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Short Communication

Occurrence of glucocorticogenic activity in various surface waters in The Netherlands

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HIGHLIGHTS

- Glucocorticogenic compounds are emerging contaminants in the environment.
- A powerful bioassay is available to measure glucocorticogenic effects in the field.
- The effects of synthetic glucocorticoids on aquatic biota and human health requires further attention.

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ABSTRACT

Considering the important role that surface waters serve for drinking water production, it is important to know if these resources are under the impact of contaminants. Apart from environmental pollutants such as pesticides, compounds such as (xeno)estrogens have received al lot of research attention and several large monitoring campaigns have been carried out to assess estrogenic contamination in the aquatic environment. The introduction of novel *in vitro* bioassays enables researchers to study if – and to what extent – water bodies are under the impact of less-studied (synthetic) hormone active compounds. The aim of the present study was to carry out an assessment on the presence and extent of glucocorticogenic activity in Dutch surface waters that serve as sources for drinking water production. The results show glucocorticogenic activity in the range of <LOD – 2.4 ng dexamethasone equivalents L^{-1} (dex EQs) in four out of eight surface waters. An exploratory time-series study to obtain a more complete picture of the yearly average of fluctuating glucocorticogenic activities at two sample locations demonstrated glucocorticogenic activities ranging between <LOD – 2.7 ng dex EQs L^{-1}. Although immediate human health effects are unlikely, the environmental presence of glucocorticogenic compounds in the ng L^{-1} range compels further environmental research and assessment.

1. Introduction

In the Netherlands about 40% of 1300 million m^3 (drinking)water is produced from surface waters (Versteegh and Dik, 2009) and this illustrates the importance of understanding by which pollutants major bodies of surface water such as the rivers Rhine and Meuse are contaminated. Apart from the mandatory monitoring of standard (in)organic contaminants as laid down in the Dutch drinking water act (URL1), the Dutch drinking water companies take a pro-active role in the detection of emerging (unknown) contaminants in their source waters. Within this framework, the presence of estrogenic compounds in drinking water and its environmental sources has received a lot of attention from international research groups (Belfroid et al., 1999; Bogers et al., 2007; Snyder et al., 2008; Benotti et al., 2009) as it is known that such compounds adversely impact human and wildlife/ecosystem health (Sumpter, 1998, 2009; Safe et al., 2001; Kidd et al., 2007). In addition, the Dutch government initiated a broad national investigation into the occurrence and effects of estrogenic compounds in the aquatic environment which took place in 1999–2002 (Vethaak et al., 2005, 2006). However, apart from estrogenic compounds that interfere with the hypothalic-pituitary-gonadal axis, the impact of xenobiotics on other hormonal endpoints may be of equal importance. The availability of novel technologies such as sensitive reporter gene bioassays, allows investigating the impact of chemicals and/or water extracts on multiple hormonal endpoints such as demonstrated in our earlier

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study (Van der Linden et al., 2008). The latter study was aimed at examining the utility of a panel of four CALUX reporter gene bioassays specific for androgen, estrogen, progestagen and glucocorticoid induced hormonal activity in a number of wastewater effluents in The Netherlands. An unexpected finding of the study was the observed glucocorticogenic activities found in all samples. In our follow-up high resolution LC mass spectrometric analysis in a selection of wastewaters, we revealed the presence of potent glucocorticoids such as cortisol (max. 301 ng dex EQs L^{-1}), cortisone (max. 472 ng dex EQs L^{-1}), prednisone (max. 545 ng dex EQs L^{-1}), prednisolone (max. 1918 ng dex EQs L^{-1}) and triamcinolone acetonide (max. 41 ng dex EQs L^{-1}) which are used to treat a great number of human pathologies (Schriks et al., 2010). Besides, glucocorticoids are important in regulating a number of physiological functions that enable stress response and resistance (Munck et al., 1984). The presence of hormone active compounds such as glucocorticoids in wastewaters raises the question to what extent receiving surface waters – that serve as sources for drinking water production – are impacted by such compounds. The objective of the present study was therefore to carry out an exploratory study into the presence and extent glucocorticogenic activity in Dutch surface waters that serve as sources for drinking water abstraction. To this end, eight drinking water abstraction sites in The Netherlands were sampled in spring, summer and autumn in 2007. In addition, temporal variation in hormonal activities were obtained in the period August 2007 to August 2008 at two important locations in the Dutch part of the Rhine basin, namely the river Rhine at the Dutch–German border and the hydrologically connected river Lekkanaal which serves as a major drinking water abstraction site.

