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Endocrine disruptor activity of multiple environmental food chain contaminants

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ABSTRACT

Industrial chemicals, antimicrobials, drugs and personal care products have been reported as global pollutants which enter the food chain. Some of them have also been classified as endocrine disruptors based on results of various studies employing a number of *in vitro*/*vivo* tests. The present study employed a mammalian reporter gene assay to assess the effects of known and emerging contaminants on estrogen nuclear receptor transactivation.

Out of fifty-nine compounds assessed, estrogen receptor agonistic activity was observed for parabens ($n = 3$), UV filters ($n = 6$), phthalates ($n = 4$) and a metabolite, pyrethroids ($n = 9$) and their metabolites ($n = 3$). Two compounds were estrogen receptor antagonists while some of the agonists enhanced 17β-estradiol mediated response.

This study reports five new compounds (pyrethroids and their metabolites) possessing estrogen agonist activity and highlights for the first time that pyrethroid metabolites are of particular concern showing much greater estrogenic activity than their parent compounds.

1. Introduction

An endocrine disrupting compound (ED) has been defined as '*...an exogenous substance or mixture that alters function(s) of the endocrine system and consequently causes adverse health effects in an intact organism, or its progeny, or (sub)population*' (IPCS, 2002). The adverse effects of ED exposure have already been extensively described (Diamanti-Kandarakis et al., 2009) and include infertility, cancers, disrupted thyroid function, cognitive and behavioural disorders, obesity, metabolic syndrome, type I diabetes and immune dysfunction (WHO and UNEP, 2012).

EDs include compounds of natural origin (such as phytoestrogens or mycotoxins) as well as a variety of man-made chemicals (e.g. pesticides, brominated flame retardants (BfRs), ultraviolet (UV) filters and phthalates) (WWF, 2006). We are exposed to EDs via our environment and through our diet as these compounds may gain entry to the food chain (Connolly, 2009). Many of these compounds have been shown to biomagnify in the food chain and some have been reported as emerging contaminants in its lower levels (Ferrer et al., 2011; Jogsten et al., 2009; Kim et al., 2011a; Munshi et al., 2009; Ramaswamy et al., 2011; Sannino, 2010; Schecter et al., 2010). Thus it is of real importance to assess these contaminants for ED activity.

Perfluoroalkylated substances (PFCs) are extensively used in industry to repel dirt, water and oils in various consumer products including clothes, footwear, carpets and furniture (Jensen and Leffers, 2008). They also possess extreme thermal and chemical stability characteristics which leads to their persistence in the environment and consequent bioaccumulation in organisms (Fromme et al., 2009). As a result they have been detected in all parts of the ecosystem (Giesy and Kannan, 2001) and in a variety of food products such as fish, seafood, meat, poultry and fast food (Jogsten et al., 2009; Llorca et al., 2009; Tittlemier et al., 2007). However, the main exposure route is most likely through PFC usage as surfactants (Jensen and Leffers, 2008). The European Union (EU) has recognised the dangers presented by PFCs and banned the use of perfluorooctanoic acid (PFOS) in particular (EU, 2006) and included it in the Persistent Organic Pollutants (POPs) list (UNEP, 2009).

Parabens are used as preservatives in pharmaceuticals, cosmetics, toiletries and foods due to their bactericidal or fungicidal properties (Witorsch and Thomas, 2010). Their transport into the environment (Brausch and Rand, 2011) and human exposure has been described (Darbre and Harvey, 2008) and reviewed (Boberg et al., 2010).

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Triclosan (TCS) and trichlorocarban (TCC) are extensively used antimicrobial agents which can be found in tooth paste, soaps and detergents (Dann and Hontela, 2011). Over the last 30 years the widespread use of antimicrobials has led to contamination of the aquatic environment (Halden and Paull, 2004; Houtman et al., 2004; Kolpin et al., 2002; Ying and Kookana, 2007). Human exposure to these compounds has also been described (Allmyr et al., 2006a,b; Calafat et al., 2008).

