~~USES~~MONITORING, ~~,~~ SOURCES, RECEPTORS, AND CONTROL MEASURES FOR THREE EUROPEAN UNION WATCH LIST PHARMACEUTICAL COMPOUNDS IN RECEIVING WATERS – A 20 YEAR SYSTEMATIC REVIEW

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Abstract

Pollution of European receiving waters with pharmaceutically-active compounds (PhACS) is a ubiquitous phenomenon. This study specifically focused e Water Framework Directive (WFD) onhas added diclofenac (an anti-inflammatory drug, DCL) along with the natural (17-beta-estradiol (E2)) and synthetic (17-alpha-ethynylestradiol (EE2)) estrogenic hormones that were the first substances on the to theirthe European watch list in the field of water policyunder new EU legislation. AThis study conducted a systematic literature review was conducted of 3,952 potentially relevant articles over period 1995 to 2015 that produced a new EU-wide database consisting of 1,268 publications on DCL, E2 and EE2. European surface water concentrations of DCL are typically reported below the proposed annual average environmental quality standard (AA EQS) of 100 ng/l, but that exceedances frequently occur. E2 and EE2 surface water concentrations are typically below 50 ng/l and 10 ng/l respectively, but these values greatly exceed the proposed AA EQS values for these compounds (0.04 and 0.035 ng/l respectively). However, levels of these PhACs are frequently reported to be disproportionately high in EU receiving waters, particularly in effluents at control points that require urgent attention. Overall it was found that DCL and EE2 enter European aquatic environment mainly following human consumption and excretion of therapeutic drugs, and by incomplete removal from influent at urban wastewater treatment plants (WWTPs). E2 is a natural hormone excreted by humans which also experiences incomplete removal during WWTPs treatment, although livestock populations in Europe are also a significant non-point source of E2 contamination. Current laboratory-based analytical chemistry methods are sufficiently sensitive for the detection and quantification of DCL but not for E2 and EE2, thus alternative, ultra-trace, time-integrated monitoring techniques such as passive sampling are needed to inform water quality for these estrogens. DCL appears resistant to conventional wastewater treatment while E2 and EE2 have high removal efficienciesrates that occurs through biodegradation or sorption to organic matter. There is a pressing need to determine fate and behaviour of these PhACs in European receiving waters such as using GIS-modelling of river basins as this will identify pressure points for informing priority decision making and alleviation strategies. More monitoring data for these PhACs in receiving waters is urgently needed for EU legislation and effective risk management.

Key words

Water framework directive, Diclofenac, Hormones17-beta-estradiol (E2), 17-alpha-ethynylestradiol (EE2), Sources, ReceptorsOccurrences, Control, Watch list

Highlights

- Three EU wWatch list pharmaceutical compounds in receiving waters are reviewed
- Diclofenac and estrogens E2 and EE2 reported above environmental quality standards
- Under monitoring of these chemicals in many EU member countries
- Need for more sensitive estrogen detection methods to meet WFD limits
- Control measures frequently do not remove these harmful chemicals

Introduction

Pharmaceuticals are a class of emerging environmental contaminants that are widely used in human and veterinary medicine (Fent et al, 2006; Nikolaou et al, 2007). From here on, these compounds will be referred to as pharmaceutically active chemicals (PhACs), which includes not just pharmaceuticals but also their pharmaceutically active metabolites/transformation products (Heberer, 2002). PhACs~~They~~ are essential to modern healthcare, especially in the developed world; nevertheless, there are growing concerns about the negative impacts that may result from continuous contamination of the environment with PhACs. This research is important because of the potential toxic effects for aquatic biota and human health that may result from chronic exposure to PhACs (Fent et al, 2006; Kümmerer, 2009; Nikolaou et al, 2007). Characteristics specific to this class of environmental contaminants can however present significant challenges for research. For example, PhACs exhibit wide variation in function, chemical structure and physiochemical properties, making it difficult to generalize about their behaviour, persistence or impact in the environment. PhACs are also designed to be biologically active, have a specific mode of action and to be persistent in the body, meaning they can impact humans and wildlife at trace concentrations which are often hard to detect and quantify using traditional analytical methods (Fent et al, 2006).

PhACs in the aquatic environment primarily originate from use in human medicines, however certain classes are also heavily used in veterinary practices (e.g. anti-inflammatory drugs, antibiotics) (Fent et al, 2006; Zhou et al, 2009). A large number of PhACs have been detected in WWTPs influents and effluents and surface, ground and drinking water worldwide in recent years (Heberer, 2002; Nikolaou et al, 2007; Ternes, 1998; Zhou et al, 2009). In fact, ~~,~~ it is now established that throughout the developed world, PhAC~~s~~ are ubiquitous at µg to ng per litre levels in the aquatic environment (Nikolaou et al, 2007), although the concentrations of specific compounds depend on usage patterns in different countries and can vary temporally (Verlicchi et al, 2012). The impacts of chronic exposure to trace concentrations of many PhACs on wildlife and human health may be severe (e.g Verlicchi et al 2012), thus it is critical to limit as much as possible the concentrations of this class of contaminants in our waterways. Certain PhACs can specifically impact the endocrine system of humans or wildlife; such chemicals are part of ~~a larger classification of~~ emerging pollutants known as endocrine disrupting chemicals (EDCs). Much of the growing interest in this field of research stems from fears that chronic exposure to EDCs (in bathing or drinking water, for example) may be linked to adverse human health conditions such as declining male fertility, birth defects, and breast and testicular cancer (Nikolaou et al, 2007). Furthermore negative impacts of EDCs exposure on wildlife may include severe consequences such as feminisation in fish (Sumpter & Johnson, 2008). Similar to PhACs as a whole, EDCs are mainly thought to be transported into the aquatic environment via incomplete removal at WWTPs (Nikolaou et al, 2007).

Until recently, environmental regulations worldwide had not required explicit testing for any PhACs in water bodies. However given the growing concern about contamination of the aquatic environment with these compounds, legislation has recently begun to acknowledge this potential problem. The Water Framework Directive (WFD, 2000/60/EC) is an overarching piece of European environmental legislation aimed at protecting and improving water quality throughout the EU. The WFD committed EU Member States to achieve

good qualitative and quantitative status of all water bodies by 2015. In order to reach this goal, certain chemicals identified by Annex X of the WFD have been deemed priority substances; these chemicals (e.g. some pesticides, metals such as lead or mercury, organic volatile compounds and other organics such as polycyclic aromatic hydrocarbon) must be monitored by all member states and cannot exceed specific concentration thresholds in surface waters (defined by the legislation as Environmental Quality Standards, or EQSs). Furthermore, article 16(4) of this legislation requires that the list of priority substances must be reviewed and adjusted as appropriate at regular intervals. As such, directive 2013/39/EU of 12 August 2013 added a further 12 substances to Annex X of the WFD. In addition, Article 8b of Directive 2013/39/EU states that "the Commission shall establish a watch list of substances for which EU-wide monitoring data are to be gathered for the purpose of supporting future prioritisation exercises." In response to growing EU concern about the release of untreated PhACs into the aquatic environment, three compounds were~~have been~~ included in the first watch list in 2013: diclofenac (CAS# 15307-79-6, hereafter referred as DCL), 17-beta-estradiol (CAS# 50-28-2, hereafter referred as E2) and 17-alpha-ethinylestradiol (CAS# 57-63-6, hereafter referred as EE2). It is relevant to note that the European Commission implemented decision 495 of 20 March 2015 that expanded substances or groups of substances on the watch list to 10 in the field of water policy, which also comprised oxadiazon, methiocarb, 2,6-ditert-butyl-4-methylphenol, tri-allate, four neonicotinoid pesticides, 3 macrolide antibiotics, and 2-ethinylhexyl 4-methoxcinnamate. This review focuses solely on the first three pharmaceutical compounds DCL, E2 and EE2 as there is a requirement to investigate policy implications for Ireland of these PhACs in receiving waters in the first instance. The EU-wide monitoring data that will be produced in the next few years will help legislators determine whether or not these compounds are ultimately added to the list of priority substances from Annex X of the WFD. The WFD requires that all EU member states prepare river basin management plans (RBMPs) to address the many issues relating to water quality and protection in a holistic manner. These RBMPs identify the main pressures and activities affecting water status and propose environmental objectives that must be achieved during certain time periods. The recent European legislation on DCL, E2 and EE2 mentioned above has been identified as potentially significant water management issue that may need to be addressed in the next round of RBMPs (due for publication in 2017).

The overall aim of this literature review was to identify and evaluate all previous relevant EU-wide studies on contamination of the aquatic environment with the three watch list pharmaceuticals DCL, E2 and EE2 in order to anticipate their entrance in the WFD priority substances list and to identify gaps in knowledge aiming at guiding future research. This review is directed towards at-risk industries, companies, researchers, regulators and any sectors that would be affected by the addition of these compounds to future iterations of the WFD priority substance list (toxicology, water treatment, chemical analysis, biology, regulation). It addresses four main research questions for each compound:

1.) What are the likely sources/entry points of these PhACs into European aquatic environment?

2.) What are the likely receptors and loadings in European waters?

3.) What monitoring methods are currently employed to measure aquatic concentrations of these PhACs, and what are the current limits of detection/quantification?

4.) What control measures (including both source control and treatment options) are effective (or potentially effective) and employed for lowering concentrations of these compounds in the aquatic environment?

2- Materials and methods

2.1 Systematic review protocol and defining search parameters

Even a cursory search of the literature reveals a vast amount of published material regarding the sources, receptors, monitoring and control measures of DCL, E2 and EE2 (Fatta-Kassinos et al, 2011b; Johnson et al, 2013; Qian et al, 2015). Consequently this literature review was carried out using a defined systematic approach that answers research questions based on the published evidence, which is gathered using a predefined protocol that was adapted from the Centre for Evidence-Based Conservation's (CEBC) "Guidelines for Systematic Review in Conservation and Environmental Management" (Pautasso, 2013; Pullin & Stewart, 2006). The protocol comprised defining search parameters (databases to be searched, search times, types of publications), selecting search terms, developing eligibility (inclusion/exclusion) criteria, and conducting the literature search and carrying out the article review and selection process to produce publication database and bibliographic analysis. The article review was a two-step process including both a title and abstract filter~~, where final publication database includes bibliographic information about full articles that were deemed eligible after the review process.~~ Studies on the sources, receptors/monitoring and control measures of DCL, E2 and EE2 were identified using the Scopus database and from professional networks that included grey literature sources or sources that would not be returned by the database search (such as PhD theses or government reports) (Pullin & Stewart, 2006).The search was limited to literature published from 1995 to 2015 to ensure the publications included in the final database were up-to-date. The mid 1990s reflected time period when this field of research was in its infancy (Qian et al, 2015). Search terms were selected to ensure all potentially relevant articles were returned from the database searches. Two separate searches were run, one for DCL and one combined search for E2 and EE2. The E2 and EE2 searches were combined due to a high percentage of overlap in these search results. For both final searches, results were limited to articles, articles in press or review papers. All 28 EU member states were included, as well as Switzerland, Norway and Turkey. Terms for each of the two searches included "water" and "wastewater" in order to focus on articles considering the PhACs in aquatic matrices. In order to cover all relevant research, search terms included the class of PhACs describing each drug of interest (i.e. "NSAID" or "estrogen") and all relevant synonyms for each specific compound. In order not to miss articles considering the veterinary usage of DCL, which can be a significant source of environmental pollution (Boxall, 2010; Hunt et al, 2015), the term "veterinary" was also included. A list of eligibility criteria was developed so that once all of the potentially relevant articles were located through the searches described above, articles for inclusion in the database could be distinguished (Table 1).

Table 1. Eligibility criteria for systematic literature review; used for title and abstract filter.

Eligibility Criteria
- Must specifically discuss at least one of the three compounds of interest
- Cannot focus exclusively on impacts of compound for human/animal/plant health
- Exclude papers that focus only on ecological/environmental/toxicological impacts unless they also discuss relevant sources, receptors/monitoring or control measures
- Exclude clinical trial studies
- Must include some specific information on sources, receptors/monitoring or control measures
- Cannot focus on exposure routes other than water
- Study cannot be purely chemical, i.e. determining a chemical coefficient
- Exclude any papers on leaching of chemicals from bottled water/plastics
- Must be peer reviewed original article or review, or article in press
- Must be published between 1995-May 2015
- Research must be conducted in Europe or by at least one author affiliated with a European country
- Article must be written in English
- Full text must be available

2.2 Article review and selection

Once all potentially relevant articles were identified through the searches, a selection process was undertaken to find articles for inclusion in the final database framed upon meeting eligibility criteria (Table 1). Title and abstract review were undertaken by two ~~postdoctoral~~ researchers with 10% overlap in order to validate consistent choices. During the abstract review, additional fields were added to the spreadsheet by the reviewer (Figure 1), which were organised into six domains namely topic of article, monitoring type, compounds studies, analytical methods used, study type, and country study was performed in. Articles with authors or fieldwork from multiple countries were counted as full publications for each country, rather than fractionally (Qian et al, 2015). These additional fields were filled in by reading the abstract, or if necessary, by downloading and reading the full-text of the article. The only exception was the analytical method employed for detection; this field was only filled out if the method was specified in the abstract. These additional fields, as well as the bibliographic information provided by Scopus, were utilized to conduct the bibliographic analysis (section 3.2.).

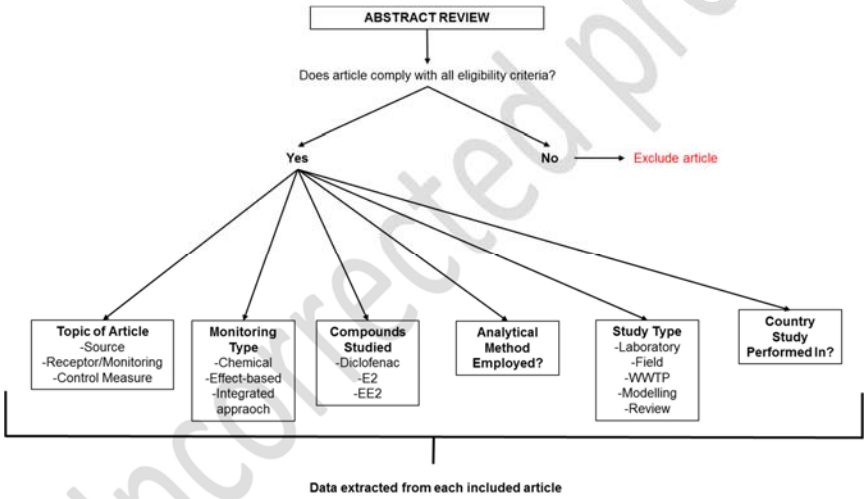

Figure 1. Multi-step abstract review and data extraction approach used in the abstract filter step of the systematic literature review. Extracted data was used to carry out the bibliographic analysis.

3- Results and discussion

The aim of this systematic literature review was to evaluate current state of knowledge on contamination of the European aquatic environment with DCL, E2 and EE2, especially in regards to sources, receptors, monitoring and control measures. The following section<u>s</u> address~~es~~ the specific research questions this systematic review was concerned with: section<u>s</u> 3.1.and 3.2 report~~s~~ the results from the bibliographic analysis, section 3.3 details the key findings on the sources of these PhACs in the aquatic environment; section 3.4 discusses the receptors and concentrations of these PhACs in a European context; and section

3.5 discusses the effectiveness and challenges associated with monitoring methods used to detect these compounds. ;F finally, section 3.6 discusses DCL, E2 and EE2 current and potential control measures.

3.1 General overview of the database

Even following strict exclusion criteria (see section 2.4), the systematic review identified a very large number of peer-reviewed publications on the sources, receptors/monitoring and control measures for DCL, E2 and EE2. Figure 2 demonstrates the enormous number of articles returned by our searches, and the number of articles excluded (and reasons for exclusion) during the title and abstract filters. The database of publications and the summary information regarding this database (bibliographic analysis, section 3.2) include 1,268 publications deemed eligible by the systematic review protocol. Published review studies were analysed for data on monitoring, source, receptors and control measures for sections 3.3 to 3.6 where Publications were evaluated and any summary data on three topics was extracted: (i) concentrations of DCL, E2 or EE2 in influent or effluent, and their removal efficiencies during various wastewater treatments; (ii) concentrations of these three PhACs in surface, ground or drinking water; and (iii) methods of detection and limits of detection (LODs) for each of the three compounds.

