., *Pharmacokinetics and metabolism of chlorambucil in man: a preliminary report.* Cancer Treatment Reviews, 1979. **6, Supplement 1**(0): p. 9-17.
71. Hans Ehrsson , M.H., Mats Ehrnebo & Miloslav Beran *Busulfan kinetics.* Clinical Pharmacology & Therapeutics, 1983. **34**(1): p. 86-89.
72. Beumer, J.H., et al., *Trabectedin (Yondelis^{TM}, formerly ET-743), a mass balance study in patients with advanced cancer.* Investigational New Drugs, 2005. **23**(5): p. 429-436.
73. Lu K, S.N., Kavanagh J, Feun LG, Burgess M, Bodey GP, Loo TL., *Clinical pharmacology of 4-demethoxydaunorubicin (DMDR).* Cancer Chemotherapy Pharmacology, 1986. **17**(2): p. 143-8.
74. Castellino, S., et al., *Human Metabolism of Lapatinib, a Dual Kinase Inhibitor: Implications for Hepatotoxicity.* Drug Metabolism and Disposition, 2011.
75. Baker, S.D., et al., *Absorption, Metabolism, and Excretion of 14C-Temozolomide following Oral Administration to Patients with Advanced Cancer.* Clinical Cancer Research, 1999. **5**(2): p. 309-317.
76. Weissbrodt, D., et al., *Mass Flows of X-ray Contrast Media and Cytostatics in Hospital Wastewater.* Environmental Science & Technology, 2009. **43**(13): p. 4810-4817.
77. Olaf Tellingen, J.H.B., Willem J. Nooijen and Auke Bult, *Tissue disposition, excretion and metabolism of vinblastine in mice as determined by high-performance liquid chromatography* Cancer Chemotherapy and Pharmacology, 1993. **32**(4): p. 286-292.
78. Bedikian, A.Y., et al., *Pharmacokinetics and Urinary Excretion of Vincristine Sulfate Liposomes Injection in Metastatic Melanoma Patients.* The Journal of Clinical Pharmacology, 2006. **46**(7): p. 727-737.
79. Edward Chu; Vincent T DeVita, J., *Physician's cancer chemotherapy drug manual 2007*2007: Sudbury, MA : Jones and Bartlett.
80. Joqueviel, C., et al., *Urinary Excretion of Cyclophosphamide in Humans, Determined by Phosphorus-31 Nuclear Magnetic Resonance Spectroscopy.* Drug Metabolism and Disposition, 1998. **26**(5): p. 418-428.
81. Hadidi, A.-H.F.A., C.E.A. Coulter, and J.R. Idle, *Phenotypically Deficient Urinary Elimination of Carboxyphosphamide after Cyclophosphamide Administration to Cancer Patients.* Cancer Research, 1988. **48**(18): p. 5167-5171.
82. McCune, J.S., et al., *Population Pharmacokinetics of Cyclophosphamide and Metabolites in Children With Neuroblastoma: A Report From the Children's Oncology Group.* The Journal of Clinical Pharmacology, 2008.
83. Angela S Ensslin, Y.S., Angelika Pethran, Andreas Pfaller, Horst Rommelt, Gunter Fruhmann, *Biological monitoring of cyclophosphamide and ifosfamide in urine of hospital personnel occupationally exposed to cytostatic drugs.* Occupational and Environmental Medicine, 1994. **51**: p. 229-233.

