



Detection of endocrine disrupting chemicals in aerial invertebrates at sewage treatment works

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ARTICLE INFO

Article history:

Received 15 May 2009

Received in revised form 21 August 2009

Accepted 23 August 2009

Available online 31 October 2009

Keywords:

Bat

Bioaccumulation

Diptera

Pipistrellus pipistrellus

17 α -ethinylestradiol

ABSTRACT

Endocrine disrupting chemicals (EDCs) constitute a diverse group of chemical compounds which can alter endocrine function in exposed animals. Whilst most studies have focussed on exposure of wildlife to EDCs via aquatic routes, there is the potential for transfer into the terrestrial food chain through consumption of contaminated prey items developing in sewage sludge and waste water at sewage treatment works. In this study, we determine levels of EDCs in aerial insects whose larval stages develop on percolating filter beds at sewage treatment works. We compare absolute concentrations of known EDCs with those collected from aquatic environments not exposed to sewage effluent outflow. Our findings document for the first time that aerial invertebrates developing on sewage filter beds take up a range of chemicals thought to be incorporated from the sewage effluent, which act as endocrine disruptors. For two synthetic chemicals (17 α -ethinylestradiol and butylated hydroxy aniline), concentrations were significantly higher in insects captured around percolating filter beds than sites over 2 km from the nearest sewage works. A number of species of insectivorous bats and birds, some of which are declining or threatened, use sewage works as principle foraging sites. We calculate approximate exposure levels for a species of bat known to forage within sewage works and suggest that further research is warranted to assess the ecological implications of consuming contaminated invertebrate prey.

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1. Introduction

A variety of natural and synthetic chemicals are known to act as endocrine disruptors by mimicking or blocking the actions of natural steroid hormones, through similarity in their chemical structure (IPCS, 2002; Tabb and Blumberg, 2006). These chemicals can have antagonistic effects, for example, preventing transcription associated with receptor activation, or agonistic effects, up-regulating transcription. One such class of endocrine disrupting chemicals (EDCs) are oestrogenic chemicals, including natural oestrogen and a number of synthetic compounds which mimic oestrogen molecules (e.g., Van Der Kraak, 1998). The possible adverse impacts of such EDCs on wildlife and, more controversially, humans is the focus of much current scientific research and debate (Preziosi, 1998; Van Der Kraak, 1998; IPCS, 2002; Sekizawa, 2008). Concerns regarding exposure of wildlife to EDCs are due primarily to documented adverse effects including the occurrence of inter-sex

fish, eggshell thinning in some bird species, and morphological deformities in crustaceans and reptiles (e.g., Ratcliffe, 1967; Bryan et al., 1986; Sumpter, 1995; Ankley and Giesy, 1998; Fox, 2001). However, almost without exception, any suspected effects of EDCs documented to date have been in animals living and/or foraging in aquatic environments.

The potential for exposure of aquatic wildlife to EDCs is high because of the widespread use of such compounds in industry, the stability of some EDCs to degradation, and the routes of contamination via, for example, treatment plants for industrial wastewater and domestic sewage (Birkett, 2002; Williams et al., 2009). For example, food containers and dental composites and sealants contain epoxy resins and polycarbonate plastics, which are manufactured using bisphenol-A, a compound which has now been detected in human urine in the United States (Calafat et al., 2005), whilst phthalates, used as plasticisers, have been found in human breast milk (Pfordt, 2004). It has been estimated that the synthetic oestrogen used in the human contraceptive pill and in hormone replacement therapy, 17 α -ethinylestradiol is excreted in the urine of approximately 16% of the UK's female population

