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Environmental fish exposure to bisphenol A: what is the level of evidence?

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Abstract. Two years ago, a directive of the European Commission banned the use of baby bottles containing the organic compound bisphenol A (BPA). Nevertheless, BPA is continuously released into the environment from polycarbonate warehouse and is regularly detected in the aquatic environment. Being highly lipophilic, BPA bioaccumulates, a phenomenon that indicates the potential risks posed on the aquatic biota (fish). Toxicological studies demonstrated that at high concentrations (>100-500 µg L^{-1}), BPA is associated with cardiotoxicity and increased mortality, as well as with severe gonadal alterations in fish embryo. Noteworthy, in the environment BPA is usually found in the form of organic micropollutants mixtures, together with other organic toxicants, and therefore additive or synergic effects cannot be excluded. Definition of a cut-off value of BPA concentration in the aquatic environment is still pending and assessment of consequences on fish are based on measurement of global estrogenicity of water samples; preliminary data suggest transcriptomic-based tests will provide evidence for more sensitive and specific assays for BPA effects.
Key Words: aquatoxicology, endocrine disruption, bisphenol A, biological indicator.

Introduction. In 2011, the European Commission adopted a directive (EC directive 2011/8/EU) to ban the use of baby bottles containing the plastic component bisphenol A (BPA) while in 2013 a total of four European countries, i. e. Belgium (Document Legislatif no. 5-338/8), Austria (327th Regulation of the Ministry of Health), Denmark (Regulation 355/1998) and Sweden (Regulation SFS2012:991) limited the use of BPA in containers which come into contact with food for children up to 3 years. According to French Law no. 2012-1442 adopted on 24 December 2012 ("the French BPA Law"), from 2015, BPA in any type of packaging which comes into contact with food will be illegal in France

BPA, a highly lipophilic substance is a main component of polycarbonate plastics to be found in baby and water bottles, food-containers, water supply pipes, cardboards, compact-discs etc. A recent evaluation of 179 toxicants in individuals from the NHANES (National Health and Nutrition Examination Survey) III study 2001-2010, a nationwide cohort from the USA, indicated an inverse relationship between the socioeconomic status of individuals and serum and urinary BPA, phtalates, lead, cadmium and antimony (Tyrrell et al 2013).

BPA is part of xenoestrogenic organic endocrine-disrupting chemicals (EDC), which represent a class of industrial man-made substances able to bind to estrogen receptors (ER) to increase estrogen-dependent gene transcription. In fact, BPA exhibits a more complex mechanism of action targeting multireceptor pathways within the endocrine system which may explain its pleiotropic effects. Apart from binding to estrogen receptors, BPA also exerts glucocorticoid hormones receptor agonistic effects and interferes with thyroid hormones binding to endogenous tissue receptors (Georgescu et al 2012). Recently, the intimate mechanism by which BPA activates ER has been elucidated and it appears to differ largely from that used by 17β-estradiol; that is BPA acts as a

selective modulator of ER (SERM) meaning that it may display estrogen agonist actions in some tissues but not in others (Delfosse et al 2012). In human, modulators of estrogen receptor activity (SERMs) are presently used in the treatment of breast cancer (e.g. tamoxifene, as an ER antagonist in breast tissue but ER agonist in the uterus and the bone) or postmenopausal osteoporosis (e.g. raloxifene, as an ER agonist in the bone but ER antagonist in the uterus and breast). Recognising this mode of action for BPA implies that cells or animal models used in which the risk of BPA exposure to human health is assessed shoud be carefully selected. Even though there is no evidence of BPA binding directly to the androgen hormones receptor, there is evidence that BPA decreases the activity of enzymes involved in testosterone metabolic pathways and exhibits affinity for sex hormone-binding globulin (SHBG) and temporarily increases the levels of serum free testosterone (Hanioka et al 1998). On the other hand, androgens clearly influence BPA metabolism by impacting on BPA clearance by hepatic uridine diphosphate-glucuronosyltransferase activity, thereby increasing serum levels of BPA (Takeuchi et al 2006).

