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Acute effects of Benzo[a]pyrene, anthracene and a fuel oil on biomarkers of the common goby *Pomatoschistus microps* (Teleostei, Gobiidae)☆

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ABSTRACT

The objective of this study was to investigate the effects of two different PAHs and a complex petrochemical mixture on the common goby, *Pomatoschistus microps*, using selected biomarkers as effect criteria. Benzo[a]pyrene (BaP) and anthracene were used as reference substances, while the water accommodated fraction of #4 fuel-oil (#4 WAF) was used as an example of a petrochemical mixture. *P. microps* was used since it is both a suitable bioindicator and a good test organism. Groups of fish were exposed to different concentrations of each of the test substances for 96 h and the activities of several enzymes commonly used as biomarkers were determined at the end of the bioassays. All the substances inhibited *P. microps* acetylcholinesterase (AChE) indicating that they have at least one mechanism of neurotoxicity in common: the disruption of cholinergic transmission by inhibition of AChE. An induction of lactate dehydrogenase (LDH) activity was found in fish exposed to BaP or to anthracene, suggesting an increase of the anaerobic pathway of energy production. On the contrary, inhibition of LDH was found in fish exposed to #4 WAF, suggesting a distinct effect of the mixture. An induction of *P. microps* glutathione S-transferase (GST) activity was found in fish exposed to BaP or to #4 WAF, while an inhibition was observed after exposure to anthracene. These results suggest that GST is involved in the detoxification of BaP and #4 WAF, but not of anthracene. All the substances increased catalase activity and isolated PAHs also increased superoxide dismutase, glutathione reductase and glutathione peroxidase activities, while #4 WAF did not cause significant alterations on these enzymes. These results suggest that all the substances may induce oxidative stress on *P. microps*, with BaP and anthracene apparently having more oxidative stress potential than #4 WAF.

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1. Introduction

Coastal and estuarine areas are productive ecosystems with a high biodiversity, and, thus, they are considered of great ecologic and economic value. Petrochemical products may enter into aquatic ecosystems as a result of harbour activities, petrochemical industry, shipping transport and other anthropogenic activities, as well as from natural sources. In the last decades, fuel oil spills such as the recent accident with the tanker *Prestige* in the Galician coast, have highlighted the ecological and social-economic problems inherent to this class of contaminants.

The NW coast of Portugal belongs to the so-called "risk" area of the Iberian coast regarding shipping accidents due to adverse sea conditions in some periods of the year, maritime currents and characteristics of the coast that make it particularly dangerous for navigation (Lima et al., 2007). Therefore, it is very important to recognize in advance the effects of fuel oils, polycyclic aromatic hydrocarbons (PAHs) and other components of petrochemical products on native organisms, considered suitable for use in the assessment of the impact of potential accidents. Basic knowledge about the potential adverse effects on wild species is also crucial to mitigate effects and to help in population recovery if necessary.

Among petrochemical products, fuel-oils are of special concern because they are widespread in aquatic ecosystems and have been found to have a high toxicity to aquatic organisms. They are complex mixtures that contain PAHs, metals and other compounds (Albaigés and Bayona, 2003). PAHs are known to be determinant for the toxicity elicited by these environmental contaminants to aquatic organisms (Anderson, 1977; Connell and Miller, 1981; Spies, 1987).

Petrochemical products and/or PAHs have been found to induce adverse effects on fish growth (Hannah et al., 1982; Ostrander et al., 1990), reproduction (Thomas, 1988; White et al., 1999; Monteverdi and Di Giulio, 2000) and survival (Collier and Varanasi, 1991; Hawkins et al., 1991). Furthermore, after biotransformation, these compounds may originate reactive products that bind to DNA and may cause mutations or other alterations on the genetic material (Hall and Glower, 1990; Marvin et al., 1995; Woodhead et al., 1999). For example, in fish, the PAH benzo[a]pyrene (BaP) was found to cause mutations in the oncogene *ras* (Rotchell et al., 2001), while the PAH anthracene was found to alter gene expression in the mummichog (*Fundulus heteroclitus*) (Peterson and Bain, 2004). In this species and in the steelhead trout (*Salmo gairdneri*), carcinogenic effects induced by BaP exposure were also found (Black et al., 1988).

