THE POTENTIAL FOR ESTRADIOL AND ETHINYLESTRADIOL DEGRADATION IN ENGLISH RIVERS

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Abstract—Water samples were collected in spring, summer, and winter from English rivers in urban/industrial (River Aire and River Calder, Yorkshire, UK) and rural environments (River Thames, Oxfordshire, UK) to study the biodegradation potential of the key steroid estrogen 17ß-estradiol (E2) and its synthetic derivate ethinylestradiol (EE2). Microorganisms in the river water samples were capable of transforming E2 to estrone (E1) with half-lives of 0.2 to 9 d when incubated at 20°C. The E1 was then further degraded at similar rates. The most rapid biodegradation rates were associated with the downstream summer samples of the River Aire and River Calder. E2 degradation rates were similar for spiking concentrations throughout the range of 20 ng/L to 500 µg/L. Microbial cleavage of the steroid ring system was demonstrated by release of radiolabeled CO2 from the aromatic ring of E2 (position 4). When E2 was degraded, the loss of estrogenicity, measured by the yeast estrogen screen (YES) assay, closely followed the loss of the parent molecule. Thus, apart from the transient formation of E1, the degradation of E2 does not form other significantly estrogenic intermediates. The E2 could also be degraded when incubated with anaerobic bed sediments. Compared to E2, EE2 was much more resistant to biodegradation, but both E2 and EE2 were susceptible to photodegradation, with half-lives in the order of 10 d under ideal conditions.

Keywords—Estrogen Estradiol Ethinylestradiol Degradation River

INTRODUCTION

International concern has arisen that estrogenic substances are entering the freshwater environment and having disruptive effects on the indigenous fauna. Many sewage treatment work effluents in the United Kingdom [1–6], Germany [7–9], Italy [10], the Netherlands [10,11], Sweden [12], the United States [13,14], Canada [9,15], and Israel [16] have been shown to contain potentially estrogenic components. The compounds found to be responsible for the majority of the in vitro estrogenicity of primarily domestic sewage treatment works have been the natural estrogens E1 and E2 and the synthetic E2 derivate EE2 [3]. Concentrations of steroid estrogens have usually been in the low-ng/L range [3,4,7–16], but even at such low concentrations these compounds can be extremely potent. For example, less than 1 ng/L EE2 can stimulate male rainbow trout to produce vitellogenin, an egg yolk protein normally only associated with sexually mature females [6], and 4 ng/L caused male fathead minnows to fail to develop normal secondary sexual characteristics [17].

Natural estrogens such as E2 are excreted by females and to a lesser extent males, mainly through urine. Most of the excreted estrogen is in a conjugated form as estrogen glucuronides or sulfonides, which makes them more water soluble and also renders them virtually inactive as hormones [18,19]. However, it is believed that through microbial processes in the sewer and in the sewage treatment works, many of these conjugates become cleaved [11,20,21], thus returning the free (active) estrogens into solution. The process of conjugation in the body, excretion of the conjugated form, and microbial deconjugation also applies to synthetic steroid estrogens such as EE2, the active ingredient of the contraceptive pill.

The fact that both natural and synthetic steroid estrogens have been found in the outlets of sewage treatment works [3,4,7–16] and occasionally in the receiving waterways [4,9,11,14] indicates that these substances are not (always) completely broken down during the treatment process. In densely populated areas, such as large parts of England, sewage treatment work effluents can make up a large proportion of the river flow. This could lead to significant estrogen inputs, especially during dry periods, when low flow rates mean little dilution [22]. Despite their importance, very little is known about the fate of steroid estrogens in rivers.

In this paper, new data are provided on the potential for steroid estrogens to degrade in a range of river water samples. The compounds selected for study were E2 as an example of a natural steroid estrogen and EE2 as a common synthetic estrogen. The results presented relate to samples collected in England from two types of riverine systems: urban/industrial and rural. The Yorkshire rivers Aire and Calder run through urban and industrial landscapes that have been studied under the Land Ocean Interaction Study [23]. In contrast, the River Thames at Wallingford is an agriculturally impacted stretch of the river [24].

