Catchment approach to passive sampling of Irish waters

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I. INTRODUCTION

In a catchment, or river basin, water is collected by the natural landscape and all rain and run-off eventually flow into a single point at a lower elevation i.e. a river, lake, ocean or groundwater system. The EU Water Framework Directive (WFD, 2000/60/EC) [1] introduced an integrated river basin management plan for Europe in 2000 into legislation thus taking a comprehensive catchment based approach to water management. Ireland has been divided into eight river basin districts under the WFD and a River Basin Management Plan and Programme of Measures has been developed for each river basin district [2].

In the UK and Ireland catchment monitoring is being used effectively to identify major pollutant sources and pathways. The Scottish Environmental Protection Agency (SEPA) have developed a comprehensive risk based approach to water quality monitoring and select catchments for targeted monitoring based on 'water bodies or protected areas that are significantly failing standards due to rural diffuse pollution' [3]. Other European studies have also demonstrated that a catchment approach can allow for more complex and a more holistic approach to water quality management [4].

The challenges of monitoring our waters for compliance with WFD and the expansion of the list of organic chemicals that are to be added for monitoring, provides impetus for investigation of alternative monitoring approaches such as passive sampling. This study focused on investigating a potential role in the establishment of a capability to utilise passive sampling to support polar (oestrogenic) pollutant monitoring programmes in Ireland. By completing this in the catchment approach pollutant sources and pathways can be identified allowing for a more targeted approach to monitoring of emerging and priority compounds in water.

Many compounds may interfere with the hormonal system of exposed organisms. Among these compounds, the natural estrogens oestrone (E1) and 17β oestradiol (E2) and the synthetic oestrogen 17α ethynylestradiol (EE2) have the highest potencies [5, 6]. They have been shown to exert estrogenic effects in the laboratory [7, 8], and in the environment [9, 10]. Recently, E2 and EE2 have been included in a WFD watch list of substances for which Union-wide monitoring data are to be gathered for the purpose of supporting future prioritisation exercises (Directive 2013/39/EU). When these substances were first considered for inclusion on the priority substances list, annual average (AA) environmental quality standards (EQS) in other surface waters of 0.08 and 0.007 ng L-1 respectively were proposed.

In order to evaluate the benefit of the catchment approach to identify point sources and pathways of pollution, the River Lee catchment in Cork was selected for the study of potential impact from the natural oestrogens oestrone (E1) and 17β oestradiol (E2) and the synthetic oestrogen 17α ethynylestradiol (EE2). The river Lee flows from an area of low anthropogenic activity, through agricultural land to Cork City entering the Celtic Sea via the industrialised Cork Harbour (100 km2 surface). Within the study area Lough Mahon and the River Lee in Co. Cork received a poor WFD classification status for fish. Evidence of pollutant stress in mussels has also been revealed by scope for growth measurements, potentially due to untreated wastewater from population centres such as Cork City [11]. Secondary treated waste enters Cork

Harbour from Cork City (333,000PE) [12] and from a number of other potential inputs ranging including industrial waste and riverine inputs of agricultural run-off and untreated waste from the River Lee catchment.

Liquid chromatography tandem mass spectrometry (LC-MS/MS) is well documented as a leading method of analysis for the steroid oestrogens due to the high sensitivity and specificity and rapid sample throughput [13] with ionisation in negative electrospray mode (ESI) the most commonly used technique [14]. The detection of these compounds at and below the proposed EQS values using traditional spot water sampling and MS methods has often proved challenging for marine samples due to the low instrument sensitivities required, volumes of water required, increased dilution effects and sampling representivity in what is generally a dynamic environment. Passive sampling (PS) technologies are emerging as sensitive cost effective techniques to detect time-integrated trace levels of pollutants in water and have been suggested as complementary methods for WFD surveillance, operational and investigative monitoring [15].

This study used the polar organic chemical integrative sampler (POCIS) which is capable of sequestering polar compounds (Log Kow <4), including estrone (E1), E2 and EE2 from the water phase [16-18]. Sampling rates which consider all environmental factors are not yet viable, thus POCIS is generally applicable for use as a qualitative/screening device whereupon focused monitoring may evolve based on compound detection. Surface water samples were also collected and analysed by LC-MS/MS.

