Occurrence and geodatabase mapping of three contaminants of emerging concern in receiving water and at effluent from waste water treatment plants – a first overview of the situation in the Republic of Ireland

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Abstract

This constitutes the first study to address occurrence and geodatabase mapping of the anti-inflammatory drug diclofenac (DCL) and the natural (17-beta-estradiol or E2) and synthetic (17-alpha-ethynylestradiol or EE2) estrogenic hormones in Republic of Ireland receiving waters over the period 1999 to 2015. Among these data, 317 samples came from concentration studies, while 205 were from effect-based studies. Monitoring data came from 16 waste water treatment plants (WWTPs), 23 water bodies (including rivers, lakes, marine and transitional waters) and 7 from domestic locations. Out of approximately 1000 WWPTs in the Republic of Ireland, only 16 have been monitored for at least one of these compounds of emerging concern (CECs). Diclofenac is found in treated effluents from 5 WWTPs at levels at least as high as other European WWPTs, and sometime higher. Measurements of E2 and EE2 in WWPT effluents were rare and effluents were more often evaluated for total estrogens; these CECs were generally not detected using conventional analytical methods because of limits of detection being too high compared to environmental concentrations and WFD environmental quality standards. There was good agreement between occurrence of these CEC and regional drug dispensing data in Ireland. Mapping the aforementioned data onto appropriate river basin catchment management tools will inform predictive and simulated risk determinations to inform investment in infrastructure that is necessary to protect rivers and beaches and economic activities that rely on clean water. There is a pressing commensurate need to refine/develop new analytical methods with low levels of detection for future CEC intervention.

Keywords: EU watch-list substances, diclofenac, estrogens, occurrence, monitoring, receiving water, control points, Republic of Ireland

Highlights:

- Occurrence and mapping of 3 EU Watch list substances in Irish aquatic environment
- Lack of monitored data for CECs given number of river basin catchments and control points
- Need for new analytical techniques with low appropriate levels of detection to meet WFD limits
- Control measures frequently do not fully remove these harmful chemicals
- Mapping of CECs will strategically inform future upgrades to important control points

Introduction

Pollution of European receiving waters containing pharmaceuticals is a ubiquitous phenomenon (Verlicchi and Zambollo, 2016; Barbosa et al., 2016; Tiedeken et al., 2017). Until recently environmental regulations worldwide had not required explicit testing of these contaminants of emerging concern in water bodies. However, given concern about contamination of aquatic environment with these substances, legislation such as the Water Framework Directive (WFD) and the Environmental Quality Standards Directive (EQSD) at a European level and associated legislation at a local level has recently begun to acknowledge this problem (Tiedeken et al., 2017; Tahar et al., 2017) The identification of these contaminants and associated analytical methods may inform pressure points and efficacy of appropriate interventions for consideration in future WFD-monitoring programmes and regulations. Pharmaceuticals are a class of emerging environmental contaminants that are widely used in human and veterinary medicine (Tahar et al., 2017). From here on, these substances of emerging concern will be referred to as pharmaceutically active chemicals (PhACs) that includes not just pharmaceuticals but also their pharmaceutically active metabolites/transformation products. This research is important because of the potential toxic effects for aquatic biota and human health that may result from chronic exposure to PhACs (Streck, 2009; Kümmerer, 2009; Kosma et al., 2014). 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 (Kosma et al., 2014). A large number of PhACs have been detected in wastewater treatment plants (WWTP) influents and effluents and surface, ground and drinking water worldwide in recent years (Cirja et al., 2008; Streck, 2009; Zhou et al., 2009; Verlicchi and Zambolla, 2016). It is now established that throughout the developed world, PhACs are ubiquitous at µg to ng per litre levels in the aquatic environment (Streck, 2009). The impacts of chronic exposure to trace concentrations of many PhACs on wildlife and human health may be severe (Verlicchi et al., 2012); thus, it is critical to limit as much as possible the concentrations of this class of contaminants in our waterways.

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 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. In response to growing EU concern about the release of untreated PhACs into the aquatic environment, three compounds were included on in the first EU 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) (Barbosa et al., 2016). Annual average environmental quality standard (AA-EQS) were defined for these 3 compounds as being the concentrations defining the boundary between good and moderate WFD status. The respective AA EQS in surface water for DCL, E2 and EE2 are 100 ng/L, 0.035 ng/L and 0.4 ng/L. EE2 and E2 can impact the endocrine system of humans or wildlife (Verlicchi et al., 2012). There are growing fears that chronic exposure to these endocrine disrupting chemicals or 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.[5] Similar to PhACs as a whole, EDCs are mainly thought to be transported into the aquatic environment via incomplete removal at WWTPs (Streck, 2009). 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 (Barbosa et al., 2016). Also, following the timetable for the common implementation of the WFD, the first management cycle ended in 2015, and the second river basin management plan combined with the first flood management plan is due to end in 2021.

A systematic review of these three first EU watch list PhACs in receiving waters was recently published, which reviewed 3945 potentially relevant articles over period 1995 to 2015 publications on uses, sources, monitoring and control measures to produce a EU-wide database (Tiedeken et al., 2017). Overall, European surface water concentrations of DCL are typically below reported annual proposed AA EQS of 100 ng/L, but 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 (0.4 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. In addition, the EPA reported in October 2017 that in 42 locations in the Republic of Ireland, sewage is discharged untreated, putting rivers and bathing areas at risk of pollution: 44 of 170 large urban areas did not comply with EU water quality standards (EPA, 2017). The review of Tiedeken et al. (2017) highlighted that there is a pressing need to conduct detailed mapping of the occurrences and control measures for CECs at a national scale that provides a platform for EU orientation so as to inform policy and decision-making on improving and protecting water quality. Thus, the aim of this case study was to evaluate best-published data on these three EU Watch list PhACs so as to geographically map their occurrence in Irish receiving waters and in effluent at WWTPs and to compare with regional drug dispensing data.

Materials and Methods

For each publication identified as containing relevant Irish DCL, E2 or EE2 monitoring data, several other parameters were extracted for use in mapping. First, the date water samples were taken was

identified (including day, month and year if available, but year at minimum). The type of study (i.e. a study measuring the concentration of a compound or a study using effect-based methods) as well as the compound analysed (DCL, E2, EE2 or estradiols equivalents, EEQ) was identified. The method of sampling (grab, composite, passive, or any type of assay utilised) was identified, as was the matrix studied (marine water, lake water, ground water, WWTP influent/effluent). The name and GPS coordinated of the specific location where the sampling took place as well as the county was listed. If the sampling location was a WWTP, its coordinates were recorded as the primary discharge point listed in the EPA wastewater licence (http://www.epa.ie/terminalfour/wwda/index.jsp?disclaimer=yes&Submit=Continue#.VpPcJPmLTIX). The concentration (in ng/l) of the compound recorded during each sampling event was also recorded if it was available. If multiple samples were taken at the same location during a study (i.e. repeat sampling over time), each sampling event was listed separately. However, if multiple analyses were run on the same samples (i.e. tests run in duplicate or triplicate) the individual results were not reported; instead the final value as presented by the authors of the study (usually an average) was utilised. The publication (reference) associated with each data point was recorded. Frequently some of the data described above were not available from the publications, in which case corresponding authors were emailed or otherwise contacted and a data request was made.