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(Johnson et al., 2000). In addition to these synthetic chemicals, sewage outflow can contain substantial levels of natural oestrogens (e.g., $0.1\text{--}56\text{ ng L}^{-1}$ estrone; $0.2\text{--}141\text{ ng L}^{-1}$ $17\beta\text{-estradiol}$, Gomes and Lester, 2003). Through the discharge of urine and faeces into waste water such chemicals may therefore enter the sewage treatment system and, if not broken down by the treatment process, flow subsequently into freshwater systems (Stumpf et al., 1996; Teske and Arnold, 2008). Reported concentrations of $17\alpha\text{-ethinylestradiol}$ in sewage treatment work (STW) effluent and adjacent rivers vary greatly but, where detected, are usually in the low ng L^{-1} range (e.g., Desbrow et al., 1998; Ternes et al., 1999; Baronti et al., 2000; Williams et al., 2003; Johnson et al., 2005), although concentrations of up to 62 ng L^{-1} have been reported in Germany (Stumpf et al., 1996). Lipophilic steroid estrogens, such as $17\alpha\text{-ethinylestradiol}$ will readily adsorb onto sediment and small particles. Again, reported concentrations vary greatly but several studies have indicated that levels of natural (e.g., Peck et al., 2004) and synthetic estrogens may be considerably higher than that found in effluent or receiving waters and concentrations of $2.5\text{--}22.8\text{ }\mu\text{g kg}^{-1}$ $17\alpha\text{-ethinylestradiol}$ have been reported from river sediments in South Korea and Spain (e.g., Lopez de Alda and Barcelo, 2001; Duong et al., 2009).

Extensive laboratory studies confirm that steroid estrogens, including $17\alpha\text{-ethinylestradiol}$, can cause inter-sex fish (with gonads containing male and female tissue, and/or feminised reproductive ducts), a phenomenon which has been observed in wild fish at concentrations of natural and synthetic oestrogens that have been measured in effluents and in river systems (review in Gross-Sorokin et al., 2006). There is also evidence from field and laboratory studies that invertebrate fauna can be affected by exposure to EDCs discharged from sewage works (Oehlmann and Schulte-Oehlmann, 2003).

A considerable body of literature now exists quantifying the potential for wildlife to be exposed to EDCs through the sewage treatment process. Most of this work has, understandably, focussed on detecting and quantifying concentrations of chemicals entering the freshwater system (Desbrow et al., 1998; Williams et al., 2003), and assessing the effects on aquatic vertebrates, primarily fish (e.g., Sumpter, 1995; Tyler et al., 1998; Gross-Sorokin et al., 2006). An important outcome of these studies has been to develop valuable bioindicators which can be used to identify populations at risk of exposure (e.g., IEH, 1999; Tyler et al., 1999). The possibility of food chain effects has rarely been examined (although there are notable exceptions e.g., aquatic vertebrates and fish-eating mammals and birds; Giesy et al., 1994; Fox, 2001; Fossi and Marsili, 2003; Hu et al., 2005; Porte et al., 2006), and the short half-lives of some EDCs in water has led to the suggestion that these chemicals are unlikely to persist in the environment (Lee and Liu, 2002). There are, however, alternative routes for EDC exposure which include the consumption of contaminated prey items, such as invertebrates developing in sewage sludge and waste water (Markman et al., 2007). Percolating filter beds are a common type of second-

ary sewage treatment in the UK (Gray, 2005). After a screening and sedimentation process, waste water is sprayed over inert material and a microbial biomass, consisting of bacteria, fungi, protozoa and other mesofauna, develops as a film over the surface (Gray, 2005). This film supports a wide diversity of macro invertebrates including dipteran fly larvae which feed upon it (Learner and Chawner, 1998; Gray, 2005). Sewage treatment works have long been recognised as potentially valuable foraging areas for birds (e.g., Boyd, 1957; Fuller and Glue, 1980), and recent work has highlighted their potential value to foraging bats due to the availability of invertebrate prey (Park and Cristinacce, 2006).