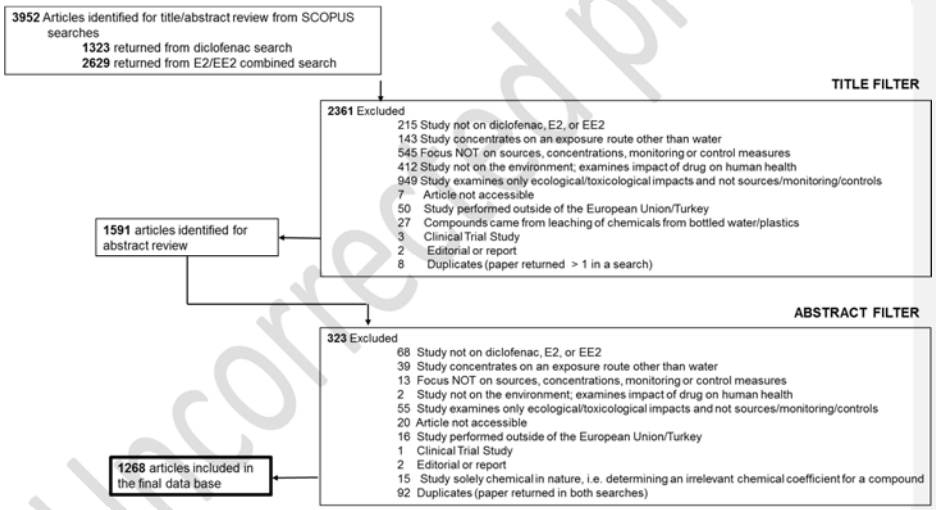

Figure 2. Publications (articles) returned from the systematic review searches; the figure demonstrates the number of publications excluded plus reasons for exclusion during the title and abstract filter, as well as the total number of publications included in the final database.

3.2 Bibliographic analysis: State of European research on DCL, E2 and EE2

Bibliographic analyses are particularly useful for fields with large bodies of research that are difficult or impossible to summarize via traditional, full-text review studies (Belter & Seidel, 2013). They are also important for defining gaps in the literature and directing future research (Qian et al, 2015). This bibliographic analysis originates from the database of publications created during the systematic review; it summarizes the state of European research on DCL, E2 and EE2 from 1995-May 2015 (details of the methodology used to create the database are provided in section 2).

3.2.1 Pharmaceuticals studied

EU database constituted of 628, 697, and 665 EU studies reported on DCL, E2 and EE2 respectively as per alignment with eligibility criteria (Fig. 1). Many of the individual studies in the database reported on more than one of these PhACs. In particular, studies that investigated hormones tended to include both the natural steroid estrogen E2 as well as the synthetic EE2. There are a large number of total studies (> 600) focused on each of these three PhACs, however slightly more research has been published on E2 and EE2 when compared with DCL; that may be due to particular concerns regarding environmental contamination with hormonal EDCs. Figure 3 demonstrates the total annual number of published articles from the database that include information on each PhAC of interest. It is clear that a large increase in research on the contamination of aquatic matrices with DCL, E2 and EE2 has occurred since the early 2000s. The annual counts of articles increased from 0 for all three PhACs in 1995 to 76, 50 and 51 for DCL, E2 and EE2 respectively in 2014. The maximum number of annual publications on DCL sources, receptors or control measures occurred in 2012 (83), while E2 and EE2 reached a maximum in 2013 (68 and 67 respectively). This figure also demonstrates that most years, slightly more articles are published on E2 and EE2 when compared with DCL, although this trend reversed itself from 2011 onwards. Finally, the majority of publications (> 84%) on these three PhACs have occurred from 2005 onward. This trend likely relates to the recent increased concern regarding DCL, E2 and EE2 in regards to EU legislation (specifically via the WFD). The apparent sharp decrease in publications from 2014 to 2015 is an artefact as the search was conducted in May of 2015, thus presumably many more articles were~~ill be~~ published on these PhACs in the second half of the year.

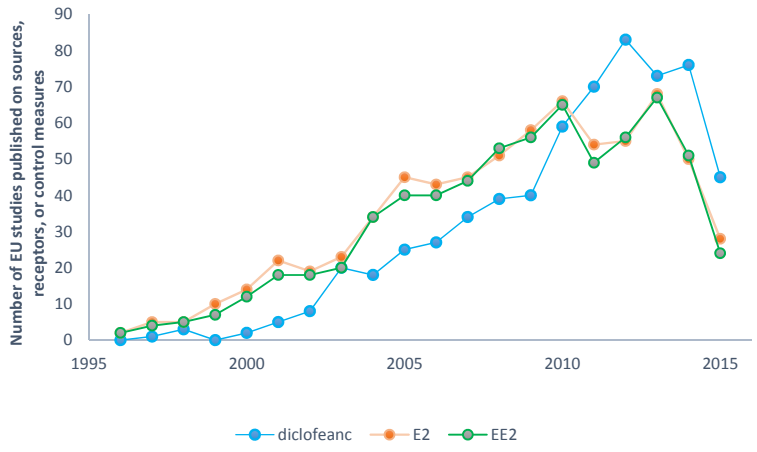

Figure 3. Total combined number of EU studies on sources, receptors or control measures for each DCL (circles), E2 (triangles) and EE2 (squares) from 1995-May 2015, by year

3.2.2 Research theme studied

This systematic review investigated three general themes regarding research on DCL, E2 and EE2 and found 595 studies for sources of contamination (section 3.3); 775 studies for receptors or monitoring methods used

to measure the levels of these compounds in the aquatic environment (section 3.4-3.5); and 651 for control measures for reducing contamination (section 3.6). Studies often focus on more than one of these themes, for example, ~~such as~~ some monitoring studies also discuss ~~on~~ removal of PhACs via wastewater treatment. Furthermore, studies focused on receptors or monitoring methods outnumber source studies by nearly 200 articles and control measure studies by over a hundred articles. Many of these monitoring articles describe analytical methods and conditions used to detect low levels of the PhACs of interest, but they often also report detected concentrations in wastewater influent and effluent; surface, ground and drinking water or other environmental matrices for validation of the developed analytical protocols (e.g. Ben Fredj et al, 2015; Lacey et al, 2008; Ronan & McHugh, 2013). Studies on sources are the least common of the three research themes and often focus on consumption rates, the contribution of municipal vs industry wastewater to total PhACs load, or contributions via agricultural or veterinary practices (e.g. Kümmerer, 2009; Rivera-Utrilla et al, 2013; Santos et al, 2010). Finally, studies on control measures occur frequently in the database, but these publications represent studies carried out on a variety of scales, from laboratory experiments, to pilot scale studies, to whole WWTP-level studies. They also include investigations of removal via primary, secondary and tertiary ~~(advanced)~~ treatment technologies. Figure 4 demonstrates the total number of studies from each research theme carried out each year, from 1995 to May 2015. While publications on all three themes of research have increased dramatically during this time period, the graph demonstrates that since 2010 studies on sources of contamination have become less popular and have begun to level out. Commensurately, the number of publications on monitoring methods have been slightly lower than the number of publications on control measures in recent years (2012 to 2015). This may indicate that while monitoring methods are still being developed and measurements of these PhACs in water matrices are still taking place, the research community is increasingly concerned with investigating mitigation methods for PhACs~~s~~ contamination. Given the potential for increased regulations regarding aquatic contamination with DCL, E2 and EE2, a further increase in control measure studies is expected.

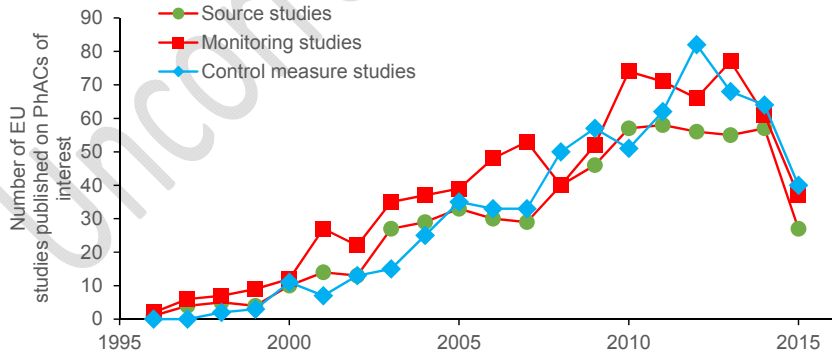

Figure 4. Number of EU studies on at least one of the three pharmaceuticals of interest (DCL, E2 or EE2) investigating: sources of contamination (circles), monitoring data or techniques (triangles), or control measures (squares), from 1995-May 2015, by year.

~~3.2.3 Pharmaceuticals and research theme studied~~

In order to understand if source, monitoring and control measure studies are conducted equally for each PhAC, Figure 5 shows the number of each type of study conducted for each compound. The difference in monitoring studies compared with source or control measure studies is accentuated for the two hormones, while DCL studies are more evenly split between the three research themes. The number of source studies is approximately equal for each of the three PhACs, however control studies are conducted more frequently for DCL. The inability of conventional WWTPs processes to remove this NSAID (see section 3.6) has likely led to more investigations of alternative or advanced treatments that may improve removal efficiencies~~rates~~.

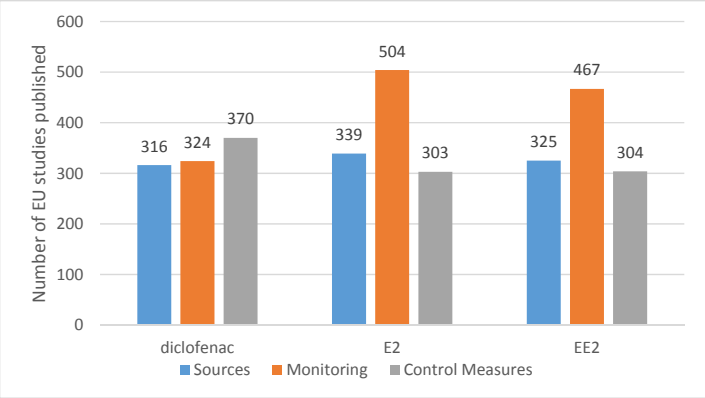

Figure 5. Total number of EU studies on each pharmaceutical of interest investigating sources, of contamination, monitoring data or techniques, or control measures, from 1995-May 2015.

3.2.4 Hormones: chemical vs biological monitoring method

In addition to traditional chemical monitoring, a variety of *in vitro,* effect-based monitoring assays can be used to identify the total estrogenic activity in environmental samples (Kunz et al, 2015). Figure 6 compares the number of E2/EE2 monitoring studies that used traditional chemical (concentration) methods vs those that used biological effects monitoring. There is also a category for integrated or combined monitoring methods. Clearly chemical methods are much more common than biological effects monitoring. This trend is apparent both during the early years of research (1999-2001) and in more recent years (2007-2015). More information on these monitoring methods are presented in sections 3.5.2 and 3.5.3. The recent spike in concentration studies is likely related to an increase in the sensitivity of recent analytical approaches for measuring estrogens. Nevertheless, detecting environmentally relevant, low concentrations of estrogens remains a challenge, thus biological effect monitoring has become more popular as the field has developed.

3.2.5 Scale of the studies

Studies on DCL, E2 and EE2 can be conducted on a variety of scales. Some studies take place at the field level, measuring PhACs concentrations in various aquatic matrices such as surface or ground water (e.g. Camacho-Muñoz et al, 2013; McEneff et al, 2014). Some take place on a laboratory scale, e.g. measuring the removal or effectiveness of monitoring methodologies of spiked water samples in the lab (e.g. Rizzo et al, 2015; Zhou & Jiang, 2015). Others are conducted on a full WWTP level, where influent/effluent concentrations and removal efficiencies are measured at specific WWTPs (e.g. Clara et al, 2005b; Lacey et

al, 2012). The concentrations of PhACs in different matrices can also be modelled (Balaam et al, 2010; Johnson et al, 2007a), and many studies are reviews of recent literature (see sections 3.3-3.6). The number of each of these study types published annually from the database results is presented in Figure 7 below. This figure demonstrates that by far, laboratory scale studies are the most common type of investigations on DCL, E2 or EE2. Laboratory studies are manageable, have controlled conditions, and can be done relatively quickly, all factors that likely contribute to the high frequency of this study type. Field studies can be more time intensive and expensive as they involve travel to a variety of locations for the collection of samples; nevertheless these types of studies have occurred with increasing frequency in the past two decades as people become more concerned with the levels of these three PhACs in the aquatic environment. WWTPs scale studies have increased slowly but steadily in frequency, and now more than 20 tend to be published each year on just these three PhACs alone. Such studies contribute to valuable meta-analyses which can provide important information regarding removal efficiencies~~rates~~ via various wastewater treatments (Miege et al, 2008; Verlicchi et al, 2012). Modelling studies have increased recently (from 2007 onward) as more data have become available in this field, and a further increase in this study type is likely. As total number of primary publications on these PhACs increases, so does the number of reviews including data on these compounds.

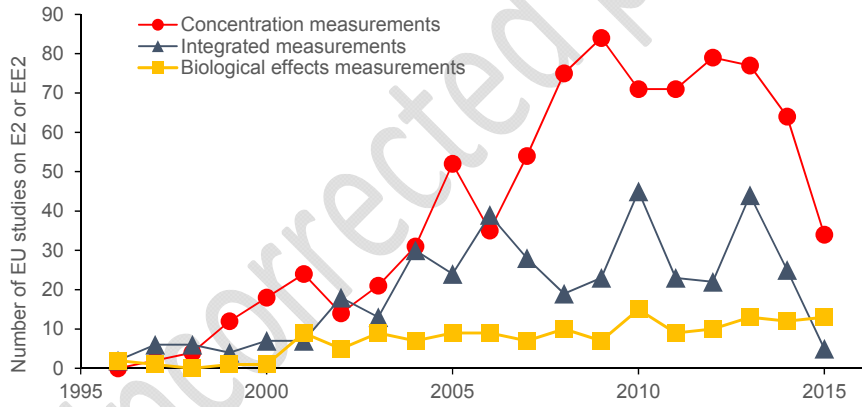

Figure 6. EU studies investigating E2 and EE2 using concentration measurements, biological effects measurements, or an integrated approach by year, 1995-May 2015

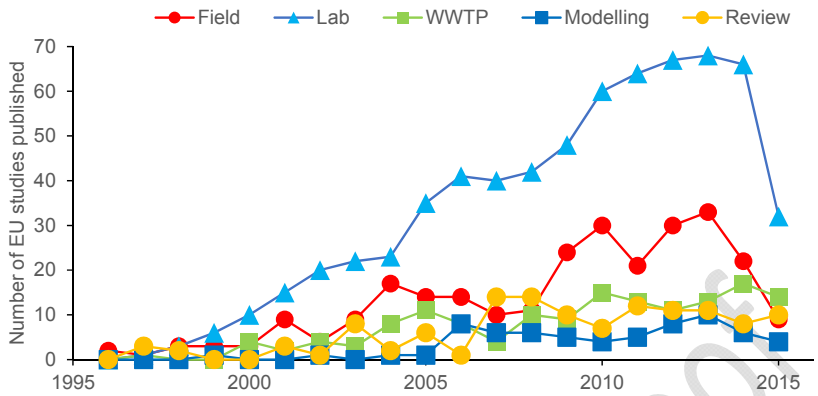

Figure 7. Number of studies on three PhACs (DCL, E2 and/or EE2) published in the EU from 1995-May 2015 broken down by type of study: field ~~(closed circle)~~, laboratory scale ~~(closed triangle)~~, wastewater treatment plant level (WWTP, ~~square~~), modelling ~~(open triangle)~~ and review ~~(open circle)~~.