Two aspects of these data were mapped for this report, (i) the distribution of the sampling events (or sampling effort) for each compound and (ii) the highest recorded concentrations of each compound at a sampling location. In order to map sampling events, the data were divided into three 5-6 year intervals: 1999-2004, 2005-2009, and 2010-2015. Results for samples that were taken at the same location, analysed for the same compound and sampled during the same interval were consolidated, and the number of sampling events at each location was specified. This allowed the sampling effort at each location to be mapped for each compound of interest. For the concentration data, effect-based studies were ignored. For some of these studies, the concentration values were not available. This was because either the study reported only presence/absence of the compound or reported average values instead of raw data. In the latter case, authors were contacted to attempt to obtain raw data values, however a small number of authors did not respond to data requests. These studies were thus excluded from concentration mapping. The remaining concentration data were then sorted by compound, location and value, and for each unique location the highest concentration recorded for each compound was identified for mapping. These concentration maps therefore represent the worst case scenario as recorded by monitoring studies to date at each location. It must be noted that this does not mean that these sites will always have such high concentrations of the compounds of interest; additional sampling events may have recorded lower values or non-detects. It should also be noted that just because a compound has not been detected at a site does not mean it will not exceed WFD proposed limits (EQS); it is possible (especially for the estrogens) that the limit of detection (LOD) may exceed the EQS limit. Nevertheless, these maps still provide an indication of which areas could be pollution “hotspots” based on currently available monitoring data.

Data were mapped using ArcGIS Desktop software (Arc Catalog and ArcMap 10.3.1, Environmental Systems Research Institute, via an ESRI single-user, one-year license). Additional data used to create the file geodatabase were downloaded from the Central Statistics Office database "StatBank Ireland," (http://www.cso.ie/px/pxeirestat/statire/Select_Table/Omrade0.asp?Planguage=0, utilised for county boundaries, city locations and population density) and the EPA's Geo Portal (http://gis.epa.ie/GetData/Download, utilised for river basin catchments, WFD river basin districts, WWTP locations and attribute data, and WFD protected areas). A map of the the Republic of Ireland river basin catchment is provided in supplementary materials (Fig S1).

Reported drug utilization or dispensing data is an important source of information on the quantities of PhACs that ultimately are released into the environment (Boxall, 2010; Rowan, 2011). Therefore, with the objective to correlate the concentration data gathered and to provide a proxy for the region where no environmental data were found, drug utilising dispensing data was provided by Ireland's Health Service Executive (HSE) for the 32 Local Health Offices (LHOs) for DCL and E2. The total volume (kg) of DCL and E2 dispensed to patients in all LHOs from 2009-2012 was provided, however data for years 2008 and 2013 were excluded as these periods were not full calendar years. EE2 is often prescribed as a combination drug, thus obtaining and summarizing dispensing data is more difficult than for DCL and E2 and was therefore not included in this study.

Results and Discussion

GIS mapping – an overview of Irish DCL, E2 and EE2 data (1999-2015)

From the literature review performed, a total of 522 unique Irish monitoring data points were identified for DCL, E2, EE2 or EEQ concentrations. Of these samples, 151 were measurements of DCL concentrations, 83 each were measurements of E2 and EE2 concentrations and 205 were measurements of EEQ concentrations. These monitoring data include samples from 50 unique locations, comprised of influent or effluent from 16 Irish WWTPs, samples from 23 unique water bodies (including rivers, lakes, marine and transitional waters) and domestic effluent from 7 locations. Figure 1 demonstrates the distribution and frequency of the national monitoring data for DCL, E2, EE2 and EEQ concentrations over the entire period reviewed by this report, from 1999-2014 (the different maps for the periods 1999-2004, 2005-2009 and 2010-2015 are presented in supplementary material). Several patterns regarding the overall distribution of these monitoring data can be observed. First, the monitoring data are distributed relatively evenly throughout the country, though they do tend to be focused around population centres (Galway, Dublin and Cork). EEQ sampling is particularly evenly distributed, having been taken for many inland and coastal surface waters, including the east, south east, south west, and west coasts, as well as one location in the north of the country. Much of the concentration data for all three compounds, however, is carried out in coastal water bodies (marine or transitional waters), rather than in inland surface waters. This is particularly true of DCL, which has

mainly been sampled in coastal waters surrounding Dublin and Galway. Concentration measurements of DCL, E2 and EE2 are particularly lacking in the midlands region. Finally, it is clear from this figure that all monitoring data on these watch list PhACs are lacking from the north, north east and northwest coasts of the republic of Ireland. A summary of all the monitoring studies across the country is provided in supplementary materials (Figures S3-S5).

In general, the estrogens/estrogenicity is better studied in the Republic of Ireland in terms of monitoring data when compared with DCL. Furthermore, in regards to the estrogens, effect data (measured by EEQ) are more common than concentration data. This is likely due to difficulties associated with obtaining sufficiently sensitive analytical methods of detecting trace amounts of estrogens in water samples. While data from effect-based studies reporting EEQ concentrations can help give an indication of the overall estrogenicity of Irish waters, the compounds responsible usually are not identified. Thus, in regards to quantifying contamination from these two specific estrogenic compounds, effect-based sampling is not as useful as concentration studies that report specific levels of E2 or EE2. Nevertheless, given the lack of sensitivity of current analytical methods, effect-based studies may have a place regarding reporting for WFD monitoring. Figure 1 illustrates that in addition to surface water samples, monitoring data also came from measurements of WWTP influent or effluent.

In the period 1999-2004, studies of endocrine disrupting chemicals and pharmaceutical contamination of aquatic environments were just beginning to gain international popularity during this time. Irish monitoring data during these early years consist mostly of results from effect-based studies, reporting EEQ concentrations. They were carried out largely in the east and southwest coasts and midlands by a project focusing on the Shannon International River Basin District (Tarrant et al., 2005; Tarrant et al., 2008). Some E2 and EE2 concentration measurements were also taken for domestic effluent at sites in the south east of the country, but LODs for these compounds were still too high to compare to WFD proposed limits (Kelly et al., 2010; Quinn-Hosey et al., 2012). No DCL data were found for this period. In the second time period, from 2005-2009, the first DCL sampling took place on the east coast of the country, although all three sampling locations were samples from WWTP influent and effluent as opposed to surface waters (Lacey et al., 2008; Lacey et al., 2012). EEQ sampling for estrogenicity continued for the Shannon International River Basin District project during this time period (Quinn-Hosey et al., 2012) and EEQ sampling began on east, south east and west coast locations for the SeaChange project (Giltrap et al., 2013). The project with the most individual EEQ sampling events (89) also took place on a dairy farm in the north of Ireland during this time period (Gai et al., 2012). In the final and most recent time period, from 2010-2015, a shift in estrogen sampling is apparent; instead of sampling more inland freshwater sources using largely effect-based studies (EEQ measurements), coastal locations are more frequently monitored. This means that instead of freshwater samples there was a focus on marine and transitional waters. This is because the SeaChange project, which was meant to rectify the lack of estrogen concentration data in marine waters, took place largely during this time period. Less individual samples tend to be taken during concentration studies, because

analyses are more expensive and time intensive than many effect-based studies; this is indicated by the smaller symbol sizes in Figure 4, as opposed to 2 and 3. For DCL, more samples were taken in surface waters in both the east and west coasts of the country (McEneff et al., 2014), these data represent the best information to date on DCL levels in surface waters in Ireland. The GIS maps associated to these periods are presented in supplementary materials II.