The recent development of a protocol for the extraction and quantification of EDCs from invertebrates revealed significantly higher concentrations of a range of EDCs in the tissue of earthworms collected from sewage percolating filter beds than those living in garden soil, and suggested that some organisms may be able to bioaccumulate EDCs (Markman et al., 2007). Furthermore, male European starlings experimentally exposed to EDCs at levels found in earthworms from sewage percolating filter beds, showed reduced immune function, altered song patterns and hypermasculinisation of the brain (Markman et al., 2008). These findings show the vulnerability of neural development to exposure to endocrine disruptors, as previously identified by Iwaniuk et al. (2006) in American Robins (*Turdus migratorius*).

Here we assess levels of EDCs in the aerial stages of insects, the larvae of which develop on percolating filter beds at sewage works. We also compare concentrations of EDCs with those collected from aquatic environments not adjacent to treatment works. Specifically, we predict that invertebrate samples collected at sewage works with percolating filter beds would show elevated levels of chemicals previously identified as EDCs and in particular, natural and synthetic oestrogens. Finally, using known foraging rates of a common bat species, we predict intake rates for individuals feeding on aerial insects emerging at the sewage treatment works.

2. Materials and methods

2.1. Invertebrate samples

Between 2003 and 2006 (April–August) we investigated EDC concentrations in aerial insects at four sewage works with percolating filter beds, and two non-sewage work sites (i.e., rural sites, adjacent to rivers or stillwater, which were >2 km from the nearest sewage treatment works) in Central Scotland within a radius of 25 miles from Stirling City (Table 1). Sampling dates were randomised with respect to site. Between two and four samples were collected at each site using a Malaise trap (Watkins and Doncaster, UK) erected adjacent to filter beds or water bodies (for non-sewage work sites) for approximately 48 h containing 70 mL of HPLC grade ethanol. Insect samples were stored at $-80\text{ }^{\circ}\text{C}$ in ethanol before analysis. In 2006, each invertebrate sample was collected alongside a “blank” sample, in which the container of ethanol was covered

Table 1
Information on sites sampled for invertebrates, in the present study: (1) Type includes STW = sewage treatment works, and non STW = a site > 2 km from the nearest sewage works. (2) Grid reference. (3) Population served indicates the Human population served by each STW site. The final column gives the (4) number of invertebrate samples and the year they were collected.

Site	Type	Site location		Population served	Year (number of samples)
		Longitude	Latitude		
Linlithgow	STW	3°37.5'W	55°59.0'N	12200	2003 (1), 2006 (1)
Bathgate	STW	3°39.8'W	55°55.0'N	10150	2003 (1), 2006 (1)
Fallin	STW	3°52.3'W	56°06.6'N	2447	2003 (1), 2005 (1), 2006 (2)
Doune	STW	4°02.9'W	56°11.0'N	1235	2003 (1), 2005 (1), 2006 (2)
Ashfield	Non STW	3°57.6'W	56°12.7'N	n/a	2006 (2)
Stirling University	Non STW	3°55.1'W	56°08.8'N	n/a	2006 (2)

with a fine mesh to prevent collection of invertebrates in order to control for contamination during collection and processing. A total of 16 samples (c. 2 g each) of aerial invertebrates was collected across the six sites, with at least two samples from each site (Table 1).

2.2. Standards

Standards of 17β -estradiol, estrone, 17α -ethinyloestradiol, cholesterol, diethylstilbestrol, bisphenol-A, diethylphthalate, dibutylphthalate, dioctylphthalate, butylated hydroxy aniline, *p*-hydroxybiphenyl, *n*-octylphenol and pentachlorophenol were purchased from Sigma, UK. Stock solutions of individual compounds were initially made up at a level of 1 mg mL^{-1} by dissolving an appropriate amount in methanol. Internal standard solution ($100 \mu\text{g mL}^{-1}$) of diethylstilbestrol was prepared in methanol. All the standard solutions were stored at 4°C prior to use.

2.3. Reagents

Acetone, chloroform, cyclohexane, ethyl acetate, hexane, iso-octane, methanol (HPLC grade) were obtained from Fisher, UK. Toluene (HPLC grade) was from Sigma-Adrich, UK. Sylon CT (5% dimethyldichlorosilane in toluene), glass wool (silane treated) and BSTFA (bis-(trimethylsilyl)-trifluoroacetamide) were purchased from Supelco. Bio-beads S-X3 (mesh size 200–400) were from Bio-Rad (Richmond, CA, USA).