The work was structured to address a series of questions. At what rate are the steroid estrogens biodegraded in river water? How does the degradation potential vary along the same river, between rivers, and in different seasons? How completely is the E2 molecule mineralized? How does estrogen concentration affect the biodegradation rate? Can E2 be degraded in anaerobic bed sediments? Does loss of estrogenic potency follow biodegradation of E2? At what rate could E2 and EE2 be photodegraded under ideal conditions?
MATERIALS AND METHODS

Collection of materials

Instantaneous bulk samples of river water were collected in 1-L glass bottles from the top 0.5 m of the water column on a number of occasions between 1997 and 2000. The pre-cleaned bottles were first rinsed with river water before filling and capping them. Collections were made from urban/industrial stretches of the River Aire near Riddlesden (20 km upstream of Leeds, National Grid Reference [NGR] SE 080 418) and Beal (25 km downstream of Leeds, NGR SE 532 256) and the River Calder near Brighouse (20 km upstream of Wakefield, near Halifax and Huddersfield, NGR SE 155 223) and Methley Bridge (16 km downstream of Wakefield, NGR 408 257) and from relatively rural reaches of the River Thames at Ifley (South Oxford, NGR SU 524 028) and near Wallingford (NGR SU 614 903). The samples were taken during low (summer 1999, flow rates exceeded 80–90% of the time), medium (spring 1999 and spring 2000, 30–70% exceedance), and high flow periods (December 1999 for Yorkshire rivers and February 3, 2000, for Thames, flow rates in the top 2–5% of the gauging stations) (National River Flow Archive: http://www.nwl.ac.uk/ih/nrfa/index.htm). The water samples were stored at 4°C and used within 4 d of collection. From the River Calder (Methley Bridge) and River Thames (Wallingford), bed sediments were collected 2 to 3 m from the bank by skimming off the top few centimeters with a bucket. The sampling bucket was thoroughly cleaned between samples but not sterilized. It was considered that any contamination on the sampling bucket would not jeopardize the experiment because of the very high quantity of bacteria that would be associated with the collected sediments. The bed sediments were then sieved (2 mm) and stored at 4°C until use.

Measurement of water properties

On return to the laboratory, pH was measured, and suspended sediment concentrations were determined as dry weight after 0.2-μm filtration (cellulose nitrate, Whatman, Maidstone, Kent, UK). Dissolved organic carbon (DOC) in river water was measured using a TOCsin II aqueous carbon analyzer (Phase Separations, Watford, Herts, UK) on a 0.2-μm-filtered sample. Each sample was acidified with 1% nitric acid and purged of CO₂ and then pumped through a heated capillary inlet tube and forced into an oxidation furnace where the DOC was converted to CO₂. The CO₂ so formed was then mixed with hydrogen over a nickel catalyst to form methane, which was measured using a flame ionization detector. To determine heterotrophic aerobic bacteria as colony-forming units, river water samples were vigorously mixed by hand before serial dilution in sterile one-fourth-strength Ringers solution (BDH, Poole, Dorset, UK) and plated on 0.3% tryptone soy agar plates; colony-forming units were counted after 2 to 3 d at room temperature (20°C). Two separate dilution rows were made, and three 0.04-ml aliquots each were counted from both replicates of each dilution. The standard deviation of the six counts so obtained was between 6 and 60% of the values.

