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EDITORIAL

PAHs and fish—exposure monitoring and adverse effects—from molecular to individual level

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Polycyclic aromatic hydrocarbons (PAHs) are a diverse family of more than one hundred compounds, containing at least two aromatic rings. In addition to parent compounds, the PAH family also includes substituted derivatives, bearing one or several alkyl groups, sulfur, or oxygen. In the environment, PAHs are ubiquitous and present as very complex mixtures. They can also be associated with metallic and/or other organic compounds. The composition of PAH mixtures depends on their origin. There are two major types of such PAH mixtures, petrogenic and pyrogenic, which enter the environment through different routes. Petrogenic mixtures originate from oils, including natural oil seeps. They enter the aquatic environment due to harbor activity or soil runoff or as a consequence of oil spills. Pyrolytic mixtures result from the incomplete combustion of organic matter, including fossil fuel, entering aquatic environments through deposits of atmospheric emissions directly into water or soil, followed by soil erosion and runoff. Directly linked to human activity, the release of PAHs into the environment has increased over the last few decades. As an example, the amount of PAHs released into the atmosphere has dramatically increased from under 50,000 tons in 1987 (Eisler 1987) to over 500,000 tons in 2004 (Zhang and Tao 2009).

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PAHs are hydrophobic molecules and are found mainly associated with suspended particulate matter in water. They tend to accumulate in sediments over time. Consequently, sediments are major sinks for PAHs and can also act as secondary sources of contamination in aquatic systems (Hylland 2006).

A large number of publications have reported the toxic effects of PAHs, observed on wild-caught fish after oil spills and after experimental exposures. Studies looking at the former revealed the toxic potential of PAHs and possible mechanisms, while work examining the latter confirmed the toxicity of PAHs, and provided very detailed information on underlying molecular mechanisms. However, in the second case, experimental designs mostly involved waterborne exposure to individual PAHs, and only a handful of studies investigated exposure via sediment and/or trophic transfer to complex PAH mixtures. Mechanisms identified include the triggering of the aryl hydrocarbon receptor (AhR) pathway and downstream molecular cascades. This pathway is, for example, involved in the activation of detoxifying enzymes such as Cyp1 (Billiard et al. 2002).

The ConPhyPoP project ("Contamination et Physiologie des Poissons exposés aux Polluants"; CES 09_002) has been funded by the French National Research Agency (ANR) to investigate contamination and effects of PAHs on early life stages or in whole life cycle experiments in three model fish species: zebrafish *Danio rerio*, Japanese medaka *Oryzias latipes*, and rainbow trout *Oncorhynchus mykiss*. The ConPhyPoP project had two main goals: to characterize the early effects of mixtures approaching situations occurring in the environment to develop early indicators suitable for pollution monitoring by hydrophobic compounds such as PAHs and to identify long-term effects on future fish performances. Besides articles produced as part of the ConPhyPoP project, this Special Issue benefits from the input of many other external contributions, also focused on these two topics.

The first three papers of this issue describe the optimization and use of a sediment-contact assay to assess the toxicity of hydrophobic pollutants. In the first, a reference artificial sediment was developed and validated using three model PAHs: fluoranthene, benz[a]anthracene, and benzo[a]pyrene (Le Bihanic et al. 2014a). Japanese medaka embryos are incubated on the artificial sediment spiked with different concentrations of the studied compounds. Classical developmental endpoints are then investigated. The main advantage of this approach is the known composition of the sediment matrix. In addition, it is totally safe for fish embryos and presents relatively high sorption capacities for hydrophobic compounds. In the two following papers, this approach was applied to evaluate the toxicity of three PAH fractions representative of different situations or sources: a pyrolytic mixture extracted from Seine sediment, a crude light oil (Arabian light), and a heavy oil (Erika oil) in two fish species, medaka and rainbow trout. In rainbow trout, *Oncorhynchus mykiss*, the degree and spectrum of toxicity were shown to vary according to the extract considered. The concentration and proportion of methylphenanthrenes and methylanthracenes appeared to drive the toxicity of the three PAH fractions tested. The minimal concentration causing developmental defects was as low as 0.7 $\mu g\ g^{-1}$ (sum of PAHs), indicating the high sensitivity of the assay with rainbow trout embryos and validating its use for toxicity assessment of particle-bound pollutants (Le Bihanic et al. 2014b). In medaka, the three PAH fractions were shown to delay hatching, to induce deformities, to disrupt larvae swimming activity, and to damage DNA at environmental concentrations. Differences in toxicity levels were observed and are likely related to differences in PAH proportions in the different extracts studied, in particular content in alkylated PAHs and low molecular weight PAHs (Le Bihanic et al. 2014c).