2.4. Treatment with DMS

Prior to extraction of the samples, all glassware involved in the analysis was de-activated using Sylon CT (5% dimethyldichlorosilane, DMS, in toluene) to minimize non-specific binding and sample loss.

2.5. Extraction of oestrogens

The samples were thawed for about 20 min at room temperature and the ethanol was filtered off. The insects were transferred to de-activated test-tubes and dried in a centrifugal concentrator (miVac, GeneVac Ltd.) before determination of weight (to 0.1 mg, Ohaus Explorer). The ethanol was also dried in the centrifugal evaporator and the residues re-dissolved in approximately 1 mL ethyl acetate. The dried insects were then transferred to a de-activated glass bottle and homogenised in 50 mL ethyl acetate. Extracts were filtered through glass wool into a large glass test-tube – the bottle and glass wool were then rinsed three times with 10 mL ethyl acetate. Extracts, rinses and re-dissolved residues were combined and the solvent was evaporated to dryness at 35°C under a gentle stream of nitrogen. The remaining extract was dissolved in 1 mL of cyclohexane–acetone (3:1, v/v) and subjected to Gel Permeation Chromatography (GPC).

2.5.1. Gel Permeation Chromatography

GPC was applied to remove the large amounts of high molecular weight lipids, which unavoidably, were extracted together with the target compounds. A GPC column ($400 \times 25 \text{ mm}$) that was resistant to solvents from Omnifit (Cambridge, UK) was packed with 70 g bio-beads S-X3 pre-swollen in and washed with cyclohexane–acetone (3:1, v/v). The column was operated under gravity flow conditions and achieved a flow rate of 4 mL min^{-1} operating with cyclohexane–acetone (3:1, v/v) as eluent. The resolution of the system was evaluated using an invertebrate sample from a non-sewage site spiked with a mixture of oestrogen standard solutions. The first 80 mL-fraction was found to contain only lipids and was discarded in further preparations. The analytes were recovered

in the following 50 mL-fraction and subjected to further analysis. No compounds of interest were detected in subsequent fractions and the GPC system was ready to use for the next sample after subsequent elutions with 50 mL of eluent, 50 mL of toluene–chloroform–methanol (2:2:1, v/v/v) and a further 50 mL of eluent. Control runs were performed with all steps identical from extraction to GPC starting with 50 mL to assess potential contamination and carry-over by and in the system.

2.5.2. Derivatisation of oestrogens

The GPC fractions were reduced to about 1 mL volume under a stream of nitrogen and transferred to 2 mL GC vials (chromacol). After adding 50 μL of internal standard (diethylstilbestrol) samples were reduced to dryness under a gentle stream of nitrogen and derivatised by adding 50 μL of BSTFA to the residues and heating at 65°C for 20 min in a water bath. Although the addition of diethylstilbestrol just before derivatisation does not account for losses during extraction and GPC, previous work using this internal standard in the same GPC system indicated that such losses are low with high recoveries $>95\%$ (Markman et al., 2007). After cooling to room temperature, 50 μL of iso-octane–acetone (99:1, v/v) were added resulting in a final sample volume of 100 μL for measurement on the GC–MS system.

One microliter of the sample was injected into the GC–MS (Agilent GC 6890 N-MSD 5973 N) at 200°C in splitless mode and analysed over a 30 m and 0.25 mm I.D. capillary column on 0.25 μm ZB5 ms (Zebron Phenomenex). The column was operated under constant pressure of 13.34 psi Helium and the following temperature programme: initial temperature 85°C for 3 min, $10^\circ\text{C min}^{-1}$ to 130°C , 3°C min^{-1} to 300°C , 300°C for 3 min. The mass spectrometer was operated at a source temperature of 230°C and an analyser temperature of 150°C . Mass spectra were recorded after a 3 min solvent delay in both Full scan and Single ion mode. Signals were integrated and normalised against internal standards and quantified against external standards using Chemstation (v D 00.00.38, Agilent) and identities of analytes were confirmed by AMDIS software (version 2.1, National Institute of Standards and Technology) and manually checked against reference spectra.

