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Short communication

Endocrine disrupter—estradiol—in Chesapeake Bay tributaries

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Abstract

Exogenous chemicals that interfere with natural hormonal functions are considered endocrine disrupting chemicals (EDCs). Estradiol (17β-estradiol or E2) is the most potent of all xenoestrogens. Induction of vitellogenin (VTG) production in male fish occurs at E2 concentrations as low as 1 ng l^{-1}. E2 reaches aquatic systems mainly through sewage and animal waste disposal. Surface water samples from ponds, rivers (Wicomico, Manokin and Pocomoke), sewage treatment plants (STPs), and coastal bays (Assawoman, Monie, Chincoteague, and Tangier Sound – Chesapeake Bay) on the Eastern Shore of Maryland were analyzed for E2 using enzyme linked immuno-sorbent assay (ELISA). E2 concentrations in river waters varied between 1.9 and 6.0 ng l^{-1}. Highest E2 concentrations in river waters were observed immediately downstream of STPs. E2 concentrations in all the coastal bays tested were 2.3–3.2 ng l^{-1}.

Keywords: Endocrine disrupters; Estradiol; Coastal Bays; Sewage treatment plants; Rivers

1. Introduction

Global concern about EDCs has been increasing for the past two decades; EDCs bring about hormone imbalance in natural systems [1,2]. Environmental estrogens have been suggested as the cause of increased incidence of male reproductive tract disorders and reduced sperm counts and also for increase in frequency of female breast cancer [3]. Exposure to xenoestrogens is also associated with abnormal physiological changes and reproductive impairments in birds, fish, shellfish, turtles, gastropods, and mammals [4]. Feminization has been observed in early life-stages of roach (*Rutilus rutilus*) exposed to estrogenic effluents during periods of sexual differentiation [5]. Furthermore, intersex imposition (simultaneous presence of both testicular and ovarian characteristics), which is believed to be a consequence of exposure to estrogens, has been observed in the gonads of wild populations of roach [6], and gudgeon – *Gobio gobio* – living in rivers downstream of STPs [7]. Data suggest that low concentrations of estrogen can also induce oocytes in the testis of the Japanese medaka [8,9]. E2 concentrations as low as 1–5 ng l^{-1} have the ability to induce the production of a female specific egg-yolk protein precursor VTG in male fish [10,11]; VTG is completely absent in male fish under natural conditions [12]. E2 concentrations can vary due to factors such as dilution, sorption by sediments and organic matter, and photo-degradation [13]. E2 is one of the most potent estrogens; the relative potency of 17β-estradiol is 10^4 to 10^6 times that of six estrogenically active alkylphenol-polyethoxylates [6]. E2 reaches aquatic environments mainly through sewage and animal waste disposal [14–16]. A woman's daily discharge of estrogen in urine is 3.0 μg of E2 [16]; livestock are frequently administered growth hormones with E2 to expedite their growth and thus add value to the carcass resulting in an increased weight gain [17].

These estradiols from agricultural land and STPs can get into rivers, tributaries, and coastal bays (including Chesapeake Bay). There is some data in the literature on estradiol concentrations in a few rivers; however, little information is available on E2 concentrations in coastal bays and tributaries in USA. The objective of this study was to measure the con-

