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Toxicity on crustaceans and endocrine disrupting activity on *Saccharomyces cerevisiae* of eight alkylphenols

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Abstract

In the last few years many concerns have been raised regarding the environmental safety of alkylphenol polyethoxylate surfactants (APnEOs).They are widely used in detergents, paints, herbicides and many other formulated products. It has been estimated that 60% of APnEOs end up in the aquatic environment; they are biodegradable and transformed into alkylphenols, such as nonylphenol and octylphenol that are hydrophobic and tend to accumulate.

In the present study, acute and chronic aquatic toxicity and the estrogenic activity of the following eight alkylphenols were assessed: 4-nonylphenol, 4-octylphenol, 4-nonylphenol-10-ethoxylate, 4-tert-octylphenol, POE (1 to 2)-nonylphenol, POE (6)-nonylphenol, POE (3)-tert-octylphenol and POE (9 to 10)-tert-octylphenol. The toxic potential was measured on the crustaceans *Daphnia magna* and *Ceriodaphnia dubia*, while the estrogenic activity was determined by using the YES-test with the strain *Saccharomyces cerevisiae* RMY326. The results showed that the exposure of crustaceans to the eight xenoestrogens investigated caused both acute and chronic effects. The EC50 values found for *C. dubia* at 48 h were compared to *D. magna* at 24 h and, gave a first indication about the toxic activity of the compounds investigated, that is better expressed in the long-term. In fact, chronic data showed a strong increase in toxicity with EC50 values one or two orders of magnitude lower than the acute values. The results of the YES-test showed that nonylphenol, octylphenol and 4-tert-octylphenol were the most estrogenic and the bioassay was able to detect their estrogenicity at very low concentrations (ng–μg/l).

Keywords: Endocrine disruptors; Alkylphenols; Acute and chronic toxicity; YES-test

1. Introduction

A wide variety of contaminants, present in the environment, are known to have adverse effects on living organisms interfering with hormone receptors and mimicking the normal endocrine functions such as development, growth and homeostatic mechanisms (Cargouët et al., 2004; Taneda et al., 2004; Brossa et al., 2005). In the last decade, these contaminants, indicated as endocrine disruptors (EDs), have received particular attention from the scientific community because they may lead to altered growth, development, or reproduction in exposed animals, and these changes may be expressed later in the life cycle or even in future generations (Larkin et al., 2003; Takamura-Enya et al., 2003; Schilirò et al., 2004; Servos et al., 2005). The EDs may have different types of activity (e.g. estrogenic, anti-androgenic), and cause their effects through different mechanisms of action (i.e. be agonists, or antagonists of endogenous hormones, or affect hormone synthesis and/or metabolism). These contaminants can be found in various environmental matrices, but they are mainly detected in the aquatic environment, particularly susceptible to pollution, because of considerable intentional release of chemicals into rivers, lakes, and sea (mainly through effluents from sewage treatment plants and factories), and accidental releases of chemicals (through spills, run-off, atmospheric deposition, etc.).

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They comprise a wide range of natural and man-made molecules including pesticides, polycyclic aromatic hydrocarbons, phthalate plasticizers, alkylphenol polyethoxylates (APnEOs), and natural and synthetic hormones (Foster et al., 2004; Brossa et al., 2005; Sumpter, 2005). Although some of these chemicals have been included among the priority pollutants of surface waters and many countries have considerably reduced their use, they still are abundantly utilized in many other states from where they might be moved and deposited from other parts of the world that still use them or from previous environmental contamination (Ferrara et al., 2005). In fact, some xenobiotic compounds, including the APnEOs, have levelled off because of their physical properties that cause accumulation in sediments, are re-released into the aquatic environment and, increase in the tissues of organisms (USEPA, 1997). In particular, human exposure to APnEOs may occur through contaminated food, inhalation and dermal absorption (Ferrara et al., 2005). Considering their occurrence in different environmental matrices and, toxicological and estrogenic activities, these compounds may play a critical role in the incidence of certain cancers and disorders in the male reproductive system, including reduced testicular size and sperm production in humans and wildlife, through both direct and indirect actions on the endocrine system (White et al., 1994; Routledge and Sumpter, 1997; Fries and Püttman, 2004). Nevertheless, other authors assert that epidemiological data do not provide consistent evidence that xenobiotics with hormonal properties adversely affect human health (Juberg, 2000). Humans are daily exposed to high concentrations of dietary phytoestrogens and endogenous estrogens. The measurement of the endocrine activity of environmental contaminants should not be confused with the assessment of risk to humans because critical factors such as exposure, potency, lifestyle risk factors (diet, smoking, sexual practices) and dose-response have also to be considered (Waddell, 1998).