2.6. Estimating rates of intake for an aerial insectivore

In order to predict exposure of aerial insectivores to EDCs contained within insect tissue, we need to know how many insects an individual may consume at a sewage treatment works. Here we present as an example, an estimate of the exposure of 17α -ethinyloestradiol, a potent synthetic oestrogen, for *Pipistrellus pipistrellus*, a bat species which has been documented foraging at sewage treatment works (Park and Cristinacce, 2006).

A daily estimated food intake of 5.2 g of insects has been made for *P. pipistrellus* (Crocker et al., 2002). In order to estimate the consumption of insects from filter beds for bats whose home range incorporates a percolating filter bed sewage treatment works, we also need to know the proportion of its nightly foraging time spent at a sewage works. Although these data do not currently exist, we can make estimates based on knowledge of *P. pipistrellus* foraging ecology, although it should be noted that, for practical reasons, most studies to date have focussed on females. Individual *P. pipistrellus* forage for between 2.5 and 5 h per night (Swift, 1980), moving between foraging areas “foraging beats” approximately every 30 min along a regular circuit (Racey and Swift, 1985). If we take an average of 225 min foraging per night and assume that a small sewage works represents a single foraging beat and a large works two foraging beats, between 13% and 26% of an individual's foraging time may be spent feeding on insects that have developed on filter beds, resulting in the consumption of between 0.68 and 1.35 g of insects from sewage works per night. These values were

multiplied by the concentrations of 17α -ethinylestradiol detected at the sewage works sampled to give a daily estimate of exposure to this synthetic oestrogen.

2.7. Statistical analysis

The power of statistical analyses was limited by the small number of sites at which invertebrates were collected (six in total). Due to the large variation we observed between consecutive samples at the same site, and the long period of time (up to 3 years) over which these samples were collected, we have consequently treated each sample (rather than site) as an independent unit giving a sample size of 12 for sewage sites, and four for non-sewage sites. Non-parametric Mann–Whitney U tests were used to determine if the concentrations of detected EDCs were higher in invertebrates collected from sewage sites than from non-sewage sites. Statistical analyses were carried out using SPSS 16.0 with a significance level of 5%, and all statistical tests conducted were two-tailed.

3. Results

A sub-sample of invertebrates collected was taken for identification – the vast majority (98%) was Diptera, with most of these consisting of Chironomidae (59% of the total). The remainders were Plecoptera and Hymenoptera.

A total of 12 natural and synthetic chemicals were detected which had previously been identified as EDCs (17β -estradiol, estrone, 17α -ethinylestradiol, cholesterol, bisphenol-A, diethylphthalate, dibutylphthalate, dioctylphthalate, butylated hydroxy aniline, *p*-hydroxybiphenyl, octylphenol and pentachlorophenol). The elution volume for these chemicals in spiked insect samples on GPC was between 80 and 130 mL. The limits of quantification were determined from the minimal area required to deliver a sufficiently large signal to noise ratio (>10) in samples and external standard calibrations, and ranged from 0.14 ng g^{-1} for octylphenol to 1.9 ng g^{-1} for butylated hydroxy aniline. The limits compare well to the limits of detection reported by Markman et al. (2007).

Some EDCs were detected in blank ethanol samples but, on average, these blanks contained approximately an 80-fold lower concentration than that detected in invertebrate samples (sewage and non-sewage works combined). Concentrations of 17β -estradiol

and dioctylphthalate, however, were similar between blank and invertebrate samples and, in case of dioctylphthalate very high concentrations were detected in some samples (up to 6400 ng g^{-1}). These findings indicate potential contamination during sampling and/or processing, and both 17β -estradiol and dioctylphthalate were consequently removed from further analysis. The majority of remaining detections in blank samples (85%) were accounted for by the other two phthalates (diethylphthalate, dibutylphthalate), cholesterol and bisphenol-A.