3.2.6 Repartition of the studies by country

This bibliographic analysis identified which European countries are producing the majority of research regarding contamination of the aquatic environment with DCL, E2 and EE2 (Table 2). As stated in section 2.3, articles with authors from multiple countries were counted as full publications for each country, rather than fractionally (Qian et al, 2015). Spain and Germany effectively contribute 528 (35.5%) of total studies where review papers evaluating such national studies have been published (González et al, 2012; Jurado et al, 2012) ~~that is also incorporated into this database~~. The top 6 EU countries including Switzerland listed in Table 2 collectively published 971 (65%) studies where metadata on these PhACs informs baseline and predictive modelling such as for river basins and catchments. However, the majority of EU countries have limited studies reported and will require ~~to undertake~~ substantial monitoring to effectively inform decision making and policy.

Table 2. Number of articles produced by each EU country along with Switzerland, Norway and Turkey on sources, monitoring or control measures for DCL, E2 or EE2: 1995-May 2015.

Country	Total number (%) of Studies
Spain	285 (19.2)
Germany	243 (16.3)
United Kingdom	179 (12.0)
France	93 (6.3)
Switzerland	87 (5.8)
Italy	84 (5.7)
The Netherlands	57 (3.8)
Sweden	51 (3.4)
Portugal	50 (3.4)
Greece	43 (2.9)

Belgium	42 (2.8)
Denmark	37 (2.5)
Poland	37 (2.5)
Czech Republic	26 (1.7)
Austria	24 (1.6)
Finland	23 (1.5)
Norway	21 (1.4)
Slovenia	21 (1.4)
Turkey	19 1.3)
Ireland	17 (1.2)
Cyprus	14 (0.9)
Hungary	11 (0.7)
Romania	7 (0.5)
Luxembourg	6 (0.4)
Croatia	3 (0.2)
Slovakia	3 (0.2)
Bulgaria	2 0.1)
Estonia	2 (0.1)
Northern Ireland	2 (0.1)
Lithuania	1 (0.06)
Latvia	0 (0)
Malta	0 (0)

3.3 Sources and vectors of DCL, E2 and EE2

As the bibliographic analysis above demonstrates, contamination of the environment with PhACs is a relatively recent research field with the majority of studies conducted in the past 15 years (Qian et al, 2015; Rivera-Utrilla et al, 2013; Santos et al, 2010). Now that researchers have been able to identify and quantify a large number of potentially harmful PhACs in the aquatic environment (Santos et al, 2010), there is increased interest in identifying sources and vectors of these compounds. Only when the sources and pathways of PhACs contamination are understood can opportunities to reduce the input of these substances into the aquatic environment be identified (Jurado et al, 2012; Kümmerer, 2010). The main sources and vectors discussed by these articles are reviewed below in a general manner, because many of them are applicable to DCL, E2 and EE2, as well as other PhACs (section 3.3.1). However, sources and vectors specific to each of the three compounds of interest are also addressed below (section 3.3.2).

3.3.1 General sources of PhACs

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The largest source of environmental contamination with PhACs comes from human use of therapeutic drugs (Kümmerer, 2009; Rivera-Utrilla et al, 2013; Santos et al, 2010). After consumption, unaltered PhACs can enter the environment via excretion in urine and faeces (Santos et al, 2010). Medicines containing the PhACs of interest in this study are almost exclusively prescription medications; this allows for relatively easy measurement of drug usage or consumption (Clouzot et al, 2008; Wise et al, 2011; Zhang et al, 2008), a critical factor for predicting the ultimate levels of environmental contamination in an area. Furthermore, review studies have noted that consumption of PhACs varies temporally and spatially. For example, significant

differences in consumption of individual compounds can occur from one country to another, often due to cultural or economic factors (Kümmerer, 2009). In addition to excretion of unaltered PhACs, parent compounds can also be converted to metabolites or conjugates through various reactions in the body. These metabolites/conjugates are then excreted and can be harmful to aquatic organisms themselves, or can be transformed or deconjugated back into the parent compound in environmental matrices (Santos et al, 2010). PhACs that are excreted by humans will ultimately end up in wastewater, and will potentially receive treatment at a municipal WWTP or via a domestic treatment system (e.g. septic tank). However, WWTPs and domestic treatment systems are generally not designed to treat PhACs (e.g. Verlicchi et al, 2012) (see section 3.6). If incomplete removal of PhACs during municipal or domestic wastewater treatment occurs, the compounds will enter the aquatic environment via WWTPs effluents discharged into receiving waters.

Another potential source of environmental contamination with PhACs comes from household disposal of unused or out-of-date medications (Kümmerer, 2009; Santos et al, 2010). These medications are either discarded through the sink/toilet, in which case they go directly to WWTPs via sewage influent, or they are disposed of via household waste. If household waste containing unused drugs is landfilled, PhACs can enter the landfill effluent (Kümmerer, 2009; Santos et al, 2010) and consequently the aquatic environment. In addition to household waste, sludge from WWTPs can also be brought to landfills. In this case, leaching of PhACs that were removed from wastewater via sorption to sludge could further increase the PhACs content of landfill effluents (Santos et al, 2010). Treated sludge (biosolids) may also be applied to soil and rececent studies have documented that PhACs may also reach the environment by this entry route (Verlicchi et al, 2012).

Industrial effluent can be another significant source of PhACs contamination (Kümmerer, 2009; Rivera-Utrilla et al, 2013; Santos et al, 2010). The effluents of pharmaceutical production facilities in particular can contain high levels of bioactive compounds (Santos et al, 2010). However, although very limited data exist, good manufacturing practices, regulatory requirements and the high value of the active ingredients in most pharmaceuticals have often led to the assumption that such emissions are negligible in a European context (Kümmerer, 2009). Another type of ~~industrial~~ wastewater that could contain high levels of PhACs is hospital effluent (Kümmerer, 2009; Rivera-Utrilla et al, 2013; Santos et al, 2010). Reviews studies indicate that while PhACs concentrations in hospital wastewater tend to be much higher than those in municipal sewage, the total contribution of this source to environmental contamination with PhACs is low because of the relatively lower volume of hospital effluent ~~(diluted by a factor of 100 according to a recent review~~ (Kümmerer, 2009)).

The use of PhACs in agriculture and aquaculture can also be sources of environmental contamination, particularly in rural environments (Boxall, 2010; Rivera-Utrilla et al, 2013; Santos et al, 2010). First, PhACs given to grazing or outdoor animals are excreted directly onto the ground or into surface waters without receiving any wastewater treatment. Furthermore, disposal of farmyard manure, slurry or litter containing unmetabolized PhACs via application onto agricultural land can lead to leaching of compounds into groundwater, or runoff into surface water (Rivera-Utrilla et al, 2013). Municipal sewage sludge is also often spread on agricultural land as a fertilizer, and can contain PhACs that were removed from wastewater during the treatment process (Santos et al, 2010). In the case of aquaculture, PhACs can be used as veterinary medicines and may be applied through many routes, including via feed, topical application or injection; all of these uses have potential to lead to contamination of surface waters (Boxall, 2010).

3.3.2 Specific sources of DCL, E2 and EE2

~~Sources of contamination of the aquatic environment with the steroid estrogens and DCL are of particular interest, because there is definitive evidence that these PhACs have negative environmental impacts (Kümmerer, 2009). Many of the general sources of PhACs contamination reviewed above (see section 3.3.1) apply to DCL, E2 and EE2; however, sources of each of these specific three compounds is reviewed below.~~

3.3.2.1 Sources of diclofenac

Sources of DCL were specifically addressed by two review articles in the database of publications (Vieno & Sillanpää, 2014; Zhang et al, 2008). DCL is an arylacetic acid NSAID. It is prescribed as oral tablets or a topical gel, and it is sold under many commercial names including Dicloabac, Diclofenbeta, Diclomex, Voltaren, among others (Vieno & Sillanpää, 2014). Vieno and Sillanpää (2014) comprehensively reviewed the human metabolism of this PhAC. They found that studies generally report that only 6-7% of the topical gel is absorbed, while the rest is washed off the skin or attaches to clothing. This is significant in regards to envrionmental contamination because a large percentage of topicaly aplied DCL will end up washed down household drains, ultimately ending up in WWTP~~s~~ influent. Vieno and Sillanpää (2014) also summarized the metabolism of the tablet form; the studies they reviewed found that between 65-75% of the orally adminsitered dose is excreted through urine and 20-30% is excreted in faeces as the parent drug or metabolites. This review also reports that both the topical and oral forms of DCL undergo almost complete biotransformation in the body, with less than 1% of the orally administerd dose being excreted as unmetabolized DCL. The World Health Organization defined daily dose for DCL as 100 mg, of which less than one mg is eliminated as DCL and 11 mg as DCL conjugates. The rest is excreted as metabolites of DCL or their conjugates (Vieno & Sillanpää, 2014). This finding demonstrates the importance of analyzing environmental matrices for metabolites and conjugates, as well as the parent drug. Diclofenac is one of the most widely used NSAID, and consumption of this compound in a variety of regions is reviewed by Zhang et al. (2008) and Ziylan and Ince. (2011). They summarized the annual consumed volumes of DCL for some European countries including Austria, France, Germany, and England. Consumption in the Zhang et al. (2008) study was compared using dose per capita, or the annual consumption of the drug in an area divided by that area's population. The authors reported that Germany had the highest dose per capita (915 mg), followed by Austria (750 mg), England (531 mg) and France (271 mg). The authors also calculated a simplified estimate of annual global DCL consumption of 940 tons. Such estimations of human consumption are critical for understanding the concentrations of this PhAC expected in aquatic matrices.

Treated municipal wastewater effluent is considered to be the major vector of contamination of the aquatic environment with DCL (Vieno & Sillanpää, 2014). DCL is considered as a recalcitrant compound, meaning its removal ~~rate~~efficiency during conventional wastewater treatment is poor (Miege et al, 2008; Verlicchi et al, 2012 and see section 3.6). Thus, concentrations of this compound in effluent are generally high (Table 6), and DCL is commonly released via this pathway into surface waters. This compound is hydrophilic, meaning it dissolves in water and does not significantly sorb onto sludge during wastewater treatment to any significant extent (Vieno & Sillanpää, 2014 and section 3.6). It is thus unlikely that DCL contamination will result from the spreading of sewage sludge on agricultural land. DCL may be found in landfill effluent, though only via disposal of the compound through household wastes, and not from sewage sludge deposited in landfills. To our knowledge, the removal of DCL in domestic treatment systems has not been investigated yet, but this could be another potential vector of environmental contamination. Veterinary use of DCL in Europe is a potential source of contamination with this PhAC, however studies evaluated did not report on

veterinary drug usage. Nevertheless, the European Medicines Agency (2014) reports that DCL is authorized for veterinary use in many member states. Increased regulations and risk assessments associated with veterinary use of DCL have been suggested, and may be implemented on a European level (European Medicines Agency, 2014).

3.3.2.2 Sources of E2

E2 is one of three naturally occurring steroid estrogens produced by the human body. Females excrete on average more E2 than males (males = 1.6 µg/day), and menstruating and pregnant women excrete particularly large amounts of this natural estrogenic compound (3.5 and 259 µg/day respectively) (reviewed in Wise et al, 2011). This natural PhAC can also be used in prescribed drugs, including hormone replacement therapy and to treat infertility in women or advanced prostate and breast cancer (reviewed in Kunz et al, 2015). Compared with the other natural estrogenic hormones, E2 has the highest potency and levels of aquatic contamination of this PhAC are therefore of great concern (Wise et al, 2011). Given that E2 is a naturally produced compound, humans represent one of the most important sources of contamination of the environment with this PhAC. Similar to DCL, effluents from WWTPs are still one of the most important vector of aquatic contamination with E2 (Burkhardt-Holm, 2010; Hecker & Hollert, 2011; Verlicchi et al, 2012; Wise et al, 2011). This compound is easily eliminated during wastewater treatment (see section 3.6), nevertheless removal of E2 is usually incomplete (Table 3). Trace concentrations of E2 are therefore released into surface waters via WWTPs effluents. E2 is also excreted by livestock, which in general excrete the same natural hormones as humans (Burkhardt-Holm, 2010; Wise et al, 2011). Research has demonstrated that surface waters downstream of agricultural land or farms often have relatively elevated levels of estrogens, including E2 (Wise et al, 2011). Sewage sludge is not thought to be a significant source of E2 contamination, again because the compound is readily biodegradable (see section 3.6). Domestic treatment systems and landfill effluent can contribute to environmental contamination of E2 according to a review by Burkhardt-Holm (2010). Finally, E2 has been used as a veterinary medication for livestock, although determining the contribution of natural versus pharmaceutical estrogens to total livestock excretions is difficult (Wise et al, 2011).

3.3.2.3 Sources of EE2

The structure of the synthetic estrogen EE2 is more similar to E2 than any other natural estrogen (Clouzot et al, 2008). EE2 is the main estrogenic ingredient in oral contraceptive pills taken by women of reproductive age (Clouzot et al, 2008; Wise et al, 2011). It is also found in other prescription medications including hormone replacement therapies, palliative treatments for breast and prostate cancer, and lotions used to prevent androgen-dependent hair loss in women (reviewed in Kunz et al, 2015). Estimation of consumption of this PhAC can be difficult because it is usually prescribed as a combination drug (usually in combination with a progestin). Wise et al. (2011) reviewed studies on the excretion of EE2, and they report that the average daily dose of this compound is 30-35 µg of EE2 per pill, and that women on oral contraceptives fully metabolize 20-48% of this dose. The rest is excreted in either its original form or as EE2 sulfate or glucuronide conjugates, but these conjugates are mostly deconjugated back to its original form in the environment (Clouzot et al, 2008; Wise et al, 2011). As with E2, effluent from municipal WWTPs is often considered to be the most important vector of environmental EE2 contamination (Burkhardt-Holm, 2010; Hecker & Hollert, 2011; Verlicchi et al, 2012). EE2 is prone to biodegradation during wastewater treatment (see section 3.6), but it is significantly more recalcitrant (and therefore has lower removal rates, see Table 3) than E2 (Miege

et al, 2008; Verlicchi et al, 2012). Because this PhAC is not completely removed by conventional wastewater treatment, it enters surface waters via WWTPs effluent discharge. Unlike E2, EE2 is not produced by livestock. Consequently, agricultural practices and livestock in particular are not currently thought to be a significant contributor to environmental contamination with EE2 in Europe. Sewage sludge transferred to landfills or spread on agricultural land may contain traces of EE2, but this compound is thought to biodegrade readily and thus these practices also may not represent significant sources of EE2 contamination. As for E2, domestic treatment systems and landfill leachate may present pathways to groundwater contamination with EE2, again, related back to human usage of this compound (Burkhardt-Holm, 2010).

3.4 Receptors and occurrence of diclofenac, E2 and EE2 in European waters

There is now evidence of contamination of the aquatic environment with hundreds of different PhACs (Kummerer, 2010) from a variety of therapeutic classes, including antibiotics, lipid regulators, psychiatric drugs, and of course, NSAIDs (e.g. DCL) and hormones (e.g. E2 and EE2) (Verlicchi et al, 2012). Levels of PhACs in the aquatic environment can vary dramatically, but are usually present in low concentrations from the nanogram to microgram per litre range depending on the location and the aquatic matrix considered (Kummerer, 2010; Verlicchi et al, 2012). In addition to global reviews (e.g. Ratola et al, 2012; Verlicchi et al, 2012; Vieno & Sillanpää, 2014) there are now also several studies summarizing the findings of PhACs occurrence in particular European countries such as Spain (González et al, 2012; Vazquez-Roig et al, 2013) and Italy (Meffe & de Bustamante, 2014). Given the importance of WWTPs as point sources of PhACs contamination (as mentioned in section 3.3), it is essential to understand the levels of compounds entering the system via influent, as well as the final concentrations in treated effluent. Many individual articles in our database measured DCL, E2, EE2 and other PhACs concentrations in wastewaters. Thus, mMany of the published review studies are devoted specifically to evaluating the typical occurrence of PhACs in WWTPs influents and effluents (e.g. Miege et al, 2008; Verlicchi et al, 2012; Vieno & Sillanpää, 2014). In this chapter,Here only inlet and outlet WWTPs concentrations for the three PhACs of interest will be discussed; some more specific information on the removal efficiencies obtained with different treatment processes and potential interpretations of the encountered removal efficiencies for DCL, E2 and EE2 will be given in the next chapter (i.e. 3.6). Other reviews focus instead on the reported concentration of PhACs in surface, ground and drinking water, as concentrations in these aquatic matrices ultimately have the most relevance for animal and human health (e.g Petrie et al, 2013; Lapworth et al, 2012; Martin & Voulvoulis, 2009). Generally, many more reviews summarize surface water concentrations than ground or drinking water concentrations, due to the low number of primary studies that consider the two later matrices. Tables 3 and 4 summarize the findings from recent review papers regarding the occurrence of DCL, E2 and EE2 in these aquatic matrices in Europe and internationally. Although concentrations of DCL, E2 and EE2 can vary a great deal in each of these aquatic matrices (even when considering each compound individually), typical concentrations (including averages and ranges) in each matrix are compared and contrasted below.

