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Endocrine disruptors in bottled mineral water: Estrogenic activity in the E-Screen☆

Martin Wagner*, Jörg Oehlmann

Department Aquatic Ecotoxicology, Faculty of Biological Sciences, Goethe University Frankfurt am Main, Siesmayerstr. 70 A, D-60054 Frankfurt am Main, Germany

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ABSTRACT

Human exposure to endocrine disruptors is well documented by biomonitoring data. However, this information is limited to few chemicals like bisphenol A or phthalate plasticizers. To account for so-far unidentified endocrine disruptors and potential mixture effects we employ bioassays to detect endocrine activity in foodstuff and consequently characterize the integrated exposure to endocrine active compounds.

Recently, we reported a broad contamination of commercially available bottled water with estrogenic activity and presented evidence for the plastic packaging being a source of this contamination. In continuation of that work, we here compare different sample preparation methods to extract estrogen-like compounds from bottled water. These data demonstrate that inappropriate extraction methods and sample treatment may lead to false-negative results when testing water extracts in bioassays.

Using an optimized sample preparation strategy, we furthermore present data on the estrogenic activity of bottled water from France, Germany, and Italy: eleven of the 18 analyzed water samples (61.1%) induced a significant estrogenic response in a bioassay employing a human carcinoma cell line (MCF7, E-Screen). The relative proliferative effects ranged from 19.8 to 50.2% corresponding to an estrogenic activity of 1.9–12.2 pg estradiol equivalents per liter bottled water.

When comparing water of the same spring that is packed in glass or plastic bottles made of polyethylene terephthalate (PET), estrogenic activity is three times higher in water from plastic bottles. These data support the hypothesis that PET packaging materials are a source of estrogen-like compounds. Furthermore, the findings presented here conform to previous studies and indicate that the contamination of bottled water with endocrine disruptors is a transnational phenomenon.

1. Introduction

In 1991 scientists from diverse disciplines gathered at the Wingspread Conference Center to structure and define the phenomenon of endocrine disruption. They came to the consensus that "a large number of man-made chemicals [...] have the potential to disrupt the endocrine system of animals, including humans" [1]. Since then, research in this multidisciplinary field and thus knowledge about endocrine disruptors is steadily expanding. Many current aspects concerning the effects of endocrine disruptors on different hormonal pathways [Kato, this issue; Watson, this issue; vom Saal, this issue; Baker, this issue; Blumberg, this issue], organs [Prins, this issue; Miyagawa, this issue], individuals [Rubin, this issue], and populations [Woodruff, this issue; Hayes, this issue] are comprehensively documented in this Special Issue of The Journal of Steroid Biochemistry and Molecular Biology.

Characterizing the exposure to endocrine disruptors has been recognized as a crucial aspect for the prediction of actual health effects in the Wingspread Consensus [1]. Two decades later, the Endocrine Society renewed the demand for making the screening for exposures a research priority in its Scientific Statement on endocrine disruptors [2]. However, understanding the complexity of human exposure to man-made chemicals, including endocrine disruptors, is compromised by the overwhelming number of compounds in use and the technical limitations in their detection. Thus, exposure science is forced to focus on few chemicals as proxies for the total exposure, like for example bisphenol A and phthalates for which excellent biomonitoring data are available [3,4]. Whether these compounds adequately represent the total exposure and consequently the total toxicity remains, nevertheless, questionable in

Abbreviations: b.d.l., below detection limit; DMEM, Dulbecco's modified Eagle's medium; DMSO, dimethyl sulfoxide; EEQ, estradiol equivalents; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; MCF7, Michigan Cancer Foundation cell line 7; PET, polyethylene terephthalate; PTFE, polytetrafluoroethylene; RPE, relative proliferative effect; SPE, solid phase extraction; YES, Yeast Estrogen Screen.

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* Corresponding author. Tel.: +49 69 798 24900; fax: +49 69 798 24748.
E-mail address: wagner@bio.uni-frankfurt.de (M. Wagner).

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face of a "universe of toxicants" as Daughton phrases it [5]. In this respect, recent advances in mixture toxicity contribute to a more holistic appraisal of the effect assessment in toxicology [6–8]. For exposure assessment on the other hand, that same holistic view is desirable but obstructed by the limitation of analytical tools to elucidate the entire chemical universe including an unknown number of yet-to-be identified compounds.

Bioanalytical techniques (i.e. bioassays) can help to overcome this shortcoming because they characterize the actual biological effect of a complex sample and thus integrate the effects of unidentified compounds and potential mixtures. Ecotoxicology takes advantage of that by routinely employing in vitro bioassays to assess the endocrine activity in environmental samples (e.g. effluents from sewage treatment). In human toxicology this practice is far less common, and only scarce data is available for endocrine activity of human matrices [9–13] or foodstuff as main route of exposure [14–16].

In this context, we focus on bottled mineral water and characterize its total estrogenic burden using bioassays. Since limited in vitro evidence for the presence of estrogen-like chemicals in bottled water is available [17–19], the present study aims to provide additional data on that issue. In our previous study, we employed a yeast-based bioassay (Yeast Estrogen Screen, YES) to determine the estrogenicity of bottled water. Therefore, we decided to reassess our findings using an additional bioassay that is based on a human cancer cell line (MCF7, E-Screen). Developing and employing an optimized sample preparation method, we here report a broad contamination of commercially available bottled water with estrogen-like compounds. When comparing water from the same spring that was packed in glass or plastic bottles, estrogenicity was significantly higher in samples from PET bottles. This corroborates our hypothesis that the plastic packaging is one source of so-far unidentified endocrine disruptors in bottled water.