Comparative biodegradation of steroid estrogens

River water samples were dispensed in 50-ml quantities into sterile (autoclaved) 125-ml polytetrafluoroethylene conical flasks (Nalgene, Rochester, NY, USA). In the first set of samples collected in spring 1999, the water was then spiked with a stock solution of 45 mg/L E2 or EE2 in methanol to give a nominal concentration of 100 μg/L E2 or EE2 (0.2% methanol). After this occasion, the method was modified so that the steroid stock solutions were added to the empty flasks and the solvent was left to evaporate at room temperature in a laminar flow hood prior to the addition of the river water. This was to eliminate the cosolvent methanol entirely from the experiment. Autoclaved water (30 min, 121°C) from the same river sites was used for sterile controls, and the samples were incubated in darkness at either 20°C or 10°C, 20°C being a common summer water temperature for central and southern English rivers [25]. The flasks were regularly sampled by withdrawing 1 ml into a syringe that already contained 1 ml methanol, mixing, and then filtering this mixture through a 0.45-μm polytetrafluoroethylene filter (Gelman Sciences, Ann Arbor, MI, USA) into a high-performance liquid chromatography (HPLC) vial. The presence of methanol prevents sorption to the filter and glass vial and inhibits further degradation during storage. The vials were sealed with polytetrafluoroethylene/rubber septa and stored in the dark at 4°C until analysis.

Low concentration biodegradation assessment

To enable the study of the degradation potential for E2 at lower concentrations, 2.5-L Winchester bottles were filled with water from the River Thames (Wallingford), and E2 was added to give a nominal concentration of 20, 50, or 100 ng/L. River water autoclaved for 30 min (121°C) was used as a sterile control. For the 20-ng/L experiment, six nonsterile 2.5-L river water samples were used. The bottles were covered with cotton wool bungs through which a glass tube was fed to allow aeration and were incubated at 10°C. Aeration was achieved by continuously bubbling moist air through each bottle at a rate of approximately 5 L/h. The six nonsterile flasks were sampled as pairs at 1- to 2-d intervals by decanting 0.5 L from each member of a pair. The two 0.5-L samples were combined to give a 1-L sample, thus providing three replicate samples at each sampling interval. The sterile flasks were sampled in the same way, but only at 0, 10, and 14 d. For the other low-concentration experiments (50 and 100 ng/L), nine nonsterile flasks were used and at the sampling intervals 0.33 L taken from each of three bottles to generate three replicate 1-L samples. To analyze the samples, suspended solids were removed by filtration through a glass-fiber filter (GF/C, 1.2 μm, Whatman), and E2 was concentrated immediately by passing the filtrate through C18 solid-phase extraction cartridges (Isolute 3 cc, 500 mg, Jones Chromatography, Lakewood, CO, USA) that had been conditioned with 3 ml each of acetone, methanol, and deionized water beforehand. After the sample had been passed through, the cartridges were cleaned with 3 ml 50% methanol and dried by passing dry air through the cartridge, and the estrogens were eluted with 1 ml pure methanol prior to high-performance liquid chromatography/mass spectrometry (HPLC/MS) analysis as described in the following. The recovery using this method was 92 to 122%.

Anaerobic biodegradation in bed sediments

Bed sediment was used from the River Calder (Methley Bridge, July 24, 2000, stored wet for 10 weeks at 4°C) and from the River Thames (Wallingford, October 20, 2000, fresh) to examine the potential for E2 to be degraded in anaerobic bed sediments. The wet sediment and water from the same site were placed in 100 ml Quickfit (Bibby Sterilin, Staffs, UK) conical flasks to make up a total of 5 g solids with 10 ml river water. The conical flasks were fitted with gas-flushing heads,
and moist nitrogen (two volume of gas changes/min) was blown through the headspace in the conical flasks for 16 h to create anaerobic conditions. The nitrogen gas flow rate was then increased to provide a positive pressure to permit the heads to be partially lifted, and 5 µg E2 in 11 µl methanol were added to give a final concentration of 0.5 mg/L (1 mg/kg). The nitrogen flow was then returned to the original rate. To achieve an even distribution of the added E2, the samples were swirled at intervals for the first 4 h after spiking. The conical flasks (three replicates for each sample interval) were connected by neoprene tubing in sequence, with a final empty flask for each row containing oxygen indicators (Oxoid, Basingstoke, Hants, UK) to confirm anaerobic conditions. The flasks were incubated at room temperature (20 ± 2°C) in a fume cupboard throughout the experiment. Autoclaved (121°C, 30 min) samples, treated in the same way, were used as sterile controls.