Monitoring studies

WWTPs monitoring and locations in Republic of Ireland

Figure 2 highlights the monitoring data which originate from the 16 sampled Irish WWTPs, and demonstrates the distribution of these sampling locations in regards to all Irish WWTPs. Details of the 16 WWTPs is shown in supplementary material (Table S1). In total, 178 (34%) of the national monitoring data points from the reviewed publications were measurements of one of the four investigated parameters (E2, EE2, DCL or EEQ concentrations) from WWTP influent or effluent. Most WWTPs were sampled for estrogenicity only. Fourteen WWTPs were sampled for estrogenicity in total, including Athlone, Ballincollig, Carlow, Clonmel, Fermoy, Kilkenny, Killarney, Leixlip, Longford, Osberstown, Ringsend, Roscommon, Tralee and Tullamore. Only two WWTPs were sampled for E2 and EE2 specifically, Ringsend and Osberstown. Five plants were monitored for DCL levels including Galway, Leixlip, Ringsend Osberstown and Swords. The two WWTPs that were sampled for all 4 compounds were Ringsend and Osberstown. The 16 WWTPs for which monitoring data for at least one of these four compounds exists make up approximately 1.45% of all of Ireland's WWTPs, indicating that more data on the concentrations of these compounds in Irish agglomerations would be useful. The WWTPs with EEQ sampling data are distributed evenly throughout the country, though measurements are missing from the northwest. DCL again has only been sampled in WWTPs surrounding the major population centres of Dublin and Galway. Data on DCL levels in WWTPs influent and effluent in the midlands, south and north are severely lacking.

Highest monitored concentrations in Republic of Ireland for the 3 targeted compounds

Figures 3-4 and supplementary material (Figure S2) were created by mapping the highest concentration value recorded for each drug at each site where it was monitored. These figures thus allow for a comparison of the maximum recorded concentrations of each particular compound at the various sites for which monitoring data currently exist. These maps provide an indication of which areas could be pollution "hotspots" based on currently available monitoring data. The maximum E2 concentrations ranged from not being detected in any samples at a site to 15.2 ng/L. The AA EQS proposed for E2 is 0.4 ng/L (European Commission, 2011); it is clear from Figure 3 that several locations had maximum values which exceed this limit, including locations near Cork, Galway and Dublin city. Given that E2 is a natural steroid estrogen, it is unsurprising that high concentrations are found near these population

centres. Out of a total of 12 sites with reliable quantitative monitoring data, 7 had maximum values that exceeded the proposed AA EQS value. Of those 7, two were domestic wastewater (that are not to be compared with AA EQS that refer to surface water after mixing), but the rest were surface waters distributed throughout the country; future studies should consider these water bodies as potential monitoring sites as it is possible their E2 levels may exceed WFD limits.

EE2 could not be detected at 9 out of 11 sites where sampling occurred, and the maximum concentration recorded for EE2 at any site was 0.32 ng/L. This relatively high value was found in a sample of domestic effluent from a home in County Wicklow as opposed to in a surface water sample. The AA EQS value proposed for EE2 is 0.035 ng/L (European Commission, 2011). From the data presented in Figure 4, it may seem like only two locations may exceed this AA EQS; however, this interpretation cannot be trusted because the sensitivity of many of the analytical methods used to detect and quantify EE2 was insufficient. Few methods exist that can reach the required LOD of 0.035 ng/L, hence it is impossible to state with certainty that EE2 is below the WFD proposed limit if the compound is not detected at any of these locations. Additional sampling of surface waters for EE2 using sufficiently sensitive analytical methods is therefore of critical importance to our understanding of how concentrations of this compound in Irish waters compare to WFD limits.

For DCL there were clearly less quantitative available data in surface waters, even though analytical methods used to detect this compound are more easily available than for the estrogenic compounds. The AA EQS value proposed for DCL is 100 ng/L, and the maximum recorded concentration at every surface water sampling location except for one exceeded this value, including samples from Galway and Dublin Bay. Among all monitoring locations, the highest recorded values at two locations actually exceed 1000 ng/L, however these were effluent samples from WWTPs; however, these effluent waters are diluted in receiving waters and the expected concentrations in receiving water would be reduced. Nevertheless, it appears that certain locations in Ireland can have very high levels of DCL at times, indicating that additional monitoring for this compound is necessary.

Wastewater treatment plants and receiving waters monitoring studies

Table 2 gives a summary of the monitoring data found for the Republic of Ireland for the three compounds of interest. The first study investigated DCL in WWTPs for three agglomerations in the greater Dublin area (Lacey et al., 2008). Twenty-four hour composite samples were collected from the influent and effluent of each WWTP. The authors report that DCL was not detected in the influent samples (limit of detection or LOD = 0.855 µg/L), but was present in effluent samples (LOD = 0.743 µg/L), although not above the study's limit of quantification (LOQ) for this compound (LOQ in effluent = 2.478 µg/L). A second study by the same research group analysed monthly influent and effluent samples in three WWTPs for a full year (Lacey et al., 2012). Diclofenac was again detected in at least one effluent sample from each plant (detected in 5 effluent samples in total, LOD ranged from 0.5-2.95 µg/L). Similar to the previous study however, DCL was not detected in any influent samples (Lacey et

al., 2012). The authors suggest this could be due to deconjugation of conjugated metabolites during the treatment process, which has been observed in other studies (Zhang et al., 2008), this could also be due to possible higher interferences in the analysis of the inlet water due to higher organic matter content that could have reduced the sensitivity of the chemical analysis; however, it is possible that DCL was present in the influent, but at levels below the LOD. In fact in comparison to other European studies, the LODs for both of the Lacey and co-workers (2012) studies were high. LOD in the ng/L range for DCL are currently standard in the international literature. These were the only studies found that investigated removal efficiencies of DCL, E2 or EE2 in Irish WWTPs.

However, some studies of E2 and EE2 in Irish WWTPs used biomarkers or *in vitro* bioassays to quantify total estrogenic activity (reported as estradiols equivalents, EEQ) instead of direct chemical analysis. The first study to evaluate Irish WWTP effluent for estrogenicity was carried out in 2004 by Quinn and co-workers (2004). An effluent sample from Athlone WWTP was analysed for the presence of estrogenic chemicals using the yeast estrogen screen (YES) assay, and HPLC and GC-MS were used for chemical analyses. Lake water from a marina in Killenure Lough, Lough Ree (Co. Westmeath) was also sampled and analysed (Quinn et al., 2004). This study confirmed the presence of a complex mixture of compounds with estrogenic activity in the WWTP effluent. This estrogenicity was attributed mostly to E2, EE2 and bisphenol A. This was also the first study to demonstrate the presence of EDCs in lake water in Ireland. However, results in both effluent and lake water were limited; attempts to identify E2 and EE2 with GC-MS were unsuccessful due to relatively high LOQs associated to the chemical analysis, and the authors stated that further work was necessary to confirm their presence in these aquatic matrices. At approximately the same time that the Quinn study was performed (Quinn et al., 2004), the Tarrant et al.. (2005) also began investigating Irish WWPT effluent for estrogenicity. They used the YES assay to screen undiluted effluent from Osberstown (Co. Kildare) and Ballincollig (Co. Cork) WWTPs, and found estrogen levels at 17.2 ± 3.8 and 3.2 ± 1.1 ng/L EEQ respectively. Ballincolig effluent levels were diluted upon entering the River Lee to a level that was below the threshold required to induce vitellogenesis in fish. The effluent form Osberstown WWTP, however, was not diluted enough by the River Liffey to reach levels below this threshold. Accordingly, the study also found raised vitellogenin in male brown trout from this study site, indicating the water was estrogenic enough to have an impact on wild fish (Tarrant et al., 2005). It should be noted however, that the compounds responsible for the estrogenicity of the water were not identified analytically; however, the authors hypothesized EE2 might be responsible because a component of Osberstown WWTP industrial effluent came from a manufacturer of the contraceptive pill. In the work described in Tarrant and co-workers (2008) estrogenicty using the YES assay was also measured for 8 additional Irish WWTPs and some of their receiving waters. Again, results are reported in EEQ; effluents ranged from 1.1-16.0 ng/L and receiving waters ranged from 0.9-2.9 ng/L. These results indicated that (besides a few notable exceptions in Co. Dublin), levels of estrogenic compounds were high in some Irish WWTP effluents,

but that they were sufficiently diluted in most receiving waters so as not to cause an immediate threat to aquatic wildlife (Tarrant et al., 2008).