In fish, BaP and PAHs in general, are subject to biotransformation in a first step by enzymes of the P450 system. An induction of cytochrome P4501A (CYP1A) has been found in several species exposed to these xenobiotics, including in the Arctic charr (*Salvelinus alpinus*) (Wolkers et al., 1996), in the common carp (*Cyprinus carpio*) (Van der Weiden et al., 1993), in the european eel (*Anguilla anguilla*) (Lemaire-Gony and Lemaire, 1992) and in the turbot (*Scophthalmus maximus*) (Peters et al., 1997). In this first step of the biotransformation of these compounds, several metabolites are formed, some of which are subject to further transformation by conjugation with endogenous substances. A possible pathway is the conjugation with glutathione, a reaction catalysed by glutathione S-transferases (GST), a family of enzymes that is also involved in the prevention of lipid peroxidation (LPO). Glutathione conjugation seems to be an important pathway of detoxification of BaP, at least in some species, since an induction of GST activity has been found in fish exposed to this xenobiotic, including in the Japanese sea bass (*Lateolabrax japonicus*) (Jifa et al., 2006) and in the sea bass (*Dicentrarchus labrax*) (Gravato and Guilhermino, in press). However, inhibition of GST activity after exposure to BaP has also been found, for example in the rockfish *Sebastiscus marmoratus* (Wang et al., 2006). Therefore, the role of this enzyme on PAHs detoxification in fish deserves further research.

PAHs have been also found to induce oxidative stress and to cause lipid peroxidation (LPO) in several fish species (Orbea et al., 2002; Reid and MacFarlane, 2003; Jifa et al., 2006; Gravato and Guilhermino, in press). However, distinct and even contradictory effects of PAHs and fuel oils on anti-oxidant enzymes have been reported. For example, catalase (CAT) activity was found to be increased in the sea bass (*D. labrax*) exposed to BaP (Gravato and Guilhermino, in press) but no changes were found in the same species exposed to 3-methylcholanthrene (3MC) (Lemaire et al., 1996), suggesting that distinct substances may have different effects on this enzyme. In addition, an opposite answer of anti-oxidant enzymes over time has been also reported. For example, during the first days of exposure of the goldfish (*Carassius auratus*) to the water-soluble fraction of a diesel oil, an increase of superoxide dismutase (SOD) activity was observed, while in the next days a gradual decrease was recorded (Zhang et al., 2004), indicating that the time of exposure may also induce different answers from anti-oxidant enzymes. Furthermore, a sort of bell-shaped pattern for these enzymatic activities in response to the increase of the concentration of PAHs has been reported for several fish, including the sea bass (*D. labrax*) and the rockfish (*S. marmoratus*): the activity increases until a certain concentration and then progressively decreases despite the increase of the exposure concentration (Wang et al., 2006; Gravato and Guilhermino, in press). Therefore, since anti-oxidant enzymes of fish have been used as biomarkers in areas polluted with petrochemical products, it is convenient to clarify their pattern of answer to petrochemical products and their components.

Another enzyme that has been used as an environmental biomarker is lactate dehydrogenase (LDH) which is a key enzyme in the anaerobic pathway of energy production, being particularly important for muscular physiology in conditions of chemical stress when high levels of energy may be required in a short period of time (De Coen et al., 2001). Also in the case of fish LDH, contradictory answers to PAHS and fuel oils exposure can be found in the literature. For example, Tintos et al. (2008) observed no significant effects of BaP on rainbow trout (*Oncorhynchus mykiss*) LDH activity, while an increase of LDH activity was found in the crimson-spotted rainbowfish (*Melanotaenia fluvialis*) exposed to the WAF of a dispersed crude oil (Pollino and Holdway, 2003) and inhibition of this enzymatic activity was found in the Atlantic salmon (*Salmo salar*) exposed to the WAF of "Bass Strait" crude oil (Gagnon and Holdway, 1999). Thus, the effects of PAHs on LDH also require more investigation.