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Chemical Analysis and Ecotoxicological Effects of UV Absorbing Organic Chemicals in Aquatic Environments

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Abstract: Substances that exert adverse effects on reproduction and fertility are a cause of concern. UV filters used in sunscreens and in the protection of materials may enter aquatic environments. Properties of UV filters such as environmental persistency and high lipophilicity make an environmental risk assessment desirable, in particular as some of them show hormonal activity at high concentrations. In this research project conducted within the National Research Programme NRP50, hormonal activities of UV filters are evaluated *in vitro* in recombinant yeast system and *in vivo* in fish and frogs.

Keywords: Ecotoxicology · Endocrine disrupters · Estrogenic activity · UV filters

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Hormonally Active Substances in the Environment

Substances that are active towards the hormone system are of concern, as they may have adverse effects on reproduction and fertility in man and wildlife. The identification of at least 150 compounds possessing hormonal activity has increased our knowledge about possible candidates and adverse effects [1]. Most of the hormonally active compounds identified so far act *via* estrogen receptors (ER). They comprise many structurally diverse chemicals, of which some share the major structural features associated with estrogenic activity. For ER binding, a similar pattern of atoms or functional groups in the chemical's structure is required. A ligand with a phenolic group seems to be a common feature of many, but not all, molecules that display estrogenic activity [2], and molecular symmetry enhances potency [3]. In addition, other environmental compounds have been found to exhibit estrogenic activity *in vitro* and *in vivo*, namely steroid hormones, phytoestrogens, degradation products of detergents such as nonyl- and octylphenol, preservatives such as parabens, some pesticides, cadmium and other heavy metals, and many others [1][4][5]. Assessment of estrogenic activity has been performed by the use of several *in vitro* systems including the recombinant yeast reporter gene systems (YES) carrying the human ERα [6], and *in vivo* in laboratory animals and wildlife, in particular in fish. Wild fish exposed to estrogenic compounds originating from wastewater entering rivers show estrogenic effects in juvenile and male fish such as induction of yolk precursor protein vitellogenin and ovarian tissue in gonads [7]. Estrogenic effects of environmental chemicals have been demonstrated in numerous laboratory experiments *in vitro* [8] and *in vivo* [7].

UV Filters in the Environment

Organic compounds used as UV absorbers (UV filters) are added to sunscreen products for human skin protection and in cosmetics, but also in a variety of commercial and industrial products including polymers as light stabilizers (Fig. 1). A variety

of structurally diverse compounds are used to absorb UV light, but only little is know about their occurrence in the environment and their ecotoxicological effects. Because of growing public concern about skin damage by UV light and skin cancer, the use of UV filters is recommended, and hence increasing. In Switzerland, many UV absorbing organic compounds are in use, of which the six most important ones make up 88% of the total amount used in sunscreens [9]. Estimated annual consumption in Switzerland for some of the UV filters lies in the range of 16–20 t for 4-methylbenzylidene camphor (4-MBC), 14–20 t for octyl methoxycinnamate (OMC), and 5–10 t for butyl methoxydibenzoyl methane [9]. Through application in sunscreens, these compounds are introduced directly into the environment in particular during summer, but they can also reach the aquatic environment *via* wastewater and application in materials protection. These compounds are moderately to highly lipophilic and some of them are not readily degradable. As a consequence, they are prone to occur in the aquatic environment and to lead to bioaccumulation in organisms.

In fish, residues of different UV filters were detected in Germany at total concentrations of 2 and 0.5 mg/kg lipid (or 45 and 6.8 μg/kg fish) in perch and roach, respectively [10]. A monitoring study in Lake Zürich and Lake Hütten demonstrated the occurrence of 4-MBC, OMC, octocrylene, and benzophenone-3 in surface waters of up to 82, 26, 27, and 125 ng/l, respectively [9]. A recent survey by these authors confirmed the occurrence of UV filter residues in fish in Switzerland [11].

Acute and subchronic toxicity of UV filters in man and mammals is considered to be rather low. However, a recent study demonstrates estrogenic activity of some UV filters in mammals both *in vitro* and *in vivo* [12]. Estrogenic activity was found *in vitro* in MCF-7 cells for five out of six frequently used UV filters, and for three out of six compounds *in vivo* in rats. Recently, estrogenic activity of 4-MBC, OMC and propyl paraben were indicated at high exposure concentrations in laboratory experiments in fish as well [13]. Whether or not this activity occurs at environmentally relevant concentration remains to be shown. At present, a hazard and risk assessment of UV filters is not possible due to the lack of data on potential ecotoxicological effects of these compounds, in particular to the endocrine system and reproduction of aquatic organisms.

