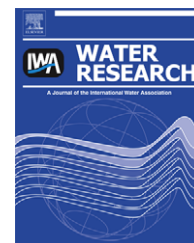


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Do suspended sediments modulate the effects of octylphenol on rainbow trout?

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ARTICLE INFO

Article history:

Received 23 September 2008

Received in revised form

5 December 2008

Accepted 9 December 2008

Published online 24 December 2008

Keywords:

Alkylphenols

Suspended sediments

Rainbow trout

Octylphenol

Endocrine disruptors

Partition coefficient

ABSTRACT

A system was devised which allows particles to remain in suspension in a conventional 60 L aquarium without undue disturbance to resident fish. Using this system, juvenile rainbow trout were exposed for one week to 4-tert-octylphenol (OP, 10–1000 µg/L) with or without the presence of suspended sediments (10–20 mg/L of natural suspended sediments from the River Calder, UK). About 8% of the added OP partitioned to the solid phase. Vitellogenin levels were determined in the plasma of the exposed rainbow trout and showed a dose-dependent increase with regards to OP exposure concentration. Considerable variation in the vitellogenin response was observed between separate runs with the same OP concentration. There was no statistically significant (at $P < 0.05$) difference in plasma VTG levels between the OP treatments with or without suspended sediments. This suggests that the dissolved concentration is the key factor and natural suspended sediment neither protects against, nor exacerbates, the endocrine disrupting effect of OP on fish.

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1. Introduction

Most experimental studies of endocrine disruption in aquatic organisms have involved the exposure of animals to single pure chemicals dissolved in water. But in the real world, the aquatic environment consists not only of mixtures of chemicals, but also of a natural suspension of particles in the aqueous medium, at concentrations extending into the grams per litre range depending upon hydrological conditions (Wass and Leeks, 1999). Thus, there is the potential for suspended solids to modulate the effects of endocrine disruptors on susceptible organisms.

Although, after the restrictions on the use of alkylphenol polyethoxylate surfactants in the EU and some other

countries (Directive 2003/53/EC, 2003; Soares et al., 2008), much of the focus on endocrine disrupting chemicals in the aquatic environment has now turned to the ubiquitous steroid estrogens (Desbrow et al., 1998; Sheahan et al., 2002a), there are still rivers where alkylphenols from the degradation of their ethoxylates play a significant role, in particular with respect to estrogenic chemicals associated with solid matter (Fenet et al., 2003; Petrovic et al., 2002; Sheahan et al., 2002b). It is frequently found that while estrogenic activity measured in the water phase of sewage effluents, or receiving waters, is mostly caused by steroid estrogens, a large part of the estrogenicity extracted from solids, such as sediments and sewage sludge, can be explained by alkylphenols (Bolz et al., 2002; Fenet et al., 2003). Furthermore, since in lakes with stratified

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doi:10.1016/j.watres.2008.12.010

sediment deposition, the highest NP concentrations were found in bed sediments from the 70s or 80s (Giger and Alder, 2002; Isobe et al., 2001), re-suspension of historic bed-sediments may provide a secondary source of alkylphenol-contaminated solids. The most common alkylphenols nonylphenol and octylphenol have octanol–water partitioning coefficients ($\log K_{ow}$) in the range of 4–5 (Ahel and Giger, 1993; McLeese et al., 1981), which is somewhat higher than the steroid estrogens, which have $\log K_{ow}$ values between 2.5 and 4 (Hansch, 1995; Holthaus et al., 2002). Furthermore, distribution coefficients (K_d) observed in the field are often higher than those measured in laboratory experiments or predicted from $\log K_{ow}$ data (Isobe et al., 2001; Patrolecco et al., 2006).

In environmental samples, a significant proportion of alkylphenols may be bound to suspended sediments. For example, in the Tokyo area Isobe et al. (2001) found an average of 85% of nonylphenol (NP) and 61% of OP in primary sewage effluent and 23% of NP and 8% of OP in river water were bound to suspended sediments. The proportion of NP on suspended particles was up to 86% in treated sewage and water from the River Aire (Blackburn and Waldock, 1995; Sheahan et al., 2002a), 12–58% in water samples from a range of other UK rivers (Blackburn et al., 1999), 6–25% and 2–42% in samples from the Italian rivers Lambro and Tiber respectively (Patrolecco et al., 2006; Polesello et al., 2003). In a Korean lake and its tributary creeks, Li et al. (2004) found between 5 and 78% of the measured NP in the suspended particles and this proportion tended to be higher in winter than in summer. Thus, despite the relatively low K_{ow} , a significant proportion of alkylphenols may sometimes be found on suspended sediments in a real environmental situation.

