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Multiple hormonal activities of UV filters and comparison of *in vivo* and *in vitro* estrogenic activity of ethyl-4-aminobenzoate in fish

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Abstract

UV filters have been detected in surface water, wastewater and fish, and some of them are estrogenic in fish. At present, little is known about their additional hormonal activities in different hormonal receptor systems despite their increasing use and environmental persistence. Besides estrogenic activity, UV filters may have additional activities, both agonistic and antagonistic in aquatic organisms. In our study, we investigate a series of UV filters for multiple hormonal activities *in vitro* in human receptor systems and evaluate the predictive value of these findings for the activity in fish *in vitro* and *in vivo*. First we systematically analysed the estrogenic, antiestrogenic, androgenic, and antiandrogenic activity of 18 UV filters and one metabolite *in vitro* at non-cytotoxic concentrations with recombinant yeast systems carrying either a human estrogen (hERα) or androgen receptor (hAR). All 19 compounds elicited hormonal activities, surprisingly most of them multiple activities. We found 10 UV-filters having agonistic effects towards the hERα. Surprisingly, we identified for the first time six UV filters with androgenic activities and many of them having pronounced antiestrogenic and antiandrogenic activities. As much as 17 compounds inhibited 4,5-dihydrotestosterone activity in the hAR assay, while 14 compounds inhibited estradiol activity in the hERα assay, indicating antiandrogenic and antiestrogenic activity, respectively. In particular, the antiandrogenic activities of phenyl- and benzyl salicylate, benzophenone-1 and -2, and of 4-hydroxybenzophenone were higher than that of flutamide, a known hAR antagonist.

In a second series of experiments, we investigated the predictive power of the hERα assay for aquatic organisms by further investigating the estrogenic UV filter ethyl 4-aminobenzoate (Et-PABA) *in vitro* and *in vivo* in fish. Et-PABA showed estrogenic activity in a recombinant yeast system carrying the rainbow trout estrogen receptor (rtERα) with higher activity than in the hERα assay. In addition, Et-PABA induced vitellogenin after 14 days of exposure in juvenile fathead minnows at 4394 μg/L. Our study shows estrogenic activity of this UV filter in fish both *in vitro* and *in vivo*. In conjunction with *in vitro* human receptor-based systems our results give a more detailed picture about distinct hormonal activities of UV filters occurring in aquatic systems. We conclude that receptor-based assays are important for *in vitro* assessment of UV-filters prior to or concurrently with *in vivo* assays, which ultimately provide data for the environmental risk assessment of these important personal care products.

Keywords: UV filters; Ethyl 4-aminobenzoate; Anaesthetic; Estrogenicity; Antiestrogenicity; Androgenicity; Antiandrogenicity; hERα; rtERα; hAR; Fathead minnow; Vitellogenin

1. Introduction

Thus far, endocrine disruption has been mostly concerned with estrogenic compounds because of the discovery that certain chemicals (Soto et al., 1991) and sewage effluents were estrogenic and field studies showed that estrogenic effects occur in wild fish (Tyler et al., 1998). Only in the last few years, antiandrogenic compounds (Kelce et al., 1995; Vinggaard et al., 2005), and androgenic activities in the aquatic environment (Thomas et al., 2002) were identified, originating for example from kraft mill effluents (Parks et al., 2001). In fish polycyclic musks have found to be antiestrogenic (Schreurs et al., 2004), but better known are antiandrogenic activities of compounds like *p*,*p*′-DDE,

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vinclozolin (Kelce and Wilson, 1997) and fenarimol (Vinggaard et al., 2005). Thus far, relatively few environmental contaminants have been shown to exhibit antagonistic besides agonist activity at steroid hormone receptors. For instance, alkylphenols elicited estrogenic and antiandrogenic activity *in vitro* (Sohoni and Sumpter, 1998), similarly to bisphenol A (Lee et al., 2003) and methoxychlor (Maness et al., 1998), to name a few chemicals. In the fields of endocrinology and pharmacology, on the other hand, multiple hormonal activities were investigated extensively, especially on the receptor level. For instance drugs targeted to the ER were found to be selective receptor modulators (SRM's, for a detailed review see Smith and O'Malley (2004)) with partial agonistic activities towards the receptor, like the weakly estrogenic tamoxifen, which acts antiestrogenic in the presence of E2 (Jordan et al., 1987). Few studies have investigated multiple endocrine disrupting mechanisms of environmental contaminants. The pyrethroid insecticide fenvalerate was found to possess estrogenic as well as anti-progesteronic activity *in vitro* (Garey and Wolff, 1998). Pesticides have been shown to have agonist and/or antagonists activities with one or more receptors (Andersen et al., 2002; Kojima et al., 2004). Despite this knowledge, most chemicals found in the environment have not been analysed systematically either for agonist and antagonist activities, or different steroid receptors. Therefore, it remains elusive to what extent multiple hormonal activities are a common feature of hormonally active compounds.