UV filters are widely used in personal care products due to growing concerns about skin cancer. There are 27 UV filters permitted for use by the EU (Giokas et al., 2007) including compounds such as 4-methylbenzylidene camphor (4-MBC), ethylhexyl cinnamate (OMC) or benzophenone-3 (Bp-3). They may be present in sunscreen products at concentrations up to 10% (EU, 1976) and are also widely found in creams, lip balms and shampoos (Schlumpf et al., 2001). Contamination of the aquatic environment may occur through release from skin during bathing and swimming or via insufficiently treated effluent from waste water treatment plants (Poiger et al., 2004). Contamination of surface waters and biota with UV parent compounds and their metabolites has been documented (Diaz-Cruz et al., 2009; Sabaliunas et al., 2010; Schlumpf et al., 2008) as well as their transfer to human milk after dermal absorption (Schlumpf et al., 2008).

Insecticides fall into four main classes of chemicals; organochlorines, organophosphates, carbamates and pyrethroids (McCarthy et al., 2006). In the last decade a major shift from the use of organochlorines and organophosphates to carbamates and pyrethroids has been recorded (FAO; Withers et al., 2008) with the latter now being widely used both in agriculture and household products. Pyrethroids have a similar mode of action to pyrethrin, one of the oldest known insecticide (Hodgson, 2010), but are more persistent. They are regarded as safe (in contrary to organochlorines and organophosphates) and are effective in low doses (Hodgson, 2010). Parent compounds are monitored in food and have been detected in fruit, vegetables and tea (Mortimer and Shields, 1995; Teagasc, 2005; Tsumura et al., 1994). Their increased presence in the environment has resulted in exposure of both wildlife (Alonso et al., 2012) and humans (Le Grand et al., 2012; Tao et al., 2013; Ueyama et al., 2010). The environmental degradation and metabolism of pyrethroids is well understood (Fernandez-Alvarez et al., 2007; McCarthy et al., 2006) and numerous studies employ pyrethroid metabolite quantitation in urine as a measure of human exposure (Le Grand et al., 2012; Tao et al., 2013; Ueyama et al., 2010).

Phthalates are a group of chemicals used in plastics manufacture to improve flexibility and are found in more than 60% of polyvinyl chloride (PVC) polymers (EU, 2000). They are dispersed in polymer pores and can migrate to the surrounding environment. Even though five of the most abundantly used phthalates have been restricted by the EU in food contact materials (EU, 2007), they can be still found in many other products. Their release and environmental fate, as well as the exposure levels of the human population have been previously described (Huang et al., 2008; Kelly et al., 2010; Liu et al., 2012; Teuten et al., 2009). Consumers may be exposed via fat containing foods such as sausages, ham, milk, and oil or aqueous based beverages (Fierens et al., 2012; Page and Lacroix, 1995; Sannino, 2010; Sorensen, 2006).

BfRs are a diverse group of chemicals added during the manufacture of plastics, foams and textiles in order to decrease their flammability. Reactive BfRs are added during polymer production and become part of its matrix, while additive BfRs are mixed with the polymer after production, making them more likely to leach out during their lifetime (Sjodin et al., 2003). Polybrominated diphenyl ethers (PBDEs) and hexabromocyclododecane (HBCD) are examples of additive BfRs, whereas tetrabromobisphenol A (TBBPA) and its derivatives may be categorised in both groups. Numerous studies report vast environmental contamination with BfRs resulting in exposure of the human population mainly through inhalation and diet (Covaci et al., 2011; Frederiksen et al., 2009; Hites, 2004; Sjodin et al., 2003). Consequently, the production and usage of the most commercially produced PBDE has been restricted by the EU (EU, 2003) while other mixtures have been included in the POPs list for production elimination (UNEP, 2009). However, neither HBCD nor TBBPA and its derivatives have been restricted even though their POP-like properties have been suspected (Covaci et al., 2011) or already recognised (Roosens et al., 2010). Additionally, in the coming years, BfRs will continue to leach from products which are still in use or from items which have been disposed of in landfills (Guerra et al., 2008). BfRs are still being detected in meat, poultry, fish, shellfish, eggs, milk, eggs, cheese, fruit and vegetables (Bocio et al., 2003; Perez-Fuentetaja et al., 2010; Petro et al., 2010; Schecter et al., 2010).