All the EDCs, for which we tested, were found in at least one of the 12 sewage samples and all, excluding butylated hydroxy aniline, were also detected in at least one of the four non-sewage samples (Fig. 1; estrone is not included in this figure since the median and 75th percentile were zero for both STW and non-sewage sites).

Levels of butylated hydroxy aniline and 17α -ethinylestradiol were significantly higher in samples collected from sewage sites than non-sewage sites (Fig. 1a and c). The median (25th, 75th percentiles) concentration of 17α -ethinylestradiol was 42.4 ng g^{-1} (12.6, 140.3) at the STW and 2.6 ng g^{-1} (0, 9.0) at non-sewage sites. For butylated hydroxy aniline, the median concentration was 8.5 ng g^{-1} (0.2, 22.8) at the STW, with none detected at the non-sewage sites. Although there was a strong trend for some of the other EDCs detected (e.g., diethylphthalate, bisphenol-A) to have higher concentrations in samples from the sewage sites, these trends were not statistically significant (Fig. 1g).

3.1. Predictions of intake rates for bats

Multiplying the estimates of insects from filter beds that could be consumed by individual *P. pipistrellus* per night with the median (25th, 75th percentiles) concentration of 17α -ethinylestradiol detected in these insects (given above) gives a daily dose of 28.8 ng ($8.6, 95.4 \text{ ng g}^{-1}$) for bats with only one foraging beat at a sewage works or 57.2 ng ($17.0, 189.4 \text{ ng g}^{-1}$) for those with two foraging beats at a sewage works.

4. Discussion

These findings document for the first time that aerial invertebrates, primarily Diptera, whose larval stages develop on sewage filter beds take up a range of endocrine disruptors. For two syn-