Organic ultraviolet absorbing chemicals (UV filters) are currently of concern because of the demonstrated estrogenicity of some of these compounds (Miller et al., 2001; Schlumpf et al., 2001). UV filters are widely used in sunscreens for direct protection of the skin against erythema and cancer, in a variety of cosmetic products and in the UV protection of plastic products.

Thus it is no surprise that humans can be exposed to UV filters via dermal absorption or food. Residues of benzophenone-3 (BP3) and octyl-methoxycinnamate (OMC) were found in human breast milk samples (Hany and Nagel, 1995). In human urine, BP3 and its metabolite benzophenone-1 (BP1) have been detected 4 h after application of commercially available sunscreen products to the skin (Felix et al., 1998). But the high consumption volumes combined with critical physicochemical properties such as environmental persistence and high lipophilicity, make UV filters also of potential environmental concern. Recently, residues of UV filters have been found in lake and river water in the range of 2–80 ng/L (Poiger et al., 2004) and in treated wastewater up to 2700 ng/L (Balmer et al., 2005). In lake fish four different UV filters were identified in a recent monitoring study at concentrations of up to 166 ng/g (lipid) of 4-methylbenzylidene camphor (4MBC), of 123 ng/g BP3 and 64 ng/g of OMC (Balmer et al., 2005; Buser et al., 2006).

Besides being present in the environment, UV filters recently gained importance because of their reported estrogenic activity *in vitro* (Miller et al., 2001; Schlumpf et al., 2001; Schreurs et al., 2002; Mueller et al., 2003). In juvenile fathead minnows 3BC, BP1 and benzophenone-2 (BP2) led to vitellogenin (VTG) induction (Kunz et al., 2006) and 3-benzylidene camphor (3BC) did as well in rainbow trout (Holbech et al., 2002), whereas 4MBC and OMC were found to be estrogenic in male medaka (Inui et al., 2003). An indication for VTG expression in rainbow trout was found after the exposure to BP3 (Daniel Schlenk, personal communication). BP3 was also present in wastewater from New York City and the exposure of male medaka to wastewater fractions lead to VTG induction (Sapozhnikova et al., 2005). BP3 also occurred in marine sediment and exposure of male California halibut to this sediment lead to VTG induction (Schlenk et al., 2005). In rats 3BC, 4MBC, OMC, BP2, BP3 and 4,4-dihydroxybenzophenone (4DHB) elicited estrogenic effects (Schlumpf et al., 2001; Yamasaki et al., 2003; Schlecht et al., 2004). Besides being estrogenic, some UV filters such 4MBC, homosalate (HMS) and BP3 showed antiestrogenic activity (Schreurs et al., 2002; Mueller et al., 2003), and BP3 and HMS had antiandrogenic activity *in vitro* (Ma et al., 2003; Schlumpf et al., 2004). However, it is not known whether multiple hormonal activities are a common feature of these compounds. In order to shed some light on other possible endocrine properties of UV filters, especially as these compounds are found in surface waters and fish (Balmer et al., 2005; Buser et al., 2006), we screened 19 compounds for additional agonistic and antagonistic activities besides estrogenicity. Therefore we employed a recombinant yeast system in order to investigate, in a systematic manner, the estrogenicity, antiestrogenicity, androgenicity and antiandrogenicity of commonly used UV filters. We used this approach, because earlier results on estrogenicity of UV filters demonstrated that the yeast assay is particularly useful for screening this group of compounds. First results indicate that it identifies possible estrogenic compounds and separates them from compounds with no estrogenic activity, averting false negative results (Kunz et al., 2006). Therefore we furthermore investigated one UV filter, ethyl 4-aminobenzoate (Et-PABA), that was found to be estrogenic in the hERα assay, for its estrogenicity in a recombinant yeast assay carrying a rainbow trout ER (rtERα). Et-PABA is also used as an anaesthetic in veterinary medicine. In addition, we analysed this compound in juvenile fathead minnows for vitellogenin induction. With this approach we were able to validate whether our findings for the hER are transferable to the rtER *in vitro*, and fish *in vivo*.

Our results reveal a novel picture of the hormonal activities of these environmentally relevant chemicals and address the question of their modes of action. All 19 compounds analysed showed hormonal activities, surprisingly most of them multiple activities. Moreover, antiestrogenicity and antiandrogenicity of UV filters are possibly even more environmentally relevant than estrogenicity. Et-PABA displayed its estrogenic activity not only towards the hERα but also toward rtERα and *in vivo* in fish. These findings raise the question whether UV filter residues found in the environment are potentially able to adversely affect aquatic organisms with their multiple hormonal activities. Forthcoming studies on their effects on fish reproduction will show whether multiple hormonal activities will translate into *in vivo* effects.