Project in the National Research Programme NRP50

Sponsored by the National Science Foundation within the National Research Programme NRP50, our project is aimed at filling these gaps by the use of a series of ecotoxicological *in vitro* and *in vivo* techniques combined with analytical chemical determinations. The overall goal is to evaluate the hormonal activity of selected UV filters *in vitro* and *in vivo* in order to assess the hormonal activity and ecokinetics of these compounds in representative aquatic organisms, in particular in fish and frog. Eventually, these data serve for an environmental risk assessment of these important chemicals. The project consists of an interactive venture between the Institute of Environmental Technology and Department of Chemistry of the University of Applied Sciences of Basel (FHBB) and Springborn Smithers Laboratories (Europe) AG. The project has the following objectives:

- To analyze and to compare the estrogenic and androgenic activity of commonly used UV filters *in vitro* in recombinant yeast carrying a human ERα (YES) or human androgen receptor (YAS), respectively.
- To assess the interaction of mixtures of estrogenic compounds *in vitro*, as UV filters occur in mixtures and mixture toxicity is largely unknown.
- To investigate the estrogenic activity *in vivo* of important UV filters in short-term and long-term laboratory experiments in fish. Estrogenic activity in fish and potential effects on fish reproduction will be assessed. In addition, bioaccumulation and ecokinetics of one relevant UV filter will be evaluated.
- To investigate possible activities of UV filters to the thyroid hormone system and sex determination during development of tadpoles of frogs *Xenopus laevis*.

Preliminary Results

The YES was used to assess the estrogenic activity of UV filters *in vitro*. Reporter gene activity is elicited after binding of agonists to the ERα, which in turn bind to specific response elements on a reporter plasmid resulting in the expression of β-galactosidase. Estrogenic or androgenic activities are assessed by determination of enzyme activity. We have determined the estrogenic activities of 18 single UV filters. A series of eight UV filters displayed no detectable estrogenicity, whereas three UV filters including, benzophenone-1, benzophenone-2 and 4-dihydroxybenzophenone showed estrogenic activity exhibiting full dose-response curves. The estrogenicity was several orders of magnitude lower than that of the female sex hormone estradiol, however. Some UV filters exhibited lower efficacy showing submaximal activity. Currently, we are analyzing combinations of binary mixtures of UV filters to evaluate their interaction.

For an ecotoxicological hazard and risk assessment, it is important to investigate whether UV filters active *in vitro* also act *in vivo* in aquatic animals. We have performed first exposure experiments with the frog *Xenopus laevis* and fish. For determination

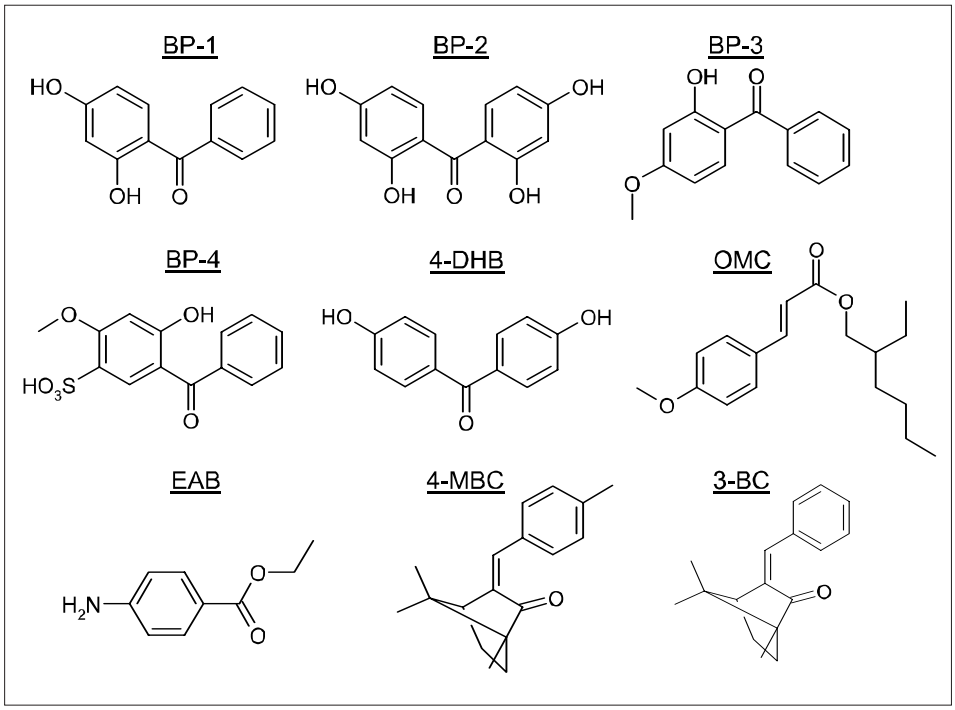

Fig. 1. Some UV filters analyzed in our project in the programme NRP50 for endocrine effects in aquatic animals. EAB: ethyl-4-aminobenzoate (benzocaine) is used as an anaesthetic.

