Environmental Research 164 (2018) 430–443

Contents lists available at ScienceDirect

Environmental Research

journal homepage: www.elsevier.com/locate/envres

The influence of microplastics and halogenated contaminants in feed on toxicokinetics and gene expression in European seabass (*Dicentrarchus labrax*)

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ARTICLE INFO

Keywords:
PCB
PBDE
Microplastics
Elimination
Gene expression

ABSTRACT

When microplastics pollute fish habitats, it may be ingested by fish, thereby contaminating fish with sorbed contaminants. The present study investigates how combinations of halogenated contaminants and microplastics associated with feed are able to alter toxicokinetics in European seabass and affect the fish. Microplastic particles (2%) were added to the feed either with sorbed contaminants or as a mixture of clean microplastics and chemical contaminants, and compared to feed containing contaminants without microplastics. For the contaminated microplastic diet, the accumulation of polychlorinated biphenyls (PCBs) and brominated flame retardants (BFRs) in fish was significantly higher, increasing up to 40 days of accumulation and then reversing to values comparable to the other diets at the end of accumulation. The significant gene expression results of liver (*cyp1a, il1β, gstα*) after 40 days of exposure indicate that microplastics might indeed exacerbate the toxic effects (liver metabolism, immune system, oxidative stress) of some chemical contaminants sorbed to microplastics. Seabass quickly metabolised BDE99 to BDE47 by debromination, probably mediated by deiodinase enzymes, and unlike other contaminants, this metabolism was unaffected by the presence of microplastics. For the other PCBs and BFRs, the elimination coefficients were significantly lower in fish fed the diet with contaminants sorbed to microplastic compared to the other diets. The results indicate that microplastics affects liver detoxification and lipid distribution, both of which affect the concentration of contaminants.

1. Introduction

Since the 1950s the growth in the world's plastic production has been increasing, comprising in 2015 ~ 300 million tons per year (Plastic Europe, 2016), some of these plastics will end up in the marine environment and with time it degrades to microplastics through physical, chemical and biological processes. Microplastics is becoming an ecotoxicological problem of growing proportions, especially for the aquatic environment (Avio et al., 2017).

Aquatic animals can accidentally ingest microplastic particles that are suspended in water (Ivar do Sul, Costa, 2014). Marine fish drink considerable amounts of seawater to regain the water loss by osmosis. For example salmon smolts drink approximately 10–15% of their body weight per day (Fuentes and Eddy, 1997; Usher et al., 1988; Carroll

Abbreviations: α, assimilation efficiency; α–HBCD, α–hexabromocyclododecane; BFR, brominated flame retardant; $C_{control\ feed}$, contaminant concentration in control feed; C_{feed}, contaminant concentration in feed; C_{fish}, contaminant concentration in fish fillet; *cyp1a*, cytochrome-P450-1A1; DNA, deoxyribonucleic acid; *ef1a*, elongation factor α; F, feeding rate; FCR, feed conversion ratio; GC, gas chromatography; GF, growth factor; GI, gastro intestinal system; *gstα*, glutathione S transferase α; *il1β*, interleukin β; k_{el}, elimination coefficient; $k_{el\ adj}$, elimination coefficient, adjusted for contaminants from control feed; LC, liquid chromatography; HSI, hepatosomatic index; MS, mass spectrometry; PE-HD, high density polyethylene; PE-LD, low density polyethylene; PAH, polyaromatic hydrocarbons; PCB, polychlorinated biphenyls; PBDE, polybrominated diphenyl ethers; Pit-tagging, passive integrator transponder tagging; qRT-PCR, quantitative real-time polymerase chain reaction; RNA, ribonucleic acid; SGR, specific growth rate; $t_{½}$, halflife; w, weight

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https://doi.org/10.1016/j.envres.2018.02.035
Received 15 July 2017; Received in revised form 22 February 2018; Accepted 25 February 2018

et al., 1994), hence they can ingest microplastic particles when drinking. Fish can also ingest microplastic particles when they are inadvertly mistaken by prey (Wright et al., 2013), e.g. de Sá et al. (2015) showed in a trial that fish confused microplastics by prey when exposed to polyethylene (PE)- microplastic particles of similar size and abundance as prey, causing reduced feeding efficiency.

Microplastics can cause physical damage to fish that ingests it, and the type of damage depends on the size of the particles. Microplastic particles in the environment may occur in the defined size range from < 5 mm down to where they become nanoplastic particles (< 100 nm). In the upper size range, microplastic may cause intestinal blockage with decreased nutrition and suffocation (Choy and Drazen, 2013), but smaller microplastic particles are expected to pass through the fish's gastrointestinal (GI) system. However, if the microplastic particles are very small (< 10 μm) they may be transported across the GI system, as was shown in common mussels, *Mytilus edulis* (Browne et al., 2008). The effects of microplastic particles < 10 μm and nanoplastic particles (< 100 nm) on marine organism are not yet fully elucidated. However, Besseling et al. (2014) showed that feeding nanopolystyrene particles to the zooplankton *Daphnia magna* affected their reproduction.

