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Short Communication

AN ASSESSMENT OF THE BIOACCUMULATION OF ESTRONE IN *DAPHNIA MAGNA*

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Abstract—The bioaccumulation of estrone by *Daphnia magna* was determined. Direct uptake via the aqueous medium occurred within the first 16 h. A bioconcentration factor of 228 was established over all temporal periods. Ingestion via *Chlorella vulgaris* gave a partitioning factor of 24, which may approximate to a biomagnification factor assuming steady state conditions. These preliminary results indicate that the partitioning to *Daphnia magna* via the food source, *C. vulgaris* is less significant than bioconcentration.

Keywords—Estrone *Daphnia magna* *Chlorella vulgaris* Bioconcentration Biomagnification

INTRODUCTION

Steroid estrogens have been identified as the key chemicals responsible for endocrine disruption in fish and are priority pollutants in the United Kingdom aquatic environment [1,2]. Current studies have been limited to fish and the accumulation of estrogens in organisms at the lower trophic levels has not been assessed. Invertebrates constitute 95% of the faunal species and are key components of all ecosystems. However, relatively little is known about their endocrine system or their susceptibility to environmental endocrine disruption. The paucity of research into the impact of endocrine disrupting substances on invertebrates has recently been highlighted [3,4]. The determination of the bioaccumulation properties of all estrogens is essential to fully assess their impact and risk in the aquatic environment [5,6].

The presence of estrone (E1), 17β-estradiol, estriol and 17α-ethinylestradiol in the aqueous environment have been attributed to incomplete removal from sewage treatment works [7,8]. Interconversion between the steroids E1 and 17β-estradiol occurs, favoring E1, and partly explains why concentrations of E1 are generally the greatest of all steroids in aqueous samples [9]. Levels of E1 in sewage effluents have been detected up to 0.220 μg/L, though are typically in the range of 0.005 to 0.02 μg/L [10]. Dilution, sorption, and biodegradation processes in surface waters decrease these concentrations to the low ng/L range. E1 has been detected up to 0.014 μg/L [10] in surface waters and at 11.8 μg/kg in sediment [11].

The uptake and accumulation of steroid estrogens by the algae *Chlorella vulgaris* have been studied and a bioconcentration factor (BCF) of 27 established for E1 over 48 h under light and dark conditions [12]. *Daphnia magna* is a primary consumer of algae and represents the subsequent step in the food chain. The assessment of bioaccumulation through both the direct route (via the aqueous medium) and via the trophic route will determine the significance of E1 biomagnification for *D. magna* and indicate the level of exposure.

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MATERIALS AND METHODS

Test material

Individual stock solutions of E1 with a purity of >99% (Sigma-Aldrich, Poole, UK) of 1000 μg/mL were prepared in high-performance liquid chromatography grade acetone (Rathburn, Walkerburn, UK). The stock solution was diluted in 50:50 acetonitrile (Rathburn) and ultrapure water (Maxima Ultrapure water generator, USF Elga, Bucks, UK) to create working solutions from 1 μg/L to 10,000 μg/L. Analysis was by negative ionization, electrospray liquid chromatography/mass spectrometry (LC/MS).

Test cultures

Initial cultures of *C. vulgaris* and *D. magna* were purchased from the Culture Collection of Algae and Protozoa (Windermere, UK). *Chorella vulgaris* was grown in Jaworski's Medium with constant aeration and maintained at 17 ± 1°C under constant cool fluorescent light at 300 lx provided by Osram L 58W/23 tubes (Langley, UK). *Daphnia magna* was cultured in 4-L polypropylene containers (Whitefurze, UK) containing synthetic pond water and maintained under natural light. The water temperature was measured by thermometer and maintained at 18 ± 2°C in a temperature-controlled environment. The synthetic pond water was made to the following composition $NaHCO_3$ (192 μg/L), $CaSO_4 \cdot 2H_2O$ (120 μg/L), $MgSO_4$ (120 μg/L), and KCl (8 μg/L) using ultrapure water (SCIENTO Culture instructions, Culture Collection of Algae and Protozoa). The dry weight of the *C. vulgaris* was calculated by measuring the optical density (OD_{680nm}) using a spectrometer (SP6–200 Spectrometer, Unicam, Cambridge, UK) and converted to dry weight using 1.0769 mg/mL/OD [12].