84. Nath, C.E., et al., *Melphalan pharmacokinetics in children with malignant disease: influence of body weight, renal function, carboplatin therapy and total body irradiation.* British Journal of Clinical Pharmacology, 2005. **59**(3): p. 314-324.
85. Hilger, R.A., et al., *Investigation of bioavailability and pharmacokinetics of treosulfan capsules in patients with relapsed ovarian cancer.* Cancer Chemotherapy and Pharmacology, 2000. **45**(6): p. 483-488.
86. Hilger, R.A., et al., *Clinical pharmacokinetics of intravenous treosulfan in patients with advanced solid tumors.* Cancer Chemotherapy and Pharmacology, 1998. **42**(2): p. 99-104.
87. S. Feyerabend, G.F., J. Krug, A. Kassen and A. Stenzl, *Cytotoxic effects of treosulfan on prostate cancer cell lines.* . Anticancer Reseach, 2007. **27**: p. 2403-2408.
88. Scheulen, M.E., et al., *Clinical Phase I Dose Escalation and Pharmacokinetic Study of High-Dose Chemotherapy with Treosulfan and Autologous Peripheral Blood Stem Cell Transplantation in Patients with Advanced Malignancies.* Clinical Cancer Research, 2000. **6**(11): p. 4209-4216.
89. LePage, G.A. and J.P. Whitecar, *Pharmacology of 6-Thioguanine in Man.* Cancer Research, 1971. **31**(11): p. 1627-1631.
90. Estelle R. Lefkowitz, W.A.C., Paul Calabresi, et al., *Clinical and Pharmacologic Effects of Combinations of 6-Thioguanine and Duazomycin A in Patients with Neoplastic Disease.* Cancer Research, 1965. **25**: p. 1207-1212.
91. Houk, B.E., et al., *A Population Pharmacokinetic Meta-analysis of Sunitinib Malate (SU11248) and Its Primary Metabolite (SU12662) in Healthy Volunteers and Oncology Patients.* Clinical Cancer Research, 2009. **15**(7): p. 2497-2506.
92. Kurowski V, W.T., *Urinary excretion of ifosfamide, 4-hydroxyifosfamide, 3- and 2-dechloroethylifosfamide, mesna, and dimesna in patients on fractionated intravenous ifosfamide and concomitant mesna therapy.* Cancer Chemotherapy and Pharmacology, 1997. **39**(5): p. 431-9.
93. V Gilard, M.C.M.-M., M De Forni, U Niemeyer, J C Ader, R Martino, *Determination of the urinary excretion of ifosfamide and its phosphorated metabolites by phosphorus-31 nuclear magnetic resonance spectroscopy.* Cancer Chemotherapy and Pharmacology, 1993. **31**(5): p. 387-394.
94. Catapano CV, G.A., Broggini M, Corada M, Bartosek I, Italia C, Donelli MG., *Hepatobiliary metabolism and urinary excretion of 4-demethoxydaunorubicin as compared to daunorubicin in rats.* Anticancer Reseach, 1988. **8**(4): p. 725-31.
95. Blum, R., J.F. Seymour, and G. Toner, *Significant impairment of high-dose methotrexate clearance following vancomycin administration in the absence of overt renal impairment.* Annals of Oncology, 2002. **13**(2): p. 327-330.
96. Sweeney, C.J., et al., *Two Drug Interaction Studies Evaluating the Pharmacokinetics and Toxicity of Pemetrexed When Coadministered with Aspirin or Ibuprofen in Patients with Advanced Cancer.* Clinical Cancer Research, 2006. **12**(2): p. 536-542.
97. Sottani, C., et al., *Simultaneous determination of cyclophosphamide, ifosfamide, doxorubicin, epirubicin and daunorubicin in human urine using high-performance liquid chromatography/electrospray ionization tandem mass spectrometry: bioanalytical method validation.* Rapid Communications in Mass Spectrometry, 2008. **22**(17): p. 2645-2659.