2. Materials and methods

2.1. Sample collection, treatment and extraction

Surface water samples were collected from eight different drinking water abstraction sites (Table 1). All locations were sampled once in May, August and November 2007, except for Loenen which was only sampled in August and November 2007 and Nieuwegein which was sampled once in May 2007 and subsequently twice a month from August 2007 through July 2008. Additionally, in May 2007 surface water from sample location Lobith was collected once and subsequently twice a month from August 2007 through July 2008.

At the day of sampling, surface water samples were collected by immersion of 1 L ultra-cleaned dark glass bottles approximately 25 cm below the water surface. After collection, the samples were immediately stored at 4 °C until extraction, which followed within 48 h. To prevent any contamination during sample treatment, all glassware was extensively washed with distilled acetone followed by petroleum ether and dried under ambient conditions. Prior to sample extraction, glass Oasis (200 mg) Hydrophilic–Lipophilic Balance (HLB) solid phase extraction (SPE) columns (Waters, Netherlands) were conditioned twice with ethyl acetate (Baker, Netherlands) followed by conditioning with methanol (once) and Evian mineral water (twice). A glass filtration column filled with ignited sea sand (Mallinckrodt Baker, Netherlands) was placed on top of the extraction column and the samples (1 L) were isolated at a flow rate of approximately 10 mL min^{-1}. After drying, the column was eluted three times with 2.5 mL of ethyl acetate. The ethyl acetate fraction was transferred into a glass tube and subsequently evaporated at 56 °C under a gentle stream of nitrogen to a volume of approximately 3 μL. The last microliters were left to evaporate spontaneously and the extracts were redissolved in 50 μL of DMSO (Acros, Belgium). All extracts were stored at −18 °C until further analysis. Evian mineral water from glass bottles was used as a procedural blank.

2.2. CALUX bioassays

The CALUX bioassays were carried out as described before (Sonneveld et al., 2005; Van der Linden et al., 2008; Schriks et al., 2010). Briefly, human U2OS osteosarcoma cells (stably transfected with a luciferase gene under transcriptional control of response elements for activated hormone receptors) were seeded into 96 well plates with DF medium (without phenol red and supplemented with dextran coated charcoal stripped serum). After 24 h of incubation (37 °C, 7.5% CO_2), the medium was replaced by medium containing sample extracts (max. 0.4% DMSO) for activity testing. After 24 h of exposure in triplicate, the medium was removed and the cells were lysed in 30 μL of Triton-lysis buffer. The amount of luciferase activity was quantified using a luminometer (Lucy 2, Anthos, Austria). Data analysis was carried out as described by Van der Linden et al. (2008). Briefly, dilution series of water extracts in DMSO were analyzed in triplicate. On all plates, a concentration response curve of the respective reference compound was included for adequate quantitative expression of the observed response into nanograms reference compound equivalents per liter of sample (ng EQs L^{-1}). The concentration response curves were modeled using a sigmoidal fit with variable hill slope (log(agonist vs. response – variable slope)) in Graphpad Prism 5. The method limit of detection (LOD) was derived from the dexamethasone concentration response curve and equaled 0.4 ng dexamethasone equivalents per liter of surface water.

Table 1
Characteristics of the sample locations.

Sample location	Surface water	Code	Main feeding water supply	Drinking water abstraction volume (million m^3 y^{-1})[a]	Drinking water supply area in The Netherlands
Andijk	IJsselmeer (lake)	AND	River Rhine	25	North Holland (province)
Brakel	Afgedamde Maas (river)	BRA	River Meuse	75	The Hague (city)
De Punt	Drentsche Aa (creek)	PUN	Creek Drentsche Aa	5	Groningen (province)
Heel	Lateraal kanaal (canal)	HEE	River Meuse	15	Limburg (province)
Keizersveer	Meuse (river)	KEI	River Meuse	90	Rotterdam (city)
Lobith	Rhine (river)	LOB	River Rhine	*NA*	Monitoring station at the Dutch-German border
Loenen	Amsterdam-Rijnkanaal (canal)	LOE	River Rhine	*NA*	Monitoring station for drinking water supply of Amsterdam (city)
Nieuwegein	Lekkanaal (canal)	NIE	River Rhine	95	Amsterdam (city)
Ouddorp	Haringvliet (estuary)	OUD	River Rhine (~75%) and river Meuse (25%)	7	Zeeland (province)

NA – Not Applicable.
[a] Data derived from the REWAB data set (restricted water-quality data from the Dutch water companies).