Environment International 81 (2015) 26–44

Contents lists available at ScienceDirect

Environment International

journal homepage: www.elsevier.com/locate/envint

Review

Phytoestrogens and mycoestrogens in surface waters — Their sources, occurrence, and potential contribution to estrogenic activity

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ARTICLE INFO

Article history:
Received 7 September 2014
Received in revised form 27 March 2015
Accepted 31 March 2015
Available online xxxx

Keywords:
Phytoestrogen
Mycoestrogen
Estrogenicity
Sterol
Phytosterols
Flavonoids
Surface waters
Estrogenic equivalent
Relative potency

ABSTRACT

This review discusses the potential contribution of phytoestrogens and mycoestrogens to *in vitro* estrogenic activities occurring in surface waters and *in vivo* estrogenic effects in fish. Main types, sources, and pathways of entry into aquatic environment of these detected compounds were summarized. Reviewed concentrations of phyto/mycoestrogens in surface waters were mostly undetectable or in low ng/L ranges, but exceeded tens of μg/L for the flavonoids biochanin A, daidzein and genistein at some sites. While a few phytosterols were reported to occur at relatively high concentrations in surface waters, information about their potencies in *in vitro* systems is very limited, and contradictory in some cases. The relative estrogenic activities of compounds (compared to standard estrogen 17β-estradiol) by various *in vitro* assays were included, and found to differ by orders of magnitude. These potencies were used to estimate total potential estrogenic activities based on chemical analyses of phyto/mycoestrogens. *In vivo* effective concentrations of waterborne phyto/mycoestrogens were available only for biochanin A, daidzein, formononetin, genistein, equol, sitosterol, and zearalenone. The lowest observable effect concentrations *in vivo* were reported for the mycoestrogen zearalenone. This compound and especially its metabolites also elicited the highest *in vitro* estrogenic potencies. Despite the limited information available, the review documents low contribution of phyto/mycoestrogens to estrogenic activity in vast majority of surface waters, but significant contribution to *in vitro* responses and potentially also to *in vivo* effects in areas with high concentrations.

Contents

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http://dx.doi.org/10.1016/j.envint.2015.03.019
0160-4120/

1. Introduction

Fish feminization downstream of wastewater treatment plants (WWTPs) as well as some other endocrine-disruptive effects on aquatic organisms have been observed worldwide (WHO and UNEP, 2013). Estrogenic compounds have been shown to play an important role in these effects (Desbrow et al., 1998; Sumpter and Johnson, 2008). *In vitro* assays evaluating estrogenic activity are widely used nowadays to monitor a variety of environmental waters (e.g., Kinnberg, 2003; Leusch et al., 2010). Compared to *in vivo* studies and instrumental analyses of estrogenic compounds, *in vitro* assays are more often used for large-scale monitoring as some have relatively lower cost and higher throughput capacity (Leusch et al., 2010; Liu et al., 2010). The results of *in vitro* assays reveal the overall estrogenic activity (estrogenicity) of tested samples and are expressed as equivalent concentrations of 17β-estradiol (E2) which would cause the same response as the samples (Estrogenic Equivalents, EEQs). However, the estrogenic potencies of various compounds relative to E2 (i.e., the sensitivity of a particular assay toward a particular estrogen) often differ among bioassays. As a result, the measured values of EEQ in environmental samples can differ greatly among different bioassays (Jarosova et al., 2014; Liu et al., 2010).

In vitro estrogenic activity downstream of municipal WWTPs and animal farms can usually be explained by human/animal-excreted steroid estrogens (Sumpter and Johnson, 2008). Alkylphenolic compounds have been identified as major contributors to the estrogenic activity of industrial waste waters (e.g., Johnson et al., 2005). Much less information is available on compounds responsible for estrogenic activity in surface waters at greater distances from WWTP discharges or in headwaters above any WWTP discharges. Moreover, many studies, especially in freshwaters, document that the known man-made xenoestrogens or human/animal-excreted steroid estrogens can explain only a proportion of the measured estrogenic activities (e.g., Fernandez et al., 2007; Sun et al., 2008; Jarosova et al., 2012). Several studies suggested that phyto/mycoestrogens could contribute to the detected activities (Liu et al., 2010). For example, genistein, an isoflavone of plant origin, was identified as the main estrogenic chemical in the Kanzaki River in Japan (Kawanishi et al., 2004).

Phytoestrogens can be defined as any plant compounds structurally and/or functionally similar to ovarian and placental estrogens and their active metabolites (Whitten and Patisaul, 2001). They are usually synthesized by plants as metabolites for protection against pathogens and herbivores (Kiparissis et al., 2001), and they also contribute to floral coloration (Clotfelter and Rodriguez, 2006). Phytoestrogens have attracted considerable attention since 1940, when infertility in sheep grazing on pastures rich in subterranean clover (*Trifolium subterraneum*) in Western Australia was observed (Bennetts et al., 1946). Consequently, a number of phytoestrogens present in plants serving as food for animals and humans have been studied (Murkies et al., 1998; Jefferson et al., 2012).

