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Evaluation of estrogenic and antiestrogenic activity in sludge and explanation of individual compound contributions

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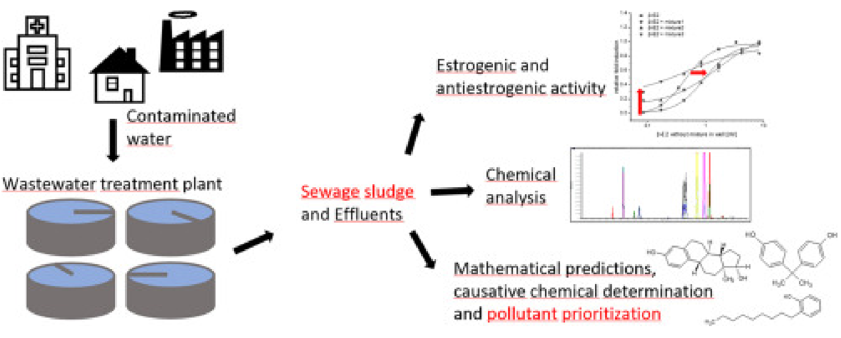
Abstract

Mixture toxicity, including agonistic and antagonistic effects, is an unrevealed environmental problem. Estrogenic endocrine disruptors are known to cause adverse effects for aquatic biota, but causative chemicals and their contributions to the total activity in sewage sludge remain unknown. Therefore, advanced analytical methods, a yeast bioassay and mixture toxicity models were concurrently applied for the characterization of 8 selected sludges with delectable estrogenic activity (and 3 sludges with no activity as blanks) out of 25 samples from wastewater treatment plants (WWTPs). The first applied full logistic model adequately explained total activity by considering the concentrations of the monitored compounds. The results showed that the activity was primarily caused by natural estrogens in municipal WWTP sludge. Nevertheless, activity in a sample originating from a car-wash facility was dominantly caused by partial agonists – nonylphenols – and only a model enabling prediction of all dose-response curve parameters of the final mixture curve explained these results. Antiestrogenic effects were negligible, and effect-directed analysis identified the causative chemicals.

Graphical Abstract



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Introduction

A broad spectrum of toxic compounds is present in the environment and may pose a risk to biota (Bradley et al., 2017). Endocrine disruption in the water environment is a broadly discussed topic; in particular, estrogenic activity is one of the most studied and most frequent disruptions among hormonal activities in wastewater treatment plant (WWTP) effluents (Itzel et al., 2019, Kakaley et al., 2020, Leusch et al., 2018, Pedrazzani et al., 2019). However, estrogenic activity can be detected in various parts of the environment, and research should not be focused only on WWTP effluents (Conley et al., 2017a). Apart from effluents, thousands of tons of treated sludge are generated annually in WWTPs and applied to cropland in many European countries to amend soil properties (Seleiman et al., 2020). Sludge is known for containing pollutants such as pharmaceuticals and steroids that differ in polarity and other properties determining their persistence, partitioning, etc. (Verlicchi and Zambello, 2015).

Estrogenic steroid hormones are often detected in aquatic samples, and at the same time, they are present in sediment and biosolids (Lee et al., 2003, Yost et al., 2014). They migrate in the soil (Alves et al., 2020) and are transformed to more or less active metabolites therein (Mashtare et al., 2013). Furthermore, chemicals from biosolids can enter pore water and leach or run off to ground or surface water (Seleiman et al., 2020, Verlicchi and Zambello, 2015, Yang et al., 2021a, Yang et al., 2021b). Due to steroid sorption onto the soil, slow leaching can be expected at low concentrations. Desorption is the crucial factor for further water contamination (Andaluri et al., 2012). For instance, the estrogenic biomolecules equol (EQ) and estrone (E1) can persist in soil for more than 2 months after application of sludge (Caron et al., 2010, Yang et al., 2021a, Yang et al., 2021b, Yost et al., 2014). For these reasons, emphasis should also be given to the characterization of sludge as a source of endocrine disruptors in the environment.