Microplastics can also cause damages in combination with chemicals. For example, microplastic particles can concentrate environmental contaminants and 'carry' them to fish, where they may be bioaccessible and accumulate for example in the lipid fraction of fish fillets (Teuten et al., 2007). Pedá et al. (2016) found histological alterations in the distal part of the intestine after exposing European seabass, *Dicentrarchus labrax* to 0.1% environmentally contaminated polyvinyl chloride (PVC) microplastic particles < 0.3 mm via feed, with the intestinal functions in some cases being totally compromised during the 90 days exposure. Rochman et al. (2014) demonstrated that the brominated flame retardants (BFRs) polybrominated diphenyl ethers (PBDEs) can be transferred from plastic to fish upon microplastic ingestion. The affinity and sorption of chemicals with high log K_{ow} to microplastic is very high, with pre-concentration up to 10^6 times (Mato et al., 2001), the affinity being polymer dependent. Polychlorinated biphenyls (PCBs) for example revealed the highest affinity to PE, followed by polypropylene (PP), compared to other polymer types (Rochman et al., 2013a). Chemicals that exhibit the highest affinity for PE microplastics are also some of those that bioaccumulate and biomagnify in marine organisms and humans, such as PCBs and BFRs. How important microplastics is for the transfer of chemical contaminants into fish, and the extent to which they affect the bioaccessibility have not yet been fully studied. However, model systems have shown that one important parameter for desorption of contaminants from microplastic in the intestine is the presence of digestive surfactants, such as cholesterol-derived bile salts (Teuten et al., 2007; Bakir et al., 2014).

There are currently doubts whether to consider microplastics as a food safety issue. Microplastics is generally found in the guts of aquatic animals that ingest it. For this reason, animals of which the guts are eaten (such as mussels or crabs, for example) may represent a risk to human health. However, exposure studies related to microplastics and their additives/contaminants carried out with mussels confirm that microplastics and its sorbed contaminants do not represent a risk for consumers as they would increase only in insignificant amount the concentration of Bisphenol A, PCBs and polyaromatic hydrocarbons (PAHs) (Li et al., 2016). Moreover, several authors state that the contribution of microplastics to the dispersion of chemical contaminants in the environment where they already are abundant does not seem to be significant (Koelmans et al., 2013, 2016; EFSA, 2016). Some studies, however, highlighted that the effect of chemical contaminants is enhanced by the presence of microplastics (Pedá et al., 2016; Rainieri et al., 2018). In this context, this aspect should be thoroughly considered as it might influence risk assessment evaluation of chemical compounds.

The aim of the present study was to investigate if the presence of microplastics can influence the toxicokinetics, including the transfer of contaminants from feed to fish. Furthermore, this study also estimates if microplastics can influence the adverse effects of a mixture of commonly occurring halogenated contaminants in a commercially important seafood species. Specifically, it assesses if microplastics with sorbed contaminants are able to produce distinct or exacerbated effects compared to clean microplastics in the presence of chemical contaminants.

To reach this aim we performed a bioaccumulation study with European seabass (*Dicentrarchus labrax*) by feeding them with three contaminated feeds and one control. We then evaluated and compared toxicokinetic parameters, hepatosomatic indexes and the differential expression of selected genes involved in the detoxification process and in the immune response.

2. Materials and methods

2.1. Seabass trial

The experiment took place between March 2016 and August 2016 at The North Sea Science Center, Hirtshals, Denmark. The approval (No. 2014-15-2934-01041) of the experiment was given by the Animal Experiment Directorate, The Ministry for the Environment and Food of Denmark. The experiment consisted of a 80 days accumulation period, where European seabass were fed with one of the four diets described below (three contaminated diets + one control) followed by an 51 days depuration period, where all fish were fed the control diet. Juvenile seabass were obtained in February 2016 from a seabass breeder (Ecloserie Marine de Gravelines Ichtus) in Graveline, France, and kept for four weeks in a large holding tank (2 m × 2 m, 4000 L) under flow-through condition with seawater (33 ppt) and heated by a heat exchanger to ~ 20 °C. They were fed to satiation, initially using commercial diets from Biomar A/S.

After four weeks, fish of similar size were transferred to the experimental facilities, which consisted of clear thermoplastic round tanks (diameter 0.81 m, water column height ~ 1.25 m, total water volume ~ 465 L) with the lower third being conical and separated from the rest of the tanks by a grid, allowing rapid sedimentation of faeces and uneaten feed pellets. Heated seawater was pumped to a storage tank, oxygenated with pure oxygen and pumped to the eight experimental tanks at a flow rate of about 110 L h^{-1} per tank. In addition, each tank was equipped with a filter unit to which the water within the tank was pumped at a flow rate of about 500 L h^{-1}, i.e. the water was continuously recirculated within each tank. It served primarily to aerate the water further and to create a current within the fish tank. From the tank outlet the water was led to the sewage system. Seabass were kept at an automated 15 h light/9 h dark cycle, where the light was switched between 7:00 and 7:30 h (using light dimmer) and between 22:00 and 22:30 h. Feeding was done by belt feeders for a period of ~ 4 h per day, starting at 9:00 h. Uneaten feed pellets were collected and counted each day at the end of the feeding period. The temperature was monitored on a daily basis and showed an average temperature of 20.6 ± 1.0 °C throughout the experiment. Oxygen saturation varied during the day due to an increased activity level during feeding hours, where saturation could decrease to about 60%, but increase again to about 90%. Upon transfer to the experimental tanks, fish were anesthetised in benzokain (BENZOAK® VET, 200 mg L^{-1}, diluted as 15 mL per 100 L water), sorted and those of similar size were pit-tagged with 12 mm RFID glass tags (Loligo systems, DK) and randomly distributed to the eight tanks.

A total of 482 fish were transferred, i.e. 60–61 fish per tank, around 5.3 kg per tank (mean ~ 88 g per fish). They were allowed to recover from passive integrator transponder (pit)-tagging and to acclimatise to the experimental tanks for two weeks, during which they were fed the experimental control diet. Pit-tagging did not cause any problems, i.e. no signs of infections, scale loss, or redness of skin. The two week