université de BORDEAUX

THÈSE PRÉSENTÉE

POUR OBTENIR LE GRADE DE

DOCTEUR DE

L'UNIVERSITÉ DE BORDEAUX

ÉCOLE DOCTORALE SCIENCES DU VIVANT, GÉOSCIENCES, SCIENCES DE L'ENVIRONNEMENT

SPÉCIALITÉ : Géochimie et Écotoxicologie

Par Bettie CORMIER

TOXICITÉ DES MICROPLASTIQUES CHEZ LES POISSONS, AU-DELA DE SIMPLE VECTEURS DE POLLUANTS ?

Microplastics toxicity for fish: beyond simple vectors for pollutants?

Sous la direction du Dr Jérôme CACHOT

et la codirection du Dr Steffen KEITER

Membres du jury :

M. HENNER, Hollert	Professeur, Goethe-Universität Frankfurt am Main	Rapporteur
Mme. CARAJAVILLE, Miren	Professeur, Universidad del País Vasco	Rapporteuse
Mme. BAUDRIMONT, Magalie	Professeur, Université de Bordeaux	Présidente
M. GASPÉRI, Johnny	Directeur de recherche, Université Gustave Eiffel	Examinateur
M. COUSIN, Xavier	Chargé de recherche, INRA	Examinateur
Mme BÉGOUT, Marie-Laure	Chercheur, IFREMER	Invitée
Mme MORIN, Bénédicte	Maître de conférences, Université de Bordeaux	Invitée

Titre: Toxicité des microplastiques chez les poissons, au-delà de simple vecteurs de polluants?

Résumé:

Au cours des dernières années, les plastiques ont suscité un intérêt grandissant de la part des médias, du grand public et des scientifiques. Les plastiques ont été classés parmi les polluants émergents, notamment dans le milieu marin, en particulier les microplastiques (< 5mm, MP). De nombreuses études ont rapporté la présence de MP dans le milieu marin (eau de surface et sédiments). Cependant, il n'existe pas de méthodes standards pour évaluer leur toxicité potentielle ainsi que leur rôle en tant que vecteur de polluants au sein des organismes. Cette thèse avait pour objectifs, (1) l'étude de la sorption de polluants organiques modèles sur HDPE et PVC, (2) l'étude de la toxicité de ces composés adsorbés artificiellement sur différents stades de vie du poisson zèbre (*Danio rerio*) ainsi que (3), la caractérisation et l'étude de la toxicité de MP environnementaux. La première partie de cette thèse avait pour but d'étudier les processus de sorption de trois composés modèles : l'acide perfluorooctanesulfonique (PFOS), le benzo[a]pyrene (BaP) et l'oxybenzone (BP3) sur des particules de HDPE de différentes tailles. La seconde partie concernait l'étude de la vectorisation de ces 3 composés modèles adsorbés sur des particules de HDPE en utilisant des stades précoces, des juvéniles et des adultes de poisson zèbre, au travers d'exposition directe ou via des expositions trophiques. La dernière partie concernait la toxicité de MP environnementaux collectés sur deux plages de sable de Guadeloupe (France), en utilisant les mêmes procédures que pour l'évaluation de la toxicité de MP industriels. La caractérisation polymérique et chimique des échantillons environnementaux a également été étudiée. L'étude de la sorption des polluants a permis de montrer des différences de réponses (cinétiques et efficacité de la sorption) dépendant de la taille des particules ainsi que des propriétés chimiques des composés. La toxicité des MP artificiellement dopés avec des composés modèles a d'abord été évaluée selon la ligne directrice de l'OCDE 236 ainsi que lors d'une exposition trophique chronique, débutant à l'état larvaire jusqu'à des poissons âgés de 5 mois. Des biomarqueurs à l'échelle individuelle et moléculaire tel que la croissance, le comportement, l'activité EROD ou la reproduction, ont été suivis au cours de l'exposition. Les résultats montrent une faible toxicité aiguë sur les stades précoces de développement (embryons et larves) exposés aux particules, aux extraits organiques mais également aux lixiviats, de MP industriels dopés ou non, ainsi qu'au MP environnementaux. Néanmoins, l'ingestion des MP au cours de l'exposition trophique a conduit à des effets à long-terme, avec des intensités différentes selon les composés chimiques adsorbés. Les effets toxiques observés incluaient des altérations de la croissance (taille et poids des poissons), des réductions de taux de succès de reproduction, ainsi que des changements d'activité natatoire des larves de la génération F1. En conclusion, cette thèse a permis de mettre en évidence le fait que (1) les MP se comportent comme des vecteurs de polluants mais qu'ils peuvent également (2) induire des effets sublétaux lors d'expositions chroniques par voie trophique sur des juvéniles et adultes de poisson zèbre mais également sur la progéniture F1.

Mots-clés: microplastiques, processus de sorption, poisson zèbre, test embryo-larvaire, toxicité à long-terme, effets sur la croissance et la reproduction

---

Environnements et Paléoenvironnements Océaniques et Continentaux

UMR CNRS 5805

Title: Microplastic toxicity for fish: beyond simple vectors for pollutants?

Abstract:

During the last decades, plastics have gained interest from media, public and scientists. Plastics have been categorized as emerging pollutants particularly in the marine, particularly microplastics (< 5 mm, MPs). Numerous studies reported the occurrence of MPs in the marine environment (surface water, sediments). However, neither standardized nor harmonized methods exist to evaluate their potential toxicity and their role as vector of hazardous chemicals into organism. This thesis had three main objectives, (1) the investigation of sorption of model organic pollutants on HDPE and PVC, (2) the study of the toxic potential of these sorbed chemicals on zebrafish and (3) the characterization and assessment of the toxicity of environmental MPs. The first part of the thesis aimed at the investigation of the sorption processes of three model pollutants; perfluorooctanesulfonic acid (PFOS), benzo[a]pyrene (BaP) and oxybenzone (BP3); on pristine industrial HDPE particles using different sizes and polymer types. The second part studied the vectorization of the three compounds sorbed on HDPE MPs toward zebrafish (*Danio rerio*) embryos, juveniles and adults through direct or trophic exposures, and their toxicities. The third part investigated the toxicity of environmental samples of MPs collected on two sandy beaches from Guadeloupe Island (France), using the same procedure as above, as well as the characterization of additives and adsorbed chemicals through a non-target approach. Study of pollutants sorption demonstrated differences in pattern (e.g. kinetics, sorption efficiency), depending on size particles and chemicals. Potential adverse effects of MPs associated with chemicals were investigated in zebrafish embryos using OECD 236 guideline and by using a chronic trophic exposure from larvae or juveniles up to 5-months old adults. Molecular and individual toxicological endpoints were monitored over the exposure, such as growth, swimming activity, EROD activity or reprotoxicity. Main findings were the low acute toxicity of MPs on early life stages (embryos and larvae) exposed to particles, organic extracts or leachates. Nevertheless, the ingestion of MPs by juveniles and adults led to a significant long-term toxicity, for all tested MPs, albeit with different intensities, including growth alteration, decrease of successful reproduction, as well as hyperactivity observed in offspring F1 of exposed fish. In conclusion, the present work revealed that (1) MPs may play a role in the vectorization of pollutants and (2) may induce significant sublethal effects in juveniles of zebrafish chronically fed with pristine MPs or MPs artificially spiked with pollutants. Same conclusion was observed (3) with environmental MPs.

Keywords: microplastics, sorption processes, zebrafish, embryo-larval assay, long-term toxicity, reproduction and growth effects

---

Environnements et Paléoenvironnements Océaniques et Continentaux

UMR CNRS 5805

Résumé substantiel

Contexte de l'étude

Depuis plusieurs décennies, le plastique est devenu omniprésent et sa production en 2017 a atteint 348 million de tonnes, due à une forte demande ainsi qu'une utilisation dans tous les domaines quotidiens (Andrady and Neal, 2009). Les emballages représentent approximativement 40% de la production globale (PlasticEurope, 2018). Les plastiques sont définis comme des chaînes de polymères obtenus par un processus chimique : la polymérisation, qui consiste à associer des unités appelés monomères. Cependant, les plastiques ne se limitent pas aux polymères et ils peuvent contenir différents additives tels que des plastifiants, retardateurs de flammes, colorant, stabilisateurs, etc. Les additifs sont ajoutés pour modifier et améliorer les propriétés physicochimiques du produit fini tel que sa dureté, sa densité mais également pour protéger le plastique de la dégradation liée à la chaleur, aux rayons UV ou encore à l'oxidation (Andrady, 2011).

L'utilisation excessive d'objets plastiques couplée à une mauvaise gestion et recyclage des déchets, conduisent à un relargage important de débris plastiques dans les milieux aquatiques. En 2010, une évaluation a estimé que 4,8 à 12,7 million de tonnes de plastiques, ont été introduits dans l'océan dû à des défaillances dans la gestion des déchets (Jambeck et al., 2015). Par ailleurs il a été estimé à 5 milles milliards, le nombre de plastiques flottant à la surface de l'ensemble des océans (Eriksen et al., 2014). C'est dans les années 1970 que des petites particules de plastique (0,25-0,5 cm) ont été décrites pour la première fois dans l'environnement marin (Carpenter and Smith 1972).

Des fragments de plastique, appelés microplastiques (MP) ont été décrits en 2004 par Thompson et son équipe, qui ont amené à s'intéresser à ces polluants émergents (Thompson et al., 2004). Les MP ont été définis comme étant des particules de plastique ayant une forme sphérique, granulaire, fragmentaire ou encore sous forme de film ou fibres synthétiques, avec une dimension inférieure à 5 mm (Arthur et al., 2009). Les MP ont ensuite été catégorisés selon leur source, et classifiés selon 2 catégories : primaire et secondaire. Les MP primaires correspondent à des particules fabriquées à l'échelle millimétrique pour la production de plastique par l'industrie de la plasturgie (granulés industriels) ou sous forme de microbilles utilisés pour les cosmétiques (crèmes exfoliantes). Les MP secondaires quant à eux résultent de la dégradation de macroplastiques. Cette dégradation peut être physique, chimique ou biologique (Derraik, 2002; Thompson et al., 2004). Les fibres synthétiques sont également

classées parmi les MP secondaires car elles sont principalement issues du lavage des textiles en machine à laver, et déverser dans les systèmes aquatiques via les stations d'épuration (Browne et al., 2011).

De nombreux additifs sont connus pour leur toxicité et, du fait de leur potentiel relargage à partir des particules de plastiques, peuvent causer des dommages biologiques (Bakir et al., 2014). Au-delà de la présence d'additifs, les MP sont désormais reconnus pour sorber des quantités importantes de polluants organiques comme les polychlorobiphényles (PCB), les hydrocarbures aromatiques polycycliques (PAH), les pesticides, les dioxines ou encore les cosmétiques et produits de soins, présents dans l'environnement (Barnes et al., 2009; Koelmans et al., 2016; Mato et al., 2001; Rios et al., 2007, Rochman, Hoh, Hentschel et al., 2013; Rochamn, Hoh, Kurobe et al., 2013; Ziccardi et al., 2016). Les processus de fragmentation des particules par de nombreux processus physiques, chimiques et biologiques peuvent entrainer la formation de particules plus petites, conduisant à augmenter la surface spécifique des particules et donc la sorption des polluants (Koelmans et al., 2013; Lee et al., 2014; Teuten et al., 2007). Si la sorption des polluants par les MP est un processus déjà connu, la contribution des MP dans la vectorisation des polluants organiques depuis le milieu aquatique vers les organismes reste controversée (Bakir et al., 2016; Koelmans et al., 2016; Lohmann, 2017).

Deux questions scientifiques principales sous-tendent ce travail de thèse : (1) la sorption de polluants peut-elle conduire à transporter les composés dans les organismes aquatiques, et (2), si la vectorisation est avérée, les MP associés aux contaminants peuvent-ils être désorbés dans le biote et conduire à des effets toxiques ?

Diverses études ont mis en avant des effets toxiques liés à l'exposition d'organismes aquatiques aux MP. Par exemple, les microalgues marines Skeletonema costatum exposées à des microparticules de polychlorure de vinyle (PVC) ont vu une inhibition de leur croissance et de leur photosynthèse (Zhang et al 2017). Les niveaux trophiques supérieurs peuvent également être affectés par la contamination aux MP. C'est notamment le cas des huîtres exposées aux MP de polystyrène, pour lesquelles une diminution du nombre d'ovocytes et une diminution de la vitesse des spermatozoïdes ont été démontrées (Sussarellu et al., 2016). Les vertébrés peuvent également être impactés par l'exposition aux microparticules de plastiques. Des particules de MP ont été détectées dans le tractus digestif de bars (*Dicentrarchus labrax*) exposés en laboratoires au PVC (Peda et al., 2016). L'effet de vectorisation de polluants via les MP a été démontré avec l'utilisation de pyrène adsorbé sur des particules de polyéthylène (PE) et polystyrène (PS). Des moules (*Mytilus galloprovincialis*) ont été exposées à ces particules et

un transfert ainsi qu'une accumulation de pyrène dans les glandes digestives ont été observés (Avio et al., 2015), ainsi que la facilitation du transport de contaminants du sédiment à l'arénicole (*Arenicola marina*) ou le medaka (*Oryzias latipes*) (Teuten et al., 2007; Rochman et al., 2013).

Malgré un intérêt scientifique grandissant sur la menace des MP pour les écosystèmes aquatiques, des manques de connaissances persistent, notamment sur les processus de sorption ainsi que les interactions entre les polluants organiques et les MP, et leur potentiel rôle en tant que vecteur de polluants.

Dans cette thèse, les MP ont été utilisés pour étudier leur potentiel rôle en tant que vecteur pour 3 polluants modèles, mais également pour évaluer leur toxicité sur différents stades de vie du poisson zèbre (*Danio rerio*). Les composés modèles ont été sélectionnés en raison de leur présence dans les environnements aquatiques, leur toxicité spécifique mais également pour leur différence de structures et de propriétés chimiques. L'acide perfluorooctanesulfonique (PFOS), le benzo[a]pyrene (BaP) et l'oxybenzone (BP3) sont les 3 composés sélectionnés, et ont été utilisés à 2 concentrations, une concentration environnementale et une concentration toxique basée sur la concentration efficace 50% (EC_{50}) sur embryons de poissons. Parallèlement, des échantillons environnementaux de MP collectés sur 2 plages en Guadeloupe (France), ont été utilisés pour étudier leur toxicité sur *D. rerio*. Différents stades de poisson zèbre, à savoir embryons, larves, juvéniles et adultes ont été exposés aux MP. Afin de mieux comprendre la toxicité des microplastiques sur les poissons, au-delà de simples vecteurs de polluants, cette thèse a été réalisée dans le cadre du projet EPHEMARE de l'appel à porjets JPI Oceans.

Démarche méthodologique

Afin d'étudier les MP en tant que vecteur de polluants, l'étude a été partagé en 3 parties distinctes mais complémentaires, à savoir, (1) l'étude des processus de sorption de composés modèles sur des MP industriels, (2) l'évaluation de la toxicité des MP dopés artificiellement avec les composés modèles sélectionnés sur le poisson zèbre et enfin (3) la caractérisation chimique de MP environnementaux et l'étude la toxicité de ces MP environnementaux sur le poisson zèbre.

1) Processus de sorption artificielle de polluants sur MP industriels

Deux types de MP ont été sélectionnés, le PE (Micro Powders, NY, USA) et le PVC (Fainplast, Ascoli Piceno, Italy), pour leur présence majoritaire dans les eaux de surface et les sédiments (Erni-Cassola et al., 2019). Plusieurs tailles de PE (125-500; 20-25; 11-13; 4-6 µm) ont été sélectionnées pour étudier l'impact de la taille des MP sur les processus de sorption des polluants. La sélection des polluants (PFOS, BaP et BP3) a été guidée par le besoin d'étudier 3 composés aux modes d'action distincts et induisant des effets toxiques spécifiques. Les composés sélectionnés ont précédemment été rapportés comme étant présents dans les milieux aquatiques et étant toxiques pour les poissons. Leur présence dans les environnements aquatiques est liée à leurs sources ou leurs usages importants, PFOS en tant qu'imperméabilisant de textiles, mobiliers ou emballages alimentaires, le BaP provient de la combustion incomplète de la matière organique et le BP3 quant à lui est utilisé comme filtre ultraviolet (UV) dans les crèmes solaires mais aussi comme additif pour protéger le plastique des rayonnements UV. Leur présence dans les eaux continentales et marines de l'ensemble du globe et leur propriété hydrophobe explique leur présence à la surface des déchets plastiques retrouvés en mer.

La sorption des composés s'est faite par mise en contact de différentes concentrations de MP et polluants individuels, dans de l'eau milliQ, pour des durées variables avec une agitation continue par rotation (20 rpm). Une isotherme (BaP et PFOS) a été réalisée, consistant à l'exposition de 2,5 g/L de différentes tailles de PE avec des concentrations variant de 0 à 100 µg/L de BaP ou PFOS (0, 25, 50, 75 et 100 µg/L), pendant 7 jours. Une cinétique portant sur 6 mois a été menée, utilisant du PE (125-500 µm) en contact avec 200 mg/L de PFOS ou 2,5 mg/L de BaP, et des échantillons ont été prélevés après 2, 7, 30, 90 et 180 jours. Des particules de PE ont été également enrobées avec les mêmes composés à des concentrations environnementales afin de permettre l'exposition de poissons pour les tests de toxicité. En plus des processus de sorption, le processus de désorption a aussi été étudié, en simulant artificiellement la désorption de MP dopés au PFOS en utilisant un fluide intestinal artificiel de poisson (basé sur une solution de 15 mM de taurocholate de sodium dans de l'eau de mer artificielle à 35 ‰ avec un pH de 6,7). Toutes les analyses chimiques ont été réalisées sur les liquides (eaux ou fluide de digestion) et les MP avant et après sorption/désorption, afin de quantifier les quantités de polluants dans chacune des phases, après extraction.

2) Exposition des organismes aux MP

Le protocole standardisé OCDE 236 a été le premier protocole utilisé dans cette thèse afin d'évaluer la toxicité aigüe des particules, mais également des extraits organiques obtenus avec du diméthylsulfoxyde (DMSO) et des lixiviats préparés avec ces mêmes MP. Les embryons ont été exposés dès 3 heures post-fécondation (hpf), et jusqu'à 96 hpf, en mesurant le taux de mortalité, les malformations observées au cours du développement, l'éclosion ainsi que l'activité EROD, les dommages à l'ADN (test des comètes) et l'analyse de l'activité de nage. En parallèle, les poissons zèbre ont été nourris avec de la nourriture supplémentée à 1% de MP, à partir du 1er repas (14 dpf) pendant 4,5 mois. La mortalité et la croissance ont été suivis tout au long de l'exposition. A la fin de l'exposition, des prélèvements de poissons ont permis l'analyse de différents biomarqueurs de toxicité tel que l'activité acétylcholinestérase (AChE) liée à la transmission de l'influx nerveux dans les neurones cholinergiques, l'activité EROD liée à la métabolisation des polluants organiques hydrophobes et le stress oxydant mesuré par la peroxydation des lipides (TBARs). L'anxiété a été évaluée par un test comportemental (novel tank) à la fin de l'exposition. A partir de 3 mois, la reproduction des poissons a été étudiée via l'évaluation du succès de ponte, le taux de fécondation et l'effort reproducteur mesuré par le nombre d'œufs/ponte. Les poissons exposés ont été prélevés pour quantifier la présence de polluants dans l'organisme entier et valider la vectorisation par les MP. A la fin de l'exposition, la génération F1, n'ayant jamais été exposée autrement que par les parents F0, a été suivie afin d'étudier la survie et la croissance des larves et estimer la neurotoxicité par un test d'activité natatoire (LPMR).

3) Microplastiques environnementaux

Des MP environnementaux ont été collectés sur deux plages de Guadeloupe (France) en octobre 2017 à l'occasion de l'Odyssée 2017-2021 de la fondation Race for Water. Cette île a été sélectionnée pour sa localisation à proximité du gyre de l'Atlantique Nord. Les débris ont été collectés sur la plage de Petit-Bourg (PB) sur Basse-Terre, une plage proche de la plus importante zone industrielle et portuaire de l'île et sur une plage de Marie-Galante (MG), une île peu peuplée et très préservée de l'archipel de Guadeloupe. Les polymères ont été caractérisés par infrarouge à transformée de Fourrier (FT-IR), et broyés par cryobroyage avant d'être tamisés à 100 et 53 µm. Une analyse granulométrique a également été faite pour établir un spectre granulométrique des fractions obtenues. L'empreinte chimique des échantillons a été

mesurée par une approche analytique non ciblée par chromatographie gazeuse pour les composés organiques (pesticides, hydrocarbures dont HAP, dioxines et PCB), ainsi que par spectrométrie de masse par plasma à couplage inductif pour les métaux. Pour évaluer la toxicité des échantillons environnementaux, les mêmes protocoles que ceux décrient précédemment ont été utilisés.

Résultats de l'étude

Cette thèse avait pour but l'étude des MP en tant que potentiel vecteurs de polluants au sein des organismes aquatiques. L'étude portait plus spécifiquement sur la cinétique de sorption de polluants modèles (BaP, PFOS et BP3) sur des MP de PE et PVC, ainsi l'évaluation de leurs toxicités sur le poisson zèbre et l'imprégnation chimique et la toxicité d'échantillons environnementaux de MP.

Tout d'abord, les polluants modèles ont été adsorbés sur des particules industrielles de PE et PVC, et nous avons observé différents modèles de sorption en fonction de la nature du polluant et du type de polymères utilisés. Cependant, du fait d'un manque de temps au cours de la thèse, le processus de sorption étudié sur le long terme (180 jours) n'a été réalisé que pour le PFOS, tout comme les cinétiques de sorption. Les résultats obtenus ont démontré qu'au cours des 180 jours d'exposition, la concentration de PFOS adsorbée à la surface des MP n'a cessé d'augmenter. De plus, la désorption a été simulée en utilisant du taurocholate de sodium et des MP artificiellement contaminés avec du PFOS pendant 4 jours et nous avons démontré que la désorption avait lieu à hauteur de 70 à 80%. Il apparaît donc que le taurocholate de sodium qui mime le fluide intestinal de poisson, augmente la biodisponibilité du PFOS adsorbé sur les MP. Afin d'approfondir l'étude de désorption, plusieurs prélèvements de MP et de milieu de désorption devraient être pris en compte, ainsi que l'étude de la cinétique de désorption. La méthode développée pourrait ainsi être utilisée comme une nouvelle approche simplifiée pour la compréhension des processus de désorption de polluants depuis les MP au cours de la digestion. Cependant, cette présente étude est limitée dans l'utilisation d'un unique polymère, le PE, avec des concentrations de polluants et MP non environnementales. Des études plus approfondies devraient être menées en utilisant différent types de polymères tel que le PS, le PP ou le PVC, avec des concentrations environnementales de MP ainsi que différents polluants, seuls ou en mélange. Des études sur différents polluants émergents en combinaison avec d'autres polymères sont nécessaires car les processus de sorption et de désorption dépendent à la fois du type de plastique et du polluant.

Afin d'évaluer la toxicité des MP, les stades précoces de développement (SPD) du poisson zèbre ont été exposés aux MP industriels contaminés ou non avec des polluants modèles (PFOS, BaP et BP3) ainsi qu'à des échantillons de MP environnementaux. Cette thèse a permis de démontrer que l'exposition des SPD aux particules de PE de différentes tailles, contaminées ou non avec les polluants modèles choisis, n'ont pas mis en évidence de toxicité aiguë ou d'effets subléthaux en suivant le protocole standardisé de l'OCDE 236. Cependant, l'ajout d'autres critères de toxicité tels que la mesure de l'activité EROD ou l'activité de nage ont montré des effets significatifs des MP sur les SPD de poissons. Par conséquent, le test standard OCDE 236 semble inapproprié pour évaluer la toxicité des MP de type PE et PVC sur embryons de poisson zèbre. Il faut préciser que l'activité de nage des larves n'a pas été altérée après exposition aux particules de MP, mais ce sont les extraits organiques des MP environnementaux qui ont conduit à une hypoactivité des larves. La composition chimique des MP environnementaux semble être responsable de la toxicité observée. En effet, l'échantillon de MP de Petit-Bourg s'est avéré contaminé par un grand nombre d'hydrocarbures et d'éléments traces métalliques (cuivre et zinc), probablement lié à la proximité de la zone industrielle et portuaire de Pointe-à-Pitre. De la même façon, l'exposition des SPD aux lixiviats de ces MP environnementaux a démontré des effets sublétaux, renforçant l'idée d'une toxicité liée aux polluants adsorbés et aux additifs plus qu'aux particules elles-mêmes. Il apparaît donc que le test embryonnaire sur poisson zèbre OCDE 236 soit inadéquat pour évaluer et caractériser les effets toxiques des MP, l'ajout d'autres critères de toxicité subaiguë est nécessaire pour accroitre la sensibilité du test. De plus, l'utilisation d'autres poissons modèles avec un développement embryonnaire et larvaire plus long, tel que le médaka japonais ou le médaka marin, peuvent être nécessaires pour mieux caractériser la toxicité d'échantillons de MP.

Pour finir, une contamination trophique a été menée en exposant des juvéniles de poisson zèbre aux différents type de MP, pendant 4,5 mois. Cette exposition a induit des effets délétères autant sur les adultes exposés que sur la progéniture non-exposée F1. En effet, la croissance des femelles a été réduite associée à une diminution du succès de reproduction. Ces effets peuvent être liés à certains additifs présents dans les MP et/ou aux polluants sorbés, qui peuvent être directement biodisponibles lors de l'ingestion. Les effets observés (diminution de la survie et/ou modification de l'activité de nage) pour la progéniture F1 peuvent avoir été induits par un transfert parental des polluants ou par des effets directs sur la physiologie des parents menant à une qualité moindre des embryons de la génération F1. La composition chimique des MP peut expliquer la différence de toxicité observée entre les différents MP testés. Cette étude soulève

la question du risque environnemental lié aux MP environnementaux sur les organismes aquatiques, et particulièrement sur les effets intergénérationnels des MP.

Pour conclure, les principaux objectifs de cette thèse ont été remplis, bien que certains résultats doivent encore être confirmés ou complétés. En effet, les différents processus de sorption de polluants modèles sur des particules de MP ont démontré une capacité de ces MP à sorber et donc à transporter des polluants à des concentrations importantes. Par ailleurs, la désorption du PFOS a été observée après simulation artificielle de la digestion. L'utilisation des SPD du poisson zèbre en utilisant les critères de toxicité de la ligne directrice de l'OCDE 236 semble insuffisamment sensible pour conclure sur la toxicité des MP. Cependant, les résultats obtenus après exposition des SPD aux extraits organiques et aux lixiviats de MP environnementaux ont permis de démontrer la toxicité des polluants sorbés à la surface de ces MP sur les stades précoces de développement de *D. rerio*. Enfin, l'exposition trophique a confirmé la toxicité des différents MP testés en induisant des effets délétères chez les adultes exposés ainsi que pour la progéniture non exposée, confirmant le rôle des MP en tant que vecteur de polluants.

References

Andrady A. L. (2011). Microplastics in the marine environment. Marine Pollution Bulletin. 62, 1596-1605

Andrady A. L., Neal M. A. (2009). Applications and societal benefits of plastics. Philosophical Transactions of the Royal Society B: Biological Sciences. 364, 1977-1984

Arthur C., Baker J. E., Bamford H. A. (2009). Proceedings of the International Research Workshop on the Occurrence, Effects, and Fate of Microplastic Marine Debris, September 9-11, 2008, University of Washington Tacoma, Tacoma, WA, USA.

Avio C. G., Gorbi S., Milan M., Benedetti M., Fattorini D., d'Errico G., Pauletto M., Bargelloni L., Regoli F. (2015). Pollutants bioavailability and toxicological risk from microplastics to marine mussels. Environmental Pollution. 198, 211-222

Bakir A., O'Connor I. A., Rowland S. J., Hendriks A. J., Thompson R. C. (2016). Relative importance of microplastics as a pathway for the transfer of hydrophobic organic chemicals to marine life. Environmental Pollution. 219, 56-65

Bakir A., Rowland S. J., Thompson R. C. (2012). Competitive sorption of persistent organic pollutants onto microplastics in the marine environment. Mar Pollut Bull. 64, 2782-2789

Barnes D. K. A., Galgani F., Thompson R. C., Barlaz M. (2009). Accumulation and fragmentation of plastic debris in global environments. Philosophical Transactions of the Royal Society B: Biological Sciences. 364, 1985-1998

Browne M. A., Crump P., Niven S. J., Teuten E., Tonkin A., Galloway T., Thompson R. (2011). Accumulation of Microplastic on Shorelines Woldwide: Sources and Sinks. Environmental Science & Technology. 45, 9175-9179

Carpenter E. J., Smith K. L. (1972). Plastics on the Sargasso Sea Surface. Science. 175, 1240

Derraik J. G. B. (2002). The pollution of the marine environment by plastic debris: a review. Marine Pollution Bulletin. 44, 842-852

Eriksen M., Lebreton L. C. M., Carson H. S., Thiel M., Moore C. J., Borerro J. C., Galgani F., Ryan P. G., Reisser J. (2014). Plastic Pollution in the World's Oceans: More than 5 Trillion Plastic Pieces Weighing over 250,000 Tons Afloat at Sea. PLOS ONE. 9, e111913

Erni-Cassola G., Zadjelovic V., Gibson M.I., Christie-Oleza J.A. (2019). Distribution of plastic polymer types in the marine environment; A meta-analysis. Journal of Hazardous Materials. 369, 691-698

Jambeck J. R., Geyer R., Wilcox C., Siegler T. R., Perryman M., Andrady A., Narayan R., Law K. L. (2015). Plastic waste inputs from land into the ocean. Science. 347, 768

Koelmans A. A., Bakir A., Burton G. A., Janssen C. R. (2016). Microplastic as a Vector for Chemicals in the Aquatic Environment: Critical Review and Model-Supported Reinterpretation of Empirical Studies. Environmental Science and Technology. 50, 3315-3326

Koelmans A. A., Besseling E., Wegner A., Foekema E. M. (2013). Plastic as a Carrier of POPs to Aquatic Organisms: A Model Analysis. Environ Sci Technol. 47, 7812-7820

Lee H., Shim W. J., Kwon J.-H. (2014). Sorption capacity of plastic debris for hydrophobic organic chemicals. Science of The Total Environment. 470-471, 1545-1552

Lohmann R. (2017). Microplastics are not important for the cycling and bioaccumulation of organic pollutants in the oceans—but should microplastics be considered POPs themselves? Integrated Environmental Assessment and Management. 13, 460-465

Mato Y., Isobe T., Takada H., Kanehiro H., Ohtake C., Kaminuma T. (2001). Plastic Resin Pellets as a Transport Medium for Toxic Chemicals in the Marine Environment. Environmental Science & Technology. 35, 318-324

Pedà C., Caccamo L., Fossi M. C., Gai F., Andaloro F., Genovese L., Perdichizzi A., Romeo T., Maricchiolo G. (2016). Intestinal alterations in European sea bass *Dicentrarchus labrax* (Linnaeus, 1758) exposed to microplastics: Preliminary results. Environmental Pollution. 212, 251-256

PlasticsEurope (2018). PlasticsEurope: Annual review _ The Facts 2017-2018.

Rios L. M., Moore C., Jones P. R. (2007). Persistent organic pollutants carried by synthetic polymers in the ocean environment. Marine Pollution Bulletin. 54, 1230-1237

Rochman C. M., Hoh E., Hentschel B. T., Kaye S. (2013). Long-Term Field Measurement of Sorption of Organic Contaminants to Five Types of Plastic Pellets: Implications for Plastic Marine Debris. Environmental Science & Technology. 47, 1646-1654

Rochman C. M., Hoh E., Kurobe T., Teh S. J. (2013). Ingested plastic transfers hazardous chemicals to fish and induces hepatic stress. Scientific Reports. 3, 3263

Sussarellu R., Suquet M., Thomas Y., Lambert C., Fabioux C., Pernet M. E. J., Le Goïc N., Quillien V., Mingant C., Epelboin Y., et al. (2016). Oyster reproduction is affected by exposure to polystyrene microplastics. Proceedings of the National Academy of Sciences. 113, 2430

Teuten E. L., Rowland S. J., Galloway T. S., Thompson R. C. (2007). Potential for Plastics to Transport Hydrophobic Contaminants. Environmental Science & Technology. 41, 7759-7764

Thompson R. C., Olsen Y., Mitchell R. P., Davis A., Rowland S. J., John A. W. G., McGonigle D., Russell A. E. (2004). Lost at Sea: Where Is All the Plastic? Science. 304, 838

Zhang C., Chen X., Wang J., Tan L. (2017). Toxic effects of microplastic on marine microalgae *Skeletonema costatum*: Interactions between microplastic and algae. Environmental Pollution. 220, 1282-1288

Ziccardi L. M., Edgington A., Hentz K., Kulacki K. J., Driscoll S. K. (2016). Microplastics as vectors for bioaccumulation of hydrophobic organic chemicals in the marine environment: A state-of-the-science review. Environ Toxicol Chem. 35, 1667-1676



Sea Globe
Max Liboiron
2014

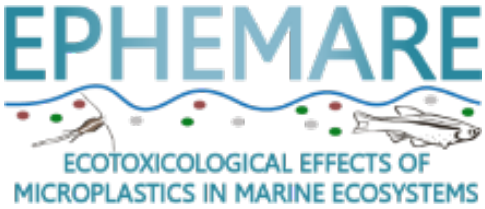
The greatest threat to our planet is the belief that someone else will save it.

Robert Swan

Funding

This work was developed under the EPHEMARE project (Ecotoxicological effects of microplastics in marine ecosystems), supported by national funding agencies within the framework of JPI Oceans (FCT JPIOCEANS/0005/2015; FORMAS, 2015-01865; ANR-15-JOCE-0002-01).

I was directly supported by an IdEx grant (Initiative d'excellence de l'Université de Bordeaux) from the University of Bordeaux.





















Aknowledgments // Remerciements

First, I would like to thank my thesis examiners, Miren Carajaville and Henner Hollert. You spent time on reading my PhD manuscript and you gave me the opportunity to get a constructive discussion during my defense. Also, a special thanks to Magalie Baudrimont, Johnny Caspéri, Bénédicte Morin, Marie-Laure Bégout and Xavier Cousin for being part of my PhD defense and for your interesting contribution to my PhD defense.

I will continue in English, for my Swedish team, starting with Steffen. In August 2016, I came to Sweden, without knowing the university, the city nor the country! I was your first Licenciate student in Sweden so we had to learn almost everything together. It was not always as easy as I expected, but we finally did it and I obtained my Licenciate, and now, my PhD. Thanks for giving me the opportunity to join the Swedish team; you let me develop myself as well as my scientific project.

Anna Kärrman, Magnus Engwall, Maria Larsson and Leo Yeung, I would like to thank you for your scientific support and the time we spent together. The PhD was not only about work, and thanks to Sweden, I met awesome persons! Nikolai, thanks for everything we shared together, unfortunately with the Covid-19, you were not able to come to Bordeaux to celebrate together, but in France, we say, "Ce n'est que partie remise", meaning "let's do it another time", See You Soon!

Special thanks goes to my office mate, Christine. Darling, we had so much fun together, sunbathing behind the window in our office, the (fake) proposal, our teatimes, language exchanges, the "sampling" of microplastics in Gotska Sandön…! I wish you all the best in your future life, and I hope we will meet, in France, in Germany or somewhere else…! In addition, thanks to all of my colleagues in Sweden, with a special thought for my 3 Swedes: Greta (and Vega), Mio and Daniel. I wish you all the best with your family, and the best in your scientific choices. I hope to see you all when I will come back to Örebro!

In Sweden, I also met friends, Marta, Han and Ravi, you were not part of my lab, but we spent a lot of time together, and thanks to all of you, I really appreciated my free time in Sweden (Midsommar, Kårhus, Boda Borg, Strömpis…).

I would like to thanks all of my EPHEMARE colleagues, with special thanks to Francesca Garaventa, Stefania Gorbi and Francesco Regoli, with whom I spent unforgettable days in Guadeloupe! I take advantage of the Guadeloupe period, to thanks the Race For Water team, for their collaboration. It was a pleasure to spend time on this impressive boat, and to participate to the Race for Water Odyssey from the inside! Special thanks to Kim, Pascal, Annabelle, Bunny, Anne, Basile and Peter, it was an awesome journey and I will never forget it!

※※※※※※※※※※※※※※※※※※※※※※※※※

Avant toute autre personne, cette thèse a été possible car il y a 4 ans, Jérôme, tu as cru en moi et tu m'as proposé un stage « sur mesure » pour que je découvre l'Ecotoxicologie dans le monde de la recherche, et après quelques semaines de stage, tu me parlais déjà de la continuité en thèse. C'est grâce à toi, parce que tu as cru en moi et que tu m'as offert l'opportunité de postuler pour cette thèse, que j'en suis là aujourd'hui. Alors un grand merci, car cette épreuve qu'est la thèse, restera un très bon souvenir. Au-delà de l'aspect scientifique où tu m'as soutenue, il y a eu aussi tous les moments à côté, qui sont tout aussi importants. Notamment les moments passés en conférences et une pensée spéciale pour notre séjour en Guadeloupe ! Aussi, je pense que peu de doctorants fêtent l'anniversaire de leur directeur de thèse (promis je ne dirai pas ton âge !) en allant observer les grues cendrées, suivi d'un super repas au restaurant avec toute l'équipe, comme une famille ! Parce que plus largement, le B2 ça a été ça, comme une grande famille ! Nos galettes/crêpes du jeudi, nos pauses thés et gâteaux, nos déjeuners tous ensemble, les repas de fin d'année ou de célébrations, nos discussions, nos fous rires et j'en passe. Même si je n'ai pas la prétention d'avoir marqué le B2 avec ma thèse, j'ai au moins la prétention d'avoir marqué le B2 avec les rillettes et la FAMEUSE tarte aux rillettes ! Et ça, j'en suis sure, ça restera dans les annales du B2 !

Merci Béné d'avoir été présente, pour nos discussions, ton aide pour tenter de développer le test comète sur poisson zèbre, nos différents moments passés à bord du Race For Water à travailler, bronzer, manger et rigoler ! Blandine, l'amoureuse des chats, notre grand point commun, merci pour nos longues discussions intéressantes le midi, et merci encore pour le lys qui a trouvé sa place chez mes parents et qui donne de magnifiques fleurs ! Christelle, ma « superwoman du laboratoire », je me répète parce que je le pense, mais sans toi, le B2 ne serait plus le même. Merci à toi d'avoir été présente, de m'avoir toujours soutenue, quand je baissais les bras, tu étais là pour me dire de ne pas abandonner et d'aller jusqu'au bout ! Merci pour tout ton réconfort, et tous les partages sur les voyages, les T4, et tout le reste. Prends soin de toi, des tiens, et continue de lâcher prise car la vie, la vraie et l'intense, se trouve en dehors du labo, et tu le sais aussi bien que moi. Continue de voyager et de nous faire rêver avec tes récits d'aventures ! Merci également à toi Florane, on a partagé les galères du projet ensemble, mais pas que. Avec ton recul d'ancienne doctorante au B2, tu m'as aiguillé plus d'une fois et tu m'as aidé à me développer en tant que doctorante au labo. Merci à toi, et je te souhaite de réussir ton concours, et de t'épanouir à 200 % dans ton boulot, et dans ta vie de famille, ici ou ailleurs. J'ai également une pensée pour Charlotte, la relève des doctorants du B2. Nous nous sommes vraiment découvertes à Lanzarote, et ça a été un plaisir de partager ces quelques jours, les 2 privilégiées sur l'île. Je te souhaite le meilleur pour ta thèse, n'oublies pas de profiter de cette période, longue et courte à la fois, c'est une très belle aventure, bien que compliquée parfois, mais tu vas y arriver, courage à toi ! Et bisous à Léon et aux poissons, prends bien soin d'eux, et de la Grosse Bettie !

Au cours de ma thèse j'ai également eu un super soutien de la part de Xavier et Marie-Laure. Vous n'allez pas le découvrir ici, mais votre présence et votre soutien au cours de ma thèse ont été hyper

importants ! Quand rien n'allait, vous étiez les premiers au courant, et toujours prêt à me remonter le moral… Double peine pour vous, car il y avait Mel et moi, dans le même bateau, mais vous avez toujours été bienveillants, et très présents. Merci aussi pour tout ce qui s'est passé en extérieur, les pizzas partagées de l'Houmeau, les repas et l'accueil chez vous, les conférences etc. Mais surtout, merci mille fois pour le réveil avec Cloclo, ça restera un des grands fous rires de ma thèse !!! En bref, merci, merci, merci à tous les 2 pour votre accueil à l'IFREMER ou chez vous, et pour tout le reste ! Vous êtes 2 personnes formidables, et pas uniquement des supers encadrants, mais bien des chouettes personnes !

Au milieu de ces personnes qui avaient choisi la voie de la recherche depuis des années, j'ai également eu la chance de partager mon projet (et aussi mes galères !) avec plusieurs stagiaires, et je voulais vous remercier pour votre contribution. Ezvin, le premier et l'unique, malgré ton mauvais Karma avec les zebra, j'ai beaucoup aimé les moments partagés ensemble, à écoper la salle d'élevage, à observer les œufs devenir blancs, mais aussi à fêter Midsommar ensemble et à te voir t'échouer avec ta bouée. Merci d'être toi et d'être venu partager mes galères en Suède, et tout le reste. Puis, retour en France, et Sarah tu as ressurgi dans ma vie, improbable. Une rencontre au début de nos études (DUT au Mans !), 2 chemins qui se sont séparés, et au cours de ma thèse, un SMS pour une recherche de stage, et voilà que tu es devenue ma stagiaire. Merci à toi, car tu as été une scientifique de qualité, toujours des remarques pertinentes et une rigueur scientifique à toute épreuve (même le soir et les weekends), les (trop) nombreuses étiquettes colorées collées et décollées. Tu ne t'es jamais plainte des horaires, de la charge de travail etc. Mais nous avons également partagé de chouettes moments ensemble, pour que tu découvres Bordeaux et ses alentours, et que ton stage ne corresponde pas uniquement qu'aux semaines de boulot. Je suis contente de la suite que tu as donné à ce stage avec ta nouvelle vie au Québec, et je te souhaite pleins de belles choses à vivre là-bas, et j'espère bien pouvoir venir te voir rapidement ! Le dernier, celui qu'on ne peut pas oublier… Francesco ! Tu n'étais pas mon stagiaire, mais un stagiaire du B2, et pas des moindres. Ta joie de vivre, ton accent, ton humour, j'ai tout aimé chez toi, et je t'ai pris sous mon aile, comme un petit frère. Tes débuts en France ont été difficiles, mais j'espère avoir réussi à apporter un peu de bonheur dans ton passage à Bordeaux, en partageant des restos, toutes nos pauses clopes, le weekend à la Rochelle et nos journées au labo. Merci d'être venu au B2, tu as rendu mon année en France que plus belle par ta présence ! J'ai rencontré une très belle personne, tu m'as beaucoup aidé pendant ma thèse, sans rien attendre en retour, juste un sourire et de beaux moments partagés. A presto amico e grazie !

Grâce au B2, avant ou pendant ma thèse, j'ai eu la chance d'avoir un vrai clan autour de moi : Pauline, Shannon, Béber et Perrine. Perrine, nous n'avons passé que très peu de temps ensemble au B2, mais c'est par la suite que nous avons partagé du temps, au milieu de notre Clan du B2, merci pour tes conseils sur la thèse et de m'avoir montré que la vie d'après thèse était à portée de main ! Beber, je ne sais pas à quel moment on s'est rencontré et devenus amis, mais ce n'est pas important. Nous avons partagé les galères de la thèse, mais aussi et surtout les points positifs. Tu as réussi à terminer ta thèse

malgré toutes les péripéties que tu as vécu, tout en continuant de m'encourager. Merci de m'avoir permis d'être à vos côtés (toi et Bérangère) pour votre mariage et d'avoir fait vivre notre amitié au-delà du labo. Merci aussi de m'avoir hébergée à mon retour en France : un canapé, les pets de Lupin, un paquet de clope, du pain et toi, étaient suffisant à mon bonheur ! Tu es un grand fou, mais merci d'être ce fou car nos délires m'ont permis de me vider la tête et d'avancer au cours de ces 4 années. Ma Shannon, le rayon de soleil du B2, la gentillesse incarnée, la personne la plus adorable que j'ai eu la chance de rencontrer, et c'est avec toi que j'ai commencé la recherche. Tu m'as offert la possibilité de partager ton projet de recherche et de devenir ton amie. Merci pour tous tes petits mots, merci pour les nombreux éclairs au café que tu m'as ramené quand j'étais déprimée au bureau. Tu es une personne formidable qui cherche à faire le bien autour d'elle, et tu y arrives parfaitement bien, et j'ai eu la chance de faire partie de ton entourage. Pour finir, ma Pauline, un seul merci ne suffirait pas. Tu as été une collègue, mais très rapidement une copine, puis une véritable amie. Tu es d'une simplicité et d'une gentillesse sans faille. Merci pour tes conseils et encouragements dont ta fameuse phrase : « la thèse n'est pas un sprint, mais un marathon, le but c'est de tenir sur la durée et de pas s'épuiser dès la première année ». Et s'il y a une phrase qui a marqué ma thèse c'est bien celle-là ! Merci de ton soutien, ton aide, et de ton amitié. Une grosse pensée à toutes les « pièces rapportées » de ce clan : Klet, Eudis, Bérangère et Romain, sans oublier les bébés (qui ne sont plus des bébés mais je les vois pas grandir !) : Priam et Kiara (et aussi Lupin !). Pour finir, merci encore à mon clan du B2 pour nos weekends tous ensemble : à Orléans, à Bordeaux, à Wargnies-le-Grand et à Disney ; vous êtes des amis formidables !

Merci également à mes copines de master, et copines de galère en thèse ! Ma Cannelloni, au cours de ma thèse nous nous sommes retrouvées partout autour du monde, Costa Rica, Guadeloupe… Merci de m'avoir soutenue et de m'avoir fait découvrir ton île, d'avoir partagé la mission scientifique avec nous, et pour toutes les soirées à tes côtés ici et ailleurs ! Merci également à ta maman de m'avoir accueillie comme sa fille pendant plusieurs jours chez elle. Merci ma copine, et comme on s'est toujours dit, « à très vite, dans un nouveau pays » ! Merci aussi à Eli, ce petit bout de femme venu de Brno pour un semestre de master, et qui n'est finalement jamais repartie. On a partagé le master mais aussi et surtout la période de thèse. Discrète mais toujours présente, merci pour tous les partages de culture, de langage et de spécialités de chez toi et de chez moi. Nos échanges étaient riches et intéressants, et c'est aussi un des aspects positifs que je retiens de ma thèse, l'interculturalité. Merci d'avoir fait partie de ma thèse, et bon courage à toi pour la fin de la tienne, c'est la dernière ligne droite ! Ma Tia, que dire de toi… Une personne formidable, avec ton sourire ancré sur ton visage rayonnant. Ton accent, ta bonne humeur (peu importe les circonstances de la vie), ta présence. Merci pour tout ça, tu m'as apporté de très belles leçons de vie au cours de ces 6 années, ne jamais perdre le moral car la vie continue et il faut se battre. Tu es une très belle personne ma Tia, je suis fière de t'avoir comme amie, et encore plus fière de la femme que tu es devenue, forte et courageuse. Ne lâche rien ma jolie Tia, c'est également la dernière ligne droite pour toi, avant de retrouver ton île bijou et tes proches, où j'espère pouvoir venir te voir et

découvrir ta vie de sirène ! Pour finir ma Flo, une amitié débutée en master qui n'a fait que grandir. Je me rappelle encore de ton appel pour m'annoncer que tu étais prise en thèse à IRSTEA, j'étais aussi heureuse que le jour où j'ai su que j'étais prise en thèse. J'espère que tu ne m'en voudras pas avec ta fameuse phrase : « je ne sais pas si je dois dire grâce ou à cause de Bettie, j'ai fait ma thèse » ☺. Nous avions fini major et seconde de promo de master, en se soutenant et révisant ensemble pendant ces 2 années, et cette complicité s'est confirmée en thèse. Nous nous sommes soutenues, et nous étions présente l'une pour l'autre au moment de la soutenance, dans nos conditions si particulières, merci le Covid ! Merci d'avoir été présente pendant ces 6 années, et merci de continuer à être mon amie, au-delà de nos études. Loïse, merci aussi pour tes gazouillis ma petite pepette, tu m'as fait rire, et tu as rendu mes venues à Bordeaux plus sereines et familiales !

La Suède m'a fait rencontrer 2 personnes en particulier, mes 2 acolytes : Mel et Flo. Merci commun pour les moments ensemble : la piscine (sauna, jacuzzi et hammam, et aussi pesées hebdomadaires), Pinchos, Khana Khazana, Fratteli, Espresso House… Bref on a passé notre temps à bouffer, mais c'était bon pour le corps et pour l'esprit alors aucuns regrets, surtout avec nos séances au gymnase ! Flo, mon Loulou, dès ton arrivée en Suède, on s'est rapproché comme les 2 nouveaux français débarquant à Örebro. Et une vraie amitié est née de cette rencontre, nous nous sommes soutenus l'un et l'autre, on a partagé notre bureau, et nos moments de joie et de désillusions. Merci d'avoir été présent pendant toutes ces années, et un énorme merci pour la surprise à mon retour du Costa Rica, avec tous les mots de mes proches pour me remonter le moral, ça restera un moment très fort de ma thèse. Tu as pris soin de moi, pour que je me sente toujours bien au cours de ces années, en tant qu'ami, alors merci. Mel, que dire de toi… Tout mon entourage te connaît parce que j'ai passé 4 ans à parler de toi ! Nous étions dans la même galère toutes les 2, avec la même direction, les mêmes problèmes liés aux poissons et tout le reste. Ça nous a rapprochées, mais ce n'est pas tout, car nous avons appris à nous connaître et à nous aimer, avec nos ressemblances mais aussi nos différences. Tu as été un moteur pendant la thèse, nous n'avons pas baissé les bras, on en a chié faut le dire, mais on a réussi. Tu as été celle qui me comprenait le mieux, et donc tu as été ma plus grande (tu vois que tu n'es pas petite) confidente ! Et malgré les difficultés qu'on a rencontrées, on en sortira que plus fortes et aussi plus soudées. Grâce à nos thèses, en 2021 je serai à tes côtés pour le jour le plus important de ta vie, en étant ta témoin de mariage, une belle preuve de l'importance de notre amitié. Merci à toi pour tous les bons moments (bien trop nombreux à citer) partagés avec toi : nos nombreuses conférences ensemble, nos virées en camion (Ännaboda, Öland, Sälen), mais aussi la zumba et tout le reste ! Autant de moments partagés pour se vider la tête et profiter du temps ensemble. Merci mille fois ma copine pour tout ça, tu es une vraie amie. Merci aussi à bébé Milane, mon petit chat d'amour en Suède ! Merci pour tous tes câlins et tes ronrons, mais aussi pour ton attitude de grognasse quand tu voulais qu'on s'occupe de toi, miss Ronchon d'amour, on dirait ta mère !

Merci aux amis, extérieurs à la thèse, qui ont pris le temps de m'écouter me plaindre (ou pas), qui m'ont permis de me sortir la tête de tout ça grâce à de nombreuses soirées, des weekends ici ou ailleurs etc. Elo, ma copine d'enfance, à chaque retour aux sources à La Guierche, tu étais présente, et pendant une soirée ou plus, je ne parlais et ne pensais pas à ma thèse, mais juste à la vie d'avant, de maintenant et d'après. Merci pour tous ces moments, et merci de m'avoir soutenue depuis plus de 20 ans ! Alex et Gaël, les 2 couillons par excellence, alors avec vous, pas de prise de tête, pas de discussion boulot parce que « ça fait chier » et « franchement, 8 ans d'études pour dire que le plastique c'est de la merde, bah faut être con, parce que moi aussi je peux le dire, et j'ai pas bac+8 pour ça ! ». Juste merci à tous les 2, à chaque fois que je vous voyais, je me disais « et merde, je ne vais pas avancer sur ma thèse », suivi d'un « et bah tant pis ! ». Alors rien que pour ça, merci, parce que vous avez su, grâce à vos conneries, à me faire rire de tout, mais surtout de rien, et à me rappeler que la vie, aussi simple soit elle, n'est pas centrée sur le boulot, et que le reste est bien plus important.
Et finalement, merci à ma Ninette, ma meilleure amie depuis le lycée. A distance, tu as toujours été là pour m'écouter et me remonter le moral, même si parfois tu ne comprenais pas la situation avec mes mots compliqués, peu importe, tu m'as toujours épaulée dans toutes les situations. Merci à toi ma Ninette, d'avoir été présente pour tous les moments de ma thèse, et plus largement, de mes études supérieures ! J'espère que nos projets à chacune vont se concrétiser, et qu'on continuera d'échanger autour de ces sujets pour enrichir nos vies respectives.

※※※

comme ça, mais j'aimerai bien te voir quand même ma Grande Bettie ». Et bien maintenant, je te promets qu'on se verra plus souvent, sans attendre Noël ! J'ai été également soutenue par mes 2 petites mamies. Malheureusement, mamie Micheline, tu es partie 5 jours avant le dépôt de mon manuscrit et donc tu ne sauras jamais que j'ai réussi, que je suis allée au bout, et que je n'ai rien lâché, mais je sais qu'au fond de toi, tu as toujours su que j'y arriverai. Alors merci pour ton soutien, car jusqu'à tes 89 ans, tu as suivi mes études et tu m'as encouragée en découpant des articles de journaux, en me les envoyant avec un petit mot « pensée pour toi car ça parle de poisson », « tiens ma petite, c'est pour toi, c'est sur la pollution des eaux ». C'était des attentions toutes simples mais qui me redonnaient toujours le sourire. Merci aussi à mamie Yvette, pour les (trop) nombreux goûters et déjeuners chez toi en me disant « ça remonte le moral de bien manger, allez tu veux quoi ? ». Tu m'as encouragée à finir, et du haut de tes 90 ans, tu m'as toujours dit que je faisais les choses bien et qu'avec ce que je faisais, je pourrai peut-être changer le monde, ou pas, mais qu'au moins j'apportais ma pierre à l'édifice, alors merci Mamie. Dans mon cercle familial, j'ai eu le soutien de mes frangins, Ludo, Steph et Doudou. Merci à vous pour votre soutien, et vos conseils, ne rien lâcher pour être fière de moi, mais aussi de savoir lâcher prise pour apprécier la vie. Je n'ai pas toujours réussi à appliquer vos conseils, mais j'ai fait de mon mieux, et je n'ai aucuns regrets. Doudou, mon p'tit frère (oui même avec ton mètre 90 !), on a évolué en parallèle, et on a fait nos études ensemble. On s'est soutenu l'un et l'autre, quand l'un doutait, l'autre le rassurait ; quand l'un baissait les bras, l'autre le relevait pour continuer à avancer. Et nous voilà aujourd'hui, Docteur Bettie Cormier, et Médecin Marius Cormier (bientôt Docteur !). Je suis très fière de toi, et heureuse de t'avoir eu comme soutien au cours de mes 8 années d'études, mais aussi de mes 27 années de vie ! Je te renvoie à cette chanson que j'aime tant et tu le sais, Ben Mazué « Let Me ». Ludo et Steph, je vous ai toujours admiré pour vos métiers passions, vous avez réussi à vous épanouir à 200 % en gagnant vos vies tout en réalisant vos passions. J'espère, dans les années à venir, réussir à suivre vos pas, avec ou sans diplôme, mais vivre de mes passions. Muchas gracias también a Claudia, mi querida cuñadita, me apoyaste mucho durante mis estudios! Et merci à mes neveux, les 4 monstres, Wayra, Hugo, Amaru et Thibault, mes Loulous d'amour, même s'ils ne liront jamais ce pavé (et je les comprends !!), malgré eux, ils ont joué un rôle dans ma thèse, en prenant de mon temps libre pour m'occuper d'eux et jouer avec eux. Vos rires et vos câlins sincères, m'ont, plus d'une fois, remonté le moral pour continuer !

Papa et Maman, mes 2 battants et mes plus grands supporters ! Alors vous 2, avec votre soutien depuis 27 ans, depuis mes premiers pas jusqu'à la concrétisation de 8 ans d'étude et du titre de Docteur, vous avez toujours été derrière moi. Vos mots ont été les plus importants pendant cette « putain de thèse » comme j'ai aimé vous le rappeler ! « Ton monde intérieur est le plus beau paysage que je connaisse » et « La vie n'a de sens qu'en accomplissant ses passions. Va, vis, vogue, vole et surtout sache que quoi que tu fasses, où que tu ailles, je serai toujours là ». Avec deux êtres aussi exceptionnels à mes côtés, je ne pouvais aller que de l'avant, pour vous rendre fiers, et vous remercier pour ces années à tout faire

pour vos enfants. Je sais qu'aujourd'hui vous êtes fiers de Doudou et moi, mais n'oubliez jamais que c'est en grande partie grâce à vous, l'éducation que vous nous avez donné, les valeurs que vous nous avez inculqué, le soutien infaillible. Bref, ce titre de Docteur, c'est à vous que je le dédie, cette thèse elle est pour vous, pour que vous puissiez prendre conscience que ces 27 années à m'éduquer m'ont permises de devenir Docteur, et que sans vous, je n'aurai jamais réussi à venir à bout de ma « putain de thèse », alors Merci infiniment, je vous aime démesurément.

Merci à mon petit Loup, Nordik. Tu es arrivé au moment de ma rédaction, et j'ai eu besoin de toi pour finir, et tu m'as soutenu en posant ta grosse tête sur moi ou en me sautant dessus, pour me rappeler que je devais m'occuper de toi et sortir prendre l'air, parce que la thèse ça va 5 minutes, mais la balade…! Je t'aime mon gros Loup.

Après des jours à écrire mes remerciements (en me limitant dans le nombre de pages !), j'arrive à la dernière personne que je souhaite vraiment mettre en avant, Gab. Je pense qu'au début tu t'es lancé dans l'aventure avec moi sans vraiment prendre conscience de l'ampleur « des dégâts ». Ça n'a pas toujours été facile pour moi, mais j'ai souvent sous-estimé les impacts que ça pouvait avoir sur toi, tel une éponge qui capte toutes mes émotions, positives mais surtout négatives, sans jamais te plaindre. Tu as été un réel pilier, et au-delà du soutien moral pour que je ne lâche pas pour partir élever des chèvres à l'autre bout du monde, tu as aussi participé activement à ma thèse en venant nourrir les poissons avec moi, en collant des étiquettes sur des tubes ou en m'affichant un « planning et règles de rédaction » pour que je reste concentrée jusqu'au bout car « 3 mois sur 4 ans, c'est rien ». Tu mérites ton titre de « Assistant Docteur », et moi je te le décerne, avec mes félicitations ! Merci aussi pour les moments de déconnexion forcée, où tu prenais mon téléphone pour que je n'ai aucun accès à mes mails pendant quelques heures/jours, afin de me rappeler que rien n'est urgent, si je le décide. Merci d'être venue avec moi en Scandinavie et d'avoir parcouru ces merveilleux 12 000 km en été et pas moins de 3500 km en hiver avec bébé camion. Tu as su me soutenir comme personne, car c'est un soutien quotidien dont tu as fait preuve, me rappelant que je devais être fière de moi tous les jours, et que je ne devais rien regretter, et c'est chose faite. Tu as attendu la fin de ma thèse pendant plus de 2 ans, et ça y est, on y est. Les derniers mots que j'écris dans cette thèse sont pour toi, c'est un symbole fort pour moi ! Maintenant on peut commencer notre nouvelle vie, pleine d'idées et de projets, ici et ailleurs. Et c'est à mon tour de te soutenir dans ta formation, ON ne va rien lâcher et ON ira au bout ensemble, comme ON l'a fait pour la thèse, t'es le meilleur mon mec ! Merci à toi, Je t'aime.

Grosses pensées pour,

Papy Bebert, Papy Gabriel, Mamie Micheline, Léa et Fefer.

Je sais que Mes Etoiles brillent au-dessus de moi et m'ont souvent donné la force de persévérer,

Merci à vous Mes Etoiles !