Given that previous studies mostly focused on surface waters near Dublin or Cork, in 2010 a study investigating estrogenicty in the Shannon International River Basin District (SIRBD) was carried out (Kelly et al.., 2010). Five rivers were sampled and estrogenicity was assessed using the YES assay. Not only was estrogenicity evident in the rivers at all sites downstream of WWTPs, it was also present at two control locations. The EEQ values ranged from 0.53-2.67 ng/L, within the concentration range of E2 required to induce vitellogenesis in male rainbow trout (Kelly et al., 2010). This study also demonstrated that levels of EDCs in Irish rivers were elevated in comparison to EEQ values reported by previous studies (Tarrant et al., 2005). An additional study was carried out by the same research group further investigating EDCs in the Border, Midland and Western (BMW) region of Ireland (DoELG, 1999). In this work, influent and effluent samples were collected from inlet and outlet pipes at 4 different WWTPs, and corresponding upstream (control) and downstream water samples were taken from the Rivers Hind, Camlin, Shannon (at Athlone) and Silver (at Tullamore). Grab samples were taken over two year period to investigate seasonal changes. The results demonstrated that some WWTPs in this region were highly efficient in removing estrogen contamination; EEQ removal achieved was 98% and 92% respectively for Tullamore and Longford WWTPs. However, the EEQ levels in receiving water samples from downstream and upstream of the WWTPs demonstrated that this region of Ireland can obtain heavy loads of EDCs (maximum EEQ = 16.21 ng/L in a downstream sample from the River Camlin) (DoELG, 1999).

The SeaChange project provides the most recent monitoring data of E2 and EE2 in Irish waters (Giltrap et al., 2013). For example, in one study, water and mussel samples were collected from three locations on the Irish coast in June 2010: (i) in the estuary of the River Liffey (Dublin site), (ii) in Galway Bay (Galway site) and (iii) in Redbank hatchery, Aughinish Co. Clare (Clare site, mussels only analysed). Samples were analysed by LCMS/MS for estrone (E1), E2 and EE2. This study provided the first detection of E1 by LC/MS/MS in Irish marine waters (Dublin Bay, 0.76 ng/L) (Kunz et al., 2015). E2 and EE2 were not detected in the water samples from Galway Bay or in any mussel samples from the Dublin, Galway or Clare sites (Kunz et al., 2015). E2 was detected in a sample from Dublin Bay, but levels were low (0.13 ng/L) and three additional samples were non-detects. Therefore the annual average value was below the proposed WFD AA EQS.In general, the authors of the report concluded that the levels and associated endocrine disrupting effects were generally low at their study sites, and there was likely a low risk of estrogen-caused endocrine disruption to resident species; however areas with significant anthropogenic pressures are at higher risk and additional monitoring was suggested by the authors at such sites (e.g. Dublin Bay) (Giltrap et al., 2013). Further sampling for estrogens at additional sites along the Irish coastline was carried out during the SeaChange project using passive sampling and effect-based monitoring, however these results are not as easily compared with the proposed standards for the WFD.

The most recent data evaluating DCL levels in Irish wastewater effluent or surface waters come from Schmidt and co-workers (2013). In a year-long study, sewage effluent, receiving marine waters and marine bivalves were analysed for DCL and other pharmaceutical residues. Effluent was sampled (24-h composite samples) from one WWTP on both the east and west coast of Ireland. Mussels were deployed in a year-long cage experiment in a control site and two effluent exposure sites of the east and west coast, and grab samples of surrounding marine surface waters were also analysed. LOQ for DCL were 225 ng/L, 22 ng/L, and 29 ng/g respectively in effluent, marine water, and mussels. The highest DCL concentrations detected in effluent were 1.69 and 2.63 µg/L in the eastern and western WWTP respectively. In marine surface waters the highest values were 0.46 and 0.55 µg/L in the east and west. Diclofenac was not detected in mussels (Wille et al., 2012). The concentrations of DCL in effluents and surface waters are comparable to those found in other EU monitoring programs, but surface water values in particular are on average higher than DCL concentrations detected in other European marine waters (Wille et al., 2012). Furthermore, the highest marine surface water value detected in this study was approximately 5 times higher than the current proposed AA EQS for DCL. In a later experiment associated with the same project, wild mussels from a pristine site off the west of Ireland and a highly contaminated site on the east coast were analysed for several PhACs; again, DCL was not detected in any samples (McEneff et al., 2013). An additional study investigated PhACs residues (including DCL) in cooked and uncooked marine mussel tissue, and found that cooking increased PhACs residues in contaminated tissue.[30] Although this does provide evidence for the potential exposure of humans to DCL via bioaccumulation, mussels in this experiment were artificially exposed in the lab and so this study did not provide monitoring data.

Monitoring of on-site non collective treatment systems

In Ireland the domestic wastewater of more than one-third of the population is treated by on-site systems (DoELG, 1999). Considering that human excretions represent a major source of PhACs contamination (Buckberge, 2011) on-site wastewater treatment systems could thus be an important source requiring consideration in Ireland. Work reported by Gill and co-workers (2009) which investigated the effectiveness of septic tank and secondary treatment on-site wastewater systems, produced two publications specific to EDC removal. The first study sampled domestic effluent at 4 sites in Ireland, two with effluent discharged following primary treatment (i.e. septic tank) and two with secondary treatment (i.e. peat filter) systems (Ó Súlleabháin et al., 2009). EDCs were found at all 4 sites, but E2 and EE2 were each found at two out the four sites with no straightforward relationship with the type of treatment. The sensitivity of the chemical analysis was poor in this study; EE2 was not determined quantitatively and E2 had a high LOD (2 µg/L). The second study aimed to answer similar questions using analytical methods with increased sensitivity (e.g. LOD for E2 = 0.05 µg/L). Gill et al. (2009) again investigated the natural attenuation of EDCs in the most common on-site treatment system in Ireland, the septic tank and subsoil percolation area. The study focused on the transport of EDCs,

including E2 and EE2, through the soil at three sites in the east of Ireland. Overall, the authors found that E2 and EE2 were significantly degraded with depth to sub ng/L levels at all sites investigated. These results are the only indication of how efficient on-site systems are at EDC removal in Ireland, and no equivalent study has investigated DCL removal.

