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Parental trophic exposure to three aromatic fractions of polycyclic aromatic hydrocarbons in the zebrafish: Consequences for the offspring

Prescilla Perrichon [a,b,*], Farida Akcha [a], Karyn Le Menach [c], Manon Goubeau [a], Hélène Budzinski [c], Xavier Cousin [a,d], Paco Bustamante [b]

[a] Ifremer, Laboratoire d'Écotoxicologie, L'Houmeau/Nantes, France
[b] Littoral Environnement et Sociétés (LIENSs), CNRS-Université de La Rochelle, UMRi 7266, 2 rue Olympe de Gouges, F17042 La Rochelle Cedex 01, France
[c] Université de Bordeaux (EPOC, LPTC, UMR CNRS 5805), 351 Cours de la Libération, F33405 Talence Cedex, France
[d] INRA, Laboratoire de Physiologie et Génomique des Poissons, Campus Beaulieu, 35042 Rennes Cedex, France

HIGHLIGHTS

- We examine the toxicity of organic pollutants in a temporal dynamic (multigenerational study).
- We examine the consequences of parental trophic exposure to the first offspring generation at different biological level with exposures at environmentally relevant concentrations.
- We highlight the alteration of two vital functions in fish: the swimming and cardiac performances.
- Increasing information in the next generation will increase the risk prediction.
- The multigenerational studies should be widely undertaken to evaluate the potential for exposed-population to maintain in the future.

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ABSTRACT

In recent decades, PAH emissions due to extensive anthropogenic activities have risen sharply causing considerable pollution of aquatic ecosystems. This pollution represents a threat for organisms, among them are fish. Consequently, prenatal stress can have important repercussions, and may impact survival and population recruitment. To investigate this point, eggs were collected from zebrafish exposed during 6 months by trophic route to three aromatic fractions from two different origins, pyrolytic (PY) and petrogenic (light (BAL) and heavy (HFO) fractions) sources. Chronic dietary exposure of the parents was performed at environmentally relevant concentrations (0.3×, 1× and 3×; 1× represents an environmental concentration measured in French estuary). In order to explore the consequences of parental exposure for the next first generation, toxic responses were studied in both embryos and larvae using a multiscale approach. Toxic effects were assessed by looking at hatching success, developmental abnormalities, photomotor response and heartbeat. The level of PAH metabolites and EROD activity in fish larvae were measured to assess exposure to PAHs. Egg production of parents was significantly reduced compared to the Control; hence little information was available for BAL and HFO offspring. The size of larvae from PY parents was found to increase despite a reduced yolk sac compared to Control larvae. Furthermore, a high level of behavioral stress was observed in larvae originating from parents exposed to threefold the environmental concentration. The cardiac activity was reduced in a concentration-dependent manner for the PY exposure group. No effect was however observed on biotransformation markers (*cyp1a*, EROD), nor on the level of DNA damage for all PY, BAL and HFO offspring. The absence of significant differences in metabolite levels may indicate a potential early depuration of transferred compounds or no PAH-transmission. The disruptions observed at the individual level in the next generation could impact on the longer-term, surviving population.

1. Introduction

In recent decades, increasing industrialization and urbanization has resulted in significant chemical pollution of the aquatic environment (Shen et al., 2013). Among organic pollutants, polycyclic aromatic hydrocarbons (PAHs) are ubiquitous and semi-persistent pollutants in

Abbreviations: BAL, Brut Arabian Light; EROD, ethoxyresorufin-O-deethylase; dpf, days post-fertilization; HFO, heavy fuel oil; HMW, High Molecular Weight; hpf, hours post-fertilization; LMW, Low Molecular Weight; PAHs, polycyclic aromatic hydrocarbons; PY, pyrolytic; PMR, PhotoMotor Response; RT-PCR, real-time polymerase chain reaction.

* Corresponding author at: Littoral Environnement et Sociétés (LIENSs), Université de La Rochelle, UMRi 7266, 2 rue Olympe de Gouges, F17042 La Rochelle Cedex 01, France.
E-mail address: prescilla.perrichon@gmail.com (P. Perrichon).