List of tables

Chapter 2

Table 1: Chemical characteristics of the selected compounds **-46-**

Table 2: Variable concentration of PFOS added into water, depending on MPs used for the sorption experiment **-49-**

Table 3: Husbandry conditions of zebrafish in the different participating laboratories **-53-**

Chapter 3

Article 1

Table 1: PFOS concentration in water to aim a high and a low concentration of PFOS to perform the sorption experiment **-67-**

Table 2: PFOS adsorbed on HDPE particles with high and low concentration of chemicals after 7 days. **-71-**

Table 3: Distribution coefficient (Kd, L/kg) values of PFOS concentrations for tested PE MPs exposed to 100 µg/L of PFOS for 7 days **-75-**

Table 4:.Sorbed versus desorbed PFOS concentrations before (d=0 days) and after (d=4 days) exposure to artificial gut fluid (AGF). Two quantities (0.3 and 1 %) of PFOS-spiked MPs (PPE) were applied (n=3) and digested in two replicates (A and B) **-75-**

Table 1, SM: Determination of PFOS concentrations in freshly opened solvent ; pipettes and in all steps of the preparation and extraction protocol ; e.g. transfer of MeOH with 3 different plastic or glass pipettes, transfer of MeOH with same glass pipette, sonication bath and centrifuge **-86-**

Extra study

Table 1: BaP adsorbed on LDPE particles with high and low concentration of chemicals **-91-**

Chapter 4

Article 2

Table 1: Husbandry conditions of zebrafish in the three participating laboratories **-103-**

Table 2: Overview about the test conditions and solutions for the zebrafish embryo toxicity test used by the three different laboratories **-103-**

Table 3: Exposure scenarios tested by the three laboratories for MPs, adsorbed MPs (MP + pollutant(s)) and pollutants alone **-104-**

Table 4: Concentrations of MPs and pollutants (mean ± SD) in solution and the resulting concentration of the pollutants on the PE particles **-104-**

Table S1: Sequences of primers used for qPCR experiment with associated amplicon size and efficiency **-114-**

Article 3

Table 1: Concentrations of PFOS, BaP (µg/g) and BP3 (ng/g) extracted from PE (11-13 µm) and PVC (125-500 µm) **-123-**

Table 2: Results of the logrank test of larval survival, for all conditions, including chi2, p-value and the significance **-133-**

Chapter 5

Article 4

Table 1: Characteristics and GPS coordinates of the sampled beaches in Guadeloupe **-154-**

Table 2: Polymer composition of microplastics samples from two different beaches in Guadeloupe (% in mass) **-161-**

Table 3: Toxicity of leachates from microplastic samples (<250µm) of a 'virgin' polyethylene resin obtained from Rotogal (PE Rotogal), Petit-Bourg Viard (PB), Marie Galante Island (MG) assessed using the Paracentrotus lividus embryo test **-163-**

Table 1, SM: Fold change comparison between PB and MG samples. Confidence level were selected according to Schymanski et al., 2014 **-175-**

Article 5

Table 1: Characteristics and GPS coordinates of the sampled beaches in Guadeloupe **-181-**

Table 2: Polymer composition of MPs samples from two different locations in Guadeloupe, % based on the mass **-189-**

List of figures

Chapter 1

Figure 1: Polymer types in demand in Europe, 2014. (PlasticsEurope, 2015) **-9-**

Figure 2: Plastic polymers classified following their densities, PVC: polyvinyl chloride; PET: polyethylene terephthalate; PP: polypropylene; PE: polyethylene; PS: polystyrene; PA: polyamide; ABS: acrylonitrile butadiene styrene; POM: polyoxymethylene; PC: polycarbonate; PUR: polyurethane (Brydson, 1999) **-10-**

Figure 3: Table of Resin Identification Codes (RIC), to classify plastic products in function of polymer types and possible recycling (source: sustainablepackaging.org) **-12-**

Figure 4: Conceptual model of the environmental compartments and average on polymer composition with transport pathways of material between compartments (black arrow). Arrow thickness represents importance of the transport (according to Schwarz et al. (2019)) **-14-**

Figure 5: Different sources of microplastics entering the aquatic environment. (Riccardo Pravettoni (2018)) **-16-**

Figure 6: Map showing the oceanic gyres (red: warm currents; blue: cold currents). **-18-**

Figure 7: Model prediction of global count density (particles/km2; see color bar) for each of four size classes (0.33–1.00 mm, 1.01–4.75 mm, 4.76–200 mm, and > 200 mm). (Eriksen et al., 2014) **-18-**

Figure 8: Chemical structure of PFOS (SymyxDraw 4.0) **-22-**

Figure 9: Chemical structure of BaP (SymyxDraw 4.0) **-24-**

Figure 10: Chemical structure of oxybenzone (SymyxDraw 4.0) **-26-**

Figure 11: Life cycle of the zebrafish (D'Costa and Shepherd, 2009) **-35-**

Figure 12: Work packages of the EPHEMARE project **-41-**

Chapter 2

Figure 13: Images of the different HDPE particles used **-43-**

Figure 14: Study area showing the sampled sites in Guadeloupe (France): PB (red star) indicates Petit-Bourg site located on the main island and MG (red circle) indicates Marie-Galante site located on the second island of the archipelago **-45-**

Figure 15: Shaking a rotary shaker (Heidolph Instruments GmbH & CO. KG, Schwabach, Germany) at 20 rpm for the preparation of MPs spiked with PFOS. **-49-**

Figure 16: Solid phase extraction principle, conditioning (A); loading sample (B); washing step (C) and elution of the compound (D) (Andrade-Eiroa et al., 2015) **-51-**

Figure 17: Design of the feeding experiment. Red lines correspond to the starting of the exposure according to polymer types. Blue line corresponds to the period used to evaluate the reproduction. Dashed vertical lines represent the sampling time with biomarkers analyzed (biometry: growth and weight; chemistry: fish sampled for pollutants quantification; behavior: "novel tank"). **-54-**

Chapter 3

Article 1

Figure 1: Concentration of PFOS in µg/g, adsorbed on MPs (HDPE 250-500 µm) after 7, 30, 90 and 180 days of exposure to 200 mg/L of PFOS **-72-**

Figure 2: Concentration of PFOS in µg/g adsorbed on particles of HDPE (4-6; 11-13; 20-25 and 250-500 µm) after exposure to 25, 50, 75 and 100 µg/L of PFOS for 7 days **-74-**

Figure 3: PFOS concentration (µgPFOS/mlAGF) sorbed onto 0.3 and 1 % PE microplastics (d=0 days) and in exposed artificial gut fluid (replicate A and B) after d=4 days **-76-**

Figure 1, SM: Molecular structure of sodium taurocholate (left) and PFOS (right). Hydrophobic chains and polar functional groups are marked in both compounds in blue and red, respectively **-86-**

Extra study

Figure 1: Concentration of BaP in µg/g, adsorbed on MPs (LDPE 250-500 µm) after 7, 30, 90 and 180 days of exposure to 1 mg/L of BaP (0.05% of toluene) **-92-**

Figure 2: Concentration of BaP in ng/g, adsorbed on different size particles of LDPE (250-500 µm) after exposure of 25, 50, 75 and 100 µg/L of chemical pollutant for 7 days **-93-**

Chapter 4

Article 2

Figure 1: Sublethal and lethal endpoints of zebrafish (*Danio rerio*) embryos after 24, 48, 72 and 96 h exposure to microplastics (MP) and microplastics spiked with benzophenone 3 (MP + BP3), benzo(a)pyrene (MP + BaP), perfuorooctane sulfonate (MP + PFOS) as well as a mixture of the 3 pollutants (MP + 3 POPs) relative to the negative control (CTRL) **-105-**

Figure 2: Hatching rate of zebrafish (*Danio rerio*) embryos after 24, 48, 72 and 96 h exposure to microplastics (MP) and microplastics spiked with benzophenone 3 (MP + BP3), benzo[a]pyrene (MP + BaP), perfuorooctane sulfonate (MP + PFOS) as well as a mixture of the 3 pollutants (MP + 3POPs) in comparison to the control (CTRL) **-106-**

Figure 3: EROD activity in 96 h old zebrafish (*Danio rerio*) embryos exposed to microplastic (MP) and microplastic spiked with benzophenone 3 (MP + BP3), benzo[a]pyrene (MP + BaP), perfuorooctane sulfonate (MP + PFOS) and ß-naphthoflavone as a positive control (PC BNF) after 3 h pulse (A) or 96 h continuous exposure (B) **-107-**

Figure 4: Induction of cyp1a gene transcription in 96 hpf zebrafish (*Danio rerio*) embryos after exposure to 10 mg/L of microplastics (MP), microplastics spiked with oxybenzone (MP + BP3), microplastics spiked with benzo[a]pyrene (MP + BaP), microplastics spiked with perfuorooctane sulfonate (MP + PFOS) as well as a positive control (75 µg/L β-naphthoflavone). Transcription of cyp1a is given as fold-change relative to the negative control (dashed line) after 3h exposure to MP + pollutant starting at 96 hpf **-107-**

Figure 5: Larval photomotor response in 5 d old zebrafish (*Danio rerio*) embryos after exposure to 10 mg/L of microplastics. Distance travelled over 5 min periods including two light-on periods (LON-1, LON-2) and one light off period (LOFF) **-108-**

Figure S1: Standard length (mm) of 5 d old zebrafish (*Danio rerio*) exposed to 10 mg/L microplastics (MP) and microplastics spiked with oxybenzone (MP + BP3), benzo[a]pyrene (MP + BaP), perfluorooctane sulfonate (MP + PFOS) as well as a mixture of all 3 pollutants (MP + 3POPs) versus negative controls **-114-**

Article 3

Figure 1: PFOS concentration in zebrafish exposed for 4.5 months to pristine MPs (PE, PVC) or to spiked MPs (PE-PFOS, PVC-PFOS) **-128-**

Figure 2: Relative proportions of successful (green) and failed (red) attempts to obtain eggs from fish exposed PE and spiked PE (on the left) and to PVC and PVC spiked (on the right). Stars indicate significant difference with their respective CTRL **-129-**

Figure 3: EROD activity, expressed in pmol per minute and per mg of proteins **-130-**

Figure 4: TBARs activity, measured in the different conditions, expressed in nmol per mg of protein **-130-**

Figure 5: AChE activity (in nmol of AChE per minute and per mg of proteins) **-130-**

Figure 6: Individual standard length of larvae from each condition, in mm **-131-**

Figure 7: LPMR in 5 d old larvae issued from fish exposed to PE and spiked PE. Distance travelled over 5 min periods. **-132-**

Figure 8: LPMR in 5 d old larvae issued from fish exposed to PVC and spiked PVC. Distance travelled over 5 min periods. **-132-**

Chapter 5

Article 4

Figure 1: Map of Guadeloupe archipelago (Caribbean Island) with the two sampled beaches, Petit-Bourg (PB) on the main island and Capesterre in Marie-Galante island (MG) **-155-**

Figure 2: GC chromatograms as relative abundance expressed as the total ion current intensity with the retention time (RT, minute). MG in red and PB in blue **-162-**

Figure 3: Larvae of Paracentrotus lividus length increase with control corrected (ΔLc) in serial dilutions of leachates from microplastic samples. Virgin polyethylene resin obtained from Rotogal (PE Rotogal), Petit-Bourg Viard (PB) and Marie-Galante Island (MG). **-163-**

Figure 4: Alteration of frequency of pulsation Afp, after 48 h of exposure in semi-dynamic conditions to MG and PB samples at 1 and 10 mg/L, using ephyrae of *Aurelia aurita* **-164-**

Figure 5: Alteration of frequency of pulsation Afp, after 48 h of exposure in rotary wheel to MG and PB samples from 1/30x to 1x (equivalent to 1000 mg/L), using ephyrae of *Aurelia aurita* **-164-**

Figure 6: Number of deformed zebrafish larvae after 48h-exposure to MPs leachate from Marie-Galante and Petit Bourg **-165-**

Figure 7: Environmental MPs did not induce growth retardation in zebrafish early life stages. Zebrafish embryos were exposed after hatching, for two days to E3 medium (control), MG and PB samples at 10 and 50 g/L and positive control (PC). Total length and head length (mm) were measured **-166-**

Figure 8: In vivo EROD activity in Zebrafish larvae exposed for 48 hours to MPs leachates. EROD Activity (pmole per min and per larvae) was measured at 5 dpf zebrafish embryos exposed to negative control, MPs leachates and positive control (PC) **-166-**

Figure 9: Larval photomotor response in 5 day-old zebrafish (*Danio rerio*) after exposure to negative control, MG and PB samples at 10 and 50 g/L, from hatching time. Distance travelled over 5 min periods including two light on periods (LON1 and LON2) with one light-off period (LOFF) **-166-**

Article 5

Figure 1: Map of Guadeloupe (Caribbean Island) with the two sampled beaches, Petit-Bourg (PB) on the main island and Marie-Galante (MG) on a secondary island **-182-**

Figure 2: Experimental design for the feeding of zebrafish. The 1st contamination started at 30 days post fertilization, until the end of the exposure. Pellets size from 100 to 300 µm were SDS and 500 µm were InicioPlus **-186-**

Figure 3: GC chromatograms expressed as relative abundance with the retention time (RT, minute) for MG (red chromatogram) and PB (blue chromatogram) **-189-**

Figure 4: EROD activity in zebrafish larvae exposed to the equivalent of 1, 10 or 100 mg/L of MPs from MG or PB location. EROD activity is given relative to the control **-191-**

Figure 5: Larval photomotor response in 5 days-old zebrafishafter exposure to organic extracts of MG or PB MPs at an equivalent concentration of 1, 10 or 100 mg/L of MPs. Distance travelled over 5 min periods including two light-on periods (LON1, LON2) and one light off period (LOFF) **-192-**

Figure 6: Zebrafish body weight (mg) after feeding for 4.5 months with diet containing 1% of MG or PB MPs. Treatments correspond to plain diet (ctrl), MG and PB MPs spiked diet at a ratio of 1% **-193-**

Figure 7: Zebrafish reproductive success after feeding for 4.5 months with diet containing 1% of MG or PB MPs. The reproductive success is the total number of spawning events obtained, relative to attempts number (red: failure ratio; green: success) **-193-**

Figure 8: Characteristics of spawns obtained after feeding for 4.5 months with diet containing 1% of MG or PB MPs **-194-**

Figure 9: Larval survival (%) of the F1 generation, monitored for 14 days following feeding of parents for 4.5 months with diet containing 1% of MG or PB MPs MG **-195-**

Figure 10: Larval photomotor response in F1 generation, 5 d old zebrafish (*Danio rerio*) after exposure of F0 generation to control (Ctrl) and MPs treatments (MG and PB). Distance travelled over 5 min periods including two light-on periods (LON1, LON2) and one light off period (LOFF) **-195-**

Abbreviation list

7-ER: 7-ethoxyresorufin

ABS: acrylonitrile butadiene styrene

ACh: acetylcholine

AChE: acetylcholinesterase activity

AGF: artificial gut fluid

AhR: aryl hydrocarbon receptor

BaP: benzo[a]pyrene

BP1: benzophenone-1

BP3: benzophenone-3

CYP: cytochromes P450

DMSO: dimethyl sulfoxide

DNA: deoxyribonucleic acid

dpf: days post fertilization

ELS: early life stage

EROD: ethoxyresorufin-o-deethylase

EU: European Union

FET: fish embryo toxicity

FOSA: perfluorooctane sulfonamide

FT-IR: Fourier Transform InfraRed spectroscopy

GC: gas chromatography

GST: glutathione s-transferase

HDPE: high-density polyethylene

HOCs: hydrophobic organic compounds

hpf: hours post fertilization

Koc: carbon water partition

LC: liquid chromatography

LDPE: low-density polyethylene

LOD: limit of detection

LOFF: light OFF

LogKow: octanol water coefficient partition

LON: light ON

LPMR: larval photomotor response

MeOH: methanol

MFSD: Marine Strategy Framework Directive

MG: Marie Galante

MPs: microplastics

MTBE: methyl tert-butyl ether

OECD: Organization for Economic Cooperation and Development

PA: polyamide

PAHs: polycyclic aromatic hydrocarbons

PB: Petit-Bourg

PC: polycarbonate

PCBs: polychlorinated biphenyls

PE: polyethylene

PET: polyethylene terephthalate

PFOS: perfluorooctanesulfonic acid

POM: polyoxymethylene

POPs: persistent organic pollutants

PP: polypropylene

PS: polystyrene

PUR: polyurethane

PUR: polyurethane

PVC: polyvinyl chloride

REACh: Registration, Evaluation, Authorization and Restriction of Chemicals

RIC: Resin Identification Code

ROS: reactive oxygen species

SPD: stade précoce de développement

SPE: solid phase extraction

TBARs: thiobarbituric acid reactive substances

TG: technical guideline

UPLC: ultra-performance liquid chromatography

US-EPA: United State, Environmental Protection Agency

UV: ultraviolet light

v/v: volume/volume

VTG: vitellogenin

WFD: Water Framework Directive

WP: work package

WWTPs: wastewater treatment plant

Publications and conference presentations

List of publications

- Batel A, Baumann L, Catarci Carteny C, **Cormier B**, Keiter S.H, Braunbeck T (2020). Histological, enzymatic and chemical analyses of the potential effects of differently sized microplastic particles upon long-term ingestion in zebrafish (*Danio rerio*). *Marine Pollution Bulletin, Volume 153, 111022*
- Le Bihanic, F, Clérandeau C, **Cormier B**, Crebassa JC, Keiter S.H, Beiras R, Morin B, Bégout M.L, Cousin X, Cachot J (2020). Organic contaminants sorbed to microplastics affect marine medaka fish early life stages development. Marine Pollution Bulletin. 111059
- O'Donovan S, Mestre NC, Abel S, Fonseca TG, Carteny CC, Williems T, Prinsen E, **Cormier B**, Keiter SH, Bebianno MJ (2020). Effects of the UV filter, oxybenzone, adsorbed to microplastics in the clam *Scrobicularia plana*. *Science of The Total Environment. 733. 139102*
- **Cormier B**, Batel A, Cachot J, Bégout M-L, Braunbeck T, Cousin X and Keiter SH (2019). Multi-Laboratory Hazard Assessment of Contaminated Microplastic Particles by Means of Enhanced Fish Embryo Test With the Zebrafish (*Danio rerio*). *Front. Environ. Sci*. 7:135.
- Beiras R, Bellas J, Cachot J, **Cormier B**, Cousin X, Engwall M, Gambardella C, Garaventa F, Keiter S, Le Bihanic F, López-Ibáñez S, Piazza V, Rial D, Tato T, Vidal-Liñán L (2018). Ingestion and contact with polyethylene microparticles does not cause toxicity on marine zooplankton. *Journal of Hazardous Materials* 360, 452-460
- O'Donovan S, Mestre NC, Abel S, Fonseca TG, Garcia AR, Ilharco LM, Carteny CC, Groffen T, Blust R, **Cormier B**, Keiter S, Bebianno MJ (2018). Ecotoxicological effects of organic contaminants adsorbed to microplastics in the clam Scrobicularia plana. *Frontiers in Marine Science, section Marine Pollution* 5 (143), 1-15
- Pittura L, Avio1 CG, Giuliani ME, d'Errico G, Keiter SH, **Cormier B**, Gorbi S, Regoli F (2018). Microplastics as Vehicles of Environmental PAHs to Marine Organisms: Combined Chemical and Physical Hazards to the Mediterranean Mussels, Mytilus galloprovincialis. *Frontiers in Marine Science, section Marine Pollution* 5 (103), 1-15

List of Poster presentation

- **Cormier B**., Le Bihanic F., Keiter S., Engwall M., Larsson M., Yeung L., Clerandeau C., Crebassa J.-C., Morin B., Bégout M.-L., Cousin X., Cachot J. (2018) EPHEMARE : Méthodologie pour étudier les effets toxiques de microplastiques, en tant que vecteur de polluants, en utilisant les poissons modèles : poisson zèbre (*Danio rerio*) et médaka marin (*Oryzias melastigma*). 14ème colloque international francophone en écotoxicologie aquatique, EcoBIM 2018, Bordeaux 22-25 mai 2018.
- **Cormier B**., Larsson M., Yeung L., Clérandeau C., Kärrman A., Morin B., Engwall M., Bégout M.-L., Cousin X., Keiter S., Cachot J. (2018) Toxic effects of industrial primary microplastics, spiked with model pollutants, using zebrafish (*Danio rerio*). Colloque Polymères et Océans, 15-17 janvier 2018, Montpellier.

List of Platform presentation

- Keiter S., **Cormier B**., Bégout M.-L., Cachot J., Cousin X., Larsson M., Engwall M. (2019) Ecotoxicological effects of microplastic contaminated with benzo[a]pyrene - Results from the Ephemare project. International Symposium on Polycyclic Aromatic Compounds (ISPAC), September 9-12, 2019, Örebro, Sweden.

- **Cormier B**., Le Bihanic F., Joassard L., Keiter S., Cachot J., Bégout M.-L., Cousin X. (2019) Conséquences physiologiques d'une exposition chronique de poissons à des microplastiques vierges ou contaminés. Première rencontres nationales du GDR Polymères et Océans, 24-26 juin 2019, Université Paris-Est Créteil.

- M. Albentosa, C.G. Avio, A. Batel, M. Bebianno, M.L. Begout, R. Beiras, J. Bellas, R. Blust, T. Braunbeck, J. Cachot, C.C. Carteny, **B. Cormier**, X. Cousin, A. Cuesta, M. Faimali, F. Garaventa, S. Gorbi, L. Guilhermino, K. Hylland, S. Keiter, K. Kopke, B. Morin, L. Pittura, F. Regoli, R. Town (2019) Ecotoxicicological effects of microplastics in marine ecosystems: insights from the EPHEMARE project. Pollutants Responses In Marine Organism 20 (PRIMO 20), May 19-22, 2019, Charleston, South Carolina, USA.

- **Cormier B**., Cabar M., Zapata S., Lagarde F., Morin B., Dubocq F., Clérandeau C., Keiter S. H., Bégout M.-L., Cachot J., Cousin X. (2019) Toxicological effects in different stages of zebrafish after direct and trophic exposures to microplastics collected from two Guadeloupe beaches. 8th Young Environmental Scientists (YES) SETAC – February 5-10 2019, Ghent, Belgium.

- **Cormier B**., Le Bihanic F., Zapata S., Keiter S., Bégout M.-L., Cousin X., Cachot J. (2018) Toxicity assessment of microplastics using early life stage of zebrafish (*Danio rerio*) and marine medaka (Oryzias melastigma). MICRO 2018, November 19–23, 2018, Arrecife, Lanzarote, Spain.

- Keiter S, **Cormier B**, Borchert F, Hylland K, Kärrmann A, Larsson M, Yeung L, Engwall M (2018). Sorption and bioavailability of model pollutants on microplastics. MICRO 2018, November 19-23, 2018, Arrecife, Lanzarote, Spain

- Le Bihanic F., **Cormier B**., Misurale F., Joassard L., Keiter S., Cachot J., Bégout M.-L., Cousin X. (2018) Physiological consequences of chronic exposure of marine medaka to microplastics. MICRO 2018, November 19–23, 2018, Arrecife, Lanzarote, Spain.

- Le Bihanic F., **Cormier B**., Misurale F., Bringer A., Joassard L., Keiter S., Cachot J., Bégout M.-L., Cousin X. (2018) Conséquences physiologiques de l'exposition de medaka marin à des microplastiques. 14ème colloque international francophone en écotoxicologie aquatique, EcoBIM 2018, Bordeaux 22-25 mai 2018.

- Le Bihanic F., **Cormier B**., Clerandeau C., Crebassa J.-C., Morin B., Bégout M.-L., Cousin X., Cachot J. (2018) Toxicité comparée des microplastiques pour les premiers stades de développement de deux poissons modèles le medaka marin (*Oryzias melastigma*) et le poisson zebre (*Danio rerio*). 14ème colloque international francophone en écotoxicologie aquatique, EcoBIM 2018, Bordeaux 22-25 mai 2018.

- **Cormier B**., Larsson M., Yeung L., Clérandeau C., Kärrman A., Morin B., Engwall M., Bégout M-L., Cousin X., Cachot J., Keiter S. (2018). Sorption of model pollutants on microplastics and toxicity assessment using early life stage of zebrafish (*Danio rerio*). SETAC Europe, 28th Annual Meeting 2018, Rome, Italy, 13-17 May 2018.

- **Cormier B**., Le Bihanic F., Clérandeau C., Crebassa J.-C., Morin B., Bégout M.-L., Cousin X., Cachot J. (2018) Quelle est la toxicité des microplastiques pour les premiers stades de développement des poissons ? Colloque 2018 de la Société Française d'Ecotoxicologie Fondamentale et Appliquée, 27-28 juin 2018, Montpellier.

- Le Bihanic F., **Cormier B**., Clérandeau C., Kärrman A., Morin B., Bégout M.-L., Cousin X., Keiter S., Cachot J. (2018) Toxicité des microplastiques pour les premiers stades de développement d'un poisson modèle : le medaka marin. Colloque Polymères et Océans, 15-17 janvier 2018, Montpellier.

List of workshops, stakeholder meetings

- Keiter SH, Albentosa M, Avio CG, Batel A, Bebianno M, Begout ML, Beiras R, Bellas J, Blust R, Braunbeck T, Cachot J, Carteny CC, **Cormier B**, Cousin X, Cuesta A, Faimali M, Garaventa F, Gorbi S, Guilhermino L, Hylland K, Kopke K, Morin B, Pittura L, Regoli F, Town R (2018): Knowledge gaps for the ecotoxicological assessment and modelling of microplastics in the sea – Conclusions of the EPHEMARE project. ECHA Stakeholder Workshop on Microplastic, Helsinki, Finland

- Keiter SH, Albentosa M, Avio CG, Batel A, Bebianno M, Begout ML, Beiras R, Bellas J, Blust R, Braunbeck T, Cachot J, Carteny CC, **Cormier B**, Cousin X, Cuesta A, Faimali M, Garaventa F, Gorbi S, Guilhermino L, Hylland K, Kopke K, Morin B, Pittura L, Regoli F, Town R (2017): EU-projektet EPHEMARE- ekotoxikologiska effekter av mikroplaster i marina ekosystem. Renare Mark Symposium – Framtida problem, Östersund, Sweden

- **Cormier B**, Larsson M, Engwall M, Keiter SH, 2017. WP1: Adsorption and equilibrium partition of persistent pollutants to microplastics (MPs). 2nd EPHEMARE Meeting report. Genoa, Italy

List of Articles

Article 1: **Sorption and desorption kinetics of PFOS to pristine microplastic under different conditions** _ Bettie Cormier, Flora Borchet, Anna Kärrman, Marta Szot, Leo Yeung, Steffen H. Keiter. **-63-**

Extra-study: **Sorption processes of BaP to pristine microplastic under different conditions** Bettie Cormier **-91-**

Article 2: **Multi-Laboratory Hazard Assessment of Contaminated Microplastic Particles by Means of Enhanced Fish Embryo Test With the Zebrafish (*Danio rerio*)** _ Bettie Cormier, Annika Batel, Jérôme Cachot, Marie-Laure Bégout, Thomas Braunbeck, Xavier Cousin, Steffen H. Keiter. (*Published in Frontiers in Environmental Science, 2019*) **-105-**

Article 3: **Chronic feeding exposure of pristine microplastics spiked with model pollutants on zebrafish (*Danio rerio*)** _ Bettie Cormier, Mathieu Cabar, Sarah Zapata, Mélanie Blanc, Maria Larsson, Florian Dubocq, Leo Yeung, Christelle Clérandeau, Florane Le Bihanic, Bénédicte Morin, Steffen.H Keiter, Jérôme Cachot, Marie-Laure Bégout, Xavier Cousin. **-123-**

Article 4: **Toxicity assessment of environmental microplastics on early life stages of three aquatic organisms** _ Bettie Cormier, Chiara Gambardella, Tania Tato, Florian Dubocq, Quentin Perdriat, Elisa Costa, Anna Kärmann, Kim Van Arkel, Soizic Lemoine, Fabienne Lagarde, Francesca Garaventa, Marco Faimali, Marie-Laure Bégout, Xavier Cousin, Ricardo Beiras, Jérôme Cachot. **-161-**

Article 5: **Chronic exposure to environmental microplastics reduce growth and reproduction efficiency in F0 and F1 survival in zebrafish, *Danio rerio*** _ Bettie Cormier, Jérôme Cachot, Mathieu Cabar, Sarah Zapata, Mélanie Blanc, Christelle Clérandeau, Florane Le Bihanic, Marie-Laure Bégout, Xavier Cousin. **-189-**

Contents

Résumé substantiel iv

Funding xv

Aknowledgments // Remerciements xvi

List of tables xxiv

List of figures xxvii

Abbreviation list xxxiii

Publications and conference presentations xxxv

List of Articles xxxviii

Introduction ..... - 5 -

Chapter 1: General Context ..... - 8 -

1) Plastic: background information ..... - 9 -

2) Plastic pollution in aquatic ecosystems ..... - 14 -

a. Microplastics as an emerging contaminant ..... - 15 -

b. Dispersion of microplastics in aquatic environment ..... - 17 -

3) Combination of pollutants and microplastics ..... - 19 -

a. Regulations concerning pollutants ..... - 20 -

b. Selected compounds ..... - 22 -

4) Ecotoxicological effects of plastic pollution in aquatic environments ..... - 27 -

a. Physical impacts of plastic debris in ocean ..... - 27 -

b. Microplastics toxicity in marine organisms: mechanisms involved ..... - 28 -

c. Combination of microplastics with additives/contaminants ..... - 30 -

d. Toxicity of MPs related to fish ..... - 31 -

5) Bioassays and biomarkers using zebrafish (*Danio rerio*) ..... - 32 -

a. The “3Rs” principle ..... - 32 -

b. Biological model ..... - 33 -

c. Life stages ..... - 33 -
d. Ecotoxicological biomarkers using fish ..... - 35 -
6) EPHEMARE project ..... - 39 -
Chapter 2: Materials and methods ..... - 42 -
1) Microplastics & Chemicals ..... - 43 -
a. Selection of industrial microplastics ..... - 43 -
b. Environmental microplastics ..... - 44 -
c. Selection of chemicals ..... - 46 -
2) Sorption of pollutants ..... - 47 -
a. Chemical analysis ..... - 47 -
b. Study of the sorption of pollutants onto microplastics particles ..... - 48 -
c. Sorption kinetics of PFOS and BaP ..... - 50 -
d. Isotherm sorption PFOS and BaP ..... - 50 -
e. Particle digestion under simulated physiological conditions ..... - 50 -
3) Biological model, zebrafish (*Danio rerio*) ..... - 51 -
4) Experimental designs ..... - 53 -
a. Exposure of zebrafish embryo for acute toxicity test ..... - 53 -
b. Trophic exposure ..... - 53 -
5) Endpoints analyzed ..... - 55 -
a. Standardized FET ..... - 55 -
b. In vivo ethoxyresorufin-O-deethylase (EROD) assay ..... - 55 -
c. Cyp1a gene transcription analysis ..... - 55 -
d. Acetylcholinesterase activity (AChE) ..... - 56 -
e. Lipid peroxidation ..... - 56 -
f. Behavioral response: Larval photomotor response (LPMR) ..... - 56 -
g. Reprotoxicity ..... - 56 -
h. Chemical analysis ..... - 57 -

..... - 59 -

Chapter 3: Sorption processes ..... - 59 -

Article 1 ..... - 61 -

Extra-study ..... - 87 -

Chapter 4: Toxicity of artificial microplastics ..... - 97 -

Article 2 ..... - 99 -

Article 3 ..... - 115 -

Chapter 5: Toxicity of environmental microplastics ..... - 148 -

Article 4 ..... - 150 -

Article 5 ..... - 176 -

Chapter 6: General discussion ..... - 209 -

1) Sorption of organic pollutants on plastics ..... - 210 -
2) Toxicity of microplastics using early life stages of zebrafish ..... - 214 -
3) Toxicity following ingestion of microplastics ..... - 217 -

Chapter 7: Conclusion and Perspectives ..... - 222 -

References ..... - 225 -

Introduction

Over the last few decades, plastic production has increased to around 348 million tons (PlasticsEurope, 2018) due to ever-growing demand and use in everyday life (Andrady and Neal, 2009). Packaging accounts for around 40 % of all worldwide plastic production (PlasticsEurope, 2018). Because most plastic items are of the single-use variety, a large amount of waste, such as plastic bags, plastic bottles, synthetic fibers and thin films, end up in the aquatic ecosystem. Plastics are defined as polymer compounds formed through a chemical process in which monomer chains are fused. However, plastic polymers are not made up only of monomers, and may contain different additives, such as plasticizers or flame retardants, added to improve specific physiochemical properties like hardness and density, as well as protecting plastics from damage, e.g., heat, UV and oxidation (Andrady, 2011).

Due to excessive use of plastic items and poor waste management, a large amount of plastic debris ends up in aquatic environments. In the 1970's, small plastic particles (0.25 - 0.5 cm) were first reported in the marine environment (Carpenter and Smith, 1972). Thompson and coworkers (2004) were among the first to observe small plastic particles in the environment, which later came to be known as (MPs). MPs have been defined as plastic particles shaped like spheres, pellets, films, fragments or fibers, with maximum diameter of 5 mm (Arthur et al., 2009). Plastic debris larger than 5 mm are referred to as macroplastics (Jambeck et al., 2015). MPs are categorized according to their origin. Particles manufactured at millimetric scale, such as preproduction pellets (plastic industry) and microbeads (exfoliating agents or facial scrubs used in cosmetics), are called primary MPs. Secondary MPs are the result of the breakdown of macroplastics via physical, chemical or biological degradation (Derraik, 2002; Thompson et al., 2004), synthetic fibers are also part of the secondary MPs (Browne et al., 2011).

In 2010, it was estimated that between 5 and 13 million were introduced into the world's oceans as a result of mismanaged plastic waste (Jambeck et al., 2015). In 2014, it was estimated that approximately 5 trillion plastic pieces, such as MPs, were floating in global waters (Eriksen et al., 2014).

Numerous additives, such as plasticizers or flame retardants, are known to be toxicants which may cause adverse health effects (Bakir et al., 2014). Weathering, as along with mechanical fragmentation of plastic debris, lead to smaller particles, MPs, and may increase the specific reactive surface area of particles, facilitating the sorption of pollutants (Koelmans, 2015; Lee et al., 2014; Teuten et al., 2007). Different microplastic particles have been shown to adsorb

diverse classes of pollutants such as per- and polyfluorinated compounds, polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), pesticides and personal care products (Rochman, Browne, et al., 2013; Rochman, Hoh, Hentschel, et al., 2013; Ziccardi et al., 2016). However, the possible contribution of microplastics to act as vector of organic pollutants to biota has for long been subject to controversy (Bakir et al., 2016; Koelmans et al., 2016; Lohmann, 2017).

The presence of microplastics in the environment leads to two main issues: (1) their small size favors an uptake of particles by biota, and can result in effects (physical obstruction in the digestive system and sense of false satiation); (2) MPs can also adsorb pollutants on their surfaces (Rillig, 2012) and act as a vector of pollutants. Different studies have highlighted the diverse toxic effects of MPs on freshwater and marine biota at all levels. For example, producer level can be affected, such as with the marine microalgae *Skeletonema costatum* exposed to polyvinyl chloride (PVC) microparticles (Zhang et al., 2017). Consumer level is also affected by MPs contamination, primarily for invertebrates, as oysters exposed to polystyrene microplastics showed a decreased oocyte number and sperm velocity (Sussarellu et al., 2016), and the uptake of high density polyethylene MPs by marine mussel, *Mytilus edulis* (von Moos et al., 2012). Vertebrates are also affected by exposure to plastic microparticles in marine environments, with the presence of MPs in fish after laboratory exposure, as illustrated by the intestinal perturbation observed in *Dicentrarchus labrax* exposed to PVC particles (Pedà et al., 2016). The vector property of MPs has been proved using pyrene adsorbed on polyethylene and polystyrene to *Mytilus galloprovincialis* with a transfer and accumulation of pyrene in digestive tissues (Avio et al., 2015), as well as a facilitation in the transport of contaminants to the sediment dwelling lugworm *Arenicola marina* or the amphidromous medaka fish, *Oryzias latipes* (Rochman, Hoh, Kurobe, et al., 2013; Teuten et al., 2007). An experimental food chain using zebrafish, *Danio rerio*, fed with *Artemia* nauplii loaded with benzo[a]pyrene resulted in an efficient transfer of chemicals into zebrafish (Batel et al., 2016).

Despite increased scientific interest in the dangers posed by microplastics, there are still significant gaps in knowledge in this field, particularly relating to sorption processes between organic pollutants and microplastics, and their role as vectors of pollutants. On the specific issue of vectorization, no scientific consensus appears to have been reached as yet. In the present study, microplastics were studied to identify their potential role as a vector for pollutants, but also to investigate their toxicity for zebrafish (*D. rerio*). Three model pollutants were selected due to their presence in the aquatic environment, their known toxicity to fish but also because

of differences of chemical structures and expected properties: perfluorooctane sulfonate (PFOS), benzo[a]pyrene (BaP) and oxybenzone (BP3). Different industrial microplastics, of varying types and sizes, were used to investigate their potential interference with microplastic/pollutant interactions. Different life stages of zebrafish, e.g., early life stages (embryos) and juveniles/adults, and exposure durations, were used to investigate the toxicity of microplastics. Alongside artificial microplastics, environmental samples from sandy beaches (Guadeloupe, France) were also used to study the potential toxicity of environmental microplastics to fish. The present study has been carried out in the frame of EPHEMARE project, funded by JPI Oceans.

In this context, the different aims of the PhD project were:

- To develop and implement protocols to artificially sorb model pollutants, PFOS and BaP, to different size ranges of high-density polyethylene and polyvinyl chloride microparticles
- To investigate the ability of microplastics to act as a vector of pollutants by using industrial particles spiked with model pollutants (PFOS, BaP and BP3) using early life stages, juveniles and adults of zebrafish
- To study the toxicity of environmental microplastics (sandy beaches, Guadeloupe, France) on different life stages of zebrafish

Chapter 1 provides a broad review of the issue of global plastic pollution, with a focus on microplastics as emerging contaminants in aquatic environments, their ability to adsorb or transport pollutants, and their toxicity for aquatic organisms.

The biological model used in the present study e.g. *Danio rerio* as well as the use of zebrafish in ecotoxicological studies are reported in this chapter. Experimental designs, analysis and toxicity bioassays/biomarkers are described in **Chapter 2**, material and methods. **Chapters 3, 4** and **5** present the results of the PhD project, written in the form of journal articles. Chapter **3** concerns the artificial sorption of pollutants onto industrial microplastics, **Chapter 4** describes the ecotoxicity of artificial spiked microplastics, and **Chapter 5** is about the ecotoxicity of environmental microplastics collected on sandy beaches (Guadeloupe Island, France). Finally, **Chapter 6** is a general discussion relating to the subject of microplastics as vectors of pollutants, and their toxicity for aquatic organisms, in comparison with previous scientific studies.

Chapter 1:
General Context

1) Plastic: background information

The term “plastic” comes from the Greek “*plastikos*” which was used to describe an object able to be molded or for molding (PlasticsEurope, 2018). The use of the term “plastic” existed long before the first plastic material, known today as “Bakelite” and created in 1907 (Cole et al., 2011). Plastics are produced by the polymerization of monomers, leading to a polymer. Numerous types of plastics exist, and they are used for a variety of purposes, such as packaging, e.g. plastic bags, plastic bottles etc. (Figure 1).

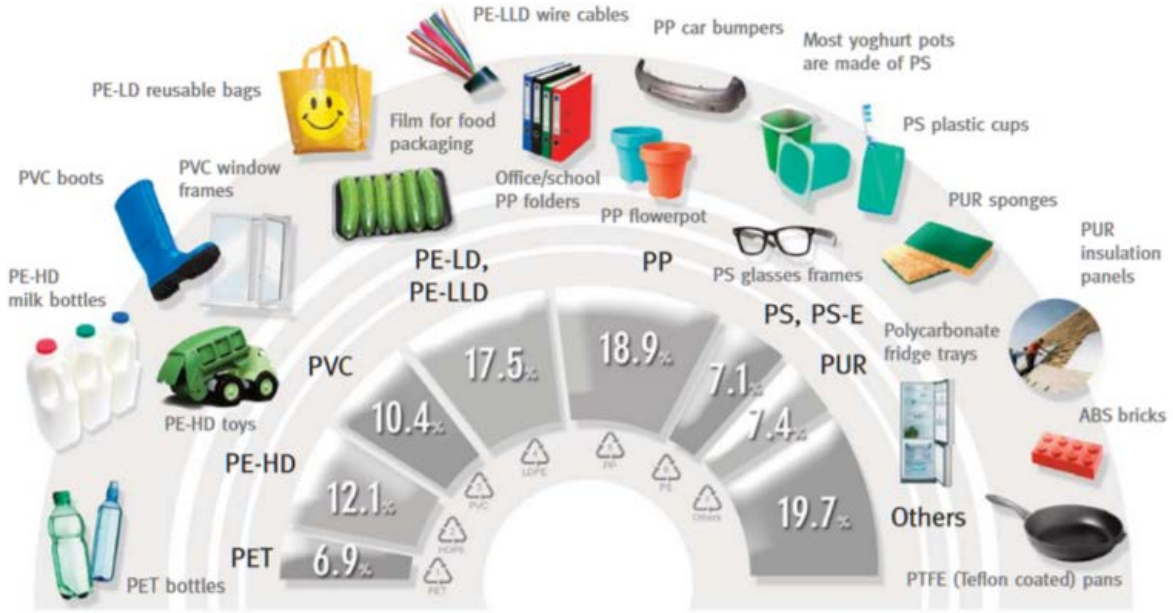


Figure 1: Polymer types in demand in Europe, 2014. (PlasticsEurope, 2015)

Different types of plastics are defined by their polymeric structure. A polymer consists of a repetition of monomers, the same chemical structure, to create chains. The use of different monomers leads to differences in the physicochemical characteristics of these polymers, and in turn, different forms of plastic. For example, the length of the polymer chain modifies the strength of the plastic, meaning that the stronger a plastic, the more force is required to break the chain. Strength can be also increased with the presence of polar groups, leading to hydrogen bonding, which favors attraction between chain polymers. Another difference between polymer types is their melting point, which can lead to different properties. A larger number of covalent bonds will make a polymer harder, and increase its melting point. Plastics can be placed in two thermal categories: thermosetting resins (thermally hardened which cannot become soft again) and thermoplastic resins (can be softened by heating). Many other physicochemical parameters

can be used to characterize and classify plastic polymers. For instance, one of the most common parameters is density, varying from 0.9 to 1.45 g/cm^3 (Figure 2). The same chain of monomers, e.g. polyethylene (PE), may have different properties due to its branching chains: low-density PE (LDPE) has long-chain branching while high-density PE (HDPE) is composed of straight chains, making it stronger and denser than LDPE.

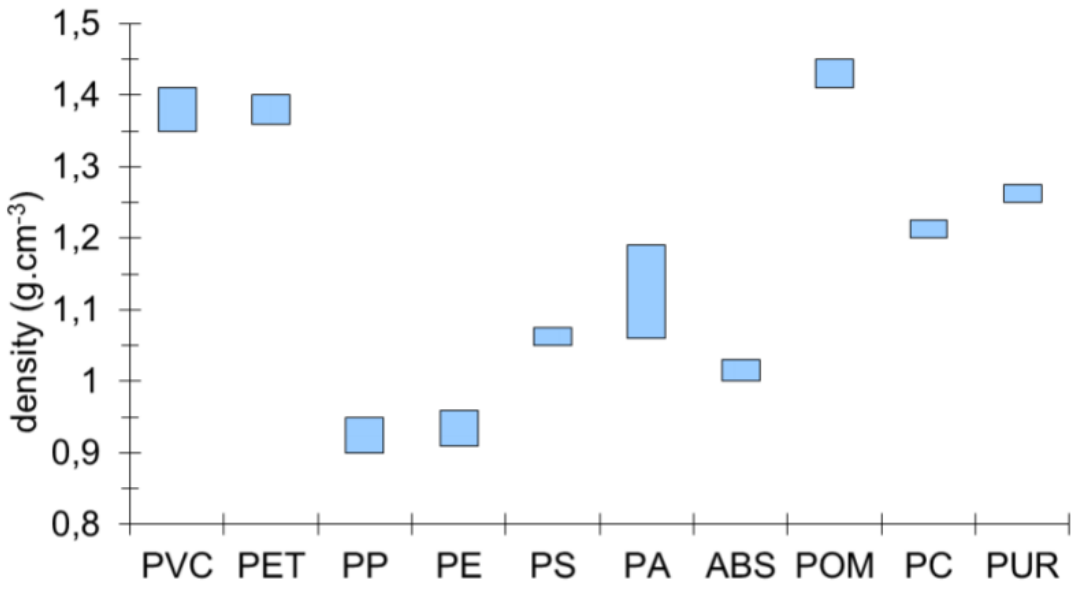


Figure 2: Plastic polymers classified following their densities, PVC: polyvinyl chloride; PET: polyethylene terephthalate; PP: polypropylene; PE: polyethylene; PS: polystyrene; PA: polyamide; ABS: acrylonitrile butadiene styrene; POM: polyoxymethylene; PC: polycarbonate; PUR: polyurethane (Brydson, 1999)

The Resin Identification Code (RIC) was developed by the plastics industry to provide uniform classification of plastics. Recycling has been made easier due to early separation before the melting process. It described plastics as categories with a reference code associated with plastic materials used in industry (Brydson, 1999), Figure 3.

PET: Polyethylene terephthalate is a polymer composed of a linear polyester (polymer composed of an ester, -COO-), firstly developed in 1941. PET is the basis of synthetic polyester fibers and it is commonly used in clothes, ropes, carpets fibers, but also in beverage bottles. Due to its thermoplastic property, items made of PET can be recycled.

PE: Polyethylene is the simplest chain polymer. It is a linear polymer with ethylene (-CH_2-CH_2-) as monomer. It was first used in 1933. Due to its low price and numerous qualities (electrical insulation, chemical resistance, flexibility, transparency etc.), it is the most commonly produced plastic. There are two types of PE, High-density polyethylene (HDPE) and

low-density polyethylene (LDPE). HDPE is used for bottles; toys etc., while LDPE is mainly used in light packaging (e.g. film wraps or plastic bags).

PVC: Polyvinyl chloride, is a fairly unstable polymer, requiring additives to remain stable. PVC was first reported in 1872, with the polymerization of vinyl chloride exposed to sunlight. It was first manufactured in 1926. Today, PVC is used for construction (pipes, doors or windows) and an array of day-to-day items regular items (bottles, non-food packaging etc.).

PP: Polypropylene has similar properties to polyethylene, with greater hardness, and is more resistant to heat. The process of PP polymerization dates back to 1954, with the first commercially produced products appearing just a few years later. It is the second most widely produced plastic, and commonly used in packaging and labeling. Resistant enough to withstand the heat in an autoclave, PP is often used for medical receptacles and other similar items.

PS: Polystyrene can exist in solid or foam form, and can be used in many types of products (CD packaging, containers, lids, construction). PS is a cheap material with various desirable characteristics, e.g., electrical insulation, rigidity, ease of molding.

Other: The last category combines a variety of materials, including polyamides (PA) or polycarbonates (PC) and others copolymers composed of more than one monomer. Polyurethane (PUR) is also part of this other category. Besides petrochemical polymers, bioplastic and natural plastics are also listed in category. These materials are made of carbons derived from renewable biomass and not from fossil resources. Rice, corn, potatoes or sugar cane are used to produce bio-based materials, but the origin of carbons is not linked to biodegradation potential, and a bio-based plastic is not always recyclable.

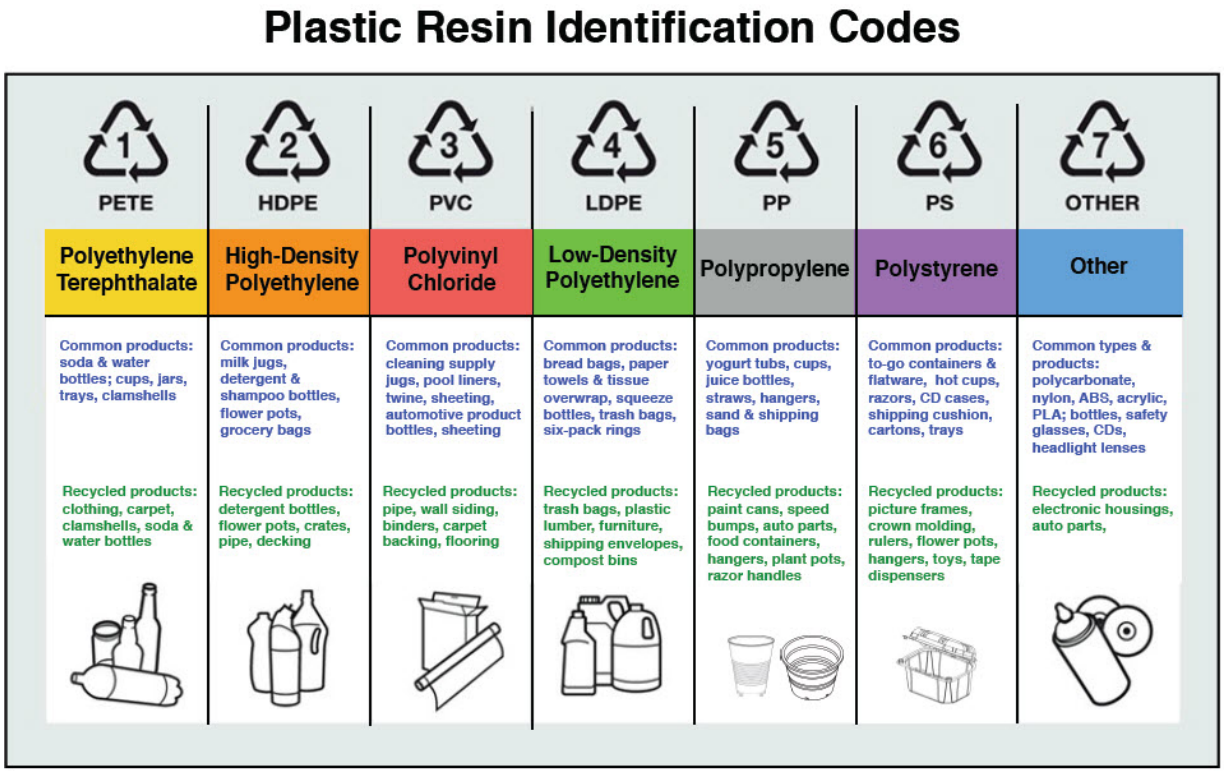


Figure 3: Table of Resin Identification Codes (RIC), to classify plastic products in function of polymer types and possible recycling (source: sustainablepackaging.org)

Since the mass manufacture of plastics began in the 1950s, worldwide production has been continuously on the rise, reaching 348 million tons in 2017 (PlasticsEurope, 2018).

Plastics have many social and technological benefits, aiding the development of new technologies, and reducing transport costs (Thompson et al., 2009; Zarfl and Matthies, 2010). They have become a fixture of everyday life (Andrady and Neal, 2009). However, despite their many advantages, plastics are also having an increased impact on the environment (Zarfl and Matthies, 2010). Authors have estimated that between 2 and 4 % of the plastics produced end up in the marine environment because of poor waste management, among others (Jambeck et al., 2015).

Plastics do not contain only polymers. . They also contain numerous additives used to improve their characteristics, or to modify the chemical affinity between molecules. Additives include plasticizers, flame-retardants, antioxidants, light and heat stabilizers, pigments, etc. Each additive has a specific role in the structure and functional properties of a plastic product (Hahladakis et al., 2018). Additives can be divided into four distinct categories (Hansen et al., 2013):

- Functional additives (stabilizers, antistatic agents, flame retardants, plasticizers, lubricants, slip agents, curing agents, foaming agents, biocides, etc.)
- Colorants (pigments, soluble azo dyes, etc.)
- Fillers (mica, talc, kaolin, clay, calcium carbonate, barium sulfate)
- Reinforcements (e.g. glass fibers, carbon fibers).

For the most part, additives are not chemically bound to polymers. Only the reactive organic additives (flame-retardants) are polymerized with the plastic molecules and are becoming part of the polymer chain (Hahladakis et al., 2018). Therefore, additives are unstable in the polymer, and can be released into the environment (Staples et al., 1997). Recent studies demonstrated the presence of plastic additives in aquatic organisms, which can be released from the plastic after ingestion (Engler, 2012; Lithner et al., 2011). The desorption rate of plastic additives, such as diethylhexyl phthalate, is higher in the gut than in seawater, according to Bakir et al. (2014). Plastic additives are used at low concentrations (ng/L or µg/L), and may have harmful effects on mollusks, crustaceans, fish and amphibians (Oehlmann et al., 2009).

Toxicity of additives has been studied, and released additives may lead to the disruption of biological processes. Toxicity mechanisms depend on additives. Some additives interfere with endogenous hormones causing endocrine disruption (Barnes et al., 2009; Lithner et al., 2009). Phthalates, used as plasticizers, are known to induce toxic effects at the molecular, cellular and organ levels in fish or marine invertebrates, as well as leading to behavioral alterations in fish (Oehlmann et al., 2009). For example, the presence of benzyl butyl phthalate in water disrupted the shoaling and feeding behavior of the three-spined stickleback (*Gasterosteus aculeate*). Behavioral alterations have also been shown in the common carp exposed to diethyl phthalate (Barse et al., 2007). Marine mammals are also affected by the exposition to plastic additives, with an endocrine disruption of the baleen whale exposed to two kind of phthalates (Fossi et al., 2012). Endocrine system can be altered by the presence of bisphenol A, depending on the concentration and the species (Oehlmann et al., 2009), with ovulation delays or reduction in sperm quality (Lahnsteiner et al., 2005). Majority of studies reported effects on invertebrates and fish, while few studies are reporting effects on marine seabirds and mammals.

2) Plastic pollution in aquatic ecosystems

Increased plastic production and anthropic activities taking place in coastal areas, coupled with poor waste management, has led to ever-greater quantities of plastics ending up in aquatic areas (rivers, lakes, oceans etc.). These can come from either land-based sources (plastic bags, food packaging, industries, cosmetics etc.) or ocean-based sources (transport of plastic materials via boats, accidental discharge of raw plastic). The most commonly found polymer in aquatic ecosystems (ocean and freshwater) is PE (in most of abiotic compartments; e.g. beach, epipelagic zone and sediments) followed by PP (Figure 4) (Cheang et al., 2018; Fossi et al., 2017; Hidalgo-Ruz et al., 2012; Karthik et al., 2018). Plastics are transported via ***wind*** action (from water or coastal areas to beaches), ***sedimentation*** (from water to sediments), ***resuspension*** (biological and mechanic movements), ***wash-up*** (dredging of sediments for beach nourishment) and in sediments. Plastics in freshwater are also transferred to oceanic ecosystems via riverine horizontal transportation (water and sediments).

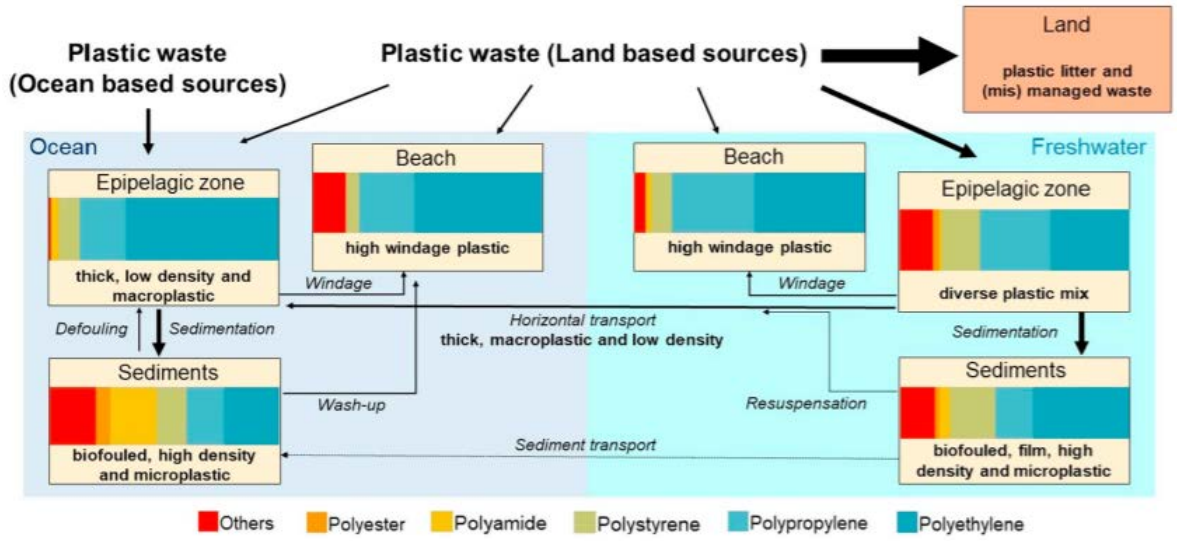


Figure 4 : Conceptual model representing environmental compartments and average on polymer composition with transport pathways of material between compartments (black arrow). Arrow thickness represents importance of the transport (according to Schwarz et al. (2019))

Various types of plastic can be found in the environment, in varying shapes and sizes. The scientific community generally divides them up into categories. Size is one common variable, with plastics found in the environment being categorized as macro, micro, and nano.

A macroplastic is defined as anything larger than 5mm. This category includes products such as plastic bags, straws, and bottles.

In 2010, it was estimated that between 5 and 13 million tons of plastic were introduced into world's oceans (Jambeck et al., 2015), and that approximately 5 trillion pieces of microplastic were present (Eriksen et al., 2014).

Over the years, plastic debris has built up in oceans because of dumping and accidental releases of plastic items. Based on their size, plastic debris can be divided up into four categories: megaplastics (> 50 cm), macroplastics (5-50 cm), mesoplastics (0.5-5 cm) and microplastics (< 0.5 cm) (Andrady, 2011; Eriksen et al., 2014; Jambeck et al., 2015; Koelmans et al., 2017; Lebreton et al., 2018). Plastic debris in the oceans may persist and accumulate (Law and Thompson, 2014). Macro- and mesoplastic refers to plastic particles including nets, bags, bottles etc. Beyond being an eyesore, they have been known to injure and kill a wide range of aquatic organisms that either eat or become entangled in them. Traces of plastics have been found in the stomachs of sea turtles, whales and albatross, among others (Mrosovsky et al., 2009). Ingestion or entanglement result in impaired movement and feeding, a reduction of reproductive output, lacerations, ulcers and deaths in invertebrates, turtles, fish, seabirds and mammals (Barnes et al., 2010; Derraik, 2002; Laist, 1997; Rochman, Browne, et al., 2013). One of the more visible impacts is entanglement of sea turtles in discarded and lost plastic netting or rope from commercial fishing (Laist, 1997). Ingestion of plastic occurs at sea with the confusion between jellyfish and plastic bags for examples (Laist, 1997; Robards et al., 1997). It has been estimated that more than 100,000 sea animals die annually due to the presence of plastic debris in aquatic ecosystems, by ingestion or entanglement (Wilks, 2006).

a. *Microplastics as an emerging contaminant*

Primary MPs particles are particles manufactured at millimetric scale: preproduction pellets and microbeads. Pellets are used in the plastic industry, first as raw resins and then rounded with a diameter between 2 and 5 mm (Costa et al., 2010). Microbeads are used in a range of everyday products: exfoliating agents in facial cleansers, facial scrubs, pesticides, paint stripper, etc.

Secondary MPs particles, known as small plastic fragments, are derived from the breakdown of macroplastics via physical, chemical or biological degradation (Andrady et al., 2003; Cooper and Corcoran, 2010; Derraik, 2002; Thompson et al., 2004). Physical fragmentation of plastics generally occurs due to waves, wind or ultra violet (UV) light (Galgani et al., 2015), while biological degradation consists of enzymatic oxidation or hydrolysis of plastics by

microorganisms (Shah et al., 2008). Plastic particles degrade more quickly when they are deposited on beaches or inland rather than in the open ocean due to a higher exposition to UV radiation and wind processes (Biesinger et al., 2011; Corcoran et al., 2009).

Synthetic fibers may also be part of secondary MPs, entering the environment at microscopic size due to the absence of effective filters in washing machines and wastewater treatment plants (WWTPs) (Browne et al., 2011).

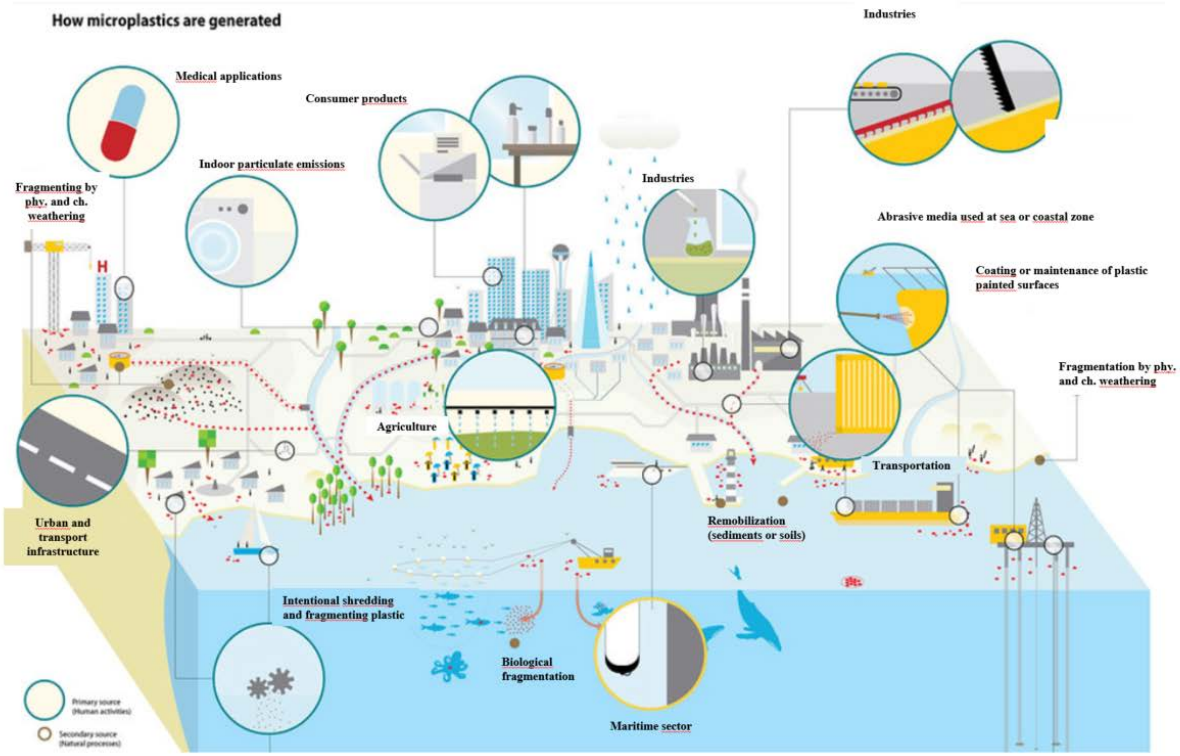


Figure 5: Different sources of microplastics entering the aquatic environment. (Riccardo Pravettoni (2018))

Figure 5 shows different sources of MPs in the aquatic environment are represented. In the marine environment, about 18% of MPs entered the aquatic system via fishing activities (Andrady, 2011; Cole et al., 2011; Derraik, 2002) and approximately 80% of marine plastic debris originate from terrestrial sources (Jambeck et al., 2015; Mani et al., 2015). Specific weather conditions as floods or hurricanes can increase the transfer of MPs from lands to aquatic systems (Barnes et al., 2009). While WWTPs filter some MPs from domestic effluents, they are not designed or equipped to remove all plastic particles. WWTPs are therefore a frequent source of contamination of the aquatic environment (Fendall and Sewell, 2009; Wolff et al., 2019). MPs of both types are considered to be a potential threat for the aquatic environment (Cole et al., 2011; Thompson et al., 2009).

b. *Dispersion of microplastics in aquatic environment*

MPs are present throughout our oceans, the Atlantic to the Pacific Ocean, and in the Caribbean and Mediterranean seas (Cózar et al., 2014; Law and Thompson, 2014; Ory et al., 2018; Welden and Lusher, 2017). MPs have been detected at up to 96% of all water samples in the Pacific Ocean (Moore et al., 2001). Cozar and collaborators studied surface water samples from different locations and demonstrated the extensive presence of MPs by showing that 88% samples contained MPs with high variations in concentrations (Cózar et al., 2014). The worldwide distribution of MPs in the open seas has been demonstrated by the presence of particles in ice cores in the remote Polar areas of the Arctic Ocean and in the deep oceans (Obbard et al., 2014), with a frequency of occurrence of 93-95% in Arctic waterbodies. Accumulation can also occur in gulfs, closed bays and areas close to areas of dense human population (Eriksen et al., 2014), as the Mediterranean (Cózar et al., 2014; Suaria et al., 2016).

The abundance and dispersion of MPs have increased over the last few decades (Barnes et al., 2009; Lebreton et al., 2018; Pham et al., 2014). However, concentration of MPs in surface water is not uniform, with significant variations, ranging from 0.34 particles/m^3 in the Barents sea (Lusher et al., 2015) up to 102 000 particles/m^3 in coastal waters of Sweden (Norén and Naustvoll, 2010). In 1997, Charles Moore discovered the Great Pacific Garbage Patch in the North Pacific Ocean gyre. Tidal rhythms and currents tend to focus on plastic debris inside ocean gyres, leading to accumulation zones (Matsumura and Nasu, 1997). The abundance of MPs is more evident in areas with converging currents (Eriksen et al., 2014; Law and Thompson 2014; Moore et al., 2001). Figure 6 shows the five most important gyres: the Indian Ocean gyre, the North Atlantic gyre, the North Pacific gyre, the South Atlantic gyre, and the South Pacific gyre. Figure 7 shows the predicted number of MPs (particles/km^2) in surface waters, with a high accumulation (red color) in oceanic gyres (Eriksen et al., 2014). Consequently, trends in plastic accumulation in marine environment are not uniformly increasing while average size of plastic particles seems to be decreasing (Eriksen et al., 2014).

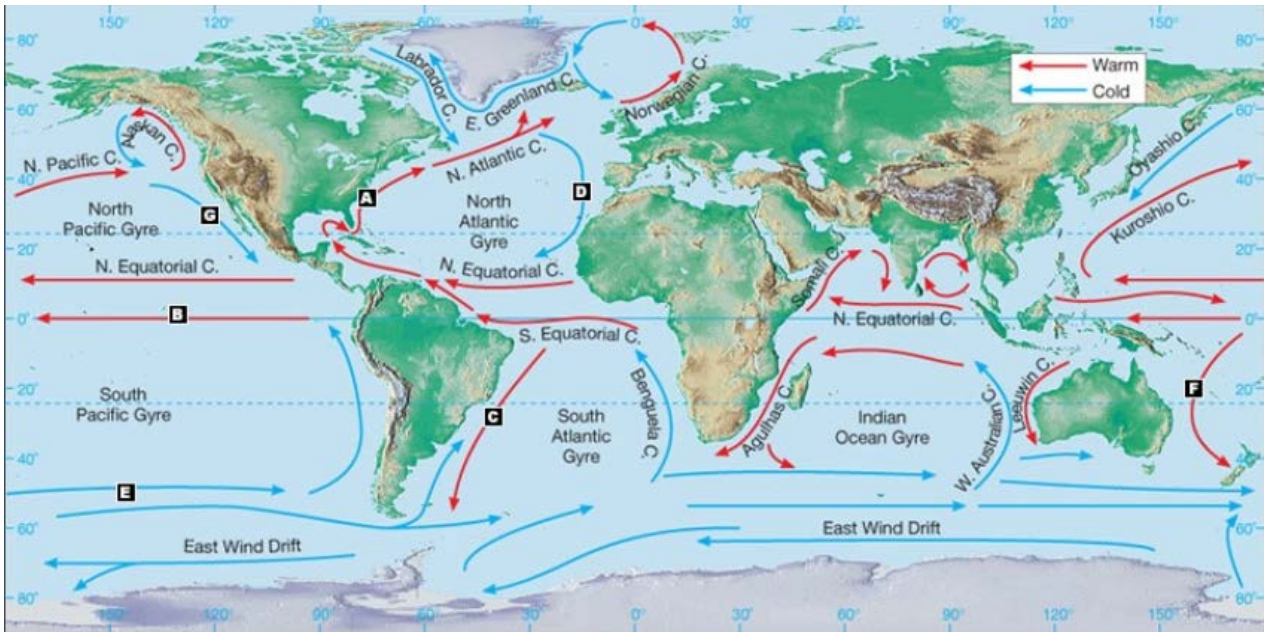


Figure 6: Map showing the oceanic gyres, with warm currents (red) and cold currents (blue). (Source: Expedition 7e continent)

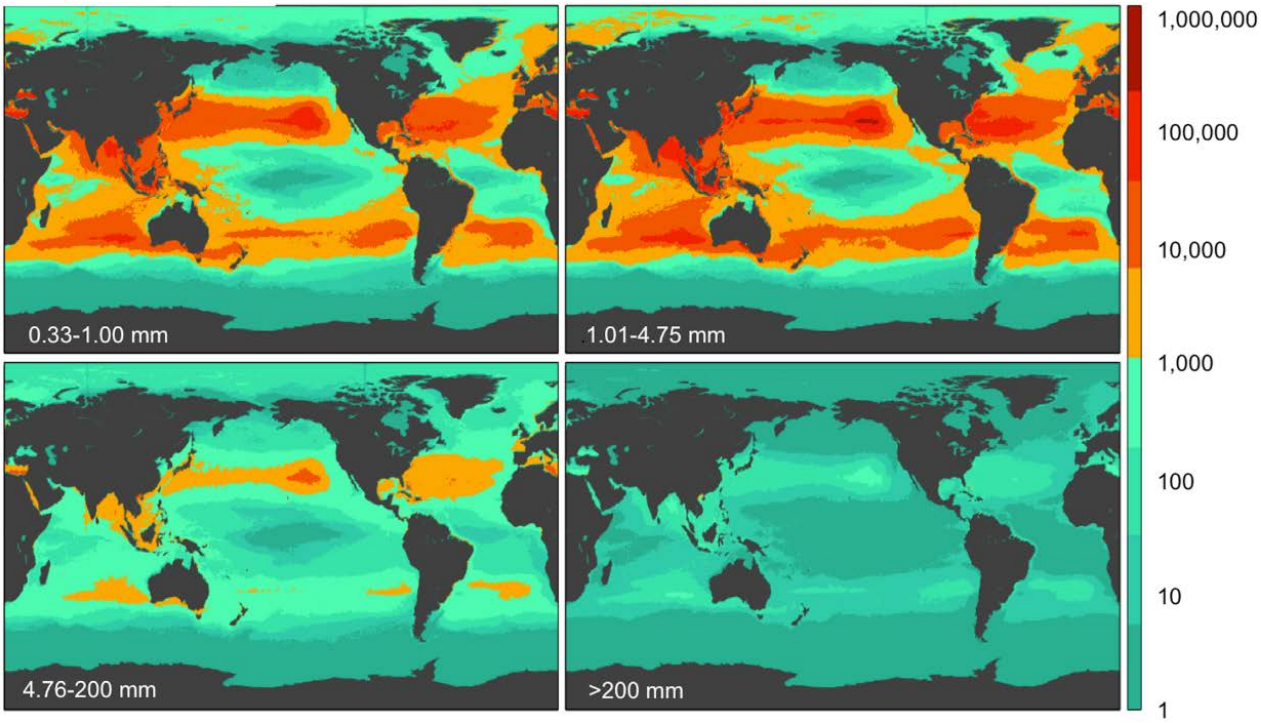


Figure 7: Model prediction of global count density (particles/km^2; see color bar) for each of four size classes (0.33–1.00 mm, 1.01–4.75 mm, 4.76–200 mm, and > 200 mm). (Eriksen et al., 2014)

Plastic items are found in the water column and in sediments, depending on their densities. Plastics with a density > 1, such as PS, PET and PVC, have a tendency to sink to the bottom, whereas PE and PP tend to float to the surface of water. However, the density of plastic debris can be modified once in the marine environment, for example, low density plastic can also be found on the seafloor, due to the development of a microbial biofilm on its surface, which can be followed by colonization by invertebrates and algae, which increase the density (Andrady, 2011). Biofilm development on plastic bags has been demonstrated after 7 days of immersion and show a significant increase of density after 3 weeks of immersion (Lobelle and Cunliffe, 2011). The growth of the biofilm is not the only phenomena that can change the position of plastic items in the aquatic environment: ocean swells can re-suspend particles in the water column (Cole et al., 2011). Indeed, the distribution of particles is not only driven by the density of inert polymers, but also by the currents and movement of the water column.

3) Combination of pollutants and microplastics

As well as numerous additives used in plastic production, MPs offer a surface where many waterborne-pollutants can be adsorbed, including metals (Rochman, Hentschel, et al., 2014) and persistent organic pollutants (Rios et al., 2007; Rochman, Hoh, Hentschel, et al., 2013). MPs are believed to act as vectors and carriers for a wide range of pollutants (Andrady, 2011; Koelmans et al., 2014).

These MPs associated with persistent organic pollutants (POPs) can then act as reservoirs and/or vectors for those pollutants (Barnes et al., 2009; Thompson et al., 2004). The processes of sorption of organic pollutants onto MPs are very complex and poorly understood. The equilibrium kinetics of contaminants on MPs depends on the intrinsic properties of the chemicals, but also on the size, density and nature of the MPs. For example, the diffusion rate for PAHs into polymeric material correlates to a high degree with their molecular weight (Hong and Luthy, 2008) and has been shown to take up to a month. A higher diffusion coefficient can be derived for LDPE than for HDPE, indicating a greater sorption velocity for LDPE (Fries and Zarfl, 2012). In addition, adsorption of PAHs, PCBs, and dioxins to PE correlates with their octanol-water partition constants (logKow) (Adams et al., 2007).

MPs particles were shown to sorb significant amounts of POPs from the surrounding environment (Barnes et al., 2009; Koelmans et al., 2016; Mato et al., 2001; Rios et al., 2007). The weathering and fragmentation of MPs lead to smaller particles, and may increase reactive

specific surface area, thus further facilitating the sorption of such pollutants (Lee et al., 2014; Teuten et al., 2007). The importance of the role of MPs in transport of POPs in biota is still controversial (Bakir et al., 2016; Koelmans et al., 2016; Lohmann, 2017). However, under both laboratory and field conditions, different classes of organic pollutants such as per- and polyfluorinated compounds, PAHs, PCBs, pesticides or pharmaceutical and personal care products adsorb to different plastic materials (Rochman, Hoh, Hentschel, et al., 2013; Ziccardi et al., 2016). In the present study, three different organic pollutants were selected from different chemical groups, known to cause specific toxic effects, but also due to their use in plastic production or their presence in the aquatic environment. These compounds were studied for their sorption properties and to investigate how they are transferred in the environment using MPs as vector.

The sorption of pollutants to MPs is still poorly understood. Sorption of contaminants by plastic has been reported, and some studies have shown easier sorption of contaminants to plastic rather than to natural sediments (Teuten et al., 2007; Wardrop et al., 2016). However, Koelmans (2015) described that while chemicals accumulate on their surface; the role of plastics in chemical bioaccumulation by organisms seems relatively low compared to the bioaccumulation from natural particles or direct uptake from the environment.

a. ***Regulations concerning pollutants***

i. *European Frameworks on Water quality*

To monitor and protect aquatic environments, the European Union (EU) has adopted two distinct directives: the Water Framework Directive (WFD) and the Marine Strategy Framework Directive (MSFD).