Sediments are generally considered as sinks for persistent organic pollutants and a possible secondary source of contamination for aquatic species. To elucidate the effects of sediment-bound organic pollutants such as PAHs, juvenile rainbow trout were exposed to three resuspended natural sediments with different contamination levels (Hudjetz et al. 2013). Significant differences in bile metabolite concentrations as well as in 7-ethoxyresorufin O-deethylase (EROD) induction compared to controls were observed for all exposure scenarios. The ratio between 1-hydroxypyrene in bile from fish exposed to the three different contamination levels correlated well with the ratio of pyrene concentrations in corresponding sediments and suspended particulate matter. In contrast, hepatic lipid peroxidation and micronuclei formation showed a less conclusive link with contamination. The results of this study clearly demonstrate that firmly bound PAH from aged sediments can become bioaccessible upon resuspension under flood-like conditions and are readily absorbed by aquatic organisms such as rainbow trout.

Oil spills are another major generator of PAHs in certain aquatic environments. In their study, Sturve et al. (2014) reported that eelpout exposed to a bunker oil—both in the field and in controlled laboratory conditions—showed elevated levels of EROD activity and DNA adducts, as well as an increased level of PAH metabolites. Likewise, Danion et al. (2014) showed that sea bass (*Dicentrarchus labrax*) juveniles chronically exposed to a water-soluble fraction of Arabian light exhibited a significant increased EROD activity in their liver, along with a drop in SOD activity, and a greater gill Glutathione content.

Two other articles report analysis of molecular responses after exposure to PAHs and other chemicals. In one case, mature polar cods are exposed to oil (the same Arabian light oil mentioned above), which is either mechanically or chemically dispersed. The authors report reduced O_2 consumption by permeabilised cardiac muscle fibers, indicative of inhibition of complexes I and IV of the respiratory chain in polar cod exposed to mechanically dispersed oil. They also show that dispersant did not increase oil toxicity (Dussauze et al. 2014). In the second article, European flounder juveniles were exposed through diet to a mixture of PAHs and PCBs. Short-term exposure led to expression of detoxification biomarkers, DNA damage, and deregulation of the immune system. Detoxification process biomarkers remained activated for the highest concentration after 2 weeks of recovery (Dupuy et al. 2014).

A number of articles refer to experiments carried out based on ConPhyPoP project specifications, with long-term exposure of zebrafish to the same three environmental PAH mixtures as described above. Exposures were performed at the PEP platform (http://wwz.ifremer.fr/pep) through spiked diets from the first meal and up to more than 1 year. Their effects on survival and growth were monitored throughout the exposure period (Vignet et al. 2014c). This article also report that jaw morphology disruptions observed after embryonic waterborne exposure to AhR agonists also occurred when exposure began later in the subjects' development. This can contribute to growth impairment in addition to observed digestive metabolism disruptions. A second article describes behavioral disruptions in juveniles and adults and shows modification of mobility, a reduction in exploratory activity, and an increase in anxiety levels (Vignet et al. 2014b). A third article describes carcinogenesis occurring in the same fish and revealed a time- and dose-dependent increase in neoplastic disorders with bile duct described as the main target. Paradoxically, no DNA damage has been observed with the comet and micronucleus assays. This apparent discrepancy may be due to the fact that it has been assayed in blood, following an overnight starvation, while a kinetic analysis revealed a quick but transient induction of *cyp1a* expression in hours following feeding (Larcher et al. 2014). A common finding of these three articles is that all three mixtures tested produced disruptions at varying degrees of severity. Heavy oil produced the greatest disruption, followed by light oil, while pyrolytic extract was the least disruptive. This ranking is consistent with that resulting from

the short-term exposures described above. This underlines the major role played by alkylated (particularly methylated PAH) derivatives in the toxicity of these mixtures.