3.4.1 Occurrence of diclofenac

Compared with E2 and EE2, DCL tends to be present in high concentrations in WWTPs influents. This finding is common for compounds in this therapeutic class; in a recent meta-analysis Miege et al. (2008) found that NSAIDs had the highest WWTPs influent concentrations when compared with other drug classes (e.g. antibiotics, beta-blockers, lipid regulators, vasodilators). In the review papers evaluated, average DCL values

varied from 80 to 2100 ng/l in this aquatic matrix (Table 3). The minimum DCL influent value reported by any of the reviews was 2 ng/l (Santos et al, 2010), while the maximum was 203,000 ng/l (Ratola et al, 2012). These large variations in reported influent concentrations may be partially explained by differences in consumption of DCL between and within countries (see section 3.3.3.1), and also by the differences in analytical methods employed (see section 3.5). Such differences can make describing or predicting DCL influent concentrations difficult (Zhang et al, 2008).

Meta-analyses that evaluate multiple PhACs repeatedly found that DCL is among the most frequently detected compound in WWTPs effluents (Miege et al, 2008; Verlicchi et al, 2012). DCL is rarely completely eliminated during wastewater treatment, especially using conventional treatment processes (Table 3). As a result, this recalcitrant compound rarely falls below the LODs of a few ng/l in WWTPs effluents (Zhang et al, 2008). In the reviews evaluated, mean DCL concentrations in effluents varied widely, from <2 to 2500 ng/l. These values do tend to be slightly lower than the average influent values reported in Table 3. Nevertheless, it is clear that high nanogram to microgram per litre levels of DCL in WWTPs effluents are common throughout Europe. Occasionally individual studies found that DCL showed negative removal rates during WWTPs treatment, i.e. concentrations are actually higher in effluent than influent (e.g. Clara et al, 2005b; Lacey et al, 2012; Lacey et al, 2008). Besides the impact of analytical uncertainty, two mechanisms have been proposed to explain this phenomenon, deconjugation of glucuronidated or sulphated DCL, or desorption of this compound from particles (Verlicchi et al, 2012; Vieno & Sillanpää, 2014). It should be noted that many review papers do not include these negative removal rates when calculating average removal via WWTPs processes (see removal efficiency, Table 3). Verlicchi et al. (2012) conducted the most comprehensive, recent meta-analysis of PhACs concentrations in municipal WWTPs found in our database, and DCL was one of the compounds included. PhACs concentrations of raw influent at more than 200 municipal WWTPs (all utilizing conventional activated sludge (CAS) systems) were compared with the concentrations in secondary effluents in order to calculate global removal efficiencies. The average concentration of DCL in influent was 1.0 µg/l, but even in this one review, the minimum and maximum reported values varied over an order of magnitude. The average concentration of DCL in effluent was 0.8 µg/l, but again the values ranged greatly; in one study, DCL was found in WWTP effluent at 11 µg/l, one of the highest absolute effluent concentrations found for all 118 PhACs included in the study. In another study, Loos et al. (2013) analysed effluents from 90 WWTPs across Europe for 156 polar organic chemical contaminants and showed that DCL had a frequency of detection of 89%. The maximum concentration of DCL found was 174 ng/l and the median concentration was 43 ng/l. These levels are relatively low when compared with levels found in similar studies; the most recent review of DCL found that mean concentrations in wastewater effluents were usually above 100 ng/l, however mean values as low as 2 ng/l have been found also (Vieno & Sillanpää, 2014). Loos and co-workers (2013) hypothesize that the low levels could have been due to problems with different analytical standards.

DCL is frequently detected in surface waters throughout Europe (Table 4). This fact is not surprising given the high levels often found in WWTPs effluents. According to the most recent review, DCL concentrations in surface waters are generally reported below 100 ng/l (Vieno & Sillanpää, 2014). Other reviews however include maximum values as high as 1030 ng/l (Ziylan & Ince, 2011) or 1200 ng/l (Rivera-Utrilla et al, 2013). Still, such high levels are the exceptions rather than the rule in regards to concentrations of DCL in surface waters. Surface waters in the UK range in DCL concentrations from <0.5 to 261 ng/l while the same author reports a range of <12 to 154 ng/l for mainland Europe (Petrie et al, 2013). Similarly, an Italian review study found a maximum concentration of 158 ng/l of DCL in surface waters (Meffe & de Bustamante, 2014). Levels

in protected areas may be lower, as was demonstrated by a review of DCL levels in Spanish wetlands, estuaries and watersheds where levels ranged from 1 to 90 ng/l (Vazquez-Roig et al, 2013). Given that the predicted no effect concentration (PNEC) for DCL is reported in the literature as approximately 14 µg/l (Santos et al, 2007), the data in Table 4 suggest that typical surface water concentrations in Europe do not usually pose a significant environmental threat. However, point sources of pollution can lead to concerning levels of DCL contamination in European surface waters.

Levels of DCL in groundwater tend to be much lower than those in surface water (Table 4). The most recent review of DCL states that levels in groundwater are typically low or below LODs (of generally a few ng/l for this type of water, see section 3.5) (Vieno & Sillanpää, 2014). According to a recent review, no Italian study has detected DCL in groundwater to date with LODs generally in the ng/L range (Meffe & de Bustamante, 2014, see section 3.5.1), however Spanish studies have found a maximum concentration of 477 ng/l in groundwater (Jurado et al, 2012). In a review of international studies, Lapworth et al. (2012) found a mean groundwater concentration of 121 ng/l, while Santos et al. (2010) found values ranging from <10 to 50 ng/l. Finally, concentrations in drinking water appear to be even lower; only two review studies report on DCL levels in drinking water, and they state that international studies demonstrate levels between 1 to 7 ng/l (Vieno & Sillanpää, 2014) and <0.25 to 7 ng/l (Santos et al, 2010).

3.4.2 Occurrence of E2

Levels of E2 in WWTPs influents tend to be in the nanogram per litre range (Miege et al, 2008; Pereira et al, 2011; Ratola et al, 2012; Verlicchi et al, 2012). Of the reviews evaluated, mean E2 concentrations in influents ranged from 27.4 to 250 ng/l, considerably lower than those reported for DCL (Table 3) (Miege et al, 2008; Verlicchi et al, 2012). Similar to DCL, however, the range of E2 values the reviews report for influents are high; the lowest reported influent value in any review paper was 0.3 ng/l (Santos et al, 2010) and the highest was 3000 ng/l (Verlicchi et al, 2012). In the Verlicchi et al. meta-analysis (2012), E2 in influent presented the highest absolute concentration and the highest average observed value among any of the hormones studied. In contrast, in a meta-analysis performed by Miege et al. (2008), the mean E2 value was lower, 27.4 ng/l, and the range was much smaller (min = 2.5 to 48.4 ng/l). The Verlicchi et al. review included three studies with extremely high E2 influent concentrations (> 1000 ng/l), which drove the overall reported mean value up considerably. In general however, European influent concentrations of E2 are much less than 1000 ng/l.

A greater number of reviews provide summary information on E2 concentrations in WWTPs effluents than in influents (Table 3). These reviews demonstrate that levels of E2 in WWTPs effluents are also usually found in the low nanogram per litre range. Furthermore, reported E2 concentrations in effluents are generally lower than average influent concentrations. For example, the Verlicchi et al. meta-analysis (2012) reported a mean E2 concentration of 10 ng/l in effluent, 25 times less than the mean concentration in influent. Similarly the Miege et al. meta-analysis (2008) reported a decrease in E2 effluent concentrations when compared with influent concentrations (1.8 ng/l vs 27.4 ng/l respectively). This decrease in E2 concentrations in effluent is likely due to the high removal rates of E2 during many wastewater treatment processes (often > 90%, see Table 3 and section 3.6.4.2). In contrast, Pereira et al. state in their 2011 review paper that estrogen concentrations in effluent wastewaters are similar to those found in influent wastewaters; however, the values they report for each matrix do indicate a slight decrease in effluent levels for E2 specifically (Table 3).

The presence of estrogenic compounds (including E2) in surface water has been widely investigated (Table 4), supposedly largely due to concerns about the endocrine disrupting effects of these compounds. We found

644 that the majority of recent review studies report surface water E2 concentrations of less than 50 ng/l (Meffe
645 & de Bustamante, 2014; Pereira et al, 2011), although in some studies the maximum values extend as high
646 as 200 ng/l (Martin & Voulvoulis, 2009; Santos et al, 2010). E2 surface water concentrations can reach these
647 high levels of >100 ng/l when measurements are taken directly downstream from WWTPs effluent discharge
648 (Pereira et al, 2011). However it is also not uncommon for studies to report that E2 is below the LOD in
649 surface waters (generally a few ng/l or below in this type of water, see section 3.5). For example, in a review
650 of studies conducted in the Llobregat River (Spain), Gonzalez et al. (2012) find no reports of E2 exceeding
651 LODs (generally in the ng/L range, see section 3.5.2). Similarly Santos et al. (2010) and Martin & Voulvoulis
652 (2009) report that some of the studies they reviewed did not detect E2 in surface waters. Nevertheless, very
653 low concentrations (i.e. sub ng/L range) of EDCs such as E2 can have a negative impact on aquatic
654 organisms, especially via chronic exposure; thus even though on average, surface water concentrations of
655 E2 are lower than many other PhACs, the environmental impact of this compound should not be
656 underestimated (Burkhardt-Holm, 2010; Abargues Llamas et al, 2012b).
657 Reviews examining the occurrence of E2 in the aquatic environment often consider levels in groundwater,
658 but less frequently discuss levels in drinking water (Table 4). Measuring the low concentrations in drinking
659 water can present a serious analytical challenge in terms of the sensitivity of the method (see section 3.5),
660 thus there are not as many primary studies that are able to investigate this aquatic matrix. Out of all of the
661 reviews evaluated, the highest E2 concentration reported for groundwater was 120 ng/l (Lapworth et al,
662 2012), however most values were much lower than this (i.e. a few nanograms per litre), especially in reviews
663 that excluded outliers (Pereira et al, 2011; Santos et al, 2010). Several reviews reported that E2 is often
664 present in concentrations below detection levels in groundwater (Jurado et al, 2012; Martin & Voulvoulis,
665 2009; Pereira et al, 2011). Concentrations of E2 in drinking water usually are reported as even lower,
666 reaching only a few ng/l according to most reviews (Table 4).
667
668 *3.4.3 Occurrence of EE2*
669 Reviews that consider occurrence of E2 in aquatic matrices often also include figures for the synthetic
670 estrogen EE2 (Pereira et al, 2011; Ratola et al, 2012; Verlicchi et al, 2012). According to reviews included in
671 our study, EE2 concentrations in WWTPs influents range from <0.2 to 50 ng/l, with mean values ranging from
672 1.5 to 20 ng/l (Table 3). As with E2, two recent meta-analyses provide the best information on likely
673 concentrations of these compounds in European WWTPs influents and effluents (Miege et al, 2008; Verlicchi
674 et al, 2012). Verlicchi et al. (2012) include a small number of studies in their analyses with higher EE2 influent
675 values (> 10 ng/l), whereas Miege et al. (2008) report a maximum concentration of 5.2 ng/l EE2 in WWTPs
676 influents. Both meta-analyses report lower concentrations of EE2 in influents compared with the natural
677 hormone E2. The low concentrations of EE2 in WWTPs influents, as well as other aquatic matrices, makes
678 it difficult to quantify or even detect this compound using standard analytical methods; this can limit the
679 discussion about EE2 levels and removal during wastewater treatment (Clouzot et al, 2008 and see section
680 3.5). Similar to influent concentrations, effluent concentrations are usually just a few nanograms EE2 per
681 litre (Table 3). In the review studies evaluated, mean EE2 effluent concentrations ranged from 0.6 ng/l (Miege
682 et al, 2008) to 3 ng/l (Verlicchi et al, 2012). As with influent concentrations, these values are lower than the
683 corresponding mean E2 effluent concentrations. The minimum reported value in effluent is < 0.02 ng/l (below
684 LOD) (Clouzot et al, 2008), while the maximum value is 60 ng/l (Pereira et al, 2011). Generally average

685 effluent concentrations are less than influent concentrations, however EE2 is known to be slightly more
686 recalcitrant than E2, especially in regards to conventional WWT processes (Petrie et al, 2013).
687 Generally reviews of EE2 indicate that surface water concentrations are very low, often below LODs
688 (González et al, 2012; Jurado et al, 2012; Martin & Voulvoulis, 2009). According to the reviews evaluated
689 (Table 4), surface water concentrations of EE2 range from 0.04 ng/l (Kralchevska et al, 2013) to as high as
690 831 ng/l (Martin & Voulvoulis, 2009). The Martin and Voulvoulis review (2009), which reported the highest
691 EE2 surface water concentration of all the studies evaluated, is the only review to report a maximum value
692 above 100 ng/l. In contrast, most reviews state that EE2 concentrations in surface waters do not exceed 10
693 ng/l (Clouzot et al, 2008; Meffe & de Bustamante, 2014; Rivera-Utrilla et al, 2013; Wise et al, 2011).
694 Compared with other steroid estrogens such as E2 and estrone (E1), EE2 is detected in surface waters with
695 the lowest frequency and at the lowest concentrations (Wise et al, 2011). Nevertheless, extremely low
696 concentrations of EE2, even levels below most LODs, are known to cause endocrine disruptions such as
697 intersex fish or vitellogenin induction (Clouzot et al, 2008). Thus similar to E2, the environmental risk of EE2
698 should not be underestimated just because surface water levels are low compared with other PhACs.
699 As of 2011, only a small number of studies had measured EE2 in drinking water (Wise et al, 2011). Wise et
700 al. (2011) reviewed these studies and found that in the UK, the EE2 levels were usually below reported LODs.
701 Since then, a few more studies have reviewed concentrations of EE2 in drinking water and have found
702 similarly low levels, ranging from 0.15 to 3 ng/l (Kralchevska et al, 2013; Pereira et al, 2011). Groundwater
703 concentrations of EE2 have been reviewed by four studies; two reviews found no studies that detected EE2
704 in groundwater (Jurado et al, 2012; Meffe & de Bustamante, 2014), while two found values that ranged from
705 0.5 to 5 ng/l (Pereira et al, 2011; Santos et al, 2010).

Table 3. Summary of influent and effluent concentrations and removal efficiencies following various wastewater treatments throughout Europe. All values for influent and effluent concentrations reported in ng/l. Values reported as minimum, maximum, range or mean, depending on what was reviewed by the reference. Removal efficiencies could be determined using lab, pilot or whole plant scale studies. Removal efficiencies are also given for a variety of secondary or tertiary treatments. Note: removal efficiencies represent global removal, and are not based on direct comparisons between the listed influent and effluent concentrations. These values do not represent central tendencies of removal efficiencies unless specified; furthermore they may be influenced by factors such as artefacts of the analytical (detection) methods used. Data originate from summary information provided by review studies from published database: specific references cited for each PhAC.