Sampling was carried out by increasing the nitrogen flow and partially lifting the heads of the flasks to allow 90 ml of methanol to be added (9:1 ratio of methanol to water) to the last flasks in the row (three replicates each time). The flasks were then removed from the nitrogen line and shaken for 24 h at room temperature to extract E2 from the sediments. The sediments were then allowed to settle before sampling and filtering (0.45-µm polytetrafluoroethylene syringe filters) the supernatant liquid for analysis by HPLC/MS. To compare anaerobic versus aerobic degradation in the bed sediments, another set of sacrificial samples (River Calder) and sterile controls was set up in the same way, but without nitrogen flushing.

Analytical technique for measuring steroids using HPLC/MS

Samples were analyzed by HPLC/MS with a C18 column (Columbus 250 × 2 mm, 5 µm, injection volume 150 µl, Columbus, OH, USA) and acetonitrile:water 36:64 as mobile phase. Quantification was with a mass spectrometer (HP 1100 MSD, Hewlett-Packard, Avondale, PA, USA) in electrospray negative mode at 271.1, 269.1, and 295.1 mass units for E2, E1, and EE2, respectively. For a few samples, an ultraviolet absorption detector was used instead of the mass spectrometer, another set of sacrificial samples (River Calder) and sterile controls was set up in the same way, but without nitrogen flushing.

Mineralization of estradiol phenol ring A

To study the potential for E2 to be completely mineralized, the evolution of 14CO2 from position 4 in the A ring of E2 was monitored using water samples from the River Aire (Beal, July 18, 1997) and River Calder (Methley Bridge, July 18, 1997) that had been refrigerated for eight weeks and a fresh River Thames (Wallingford, September 9, 1997) sample. Triplicate 40-ml samples were placed in 150-ml Quickfit conical flasks with gas-flushing heads (Bibby Sterilin, Staffordshire, UK). One sample from each river was autoclaved for 30 min to act as a sterile control. The water samples were spiked with a mixture of unlabeled E2 (490 µg/L from a stock solution of 6.74 g/L in methanol) and 10 µg/L radiolabeled E2 (Du Pont, Boston, MA, USA; 7.4 MBq/mg, 100 mg/L in ethanol). The conical flasks were placed in an incubator at 20°C and connected via neoprene tubing between an air pump and a CO2 trap containing NaOH. Twice weekly, carbon dioxide–free moist air was passed over the samples for a 3-h period (120 air changes in the headspace of the conical flasks) and bubbled into a 50-mM NaOH solution to capture the evolved carbon dioxide. The amount of 14CO2 captured in the NaOH solution was assessed at intervals by withdrawing 1 ml, mixing it with 5 ml scintillant (Ultima Gold, Canberra Packard, Mississauga, ON, Canada), and counting for 20 min with a liquid scintillation counter (Beckman LS 6500, Beckman Instruments, Fullerton, CA, USA). To capture volatilized E2 or its by-products, a tube with granulated activated charcoal was inserted between the sample and the NaOH solution. At the end of the experiment, a mass balance was undertaken for the radiolabel by measuring it in the different compartments in the following way.

The remaining radioactivity in the batches was measured by sampling 1 ml into the scintillation vials and counting them as usual. The remaining liquid was then passed under gravity through solid-phase extraction cartridges (Varian Mega Bond Elut, Middelburg, The Netherlands; C18, 1 g, 6 cc), and the radioactivity that passed through them was used to produce a measure for hydrophilic by-products containing the radiolabel. To measure the radioactive carbon trapped on the charcoal, the charcoal was heated to 850°C for 1 h to completely convert it to CO2, and this was trapped in a 1-M-NaOH solution and measured as described previously. This method gave a recovery of 60% or better for estradiol sorbed to the charcoal. Finally, the glassware was washed with a known amount of methanol that was then also checked for radioactivity.