The impact of this study may lie in the identification of a potential role for the combination of catchment based approaches and focused water and passive sampler analysis for the surveillance monitoring in of estrogenic compounds in Irish water bodies and to establish the potential role for inclusion of PS as a support tool to polar pollutant monition in Ireland.

Figure 1 Cork sampling sites (1. Inchigealagh, 2. Inniscarra, 3. Shandon, 4. Lough Mahon, 5. Outer Harbour)

II. MATERIALS AND METHODS

A. Sampling approach

A catchment approach was used to investigate the presence and levels of natural and synthetic steroid estrogens in the River Lee, Co. Cork at two different times of the year (summer and spring). Passive sampling devices were deployed for a period of one month at five sites along the river Lee from July to August 2013 and March to April 2014. Surface water samples were collected at deployment and retrieval of the devices. The river Lee rises in the Shehy Mountains on the western border of Co. Cork and flows eastwards through Inchigealagh (site 1) and Inniscarra (site 2) and through Cork City (Shandon, site 3). Here it mixes with seawater and continues through the estuarine Lough Mahon (site 4) to the more saline Outer Harbour (site 5) emptying into the Celtic Sea (Figure 1). Cork Harbour is one of the largest natural harbours in the world (100 km^2 surface). The catchment area of the River Lee is 1,253 km. The longterm average flow rate is 40.4 m^3/s [19].

B. Measurement approaches (Passive samplers and water analysis)

POCIS devices (consisting of a layer of Oasis HLB sorbent) used in this study were as developed by Alvarez et al. [16] and were supplied by the National Laboratory Service (NLS), Environment Agency, United Kingdom. A POCIS device (3 POCIS discs) and one single POCIS disc for use as a field blank were stored in an airtight metal canister at -30˚C and transported to the site in cold conditions. POCIS devices were deployed at 1 m depth at selected sites for a period of one month. The field blank was exposed to the environment during deployment and retrieval. Samplers and field blanks were stored at -30˚C prior to transport (in cold conditions) to the NLS, UK, for extraction and analysis.
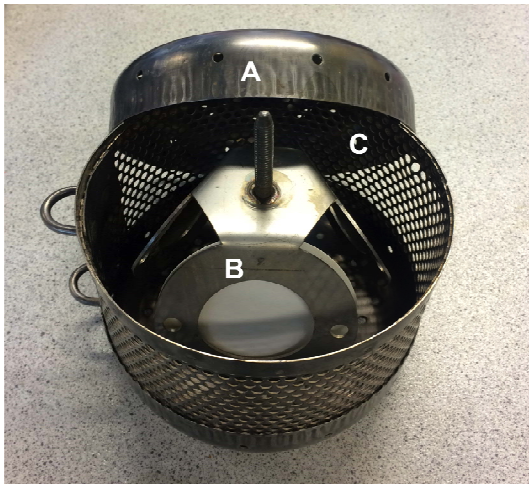
Figure 2 POCIS device; A-lid, B-secure disk holder containing POCIS sampling disk, the device is equipped with 3 holders, C-protective outer casing of sampler which allows water to move freely through the device.

C. LC-MS/MS analysis of water

Water samples (five L) were extracted as per Ronan and McHugh [20]. Briefly, samples were filtered with 0.45 µm glass fibre filters and cleaned-up using Oasis hydrophilic-lipophilic-balanced (HLB) solid phase extraction (SPE) cartridges (Waters, Milford, MA 01757, USA). Cartridges were rinsed and conditioned prior to elution of sample in 2 mL CH_3OH under a gentle vacuum. Analysis of this solution was then completed on an Agilent 1200 Series Game Pad system using a Kinetex 4.6 x 50 mm, 2.1 mm ID C_{18} 2.5 µm particle size column. For LC-MS/MS experiments, an Applied Biosystems 3200 Q-TRAP was used with an ESI source (Turbo-Ionspray, Applied Biosystems) operated in negative mode at 550°C and -4,400 V. Quantification was performed using aqueous standards for water samples.