The alkylphenol polyethoxylates are an important group of non-ionic surfactants (more than 300 000 tons of APnEOs are produced worldwide annually) (Warhurst, 1995; Jobling et al., 1996; Tsuda et al., 2001) commonly used in household detergents, industrial detergents and tanneries, as well as in paints, herbicides, and many other formulated products. During sewage treatment, APnEOs are initially biodegraded via a shortening of the hydrophilic chain, forming increasingly lipophilic and persistent metabolites, including short chain alkylphenol ethoxylates (e.g. nonylphenoldiethoxylate), alkylphenol carboxylic acids (e.g. nonylphenoxycarboxylic acid), and finally alkylphenols such as nonylphenol (NP) and octylphenol (OP). These last compounds are the mainly used alkylphenols. Nonylphenol polyethoxylates (NPnEOs) account for about 80% of the world market and octylphenol polyethoxilates the remaining 20% (Warhurst, 1995; Tsuda et al., 2001). It has been estimated that 60% of parent nonylphenol polyethoxylates end up in the aquatic environment. Nonylphenol has been found in the amounts of 330 μg/l in sewage effluents, 180 μg/l downstream wastewater treatment plants and 40 μg/l in surface waters (Hill et al., 2003), while octylphenol is usually detected at concentrations of about one order of magnitude lower (Bennie, 1999).

In this study, we will report data on the acute and chronic aquatic toxicity and estrogenic activity of three major degradation products of non-ionic surfactants, 4-nonylphenol, 4-octylphenol, 4-tert-octylphenol, and five APnEOs, 4-nonylphenol-10-ethoxylate, POE (1 to 2)-nonylphenol, POE (6)-nonylphenol, POE (3)-tert-octylphenol and POE (9 to 10)-tert-octylphenol. The POEs are the commercial formulations of complex mixtures of homologues, oligomers and isomers.

The toxic potential was measured on the crustaceans: *Daphnia magna* and *Ceriodaphnia dubia*, two primary consumers in the aquatic chain. We preferred to employ invertebrates because many more species exist when compared to vertebrates and, tests based on invertebrates are generally favoured in a testing battery (Rasmus Andersen et al., 2001). Particularly, the choice was focussed on *D. magna* because it is one of the most sensitive organisms towards NP in short term experiments (Comber et al., 1993) and, because the effect of these compounds on *C. dubia* has never been studied before on these compounds and it is a useful organism to detect chronic toxicity. The estrogenic activity was determined using an estrogen-inducible yeast-screening bioassay (YES-test) (Takamura-Enya et al., 2003). The yeast cells contain most of the transcriptional equipment required for the receptors to bind to the ligand and to activate transcription. This method employs the yeast strain RMY326 of the *Saccharomyces cerevisiae*, transfected with a human α-estrogen receptor and an estrogen-responsible element linked to the reporter gene *lac-Z*, encoding the enzyme β-galactosidase. The use of this bioassay allowed us to reproduce the effect that the compounds investigated have on vertebrates in yeast cells even if vertebrate cells modulate the hormone response with complex systems of co-activation and co-repression. Furthermore, the YES-test may be considered as a complement for the toxicity detection of the compounds that, sometimes, exceed their presumed endocrine effects. In fact, the bioassay utilized is sensitive in assessing the estrogenic activity of chemicals at very low concentrations while at high concentrations responds by inhibiting the yeast transcriptional system.

2. Materials and methods

2.1. Test substances

4-nonylphenol [**1**] and 4-octylphenol [**2**] were purchased from Sigma Chemical, St. Louis, MO, USA; 4-nonylphenol-10-ethoxylate [**3**] was purchased from Carlo Erba, Milano, Italy; 4-tert-octylphenol [**4**], POE (1 to 2)-nonylphenol [**5**], POE (6)-nonylphenol [**6**], POE (3)-tert-octylphenol [**7**] and POE (9 to 10)-tert-octylphenol [**8**] were