Drug	Influent concentration (ng/L)				Effluent concentration (ng/L)				Removal efficiency (%)		Comment	Reference
	mean	*max*	*min*	*range*	*mean*	*max*	*min*	*range*	*mean*	*range*		
Diclofenac										90 - 100	Ozonation	
										91 - 99	Bank filtration and soil aquifer treatment [Germany]	Jekel et al, 2015
	80 - 2300	150 - 7100			<2 - 2500	120 - 4700			36		CAS	
									36		Activated sludge with BNR	Vieno & Sillanpää, 2014
									48		MBR [	
	250				215					28 - 46	Activated carbon [Italy, Belgium, UK, Ireland, Germany]	
									100		O3 based AOPs	
									> 80		AOPs based on UV radiation [the Netherlands]	Rivera-Utrilla et al, 2013
										62.9 - 85	Gamma radiation, various parameters [Italy]	

	nd - 203,000	nd – 19,200			Ratola et al, 2012
			43 - 77	Generally <50% DCL removed (CAS at varying SRTs) [Austria]	Petrie et al, 2013
			23 - 76	Biofiltration processes [Spain]	
			92 - 99	Ozonation [Spain, Austria]	
			69 - 98	Sorption processes [UK]	
	105 - 4110	5 - 5450	9 - 60	Activated sludge plants, various treatment operations	Ziylan & Ince, 2011
			96	Ozonation	
			29	UV radiation	
	2 - 3600	0.3 - 2400		Luxembourg	Santos et al, 2010
			< 30	Conventional wastewater treatment, 66% of reviewed studies found removal rates of < 30%)	Oulton et al, 2010
			6 - 96	Removal efficiency of CW; WWTP removal for comparison was 24% [Spain]	Matamoros & Bayona, 2008
			59 - 75	Full scale WWTPs, treatments not specified; high removal rates due to elimination of sludge during primary treatment and/or enhanced sorption to sludge during secondary treatment upon	Suárez et al, 2008

										addition of inorganic salts for P precipitation [Spain]	
	882	4110	105	477	1720	35		35		WWTPs with activated sludge processes {France]	Miege et al, 2008
							< 100 - 1750		0 - 80	Mainly 21 to 40% (Europeaninternational studies, various treatment processes including Austria, Denmark, France, Greece, Italy, Spain, Sweden, UK)	Zhang et al, 2008
	1000	1200		800	1100			29		International studies, CAS	Verlicchi et al, 2012
								60		International studies, MBR	
E2							2.7 - 48			UK	Kralchevska et al, 2013
			2.5 - 125				0.3 - 30				Ratola et al, 2012
			4 - 30				0.1 - 60		39 - 100	Removal of various estrogens via various oxidative treatments	Pereira et al, 2011
									94 - 100	Removal of various estrogens via ozonation	
			0.3 – 102 10 – 31				< 0.3 – 85 3 – 8			Luxembourg Italy-	Santos et al, 2010

								> 90		CAS, 69% of reviewed studies found removal rates of > 90%	Oulton et al, 2010
							1 - 10			Germany, UK	Burkhardt-Holm, 2010
								36		Removal efficiency of constructed wetlands; WWTP removal for comparison was 85 - 99%) [Spain]	Matamoros & Bayona, 2008
							0 - 50			Median = 2 ng/L [UK]	Martin & Voulvoulis, 2009
									30 - 100	Full scale WWTPs, treatments not specified [Spain]	Suárez et al, 2008
	27.4	48.4	2.5		1.8	5.2	0.3	85		WWTPs with activated sludge processes [France]	Miege et al, 2008
	250	3000			10	80		80		International studies, CAS	Verlicchi et al, 2012
								99		International studies, MBR	
EE2							0.1 - 8.9			Germany	Kralchevska et al, 2013
									7 - 82	Biofiltration processes [Sweden]	Petrie et al, 2013
									> 50 - > 66	Ozonation [Sweden]	
									> 43	Sorption processes [UK]	
				1.5 - 17.2			0.1 - 3.1				Ratola et al, 2012
				7 - 50			2 - 60		39 - 100	Removal of various estrogens via various oxidative treatments	Pereira et al, 2011

									94 - 100	Removal of various estrogens via ozonation [Spain]	
			< 1.6 - 24				< 1.1 - 1.7				Santos et al, 2010
								41		Removal efficiency of constructed wetlands; WWTP removal for comparison was 71 - 78%) [Spain]	Matamoros & Bayona, 2008
							nd - 10			Germany, UK	Burkhardt-Holm, 2010
									(-18) - 98	Full scale WWTPs, treatments not specified [Spain]	Suárez et al, 2008
							0 - 25			Median = 1 ng/L [UK]	Martin & Voulvoulis, 2009
1.5	2.8	0.8		0.6	1.4	0.2		95		WWTPs with activated sludge processes [France]	Miege et al, 2008
	13	< 0.2	1.4 - 6.1		42	< 0.02	< 0.2 - 9		< 80	Activated sludge processes [Denmark]	Clouzot et al, 2008
20	50			3	10			78		International studies, CAS	Verlicchi et al, 2012
								60		International studies, MBR	

CAS = conventional activated sludge; BNR = biological nutrient removal; AOP = advanced oxidation process; SRT= solids retention time; MBR = membrane bioreactor; nd.= not detected

Table 4. Concentrations of each PhAC of interest in EU waters, including surface, ground and drinking water. Data originate from summary information provided in review studies from publication database; specific references listed for each PhAC. All values reported in ng/l. Values reported as minimum, maximum, range or mean, depending on what was reviewed by the reference.

* Indicates value was estimated from a figure

Drug	Concentrations in water matrices (ng/L)			Comment	Reference
	Surface water	Ground water	Drinking water		
Diclofenac	158	nd		Max in Italian studies	Meffe & de Bustamante, 2014
	< 100	< LODs	< LODs	Surface water: generally below 100 ng/L, almost always below 500 ng/L [UK, Spain, Italy, Germany, Sweden, Finland) Ground water: generally low or below detection limits, max = 380 ng/L [Spain, Italy, UK) Drinking water: generally low or below detection limits, range = 1-7 ng/L (Italy, Spain, France)	Vieno & Sillanpää, 2014
	1200			Max of international studies between 1999-2004	Rivera-Utrilla et al, 2013
	< 0.5 - 261 < 12 - 154			Range in UK Range in ~~Range in mainland Europe~~Austria	Petrie et al, 2013
	1 - 90			Range in Spanish protected areas (wetlands, estuaries, watersheds)	Vazquez-Roig et al, 2013
		477		Max in Spanish studies	Jurado et al, 2012
		121		Mean value, ~~international studies~~ the Netherlands (max = 590 ng/L, min = 2.5 ng/L)	Lapworth et al, 2012
	1 - 1030			Range ~~of international studies~~i	Ziylan & Ince, 2011
	0.3 - 147	< 10 - 50	< 0.25 - 7	Range ~~of international studies~~in UK, Germany, Slovenia	Santos et al, 2010
	15 - 135			Range in Germany~~of international studies~~	Díaz-Cruz & Barceló, 2008
	< 50 - 290			Mean value, range of international studies	Zhang et al, 2008
E2	12.9			Max in Italian studies	Meffe & de Bustamante, 2014
	0.11		0.2 - 2.1	European studies [Italy and Germany]	Kralchevska et al, 2013
	nd			Studies in Llobregat River (Spain)	González et al, 2012
		nd		Max in Spanish studies	Jurado et al, 2012

		31		Mean value, ~~international studies~~the Netherlands (max = 120 ng/L, min = 0.79 ng/L)	Lapworth et al, 2012
	0.2 - 50	0.08 - 2	nd	Range of international studies, excluding outliers	Pereira et al, 2011
	< 0.2 - 100	0.3 - 1.3	nd	~~Range of international studies~~in Germany France	Santos et al, 2010
	nd - 200	nd - 45	nd - 2	Range ~~of international studies~~in the Netherlands, France, Germany	Martin & Voulvoulis, 2009
	0.15 - 17		0.2 - 17	~~Range of international studies~~Germany, UK, the Netherlands	Wise et al, 2011
EE2	2.7	nd		Max in Italian studies	Meffe & de Bustamante, 2014
	4.3			Max of international studies between 1999-2004	Rivera-Utrilla et al, 2013
	0.04		0.15 - 2.4	Italy, Germany~~European studies~~	Kralchevska et al, 2013
	nd			Studies in Llobregat River (Spain)	González et al, 2012
		nd		Max in Spanish studies	Jurado et al, 2012
	0.5 - 50	0.7 - 5	1 - 3	Range of international studies, excluding outliers	Pereira et al, 2011
	< 0.2 - 73	0.5 - 3	< 0.1	~~Range of international studies~~Germany, France	Santos et al, 2010
	nd - 831		nd - 0.5	~~Range of international studies~~the Netherlands, UK, France	Martin & Voulvoulis, 2009
	< 0.1 - 5.1		0.15 - 1.4	~~Range of international studies~~Germany	Wise et al, 2011

3.5 Monitoring for DCL, E2 and EE2

~~Many different techniques and methods exist for monitoring the presence and effects of pharmaceutical pollutants in aquatic environments (Hecker & Hollert, 2011; Olives et al, 2012; Streck, 2009; Vazquez-Roig et al, 2013). In fact t~~The majority of the review studies evaluated by this systematic literature review were summaries of various methods for monitoring PhACs in different environmental matrices. Variation in monitoring techniques can greatly influence the results of studies that report levels of PhACs in the aquatic environment (Vazquez-Roig et al, 2013). This variation is certainly one component responsible for the wide range of DCL, E2 and EE2 concentrations in different matrices reported in section 3.4 above. The following section is by no means a comprehensive review of PhACs monitoring and analyse techniques; instead, it specifically focuses on some of the most common methods and problems for evaluating the presence, the concentrations and effects of DCL, E2 and EE2 in the context of WFD monitoring. It also addresses some major issues and concerns related to monitoring techniques for priority substances in general.

In order to evaluate and regulate the levels of priority substances in water, the WFD has defined environmental quality criteria (Environmental Quality Standards, EQSs) (European Parliament and Council of the EU, 2008). Two forms of EQSs are used, the annual average (AA) EQS and the maximum allowable concentration (MAC) EQS (units of both are µg/l or ng/l). The arithmetic mean of the concentrations of a given priority substance recorded during all representative monitoring points in a water body for a given year must not exceed the defined AA-EQS. In contrast, the measured concentration at any monitoring point within a water body may not exceed the WFD-defined MAC-EQS. EQS values can be proposed for inland surface waters (which encompass rivers and lakes and related artificial or heavily modified water bodies) as well as "other" surface waters (European Parliament and Council of the EU, 2008). To date, AA-EQS values for both inland and other surface waters have been proposed by the WFD for DCL, E2 and EE2 (European Commission, 2011). Compliance with EQSs is necessary to achieve a good chemical status of surface waters with regards to the chemicals on the EU list of priority substances, which could soon include DCL, E2 and EE2. The EQS values set by the WFD legislation will therefore directly impact which monitoring techniques will be acceptable for reporting purposes for a given compound, and will dictate the required level of sensitivity of those monitoring methods (Kunz et al, 2015).

3.5.1 Monitoring of Diclofenac

The AA-EQS values proposed by the European Commission for DCL are 100 ng/l for inland surface waters and 10 ng/l for other surface waters (European Commission, 2011). The methods for detecting NSAIDs such as DCL were recently reviewed by Olives et al. (2012). These authors report that common identification and quantification methods include gas chromatography-mass spectrometry (GC-MS) and liquid chromatography (LC) coupled with a variety of detection methods, including ultraviolet (UV) detection, diode array detection, florescence detection and tandem MS. Because DCL is a polar compound, it is more suitable for analysis by LC as opposed to GC (Vazquez-Roig et al, 2013). Furthermore, in the review studies evaluated, LC was most often coupled with MS, a highly specific technique which can detect target compounds with high accuracy (Fischer et al, 2012; Hernández et al, 2014; Vazquez-Roig et al, 2013). Another recent review from the database of publications states that there is a clear trend towards the use of LC-MS over alternative detection methods for this class of emerging contaminants (Hernández et al, 2014). LC-MS/MS (liquid chromatography with tandem MS/MS detection) is preferred over LC-MS because the former method has greater analytical sensitivity and selectivity in the analysis of drug residues in complex samples (Olives et al,

2012). Table 5 shows that recent reviews from the database of publications indicate that when using these state-of-the-art analytical methods, the LOD for DCL are typically only a few nanograms per litre (Vieno & Sillanpää, 2014). It is therefore the case that current chemical analysis techniques can usually achieve the sensitivity required to detect DCL at the concentrations required for WFD reporting.

3.5.2 Monitoring of E2 and EE2

The AA-EQS values for E2 are 0.4 ng/l in inland surface waters and 0.08 ng/l in other surface waters (European Commission, 2011). For EE2, the AA-EQS values are even lower, 0.035 ng/l and 0.007 ng/l in inland and other surface waters respectively (European Commission, 2011). The WFD-proposed AA-EQS values are derived based on species sensitivity distribution studies using the most sensitive taxonomic groups, which in this case are fish and amphibians (Kunz et al, 2015). Because even very low concentrations of E2 and EE2 can have endocrine disrupting effects for some aquatic organisms (reviewed in Burkhardt-Holm, 2010), the proposed AA-EQs values for these two compounds are low in order to provide adequate protection for the aquatic environment and human health (Kunz et al, 2015). The implications of these low standards for monitoring methods and reporting, however, are significant.

In comparison to DCL, many more review studies in our database of publications focused on monitoring methods for measuring the effects and concentrations of estrogens in aquatic matrices (Briciu et al, 2009; Kozlowska-Tylingo et al, 2010; Kunz et al, 2015; Simon et al, 2015; Sosa-Ferrera et al, 2013; Streck, 2009; Tomšíková et al, 2012). Similar to DCL, techniques for the separation of steroid estrogens are usually based on LC or GC (Briciu et al, 2009; Streck, 2009; Tomšíková et al, 2012). Detection of these compounds is also carried out using various techniques, including UV detection, florescence detection, diode detection, MS detection and tandem MS (MS/MS) (Streck, 2009; Tomšíková et al, 2012). It is difficult to achieve the required sensitivity with UV, diode or florescence detection, whereas GC-MS, GC-MS/MS, LC-MS and LC-MS/MS have much lower LODs (Briciu et al, 2009; Streck, 2009; Tomšíková et al, 2012). The specificity and sensitivity of LC-MS/MS techniques are especially required for analysis of environmental samples with steroid estrogens because of the presence of endogenous steroids in biota; that LC-MS/MS can accurately identify endogenous and exogenous estrogens is a major advantage of this technique, and has led to it being the preferred method of choice for steroid-hormone analysis (Briciu et al, 2009; Sosa-Ferrera et al, 2013; Tomšíková et al, 2012). Even LC-MS analyses typically fail to provide the required level of sensitivity to detect and quantify trace concentrations of these compounds in environmental samples (Streck, 2009), thus the most sensitive methodology for the identification and quantification of steroid estrogens is widely recognized as LC-MS/MS.