Every environmental sample should be analyzed as an unknown mixture of toxic compounds to obtain a comprehensive picture of environmental contamination (Petrie et al., 2015). Moreover, the toxic effect has to be analyzed in a context. *In vitro* and in vivo experiments suggest that the total toxic effect arises from a balance of agonistic and antagonistic activities of mixture constituents (Ihara et al., 2014, Ihara et al., 2015, Pannekens et al., 2019). However, the risk of a specific endpoint can be mainly driven by a few chemicals that need to be identified (Pedrazzani et al., 2019). While endogenous steroid hormones directly mediating the estrogen receptor (ER) are usually considered to cause most of the hormonal activity in WWTP effluents (Avbersek et al., 2011, Konemann et al., 2018); to our knowledge, the possible individual contributors to total estrogenic activity in sludge from municipal WWTPs have not been fully described. Sludge samples were studied only in anaerobic lagoons receiving waste from animal feeding operations (AFOs) (Yost et al., 2013, Yost et al., 2014) or to identify the contribution of bisphenols to overall estrogenic activity in municipal WWTPs (Ruan et al., 2015, Yu et al., 2015).

There are several approaches allowing evaluation of either a single bioactive compound or the toxic effect of a complex mixture. The mixture effect can be predicted based on the concentrations and relative potencies of single compounds. For this purpose, mathematical models for a mixture of toxicants acting through the same mode of action are mainly utilized (Altenburger et al., 2018, De Zwart and Posthuma, 2005). Concentration addition (CA)-based models, such as the toxic equivalent (TEQ) model, are mostly used for environmental samples (Balsiger et al., 2010, Ihara et al., 2015, Neale et al., 2017, Valitalo et al., 2016). Nevertheless, the TEQ model is unable to correctly include partial agonists because only the concentration (dose) and the effective concentration (EC), but not the maximum effect, of an individual substance and the slope of the dose-response curve are used in the model. However, some new and more accurate models (Schlotz et al., 2017) have been published in recent years. The generalized concentration addition model (GCA) represents a certain improvement as it extends its applicability to partial agonists; nevertheless, the slope parameter of the inverse Hill function is fixed to 1 (Howard and Webster, 2009). A model published by Schindler (2017) assumes that a mixture of chemicals with individual dose-response curves can be described by an n-dimensional logistic function. This model provides similarly reliable results as the full logistic model (FLM) used in our current study (Ezechias and Cajthaml, 2016). The FLM and Schindler´s model are similar, they can both calculate the mixture effects from the individual curves with any shape or maximum covering other dose–response curve parameters.It is noteworthy that discrepancies between the predicted toxicity based on chemical analysis and measured bioassay-based toxicity were described in some studies in which the models were applied to environmental samples. The suggested sources of this discrepancy are high limits of detection (LODs) in chemical analyses (Valitalo et al., 2016), the antagonistic activity of mixture constituents (Weiss et al., 2009) or the presence of unknown chemicals in the mixture (Conley et al., 2017b). Consequently, effect-directed analysis (EDA) has been considered a suitable approach for the identification of new pollutants in mixtures in recent years. Key steps of this approach consist of sample fractionation, further bioassay measurement and nontarget chemical analysis of the individual fractions (Brack et al., 2016, Hashmi et al., 2018, Hollert et al., 2005, Kim et al., 2019).

The aim of this study was to evaluate the contributions of estrogenic compounds to the overall activity in WWTP sludge samples with respect to a balance between agonistic and antagonistic activity. For this purpose, 25 WWTPs were monitored for estrogenic activity. Selected municipal WWTP sludges were comprehensively analyzed using a target chemical analysis, and estrogenic and antiestrogenic activities were also determined. To evaluate the contribution of causative substances to estrogenic activity, the analytical data were processed using FLM, which enables precise modeling and prediction of mixture toxicity. Values of 17β-estradiol (β-E2) equivalent (EEQ) were predicted based on chemical analysis (chemEEQ) and compared with the EEQ results obtained with the bioassay (bioEEQ). Additionally, compounds present at levels below their respective analytical LODs were also considered by calculating possible maximum adverse effects of the undetected analytes (lodEEQ). EDA was applied to reveal any masking effects and nontarget analytes.