The WFD (2000/60/CE) was validated on October 23, 2000 (EU, 2010) and improved on August 12, 2013 (European Parliament and of the Council). The present framework aims to introduce a global policy of achieving good qualitative and quantitative status of all water systems by 2015. The framework applies to all member states of the EU. Among all substances used, 45 have been selected as priorities, and among those, 21 are priority hazardous substances, including PAHs.

The MSFD (Directive 2008/56/EC) is complementary to the WFD, and has the aim of achieving and maintaining good environmental status in European oceans, seas and coasts. Descriptor 10, relating to marine litter, includes pollution by plastics and MPs. The directive aims to eliminate

certain single-use plastic products by 2020, as well as decreasing the use of plastic food and beverage containers.

ii. REACh

Besides environmental regulations, a specific regulation, REACh (Registration, Evaluation, Authorization and Restriction of Chemicals) dating from December 2006 and establishing a European Chemicals Agency, evaluates chemical substances before they can be sold in the EU. REACh draws up lists relating to the production and use of chemical substances, as well as their potential impacts on human health and the environment. Industries are legally obliged to register produced and imported chemicals and to provide information on production process, physical and chemical properties, toxicological characteristics and the potential impacts on human and environmental health. The amount of information required depends on the quantity of the product that is produced or imported. The fish test (OECD, 2013), using early life stage zebrafish, is required for chemicals that are imported/produced at volumes greater than 100 tons. For highly toxic substances, REACh aims to replace or restrict their use.

iii. Persistent Organic Pollutants (POPs)

POPs are a group of anthropogenic chemicals used in various industrial and consumer applications. Their intensive has become an environmental issue because of their resistance to degradation, bioaccumulation, long-range transport and their potential toxic effects (UNEP, 2010). According to the United States Environmental Protection Agency (US-EPA), the first POPs were produced in the 1920s. Since then, production has increased on an industrial scale. POPs are produced for a variety of applications, including as additives in agriculture, flame-retardants, pesticides and firefighting foams (UNEP, 2010). In 2004, the Stockholm Convention was created to deal with increasing levels of POPs in the environment and their toxic effects on environmental and human health. The major goal of this environmental treaty is to eliminate or restrict the production and use of POPs. Initially, there were twelve chemicals listed in the Stockholm Convention, today there are 26 chemicals listed for elimination, 2 chemicals under use and production restrictions and 7 chemicals listed in a specific annex to reduce the unintentional releases (UNEP, 2010).

b. *Selected compounds*

Three different compounds were selected from the EPHEMARE project, each with its own mode of action and set of toxic effects: PFOS is known to affect the liver and the immune system; BaP is a mutagenic and highly carcinogenic compound and BP3 is an endocrine disruptor. Besides their specific toxicities, their octanol/water partition (logKow) and carbon/water partition (Koc) cover a large range, which can in turn influence the sorption processes.

In this thesis, the three compounds were investigated:

- to establish their different sorption/desorption processes onto different MP particles
- to determine whether, once spiked on MPs, the toxicants are still bioavailable
- to better understand the inherent toxicities of each compound to zebrafish embryos and adults would be modified by the presence of MPs, by direct exposure (waterborne exposure) or by trophic exposure (MPs mixed with regular food).
-

i. Perfluorooctane sulfonic acid

PFOS is an organic compound, first produced in 1949, using the electro-chemical fluorination process. Known for its chemical stability and surface tension properties, PFOS was widely produced up until the 1990s. Olsen et al. (2005), reported a 5-fold increase in PFOS production in 14 years (1975-1989), which remained constant during the 1990s. It was used for a variety of purposes: fire-fighting foams, coating formulations, inks, varnishes, lubricants, oil and water repellents for leather, paper and textiles. The compound is a $C_8HF_{17}O_3S$ (Figure 8), composed of two subunits: the C_8F_{17} is hydrophobic and lipophobic, while the sulfonic acid group (SO_3H) adds polarity. The fluorinated compound is highly stable due to its carbon-fluorine bonds.

3M, the biggest manufacturer of PFOS and assimilated, produced approximately 3665 tons of PFOS, representing 78 % of the estimated worldwide volume PFOS, between 1949 and 2002, date when the company decided to stop the production of PFOS and similar compounds.



Figure 8: Chemical structure of PFOS (SymyxDraw 4.0)

Due to its diverse range of applications, such as treatment of carpets (impart stain and dirt repellence), textiles (water repellence), paper and packaging (oil and grease repellence) or fire-fighting foams, PFOS enters the environment in two main ways. It can enter indirectly, either from precursor compounds degradation, or directly through a release to the environment during manufacturing and application (Ahrens and Bundschuh, 2014). PFOS is often used as a surfactant for consumer and industrial products. 3M itself estimated that the majority (around 85%) of PFOS found in the environment is the result of losses from the consumer products mentioned above. The rest may be the result of manufacturing releases from secondary applications.

PFOS is a very persistent fluorinated pollutant that does not photolyze, hydrolyze and biodegrade under any environmental conditions and can be transported easily through all environmental media (aquatic, air and terrestrial). PFOS has been found in water, sediment, sludge and soil (Giesy and Kannan, 2002). In ocean surface waters, levels of PFOS and its precursors have been estimated at between 235 and 1,770 metric tons (Paul et al., 2009). PFOS is also known to be toxic and bioaccumulative in vertebrates, showing a broad range of toxic effects (Giesy and Kannan, 2002; Huang et al., 2010).

Given its wide distribution, PFOS has attracted the most attention of all perfluorinated substances, since it represents the main perfluorinated alkylated compound detected in the aquatic environment (Keiter et al., 2016), as well as in wild species (Houde et al., 2011). Accumulation of PFOS in marine mammals is common in the northern hemisphere (> 1.2 mg/g wet weight in liver) while, in the southern hemisphere, accumulation levels are much lower (Houde et al., 2011). Even in Antarctica, PFOS has been found in elephant seal blood at concentrations from 0.08 to 3.52 ng/mL but also in eggs (2.1-3.1 ng/g) and blood (<0.24-1.4 ng/mL) of polar skua (Tao et al., 2006). PFOS and its precursors were added to the Annex B list of the Stockholm Convention on POPs in 2010, to restrict their production and use (Paul et al., 2009). Even with this restriction, no significant change of PFOS level in osprey (*Pandion haliaetus*) eggs was observed between 1997, 2001 and 2013 (Eriksson et al., 2016).

PFOS is known to enter the aquatic environment. However, until now only a few studies have investigated the sorption behavior of PFOS on MPs. The sorption of PFOS onto MPs has been only investigated in (2015) by Wang, Shih and collaborators that studied the sorption characteristics of PFOS and FOSA (perfluorooctane sulfonamide, a PFOS precursor) on three different MPs types, including PE. They demonstrated linear sorption mechanisms and a difference of sorption rate depending on the type of polymer (Wang, Shih, et al., 2015).

ii. Benzo[a]pyrene

BaP is one of the PAHs and results from incomplete combustion, between 300 and 600 °C, of organic material (Collins et al., 1991). BaP is not used in the industry, but is found in organic material such as fossil fuel. The presence of the compound in the aquatic environment is due to atmospheric transport (exhaust emissions, heating system using derived from fossil fuel combustion, waste incineration…) or by accidental release, e.g. during an oil spill.

BaP is composed only of carbon and hydrogen atoms, $C_{20}H_{12}$ (Figure 9). Due to its chemical structure, BaP is a hydrophobic compound, explaining its distribution in soils and sediments. BaP is also widely present in coastal and offshore systems (Antunes et al., 2013; Duan et al., 2015).

The US-EPA has prioritized 16 PAHs as priority pollutants in aquatic ecosystems. While PAHs are not included in the Stockholm convention, there were listed in the Aarhus protocol, to reduce or eliminate discharge, emissions and losses of pollutants in Europe and the United States (UNECE, 2014).

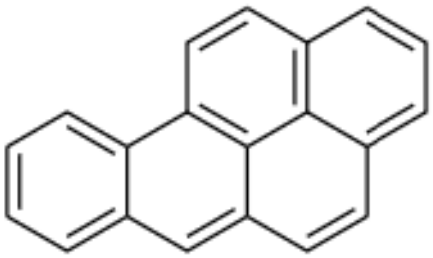


Figure 9: Chemical structure of BaP (SymyxDraw 4.0)

BaP is a compound known to affect fish from developmental stages by entering organisms and passing through the chorion, as it has been demonstrated in Japanese medaka (Hornung et al., 2007). It is mutagenic, genotoxic and carcinogenic effects have been widely studied. The BaP is also a ligand for aryl hydrocarbon receptor (AhR) that triggers its own metabolization, and its bioactivation in diol epoxide metabolite via the induction of CYP1A. BaP is known for its pro-genotoxic and pro-carcinogenic activities, and is one of the most toxic PAHs. It has been classified by the International Agency for Research on Cancer as a human carcinogen (Liu et al., 2015).

Toxic effects in fish exposed to BaP extend to include growth, teratogenicity, behavior (Vignet et al., 2014), cardiac function (Incardona et al., 2011), cardiorespiratory function (Gerger and

Weber, 2015), embryotoxicity and genotoxicity (Vicquelin et al., 2011; Wessel et al., 2007). Immunotoxic effects of BaP have also been reported in fish (Carlson et al., 2004). Cell lines have been used to investigate BaP toxicity, and deoxyribonucleic acid (DNA) damage, and increases in ethoxyresorufin-O-deethylase (EROD) activity. Metabolic modifications and cytotoxicity have also been observed at high concentrations (Kienzler et al., 2012; Nehls and Segner, 2001; Schirmer et al., 1998).

It is known that PAHs, including BaP, adsorb at high rates to different types of plastics in seawater, particularly PE (Rochman, Hoh, Hentschel, et al., 2013; Schönlau et al., 2019; Ziccardi et al., 2016). BaP adsorption to MPs has been documented to be transferred via trophic transfer to biota (Batel et al., 2018; Batel et al., 2016; Bour et al., 2018; O'Donovan et al., 2018; Pittura et al., 2018). Bioaccumulation of the compound in invertebrates is , with a logKow = 6.13.

iii. Benzophenone-3

The third compound in the present study is the widely-used benzophenone-3, also known as oxybenzone. The compound is relatively stable under UV light and artificial sunlight (Gago-Ferrero et al., 2011; Rodil et al., 2009). It has been used as a sunscreen agent for more than 40 years (Kim and Choi, 2014). Due to its chemical structure (Figure 10), BP3 is a lipophilic and potentially bioaccumulative compound with a relatively high logKow value of BP3. This suggests a tendency to adsorb to suspended solids and sediments, as well as others particles such as microplastics.

Due to its extensive use, BP3 is considered an “emerging environmental contaminant of concern” by the US-EPA. The run-off of this UV filter caused by its removal from the skin during recreational activities such as swimming or bathing, leads to massive contamination of aquatic environments (Gago-Ferrero et al., 2011), such as lakes and rivers (Balmer et al., 2005; Kameda et al., 2011; Loraine and Pettigrove, 2006). In surface water, BP3 has been measured at up to 125 ng/L (Poiger et al., 2004), and has been quantified in even higher concentrations in WWTP effluents (Loraine and Pettigrove, 2006), coming from indirect inputs such as laundry or showering processes (Balmer et al., 2005; Langford and Thomas, 2008). During summer 2011, BP3 reached levels of 577.5 ng/L in coastal waters off Majorca (Tovar-Sánchez et al., 2013). After 28 days in water, BP3 has been shown to degrade by about 4% (MITI, 1992) indicating its relative persistence in aquatic environment. The half-life of BP3 in surface water

has been estimated at a few weeks in summer, while in winter, the persistence appears to be 7 to 9 times higher under mid-latitude conditions (Vione et al., 2013). The presence of BP3 in aquatic systems has caused the scientific community to evaluate its potential impacts on aquatic organisms.

While BP3 has been frequently reported in the context of endocrine disruption (Fent et al., 2010; Heneweer et al., 2005; Schreurs et al., 2002; Sieratowicz et al., 2011), only a few studies have investigated its toxicity for fish. The estrogenic activity of BP3 reported in aquatic species, may be due to its metabolic product, i.e., benzophenone-1 (BP1), rather than BP3, which has shown only slight estrogenic activities in *in-vitro* studies (Molina-Molina et al., 2008). However, studies using fish demonstrated that BP3 tends to interfere with reproduction and sex hormone signaling, inducing reproducing pathologies and an alteration of the reproductive fitness (Blüthgen et al., 2012; Coronado et al., 2008; Fent et al., 2010). For example, in adult zebrafish, the bioaccumulation of BP3 resulted in a reduction of egg production and hatching. In zebrafish males, this bioaccumulation led to an increase in levels of vitellogenin (VTG), which can be indicative of gender shifts (Coronado et al., 2008; Nimrod and Benson, 1998).

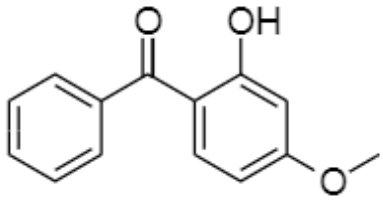


Figure 10: Chemical structure of oxybenzone (SymyxDraw 4.0)

Unlike the two other compounds, BP3 has never been studied in relation to MPs. The increase of recreational activities and populated areas around coastal zones may cause an increased BP3 level in aquatic environments in the next few years, and due to its lipophilicity, BP3 may adsorb onto MPs available already present on water surfaces or sediments, and may cause endocrine disruption to aquatic organisms. In the present study, besides PFOS and BaP, BP3 was selected to be sorbed onto MPs, and then, used to investigate its toxicity in combination with MPs toward zebrafish.

4) Ecotoxicological effects of plastic pollution in aquatic environments

a. *Physical impacts of plastic debris in ocean*

Plastic pollution affects a wide range of aquatic organisms, and different routes of exposure can be involved. Ingestion and the entanglement are among the most obvious of these. Marine mammals, turtles, birds and fish are susceptible to possible entanglement in plastic bags and packaging in general, fishing nets and ropes (ghost fishing) as well as fiber ropes, that have increased animal suffering and mortality (Gregory, 2009; Moore et al., 2001; Schrey and Vauk, 1987; Votier et al., 2011). It has been estimated that more than 130 marine species, including 6 species of sea turtles, 51 species of seabirds and 32 species of marine mammals are entangled in the marine environment (Marine-Mammal, 1996). Cetaceans are part of this ecological catastrophe, with a total of 54 % of cetaceans known to have been entangled in or have ingested plastic debris, leading to a morbidity or mortality (STAP, 2011), including whales and dolphins, listed on the International Union for Conservation of Nature Red List. Besides cetaceans, sea turtles known to be victims of ghost fishing (Kiessling, 2003) and to have plastic debris build up in their gastrointestinal tracts. Captured sea turtles are frequently found to have plastic debris in their intestine (Campani et al., 2013; Casale et al., 2016; Nicolau et al., 2016). The frequency with which turtles ingest plastic would appear indicative of increasing plastic pollution in our oceans. It also marks out the turtle as a useful sentinel organism by which marine litter trends can be monitored.

Sea turtles entangled in plastic products were found to skin lesions with ulceration, dead muscles, and even loss of flippers in some cases (Oros et al., 2005). Marine fish are can also become entangled. A study in 1928 by Gudger, reported the entanglement of a mackerel (*Scomber scombrus*) in a rubber band, while another noted the death of a shortfin mako (*Isurus oxyrhincus*) having become caught up in rubber from a car tyre (Gudger and Hoffmann, 1931). Entanglement may have impacts on animal welfare, such as death by asphyxiation, restricted feeding, and sometimes-even starvation (Butterworth et al., 2012). It can create energetic burdens with additional drag, restricted movements, and inhibited swimming (Derraik, 2002; Gall and Thompson, 2015; Laist, 1997). Ropes and plastic line can lead to loss of limbs, which in turn leaves open wounds, ripe for infection. In many cases, plastic is so durable that when an entangled animal dies, the same debris can return to the sea with the potential to entangle another animal (Acampora et al., 2016; Campani et al., 2013; Laist, 1997).

Carpenter and collaborators (1972), first documented plastic ingestion by small marine fish, with a variation in the occurrence of spherical plastic in the gut linked to PCB contamination, identifying that contamination may lead to intestinal blockage as well as a decrease in growth and mortality. Later, positive correlations were made between increased levels of MPs in oceans and ingestion by small marine fishes. From a sample of 670 planktivorous fish, an average of 2.1 MPs per gut were found, ranging from 1 to 83 pieces individual pieces (Boerger et al., 2010). Numerous studies have reported the presence of MPs in fish guts (Bellas et al., 2016; Lusher et al., 2013; Romeo et al., 2015). The tiny size of MPs makes them bioavailable to a wide range of aquatic organisms, causing a potential threat to biota (Barnes et al., 2009; Cole et al., 2011; Derraik 2002; Thompson et al., 2004). MPs have been shown to adhere to the external carapace of exposed zooplankton, leading to a decrease of algal feeding (Cole et al., 2013). The accumulation of MPs in organs and tissues of aquatic organisms may lead to an immune response, granuloma formation or foreign body reaction (Tang and Eaton, 1995). The presence of MPs in fish after laboratory exposure demonstrated an intestinal perturbation observed in *Dicentrarchus labrax* exposed to PVC particles (Pedà, Caccamo, Fossi, Gai, Andaloro, Genovese, Perdichizzi, Romeo and Maricchiolo, 2016). Digestive gland vacuoles of mussels have been shown to absorb HDPE plastic, demonstrating an internalization of MPS (von Moos et al., 2012). An accumulation of MPs inside lysosomes led to the breakdown of the lysosomal membrane, causing cell death with the release of specific enzymes, and the presence of HDPE particles expelled from the digestive tubules, led to a strong immune response (von Moos et al., 2012).

b. *Microplastics toxicity in marine organisms: mechanisms involved*

A wide array of studies have been carried out to assess the toxicity of chemicals or environmental samples. The toxicity of MPs for organisms remains almost unknown, and depends on their physical and chemical properties (Law and Thompson, 2014; Zarfl and Matthies, 2010). An increasing number of studies have also focused on the fragmentation of plastics into MPs (Thompson et al., 2004) or even nanoplastics (Gigault et al., 2018). The ability of MPs to release hazardous chemicals (Jeong et al., 2017; Maršić-Lučić et al., 2018; Oberbeckmann et al., 2018; Rani et al., 2017; Turner, 2018; Wang et al., 2017), is an important focus, but remains unclear, and scientific studies need to focus on the underlying mechanisms of their toxicity (Zhang, Wang, et al., 2018). It is important to investigate at length this aspect of MPs pollution, given that some chemicals associated with MPs as additives or after sorption

are capable of disrupting the endocrine system in vertebrates include fish and mammals (Rochman, Kurobe, et al., 2014). The impact on the trophic chain also needs to be considered, as MPs are easily ingested by zooplankton, and then transfer to higher consumers in the marine food chain, with humans as the final consumers. MPs size also seems to be a key issue, with smaller particles causing higher toxicological consequences (Browne et al., 2008).

i. Accumulation and translocation

Mussel (*Mytilus edulis*) has been used as a bio-indicator to study the accumulation of PS microparticles as well as their toxicity after ingestion (Browne et al., 2008). The study revealed that PS accumulated in the gut cavity and digestive tubules after 12 h of exposure while after 3 days, MPs translocated from the gut to the circulatory system and has been found in the haemolymph. The abundance of MPs was greater after almost 2 weeks of exposure, demonstrating an accumulation of particles with a majority of smaller particles (3 µm). Despite the translocation, MPs did not induce toxicity as oxidative stress, viability or filter-feeding activity. The permeation of PS particles into the plasma membrane of cells via alteration of the lipid bilayer structure has been modelled in 2014 by Rossi et al., and confirmed by an *in vivo* study using nematodes (Lei et al., 2018; Rossi et al., 2014).

ii. Physio-pathological alterations and reproductive abnormalities

Besides oxidative stress, MPs have demonstrated their abilities to induce toxicity on the different metabolic processes of the body. For example, oysters (*Pinctada margaritifera*) exposed to PS microbeads for 2 months were impacted throughout their physiology, including a decrease in assimilation efficiency and scope for growth. This energy balance disruption was linked to a reduction in oyster gonad size (Gardon et al., 2018). Different PS particles, in term of sizes and functionalization, affected the reproductive efficiency of Pacific oysters (*Crassostrea gigas*) with a clear decrease in fertilization success and embryo-larval development due to malformations (Tallec et al., 2018). Results for oysters were consistent with the toxicity observed in the early developmental stages of sea urchin embryos (*Paracentrotus lividus*). Sea urchin embryos exposed to PS showed an accumulation of particles in their digestive tract, with an induction of developmental defects including up-regulation of Abcb1 and cas8 genes, involved in apoptotic pathways (Della Torre et al., 2014). Studies relating to invertebrates are numerous, but vertebrates are also sensitives to MPs. Adult zebrafish exposed

to dietary nanoparticles made of PS for a week demonstrated a transfer of nanoparticles from parents to the offspring (Pitt, Trevisan, et al., 2018). Besides this transfer, PS reduced gluthatione reductase activity in the brain, muscles and testes of parents, and were detected in the yolk sac, gastrointestinal tract, liver and pancreas of the F1 generation. The antioxidant system was altered by the presence of PS, in both F0 adult tissues and F1 larvae, via a transfer from parents to the offspring but with minor physiological alterations.

iii. Oxidative stress involvement, a possible mechanism of toxicity induced by microplastics

Exposure to PS particles induced oxidative stress in zebrafish and nematode (Lei et al., 2018; Lu et al., 2016) suggesting that MPs may induced free radical formation. The reactive oxygen species (ROS) formation can modify the physiological homeostasis of cellular components, and may damage cellular macromolecules including DNA, leading to genome instability and biochemical alterations or carcinogenesis. The exposure of sceractinian coral (*Pocillopora damicornis*) to 1 µm PS increased the activities of superoxide dismutase and catalase (antioxidant enzymes) with a decrease in glutathione S-transferase (GST), a phase II biotransformation enzyme, as well as the alkaline phosphatase (immune enzyme) (Tang et al., 2018). Some genes involved in the stress responses pathway were up-regulated while genes related to the c-Jun N-terminal kinases, kinases activated by ROS, were down-regulated in coral cells (Tang et al., 2018). A study on the exposure of clam (*Scrobicularia plana*), exposed to LDPE microparticles showed an increase of GST, catalase and gluthatione peroxidase activities in gills (O'Donovan et al., 2018). These previous studies are consistent with Lei et al. (2018) study, who demonstrated that PS microparticles induced oxidative stress and the up-regulation of GST in *Caenorhabditis elegans*. Oxidative stress has been also showed on zebrafish liver by the activation of superoxide dismutase and catalase activity after an exposure to PS (Lu et al., 2018).

c. ***Combination of microplastics with additives/contaminants***

MPs hazards come not only from polymers, but also from the presence of additives and/or chemical contaminants. Sorbed contaminants can include POPs and heavy metals (Besseling et al., 2013; Maršić-Lučić et al., 2018; Nakashima et al., 2012; Oberbeckmann et al., 2018; Rani et al., 2017; Reisser et al., 2014). MPs can act as reservoirs and/or vectors for such pollutants

(Barnes et al., 2009; Thompson et al., 2004). However, there are different schools of thought relating to the uptake of chemicals released from plastics. Rochman, Lewison et al. (2014) showed an uptake of chemicals but an absence of effects from the uptake of chemicals from plastics on marine biota. A recent study using virgin PE particles and particles loaded with BP3 on early developmental stages of numerous marine taxa resulted in an absence of acute toxicity to all species used, except marine medaka (*Oryzias melastigma*) (Beiras et al., 2018).

It has been shown that plastics facilitate the vectorization of chemicals to the lugworm (*Arenicola marina*) and Japanese medaka (*Oryzias latipes*) (Rochman, Hoh, Kurobe et al., 2013; Teuten et al., 2007). While contaminants have been shown to be transported, certain studies demonstrated a modulation of the toxicity in exposed fish by reducing the toxicity for some chemicals (Oliveira et al., 2013). Batel et al. (2016) conducted an experiment to investigate the trophic transfer of PAHs spiked to MPs. The authors fed *Artemia nauplii* with pristine MPs or MPs spiked with BaP. Zebrafish fed with Artemia contaminated by MPs spiked with BaP showed effective ingestion and translocation of MPs in the chyme, villi and epithelial cell, as well as an effective transfer of BaP, but without direct stress or diseases.

The first evidence of a positive correlation between bioaccumulation of chemicals and presence of MPs was discovered using PCB-spiked PS microparticles given to *Arenicola marina*, causing weight loss, reduction in feeding activity and fitness (Besseling et al., 2013). Nickel has been used in combination with PS particles on *Daphnia magna,* resulting in differences in toxicity between metal alone, or in combination with MPs (Kim et al., 2017). Rainieri et al. (2018) exposed zebrafish to food supplemented with MPs and sorbed with PCBs, perfluorinated compounds and methylmercury. Significant alterations in the homeostasis of liver, brain, intestines and muscles have been observed for MPs combined with contaminants compared to MPs or contaminants alone.

d. *Toxicity of MPs related to fish*

Uptake of MPs can occur in two main ways: firstly the ingestion of MPs via trophic transfer, and secondly, via direct uptake from water column and/or sediments. Standardized protocols have been used to test acute toxicity for numerous marine zooplankton organisms. One study used virgin polyethylene particles and particles loaded with BP3, with early life stages (ELS) of numerous taxa, and results showed an absence of acute toxicity for all species used, except ELS of fish (*Oryzias melastigma*) at concentrations higher than environmental concentrations

(Beiras et al., 2018). MPs provide a significant surface for the sorption of other pollutants, particularly hydrophobic compounds like POPs. These MPs-POPs can then act as reservoirs and/or vectors for such pollutants (Barnes et al., 2009; Hammer et al., 2012; Thompson et al., 2004). Sorption of contaminants by plastic has been reported, and some studies showed an easier sorption of contaminants to plastic rather than to natural sediments (Teuten et al., 2007; Wardrop et al., 2016). It has been shown that plastic facilitate the vectorization of chemicals to the lugworm (*Arenicola marina*) and Japanese medaka (*Oryzias latipes*) (Rochman, Hoh, Kurobe et al., 2013; Teuten et al., 2007). As described before, Batel et al. (2016) conducted an experiment to investigate the trophic transfer of chemical spiked to the surface of MPs. The authors used *Artemia nauplii* that were fed with MPs and MPs spiked with BaP, and those artemia where then fed to zebrafish. Zebrafish showed effective ingestion and translocation of MPs in the chyme, villi and epithelial cell, but did not induce direct stress or diseases.

Only a handful of existing studies cover the *in-vitro* toxicity of MPs. On gilthead seabream (*Sparus aurata*) and European seabass (*Dicentrarchus labrax*) head-kidney leucocytes, PE MPs failed to alter cell viability or immune parameters (e.g., phagocytosis), and did not induce significant expression of genes related to inflammation, oxidative stress or metabolism of xenobiotics (Espinosa et al., 2018).

5) Bioassays and biomarkers using zebrafish (*Danio rerio*)

Pollution in aquatic environments has led to the development and improvement of new biological tools to evaluate toxic effects on aquatic organisms. Research in the fields of toxicology and ecotoxicology has traditionally been based on *in vivo* models (Castaño et al., 2003). Under European rules, the "3 R's" principle is mandatory in chemical testing using vertebrates.

a. *The "3Rs" principle*

EU directive 2010/63/UE was designed to protect animals used for scientific purposes, based on a set of ethical and welfare standards. The directive protects live non-human vertebrates, including independently feeding larval forms, fetal forms of mammals from the last 1/3 of their normal development as well as cephalopods mollusks. Breeding facilities and conditions, as well as the use of scientific animals, are approved by a scientific committee. As its name suggests, the directive uses a principle of 3 "R's) (Reduce, Refine and Replace), as defined by William Russel and Rex Burch in 1959 (Russell and Burch, 1960). These three goals aim to:

- Reduce the number of animals used in scientific studies
- Refine tends to improve conditions and techniques employed to limit pain, suffering, and stress in animals used
- Replace the use of animals in research with alternative methods, such as the use of *in-vitro* models, or the use of predictive models, such as quantitative structure-activity relationships.

For aquatic systems, directive 2010/63/UE aims to protect advanced fish and other aquatic vertebrates, and to limit their use in scientific research. The directive defines protected fish as fish with external feeding. Unprotected stages include early life stages e.g. fish using vitellus as feeding and also gametes and eggs. Use of embryo-larval assays is in agreement with the 3Rs principle, to replace use of animals.

b. *Biological model*

Zebrafish (*Danio rerio*) is a freshwater fish belonging to the Cyprinidae family. Originating in South Asia (India, Pakistan, Bangladesh, Nepal and Bhutan), it was later introduced to different parts of the United States. Zebrafish is mainly found in moderately flowing and shallow waters, such as rice paddies, with some vegetation. Zebrafish can be found in water ranging from 16.5 to 34 °C and pH ranging from 6.5 to 8. Adult zebrafish measured between 3 and 5 cm (around 100 days), with a sexual dimorphism between males and females. Males are smaller than females, and females have a larger whitish belly and possess silver stripes, as opposed to gold stripes for males. The presence of a genital papilla in front of the anal fin is also characteristic of female zebrafish.

c. *Life stages*

Reproductive behavior is stimulated by light. The female releases ovocytes, whereas the male releases sperm into the medium for the fertilization. Eggs are protected by chorion, and development is divided into 8 stages (Kimmel et al., 1995), Figure 11.

- 0 – 0.45 hours post fertilization (hpf): zygote period, with the activation of cytoplasmic movements due to fertilization
- 0.45 – 2.15 hpf: cleavage phase. The first cell division occurs about 45 minutes after fertilization, with new divisions occurring every 15 minutes.

- 2.15 – 5.15 hpf: blastula , durng which the 128 cell stage starts with the beginning of epiboly.
- 5.15 - 10 hpf: gastrula period, when embryonic axis are formed. This period ends when the epiboly is completed and the tail bud is formed.
- 10 - 24 hpf: segmentation period, during which somites and the basis of the primary organs are formed. The tail bud becomes more prominent, and the first embryonic movements are visible. The yolk shrinks over time, as it serves as a source of nutrients for the fish during theirk first 3 days.
- 24 - 48 hpf: pharyngula period: development of pharyngeal arches, pigmentation, and thheartbeat, to distribute blood via a closed set of channels.
- 48 - 72 hpf, hatching period: , jaws, gills and pectoral fins develop. After 3 days post fertilization (dpf), the last development period, known as the larval period, begins.

At the end of these periods, growth will continue, but the morphogenesis is completed (Kimmel et al., 1995; Nüsslein-Volhard and Dahm, 2002). At 3 dpf, the first germinal cells are formed and, with larvae measuring approximately 3.5 mm. Opened-mouth starts at 4-5 dpf, and the larvae begin to feed on vitellus in the yolk sac. An optical response is observed at 3 dpf, and a day later, vision achieves adult levels (Nüsslein-Volhard and Dahm, 2002). Zebrafish are sexually mature after 3-4 months post fertilization.

D.rerio is a model fish species used extensively in scientific research. Standardized bioassays have been developed using zebrafish for toxicity testing (OECD, 1998; OECD, 2013; OECD, 2019). The species can be easily bred in laboratories, due to its small size. It tolerates a wide variety of water conditions. Thanks to these characteristics, it is possible to rear large numbers of zebrafish in a relatively small facility. The short life cycle of zebrafish allows it to be used use in multigenerational or transgenerational toxicological experiments, to evaluate long-term effects on populations exposed to toxic agents. Their external embryonic development provides an effective way by which to study possible anomalies and lesions affecting fish development, using a simple dissecting microscope ((OECD, 1998; OECD, 2013; OECD, 2019).

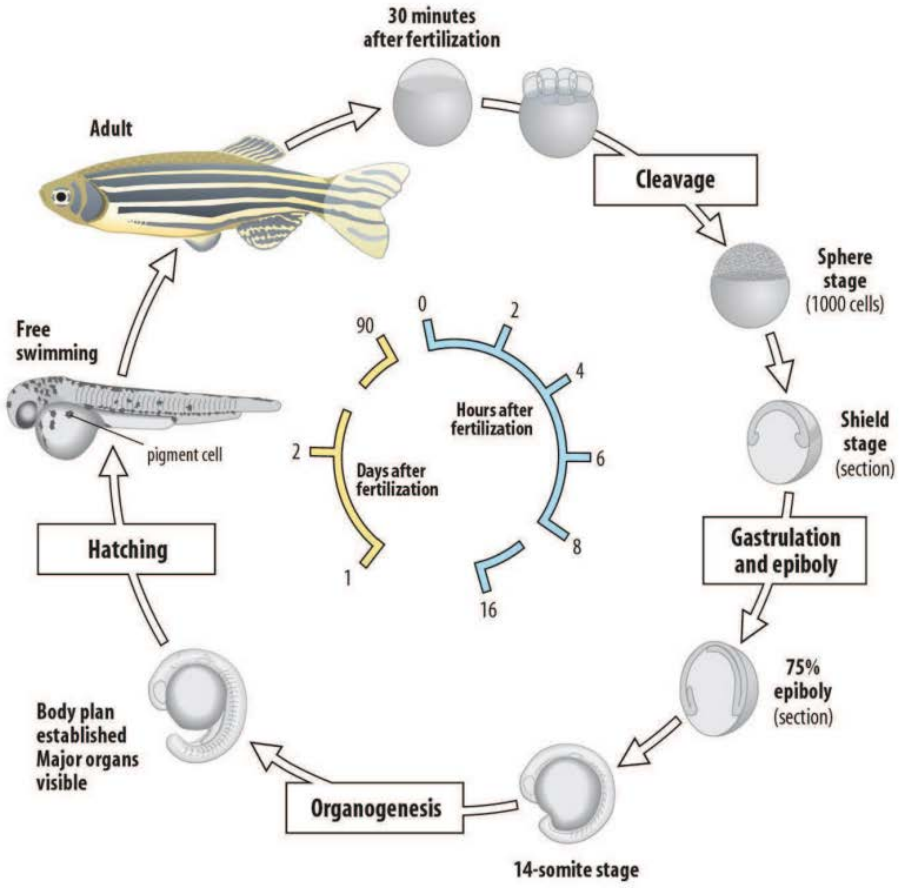


Figure 11: Life cycle of the zebrafish (D'Costa and Shepherd, 2009)

In a previous study, the Zebrafish genome was totally sequenced, showing significant similarities with the human genome, and highlighting that approximately 70 % of human genes have at least one obvious zebrafish orthologue, making them a useful tool with which to study diseases affecting humans (Howe et al., 2013).

In the present study, which combined a variety of laboratories and scientific teams, zebrafish was the reference biological model bred in each lab, allowing us to test MP effects as part of a multi-generational experiment.

d. *Ecotoxicological biomarkers using fish*

Biomarkers are biological parameters that have been developed as warning signals of chemical toxicity (Bucheli and Fent, 1995). In an environmental context, biomarkers are sensitive

indicators which reveal the internalization of a particular contaminant, its distribution into different tissues, and its inherent toxicity (Shugart et al., 1992). In 1997, Lagadic and coworkers defined a biomarker as an observable and/or measurable change at molecular, biochemical, cellular, physiological or behavioral level, revealing exposure of an organism to a chemical substance (Lagadic et al., 1997). Biomarkers are divided into three ecotoxicological categories: exposure biomarkers, effects and sensitivity. Exposure biomarkers give information on the presence of a pollutant within the body, through the bioaccumulation of a compound detected in a targeted fluid as blood, tissues or gametes (van der Oost et al., 1996). Effect biomarkers identify the toxic effects of a given substance once it has been ingested. Chemical stress is known to alter development, growth, or reproduction of fish (Miranda et al., 2008). A particularity of biomarker of effects is that, even if the biomarker shows a toxic effect at the cellular or subcellular level, it is not always observable at physiological level (mortality, deformities, or behavioral modifications).

In the present study, different biomarkers have been used to characterize the toxicity of MPs with and without adsorbed pollutants in zebrafish: embryotoxicity, teratogenicity and biometry, xenobiotic metabolism using enzymatic biomarkers, genotoxicity, behavioral studies and reproductive toxicity.

i. Embryotoxicity

Embryonic survival is a common biomarker used to assess the toxicity of a compound. Fish ELS are sensitive to water pollution. Due to their inability to escape pollution, they are also widely used to determine environmental standards (Frantzen et al., 2012; Vignet et al., 2014).

Standardized protocols have been developed for toxicity assays by the Organization for Economic Cooperation and Development (OECD) as technical guidelines (TG). TG using fish ELS are OECD TG 210, 212 and 236, which vary according to test conditions, generally in terms of duration. To achieve maximum throughput, these tests have been developed using endpoints that are easy to monitor. As an example, for TG236, death is recorded, and defined as at least one of four criteria from among egg coagulation, a lack of somite, no cardiac activity, or an undetached tail. While these criteria are fairly basic, they are efficient in identifying short-term acute toxicity. They also include both negative and positive controls, which are (for TG236) less than 10 % mortality for negative control and more than 30 % mortality for positive control (4 mg/L 3,4-dichloroaniline, 3,4-DCA). These tests can be enriched using additional

endpoints such as sub-lethal endpoints (*e.g*. body malformation, behavioral defects), some of which will be described later.

In the present study, the OECD TG 236 was used as a basis to assess the toxicity of different samples of MPs.

ii. Teratogenicity and biometry

Some pollutants, such as PAHs, are known to have teratogenic effects *e.g.* embryo-larval deformities (Incardona et al., 2006; Le Bihanic, Clérandeau, et al., 2014; Le Bihanic, Morin, et al., 2014). Developmental abnormalities can occur after pollutant exposure and can be expressed by malformations (spinal malformations, cardio-vascular or craniofacial anomalies) and lesions (edemas, yolk-sac malabsorption, blood circulation defects) or growth retardation (total length or weight) (Vignet et al., 2014; Weeks-Santos et al., 2019). The teratogenicity of a given substance is expressed using the teratogenicity index, which is the ratio of the percentage of dead or malformed individuals, divided by the percentage of dead individuals. An index superior to 1 indicates a teratogen substance. In the present study, we measured body length of fish in all experiments, as well as abnormalities and lesions in embryos, larvae, juveniles and adult zebrafish. Head length was recorded as a supplementary parameter for larvae.

iii. Xenobiotic biotransformation: ethoxy-resorufin-O-deethylase activity (EROD)

Biotransformation is a process of detoxification, able to transform an organic lipophilic compound into metabolites to facilitate their excretion by increasing the polarity and their hydrophilic properties. Biotransformation is composed of two phases: functionalization by oxidation, reductive or hydrolytic reactions to introduce polar groups, and conjugation: covalent attachment of small hydrophilic molecules (e.g. glucuronic acid, sulfate). Enzymatic systems associated with xenobiotic biotransformation pathways can be used as biomarkers of exposure to some substances, as for example the induction of EROD, a biomarker of exposure to substances including PAHs, dioxins or some PCBs.

The metabolic reactions of pollutants mainly involve cytochromes P450 (CYP) in the functionalization phase. Measurements of CYP transcription or their enzymatic activities are commonly used as biomarkers of exposure for the aquatic environmental pollution (Bouraoui

et al., 2009). EROD activity is one of the enzymatic activities of the CYP1A protein. In fish, EROD induction occurs in liver tissue exposed to aquatic pollution by PAHs and halogenated compounds (van der Oost et al., 2003). EROD activity can be increased or decreased, depending on the compound, its concentration, bioavailability and the time of exposure but also to biotic parameters (Klumpp et al., 2002; Lemaire-Gony et al., 1995; Milinkovitch et al., 2011).

iv. Neurotoxicity: acetylcholinesterase activity (AChE)

Only a few biomarkers of neurotoxicity are commonly studied and the most common is the measure of the acetylcholinesterase (AChE) activity. Acetylcholine (ACh) is a neurotransmitter of cholinergic neurons, which is delivered in the synaptic cleft upon arrival of nervous stimulus. Binding of ACh to receptors causes transmission of post-synaptic signals. AChE located in the synaptic cleft hydrolyses ACh to complete signal transmission. Some chemicals can interfere with AChE activity, such as organophosphorus pesticides. Measuring AChE inhibition has been reported as a biomarker of exposure to some neurotoxic compounds in vertebrates (Bocquené et al., 1997; Mineau, 1991).

v. Oxidative stress: thiobarbituric acid reactive substances (TBARs)

ROS are naturally produced in organisms at a basal level, allowing cell communication, as well as reinforcement of the immune system against exogenous substances (Iversen et al., 2011). ROS are highly biologically reactive and can interact with different biological substrates such as lipids, proteins or DNA. Lipid peroxidation occurs when a ROS reacts with a lipid, leading to oxidative degradation. Due to the short half-life of ROS, it is easier to measure damaged lipids than a specific amount of ROS. Thiobarbituric acid reactive substances (TBARs) are formed as a byproduct of lipid peroxidation and can be measured in fish following exposure to pollutants generating ROS (Barhoumi et al., 2014; Weeks-Santos et al., 2019).

vi. Monitoring of behavior

Behavioral studies are commonly used to investigate the toxicity of a chemical or a biological stress (Ali et al., 2012; Chen et al., 2011; Egan et al., 2009; Pannetier, Morin, et al., 2019; Sackerman et al., 2010; Vignet et al., 2014). Monitored behaviors include swimming behavior but also on the activity, anxiety, social communication, feeding behavior, exploration behavior,

escape strategies, learning abilities or reproductive behavior of fish (Vignet et al., 2014). Behavioral disruptions can be the result of a neurotoxic contaminant but also -since they form an integrative endpoint - disruption of development, growth, and metabolism (Legradi et al., 2018). Zebrafish is a diurnal fish, which reacts to changes in light intensity. A common behavioral test is to monitoring swimming activity after a dark/light stimulation to induce stress and thus a change in swimming activity. This test, generally performed on larvae, is based on a published method (Emran et al., 2008), which was refined and shown to be suitable to evaluate anxiety level.

vii. Reproductive toxicity

Reprotoxicity is an important criterion to evaluate the biological consequences of chemical exposure, as the reproduction does not affect an individual organism, rather the population as a whole. Some pollutants, such as PAHs, have been show to be transported to ovaries through the VTG (Monteverdi and Di Giulio, 2000) and/or the lipovitellin (Lee et al., 1993). An inhibition of VTG synthesis can result in stunted ovary maturation, and may increase apoptosis in gonadic cells (Marty et al., 1997). Direct damages to oocytes or sperm can cause a decrease in fertilized eggs and larval survival, leading to population decline (Kime, 1995). Biomarkers of reprotoxicity include changes in number of eggs layed per female, fertilization rates, quality of development (Vignet et al., 2014).

6) EPHEMARE project

My PhD thesis was written as part of the EPHEMARE project, funded by the JPI-Ocean. The EPHEMARE project aims to investigate the adsorption of pollutants on microplastics, their ingestion, trophic transfer and chemical release, and ecotoxicological effects on a wide range of aquatic organisms including invertebrates and vertebrates. The project was divided into 7 work packages (WP), as listed below:



- **WP1**: To investigate the sorption equilibrium kinetics of model persistent pollutants to MPs, to test the potential role of MPs as vectors for marine pollutants.
- **WP2**: To provide information concerning the uptake, accumulation and elimination of MPs spiked with pollutants to a variety of aquatic species.
- **WP3**: To study the consequences of MPs and organic or metallic pollutants on several key physiological functions for recruitment: survival, growth, reproduction as well as behavior.
- **WP4**: To evaluate the effect of MPs and the adsorbed pollutants in different biological models (from cell lines to vertebrates) by a wide set of biomarkers from a very low level of organization such as gene expression, to the highest such as individual energy balance.
- **WP5**: To find out whether MPs accumulate along food chains and serve as vectors for persistent pollutants and to lead to adverse effects at molecular, cellular, physiological and organism levels.
- **WP6**: To support results in the previous WPs by using relevant field data describing the occurrence, distribution, typology and chemical loads of MPs in representative biota of defined geographical areas.
- **WP7**: To disseminate and communicate scientific findings

Figure 12 provides an overview of the structure and organization of the EPHEMARE project. Experiments presented in this PhD manuscript were part of the WP1 for the sorption/desorption studies, while the short and long-term exposures were part of WP3 and WP4.

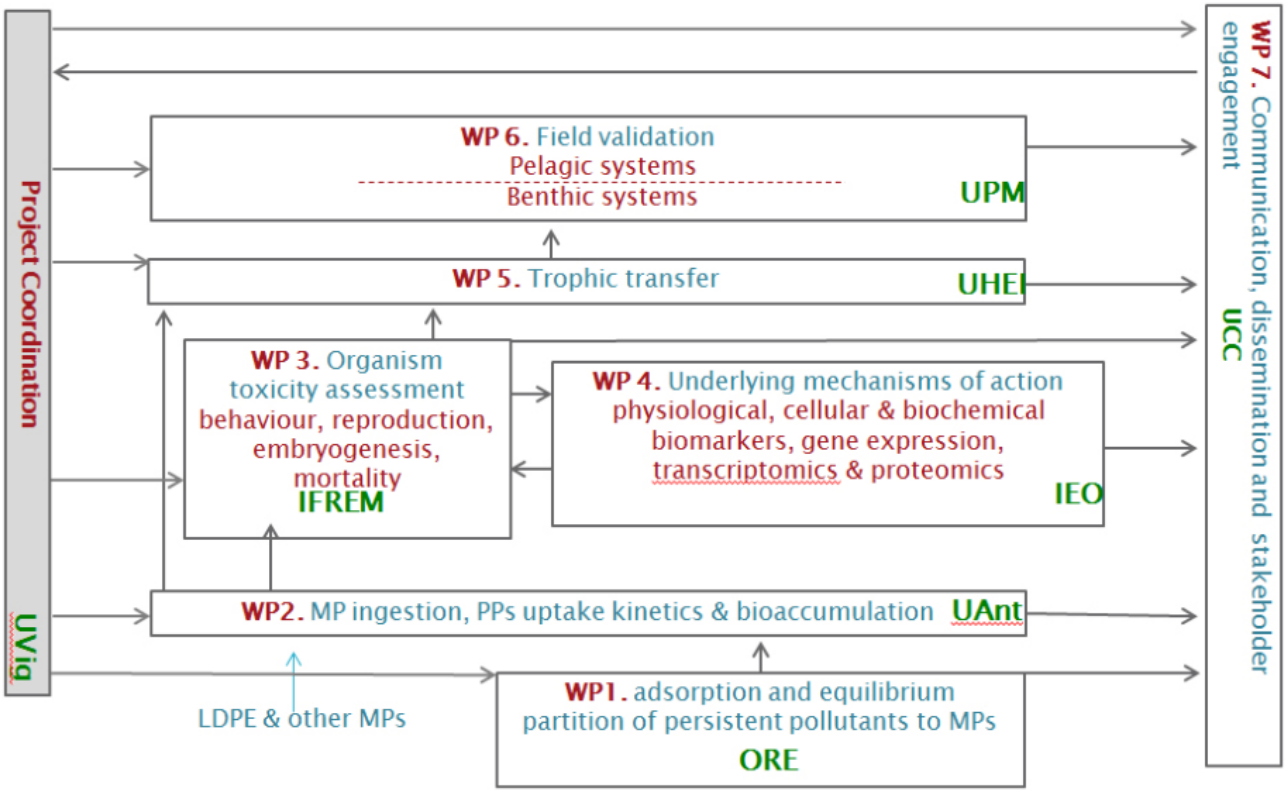


Figure 12: Work packages of the EPHEMARE project

Chapter 2: Materials and methods

1) Microplastics & Chemicals

a. *Selection of industrial microplastics*

Two industrial-type were selected at the beginning of the project, on the basis of their frequency and environmental relevance. Polyethylene (PE; $-(-CH_2-CH_2-)_n-$) is most-produced polymer worldwide (PlasticsEurope, 2015), as well as being the most frequently found in the open sea, when comparted to other polymer materials (Dris et al., 2015). To provide a wide representation of plastic pollution, polyvinyl chloride (PVC; $-(-C_2H_3Cl-)_n-$) is also used. Because PVC is heavier than water, it generally appears in sediment.

For PE (density of 0.96 kg/m3), 4 different size ranges were used: 125-500 µm, 20-25 µm, 11-13 µm and 4-6 µm (Figure 13). PVC MPs were measured between 100 and 250 µm

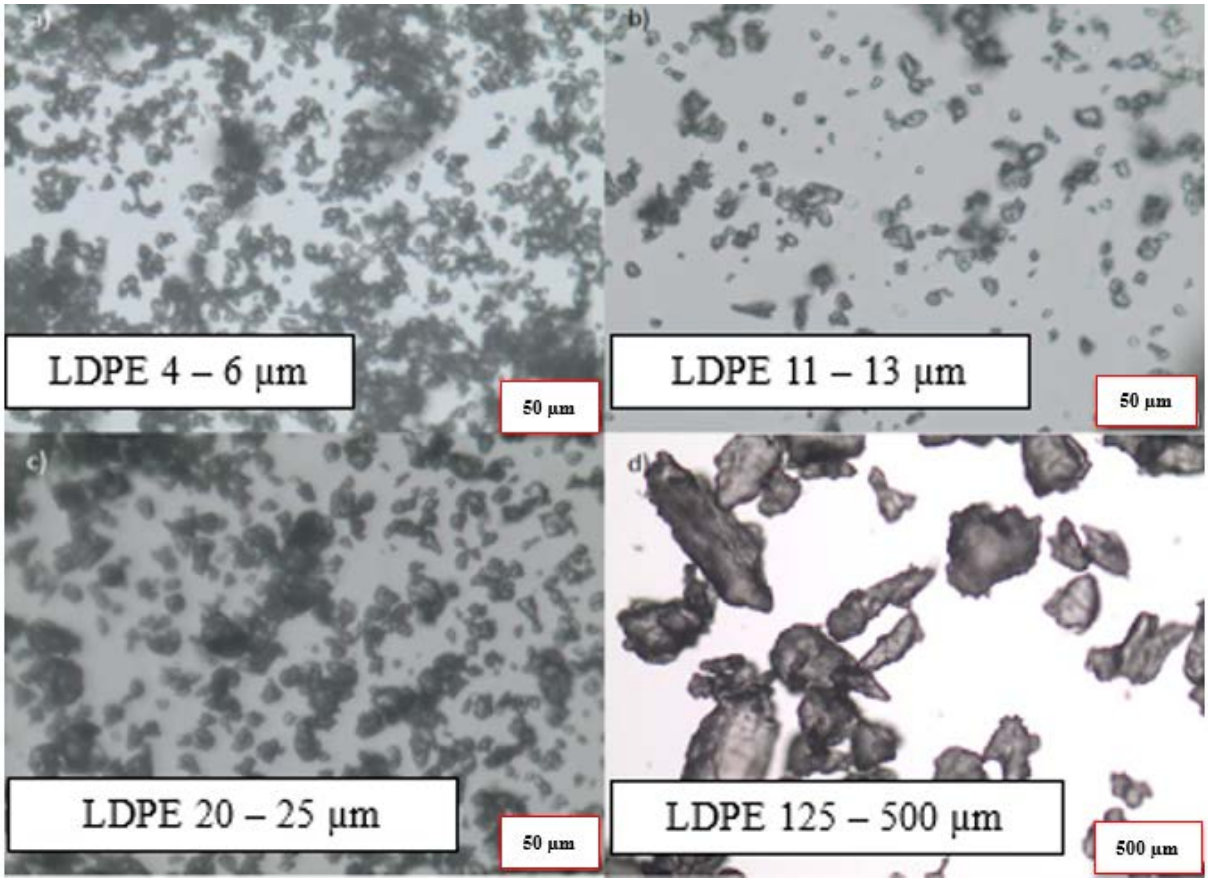


Figure 13: Images of the different HDPE particles used

As can be seen in Figure 13, whatever their size, PE and PVC MPs are not spherical, and have irregular shapes.

b. *Environmental microplastics*

Environmental MPs were collected during the Race for Water Odyssey in 2017, in two different sandy beaches in Guadeloupe (France), following the standardized NOAA protocol (Lippiatt et al., 2013). The location of Guadeloupe was chosen due to its position in the North Atlantic Gyre, an open ocean highly contaminated with MPs (Law et al., 2010; Ter Halle et al., 2017). Samples of meso- and MPs were collected in October 2017, after hurricane Maria with the combination of rough seas and strong winds, which led to accumulation of plastic debris on sandy beaches. Two sample sites were chosen (Figure 14); Viard Petit-Bourg (site PB), a beach on the eastern side of Basse-Terre island, a densely populated area nearby the largest industrial zone of the archipelago; and Capesterre de Marie-Galante (site MG), a sandy beach on the southeast of Marie Galante island, a small island faintly populated. Debris were selected visually at the different sites, and weighed later. Polymer types were characterized by using Fourrier-transform infrared spectroscopy with a spectrum Vertex 70v (Bruker). To obtain particles with the same size range, debris were ground using a cryogenic mill 6770 Freezer/Mill. Powder obtained was sieved for 12 hours at ambient temperature through 100 and 53 µm sieves, and the granulometry of particles was measured.

Chemical analyses were performed to identify the chemical composition of each sample (additives and pollutants). This involved non-target analysis, after liquid/solid extraction in hexane (≥ 98 %, SupraSolv; Merck, Darmstadt, DE), followed by sonication centrifugation. Extracts were filtered through glass wool with sodium sulfate, and finally reduced to 25 µL for injection. Analysis was performed using gas chromatography (GC) coupled with an orbitrap (Q-Exactive, ThermoFisher) with a resolution of 60 000 at m/z 200 with a fullMS between 53.4 and 800 m/z. Plastic samples were analyzed using mass defect-plot for chlorinated and brominated compounds (HaloSeeker), PAHs and hydrocarbons (CH_2). TraceFinder (ThermoFisher) was used for data processing, fold change and elemental composition analysis. Fold change results were then sorted, with the 50 highest fold change features being analyzed. Chromatogram peaks were then checked, and only symmetric peaks with widths below 0.5 min were retained. Finally, 26 features were selected for further analysis for elemental composition analysis.



Figure 14: Study area showing the sampled sites in Guadeloupe (France): PB (red star) indicates Petit-Bourg site located on the main island and MG (red circle) indicates Marie-Galante site located on the second island of the archipelago

c. *Selection of chemicals*

Given the wide range of chemicals, three different model organic pollutants were selected:

- Perfluorooctane sulfonate (PFOS)
- Benzo[a]pyrene (BaP)
- Benzophenone-3 (BP3)

Table 1 summarizes physico-chemical characteristics of each compound.

Table 1: Chemical characteristics of the selected compounds (information from substances.ineris.fr)

	PFOS	**BaP**	**BP3**
IUPAC name	1-octanesulfonicacid, 1,1,2,2,3,3,4,4,5,5,6,6,7, 7,8,8-heptadecafluoro	Benzo[a]pyrene	2-benzoyl-5-methoxyphenol
CAS number	1763-23-1	50-32-8	131-57-7
Molar mass	500.13 g/mol	252.31 g/mol	228.247 g/mol
Boiling point (°C)	133 °C	475 °C	300 °C
Water solubility	519 mg/L (at 20 °C)	0.0038 mg/L (at 25 °C)	6 mg/L (at 25 °C)
Vapor pressure	$3.31x10^{-4}$ Pa	$7.3x10^{-7}$ Pa	$1x10^{-3}$ Pa
LogKow	4.88	6.07	3.45 (at 40 °C)
Koc (20 °C)	26.6 L/kg	5000000 L/kg (sediments)	954.8 L/kg
Chemical formula	$C_8HF_{17}O_3S$	$C_{20}H_{12}$	$C_{14}H_{12}0_3$

As said previously, as part of the EPHEMARE project, three chemicals were chosen based on their differing levels of toxicity and modes of action, from different chemical groups. A major aim of the study was to investigate MPs as vectors of pollutants for fish. The three compounds have previously been reported in different aquatic environments because of their anthropogenic uses, PFOS as surfactants, BaP is a PAH produced by incomplete combustion (fossil fuel combustion) and BP3 as UV-filter used in cosmetics. Their presence in environmental waters may facilitate the contact and the adsorption of pollutants to MPs surfaces. Laboratory studies have shown that is possible to artificially sorb these compounds to MPs, under experimental conditions. Besides artificial sorption, analyses and characterization of environmental MPs samples have shown the sorption of these compounds to MPs surfaces.

Besides experimental work, I was responsible for producing the spiked MPs (spiked PE/PVC) used in EPHEMARE. Protocols to prepare spiked MPs were developed to provide materials for partners, to test the toxicity of MPs spiked on a broad range of aquatic organisms.

2) Sorption of pollutants

Sizes of MPs and pollutants are important both for sorption level and interaction mechanisms. To study sorption characteristics and the associated sorption mechanisms of two model pollutants (PFOS and BaP), four different PE size ranges were investigated. The sorption isotherms were first evaluated, and the influence of time on sorption levels was then studied.

Before each experiment, polymer particles were analysed in order to check the background contamination for the three different pollutants. The analysis was carried out on 1g of MP in each size range, in triplicate.

a. *Chemical analysis*

PFOS

Chemical analysis of PFOS was carried out according to Eriksson et al. (2016), with minor modifications. MPs were extracted in methanol (MeOH) by ultra-sonication followed by centrifugation (7000 rpm). Extracts were filtered using a polyethylene syringe with a filter of 0.2 µm. Liquid chromatography (LC) vials were prepared with recovery standard (mass-labelled PFOS). Samples were diluted using 40/60% (v/v) of MeOH/ammonium acetate (v/v). Analysis was performed on an Acquity ultra performance liquid chromatography (UPLC) system coupled to a Xevo TQ-S quadrupole MS (Waters, Milford, USA), with a guard column inserted between the pump and the injector to prevent contamination from the system. PFOS were separated on 100 mm Acquity BEH C18 column using MeOH and water as mobile phases, both with 2 mM ammonium acetate. Quantification of total PFOS was achieved using the sum of linear PFOS and individual branched PFOS calculated against an external calibration curve using technical PFOS as standard. Recoveries were assessed using labelled internal standards. The mean recoveries of labelled PFOS internal standards were 83-96%. The limit of detection (LOD) was 10 pg/g. Procedure blanks were included in all batches.

BaP

BaP analysis wasconducted according to Larsson et al. (2013) with minor modifications. Samples were extracted in hexane with addition of internal standard (BaP D12 in toluene) by ultra-sonication and centrifugation at 2000 rpm. Extracts were filtered through fiberglass and transferred to toluene. GC vials for analysis consisted in a mix of recovery standard perylene D12 and the extract. Concentrations of BaP were quantified using a Micromass Autospec Ultima high-resolution GC/MS system (Agilent Technologies), separation on a DB-5MS column (30 meters, 0.25 mm internal diameter, 25 µm film thickness). Procedure blanks were included in all batches. Concentration of BaP was calculated using the internal standard method. LOD was defined as mean concentration in blanks plus three times the standard deviations and was calculated as 5 pg/g.

BP3

Analysis of BP3 was performed by Eurofins Germany by using an aliquot of the sample spiked with internal standards (i.e., 3,4,5,6-pentadeuteriobenzoic acid, 1-bromo-4-phenylbenzene, 1,2,3,4,5,6,7,8-octadeuterionaphthalene, 1,2,3,4,5-pentachloro-6-(2,3,4,5,6-pentachlorophenyl)benzene, and 1,2,3,4,5,6,7,8,9,10-decadeuteriophenanthrene) and concentrated with a factor of 10. The samples were extracted with a 1:1 solvent mixture of ethyl acetate and cyclohexane. Additionally, an external calibration standard and a blank sample were prepared. Extracts were measured with GC/MS/MS, electron impact ionization (GC: Agilent7890 A, MS/MS: Agilent 7000 multiple-reaction monitoring mode) using a splitless injection of 2 µl (column DB5-MS, 30m×0.25mm×0.25 µm). Identification and quantification were performed against the retention time and the external calibration using the BP3 specific selected reaction monitoring (one quantifier and one qualifier).

b. *Study of the sorption of pollutants onto microplastics particles*

The sorption of the selected chemicals was investigated with the aim of providing similar particles at environmental or toxic concentrations to all partners involved in the EPHEMARE project, for toxicity testing and comparison of results obtained on numerous aquatic organisms within the scientific consensus.



Figure 15: Shaking a rotary shaker (Heidolph Instruments GmbH & CO. KG, Schwabach, Germany) at 20 rpm, for the preparation of MPs spiked with PFOS (polypropylene bottles).

The sorption of PFOS on PE and PVC was conducted using 50 g/L and 200 g/L of plastic, respectively, placed into polypropylene bottles containing double-deionized water. Various amounts of PFOS were added (Table 2). Bottles were placed on a rotary shaker (Figure 15) for 7 days at 20 rpm. For the sorption of BaP and BP3, 125 g/L of PE or PVC was used in amber glass bottles filled with double-deionized water, to avoid UV degradation. BP3 concentration in the solutions was 20 µg/L, independently of MPs used, while BaP concentration was set up to 2500 µg/L for PE and 5000 µg/L for PVC (from stock solutions prepared in toluene). Bottles were placed on a rotary shaker for 2 days at 20 rpm. MPs particles were filtered using 1 µm Whatman® glass microfiber filter, and subsequently, the particles were rinsed with double deionized water, before chemical analysis.

Table 2: Variable concentration of PFOS added into water, depending on MPs used for the sorption experiment

	PE				**PVC**
Size	125-500 µm	20-25 µm	11-13 µm	4-6 µm	100-250 µm
[PFOS] (mg/L)	600	30	20	10	200

Sorption experiments, such as kinetics and isotherms, were performed on PFOS and BaP only, due to the level of lab access available.

c. *Sorption kinetics of PFOS and BaP*

The sorption processes of chemicals to MPs is poorly understood. Testing of sorption kinetics using MPs has only been tested for short-term exposure (a few weeks). We aimed to investigate the sorption process and the equilibrium between pollutants and MPs for a long-term exposure of 6 months, to achieve a realistic scenario. Long-term sorption kinetic experiments were conducted in 50 mL PP centrifuge tubes or 40 mL amber glass tubes, for PFOS and BaP, respectively. Each tube was filled with 0.4 g of MPs and spiked with either 200 mg/L of PFOS or with 2.5 mg/L of BaP (prepared in toluene). Tubes were continuously shaken on a rotary shaker at 20 rpm for 180 days, at room temperature (20 ± 1 °C). Some samples were collected after 2 days, 7 days, 30 days, 90 days and 180 days, and both water and MPs were analysed as described above.

d. *Isotherm sorption PFOS and BaP*

Isotherm sorption was carried out over 7 days, for both pollutants. Containers for the isotherm experiments were the same as those used for sorption kinetics. Tubes contained 0.1 g of MPs and 40 mL of double-deionized water solution with different PFOS or BaP concentrations ranging from 25 µg/L to 100 µg/L. The tubes were shaken at 20 rpm on a rotary shaker and kept at 20°C for 7 days and 2 days, for PFOS and BaP, respectively. All experiments were conducted as three independent replicates. The major objective of this experiment was to investigate the effect of size on sorption efficiency, influenced by the initial concentration of the compound.

e. *Particle digestion under simulated physiological conditions*

One aim of the present study was to investigate whether MPs could be potential vectors for pollutants. Besides sorption experiments, the desorption process is also an important part of evaluating the potential transfer of pollutants to aquatic organisms. To test desorption, an artificial gut fluid (AGF) was used to simulate biological digestion by fish, to analyze the quantity of a selected compound, PFOS, on MPs after digestion as well as in the digestive fluid.

A digestion scenario was set up for an Atlantic Cod (*Gadus morhua*) which ingests 0.0126 g food/g bodyweight per day (1.26 %). Since our study focuses on the uptake of MPs through food, quantities of 0.3 % (Jovanović et al., 2018) and 1 % of plastic particles related to ingested food per day were chosen. Gut retention time was assumed four days on average (Daan, 1973).

PE spiked with PFOS was artificially digested in 10 ml AGF, prepared based on the protocol of Voparil and Mayer (2004) in a PP tube. Three replicates of 10 ml pure AGF and 10 ml AGF containing 1 % PE were run as controls in parallel. Samples were incubated in darkness for 4 days at 20 rpm on a rotary shaker at room temperature. Exposure was stopped by filtering MPs out of AGF using a glassfiber filter. Filtrates and MPs were transferred to new PP tubes, prior to extraction. PFOS was extracted from AGF and PE control samples after exposure using solid phase extraction (SPE) (**Error! Reference source not found.**).

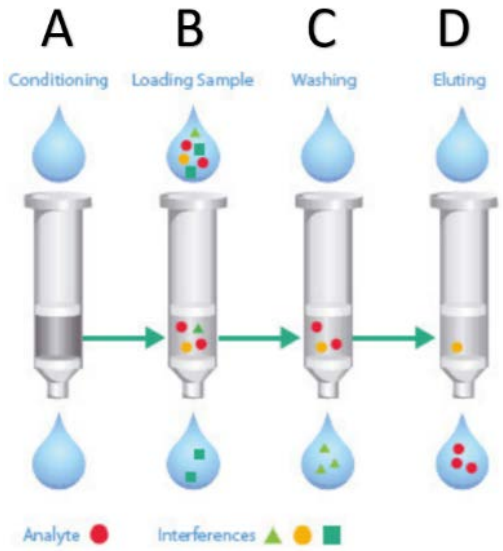


Figure 16: Solid phase extraction principle, conditioning (A); loading sample (B); washing step (C) and elution of the compound (D) (Andrade-Eiroa et al., 2015)

SPE cartridges (Oasis® WAX 150 mg 6 cc, Waters®) were preconditioned with 0.1 % ammonium hydroxide (NH_4OH) in MeOH, pure MeOH and double deionized water (Figure 16-A), then samples were loaded (Figure 16-B). Columns were washed using double deionized water, ammonium acetate buffer (pH 4) and 20 % and 100 % of MeOH (Figure 16-C). Subsequently, columns were eluted using 0.1 % NH_4OH in methanol (Figure 16-D).

3) Biological model, zebrafish (*Danio rerio*)

Zebrafish (*Danio rerio*) was selected to evaluate the toxicity of MPs associated with pollutants, due to its characteristics, which lend themselves particularly well to the study of development. Fertilization and embryonic development are external, large amounts of eggs are produced in each breeding period. Also, embryos are relatively large, and their development can be observed easily through the chorion, showing developing organs.. Embryonic development is rapid, with

progression from egg stage to larvae stage taking less than three days. Right after hatching, zebrafish larvae are able to swim. First feeding occurs on or around the fifth day. Zebrafish were selected due to their current use for toxicity testing based on OECD standard protocols: *e.g*. OECD 212, 210 and 236.

Experiments were conducted at Örebro University (Sweden), Bordeaux University (France) and IFREMER La Rochelle (France). While conditions differ at each of the sites, (Table 3) they remain within the recommendations laid out by OECD, and conform to international standards (OECD, 2013; Westerfield, 2000). Fish were fed daily, with commercially available fish food in the morning and evening, with freshly hatched brine *Artemia nauplii* in the afternoon. Constant filtering conditions guaranteed that ammonia, nitrite, and nitrate were kept below detection limits in laboratories (0 - 5, 0.025 – 1 and 0 - 140 mg/L, resp.). Zebrafish eggs were collected according to OECD guidelines TG 236 (OECD, 2013).

The laboratory animal facilities at MTM Research Centre, Örebro University (#5.2.18.-12707/17) have permission to use (5.12.18-12628-17) and breed laboratory animals (5.2.18-12630-17). These permits are issued by the Swedish Board of Agriculture, Jönköping, Sweden. The Swedish facilities also hold ethical permission (# 5.2.18-861/15) from the same board in Linköping, Sweden. The laboratory animal facilities at Ifremer Research Centre, L'Houmeau, have authorisation to use and breed laboratory animals (EEA 171901).The project took place under the approval of the Animal Care Committee of Poitou-Charentes # 84 COMETHEA (France) with project authorization number APAFIS 10883 and followed the recommendations of Directive 2010/63/EU.

The study was conducted at the laboratory animal facilities at Ifremer Research Centre, L'Houmeau, holding permission to use and breed laboratory animals (EEA 171901) and the project was performed under the approval of the Animal Care Committee of Poitou-Charentes # 84 COMETHEA (France) with project authorization number APAFIS 10883 and followed the recommendations of Directive 2010/63/EU.