Studies on alternative control measures

Studies investigating DCL, E2 and EE2 in wastewater effluent such as those reviewed above (Quinn et al., 2004; Tarrant et al., 2005; Tarrant et al., 2008; Lacey et al., 2008; Gill et al., 2009; Lacey et al., 2012; Wille et al., 2012; McEneff et al., 2013; Loos et al. 2013; Schmidt et al., 2013; Jarošová et al., 2014; Tiedeken et al., 2017) demonstrate that these compounds are often not removed completely during treatment at Irish WWTPs. Some Irish studies have therefore examined advanced treatment options which may remove DCL, E2 or EE2 from water more efficiently. For example, a study in 2010 examined the feasibility, kinetics and efficiency of using liquid-core microcapsules as a novel methodology for removal of DCL from water (Whelan et al., 2010). The work demonstrated that liquid-core microcapsules are capable of rapid extraction of DCL (100% within 50 min of capsule addition to contaminated water) and other common PhACs. Another study investigated the temporal removal of estrogenic activity of several estrogens (including E2 and EE2) by UVA irradiation over an immobilised titanium dioxide (TiO_2) catalyst (Coleman et al., 2004). UVA photolysis over the catalyst was equally effective at removing estrogenic activity of E2, EE2 and E1; the study demonstrated a 50% reduction in estrogenicity in samples treated for ten minutes. Also, the work reviewed above by Cai and co-workers (2013) investigated removal of hormones from dairy farm wastewater with the aim of evaluating the efficiency of CWs to reduce estrogenic hormone concentrations in dairy wastewater as it was demonstrated in a study carried out in the UK that dairy cows were identified as the largest contributors to excreted estrogens (Johnson et al., 2006). Over the course of a year, monthly samples were taken at seven locations on the farm (i.e. the inflow pond, a plant covered area, close to the outlet of ponds, a lake on the farm, the receiving river, and a groundwater monitoring well) and analysed via a reporter gene assay (RGA). An average removal efficiency for estrogenic compounds of 95.2% was found, indicating that the CW was efficient at removal of such compounds; however, the lowest removal rate during the year was 83.7%, and the concentration at the final pond was 18.8 EEQ ng/L, which is above the proposed lowest observable effect concentration of 10 ng/l (Cai et al., 2012). These authors found that CWs currently employed in Ireland can eliminate hormones in dairy wastewater to low levels often acceptable for effluent. Cai et al. (2013) was also reported that advanced treatments, such as the employment of reactive and sorptive materials (granulated activated carbon, organoclay, etc.), can further improve CWS treated dairy farm wastewater quality, which may be particularly important in enhancing removal efficiency of peak hormone concentrations Additional studies on effective treatment options that can function in an Irish context are needed and could improve the effluent quality in regards to DCL, E2 and EE2 levels.

457 ***Comparison of Irish and EU monitoring data***

458 In 2010, two Irish WWTPs (one in Co. Kildare and one in Co. Dublin) were surveyed (via grab samples)
459 for a wide range of PhACs, including DCL, E2 and EE2. This monitoring was part of a Joint Research
460 Centre (JRC) pan-European campaign designed to provide the first concise overview of concentrations
461 of many emerging pollutants in WWTP effluents across Europe (Jarošováet al., 2014). Along with
462 conventional analytical techniques, effect-based monitoring was also carried out in order to determine
463 total estrogenicity of the effluents, reported as EEQ. The study's results for Ireland found no steroid
464 estrogens above their LOQ (i.e. 10 ng/L for steroidal estrogens) via chemical monitoring. In addition,
465 the detected EEQ for both Irish WWTPs were <0.5 ng/L, even though one third of the municipal WWTP
466 effluents from across Europe that were included in the study had values greater than 0.5 ng/L. This
467 finding indicates that the two Irish WWTP effluents have relatively low estrogenicity in comparison to
468 many European countries. One of the Irish WWTPs was one of only 4 municipal WWTPs in the study
469 that utilized a tertiary treatment step (UV light); this advanced treatment could have contributed to the
470 observed low levels of estrogenic contamination (Loos et al., 2013). The same year, another study based
471 on the same Irish sampling events was published which analysed effluents for additional PhACs,
472 including DCL (Loos et al., 2013). The study found that throughout Europe, DCL was among the
473 compounds with the highest median concentration levels (it was found in 89% of all samples, max =
474 174 ng/L, median = 43.3 ng/L). In the Irish samples specifically, DCL was detected at concentrations
475 of 80.3 and 144.3 ng/L for the two investigated WWTPs respectively (Loos et al., 2013).

476 Another European level study in 2013 used the GWAVA model to predict water concentrations
477 of DCL, E2 and EE2 in rivers throughout Europe. The study recognized that the levels of these
478 compounds found in receiving waters would vary considerably between European nations depending
479 on available dilution of sewage effluent. Overall, the model predicted that 12%, 1% and 2% by length
480 of Europe's rivers would exceed the EE2, E2 and DCL proposed annual average EQSs. For all three
481 compounds however, less than 10% of Irish river length was predicted to exceed EQSs. There are
482 significant sources of uncertainty in the model that should be noted; as far as we can tell, estimates are
483 based on Northern Irish data only, and the parameters determining effluent concentrations, a critical
484 component of estimating river concentrations, have additional uncertainty (Johnson et al., 2013).

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486 **Relationship with drug utilizing dispensing data**

487 Annual dispensing data for each LHO throughout the country is mapped for DCL and for E2 (heat maps
488 and dispensing data presented in supplementary material. Similar trend emerged where LHOs
489 exhibiting higher dispensing data for DCL also matched locations reported previously for upper
490 concentrations of the same compound using monitoring techniques (supplementary material). However,
491 this observation should be taken with care considering the low number of monitoring data referenced
492 for DCL in surface waters. This trend was also evident for E2 where mapped baseline drug dispensing
493 data from LHO's exhibiting upper concentrations of this estrogen were similar to locations identified

494 for upper values using monitoring techniques (Figure 3). While this proxy baseline dispensing data is
495 useful for possibly intimating indicator areas that make disproportionately high contributions to overall
496 pollution load, these "hotspots" may vary spatially and temporality and may occur due a variety of other
497 contributing sources such as discharge points of high-risk WWTPs, high densities of livestock near
498 water sources, effluent from hospitals and residential homes, and/or pharmaceutical producers
499 (Verlicchi et al., 2012; Tiedeken et al., 2017).
500
501 **Conclusion and perspectives**
502 The Water Framework Directive (EC, 2000) and Irish River Basin Management Plans (RBMPs)
503 establish both legal and operational frameworks to protect and restore clean water and to ensure its
504 long-term, sustainable use. These goals require an integrated approach to the sustainable management
505 and protection of water resources. Critical shortfalls in existing Irish RBMPs highlight the importance
506 of affordability and prioritisation considerations, particularly given the economic and social value of a
507 clean and protected water supply. Therefore, the overall aim of this study was to provide a baseline
508 study for Ireland exploring the implications of the addition of DCL, E2 and EE2 to the WFD priority
509 substances list. This study mapped all national concentration data and concludes that DCL
510 concentrations found in surface waters are generally below the limits proposed by the WFD, but that
511 exceedances have occasionally been reported as it is the case in several European countries. In
512 comparison, E2 and EE2 surface water concentrations are generally much lower, however reported
513 values still commonly exceed the WFD proposed limits for these bioactive compounds. Perhaps most
514 notably, while current standard, laboratory-based analytical chemistry methods are sufficiently
515 sensitive for the detection and quantification of DCL, limits of detection for E2 and EE2 are often higher
516 than proposed EQSs. This issue presents serious analytical challenges in regards to chemical monitoring
517 methods and reporting for these two PhACs, and impacts Ireland's ability to meet European reporting
518 requirements for these estrogenic compounds. The mapping work conducted during this study
519 demonstrated that more monitoring data on DCL, E2 and EE2 in Irish waters is required. Nevertheless,
520 based on the limited Irish data extracted from the literature and mapped in this study, it appears that the
521 majority of Irish surface waters may not exceed WFD proposed limits for DCL, E2 and EE2, but that
522 point sources of pollution could lead to occasional hotspots exceeding European limits. It must be noted
523 that this prediction is based upon the use of very limited data, and is especially uncertain because of a
524 lack of sufficiently sensitive analytical detection methods. This observation will resonate with the
525 majority of EU countries in terms of current levels of CEC monitoring in respective receiving waters
526 and at control points. Conventional analytical methods are sufficiently sensitive for the detection and
527 quantification of DCL, but not for E2 and EE2, thus alternative, ultra-trace, time-integrated monitoring
528 techniques such as passing sampling are needed to inform water quality for these estrogens. Another
529 emerging potential solution to the problem of low EQS values of E2 and EE2 is the use of biological
530 effects monitoring techniques (Streck, 2009; Kunz et al., 2015; Simon et al., 2015).

