



Effects of bisphenol A on different trophic levels in a lotic experimental ecosystem



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ABSTRACT

Bisphenol A (BPA) is commonly used by manufacturers and can be found in many aquatic ecosystems. Data relative to BPA ecotoxicity are only available for studies in laboratory conditions on macro-invertebrates and fish. There is thus a lack of information for other trophic levels such as macrophytes. Moreover, the impacts of BPA within an ecosystem context, *i.e.* with populations from different trophic levels studied at long term in environmental conditions, have never been assessed. We carried out a long-term lotic mesocosm study in 20 m long channels under three exposure concentrations of BPA (nominal concentrations of 0, 1, 10 and 100 µg/L) delivered continuously for 165 days. Three trophic levels were followed: macrophytes, macro-invertebrates (with a focus on *Radix balthica*) and fish (*Gasterosteus aculeatus*). Significant effects were shown at 100 µg/L BPA on the three trophic levels. BPA had a direct impact on macrophyte community structure, direct and indirect impacts on macro-invertebrates and on fish population structure. Gonad morphology of fish was affected at 1 and 10 µg/L of BPA, respectively for female and male sticklebacks. In addition to these ecotoxicity data, our results suggest that fish are good integrators of the responses of other communities (including macro-invertebrates and macrophytes) in mesocosm systems.

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1. Introduction

Most of the domestically produced bisphenol A (BPA; 4,4'-dihydroxy-2,2-diphenylpropane) is used by manufacturers as an intermediate in the production of polycarbonate and epoxy resins, flame retardants, and other specialty products. Final products include adhesives, protective coatings, powder paints, automotive lenses, protective window glazing, building materials, compact disks, optical lenses, thermal paper, paper coatings, as a developer in dyes, and products designed for encapsulation of electrical and electronic parts (Staples et al., 1998). Due to its endocrine disrupting properties, the use of BPA is now restricted and more particularly banned in baby bottles in the European Union (European Council, 2011). Moreover, in France, in 2013, BPA is planned to be banned in all containers used for children under 3.

In 2006, the global BPA production volume in Western Europe was estimated at 1.150×10^3 tonnes/year (European Union, 2008). In 1993, around 0.017% of the produced BPA was released to air, surface water or wastewater treatment plants. An additional 0.085% was recycled, landfilled or incinerated (Staples et al., 1998). Although BPA degrades rapidly in the environment (aquatic half-lives from 0.5 to 6 days), as demonstrated in both standard biodegradation tests and in environmentally relevant river die-away studies (Klečka et al., 2001; West et al., 2001), exposure to low concentrations of BPA occurs in aquatic systems primarily from discharges into wastewater treatment plants during its manufacturing and use (Cousins et al., 2002; Staples et al., 1998). BPA was found in North American and European freshwaters (Klečka et al., 2009). In the River Elbe in Germany, BPA was measured at 0.105 ± 0.204 µg/L (Heemken et al., 2001). In Dutch surface waters, BPA was measured at levels up to 0.33 µg/L, with one occasional observation of 21 µg/L (Belfroid et al., 2002). This latter value substantially exceeds the predicted environmental concentration of 0.12 µg/L for water as indicated in the EU's risk assessment report for BPA (European Union, 2003). BPA was also detected in animal

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Table 1
Partial review of BPA effects on fish.

Fish species	LOEC (µg/L)	Duration (d)	Endpoint	References
<i>Dicentrarchus labrax</i>	10	15	VTG induction	Flint et al. (2012)
<i>P. promelas</i>	64	164	VTG induction	Mihaich et al. (2012)
<i>P. promelas</i>	160	71	VTG induction	Sohoni et al. (2001) and Staples et al. (2011)
<i>P. promelas</i>	1	164	The percentage of spermatocytes increased	Flint et al. (2012)
<i>Cyprinus carpio</i>	1	15	Modification of the gonad structure in males and increased oocytes atresia	Flint et al. (2012)
<i>Salmo trutta fario</i>	1.75	100	Reduction of sperm quality; delayed ovulation	Flint et al. (2012)
<i>Orizias latipes</i>	10	–	Ova-testis	Metcalfe et al. (2001)
<i>P. promelas</i>	16	164	Proportion of sex cells types in the testis is altered	Mihaich et al. (2012)
<i>P. promelas</i>	160	164	Frequency of spermatocytes decreased	Mihaich et al. (2012)
<i>Danio Rerio</i>	375	–	Gonad histology is modified	Segner et al. (2003)
<i>P. promelas</i>	640	–	Hatchability reduced	Sohoni et al. (2001) and Staples et al. (2011)
<i>P. promelas</i>	1280	–	Eggs production inhibited	Sohoni et al. (2001) and Staples et al. (2011)
<i>O. latipes</i>	2280	–	Decreases the number of eggs and hatchings	Shioda and Wakabayashi (2000)
<i>C. carpio</i>	1	15	Decreased estrogen and androgen ratios	Flint et al. (2012)
<i>P. promelas</i>	640	–	Effects on survival	Mihaich et al. (2012) and Staples et al. (2011)

tissues, in the Rhone River in France. Miège et al. (2012) quantified BPA in 38% of their 32 fish samples of 4 different species, with mean measured concentrations of BPA of 0.137 ± 0.211 µg of BPA/g of dry weight.