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the environment (Laflamme and Hites, 1978). They are lipophilic compounds with two or more fused benzene rings. PAHs can be divided into two groups: the Low-Molecular-Weight (LMW) PAHs (PAHs with 2 or 3-benzenic rings) and the High-Molecular-Weight (HMW) PAHs (PAHs presenting more than 3-benzenic rings). PAHs are produced from three pathways: i) pyrolytic processes such as incomplete combustion of organic matter from natural or anthropogenic sources (pyrogenic origin), ii) diagenesis of sedimentary organic matter giving form to fossil fuel (petrogenic origin) and iii) natural precursor transformations during early diagenesis processes (diagenetic origin) (Neff, 1979). Although they may be produced naturally, their predominant emission is from anthropogenic sources, including burning of fossil fuel, coal tar, wood, waste incineration, urban runoff, maritime transport discharges and petroleum spills (Neff, 1979). Depending on their origin, PAHs are generally present in complex mixtures that can be characterized by their molecular fingerprint. Indeed, a predominance of HMW compounds associated with the presence of less or non-alkylated PAHs is representative of a pyrolytic mixture. Conversely, a petrogenic mixture is characterized by a majority of LMW PAHs and the presence of methylated compounds (Abrajano et al., 2003; Sakari, 2012).

Due to their physico-chemical properties, most PAHs exhibit hydrophobic and lipophilic properties making them relatively insoluble in water. They have a high capacity to adsorb onto suspended particles or non-polar matrices which respectively favor their accumulation in sediments and in the lipid tissue of organisms. Fish have the ability to biotransform PAHs to more water-soluble metabolites, hence reducing bioaccumulation and transfer efficiency in higher levels of marine food chains (Meador et al., 1995). Nonetheless, benthic invertebrate prey represent important pathways of PAH exposure for demersal fish. Indeed, many invertebrate species such as mollusks can bioaccumulate high PAH concentrations in their fatty tissues (Peterson et al., 2003; Varanasi et al., 1985) due to the lower biotransformation capability (Bustamante et al., 2012; Lee et al., 1972; Palmork and Solbakken, 1981). PAH toxicity in fish can be directly linked to biotransformation by enzymatic systems such as those from cytochrome P450, which metabolize PAHs into reactive metabolites (Meador et al., 1995; van der Oost et al., 2003). Some of the metabolites produced are more toxic than the parent compounds, presenting, for example, a high reactivity for the DNA leading to genotoxic effects (Le Dû-Lacoste et al., 2013; Wessel et al., 2010, 2012). However, DNA damage is not necessary linked to activation of enzymatic systems such as ethoxyresorufin-O-deethylase (EROD) activity. A case in point is fluoranthene which is not a potential inducer of EROD activity, while its produced-metabolites exhibit genotoxic effects (Wessel et al., 2012). Furthermore, PAH metabolization can also lead to the production of reactive oxygen species (ROS) which may induce an oxidative stress (Frenzilli et al., 2009).