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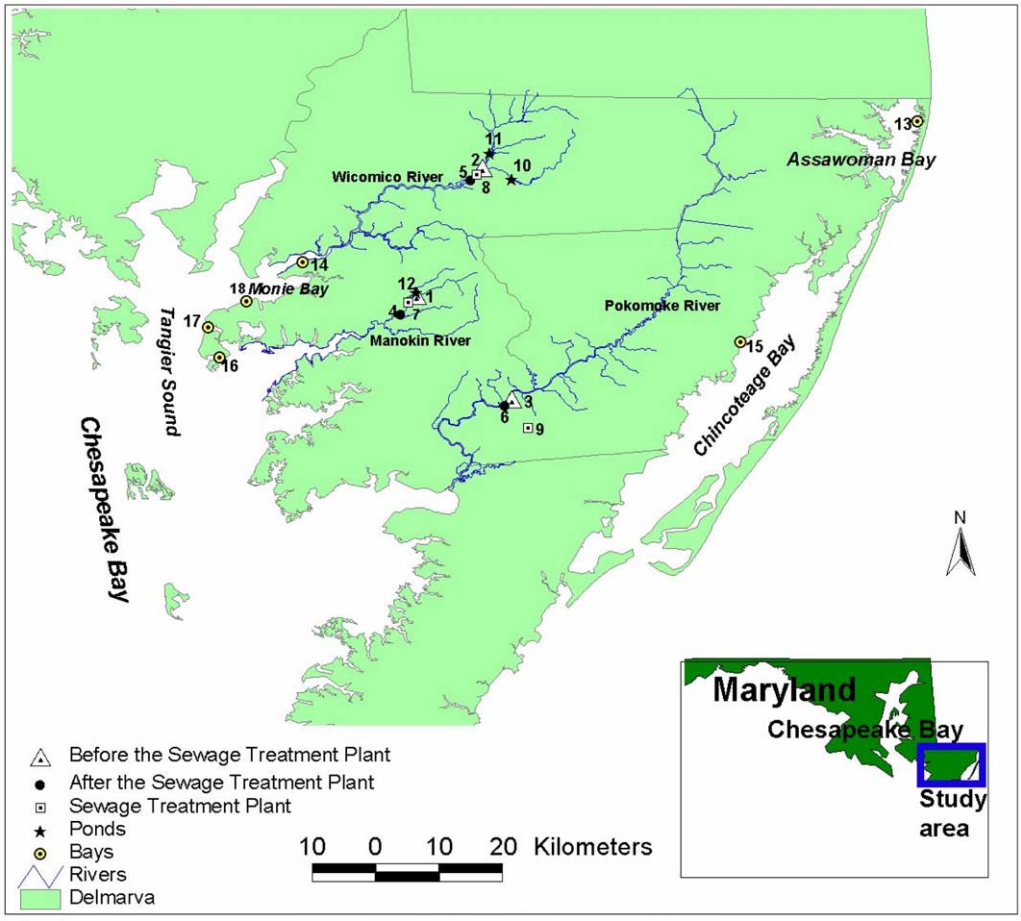

Fig. 1. Eastern Shore of Maryland/Chesapeake Bay study area and sampling sites.

centration of estradiol in the rivers, STPs on these rivers, ponds and Coastal Bays on the Chesapeake Bay Watershed on the Eastern Shore of MD.

Goda et al. [18] have developed an ELISA method for detection of hormone-disrupting chemicals including estradiol. ELISA method for E2 measurements have often been used [19,20]; ELISA and gas chromatograph (GC) methods were correlated ($r = 0.82$). The cross-reactivity of the ELISA kits for E2 is <7% for estrone, 0.3% for estriol and 0.1% for testosterone. Reliable ELISA kits for E1 analyses are not available; therefore E2 is commonly measured.

2. Materials and methods

Grab samples were collected from rivers (Wicomico, Manokin and Pocomoke on the Eastern Shore of MD which are tributaries of the Chesapeake Bay; Fig. 1) at a depth of

Table 1
E2 concentrations (ng l^{-1}) in rivers

Location	Concentration	GPS coordinates
Upstream sewage treatment plant[a]		
Manokin River, Princess Anne, MD (1)[b]	1.9 ± 0.4 a	38°12.305′N 75°41.328′W
Wicomico River, Salisbury, MD (2)	2.0 ± 0.4 a	38°21.861′N 75°36.391′W
Pocomoke River, Pocomoke, MD (3)	2.0 ± 0.9 a	38°04.594′N 75°34.208′W
Downstream sewage treatment plant[c]		
Manokin River, Princess Anne, MD (4)	5.4 ± 0.5 b	38°10.918′N 75°42.436′W
Wicomico River, Salisbury, MD (5)	6.0 ± 0.6 b	38°20.935′N 75°37.248′W
Pocomoke River, Pocomoke, MD (6)	2.3 ± 0.1 c	38°04.088′N 75°34.698′W

Values followed by the same letter are not significantly different from each other ($p \leq 0.05$) according to Tukey's mean comparison test.
[a] Critical value for comparison = 1.01.
[b] Number in parenthesis refers to the sampling site shown in Fig. 1.
[c] Critical value for comparison = 1.66.