Effects of BPA in toxicological studies in fish. In order to assess potential health risk linked to BPA contamination, toxicology experiments were done in several fish models such as the zebrafish (*Danio rerio*), the medaka (*Oryzias latipes*), the rainbow trout (*Oncorhynchus mykiss*), the fathead minnow (*Pimephales promelas*), the hybrid catfish *Clarias gariepinus*♀ *x Heterobranchus longifilis*♂ *(Heteroclarias)* (Ndome et al 2013) or the common carp (*Cyprinus carpio*). One common trait associated to BPA exposure in fish is increased vitellogenin (VTG) production in males, in a dose-dependent manner, which is a hallmark for the estrogen-mimetic effect of BPA (Keiter et al 2012). The zebrafish is one widely used animal model and suits well for toxicological and toxicogenomic studies and drug screening research. Moreover, the Organisation for Economic Cooperation and Development (OECD) accepts the zebrafish embryo as a valuable biological indicator in the evaluation of EDC (OECD 2006). Phenotipic evaluation of early-life exposure toxicity to BPA in wild-type zebrafish induced significant increased mortality rate and cardiac edema at drinking water concentrations between 1500 and 4500 µg L^{-1}, however, differences were not significant at BPA levels of 500 µg L^{-1} or lower (Lam et al 2012). Other consequences were represented by cardio-facial malformations (brahiocephaly), gastro-intestinal abnormalities, failure of swimmbladder inflation and poor tactile response. The „curved-tail-down" phenotype, suggesting neuromuscular problems was also reported following BPA exposure (Lam et al 2012). Exposure of zebrafish to BPA 1-1000 µg L^{-1} resulted in accumulation of BPA in fish tissues, and this increased as the BPA concentrations to which the fish were exposed did, however, with normal appearance of gonads at external appearance. At high concentrations of BPA (100-1000 µg L^{-1}), an increase in the percentages of atretic follicles was observed along with cell components degeneration (Molina et al 2013). Ecotoxicogenomics studies indicated the fathead minnow might represent a more sensitive species to BPA action compared to the zebrafish, as VTG gene induction even at low BPA concentrations of 10 µg L^{-1} induced a four thousands-fold increase in VTG gene expression as compared to the zebrafish (Villeneuve et al 2012). Further, microarray data analysis of early-life exposure of zebrafish to BPA, tested at pharmacological concentrations of 500-4500 µg L^{-1} strongly evidenced dysregulation of genes involved in brain development, muscular activity and reproduction (Lam et al 2012).

Environmental exposure of fish to BPA. Environmental contamination of fish results from industrial and/or municipal inputs on the aquatic environment. Plasticizers have the potential to enter the water supply though treated and untreated sewage. Relevant BPA concentrations were confirmed in the aquatic environment of the Greater Pittsburgh Area, with levels up to 120 pg g^{-1} and a direct correlation between estrogenicity and BPA (Renz et al 2013). In China, around the Tahu Lake area, BPA was detected in most of the samples of male goldfish (*Carassius auratus*) together with 17β-estradiol, diethylstilbestrol (DES) and other estrogenic compounds; accordingly, reduced gonadosomatic index and elevated serum VTG and estradiol levels were observed in fish

samples in correlation with the cumulated estrogenicity - total estradiol equivalent (EEQ) (Yan et al 2012). In a Spanish study, BPA, phtalates and alkylphenols were identified as the main contributors to the sum of organic micropollutants in the coastal sea waters of NW Mediterranean Sea resulting in a significant pollution risk for fish and other seawater organisms (Sánchez et al 2012). BPA was detected in marine biota samples from coastal waters of Malaysia (Santhi et al 2012) and water samples from the Upper Danube River, Germany, the Mondego River estuary, Portugal, the Swedish coastal waters (Pettersson et al 2007) etc. In contrast, in a large investigation on Austrian surface and ground waters, the environment risk assessment indicated no significant risk upon aquatic environment (fish) (Bursch et al 2004).

Assessment methods of BPA contamination in fresh- and seawater. In the aquatic environment, BPA can be directly quantitated in both drinking water and sewage. Nevertheless, indirect methods are also available, applicable to the detection and quantitation of estrogenic activity (Table 1).

Table 1

Methods for testing estrogenicity of EDC in fish

Method	*Assay*	*OECD recommendation*
	In vivo methods	
Physicochemical analyses	Direct measurement of BPA in water or tissue extracts by LC/MS* or GC/MS*	yes
Sex differentiation studies	Evidence of sex inversion in male fish embryo	yes
Biomarkers of estrogenicity	VTG gene expression in fish tissues	yes
	In vitro methods	
Proliferation studies on cell culture lines: - the human breast cancer cell line MCF-7 (E-SCREEN test) - the recombinanat yeast cell line	Measurement of proliferation rate of MCF-7 cells; by comparing effects of an BPA containing EDC mixture, the relative estrogenic potency can be determined	yes
Competitive receptor binding assays		
Functional reporter gene assays for screening of hormonal activity	Transfection of cells with an estrogen receptor-mediated luciferase gene construct followed by the analyses of an BPA containing EDC mixture	
Transcriptomics-based assays	target-genes expression	

*LC/MS – liquid cromatography/mass spectrometry; *GC/MS – gas cromatography/mass spectrometry.

Particularly, these methods are useful to assess the global (total) estrogenic activity when more than one compound with endocrine-disrupting activity contaminates the aquatic environment. In the laboratory, cell culture and transcriptomics-based assays provide insights into differential regulation of genetic control pathways by various environmental EDC including BPA. Apart from traditional, less specific tests such as the E-SCREEN test, efforts are recently oriented towards detection of sensitive genes to be employed for screening of BPA activity. In that sense, a group of 6 genes (*ncl1, apoeb, mdm1, mycl1b, sp4, U1SNRNPBP* homolog), apparently involved in the brain

development were found to be highly sensitive biomarkers for BPA early-life exposure toxicity in zebrafish (Lam et al 2012).

Conclusions. The aquatic environment represents one large reservoir for EDC. BPA, one man-made xenoestrogen which received interdiction of use on the EU market in baby bottles and in any food containers in few EU countries, is widely detected in water and fish samples. Further studies will clarify the dose-effect relationship of long-standing use of BPA.

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