In aquatic organisms, exposure to PAHs can thus lead to detrimental defects from a developmental, physiological and behavioral point of view (Le Bihanic et al., 2015; Incardona et al., 2004, 2011; Perrichon et al., 2014). PAHs' impact on reproductive success is particularly studied due to its consequence on population recruitment. Indeed, PAHs can act as endocrine disruptors that interfere and thus lead to adverse reproductive and developmental effects (Cooper and Kavlock, 1997; Hawliczek et al., 2012; Monteiro et al., 2000a,b). Previous studies showed a reduction of the plasma level of gonad hormones in fish after PAH exposure (Monteiro et al., 2000a; Thomas and Budiantara, 1995). Thomas and Budiantara (1995) highlighted a blockage of sexual maturation, impairments of ovarian recrudescence and induction of oocyte atresia in female Atlantic croaker (*Micropogonias undulatus*) following exposure to water-soluble fractions of diesel fuel and naphthalene. Similarly, a delay in gonad maturation has been reported in female English sole from sites with high concentrations of PAHs (Casillas et al., 1991; Johnson et al., 1988, 2002) as well as in female Atlantic croaker exposed in the laboratory to benzo[a]pyrene (Thomas, 1990). An exposure to a PAH-contaminated sediment has been shown to reduce spawning in sole (Johnson et al., 2002). PAHs can hence have an effect on the parents themselves but also on their offspring. The physiological condition of a female at the time of vitellogenesis was reported as a modulation source for the offspring generation. Prenatal stress (i.e., chemical contamination) could thus affect the optimal development of the early life stage in the future generation (Eriksen et al., 2006; Gagliano and McCormick, 2009; Mccormick, 1999). Uptake of pollutants can occur during ovogenesis and consequently accumulate in the vitellin reserve of the embryos. Relatively few studies reported potential parental transmission of PAHs in offspring generation. Following maternal exposure, the transfer of anthracene in the vitellus of fathead minnow eggs has been shown by Tilghman Hall and Oris (1991). More recently, Pollino et al. (2009) demonstrated a positive correlation between the occurrences of abnormal larvae in offspring with naphthalene-exposed female rainbowfish (*Melanotaenia fluviatilis*). The transfer of PAHs to the embryos was associated with mortality, hatching delay and growth alterations (Hose et al., 1981; Nye et al., 2007; Tilghman Hall and Oris, 1991).

This study aimed to explore the consequences of parental exposure (F_0) to PAHs for the next first offspring generation (F_1) under chronic dietary exposure conditions. Parents were chronically exposed during their whole lifecycle, through diet, to three aromatic fractions of pyrolytic and petrogenic origin, at concentrations which were relevant from an environmental point of view. This study focused on the early life stages of offspring. Multiscale approach was undertaken in order to assess a wide range of fish toxic response, using phenotypic markers (hatching success, developmental abnormalities), behavioral (PhotoMotor Responses), physiological (e.g., heartbeat) and cellular/subcellular markers (DNA damage, apoptosis, oxidative stress). In addition to these effect markers, the degree of exposure to PAHs was evaluated with the level of EROD activity, *cyp1a* induction and PAH-metabolites in offspring larvae.

2. Materials and methods

2.1. Chemicals

The *Arabian light* crude oil (BAL 100) and the heavy fuel oil from the *Erika* fuel oils (No. 2) used for this study were provided by *Cedre* (Centre of Documentation, Research and Experimentation on Accidental Water Pollution, France). PAH spiking of food and chemical analysis were performed with dichloromethane solvent from Acros Organics (Thermo Fisher Scientific, Geel, Belgium). The ethyl-4-aminobenzoate (benzocaïne) was purchased from Sigma-Aldrich (St Quentin Fallavier, France).

2.2. Preparation of PAH-contaminated food

Four sizes of food were prepared (i.e., ≤125 μm, 125–315 μm, 315–500 μm, ≥500 μm) from ground commercial food INICIO Plus 0.5 mm (Biomar, France), in order to adapt the food to the fish morphology throughout the lifecycle (from larvae to adult stages). Each food lot was contaminated by the PAH aromatic fractions as described hereafter. The aromatic fractions were a pyrolytic fraction (PY) extracted from sediments collected in the highly polluted site of Oissel (Seine estuary, France) and two petrogenic fractions extracted from *Brut Arabian Light* oil (BAL) and heavy *Erika* fuel oil (HFO). Extraction and quantification of these aromatic fractions based on the 16 priority PAHs listed by US-EPA were performed according to procedures described in Budzinski et al. (1997) and Cachot et al. (2007). PAH and alkylated PAH concentrations were reported in Vignet et al. (2014) and given in Fig. 1 Supplementary data. Dry food was hence spiked with the three PAH fractions according to Vicquelin et al. (2011). Briefly, the amount of food required for the experiment was mixed with dichloromethane (Solvent Control) and each tested aromatic extract in a round-bottom glass flask. Then, solvent was completely evaporated with a rotary evaporator (RV10