The study was conducted at the laboratory animal facilities at Bordeaux University, holding permission to use and breed laboratory animals (A33-522-7).

Table 3: Husbandry conditions of zebrafish in the different participating laboratories

	Örebro University, Sweden	*IFREMER La Rochelle, France*	*Bordeaux University, France*
Zebrafish strain	AB strain	TU strain	TU strain
Fish group	100 ± 10	28 ± 5	16 ± 2
Tank size (L)	60 L	10 L	8 L
Temperature (°C)	26 ± 1	27 ± 1	26.5 ± 1
pH	7.2 ± 0.2	7.8 ± 0.2	7.5 ± 0.2
Conductivity (µS)	380 ± 50	485 ± 130	410 ± 80
Day/night cycle	14/10	14/10	14/10

4) Experimental designs

a. *Exposure of zebrafish embryo for acute toxicity test*

The zebrafish embryo acute toxicity test (FET) was performed according to OECD TG 236, with minor modifications. The use of the standardized protocol is designed to evaluate the acute toxicity of MPs for non-protected stages of fish as an alternative to animal experimentation.

Embryos were exposed either to MPs as particles, organic extracts (dimethyl sulfoxide, DMSO extracts), or lixiviates (water extracts). Glass beakers were filled with the different test solutions, prepared in ISO 7346/3 water. Each beaker was populated with five embryos of 3 to 4 hpf. Exposure to MPs was performed by adding MPs at final concentrations of either 1, 10 or 100 mg/L. Waterborne exposure was also performed t for the three pollutants in DMSO (0.01%), at final concentrations, resulting in complete desorption in the case of an exposure to 10 mg/L of MPs (0.8 ng/L BP3, 170 ng/L BaP and 700 ng/L PFOS). Each test included negative, solvent and positive controls using the specific media without pollutant (with 0.01% of DMSO for organic extracts) and 3,4-DCA, 4 mg/L, respectively. For all test solutions, embryos were exposed for 96 h under semi-static conditions (OECD, 2013) with a medium exchanged every 24 h. All exposures were performed in triplicate.

b. *Trophic exposure*

The trophic experiment aims to assess MPs as vectors of pollutants for zebrafish, and their potential to cause long-term toxicity. For trophic exposure, eggs were collected at IFREMER (La Rochelle) and raised in 1-litre tanks with daily renewal for one month, then transferred to 10-litre tanks for 3.5 additional months. All fish were fed from the 5th day, after hatching.

Zebrafish were exposed via contaminated food, spiked with 1% of MPs. Fish exposed to HDPE polymers (11-13 µm) were exposed to MPs from the 5th day post fertilization while, fish exposed to PVC (100-250 µm) started to be fed with polymers after 2 months due to a bigger size particles. Size particles of environmental samples (< 53 µm) were prepared to feed zebrafish after 1-month post fertilization. After 2 months, bigger particles (53-100 µm) were used. Figure 17 summarizes the design of the trophic experiment.

After 1, 2 and 4.5 months, fish were anesthetized to record growth (length and weight) and some fish were sampled to quantify the amount of pollutants within them. After 3 months, reproduction parameters such as spawning success, fertilization ratio and reproductive effort (number of eggs/spawning) were evaluated. Some spawns were used to produce F1 generation that was monitored for behavior and survival. At 4.5 months, fish were sampled to evaluate different toxicity biomarkers.

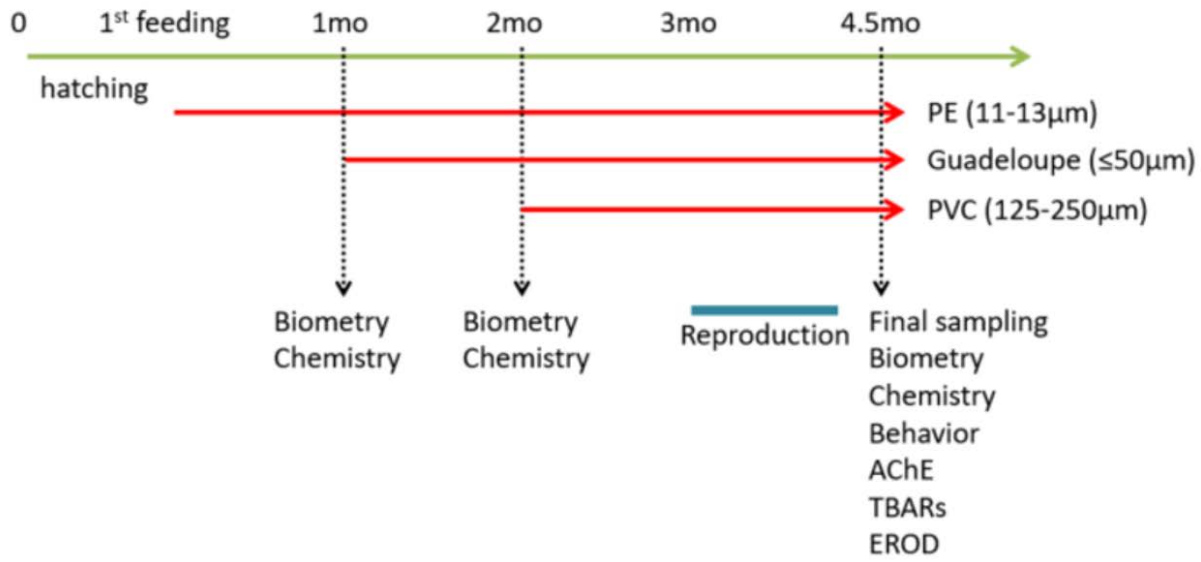


Figure 17: Design of the feeding experiment. Red lines correspond to the starting of the exposure according to polymer types. Blue line corresponds to the period used to evaluate the reproduction. Dashed vertical lines represent the sampling time with biomarkers analyzed (biometry: growth and weight; chemistry: fish sampled for pollutants quantification; behavior: "novel tank").

For each polymer type (PE and PVC), pristine MPs and MPs spiked with PFOS, BaP and BP3, were used, to identify toxicity of MPs or MPs-spiked pollutants. In addition to MPs spiked with one pollutant, additional diets were produced by mixing equal proportions of MPs spiked with three pollutants, keeping the MP ratio to 1% (called PE-3POP and PVC-3POP). Two additional conditions involved MPs prepared from plastic taken from beaches in Guadeloupe, MG and

PB. With control, this resulted in thirteen conditions. In the present study, results for PE-3POP and PVC-3POP are not presented.

5) Endpoints analyzed

a. *Standardized FET*

Embryos were analysed every day for lethal indicators (OECD, 2013), *e.g.* hatching rate, coagulation of embryos, lack of somite formation, non-detachment of the tail, and lack of heartbeat. Additionally, length was measured at 5 dpf, using lateral photography. Larval standard length was measured using ImageJ (Schneider et al., 2012). The standardized protocol was improved by the addition of other endpoints, which are described below.

b. *In vivo ethoxyresorufin-O-deethylase (EROD) assay*

Measurement of EROD activity is based on the transformation of 7-ethoxyresorufin (7-ER) into resorufin, a fluorescent molecule. This reaction is catalyzed by the P450 cytochrome, CYP1A. Fluorescent intensity is proportional to the resorufin produced by EROD activity (Whyte et al., 2000; Whyte and Tillitt, 1995). Analyses were performed along the same lines as Kais et al., (2018). Samples of fish were incubated in 7-ER (Sigma Aldrich, Deisenhofen, Germany). Fluorescence emitted by resorufin was measured with an epifluorescence microscope at an excitation of 560 ± 20 nm and an emission of 630 ± 30 nm. As a positive control, embryos were incubated at 96 hpf for 3 h in 75 µg/L β-naphthoflavone or BaP. Three replicates were run for each test and treatment group.

c. *Cyp1a gene transcription analysis*

For analyses of *cyp1a* induction, 96 hpf larvae were exposed for 3 h to MPs as described above. Exposures were performed in triplicate with 15 larvae per replicate. In a similar manner, 96 hpf larvae incubated for 3 hours in 75 µg/L β-naphthoflavone (Boehler et al., 2018) were used as positive control. After incubation, larvae were euthanatized in benzocaine and embryos were placed in a lysis buffer and being mechanically disrupted. Total RNA extraction was performed, and subsequently, cDNA was synthesized from total RNA. The amplification protocol used was as follows: initial denaturation (20 s at 95 °C) followed by 40 cycles of 3 s at 95 °C and 30 s at 60 °C. Technical triplicates were run for each biological replicate. The analysis software Relative Expression Software Tool (Pfaffl, 2001; Pfaffl et al., 2002) automatically calculated fold changes in expression relative to negative control using the 3 most stable references genes

between all groups, and combined them into an index via the free access BestKeeper software. Reference genes were *g6pd*, *actb1* and *rpl13a*.

d. *Acetylcholinesterase activity (AChE)*

AChE was performed on 4.5-month-old zebrafish brains. Extraction was carried out following Lowry et al., 1951 and quantification of AChE was performed following Ellman et al., 1961 (Ellman et al., 1961; Lowry et al., 1951).

e. *Lipid peroxidation*

The amount of ROS needed to evaluate oxidative stress is difficult to measure, due to their short half-life, so monitoring of byproducts of lipid peroxidation such as TBARs can be used (Buege and Aust, 1978). TBARs have been measured in zebrafish muscles sampled at 4.5 months, and following the colorimetric protocol developed by Buege and Aust (1978).

f. *Behavioral response: Larval photomotor response (LPMR)*

LPMR has been used to monitor the swimming behaviour of zebrafish larvae, in response to a light stimulus. Larval behavior was monitored at 120 hpf using LPMR test as described in Vignet et al. (2014). Larvae were individually transferred into one well of a 24-well plate and plates were transferred to an enlighten incubator at 27 ± 1°C. Plates were successively placed into DanioVisionTM (Noldus, NL) in the dark for 10 minutes of acclimation before the LPMR test which included the following 5-min steps: Light on–1 (LON1, 70 lux), Light off (LOFF, <1 lux) and Light on–2 (LON2, 70 lux) with constant infra-red light maintained during video recording. Distance travelled (cm), mobility and velocity (cm/s) were recorded and summed for each 5-min step.

g. *Reprotoxicity*

Starting at 3 months, fish were paired late in the afternoon in spawning boxes. To limit stress due to manipulation, fish from one tank were only solicited once a week. In the morning, switching on lights was the stimulus used to initiate spawning. After two hours, spawning boxes were inspected, and spawning success (eggs obtained) was recorded. After that, fish were returned to their tank and each spawn qualified (eggs number, fertilization rate). For a subset

of spawns, fertilized embryos (50 fertilized eggs/spawning) were placed into E3 medium to evaluate hatching success, larval survival without external feeding.

h. *Chemical analysis*

During long-term exposure, fish were sampled to quantify PFOS, BaP or BP3 levels. Pooled fish (1 and 2 months) and individual fish (4.5 months) have been used, and the F1 generation has been sampled at embryonic stages (3-5 hpf), to quantify the transfer of pollutants from genitors to offspring.

PFOS

To analyze PFOS in fish, ion pair extraction was performed, with alkaline digestion (So et al., 2006; Yoo et al., 2009). Fish were grinded in a mortar with the addition of 20 mM of sodium hydroxide, sonicate for 30 min and placed on a horizontal shaker overnight (16 h). pH was neutralized, and 0.5 M of tetrabutylammonium sulfate and 10 ng of internal standard were added. Samples were then sonicated for 15 minutes. Extraction was performed three times with 2 mL MTBE (15 min of sonication followed by centrifugation 10 min at 8000 rpm). Extracts were evaporated down to dryness with nitrogen and then, MeOH was used to dissolve extracts, and 2 ng of RS was added. Chemical analysis was performed as previously described.

BaP

Samples were firstly homogenized in a mortar to obtain a homogenous and representative sample. Anhydrous sodium sulfate in a 1:5 ratio was added to eliminate water in the sample, as well as 1 ng of BaP d12. Deactivated mini-silica columns were prepared with 10 % of deactivated silica and anhydrous sodium sulfate, washed with n-hexane:dichloromethane (3:1, v/v). Samples were eluted with 8 mL of n-hexane:dichloromethane (1:1, v/v), and reduced to 200 µL before a transfer to toluene (reduced to 500 µL), with the addition of 2 ng of perylene d12. Chemical analysis was performed as previously described.

BP3

Samples were grinded in a mortar with 1 mL of acetonitrile, followed by 15 min of sonication, 15 min of horizontal shaking and 5 min on a vortex. Samples were subsequently centrifuged for 15 minutes at 3900 rpm, and passed through a filter (GHP acrodisc 13 mm). Filtrates were stored in a freezer (- 20 °C) overnight (16 h) to precipitate proteins. After precipitation, samples were centrifuged and the supernatant was evaporated down to 100 µL with nitrogen gas stream.

Analysis was performed using Acquity UPLC I-class coupled to a quadrupole time-of-flight mass spectrometer (Xevo G2-XS QToF MS, Waters Corporation, Milford, USA) using Acquity UPLC BEH C18 column (L 100 mm * ID 2.1 mm, particles of 1.7 µm) with a set temperature of 50°C and an injection volume of 10 µL. Sample temperature was set at 10 °C to avoid compound degradation during analysis. Analysis was performed with an electrospray ionization in positive mode with an MSE method between m/z 50 and m/z 600. Mobile phase was composed of 0.1 % formic acid in water for the aqueous phase and 0.1 % formic acid in acetonitrile for the organic phase. BP3 and BP1 concentrations were calculated using BP-d10 as internal standard. Calibration curves (corrected response (analyte response * IS concentration / IS response) against the analyte concentration) were plotted for a concentration of BP1 and BP3 of 0.4; 2; 8; 20; 40 and 80 pg/µL with an internal standard concentration of 10 pg/µL. Each calibration curve concentration was injected in triplicate. Quantification limits were calculated from the 0.4 pg/µL calibration curve point for BP3 and BP1 as 0.031 pg/µL and 0.063 pg/µL respectively. Procedure blanks were included in all batches.

Chapter 3: Sorption processes

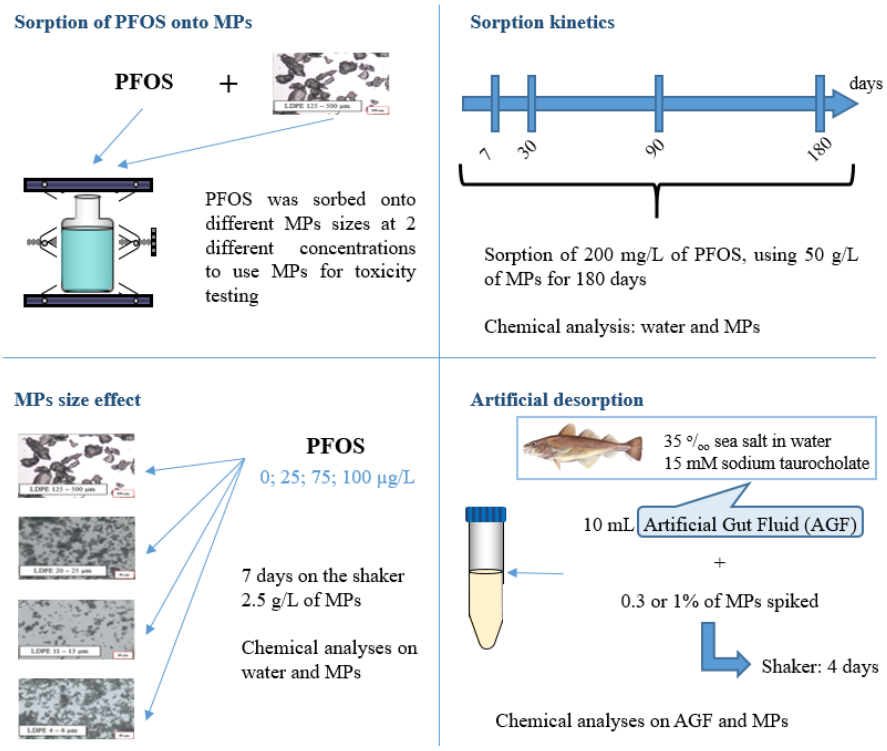
Chapter 3 – Presentation

Context

Sorption of contaminants by microplastics (MPs) is known to influence the distribution, persistency and the bioavailability of the pollutants in aquatic ecosystems. Previous studies demonstrated that numerous organic and metallic contaminants sorbed onto MPs, and particularly to PE particles. Aims of the present chapter were to investigate different sorption processes of PFOS onto PE, such as the sorption kinetics over 180 days or the influence of particles sizes on the sorption. Besides sorption processes, a simulation of the desorption using artificial gut fluid (AGF) was performed to evaluate the potential desorption of PFOS in fish gut.

Method

Sorption processes of PFOS and BaP on PE MPs were investigated. Sorption efficiencies have been studied on four sizes range of MPs (4-6; 11-13; 20-25 and 125-500 µm), since the **role of particle sizes** is poorly understood. **Kinetic of sorption** for 180 days was also studied by using one size range of PE (125-500 µm). Besides sorption experiments, the **kinetic of desorption** of pollutants from MPs under artificial digestive conditions has been carried out by using artificial gut fluid mimicking intestinal juice of the Atlantic cod (*Gadus morhua*), since little is known about the desorption of pollutants or additives from plastic during fish digestion.



Article 1

Sorption and desorption kinetics of PFOS to pristine microplastic under different conditions

Bettie Cormier, Flora Borchet, Anna Kärrman, Marta Szot, Leo Yeung, Steffen H. Keiter.

Sorption and desorption kinetics of PFOS to pristine microplastic under different conditions

Bettie Cormier[1,2,*], Flora Borchet[3], Anna Kärrman[1], Marta Szot[1], Leo Yeung[1], Steffen H. Keiter[1*]

[1] Man-Technology-Environment Research Centre, School of Science and Technology, Örebro University, Fakultetsgatan 1, 701 82 Örebro, Sweden

[2] Bordeaux University, EPOC, UMR CNRS 5805, Avenue des Facultés, 33400 Talence, France

[3] RWTH Aachen, Institut für Umweltforschung (Biologie V), Worringerweg 1, 52074 Aachen, Germany

Keywords: Microplastics, polyethylene, sorption, PFOS

Abstract

Microplastics (MPs) has become one of the major pollution concerns in the last decades due to the intensive use of plastics in our daily life. MPs are entering the aquatic environment and by that, they can concentrate and transport chemicals. Sorption of contaminants by MPs is known to be able to influence the distribution and the bioavailability of the pollutants. In the present study, sorption processes of perfluorooctane sulfonate (PFOS) on low-density polyethylene MPs were investigated. Thus, PFOS sorption has been studied in four sizes range of MPs, since the role of particle sizes is poorly understood. Kinetic sorption for 6 months was also studied by using one size range of MPs. Besides sorption experiments, the desorption of PFOS from MPs under digestive conditions has been carried out by using artificial gut fluid mimicking intestinal juice of the Atlantic cod (*Gadus morhua*), since little is known about the desorption of pollutants or additives from plastic during fish digestion. The investigation of size effects of particles demonstrated an increase of PFOS concentration sorbed onto MPs with a decrease of the size particle, due to an increase of the surface area. Over 6 months, PFOS sorption was linearly increasing, without reaching a maximum sorbed concentration. PFOS desorbed from MPs into artificial gut fluid corresponded to a desorption rate of 70 to 80 %. Thus, our findings implicate efficient sorption of PFOS onto MPs particles from different sizes, with higher sorption when MPs are the smallest. Besides, a longer exposure of MPs to PFOS leads to a higher concentration adsorbed by low-density polyethylene MPs, which can favor the ingestion of higher concentration of PFOS and so, a higher risk of polyethylene MPs to transfer relevant concentrations of PFOS during digestion. The overall goal of this investigation aimed for a better understanding of sorption and desorption processes of PFOS from MPs, which may also contribute to a more comprehensive environmental risk assessment of MPs pollution.

Introduction

The use of plastic has become unavoidable in our society with global production rising; the production was estimated to be 348 million tons in 2017 (Geyer et al., 2017). The need for plastics is evident with many societal benefits and it offers the development of new technologies, improved consumer health, and reduced transportation costs (Andrady and Neal, 2009; Thompson et al., 2009; Zarfl and Matthies, 2010). Besides the numerous benefits of plastic products, it becomes obvious that plastics have become one of the major impacts on the environment (Zarfl and Matthies, 2010). Plastics may persist and accumulate in the oceans (Law and Thompson, 2014) as either macroplastic (> 5 mm) or a microplastic (MPs; < 5 mm) (Jambeck et al., 2015). Microplastics (MPs) exist in two types; primary MPs particles that consist of plastic granules produced on purpose, e.g., in cosmetics but also from waste of plastic production; and secondary MPs known as small plastic fragments derived from the fragmentation of macroplastics via physical, chemical or biological degradation (Derraik, 2002; Thompson et al., 2004). Both types are considered a potential threat to the aquatic environment (Cole et al., 2011; Thompson, Moore, vom Saal and Swan, 2009). Ubiquitous occurrence of MPs have been observed in oceans, even in the polar areas and in the deep sea (Obbard et al., 2014). Cozar and collaborators (2014) studied 141 water samples and demonstrated the omnipresence of MPs showing that 88 % of the water samples contained MPs, with large variation in concentrations. Through different degradation processes, plastics fragments are dispersed in the ocean and converged in gyres (Eriksen et al., 2014; Law and Thompson, 2014). Therefore, the abundance of MPs has increased over the last few decades (Barnes et al., 2009). On the other hand, trends in macroplastic accumulation in the marine environment are not uniformly increasing while the average size of plastic particles seems to be decreasing (Eriksen, Lebreton, Carson, Thiel, Moore, Borerro, Galgani, Ryan and Reisser, 2014).

Polyethylene (PE; $-(-CH_2-CH_2-)_n-$) represents the polymer with the greatest global production for plastic manufacturing (PlasticsEurope, 2018). LDPE represents 17.2 % of total plastic consumption with different utilization, e.g., film for food packaging or reusable plastic bags (PlasticsEurope, 2015). Dris and co-workers showed that PE was one of the most abundant polymers found in aquatic ecosystems (Dris et al., 2015) caused by the extensive use of plastic and poor waste management. PE used as passive sampler has been firstly studied in semipermeable polymeric membrane device, made of PE tubing containing lipids (Huckins et al., 1990). Since then, passive samplers have been implemented, but PE sheets and simple PE remain widely used (Adams et al., 2007; Rusina et al., 2007; Vrana et al., 2006). Presence of

low-density plastics, have been described as the main polymers composing MPs contamination in the environment (Heo et al., 2013; Hidalgo-Ruz et al., 2012; Martins and Sobral, 2011). Besides its intensive use as passive samplers for hydrophobic organic pollutants, PE has been commonly studied as one of the reference polymers to investigate chemical sorption to MPs (Hüffer and Hofmann, 2016; Lee et al., 2014; Li et al., 2018; Xu et al., 2018a).

Plastic polymers are commonly considered as biologically inert and thus non-toxic. However, plastic production includes additives as plasticizers for example, to improve resistance or flexibility, dyes or fire retardants, and these additives can be toxic (Barnes, Galgani, Thompson and Barlaz, 2009; Lithner et al., 2011). In addition to the potential toxicity caused by the numerous plastic additives, MPs offer a surface where many waterborne pollutants can be adsorbed, including aqueous metals (Rochman, Hentschel, et al., 2014) and persistent organic pollutants (POPs) (Rios et al., 2007; Rochman, Hoh, Hentschel et al., 2013). In the marine environment such chemicals are typically found at their highest concentrations in the surface microlayer, where low-density MPs are most abundant as well (Ng and Obbard, 2006; Rios, Moore and Jones, 2007; Teuten et al., 2007). Thus, MPs are hypothesized to act as vectors and carriers for a wide range of pollutants in the marine environment (Andrady, 2011; Koelmans et al., 2016).

These MPs associated with POPs can then act as a reservoir and/or vector for those pollutants (Barnes, Galgani, Thompson and Barlaz, 2009; Thompson, Olsen, Mitchell, Davis, Rowland, John, McGonigle and Russell, 2004). The processes of the sorption of organic pollutants onto MPs are very complex and poorly understood. The equilibrium kinetics of contaminants on MPs depends on the inherent properties of the chemicals, but also on the size, density and quality of the MPs (Wang, Shih, et al., 2015; Wang et al., 2019).

In recent years, perfluoroalkyl acids (PFAAs) have attracted attention due to their high-energy carbon–fluorine (C–F) bonds (Giesy and Kannan, 2002) and their toxicity to aquatic organisms (Ahrens and Bundschuh, 2014; Huang et al., 2010; Keiter et al., 2016). Perfluorooctanesulfonate (PFOS), a commonly found PFAAs in the environment, has been listed as a POP in Annex B of the Stockholm Convention in 2009 (Paul et al., 2009). PFOS has been restricted due to its bioaccumulation and biomagnification in organisms (Houde et al., 2008; Martin et al., 2003; Powley et al., 2008) and the food webs, respectively. However, PFOS is also ubiquitously present in different ecosystems (Ahrens and Bundschuh, 2014; Zareitalabad et al., 2013), in wildlife up to ng/g to µg/g levels (Giesy and Kannan, 2001) and human blood at ng/mL levels (Kärrman et al., 2006; Olsen et al., 2005). Mostly detected in a ng/g range

(Giesy and Kannan, 2001; Kannan et al., 2004; Zareitalabad, Siemens, Hamer and Amelung, 2013); however, concentrations in soil can reach up to 230 µg/g in highly contaminated sites where firefighting foams were used (Baduel et al., 2015). Although PFOS is well-studied in different environmental compartment, it was barely examined along with MPs. Bakir et al. (2014) investigated the (de)sorption behavior of PE and polyvinyl chloride (PVC) in combination with perfluorooctanoic acid (PFOA), showing a faster desorption rate using a gut surfactant compared to seawater alone, bringing the interest to investigate the desorption process. Wang, Shih, et al (2015) studied the sorption of PFOS onto different types of polymer, demonstrating a linear sorption as well as a higher sorption affinity to PE rather than to PVC and PS, but the investigation of size effects of the particles on the sorption behavior of PFOS remains unknown.

The present study was part of the JPI Ocean project EPHEMARE, which aimed to investigate the distribution, fate, and toxicity of MPs. A major part of the project was to investigate the role of MPs as vector of pollutants; therefore, PFOS was sorbed on HDPE of different size ranges. Moreover, a desorption experiment using an artificial gut fluid (AGF) has been performed to evaluate the potential desorption of PFOS from MPs artificially spiked. For the sorption, we aimed for two concentrations; a low concentration, representing environmental relevant level and a higher concentration level which may cause toxic effects. Since there are several overlooked information of the behavior of MPs and PFOS, the present study aimed to investigate the sorption processes between PFOS and HDPE microparticles using different size range that may cover a wide range of MPs found in the ocean (Barnes, Galgani, Thompson and Barlaz, 2009; Ng and Obbard, 2006).

Materials and methods

Materials and chemicals

Four size ranges of HDPE were used as the sorbents, purchased from Micro Powders (New York, USA). MPs were sieved to size range of 125-500 µm; 20-25 µm; 11-13 µm and 4-6 µm, and all particles possessed a density of 0.96 kg/m^3. MPs were kindly provided within the JPI Oceans project EPHEMARE as dry powder by Marina Albentosa from the Instituto Español de Oceanografía Centro Oceanografico de Murcia, Spain. Sea salt was obtained from Red Sea Fish Pharm LTD., Israel and sodium taurocholate was from Merck, Germany (NaTC, CAS 345909-26-4). Perfluorooctane sulfonate (PFOS; solid powder; IUPAC: 1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-heptadecafluorooctane-1-sulfonic acid, CAS 2785-37-3; purity

≥ 98 %) was purchased from Sigma Aldrich (Stockholm, Sweden). Ammonium solution (NH_4OH) was from Fischer Scientific, Ottawa, ON, Canada. All other chemicals and reagents were purchased at the highest purity available from Sigma-Aldrich (Stockholm, Sweden), unless stated otherwise. Analytical standards including PFOS technical mixture, mass labelled standards of PFOS (13C8 PFOS and 13C4 PFOS) were purchased from the Wellington Laboratories (Guelph, ON, Canada).

Sorption of pollutants onto MPs particles

Three time points were used to complete sorption of PFOS. 2 days, 5 days and 7 days were investigated to assess the sorption time, based on previous studies (Wang, Shih, et al., 2015). The sorption time of 7 days was maintained because of a low standard deviation and the highest concentration of PFOS adsorbed on particles compared to other time points, results are not shown. For all size ranges used, PFOS concentration in water was experimentally adapted to the different size ranges (Table 1).

Table 1: PFOS concentration in water to aim a high and a low concentration of PFOS to perform the sorption experiment

	High [PFOS] in water	**Low [PFOS] in water**
LPDE (500-125 µm)	600 mg/L	600 µg/L
LPDE (25-20 µm)	30 mg/L	30 µg/L
LPDE (13-11 µm)	20 mg/L	20 µg/L
LPDE (6-4 µm)	10 mg/L	10 µg/L

The sorption of PFOS on PE was performed using 50 g/L of MPs (125-500 ; 20-25 ; 11-13 and 4-6 µm HDPE) placed into 1L polypropylene (PP) bottle (Lamaplast; Sesto San Giovanni, Italy) with 500 mL of MilliQ water (Millipore, Billerica, MA, USA), and adjusted concentration of PFOS (Table 1) was added. Bottles were placed on a rotary shaker (Heidolph Instruments GmbH & CO. KG, Schwabach, Germany) at 20 rpm for 7 days. After 7 days, MPs particles were filtered using 1 µm Whatman® glass microfiber filter (GE Healthcare Life Sciences; Uppsala, SE). Subsequently, the particles were rinsed with MilliQ water and chemical analyses were performed as follows.

Sorption kinetics

Long-term sorption kinetics experiments were conducted in 50 mL polyprolpylene centrifuge tubes filled with 50 g/L of MPs (125-500 µm HDPE), 50 mL of MilliQ water and 200 mg/L of PFOS. Tubes were continuously agitated on a rotatory shaker at 20 rpm for 180 days. Samples were collected after 2, 7, 30, 90 and 180 days, and both water and MPs were extracted and analyzed as described in the chemical analysis section.

Size effect of MPs on PFOS

Sorption isotherm experiments was conducted for 7 days, based on the results obtained from the previous described kinetics experiments. Containers for the isotherms experiments were the same as the one used for the sorption kinetics. Tubes contained 0.1 g of MPs (4-6 ; 11-13 ; 20-25 or 125-500 µm) and 40 mL of milliQ water solution with different PFOS concentration ranging from 0 µg/L to 100 µg/L (0, 25, 50, 75 and 100 µg/L, HDPE). Tubes were shaken at 20 rpm and kept at 20 ± 1 °C for 7 days. All experiments were conducted as three independent replicates.

Desorption of PFOS from MPs by artificial gut fluid (AGF)

Based on the protocol of (Voparil and Mayer, 2004), AGF was prepared by resolving 35 ‰ sea salt and 15 mM sodium taurocholate in MilliQ water, pH was adjusted to 6.7 by using 1 M hydrochloric acid (Sigma-Aldrich, Stockholm, Sweden) (Grosell et al., 2001). The digestion has been performed according to the model used by Koelmans et al. (2014). The digestion scenario was set up for a model of 3300 g Atlantic Cod (*Gadus morhua*) which ingests 0.0126 g food/g bodyweight per day (1.26 %). Since our study focused on the uptake of MPs by food, quantities of 0.3 % (Jovanović et al., 2018) and 1 % of MPs related to ingested food per day were chosen. Gut retention time was assumed to be 4 days on average (Daan, 1973). Thus, MPs (11-13 µm) spiked with PFOS were artificially digested in 10 ml AGF in PP tubes. Controls consisted in replicates of 10 ml pure AGF and 10 ml AGF containing 1 % MPs. Samples were incubated in darkness for 4 days on a rotary shaker at 20 rpm at room temperature (20 ± 1 °C). Samples were filtered by using a glass fiber filter on a funnel connected to vacuum as described before. Exposure tubes and particles were rinsed with 5 ml and 1 ml of milliQ water, respectively. Samples were extracted by using 3 ml of filtered AGF spiked with internal standard, and were subsequently evaporated to 3 ml (1:1) after SPE. 200 µl extract were prepared for chemical analysis as described before.

Chemical analysis of PFOS

Sorption kinetics

Chemical analysis of PFOS in MPs has been conducted according to Eriksson et al. (2016) with minor modifications.

Analysis of PFOS in water

The water extracts were filtered using a PE syringe (Norm-Ject®; Henke Sass Wolf, Tuttlingen, Germany) with a filter of 0.2 µm (13 mm, 0.2 µm AcrodiscGHP; Pall, Dreieich, Germany). The volume of the water extracts were adjusted to 5 mL before transferring to LC vials for analysis. In the LC vials, 2 ng of mass-labelled 13C8 PFOS, 300 uL of the water extracts and 200 uL of organic mobile phase (2 mM ammonium acetate) were added. The LC vial was vortex for 30 s and ready for instrumental analysis.

Analysis of PFOS sorbed on MPs

In a 15 mL PP tube, 0.3 g of MPs were extracted in 2 mL of methanol (MeOH HPLC grade, > 99.9 % purity, Fisher Scientific, Sweden) by ultra-sonication for 15 min following by 10 min of centrifugation at 7000 rpm. The extraction procedure was repeated twice and combined. Extracts were then filtered using a PE syringe (Norm-Ject®; Henke Sass Wolf, Tuttlingen, Germany) and a filter of 0.2 µm (13 mm, 0.2 µm AcrodiscGHP; Pall, Dreieich, Germany), and the extract was concentrated to 200 uL under a gentle stream of nitrogen. The 200 uL extract was then transferred to the LC vial. In the LC vial, 2 ng of mass-labelled 13C8 PFOS, 300 uL of aqueous mobile phase (2 mM ammonium acetate) were added. The LC vial was vortex for 30 s and ready for instrumental analysis.

Desorption of PFOS in AGF

Chemical analysis of AGF used solid-phase extraction (SPE). First, 10 mL of AGF in triplicates were adjusted to pH 4 using acetic acid (97 %, Sigma-Aldrich, Stockholm, Sweden) and spiked with 1 ng mass-labelled 13C4 PFOS (Wellington Laboratories Inc., Canada). SPE cartridges (Oasis® WAX 150 mg 6 cc, Waters®) were preconditioned with 4 mL of 0.1 % of NH_4OH (25 %, Fisher Scientific) in methanol, followed by 4 mL of methanol and finally 4 mL of MilliQ water. Then, samples were loaded on the cartridges. The cartridges were washed using 20 mL of MilliQ water, 4 mL of ammonium acetate buffer (pH 4) and 4 mL of methanol. Target compound was eluted using 4 mL of 0.1 % NH_4OH in methanol, and the extracts were evaporated down to 200 µl by nitrogen stream. 1 ng of mass-labelled 13C8 PFOS was added

and samples were transferred to LC vial containing 300 µL of 2 mM ammonium acetate in MilliQ water. Samples were analyzed using UPLC – MS/MS system described below

Analysis of MPs followed the method as described above (Anlaysis of PFOS sorbed on MPs).

Instrumental analysis

Analysis were performed on an Acquity UPLC system coupled to a Xevo TQ-S quadrupole MS (Waters, Milford, USA). PFOS were separated on Acquity BEH C18 column (2.1x100 mm, 1.7 um) using MeOH and water as mobile phases, both with 2 mM ammonium acetate. Column temperature was kept at 50°C. Different transitions were monitored for the quantifications of PFOS isomers. Quantification of total PFOS was performed by the summation of linear PFOS and individual branched PFOS calculated against an internal calibration curve using technical PFOS and corresponding mass labelled PFOS standards as internal standard.

Quality assurance and quality control (QA/QC)

In order to avoid contamination from the LC system that might interfere with the samples, an isolator column (PFC isolator, Waters) was inserted between the pump and the injector. All the glassware and consumables had been rinsed with MeOH. The extraction efficiency of the MPs was assessed based on the peak area of same amounts of the mass-labelled 13C4 PFOS and mass-labelled 13C8 PFOS; they were found to be 83-96%. Background contamination in MPs were also evaluated using 1g of each MPs size range, in triplicates and conducted according to the description above. Background contamination of PFOS in PE was observed in the range of 10 – 15 pg/g and the spiking was at ug/g levels (Table 1). Background check on the AGF was conducted prior to experiments in order to determine any background contamination of PFOS in the pure fluid and to validate the extraction method; no detectable PFOS was found in the AGF. Procedure blanks (without any MPs) were included in all batches; no detectable levels of PFOS were found in the procedure blanks. The limit of detection was calculated as 10 pg/g.

Statistics

The amount of PFOS sorbed per unit mass of MPs were analysed, and a distribution coefficient (Kd, L/kg) for each individual data point within the linear range of sorption isotherms was calculated as $K_d = C_m/C_e$, with the concentration sorbed to the MPs (C_m) and C_e as the final equilibrium concentration in the water solution. A one-way ANOVA or a non-parametric test, Kruskall Wallis was performed on data to statistically analyze the difference of the data sets.

Parameters for sorption experiments were determined using Microsoft Excel 2010 and GraphPad Prism 8.1.

Results and discussion

Sorption of PFOS

Table 2 shows the results of PFOS sorbed to MPs particles. Concentrations of PFOS were in the same range for all particle sizes, 41.2 to 73.6 µg/g for high aimed concentration on MPs and 20.6 to 63.3 ng/g for low aimed concentration on MPs. The initial PFOS concentration in water was held approximately constant with respect to the particles size, with a concentration to particle size ratio of 0.6 to 0.8.

Table 2: PFOS adsorbed on HDPE particles with high and low concentration of chemicals after 7 days. Concentration mean of 3 independent samples ± SD, n=3

	(500-125µm)	**(25-20µm)**	**(13-11µm)**	**(6-4µm)**
High $[PFOS]_{MPs}$ (µg/g)	41.20 (± 9.2)	54.02 (± 1.12)	46.26 (± 4.28)	73.61 (± 7.13)
Low $[PFOS]_{MPs}$ (ng/g)	63.30 (± 9.1)	20.57 (± 1.61)	30.23 (± 0.50)	22.59 (± 23.62)

The effect of particle size on the sorption capacity of MPs is poorly understood, but it has been shown that a decrease in particles size, at micron- and submicron- sized, is increasing the sorption rates of contaminants (Wang, et al., 2019). The low concentration of PFOS obtained on MPs particles correspond to an environmental concentration that can be found in sewage sludge (Becker et al., 2008; Guo et al., 2009; Loganathan et al., 2007). Whereas, the high PFOS concentrations tends to represent an equivalent concentration inducing acute toxicity on zebrafish embryos exposed to the pure compound (Ye et al., 2009).

In general, the (de)sorption behavior varies depending on sorbent and sorbate (Bakir et al., 2012). For PFAAs, it is assumed that hydrophobic and/or electrostatic interaction are two main sorption processes onto organic material (Higgins and Luthy, 2006). Differences in sorption efficiencies can be attributed to differences in functional groups. For example, the sulfonate group of PFOS bears a stronger polarity than the carboxylic group of PFOA. Hence, the resulting stronger electrostatic repulsion suggests an even lower sorption affinity of PFOS to PE compared to PFOA (Bakir, Rowland and Thompson, 2014). In the present study PFOS was successfully sorbed onto PE. Inconsistent sorption phenomena might partly derive from different size and morphology of the investigated plastic particles. Bakir et al. (2014) tested particles with a size range of 200-250 µm. Further, PFASs are amphiphilic substances and

might therefore show a highly substance-specific sorption behavior. Inconsistent or a general lack of reliable data from laboratory studies (Wang, Shih et al., 2015) call for further research on the interaction behavior between PFOS or other PFASs and MPs particles.

To date, studies investigating the sorption behavior of PFOS or other PFASs along with MPs are rather scarce. Bakir and collaborators (2014) investigated the (de)sorption behavior of PE in combination with perfluorooctanoic acid (PFOA), a structurally similar molecule to PFOS. Due to their comparable molecular structure, a similar sorption behavior was expected for PFOA and PFOS.

Sorption kinetics

Sorption kinetics are a relevant insight into the mechanisms involved in the vectorization of chemicals toward sorbents (Azizian, 2004). In general, sorption kinetics using MPs are performed on a small-scale for many hours (Zhan et al., 2016; Zhang, Wang, et al., 2018). In the present study, a long-term sorption experiment was conducted using PFOS to investigate sorption onto HDPE particles (125-500 µm) over 180 days. Samples were taken at 7, 30, 90 and 180 days.

Figure 1 shows the sorption kinetics of PFOS onto 125-500 µm HDPE particles. Experimental data were plotted, and a linear regression was performed ($F=52.80$, $R^2=0.9635$), due to previous results showing a linear sorption (Wang, Shih, et al., 2015).

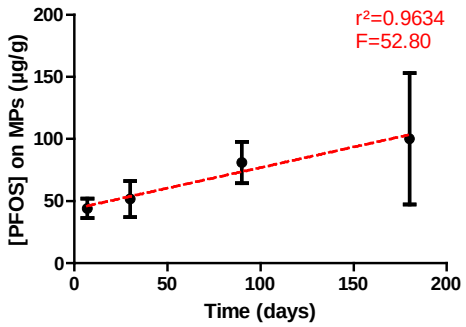


Figure 1: Concentration of PFOS in µg/g, adsorbed on MPs (HDPE 250-500 µm) after 7, 30, 90 and 180 days of exposure to 200 mg/L of PFOS. Mean ± SD; n=3.

The linearity of the observed sorption is in line with a previous study on PFOS sorption conducted by Wang, Shih, et al. (2015), on PE, polystyrene and PVC. However, PFOS has not been studied in any kinetic experiment using MPs, before. Equilibrium of PFOS to activated sludge and carbon nanotubes were reached within 11h and 2h, respectively (Deng et al., 2013;

Zhou et al., 2010). PFOS concentration adsorbed on MPs represented 1% up to 2.5% of the initial amount of PFOS introduced into water, after 7 and 180 days respectively, PFOS has demonstrated to adsorb to MPs at lower ratio in comparison to the sorption of its precursor FOSA, due to different functional groups (Wang, Shih, et al., 2015). The limited sorption of PFOS onto PE might be due to physical properties of the compound, nevertheless, the sorption rate was increasing over time, and after 180 days, a high variability has been observed. The high variation after 180 days might be due to (1), a high initial concentration of PFOS. But also to (2) the aging of particles remaining in solution for 6 months and leading to a degradation of MPs surfaces with modification of the sorption sites. The partitioning via hydrophobic interaction but also electrostatic interaction seemed to be two potential sorption mechanisms for PFOS on sediments (Higgins and Luthy, 2006). In the present study, anionic form of PFOS was the main ion present in solution; thus, the low sorption rate of PFOS could be explained by electrostatic repulsion from the surface of PE (Wang, Shih, et al., 2015).

Size effect of MPs on PFOS

The sorption of PFOS on different sizes of MPs in the present study conducted to the results presented in Figure 2, with equilibrium dissociation constants calculated in table 3. On the figure 2, a tendency can be observed, sorption efficiencies are higher for small particles rather than for bigger particles, in ascending order, for all tested concentrations. However, only the highest concentration, 100 µg/L of PFOS, led to significant differences between particle sizes with an increase of sorption efficiency link to the decrease of particles size. The table 3 confirms the tendency, that the particle size affects the sorption rate of PFOS at the highest concentration with a decrease in Kd value in parallel with a decrease of size particles. A previous study observed similar distribution coefficient of K_d = 32.8 L/kg determined for PE (150 µm) and PFOS (Wang, Shih, et al., 2015). The effect of functional groups on the sorption has been demonstrated for PFOS and its precursor, FOSA (Wang, Shih, et al., 2015). In addition, the decrease of the sorption efficiency of PFOS with an increase of initial PFOS concentration from 25 ug/L to 50-75 ug/L (Figure 2) might be explained by saturation of the sorption sites of MPs (Wang and Wang, 2018a). The polymer type might also affect the sorption rate of a compound, PE particles have a high segmental mobility and free volume which can support the sorption of chemicals (Karapanagioti and Klontza, 2008; Pascall et al., 2005). Therefore, differences in particle sizes, inducing a modification of surface areas, could have contributed to the difference in chemicals uptake, as well as chemical properties of pollutants. Numerous studies have shown effects of the particle size of sorbents on the sorption efficiency of different chemicals

(Waychunas et al., 2005; Zhang and Moore, 1999). For example, the sorption of adipic acids on TiO2 was increased by almost 70-fold when increasing the particle diameter from 16 nm to 6 nm (Zhang et al., 1999). Unlike the previous studies, Mckay et al. (1982) found that particle size of chitin had no effect or only a little impact on the sorption efficiency of a dye. Chiou and Li (2002) have been reported an absence of clear effect size with the sorption of dyes onto chitosan beads. No scientific consensus has been highlighted on the effect of the particle size of a sorbent on the sorption efficiency of contaminants. Since this effect of size depending sorption processes on MPs barely have been studied yet, it remains unclear if a decrease of particle sizes is inducing an increase of the sorption rate of a contaminant. In addition, external factors such as temperature, pH, salinity and composition of the water phase (e.g., dissolved organic matter, ion concentrations) can also influence the sorption efficiency of compounds onto MPs (Endo and Koelmans, 2019; Wang, Shih et al., 2015).

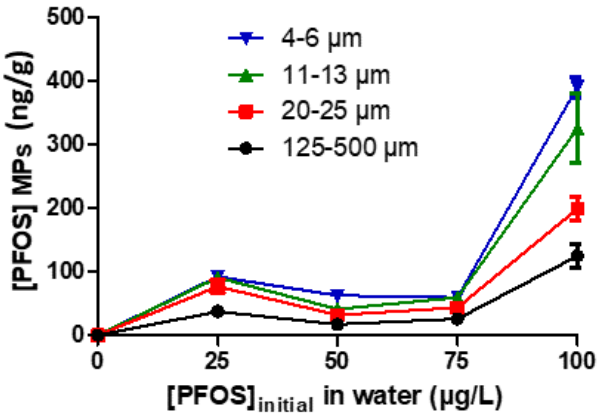


Figure 2: Concentration of PFOS in µg/g adsorbed on different size particles of HDPE (4-6; 11-13; 20-25 and 250-500 µm) after exposure to 25, 50, 75 and 100 µg/L of PFOS for 7 days. Mean ± SD; n=3

Table 3: Distribution coefficient (Kd, L/kg) values of PFOS concentrations for tested PE MPs exposed to 100 µg/L of PFOS for 7 days. Different letters indicate significant differences (ANOVA, $p < 0.05$).

	Kd, PFOS (L/kg)
HDPE 4-6 µm	62.6[a]
HDPE 11-13 µm	54.2[a]
HDPE 20-25 µm	33.8[b]
HDPE 125-500 µm	20.9[c]

Desorption of PFOS from MPs by AGF

PFOS-spiked PE particles were exposed to AGF to simulate the digestion of contaminated MPs in Atlantic Cod (*Gadus morhua*). Background levels of PFOS in AGF was negligible ($2.5.10^{-5}$ ± $1.1.10^{-6}$ $\mu g_{PFOS}/ml_{AGF}$). PFOS-spiked particles of 0.3 and 1 % respective to the total mass of daily food uptake for Atlantic Cod (*Gadus morhua*) were digested and two independent replicates (A and B) of artificial digestion resulted in reproducible high desorption rates. Regardless the quantity of exposed PFOS-spiked particles, desorption ranged between 70 and 80 %. Initial PFOS concentrations (d = 0 days) encompassed 0.72 and 2.40 $\mu g_{PFOS}/ml_{AGF}$ (PFOS sorbed on the particles, Figure 3) for 0.3 and 1 % spiked particles, respectively. Therefore, 0.51 ± 0.03 $\mu g_{PFOS}/ml_{AGF}$ and 1.80 ± 0.26 $\mu g_{PFOS}/ml_{AGF}$ desorbed into the gut fluid of replicate A after d = 4 days, and corresponds to a desorption rate of 70.8 and 75.1 % for 0.3 and 1 % spiked MPs, respectively. The second replicate (replicate B) showed similar results of 0.60 ± 0.11 (0.3 %) and 1.92 ± 0.05 $\mu g_{PFOS}/ml_{AGF}$ (1 %) desorbed PFOS which corresponds to a desorption rate of 82.8 and 80.0 %, for 0.3 and 1 % spiked MPs, respectively (table 4 and figure 3).

Table 4. Sorbed versus desorbed PFOS concentrations before (d=0 days) and after (d=4 days) exposure to artificial gut fluid (AGF). Two quantities (0.3 and 1 %) of PFOS-spiked MPs (PPE) were applied (n=3) and digested in two replicates (A and B).

Exposed PFOS-spiked MP (PPE) quantity		sorbed PFOS onto MPs, d=0 ($\mu g_{PFOS}/ml_{AGF}$)	desorbed PFOS into AGF, d=4 ($\mu g_{PFOS}/ml_{AGF}$)	% desorption
Replicate A	PPE 0.3%	0.72 ± 0.08	0.51± 0.03	70.8
	PPE 1%	2.40 ± 0.28	1.80 ± 0.26	75.1
Replicate B	PPE 0.3%	0.72 ± 0.08	0.60 ± 0.11	82.8
	PPE 1%	2.40 ± 0.28	1.92 ± 0.05	80.0

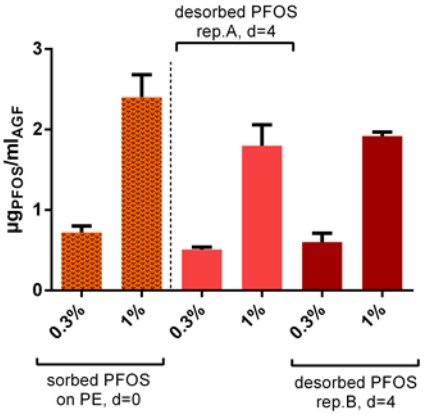


Figure 3: PFOS concentration (µgPFOS/mlAGF) sorbed onto 0.3 and 1 % PE microplastics (d=0 days) and in exposed artificial gut fluid (replicate A and B) after d=4 days

The high desorption rate between 70 and 80 % is most likely attributed to molecular properties and chemical interactions between the bile salt sodium taurocholate and PFOS. Both compounds are amphiphilic chemicals consisting of a hydrophobic and a polar functional group (*Figure 1, SM*). Thus, either the bioavailability of PFOS might be enhanced by the inclusion into bile salt micelles or into their micellular layer, as is was shown for lecithin (Mazer et al., 1980). Enhanced desorbing effects mitigated through bile have been already demonstrated in previous studies for different compounds and matrices. For example, a mixture of 30 to 40 % of polychlorinated biphenyls (PCBs) and 57 % of lindan were desorbed from soil after *in vitro* digestion with chicken bile (Oomen et al., 2000). Another study by Hack and Selenka (1996) investigated the digestion of soil contaminated with polycyclic aromatic hydrocarbons (PAHs) and PCBs. Their findings revealed a mobilization of these substances after gastro-intestinal digestion up to two times higher than a gastric digestion without bile (Hack and Selenka 1996). This emphasizes the important role of bile for allocating nutrients and toxicants at the same time. Additionally, Hack and Selenka (1996) measured a maximum desorption of 40 % for PCBs and PAHs within 6 hours. In the present study, PFOS showed even a higher desorption rate of 80 % over a period of 4 days. PFOS is less hydrophobic but more amphiphilic than hydrophobic POPs like PCBs and PAHs. Hence, our results suggest that the desorption efficiency of bile depends on hydrophobic and/or amphiphilic properties of the particle-bound toxicant.

There are only few investigations on the desorption behavior of MPs sorbed chemicals under physiological conditions and mostly focused on common hydrophobic organic compounds

(HOCs) (Teuten et al., 2009; Ziccardi et al., 2016). Despite of particular affinities between different HOCs and MPs, Bakir et al. (2014) demonstrated a significant increase of desorption rates in the presence of gut surfactant between 2 to 20 times higher compared to sea water. Similar results were presented by Teuten et al. (2007), for phenanthrene (Phe) in combination with PE and polypropylene (PP). Even though desorption rate constants of Phe from natural sediment significantly surpassed the ones determined from MPs, the sediment's sorption capacities were remarkably lower. It is proposed that even though contaminants desorb more slowly from plastics than from sediment, the relative amount desorbing from plastics under physiological conditions might exceed the quantities desorbing from natural sediment particles (Bakir et al., 2014). Considering a longer gut retention time in carnivorous vertebrates as well as chronic exposure to MPs contaminated prey, a desorption between 70 to 80 % suggests a higher threat of MPs to contribute to the PFOS burden of marine predator fish such as Atlantic Cod (*Gadus morhua*). In the present study, AGF was used to simulate the digestion of PFOS-spiked MPs. The most commonly used protocol was developed to mimic the physiological conditions in the marine lugworm *Arenicola marina* (Voparil and Mayer, 2004) containing a model protein (bovine serum albumin, BSA) and sodium taurocholate as the only bile salt, which is a taurine-conjugated C_{24} bile salt and actually produced in vertebrates (Haslewood and Tökés, 1969). In the present approach, no proteins were added to the AGF to focus on the role of bile during digestion and to eliminate an additional factor of uncertainty since composition of nutrients in food varies among species and their position in the food chain. Interestingly, sodium taurocholate is the salt that better imitates digestive fluids of invertebrates (Voparil and Mayer, 2004). Moreover, comparable levels between average 5 and 15 mM of sodium taurocholate were found in vertebrate (human) intestine (Mithani et al., 1996). Among teleost fish, approximately 60 % of species produce no other than C_{24} bile salts for their digestive juices (Hofmann et al., 2010). In addition, taurine conjugates, such as sodium taurocholate, are the most dominant C_{24} bile salts among carnivore species (Moschetta and Kliewer, 2005). This promotes sodium taurocholate as a suitable model bile salt in AGF of Atlantic Cod (*Gadus morhua*). It provides a low-cost and feasible approach to assess the desorption behavior of MP-associated toxicants in a broad range of species.

Conclusion

The sorption behavior of perfluorinated compounds has been investigated in different natural soils and sediments (Chen et al., 2016; Higgins and Luthy, 2006). However, to our knowledge, sorption of PFAAS has been barely studied. Llorca et al. (2018) revealed that PFAAS tends to adsorb more to polystyrene particles rather than to PE. And more specifically, MPs adsorption capacity for PFOS showed a low adsorption ratio on 3 types of polymers, including PE, compared to its precursor, perfluorooctanesulfonamide (FOSA) (Wang, Shih, et al., 2015). Sorption behaviors of PFOS, an environmental contaminant on different sizes of HDPE MPs particles were investigated. A long-term sorption demonstrated a linear sorption of PFOS over 6 months. A decrease of the particle sizes increased the sorption efficiency of PFOS, due to a higher surface area of smaller particles. To our knowledge, the present study is the first to investigate the desorption behavior of PFOS spiked on MPs under simulated physiological conditions. We showed that the bile salt is desorbing PFOS from PE within 4 days during artificial digestion. Regardless the quantity of 0.3 and 1 % digested PFOS-spiked MPs, the desorption accounted for 70 to 80 %. Thus, sodium taurocholate enhanced the bioavailability of PFOS associated to HDPE MPs. To further investigate the desorption efficiency of sodium taurocholate, desorbed quantities of PFOS should be measured at more time points to determine desorption kinetics. The developed method might be used as an approach to a more appropriate understanding of the desorption of toxicants from MPs during digestion.

This study is limited in using one single polymer type, (i.e., PE) with irrelevant environmental concentration of MPs and relatively high contaminants concentrations. Further studies should be conducted by using different types of polymers (e.g. PS, PP) and environmental relevant concentrations of MPs as well as contaminants concentrations. Investigations on emerging pollutants and further PFASs in combination with other plastic types are needed since adsorption and desorption behavior depends both on the plastic type and the pollutant. Future projects should also make use of higher tier studies to evaluate the burden of MPs as vectors for toxic substances as well as the reliability of artificial digestion experiments.

Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Author Contributions

AK, BC, ML and SK: Designed the experiment; BC, FB, MS: Performed the experiment and BC, FB, LY contributed to the data analysis; BC: wrote the manuscript. All co-authors corrected the manuscript.

Funding

This work was developed under the EPHEMARE project (Ecotoxicological effects of microplastics in marine ecosystems), supported by national funding agencies in the framework of JPI Oceans (FCT JPIOCEANS/0005/2015; FORMAS, 2015-01865). Bettie Cormier was directly supported by IdEx grant from University of Bordeaux.

Acknowledgments

The authors would like to thank Marie-Laure Bégout, Jérôme Cachot and Xavier Cousin for their skillful collaboration to this study and their supervision.

References

Adams R. G., Lohmann R., Fernandez L. A., MacFarlane J. K. (2007). Polyethylene Devices: Passive Samplers for Measuring Dissolved Hydrophobic Organic Compounds in Aquatic Environments. Environmental Science & Technology. 41, 1317-1323

Ahrens L., Bundschuh M. (2014). Fate and effects of poly- and perfluoroalkyl substances in the aquatic environment: A review. Environmental Toxicology and Chemistry. 33, 1921-1929

Andrady A. L. (2011). Microplastics in the marine environment. Marine Pollution Bulletin. 62, 1596-1605

Andrady A. L., Neal M. A. (2009). Applications and societal benefits of plastics. Philosophical Transactions of the Royal Society B: Biological Sciences. 364, 1977-1984

Azizian S. (2004). Kinetic models of sorption: a theoretical analysis. Journal of Colloid and Interface Science. 276, 47-52

Baduel C., Paxman C. J., Mueller J. F. (2015). Perfluoroalkyl substances in a firefighting training ground (FTG), distribution and potential future release. Journal of Hazardous Materials. 296, 46-53

Bakir A., Rowland S. J., Thompson R. C. (2012). Competitive sorption of persistent organic pollutants onto microplastics in the marine environment. Mar Pollut Bull. 64, 2782-2789

Bakir A., Rowland S. J., Thompson R. C. (2014). Enhanced desorption of persistent organic pollutants from microplastics under simulated physiological conditions. Environmental Pollution. 185, 16-23

Barnes D. K. A., Galgani F., Thompson R. C., Barlaz M. (2009). Accumulation and fragmentation of plastic debris in global environments. Philosophical Transactions of the Royal Society B: Biological Sciences. 364, 1985-1998

Becker A. M., Gerstmann S., Frank H. (2008). Perfluorooctane surfactants in waste waters, the major source of river pollution. Chemosphere. 72, 115-121

Chen H., Reinhard M., Nguyen V. T., Gin K. Y.-H. (2016). Reversible and irreversible sorption of perfluorinated compounds (PFCs) by sediments of an urban reservoir. Chemosphere. 144, 1747-1753

Chiou M.-S., Li H.-Y. (2002). Equilibrium and kinetic modeling of adsorption of reactive dye on cross-linked chitosan beads. Journal of Hazardous Materials. 93, 233-248

Cole M., Lindeque P., Halsband C., Galloway T. S. (2011). Microplastics as contaminants in the marine environment: A review. Marine Pollution Bulletin. 62, 2588-2597

Cózar A., Echevarría F., González-Gordillo J. I., Irigoien X., Úbeda B., Hernández-León S., Palma Á. T., Navarro S., García-de-Lomas J., Ruiz A., et al. (2014). Plastic debris in the open ocean. Proceedings of the National Academy of Sciences. 111, 10239

Daan N. (1973). A quantitative analysis of the food intake of North Sea cod, Gadus Morhua. Netherlands Journal of Sea Research. 6, 479-517

Deng S., Niu L., Bei Y., Wang B., Huang J., Yu G. (2013). Adsorption of perfluorinated compounds on aminated rice husk prepared by atom transfer radical polymerization. Chemosphere. 91, 124-130

Derraik J. G. B. (2002). The pollution of the marine environment by plastic debris: a review. Marine Pollution Bulletin. 44, 842-852

Dris R., Gasperi J., Rocher V., Saad M., Renault N., Tassin B. (2015). Microplastic contamination in an urban area: a case study in Greater Paris. Environmental Chemistry. 12, 592-599

Endo S., Koelmans A. A. 2019. Sorption of Hydrophobic Organic Compounds to Plastics in the Marine Environment: Equilibrium. In: Hazardous Chemicals Associated with Plastics in the Marine Environment. Cham: Springer International Publishing. p. 185-204.

Eriksen M., Lebreton L. C. M., Carson H. S., Thiel M., Moore C. J., Borerro J. C., Galgani F., Ryan P. G., Reisser J. (2014). Plastic Pollution in the World's Oceans: More than 5 Trillion Plastic Pieces Weighing over 250,000 Tons Afloat at Sea. PLOS ONE. 9, e111913

Eriksson U., Roos A., Lind Y., Hope K., Ekblad A., Kärrman A. (2016). Comparison of PFASs contamination in the freshwater and terrestrial environments by analysis of eggs from osprey (Pandion haliaetus), tawny owl (Strix aluco), and common kestrel (Falco tinnunculus). Environmental Research. 149, 40-47

Geyer R., Jambeck J. R., Law K. L. (2017). Production, use, and fate of all plastics ever made. Science Advances. 3, e1700782

Giesy J. P., Kannan K. (2001). Global Distribution of Perfluorooctane Sulfonate in Wildlife. Environmental Science & Technology. 35, 1339-1342

Giesy J. P., Kannan K. (2002). Peer Reviewed: Perfluorochemical Surfactants in the Environment. Environmental Science & Technology. 36, 146A-152A

Grosell M., Laliberte C. N., Wood S., Jensen F. B., Wood C. M. (2001). Intestinal HCO3− secretion in marine teleost fish: evidence for an apical rather than a basolateral Cl−/HCO3− exchanger. Fish Physiology and Biochemistry. 24, 81-95

Guo L., Zhang B., Xiao K., Zhang Q., Zheng M. (2009). Levels and distributions of polychlorinated biphenyls in sewage sludge of urban wastewater treatment plants. Journal of Environmental Sciences. 21, 468-473

Hack A., Selenka F. (1996). Mobilization of PAH and PCB from contaminated soil using a digestive tract model. Toxicology Letters. 88, 199-210

Haslewood G. A., Tökés L. (1969). Comparative studies of bile salts. Bile salts of the lamprey Petromyzon marinus L. Biochem J. 114, 179-184

Heo N. W., Hong S. H., Han G. M., Hong S., Lee J., Song Y. K., Jang M., Shim W. J. (2013). Distribution of small plastic debris in cross-section and high strandline on Heungnam beach, South Korea. Ocean Science Journal. 48, 225-233

Hidalgo-Ruz V., Gutow L., Thompson R. C., Thiel M. (2012). Microplastics in the Marine Environment: A Review of the Methods Used for Identification and Quantification. Environmental Science & Technology. 46, 3060-3075

Higgins C. P., Luthy R. G. (2006). Sorption of Perfluorinated Surfactants on Sediments. Environmental Science & Technology. 40, 7251-7256

Hofmann A. F., Hagey L. R., Krasowski M. D. (2010). Bile salts of vertebrates: structural variation and possible evolutionary significance. J Lipid Res. 51, 226-246. Epub 2009/07/28

Houde M., Czub G., Small J. M., Backus S., Wang X., Alaee M., Muir D. C. G. (2008). Fractionation and Bioaccumulation of Perfluorooctane Sulfonate (PFOS) Isomers in a Lake Ontario Food Web. Environmental Science & Technology. 42, 9397-9403

Huang H., Huang C., Wang L., Ye X., Bai C., Simonich M. T., Tanguay R. L., Dong Q. (2010). Toxicity, uptake kinetics and behavior assessment in zebrafish embryos following exposure to perfluorooctanesulphonicacid (PFOS). Aquatic Toxicology. 98, 139-147

Huckins J. N., Tubergen M. W., Manuweera G. K. (1990). Semipermeable membrane devices containing model lipid: A new approach to monitoring the bioavaiiability of lipophilic contaminants and estimating their bioconcentration potential. Chemosphere. 20, 533-552

Hüffer T., Hofmann T. (2016). Sorption of non-polar organic compounds by micro-sized plastic particles in aqueous solution. Environmental Pollution. 214, 194-201

Jambeck J. R., Geyer R., Wilcox C., Siegler T. R., Perryman M., Andrady A., Narayan R., Law K. L. (2015). Plastic waste inputs from land into the ocean. Science. 347, 768

Jovanović B., Gökdağ K., Güven O., Emre Y., Whitley E. M., Kideys A. E. (2018). Virgin microplastics are not causing imminent harm to fish after dietary exposure. Marine Pollution Bulletin. 130, 123-131

Kannan K., Corsolini S., Falandysz J., Fillmann G., Kumar K. S., Loganathan B. G., Mohd M. A., Olivero J., Wouwe N. V., Yang J. H., et al. (2004). Perfluorooctanesulfonate and Related Fluorochemicals in Human Blood from Several Countries. Environmental Science & Technology. 38, 4489-4495

Karapanagioti H. K., Klontza I. (2008). Testing phenanthrene distribution properties of virgin plastic pellets and plastic eroded pellets found on Lesvos island beaches (Greece). Marine Environmental Research. 65, 283-290

Kärrman A., van Bavel B., Järnberg U., Hardell L., Lindström G. (2006). Perfluorinated chemicals in relation to other persistent organic pollutants in human blood. Chemosphere. 64, 1582-1591

Keiter S., Burkhardt-Medicke K., Wellner P., Kais B., Färber H., Skutlarek D., Engwall M., Braunbeck T., Keiter S. H., Luckenbach T. (2016). Does perfluorooctane sulfonate (PFOS) act as chemosensitizer in zebrafish embryos? Science of The Total Environment. 548-549, 317-324

Koelmans A. A., Bakir A., Burton G. A., Janssen C. R. (2016). Microplastic as a Vector for Chemicals in the Aquatic Environment: Critical Review and Model-Supported Reinterpretation of Empirical Studies. Environmental Science and Technology. 50, 3315-3326

Koelmans A. A., Besseling E., Foekema E. M. (2014). Leaching of plastic additives to marine organisms. Environmental Pollution. 187, 49-54

Law K. L., Thompson R. C. (2014). Microplastics in the seas. Science. 345, 144

Lee H., Shim W. J., Kwon J.-H. (2014). Sorption capacity of plastic debris for hydrophobic organic chemicals. Science of The Total Environment. 470-471, 1545-1552

Li J., Liu H., Paul Chen J. (2018). Microplastics in freshwater systems: A review on occurrence, environmental effects, and methods for microplastics detection. Water Research. 137, 362-374

Lithner D., Larsson Å., Dave G. (2011). Environmental and health hazard ranking and assessment of plastic polymers based on chemical composition. Science of The Total Environment. 409, 3309-3324

Llorca M., Schirinzi G., Martínez M., Barceló D., Farré M. (2018). Adsorption of perfluoroalkyl substances on microplastics under environmental conditions. Environmental Pollution. 235, 680-691

Loganathan B. G., Sajwan K. S., Sinclair E., Senthil Kumar K., Kannan K. (2007). Perfluoroalkyl sulfonates and perfluorocarboxylates in two wastewater treatment facilities in Kentucky and Georgia. Water Research. 41, 4611-4620

Martin J. W., Mabury S. A., Solomon K. R., Muir D. C. G. (2003). Dietary accumulation of perfluorinated acids in juvenile rainbow trout (Oncorhynchus mykiss). Environmental Toxicology and Chemistry. 22, 189-195

Martins J., Sobral P. (2011). Plastic marine debris on the Portuguese coastline: A matter of size? Marine Pollution Bulletin. 62, 2649-2653

Mazer N. A., Benedek G. B., Carey M. C. (1980). Quasielastic light-scattering studies of aqueous biliary lipid systems. Mixed micelle formation in bile salt-lecithin solutions. Biochemistry. 19, 601-615

McKay G., Blair H. S., Gardner J. R. (1982). Adsorption of dyes on chitin. I. Equilibrium studies. Journal of Applied Polymer Science. 27, 3043-3057

Mithani S. D., Bakatselou V., TenHoor C. N., Dressman J. B. (1996). Estimation of the Increase in Solubility of Drugs as a Function of Bile Salt Concentration. Pharmaceutical Research. 13, 163-167

Moschetta A., Kliewer S. A. (2005). Weaving betaKlotho into bile acid metabolism. J Clin Invest. 115, 2075-2077

Ng K. L., Obbard J. P. (2006). Prevalence of microplastics in Singapore's coastal marine environment. Marine Pollution Bulletin. 52, 761-767

Obbard R. W., Sadri S., Wong Y. Q., Khitun A. A., Baker I., Thompson R. C. (2014). Global warming releases microplastic legacy frozen in Arctic Sea ice. Earth's Future. 2, 315-320

Olsen G., W., Huang H.-Y., Helzlsouer Kathy J., Hansen Kristen J., Butenhoff John L., Mandel Jeffrey H. (2005). Historical Comparison of Perfluorooctanesulfonate, Perfluorooctanoate, and Other Fluorochemicals in Human Blood. Environmental Health Perspectives. 113, 539-545

Oomen A. G., Sips A. J. A. M., Groten J. P., Sijm D. T. H. M., Tolls J. (2000). Mobilization of PCBs and Lindane from Soil during in Vitro Digestion and Their Distribution among Bile Salt Micelles and Proteins of Human Digestive Fluid and the Soil. Environmental Science & Technology. 34, 297-303

Pascall M. A., Zabik M. E., Zabik M. J., Hernandez R. J. (2005). Uptake of Polychlorinated Biphenyls (PCBs) from an Aqueous Medium by Polyethylene, Polyvinyl Chloride, and Polystyrene Films. Journal of Agricultural and Food Chemistry. 53, 164-169

Paul A. G., Jones K. C., Sweetman A. J. (2009). A First Global Production, Emission, And Environmental Inventory For Perfluorooctane Sulfonate. Environmental Science & Technology. 43, 386-392

PlasticsEurope (2015). Plastics - the Facts 2015, An analysis of European latest plastics production, demand and waste data.