The aim of the present study was to assess a wide range of known and emerging food chain contaminants (listed in Table 1) for potential endocrine disrupting effects at the level of estrogen nuclear receptor transcriptional activity using a mammalian reporter gene assay (RGA).

2. Materials and methods

2.1. Chemicals

Methanol and dimethyl sulfoxide (DMSO) were obtained from Sigma–Aldrich, St Louis, MO, USA. The following standards were purchased from Sigma–Aldrich, St Louis, MO, USA: 17β-estradiol (E2), 3,4,4′-trichlorocarbanilide (TCC), irgasan/triclosan (TCS), methyl 4-hydroxybenzoate (PB–Me), ethyl 4-hydroxybenzoate (PB–Et), propyl 4-hydroxybenzoate (PB–Pr), butyl 4-hydroxybenzoate (PB–Bu), di-*n*-butyl phthalate (DBP), benzyl butyl phthalate (BBP), bis(2-ethylhexyl) phthalate (DEHP), diisodecyl phthalate (DiDP), 2-hydroxy-4-methoxybenzophenone (Bp-3), homosalate (HMS), 3-(4-methylbenzylidene)camphor (4-MBC), 2-ethylhexyl 4-methoxycinnamate (OMC), BDE No 47 solution (47-BDE), tridecafluorohexane-1-sulfonic acid potassium salt (PFHxS K-salt), heptadecafluorooctanesulfonic acid potassium salt (PFOS K-salt), perfluoropentanoic acid (PFPA), undecafluorohexanoic acid (PFHxA), perfluoroheptanoic acid (PFHpA), perfluorooctanoic acid (PFOA), perfluorononanoic acid (PFNA), perfluorodecanoic acid (PFDA), perfluoroundecanoic acid (PFUA), tricosafluorododecanoic acid (PFDDA), 2-(2-hydroxy-5-methylphenyl) benzotriazole (UV-P), 2-(2*H*-benzotriazol-2-yl)-4-methyl-6-(2-propenyl) phenol (UV-9), 2-(2*H*-benzotriazol-2-yl)-4,6-bis (1-methyl-1-phenylethyl)phenol (UV-234), 2-*tert*-butyl-6-(5-chloro-2*H*-benzotriazol-2-yl)-4-methylphenol (UV-326), 2,4-di-*tert*-butyl-6-(5-chloro-2*H*-benzotriazol-2-yl)phenol (UV-327), 2-(2*H*-benzotriazol-2-yl)-4,6-di-*tert*-pentylphenol (UV-328), 2-(2*H*-benzotriazol-2-yl)-4-(1,1,3,3-tetramethylbutyl)phenol (UV-329), cyphenothrin, cyfluthrin, flumethrin, tetramethrin, fenvalerate, permethrin, cyhalothrin, τ-fluvalinate, deltamethrin, resmethrin, cypermethrin. 2,2′,6,6′-tetrabromobisphenol A diallyl ether (TBBPA-de), tetrabromobisphenol A dibromopropyl ether (TBBPA-dbpe), 3,3′,5,5′-tetrabromobisphenol A (TBBPA), 3-phenoxybenzoic acid (3-PBA), methyl-3-(2,2-dichlorovinyl)-2,2-dimethyl (cyclopropane)carboxylate (DCCA), 4-fluoro-3-phenoxybenzoic acid (3-F-3-PBA) were purchased from Dr. Ehrenstorfer GmbH (Augsbusrg, Germany). β-hexabromocyclododecane (β-HBCD), diallyl phthalate (DaP), dicyclohexyl phthalate (DCHP), diethyl phthalate (DEP), dimethyl phthalate (DMP), monobenzyl phthalate (mBzP), monoethyl phthalate (mEP), monomethyl phthalate (mMP) were obtained from the Accustandard (New Haven, CT, USA). *Cis*-3-(2-chloro-3,3,3-trifluoro-1-propenyl)-2,2-dimethyl-cyclopropanecarboxylic acid