Biodegradation of E2 and the yeast estrogen screen assay

A biodegradation experiment was set up, as described in the section Comparative Biodegradation of Steroid Estrogens, using water collected from the River Thames in March 2000. Three 50-ml river water samples were spiked to a nominal concentration of 100 µg/L E2 and incubated at 20°C for 14 d. Controls were autoclaved before spiking. At 1- or 2-d intervals, samples were taken to be analyzed in the recombinant yeast estrogen screen (YES) assay following the procedures of Routledge and Sumpter [26]. In this assay, a yeast strain is used that has been modified to contain a human estrogen receptor. Estrogens stimulate the yeast to produce an enzyme that changes a yellow compound (chlorophenol red-β-D-galactopyranoside) into a red product that can easily be measured photometrically.

Serial 1:2 dilutions of each sample were made with 50/50 methanol/river water. Aliquots of 10 µl of each dilution were transferred into a microtiter assay plate and left to dry before addition of 200 µl assay medium, containing chlorophenol red-β-D-galactopyranoside and yeast cells. After mixing, the plates were incubated at 20°C for 3 d. The absorbance at 540 nm was measured and corrected for turbidity by the absorbance at 620 nm. The absorbance of the samples was compared to E2 or E1 standards treated in the same way. In addition to the YES assay, a subset of the same samples was analyzed by HPLC for E2 and E1.

Photolysis of estradiol and ethinylestradiol

The Suntest CPS (Heraeus Instruments, Hanau, Germany) is equipped with a polychromatic light source, produced from a filtered Xenon lamp, to provide a spectral distribution similar to natural sunlight. The design of the apparatus is such that
Temporal and spatial variation in E2 biodegradation potential in river waters

For the River Aire and River Calder, the downstream sites had in most cases higher DOC and sediment loads but similar viable counts to their upstream sites (Table 1). In all the non-sterile river water samples, E2 concentration declined and was associated with the transient formation of E1 (Table 1). No significant loss of E2 was observed with the sterile controls. It was not expected that sorption of E2 to suspended sediments would be an important factor in reducing the aqueous concentration [27]. To compare the degradation rates for all the river samples, the data (averages of three replicates) were entered into the Model Manager program (Cherwell Scientific, Oxford, UK) to calculate the half-lives and give confidence levels for E2 and E1 in a simple first-order model for parent with one metabolite. The values in brackets give the 95% confidence interval of a first-order degradation model for parent and metabolite.

RESULTS AND DISCUSSION

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$$M_p(t) = M_0 \exp(-k_p t)$$

$$M_m(t) = CM_0 \int_0^t k_m \exp(-k_m t') \exp[-k_e(t - t')] dt'$$

where $M_p$ is the concentration of the parent E2, $M_m$ is the concentration of the metabolite E1, $M_0$ is the initial concentration of E2, $C$ is the ratio of the molecular weights of E1 and E2 = 0.993, and $k_p$, $k_m$, $k_e$ are the first-order rate constants of the parent and the metabolite, respectively.