In comparison to other 28 EU countries along with Switzerland, Norway and Turkey, Ireland produced 21 studies on these three contaminants of emerging concern over 15-year systematic review period (Tiedeken et al., 2017) where Spain, Germany and the United Kingdom contributed 707 (56%) of all reports. However, 24 and 16 EU countries produced under 50 and 20 articles respectively on these PhACs in their national receiving waters; consequently, very few countries have reported on use predicted or measured environmental concentrations to underpin modelling or to inform risks in their river basins (ter Laak et al., 2010; Guillén et al., 2012; Murphy et al., 2017). Overall, this Irish study supported main tenets of Tiedeken and co-workers (2017)) which 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. E2 is a natural hormone excreted by humans, which also experiences incomplete removal during WWTP treatments.

Future Irish-specific work in this research field is essential in order to ensure PhACs do not threaten our water supplies. Irish studies evaluating PhACs levels in WWTPs influents and effluents are also lacking; these are needed in order to develop effective and economic control measures for PhAC removal from wastewaters. The present study also found that on-site treatment systems could potentially be major sources of PhACs contamination in an Irish context, thus future research should address this issue. Given the positive results and outcomes from studies that utilize effect-based (biological) monitoring, passive sampling or an integrated monitoring approach (combined use of chemical and biological monitoring methods), thus Ireland along with other EU member-states must consider broader acceptance of these types of methodologies for WFD priority substance reporting. Future projects evaluating concentrations in aquatic and other environmental matrices (sludge, sediment, biota) must be supported, particularly for compounds that are not yet considered priority substances (current and potential future watch list compounds). Furthermore, more data should be collected on prescriptions written and dispensed by public and private health agencies in Ireland, and such data should be made available to research projects. Another issue is the unavailability of commercially sensitive data such as PhAC sales/production information. This information would facilitate a more accurate determination of emission sources in different catchments. Now that the limitations of these existing monitoring data are understood, additional types of data that can help inform regulators and policy makers about the levels of these substances in Irish waters should be explored. In particular, it would be useful to apply established European models for predicting fate, behaviour and concentrations of chemical pollutants in surface waters in an Irish context including considerations for influence of climate change given that 2015 was the wettest year recorded in Ireland over 250 years of annual measurements (Murphy et al., 2017). To this aim, longer term projects that utilise European software and models to predict fate of watch list compound concentrations in whole Irish watersheds should be carried out such development of spatially explicit Geography-Referenced Regional Exposure Assessment Tool for European River Basins (GREAT-ER).

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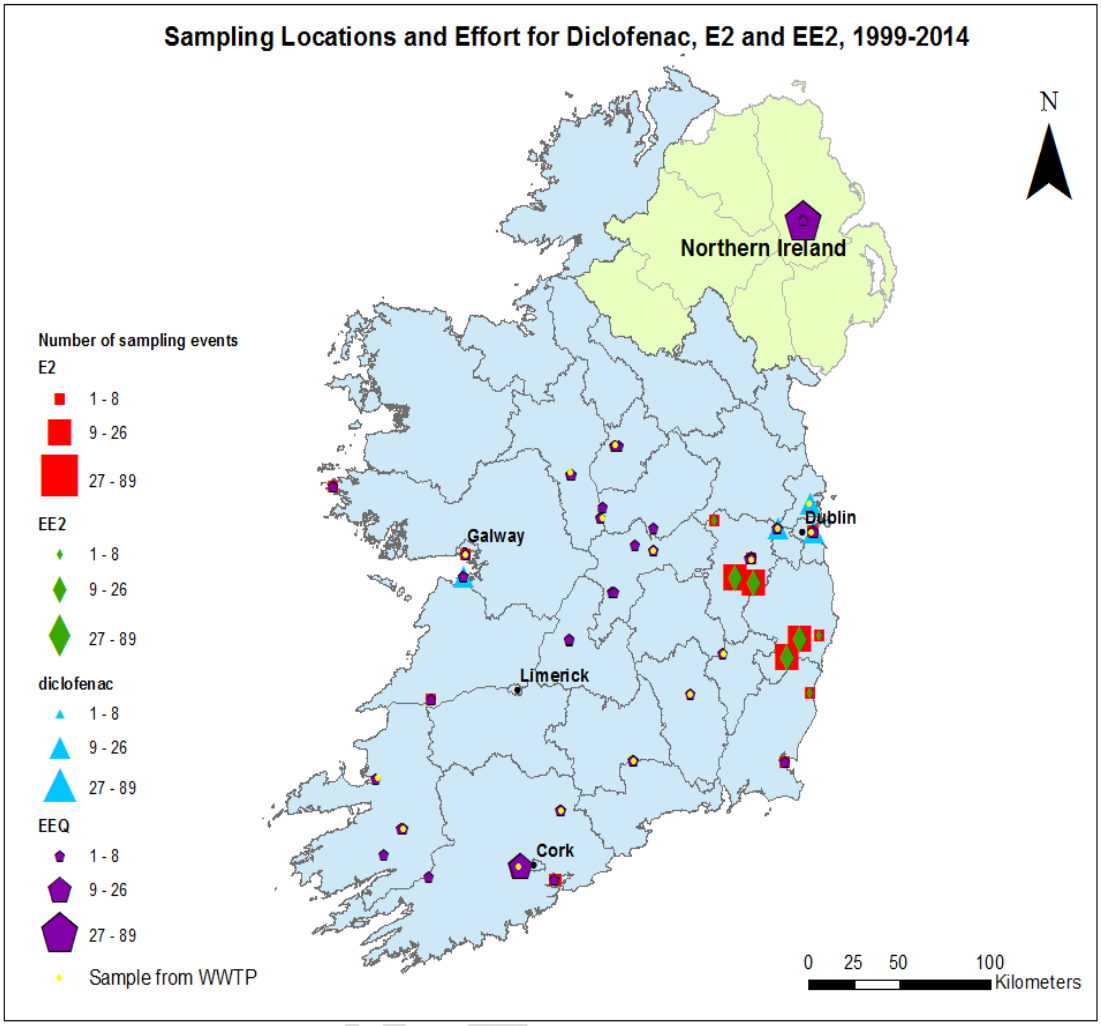

Figure 1. Summary of national monitoring distribution and frequency for diclofenac (blue triangles), E2 (red squares), EE2 (green diamonds), and estradiols equivalents (purple pentagons) in Ireland from 1999-2015. Symbol size increases with increasing number of samples taken at each location.