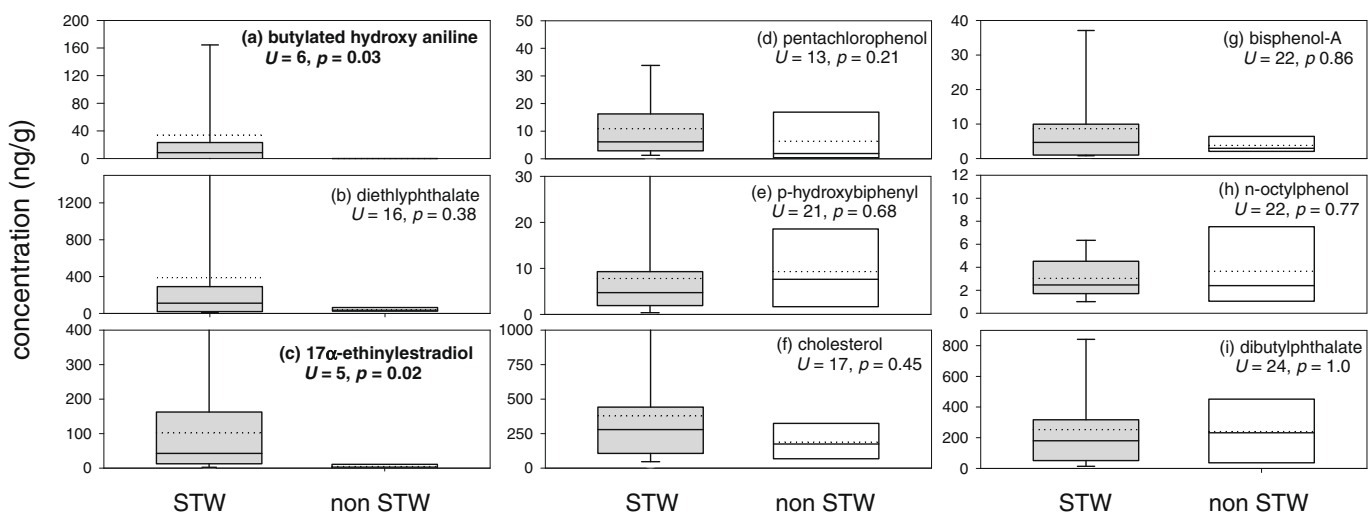


Fig. 1. The concentration of endocrine disrupting chemicals found (ng per g of invertebrates; boxes represent median, 25th, 75th percentiles, whiskers are 10th, 90th percentiles, and the mean value is indicated by the dotted line) in wet weight invertebrate tissue at sewage treatment works (STW, grey bars) and non-sewage sites (non STW, white bars). Number of invertebrate samples for all comparisons was 12 (from STW) and 4 (from non STW). The Mann–Whitney U test statistic is given and significant differences ($p < 0.05$) in concentration between site types were found in (a) butylated hydroxy aniline and (c) 17α -ethinylestradiol, as indicated in bold.

thetic chemicals with known oestrogenic properties (17 α -ethinylestradiol and butylated hydroxy aniline), concentrations were significantly higher in insects captured around percolating filter beds than from sites remote from sewage processing.

A number of studies have quantified the concentrations of 17 α -ethinylestradiol in the final effluent of a sewage works across Europe – at several sites no detectable levels of 17 α -ethinylestradiol were identified, and where it was, concentrations were usually in the low ng L⁻¹ range (e.g., Desbrow et al., 1998; Ternes et al., 1999; Baronti et al., 2000; Williams et al., 2003; Johnson et al., 2005). Although the concentrations in liquid and solid samples cannot be directly compared, the concentrations of 17 α -ethinylestradiol identified by our study in 1 g of aerial invertebrates greatly exceeds those previously reported in 1 mL of sewage treatment water (approximately 700-fold of even the higher reported concentrations; Stumpf et al., 1996).

Although many EDCs have short half-lives in water under particular environmental conditions (e.g., Ying et al., 2003), invertebrates developing and living on sewage filter beds are constantly exposed to the input of new EDCs from trickling sewage water. Little is known about the metabolism of EDCs in invertebrate tissue and this is likely to depend on the invertebrate species involved. The wastewater in which Diptera develop at filter beds may contain considerably higher concentrations of 17 α -ethinylestradiol than has been quantified to date in final effluent (treated water, ready to leave the sewage works), as it is only partially treated, and reductions in concentration from influent to effluent during treatment have been estimated as between 64% and 85% (Ternes et al., 1999; Baronti et al., 2000). In addition, some of the reported concentrations in sediment are considerably greater than those for water (e.g., Duong et al., 2009). Nevertheless, the large discrepancy between concentrations detected in water or sediments and invertebrate tissue suggests that these invertebrates may be accumulating the chemicals from the water and/or from the biofilm and/or the sediment of the sewage treatment works.

Two experimental studies have shown that three invertebrate species (*Lumbriculus variegatus* (class Clitellata), *Hyaella azteca* (class Malacostraca), and *Chironomus tentans* (class Insecta)) are capable of bioaccumulating 17 α -ethinylestradiol: Liebig et al., 2005; Dussault et al., 2009) and highlight the possibility of food chain effects involving the bioaccumulation of EDCs. Our study did not measure effluent levels at the same time as invertebrate concentrations, however, so we cannot provide conclusive proof of bioaccumulation from this study. However the values obtained strongly suggest that this is a possibility and that further work is needed to clarify this. The only other study to quantify EDCs from invertebrates (earthworms) developing on sewage filter beds did not detect any 17 α -ethinylestradiol (Markman et al., 2007). They did, however, detect 17 β -estradiol in invertebrate tissue which is at substantially higher concentrations than that reported in studies assessing this chemical in final effluent (0.9–100 ng L⁻¹; Desbrow et al., 1998; Williams et al., 2003; Jiang et al., 2005), and suggested that this may be the result of bioaccumulation (Markman et al., 2007). In the present study, some EDCs were also detected in blank ethanol samples left alongside the traps catching insects. The majority of these detections were accounted for by the three phthalates, cholesterol and bisphenol-A, which is not surprising as all four compounds are used as plasticizers and/or are ubiquitous in the environment, so are very difficult to exclude completely in such analyses.