Bioconcentration of estrone

In a 400-ml Pyrex glass beaker (VWR International, Poole, UK), 200 ml of synthetic pond water was spiked to 400 μg/L of E1 (quantified by LC/MS). Fifty adult *D. magna* (over 10 d old), which had not been fed for 24 h, were placed in the pond water containing E1. Uptake of E1 by the *D. magna*

was measured at 4, 16, and 24 h and the final concentration of E1 in the pond water was analyzed by LC/MS at each time point. The experiment was repeated at a lower concentration of E1 (40 μg/L) and uptake in the *D. magna* and concentration of E1 in the water was determined after 4 h. All bioconcentration experiments were carried out in triplicate. On determining the concentration of E1 in the *D. magna* and the final pond water, the BCF was calculated using Equation 1.

$$\text{BCF} = \frac{\text{E1 concentration in } D.\ magma}{\text{E1 concentration in pond water}} \quad (1)$$

Biomagnification of estrone

Chorella vulgaris was spiked to 2.5 μg/L of E1 and rotary shaken in the dark for 6 h. Dark conditions were used as algae transforms E1 to 17β-estradiol in the light [12]. The algae were filtered through a 0.22 μm membrane filter (Scheicher and Schuell, London, U.K.) and rinsed off the filter paper into fresh pond water. The concentration of E1 in the algal extract spiked to 2.5 μg/L was previously determined to be 0.055 μg/g (dry wt) [12]. The suspended algae were diluted to 200 mL with pond water. Sixty *D. magna* which had been starved for the previous 24 h, were placed in the new medium with the suspended algae for a period of 48 h. All biomagnification experiments were carried out in triplicate. On determining the E1 concentration in *D. magna*, the partitioning factor was calculated using Equation 2.

$$\text{partitioning factor} = \frac{\text{E1 concentration in } D.\ magma}{\text{E1 concentration in } C.\ vulgaris} \quad (2)$$

Estrone determination in D. magna

The *D. magna* were removed from the pond water and dipped into clean water to rinse off excess spiked pond water. The *D. magna* were then placed in a 20-mL screw-cap glass container containing 10 mL acetone and shaken vigorously for 10 min. The sample was filtered through a 0.22-μm Durapore membrane filter (Millipore, Watford, UK), transferred to a round bottom flask, and rotary evaporated. The recovery of E1 for the acetone extraction was determined to be 88.94 ± 5.56% (n = 3) and results were adjusted to account for the recovery. Final samples were made up to 1 mL in 50:50 acetonitrile:water. The pond water filtrate was collected at each time point and 1-mL subaliquots were analyzed by LC/MS to determine the final concentration of E1 in the aqueous medium. The filtered *D. magna* were dried at 90°C for 24 h and measured to determine the dry weight. A control experiment was conducted to assess whether extraction of *D. magna* with acetone for 10 min affects the subsequent determined dry weight. The dry weight after acetone extraction was 90.5% (n = 6) of the dry weight after shaking with water under the same conditions. After acetone extraction, the subsequent dry weight was adjusted to account for the recovery. Dividing the E1 concentration in *D. magna* by the dry weight of the *D. magna* allowed for comparison between samples.

LC/MS analysis

All E1 analysis in this study was by LC/MS, a Perkin-Elmer Series 200 LC System (Bucks, UK) connected to a SCIEX API 150 EX with TurboIonSpray interface (Applied Biosystems, Warrington, Cheshire, UK). Liquid chromatographic separation was achieved using a Hypersil BDS C18 column (100 × 2.1 mm, 5 μm) with an injection volume of 10 μL. The mobile phases, ultrapure water, and high-performance liquid chromatography grade acetonitrile were delivered at 200 μL/min on a 50:50 gradient. Analysis of E1 was in single ion monitoring at mass 269.4 [M-H$^-$], eluting at a retention time of 8.59 min. Detection and quantification limits of E1 were 10 pg and 50 pg on column, respectively.

Table 1. Estrone (E1) concentration in *Daphnia magna* and the aqueous medium after different exposure times and calculated bioconcentration factors (BCFs). Values are determined from three replicates

Duration of experiment (hours)	Average E1 concentration in *D. magna* (μg/g)	Average E1 concentration in aqueous medium (μg/mL)	BCF
4	4.24 ± 2.96	0.018 ± 0.005	241
4	65.00 ± 5.64	0.234 ± 0.032	278
16	63.58 ± 20.82	0.278 ± 0.120	229
24	56.80 ± 13.05	0.343 ± 0.012	165

RESULTS AND DISCUSSION

Bioconcentration of estrone

The results of the temporal study indicate that accumulation of E1 into the *D. magna* occurred within the first 16 h. Within the closed system, the BCF decreased after 24 h (Table 1). This may be attributed to an increase in depuration or degradation of E1 by the *D. magna*, which may involve an acclimation lag phase. The downward trend may also be attributed to changes in solution due to increased concentration of dissolved organic carbon in the aqueous phase, similar to that observed for steroid estrogens when binding to sediment [13]. The relationship between E1 concentration in *D. magna* to that in the aqueous phase over all temporal points was r^2 = 0.75. However, the significant positive correlation increased to 0.93 when results from the 24-h experiments were excluded (Fig. 1). The equation of the line (y = 243.09x) in Figure 1 also equates to BCF.