The toxicity of PAH derivatives has also been evaluated in two other articles featured in the Special Issue, oxygenated PAHs and methylpyrene. In one article, the authors exposed Japanese medaka embryos to sediment spiked with pyrene and methylpyrene and revealed similar responses after exposure to both compounds, leading to embryotoxicity characteristics of AhR activation, as well as some gene activation at relevant environmental concentrations (Barjhoux et al. 2014). In addition, methylpyrene also activated the expression of genes involved in cell cycle control, oxidative DNA damage repair, and the retinoid pathway. Oxy-PAH can also be detected in aquatic sediments but knowledge about their toxicity is currently very limited. A screen of seven oxy-PAHs revealed that these pollutants can induce developmental abnormalities, including jaw, heart, and tail cartilage anomalies in exposed Japanese medaka embryos (Dasgupta et al. 2014). Certain oxy-PAHs were also shown to significantly increase DNA damage as revealed by the comet assay. Comparisons between the genotoxic potential of these oxy-PAHs, their corresponding parent PAHs, and the potent mutagenic PAH, benzo[a]pyrene, indicated similar potency. This study pointed out the need for additional data about the environmental occurrence and biologic effects of oxy-PAHs.

Finally, three articles tackle the question of long-term and trans-generational effects. In one, the authors exposed zebrafish embryos to a sediment spiked with a mixture of three PAHs and bred fish until adulthood in clean medium. This early and short-term exposure produced a late disruption of growth and a trend toward a lower reproductive ability. In addition, adults displayed lethargic and/or anxiety-like behaviors. This latter behavior was also identified in offspring (F1) at the larval stage (Vignet et al. 2014a). A second one reports an increase of cardiac frequency in larvae originating from fish exposed to pyrolytic mixture. This is associated to an increase in ATPase, cardiac transporting *atp2a2a* (Lucas et al. 2014). In the third article, Clark et al. pursued their study of a killifish population exposed to PAHs for several decades (Atlantic Wood Industries Superfund; AW). This population had developed remarkable resistance to the acute effects and teratogenesis caused by AhR agonists. In this article, they compared sensitivity to AhR inducers of AW F1 and F2 embryos to embryos obtained from a control unexposed population. In this way, they revealed an impressive resistance of F1 and F2 embryos to express cardiac teratogenesis and to activate expression of *cyp1* genes. It is noteworthy that despite strong resistance to cardiac teratogenesis, repression of *cyp1* and EROD activation is intermediate in AW F2 compared to AW F1. The authors conclude resistance to cardiac teratogenesis in AW fish is conferred by multiple factors, not all of which appear to be fully genetically heritable (Clark et al. 2013).

Taken together, these articles increase our knowledge of the effects of PAHs in fish at several developmental stages and via a number of different routes of exposure. They all lead to the conclusion that whatever the route and the mixture used, exposure to environmental concentrations of PAHs reduces fish fitness and participation in recruitment (i.e., their ability to contribute to the next generation). They also pave the way for new important research, such as that examining mixtures of PAHs and PAHs associated with other chemical and non-chemical stressors, e.g., photoactivation by UV, as well as the need for studies on the effects of PAH derivatives and long-term and trans-generational effects. Considering the major adverse effects of PAHs on reproduction success and development and survival of early life stages, we believe that it is of paramount importance to investigate the effects of PAHs on recruitment and dynamics in fish populations.

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