Effects of BPA have been studied in many species at different trophic levels. Flint et al. (2012) propose an extensive review. Just a few papers mentioned macrophytes in their studies (European Union, 2003; Mihaich et al., 2009; Oehlmann et al., 2009; Staples et al., 1998). Effects have been assessed on growth of green algae, *Selenastrum capricornutum* (96 h EC₅₀ based on cell volume: 3100 µg/L) and macrophytes *Lemna gibba* (NOEC based on growth: 7800 µg/L) (Alexander et al., 1988 and Putt in Staples et al., 2008).

Molluscs (Duft et al., 2003; Flint et al., 2012; Jobling et al., 2004; Schirling et al., 2006), crustaceans (Andersen et al., 1999; Flint et al., 2012; Watts et al., 2001b), insects (Flint et al., 2012; Hoshi et al., 2003; Kohra et al., 2002), cnidarians (Fukuhori et al., 2005; Pascoe et al., 2002), rotifers (European Union, 2008) and amphibians (Levy et al., 2004) appear to be affected by BPA, with biological effects observed at relevant environmental exposures in the low ng/L to µg/L range (Oehlmann et al., 2009). In a review of Flint et al. (2012), the nematode *Caenorhabditis elegans* appears to be one of the most sensitive invertebrate species as an exposure to 10^{-9} M (0.228 µg/L) of BPA during 6 days resulted in a significant increase of the number of germ cells (Hoshi et al., 2003). A reduction of its feeding behaviour was observed after 24 h of exposure to a concentration of 100 nM (228 µg/L) (Kohra et al., 2002). Another sensitive class is gastropods for which a significant decrease of the length of penis and prostate gland of *Nucella lapillus* and an induction of a complex syndrome of alterations in female *Marisa cornuarietis* have been shown at 1 µg/L by Oehlmann et al. (2000). An increase of the embryo production has also been shown for *Potamopyrgus antipodarum* exposed to 5 µg/L (Jobling et al., 2004). In the EU's risk assessment of BPA (European Union, 2003), they did not consider the data by Oehlmann et al. in the derivation of a PNEC in view of the apparent instability of the substance under the exposure conditions used, and the possible overlap with natural changes. However, due to the concern from the apparent sensitivity of snails to BPA, they propose a factor of 10 to derive a "conservative" PNEC and ask for further studies on snails.

The effects of BPA on fish were also reviewed (Table 1). To sum up this review, BPA has effects on the structure and composition of gonads at 1 µg/L, VTG was induced at 10 µg/L and egg production and hatchability were affected at 640 µg/L.

Considering the available literature data, there appears to be a clear lack of information for some biological compartments

of aquatic ecosystems, especially macrophytes. Moreover, the impacts of BPA in an ecosystemic context, i.e. with populations from different trophic levels studied at long term in environmental conditions, have never been assessed. Long term studies encompassing a larger portion of the life cycle of the species exposed is expected to provide much more relevant data for ecological risk assessment than laboratory studies (Forbes et al., 2011).

In the work we present here, we studied the long term effects of BPA in aquatic mesocosms. Mesocosms provide the opportunity to simultaneously identify direct and indirect effects of toxicants and to investigate responses at many levels of biological organization in fairly controlled conditions of exposure (Caquet et al., 2000). As ecotoxicological information on the effects of BPA on macrophytes is scarce and considering that submersed macrophytes have major effects on productivity and biogeochemical cycles in freshwaters and as these aquatic plants occupy key interfaces in ecosystems (Carpenter and Lodge, 1986), part of the focus in our study was on this community. We also focused on macro-invertebrates based on their expected sensitivity to BPA, and more particularly on the gastropod *Radix balthica* which is probably the most common freshwater snail in Europe (reviewed by Islam et al., 2001). Finally, we followed the only fish species in our system, *Gasterosteus aculeatus*, as it is the top-species in our mesocosms. The stickleback is a convenient species for mesocosms studies (Roussel et al., 2007a,b). In addition, this fish species offers a potential for the assessment of endocrine disruption (Bernhardt et al., 2006; Björklom et al., 2007; Brian et al., 2006; Jolly et al., 2009; Katsiadaki et al., 2002) and no study on this species has ever been performed to determine the effects of BPA at the population level.

2. Materials and methods

2.1. Mesocosm experiment

2.1.1. Description of the experimental system

The experiment was performed using 12 lotic mesocosms located in the North of France (INERIS, Verneuil-en-Halatte, France, 49.3° N–2.52° E). A detailed description of the mesocosms is provided in Roussel et al. (2007a,b). Each mesocosm is 20 m in length and 1 m in width and is divided into three sections: the upper section (0–9 m), the slope (9–10 m) and the lower section (11–20 m). Prior to the layout of the sediments, each mesocosm was lined with an inert high density polyethylene film (thickness of 0.2 mm). In order to reduce experimental inter-mesocosm variability, artificial fine grain sediments were then placed in the upper and lower

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