The high densities of invertebrates emerging from percolating filter beds attract large numbers of foraging aerial insectivores such as hirundines (e.g., barn swallows *Hirundo rustica*, sand martins *Riparia riparia*; Fuller and Glue, 1980) and bats (common and soprano pipistrelles, *Pipistrellus pipistrellus*, *P. pygmaeus* respectively; Park and Cristinacce, 2006). The sub-sample of inverte-

brates collected at filter beds for identification purposes consisted largely of insects known to be regularly taken by both pipistrelle species (Vaughan, 1997). This suggests that aerial insectivores may be consuming large numbers of invertebrates, which can contain relatively high levels of EDCs, and that this may represent a previously unrecognised route of vertebrate exposure to endocrine disruptors.

The calculated daily intake rates for an individual *P. pipistrellus*, which incorporates a sewage works as part of its home range, are speculative and make assumptions about the proportion of time a bat spends feeding at sewage works. Field observations on marked individuals are required to quantify the variation in individual exposure rates. However, our data provide the first step in this process. Given the high activity levels of insectivorous bats and birds at many sewage works (Park and Cristinacce, 2006; Fuller and Glue, 1980), and the fact that our study quantifies levels of EDCs from several sewage works, the values used here do not seem unreasonable. It is not known whether the quantities of EDCs ingested via this route pose a risk either to the health of the individual animal or exert population-level effects. Several studies have measured concentrations of organochlorine pesticides and polychlorinated biphenyls in the tissue of bats (e.g., Senthilkumar et al., 2001; Eidels et al., 2007). The mortality of bats exposed to high levels of such compounds is well documented (e.g., Luckens and Davis, 1964; Bennett and Thies, 2007), and one experimental study indicated sublethal effects of the organochlorine, gamma hexachlorocyclohexane, on the bat, *P. pipistrellus* (Swanepoel et al., 1999). Adverse effects of chemical pollutants, however, may be due to toxically high levels, rather than via endocrine disruption, and to our knowledge there have been no studies of chemically-mediated endocrine disruption in bats. Recent experimental work on birds, however, found marked changes in neural characteristics of the brain, immune function and song characteristics of male European starlings (*Sturnus vulgaris*) fed on mealworms at concentrations of 200 ng 17 β -estradiol per day (Markman et al., 2008). The estimates of daily exposure calculated here for bats who have 1–2 foraging beats within a filter bed sewage works range from 9 to 159 ng g⁻¹ 17 α -ethinylestradiol. In vivo assays to assess the comparative potencies of 17 β -estradiol and 17 α -ethinylestradiol suggest they are similar, whereas in vitro assays estimate a 30-fold higher potency of 17 α -ethinylestradiol (Van den Belt et al., 2004). Our results, suggest that detrimental effects of EDCs on foraging bats is certainly plausible. For a small mammal, potential effects of 17 α -ethinylestradiol exposure include reduced egg production and fertilisation success, with consequential reductions in reproductive rates.

EDCs are recognised as a potentially serious threat to wildlife populations (e.g., Tyler et al., 1998). Here, for the first time, we identify a potential risk for aerial insectivores foraging at sewage treatment works on contaminated prey, including bats. Our study suggests that intake rates of identified EDCs could be sufficient to alter behaviour and physiology and we suggest that further research is required to quantify the importance of any effects.

Acknowledgements

We thank Sara Barnsley, Irina Guchina and Mike O'Reilly for analytical assistance, and Scottish Water for access to the sites. This work was supported by funding from The Leverhulme Trust, The Royal Society, British Ecological Society, BBSRC Grant S18938 and The University of Stirling.

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