When the E2 degradation potential of water samples from the rivers Aire, Calder, and Thames were compared in different seasons, a trend of faster degradation in samples taken in summer and from downstream sites can perhaps be identified for the River Aire and River Calder samples (Table 1 and Fig. 1). These rapid degradation rates associated with the summer samples can be correlated at the $F < 0.5\%$ level (not including the outlier sample of the Thames, August 3, 1999; see the following discussion) with lower pH ($R^2 = 0.48$) values and higher DOC ($R^2 = 0.57$) and suspended sediments ($R^2 = 0.41$) but not with numbers of culturable heterotrophic bacteria. One
possible explanation is that higher nutrient concentrations due to low dilution and higher summer temperatures encourage faster metabolic rates in the degrading community; thus, when the bacteria are transferred to the test system, they are initially more active than those collected in spring or winter. As part of a previous research project (unpublished data from the NERC Land Ocean Interaction Survey project, held at Wallingford, UK), dissolved oxygen levels were monitored at Aire Beal and Calder Methley Bridge from January 1995 to December 1996. These data showed that at both those sites, the oxygen levels were about 80 to 90% saturation during the winter, dropping to 40 to 50% in the summer months. The decline in dissolved oxygen in these urban/industrial-impacted rivers suggests that a particularly active microbiota is present during these summer months. At the Thames sampling site, the average dissolved oxygen levels are close to 100% throughout the year, but with a typical high diurnal variation due to algal production in the summer [24].

No correlation between the time of year and the half-life was found in the River Thames samples, where E2 had the greatest persistence in the sample collected in August (this sample is not included in Fig. 1, as it would be off scale). However, in this case the first-order degradation model yielded a particularly bad fit (very large 95% confidence interval), so perhaps this calculated half-life is not reliable. Overall, the River Thames samples had always fewer bacteria than the other samples.

On one occasion, the degradation rates of E2 in River Thames water was compared for incubation temperatures of 20°C and 10°C. Although 20°C is frequently reached in summer, 10°C would be a more common water temperature throughout the year in these rivers. As expected for a temperature difference of 10 K, the half-lives were roughly twice as long at the lower temperature (Table 1).

Influence of concentration on biodegradation rate

The degradation of E2 with a spiking concentration of 100 µg/L compared with 0.1 µg/L (100 ng/L) was examined for water collected on the same day from the River Thames. Despite the 1,000-fold difference in concentration, the rate of removal was similar for the two concentrations (Fig. 2a), and indeed the concomitant generation of E1 as first metabolite (Fig. 2b) suggests that degradation may even be slightly faster at the low concentration. To ensure that differences between the results for the different concentrations could not be attributed to differences in O2 content (bubbles vs exchange over the surface), additional tests were made. When 100 µg/L E2 were added to 2.5 L water (River Thames, June 17, 2000) with air bubbling through (as in the low-concentration experiment) or to 50 ml in conical flasks (as in the usual setup for 100 µg/L), E1 was not degraded faster in the larger samples (data not shown). Ternes et al. [20] also observed a faster E2 degradation rate at the lower concentration when the degradation of 1 µg/L E2 in diluted activated sludge was compared to 1 mg/L.

The degradation of E2 at the lower concentrations of 20 and 50 ng/L was also tested. Even at the lowest tested concentration of 20 ng/L, E2 was rapidly degraded (Table 2). While 20 to 100 ng/L is still higher than literature values for river water concentrations [7,9,11,14], these results suggest that, even when insufficient E2 is present to stimulate the multiplication of bacteria, E2 will still be transformed. The similarity of degradation rates for the different spiking concentrations indicates that the rates calculated for the 100-µg/L E2 concentration (Table 1) would be applicable to river water exposed to E2 at the environmentally relevant low ng/L level.

Comparison of E2 and EE2 degradation rates in river water

The aerobic biodegradation of EE2 was much slower than that of E2 under the same incubation conditions for a water sample collected from the River Thames in spring 2000 (Fig. 3). While the half-life for E2 has been calculated as 1.2 d in
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Anaerobic degradation of E2 in bed sediments

Sorption experiments have shown that a proportion of E2 and EE2 in the water will be attracted to the riverbed sediments [27,31]. The riverbed sediments may be partly or, in the case of heavily polluted rivers, completely anaerobic. It is therefore important to assess the persistence of steroid estrogens in this environment.