Table 2
E2 concentrations (ng l^{-1}) in ponds

Location	Concentration	GPS coordinates
Schumacher Pond, Salisbury, MD (10)[a]	1.7 ± 0.9 m	38°21.092′N 75°34.216′W
Johnson Pond, Salisbury, MD (11)	2.3 ± 0.4 m	38°23.020′N 75°35.869′W
UMES Pond, Princess Anne, MD (12)	7.6 ± 2.1 p	38°12.621′N 75°41.346′W

Critical value for comparison = 1.03. Values followed by the same letter are not significantly different from each other ($p \leq 0.05$) according to Tukey's mean comparison test.
[a] Number in parenthesis refers to the sampling site shown in Fig. 1.

Table 3
E2 concentrations (ng l^{-1}) in sewage treatment plants

Location	Concentration	GPS coordinates
Sewage treatment plant influent[a]		
Princess Anne, MD (7)[b]	60.6 ± 0.7 d	38°11.846′N 75°41.932′W
Salisbury, MD (8)	71.2 ± 1.5 e	38°21.863′N 75°36.390′W
Pocomoke, MD (9)	18.9 ± 2.7 f	38°02.501′N 75°21.710′W
Effluent[c]		
Princess Anne, MD (7)	6.5 ± 0.3 g	38°11.846′N 75°41.932′W
Salisbury, MD (8)	53.1 ± 1.7 h	38°21.863′N 75°36.390′W
Pocomoke, MD (9)	11.4 ± 0.5 k	38°02.501′N 75°21.710′W

Values followed by the same letter are not significantly different from each other ($p \leq 0.05$) according to Tukey's mean comparison test.
[a] Critical value for comparison = 5.24.
[b] Number in parenthesis refers to the sampling site shown in Fig. 1.
[c] Critical value for comparison = 3.8.

1 and 1.6 m from the shore; influent and effluent samples were collected from STPs on these rivers. River water sampling was carried out about 1 km before (upstream) and after (downstream) the effluent outfall of the STPs located on these rivers. Sampling was also done at three ponds, and at Coastal Bays (Monie, Assawoman, Chincoteague, and Tangier Sound) that empty into Chesapeake Bay. Global Positioning System (GPS) coordinates for each of the sampling sites are shown in Tables 1–4; numbers for each site in the map are indicated in each of the tables. Samples were collected in February 2004 between 10:00 a.m. and 2:00 p.m. with no rainfall or high tide events.

In the present study water samples were collected in triplicate from each location and were extracted within 2 h after collection. Suspended particulate matter was removed by filtering through a 1.2 μm (Whatman GF/C) glass fiber filter. Hewlett Packard Preparation System (Model 7686) was used for extraction. The C18 cartridges were conditioned with 10 ml of methanol and then 3 ml of water. Samples (1.8 ml) were injected in to the cartridges. Water in the cartridge was removed by purging with nitrogen for 2 min. E2 was eluted from the cartridge by addition of 0.5 ml methanol repeated three times. The eluent was evaporated to 50 μl at 35 °C under a gentle stream of nitrogen. Extracted E2 was measured using ELISA kits (Cayman Chemical Company, MI, USA) by dissolving in 400 μl of enzyme immuno-assay buffer and the analyses was carried out in a commercial 96-well microtiter plates. A monoclonal antibody, tracer, antiserum, and 50 μl of either standard or extracted sample were added to each well, and incubated at 21 ± 1 °C for 1 h. The wells were washed to remove any unbound reagent and the amount of tracer bound to the immobilized antibodies in the wells was detected by the addition of a substrate [Ellman's Reagent; 5,5-dithio-bis-2-nitrobenzoic acid]. The intensity of the color in each well, determined spectrophotometrically (405 nm), is proportional to the amount of tracer bound to the well. The recovery of E2 (from known standard solutions) was 92%; the detection limit was 0.5 ng l^{-1}. The ELISA kits are specific for free E2; E2 in conjugated form cannot be detected by this analysis. All experiments were replicated thrice. Sta-