Table 5 contains summary information about the LODs for E2 and EE2 from the recent review articles in the database of publications. It is clear from this summary table that oftentimes, even when using the advanced analytical detection methods described above, current monitoring techniques are not sensitive enough to detect E2 and EE2 levels in the low ng/l or pg/L range. This can result in many studies reporting no detects for these two compounds, which makes discussions of their levels and removal rates in environmental matrices difficult. What is especially problematic is that the LODs for the most advanced analytical detection methods are usually higher than the proposed WFD EQS values. This results in a serious problem regarding monitoring and reporting of E2 and EE2 concentrations in surface waters for WFD compliance. In fact a recent review study demonstrated that only 35% of published methods are able to detect E2 at the AA-EQS

804 value of 0.4 ng/l, and only one published method exists that can detect EE2 at the AA-EQS value of 0.035
805 ng/l (Kunz et al, 2015; Tomšíková et al, 2012).
806
807 *3.5.3 Possible alternatives in the monitoring of PhACs*
808 Unlike the situation for DCL, current analytical detection methods are often insufficiently sensitive or
809 robust for monitoring E2 and EE2 given the proposed WFD standards. Under this directive, methods
810 of analysis must be able to achieve limits of quantitation (LOQ) equal to or below 30% of the associated
811 EQS. For these emerging compounds extremely low EQS values, especially for marine waters, have
812 been set which provide a great challenge to the analyst. One potential support technique for future
813 monitoring lies in the application of passive sampling (PS) techniques in investigative and surveillance
814 monitoring. Passive samplers are specifically designed to be deployed over a period of days to weeks,
815 so that time-weighted average (TWA) concentrations of compounds in aquatic environments can be
816 obtained (Wille et al, 2012). PS as a technique is based on the free flow of analyte molecules from a
817 medium being sampled to a receiving medium due to a difference in chemical potentials (Mièege et
818 al., 2010).
819
820 PS is proving to be a valuable tool for the monitoring of a range of priority substances in water,
821 sediment and biota, and can generally provide more representative profile information than
822 infrequent spot sampling on the concentrations of pollutants in water bodies, particularly where
823 concentrations fluctuate markedly in time. PS is rapidly gaining general acceptance as being applicable
824 to monitoring the behaviour and (eco)toxicological effects and fate of polar compounds including,
825 DCL, E2 and EE2 in the water column and generally can often enable much greater analytical sensitivity
826 than can be achieved by "traditional" spot-sampling, potentially improving detection capabilities by
827 orders of magnitude . While a variety of PS devices are now commercially available, several review
828 studies describe the use of the Polar Organic Chemical Integrative Sampler (POCIS) (Buchberger, 2011;
829 Vermeirssen et al, 2008; Wille et al, 2012). The POCIS has a polymer component sandwiched between
830 two thin polythersulfone membranes. PhACs with particular physiochemical properties will sorb onto
831 this polymer while the device is deployed, and can then be extracted and analysed in the laboratory
832 using analytical techniques (Buchberger, 2011; Vermeirssen et al, 2008). In addition to providing
833 estimates of TWA concentration of compounds, passive samplers can be a potential solution for the
834 problem presented by the low AA-EQS values for E2 and EE2. These compounds may accumulate in
835 passive sampling devices over time, allowing for current analytical techniques to detect and quantify
836 E2 and EE2 levels. Several recent review studies refer to the use of passive sampling to monitor various
837 environmental matrices for DCL, E2 and EE2, especially as a potential useful screening method in
838 regards to WFD monitoring (Buchberger, 2011; Vermeirssen et al, 2008; Wille et al, 2012).
839
840 While passive sampling shows potential in future monitoring of concentrations and fate of emerging
841 contaminants, application of the technique (particularly in the case of polar compounds) does face
842 some obstacles before passive sampling is considered as a viable sampling method for the WFD or
843 other legislation. Although the risk of toxicity for aquatic organisms is based on the bioavailable, or
844 dissolved pollutants in a water body, the EQS set out in the WFD for the priority substances, (with the
845 exception of trace metals), are expressed as concentrations in 'whole water'. This means that current
846 analysis must include both the dissolved fraction and any suspended matter when used in compliance
847 monitoring. However, for samples in which the level of suspended solids are low, it is often very
848 difficult to reach the required limits of detection (LODs) by conventional means, and in this situation

passive sampling could provide a useful alternative since they will take up the freely dissolved analytes in the water and have been shown to reach generally lower LODs than conventional grab samples. PS is also affected by environmental variables (temperature, water flow rate, salinity) and on the development of biofilms on the surface of the device which as an external factor can impede the uptake rate. Ongoing research is required to further develop the area of performance reference compounds (PRCs) to generally account for such effects however currently in the case of polar compounds the reliability of PRC information is limited and thus use of polar passive samplers is primarily restricted to use as a screening tool. As noted throughout this review, generation of accurate concentration information on levels of pharmaceuticals and NSAIDs in aquatic environments is becoming much more relevant in respect of greater legislative monitoring requirements and/or in terms of the generation of accurate data to support consumer or ecosystem risk exposure assessments. ~~In future years improvement of existing procedures (and in the availability of new passive sampling materials), p~~Passive sampling exhibits great potential for application in future monitoring programs for the screening of current priority and emerging compounds in water, identification of "new" pollutants of concern, source identification and its potential role in operational, investigative and surveillance monitoring under the WFD and for other legislation source attribution and fate studies of any other potential solution to the problem presented by the low EQS.

Another potential solution to the problem presented by the low EQS values of E2 and EE2 is the use of biological effects monitoring techniques (Kunz et al, 2015; Simon et al, 2015; Streck, 2009). In the case of the estrogens in particular, a variety of *in vitro* assays for effect monitoring can identify the total estrogenic activity in environmental samples, which is reported as E2 equivalent (or EEQ) concentrations (Kunz et al, 2015). Streck (2009) reviewed several *in vitro* bioassays to measure endocrine disruption and he categorizes them into three groups: ligand-binding assays; recombinant receptor-reporter assays; and assays based on the measurement of cell proliferation induced by endocrine active compounds. Effect-based monitoring techniques are particularly useful in the context of the WFD for two reasons: (i) they could be used in future elaborations of monitoring programs to provide a link between chemical and ecological assessments of water quality, and (ii) they are an excellent method for analysing the overall impact of mixtures of xenoestrogens present in many water bodies (Kunz et al, 2015; Streck, 2009). Furthermore, for compounds with extremely low EQSs, effect-based techniques can provide increased sensitivity, and may be used as a screening tool in monitoring programs. Integrated monitoring is currently the recommended approach according to experts in the field, and future iterations of European and national PhACs monitoring programmes will thus likely incorporate both chemical and biological monitoring techniques (Hecker & Hollert, 2011; Kunz et al, 2015; Simon et al, 2015).

Table 5. Proposed WFD annual average environmental quality standard (AA EQS) values for each of the three PhACs of interest vs current detection limits reported in literature reviews. Data originate from review studies from publication database, specific references are listed for each PhAC (LOD/MDL values in ng/l). If no method of detection is provided with a LOD value, values represent summary (range or average) LODs for a variety of different methods.

Drug	Proposed AA-EQS (ng/L)	LOD (ng/L)	Analytical method	Comment	Country	Review Reference
Diclofenac	**100**	a few	LC-MS	WWT effluent		Vieno & Sillanpää, 2014

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		6	Immuno-assay	~~For immunoassay.~~ Surface water; WWT effluent~~s~~	Germany	Buchberger, 2011
		~~0.25 – 100~~0.3	LC-MS	~~Range in international studies~~STP~~s~~ effluent	Luxemburg	Santos et al, 2010
		20	LC-MS	STP influent	UK	
		7	HPLC-MS	STP effluent	Spain	
		30	HPLC-MS	Hospital effluent	Spain	
		29	GC-MS	Groundwater	Germany	
		100	GC-MS	STP effluent	Spain	
		6	GC-MS	STP effluent	Switzerland	
		1	GC-MS	STP effluent	Greece	
E2	**0.4**	0.008 - 40	In vitro bioassays	Surface water	Switzerland	Kunz et al, 2015
		0.6 – ~~–~~ 3.5~~< 26~~	LC-MS	Sewage sludge	Spain	Sosa-Ferrera et al, 2013
		0.0~~42~~ – 2.01~~40~~	LC-MS	~~For measurement of steroids~~Environmental water	Spain	Tomšíková et al, 2012
		0.~~301~~ – 2~~500~~	LC-MS	~~For measurement of steroids~~Surface water; STP effluent	Luxembourg	
		0.6 – 1.6	LC-MS	STP effluent	Germany	
		0.01~~0.01~~ – 2~~5~~	LC-MS	~~Range in international studies~~Groundwater	France	Santos
		1	LC-MS	STP effluent	Luxembourg	
		1.6	LC-MS	STP influent	Italy	
		1.1	LC-MS	STP effluent	Italy	
		0.4	LC-MS	Surface water	Italy	
		1.6	LC-MS	STP influent	Germany	
		0.4	LC-MS	STP effluent	Germany	
		0.2	LC-MS	Surface water	Germany	
		0.003 - 15~~200~~	LC-MS	~~For measurements of estrogens~~ STP effluent, River Waters	Italy	Briciu et al, 2009
		2.3 – 10.6~~0.03 – < 100~~	LC~~GC~~-MS	~~For measurements of estrogens~~River water	Belgium	
		0.1 – 0. 2	HPLC-MS	WWT effluent	UK	
EE2	**0.035**	0.01 - 50	In vitro bioassays	Surface water	Switzerland	Kunz et al, 2015
		~~0.02 – 40~~0.04 – 2.01	LC-MS	~~For measurement of steroids~~Environmental water	Spain	Tomšíková et al, 2012
		0.3	LC-MS	STP effluent	Luxembourg	
		0.~~601 – 1.6500~~	LC-MS	~~For measurement of steroids~~STP effluent	Germany	
		0.01 - 0.2	Immuno-assay	~~For immunoassays.~~ Surface water; WWT effluent	Germany	Buchberger, 2011

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2.0~~0.2 – 25~~	LC-MS	~~Range in international studies~~STP effluent	Luxembourg	
2.0	LC-MS	STP influent	Germany	
0.4	LC-MS	STP effluent	Germany	
0.2	LC-MS	Surface water	Germany	Santos et al, 2010
1.6	LC-MS	STP influent	Italy	
1.1	LC-MS	STP effluent	Italy	
0.4	LC-MS	Tibre river water	Italy	
0.2	LC-MS	Groundwater	France	
0.6 -3.5	LM-MS	Sewage sludge	Spain	Sosa-Ferrera et al, 2013
0.02~~0.003 – 4~~		~~For measurements of estrogens~~STP effluent;		
15~~200~~	LC-MS	river water	Italy	
2.3 – 10.6	LC-MS	River water	Belgium	Briciu
0.1 – 0.2~~0.03 – < 100~~	HPL~~C~~C-MS	~~For measurements of estrogens~~WWT effluent	UK	
0.3 - 4.1	various	WWT effluents	Denmark	Clouzo

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LOD = limit of detection. Sensitivity of various analytical techniques deployed is influenced by sample preparation method and volume used for extraction.

[1] AA EQS values are annual average environmental quality standards for inland surface waters, which according to WFD legislation encompass rivers and lakes and related artificial or heavily modified water bodies.

3.6 Control Measures

This section reviews how the specific physiochemical properties of DCL, E2 and EE2 impact their removal from wastewater. It also discusses the control measures found to be effective for removal of these specific PhACs. This section addresses three main issues: (i) how the chemical properties of DCL, E2 and EE2 impact their removal during wastewater treatment, (ii) how these three PhACs respond during conventional secondary wastewater treatment (specifically in CAS plants) where we focused on the main elimination pathways (i.e. sorption and biodegradation), and (iii) which tertiary or advanced treatments are effective against each PhAC of interest. For the later, we focused on the 4 main categories of advanced treatments, oxidation technologies, membrane technologies, activated carbon (AC) technologies and constructed wetlands (CWs).

3.6.1 Control measures of diclofenac

3.6.1.1 Chemical properties of diclofenac impacting removal

Diclofenac is weakly soluble in water (water solubility = 2.37 mg/L at 25 °C, (DrugBank, 2015a)), with a octanol–water coefficient ($\log K_{ow}$) of 4.51 (SRC, 2013). The pK_a of DCL is 4.15 (DrugBank, 2015a; SRC, 2013). DCL has a carboxylic acid portion in its molecular structure, and this region becomes negatively ionized at a neutral pH. At acidic pH, DCL becomes electronically neutral, which increases its capacity for sorption (Vieno & Sillanpää, 2014). Thus, DCL is a compound for which D_{ow} is a better predictor of hydrophobicity. Because D_{ow} is pH dependent, the matrix in which it is measured must be specified. The

$LogD_{ow}$ value for DCL at a pH typical of wastewater treatment (approximately 8) is 2.51 (De Ridder et al, 2011). $LogK_d$ values for DCL are reviewed in Vieno and Sillanpää (2014), and listed in Table 6 As these values are typically less than three, very little removal of DCL due to sorption is predicted by this physiochemical property (Ternes et al, 2004).

According to biodegradability studies, DCL biodegradation is slow or non-existent (Joss et al, 2005; Quintana et al, 2005). Studies investigating the biodegradation constant of DCL conclude that it is almost always less than 0.1 $l\ g^{-1}\ ss\ d^{-1}$, indicating no substantial biodegradation (Joss et al, 2005; reviewed in Vieno & Sillanpää, 2014).

3.6.1.2 Removal of diclofenac during secondary treatment

In general, the physiochemical properties of DCL (summarized above in section 3.6.1.1) lead to low removal via sorption (Joss et al, 2005; Martín et al, 2012; Radjenović et al, 2009; Suárez et al, 2012; Ternes et al, 2004). On average, DCL's sorption to secondary sludge is less than 5%, while its sorption to primary sludge is in the region of 5%-15% (Ternes et al, 2004). These removal percentages are actually often lower than would be predicted based on $LogD_{ow}$ values (De Ridder et al, 2011). Furthermore, DCL is poorly biodegradable (Joss et al, 2005; Joss et al, 2006; Quintana et al, 2005). As a result of its low removal via sorption and biodegradation, incomplete elimination of DCL can be expected during conventional activated sludge treatment (Table 7, Luo et al, 2014; Vieno & Sillanpää, 2014).

A study by Patrolecco et al. (2015) identified DCL as one of the PhACs that exhibited the most persistence to removal at four WWTPs in Rome. Mainly primary and CAS secondary treatments were performed at the plants investigated. DCL showed high concentrations at the four treatment plants tested in both the influent and effluent samples (range = 519-2230 ng/l in influent and 321-1424 ng/l in effluent), and had the lowest removal efficiency out of all of the PhACs studied. Mean removal efficiencies~~rates~~ for DCL were 36% removal in spring and 39% removal in winter. These values are consistent with other CAS plants according to a recent review (Vieno & Sillanpää, 2014).

Similarly to the Patrolecco et al. study (2015), Martin et al. (2012), found that DCL had the poorest removal of any of the NSAIDs studied (mean removal efficiency~~rate~~ = 14%). The authors hypothesized that the poor removal could be due to DCL's poor degradation in wastewater. They also hypothesized that low removal efficiencies~~rates~~ could be a consequence of the release of further DCL molecules by de-conjugation of glucoronidated or sulfated DCL and/or desorption from particles. Furthermore, in this study the PhACs that were detected in the wastewater were also detected in the sludge, indicating partial removal from wastewater through sorption; DCL, however, was only detected in wastewater confirming its low potential for sorption onto sludge.

Studies have shown that elimination of DCL can be enhanced during secondary treatment by changing process configuration (reviewed in Vieno & Sillanpää, 2014). There is limited evidence that membrane bioreactors (MBRs) can increase removal efficiencies~~rates~~ compared with CAS (Radjenović et al, 2009). This may be due to the higher biomass content and longer sludge retention time (SRT) applied in MBR. However, some studies show no increase in removal of DCL from wastewater when comparing MBR to CAS (e.g. Clara et al, 2005a). CAS with biological nutrient removal (BNR) utilises a combination of aerobic, anaerobic and anoxic treatment units in order to remove excess nutrients from wastewater. The use of BNR processes has been shown to sometimes increase removal of DCL from wastewater. However it should be noted that in a recent review of the impact process configuration has on DCL removal, MBR, BNR and CAS average removal

efficiencies~~rates~~ were very similar (48%, 36% and 36% average removal efficiencies~~rates~~ respectively (Vieno & Sillanpää, 2014)).

Elimination of DCL during conventional secondary treatment can also be enhanced by altering process parameters such as hydraulic retention time (HRT) and SRT. Increasing HRT to more than 2–3 days would increase the contact time of water with the biomass, leading to higher removal efficiencies~~rates~~ (Suárez et al, 2012). However such an alteration would is likely to be unrealistic at an operational level due to the resulting need of increasing the volumetric capacity of the WWTP and high investment and operating costs associated. Moreover, enriching the bioreactor with DCL degrading microbes may also enhance elimination. This could be achieved by applying an SRT of greater than 150 days; however, this may also not be a realistic option at full scale WWTPs (Fernandez-Fontaina et al, 2012). Bioaugmentation, which is the addition of cultured microbes possessing the ability to degrade DCL into the biological process, could be used, but this approach requires further research (Vieno & Sillanpää, 2014).