Unlike the xenobiotic endocrine disrupter octylphenol [25], E2 was degraded in anaerobic bed sediments (Table 3). The bed sediments were obtained from a urban/industrial stretch of the River Calder (Methley Bridge), where a large and flourishing community of anaerobic bacteria may be expected, and from the River Thames (Wallingford) site, which has relatively good water quality [24]. Under anaerobic conditions, E2 was fairly rapidly converted to E1 in both samples. Over the 2-d incubation period, the loss of E2 could be almost entirely accounted for by the formation of E1. Together with the results mentioned in the previous sections, this suggests that E2 would be eliminated from the freshwater system as a whole but that E1 may accumulate as a by-product in anaerobic bed sediments. Further research is needed to confirm this. Incubating the River Calder bed sediment sample under aerobic compared to anaerobic conditions (Table 3) showed faster removal of E2 (estimated half-life 0.11 d compared to 0.37 d). The potential for EE2 to degrade in bed sediments has not been tested, but indications exist that it may accumulate in bed sediments because of its slightly higher sorption coefficients compared to E2 [31] and a previously found poor degradability under anaerobic conditions [27].

Mineralization of E2

While previous experiments had demonstrated the transformation of E2 and E1 by the indigenous microorganisms, it was not clear whether these molecules could be completely mineralized or leave persistent by-products. Studying the evolution of $^{14}$CO$_2$ from E2, which was radiolabeled in the 4 position of the A ring, enabled the mineralization of this central part of the molecule and therefore the breakup of the steroid structure to be followed. The results (Fig. 4) show that it is possible for the A ring of E2 to be cleaved by microorganisms present in river water, as has also been shown in sewage [32]. This experimental setup measured only the CO$_2$ end product and does not identify intermediate metabolites, but a possible pathway by which the steroid ring could be cleaved was postulated by Coombe et al. [33]. No significant change occurred with the sterile controls, indicating that radioactivity in the trapping solutions was not due to E2 volatilization or abiotic breakdown. The relative mineralization rates are consistent with the degradation experiments, which looked at loss of parent, with the River Thames being slower than the River Aire and River Calder (compare Table 1). After about 25 d, when 24 to 45% of the radiolabel had been evolved as CO$_2$, the mineralization rates began to slow. It may be that the ring metabolism is cometabolic and cannot continue if the cometabolic substrate has been exhausted or that the system is limited in essential nutrients, but this cannot be confirmed on the basis of these data. There was no apparent loss of the radiolabel, a mass balance was carried out at the end of the experiment. Between 10 and 23% of the parent compound was converted into unknown hydrophilic (not binding to C18 SPE cartridge) by-products, 18 to 32% was left as E2 or hydrophobic by-products, and less than 5% was found sorbed to glassware or the charcoal. The total recovery of the radiolabel was about 80% for the samples and 99.8% for the sterile controls. The 20% of radiolabel unaccounted for was probably converted to volatile organic by-products that the charcoal filter failed to capture.

Biodegradation of E2 and the YES assay

The river water sample rapidly transformed the E2 to E1 (Fig. 5). The E1 is only a transient by-product, as it too is
soon degraded (Fig. 5) via unknown intermediate products eventually to CO₂, as shown by the mineralization experiment. From the YES standard curve for E2 and E1 (not shown), it was evident that E1 is less estrogenic than E2, but only by a factor of about 1.5. Therefore, both E2 and its degradation product E1 are expected to contribute to the overall estrogenicity of the samples. The previous experiments showed a relatively fast degradation of E2 and its first metabolite E1 but a much slower breakup of the A ring, as shown in the mineralization experiment. Since the A ring is essential for binding to the estrogen receptor [34], it would be expected that the estrogenic properties are lost during the degradation process at some stage between the loss of E1 and the breakup of the A ring. Combining the YES assay with a degradation experiment would indicate how fast this loss of estrogenicity happens.