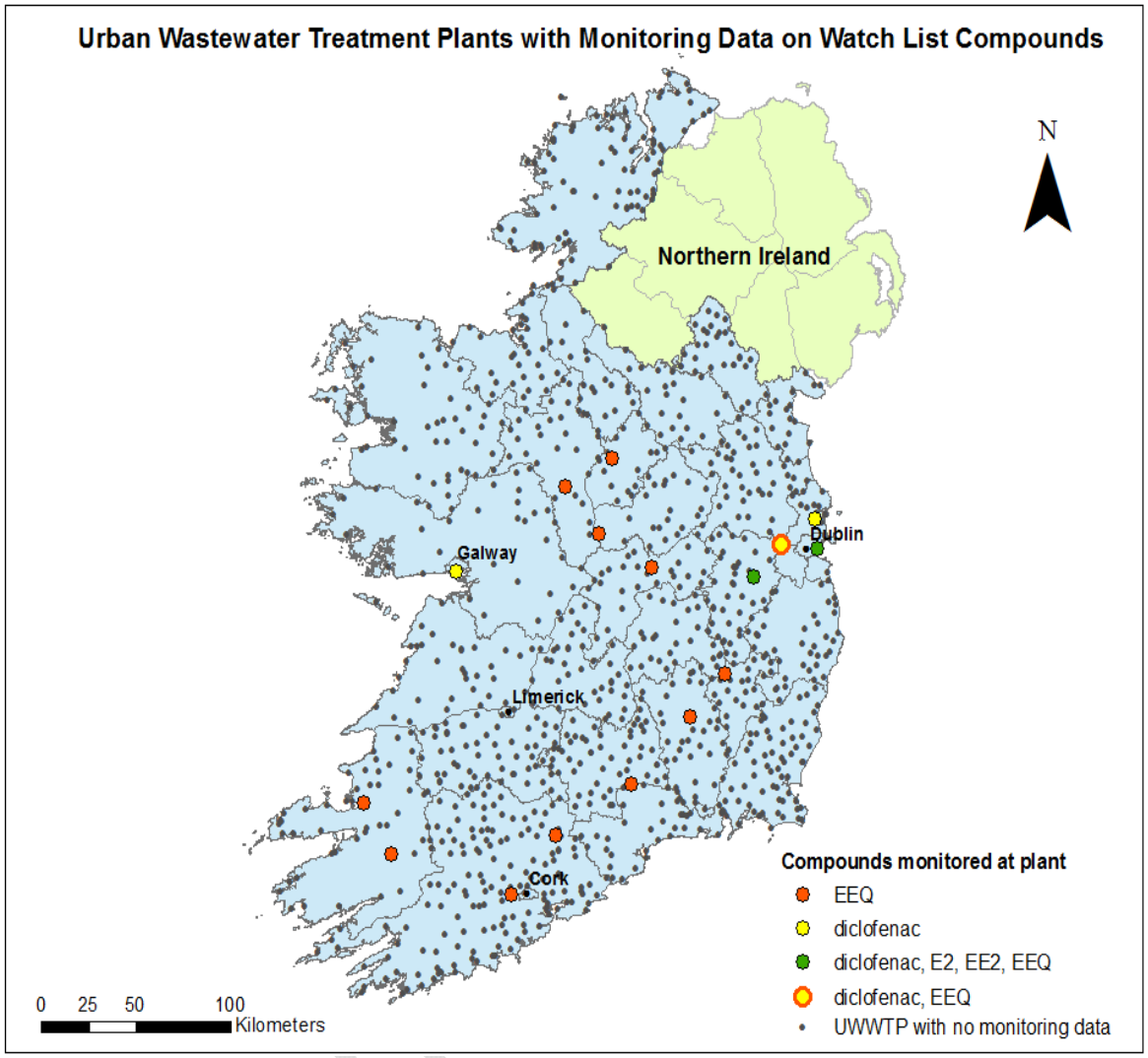

Figure 2. Distribution of urban wastewater treatment plants (UWWTPs) with existing monitoring data on diclofenac, EE2 and/or estradiol equivalents (EEQ). Orange dots represent agglomerations with only EEQ measurements; yellow dots represent plants with only diclofenac monitoring data; green dots represent plants that have been monitored for all four compounds; yellow dots with orange outlines represent plants that have been monitored for diclofenac and EEQ only; and black dots represent the locations of agglomerations with no monitoring data for these compounds.

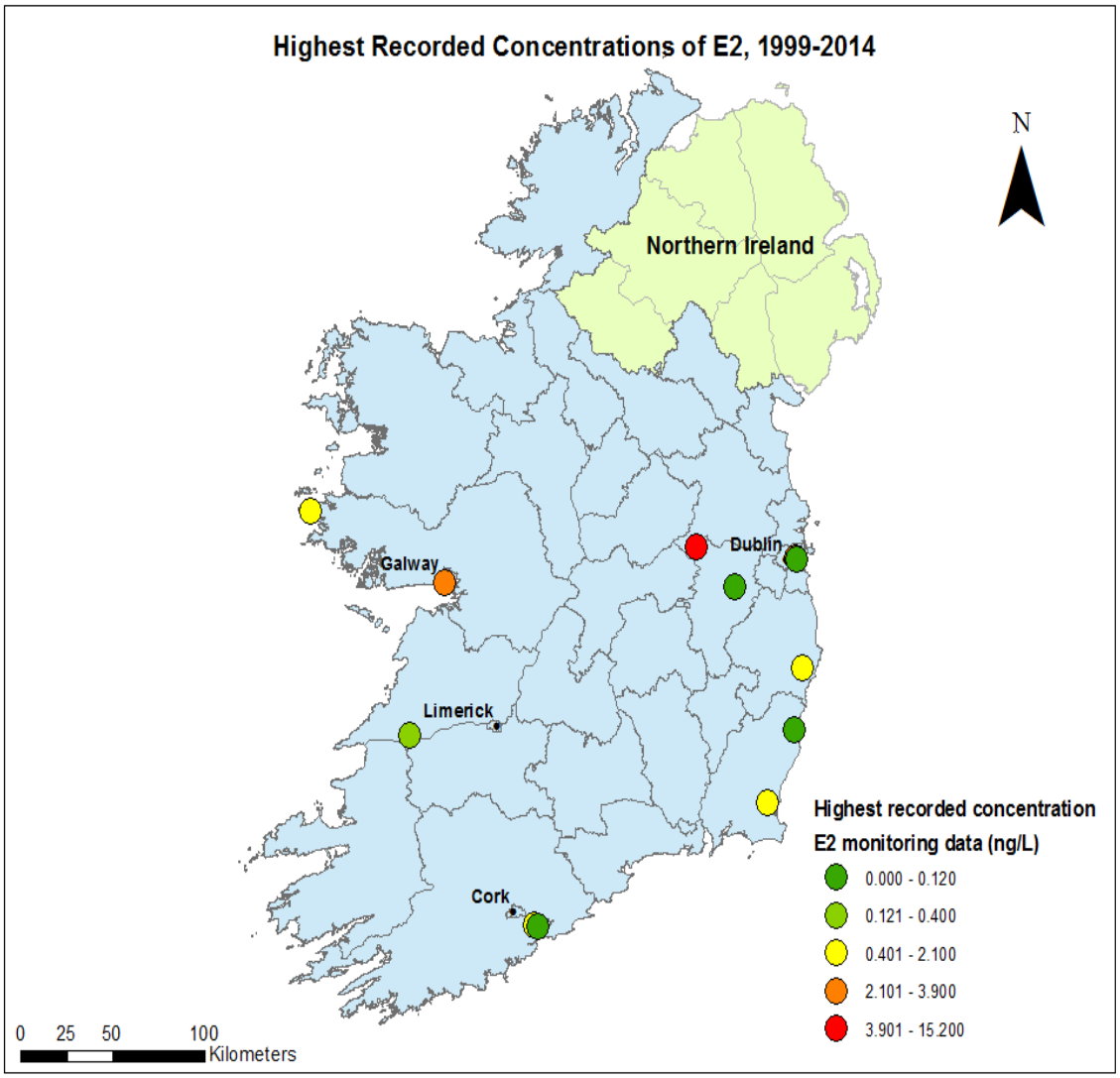

Figure 3. Highest recorded concentrations (ng/L) of E2 at each sampling site where concentration monitoring data were collected. Relative concentration values are indicated by the symbol colour, where low concentrations are indicated by greens and high by reds. Yellow, orange and red indicate sites where the highest recorded concentration was greater than the proposed WFD AA-EQS value for E2 (0.4 ng/L).