D. LC-MS/Time of Flight (TOF) analysis of POCIS

Passive sampler extracts were analysed as per the Environment Agency (UK) Blue Book 220, method B [21]. The POCIS sorbent was transferred with CH_3OH into a glass chromatography column fitted with a glass wool plug and a stopcock. The CH_3OH used to rinse the sorbent into the column was collected to be pooled with the SPE eluent. The sample was eluted with 40 mL CH_3OH and the collected eluate and rinse were evaporated by rotary evaporation to approximately 1 mL prior to clean-up through a styrene divinyl benzene polymer SPE. The resulting extract was again evaporated to incipient dryness before being dissolved in 50% aqueous CH_3OH prior to fractionation using reverse phase chromatography with a standard C18 phase column. The resulting extract was evaporated to incipient dryness and dissolved in 10% aqueous CH_3OH. Analysis of 100 µL of this was completed using high performance liquid chromatography with negative ion atmospheric photo-ionisation interface and time of flight mass spectrometric detection on an Agilent 1100 Series Game Pad system fitted with a Luna phenyl hexyl 2 x 150 mm, 3 µm particle size column with results are expressed as ng per device. For HPLC-MS/MS experiments, an Applied Biosystems 3200 Q-TRAP was used with deionised water (A) and 0.025 % TEA in 95:5 CH_3OH: acetone (B) mobile phases as per the Environment Agency Blue Book 220 methods.
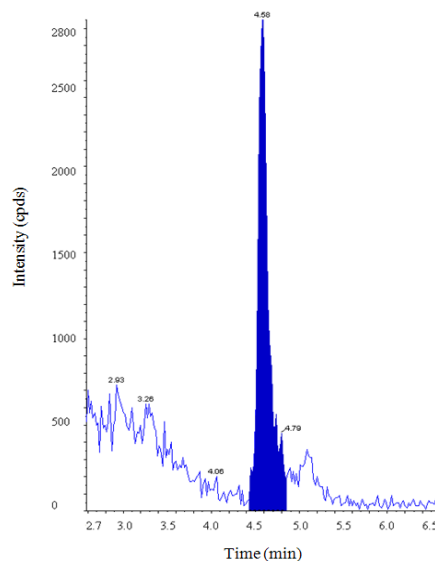

Figure 2: E1 in a water sample from Inniscarra

E. Legislation and data reporting

EQS are not currently set for watch list substances, however during the process of selection for inclusion on the list, provisional EQS in total water of 0.08 and 0.007 ng L^{-1} for E2 and EE2, respectively, in other surface waters were proposed. These values have been used in this assessment however they are not definitive limits so any inference of risk is assigned with caution. Such low values present analytical

challenges. While the LC-MS/MS method for water is sufficiently sensitive to detect E2 at these concentrations (LOD 0.07 ng L^{-1}), it was not sufficiently sensitive to detect EE2 (LOQ 0.11 ng L^{-1}). This was one of the main drivers for using passive samplers as an alternative method.

The POCIS device allows for sampling of these polar compounds over a four week sampling period. The NLS in the UK provides analysis for the devices and their analytical range for these compounds is from the minimum reporting value to 20 ng L^{-1} and is extended by dilution. Therefore the LODs are: EE2 (0.21 ng L^{-1}), E2 (0.45 ng L^{-1}) and oestrone (1.5 ng L^{-1}). The LOQs are 10 x the within batch standard deviation of measurements (according to ISO 13530) and are EE2 (0.45 ng L^{-1}), E2 (0.97 ng L^{-1}) and oestrone (3.23 ng L^{-1}). The value detected is then multiplied by the sampling rate to approximate an average upperbound value of the compound in the water body. Sampling rates for POCIS samplers are not as representative as samplers which use performance reference compounds (PRCs), which are not amenable to polar sampling, and assume that water bodies are moving at a constant speed. As such it is the upperbound value presented in this study. These values are then compared to the provisional EQS values assigned to the compounds for evaluation. The POCIS samplers allow us to detect concentrations of these polar compounds at the required concentrations when deployed for appropriate lengths of time, which this study found to be four weeks.