98. Mahoney, B.P., et al., *Tumor acidity, ion trapping and chemotherapeutics: I. Acid pH affects the distribution of chemotherapeutic agents in vitro.* Biochemical Pharmacology, 2003. **66**(7): p. 1207-1218.
99. Wu, Z.-Y., B.M. Smithers, and M.S. Roberts, *Tissue and Perfusate Pharmacokinetics of Melphalan in Isolated Perfused Rat Hindlimb.* Journal of Pharmacology and Experimental Therapeutics, 1997. **282**(3): p. 1131-1138.
100. Tannock, V.V.a.I.F., *Influence of low pH on cytotoxicity of paclitaxel, mitoxantrone and topotecan.* Br J Cancer, 1997. **75**(8): p. 1167-1172.
101. *Assessment report for temomedac*, 2010: London.
102. *The hazardous substances databank*, TOXNET.
103. Deguchi, Y., et al., *Brain distribution of 6-mercaptopurine is regulated by the efflux transport system in the blood-brain barrier 1.* Life Sciences, 2000. **66**(7): p. 649-662.
104. Marín, D., C. Teijeiro, and J.J. Piña, *Electrochemical behaviour and micromolar determination of the antineoplastic agent 5-azacytidine and its mixtures with the nucleoside cytidine.* Journal of Electroanalytical Chemistry, 1996. **407**(1-2): p. 189-194.
105. Mouchard-Delmas, C., B. Gourdier, and R. Vistelle, *Determination of vinorelbine in rabbit plasma by high-performance liquid chromatography with coulometric detection.* Journal of Chromatography B: Biomedical Sciences and Applications, 1995. **663**(2): p. 390-394.
106. Owellen, R.J., et al., *Correlation of biologic data with physico-chemical properties among the Vinca alkaloids and their congeners.* Biochemical Pharmacology, 1977. **26**(13): p. 1213-1219.
107. Weylandt, K.H., et al., *ClC-3 expression enhances etoposide resistance by increasing acidification of the late endocytic compartment.* Molecular Cancer Therapeutics, 2007. **6**(3): p. 979-986.
108. Vives, M., et al., *Spectroscopic study of the interaction of actinomycin D with oligonucleotides carrying the central base sequences -XGCY- and -XGGCCY- using multivariate methods.* Analytical and Bioanalytical Chemistry, 2007. **387**(1): p. 311-320.
109. Gallois, L., M. Fiallo, and A. Garnier-Suillerot, *Comparison of the interaction of doxorubicin, daunorubicin, idarubicin and idarubicinol with large unilamellar vesicles: Circular dichroism study.* Biochimica et Biophysica Acta (BBA) - Biomembranes, 1998. **1370**(1): p. 31-40.
110. Kimura, E., et al., *pH-dependent coordination mode of new bleomycin synthetic analogs with copper(II), iron(II), and zinc(II).* Inorganic Chemistry, 1992. **31**(21): p. 4314-4321.
111. Zimmermann, T., J. Leszczynski, and J. Burda, *Activation of the cisplatin and transplatin complexes in solution with constant pH and concentration of chloride anions; quantum chemical study.* Journal of Molecular Modeling, 2011: p. 1-9.
112. Rosing, H., et al., *High-performance liquid chromatographic determination of the novel antitumour drug topotecan and topotecan as the total of the lactone plus carboxylate forms, in human plasma.* Journal of Chromatography B: Biomedical Sciences and Applications, 1995. **668**(1): p. 107-115.
113. Yu, J.T., E.J. Bouwer, and M. Coelhan, *Occurrence and biodegradability studies of selected pharmaceuticals and personal care products in sewage effluent.* Agricultural Water Management, 2006. **86**(1-2): p. 72-80.

114. Gómez-Canela, C., et al., *Occurrence of cyclophosphamide and epirubicin in wastewaters by direct injection analysis–liquid chromatography–high-resolution mass spectrometry.* Environmental Science and Pollution Research, 2012. **19**(8): p. 3210-3218.
115. Thomas, K.V., et al., *Source to sink tracking of selected human pharmaceuticals from two Oslo city hospitals and a wastewater treatment works.* Journal of Environmental Monitoring, 2007. **9**(12): p. 1410-1418.
116. Ashton, D., M. Hilton, and K.V. Thomas, *Investigating the environmental transport of human pharmaceuticals to streams in the United Kingdom.* Science of The Total Environment, 2004. **333**(1–3): p. 167-184.
117. Coetsier, C.M., et al., *Discharge of pharmaceutical products (PPs) through a conventional biological sewage treatment plant: MECs vs PECs?* Environment International, 2009. **35**(5): p. 787-792.
118. Roberts, P.H. and K.V. Thomas, *The occurrence of selected pharmaceuticals in wastewater effluent and surface waters of the lower Tyne catchment.* Science of The Total Environment, 2006. **356**(1–3): p. 143-153.

Figure 1
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Figure 1: Schematic representation of the methodology used to select priority chemicals