In this work, OP rather than the more ubiquitous NP was chosen, because unlike NP it exists as a single isomer rather than as a mixture. There are, however, less data available for OP because it is less widely used than NP and therefore often not analysed or its concentration is below the detection limit. Earlier work (Johnson et al., 1998) suggested that a significant proportion of OP could bind to suspended sediments, thus reducing the concentration in the aqueous phase, but Isobe et al. (2001) found only an average of 8% of the OP in rivers to be bound to suspended sediments. It is important to note that the suspended sediments in a river do not remain of a consistent composition during the year. For example, in slow flowing areas in summer a large proportion of suspended sediments may consist of algae, whereas rainstorms can flush out high concentrations of clay particles and at other times, the most significant contribution may be from decaying detritus.

For an aquatic organism, sorption of alkylphenols to suspended sediments could mean a reduced exposure relative to inputs unless the chemical is taken up from the particles, for example by accidental or deliberate ingestion. Ra et al. (2008) have shown that the sorption of OP and pentachlorophenol to (artificial) suspended particles did indeed reduce the toxic effects on *Daphnia magna* and *Vibrio fischeri*, but because the toxicity tests were carried out after the contaminated particles were removed from the system by filtration, uptake of chemicals from the particles was precluded. Uptake from sediments may be important because, at least with NP-contaminated bed sediments, about 95% of the chemical on

the solids appeared to be bioavailable (de Weert et al., 2008). It is well known that the uptake of (xeno)estrogens via food can have endocrine effects on fish: increased vitellogenin concentrations in immature rainbow trout were, for example, measured after dietary exposure to E2 (Carlson and Williams, 1999) and OP (Pedersen et al., 2003). In the present study, the contaminated particles were not removed, so the animals were exposed to OP and suspended sediments at the same time.

The question posed in this study was therefore: what effect, if any, does the partitioning of OP to suspended sediments have? Does it reduce the chemical's effect on fish by reducing the bioavailable aqueous concentration or does it increase its effect through ingestion of suspended sediments, which contain a higher concentration of the chemical than the water?

2. Material and methods

2.1. Design of aquarium agitator

In order to study the effect of suspended sediments, they must be maintained in suspension within the water column without harming or unduly stressing the animals involved in the study. Several systems to expose animals to suspended sediment have been described in the literature. Generally the volume of water available to the animals is very limited compared to the size of the whole apparatus, which makes these designs either only suitable for small animals, such as invertebrates or fry (e.g. Chilton, 1991; Schmidt-Dallmeyer et al., 1992; Schrap and Opperhuizen, 1989; Servizi and Martens, 1991) or the apparatus requires considerable space, limiting the practicability of the design (Cope et al., 1996). Some authors have suggested a flow-through system with serial dilution of suspended sediments (e.g. Sved and Roberts, 1995), a design which suffers from the practical problem of collecting sufficient amounts of natural suspended sediments to sustain such a setup. Therefore, a new compact apparatus was developed which fits into a conventional glass aquarium (l: 60 cm, w: 30 cm, h: 40 cm) filled with 60 L Windermere Lake water with the space available to the fish within the mesh basket being approximately 35 L (Fig. 1). The device consists of a motor-driven bar that runs laterally back and forth along the base of the aquarium at a speed of about 8 cm/s, combined with a wire mesh basket within which the experimental animals were held. Preliminary tests of this equipment showed that OP sorption to glass and steel was negligible (less than 1% in the entire aquarium setup), so all parts of the stirring mechanism to be immersed were constructed from stainless steel. However, there was a significant loss of chemical due to sorption to the silicone sealant used in aquaria; this was monitored in each experiment by including control aquaria containing no fish. Although the device created sufficient motion to prevent particulate material from settling, the suspended sediments started to stick to the surfaces (even vertical surfaces) of both the aquarium itself and the mesh basket. These particles were re-suspended once each day by disconnecting the stirrer bar from the motor and manually moving it along the aquarium at increased speed

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