Section snippets

Analytical standards

Bis(2-ethylhexyl) phthalate (DEHP, 99.7%), bisphenol A (BPA; 99+%), bisphenol F (BPF; ≥98%), bisphenol S (BPS; ≥98%), coumarin (CMR, n.a.), diethyl phthalate (DEP, 99.5%), diisobutyl phthalate (DIBP, 99%), di-n-butyl phthalate (DBP, ≥99%), di-n-octyl phthalate (DnOP, 99%), equilin (EQN; ≥98%), estriol (E3; ≥97%), E1 (≥99%), 17α-ethinylestradiol (EE2, 99.4%), β-E2 (≥98%), 1,2-hydroxy-naphtalene (1,2-OH-NAP, technical grade), 1,3-hydroxy-naphtalene (1,3-OH-NAP, ≥99%), 1-hydroxy-pyrene (OH-PYR,…

Pure compounds and their estrogenic activity

A group of 45 organic pollutants suspected of estrogenic activity (Blair et al., 2000, Hasenbrink et al., 2006, Ruan et al., 2015, Sievers et al., 2013, Witorsch, 2014) and recommended by legislation (European Union, 2018/840; Drinking Water Contaminant Candidate List by US EPA, 2016) was selected, and all compounds were tested for their potential estrogenic activity with the bioluminescence yeast assay. Only 14 compounds out of this group were found to be relevantly estrogenic (Fig. 1) using…

Conclusion

The results of this study revealed several important aspects that should be considered when evaluating the hormonal activities of real samples containing a complex matrix. Notably, this is the first study employing an advanced mixture toxicity model (FLM) to evaluate the estrogenic activity in WWTP sludge that can cause secondary pollution, especially when applied as fertilizers. The results revealed that estrogenic activity in typical municipal WWTP sludges originating from the Czech Republic…

CRediT authorship contribution statement

Tereza Černá: Conceptualization, Methodology, Formal analysis, and Writing – review & editing. **Martin Ezechiáš:** Methodology and Data Interpretation. **Jaroslav Semerád:** Formal analysis and Methodology. **Alena Grasserová:** Methodology. **Tomáš Cajthaml:** Supervision, Conceptualization, and Writing – review & editing, Funding acquisition.…

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.…

Acknowledgements

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Toxicol. Vitr. (2018)

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Toxicology (2016)

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Endocrine disrupting activity in sewage sludge: Screening method, microbial succession and cost-effective strategy for detoxification
2023, Journal of Environmental Management

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2023, Chemosphere

> *Citation Excerpt :*
> …Archer et al. (2020) also observed antiestrogenic activity in effluents from South Africa, which might have masked the agonist effect in the YES assay. However, Černá et al. (2022) found negligible antiestrogenic activity in WWTP sludges from Czech Republic, although it was attributed to non-competitive antagonists. Finally, Westlund and Yargeau (2017) concluded that pesticides may impact the evaluation of (anti)estrogenic and (anti)androgenic activities of WWTP influents and effluents with yeast bioassays.…

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Citation Excerpt :

…To date, a systematic procedure for separation and purification is still in great need due to the complexity and variety of biological samples. Apart from water and biological samples, EDA studies have also been conducted on other samples including soil [122–124], sludge [125,126], paper [127,128], plastics [129,130], coals [131] and beers [132], which are more directly related to anthropogenic activities. The sampling and sample preparation of recent EDA studies of these samples are briefly summarized in Table 3.…

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Citation Excerpt :

…A common method for analysing a mixture dose-response curve and expressing the overall toxicity of an environmental sample is to compare the measured effect elicited by the extract with the effect caused by the natural ligand. In the case of oestrogenic assays, the 17β-estradiol equivalent concentration (EEQ) is widely used (Argolo et al., 2021; Černá et al., 2022; Escher et al., 2011; Rutishauser et al., 2004; Snyder et al., 2001; Zhao et al., 2015). The EEQ represents a concentration of 17β-estradiol that would elicit the same effect as does the tested sample.…

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