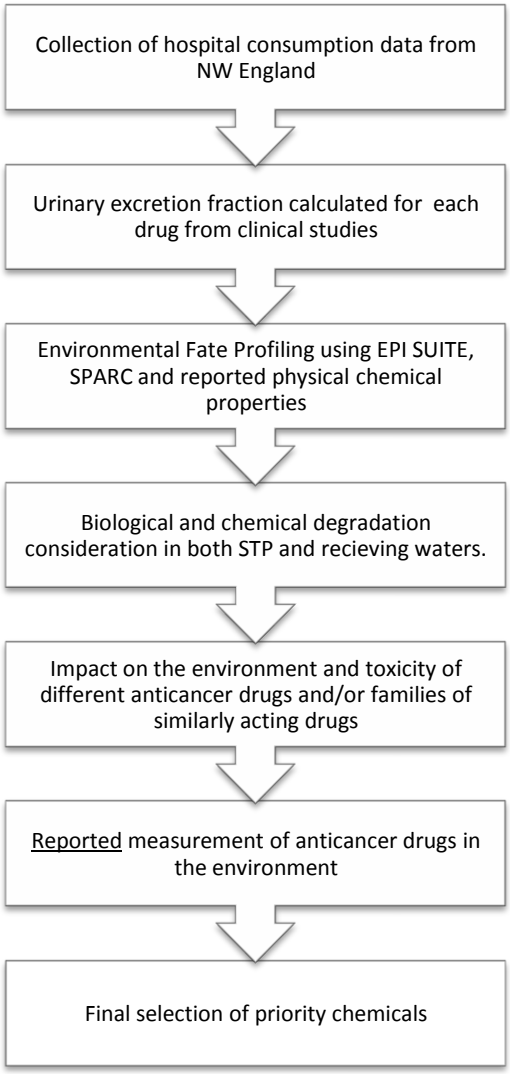

Figure 2

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Figure 2: Calculated proportions of neutral an ionized forms of selected anticancer drugs as a function of pH a) methotrexate, b) pemetrexed, c) fludarabine, d) imatinib, e) topotecan, f) bendamustine