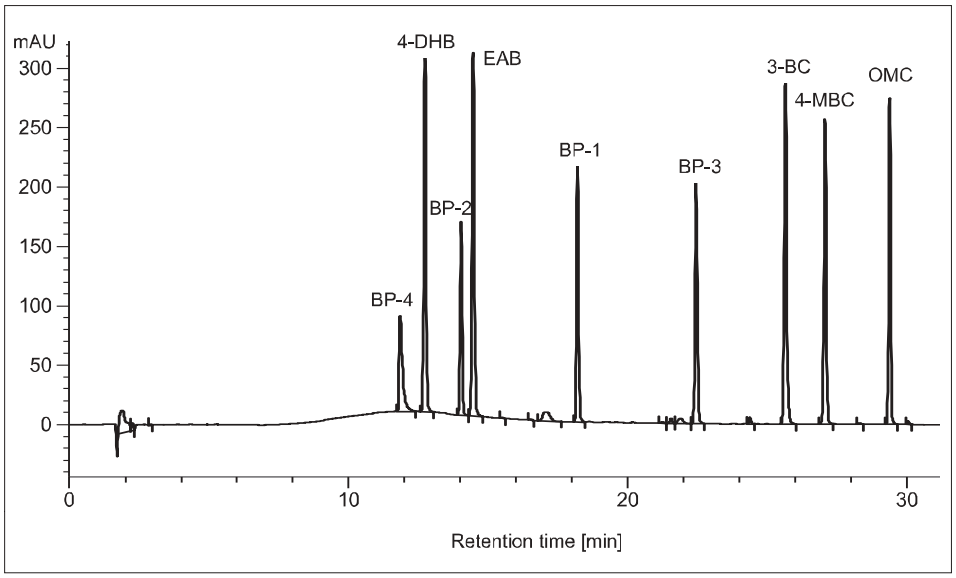

Fig. 2. HPLC chromatogram of nine UV filters in ethanol, measured at 290 nm. BP-4: benzophenone-4, 4-DHB: 4-dihydroxy benzophenone, BP-2: benzophenone-2, EAB: ethyl-4-aminobenzoate, BP-1: benzophenone-1, BP-3: benzophenone-3, 3-BC: 3-benzylidene camphor, 4-MBC: 4-methyl benzylidene camphor, OMC: octyl methoxycinnamate.

of effect concentrations, analysis of actual concentrations in exposure experiments is a prerequisite. For this purpose, a novel method for analysis of UV filters in water was developed. It is based on a solid-phase extraction procedure with analysis by either GC/MS, or HPLC with UV detection. Analysis of 4-MBC and 3-benzylidene camphor (3-BC) was performed by GC/MS, or by HPLC, the latter allowing the determination of nine UV filters simultaneously (Fig. 2).

For evaluation of potential effects on the endocrine system of frogs, *Xenopus laevis* tadpoles were exposed to 4-MBC and 3-BC close to their environmental concentrations and interference with the thyroid and sex hormone system during frog metamorphosis was assessed. Exposure to 1, 5, and 50 μg/l of both compounds for 35 days over development stages 52–66 neither changed the rate of metamorphosis, nor the sex ratio, or gross gonad morphology of *Xenopus*, respectively [14]. However, these data do not rule out the possibility that endocrine disruption may take place at higher concentrations.

Furthermore, juvenile fish, *Pimephales promelas,* were exposed for 14 days to UV filters. Potential estrogenicity is determined by means of vitellogenin induction, a well-established biochemical marker. Exposure concentrations were determined by chemical analysis and results of these experiments are currently being processed.

This project involving both *in vitro* and *in vivo* experiments in conjunction with chemical analysis of experimental waters allows the evaluation of potential ecotoxicological effects of commonly used UV filters and its environmental risk assessment. The venture involving different universities and laboratories guarantees interdisciplinary environmental research at the University of Applied Sciences.

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