III. RESULTS AND DISCUSSION

Results of water and passive sampler extracts are presented in Table 1. To evaluate the risk posed by detected levels of E2 and EE2, the proposed annual average (AA) environmental quality standards (EQS) in other surface waters were used (0.08 and 0.007 ng L^{-1} for E2 and EE2, respectively). Effective sampling rates of 0.39, 0.46 and 0.235 ng/sampler/day for E1, E2 and EE2, respectively, (National Laboratory Service, Environment Agency, UK) were applied to POCIS results to estimate water concentrations.

Table 1: Water and estimated POCIS derived dissolved water concentrations for E1, E2 and EE2 in the Cork catchment (ng L^{-1}) P – POCIS, W – Water. Ig – Inchigeelagh, Ia – Iniscarra, Sn – Shandon, LM – Lough Mahon, OH – Outer Harbour

		Upstream				Downstream
	Matrix	Ig	Ia	Sn	LM	OH
Analyte		2013				
EE2	P*	<0.2	1.39	<0.2	<0.2	<0.2
E2		<0.5	<0.5	<0.5	2.36	1.98
EE2	W**	nd	nd	nd	nd	nd
E2		nd	nd	nd	nd	nd
Analyte		2014				
EE2	P*	< 0.12	< 0.04	< 0.04	< 0.04	0.07
E2		< 0.13	< 0.04	< 0.04	0.06	0.09
E1		< 0.51	0.24	0.37	0.48	0.37
EE2	W**	nd	nd	nd	nd	nd
E2		nd	nd	nd	nd	nd
E1		nd	0.41	nd	0.41	0.54

*Effective sampling rates POCIS (ng/sampler/day): E1: 0.39, E2: 0.46, EE2: 0.235.
**LOD water samples (n=2, 5 L) by LC-MS/MS: E1: 0.07 ng L^{-1} E2: 0.07 ng L^{-1}, EE2, 0.11 ng L^{-1}.
*** Provisional EQS in other surface waters of 0.08 and 0.007 ng L^{-1} (E2 and EE2) respectively

E2 and EE2 were not detected in water samples in 2013 (summer) and 2014 (early spring). Oestrone (E1) was added to the suite of compounds in 2014, and was detected in water samples from Lough Mahon and the Outer Harbour at similar concentrations as those calculated in POCIS. E1 was also detected in water at Iniscarra and in POCIS in Iniscarra and Shandon. When estimated sampling rates were applied to the POCIS results the proposed EQS for E2 were potentially exceeded in Lough Mahon in both 2013 and 2014 and in the Outer Harbour in 2014. In the POCIS results the analytes were often below the limits of detection over the sampling period. In cases where the detected level exceeds the provisional EQS values in place for these compounds this is an upperbound estimated value using the detected level and the sampling rate.

One potential but unconfirmed source of estrogenic compounds to the gradient is the Carrigrennan Little Island WWTP which caters to Cork City and consists of secondary wastewater treatment and solids treatment with the sludge produced onsite being used in agriculture. This site (which is located between Shandon and Lough Mahon) treats for both industrial and agricultural wastewater and includes treatment of hospital water which is a potential source of E2 and EE2. Secondary

treatment processes have been shown to remove up to 90 % of E1, E2 and EE2 from wastewater during treatment [22, 23] however deconjugation by bacterial enzymes in WWTPs and in the environment reactivates these conjugates into biologically active parent compounds [24, 25] and they have been detected in surface waters and in wastewater influent and effluent [26-29]. The residence time in Lough Mahon is 15.9 days [30] which could allow for ultra trace concentrations of steroid estrogens to accumulate to detectable levels in POCIS. While this may be a potential sources of estrogens to Lough Mahon and the Outer Harbour, the levels detected particularly in Inniscarra require further investigation, particularly the as yet unexplainable elevated level of EE2 detected in Iniscarra, a remote upstream site. Potential sources include inputs of untreated wastewater and agricultural run-off. Concentrations of E2 in POCIS samplers from Lough Mahon the Outer Harbour were highest in 2013. The higher levels detected in summer 2013 may be due to reduced rainfall and thus reduced dilution at these sites.