2. Materials and methods

2.1. Samples

Bottled mineral water was purchased at local retailer stores. In total, the analyzed water samples comprised 18 products (coded as samples 1 to 18) from 13 different companies, including water from five bottlers that was packed in glass and plastic bottles made of PET (samples 1 + 2, 3 + 4, 5 + 6, 7 + 8, 9 + 10). With the exception of one so-called table water (bottled tap water), the products are marketed as so-called mineral water. These products originate from natural springs and are not processed or altered beyond deferrization. The springs of the products are located in different geographic regions in France, Germany, and Italy. Of each product, a sufficient number of bottles from the same lot (n = 10–12) was purchased and stored at 4 °C prior to analysis.

2.2. Optimization strategy for sample preparation

Sample preparation methods, like the extraction of water samples by solid phase extraction (SPE), are normally optimized for the analytical detection of specific chemicals. In case of bioassays, that also include effects of unknown compounds and mixtures, an adaptation of those methods is needed. Here, we apply a tiered approach to develop an optimized sample preparation procedure by comparing different methods of (1) sample treatment and (2) solid phase extraction and (3) apply the optimized procedure to a broader range of samples. The first two steps were carried out with tap water as procedural blank and one bottled water (sample 18) that has been repeatedly shown to be estrogenic in previous experiments.

2.3. Sample treatment

Evaporation of sample extracts is a common procedure to reduce the extract volume or exchange a solvent. In in vitro bioassays extracts are often evaporated directly on the microtiter plates to eliminate a particular solvent. To investigate whether evaporation of extracts during sample preparation results in a loss of estrogenic activity, we extracted tap water and bottled water (sample 18) via SPE using reversed phase C18 columns (C18-HD, 24 mg, 3 M, St. Paul, MN). The SPE columns were conditioned twice with 4 mL acetone and equilibrated twice with 4 mL tap water. 1.5 L water sample was loaded on each column using a vacuum manifold and a maximum flow rate of 12 mL/min. One set of columns containing tap water or bottled water was dried under a gentle stream of nitrogen for 30 min. After that, these columns were eluted with 4 mL acetone, and extracts were evaporated to dryness under nitrogen and redissolved in 100 μL dimethyl sulfoxide (DMSO, method A). From the second set of columns residual water was removed by applying vacuum for 1 min (method B). Compared to method A, 100 μL DMSO was added to the extracts before evaporation. Due to its high melting point DMSO functions as a so-called keeper that retains volatile compounds during evaporation [20]. Again, nitrogen was used to remove acetone yielding residual extracts in 100 μL DMSO. All extracts were stored in glass vials with PTFE caps at −20 °C prior to analysis in the E-Screen.

2.4. Comparison of different solid phase extraction methods

In the next step of optimization six different SPE sorbents were compared. In addition to the silica phase (C18) described above, copolymer sorbents were used because of their higher capacity and selectivity for polar compounds. These SPE sorbents include the copolymers N-vinylpyrrolidone-divinylbenzene (Oasis HLB, 200 mg, Waters, Milford, MA) as well as styrene-divinylbenzene (Bakerbond SDB[1], 200 mg, J.T. Baker, Deventer, Netherlands; SDB[XC], 15 mg, 3 M, St. Paul, MN) and its hydroxylated form (Isolute ENV+, 200 mg, Biotage, Uppsala, Sweden). The sixth sorbent consists of an amorphous carbon molecular sieve (ENVI-Carb Plus, 400 mg, Supelco, Bellefonte, PA) that is optimal for the enrichment of highly polar compounds from water samples.

The general procedure for solid phase extraction of tap and bottled water was performed with the six different sorbents as described above. Each sorbent was conditioned according to the manufacturer's recommendation: C18 (2 × 4 mL acetone, 2 × 4 mL tap water), HLB/ENV+/SDB[1]/Carb (2 × 4 mL methanol, 2 × 4 mL tap water), and SDB[XC] (4 mL 1:1 ethyl acetate:methylene chloride, 2 × 4 mL methanol, 2 × 4 mL tap water). 1.5 L tap water or bottled water was applied to each column. In case of bottled water (sample 18) the content of twelve individual bottles was mixed in equal parts to create one uniform sample for all extraction procedures. Columns were shortly dried under vacuum and eluted with 4 mL acetone (C18) or 4 mL methanol (all other sorbents). The resulting sample extracts (containing 100 μL DMSO as keeper) were concentrated under nitrogen and kept in glass vials with PTFE caps (−20 °C) prior to analysis in the E-Screen.

2.5. Optimized sample preparation procedure

Based of the previous experiments, an optimized method was used to extract a broader spectrum of bottled water. 1.5 L of 18 different products were degassed in an ultrasonic bath. C18 columns were conditioned with 2 × 4 mL acetone and 2 × 4 mL tap water. Water samples were drawn through the columns with a flow rate of 12 mL/min and directly eluted with 4 mL acetone and 4 mL methanol consecutively in glass vials containing 50 μL DMSO. Ace-