Mycoestrogens are estrogens produced as secondary metabolites of fungi. The well known mycoestrogens are zearalenone (ZEN) and its metabolites primarily produced by the mold *Fusarium* growing on a variety of crops (e.g., Massart and Saggese, 2010; Metzler et al., 2010). Similarly to phytoestrogens, influence of exposure to mycoestrogens on human/mammalian health via food has been extensively studied (e.g., Massart and Saggese, 2010). In contrast, this study focuses on phyto/mycoestrogens in surface waters and their possible impacts in aquatic environment. Human exposure by this source is negligible compared to exposure by food.

The main aim of this paper was to review available information on phyto/mycoestrogens in fresh waters in order to evaluate whether these compounds can substantially contribute to *in vitro* estrogenic activity and to *in vivo* effects in surface waters. To address these questions, the relative potencies of a wide range of phytoestrogens and mycoestrogens in a large number of *in vitro* systems were examined. Furthermore, *in vivo* effective concentrations of waterborne phyto/mycoestrogens were reviewed. Knowledge on the concentrations of these compounds detected in surface waters was summarized. The potential contribution of phyto/mycoestrogens to the total estrogenic activity found in surface waters was estimated on the basis of the summarized information.

2. Main types and sources of phyto/mycoestrogens

2.1. Main types of phyto/mycoestrogens

Most known phytoestrogens belong to the flavonoid chemical group, which is structurally characterized by substituted phenols (at least diphenols) and contains several subclasses (Dixon, 2004). The most well-known subclasses are isoflavones and coumestans (Bacaloni et al., 2005). In the present paper, steroidal flavonoids are classified within another group of phytoestrogens — phytosterols. Lignans comprise another main group of phytoestrogens. Plant lignans are polyphenolic substances derived from phenylalanine (Dixon, 2004). The only so far identified mycoestrogens are ZEN and its metabolites and these are resorcyclic acid lactones (Bucheli et al., 2008; Bakos et al., 2013). These compounds belong within a structurally diverse class of mycotoxins (Murkies et al., 1998). The chemical structures of the most well-known phytoestrogens and mycoestrogens occurring in environmental waters are presented in Fig. 1. In contrast to the abovementioned classification of phytoestrogens, some other authors (e.g., Dixon, 2004; Erbs et al., 2007) define phytoestrogens in a narrower way as only nonsteroidal polyphenols, and, therefore, phytosterols are sometimes considered as a separate group of compounds.

2.2. Primary sources of phyto/mycoestrogens

Phytoestrogens occur in numerous plants, including edible species, at rather high concentrations (Liggins et al., 2000; Dixon, 2004). Various types of phytoestrogens can occur in the same plant species and their contents usually differ among different parts of plants (Rochester and Millam, 2009). Here, examples of plants containing high amounts of phytoestrogens are presented. Flavonoids such as genistein, daidzein, and glycitein, and their glycosylated forms, genistin, daidzin, and glycitin, and partially also biochanin A and formononetin have been found at high concentrations in soybean products. Coumestans, biochanin A, and formononetin have been detected mainly in clover (Hoerger et al., 2009; Jefferson et al., 2012). Other plants containing flavonoids are most species of legumes, fruits, and cabbages and some heartwoods of tree species (Murkies et al., 1998; Dixon, 2004; Bacaloni et al., 2005). Phytosterols are plant lipids and therefore mostly occur in plant oils, such as soy, palm, chestnut, and sesame oils (Clotfelter and Rodriguez, 2006). Some phytosterols, particularly sitosterol, have also been found in pulp mill effluents at levels that were shown to cause effects on biota (Mattsson et al., 2001; Leusch and MacLatchy, 2003). Lignans occur in edible plants (i.e., in vegetables such as legumes, fruits, berries, cereals, nuts, and oilseeds) as well as in tree species (Smeds et al., 2007). Their richest food sources are flax seeds and sesame seeds (Smeds et al., 2007). In the intestine, plant lignans are known to be converted by intestinal microbiota to enterolignans, mainly enterodiol and enterolactone (Smeds et al., 2007; Rochester and Millam, 2009).

Other sources of phytoestrogens to aquatic ecosystems could be algae, cyanobacteria or aquatic macrophytes forming high biomass in water bodies or some less investigated plants naturally occurring close to surface waters, which contribute plant material into water. However, there is very limited information on the importance of these potential sources. Some marine macroalgae are currently being used as food sources of phytosterols (e.g., Andrade et al., 2013; Kazłowska et al., 2013). Freshwater algae and cyanobacteria have also been shown to contain phytoestrogens (flavonoids and sterols), although information about water concentrations of phytoestrogens produced by these