Table 4
E2 concentrations (ng l^{-1}) in coastal bays

Location	Concentration	GPS coordinates
Assawoman Bay, Ocean City, MD (13)[a]	2.3 ± 0.3 q	38°25.339′N 75°03.947′W
Monie Bay, Princess Anne, MD (14)	2.3 ± 0.4 q	38°14.880′N 75°49.797′W
Chincoteague Bay, Pocomoke, MD (15)	3.2 ± 0.3 q	38°08.912′N 75°17.148′W
Tangier Sound of Chesapeake Bay (16)	3.2 ± 0.8 q	38°07.801′N 75°56.000′W
Tangier Sound of Chesapeake Bay (17)	2.4 ± 0.3 q	38°10.043′N 75°56.818′W
Tangier Sound of Chesapeake Bay (18)	3.2 ± 1.1 q	38°11.948′N 75°53.980′W

Critical value for comparison = 1.09. Values followed by the same letter are not significantly different from each other ($p \leq 0.05$) according to Tukey's mean comparison test.
[a] Number in parenthesis refers to the sampling site shown in Fig. 1.

tistical analysis was done using Tukey's mean comparison test.

3. Results and discussion

E2 concentration in the waters of the three rivers, up-stream of STPs (Table 1), was 2.0 ng l^{-1}; these concentrations were not significantly different from each other. Shore et al. [14] and Snyder et al. [10] reported E2 concentration in US streams (few up-streams of STPs) between 0.8 and 3.7 ng l^{-1}. Significantly higher E2 concentrations were observed in water samples collected downstream from the STPs on Wicomico and Manokin Rivers compared to the samples from Pocomoke River (Table 1). E2 concentration in Wicomico River water near the river origin (Johnson Pond and Schumacher Pond – Table 2, with no agricultural land run-off) was 1.7–2.3 ng l^{-1}. A few of the older houses near these ponds use septic tanks; also there is a small population (30–40) of ducks and Canada geese (resident and migratory) on these ponds as is the case with many of the ponds on the Eastern Shore of MD. Similar concentrations of steroid estrogens upstream of STPs have been reported [21]; spawning fish can also contribute steroid hormones to surface waters [22]. Using the above mentioned ELISA test we found the amount of E2 (15 ng g^{-1}) in dried Canada geese waste; E2 from such sources can be significant but E2 concentrations can vary due to factors such as dilution, sorption by sediments and organic matter, and photo-degradation [13]. From these data it appears that E2 in Wicomico River water is from the STP effluent. Animal (poultry) waste is spread on land during the warmer season (April) and run-off from agricultural fields is not expected to contribute any E2 at the time water samples were collected for analyses.

E2 concentrations (Table 3) in STP influent (71.2 ng g^{-1}) and effluent (53.1 ng g^{-1}) were highest for the Salisbury, MD plant on Wicomico River, which is considered as one the most polluted rivers (in MD) based on nutrients, metals, micro-organisms and similar pollutant concentrations [23]. This STP has a trickling filter system as secondary waste treatment and receives wastes also from a poultry production facility; this perhaps contributes to the high influent concentration (71.2 ng g^{-1}) of E2 compared to the other two STPs.

The STP in Princess Anne, MD was recently upgraded with nitrogen removal capabilities (aeration and biological nutrient removal) and the effluent from this plant showed the lowest E2 concentration of the three STPs. The STP in Pocomoke, MD imparts only primary treatment with two large lagoons where the wastewater is stored for 70 days and then released to the river after chlorination. (The other two plants also chlorinate their effluents before releasing to the river.) The influent in this plant had significantly lower E2 concentrations compared to E2 concentrations in the other two STPs. Out of an estimated daily flow of 26 million l of wastewater in the Salisbury STP about 19 million l come from the poultry production facility. The daily wastewater flow rate in the other two STPs is close to 2.3 million l each.

E2 concentrations in two of the ponds (Table 2) were not significantly different from each other; Schumacher and Johnson Ponds form the origin of Wicomico River. In the third pond (a closed pond) the high E2 concentration can be attributed to a very large Canada geese population (over 100) and also to the very small size of this pond. E2 concentrations in the waters of all the coastal bays tested (Table 4) were not significantly different from each other and from the water in the nearby tributaries upstream of STPs.

These results show that E2 concentrations in the various surface waters in the Chesapeake Bay watershed on the Eastern Shore of MD are above 1 ng g^{-1}. This small concentration, however, appears to be sufficient to induce estrogenic effects in aquatic organisms [3–12]. There is a need to study the aquatic organisms' health in these waters and for more information on E2 concentrations in STPs influents and effluents, rivers and coastal bays in USA.

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