After 4 h of exposure to E1, the average BCF in *D. magna* was 272 (n = 6). This compares to a recorded BCF of 37 under similar conditions, for the uptake of E1 in *C. vulgaris* [12]. The BCFs for E1 are relatively small compared to other endocrine disrupting substances, such as nonylphenol and its mono- and di-ethoxylates [14]. However, due to the high potency of estrogens, low levels of bioaccumulation potentially can have significant effects. Previous studies have determined that bioconcentration increases at higher trophic levels reported to be due to increased lipid content and decreased chemical elimination efficiency up the food chain [15]. A log BCF of 2.36 was determined in the present study. This compares to a modeled log BCF of 1.77 for daphnids and 2.31 and 2.56 for nonspecies-specific data [16]. The log BCFs having been derived from physico-chemical equations using the log octanol/water partition coefficient (K_{ow}) of 3.43 for E1.

The acute median lethal concentration value for E1 on *D. magna* has not been determined. However, for the synthetic estrogen 17α-ethinylestradiol, the effective concentration in Daphnia (species not stated) was found to be 5700 μg/L (effect not stated) with a no-observed-effects concentration of 10 μg/L [17]. In the current study, *D. magna* were exposed to E1 concentrations ranging from 363 μg/L to 15 μg/L. Due to the limitations of current detection limits, the test concentrations

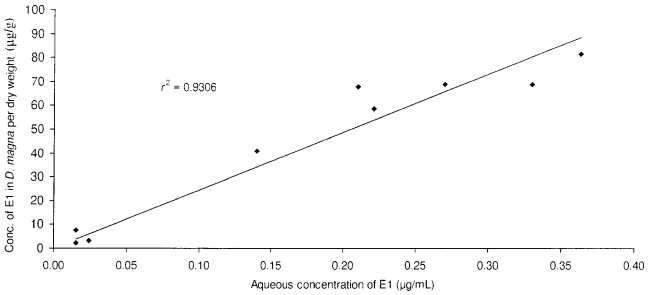

Fig. 1 Relationship between estrone (E1) concentrations in the *Daphnia magna* and the aqueous medium for the 4- and 16-h exposure experiments. The equation of the line is $y = 243.09x$, which also approximates to bioconcentration factors.

used here are a minimum of 2000 times in excess of those found in the environment. Thus extrapolation to the aquatic environment is not without the attendant risk of error.

Biomagnification of estrone

Daphnia magna were maintained in culture with *C. vulgaris* for 48 h, with the algae containing an average E1 concentration of 0.055 μg/g. E1 was detected in all *D. magna* samples, with an average concentration of 1.32 ± 0.21 μg/g ($n = 3$). Uptake via a food source will depend on the feeding rate; the concentration of the toxicant in the food and the rate that the food is processed; and the amount absorbed compared to the amount excreted [18]. Using Equation 2, the average partitioning from food source to *D. magna* was calculated to be 23.98 ± 3.79. The system had not been assessed to determine a steady state and the possible desorption of E1 from the algae (resulting in bioconcentration into the *D. magna* as opposed to biomagnification) was not eliminated as a possibility. This is a first estimation of partitioning between the food source, *C. vulgaris*, and *D. magna* and may approximate to the biomagnification factor assuming steady state conditions.

The data presented here indicate that the uptake via the trophic route is likely to be less significant compared to bioconcentration from the aqueous medium; this agrees with another study [19]. Even if the concentration in the food source is greater than that of the water, uptake from the water is more significant. This is a consequence of the volume (quantity) of food ingested being much smaller than the volume of water passing through the gills [20].

CONCLUSION

Accumulation of E1 from the aqueous medium into the *D. magna* occurs within the first 16 h with a decrease in BCF being observed after 24-h exposure. The log BCF of 2.36 obtained from the present study is slightly greater than predicted by the K_{ow} equations for Daphnids but is similar to the non-species specific physico-chemical equations. A linear relationship between the concentration of E1 in the aqueous medium to that in the *D. magna* was obtained ($r^2 = 0.93$) for the 4- and 16-h temporal experiments.

Biomagnification of E1 in *D. magna* via the algae, *C. vulgaris*, is nine times less than the bioconcentration from the aquatic environment, with a partitioning factor of 24. The partitioning factor may equate to the biomagnification factor assuming steady state conditions, which were not assessed for this study. This supports other recent studies that state, with the exception of extremely hydrophobic chemicals, biomagnification is less significant compared to uptake by bioconcentration. This preliminary study is one of the first to investigate the interaction of steroid estrogens with invertebrates. The determination of the bioaccumulation properties of all estrogens at different trophic levels is essential to fully assess their impact in the aquatic environment.

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