3.6.1.3 Removal of diclofenac during tertiary treatment

The recalcitrant nature of DCL during conventional wastewater treatment has led to a large body of research investigating further removal of this compound from treated wastewater via tertiary treatments. Much of this research has focused on oxidation technologies, which have been found effective at mineralizing many NSAIDs (Malato, 2008; Oulton et al, 2010; Suárez et al, 2008; Ziylan & Ince, 2011). Ziylan and Ince (2011) compared the relative efficiencies of some basic advanced treatment processes and found that ozonation was among the most effective in terms of achieving the complete disappearance of NSAIDs, including DCL; they report that 95-100% of residues can be destroyed using this treatment. Some oxidation technologies that have been found to effectively degrade DCL in treated wastewater are gamma ray irradiation (Liu et al, 2011), ionizing radiation (Kimura et al, 2012) and UV or UV/H_2O_2 (Lekkerkerker-Teunissen et al, 2012), among others (reviewed in Ziylan & Ince, 2011). Operating conditions, however, can impact DCL removal efficiencies~~rates~~ when considering oxidation technologies (Malato, 2008; Ziylan & Ince, 2011). For example, initial DCL concentration (Liu et al, 2011) operation pH (Malato, 2008), TSS loading (Oulton et al, 2010) and oxidant dose and contact time (Oulton et al, 2010; Rivera-Utrilla et al, 2013) have all been shown to impact DCL removal efficiencies~~rates~~. Differences in such operating conditions can explain the range of removal efficiencies~~rates~~ reported for a vast number of oxidation technologies. Combined homogenous advanced oxidation processes (AOPs) in particular (for example, UV/H_2O_2, O_3/UV, Fe^{2+}/H_2O_2 (Fenton) and UV/Fenton oxidation) are thought to be very promising, and have shown great efficacy for DCL removal from treated wastewater (Ribeiro et al, 2015; reviewed in Ziylan & Ince, 2011). However, the major drawback of oxidation technologies for treating PhACs remains the potential formation of toxic or persistent by-products if a compound fails to be completely mineralized (Oulton et al, 2010). DCL is one of the compounds that has specifically been shown to produce by-products after treatment, especially if the oxidant dose or contact time are not adequate (Sein et al, 2008). The toxicity of these compounds must be evaluated in order to fully assess the potential of any oxidation technology for treating this particular PhAC (Andreozzi, 2004).

The use of membrane filtration technologies has also been explored as a possibility for removing DCL from treated wastewater (Kimura et al, 2003; Snyder et al, 2007; reviewed in Suárez et al, 2008; Xu et al, 2005). In general, the effectiveness of membrane filtration for DCL removal greatly depends on the type of technology considered. For example, it has been shown that DCL is poorly eliminated by microfiltration or ultrafiltration membranes (Snyder et al, 2007), making these technologies poor choices for the removal of

this PhAC from treated wastewater. However studies show that nanofiltration and reverse osmosis membranes can eliminate DCL very effectively (i.e. >90%) (Kimura et al, 2003; Snyder et al, 2007; Suárez et al, 2008), although lower elimination efficiencies~~rates~~ (60%) have also been reported (Röhricht et al, 2009). Snyder et al. (2007) specify that charged compounds (including DCL), had high rejection efficiencies for the nano and reverse osmosis membranes utilized in their study due to electrostatic exclusion between the anionic compounds and the negatively charged membranes. Nevertheless, rejection efficiency via membrane filtration has been found to decrease as the concentration of DCL in treated wastewater decreases (Kimura et al, 2003). Biofouling of membranes can also impact the rejection efficiencies of some organic compounds; however, the physiochemical properties of contaminants can have an impact on their behaviour in regards to biofouling. In one study Botton et al. (2012) found that the rejection efficiencies of negatively charged compounds (including DCL) were no different when comparing virgin and biofouled nanofiltration membranes. Although the use of membrane filtration processes for removal of DCL is technically feasible and effective, some studies report that it may not be economical for wastewater treatment given high operational and investment costs (Röhricht et al, 2009; Suárez et al, 2008).

The use of both powdered AC (PAC) and granular AC (GAC) can also result in the removal of many PhACs -including DCL- from water (Delgado et al, 2012; Rivera-Utrilla et al, 2013; Snyder et al, 2007). Because removal via this technology type is based largely on sorption, the physiochemical properties of specific compounds influences their removal efficiencies~~rates~~ (Baccar et al, 2012). For example, as sorption mechanisms are mostly hydrophobic when using AC materials (Delgado et al, 2012), $\log D_{ow}$ values can sometimes be good indicators of compound removal by AC. Although this type of tertiary treatment can partially remove DCL from water, in a recent review by Delgado et al. (2012), DCL was repeatedly cited as one of the most difficult compounds to remove using AC (e.g. below 85% at 35 mg PAC/L in Snyder et al, 2007). Removal efficiencies~~rates~~ for DCL are also variable and can depend on factors such as contact time, pH, concentration of natural organic matter and AC dose (Baccar et al, 2012; Delgado et al, 2012; Snyder et al, 2007). Removal efficiencies~~rates~~ of DCL can be enhanced when AC is used in combination with other technologies, such as AOPs. In this case, by-products or intermediates produced from the oxidation process can be removed via sorption onto the AC (Rivera-Utrilla et al, 2013).

The ability of CWs to remove PhACs like DCL has been studied more extensively in the past decade (Hijosa-Valsero et al, 2010; Hijosa-Valsero et al, 2011; Matamoros & Bayona, 2008; Matamoros & Bayona, 2006). Although many PhACs can be removed from wastewater extremely efficiently through the use of CWs, DCL is commonly cited by studies as a particularly recalcitrant compound in these systems (Hijosa-Valsero et al, 2010; Hijosa-Valsero et al, 2011; Matamoros & Bayona, 2008; Matamoros & Bayona, 2006; Oulton et al, 2010). Mean removal efficiencies~~rates~~ for DCL in CW systems are very variable, ranging in just one study from 0 to 45%. This variability is similar to removal efficiencies~~rates~~ for this compound in conventional wastewater treatment (Matamoros & Bayona, 2006 and see review in section 3.6.1.2). Many factors can impact DCL removal in CWs, including process configuration (surface vs subsurface designs (Matamoros & Bayona, 2008; Oulton et al, 2010)); design parameters (water depths, presence of vegetation, plant species, etc. (Hijosa-Valsero et al, 2011; Matamoros & Bayona, 2006)); and environmental parameters (initial concentration of the compound, oxygen availability or the season (Hijosa-Valsero et al, 2011; Matamoros & Bayona, 2008)). These variables are obviously not independent as configuration and design parameters will impact many of the environmental conditions at a given treatment site. If these systems are going to be utilized with the aim of achieving significant DCL removal from wastewaters, specific parameters that have

been shown in the literature to increase removal efficacy should be implemented. For example, recent research has demonstrated that high redox potential and the presence of plants appears to favour DCL removal (Hijosa-Valsero et al, 2011). It should also be kept in mind that removal efficiencies of PhACs at CWs can vary seasonally, with some evidence of lower removal in winter months due to lower bacterial activities at low temperatures (Hijosa-Valsero et al, 2011). Furthermore, the use of low-cost alternative sorbent materials (e.g. expanded clay, zeolite), as opposed to conventional inert materials such as sand and gravels or advanced materials such as AC, was shown to have a great potential for the removal of DCL in CW with removal efficiencies up to 90% (Dordio et al, 2013; Tahar et al, 2014); however despite a great potential these studies were performed at pilot scale and the results need to be confirmed in real scale experiments.

3.6.2 Control measures of E2 and EE2

3.6.2.1 Chemical properties of E2 and EE2 impacting removal

EDCs such as E2 and EE2 are mostly hydrophobic organic molecules, meaning they have a tendency to distribute in organic phases (Ben Fredj et al, 2015). E2 has a $\log K_{ow}$ of 4.0 (Ternes, 2006) and EE2 has a $\log K_{ow}$ of 4.2 (Ternes, 2006). The $\log K_d$ values for E2 fall between 2.3 and 2.8 (Carballa et al, 2008) and as high as 3.54 for EE2 (Table 6) (Martín et al, 2012). When $\log K_d$ values are approximately 3-5, the compounds can be expected to have moderate potential for sorption to sludge (Ternes et al, 2004); even though values below three have been reported for these estrogens under specific conditions, they are close enough to this threshold that moderate sorption potential can be expected for E2 and EE2. Indeed, studies found that these two hormones tend to gather on underwater fauna, sediments or WWTP sludge when in aquatic matrices (Zhang & Zhou, 2008). Nevertheless, biodegradation is accepted as their foremost removal pathway (Petrie et al, 2014); the K_{biol} constants for E2 and EE2 are much higher than that of DCL (300-800 and 7-9 respectively according to a review by Suárez et al (2008)).

3.6.2.2 Removal of E2 and EE2 during secondary treatment

E2 and EE2 are both generally biodegraded very effectively in WWTPs under aerobic and anaerobic conditions (Table 7, Abargues Llamas et al, 2012b). According to Alvarino et al. (2014), higher biodegradation of both compounds is achieved under aerobic conditions. Cometabolism (i.e. when an organic compound is transformed by microorganisms that cannot use the compound or its transformation products as a source energy (Grady et al, 1999)) has been shown to be the main mechanism in the removal of EE2 under nitrifying conditions, through the enzyme ammonium monooxygenase. Alvarino et al. (2014) found that in addition, other aerobic bacteria could be contributing to EE2 removal via biodegradation. As well as removal via biodegradation, the low polarity of these estrogenic compounds means sorption onto sludge may also be partially responsible for their removal from wastewater (Martín et al, 2012).

In a study by Petrie et al. (2014), the potential of CAS processes to simultaneously remove multiple micropollutants was evaluated. The study utilized a pilot-scale activated sludge plant in order to ensure process control and avoid variations in receiving sewage composition and flow; they then controlled SRT and HRT in order to evaluate the impact of these process parameters on micropollutant (including the estrogens E2 and EE2) removal. First, they evaluated whether an increase in SRT had an influence on removal at a fixed HRT of 8 hours. The authors recorded maximum achievable micropollutant removal for all chemicals, including the estrogens, when at the maximum SRT studied (27 days). Furthermore, removal efficiencies

were increased when the HRT was optimised by extending it from 8 hours to 24 hours. Most notably in the study was the improvement in the removal of the persistent EE2 (increased from 41% to 65% ± 19% at the 24 hour HRT). Improved removal of E2 (≥93%) was also demonstrated following this operational process change. Lengthening of the HRT saw a decrease in the food to microorganism ratio (F: M). A lower F: M ratio is indicative of a substrate limitation which in turn can lead to less-favoured carbon substrates like steroid estrogens being biodegraded as the primary food source (Aubenneau et al. 2010). This together with an increased contact time for biodegradation might explain the improvement in the observed biodegradation at the longer HRTs (Petrie et al, 2014).

Similar to DCL, process configuration can have an impact on E2 and EE2 removal. In a 2011 study, the removal of E2 and EE2 was determined in four different WWTPs in the UK (Ifelebuegu, 2011). Removal ranges were 83-97% for E2 and 41-58% for EE2, demonstrating again that EE2 is often more persistent when compared with other estrogenic compounds. In this study, activated sludge plants that were configured for BNR showed better removal of the estrogens compared with other CAS plants. Again, both biodegradation and sorption to sludge were recognized as the primary pathways for removal (Ifelebuegu, 2011).

Jarošová et al. (2014) conducted a pan-European monitoring campaign of WWTPs effluents which included an effect-based assessment to determine estrogenicity. They found that one third of the tested municipal WWTPs effluents had EEQ values greater than 0.5 ng/l, and that the values ranged from 0.53 to 17.9 ng/l EEQ. Overall this study shows that although removal efficiencies~~rates~~ of E2 and EE2 (and other estrogenic compounds) are usually quite high, incomplete removal could still pose a threat to the environment; thus everything possible should be done at conventional wastewater treatment facilities to increase removal of these potent estrogenic compounds.

3.6.3.3 Removal of E2 and EE2 during tertiary treatment

Unlike DCL and as discussed above, E2 and EE2 are not particularly resistant to conventional wastewater treatment; however, tertiary treatment options for removing trace concentrations of these compounds are still being investigated because of their potential to negatively impact wildlife and humans even at the low levels found in conventional wastewater effluent (Burkhardt-Holm, 2010).

According to the literature, oxidative treatments (including ozonation and AOPs) are extremely efficient at eliminating estrogens from treated wastewater (Clouzot et al, 2008; Oulton et al, 2010; Pereira et al, 2011; Pereira et al, 2012; Ribeiro et al, 2015; Suárez et al, 2008). In a recent review, Pereira et al. (2011) found that estrogenic compound levels (including E2 and EE2) can be reduced between 94-99% using various AOP technologies. In fact ozone is even more reactive with E2 and EE2 than with DCL, thus almost complete transformation of these compounds is expected following treatment (Suárez et al, 2008). However, removal of estrogens from treated wastewater through ozonation is pH dependent, and higher pH values reportedly lead to better reactivity of these compounds with ozone (Pereira et al, 2011). Furthermore the ozone reaction slows down at estrogen concentrations less than 100 ng/l, which is significant because such low levels are often found in water requiring treatment. In addition the presence of other compounds in treated wastewater can reduce reaction efficiencies~~rates~~ between estrogenic compounds and oxidants (reviewed in Koh et al, 2008; Pereira et al, 2011). According to Pereira et al. (2011) some of the best oxidative technologies for the removal of estrogens are ozonation, ferrate oxidation, and disinfection with chlorine dioxide. In contrast, E2 and EE2 are poorly removed via direct phototransformation (Oulton et al, 2010). The presence of toxic by-products and intermediates produced through the treatment of estrogens with oxidation technologies is a

growing concern. Although some studies suggest that the by-products produced are less estrogenic than the parent compounds (reviewed in Clouzot et al, 2008), more work is needed to identify and evaluate these by-products. If oxidation technologies are used to reduce the estrogenicity of wastewater, at the very least operating parameters (such as oxidant dose, contact time, and water pH) should be evaluated and adjusted in order to reduce by-product production (Pereira et al, 2011).

The ability of membrane filtration technologies to remove estrogenic compounds has also been investigated, and similar to DCL, removal efficiencies~~rates~~ depend on the technology employed (reviewed by Koh et al, 2008; Oulton et al, 2010; Suárez et al, 2008). One in depth study found that microfiltration and ultrafiltration, while inefficient for the removal of most PhACs, were very effective at removing steroid hormones (including E2 and EE2 (Snyder et al, 2007)). In general, however, microfiltration and ultrafiltration are not thought to perform as well as nanofiltration and reverse osmosis membranes (Koh et al, 2008), which are considered effective at removing estrogenic hormones from water (Braeken & Van der Bruggen, 2009; Dudziak & Bodzek, 2009; Koh et al, 2008; Snyder et al, 2007). In a review of the treatment and control strategies for removing estrogens from wastewater, Koh et al. (2008) state that nanofiltration and reverse osmosis can achieve up to 90% removal of estrogens. These figures however are variable, and lower estrogen removal via nanofiltration has been reported in other studies (between 60-85%) (Braeken & Van der Bruggen, 2009; Dudziak & Bodzek, 2009). This variability can be caused by differences in the properties of specific membranes (e.g. molecular weight cut-off, hydrophobicity, surface roughness or charge); the physiochemical properties of specific compounds (e.g. molecular size/weight, the acid dissociation constant, $\log K_{ow}$ values or polarity); or the characteristics of the wastewater (e.g. concentration of the compound, pH, presence of additional organic matter) (Dudziak & Bodzek, 2009; reviewed by Oulton et al, 2010). Finally, when considering E2 and EE2 in regards to membrane filtration technologies, both hydrophobic adsorption and size exclusion should be considered as potential mechanisms of removal, and if ultra or microfiltration are used in MBR, biodegradation may also play an important role in the removal process (Koh et al, 2008; Oulton et al, 2010).