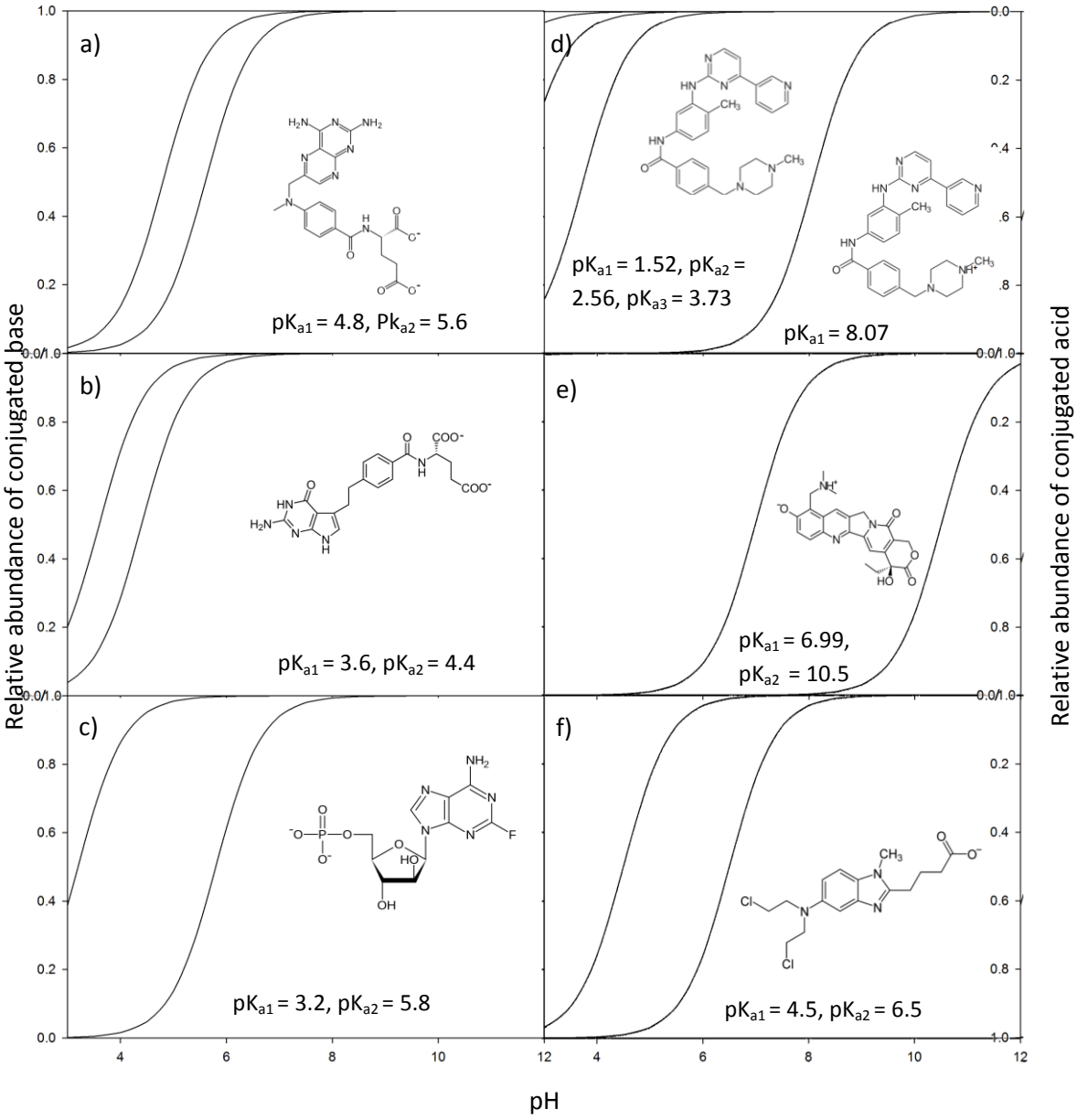

Figure 3
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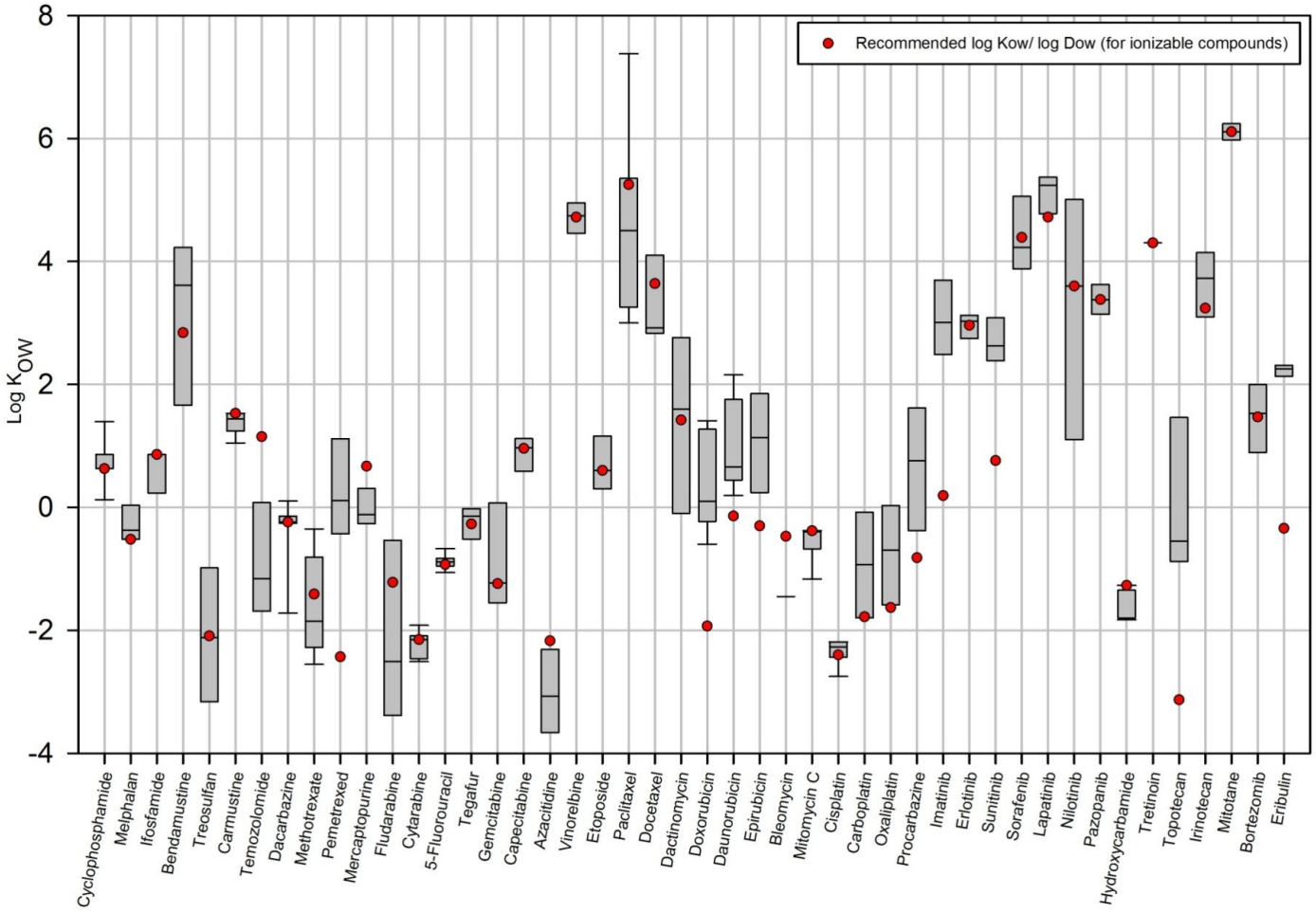
Figure 3: Box and whisker plot of log K_{ow} values for a wide number of anticancer drugs. For each chemical the K_{ow} values were obtained from the literature (i.e. empirically observed) or calculated (*n*=1-22)



Recommended log K_{ow} based on the most reliable data sources with consideration of D_{ow} for ionisable compounds