It should be noted that while WFD EQS are set for total water, filtration of water samples was not found to affect recoveries of the polar compounds E1, E2 and EE2 [20]. López de Alda and Barcélo [31] also demonstrated that filtration does not lead to significant losses of estrogens from water. Analysing the dissolved fraction for these water samples enabled a more direct means of comparison to POCIS, which samples the dissolved phase. It is arguable that analyzing the dissolved phase is a more biologically relevant measurement as it more comparable to mimicking the respiratory exposure of aquatic organisms [32] and providing more biologically relevant data.

There was a good similarity between POCIS and water for E1, with POCIS detecting E1 at four out of the five sites and spot sampling detecting it at 3 sites. E2 and EE2 were detected in POCIS at potentially biologically relevant levels while they were not detected using traditional spot sampling methods. This may be due to the longer exposure time of POCIS and resultant capacity to detect episodic events and sample a larger volume of water, or due to the variability of spot sample collection. For example a study by [33] showed that daily spot samples of waters taken from a river had a wide variance in daily E1 concentrations, ranging from 0.32 to 2.5 ng L^{-1}. It is difficult to ascertain

the potential risks to resident species as a result of these concentrations. There is a wide variation in the range of sampling rates reported for POCIS in the literature [18]. Reported sampling rates vary between 0.02 L/day [34] and 0.85 L/day [35], suggesting that between 0.6 L and 25.5 L may be sampled in 30 days deployment if using the lowest and highest sampling rates reported in the literature, respectively. These considerations have major implications for the assessment and comparison of both spot water and POCIS derived water concentrations, however in the case of POCIS applying a sampling rate which shows a worst case scenario may provide a means for using POCIS as both a screening and monitoring tool for WFD purposes, whereby exceedance of the EQS using POCIS results in follow up with labour intensive traditional methods.

Passive sampling has advantages over spot sampling in that it provides a reproducible (time weighted) means for monitoring (screening/surveillance) of contaminant levels [36]. It is also clear that data derived from spot samples may also be unrepresentative as spot samples provide only a snapshot of a single moment in what is generally a dynamic environment while passive samplers provide time integrated data, taking account of transient pollution events. While WFD compliance legislation is still based on spot water sampling and analysis, the cost of a comprehensive spot sampling programme can often preclude the collection and analysis of a sufficient number of samples to mitigate these effects. Until accurate sampling rates can be defined for POCIS, coupling POCIS with water samples taken *in-situ* will generally only be capable of providing data to support surveillance/screening programs and not truly in support of compliance monitoring.

A catchment approach can identify locally important pressures and ecosystem services to target resources and activities. In this study the catchment approach enabled the identification of a potential source of EE2 to the Lee catchment from what was otherwise considered a remote site. The concentrations of E1 and E2 as detected by POCIS appeared to increase along the catchment to Cork City with a spike in concentration in Lough Mahon and a slight reduction in the more expansive and thus dilute Outer Harbour. Traditional grab sampling methods cannot currently achieve the limits of detection required by legislation for E2 and EE2

and the adhoc nature of spot sampling may potentially have led to this increasing concentration through the catchment not having been detected. Therefore a combination of passive sampling and a catchment approach was found to be more useful in such surveillance monitoring of this catchment.

IV. Conclusions

The catchment approach used in this study enabled the identification of potential areas of concern with respect to E2 and EE2, and highlighted a general decrease in the levels of these contaminants from summer 2013 to spring 2014. This small-scale study deploying passive sampling devices at intervals along this catchment highlighted approximate locations in which estrogens may enter the system, with concentrations of E2 and EE2 increasing through the catchment. Passive samplers were more successful than traditional spot water sampling in the accumulation of E2 and EE2 which may be due to a number of factors including a greater volume of water sampled, greater sensitivity or the ability of passive samplers to detect episodic events. A follow up study deploying passive samplers 6-8 times during the year in areas of could identify if the data from this study were the result of transient or continuous pollution pressures. Passive sampling devices could be a useful, cost effective, time weighted supporting technique in a toolbox for surveillance monitoring within the WFD and other environmental programs and especially so in identifying areas of concern for additional follow up monitoring. From the results presented above it is clear that passive sampling can play an important role in screening of waters for emerging contaminants and it has a role to play in trend monitoring to illustrate where waters are changing in quality over time.

V. References

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