The removal of estrogenic compounds using AC has also been shown to be very effective (Clouzot et al, 2008; Delgado et al, 2012; Koh et al, 2008; Snyder et al, 2007; Suárez et al, 2008). In a comprehensive study by Snyder et al. (2007), both PAC and GAC were capable of removing E2 and EE2 to high levels (up to 100%). It is also specified in this study and elsewhere that the efficacy of AC for removing estrogens is reduced when natural organic matter is present, because it competes for binding sites thereby limiting removal (Koh et al, 2008; Snyder et al, 2007).

Finally, constructed wetlands (CWs) have been evaluated for their ability to remove estrogenic compounds from wastewater. A comprehensive review on this subject was carried out by Matamoros and Bayona (2008). They report that CWs can remove estrogens to similar extents as conventional wastewater treatment plants, and that certain configurations achieve >90% removal. The authors also suggest that the main mechanism of estrogen removal in CWs is sorption, and therefore subsurface flow configurations will be preferable to surface flow systems.

3.6.3 Practicalities of implementing various tertiary technologies for wastewater treatment

All four of the tertiary treatment types discussed above (oxidation technologies, membrane filtration, AC and CWs) have shown some efficacy for the removal of DCL, E2 and/or EE2 (Table 8). However, whether or not

tertiary treatments will ultimately be implemented at WWTPs depends on a number of factors besides their efficacy for removing micropollutants. Instillation and running costs, increases in consumer payments, overall environmental footprint, stage of development of different technologies, and general drawbacks associated with each type of treatment must be considered and weighed against potential benefits (Jones et al, 2007). Life cycle analysis (LCA) is currently a popular tool for evaluating the costs and benefits of products, services or processes in a number of sectors, and it recently has been applied to the wastewater treatment industry (reviewed in Corominas et al, 2013). LCA is unique in that it allows for a "cradle-to-grave" analysis of the technologies in question. LCA has been used to compare emerging technologies with conventional wastewater treatments (e.g. Igos et al, 2012; Kalbar et al, 2013; Machado et al, 2007). Researchers have also implemented it to evaluate the use of advanced treatment options for micropollutant (including PhACs) removal (Hoibye et al, 2008; Rodríguez et al, 2012; Wenzel et al, 2008). This field of research is still relatively new (for example no LCA studies evaluating constructed wetlands~~CWs~~ in this context were found), but LCA studies for oxidation technologies (Hoibye et al, 2008; Rodríguez et al, 2012; Wenzel et al, 2008), AC (Igos et al, 2013) and membrane filtration technologies (Hoibye et al, 2008; Wenzel et al, 2008) regarding micropollutant removal do exist. A recent review of such studies states that overall, most findings indicate that there is little to no environmental benefit from the removal of PhACs achieved by most advanced treatment technologies (Corominas et al, 2013). However, this is largely due to the uncertainty regarding the environmental impacts of PhACs at very low concentrations; a better understanding of the implication of contamination of waters with trace concentrations of PhACs is therefore necessary to improve LCA studies in this field. Additionally, economic analyses have also indicated that treating wastewater with advanced technologies for the purposes of micropollutant removal may not be feasible; Jones et al. (2007) suggest that the high costs of adopting tertiary treatments at wastewater facilities would most likely be passed onto industrial and domestic consumers. To avoid this phenomenon, they suggest that instead, parameters in conventional wastewater treatment plants should be adjusted to maximize PhACs removal.

1193 Table 6. Chemical parameters of DCL, E2 and EE2 potentially impacting removal from wastewater.

Drug	Chemical Formula	CAS no	Molecular Mass	Water solubility (experimental)	pK_a	Log K_{ow}	Log K_d	Proposed WFD AA EQS (inland surface waters)	Summary
Diclofenac	$C_{14}H_{10}Cl_2NO_2$ (DrugBank, 2015a)	15307-86-5 15307-79-6 (disodium salt)	296.15 (DrugBank, 2015a)	2.37 mg/L (at 25 °C) (DrugBank, 2015a)	4.15 (DrugBank, 2015a; SRC, 2013)	4.51 (SRC, 2013)	$logK_{d,primary\ sludge}$ 2.7 (Ternes et al, 2004) 2.3 (Radjenović et al, 2009) $logK_{d,secondary\ sludge}$ 1.2 (Ternes et al, 2004) 2.1 (Radjenović et al, 2009)	100 ng/l (European Commission, 2011)	Fairly soluble in water, moderately low octanol–water coefficient; Ionization at neutral pH, becomes electronically neutral at acidic pH (reviewed in Vieno & Sillanpää, 2014)
E2	$C_{18}H_{24}O_2$ (DrugBank, 2015b)	50-28-2	272.38 (DrugBank, 2015b)	3.6 mg/L (at 27 °C) (DrugBank, 2015b)	10.4 (Ternes, 2006) (DrugBank, 2015b)	4.0 (Ternes, 2006)	$logK_{d,\ sludge}$ 2.3-2.8 (Carballa et al, 2008)	0.4 ng/l (European Commission, 2011)	E2 is weakly soluble in water, has high pK_a, fairly hydrophobic (Ben Fredj et al, 2015)
EE2	$C_{20}H_{24}O_2$ (DrugBank, 2015c)	57-63-6	296.40 (DrugBank, 2015c)	11.3 mg/L (at 27 °C) (DrugBank, 2015c)	10.4-10.7 (DrugBank, 2015c; Ternes, 2006)	4.2 (Ternes, 2006)	$logK_{d,primary\ sludge}$ 2.28-2.67 (Martín et al, 2012) $logK_{d,secondary\ sludge}$ 2.77-3.54 (Martín et al, 2012)	0.035 ng/l (European Commission, 2011)	EE2 is weakly soluble in water, has high pK_a; high $logK_d$ indicates it tends to be retained onto sludge, consistent with high pK_a value (Martín et al, 2012)

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Table 7. Impact of conventional activated sludge on removal of PhACs of interest during wastewater treatment.

PhAC	Sorption to sludge	Degradation potential	HRT	SRT	Removal efficiency (conventional activated sludge)
Diclofenac	**Low potential** Sorption to sludge observed to a low degree (Martín et al, 2012; Patrolecco et al, 2015; Radjenović et al, 2009; Suárez et al, 2012; Ternes et al, 2004)	**Low potential** Poorly biodegradable (Joss et al, 2005; Joss et al, 2006; Quintana et al, 2005)	Elimination of diclofenac could be enhanced by increasing HRT to more than 2–3 days; would increase the contact time of water with the biomass (Suárez et al, 2012)	Enriching the bioreactor with diclofenac degrading microbes may enhance elimination; could be achieved by applying a SRTs > 150 days (Fernandez-Fontaina et al, 2012)	**Variable but generally poorly removed; 0-81.4% (Luo et al, 2014)** Mean concentrations in European municipal influents between 0.11 and 2.3 µg/l (110 and 2300 ng/l), effluents between 0.002 and 2.5 µg/l (2 and 2500 ng/l) (Vieno & Sillanpää, 2014)
E2 & EE2	**Moderate potential** Susceptible to removal by sorption (Ben Fredj et al, 2015; Carballa et al, 2008; Martín et al, 2012; Ternes, 2006; Zhang & Zhou, 2008)	**High potential** Generally biodegraded very effectively in WWTP processes under aerobic and anaerobic conditions (Abargues Llamas et al, 2012b; Alvarino et al, 2014; Petrie et al, 2014)	Biodegradation was increased when the HRT was optimised by extending it from 8 to 24 hours (Petrie et al, 2014)	Maximum achievable removal when at the maximum SRT studied (27 days) (Petrie et al, 2014) Critical SRT of 10 days for removal of natural estrogens and some micropollutants suggested (Clara et al, 2005a)	**Highly removed:** **E2: 92.6-100%** (Luo et al, 2014) **EE2: 43.8-100%** (Luo et al, 2014) Reduced by ~85%. Final effluents normally contain nanogram per litre concentrations (Griffith et al, 2014) EE2 typically more recalcitrant than E2 (Petrie et al, 2014)

HRT: hydraulic residence time; SRT: solids retention time

Table 8. Impact of various tertiary treatment types on removal of PhACs of interest during wastewater treatment.

Technology	Diclofenac removal	E2/EE2 removal	Costs	By-product danger
Membrane filtration technologies	Highly dependent on filtration technology; poor for micro and ultra filtration, can be efficient for nano and reverse osmosis (Kimura et al, 2003; Snyder et al, 2007; Suárez et al, 2008)	Variable depending on technology; removal via nanofiltration ranges from >50%-90% (Braeken & Van der Bruggen, 2009; Dudziak & Bodzek, 2009; Koh et al, 2008)	Capital costs include construction, engineering, materials costs, operational and management costs include replacing membranes and power to pump wastewater (US EPA, 1999a)	No (US EPA, 1999a)
Activated carbon	Can be efficient, depending on operational variables (Delgado et al, 2012)	Can be efficient, depending on operational parameters and wastewater characteristics (Delgado et al, 2012; Koh et al, 2008)	Dependent on different carbon contactor configurations and the cost of on-site vs off-site regeneration, as well as site and wastewater characteristics Capital costs include carbon contactors, storage tanks, regeneration systems (etc.) and operational costs include purchase of carbon, electrical power, flushing of carbon slurry piping, etc. (US EPA, 2000b)	No (Rivera-Utrilla et al, 2013)
Oxidation Technologies	Highly efficient processes for DCL removal (>90%) (Ribeiro et al, 2015; Ziylan & Ince, 2011)	Highly efficient process for estrogen removal (94-99%) (Pereira et al, 2011)	Dependent on technology type, capacity of the plant, wastewater characteristics, manufacturer and the site; e.g. ozonation costs generally high compared with other technologies, while UV can be competitive (US EPA, 1999b; c)	High for DCL (Sein et al, 2008) and estrogenic compounds (Pereira et al, 2011)
Constructed wetlands	Very variable removal efficiencies~~rates~~, DCL considered recalcitrant compound (Matamoros & Bayona, 2008; Oulton et al, 2010)	Variable removal efficiencies~~rates~~ but can be effective (>90%) depending on configuration and design parameters (Matamoros & Bayona, 2008)	Major capital costs include purchasing land, liner costs, engineering, etc., but both capital and operational and management costs tend to be much lower than conventional wastewater treatments (US EPA, 2000a; c)	

4. Conclusions and Future Research Needs

The overall aim of this study was to provide a baseline study for Europe exploring the implications of the addition of the three watch list compounds DCL, E2 and EE2 to the Water Framework Directive (WFD) priority substances list. This study utilized a systematic literature review to summarize the European state of knowledge in regards to the sources and prevalence of these PhACs. Finally, a critical analysis of the effectiveness of potential control measures was carried out based on best-published information. Below, the main conclusions from each of these components of the study are summarized and future research needs are established.

The bibliographic analysis carried out by this study determined that the annual output of European research on DCL, E2 and EE2 has increased steadily from 1995-2015, with approximately 84% of all articles on aquatic contamination with these PhACs published since 2005. More studies are performed on the estrogens than DCL annually, and studies focused on monitoring are more common than those on sources of contamination or control measures, though control measure studies are on the rise in recent years. Laboratory scale studies are the most common, while more realistic field and wastewater treatment plant (WWTP) level studies are rarer. This can likely be attributed to a lack of sensitive analytical techniques or accurate sensors. Spain and Germany are the European leaders in the field. The systematic literature review conducted by this study investigated the sources, receptors and monitoring methods for the three PhACs of interest. Overall it was found that DCL and EE2 enter the European aquatic environment mainly following human consumption and excretion of therapeutic drugs, and incomplete removal from influent at urban WWTPs. E2 is a natural hormone excreted by humans which also experiences incomplete removal during WWTPs treatment, although livestock populations in Europe are also a significant non-point source of E2 contamination. In regards to receptors, throughout Europe DCL has on average higher concentrations (high ng/l or µg/l levels) in all aquatic matrices compared with the hormones E2 and EE2 (ng/l range); however, this does not necessarily translate to higher negative environmental impact/risk to aquatic organisms. Diclofenac concentrations found in European surface waters are generally below the annual average environmental quality standard (AA EQS) proposed by the WFD (100 ng/l), but several review studies report values exceeding this limit in the UK, Italy and other mainland European countries. E2 European surface water values are usually less than 50 ng/l but nevertheless such values still greatly exceed the proposed AA EQS value (0.04 ng/l) of this bioactive compound. Similarly, EE2 is either not detected or found at low levels in European surface waters (usually below 10 ng/l), but reported values are often still higher than the proposed AA EQS value (0.035 ng/l). Finally, current standard, laboratory-based analytical chemistry methods are sufficiently sensitive for the detection and quantification of DCL, but limits of detection for E2 and EE2 are often higher than proposed AA EQS values, presenting serious analytical challenges in regards to chemical monitoring methods and reporting for these two PhACs.

The systematic literature review results were expanded to analyse potential control measures that may be implemented at WWTPs to decrease levels of DCL, E2 and EE2 in final effluents. The review revealed that physiochemical properties or experimentally determined constants that can be used to predict the removal of PhACs during wastewater treatment include the octanol–water partition coefficient (K_{ow}), n-octanol–water partition coefficient (D_{ow}), solid-water distribution coefficient (K_d), half-life and the biodegradation constant,

(K_{bio}). PhACs with high water solubility and low biodegradability are the most recalcitrant during wastewater treatment. Studies showed that DCL is poorly removed during conventional wastewater treatment; removal percentages are variable but generally fall between 21 to 40%. Mean concentrations in European municipal influents are between 0.11 and 2.3 µg/l (110 and 2300 ng/l) and effluents between 0.002 and 2.5 µg/l (2 and 2500 ng/l). In contrast, E2 and EE2 are generally highly removed during conventional wastewater treatment: removal percentages generally are 85% or greater. Final European effluents normally contain nanogram per litre concentrations of these compounds, but EE2 is consistently more recalcitrant than E2.

Where secondary treatments are deemed insufficient, tertiary treatments may be used to further improve the quality of the final effluent. In particular, recent studies have mostly investigated four types of tertiary treatment technologies for removal of PhACs from treated wastewater~~,~~ including oxidation technologies, membrane filtration, the use of activated carbon (AC) and constructed wetlands (CW). Oxidation technologies are considered highly efficient at DCL removal. Membrane filtration can also be efficient at removing DCL from treated wastewater, but it depends on the technology used; micro and ultra-filtration are typically ineffective while nano and reverse osmosis filtration are very efficient for this particular PhAC. The application of AC can effectively reduce DCL concentrations in treated wastewater, but removal efficiencies~~rates~~ largely depend on operational variables. Finally, CWs demonstrate variable removal of DCL, but this compound is generally considered recalcitrant in these systems. For E2 and EE2 removal, oxidation technologies are considered very effective treatments. Membrane filtration technologies, while exhibiting more variation than oxidation technologies, can also be extremely effective. The use of AC is also appropriate for estrogen removal, but similar to DCL removal efficiencie~~rate~~s depend on operational variables and wastewater characteristics. Finally, CWs can perform estrogen removal, but the effectiveness depends upon the configuration and design of the system. Although more information is needed to accurately model the benefits of using tertiary treatments to reduce PhAC concentrations in treated wastewaters, in general the literature suggests that the environmental benefits may not outweigh the costs. Some sources suggest that it may currently be more economically to adapt conventional wastewater treatment operational variables to decrease PhACs emissions, rather than incur the costs/complications of adding tertiary treatments.

This study has highlighted areas for future research attention to include (a) development of more sensitive and validated analytical methods for different environmental samples (especially for the steroid estrogens) in order to be able to comply with WFD reporting requirements. Given the extremely low AA EQSs proposed for these compounds, it is also necessary to continue to investigate alternatives to chemical analyses, such as passive sampling, use of appropriate biological surrogates or effect-based monitoring techniques; (b) intensification of technology-focused studies for effective and efficient control measures for PhACs removal particularly at areas showing disproportionally high levels of these PhACs in terms of load; (c) ~~to~~ evaluation of~~e~~ validated, bolt-on or mobile technologies effective for the removal of PhACs in wastewater,. (d) identify and quantify population level effects of wild biota from endocrine disrupting chemical exposure; and (e) investigate seasonal variations in PhAC loading and removal efficiencies in future studies with particularly addressing influence of climate change.

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