To simplify the graph, the absorbance of just one of the dilution steps of the YES assay is given in Figure 5. This showed that concurrent with the elimination of E2 and its product E1, more than 99% of the estrogenicity was lost over two weeks. However, while neither E2 nor E1 were detectable after 8 d (<10 µg/L), the YES assay still showed some estrogenicity in the less diluted samples (about 2–3% of original) up to 12 d but not after 14 d. Perhaps some small quantities of E1 were still present that were not detected by chemical analysis or a breakdown product of E1 had some estrogenic activity. Thus, the elimination of E2 from river water would not initially remove the estrogenic signal; this would follow perhaps a few days later with the removal of the E1 by-product. In an experiment that combined mineralization of position 4 radiolabeled E2 in activated sludge with the YES assay, Layton et al. [32] found that the estrogenicity of E2 was completely removed while some radiolabel still remained in the sample. This suggests that the loss of estrogenicity is not dependent on the mineralization of the A ring.

**Photolysis of estradiol and ethinylestradiol**

The measured concentration of E2 and EE2 in irradiated samples and dark controls is shown in Figure 6. Photolytic degradation of both steroids was observed, but this proceeded at a slow rate, with E2 and EE2 being present at approximately 40% of their initial concentration after 144 h continuous irradiation. No degradation of either compound occurred in the dark controls. The rates of photodegradation for E2 and EE2 were estimated from the slope of a plot of log change in concentration versus time, where the half-life is expressed as

$$t_{1/2} = -0.693/slope$$

and was calculated as 124 and 126 h for E2 and EE2, respectively. Twelve hours of irradiation in this test system are equivalent to approximately a 12-h day of British summertime daylight. This would suggest that the half-life for photolysis of E2 and EE2 is at least 10 d (assuming 12 h of sunlight per day and no reduction in the light levels due to turbidity and coloring of the water). Photolysis is slow compared to biodegradation for E2 and would not be expected to be a very significant removal mechanism in English rivers considering the short transit times. Overall, photodegradation may be more significant in removing the more recalcitrant EE2 from river water during the summer months.
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Fig. 6. Photodegradation of 17β-estradiol (E2) (● ● ● ●) or ethinyloestradiol (EE2) (● ● ● ●) (means of two replicates; the standard deviation of the replicates was <3%), compared to dark controls (open symbols).

CONCLUSIONS

The E2 at a test concentration of 100 to 500 µg/L can be degraded in river water via E1 under aerobic conditions at 20°C to give half-lives between several hours and 9 d. With the River Aire and River Calder, the most rapid biodegradation occurred with the water samples collected in summer (half-lives of 4–5 h). Similar degradation rates were achieved when the E2 concentrations were reduced from 100 µg/L to 20 to 100 ng/L. The river water samples were capable of cleaving the steroid ring system of E2. The E2 could be degraded under anaerobic conditions in bed sediments, but only as far as E1 over a 2-d period. Estrogenicity, as measured by the YES assay, was almost entirely lost with the transformation of E2 and its first degradation product E1. According to the test on the River Thames sample, EE2 was much more resistant to degradation in river water than E2. Both E2 and EE2 are susceptible, but only slowly, to photodegradation, with an estimated half-life of 10 d given clear water and 12 h of bright sunshine per day.

While in most cases degradation appears to be fastest in summer, the dilution is generally much lower than in spring or winter. Although E2 degradation may not be as rapid in winter as we would like, dilution would certainly reduce the concentrations very quickly. However, it may be that newly hatched fish are most sensitive to endocrine disruption; E2 concentrations similar to those found in at least some sewage treatment works are sufficient to completely feminize Japanese medaka populations if they are exposed to the estrogen during early life [35]. So, it could be hypothesized that endocrine disruption (which could have lasting effects) occurs predominantly during a short period in spring. Outside that period, perhaps the fish are less sensitive and/or concentrations too low, because of either dilution or degradation, to have an effect. More work needs to be done on the persistence and bioavailability of endocrine disrupters in bed sediments and their impact on invertebrates and demersal fish. Early indications suggest that EE2 persistence in English rivers may be a problem given the relatively short transit times.

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