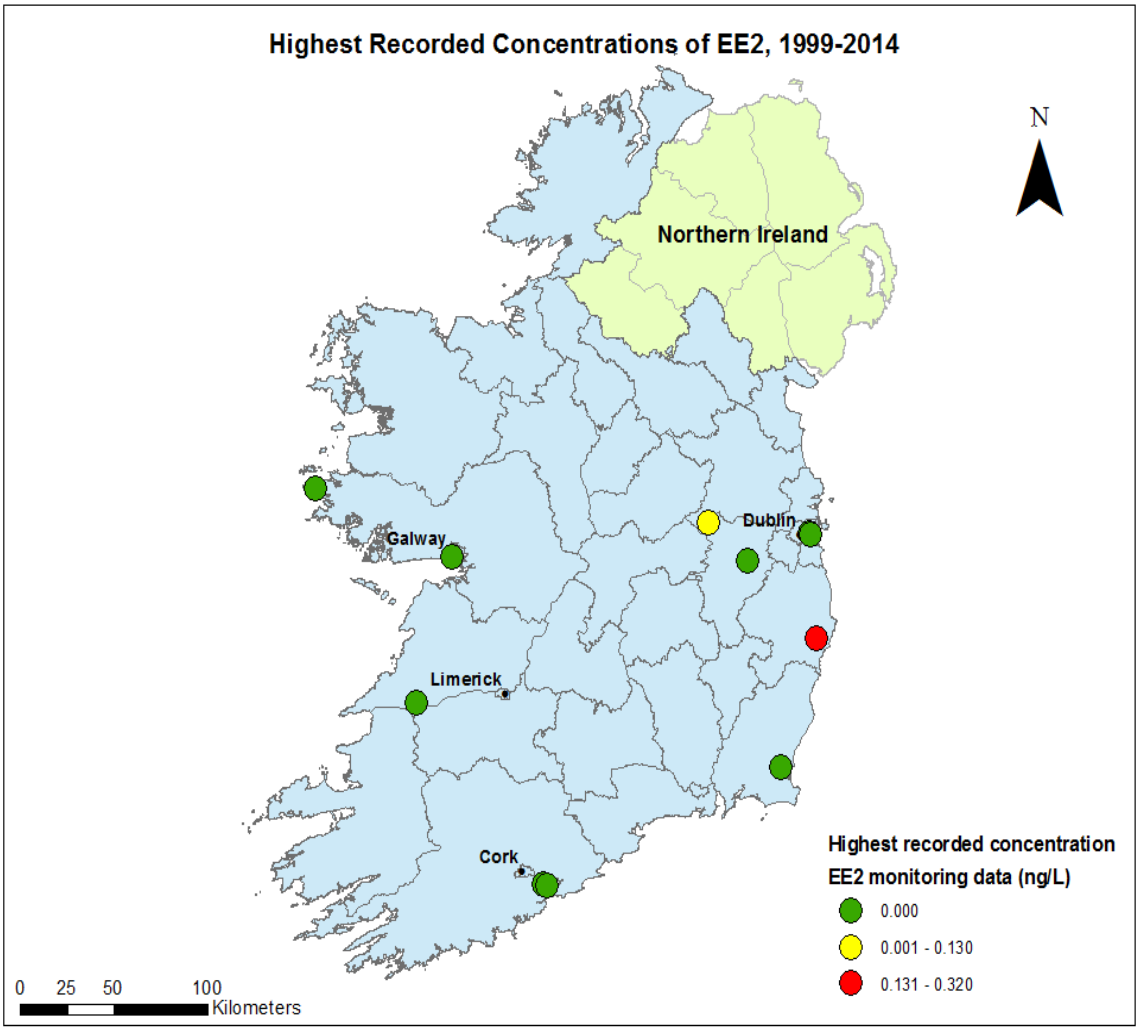

Figure 4. Highest recorded concentrations (ng/L) of EE2 at each sampling site where concentration monitoring data were collected. Relative concentration values are indicated by the symbol colour, where low concentrations are indicated by greens and high by reds. Zero values represent no detects. There are two green dots near Cork and Dublin respectively, although only one is visible at this scale

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773 **Table 1**. Sixteen WWTPs for which published monitoring data on diclofenac, E2, EE2 or estradiols
774 equivalents is available in Republic of Ireland.
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WWTP name	County	Catchment	WFD river basin district	Size (PE)	Type of secondary treatment
Carlow	Carlow	Barrow	South-eastern	39043	Extended Aeration
Ballincollig	Cork	Lee	South-western	27697	Extended Aeration
Fermoy	Cork	Blackwater	South-western	18608	CAS
Ringsend	Dublin	Coastal	Eastern	2124000	Sequence Batch Reactor
Swords	Fingal	Broad Meadow Water	Eastern	77014	Extended Aeration
Galway	Galway	Coastal	Western	213424	CAS
Killarney	Kerry	Laune	South-western	41836	CAS
Tralee	Kerry	Coastal	Shannon	35149	CAS
Leixlip	Kildare	Liffey	Eastern	100309	CAS
Osberstown	Kildare	Liffey	Eastern	104723	Sequence Batch Reactor
Kilkenny	Kilkenny	Nore	South-eastern	51988	CAS
Longford	Longford	Upper Shannon	Shannon	11672	CAS
Tullamore	Offaly	Lower Shannon	Shannon	24055	CAS
Roscommon	Roscommon	Upper	Shannon	6989	CAS
Clonmel	Tipperary	Suir	South-eastern	34909	Extended Aeration
Athlone	Westmeath	Upper Shannon	Shannon	21155	Extended Aeration

CAS = conventional activated sludge; PE = population equivalents

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785 **Table 2**: Summary of monitoring data for the three compounds of interest in the Republic of Ireland

	concentration (ng/L)	matrix	experiment	reference
DCL	nd (LOD = 855 ng/L)	WWTP influent	3 WWTPs sampled once	Lacey et al., 2008
	nd (LOD = 743 ng/L)	WWTP effluent		
	nd (LOD = 855 ng/L)	WWTPs influent	3 WWTPs sampled monthly during a year	Lacey et al., 2012
	detected in 5 samples (LOD = 500 - 2950 ng/L)	WWTP effluent		
	2630 (max value over a year long study)	WWTP effluent	2 WWTPs sampled (East and West coast) + receiving sea waters + mussels	Schmidt et al., 2013
	550 (max value over a year long study)	WWTP receiving sea water		
	nd (LOD = 29 ng/g)	mussels tissues		
	80.3	WWTP effluent	Pan EU campaign	Loos et al., 2013
	144.3			
E2	nd (LOQ = 10 ng/L)	WWTP effluent (2 plants)	Pan EU campaign	Jarošová et al., 2014
EE2	nd (LOQ = 10 ng/L)	WWTP effluent (2 plants)	Pan EU campaign	Jarošová et al., 2014
EEQ	17.2	WWTP effluent (Co. Kildare)	YES assay	Tarrant et al., 2005
	3.2	WWTP effluent (Co. Cork)		
	1.1-16.0	WWTPs effluents (8 plants)	YES assay	Tarrant et al., 2008
	0.9-2.9	WWTPs receiving waters		
	0.53-2.67	WWTPs receiving waters + 2 control locations	YES assay	Tiedeken et al., 2016
	16.21 (max value over a 2-years survey)	WWTPs receiving waters		
	< 0.05	WWTP effluent (2 plants)	Pan EU campaign	Jarošová et al., 2014

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