PlasticsEurope (2018). PlasticsEurope: Annual review _ The Facts 2017-2018.

Powley C. R., George S. W., Russell M. H., Hoke R. A., Buck R. C. (2008). Polyfluorinated chemicals in a spatially and temporally integrated food web in the Western Arctic. Chemosphere. 70, 664-672

Rios L. M., Moore C., Jones P. R. (2007). Persistent organic pollutants carried by synthetic polymers in the ocean environment. Marine Pollution Bulletin. 54, 1230-1237

Rochman C. M., Hentschel B. T., Teh S. J. (2014). Long-Term Sorption of Metals Is Similar among Plastic Types: Implications for Plastic Debris in Aquatic Environments. PLOS ONE. 9, e85433

Rochman C. M., Hoh E., Hentschel B. T., Kaye S. (2013). Long-Term Field Measurement of Sorption of Organic Contaminants to Five Types of Plastic Pellets: Implications for Plastic Marine Debris. Environmental Science & Technology. 47, 1646-1654

Rusina T. P., Smedes F., Klanova J., Booij K., Holoubek I. (2007). Polymer selection for passive sampling: A comparison of critical properties. Chemosphere. 68, 1344-1351

Teuten E. L., Rowland S. J., Galloway T. S., Thompson R. C. (2007). Potential for Plastics to Transport Hydrophobic Contaminants. Environmental Science & Technology. 41, 7759-7764

Teuten E. L., Saquing J. M., Knappe D. R. U., Barlaz M. A., Jonsson S., Björn A., Rowland S. J., Thompson R. C., Galloway T. S., Yamashita R., et al. (2009). Transport and release of chemicals from plastics to the environment and to wildlife. Philosophical Transactions of the Royal Society B: Biological Sciences. 364, 2027-2045

Thompson R. C., Moore C. J., vom Saal F. S., Swan S. H. (2009). Plastics, the environment and human health: current consensus and future trends. Philos Trans R Soc Lond B Biol Sci. 364, 2153-2166

Thompson R. C., Olsen Y., Mitchell R. P., Davis A., Rowland S. J., John A. W. G., McGonigle D., Russell A. E. (2004). Lost at Sea: Where Is All the Plastic? Science. 304, 838

Voparil I. M., Mayer L. M. (2004). Commercially Available Chemicals That Mimic a Deposit Feeder's (Arenicola marina) Digestive Solubilization of Lipids. Environmental Science & Technology. 38, 4334-4339

Vrana B., Mills G. A., Dominiak E., Greenwood R. (2006). Calibration of the Chemcatcher passive sampler for the monitoring of priority organic pollutants in water. Environmental Pollution. 142, 333-343

Wang F., Shih K. M., Li X. Y. (2015). The partition behavior of perfluorooctanesulfonate (PFOS) and perfluorooctanesulfonamide (FOSA) on microplastics. Chemosphere. 119, 841-847

Wang J., Liu X., Liu G., Zhang Z., Wu H., Cui B., Bai J., Zhang W. (2019). Size effect of polystyrene microplastics on sorption of phenanthrene and nitrobenzene. Ecotoxicology and Environmental Safety. 173, 331-338

Wang W., Wang J. (2018a). Different partition of polycyclic aromatic hydrocarbon on environmental particulates in freshwater: Microplastics in comparison to natural sediment. Ecotoxicology and Environmental Safety. 147, 648-655

Waychunas G. A., Kim C. S., Banfield J. F. (2005). Nanoparticulate Iron Oxide Minerals in Soils and Sediments: Unique Properties and Contaminant Scavenging Mechanisms. Journal of Nanoparticle Research. 7, 409-433

Xu B., Liu F., Brookes P. C., Xu J. (2018a). Microplastics play a minor role in tetracycline sorption in the presence of dissolved organic matter. Environmental Pollution. 240, 87-94

Ye L., Wu L., Jiang Y., Zhang C., Chen L. (2009). Toxicological study of PFOS/PFOA to zebrafish (*Danio rerio*) embryos. Chin J Environ Sci. 30, 1727-1732

Zareitalabad P., Siemens J., Hamer M., Amelung W. (2013). Perfluorooctanoic acid (PFOA) and perfluorooctanesulfonic acid (PFOS) in surface waters, sediments, soils and wastewater – A review on concentrations and distribution coefficients. Chemosphere. 91, 725-732

Zarfl C., Matthies M. (2010). Are marine plastic particles transport vectors for organic pollutants to the Arctic? Marine Pollution Bulletin. 60, 1810-1814

Zhan Z., Wang J., Peng J., Xie Q., Huang Y., Gao Y. (2016). Sorption of 3,3′,4,4′-tetrachlorobiphenyl by microplastics: A case study of polypropylene. Marine Pollution Bulletin. 110, 559-563

Zhang D., Moore W. R. (1999). Wheat bran particle size effects on bread baking performance and quality. Journal of the Science of Food and Agriculture. 79, 805-809

Zhang H., Wang J., Zhou B., Zhou Y., Dai Z., Zhou Q., Chriestie P., Luo Y. (2018). Enhanced adsorption of oxytetracycline to weathered microplastic polystyrene: Kinetics, isotherms and influencing factors. Environmental Pollution. 243, 1550-1557

Zhou Q., Deng S., Zhang Q., Fan Q., Huang J., Yu G. (2010). Sorption of perfluorooctane sulfonate and perfluorooctanoate on activated sludge. Chemosphere. 81, 453-458

Ziccardi L. M., Edgington A., Hentz K., Kulacki K. J., Kane Driscoll S. (2016). Microplastics as vectors for bioaccumulation of hydrophobic organic chemicals in the marine environment: A state-of-the-science review. Environmental Toxicology and Chemistry. 35, 1667-1676

Supplementary material

Table 1, SM: Determination of PFOS concentrations in freshly opened solvent ; pipettes and in all steps of the preparation and extraction protocol ; e.g. transfer of MeOH with 3 different plastic or glass pipettes, transfer of MeOH with same glass pipette, sonication bath and centrifuge. (n=2, concentration in pg/mL, mean ± SD)

		PFOS concentration (pg/mL)
Solvent	MeOH (HPLC grade)	0.12 (± 0.2)
	MeOH (LC/MS/MS grade)	3.24 (± 0.85)
Pipettes	Plastic pipette tips	0.69 (± 0.15)
	Glass pipette	0.45 (± 0.15)
Entire preparation	*Step*: Transfer plastic pipettes	5.26 (± 1.28)
	Step: Transfer glass pipette	2.75 (± 1.46)
	Step: Transfer of MeOH with same glass pipette	10.12 (± 4.01)
	Step: Sonication	0.16 (± 0.01)
	Step: Centrifugation	0.21 (± 0.06)

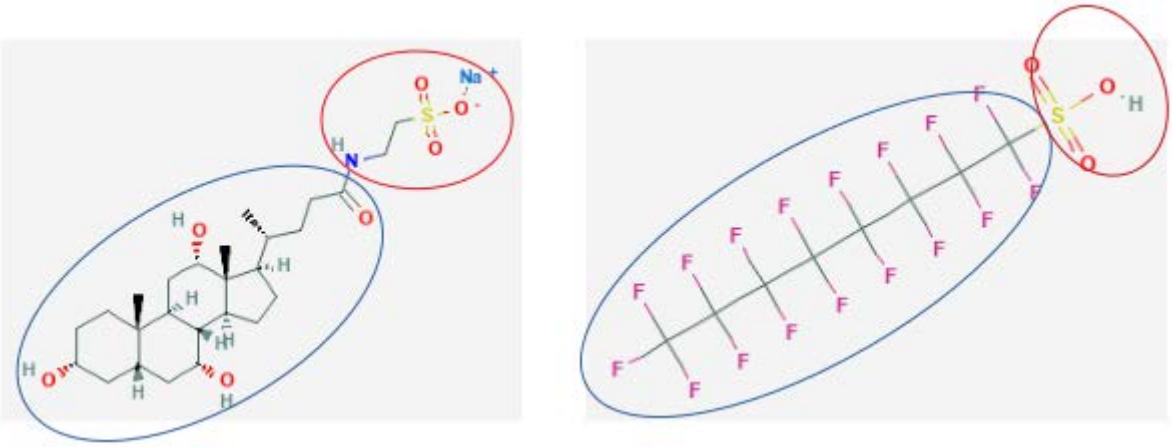


Figure 1, SM. Molecular structure of sodium taurocholate (left) and PFOS (right). Hydrophobic chains and polar functional groups are marked in both compounds in blue and red, respectively.

Extra-study

Sorption processes of BaP to pristine microplastic under different conditions

Sorption processes of BaP to pristine microplastic under different conditions

Cormier B., (2020)

Materials and methods

Materials and chemicals

Four size ranges of high density polyethylene (HDPE) were used as the sorbents, purchased from Micro Powders (New York, USA). Microplastics were sieved to size range of 125-500 µm; 20-25 µm; 11-13 µm and 4-6 µm, and all particles possess a density of 0.96 kg/m^3. Microplatics were kindly provided within the JPI Oceans project EPHEMARE as dry powder by Marina Albentosa from the Instituto Español de Oceanografía Centro Oceanografico de Murcia, Spain.

Benzo[a]pyrene (BaP; solid powder; IUPAC: benzo[a]pyrene, CAS 50-32-8; purity ≥96%) was purchased from Sigma Aldrich (Stockholm, Sweden). All other chemicals and reagents were purchased at the highest purity available from Sigma-Aldrich (Stockholm, Sweden), unless stated otherwise. Standards were purchased from Wellington Laboratories (Guelph, ON, Canada).

Initially, background contamination was investigated. The analysis consisted in 1g of each MPs size range, in triplicates and conducted as described below. MassLynx V4.1 (Waters) was used to analyse data and quantify concentration of BaP.

Chemical analysis of BaP on MPs particles

BaP analysis has been conducted according to Larsson et al. (2013) with minor modifications. Samples were extracted in hexane (≥ 98 % purity, SupraSolv; Merck, Darmstadt, DE) with addition of internal standard (BaP D12 in toluene; Chiron, Tuttlingen, DE) by ultra-sonication and centrifugation at 2000 rpm. Extracts were filtered through fiberglass and transferred to toluene (purity 96 %, SOLVECO; Rosersberg, Sweden). GC vials for analysis consisted in 100 ng of recovery standard perylene D12 (Chiron) and 500 µl of extract. Concentrations of BaP

were quantified using a Micromass Autospec Ultima high-resolution GC/MS system, separation on a DB-5MS column (30 meters, 0.25 mm internal diameter, 25 µm film thickness; J&W Scientific, Folsom, USA). Procedure blanks were included in all batches. Concentrations of BaP was calculated using the internal standard method. The limit of detection (LOD) was defined as mean concentration in blanks plus three times the standard deviations and was calculated as 5 pg/g.

Sorption of pollutants onto microplastics particles

125 g/L of PE was used for the sorption of BaP. Amber glass bottles (Thermo Scientific, Lund, Sweden) were filled with double-deionized water and PE. BaP concentrations in the solutions were 20 µg/L and 2500 µg/L, respectively (from stock solutions prepared in toluene). Bottles were placed on a rotary shaker (Heidolph) at 20 rpm. MPs particles were filtered using 1 µm Whatman® glass microfiber filter (GE Healthcare Life Sciences; Uppsala, SE). Subsequently, the particles were rinsed with double deionized water. Chemical analyses were performed as previously described.

Sorption kinetics

Long-term sorption kinetics experiments were conducted in 40 mL amber glass tubes. MPs concentration was 125 g/L for BaP. Each tube was spiked with 1 mg/L of BaP (prepared in toluene, 0.05%). Tubes were continuously agitated on a rotatory shaker at 20 rpm for 180 days. Samples were collected after 2 days, 7 days, 30 days, 90 days and 180 days, and both water and MPs were extracted and analysed as described above.

Size effect of the sorption of BaP on MPs

Sorption isotherm experiments were conducted for 7 days, based on the results gained from the previous described kinetics experiments. Containers for the isotherms experiments were the same as the one used for the sorption kinetics. Tubes contained 0.1 g of MPs and 40 mL of double-deionized water solution with different BaP concentrations ranging from 25 µg/L to 100 µg/L (25, 50, 75 and 100 µg/L). Tubes were shaken at 20 rpm and kept at 20°C 2 days. All experiments were conducted as three independent replicates.

Statistical analysis

The amount of BaP sorbed per unit mass of microplastics were analysed, and a distribution coefficient (Kd, L/kg) for each individual data point within the linear range of sorption isotherms was calculated as $K_d = C_m/C_e$, with the concentration sorbed to the MPs (C_m) and C_e as the final equilibrium concentration in the water solution. A one-way ANOVA or a non-parametric test, Kruskall Wallis was performed on data to statistically analyze the difference of the data sets. Parameters for sorption experiments were determined using Microsoft Excel 2010 and GraphPad Prism 8.1.

Results and discussion

Study of background contamination from experimental process

PAHs are ubiquitous environmental contaminants and derive mainly from anthropogenic sources. Their persistence in environment is limited due to light sensitivity (Akyüz and Çabuk, 2010), biodegradation and they are characterized as compounds with low mobility because of either a rapid deposition to organic materials as soils, and a retention close to the source (Masih et al., 2012). PAHs with low vapor pressure as BaP, will tend to adsorb to particles rather than staying in the vapor phase (Abdel-Shafy and Mansour, 2016). Contrary to PFOS, BaP would tend to be degraded by light or microbial activity. The same background investigation was done to verify a possible background contamination of BaP, and results showed no background contamination on any MPs tested.

Sorption experiments

PAHs have been reported to adsorb to plastic pellets and fragments from consumer products in marine environments (Rios et al., 2007). It indicates that a natural sorption of PAHs onto MPs may occur in the oceans. Consequently, MPs can potentially act as vector of these contaminants. PAHs are known to sorb onto solid phases as sediments or soils (Tremblay et al., 2005; Wang et al., 2001; Yu et al., 2011). Due to their hydrophobicity, PAHs tends to sorb onto solid phases rather than aqueous phase, and studies demonstrated a high sorption rate of PAHs onto microplastics particles, e.g. fluoranthene, anthracene, pyrene and BaP (Fries and Zarfl, 2012; Rochman, Hoh, Hentschel, et al., 2013; Wang and Wang, 2018a). Therefore, BaP were selected as representative for PAHs to study the sorption on MPs. The sorption of BaP onto PE has never

been studied before, therefore, it was first necessary to develop a extraction method of BaP from micrcoplastic. Extraction of BaP from soils is commonly performed as liquid/solid extraction, using n-hexane and dichloromethane solvent (Larsson et al., 2013); thus, within the present study numerous preliminary experiments were performed to develop a procedure for a solvent extraction of BaP from MPs (data not shown). Finally, n-hexane only was used for extration, rather than a mixture of hexane/dichloromethane (v/v, 50/50) as it is used for soils.

Preliminary investigations have been performed to determine the time point to reach the sorption equilibrium. Based on the preliminary results, not shown in this study, BaP sorption test was performed for 2 days, and initial concentrations of BaP in water was the same for all sizes range, 15 ng/L and 2500 µg/L, to reach low and high concentrations, respectively.

Table 1: BaP adsorbed on HDPE particles with high and low concentration of chemicals. Concentration mean ± SD, n=3.

	(500-125µm)	**(25-20µm)**	**(13-11µm)**	**(6-4µm)**
HIGH Concentration of BaP on MPs (µg/g)	16.33 ± 0.79	14.59 ± 0.30	12.42 ± 0.50	16.74 ± 0.99
LOW Concentration of BaP on MPs (pg/g)	83.92 ± 2.14	72.52 ± 17.64	67.34 ± 5.81	106.94 ± 19.91

Table 1 shows the results of BaP adsorbed to MPs particles. The use of the same initial concentrations of BaP in water, caused rather similar sorption rates for the different size range of HDPE. As mentioned before, the size of MPs as factor for the sorption of chemicals is unknown for most toxicants (Lee, Hong, et al., 2013; Lee, Shim, et al., 2013; Wang et al., 2019). Therefore, more investigations are needed to study the size effect on sorption characteristics of chemicals on MPs. As for PFOS, BaP concentrations were chosen to mimic an environmental and a toxic concentration. BaP has been found at concentration ranging between 0.3 and 5.2 µg/g in suspended particulate matter of the River Tiber in Italy (Patrolecco et al., 2010), and from 4.6 to 54.4 ng/g on microplastics from Portuguese beaches (Frias et al., 2010). MPs sampled in the Northern Pacific Gyre's showed an average contamination of 242 ng/g of total PAHs in 37 samples (Rios et al., 2010). The high concentration aimed, was chosen in a range of µg/g to induce toxicity. BaP has been shown to induce toxicity, e.g. genotoxicity in rainbow trout (*O. mykiss*) liver or digestive gland of clam (*S.sachalinensis*), after 24 h and 48h of exposure, respectively (Kim and Hyun, 2006).

Sorption kinetics

Figure 1 shows the results of the sorption of BaP onto PE microparticles in deionized water after 6 months of exposure. A decrease of BaP adsorbed on MPs was observed, whereas the equilibrium sorption of a similar compound, pyrene, has been achieved after 48h of exposure to either polyethylene, polystyrene and polyvinyl chloride microparticles (Wang and Wang, 2018a). The observed decrease of BaP adsorbed onto PE might be due to the degradation of the compound, e.g. photodegradation (Akyüz and Çabuk, 2010). Decrease of BaP concentration in water is enhanced by different degradations in natural water, and half-lives can vary from 27.5 to 266.6 days, in pond water and seawater, respectively (Kot-Wasik et al., 2004). Furthermore, beside physical and chemical degradation the sorption of BaP can be influenced by adsorption to glassware with a rate of approx. 30 to 40% (Bowman et al., 2002). In the present study, sorption of BaP over 6 months seemed to be altered by other sorption processes, e.g. sorption on glass vial, and might be affected by the degradation of the compound in water.

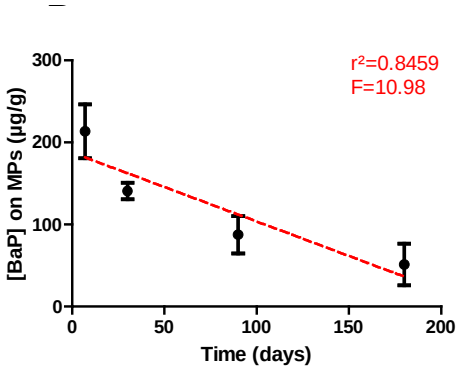


Figure 1: Concentration of BaP in µg/g, adsorbed on MPs (HDPE 250-500 µm) after 7, 30, 90 and 180 days of exposure to 1 mg/L of BaP (0.05% of toluene). Mean ± SD; n=3. Linear regression

Size effect of MPs on BaP sorption

Sorption process of BaP (Figure 2) showed a linear sorption but without any trend regarding a potential particle size effect. The effect of functional groups on the sorption has been demonstrated for PFOS and its precursor, FOSA (Wang, Shih, et al., 2015). The difference of sorption rates depending on functional groups might explain the different patterns observed in the present study, while different particle sizes were used. The polymer type might also modified the sorption rate of a compound, PE particles have a high segmental mobility and free

volume which can support the sorption of chemicals (Karapanagioti and Klontza, 2008; Pascall et al., 2005). Therefore, differences in particle sizes, inducing a modification of surface areas, could have contributed to the difference in chemicals uptake, as well as chemical properties of pollutants. No scientific consensus has been highlighted on the effect of the particle size of a sorbent on the sorption efficiency of contaminants. Since this effect of size depending sorption processes on MPs barely been studied yet, it remains unclear if a decrease of particle sizes is inducing an increase of the sorption rate of a contaminant. In addition, external factors such as temperature, pH, salinity and composition of the water phase (e.g., dissolved organic matter, ion concentrations) can also influence the sorption efficiency of compounds onto MPs (Endo and Koelmans, 2019; Wang, Shih, et al., 2015).

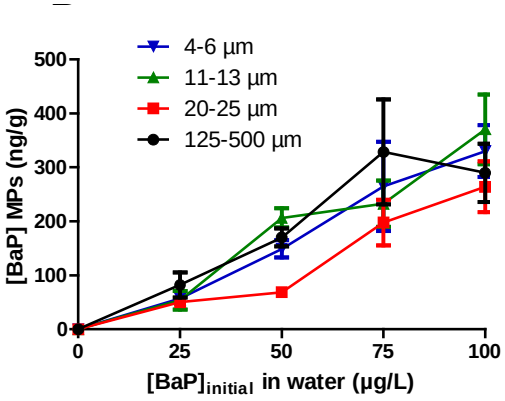


Figure 2: Concentration of BaP in ng/g, adsorbed on different size particles of HDPE (250-500 µm) after exposure of 25, 50, 75 and 100 µg/L of chemical pollutant for 7 days. Mean ± SD; n=3

Conclusion

Sorption behaviors of BaP, on different size of HDPE microplastic particles were investigated. Long-term sorption demonstrated a decrease of the concentration sorbed on MPs over 6 months. Furthermore, the decrease of the size particles did not modify the sorption of BaP. Our results showed that sorption efficiency depends on the type of contaminant (e.g. functional groups) but also on the size range of microparticles.

This study is limited in using one single polymer type, (i.e., polyethylene) with high microplastics and contaminants concentrations. Further studies should be conducted by using different types of polymers (polystyrene, polypropylene) and environmental relevant concentrations of microparticles as well as contaminants concentrations.

References

Abdel-Shafy H. I., Mansour M. S. M. (2016). A review on polycyclic aromatic hydrocarbons: Source, environmental impact, effect on human health and remediation. Egyptian Journal of Petroleum. 25, 107-123

Akyüz M., Çabuk H. (2010). Gas–particle partitioning and seasonal variation of polycyclic aromatic hydrocarbons in the atmosphere of Zonguldak, Turkey. Science of The Total Environment. 408, 5550-5558

Bowman J. C., Zhou J. L., Readman J. W. (2002). Sorption and desorption of benzo(a)pyrene in aquatic systems. Journal of Environmental Monitoring. 4, 761-766

Endo S., Koelmans A. A. 2019. Sorption of Hydrophobic Organic Compounds to Plastics in the Marine Environment: Equilibrium. In: Hazardous Chemicals Associated with Plastics in the Marine Environment. Cham: Springer International Publishing. p. 185-204.

Frias J. P. G. L., Sobral P., Ferreira A. M. (2010). Organic pollutants in microplastics from two beaches of the Portuguese coast. Marine Pollution Bulletin. 60, 1988-1992

Fries E., Zarfl C. (2012). Sorption of polycyclic aromatic hydrocarbons (PAHs) to low and high density polyethylene (PE). Environmental Science and Pollution Research. 19, 1296-1304

Karapanagioti H. K., Klontza I. (2008). Testing phenanthrene distribution properties of virgin plastic pellets and plastic eroded pellets found on Lesvos island beaches (Greece). Marine Environmental Research. 65, 283-290

Kim I.-Y., Hyun C.-K. (2006). Comparative evaluation of the alkaline comet assay with the micronucleus test for genotoxicity monitoring using aquatic organisms. Ecotoxicology and Environmental Safety. 64, 288-297

Kot-Wasik A., Dąbrowska D., Namieśnik J. (2004). Photodegradation and biodegradation study of benzo(a)pyrene in different liquid media. Journal of Photochemistry and Photobiology A: Chemistry. 168, 109-115

Larsson M., Hagberg J., Rotander A., van Bavel B., Engwall M. (2013). Chemical and bioanalytical characterisation of PAHs in risk assessment of remediated PAH-contaminated soils. Environmental Science and Pollution Research. 20, 8511-8520

Lee J., Hong S., Song Y. K., Hong S. H., Jang Y. C., Jang M., Heo N. W., Han G. M., Lee M. J., Kang D., et al. (2013). Relationships among the abundances of plastic debris in different size classes on beaches in South Korea. Marine Pollution Bulletin. 77, 349-354

Lee K.-W., Shim W. J., Kwon O. Y., Kang J.-H. (2013). Size-Dependent Effects of Micro Polystyrene Particles in the Marine Copepod Tigriopus japonicus. Environmental Science & Technology. 47, 11278-11283

Masih J., Singhvi R., Kumar K., Jain V. K., Taneja A. (2012). Seasonal Variation and Sources of Polycyclic Aromatic Hydrocarbons (PAHs) in Indoor and Outdoor Air in a Semi Arid Tract of Northern India. Aerosol and Air Quality Research. 12, 515-525

Pascall M. A., Zabik M. E., Zabik M. J., Hernandez R. J. (2005). Uptake of Polychlorinated Biphenyls (PCBs) from an Aqueous Medium by Polyethylene, Polyvinyl Chloride, and Polystyrene Films. Journal of Agricultural and Food Chemistry. 53, 164-169

Patrolecco L., Ademollo N., Capri S., Pagnotta R., Polesello S. (2010). Occurrence of priority hazardous PAHs in water, suspended particulate matter, sediment and common eels (Anguilla anguilla) in the urban stretch of the River Tiber (Italy). Chemosphere. 81, 1386-1392

Rios L. M., Jones P. R., Moore C., Narayan U. V. (2010). Quantitation of persistent organic pollutants adsorbed on plastic debris from the Northern Pacific Gyre's "eastern garbage patch". Journal of Environmental Monitoring. 12, 2226-2236

Rios L. M., Moore C., Jones P. R. (2007). Persistent organic pollutants carried by synthetic polymers in the ocean environment. Marine Pollution Bulletin. 54, 1230-1237

Rochman C. M., Hoh E., Hentschel B. T., Kaye S. (2013). Long-Term Field Measurement of Sorption of Organic Contaminants to Five Types of Plastic Pellets: Implications for Plastic Marine Debris. Environmental Science & Technology. 47, 1646-1654

Tremblay L., Kohl S. D., Rice J. A., Gagné J.-P. (2005). Effects of temperature, salinity, and dissolved humic substances on the sorption of polycyclic aromatic hydrocarbons to estuarine particles. Marine Chemistry. 96, 21-34

Wang F., Shih K. M., Li X. Y. (2015). The partition behavior of perfluorooctanesulfonate (PFOS) and perfluorooctanesulfonamide (FOSA) on microplastics. Chemosphere. 119, 841-847

Wang J., Liu X., Liu G., Zhang Z., Wu H., Cui B., Bai J., Zhang W. (2019). Size effect of polystyrene microplastics on sorption of phenanthrene and nitrobenzene. Ecotoxicology and Environmental Safety. 173, 331-338

Wang W., Wang J. (2018a). Different partition of polycyclic aromatic hydrocarbon on environmental particulates in freshwater: Microplastics in comparison to natural sediment. Ecotoxicology and Environmental Safety. 147, 648-655

Wang X.-C., Zhang Y.-X., Chen R. F. (2001). Distribution and Partitioning of Polycyclic Aromatic Hydrocarbons (PAHs) in Different Size Fractions in Sediments from Boston Harbor, United States. Marine Pollution Bulletin. 42, 1139-1149

Yu H., Huang G.-h., An C.-j., Wei J. (2011). Combined effects of DOM extracted from site soil/compost and biosurfactant on the sorption and desorption of PAHs in a soil–water system. Journal of Hazardous Materials. 190, 883-890

Chapter 3 – Conclusion

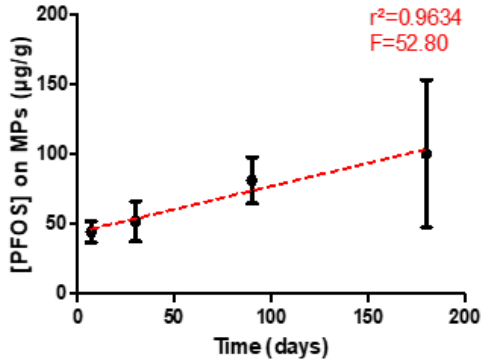
Results

Sorption of PFOS onto MPs

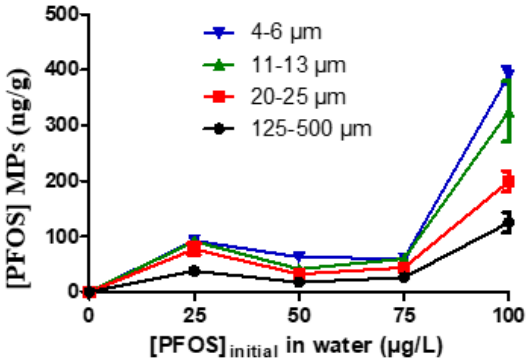
	4-6 µm	11-13 µm
[PFOS] in H_2O	10 mg/L	20 mg/L
$[PFOS]_{MPs}$ (µg/g)	73.61 (± 7.13)	46.26 (± 4.28)

	20-25 µm	125-500 µm
[PFOS] in H_2O	30 mg/L	600 mg/L
$[PFOS]_{MPs}$ (µg/g)	54.02 (± 1.12)	41.20 (± 9.20)

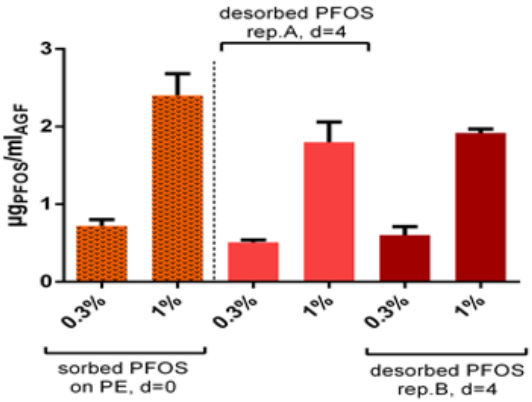
Sorption kinetics



MPs size effect



Artificial desorption



Key points

- PFOS was successfully sorbed onto PE MPs, with an effect of the particle size
- The kinetic sorption of PFOS demonstrated a linear sorption efficiency (r^2=0.96) over 180 days
- Sorption efficiencies have a tendency to be higher for smaller particles, in ascending order for different PFOS concentrations ➔ increase of sorption efficiency link to the decrease of particles size
- After 4 days, the artificial gut fluid led to a desorption rate of 70 to 80 % from the initial amount of PFOS

Chapter 4: Toxicity of artificial microplastics

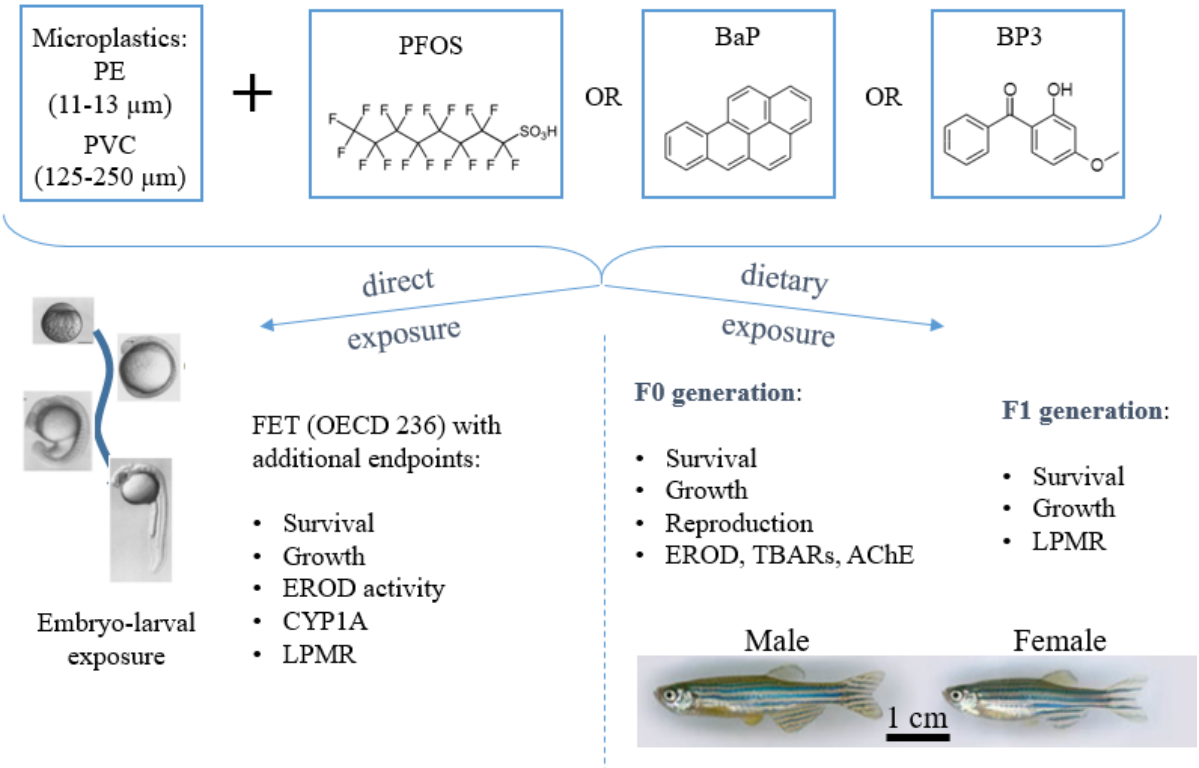
Chapter 4 – Presentation

Context

Toxicity of microplastics (MPs) to aquatic organisms have been poorly investigate to date. Toxicity of individual model pollutants are well known, and MPs are assumed to act as vector of pollutants toward fish. Industrial MPs artificially contaminated with model pollutants have been used to investigate the toxicity on embryos, juveniles and adults zebrafish (*Danio rerio*). Aims of the present chapter were to study toxic effects of virgin MPs, model pollutants (PFOS, BaP and BP3) artificially spiked onto different MPs and to evaluate their toxicities on different biological functions by using different life stages of zebrafish.

Method

In the present study, PE and PVC particles artificially spiked with perfluoroctane sulfonate (PFOS), benzo[a]pyrene or benzophenone-3 (BP3) have been used in contact with zebrafish (*D. rerio*) embryos via waterborne exposure or used to feed juveniles and adults zebrafish. Embryos were exposed to 10 and 100 mg/L of MPs with daily renewal of exposure medium from 4 hpf to 120 hpf.
The chronic experiment consisted of the feeding of zebrafish for 6 months with artificial food supplemented with 1% of MPs (starting at 5 dpf for fish exposed to PE while all PVC treatments were fed from 40 dpf). Fish were maintained in 10 L tanks at IFREMER station.



Article 2

Multi-Laboratory Hazard Assessment of Contaminated Microplastic Particles by Means of Enhanced Fish Embryo Test With the Zebrafish (*Danio rerio*)

Published in Frontiers in Environmental Science

Bettie Cormier, Annika Batel, Jérôme Cachot, Marie-Laure Bégout, Thomas Braunbeck, Xavier Cousin, Steffen H. Keiter.

frontiers in Environmental Science

ORIGINAL RESEARCH
published: 18 September 2019
doi: 10.3389/fenvs.2019.00135

Multi-Laboratory Hazard Assessment of Contaminated Microplastic Particles by Means of Enhanced Fish Embryo Test With the Zebrafish (*Danio rerio*)

Bettie Cormier[1,2], Annika Batel[3], Jérôme Cachot[2], Marie-Laure Bégout[4], Thomas Braunbeck[3], Xavier Cousin[5,6] and Steffen H. Keiter[1]**

[1] Man-Technology-Environment Research Centre, School of Science and Technology, Örebro University, Örebro, Sweden, [2] University of Bordeaux, EPOC UMR CNRS 5805, Pessac, France, [3] Aquatic Ecology and Toxicology Group, Center for Organismal Studies, University of Heidelberg, Heidelberg, Germany, [4] Laboratoire Ressources Halieutiques, IFREMER, L'Houmeau, France, [5] IFREMER, L3AS, UMR MARBEC, Palavas-les-Flots, France, [6] UMR GABI INRA, AgroParisTech, University Paris-Saclay, Jouy-en-Josas, France

OPEN ACCESS

Edited by:
Mariana Teles, University of Porto, Portugal

Reviewed by:
Gabriela Verónica Aguirre-Martínez, Universidad Arturo Prat, Chile
Lluis Tort, Autonomous University of Barcelona, Spain

***Correspondence:**
Bettie Cormier
bettie.cormier@u-bordeaux.fr
Steffen H. Keiter
steffen.keiter@oru.se

Specialty section:
This article was submitted to Marine Pollution, a section of the journal Frontiers in Environmental Science

Received: *15 February 2019*
Accepted: *02 September 2019*
Published: *18 September 2019*

Citation:
Cormier B, Batel A, Cachot J, Bégout M-L, Braunbeck T, Cousin X and Keiter SH (2019) Multi-Laboratory Hazard Assessment of Contaminated Microplastic Particles by Means of Enhanced Fish Embryo Test With the Zebrafish (Danio rerio). Front. Environ. Sci. 7:135. doi: 10.3389/fenvs.2019.00135

As wide-spread pollutants in the marine environment, microplastics (MPs) have raised public concern about potential toxic effects in aquatic organisms, and, among others, MPs were suspected to act as a vector for organic pollutants to biota. The purpose of the present study was to investigate effects by three model pollutants, oxybenzone (BP3), benzo[a]pyrene (BaP), and perfluorooctane sulfonate (PFOS) adsorbed to polyethylene MPs on the basis of a standard assay, the acute fish embryo toxicity test (FET; OECD TG 236) with zebrafish (*Danio rerio*) supplemented by additional endpoints such as induction of ethoxyresorufin-O-deethylase (EROD) activity, modification of *cyp1a* gene transcription and changes in larval swimming behavior. FET assays were performed in three laboratories using slightly different husbandry and exposure conditions, which, however, were all fully compatible with the limits defined by OECD TG 236. This allowed for testing of potential changes in the FET assay due to protocol variations. The standard endpoints of the FET (acute embryotoxicity) did not reveal any acute toxicity for both virgin MPs and MPs spiked with BP3, BaP, and PFOS. With respect to sublethal endpoints, EROD activity was increased after exposure to MPs spiked with BP3 (3 h pulse) and MPs spiked with BaP (96 h continuous exposure). *Cyp1a* transcription was increased upon exposure to MPs spiked with BP3 or BaP. For the selected combination of MPs particles and contaminants, the basic FET proved not sensitive enough to reveal effects of (virgin and spiked) MPs. However, given that the FET can easily be supplemented by a broad variety of more subtle and sensitive endpoints, an enhanced FET protocol may provide a relevant approach with developmental stages of a vertebrate animal model, which is not protected by current EU animal welfare legislation (Directive EU 2010/63).

Keywords: fish embryotoxicity test (FET), swimming behavior, EROD, *cyp1a*, perfluorooctane sulfonate, benzo[a]pyrene, oxybenzone

INTRODUCTION

Plastics have become an indispensable part of our daily life and yield important societal benefits (Andrady and Neal, 2009). Due to poor waste management, however, large portions of the manufactured plastic end up in the oceans and will eventually show up as macroplastics (>5 mm) or microplastics (MPs), i.e., particles between 1 μm and 5 mm in size (Jambeck et al., 2015). The latter consist of either plastic granules designed for purpose in e.g., cosmetics or small plastic fragments derived from the breakdown of macroplastics (Derraik, 2002; Thompson et al., 2004). MPs of both types are considered as a potential threat for aquatic ecosystems (Thompson et al., 2009; Cole et al., 2011).

Plastic materials are not restricted to the polymers themselves, but often also contain numerous additives designed to produce their specific physicochemical properties and to protect MPs from damage by heat, UV light, oxidative processes as well as from microbiological degradation (Andrady, 2011). Many additives are *per se* known as putative toxicants and, upon release from plastic materials, they may potentially cause adverse health effects (Bakir et al., 2014). MPs particles have been shown to adsorb significant amounts of organic pollutants from the surrounding environment (Mato et al., 2001; Rios et al., 2007; Barnes et al., 2009; Koelmans et al., 2016), and weathering processes as well as mechanical fragmentation of MPs lead to smaller particles and may increase the specific reactive surface area of particles and thus facilitate further the sorption of pollutants (Teuten et al., 2007; Koelmans et al., 2013; Lee et al., 2014). Under both laboratory and field conditions, different MPs have been shown to adsorb various classes of organic pollutants such as per- and polyfluorinated compounds, polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), pesticides and personal care products (Rochman et al., 2013a,b; Ziccardi et al., 2016), and the relative contribution of MPs to the transfer of organic pollutants to biota has for long been subject to controversy (Bakir et al., 2016; Koelmans et al., 2016; Lohmann, 2017).

Polyethylene (PE) represents the polymer with the greatest global production for plastic manufacturing, and—for its highly diverse uses—PE contains a wide variety of additives. PE was selected as experimental MPs for the present study because of its predominance in marine ecosystem (Andrady, 2011). As a polymer, PE *per se* is supposed to bear a relatively low hazard (Lithner et al., 2011; Karami et al., 2017); however, due to its physicochemical properties and high abundancy in aquatic environments, PE MPs have a high potential to adsorb and transfer organic pollutants (Rochman et al., 2013a; Wang F. et al., 2015). Together, chemical contaminants and PE may represent a higher risk to aquatic organisms compared to other polymers (Karami et al., 2017).

Benzophenone-3 (oxybenzone, BP3) is commonly used as a UV-filter in cosmetics such as sunscreens, but also as a light stabilizer in plastics such as PE. In addition, BP3 has repeatedly been reported to act as an endocrine disrupting chemical interfering with reproduction and sex hormone signaling in fish and mammals (Kim and Choi, 2014; Kinnberg et al., 2015; Rodríguez-Fuentes et al., 2015). BP3 shows low degradation rates in surface waters (Kim and Choi, 2014) and—due to its lipophilicity (log Kow 4.0)—may bioaccumulate in biota.

Benzo[a]pyrene (BaP) is formed by incomplete combustion of organic materials (Collins et al., 1991) and has become the most frequently studied representative of carcinogenic PAHs and a model molecule for the investigation of PAHs as inducers of aryl hydrocarbon receptor (AhR)-based metabolization pathways. BaP is ubiquitously distributed in coastal and offshore environments (Antunes et al., 2013; Liu et al., 2015; Châtel et al., 2017), and PAHs including BaP are known to adsorb at high rates to different types of MPs in seawater, particularly to PE (Rochman et al., 2013a; Ziccardi et al., 2016; Schönlau et al., 2019). Furthermore, BaP adsorbed to MPs has been documented to be transferred *via* trophic transfer to biota (Batel et al., 2016, 2018; Bour et al., 2018; O'Donovan et al., 2018; Pittura et al., 2018).

Given its wide distribution, perfluorooctane sulfonate (PFOS) has attracted most attention among perfluorinated substances, since it represents the main perfluorinated alkylated compounds detected in aquatic environments (Keiter et al., 2016). PFOS is an extremely persistent fluorinated pollutant that does not hydrolyze, photolyase or biodegrade. PFOS is also known to be toxic and bioaccumulative and undergo extensive transportation across all environmental media. As a consequence, PFOS has been documented in soil, sediment, sludge, and water bodies (Giesy and Kannan, 2002). Since, in ocean surface waters, PFOS and its precursors have been estimated at 235–1,770 metric tons (Paul et al., 2009), PFOS was added to the list of the Stockholm convention on Persistent Organic Pollutants in 2010.

A wide array of bioassays has been developed to assess the toxicity of chemicals or environmental samples. Given that early developmental stages of vertebrates that depend on the use of yolk are considered non-protected according to current European legislation on the protection of animals used for scientific purposes (EU, 2010; Strähle et al., 2012) and that fish embryonic stages have been demonstrated to be equally sensitive to chemical pollutants as intact fish (Nagel, 2002; Braunbeck et al., 2005, 2015; Lammer et al., 2009; Belanger et al., 2013). The fish embryo acute toxicity test (FET) has been designed as an alternative to conventional fish acute toxicity testing [e.g., OECD TG 203; (OECD, 1992)] and has been approved as OECD Test Guideline 236 (OECD, 2013). According to the guideline, the FET has successfully been applied to a wide range of substances exhibiting diverse modes of action, solubilities, volatilities, and hydrophobicities. However, given the particulate nature of MPs, the question arises whether the FET might equally be suited for the assessment of MPs toxicity. In fact, there are major differences between soluble pollutants and discrete objects such as MPs including, e.g., dispersion, homogeneity, localized desorption events of additives or adsorbed pollutants. The purpose of the present study was, therefore, to use the standard FET procedure to document the acute toxicity of MPs and MPs spiked with selected organic pollutants (BP3, BaP, and PFOS). Since OECD TG 236 provides a general framework and allows for substantial flexibility in test conditions, the present study was designed to test for potential differences in FET sensitivity toward MPs due to, e.g., variability in fish pre-conditioning, dark-light scenarios

or exposure conditions. Protocols used by the three laboratories involved in the present comparative study presented some differences but were all within the limits defined by OECD TG 236. In order to enhance resolution and sensitivity of FET, we also monitored additional endpoints such as EROD activity and *cyp1a* transcription as well as larval swimming behavior to reveal potential sublethal effects.

MATERIALS AND METHODS

Materials and Chemicals

Polyethylene (PE; order no. MPP-635 G) with a size range of 11–13 μm and a density of 0.96 kg/m^3 purchased from Micro Powders (New York, USA) was kindly provided within the JPI Oceans project EPHEMARE as a dry powder by Marina Albentosa (Instituto Español de Oceanografía, Centro Oceanografico de Murcia, Spain). Benzo[a]pyrene (BaP; CAS 50-32-8; purity ≥ 96%), benzophenone-3 [(2-hydroxy-4-methoxyphenyl)-phenylmethanone, BP3; CAS 131-57-7; purity ≥ 98%] and PFOS (1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-heptadecafluorooctane-1-sulfonic acid; CAS 2785-37-3; purity ≥ 98%) were purchased from Sigma Aldrich (Stockholm, SE). All other chemicals and reagents were purchased at the highest purity available from Sigma-Aldrich, unless stated otherwise.

Spiking of Microplastic Particles

Setups for the spiking, e.g., MPs and chemical concentrations were previously determined with preliminary experiments in order to determine all parameters to reach aimed concentrations, based on fish toxicity data, e.g., BP3 (Blüthgen et al., 2012; Paredes et al., 2014); BaP (Weigt et al., 2011) and PFOS (Yamashita et al., 2005). Preliminary studies demonstrated that to minimize loss of chemicals, PFOS exposure has to be prepared in polypropylene bottles (Lamaplast; Sesto San Giovanni, Italy), while for BaP and BP3, glass bottles (Thermo Scientific, Lund, SE) were the most efficient to avoid loss of compound.

According to the pretests, for the sorption of BP3 and BaP to PE, 125 g/L PE were added in 400 mL septa bottles (Thermo Scientific, Lund, SE) and bottles were filled to the maximum with double-deionized water, to avoid gas headspace in the bottle, and so avoid volatilization of compound. BP3 and BaP concentrations in the solutions were 20 and 2,500 μg/L, respectively. For the sorption of PFOS on PE, 50 g/L of plastic were weighed into 1 L polypropylene bottles (Lamaplast; Sesto San Giovanni, Italy) filled with double-deionized water and PFOS was added to a final concentration of 20 mg/L. All bottles were placed on a rotary shaker for 48 h (BP3 and BaP) or 7 days (PFOS) at 20 rpm and at room temperature (20 ± 1°C). Based on preliminary experiments, after 48 h, concentrations of BaP and BP3 adsorbed on MPs were higher than samples shaken for more than 48 h due to volatilization of compound, and an increase of the adsorption to glass bottle. While, for PFOS, the sorption time of 7 days was maintained because of the lowest standard deviation of the results and the highest concentration of PFOS adsorbed on particles, compared to other time points.

Chemical Analyses and Quality Assurance/Quality Control (QA/QC)

Concentrations of chemicals (BP3, BaP, and PFOS) were measured in corresponding spiked MPs as well as in virgin MPs to determine background contamination of each chemical.

After filtration of the MP preparations with a funnel equipped with a 1 μm Whatman® glass microfiber filter (GE Healthcare Life Sciences; Uppsala, SE), the filters were rinsed with double deionized water and dried by vacuum evaporation on a ceramic funnel.

The analysis of BP3 was performed by Eurofins Germany (Pforzheim, DE) by using an 0.3 g aliquot of the sample spiked with internal standards (i.e., 3,4,5,6-pentadeuteriobenzoic acid, 1-bromo-4-phenylbenzene, 1,2,3,4,5,6,7,8-octadeuterionaphthalene, 1,2,3,4,5-pentachloro-6-(2,3,4,5,6-pentachlorophenyl)benzene, and 1,2,3,4,5,6,7,8,9,10-decadeuteriophenanthrene) and concentrated with a factor of 10. The samples were extracted with a 1:1 solvent mixture of ethyl acetate and cyclohexane. Additionally, an external calibration standard and a blank sample were prepared. The extracts were measured with GC/MS/MS, electron impact ionization (GC: Agilent7890 A, MS/MS: Agilent 7000 multiple-reaction monitoring mode, Agilent Technologies, Kista, SE) using a split-less injection of 2 μl (column DB5-MS, 30 m × 0.25 mm × 0.25 μm). Identification and quantification were performed against the retention time and the external calibration using the BP3-specific selected reaction monitoring (one quantifier and one qualifier).

BaP analysis has been conducted according to Larsson et al. (2013): MPs were extracted in hexane (≥98%, SupraSolv; Merck, Darmstadt, DE) after addition of an internal standard (500 ng BaP D12 in toluene; Chiron, Tuttlingen, DE; prepared standard: 10 ng/μl in toluene) by sonication and centrifugation at 2,000 rpm. Extracts were filtered through fiberglass and transferred to toluene (purity 96%, SOLVECO; Rosersberg, SE). GC vials for analysis were filled with 100 ng recovery standard perylene D12 (Chiron; 2 ng/μl in toluene, 50 μl added) and 500 μl of extract. The sample volume was reduced to 500 μl using a nitrogen stream. Concentrations of BaP were quantified using a Micromass Autospec Ultima high-resolution GC/MS system, separation on a 30 meters (0.25 mm internal diameter, 25 μm film thickness) DB-5MS column (J&W Scientific, Folsom, USA). Procedure blanks were included in all batches. Target BaP was quantified by using a five-point calibration curve. Relative standard deviation of the relative response factor values was <15% for PAHs. Quantification standard replicates were analyzed after every tenth sample. Concentrations of BaP were calculated by use of the internal standard method. The limit of detection (LOD) was defined as mean concentration in blanks plus three times the standard deviations and was 5 pg/g. Samples which had concentrations exceeding the range of the calibration curve were diluted and reanalyzed. Procedure blanks were included in all batches.

Chemical analysis of PFOS has been conducted according to Eriksson et al. (2016): PFOS (linear and isomers) adsorbed to MPs were extracted in methanol (>99.9% purity, Fisher

TABLE 1 | Husbandry conditions of zebrafish in the three participating laboratories.

Laboratory	Day/night cycle [h]	Water parameters			Group sizes	Tank size	Zebrafish strain	Artificial food
		Temperature	pH	Conductivity				
Örebro University	14/10	26 ± 1°C	7.2 ± 0.2	380 ± 50 µS	100 ± 10	60 L	AB	TetraMin
IFREMER	14/10	27 ± 1°C	7.8 ± 0.2	485 ± 130 µS	28 ± 5	10 L	TU	Inicio + Biomar
Heidelberg University	16/8	26 ± 1°C	7.2 ± 0.1	360 ± 20 µS	≥150	160 L	West-aquarium	TetraMin

Provider for flake food: TetraMin (Tetra, Sweden), Tetra (Melle, Germany), Inicio + 0.5 mm (BioMar, France).

Scientific) by ultra-sonication following by centrifugation (7,000 rpm). Extracts were filtered using a 5 mL PE syringe (Norm-Ject®; Henke Sass Wolf, Tuttlingen, DE) with a filter of 0.2 µm (13 mm, 0.2 µm AcrodiscGHP; Pall, Dreieich, DE). LC vials were prepared with 2 ng of recovery standard (mass-labeled PFOS). Samples were diluted 100 times using 40% methanol (v/v) and 60% ammonium acetate (v/v). Analysis was performed on an Acquity UPLC system coupled to a Xevo TQ-S quadrupole MS (Waters, Milford, USA). A guard column (PFC isolator, Waters) was inserted between the pump and the injector to prevent contamination from the system. PFOS were separated on 100 mm Acquity BEH C18 column (2.1, 1.7 mm) using methanol and water as mobile phases, both with 2 mM ammonium acetate. Quantification of total PFOS was performed by summarization of linear PFOS and individual branched PFOS calculated against an external calibration curve using technical PFOS as standard. Recoveries were assessed using labeled internal standards. The mean recoveries of labeled PFOS internal standards was 83–96%. The LOD was calculated as above was 10 pg/g. Procedure blanks were included in all batches.

Zebrafish Husbandry and Egg Production

Fish husbandry in the three participating laboratories conditions fully complied with OECD TG 236 (Westerfield, 2007; OECD, 2013), but maintenance of adult zebrafish (*Danio rerio*) differed slightly regarding dark/light cycles, size of the rearing tanks, size of heterosexual groups, and water conditions including temperature, pH and conductivity (**Table 1**). In each laboratory, feeding was twice daily *ad libitum* with commercially available artificial diets and freshly hatched brine *Artemia* nauplii. Constant filtering or permanent flow-through conditions guaranteed that ammonia, nitrite, and nitrate were kept below detection limits (5, 1, and 140 mg/L, respectively). Zebrafish eggs were collected according to OECD TG 236 (OECD, 2013).

Toxicity Testing

Zebrafish Embryo Acute Toxicity Test

The acute hazard potential of MPs and contaminated MPs was determine on the basis of the FET guideline (OECD 236) which provides a framework for the test, but allows for some variation in parental fish breeding and exposure conditions. In order to analyze the potential impact of minor modifications of the protocol, the three laboratories participating in the present comparative study performed the test with minor differences in the rearing temperature and the dark/light cycle of the parent fish, as well as in the volume of vessels used and the shaking of the test solutions (**Table 2**); care was taken that the protocols of all three laboratories were clearly within the limits set by OECD TG 236. All other parameters of the tests themselves were identical and followed OECD TG 236.

TABLE 2 | Overview about the test conditions and solutions for the zebrafish embryo toxicity test used by the three different laboratories.

	Örebro University	IFREMER	Heidelberg University
MPs size	PE 11–13 µm		
MPs concentrations	10, 100 mg/L	10 mg/L	100 mg/L
Exposure vessels	Glass vials		
Vessel volumes	100 mL	60 mL	2 mL
Solution volumes	20 mL	10 mL	1.2 mL
Embryos per assay	5	20	20*
Shaking during exposure	No shaking	No shaking	Gentle agitation
Positive control	3,4-Dichloraniline (4 mg/L)		
Temperature	26 ± 1°C	27 ± 1°C	25 ± 1°C
Dark/light rhythm	10/14 h	10/14 h	8/16 h

**One embryo per 2 mL flat bottom argon glass vials (NeoLab, Heidelberg, DE), which were placed in 20 wells of a 24-well plate (n = 20; TPB, Trasadingen, Switzerland). Each glass vial was covered with a cap (unknown composition, caps were washed before use, but did not get into contact with the medium) to prevent evaporation.*

Embryo exposure started at a maximum of 4 hours post-fertilization (hpf) and was performed in glass containers covered with lids, under semi-static conditions with a daily renewal of exposure medium. Exposures were performed using either MPs or MPs spiked with one pollutant at 10 and/or 100 mg/L (**Table 3**). An additional treatment, later called 3POPs, was an even mixture of MPs spiked with BP3, BaP, and PFOS, also used at a final concentration of MPs of 10 or 100 mg/L. The exposure design included negative controls (virgin MPs) and positive controls (water-borne 3,4-dichloraniline, 4 mg/L). In each laboratory, all exposures were performed in triplicate. Embryos were analyzed every day for lethal (OECD, 2013) and sublethal effects as well as hatching rate.

As an additional control, the three pollutants (BP3, BaP, and PFOS) were also tested *via* waterborne exposure at final concentrations equivalent to complete desorption in the case of an exposure to 10 mg/L of MPs. Actual concentrations for BP3, BaP, and PFOS were 0.8, 170, and 700 ng/L, respectively. For waterborne exposure, DMSO was used as a solvent at a final concentration of 0.01%; as a solvent control, 0.01% DMSO was run as an additional negative control.

TABLE 3 | Exposure scenarios tested by the three laboratories for MPs, adsorbed MPs [MP + pollutant(s)] and pollutants alone.

	10 mg/L MP	100 mg/L MP	MP	MP + BP3	MP + BaP	MP + PFOS	MP + 3POPs	BP3	BaP	PFOS
Örebro University	×	×	×	×	×	×	×	×	×	×
IFREMER	×		×	×	×	×	×	–	–	–
Heidelberg University		×	×	×	×	×	–	–	–	–

TABLE 4 | Concentrations of MPs and pollutants (mean ± SD) in solution and the resulting concentration of the pollutants on the PE particles ($n = 1$ for BP3 and $n = 3$ for BaP and PFOS).

	Preparation solution		Pollutant conc. spiked to MPs (μg/g)
	PE (g/L)	Pollutants (μg/L)	
BP3	125	20	0.08
BaP	125	2,500	16.87 ± 0.22
PFOS	50	20,000	70.22 ± 12.41

In vivo EROD Assay

In vivo ethoxyresorufin-*O*-deethylase (EROD) induction in zebrafish embryos was determined according to Kais et al. (2018): Tests was performed at 96 hpf embryos, which were either incubated for 96 h with 100 mg/L of MPs (0–96 h) with daily exchange of medium as previously described, or for a 3 h short-term exposure (93–96 h) with 100 mg/L of MPs. In short, 20 embryos per treatment group and replicate were washed twice for 5 min using dilution water and incubated for 20 min in 0.6 mg/L 7-ethoxyresorufin (Sigma Aldrich, Deisenhofen, Germany). After anesthesia with 0.016% tricaine (MS-222), embryos were mounted left side down in 1% low-melting agarose (SeaKem HGT Agarose; Cambrex BioScience, Rockland, USA) supplemented with 0.016% tricaine. The fluorescence by resorufin formed from 7-ethoxyresorufin *via Cyp1a* catalysis was measured under a Nikon ECLIPSE 90i epifluorescence microscope (Nikon Instruments; Tokyo, Japan) equipped with a 10× Nikon CFI Plan Fluor water immersion objective (NA 0.17, WD 16.0 mm) at an excitation of 560 ± 20 nm (Texas Red HYQ filter) and an emission of 630 ± 30 nm with a dichroic beam splitter of 595 nm. A Nikon DS-Ri-1 camera was used for imaging. Image processing was performed with the NIS-Elements 4.0 imaging software (Nikon Instruments). As a positive control, embryos were incubated at 96 hpf for 3 h in 75 μg/L β-naphthoflavone. Three replicates were run for each test and treatment group ($n = 3 \times 20$).

Cyp1a Gene Transcription Analysis

For analyzes of *cyp1a* induction, 96 hpf embryos ($n = 15$ per exposure group) were exposed for 3 h to MPs as described above. Exposures were performed in triplicate ($n = 3 \times 15$). Ninety-six hours post-fertilization embryos incubated for 3 h in 75 μg/L β-naphthoflavone (Boehler et al., 2018) were used as a positive control. After exposure, embryos were euthanized in 500 mg/L benzocaine, thoroughly rinsed in deionized water and stored in RNALater (Qiagen, Les Ulis, France). Prior to extraction, RNALater was removed, and embryos were placed in a lysis buffer (Qiagen) before being mechanically disrupted using Beadblaster (Benchmark Scientific, Dutscher, France). The total RNA extraction followed the protocols of the RNeasy Plus Universal Mini Kit (Qiagen). Quality and quantity of the extracts were checked using electrophoresis migration and spectrophotometric dosing. Subsequently, cDNA was synthesized from 2 μg of the total RNA, using 2 μl of Superscript III Reverse Transcriptase (Invitrogen, Fisher Scientific, Illkirch, France) according to the manufacturer's protocol in a final volume of 21 μl. Prior to analysis, all cDNA was diluted 5 × in Milli-Q water. The qPCR experiments were run in a final volume of 20 μL, including an optimized primer concentration ranging between 300 and 600 nM (Eurofins, Ebersberg, Germany) and 2X Fast SYBR Green Master Mix (Applied Biosystems, Fisher Scientific, Illkirch, France). The amplification protocol used was as follows: initial denaturation (20 s at 95°C) followed by 40 cycles of 3 s at 95°C and 30 s at 60°C. Technical triplicates were run for each biological replicate. The analysis software Relative Expression Software Tool (REST; Pfaffl, 2001; Pfaffl et al., 2002) automatically calculated fold changes in expression relative to negative controls (unexposed embryos) using the 3 most stable references genes between all groups, and combined them into an index via the free access BestKeeper software (Pfaffl et al., 2004). Reference genes were *g6pd*, *actb1*, and *rpl13a*. Sequences and characteristics of all primers used are listed in **Table S1**.

Larval Behavior

Larval behavior was monitored at 120 hpf using the larval photomotor response (LPMR) test as described by Vignet et al. (2014). The morning before the LPMR, single embryos were individually transferred into one well of a 24-well plate (Krystal 24, opaque wall and clear bottom microplate, Dutscher), and plates were transferred to an enlighten incubator at the same temperature as in the testing room. Plates were successively placed into DanioVision™ (Noldus, Wageningen, NL) in the dark for 10 min of acclimation before the LPMR test which included the following 5-min steps: Light on−1 (LON1, 70 lux), Light off (LOFF, <1 lux) and Light on−2 (LON2, 70 lux) with constant infra-red light maintained during video recording. Distance traveled (cm) was recorded and summed for each 5-min step. Four embryos from each treatment were used in each plate ($n = 6$) to avoid potential bias. Embryos with tracking issues were removed resulting in 16–24 embryos analyzed per treatment. After LPMR recording, embryos were anesthetized with benzocaine (50 mg/L) and photographed in lateral view.

Larval standard length was measured using ImageJ (Schneider et al., 2012).

Statistics

For both the FET and the *in vivo* EROD assay, three independent runs were performed, and data were tested for normality with the Shapiro-Wilk and Kolmogorov-Smirnov tests. If data were normally distributed, one-way analysis of variance (ANOVA) was run in combination with a *post-hoc* Dunnett's test; otherwise, a non-parametric Kruskal-Wallis, Mann-Whitney U or Wilcoxon's matched-pairs tests were used for statistical comparison.

Data were first analyzed for differences between runs (biological replicates). Since there were no significant differences between independent runs, single data sets were merged for each laboratory, and tests on different exposure groups were performed.

In the case of LPMR, a repeated-measure ANOVA was performed to take into account the three successive periods of the test.

A p-value of 0.05 was considered statistically significant for all analyses. Graphical illustrations and statistical tests were performed with Sigma Plot 12.5 (Jandel-Systat, Erkrath, Germany).

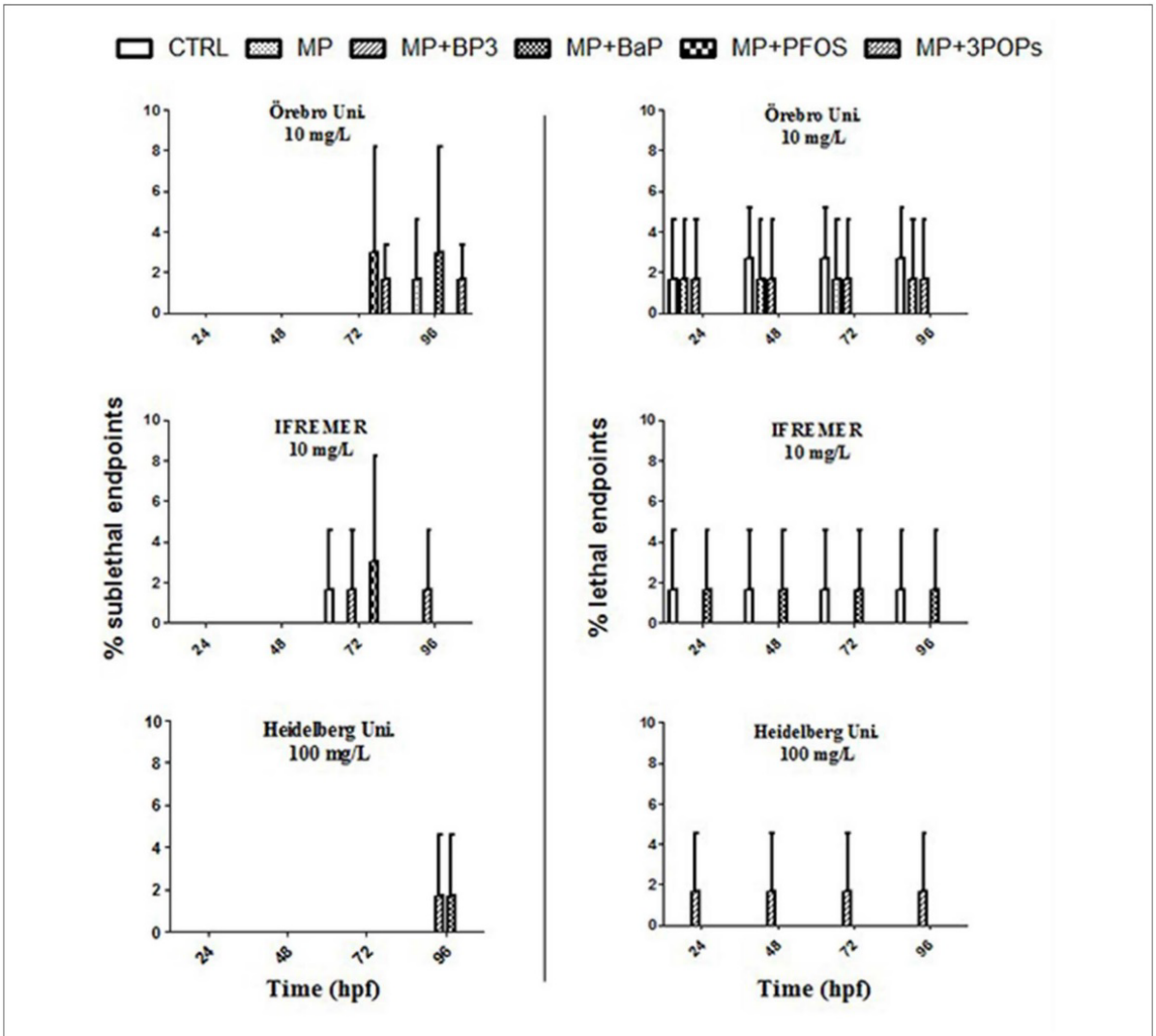


FIGURE 1 | Sublethal and lethal endpoints of zebrafish (*Danio rerio*) embryos after 24, 48, 72, and 96 h exposure to microplastics (MP) and microplastics spiked with benzophenone 3 (MP + BP3), benzo(a)pyrene (MP + BaP), perfuorooctane sulfonate (MP + PFOS) as well as a mixture of the three pollutants (MP + 3 POPs) relative to the negative control (CTRL). Endpoints are given as means ± SD from three independent experiments.

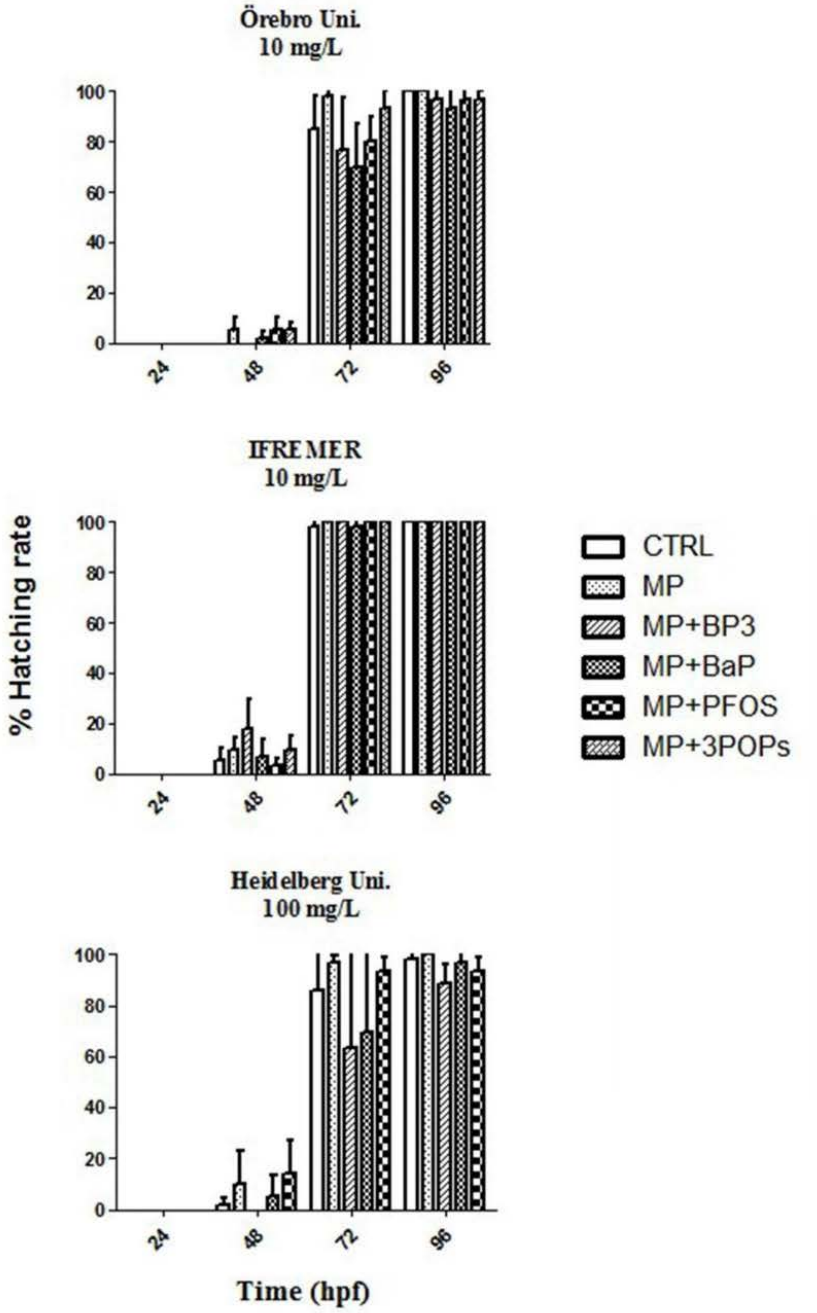


FIGURE 2 | Hatching rate of zebrafish (*Danio rerio*) embryos after 24, 48, 72, and 96 h exposure to microplastics (MP) and microplastics spiked with benzophenone 3 (MP + BP3), benzo[a]pyrene (MP + BaP), perfuorooctane sulfonate (MP + PFOS) as well as a mixture of the three pollutants (MP + 3 POPs) in comparison to the control (CTRL). Hatching rate is given as means ± SD from three independent experiments. Kruskal-Wallis analysis: $p = 0.05$.

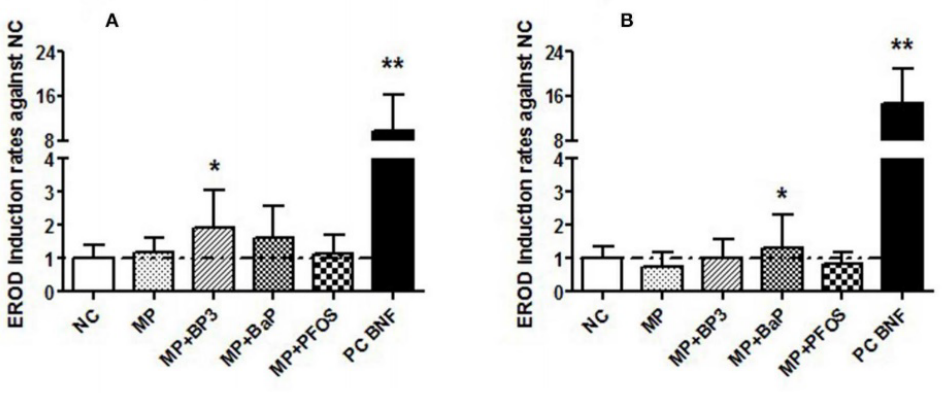


FIGURE 3 | EROD activity in 96 h old zebrafish (*Danio rerio*) embryos exposed to microplastic (MP) and microplastic spiked with benzophenone 3 (MP + BP3), benzo[a]pyrene (MP + BaP), perfuorooctane sulfonate (MP + PFOS), and β-naphthoflavone as a positive control (PC BNF) after 3 h pulse **(A)** or 96 h continuous exposure **(B)**. EROD activities are given relative to negative controls (NC) as means ± SD ($n = 3$; ANOVA on ranks: $^{*}p < 0.05$, $^{**}p < 0.01$).

RESULTS

Background Contamination and Spiking of Microplastics by the Model Pollutants

Background contamination by all three pollutants was measured on virgin microplastics. Results showed no presence of BP3 (<0.05 ng/g, LOD) and BaP (<5 pg/g, LOD). However, PFOS was found on the PE particles at a concentration of 12.1 ± 3.5 pg/g. This background concentration was taken into consideration when analyzing concentrations of PFOS adsorbed to MPs particles.

Chemical quantification of the three pollutants on PE showed that each pollutant has specific sorption efficiencies: 51% (BP3), 84% (BaP), and 18% (PFOS) of the total amount of the pollutants in the solution adsorbed to the PE particles, respectively. Concentrations of BP3, BaP and PFOS on MPs are reported in **Table 4**.

Embryotoxic and Teratogenic Effects

Embryotoxicity and teratogenicity of the selected pollutants diluted in water and DMSO without MPs were evaluated using a FET supplemented by various sublethal endpoints. The results did not show any significant effect (lethal, sublethal effects as malformations and hatching rate) between the negative control and the exposed individuals ($p > 0.05$).

The spiked MPs particles with BP3, BaP, and PFOS caused neither acute toxicity nor any teratogenic effects in the embryos using particle concentrations of 10 and 100 mg/L ($p > 0.05$; **Figure 1**). The examination of embryos exposed to the different MPs particles showed minor changes of the hatching rate at 72 hpf. Results were, however, statistically not different at any time point measured ($p > 0.05$; **Figure 2**). Moreover, measurements of the larvae standard length at 5 dpf showed no significant differences between all conditions tested ($p > 0.05$; **Figure S1**).

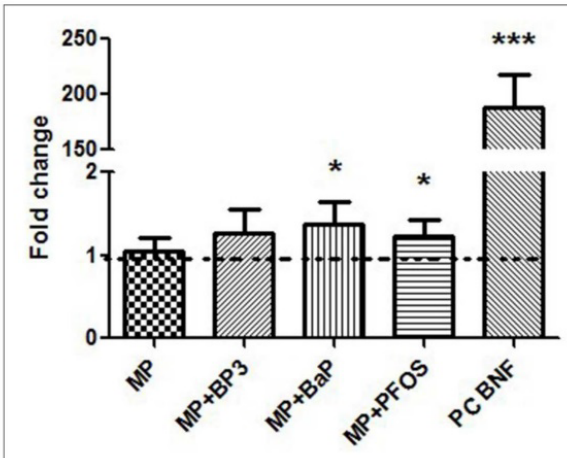


FIGURE 4 | Induction of *cyp1a* gene transcription in 96 hpf zebrafish (*Danio rerio*) embryos after exposure to 10 mg/L of microplastics (MP), microplastics spiked with benzophenone 3 (MP + BP3), microplastics spiked with benzo[a]pyrene (MP + BaP), microplastics spiked with perfuorooctane sulfonate (MP + PFOS) as well as a positive control (75 μg/L β-naphthoflavone). Transcription of *cyp1a* is given as fold-change relative to the negative control (dashed line) after 3 h exposure to MP + pollutant starting at 96 hpf. Data are given as means ± SD ($n = 4$; ANOVA on ranks: $^{*}p < 0.05$, $^{***}p < 0.0001$).

EROD Activity

EROD activity was measured in 96 hpf embryos incubated to 100 mg/L of the different MPs for either a 3 h pulse or 96 h continuously. After the 3 h pulse (**Figure 3A**), only MPs spiked with BP3 (MP + BP3) induced a significant increase in EROD activity ($p < 0.05$), whereas after 96 h of continuous exposure to MP + BP3 there was no induction of EROD activity (**Figure 3B**). In contrast, 96 h of continuous exposure to MPs spiked with BaP

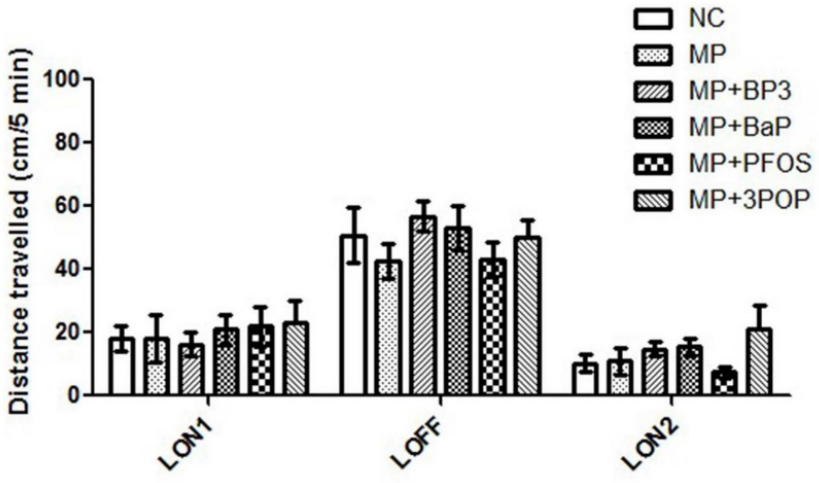


FIGURE 5 | Larval photomotor response in 5 days old zebrafish (*Danio rerio*) embryos after exposure to 10 mg/L of microplastics. Distance traveled over 5 min periods including two light-on periods (LON-1, LON-2) and one light off period (LOFF). Data are given as means ± SD (n = 16–24 larvae per treatment; repeated–measure ANOVA).

(MP + BaP) produced a significant increase in EROD activity ($p < 0.05$). MPs spiked with PFOS did not show any change in EROD activity whatever condition tested.

Gene Transcription Analysis of *Cyp1a*

Gene transcription of *cyp1a* was measured in zebrafish embryos after 3 h of exposure starting at 96 hpf. **Figure 4** shows normalized fold-change in expression of *cyp1a* relative to negative controls. Virgin MPs particles did not show any significant change of *cyp1a* transcription ($p = 0.569$). In contrast, exposure to MP + BaP and MP + PFOS induced a mild, but significant increase in *cyp1a* transcription ($p = 0.013$ and $p = 0.039$, respectively), while in the case of MP + BP3 the significance was just above the threshold ($p = 0.059$).

Larval Behavior

The larval photomotor response (LPMR) was performed at 5 dpf to monitor early behavioral disruption. Independent of the treatment, LPMR showed an increase in distance traveled during the dark phase (LOFF), if compared to both lights-on phases (LON1 and LON2; ANOVA $F_{(2,321)} = 38.07$, $p < 0.001$). Between treatments, however, there was no difference [repeated-measures ANOVA $F_{(15,281.98)} = 0.96$, $p = 0.496$; **Figure 5**].

DISCUSSION

The drastic increase in the amount of MPs in marine environments has raised concern about their potential impact on aquatic organisms (Rochman et al., 2013b; Karami et al., 2017; O'Donovan et al., 2018; Pittura et al., 2018). The adsorption of chemical pollutants from aquatic environments to MPs and the transfer to biota (Antunes et al., 2013; Bakir et al., 2016; Koelmans et al., 2016) is an additional important aspect of the potential toxicity of MPs (Batel et al., 2016; O'Donovan et al., 2018). The present study was designed to evaluate the potential hazard of contaminated PE MPs for zebrafish embryos by using the standard protocol for the acute fish embryo toxicity test (OECD TG 236) as well as enhanced protocols supplemented by additional (sublethal) endpoints such as cytochrome P450 induction and behavioral changes. In comparison to MPs levels found in aquatic environments, the microplastics concentrations selected were relatively high (10 and 100 mg/L). For ocean surface water, microplastics densities range from 0.2 to 320 μg/L (Nerland et al., 2014), and similar levels were found in freshwater ecosystems (Peng et al., 2017; Triebskorn et al., 2019). Data on environmental concentrations of BP3, BaP, and PFOS adsorbed to microplastics are either not available or scarce. Therefore, in the present study concentrations of pollutants adsorbed by PE were based on fish toxicity data from the literature, e.g., BP3 (Blüthgen et al., 2012; Paredes et al., 2014); BaP (Weigt et al., 2011), and PFOS (Yamashita et al., 2005).

In coastal waters, BP3 has been measured at relatively high concentrations, more than 2,000 ng/L, i.e., 2,500 times higher than the concentration used in the present study (Bratkovics and Sapozhnikova, 2011; Bratkovics et al., 2015). However, BaP and PFOS concentrations used in the present study were higher than quantified concentration in aquatic environment. Despite its high affinity to sediments, BaP can be found in the water column, however, the concentration used, i.e., 170 ng/L, was five times higher than concentrations found in seawater (El Nemr and Abd-Allah, 2003; Hong et al., 2016). Concentration of PFOS was also several order of magnitudes higher than concentrations detected in seawater (Ahrens et al., 2009).

Acute Embryo Toxicity and Teratogenicity

Embryotoxic and teratogenic effects were investigated for the single pollutants without MPs assuming that 100% of the

pollutants desorb from 10 mg/L of spiked MPs. Therefore, concentrations tested for BP3, BaP, and PFOS were assumed to be 0.0008, 0.17, and 0.7 μg/L, respectively. For none of the compounds, any embryotoxic effects were observed, which is in agreement with the fact that the concentrations tested were several magnitudes lower than concentrations inducing toxicity reported in the literature for different aquatic organisms. For instance, immobilization tests with *Daphnia magna* at 48 h showed an EC_{50} for BP3 between 1.67 and 1.9 mg/L (Fent et al., 2010; Sieratowicz et al., 2011). Sublethal effects of BP3 in zebrafish, rainbow trout (*Oncorhynchus mykiss*) and Japanese medaka (*Oryzias latipes*) were observed from 132 μg/L (Coronado et al., 2008; Blüthgen et al., 2012; Kim and Choi, 2014). A previous study showed that a 72 h exposure to BaP induced teratogenic and lethal effects zebrafish embryos at 63 and 126 μg/L, respectively (Weigt et al., 2011). PFOS produced sublethal and lethal effects in zebrafish embryos at 10 mg/L (Shi et al., 2008).

Likewise, PE particles up to 100 mg/L (with a diameter between 11 and 13 μm) without pollutants did not show any embryotoxic or teratogenic effects after 96 h of exposure. Similar results were found after exposure of marine medaka (*Oryzias melastigma*) using 10 mg/L of 11–13 μm PE particles (Beiras et al., 2018). Lithner et al. (2011) ranked polymers based on monomer hazard classification, and PE was classified as one of the less hazardous polymers for humans and the environment.

Regarding MPs spiked with pollutants, in the only comparable experiment so far, embryonic stages of marine medaka were exposed to 10 mg/L PE spiked with BP3 at the same concentration as in present study. Exposure to MP + BP3 induced premature hatching and a significant decrease in hatching rate (Beiras et al., 2018). The same authors also demonstrated a direct contact of MPs to villi at the surface of the marine medaka chorion, which may facilitate the uptake of toxicants. Although villi or similar structures do not exist on the zebrafish chorion, Batel et al. (2018) have shown that small MPs (1–5 μm) were able to adhere to the outer surface of the zebrafish chorion and to transfer BaP to embryos across the chorion. In order to force contact of the embryos with MPs, the exposure protocol by Ifremer used a smaller water volume than the other laboratories; yet this exposure protocol failed to induce toxic effects.

Differences have been observed between zebrafish and medaka, and beside the difference in egg surface (Hart and Donovan, 1983; Hart et al., 1984), which may interfere with MPs contact, additional factors such as exposure time might also account for the different responses observed between marine medaka and zebrafish. Indeed, different sensitivities in lethal and sublethal endpoints have been demonstrated between early life stages of Japanese medaka and zebrafish exposed to sediments spiked with two models PAHs (Perrichon et al., 2014). In the frame of FET test, one major difference between these two species is the duration of embryonic stage. For Japanese medaka, the embryonic stage lasts until days 10–11 dpf (Padilla et al., 2009), whereas this period is restricted to 48–72 hpf in zebrafish, depending on temperature (Kimmel et al., 1995). Similarly to Japanese medaka, mean hatching time for marine medaka is 11 dpf (Beiras et al., 2018). Therefore, future investigations on MPs effects in zebrafish might consider prolonged or even chronic exposure; however, this would be an animal experiment clearly requiring authorization under Directive 2010/63/EU.

Cytochrome P450 Induction and Biotransformation

CYP1A is involved in phase I of xenobiotic metabolism and is often used as a biomarker of exposure. For the investigation of sublethal effects due to MP exposures, changes in CYP1A expression was evaluated by analyzing (a) EROD activity in 96 h old zebrafish embryos exposed to 100 mg/L of MPs for 3 h (93–96 hpf) or 96 h (0–96 hpf) and (b) *cyp1a* gene transcription. Since previous studies documented short-term (3 h pulse) exposure to reference molecules to cause a more prominent EROD induction than 72 h continuous exposure (Kais et al., 2017, 2018) and has been shown to be long enough to induce *cyp1* transcription (Larcher et al., 2014), the present study tested *cyp1a* transcription levels after short-term exposure to 10 mg/L MPs for 3 h only. Independent of MP concentrations, MPs alone did not induce EROD activity nor *cyp1a* transcription.

For BP3, only the short-term exposure (93–96 hpf) to 100 mg/L MPs + BP3 significantly induced EROD activity ($p < 0.05$), while *cyp1a* transcription was slightly upregulated ($p < 0.1$) after exposure to MP + BP3 at 10 mg/L. To our knowledge, BP3 induction of EROD activity has never been shown before in embryonic stages of aquatic organisms. In adult zebrafish, Blüthgen et al. (2012) documented an increase in *cyp1a* transcription in the brain of adult zebrafish after 14 days exposure to 312 μg/L BP3.

BaP is well-known to modulate EROD activity and to induce *cyp1a* transcription in various species (Jönsson et al., 2009; Rochman et al., 2013b; Batel et al., 2016). In the present study, MPs spiked with BaP caused an induction of EROD activity after 96 h of exposure. Meanwhile, a significant induction ($p < 0.05$) of *cyp1a* transcription was also shown after 3 h of continuous exposure to BaP-spiked MPs.

MPs spiked with PFOS did not modify EROD activity, but induced *cyp1a* transcription upregulation. Both in adult marine medaka and zebrafish, previous studies showed that PFOS modifies *cyp1a* transcription after exposure to 1–16 mg/L PFOS (Fang et al., 2012; Jantzen et al., 2016).

Albeit with some differences in response with respect to exposure duration—which may reflect the need for translation or enzyme accumulation—an overall triggering of CYP1A (either as EROD activities and/or as *cyp1a* transcription) was observed after exposure to MPs spiked with pollutants, whereas virgin MPs failed to do so. Results indicate that pollutants spiked on MPs are able to desorb and reach concentrations sufficient to induce biotransformation.

Larval Behavior

An increasing number of studies uses behavioral responses to monitor sublethal effects of pollutants such as PFOS or BPA on aquatic organisms (Huang et al., 2010; Wang J. et al., 2015), and changes in, e.g., the photomotor response are commonly interpreted as evidence of potential neurotoxicity (Le Bihanic

et al., 2015; Legradi et al., 2018). Yet, our results did not show any alteration of the LPMR by any of the different MPs preparations. Similar results were shown after exposure of zebrafish embryos to pure polystyrene MPs particles by Chen et al. (2017), who spiked polystyrene MPs with EE2 (17α-ethinylestradiol) and found hypoactivity at elevated concentrations only. However, previous studies showed that microplastics may reduce other behavioral parameters such as predatory performance of fish depending on the shape of plastic particles (de Sá et al., 2015; Choi et al., 2018).

As water-borne pollutants, both PFOS (Huang et al., 2010) and BaP (Knecht et al., 2017) were shown to be able to modify the behavior of zebrafish. However, concentrations applied in these studies were several magnitudes higher than those used in this study and most likely lacked any environmental relevance. Therefore, further investigations are required to assess the toxicity of microplastics on behavioral aspects considering different endpoints and different kind of particles.

CONCLUSIONS

The current study shows that both virgin MPs and MPs spiked with BP3, BaP, or PFOS at given concentrations do not cause acute toxic effects in zebrafish embryos using the standardized protocol of OECD TG 236 (FET). Thus, the standard FET may not be sensitive enough to investigate acute embryotoxic effects of spiked MPs. Likewise, behavioral endpoints such as the LPMR were not altered under any conditions tested, even though existing literature indicate that embryonic and larval behavior of fish may be altered by MPs. Yet, given that the FET can easily be supplemented with additional (more sensitive) endpoints, enhanced FET protocols may be designed to record more subtle sublethal endpoints such as biotransformation. For the identification of adverse effects due to exposure to MPs and associated pollutants, more sensitive biomarkers or other fish models with a longer embryonic stage (i.e., Japanese or marine medaka) may thus be needed. Results indicate that sublethal toxic effects are more likely caused by pollutants associated with MPs than by the MPs themselves. This lends some support to the view that MPs might play a certain role as a vector of pollutants. Further research at more environmentally relevant MPs concentrations is needed with different types and sizes of MPs, other pollutants and adapted exposures scenarios including increased exposure duration.

DATA AVAILABILITY

The datasets generated for this study are available on request to the corresponding author.

AUTHOR CONTRIBUTIONS

AB, BC, SK, TB, and XC designed the experiment. AB, BC, and XC performed the experiments and analyzed the data. AB conducted P450 biomarker analyses. XC conducted *cyp1a* gene transcription and behavioral analyses. BC prepared and characterized the contaminated MPs, performed the chemical analyses. BC drafted the manuscript and AB, JC, M-LB, SK, TB, and XC corrected the manuscript.

FUNDING

This work was developed under the EPHEMARE project (Ecotoxicological effects of microplastics in marine ecosystems), supported by national funding agencies within the framework of JPI Oceans (FCT JPIOCEANS/0005/2015; FORMAS, 2015-01865; ANR-15-JOCE-0002-01; German BMBF 03F0735A). BC was directly supported by an IdEx grant from the University of Bordeaux; AB was awarded a scholarship by the German Academic Scholarship Foundation (Studienstiftung des Deutschen Volkes).

ACKNOWLEDGMENTS

The authors would like to thank (1) Anna Karmann and Leo Yeung for their skillful collaboration to the PFOS analyses; (2) Maria Larsson for her help and supervision with the BaP analyses; (3) Patrick Van Hees and EUROFINS Germany for their collaboration to the BP3 analyses; (4) Ezvin Le Roux for performing some FET experiments at Örebro University; and (5) Lucette Joassard at IFREMER for technical support in running the qPCR analyses.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fenvs.2019.00135/full#supplementary-material

REFERENCES

Ahrens, L., Felizeter, S., and Ebinghaus, R. (2009). Spatial distribution of polyfluoroalkyl compounds in seawater of the German Bight. *Chemosphere* 76, 179–184. doi: 10.1016/j.chemosphere.2009.03.052

Andrady, A. L. (2011). Microplastics in the marine environment. *Mar. Pollut. Bull.* 62, 1596–1605. doi: 10.1016/j.marpolbul.2011.05.030

Andrady, A. L., and Neal, M. A. (2009). Applications and societal benefits of plastics. *Philos. Trans. R. Soc. B. Biol. Sci.* 364, 1977–1984. doi: 10.1098/rstb.2008.0304

Antunes, J. C., Frias, J. G. L., Micaelo, A. C., and Sobral, P. (2013). Resin pellets from beaches of the Portuguese coast and adsorbed persistent organic pollutants. *Estuar. Coast. Shelf Sci.* 130, 62–69. doi: 10.1016/j.ecss.2013.06.016

Bakir, A., O'Connor, I. A., Rowland, S. J., Hendriks, A. J., and Thompson, R. C. (2016). Relative importance of microplastics as a pathway for the transfer of hydrophobic organic chemicals to marine life. *Environ. Pollut.* 219, 56–65. doi: 10.1016/j.envpol.2016.09.046

Bakir, A., Rowland, S. J., and Thompson, R. C. (2014). Enhanced desorption of persistent organic pollutants from microplastics under simulated physiological conditions. *Environ. Pollut.* 185, 16–23. doi: 10.1016/j.envpol.2013.10.007

Barnes, D. K., Galgani, F., Thompson, R. C., and Barlaz, M. (2009). Accumulation and fragmentation of plastic debris in global environments. *Philos. Trans. R. Soc. B. Biol. Sci.* 364, 1985–1998. doi: 10.1098/rstb.2008.0205

Batel, A., Borchert, F., Reinwald, H., Erdinger, L., and Braunbeck, T. (2018). Microplastic accumulation patterns and transfer of benzo[a]pyrene to adult zebrafish (*Danio rerio*) gills and zebrafish embryos. *Environ. Pollut.* 235, 918–930. doi: 10.1016/j.envpol.2018.01.028

Batel, A., Linti, F., Scherer, M., Erdinger, L., and Braunbeck, T. (2016). Transfer of benzo[a]pyrene from microplastics to *Artemia nauplii* and further to zebrafish via a trophic food web experiment: CYP1A induction and visual tracking of persistent organic pollutants. *Environ. Toxicol. Chem.* 35, 1656–1666. doi: 10.1002/etc.3361

Beiras, R., Bellas, J., Cachot, J., Cormier, B., Cousin, X., Engwall, M., et al. (2018). Ingestion and contact with polyethylene microplastics does not cause acute toxicity on marine zooplankton. *J. Hazard. Mater.* 360, 452–460. doi: 10.1016/j.jhazmat.2018.07.101

Belanger, S. E., Rawlings, J. M., and Carr, G. J. (2013). Use of fish embryo toxicity tests for the prediction of acute fish toxicity to chemicals. *Environ. Toxicol. Chem.* 32, 1768–1783. doi: 10.1002/etc.2244

Blüthgen, N., Zucchi, S., and Fent, K. (2012). Effects of the UV filter benzophenone-3 (oxybenzone) at low concentrations in zebrafish (*Danio rerio*). *Toxicol. Appl. Pharmacol.* 263, 184–194. doi: 10.1016/j.taap.2012.06.008

Boehler, S., Lörracher, A. K., Schubert, J., and Braunbeck, T. (2018). Comparative live-imaging of *in vivo* EROD (ethoxyresorufin-O-deethylase) induction in zebrafish (*Danio rerio*) and fathead minnow (*Pimephales promelas*) embryos after exposure to PAHs and river sediment extracts. *Sci. Total Environ.* 621, 827–838. doi: 10.1016/j.scitotenv.2017.11.101

Bour, A., Haarr, A., Keiter, S., and Hylland, K. (2018). Environmentally relevant microplastic exposure affects sediment-dwelling bivalves. *Environ. Pollut.* 236, 652–660. doi: 10.1016/j.envpol.2018.02.006

Bratkovics, S., and Sapozhnikova, Y. (2011). Determination of seven commonly used organic UV filters in fresh and saline waters by liquid chromatography-tandem mass spectrometry. *Anal. Methods.* 3, 2943–2950. doi: 10.1039/c1ay05390f

Bratkovics, S., Wirth, E., Sapozhnikova, Y., Pennington, P., and Sanger, D. (2015). Baseline monitoring of organic sunscreen compounds along South Carolina's coastal marine environment. *Mar. Pollut. Bull.* 101, 370–377. doi: 10.1016/j.marpolbul.2015.10.015

Braunbeck, T., Boettcher, M., Hollert, H., Kosmehl, T., Lammer, E., Leist, E., et al. (2005). Towards an alternative for the acute fish LC_{50} test in chemical assessment: The zebrafish (*Danio rerio*) embryo toxicity test - an update. *ALTEX* 22, 87–102. Available online at: http://www.altex.ch/resources/altex_2005_2_87_102_Braunbeck.pdf

Braunbeck, T., Kais, B., Lammer, E., Otte, J., Schneider, K., Stengel, D., et al. (2015). The fish embryo test (FET): origin, applications, and future. *Environ. Sci. Pollut. Res. Int.* 22, 16247–16261. doi: 10.1007/s11356-014-3814-7

Châtel, A., Bruneau, M., Lièvre, C., Goupil, A., and Mouneyrac, C. (2017). Spermatozoa: a relevant biological target for genotoxicity assessment of contaminants in the estuarine bivalve *Scrobicularia plana*. *Mar. Pollut. Bull.* 116, 488–490. doi: 10.1016/j.marpolbul.2016.12.062

Chen, Q., Gundlach, M., Yang, S., Jiang, J., Velki, M., Yin, D., et al. (2017). Quantitative investigation of the mechanisms of microplastics and nanoplastics toward zebrafish larvae locomotor activity. *Sci. Total Environ.* 584–585, 1022–1031. doi: 10.1016/j.scitotenv.2017.01.156

Choi, J. S., Jung, Y.-J., Hong, N.-H., Hong, S. H., and Park, J.-W. (2018). Toxicological effects of irregularly shaped and spherical microplastics in a marine teleost, the sheepshead minnow (*Cyprinodon variegatus*). *Mar. Pollut. Bull.* 129, 231–240. doi: 10.1016/j.marpolbul.2018.02.039

Cole, M., Lindeque, P., Halsband, C., and Galloway, T. S. (2011). Microplastics as contaminants in the marine environment: a review. *Mar. Pollut. Bull.* 62, 2588–2597. doi: 10.1016/j.marpolbul.2011.09.025

Collins, J. F., Brown, J. P., Dawson, S. V., and Marty, M. A. (1991). Risk assessment for benzo[a]pyrene. *Regul. Toxicol. Pharmacol.* 13, 170–184. doi: 10.1016/0273-2300(91)90020-V

Coronado, M., De Haro, H., Deng, X., Rempel, M. A., Lavado, R., and Schlenk, D. (2008). Estrogenic activity and reproductive effects of the UV-filter oxybenzone (2-hydroxy-4-methoxyphenyl-methanone) in fish. *Aquat. Toxicol.* 90, 182–187. doi: 10.1016/j.aquatox.2008.08.018

de Sá, L. C., Luís, L. G., and Guilhermino, L. (2015). Effects of microplastics on juveniles of the common goby (*Pomatoschistus microps*): confusion with prey, reduction of the predatory performance and efficiency, and possible influence of developmental conditions. *Environ. Pollut.* 196, 359–362. doi: 10.1016/j.envpol.2014.10.026

Derraik, J. G. (2002). The pollution of the marine environment by plastic debris: a review. *Mar. Pollut. Bull.* 44, 842–852. doi: 10.1016/S0025-326X(02)00220-5

El Nemr, A., and Abd-Allah, A. M. A. (2003). Contamination of polycyclic aromatic hydrocarbons (PAHs) in microlayer and subsurface waters along Alexandria coast, Egypt. *Chemosphere* 52, 1711–1716. doi: 10.1016/S0045-6535(03)00300-X

Eriksson, U., Roos, A., Lind, Y., Hope, K., Ekblad, A., and Kärrman, A. (2016). Comparison of PFASs contamination in the freshwater and terrestrial environments by analysis of eggs from osprey (*Pandion haliaetus*), tawny owl (*Strix aluco*), and common kestrel (*Falco tinnunculus*). *Environ. Res.* 149, 40–47. doi: 10.1016/j.envres.2016.04.038

EU (2010). Directive 2010/63/EU of the European parliament and of the council of 22 September 2010 on the protection of animals used for scientific purposes. *Off. J. EU.* L276, 33–79. Available online at: https://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2010:276:0033:0079:en:PDF

Fang, C., Wu, X., Huang, Q., Liao, Y., Liu, L., Qiu, L., et al. (2012). PFOS elicits transcriptional responses of the ER, AHR and PPAR pathways in *Oryzias melastigma* in a stage-specific manner. *Aquat. Toxicol.* 106–107, 9–19. doi: 10.1016/j.aquatox.2011.10.009

Fent, K., Kunz, P. Y., Zenker, A., and Rapp, M. (2010). A tentative environmental risk assessment of the UV-filters 3-(4-methylbenzylidene-camphor), 2-ethyl-hexyl-4-trimethoxycinnamate, benzophenone-3, benzophenone-4 and 3-benzylidene camphor. *Mar. Environ. Res.* 69, S4–S6. doi: 10.1016/j.marenvres.2009.10.010

Giesy, J. P., and Kannan, K. (2002). Perfluorochemical surfactants in the environment. *Environ. Sci. Technol.* 36, 146A−152A. doi: 10.1021/es022253t

Hart, N. H., and Donovan, M. (1983). Fine structure of the chorion and site of sperm entry in the egg of *Brachydanio*. *J. Exp. Zool.* 227, 277–296. doi: 10.1002/jez.1402270212

Hart, N. H., Pietri, R., and Donovan, M. (1984). The structure of the chorion and associated surface filaments in Oryzias—evidence for the presence of extracellular tubules. *J. Exp. Zool.* 230, 273–296. doi: 10.1002/jez.1402300213

Hong, W.-J., Jia, H., Li, Y.-F., Sun, Y., Liu, X., and Wang, L. (2016). Polycyclic aromatic hydrocarbons (PAHs) and alkylated PAHs in the coastal seawater, surface sediment and oyster from Dalian, Northeast China. *Ecotoxicol. Environ. Saf.* 128, 11–20. doi: 10.1016/j.ecoenv.2016.02.003

Huang, H., Huang, C., Wang, L., Ye, X., Bai, C., Simonich, M. T., et al. (2010). Toxicity, uptake kinetics and behavior assessment in zebrafish embryos following exposure to perfluorooctanesulphonicacid (PFOS). *Aquat. Toxicol.* 98, 139–147. doi: 10.1016/j.aquatox.2010.02.003

Jambeck, J. R., Geyer, R., Wilcox, C., Siegler, T. R., Perryman, M., Andrady, A., et al. (2015). Plastic waste inputs from land into the ocean. *Science* 347, 768–771. doi: 10.1126/science.1260352

Jantzen, C. E., Annunziato, K. A., Bugel, S. M., and Cooper, K. R. (2016). PFOS, PFNA, and PFOA sub-lethal exposure to embryonic zebrafish have different toxicity profiles in terms of morphometrics, behavior and gene expression. *Aquat. Toxicol.* 175, 160–170. doi: 10.1016/j.aquatox.2016.03.026

Jönsson, M. E., Brunström, B., and Brandt, I. (2009). The zebrafish gill model: induction of CYP1A, EROD and PAH adduct formation. *Aquat. Toxicol.* 91, 62–70. doi: 10.1016/j.aquatox.2008.10.010

Kais, B., Ottermanns, R., Scheller, F., and Braunbeck, T. (2018). Modification and quantification of *in vivo* EROD live-imaging with zebrafish (*Danio rerio*) embryos to detect both induction and inhibition of CYP1A. *Sci. Total Environ.* 615, 330–347. doi: 10.1016/j.scitotenv.2017.09.257

Kais, B., Schiwy, S., Hollert, H., Keiter, S. H., and Braunbeck, T. (2017). *In vivo* EROD assays with the zebrafish (*Danio rerio*) as rapid screening tools for the detection of dioxin-like activity. *Sci. Total Environ.* 590–591, 269–280. doi: 10.1016/j.scitotenv.2017.02.236

Karami, A., Groman, D. B., Wilson, S. P., Ismail, P., and Neela, V. K. (2017). Biomarker responses in zebrafish (*Danio rerio*) larvae exposed to pristine low-density polyethylene fragments. *Environ. Pollut.* 223, 466–475. doi: 10.1016/j.envpol.2017.01.047

Keiter, S., Burkhard-Medicke, K., Kais, B., Färber, H., Skutlarek, D., Engwall, M., et al. (2016). Does perfluorooctane sulfonate (PFOS) act as chemosensitizer in zebrafish embryos? *Sci. Total Environ.* 548–549, 317–324. doi: 10.1016/j.scitotenv.2015.12.089

Kim, S., and Choi, K. (2014). Occurrences, toxicities, and ecological risks of benzophenone-3, a common component of organic sunscreen products: a mini-review. *Environ. Int.* 70, 143–157. doi: 10.1016/j.envint.2014.05.015

Kimmel, C. B., Ballard, W. W., Kimmel, S. R., Ullmann, B., and Schilling, T. F. (1995). Stages of embryonic development of the zebrafish. *Dev. Dyn.* 203, 253–310. doi: 10.1002/aja.1002030302

Kinnberg, K. L., Petersen, G. I., Albrektsen, M., Minghlani, M., Awad, S. M., Holbech, B. F., et al. (2015). Endocrine-disrupting effect of the ultraviolet filter benzophenone-3 in zebrafish, *Danio rerio*. *Environ. Toxicol. Chem.* 34, 2833–2840. doi: 10.1002/etc.3129

Knecht, A. L., Truong, L., Simonich, M. T., and Tanguay, R. L. (2017). Developmental benzo[a]pyrene (B[a]P) exposure impacts larval behavior and impairs adult learning in zebrafish. *Neurotoxicol. Teratol.* 59, 27–34. doi: 10.1016/j.ntt.2016.10.006

Koelmans, A. A., Bakir, A., Burton, G. A., and Janssen, C. R. (2016). Microplastic as a vector for chemicals in the aquatic environment: critical review and model-supported reinterpretation of empirical studies. *Environ. Sci. Technol.* 50, 3315–3326. doi: 10.1021/acs.est.5b06069

Koelmans, A. A., Besseling, E., Wegner, A., and Foekema, E. M. (2013). Plastic as a carrier of POPs to aquatic organisms: a model analysis. *Environ. Sci. Technol.* 47, 7812–7820. doi: 10.1021/es401169n

Lammer, E., Carr, G. J., Wendler, K., Rawlings, J. M., Belanger, S. E., and Braunbeck, T. (2009). Is the fish embryo toxicity test (FET) with the zebrafish (*Danio rerio*) a potential alternative for the fish acute toxicity test? *Comp. Biochem. Physiol. C.* 149, 196–209. doi: 10.1016/j.cbpc.2008.11.006

Larcher, T., Perrichon, P., Vignet, C., Ledevin, M., Le Menach, K., Lyphout, L., et al. (2014). Chronic dietary exposure of zebrafish to PAH mixtures results in carcinogenic but not genotoxic effects. *Environ. Sci. Pollut. Res.* 21:13833. doi: 10.1007/s11356-014-2923-7

Larsson, M., Hagberg, J., Rotander, A., van Bavel, B., and Engwall, M. (2013). Chemical and bioanalytical characterisation of PAHs in risk assessment of remediated PAH-contaminated soils. *Environ. Sci. Pollut. Res.* 20, 8511–8520. doi: 10.1007/s11356-013-1787-6

Le Bihanic, F., Sommard, V., Perrine de, L., Pichon, A., Grasset, J., Berrada, S., et al. (2015). Environmental concentrations of benz[a]anthracene induce developmental defects and DNA damage and impair photomotor response in Japanese medaka larvae. *Ecotoxicol. Environ. Saf.* 113, 321–328. doi: 10.1016/j.ecoenv.2014.12.011

Lee, H., Shim, W. J., and Kwon, J.-H. (2014). Sorption capacity of plastic debris for hydrophobic organic chemicals. *Sci. Total Environ.* 470–471, 1545–1552. doi: 10.1016/j.scitotenv.2013.08.023

Legradi, J. B., Di Paolo, C., Kraak, M. H. S., van der Geest, H. G., Schymanski, E. L., Williams, A. J., et al. (2018). An ecotoxicological view on neurotoxicity assessment. *Environ. Sci. Eur.* 30:46. doi: 10.1186/s12302-018-0173-x

Lithner, D., Larsson, Å., and Dave, G. (2011). Environmental and health hazard ranking and assessment of plastic polymers based on chemical composition. *Sci. Total Environ.* 409, 3309–3324. doi: 10.1016/j.scitotenv.2011.04.038

Liu, T., Pan, L., Jin, Q., and Cai, Y. (2015). Differential gene expression analysis of benzo(a)pyrene toxicity in the clam, *Ruditapes philippinarum*. *Ecotoxicol. Environ. Saf.* 115, 126–136. doi: 10.1016/j.ecoenv.2015.02.007

Lohmann, R. (2017). Microplastics are not important for the cycling and bioaccumulation of organic pollutants in the oceans—but should microplastics be considered POPs themselves? *Integ. Environ. Assess. Manag.* 13, 460–465. doi: 10.1002/ieam.1914

Mato, Y., Isobe, T., Takada, H., Kanehiro, H., Ohtake, C., and Kaminuma, T. (2001). Plastic resin pellets as a transport medium for toxic chemicals in the marine environment. *Environ. Sci. Technol.* 35, 318–324. doi: 10.1021/es0010498

Nagel, R. (2002). DarT: The embryo test with the zebrafish *Danio rerio* - a general model in ecotoxicology and toxicology. *ALTEX* 19, 38–48. Available online at: http://www.altex.ch/resources/altex_2002_Supp_1_38_48_Nagel.pdf

Nerland, I. L., Halsband, C., Allan, I., and Thomas, K. V. (2014). *Microplastics in Marine Environments: Occurrence, Distribution and Effects*. Oslo: NIVA. Available online at: https://www.miljodirektoratet.no/globalassets/publikasjoner/M319/M319.pdf

O'Donovan, S., Mestre, N. C., Abel, S., Fonseca, T. G., Carteny, C. C., Cormier, B., et al. (2018). Ecotoxicological effects of chemical contaminants adsorbed to microplastics in the clam *Scrobicularia plana*. *Front. Mar. Sci.* 5:143. doi: 10.3389/fmars.2018.00143

OECD (1992). "OECD guidelines for the testing of chemicals," in *Section 2: Effects on Biotic Systems OECD Test Guideline 203: Fish, Acute toxicity Test* (Paris: OECD publishing). Available online at: https://www.oecd-ilibrary.org/environment/test-no-203-fish-acute-toxicity-test_9789264069961-en

OECD (2013). "OECD Guidelines for the Testing of Chemicals," in *Section 2: Effects on Biotic Systems Test No 236: Fish Embryo Acute Toxicity Test* (Paris: OECD publishing). Available online at: https://www.oecd-ilibrary.org/environment/test-no-236-fish-embryo-acute-toxicity-fet-test_9789264203709-en

Padilla, S., Cowden, J., Hinton, D. E., Yuen, B., Law, S., Kullman, S. W., et al. (2009). Use of medaka in toxicity testing. *Curr. Protoc. Toxicol.* 39, 1.10.11–11.10.36. doi: 10.1002/0471140856.tx0110s39

Paredes, E., Perez, S., Rodil, R., Quintana, J. B., and Beiras, R. (2014). Ecotoxicological evaluation of four UV filters using marine organisms from different trophic levels *Isochrysis galbana*, *Mytilus galloprovincialis*, *Paracentrotus lividus*, and *Siriella armata*. *Chemosphere* 104, 44–50. doi: 10.1016/j.chemosphere.2013.10.053

Paul, A. G., Jones, K. C., and Sweetman, A. J. (2009). A first global production, emission, and environmental inventory for perfluorooctane sulfonate. *Environ. Sci. Technol.* 43, 386–392. doi: 10.1021/es802216n

Peng, J., Wang, J., and Cai, L. (2017). Current understanding of microplastics in the environment: Occurrence, fate, risks, and what we should do. *Integr. Environ. Asses.* 13, 476–482. doi: 10.1002/ieam.1912

Perrichon, P., Le Bihanic, F., Bustamante, P., Le Menach, K., Budzinski, H., Cachot, J., et al. (2014). Influence of sediment composition on PAH toxicity using zebrafish (*Danio rerio*) and Japanese medaka (*Oryzias latipes*) embryo-larval assays. *Environ. Sci. Pollut. Res.* 21, 13703–13719. doi: 10.1007/s11356-014-3502-7

Pfaffl, M. W. (2001). A new mathematical model for relative quantification in real-time RT-PCR. *Nucleic Acids Res.* 29, 2002–2007. doi: 10.1093/nar/29.9.e45

Pfaffl, M. W., Horgan, G. W., and Dempfle, L. (2002). Relative expression software tool (REST (c)) for group-wise comparison and statistical analysis of relative expression results in real-time PCR. *Nucleic Acids Res.* 30:10. doi: 10.1093/nar/30.9.e36

Pfaffl, M. W., Tichopad, A., Prgomet, C., and Neuvians, T. P. (2004). Determination of stable housekeeping genes, differentially regulated target genes and sample integrity: BestKeeper - excel-based tool using pair-wise correlations. *Biotechnol. Lett.* 26, 509–515. doi: 10.1023/B:BILE.0000019559.84305.47

Pittura, L., Avio, C. G., Giuliani, M. E., d'Errico, G., Keiter, S. H., Cormier, B., et al. (2018). Microplastics as vehicles of environmental PAHs to marine organisms: combined chemical and physical hazards to the mediterranean mussels, *Mytilus galloprovincialis*. *Front. Mar. Sci.* 5:103. doi: 10.3389/fmars.2018.00103

Rios, L. M., Moore, C., and Jones, P. R. (2007). Persistent organic pollutants carried by synthetic polymers in the ocean environment. *Mar. Pollut. Bull.* 54, 1230–1237. doi: 10.1016/j.marpolbul.2007.03.022

Rochman, C. M., Hoh, E., Hentschel, B. T., and Kaye, S. (2013a). Long-term field measurement of sorption of organic contaminants to five types of plastic pellets: implications for plastic marine debris. *Environ. Sci. Technol.* 47, 1646–1654. doi: 10.1021/es303700s

Rochman, C. M., Hoh, E., Kurobe, T., and Teh, S. J. (2013b). Ingested plastic transfers hazardous chemicals to fish and induces hepatic stress. *Sci. Rep.* 3:3263. doi: 10.1038/srep03263

Rodríguez-Fuentes, G., Sandoval-Gío, J. J., Arroyo-Silva, A., Noreña-Barroso, E., Escalante-Herrera, K. S., and Olvera-Espinosa, F. (2015). Evaluation of the estrogenic and oxidative stress effects of the UV filter 3-benzophenone in zebrafish (*Danio rerio*) eleuthero-embryos. *Ecotoxicol. Environ. Saf.* 115, 14–18. doi: 10.1016/j.ecoenv.2015.01.033

Schneider, C. A., Rasband, W. S., and Eliceiri, K. W. (2012). NIH Image to ImageJ: 25 years of image analysis. *Nat. Methods* 9:671. doi: 10.1038/nmeth.2089

Schönlau, C., Larsson, M., Lam, M. M., Engwall, M., Giesy, J. P., Rochman, C., et al. (2019). Aryl hydrocarbon receptor-mediated potencies in field-deployed plastics vary by type of polymer. *Environ. Sci. Pollut. Res.* 26, 9079–9088. doi: 10.1007/s11356-019-04281-4

Shi, X., Du, Y., Lam, P. K., Wu, R. S., and Zhou, B. (2008). Developmental toxicity and alteration of gene expression in zebrafish embryos exposed to PFOS. *Toxicol. Appl. Pharmacol.* 230, 23–32. doi: 10.1016/j.taap.2008.01.043

Sieratowicz, A., Kaiser, D., Behr, M., Oetken, M., and Oehlmann, J. (2011). Acute and chronic toxicity of four frequently used UV filter substances for *Desmodesmus subspicatus* and *Daphnia magna*. *J. Environ. Sci. Health A. Tox. Hazard Subst. Environ. Eng.* 46, 1311–1319. doi: 10.1080/10934529.2011.602936

Strähle, U., Scholz, S., Geisler, R., Greiner, P., Hollert, H., Rastegar, S., et al. (2012). Zebrafish embryos as an alternative to animal experiments–a commentary on the definition of the onset of protected life stages in animal welfare regulations. *Reprod. Toxicol.* 33, 128–132. doi: 10.1016/j.reprotox.2011.06.121

Teuten, E. L., Rowland, S. J., Galloway, T. S., and Thompson, R. C. (2007). Potential for plastics to transport hydrophobic contaminants. *Environ. Sci. Technol.* 41, 7759–7764. doi: 10.1021/es071737s

Thompson, R. C., Moore, C. J., vom Saal, F. S., and Swan, S. H. (2009). Plastics, the environment and human health: current consensus and future trends. *Philos. Trans. R. Soc. B. Biol. Sci.* 364, 2153–2166. doi: 10.1098/rstb.2009.0053

Thompson, R. C., Olsen, Y., Mitchell, R. P., Davis, A., Rowland, S. J., John, A. W. G., et al. (2004). Lost at sea: where is all the plastic? *Science* 304, 838–838. doi: 10.1126/science.1094559

Triebskorn, R., Braunbeck, T., Grummt, T., Hanslik, L., Huppertsberg, S., Jekel, M., et al. (2019). Relevance of nano- and microplastics for freshwater ecosystems: a critical review. *TrAC.* 110, 375–392. doi: 10.1016/j.trac.2018.11.023

Vignet, C., Le Menach, K., Lyphout, L., Guionnet, T., Frère, L., Leguay, D., et al. (2014). Chronic dietary exposure to pyrolytic and petrogenic mixtures of PAHs causes physiological disruption in zebrafish—part II: behavior. *Environ. Sci. Pollut. R.* 21, 13818–13832. doi: 10.1007/s11356-014-2762-6

Wang, F., Shih, K. M., and Li, X. Y. (2015). The partition behavior of perfluorooctanesulfonate (PFOS) and perfluorooctanesulfonamide (FOSA) on microplastics. *Chemosphere* 119, 841–847. doi: 10.1016/j.chemosphere.2014.08.047

Wang, J., Wang, X., Xiong, C., Liu, J., Hu, B., and Zheng, L. (2015). Chronic bisphenol A exposure alters behaviors of zebrafish (*Danio rerio*). *Environ. Pollut.* 206, 275–281. doi: 10.1016/j.envpol.2015.07.015

Weigt, S., Huebler, N., Strecker, R., Braunbeck, T., and Broschard, T. H. (2011). Zebrafish (*Danio rerio*) embryos as a model for testing proteratogens. *Toxicology* 281, 25–36. doi: 10.1016/j.tox.2011.01.004

Westerfield, C. (2007). *The Zebrafish Book: A Guid for the Labortary Use of Zebrafish (Danio rerio)*. Eugene, OR: University of Oregon Press.

Yamashita, N., Kannan, K., Taniyasu, S., Horii, Y., Petrick, G., and Gamo, T. (2005). A global survey of perfluorinated acids in oceans. *Mar. Pollut. Bull.* 51, 658–668. doi: 10.1016/j.marpolbul.2005.04.026

Ziccardi, L. M., Edgington, A., Hentz, K., Kulacki, K. J., and Driscoll, S. K. (2016). Microplastics as vectors for bioaccumulation of hydrophobic organic chemicals in the marine environment: a state-of-the-science review. *Environ. Toxicol. Chem.* 35, 1667–1676. doi: 10.1002/etc.3461

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Copyright © 2019 Cormier, Batel, Cachot, Bégout, Braunbeck, Cousin and Keiter. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.

Supplementary Material

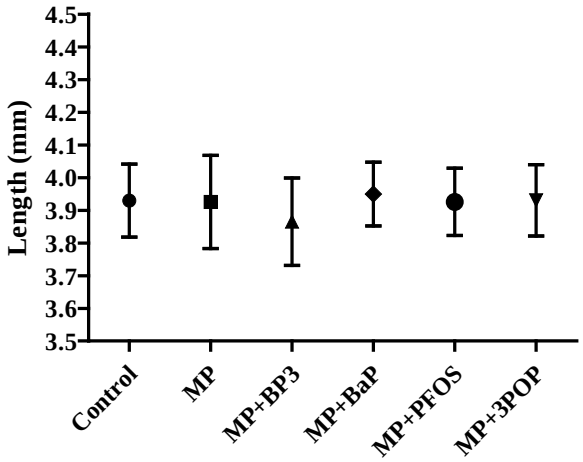


FIGURE S1 | Larvae standard length (mm) measured at 5 dpf in zebrafish (Danio rerio) exposed to a concentration of 10 mg/L of microplastics (MP) and microplastics spiked with oxybenzone (MP + BP3), benzo[a]pyrene (MP + BaP), perfluorooctane sulfonate (MP + PFOS) as well as a mixture of the 3 pollutants (MP + 3POPs) and the control. Data shown as mean ± SD, n=24 per condition. ANOVA F(5, 133)=2.1581, p=0.062.

Table S1. Sequences of primers used for qPCR experiment with associated amplicon size and efficiency (mean ± SEM).

Gene	Forward primer (5' → 3')	Reverse primer (5' → 3')	Amplicon size	Efficiency %
cyp1a	GACAGGCGCTCCTAAAACAG	CTGAACGCCAGACTCTTTCC	93	98.6 ± 2.7
actb1	CCCAGACATCAGGGAGTGAT	CACAATACCGTGCTCAATGG	123	95.3 ± 2.3
g6pd	GTCCCGAAAGGCTCCACTC	CCTCCGCTTTCCTCTC	124	95.8 ± 2.2
rpl13a	GGACTGTAAGAGGTATGCTTCCA	ACTTCCAGCCAACTTCATGG	200	97.5 ± 1.2

Article 3

Chronic feeding exposure of pristine microplastics spiked with model pollutants on zebrafish (*Danio rerio*)

Bettie Cormier, Mathieu Cabar, Sarah Zapata, Mélanie Blanc, Maria Larsson, Florian Dubocq, Leo Yeung, Christelle Clérandeau, Florane Le Bihanic, Bénédicte Morin, Steffen.H Keiter, Jérôme Cachot, Marie-Laure Bégout, Xavier Cousin.

Chronic feeding exposure of pristine microplastics spiked with model pollutants on zebrafish (*Danio rerio*)

Bettie Cormier[1,2], Mathieu Cabar[3], Sarah Zapata[1], Mélanie Blanc[2], Maria Larsson[2], Florian Dubocq[2], Leo Yeung[2], Christelle Clérandeau[1], Florane Le Bihanic[1], Bénédicte Morin[1], Steffen.H Keiter[2], Jérôme Cachot[1], Marie-Laure Bégout[3], Xavier Cousin[3,4,5]

[1] Bordeaux University, EPOC, UMR CNRS 5805, Avenue des Facultés, 33400 Talence, France

[2] Man-Technology-Environment Research Centre, School of Science and Technology, Örebro University, Fakultetsgatan 1, 701 82 Örebro, Sweden

[3] Ifremer, Univ. Montpellier, CNRS, IRD, MARBEC, 34250 Palavas-les-flots, France

[4] Université Paris-Saclay, INRAE, AgroParisTech, GABI, 78350, Jouy-en-Josas, France

[5] IFREMER, L3AS, UMR MARBEC, F-34250 Palavas, France.

Keywords: chronic feeding experiments, artificial microplastics, zebrafish, growth alteration, reprotoxic effects, larval hyperactivity

Abstract

Microplastics (MPs) can induce toxic effects in aquatic organisms, and, MPs are suspected to act as vector for organic pollutants to biota. The aim of the present study was to study polyethylene (PE) and polyvinyl chloride (PVC) as “Trojan Horse” for three model pollutants, perfluorooctane sulfonic acid (PFOS), benzo[a]pyrene (BaP) and benzophenone-3 (BP3), toward zebrafish (*Danio rerio*), on the basis of a dietary exposure. Toxic effects on adult zebrafish were evaluated as the growth, the reproduction and other biochemical biomarkers. Alongside adult zebrafish, the survival, growth, and swimming activity of offspring F1 were monitored. The accumulation of compound was demonstrated after an exposure of 4 months to MPs spiked with PFOS, while fish exposed to BaP and BP3 did not shown any contamination. Female fish growth was altered for all PE conditions expressed by a decrease in terms of weight and length. At 3 months, reproduction success was negatively affected for both MPs spiked with BP3, as well as all PVC conditions. The offspring F1 unfed demonstrated a higher mortality rate for both pristine MPs, PE and PVC, as well as PE and PVC spiked with PFOS and BP3. Also, hyperactivity in larvae from zebrafish exposed to PVC spiked with BP3 was observed. For the selected combination of MPs particles and contaminants, dietary exposure demonstrated the vectorization of pollutants through the ingestion of MPs, with diverse toxic effects, specific to the polymer type or to the compound.

Introduction

Production of plastic has increased constantly in recent decades reaching almost 350 million tons in 2017 (Geyer et al., 2017; PlasticsEurope, 2018). Despite improvements in plastic waste management in some countries effective means of disposal are still not widely available. Manufactured plastic debris ends up in the oceans, eventually showing up as macroplastics (> 5 mm) or microplastics (MPs), i.e. particles between 1 µm and 5 mm in size (Jambeck et al., 2015; Thompson et al., 2009). MPs consist of either plastic particles designed and produced for specific purpose (de Sá et al., 2018; Ogata et al., 2009) or small plastic fragments derived from the breakdown of larger pieces (Anbumani and Kakkar, 2018; Derraik, 2002; Li et al., 2018; Thompson et al., 2004).

Polymers are considered biologically inert and thus non-toxic. However, the production of plastic products uses additives to improve specific characteristics. It has been demonstrated that these additives can be toxic (Barnes et al., 2009; Lithner et al., 2011). In addition to these intrinsic chemicals, MPs particles have been shown to sorb significant amounts of persistent organic pollutants from the surrounding environment which may be of great concern because of their persistence, bioaccumulation and toxicity (Barnes et al., 2009; Koelmans et al., 2016; Mato et al., 2001; Rios et al., 2007). Weathering processes as well as fragmentation of MPs, leads to smaller particles and may increase the reactive specific surface area of particles and further facilitate the sorption of such pollutants (Koelmans, 2015; Lee et al., 2014; Teuten et al., 2007). The importance of the role of MPs in transport of POPs in biota is still controversial (Bakir et al., 2016; Koelmans et al., 2016; Lohmann, 2017).

In addition, given their persistence in the aquatic environment, MPs are globally distributed in various environments worldwide including some far from source areas (Bogdal et al., 2013; Corsolini, 2009; Rigét et al., 2016). MPs of both types are considered a potential threat for aquatic ecosystems (Cole et al., 2011; Thompson et al., 2009). Due to their size at a microscale, MPs can be ingested by organisms, and their transfer into food webs has been reported in laboratory studies (Farrell and Nelson, 2013; Pannetier et al., 2020; Setälä et al., 2014). After ingestion, MPs can cause a variety of toxic effects to aquatic organisms, such as physical effects or chemical effects. Clogging of the digestive tract (Cole et al., 2011), ulcerative lesions or gastric rupture (Law and Thompson, 2014) and intestinal alterations (Pedà et al., 2016) are some of the physical alterations observed following exposure to MPs. The ingestion of MPs may cause endocrine disruption and hepatic stress including CYP1A expression, oxidative stress, changes in metabolic parameters, decrease of enzyme activity, and cellular necrosis (Law

and Thompson, 2014; Mazurais et al., 2015; Rochman, Browne, et al., 2013; Rochman, Hoh, Kurobe, et al., 2013; Rochman, Kurobe, et al., 2014; Teuten et al., 2007). The ingestion of MPs has been demonstrated to induce toxic effects, and may compromise growth and organism development, dynamic reproduction and overall survival of a population.

Under both laboratory and field conditions, different classes of organic pollutants such as per- and polyfluorinated compounds, polycyclic aromatic hydrocarbons, polychlorinated biphenyls, pesticides and personal care products adsorb to different MPs materials (Rochman, Hoh, Hentschel, et al., 2013; Ziccardi et al., 2016). In the aquatic environment POPs are commonly found in the surface microlayer, where low-density MPs also abundant (Ng and Obbard, 2006; Rios et al., 2007; Teuten et al., 2007) or in sediments, in contact with high-density MPs such as polyvinyl chloride (PVC), or low-density MPs, whose density increases with aging or biofouling. MPs are hypothesized to act as 'Trojan Horse' to vectorize a wide range of pollutants in the marine environment (Andrady, 2011; Koelmans et al., 2014). Among POPs, perfluoroalkyl acids (PFAAs), polycyclic aromatic hydrocarbons (PAHs) and personal care products (PCPs) are three families composed of numerous chemicals found in the aquatic environment. In the present study, three different organic pollutants were selected representing these different chemical families, perfluorooctane sulfonic acid (PFOS); benzo[a]pyrene (BaP) and benzophenone-3 (BP3). These three compounds were chosen because they have a wide variety of properties, which will be described thereafter.

Given its wide distribution, PFOS has attracted the most attention of all perfluorinated substances, since it represents the most commonly detected perfluorinated alkylated compound in the aquatic environment (Keiter et al., 2016). PFOS has been found in water, sediment, sludge and soil (Giesy and Kannan, 2002). The volume of PFOS and its precursors in ocean surface waters has been estimated at between 235 and 1,770 metric tons (Paul et al., 2009). PFOS is a persistent fluorinated pollutant that does not photolyse, hydrolyze or biodegrade under any environmental conditions and can survive wide transportation across all environmental media (aquatic, air and terrestrial). While PFOS is known to enter the aquatic environment, only a few previous studies investigated the sorption behavior of PFOS on MPs. The sorption of PFOS onto MPs has been only investigated in 2015 by Wang, Shih, and collaboratos (Wang, Shih, et al., 2015) who studied the sorption behavior of PFOS and FOSA (perfluorooctane sulfonamide, a PFOS precursor) on three different MPs types, including polyethylene. They demonstrated linear sorption mechanisms and a difference of sorption rate depending on the type of polymer (Wang, Shih, et al., 2015). PFOS is also known to be toxic and bioaccumulative in vertebrates

and showed a broad range of toxic effects (Giesy and Kannan, 2002; Huang et al., 2010). PFOS and its precursors were added to the list of the Stockholm Convention on Persistent Organic Pollutants in 2010 (Paul et al., 2009).

PAHs are formed by incomplete combustion of organic material (Collins et al., 1991) and the main sources are derived from fossil fuel combustion, waste incineration and oil spills. In the environment, BaP is ubiquitously distributed, including coastal and offshore systems (Antunes et al., 2013; Liu et al., 2015). Benzo[a]pyrene is one of the PAHs known being pro-carcinogenic, as well as being one of the most toxic PAHs. It has been classified by the International Agency for Research on Cancer as a human carcinogen (Liu et al., 2015). BaP is used as a model compound to investigate the metabolic pathways of PAHs as inducers of aryl hydrocarbon receptor (AhR) metabolization pathways. It is also known that PAHs, including BaP, adsorb at high rates to different types of MPs in seawater, particularly polyethylene (Rochman, Browne, et al., 2013; Schönlau et al., 2019; Ziccardi et al., 2016). Furthermore, BaP adsorbed to MPs has been documented to be transferred via trophic transfer to biota (Batel et al., 2018; Batel et al., 2016; Bour et al., 2018; O'Donovan et al., 2018; Pittura et al., 2018).

Oxybenzone also known as benzophenone-3, is commonly used as a UV-filter in cosmetics such as shampoos and conditioners, body fragrances and sunscreens. It is also used as an additive in plastics, to prevent UV degradation. Due to its extensive use, BP3 is considered an “emerging environmental contaminants of concern” by the US-EPA. The run-off of this UV filter leads to massive contamination of aquatic environments (Gago-Ferrero et al., 2011). The presence of BP3 in aquatic systems has encouraged the scientific community to evaluate its toxicity to aquatic organisms. BP3 acts as an endocrine disrupting chemical, interfering with reproduction and sex hormone signaling, inducing reproducing pathologies, and altering reproductive fitness (Blüthgen et al., 2012; Coronado et al., 2008; Fent et al., 2010). BP3 shows low degradation rates in surface waters (Kim and Choi, 2014) and can bioaccumulate in biota. The bioaccumulation of BP3 can result in a reduction of egg production and hatching, as well as causing and increased vitellogenin level in males, which can be indicative of gender shifts (Coronado et al., Nimrod and Benson, 1998). Beiras et al. (2018) studied toxic effects of polyethylene loaded with BP3 on different marine zooplankton, showing that short-term exposure did not cause toxic effects to any organism, except fish early life stages (ELS) exposed to MPs spiked with BP3, which showed premature hatching and a decrease in hatching rates.

In this study, PE and PVC were used to evaluate MPs as vector for PFOS, BaP and BP3 toward zebrafish, via a chronic feeding exposure. Effects on adult zebrafish were studied, including

growth, reproduction and biochemical biomarkers, along with the survival, growth and swimming behavior of offspring F1, unfed. The present study aims to provide information on the comparison between the toxicity of two polymer types, PE and PVC, and the additional impact from chemicals adsorbed.

Materials and methods

Husbandry and egg production

Zebrafish (*Danio rerio*), TU strain were bred in 10 L tanks at 27 ± 1 °C with a cycle of 14/10 day/night (7.8 ± 0.2, 480 ± 130 µS, for pH and conductivity, respectively). Fish were fed twice daily *ad libitum* with Inicio + 0.5 mm (BioMar, France) and once freshly hatched brine *Artemia* nauplii. Constant filtering or permanent flow-through conditions guaranteed that ammonia, nitrite, and nitrate were kept below detection limits (5, 0.1 and 140 mg/L, respectively). Zebrafish eggs were collected within hours following spawning induction by light on in the fish room.

Experimental design

The experiment was carried out at IFREMER station, L'Houmeau. Rearing was performed as described previously (Alfonso et al., 2019; Vignet et al., 2014). A total of 2700 zebrafish embryos were placed into 27 tanks (1.5 L), following an equal distribution of eggs from different spawning to avoid genetic bias. 50 eggs obtained with a fertilization rate above 80% were evenly distributed in Petri dishes and randomly assigned to each treatment, including 3 replicates per condition. At 5 days post fertilization (dpf), larvae were transferred to 1 L tanks. At 21 dpf, they were transferred from 1 L tanks to 3 L tubes located in 10 L tanks in the rearing system. At 26 dpf, tubes were gently poured in 10 L tanks.

Fish were exposed to nine different treatment diets: control (CTRL), pristine polyethylene (PE), pristine polyvinyl chloride (PVC), spiked MPs with perfluorooctane sulfonate (PE-PFOS and PVC-PFOS), benzo[a]pyrene (PE-BaP and PVC-BaP) and oxybenzone (PE-BP3 and PVC-BP3). CTRL condition consisted of fish fed with artificial food only. Due to range of sizes of MPs, fish exposed to polyethylene (PE) and spiked PE were fed from 5 day post fertilization (dpf), while fish exposed to polyvinyl chloride (PVC) and contaminated PVC were fed from 40 dpf. All conditions were exposed until 6 months. Fish were fed twice a day by hand with

artificial food prepared as described above, supplied in two meals. An additional meal of freshly hatched *Artemia nauplii* was given once a day. At the end of the exposure, the offspring generation F1 was produced as described above but then raised without feeding. For practical and exposure management reasons, exposures to PE (pristine and spiked) and PVC (pristine and spiked) were performed sequentially, with both sets of exposures including their respective CTRL replicates.

Fish rearing and exposure were performed at the laboratory of Ifremer Research Centre, L'Houmeau, which is authorized to use and breed laboratory animals (EEA 171901). The project was performed under the approval of the Animal Care Committee of Poitou-Charentes # 84 COMETHEA (France) with project authorization number APAFIS 10883, following the recommendations of Directive 2010/63/EU.

Microplastics and diet preparation

Polyethylene (PE; order no. MPP-635 G) with a size range of 11-13 µm and a density of 0.96 kg/m^3 purchased from Micro Powders (New York, USA). Polyvinyl chloride (PVC) with a size range of 125-250 µm, purchased from Fainplast (Ascoli Piceno AP, Italia). Benzo[a]pyrene (BaP; CAS 50-32-8; purity ≥ 96%), benzophenone-3 ((2-hydroxy-4-methoxyphenyl)-phenylmethanone, BP3; CAS 131-57-7; purity ≥ 98%) and PFOS (1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-heptadecafluorooctane-1-sulfonic acid; CAS 2785-37-3; purity ≥ 98%) were purchased from Sigma Aldrich (Stockholm, SE). All other chemicals and reagents were purchased at the highest purity available from Sigma-Aldrich, unless stated otherwise.

PE and PVC were spiked with PFOS, BaP and BP3 following protocols described in Cormier et al., (2019), with minor modification for the PVC preparation. For the sorption of PFOS, 200 g/L of PVC was mixed with 200 mg/L of PFOS in 1 L polypropylene bottle (Lamaplast; Sesto San Giovanni, Italy), while for BaP and BP3, 125 g/L of PVC was added in 400 mL glass bottles (Thermo Scientific, Lund, SE) mixed with 5000 µg/L and 20 µg/L of BaP and BP3, respectively. Final concentrations of compounds on MPs are indicated in Table 1.

Table 1: Concentrations of PFOS, BaP (µg/g) and BP3 (ng/g) extracted from PE (11-13 µm) and PVC (125-500 µm). Mean ± SD, n=3.

	PFOS	**BaP**	**BP3**
PE (11-13 µm)	70.22 ± 12.41	16.87 ± 0.22	106.00 ± 6.50
PVC (125-250 µm)	159.54 ± 40.84	11.50 ± 1.35	107.00 ± 1.50

Pellet food suited to the size of fish, SDS 100-300 µm was purchased from DIETEX (France) and Inicio 500 µm from BioMar (France). To obtain a homogenous distribution of MPs in food, MPs combined with food pellets at a final ratio of 1% ww in glass tubes and mixed with a 4 mm bottlebrush fixed on a drill for 3 minutes. Afterwards, to reinforce binding of MPs to pellets, flaxseed oil was added (5µL per gram of food) and mixed for an additional 3 minutes. To allow MPs to stick to pellets, PVC was only spiked in Inicio 500 diet. This explains why exposure started with 2 month-old fish.

Chemical analysis

Fish exposed (F0 generation) to MPs were sampled at 1, 2 and 4.5 months to determine PFOS, BaP and BP3 concentrations in fish. Pooled fish (1 and 2 months) and individual fish (4.5 months) have been used. F1 embryos have been collected at 3-5 hpf to quantify the transfer of pollutants from genitors to offspring as pools of embryos or mass or spawns of at least 100 embryos.

PFOS analysis

To analyze PFOS in fish, the ion pair extraction has been performed, with alkaline digestion (So et al., 2006; Yoo et al., 2009). Frozen fish were grinded in a mortar with the addition of 20 mM of NaOH, sonicate for 30 min and placed on a horizontal shaker overnight (16 h). The pH was neutralized with hydrochloric acid, and 0.5 M of tetrabutylammonium sulfate as well as 10 ng of mass-labelled 13C4 PFOS (Wellington Laboratories Inc., Canada) were added. Samples were then were sonicated for 15 minutes. Extraction was performed three times with 2 mL methyl tert-butyl ether with 15 min of sonication followed by centrifugation 10 min at 8000 rpm. Extracts were evaporated down to dryness with N2 and then, methanol was used to dissolve extracts, 2 ng of mass-labelled 13C8 PFOS (Wellington Laboratories Inc., Canada) has been added. Chemical analysis was performed as described in Cormier et al. (2019).

BaP analysis

Frozen fish were analyzed using the homogenization in a mortar. Anhydrous sodium sulfate in a 1:5 ratio was added to eliminate water in the sample, as well as 1 ng of BaP d12. Deactivated mini-silica columns were prepared with 10 % of deactivated silica and anhydrous sodium sulfate, washed with n-hexane:dichloromethane (hex:dcm, 3:1, v/v). Samples were eluted with 8 mL of hex:dcm (1:1, v/v), and reduced to 200 µL before a transfer to toluene (reduced to 500 µL), with the addition of 2 ng of perylene d12. Chemical analysis was performed as previously described in Cormier et al., 2019.

BP3 analysis

Oxybenzone is a compound easily metabolized in organisms (Blüthgen et al., 2012; Coronado et al., 2008), leading to the formation of metabolites. Benzophenone-1 (BP1) has been reported to be one metabolite of BP3, as detected in the urine of rats and humans (Díaz-Cruz et al., 2008; Kunisue et al., 2012) as well as in zebrafish (Blüthgen et al., 2012). To determine the concentration of BP3 and BP1, frozen fish were ground in a mortar with 1 mL of acetonitrile, followed by 15 min of sonication, 15 min of horizontal shaking and 5 min on a vortex. Samples were then centrifuged for 15 min at 3900 rpm and passed through a filter (GHP acrodisc 13 mm). Filtrates were stored in a freezer (- 20 °C) overnight (16 h) to precipitate proteins. After the precipitation, samples were centrifuged and the surnatant was evaporated down to 100 µL with N2 gas stream. 500 ng of BP3 d10 was added as standard and an external quantification was used with a calibration curve of BP3 and BP1 (0.04 to 80 pg/µL). Analysis was performed using Acquity UPLC I-class coupled to a quadrupole time-of-flight mass spectrometer (Xevo G2-XS QToF MS, Waters Corporation, Milford, USA) using Acquity UPLC BEH C18 column (L 100 mm * ID 2.1 mm, particles of 1.7 µm) with a set temperature of 50°C and an injection volume of 10 µL. Sample temperature was set at 10 °C to avoid compound degradation during analysis. Analysis was performed with electrospray ionization in positive mode, with an MSE method between m/z 50 and m/z 600. The mobile phase was composed of 0.1 % formic acid in water for the aqueous phase and 0.1 % formic acid in acetonitrile for the organic phase. BP3 and BP1 concentrations were calculated using BP-d10 as internal standard. Calibration curves were plotted for a concentration of BP1 and BP3 of 0.4; 2; 8; 20; 40 and 80 pg/µL with an internal standard concentration of 10 pg/µL. Every calibration curve concentration has been injected in triplicate. Limits of quantifications were calculated for BP3 and BP1 as 0.031 pg/µL and 0.063 pg/µL respectively. Procedure blanks were included in all batches.

Biomarkers of toxicity in the F0 generation

Survival and growth

During exposure, dead larvae or fish were monitored daily. Individual standard length (mm) and body weight (g) were measured after anesthesia with benzocaine (50 mg/L, Sigma Aldrich, France) as described previously (Vignet et al., 2014). In addition, some fish were euthanized with benzocaine (500 mg/L) at different time points for biomarkers or chemical analyses. For this purpose, and depending on downstream analyses, entire fish or dissected out tissues were immediately frozen in liquid nitrogen and stored until analysis.

Reproduction

Reproduction was monitored starting at 90 dpf and for approximately one month. For this purpose, in late afternoon couples were placed in spawning boxes (AquaSchwarz, Germany) in early evening; five pairs were set up per condition per attempt. Boxes were inspected the following morning, and the presence of eggs scored as a successful attempt. In this case, eggs were collected and sorted to count the total number of eggs and to calculate fertilization rates. The general rule was to set up five pairs per condition per attempt, using fish from each replicate sequentially. Eggs were kept and placed in Petri dishes filled with 30 mL of the isotonic mixture E3 (1 L: 17.2 g NaCl, 0.76 g HCl, 2.9 g CaCl2.2H2O, 4.9 MgSO4.7 H2O) and methylene blue (250 µL/L). The hatching rate and survival of F1 without feeding were monitored to evaluate their available energy resources, with a daily medium exchange. For control fish, 23 and 53 attempts were performed at the same time as PE and PVC tests, while 30 attempts were performed for fish exposed to PE and spiked PE and 63 attempts were performed for fish exposed to PVC and spiked PVC.

EROD

Zebrafish liver tissue was homogenized in 0.1 M phosphate buffer (pH = 7.7; 0.1 M KCl). The homogenate was then centrifuged at 9000 g at 4 °C for 20 min. The supernatant (fraction S9) was then collected in a clean micro tube and stored at −80 °C before being used for all enzymatic activity measurements.

The protein concentration in the S9 fraction was measured using Bradford's method with bovine serum albumin (BSA) as standard (Bradford, 1976). Spectrophotometric measurements were performed in a Biotek Synergy HT microplate reader, and expressed as mg/mL. EROD activity was determined using the Burke and Mayer method (Burke and Mayer, 1974). The reaction

mixture consisted of a NaH_2PO_4/K_2HPO_4 buffer (0.1 M, pH 7.5), 7-ethoxyresorufin (0.15 mM) and NADPH (5.57 mM). The change in fluorescence was recorded (excitation wavelength 530 nm, emission wavelength 585 nm) and enzyme activity calculated as pmol/min/mg protein using a resorufin standard.

TBARs

The amount of reactive oxygen species (ROS) to evaluate oxidative stress is difficult to measure due to a short half-life, so the measure of thiobarbituric acid reactive substances (TBARS), an oxidative product (Buege and Aust, 1978), was studied. TBARs were measured in zebrafish muscles (4.5 months), and following the colorimetric protocol developed by Buege and Aust (1978) and adapted to a microplate reader as described in Weeks Santos et al. (2019). Results are expressed as nM of TBARs equivalents per mg of protein. Per condition, pooled of 3 males or 3 females have been used to obtain 3 measures per condition.

AChE

AChE was performed on S9 fraction of zebrafish brain (4.5 months), following Ellman et al. (1961), adapted to a microplate reader. Extraction was performed following Lowry et al., 1951, modified as in Weeks Santos et al., 2019. Each well contained 10 µL of S9, 180 µL of 5,5'-dithiobis 0.5 mM in 0.05 M of Tris Buffer (pH 7.4) and 10 µL of acetylcholine iodide. The rate of increase of optical density was measured using a spectrophotometer (Synergy HT, BioTek) at 412 nm. Results are expressed as nmol/min/mg protein. For each condition, pools of 3 males or 3 females were used to obtain 3 measures per condition.

Biomarkers of toxicity in the F1 generation

Larval behavior

Larval behavior was monitored at 120 hpf using larval photomotor response (LPMR) test as described in Vignet et al. (2014). Larvae were individually transferred into one well of a 24-well plate (Krystal 24, opaque wall and clear bottom microplate, Dutscher) and plates were transferred to an enlighten incubator at 28°C in the behavior room. Plates were then successively placed into DanioVisionTM (Noldus, NL) in the dark for 10 minutes of acclimation before the LPMR test which included the following 5-min steps: Light on–1 (LON1, 70 lux), Light off (LOFF, <1 lux) and Light on–2 (LON2, 70 lux) with constant infra-red light maintained during video recording. Distance travelled (cm) over the 5 min of each

period were then calculated using Ethovision (Noldus, NL). Larvae with tracking issues were removed resulting in 16 to 24 larvae analyzed per treatment.

Larval survival

On the F1 generation, a survival experiment was performed using unfed offspring from exposed fish. This aimed to determine whether the exposure of parents to MPs or MPs spiked, could alter the survival of their offspring larvae (F1). The monitoring of F1 larvae survival was performed using eggs collected throughout the 60 attempts, from spawns above 60 % of fertilized eggs. Thirty eggs were collected from each clutch and kept in a Petri dish in 30 mL of E3 medium at 28 °C until the end of the survival, expressed by natural death of embryos.

Statistical analyses

Each exposure condition was repeated three times. Normality of the data was checked using the Shapiro-Wilk test. Analysis of variance was performed followed by Fisher's test compared to control. ANOVA was used for normal data, and for data that did not fulfill normal distribution, the Kruskal-Wallis non-parametric test was performed. Differences in survival were assessed within each diet using Log-rank (Mantel-Cox) test and time at which 50% mortality is reached was calculated using four-parameter logistic regression, both using Prism software (Graphpad). All statistical analyzes were carried out at a 95 % level of significance and values are represented as mean ± SD.

Results

Chemical analysis of fish

Fish exposed to PE particles (11-13 µm), were sampled after 1, 2 and 4.5 months of exposure, while fish exposed to PVC particles (125-250 µm) were sampled after 4 months, since the exposure started at 2 months. Results revealed that PFOS concentration was below detection limits in control fish or exposed to pristine MPs, both PE and PVC. A similar accumulation of PFOS wass observed after 1 month (7.46 ± 3.21 µg/g) and 2 months (8.86 ± 2.41 µg/g) in fish exposed to PE-PFOS. After 4 months of exposure, fish were analyzed according to their sex. The concentration of PFOS in fish was not affected by polymer type. However, the uptake of

PFOS by males (PE 17±0.5; PVC 21±5) was significantly higher than by females (PE 12.5±2.5; PVC 13±2) (Figure 1).

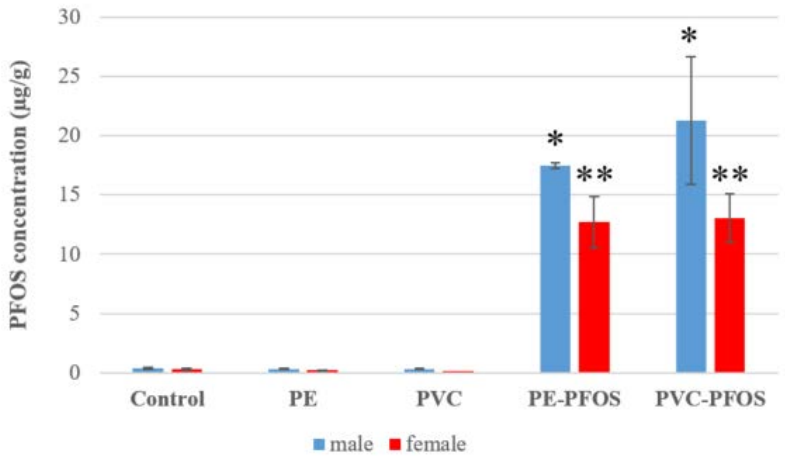


Figure 1: PFOS concentration in zebrafish exposed for 4.5 months to pristine MPs (PE, PVC) or to spiked MPs (PE-PFOS, PVC-PFOS). Stars indicates significant differences between males and females (p<0.05), however, no significant differences were observed between polymer types.

The chemical analysis of fish revealed that BaP, BP3 and BP1 concentrations were below detection limits, and so, no signs of accumulation in any cited treatment group have been demonstrated.

Toxicity in the F0 generation

Survival and growth

No significant mortality has been recorded whatever the condition and so no deviation from the control has been observed.

After 2 months of exposure (PE: 2 month-old and PVC: 4.5 month-old), no significant difference between CTRL and exposed fish was observed for total length or body mass whatever the condition and polymer type. After 4 months of exposure (PE: 4.5 month-old and PVC: 6 month-old), total length of females exposed to PE-BaP and PE-BP3 were smaller than CTRL females ($F_{(5,41)}$=2.452, p=0.049) while for other conditions for females or any conditions for males, there was no difference compared to CTRL. Regarding body mass, there was sporadic difference for males, with PE and PE-PFOS being smaller than CTRL while no difference was observed for other conditions. In the case of females however there was a

significant decrease in body mass for all exposed conditions, for PE ($F_{(5,47)}$=10.101, p<0.001) and PVC ($F_{(6,130)}$=28.3, p<0.001).

Reproduction

Spawns were obtained for all conditions. No difference between CTRL and exposed conditions was observed for either spawn size ($F_{(5,71)}$=1.404, p=0.23 for PE and ($F_{(5,72)}$=1.804, p=0.12 for PVC) or fertilization rates ($F_{(5,71)}$=0.794, p=0.56 for PE and ($F_{(5,72)}$=2.169, p=0.07 for PVC). Proportion of successful attempts was significantly reduced for PE-BP3 (78.6% for CTRL vs. 47.5% for PE-BP3; Fisher's exact test, p<0.05; Fig. 3) while there was no difference with CTRL for other PE conditions. On the contrary, attempt success was significantly reduced for all PVC conditions (50.0% for CTRL vs. 12.1 to 24.2% for PVC or spiked PVC; Fisher's exact test, p<0.05), Figure 2.

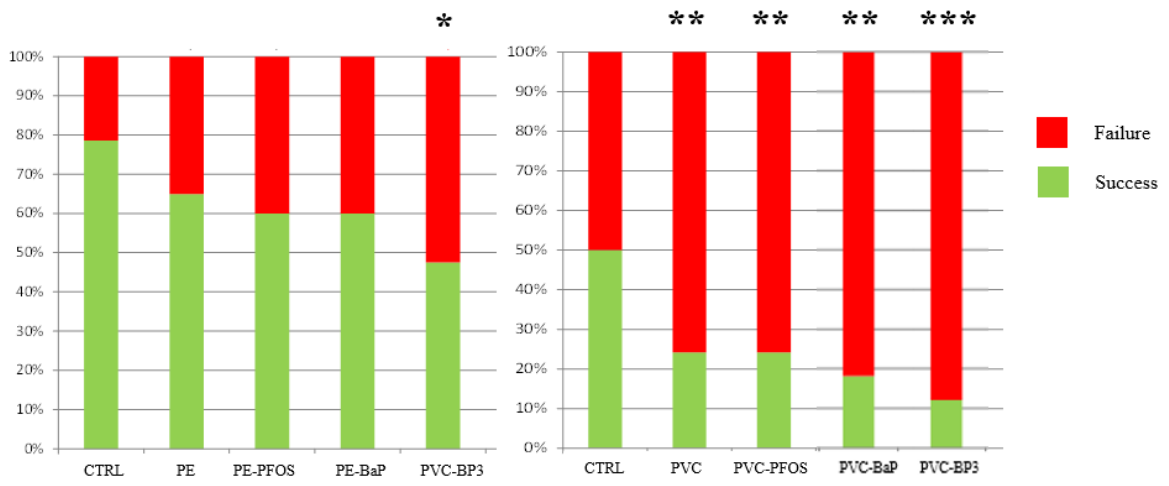


Figure 2: Relative proportions of successful (green) and failed (red) attempts to obtain eggs from fish exposed PE and spiked PE (on the left) and to PVC and PVC spiked (on the right). Stars indicate significant difference with their respective CTRL (Fisher's exact test; *: p<0.05; **: p<0.01; ***: p<0.0001).

EROD, TBARs and AChE activities

EROD activity was measured in fish liver (Figure 3). No significant difference was observed between males and females; hence, both sexes were analyzed together. There was no significant difference of EROD activity in exposed fish compared to CTRL fish.

Oxidative stress levels were evaluated using measurement of TBARs (Figure 4). No significant difference was observed between males and females; hence, both sexes were analyzed together. There was no significant difference in TBARs levels in exposed fish compared to CTRL fish.

AChE activity was measured in fish brains (Figure 5). No significant difference was observed between males and females, so both sexes were analyzed together. There was no significant difference in AChE activity in exposed fish compared to CTRL fish.

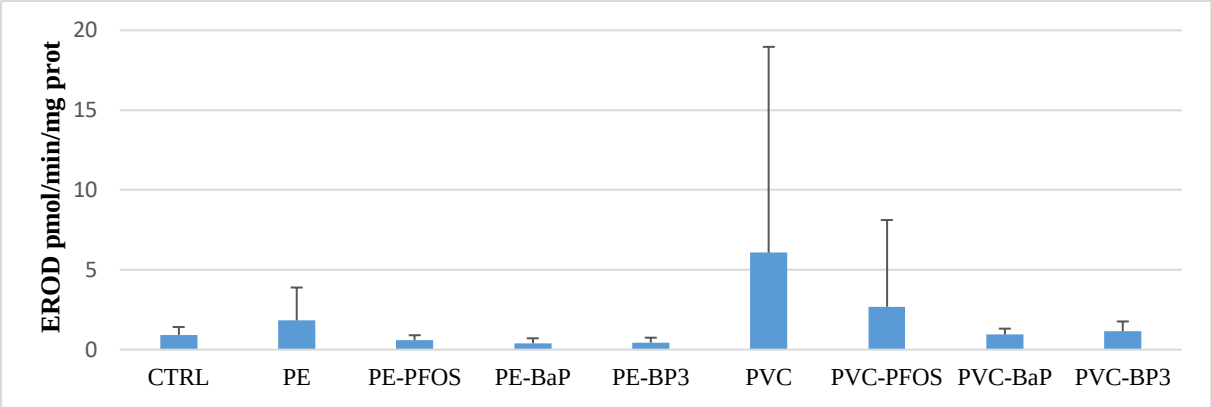


Figure 3: EROD activity, expressed in pmol per minute and per mg of proteins. Mean ± SD, n=6.

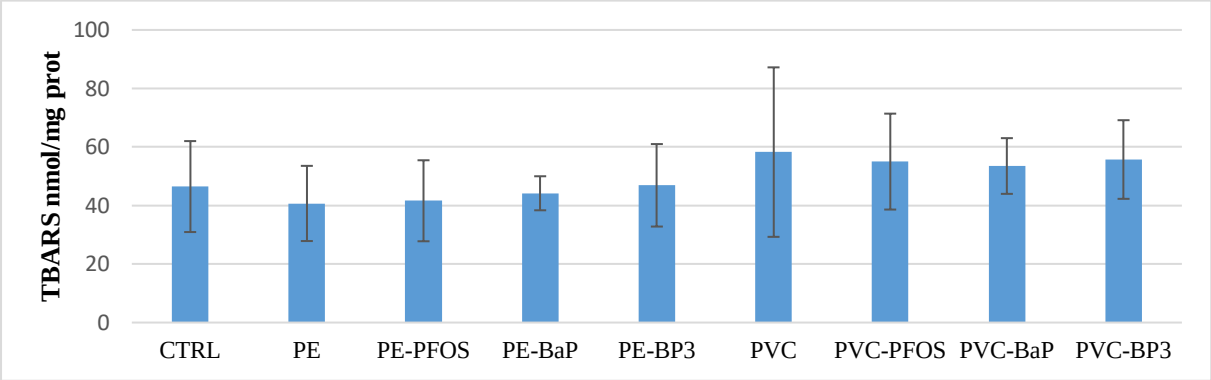


Figure 4: TBARs activity, measured in the different conditions, expressed in nmol per mg of protein. Mean ± SD, n=6.

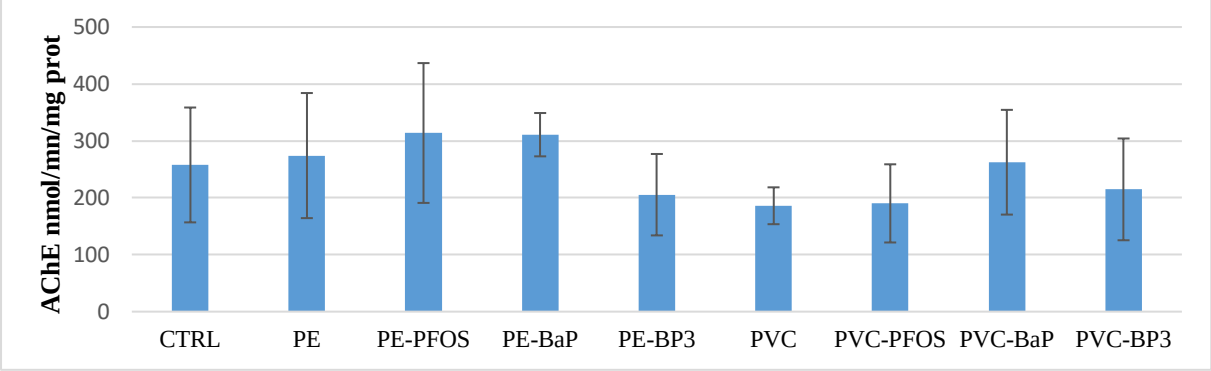


Figure 5: AChE activity, expressed in nmol of AChE per minute and per mg of proteins. Mean ± SD, n=6.

Biomarkers of toxicity in the F1 generation

Larval growth

The analysis of larval individual standard length, measured at 5 dpf did not reveal significant difference between all conditions (Kruskal-Wallis($_{12, 135}$)=16.47, p=0.170; Figure 6).

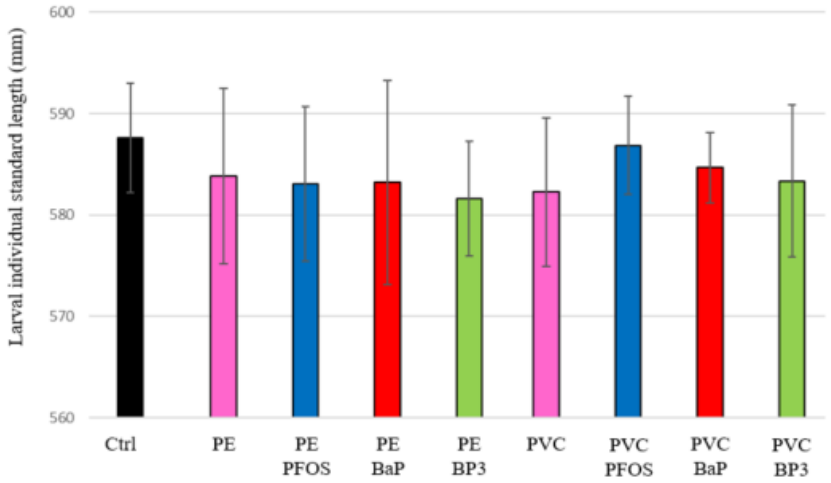


Figure 6: Individual standard length of larvae from each condition, in mm. Mean ± SD, n=15.

Larval behavior

LPMR gave the expected results for larvae from CTRL fish with an increase in distance travelled during LOFF compared to both L=ON. This was also true for larvae from all other conditions (ANOVA F(36, 2326)=3.017, p<0.001). Whatever the PE conditions, there was no difference of activity compared to CTRL within each period (Figure 7).

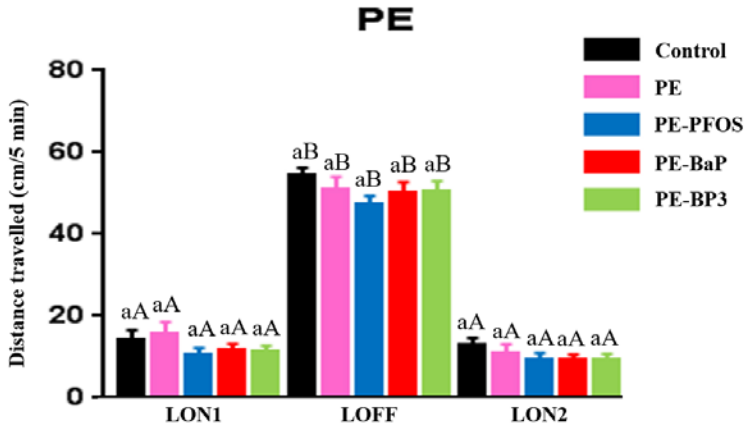


Figure 7: LPMR in 5 d old larvae issued from fish exposed to PE and spiked PE. Distance travelled over 5 min periods. Data are given as means ± SD (n = 50 to 60 larvae per treatment). Different lowercase letters indicate a significant difference between conditions within the same period. Different uppercase letters indicate a significant difference for the same condition between periods (Repeated–measure ANOVA).

In the case of PVC conditions, PVC-BP3 was the only condition differing from others, including CTRL, during LOFF ($p = 0.011$) while there was no difference during LON periods (Figure 8).

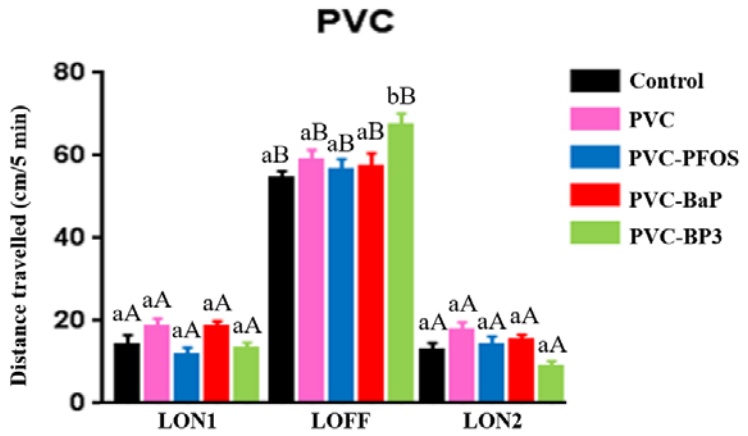


Figure 8: LPMR in 5 d old larvae issued from fish exposed to PVC and spiked PVC. Distance travelled over 5 min periods. Data are given as means ± SD (n = 50 to 60 larvae per treatment). Different lowercase letters indicate a significant difference between conditions within the same period. Different uppercase letters indicate a significant difference for the same condition between periods (Repeated–measure ANOVA).

Larval survival

Larval survival was monitored for 14 dpf on unfed larvae. Survival was first analyzed overall, then the age at which 50% of mortality was reached was calculated. According to survival

analyses, all conditions modified larval survival with the exception of PE-BaP (Chi^2 value=2.98; p=0.08) and PVC-BaP (Chi^2 value<0.01; p=0.98) (Table 2). Because the Log-rank test appeared quite permissive, we further characterized survival curves by calculating the age at which 50% of mortality occurred. This was 11.37 days [95% CI: 11.35-11.39]. Values for PE-BaP, PVC-BaP but also PE-Oxy did not differ significantly from this value. Values for PVC-PFOS and PVC-BP3 were marginally reduced by 4.08 and 3.6 hours respectively. Survival of PE was promoted with 50% of mortality occurring 13.2 hours later than CTRL while it was 12.0 and 13.2 hours earlier for F1 from PE-PFOS and PVC exposed F0 fish respectively

Table 2: Results of the logrank test of larval survival, for all conditions, including chi2, p-value and the significance.

Conditions	***Chi2 (df =1)***	***p-value***	***Significance***	
PE	86.630	< 0.0001	****	**Yes**
PE-PFOS	58.460	< 0.0001	****	**Yes**
PE-BaP	2.981	0.0843	ns	No
PE-BP3	4.012	0.0452	*	**Yes**
PVC	69.210	< 0.0001	****	**Yes**
PVC-PFOS	14.020	0.0002	***	**Yes**
PVC-BaP	0.000	0.9819	ns	No
PVC-BP3	6.107	0.0135	*	**Yes**

Discussion

Number of particles monitored in digestive tract of marine biota is generally low; however, in general, size of MPs monitored is in the higher range of MPs size (de Sà et al., 2018). Egestion of MPs by animals in general and fish in particular is quite fast, nevertheless the presence of microplastics has been observed in a large number of fish species captured in the oceans, seas and freshwater (Alomar et al., 2017; Bessa et al., 2018; Jabeen et al., 2017; Morgana et al., 2018; Pazos et al., 2017). This suggests that number of small MPs ingested by fish in the wild is largely underestimated and that exposure is almost constant over time. In this experiment, we have exposed fish to food pellet spiked with MPs at a 1% w/w. If we consider a particle of

330 µm on each side, which is the lower threshold of particles collected with the commonly used manta trawl, and considering that for an adult the daily portion delivered was 3% of the body mass, this represented between 3.5 and 2.5 particles given per day per individual, for PE and PVC respectively. Therefore, even if it is difficult to make parallel with environmental density of MPs their occurrence in fish digestive tract, exposure conditions used here fell in environmental ranges. These conditions were also comparable with ratio used in previous experiments using similar exposure route [0.2-2%] (Jovanović et al., 2018; Mazurais et al., 2015; Rainieri et al., 2018; Zhu et al., 2020).

Data on environmental concentrations of BP3, BaP and PFOS adsorbed to MPs are either not available or scarce. The present study is part of EPHEMARE project, studying the toxicity of MPs on various aquatic organisms as algae, jellyfish, sea urchin or fish. Therefore, concentrations of chemicals sorbed on MPs were selected to insure the viability of all organisms used in the project and exposed to these concentrations. Therefore, in the present study concentrations of pollutants adsorbed on PE were based on fish toxicity data from the literature, e.g. PFOS (Yamashita et al., 2005); BaP (Weigt et al., 2011) and BP3 (Blüthgen et al., 2012; Paredes et al., 2014). In coastal waters, BP3 has been measured at relatively high concentrations, more than 2000 ng/L, i.e. 2500 times higher than the concentration spiked on MPs and used in the present study (Bratkovics and Sapozhnikova, 2011; Bratkovics et al., 2015). In the case of BaP and PFOS, concentrations used in the present study were higher than detected concentrations in aquatic environment. Despite its high affinity to sediments, BaP can be found in the water column, however, the concentration used, i.e. 170 ng/L, was 5 times higher than concentrations found in seawater (El Nemr and Abd-Allah, 2003; Hong et al., 2016). Concentration of PFOS spiked on MPs and used in the present study was also several orders of magnitudes higher than concentrations found in seawater, since the environmental level of PFOS detected in seawater was in the range of ng/L (Ahrens et al., 2009).

Exposure to MPs spiked with PFOS induced the bioaccumulation of the compound into zebrafish for both polymer types. On the contrary, neither BaP nor BP3 was detected in exposed fish. In both cases, compounds are known to be metabolized (Blüthgen et al., 2012; Dévier et al., 2013; Le Dû-Lacoste et al., 2013). Actual metabolization of both compounds has been demonstrated in zebrafish (Vignet et al., 2014). In zebrafish, BP3 is partly converted in BP1 as demonstrated by exposure to high concentration of BP3 (Blüthgen et al., 2012). BP1 was also monitored but not detected in exposed fish, this may be related to the much lower concentration used in the present experiment.

Importance of MPs as vector for organic pollutants is still under discussion and proposed to be negligible based on modeling (Bakir et al., 2016; Koelmans et al., 2016), however as discussed by Hartmann et al (2017) it is likely that the actual occurrence of MPs in the environment is currently underestimated due to sampling method used and that MPs are not evenly distributed in the environment. It is therefore very likely that, at least in some situations, MPs may act as actual vector for organic pollutants. In addition, many chemicals are used as additives and can therefore be present in plastics since their productions; this is the case for PFOS and BP3. Besides, actual transfer of organic pollutants from MPs to biota has been extensively demonstrated (Batel et al., 2016; Galloway et al., 2017; González-Soto et al., 2019; Koelmans, 2015; O'Donovan et al., 2018; Pittura et al., 2018; Rainieri et al., 2018; Rochman, Hoh, Kurobe and Teh, 2013). Toxicity of MPs, either pristine or spiked has been studied in various organisms. In fish, exposure to MPs has been shown to induce oxidative stress, modification of the metabolism and cellular necrosis (Browne et al., 2013; Rochman, Hoh, Kurobe et al. 2013; Rochman, Kurobe, et al., 2014) but not always (Beiras et al., 2018, Mazurais et al., 2015). Studies reporting toxic effects at tissue level are scarce. Exposure to MPs has been shown to produce intestinal lesions in European sea bass (*Dicentrarchus labrax*) or zebrafish (Lei et al., 2018; Pedà et al., 2016) while in other case no inflammation or tissue lesion were observed in sea bass (Mazurais et al., 2015). Variability in biological response may be due, e.g. to the variety of MPs, exposure routes used, concentrations or exposure durations. Regarding the later, it is to note that concentrations are often high, poorly related to environmental situation, and exposure short. In the study reported here, exposure lasted a minimum of 4.5 month and included several developmental stages, from larvae to adult for PE and from juvenile to adult for PVC. As far as we know this is the longest experimental exposure of fish to MPs. It is of importance to note that growth defects appeared only in females and only after 4 months of exposure which likely explained why this effect has not been observed in similar experiment such as the exposure of Japanese medaka to polystyrene MPs at 0.2% in diet during 2.5 months starting with juveniles even if they observed some disruption of digestive tract morphology (Zhu et al., 2020). It is of interest to note that a similar exposure duration was necessary to observe this phenotype whatever the polymer used indicating that for growth the duration rather than the initiation of exposure is important. It is also noteworthy that the growth defect was observed for all PE and all PVC conditions suggesting that spiked chemicals did not modulate this effect. The fact that growth defects were only observed in females may be due to female specific disruption has this has been reported for some endocrine disruptors (Schneider et al., 2014). Another explanation may be related to sexual dimorphism for energy budget. In several

fish species, mature females have higher food consumption and/or metabolic costs compared with males as this has been shown in e.g. northern pike, walleye, yellow perch, catfish or zebrafish (Beaudouin et al., 2015; Diana, 1983; Rennie et al., 2008; Xie et al., 1998). Therefore disruption of energy budget due to lowered food consumption or an increase in metabolic costs reported for numerous organisms (Besseling et al., 2013; Green et al., 2016; Rist et al., 2016; Sussarellu et al., 2016; Watts et al., 2015; Wright et al., 2013; Yin et al., 2018) and integrated in a tentative adverse outcome pathway (AOP), would have larger consequences on females (Zhu et al., 2020).

Long-term exposures of oyster or Japanese medaka to polystyrene microspheres resulted in reproduction defects (Zhu et al., 2020). In the case of oyster, waterborne exposure lasted 2 months while in the case of Japanese medaka dietary exposure lasted 2.5 month. In the case of oyster, exposure resulted in a decrease in oocyte number and diameter, as well as in a decrease in sperm velocity. Reproduction output was also affected as revealed by a decrease in both D-larval yield and further larval growth (Sussarellu et al., 2016). In the case of Japanese medaka, reproduction output was slightly reduced (approximately -20%) for the highest dose used but only during the last week of exposure (week 10) but not during the previous week (Zhu et al., 2020). Other spawn parameters, i.e. fertilization rate or embryonic endpoints were not modified whatever MPs concentration. In the present study, we monitored several spawn and offspring endpoints. For spawns, in agreement with results obtained in Japanese medaka exposed to polystyrene MPs, we observed no modification of fertilization rate whatever the polymer of the spiked chemical. Regarding the two other variables we monitored they both contribute to reproductive output. We observed no change in spawn size whatever the polymer of the spiked chemical. However we observed an important decrease in spawning success in all PVC conditions, including pristine PVC while for PE, decrease was only significant for PE-BP3 condition. The total eggs number is integrative of both spawning success and spawn size; our results are in agreement with what has been reported for Japanase medaka (Zhu et al., 2020). The use of different polymers and spiked chemicals allowed going further in the analysis of the effect. Firstly, PE did not produce disruption of reproduction and neither spiked PFOS nor BaP was able to trigger additional effect. On the contrary, PE-BP3 was the only PE condition with a slight reduction of spawning success. This suggests that what is seen here is due to BP3. In the case of PVC, results suggest that PVC (or specific additives) itself is able to trigger a decrease in reproduction output. Addition of BP3, in PVC-BP3 condition, was the most reprotoxic condition with a 75% reduction of reproduction output. This is also in agreement

with results obtained for PE-BP3. In order to fully describe consequence of exposure on population recruitment of exposure, some offspring traits have been monitored. Some disruptions have been observed in F1 from exposed fish but they were not major. The slight reduction of time needed to reach 50% mortality, less than 2% for PVC-PFOS and PVC-BP3 and 4 to 5% for PE-PFOS and PVC, may be related to a decrease in the energy initially provided to eggs. This was however not translated in early development since larval growth monitored at 5 dpf was unaffected and larval behavior was not reduced. Regarding this latter trait, the increase in response to LOFF period observed in F1 from PVC-BP3 exposed fish is quite important with an increase of 24% compared to control larvae. Since this behavior during LOFF is reminiscent of an escape behavior in case of passage of a fish, potentially a predator for larvae, an excessive response may exposed larvae to this predator and be detrimental for its survival. Disruption of behavior during LOFF has also been described as an indicator of increased anxiety and/or neurotoxicity (Legradi et al., 2018).

If PE and PVC MPs (both pristine and spiked) were equally able to decrease growth after approximately 4 months of exposure, it appeared that reprotoxicity of PVC was higher since spawning was disrupted 1) for all PVC conditions versus only PE-BP3 and 2) after only 2.5 months of exposure versus 4.5 months for PE since reproduction was assessed at the same age for both polymers type. Within each polymer conditions, we also observed differences for reproduction traits between pristine MPs and spiked MPs and between chemicals spiked, with PVC-BaP and PVC-BP3 being the most reprotoxic, while PE-BP3 was the only PE condition producing reprotoxicity. PVC-BP3 was also the only condition promoting disruption in offspring by inducing hyperactivity during LOFF. Altogether, this points out the fact that BP3 appeared to be the most toxic chemical used here. BP3 is known to act as an endocrine disrupting chemical interfering with reproduction and sex hormone signaling, inducing reproducing pathologies and altering the reproductive fitness (Bluthgen et al., 2012; Coronado et al., 2008; Kunz et al., 2006).

In another experiment, we have used the same PE particles, pristine and spiked MPs, to evaluate acute toxicity using a regulatory test, the fish embryotoxicity test (Cormier et al., 2019). This test was performed according to TG236 framework (OECD, 2013) which means this was a short (4 days) waterborne exposure using zebrafish embryos. No acute toxicity was revealed even using irrelevant concentrations such as 10 or 100 mg/L and only observed effects were slight induction of *cyp1a* expression upon exposure to PE-BaP and PE-BP3 at 10 mg/L (not tested at higher concentration) and of EROD activity upon exposure to 100 mg/L of PE-BP3 or

PE-BaP depending of the exposure timing. In any case, these results obtained using the same particles as in the present study, revealed that regulatory tests are not suitable to monitor MPs toxicity. In a similar experiment but using marine medaka rather in place of zebrafish embryos, a weak but significant toxicity was obtained at the highest concentration tested, 10 mg/L for PE-BP3 (Beiras et al., 2018). Beside exposure medium salinity which can change MPs characteristics and/or chemical exchange, the major difference between two species is that marine medaka hatch at approximately 10 days post fecundation versus 2.5 for zebrafish and so that exposure lasted 12 days rather than 4 days (Beiras et al., 2018). This strongly suggests that toxicity of MPs comes from chronic exposure and so that longer exposures are necessary to properly asses their toxicity.

A reduction of growth for females and a decrease in reproduction output may have important negative consequences on individual abilities to contribute to recruitment which may lead to adverse effects at population level. Our results fit well with the tentative AOP proposed recently to link exposure to MPs to population consequences (Galloway and Lewis, 2016). It is important to note that these effects depends on polymers type (PVC being more toxic than PE), the presence or not of chemicals (BP3 being more toxic than BaP or PFOS) and the duration of exposure. Regarding this last point long-term, chronic, exposures are necessary to actually report efficiently MPs toxicity for fish. More similar comparative studies like this one are necessary to evaluate extent of generalization which could be made about these conclusions.

Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Author Contributions

BC, FLB, MLB, SK, JC and XC designed the experiment; BC, FD, ML and LY performed the chemical analysis; BC and SZ conducted the early life stages experiment; MC, MLB and XC performed the long term exposure; BC, MC, MB, MLB, CC, FLB and XC performed all the toxicological analyses. BC wrote the manuscript and all authors corrected the manuscript.

Funding

This work was developed under the EPHEMARE project (Ecotoxicological effects of microplastics in marine ecosystems), supported by national funding agencies within the framework of JPI Oceans (FCT JPIOCEANS/0005/2015; FORMAS, 2015-01865; ANR-15-JOCE-0002-01; German BMBF 03F0735A). Bettie Cormier was directly supported by an IdEx grant from the University of Bordeaux.

Acknowledgment

PE and PVC were kindly provided within the JPI Oceans project EPHEMARE as a dry powder by Marina Albentosa (Instituto Español de Oceanografía, Centro Oceanografico de Murcia, Spain) and Francesco Regoli (Università Politecnica delle Marche, Ancona, Italy), respectively.

References

Ahrens L., Felizeter S., Ebinghaus R. (2009). Spatial distribution of polyfluoroalkyl compounds in seawater of the German Bight. Chemosphere. 76, 179-184

Alfonso S., Blanc M., Joassard L., Keiter S. H., Munschy C., Loizeau V., Bégout M.-L., Cousin X. (2019). Examining multi- and transgenerational behavioral and molecular alterations resulting from parental exposure to an environmental PCB and PBDE mixture. Aquatic Toxicology. 208, 29-38

Alomar C., Sureda A., Capó X., Guijarro B., Tejada S., Deudero S. (2017). Microplastic ingestion by Mullus surmuletus Linnaeus, 1758 fish and its potential for causing oxidative stress. Environmental Research. 159, 135-142

Anbumani S., Kakkar P. (2018). Ecotoxicological effects of microplastics on biota: a review. Environmental Science and Pollution Research. 25, 14373-14396

Andrady A. L. (2011). Microplastics in the marine environment. Marine Pollution Bulletin. 62, 1596-1605

Antunes J. C., Frias J. G. L., Micaelo A. C., Sobral P. (2013). Resin pellets from beaches of the Portuguese coast and adsorbed persistent organic pollutants. Estuarine, Coastal and Shelf Science. 130, 62-69

Bakir A., O'Connor I. A., Rowland S. J., Hendriks A. J., Thompson R. C. (2016). Relative importance of microplastics as a pathway for the transfer of hydrophobic organic chemicals to marine life. Environmental Pollution. 219, 56-65

Barnes D. K. A., Galgani F., Thompson R. C., Barlaz M. (2009). Accumulation and fragmentation of plastic debris in global environments. Philosophical Transactions of the Royal Society B: Biological Sciences. 364, 1985-1998

Batel A., Borchert F., Reinwald H., Erdinger L., Braunbeck T. (2018). Microplastic accumulation patterns and transfer of benzo[a]pyrene to adult zebrafish (*Danio rerio*) gills and zebrafish embryos. Environmental Pollution. 235, 918-930

Batel A., Linti F., Scherer M., Erdinger L., Braunbeck T. (2016). Transfer of benzo[a]pyrene from microplastics to Artemia nauplii and further to zebrafish via a trophic food web experiment: CYP1A induction and visual tracking of persistent organic pollutants. Environmental Toxicology and Chemistry. 35, 1656-1666

Beaudouin R., Goussen B., Piccini B., Augustine S., Devillers J., Brion F., Péry A. R. R. (2015). An Individual-Based Model of Zebrafish Population Dynamics Accounting for Energy Dynamics. PLOS ONE. 10, e0125841

Beiras R., Bellas J., Cachot J., Cormier B., Cousin X., Engwall M., Gambardella C., Garaventa F., Keiter S., Le Bihanic F., et al. (2018). Ingestion and contact with polyethylene microplastics does not cause acute toxicity on marine zooplankton. Journal of Hazardous Materials. 360, 452-460

Bessa F., Barría P., Neto J. M., Frias J. P. G. L., Otero V., Sobral P., Marques J. C. (2018). Occurrence of microplastics in commercial fish from a natural estuarine environment. Marine Pollution Bulletin. 128, 575-584

Besseling E., Wegner A., Foekema E. M., van den Heuvel-Greve M. J., Koelmans A. A. (2013). Effects of Microplastic on Fitness and PCB Bioaccumulation by the Lugworm Arenicola marina (L.). Environmental Science & Technology. 47, 593-600

Blüthgen N., Zucchi S., Fent K. (2012). Effects of the UV filter benzophenone-3 (oxybenzone) at low concentrations in zebrafish (*Danio rerio*). Toxicology and Applied Pharmacology. 263, 184-194

Bogdal C., Scheringer M., Abad E., Abalos M., van Bavel B., Hagberg J., Fiedler H. (2013). Worldwide distribution of persistent organic pollutants in air, including results of air monitoring by passive air sampling in five continents. TrAC Trends in Analytical Chemistry. 46, 150-161

Bour A., Haarr A., Keiter S., Hylland K. (2018). Environmentally relevant microplastic exposure affects sediment-dwelling bivalves. Environmental Pollution. 236, 652-660

Bradford M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Analytical Biochemistry. 72, 248-254

Bratkovics S., Sapozhnikova Y. (2011). Determination of seven commonly used organic UV filters in fresh and saline waters by liquid chromatography-tandem mass spectrometry. Analytical Methods. 3, 2943-2950

Bratkovics S., Wirth E., Sapozhnikova Y., Pennington P., Sanger D. (2015). Baseline monitoring of organic sunscreen compounds along South Carolina's coastal marine environment. Marine Pollution Bulletin. 101, 370-377

Browne Mark A., Niven Stewart J., Galloway Tamara S., Rowland Steve J., Thompson Richard C. (2013). Microplastic Moves Pollutants and Additives to Worms, Reducing Functions Linked to Health and Biodiversity. Current Biology. 23, 2388-2392

Buege J. A., Aust S. D. 1978. [30] Microsomal lipid peroxidation. In: Methods in Enzymology. Academic Press. p. 302-310.

Burke M. D., Mayer R. T. (1974). Ethoxyresorufin: direct fluorimetric assay of a microsomal O-dealkylation which is preferentially inducible by 3-methylcholanthrene. Drug Metabolism and Disposition. 2, 583

Cole M., Lindeque P., Halsband C., Galloway T. S. (2011). Microplastics as contaminants in the marine environment: A review. Marine Pollution Bulletin. 62, 2588-2597

Collins J. F., Brown J. P., Dawson S. V., Marty M. A. (1991). Risk assessment for benzo[a]pyrene. Regulatory Toxicology and Pharmacology. 13, 170-184

Cormier B., Batel A., Cachot J., Bégout M.-L., Braunbeck T., Cousin X., Keiter S. H. (2019). Multi-Laboratory Hazard Assessment of Contaminated Microplastic Particles by Means of Enhanced Fish Embryo Test With the Zebrafish (*Danio rerio*). 7,

Coronado M., De Haro H., Deng X., Rempel M. A., Lavado R., Schlenk D. (2008). Estrogenic activity and reproductive effects of the UV-filter oxybenzone (2-hydroxy-4-methoxyphenyl-methanone) in fish. Aquatic Toxicology. 90, 182-187

Corsolini S. (2009). Industrial contaminants in Antarctic biota. Journal of Chromatography A. 1216, 598-612

de Sá L. C., Oliveira M., Ribeiro F., Rocha T. L., Futter M. N. (2018). Studies of the effects of microplastics on aquatic organisms: What do we know and where should we focus our efforts in the future? Science of The Total Environment. 645, 1029-1039

Derraik J. G. B. (2002). The pollution of the marine environment by plastic debris: a review. Marine Pollution Bulletin. 44, 842-852

Dévier M.-H., Le Menach K., Viglino L., Di Gioia L., Lachassagne P., Budzinski H. (2013). Ultra-trace analysis of hormones, pharmaceutical substances, alkylphenols and phthalates in two French natural mineral waters. Science of The Total Environment. 443, 621-632

Diana J. S. (1983). An energy budget for northern pike (*Esox lucius*). Canadian Journal of Zoology. 61, 1968-1975

Díaz-Cruz S. M., Llorca M., Barceló D., Barceló D. (2008). Organic UV filters and their photodegradates, metabolites and disinfection by-products in the aquatic environment. TrAC Trends in Analytical Chemistry. 27, 873-887

El Nemr A., Abd-Allah A. M. A. (2003). Contamination of polycyclic aromatic hydrocarbons (PAHs) in microlayer and subsurface waters along Alexandria coast, Egypt. Chemosphere. 52, 1711-1716

Ellman G. L., Courtney K. D., Andres V., Featherstone R. M. (1961). A new and rapid colorimetric determination of acetylcholinesterase activity. Biochemical Pharmacology. 7, 88-95

Farrell P., Nelson K. (2013). Trophic level transfer of microplastic: *Mytilus edulis* (L.) to Carcinus maenas (L.). Environmental Pollution. 177, 1-3

Fent K., Kunz P. Y., Zenker A., Rapp M. (2010). A tentative environmental risk assessment of the UV-filters 3-(4-methylbenzylidene-camphor), 2-ethyl-hexyl-4-trimethoxycinnamate, benzophenone-3, benzophenone-4 and 3-benzylidene camphor. Marine Environmental Research. 69, S4-S6

Gago-Ferrero P., Díaz-Cruz M. S., Barceló D. (2011). Occurrence of multiclass UV filters in treated sewage sludge from wastewater treatment plants. Chemosphere. 84, 1158-1165

Galloway T. S., Cole M., Lewis C. (2017). Interactions of microplastic debris throughout the marine ecosystem. Nature Ecology & Evolution. 1, 0116

Galloway T. S., Lewis C. N. (2016). Marine microplastics spell big problems for future generations. Proceedings of the National Academy of Sciences. 113, 2331

Geyer R., Jambeck J. R., Law K. L. (2017). Production, use, and fate of all plastics ever made. Science Advances. 3, e1700782

Giesy J. P., Kannan K. (2002). Peer Reviewed: Perfluorochemical Surfactants in the Environment. Environmental Science & Technology. 36, 146A-152A

González-Soto N., Hatfield J., Katsumiti A., Duroudier N., Lacave J. M., Bilbao E., Orbea A., Navarro E., Cajaraville M. P. (2019). Impacts of dietary exposure to different sized polystyrene microplastics alone and with sorbed benzo[a]pyrene on biomarkers and whole organism responses in mussels Mytilus galloprovincialis. Science of The Total Environment. 684, 548-566

Green D. S., Boots B., Sigwart J., Jiang S., Rocha C. (2016). Effects of conventional and biodegradable microplastics on a marine ecosystem engineer (*Arenicola marina*) and sediment nutrient cycling. Environmental Pollution. 208, 426-434

Hartmann N. B., Rist S., Bodin J., Jensen L. H. S., Schmidt S. N., Mayer P., Meibom A., Baun A. (2017). Microplastics as vectors for environmental contaminants: Exploring sorption, desorption, and transfer to biota. Integrated Environmental Assessment and Management. 13, 488-493

Hong W.-J., Jia H., Li Y.-F., Sun Y., Liu X., Wang L. (2016). Polycyclic aromatic hydrocarbons (PAHs) and alkylated PAHs in the coastal seawater, surface sediment and oyster from Dalian, Northeast China. Ecotoxicology and Environmental Safety. 128, 11-20

Huang H., Huang C., Wang L., Ye X., Bai C., Simonich M. T., Tanguay R. L., Dong Q. (2010). Toxicity, uptake kinetics and behavior assessment in zebrafish embryos following exposure to perfluorooctanesulphonicacid (PFOS). Aquatic Toxicology. 98, 139-147

Jabeen K., Su L., Li J., Yang D., Tong C., Mu J., Shi H. (2017). Microplastics and mesoplastics in fish from coastal and fresh waters of China. Environmental Pollution. 221, 141-149

Jambeck J. R., Geyer R., Wilcox C., Siegler T. R., Perryman M., Andrady A., Narayan R., Law K. L. (2015). Plastic waste inputs from land into the ocean. Science. 347, 768

Jovanović B., Gökdağ K., Güven O., Emre Y., Whitley E. M., Kideys A. E. (2018). Virgin microplastics are not causing imminent harm to fish after dietary exposure. Marine Pollution Bulletin. 130, 123-131

Keiter S., Burkhardt-Medicke K., Wellner P., Kais B., Färber H., Skutlarek D., Engwall M., Braunbeck T., Keiter S. H., Luckenbach T. (2016). Does perfluorooctane sulfonate (PFOS) act as chemosensitizer in zebrafish embryos? Science of The Total Environment. 548-549, 317-324

Kim S., Choi K. (2014). Occurrences, toxicities, and ecological risks of benzophenone-3, a common component of organic sunscreen products: A mini-review. Environment International. 70, 143-157

Koelmans A. A. (2015). Modeling the role of microplastics in bioaccumulation of organic chemicals to marine aquatic organisms. A critical review. In: Marine Anthropogenic Litter. p. 309-324.

Koelmans A. A., Bakir A., Burton G. A., Janssen C. R. (2016). Microplastic as a Vector for Chemicals in the Aquatic Environment: Critical Review and Model-Supported Reinterpretation of Empirical Studies. Environmental Science and Technology. 50, 3315-3326

Koelmans A. A., Besseling E., Foekema E. M. (2014). Leaching of plastic additives to marine organisms. Environmental Pollution. 187, 49-54

Kunisue T., Chen Z., Buck Louis G. M., Sundaram R., Hediger M. L., Sun L., Kannan K. (2012). Urinary Concentrations of Benzophenone-type UV Filters in U.S. Women and Their Association with Endometriosis. Environmental Science & Technology. 46, 4624-4632

Law K. L., Thompson R. C. (2014). Microplastics in the seas. Science. 345, 144

Le Dû-Lacoste M., Akcha F., Dévier M.-H., Morin B., Burgeot T., Budzinski H. (2013). Comparative study of different exposure routes on the biotransformation and genotoxicity of PAHs in the flatfish species, Scophthalmus maximus. Environmental Science and Pollution Research. 20, 690-707

Lee H., Shim W. J., Kwon J.-H. (2014). Sorption capacity of plastic debris for hydrophobic organic chemicals. Science of The Total Environment. 470-471, 1545-1552

Legradi J. B., Di Paolo C., Kraak M. H. S., van der Geest H. G., Schymanski E. L., Williams A. J., Dingemans M. M. L., Massei R., Brack W., Cousin X., et al. (2018). An ecotoxicological view on neurotoxicity assessment. Environmental Sciences Europe. 30, 46

Lei L., Wu S., Lu S., Liu M., Song Y., Fu Z., Shi H., Raley-Susman K. M., He D. (2018). Microplastic particles cause intestinal damage and other adverse effects in zebrafish Danio rerio and nematode Caenorhabditis elegans. Science of The Total Environment. 619-620, 1-8

Li J., Liu H., Paul Chen J. (2018). Microplastics in freshwater systems: A review on occurrence, environmental effects, and methods for microplastics detection. Water Research. 137, 362-374

Lithner D., Larsson Å., Dave G. (2011). Environmental and health hazard ranking and assessment of plastic polymers based on chemical composition. Science of The Total Environment. 409, 3309-3324

Liu T., Pan L., Jin Q., Cai Y. (2015). Differential gene expression analysis of benzo(a)pyrene toxicity in the clam, Ruditapes philippinarum. Ecotoxicology and Environmental Safety. 115, 126-136

Lohmann R. (2017). Microplastics are not important for the cycling and bioaccumulation of organic pollutants in the oceans—but should microplastics be considered POPs themselves? Integrated Environmental Assessment and Management. 13, 460-465

Mato Y., Isobe T., Takada H., Kanehiro H., Ohtake C., Kaminuma T. (2001). Plastic Resin Pellets as a Transport Medium for Toxic Chemicals in the Marine Environment. Environmental Science & Technology. 35, 318-324

Mazurais D., Ernande B., Quazuguel P., Severe A., Huelvan C., Madec L., Mouchel O., Soudant P., Robbens J., Huvet A., et al. (2015). Evaluation of the impact of polyethylene microbeads ingestion in European sea bass (Dicentrarchus labrax) larvae. Marine Environmental Research. 112, 78-85

Morgana S., Ghigliotti L., Estévez-Calvar N., Stifanese R., Wieckzorek A., Doyle T., Christiansen J. S., Faimali M., Garaventa F. (2018). Microplastics in the Arctic: A case study with sub-surface water and fish samples off Northeast Greenland. Environmental Pollution. 242, 1078-1086

Ng K. L., Obbard J. P. (2006). Prevalence of microplastics in Singapore's coastal marine environment. Marine Pollution Bulletin. 52, 761-767

Nimrod A. C., Benson W. H. (1998). Reproduction and development of Japanese medaka following an early life stage exposure to xenoestrogens (R823450). Aquatic Toxicology. 44, 141-156

O'Donovan S., Mestre N. C., Abel S., Fonseca T. G., Carteny C. C., Cormier B., Keiter S. H., Bebianno M. J. (2018). Ecotoxicological Effects of Chemical Contaminants Adsorbed to Microplastics in the Clam Scrobicularia plana. 5,

OECD. 2013. Test No. 236: Fish Embryo Acute Toxicity (FET) Test.

Ogata Y., Takada H., Mizukawa K., Hirai H., Iwasa S., Endo S., Mato Y., Saha M., Okuda K., Nakashima A., et al. (2009). International Pellet Watch: Global monitoring of persistent organic pollutants (POPs) in coastal waters. 1. Initial phase data on PCBs, DDTs, and HCHs. Marine Pollution Bulletin. 58, 1437-1446

Pannetier P., Morin B., Le Bihanic F., Dubreil L., Clérandeau C., Chouvellon F., Van Arkel K., Danion M., Cachot J. (2020). Environmental samples of microplastics induce significant toxic effects in fish larvae. Environment International. 134, 105047

Paredes E., Perez S., Rodil R., Quintana J. B., Beiras R. (2014). Ecotoxicological evaluation of four UV filters using marine organisms from different trophic levels Isochrysis galbana, Mytilus galloprovincialis, Paracentrotus lividus, and Siriella armata. Chemosphere. 104, 44-50

Paul A. G., Jones K. C., Sweetman A. J. (2009). A First Global Production, Emission, And Environmental Inventory For Perfluorooctane Sulfonate. Environmental Science & Technology. 43, 386-392

Pazos R. S., Maiztegui T., Colautti D. C., Paracampo A. H., Gómez N. (2017). Microplastics in gut contents of coastal freshwater fish from Río de la Plata estuary. Marine Pollution Bulletin. 122, 85-90

Pedà C., Caccamo L., Fossi M. C., Gai F., Andaloro F., Genovese L., Perdichizzi A., Romeo T., Maricchiolo G. (2016). Intestinal alterations in European sea bass Dicentrarchus labrax (Linnaeus, 1758) exposed to microplastics: Preliminary results. Environmental Pollution. 212, 251-256

Pittura L., Avio C. G., Giuliani M. E., d'Errico G., Keiter S. H., Cormier B., Gorbi S., Regoli F. (2018). Microplastics as Vehicles of Environmental PAHs to Marine Organisms: Combined Chemical and Physical Hazards to the Mediterranean Mussels, Mytilus galloprovincialis. 5,

PlasticsEurope (2018). PlasticsEurope: Annual review _ The Facts 2017-2018.

Rainieri S., Conlledo N., Larsen B. K., Granby K., Barranco A. (2018). Combined effects of microplastics and chemical contaminants on the organ toxicity of zebrafish (Danio rerio). Environmental Research. 162, 135-143

Rennie M. D., Purchase C. F., Lester N., Collins N. C., Shuter B. J., Abrams P. A. (2008). Lazy males? Bioenergetic differences in energy acquisition and metabolism help to explain sexual size dimorphism in percids. Journal of Animal Ecology. 77, 916-926

Rigét F., Vorkamp K., Bossi R., Sonne C., Letcher R. J., Dietz R. (2016). Twenty years of monitoring of persistent organic pollutants in Greenland biota. A review. Environmental Pollution. 217, 114-123

Rios L. M., Moore C., Jones P. R. (2007). Persistent organic pollutants carried by synthetic polymers in the ocean environment. Marine Pollution Bulletin. 54, 1230-1237

Rist S. E., Assidqi K., Zamani N. P., Appel D., Perschke M., Huhn M., Lenz M. (2016). Suspended micro-sized PVC particles impair the performance and decrease survival in the Asian green mussel Perna viridis. Marine Pollution Bulletin. 111, 213-220

Rochman C. M., Browne M. A., Halpern B. S., Hentschel B. T., Hoh E., Karapanagioti H. K., Rios-Mendoza L. M., Takada H., Teh S., Thompson R. C. (2013). Classify plastic waste as hazardous. Nature. 494, 169-171

Rochman C. M., Hoh E., Hentschel B. T., Kaye S. (2013). Long-Term Field Measurement of Sorption of Organic Contaminants to Five Types of Plastic Pellets: Implications for Plastic Marine Debris. Environmental Science & Technology. 47, 1646-1654

Rochman C. M., Hoh E., Kurobe T., Teh S. J. (2013). Ingested plastic transfers hazardous chemicals to fish and induces hepatic stress. Scientific Reports. 3, 3263

Rochman C. M., Kurobe T., Flores I., Teh S. J. (2014). Early warning signs of endocrine disruption in adult fish from the ingestion of polyethylene with and without sorbed chemical pollutants from the marine environment. Science of The Total Environment. 493, 656-661

Schneider J. E., Brozek J. M., Keen-Rhinehart E. (2014). Our stolen figures: The interface of sexual differentiation, endocrine disruptors, maternal programming, and energy balance. Hormones and Behavior. 66, 104-119

Schönlau C., Larsson M., Lam M. M., Engwall M., Giesy J. P., Rochman C., Kärrman A. (2019). Aryl hydrocarbon receptor-mediated potencies in field-deployed plastics vary by type of polymer. Environmental Science and Pollution Research. 26, 9079-9088

Setälä O., Fleming-Lehtinen V., Lehtiniemi M. (2014). Ingestion and transfer of microplastics in the planktonic food web. Environmental Pollution. 185, 77-83

So M. K., Taniyasu S., Lam P. K. S., Zheng G. J., Giesy J. P., Yamashita N. (2006). Alkaline Digestion and Solid Phase Extraction Method for Perfluorinated Compounds in Mussels and Oysters from South China and Japan. Archives of Environmental Contamination and Toxicology. 50, 240-248

Sussarellu R., Suquet M., Thomas Y., Lambert C., Fabioux C., Pernet M. E. J., Le Goïc N., Quillien V., Mingant C., Epelboin Y., et al. (2016). Oyster reproduction is affected by exposure to polystyrene microplastics. Proceedings of the National Academy of Sciences. 113, 2430

Teuten E. L., Rowland S. J., Galloway T. S., Thompson R. C. (2007). Potential for Plastics to Transport Hydrophobic Contaminants. Environmental Science & Technology. 41, 7759-7764

Thompson R. C., Moore C. J., vom Saal F. S., Swan S. H. (2009). Plastics, the environment and human health: current consensus and future trends. Philos Trans R Soc Lond B Biol Sci. 364, 2153-2166

Thompson R. C., Olsen Y., Mitchell R. P., Davis A., Rowland S. J., John A. W. G., McGonigle D., Russell A. E. (2004). Lost at Sea: Where Is All the Plastic? Science. 304, 838

Vignet C., Le Menach K., Mazurais D., Lucas J., Perrichon P., Le Bihanic F., Devier M.-H., Lyphout L., Frère L., Bégout M.-L., et al. (2014). Chronic dietary exposure to pyrolytic and petrogenic mixtures of PAHs causes physiological disruption in zebrafish - part I: Survival and growth. Environmental Science and Pollution Research. 21, 13804-13817

Wang F., Shih K. M., Li X. Y. (2015). The partition behavior of perfluorooctanesulfonate (PFOS) and perfluorooctanesulfonamide (FOSA) on microplastics. Chemosphere. 119, 841-847

Watts A. J. R., Urbina M. A., Corr S., Lewis C., Galloway T. S. (2015). Ingestion of Plastic Microfibers by the Crab Carcinus maenas and Its Effect on Food Consumption and Energy Balance. Environmental Science & Technology. 49, 14597-14604

Weeks-Santos S., Cachot J., Gourves P.-Y., Clérandeau C., Morin B., Gonzalez P. (2019). Sub-lethal effects of waterborne copper in early developmental stages of rainbow trout (Oncorhynchus mykiss). Ecotoxicology and Environmental Safety. 170, 778-788

Weigt S., Huebler N., Strecker R., Braunbeck T., Broschard T. H. (2011). Zebrafish (Danio rerio) embryos as a model for testing proteratogens. Toxicology. 281, 25-36

Wright S. L., Rowe D., Thompson R. C., Galloway T. S. (2013). Microplastic ingestion decreases energy reserves in marine worms. Current Biology. 23, R1031-R1033

Xie X., Long T., Zhang Y., Cao Z. (1998). Reproductive investment in the Silurus meridionalis. Journal of Fish Biology. 53, 259-271

Yamashita N., Kannan K., Taniyasu S., Horii Y., Petrick G., Gamo T. (2005). A global survey of perfluorinated acids in oceans. Marine Pollution Bulletin. 51, 658-668

Yin L., Chen B., Xia B., Shi X., Qu K. (2018). Polystyrene microplastics alter the behavior, energy reserve and nutritional composition of marine jacopever (Sebastes schlegelii). Journal of Hazardous Materials. 360, 97-105

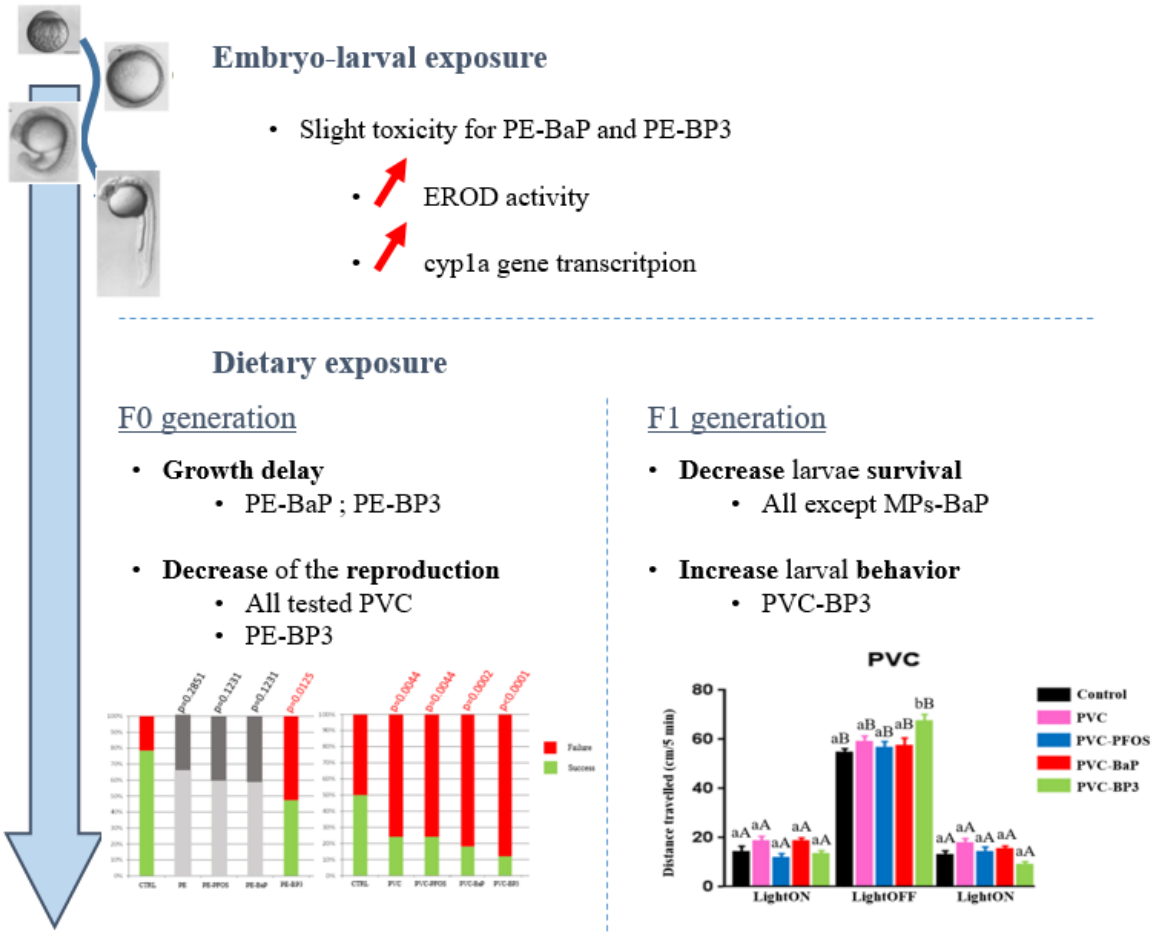
Yoo H., Washington J. W., Jenkins T. M., Laurence Libelo E. (2009). Analysis of perfluorinated chemicals in sludge: Method development and initial results. Journal of Chromatography A. 1216, 7831-7839

Zhu M., Chernick M., Rittschof D., Hinton D. E. (2020). Chronic dietary exposure to polystyrene microplastics in maturing Japanese medaka (Oryzias latipes). Aquatic Toxicology. 220, 105396

Ziccardi L. M., Edgington A., Hentz K., Kulacki K. J., Kane Driscoll S. (2016). Microplastics as vectors for bioaccumulation of hydrophobic organic chemicals in the marine environment: A state-of-the-science review. Environmental Toxicology and Chemistry. 35, 1667-1676

Chapter 4 – Conclusion

Results



Key points

- **Exposure of early life stages (ELS) of zebrafish to virgin PE MPs did not induce toxicity**
- **Exposure to** PE-MPs coated with **BaP** or **BP3 induced a significant increase of *cyp1a* transcription level and EROD activity**
- **BP3** combined with **PE** and **PVC** MPs induced **significant effects** on exposed **adult** and also on **unexposed offspring F1**

PVC MPs tend to be more toxic than PE MPs in combination with model pollutants

Chapter 5: Toxicity of environmental microplastics

Chapter 5 – Presentation

Context

Artificial MPs are not representative of the contamination of aquatic ecosystems. Environmental scenario needs to consider the cocktail of different polymer types and absorbed contaminants and also, weathering of MPs. Thus, the use of environmental samples are essential to understand the toxicity of MPs on the aquatic biota. Studies presented in the chapter were used to characterize pollutants fingerprints of environmental MPs and to investigate their toxicity on different biological functions by using different life stages of zebrafish.
In the present chapter, the first study was performed in collaboration with Vigo University and CNR IAS Genova, but only experiments using zebrafish are part of the PhD thesis.

Method

To simulate the real contamination, MPs from different beaches of Guadeloupe were grinded and non-targeted analyses were performed to characterize chemicals adsorbed at the surface, as well as the polymer composition by FTIR. ELS of zebrafish were exposed either to organic extracts using DMSO or with particles. Juveniles and adults zebrafish were exposed to particles though food, starting at 1 month and lasted for 5 months. Toxicity was evaluated on exposed adults and unexposed F1 offspring.



MPs from Guadeloupe:
- **Marie-Galante** (MG)
- **Petit-Bourg** (PB)

Milled ➔ < 50 µm

Non target analysis (GC-MS/MS)

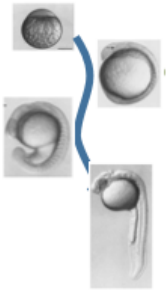
% polymer (FTIR)

Granulometry

- Organic extracts (DMSO
- Leachates
- Particles (< 50 µm)

waterborne exposure

dietary exposure



Embryo-larval Exposure ***Particles, leachates*** *and* ***Organic extracts***

FET (OECD 236) with additional endpoints:
- Survival
- Growth
- EROD activity
- CYP1A
- LPMR

Juveniles and adults exposed to ***particles***

F0 generation:
- Survival
- Growth
- Reproduction
- EROD, TBARs, AChE

F1 generation:
- Survival
- Growth
- LPMR

Article 4

Toxicity assessment of environmental microplastics on early life stages of three aquatic organisms

Bettie Cormier, Chiara Gambardella, Tania Tato, Florian Dubocq, Quentin Perdriat, Elisa Costa, Anna Kärmann, Kim Van Arkel, Soizic Lemoine, Fabienne Lagarde, Francesca Garaventa, Marco Faimali, Marie-Laure Bégout, Xavier Cousin, Ricardo Beiras, Jérôme Cachot.

Toxicity assessment of environmental microplastics on early life stages of three aquatic organisms

Bettie Cormier[1,2], Tania Tato[3], Chiara Gambardella[4], Florian Dubocq2, Quentin Perdriat[1], Elisa Costa[4], Anna Karmann[2], Kim Van Arkel[5], Soizic Lemoine[6], Fabienne Lagarde[7], Francesca Garaventa[4], Marco Faimali[4], Xavier Cousin[8,9,10], Marie-Laure Bégout[11], Ricardo Beiras[3], Jérôme Cachot[1*]

[1] Bordeaux University, EPOC, UMR CNRS 5805, Avenue des Facultés, 33400 Talence, France

[2] Man-Technology-Environment Research Centre, School of Science and Technology, Örebro University, Fakultetsgatan 1, 701 82 Örebro, Sweden

[3] Faculty of Marine Sciences, University of Vigo, E-36310 Vigo, Galicia

[4] Institute for the study of Anthropic impacts and Sustainability in marine environment – National research Council (CNR-IAS), Genova, Italy

[5] Race for Water Foundation, Lausanne, 1007, Switzerland

[6] UMR BOREA CNRS-7208, IRD-207, MNHN, UPMC, UCBN, Université des Antilles et de la Guyane, DYNECAR, Campus de Fouillole, BP 592, 97159, Pointe-à-Pitre, France

[7] Institut des Molécules et Matériaux du Mans (IMMM, UMR CNRS 6283), Université du Maine, Avenu Olivier Messiaen, Le Mans F-72085, France

[8] Ifremer, University of Montpellier, CNRS, IRD, MARBEC, 34250 Palavas-les-flots, France

[9] Université Paris-Saclay, INRAE, AgroParisTech, GABI, 78350, Jouy-en-Josas, France

[10] IFREMER, L3AS, UMR MARBEC, F-34250 Palavas, France.

[11] Ifremer, University of Montpellier, CNRS, IRD, MARBEC, 34250 Palavas-les-flots, France

Keywords: environmental microplastics, early life stages, aquatic organisms, toxicity

Introduction

Plastic became a major concern since 60 years, attracting interest from public, media and scientific (Law and Thompson, 2014). One of the main reasons behind this interest is the abundance of plastics found in the aquatic environment. Mass production of plastics began in the 1950s, with global production has rising continuously. In 2017, it was estimated that were around 348 million tons of plastic worldwide (PlasticsEurope, 2018), with millions of tons of plastic waste being accidently or intentionally discharged into aquatic ecosystems (Geyer et al., 2017; Jambeck et al., 2015).

Plastics in the environment tend to degrade and fragment into smaller particles through chemical, physical or biological processes (Barnes et al., 2009), leading to debris with a size below 5 mm in diameter, defined as microplastics (MPs) (Arthur et al., 2009). The volume of microplastics and plastic debris on the ocean surface has been estimated to be almost 270 000 tons (Eriksen et al., 2014). Due mainly to their slow rate of degradation (Cole et al., 2011), plastic debris tend to accumulate in oceanic gyres but also in coastal areas (Eriksen, Lebreton, Carson, Thiel, Moore, Borerro, Galgani, Ryan and Reisser, 2014; Law and Thompson, 2014; Lebreton et al., 2018). Beaches are major hotspots for MPs and associated pollution (Andrady, 2011). The presence of MPs on beaches has been reported in various areas around the world, including Caribbean Sea (Acosta-Coley et al., 2019; Acosta-Coley and Olivero-Verbel, 2015; Bosker et al., 2017; Bosker et al., 2018).

In 2017, a worldwide sampling campaign was carried out (Race for Water Odyssey 2017-2021) by the Race for Water Foundation, to collect MPs from the water column, subtidal sediments, beaches and living organisms. The Guadeloupe archipelago in the Caribbean Sea was selected for this study because of its location close to the North Atlantic gyre. This Caribbean archipelago is made up of 5 main islands; of which two (Basse-Terre and Marie-Galante) were considered in this study.

While plastic contamination in marine ecosystem has been widely reported (Law and Thompson, 2014), few studies have investigated the deleterious effects of MPs on aquatic organisms. The exposure of organisms to MPs can lead to both physical and chemical damage, such as blockage of the digestive tract (Cole et al., 2011; Pedà et al., 2016), false feeling of fullness (Rochman, Hoh, Kurobe, et al., 2013), growth retardation and death of fish embryos (Pannetier, Morin, et al., 2019) and impairment of development and swimming activity of oyster larvae (Bringer et al., 2020). Toxicological effects induced by chemicals sorbed on MPs

such as additives, e.g. bisphenol A, phthalates, UV stabilizer, etc. (Koelmans, 2015; Koelmans et al., 2014; Rochman, 2015) or pollutants (Pannetier, Cachot, et al., 2019; Pannetier, Morin et al., 2019) have also been documented. Chemicals adsorbed during the ageing of plastic are mostly hydrophobic organic compounds, including polychlorinated biphenyls (PCBs), polycyclic aromatic compounds (PAHs) and organochlorine pesticides (Derraik, 2002; Karapanagioti and Klontza, 2008; Koelmans et al., 2016). They may also include trace metal elements (Rochman, Hentschel, et al., 2014). Toxic effects of artificially spiked MPs have been reported, including endocrine perturbation, hepatic damage, oxidative stress induction and enzymatic activity modifications (Browne et al., 2013; Cormier et al., 2019; Mazurais et al., 2015; Pittura et al., 2018; Teuten et al., 2009).

Exposure to pristine MPs particles and pellets frequently either has no effect or causes slight alterations in exposed organisms (Beiras et al., 2018; Cormier, Batel, Cachot, Bégout, Braunbeck, Cousin and Keiter, 2019). The toxicity of MPs requires much more in-depth investigation, given that they since are widespread and easily ingested by wide variety of living organisms. To that end, leachate protocols have been developed and used for ecotoxicological assessment of MPs (Beiras et al., 2019; Bejgarn et al., 2015; Gandara e Silva et al., 2016; Lithner et al., 2009; Lithner et al., 2011; Oliviero et al., 2019). Studies carried out to date have investigated the toxicity of environmental samples of MPs are still scarce (Pannetier, Morin, et al., 2019; Pannetier et al., 2020).

Early life stages of invertebrates and fish are increasingly used in ecotoxicology, mainly due to their high sensitivity to diverse chemicals (Beiras et al., 2018; Embry et al., 2010; Lammer et al., 2009; Oliviero et al., 2019). Planktonic larvae are a critical stage in life cycle completion, as well as playing a key role in the recruitment and dissemination of populations. The *Paracentrotus lividus* sea urchin is distributed in European waters and plays an important role in coastal ecosystem maintenance. The larval stage has been reported as the most sensitive stage, and is currently used as a biological model to evaluate toxic effects of contaminants (Bellas et al., 2005; Oliviero et al., 2019; Rouchon and Phillips, 2017). The jellyfish *Aurelia aurita,* has been reported to be an innovative model organism in ecotoxicology due to its high sensitivity to pollutants, and its key position as basal metazoan (Costa et al., 2015; Faimali et al., 2014). *A. aurita* is essential for the marine food web, both as prey (Cardona et al., 2012; Titelman and Hansson, 2006) and as a consumer of zooplankton. Besides invertebrates, early life stages of zebrafish (*Danio rerio*) have been shown to be as sensitive to chemical pollutants

as adult fish (Belanger et al., 2013; Braunbeck et al., 2005; Braunbeck et al., 2015; Lammer et al., 2009; Nagel, 2002).

The purpose of this study was firstly to characterize MPs collected from two island beaches in the Guadeloupe archipelago (Basse-Terre and Marie-Galante) during the Race for Water Odyssey mission in term of polymer composition and sorbed chemicals. The toxicity of these environmental MPs samples was then investigated for the early life stages of three marine organisms, sea urchin (*Paracentrotus lividus*), jellyfish (*Aurelia aurita*) and zebrafish (*Danio rerio*), using leachates.

Materials and methods

Sample collection

Environmental MPs were collected from Guadeloupe archipelago (France), located in the Caribbean (Figure 1), during the Race for Water Odyssey 2017-2021, in October 2017. This study focused on environmental MPs samples collected on two different sandy beaches: Capesterre in Marie-Galante Island (MG), and Petit Bourg (PB) in Guadeloupe Basse-Terre island (Table 1). These sites were sampled using the standardized NOAA protocol (Lippiatt et al., 2013) adapted for millimetric plastic debris. In short, debris between 1 and 5 mm were collected along a 100 m the shoreline, at the surface of the beach. Sorting was based on a visual assessment, followed up with Raman spectroscopy. Samples were stored in aluminium tray with an aluminium lid at 4 °C.

Table 1: Characteristics and GPS coordinates of the sampled beaches in Guadeloupe (France).

Sample	GPS Coordinates	Place	Date	Description
MG (Marie Galante)	15°53'28.6"N 61°13'15.5"W	Capesterre beach in Marie-Galante island	7th October 2017	Beach in the South-east part of the well preserved Marie-Galante island near the city centre of Capesterre (3,300 inhabitants)
PB (Petit Bourg)	16°09'59.6"N 61°35'06.2"W	Viard beach in Guadeloupe Basse-Terre island	5th October 2017	Beach in the East coast of Basse-Terre closed to the main industrial zone and harbour of Pointe-à-Pitre (315,000 inhabitants for the all urban area)



Figure 1: Map of Guadeloupe archipelago (Caribbean Island) with the two sampled beaches, Petit-Bourg (PB) on the main island and Capesterre in Marie-Galante island (MG).

MPs composition and chemical characterization

Particles collected from each beach were counted, and then individually weighed and analyzed using Fourrier-transform infrared spectroscopy (FT-IR) with a spectrum Vertex 70v (Bruker) to identify polymer of each particles. Results are expressed in % mass. MPs were subsequently grinded using a cryogenic mill (SPEX, 6770 Freezer-mill) for 8 minutes at 15 cycles per second. Powders obtained were sieved for 12h at ambient temperature through 100 and 53 µm sieves. The particle-size distribution was measured using a Malvern laser diffraction particle size analyzer.

For the chemical analysis, due to the limited amount of ground MPs used for toxicological analysis, mesoplastics (particles > 1 mm) from the same beaches and sampled with the same protocol, were used. Chemical analysis of organic micropollutants was conducted using a non-target analysis with gas chromatography (GC) coupled with an orbitrap.

Liquid/solid extractions were performed using 0.3 g of mesoplastics in hexane (≥ 98 %, SupraSolv; Merck, Darmstadt, DE), followed by 30 min of sonication and centrifugation at 8000 revolutions per minute (rpm) for 10 min. Extracts were reduced to 1 mL, using nitrogen flow (6.0) and filtrated through glasswhool with sodium sulfate, and finally reduced to 25 µL for injection. Analysis was performed with a GC-orbitrap (Q-Exactive, ThermoFisher) with a resolution of 60 000 at m/z 200 with a fullMS between 53.4 and 800 m/z (3.5 and 55 minutes), and the maximum oven temperature was set-up to 350 °C. Plastic samples were analyzed using mass defect-plot for chlorinated and brominated compounds (HaloSeeker), PAHs and hydrocarbons (CH_2). TraceFinder (ThermoFisher) was used for data processing, fold change

and elemental composition analysis. Fold change values were sorted and the 50 highest fold change features were analysed. Chromatogram peaks were checked and only symmetric peaks with width below 0.5 min were kept. Finally, 26 features were selected for further analysis for elemental composition analysis. Confidence levels were attributed to each identification molecule following Schymanski et al. (2014). Levels 1, 2, 3, 4 and 5 relate to a confirmed structure, a probable structure, tentative candidate(s), unequivocal molecular formula and exact mass (m/z) of the compound, respectively (Schymanski et al., 2014).

Trace metals are going to be analysed by Inductively Coupled Plasm and Mass Spectrometry (ICP-MS) on whole particles after total digestion of plastic matrix.

Preparation of leachates

Leachates were prepared according to Beiras et al. (2019), with modifications described below for all organisms used.

Leachates protocol for sea urchin exposure

Sea urchin toxicity test followed the methodology reported in detail in Beiras et al. (2019). Microplastic samples of PB, MG and a 'virgin' Polyethylene (PE) resin obtained from Rotogal, Boiro (Spain) were tested. Stock solutions of 10 g/L of microplastic particles in artificial seawater (ASW) (Lorenzo et al., 2002) were prepared in 50 mL glass bottles and filled with no head space. The bottles were incubated during 24 h at 20 ˚C in darkness with rotational shaking (1 rpm) provided by an overhead rotator (GFL 3040, Germany). After extraction, microplastic particles were removed from leachates by means of vacuum filtration using glass microfiber filters (Whatman, GF/F) that retain fine particles down to 0.7 µm. Undiluted leachate (10,000 mg/L) and serial dilutions at 1/3 (3,333 mg/L), 1/10 (1,000 mg/L) and 1/30 (333 mg/L) in ASW were introduced in 5 mL glass vials with Teflon lined airtight lids (4 replicates per dilution and 8 replicates for blanks of filtered ASW).

Leachate protocol for jellyfish exposure

Leachates for jellyfish exposure were obtained using rotary mixing at 20 ºC for 24 h in the dark, according to the method described by Beiras et al. (2019), adapted to a solid-to-liquid proportion of 1,000 mg/L and rotation speed of 2 rpm. Leachate were then filtrated with GF/C filters.

Leachate protocol for zebrafish exposure

Leachates for zebrafish were prepared using mixing 400 mg of MPs to 4 mL of E3 medium (1-5 mM NaCl, 0.17 mM KCl, 0.33 mM CaCl2, 0.33 mM MgSO4, 10-5 % Methylene Blue). Extraction was performed in the dark, with 16 h of shaking on a horizontal mixing table (KS 501 digital IKA-Werke) at 175 rpm. After that, extracts were centrifuged at 4000 rpm for 5 minutes. Then, 100 µL of the liquid fraction were sampled with extra-long sterile tip, and re-centrifuged to remove particles. Extracts were kept in darkness at 4 °C for 7 days maximum.

Toxicological analysis using sea urchin

Sea urchin *(Paracentrotus lividus)* fertilized eggs were added to each vial at a density of 40 per mL and vials were placed in an incubator (Velp FTC 90E) for 48 h in darkness at 20 ˚C. After incubation, larvae were fixed with 40% formalin and larval length (maximum linear dimension in n=35 per vial) was recorded using an inverted microscope (Leica DMI 4000B) and Leica LAS v4.12 image analysis software. Acceptability criteria were the percentage of fertilized eggs > 98% and size increase in controls > 253 µm (Saco-Álvarez et al., 2010).

Statistical analyses were conducted using IBM SPSS statistics software (v. 24). Normal distribution and homoscedasticity of the data was checked using Shapiro-Wilk's and Levene's tests respectively**.** ANOVA with Dunnett's post hoc test was applied to determine the no observed effect concentration (NOEC) and the lowest observed effect concentration (LOEC). Effective median concentrations (EC50) and their 95% confidence limits were calculated using Probit and toxic units (TU) were calculated as TU = 1 / EC50.

Toxicological analysis using ephyrae of jellyfish

Semi dynamic conditions

Jellyfish (*Aurelia aurita*) were exposed in semi-dynamic conditions, with an oxygen supply, to undiluted leachate (10 mg/L) and 1:10 diluted leachate (1 mg/L), for 24 and 48 h. Two endpoints were measured, the immobility of jellyfish as well as the behaviour, expressed by the frequency of pulsations.

Experimental set-up: rotatory wheel

An experimental set-up was used to expose jellyfish to MPs leachates, by placing ephyrae on glass vials in a rotatory wheel and by the evaluation of two endpoints: the immobility and

frequency of pulsations after 24 and 48 h. For the specific set-up, undiluted leachate (1,000 mg/L, namely x1), 1/3 (333 mg/L, namely x1/3), 1/10 (100 mg/L, namely x1/10) and 1/30 (33 mg/L, namely x1/30) were used.

Toxicological analysis using zebrafish embryos

Husbandry and egg production

Fish husbandry conditions fully complied with OECD TG 236 (OECD, 2013; Westerfield, 2000) regarding maintenance of adult zebrafish (*Danio rerio*). Zebrafish, TU strain were bred in 10 L tanks at 27 ± 1 °C with a day/night cycle of 14h/10h (7.8 ± 0.2, 480 ± 130 µS, for pH and conductivity, respectively). Fish were fed twice-daily *ad libitum* with Inicio + 0.5 mm (BioMar, France) and freshly hatched brine shrimp *Artemia* nauplii. Constant filtering or permanent flow-through conditions guaranteed that ammonia, nitrites, and nitrates were kept below detection limits (5, 1 and 140 mg/L, respectively). Zebrafish eggs were collected according to OECD TG 236 (OECD, 2013).

Zebrafish embryo acute toxicity test

Leachate exposure started after hatching (72 hours post fertilization, hpf). Zebrafish embryos were exposed for 48 h to leachates in E3 medium until the end of the eleutheroembryo phase (120 hpf). Embryos were exposed to two equivalent concentrations of plastics 50,000 and 10,000 mg/L. The control conditions consisted of embryos placed only in the E3 medium supplemented with 0.35 mM of methylene blue as a fungicide. Positive control was added, using 4 mM of 3,4-dichloroaniline (DCA) for the test validation (OECD, 2013). Glass petri dishes (55 mm diameter) containing 20 mL of solution were used in semi-static conditions with a daily renewal of exposure medium. Twenty embryos were used for each condition, and each treatment condition was replicated 3 times. To maintain a temperature of 28 ± 1 °C, experiments were performed in temperature-controlled chambers (Snijders Scientific, Tilburg, Nederlands) with a photoperiod set up on 14:10, light:dark. Before hatching, embryos were kept in E3 medium, and then, transferred in glass beakers containing 20 mL of leachate. For the entire exposure period, dissolved oxygen was checked daily (> 85 %) with a fiber-optic oxygen mini-sensor Fibox 3 (PreSens Precision Sensor, Regensburg, Germany).

Developmental toxicity

Individuals were examined following OECD 212 (OECD, 1998). Embryos were checked daily for survival and dead embryos were counted and immediately removed to avoid modification of the medium. Abnormalities and lesions were also recorded: edema; hemorragies, skelettal axis (scoliosis, lordosis) and craniofacial deformations and cardiovascular anomalies (blood circulation) following previously published protocols for Japanese Medaka (Le Bihanic, Clérandeau, et al., 2014). Leica MZ7.5 stereomicroscope with Leica DFP420C CDD camera and Leica Microsystems software v3.8 were used to perfom these measurements (Leica, France).

Biometry

At 96 hpf, six to ten embryos per replicate were anestethized with 200 mg/L of benzocaine. Total length and head length, were measured using a Leica MZ7.5 stereomicroscope with Leica DFP420C CDD camera and Leica Microsystems software v3.8 (Leica, France), and the ratio of head/total length was calculated.

In vivo EROD assay

In vivo EROD activity was analyzed according to published methods with modifications (Jönsson et al., 2002; Le Bihanic et al., 2013). Measurements were conducted on 96 hpf embryos. Five embryos per replicate were placed into individual wells of a 48-well microplate containing 1 µM of 7-ethoxyresorufin (7-ER) in 0.5 mL of E3. After one hour of incubation in the dark, the 7-ER solution was removed and replaced by freshly prepared 1 µM 7-ER. Fluorescence of 100 µL of the incubation media was measured in duplicate in 96 white well plate at time 0 and after 4 h incubation. Resorufin formed was measured with a BMG Labtech fluorescent microplate reader (Germany) excitation and emission wavelength of 560 and 590 nm respectively. Solution of 7-ER was added to the resorufin standard used, for a final concentration of 1 nM. A batch of five control embryos was exposed to benzo[a]pyrene (70 nM) for 1h before incubation in 7-ER to obtain a positive control.

Larval behavior

Swimming behavior was investigated following the protocol of Vignet et al. (2014) with a larval photomotor response test (LPMR) to record fish behavioral responses following a light stimuli. Embryos at 96 hpf were transferred into a 48-well plate filled with 500 µL of E3 medium without methylene blue and acclimated for 20 min in the dark, followed by 5 min light, 5 min dark and 5 min light. Swimming activity was recordered with a video tracking system DanioVision (Noldus, Wageningen, The Netherlands). Swimming behavior of 10 to 12 random embryos (96 hpf) per replicate was recorded at 28 ± 1 °C (DanioVision Temperature Control Unit, Noldus). Videos were analyzed with Ethovision software 12.0 (Noldus, Wageningen, The Netherlands).

Statistical analyses

Each set of exposure conditions was repeated three times. Normality of the data was checked using the Shapiro-Wilk test. Analysis of variance was performed followed by Fisher's test compared to control. One-way ANOVA was used for data with normal distribution and for data that did not fulfil normal distribution, the Kruskal-Wallis non-parametric test was performed. All statistical analyzes were carried out at a 95% level of significance and values are represented as mean ± SD.

Results

Characterization of microplastics

The predominant shape of MPs collected was fragments (> 98%) for both sites. Pellets were not found, and only a few particles were fibers, thin films and foams (< 2%). Polymer composition was almost the same for the two beaches, with a majority of polyethylene (PE) and polypropylene (PP), few particles of polystyrene (PS) and 2 particles polyvinyl acetate only at Petit-Bourg beach (Table 2). Particle size was only measured on the MG site, due to the small amount of PB particles available. Prior to granulometry analysis, MPs were sieved on 800 µm, and no particle was larger than 800 µm. The median particle size was 33.74 µm, and the mean size particles was 87.1 µm.

Table 2: Polymer composition of microplastics samples from two different beaches in Guadeloupe (% in mass)

Polymers	Marie-Galante	Petit-Bourg
Polyethylene	78.3	74.6
Polypropylene	21.2	24.8
Polystyrene	0.1	0.4
Polyvinyl acetate	0	0.2

Pollutant fingerprint on MPs

Organic pollutants

Non-target chemical analyses were performed on samples from both sites. Figure 2 shows the two chromatograms obtained after GC analysis. Chromatograms superposition shows that the two chromatograms contain peak similarities and dissimilarities. For the chemical analysis, mass defect plot was used to detect chlorinated and brominated compounds (**Error! Reference source not found.**). Some halogenated compounds were detected in both samples. Brominated flame retardant as tribromophenol ($C_6H_3Br_3O$) and its metabolite tribromoanisole ($C_7H_5Br_3O$) were found in both samples. Tri- and di-polychlorinated biphenyl were detected as $C_{12}H_7Cl_3$ and $C_{12}H_8Cl_2$. Homologous series of pesticides from pentachlorobenzene (C_6HCl_5) and dichlorobenzene ($C_6H_4Cl_2$) were detected but only one chlorinated polycyclic aromatic compound ($C_{12}H_7Cl$), most probably chloroacenaphtylene. Analysis of PAHs was carried out using a mass defect plot with a PAHs scale, however no homologous series were detected for either MG or PB.

Finally, fold change comparisons were made between MG and PB samples using TraceFinder, 26 features were selected. The results are presented in supplementary material (Table S1). The concentrations of halogenated compounds as well as trichlorobenzene, bumetrizole and octabenzone were higher at the MG site.

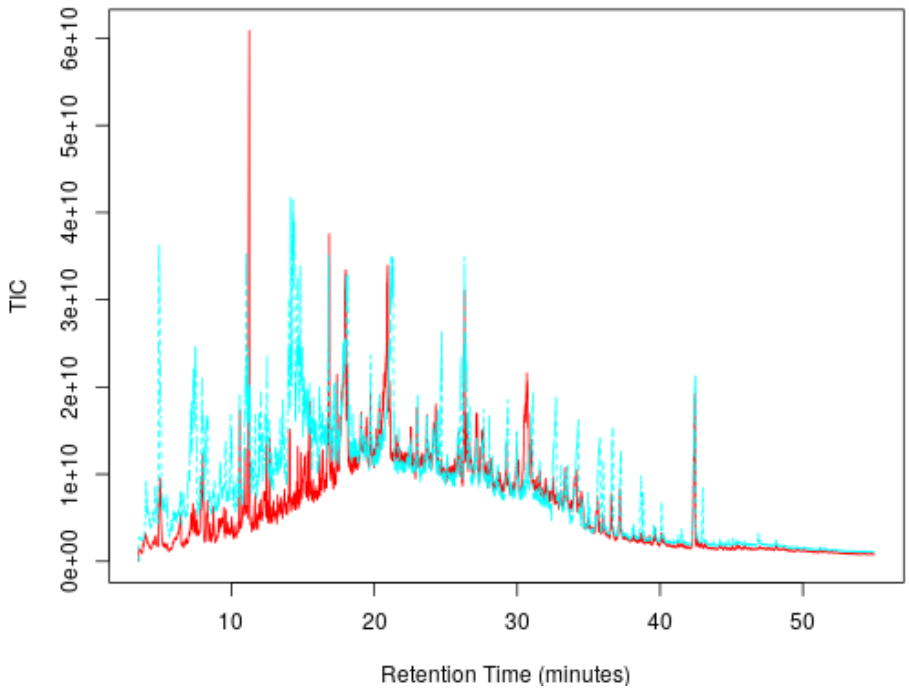


Figure 2: GC chromatograms as relative abundance expressed as the total ion current intensity with the retention time (RT, minute) for MG (red chromatogram) and PB (blue chromatogram).

Phthalates were identified in MG sample as diisobutylphtalate, dibutylphtalate, diethylphtalate, ether di-(2-ethylhexyl) phthalate and di-n-octyl phthalate.

Using mass defect plot CH_2, more hydrocarbons were detected in the PB sample than from MG, qualitatively, these compounds are mainly $C_xH_yO_z$. This could be explained by the location of PB, close to a large industrial zone. Most PB-specific peaks identified, corresponded to alkanes. The three principal peaks identified were $C_{11}H_{18}O_3^{+\cdot}$ (confidence level 4), $C_{20}H_{34}O^{+\cdot}$ (confidence level 4), seemed to correspond to a phenol C_7H_8O with an alkane chain and an isomer of octadecanoic acid $C_{18}H_{36}O_2^{+\cdot}$ (Schymanski et al., 2014).

Leachate toxicity

Sea urchin

No toxicity was found in PE 'virgin' resin and in leachates of MG plastic sample up to a concentration of 1/3. However the PB sample was highly toxic for *P. lividus* TU = 2.13 (1.82-2.45) with a NOEC of 1/10 (Table 3 and figure 3).

Table 3: Toxicity of leachates from microplastic samples (<250µm) of a 'virgin' polyethylene resin obtained from Rotogal (PE Rotogal), Petit-Bourg Viard (PB), Marie Galante Island (MG) assessed using the Paracentrotus lividus embryo test.

MPs Sample	NOEC	LOEC	Toxicity Units (TU=1/EC_{50})
PE Rotogal	1/3	1	<1
PB	1/10	1/3	2.13 (1.82-2.45)
MG	1/3	1	<1

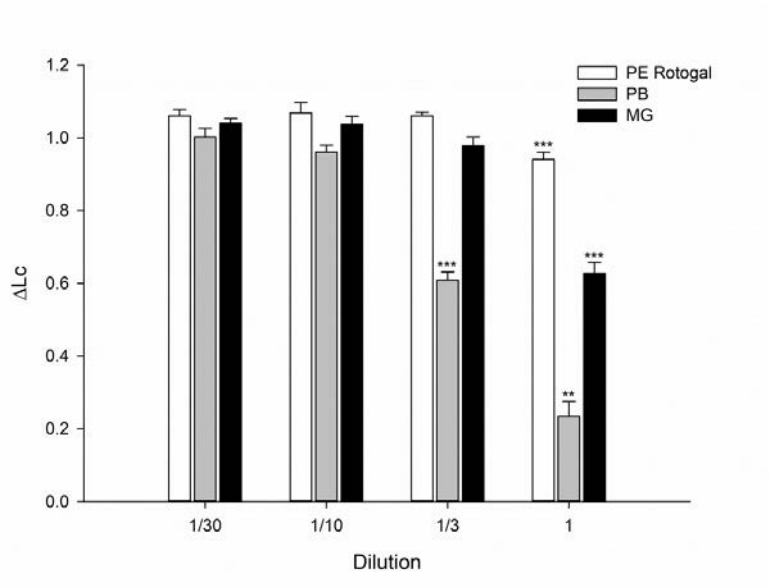


Figure 3: Larvae of Paracentrotus lividus length increase with control corrected (ΔLc) in serial dilutions of leachates from microplastic samples. *Virgin polyethylene resin obtained from Rotogal (PE Rotogal), Petit-Bourg Viard (PB) and Marie-Galante Island (MG). Bars represent mean ± SD, N=4. Asterisks refer to significant differences to the control treatment ** p<0.01 and *** p<0.001.*

Jellyfish

The first experiment performed with *A. aurita* in a semi-dynamic scenario showed no lethal effects (immobility) after the two different exposure times (24 and 48 h) at both concentrations tested (1 and 10 mg/L) and for both MPs samples. However, a significant diminution of the frequency of pulsation (Afp) of 30 and 37 % was observed in ephyrae jellyfish after 48 h of exposure to 10 mg/L MG and PB elutriates, respectively (Figure 4).

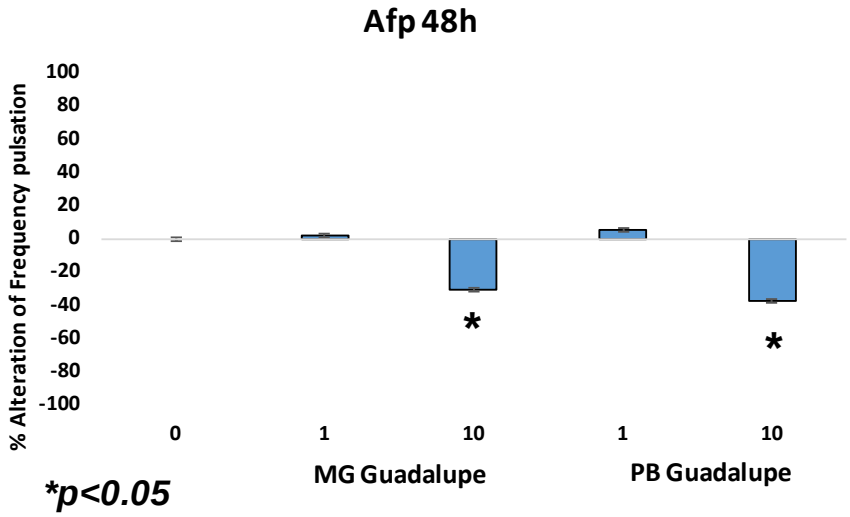


Figure 4: Alteration of frequency of pulsation Afp, after 48 h of exposure in semi-dynamic conditions to MG and PB samples at 1 and 10 mg/L, using ephyrae of Aurelia aurita. (*, p < 0.05).

The second experiment, using a rotary wheel to expose ephyrae jellyfish to environmental samples did not induce immobility or afp at 24h for all tested concentrations. After 48 hours of exposure, alterations of pulsation frequency were reported for all tested dilutions of PB, while MG affected jellyfish behaviour only from 1/10x (Figure 5).

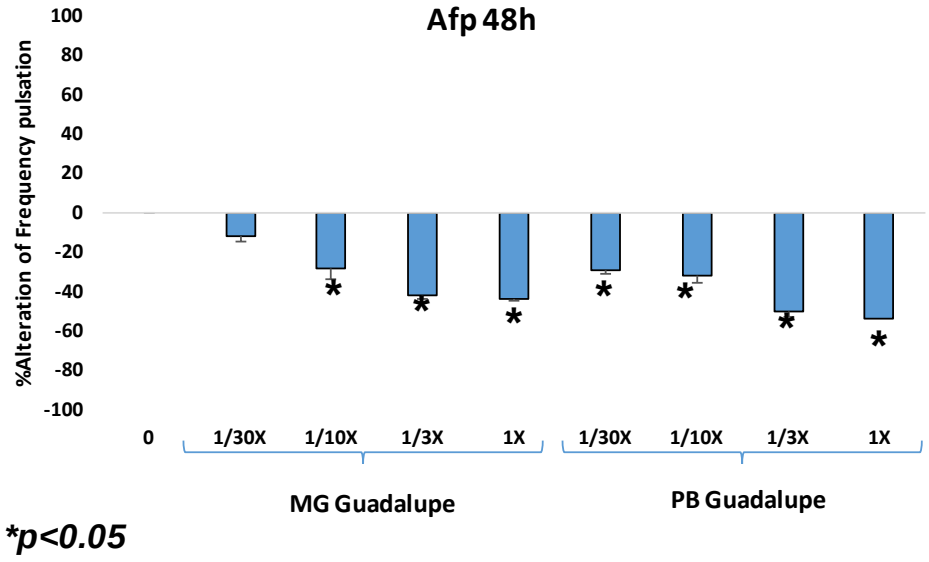


Figure 5: Alteration of frequency of pulsation Afp, after 48 h of exposure in rotary wheel to MG and PB samples from 1/30x to 1x (equivalent to 1000 mg/L), using ephyrae of Aurelia aurita. (*, p < 0.05).

Zebrafish

Zebrafish just after hatching (at 72 hpf) were exposed for 48 hours to MPs leachates. Larval survival was investigated, and no mortality was recorded, except for the positive control (40 ± 6 %). No significant developmental anomalies (skeletal and cardiac deformities) were observed except for the positive control (10 ± 2.8 %) (Figure 6). Average length of negative control larvae was 2.45 ± 0.10 mm with head length of 0.49 ± 0.01 mm. Larval size for individuals exposed to MPs leachates from the two sites at both concentrations were not significantly different from negative control larvae (Figure 7). Fish exposed to positive control were significantly smaller compared to negative control (total length 1.54 ± 0.2 mm; head length 0.23 ± 0.01 mm, Kruskall Wallis, $p < 0.05$).

No significant induction of *in vivo* EROD activity was observed in larvae exposed to MPs leachates when compared to negative control (0.021 ± 0.003 pmole/min/larvae) (Figure 8), while larvae exposed to 70 mM of BaP for 1 h showed a significant induction of EROD activity (0.039 ± 0.003 pmole/min/larvae).

The LPMR was performed at 5 dpf to monitor early behavioral disruption. There was no difference in mean velocity of larvae between treatments for a single set of light conditions (ANOVA, $p > 0.05$), (Figure 9).

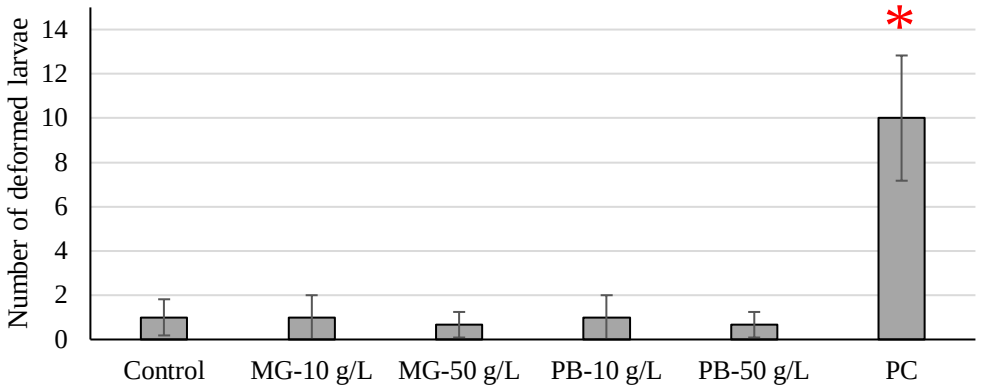


Figure 6: Number of deformed zebrafish larvae after 48h-exposure to MPs leachate from Marie-Galante and Petit Bourg. *E3 medium was used as negative control (NC) and DCA used as positive control (PC). Mean ± SD, n=30, Kruskall Wallis test, (*, p < 0.05)*

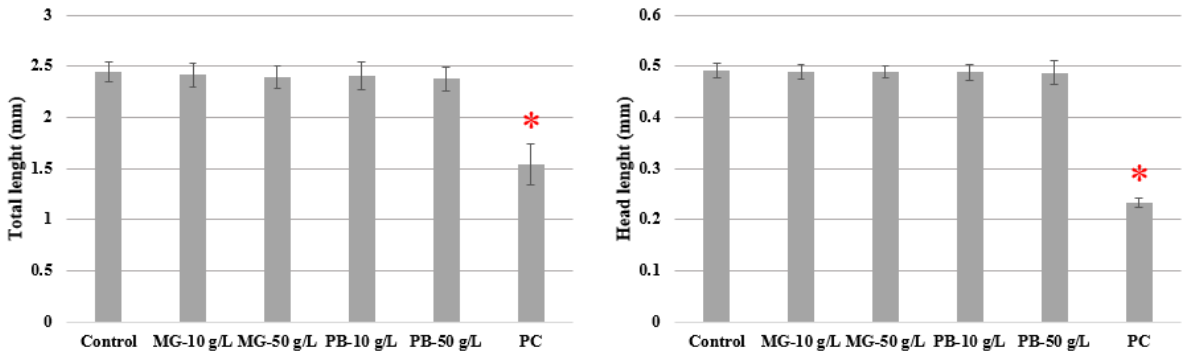


Figure 7: Environmental MPs did not induce growth retardation in zebrafish early life stages. *Zebrafish embryos were exposed after hatching, for two days to E3 medium (control), MG and PB samples at 10 and 50 g/L and positive control (PC). Total length and head length (mm) were measured. (Mean +/- SD, n=12), Kruskall Wallis (*, $p < 0.05$).*

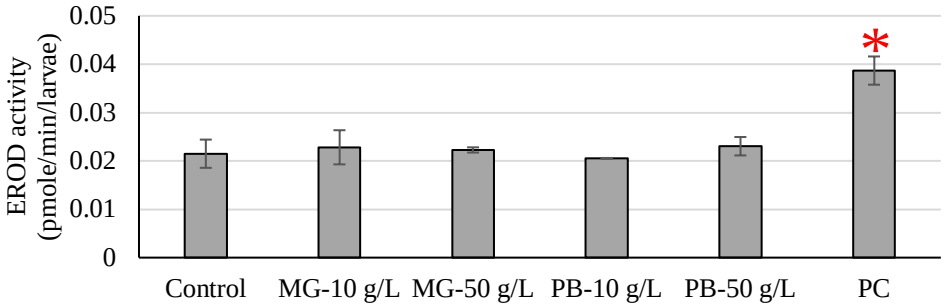


Figure 8: In vivo EROD activity in Zebrafish larvae exposed for 48 hours to MPs leachates. *EROD Activity (pmole per min and per larvae) was measured at 5 dpf zebrafish embryos exposed to negative control, MPs leachates and positive control (PC). E3 medium was used as control. Mean ± SD, n=15, one way ANOVA ($p > 0.05$).*

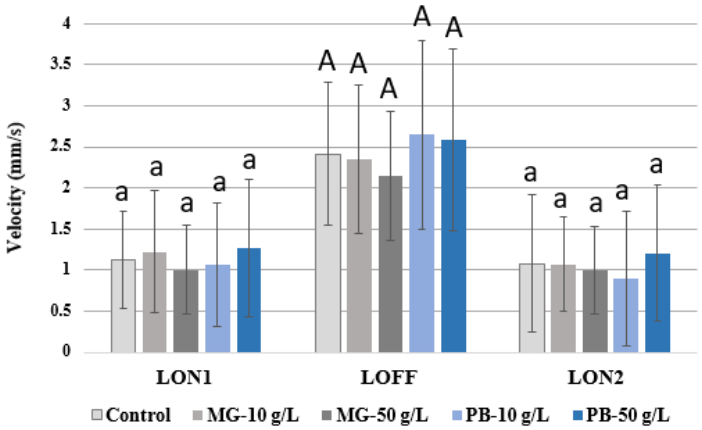


Figure 9: Larval photomotor response in 5 day-old zebrafish (Danio rerio) after exposure to negative control, MG and PB samples at 10 and 50 g/L, from hatching time. *Distance travelled over 5 min periods including two light on periods (LON1 and LON2) with one light-off period (LOFF). Data are given as mean ± SD (n = 16 to 24 larvae per treatment). The letters at the top of the bars* indicate *no significant differences between conditions for each light or black conditions (repeated–measure ANOVA).*

Discussion

Plastic characterization

MPs have been reported in the Caribbean Sea and beaches (Acosta-Coley et al., 2019; Acosta-Coley and Olivero-Verbel, 2015; Bosker et al., 2017; Bosker et al., 2018). Plastic debris have a tendency to accumulate in both oceanic gyres and coastal areas due to hydrodynamics and human activities (Eriksen et al., 2014; Law and Thompson, 2014; Lebreton et al., 2018). Beaches are known to be standing sites for marine litter, and hotspots for plastic fragmentation (Andrady, 2011; Pannetier et al., 2020). In the present study, MPs (1 to 5 mm) from both study beaches were mostly fragments (> 98 %) composed of polyethylene (PE) and polypropylene, representing more than 70 % and 20 % of the particles, respectively. Indeed, PE is the most abundant polymer found in plastic litter (Cheang et al., 2018; Fossi et al., 2017; Hidalgo-Ruz et al., 2012; Karthik et al., 2018), followed by PP and PS. The density of plastic has an influence on the location of MPs in the water column, and MPs with a density below 1 (e.g., PE, PP), tend to float (Hidalgo-Ruz et al., 2012), and are the predominant polymer reported in MPs collected on beaches. Predominance of PE and PP has previously been reported in MPs collected from several beaches across the world (Frias et al., 2010; Pannetier et al., 2020).

The chemical contamination profile of plastics collected on the two sites demonstrated similarities and dissimilarities. Halogenated compounds including brominated flame retardant, PCBs, chlorinated pesticides and chlorinated PAH (chloroacenatphthylene) were found on both sites, while unsubstituted PAHs were not detected. The major differences between the two sites consisted of higher concentrations of halogenated compounds in MG than in PB, along with the detection of phthalates (diisobutylphtalate, dibutylphtalate, diethylphtalate, ether di-(2-ethylhexyl) phthalate and di-n-octyl phthalate). MPs from PB site was characterized by the important number of hydrocarbons. This result could be explained by the proximity of the industrial area and harbour of Point-à-Pitre. Environmental samples of MPs often contained PAHs, PCBs and pesticides (Pannetier, Cachot, et al., 2019; Schönlau et al., 2019). The profile and concentration of hydrophobic organic chemicals (HOCs) are linked to the chemical exposure of particles throughout their drift history (Rochman, 2015). Koelmans et al. (2016) estimated that plastics need 2 to 4 years to reach the sorption equilibrium for all hydrophobic organic compounds (HOCs) in the marine environment, including additives and plasticizers (Koelmans et al., 2016). However, the sampling period used in this particular study was just after Hurricane Maria (October 2017) and some of the plastic debris collected on beaches came directly from inland. Indeed, it is impossible to estimate the residence time of MPs in the water,

thus making it equally impossible to evaluate the sorption equilibrium of HOCs. Sorption and desorption processes depend on multiple factors, including plastic composition and properties, plastic ageing and degradation, molecular interactions between polymer and chemicals, temperature, pH, water and biofouling (Endo and Koelmans, 2016).

Toxicity assessment using early life stages of zebrafish

Embryos and larvae of fish and invertebrates play a fundamental role in the structure and functioning of marine ecosystems. The long-term sustainability of healthy populations depends on the good health and survival of larvae (Steer et al., 2017). In addition, early life stages are particularly sensitive to pollutants (Embry et al., 2010; Lammer et al., 2009).

In the present study, the toxicity of MPs leachates produced from environmental samples of MPs have been tested on early life stages of sea urchin (*Paracentrotus lividus*), jellyfish (*Aurelia aurita*) and fish (*Danio rerio*). The main purpose of the present study was to assess the toxicity of pollutants sorbed at the surface of MPs. Results obtained demonstrated that the PB sample was highly toxic for *P.lividus* from a concentration of 1/10. Similarly, diminished pulsation of A.aurita was observed in organisms exposed for all tested dilutions of PB, while jellyfish exposed to the MG sample showed an alteration of afp only from 1/10. These results highlighted that toxicity seemed to be linked to the samples of MPs. However, in both samples, MPs were mainly fragments and the polymer composition was similar (> 70 % of PE and > 20 % of PP). The major differences were in chemical contamination. Indeed, in the present study, environmental MPs were contaminated with different pollutants and the PB sample was characterized by an important amount of hydrocarbons in comparison with MG sample. However, metal analyses are on-going, and their potential presence may explain differences in toxicity. In fact, previous leachate analyses have highlighted the chemical contamination at the surface of MPs with the presence of phthalates and metals, used as plasticizers, stabilizers or coloring agents (Oliviero et al., 2019; Omolaoye et al., 2010). Metal stabilizers and heavy-metal pigments are not chemically bound to the polymers, meaning that they can easily leak out and cause damage (Guney and Zagury, 2012; Omolaoye et al., 2010).

A study using leachates of pristine PVC in parallel with MPs obtained from plastic toys made of PVC showed that the toys had the higher level of toxicity for *P. lividus* embryo-larval (Oliviero et al., 2019). Zebrafish larvae exposed to leachates of MPs from both environmental samples in the present study dit no exhibit either lethal or sub-lethal effects. The toxicity of

industrial MPs using early life stages of zebrafish demonstrated the absence of deleterious effects on several aquatic species (Beiras et al., 2018; Cormier et al., 2019). However, leachates of environmental microplastics such as fishing nets, fishing cages and packaging collected at sea had toxic effects on the size of *Paracentrotus lividus* larvae (Beiras et al., 2018). Toxic effects were observed on sea urchin embryos exposed at high concentrations for 48 hours (Beiras et al., 2018), while in the present study, zebrafish were exposed at the larval stage. The stage of exposure and the duration of exposure is important. For example, Japanese medaka (*Oryzias latipes*) exposed for 14 or 30 days to different environmental MPs collected on beaches demonstrated a difference of sensitivity. Indeed, at 14 days, slight effects were observed while, after 30 days, toxic effects were more important (Pannetier et al., 2020). Furthermore, a recent study has shown the direct impact of HDPE microparticles on development and swimming behaviour of oyster larvae (Bringer et al, 2020).

Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Author Contributions

BC, BM, TT, CG, FG, MF, XC, MLB, RB and JC designed the experiment; BC, FD and AK performed the chemical analysis; BC and FL conducted the samples preparation and the polymer characterization; BC, CC and QP performed toxicological analysis using zebrafish; TT and RB conducted toxicological analysis using sea urchin; CG and EC conducted toxicological analysis using jellyfish; BC, BM, JC, KVA and SL participated in the sample collection. BC wrote the manuscript and all authors corrected the manuscript.

Funding

This work was developed under the EPHEMARE project (Ecotoxicological effects of microplastics in marine ecosystems), supported by national funding agencies within the framework of JPI Oceans (FCT JPIOCEANS/0005/2015; FORMAS, 2015-01865; ANR-15-JOCE-0002-01; German BMBF 03F0735A). Bettie Cormier was directly supported by an IdEx grant from the University of Bordeaux.

References

Acosta-Coley I., Mendez-Cuadro D., Rodriguez-Cavallo E., de la Rosa J., Olivero-Verbel J. (2019). Trace elements in microplastics in Cartagena: A hotspot for plastic pollution at the Caribbean. Marine Pollution Bulletin. 139, 402-411

Acosta-Coley I., Olivero-Verbel J. (2015). Microplastic resin pellets on an urban tropical beach in Colombia. Environmental Monitoring and Assessment. 187, 435

Andrady A. L. (2011). Microplastics in the marine environment. Marine Pollution Bulletin. 62, 1596-1605

Arthur C., Baker J. E., Bamford H. A. (2009). Proceedings of the International Research Workshop on the Occurrence, Effects, and Fate of Microplastic Marine Debris, September 9-11, 2008, University of Washington Tacoma, Tacoma, WA, USA.

Barnes D. K. A., Galgani F., Thompson R. C., Barlaz M. (2009). Accumulation and fragmentation of plastic debris in global environments. Philosophical Transactions of the Royal Society B: Biological Sciences. 364, 1985-1998

Beiras R., Bellas J., Cachot J., Cormier B., Cousin X., Engwall M., Gambardella C., Garaventa F., Keiter S., Le Bihanic F., et al. (2018). Ingestion and contact with polyethylene microplastics does not cause acute toxicity on marine zooplankton. Journal of Hazardous Materials. 360, 452-460

Beiras R., Tato T., López-Ibáñez S. (2019). A 2-Tier standard method to test the toxicity of microplastics in marine water using Paracentrotus lividus and Acartia clausi larvae. Environmental Toxicology and Chemistry. 38, 630-637

Bejgarn S., MacLeod M., Bogdal C., Breitholtz M. (2015). Toxicity of leachate from weathering plastics: An exploratory screening study with Nitocra spinipes. Chemosphere. 132, 114-119

Belanger S. E., Rawlings J. M., Carr G. J. (2013). Use of fish embryo toxicity tests for the prediction of acute fish toxicity to chemicals. Environmental Toxicology and Chemistry. 32, 1768-1783

Bellas J., Granmo Å., Beiras R. (2005). Embryotoxicity of the antifouling biocide zinc pyrithione to sea urchin (Paracentrotus lividus) and mussel (Mytilus edulis). Marine Pollution Bulletin. 50, 1382-1385

Bosker T., Behrens P., Vijver M. G. (2017). Determining global distribution of microplastics by combining citizen science and in-depth case studies. Integrated Environmental Assessment and Management. 13, 536-541

Bosker T., Guaita L., Behrens P. (2018). Microplastic pollution on Caribbean beaches in the Lesser Antilles. Marine Pollution Bulletin. 133, 442-447

Braunbeck T., Böttcher M., Holler H., Rudolf T. K. L. L., Seitz N. (2005). Towards an alternative for the acute fish LC(50) test in chemical assessment: the fish embryo toxicity test goes multi-species -- an update. ALTEX. 22, 87-102

Braunbeck T., Kais B., Lammer E., Otte J., Schneider K., Stengel D., Strecker R. (2015). The fish embryo test (FET): origin, applications, and future. Environmental Science and Pollution Research. 22, 16247-16261

Bringer A., Thomas H., Prunier G., Dubillot E., Bossut N., Churlaud C., Clérandeau C., Le Bihanic F., Cachot J. (2020). High density polyethylene (HDPE) microplastics impair development and swimming activity of Pacific oyster D-larvae, Crassostrea gigas, depending on particle size. Environmental Pollution. 260, 113978

Browne Mark A., Niven Stewart J., Galloway Tamara S., Rowland Steve J., Thompson Richard C. (2013). Microplastic Moves Pollutants and Additives to Worms, Reducing Functions Linked to Health and Biodiversity. Current Biology. 23, 2388-2392

Cardona L., Álvarez de Quevedo I., Borrell A., Aguilar A. (2012). Massive Consumption of Gelatinous Plankton by Mediterranean Apex Predators. PLOS ONE. 7, e31329

Cheang C. C., Ma Y., Fok L. (2018). Occurrence and Composition of Microplastics in the Seabed Sediments of the Coral Communities in Proximity of a Metropolitan Area. Int J Environ Res Public Health. 15, 2270

Cole M., Lindeque P., Halsband C., Galloway T. S. (2011). Microplastics as contaminants in the marine environment: A review. Marine Pollution Bulletin. 62, 2588-2597

Cormier B., Batel A., Cachot J., Bégout M.-L., Braunbeck T., Cousin X., Keiter S. H. (2019). Multi-Laboratory Hazard Assessment of Contaminated Microplastic Particles by Means of Enhanced Fish Embryo Test With the Zebrafish (Danio rerio). 7,

Costa E., Gambardella C., Piazza V., Greco G., Lavorano S., Beltrandi M., Bongiovanni E., Gnone G., Faimali M., Garaventa F. (2015). Effect of neurotoxic compounds on ephyrae of Aurelia aurita jellyfish. Hydrobiologia. 759, 75-84

Derraik J. G. B. (2002). The pollution of the marine environment by plastic debris: a review. Marine Pollution Bulletin. 44, 842-852

Embry M. R., Belanger S. E., Braunbeck T. A., Galay-Burgos M., Halder M., Hinton D. E., Léonard M. A., Lillicrap A., Norberg-King T., Whale G. (2010). The fish embryo toxicity test as an animal alternative method in hazard and risk assessment and scientific research. Aquat Toxicol. 97, 79-87

Endo S., Koelmans A. A. 2016. Sorption of hydrophobic organic compounds to plastics in marine environments: Equilibrium. In: Hazardous Chemicals Associated with Plastics in the Marine Environment. Springer International Publishing Switzerland. p. 185-204.

Eriksen M., Lebreton L. C. M., Carson H. S., Thiel M., Moore C. J., Borerro J. C., Galgani F., Ryan P. G., Reisser J. (2014). Plastic Pollution in the World's Oceans: More than 5 Trillion Plastic Pieces Weighing over 250,000 Tons Afloat at Sea. PLOS ONE. 9, e111913

Faimali M., Garaventa F., Piazza V., Costa E., Greco G., Mazzola V., Beltrandi M., Bongiovanni E., Lavorano S., Gnone G. (2014). Ephyra jellyfish as a new model for ecotoxicological bioassays. Marine Environmental Research. 93, 93-101

Fossi M. C., Romeo T., Baini M., Panti C., Marsili L., Campani T., Canese S., Galgani F., Druon J.-N., Airoldi S., et al. (2017). Plastic Debris Occurrence, Convergence Areas and Fin Whales Feeding Ground in the Mediterranean Marine Protected Area Pelagos Sanctuary: A Modeling Approach. 4,

Frias J. P. G. L., Sobral P., Ferreira A. M. (2010). Organic pollutants in microplastics from two beaches of the Portuguese coast. Marine Pollution Bulletin. 60, 1988-1992

Gandara e Silva P. P., Nobre C. R., Resaffe P., Pereira C. D. S., Gusmão F. (2016). Leachate from microplastics impairs larval development in brown mussels. Water Research. 106, 364-370

Geyer R., Jambeck J. R., Law K. L. (2017). Production, use, and fate of all plastics ever made. Science Advances. 3, e1700782

Guney M., Zagury G. J. (2012). Heavy Metals in Toys and Low-Cost Jewelry: Critical Review of U.S. and Canadian Legislations and Recommendations for Testing. Environmental Science & Technology. 46, 4265-4274

Hidalgo-Ruz V., Gutow L., Thompson R. C., Thiel M. (2012). Microplastics in the Marine Environment: A Review of the Methods Used for Identification and Quantification. Environmental Science & Technology. 46, 3060-3075

Jambeck J. R., Geyer R., Wilcox C., Siegler T. R., Perryman M., Andrady A., Narayan R., Law K. L. (2015). Plastic waste inputs from land into the ocean. Science. 347, 768

Jönsson E. M., Brandt I., Brunström B. (2002). Gill Filament-Based EROD Assay for Monitoring Waterborne Dioxin-like Pollutants in Fish. Environmental Science & Technology. 36, 3340-3344

Karapanagioti H. K., Klontza I. (2008). Testing phenanthrene distribution properties of virgin plastic pellets and plastic eroded pellets found on Lesvos island beaches (Greece). Marine Environmental Research. 65, 283-290

Karthik R., Robin R. S., Purvaja R., Ganguly D., Anandavelu I., Raghuraman R., Hariharan G., Ramakrishna A., Ramesh R. (2018). Microplastics along the beaches of southeast coast of India. Science of The Total Environment. 645, 1388-1399

Koelmans A. A. 2015. Modeling the role of microplastics in bioaccumulation of organic chemicals to marine aquatic organisms. A critical review. In: Marine Anthropogenic Litter. p. 309-324.

Koelmans A. A., Bakir A., Burton G. A., Janssen C. R. (2016). Microplastic as a Vector for Chemicals in the Aquatic Environment: Critical Review and Model-Supported Reinterpretation of Empirical Studies. Environmental Science and Technology. 50, 3315-3326

Koelmans A. A., Besseling E., Foekema E. M. (2014). Leaching of plastic additives to marine organisms. Environmental Pollution. 187, 49-54

Lammer E., Carr G. J., Wendler K., Rawlings J. M., Belanger S. E., Braunbeck T. (2009). Is the fish embryo toxicity test (FET) with the zebrafish (Danio rerio) a potential alternative for the fish acute toxicity test? Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology. 149, 196-209

Law K. L., Thompson R. C. (2014). Microplastics in the seas. Science. 345, 144

Le Bihanic F., Clérandeau C., Le Menach K., Morin B., Budzinski H., Cousin X., Cachot J. (2014). Developmental toxicity of PAH mixtures in fish early life stages. Part II: adverse effects in Japanese medaka. Environmental Science and Pollution Research. 21, 13732-13743

Le Bihanic F., Couillard C. M., Rigaud C., Légaré B. (2013). A simple and reliable in vivo EROD activity measurement in single Fundulus heteroclitus embryo and larva. Marine Environmental Research. 84, 17-23

Lebreton L., Slat B., Ferrari F., Sainte-Rose B., Aitken J., Marthouse R., Hajbane S., Cunsolo S., Schwarz A., Levivier A., et al. (2018). Evidence that the Great Pacific Garbage Patch is rapidly accumulating plastic. Scientific Reports. 8, 4666

Lippiatt S., Opfer S., Arthur C. (2013). Marine Debris Monitoring and Assessment. NOAA Technical Memorandum. NOS-OR&R-46

Lithner D., Damberg J., Dave G., Larsson Å. (2009). Leachates from plastic consumer products – Screening for toxicity with Daphnia magna. Chemosphere. 74, 1195-1200

Lithner D., Larsson Å., Dave G. (2011). Environmental and health hazard ranking and assessment of plastic polymers based on chemical composition. Science of The Total Environment. 409, 3309-3324

Lorenzo J. I., Nieto O., Beiras R. (2002). Effect of humic acids on speciation and toxicity of copper to Paracentrotus lividus larvae in seawater. Aquatic Toxicology. 58, 27-41

Mazurais D., Ernande B., Quazuguel P., Severe A., Huelvan C., Madec L., Mouchel O., Soudant P., Robbens J., Huvet A., et al. (2015). Evaluation of the impact of polyethylene microbeads ingestion in European sea bass (Dicentrarchus labrax) larvae. Marine Environmental Research. 112, 78-85

Nagel R. (2002). DarT: The embryo test with the zebrafish Danio rerio - A general model in ecotoxicology and toxicology. ALTEX. 19, 38-48

OECD. 1998. Test No. 212: Fish, Short-term Toxicity Test on Embryo and Sac-Fry Stages.

OECD. 2013. Test No. 236: Fish Embryo Acute Toxicity (FET) Test.

Oliviero M., Tato T., Schiavo S., Fernández V., Manzo S., Beiras R. (2019). Leachates of micronized plastic toys provoke embryotoxic effects upon sea urchin Paracentrotus lividus. Environmental Pollution. 247, 706-715

Omolaoye J. A., Uzairu A., Gimba C. E. Heavy metal assessment of some soft plastic toys imported into Nigeria from China; 2010.

Pannetier P., Cachot J., Clérandeau C., Faure F., Van Arkel K., de Alencastro L. F., Levasseur C., Sciacca F., Bourgeois J.-P., Morin B. (2019). Toxicity assessment of pollutants sorbed on environmental sample microplastics collected on beaches: Part I-adverse effects on fish cell line. Environmental Pollution. 248, 1088-1097

Pannetier P., Morin B., Clérandeau C., Laurent J., Chapelle C., Cachot J. (2019). Toxicity assessment of pollutants sorbed on environmental microplastics collected on beaches: Part II-adverse effects on Japanese medaka early life stages. Environmental Pollution. 248, 1098-1107

Pannetier P., Morin B., Le Bihanic F., Dubreil L., Clérandeau C., Chouvellon F., Van Arkel K., Danion M., Cachot J. (2020). Environmental samples of microplastics induce significant toxic effects in fish larvae. Environment International. 134, 105047

Pedà C., Caccamo L., Fossi M. C., Gai F., Andaloro F., Genovese L., Perdichizzi A., Romeo T., Maricchiolo G. (2016). Intestinal alterations in European sea bass Dicentrarchus labrax (Linnaeus, 1758) exposed to microplastics: Preliminary results. Environmental Pollution. 212, 251-256

Pittura L., Avio C. G., Giuliani M. E., d'Errico G., Keiter S. H., Cormier B., Gorbi S., Regoli F. (2018). Microplastics as Vehicles of Environmental PAHs to Marine Organisms: Combined Chemical and Physical Hazards to the Mediterranean Mussels, Mytilus galloprovincialis. 5,

PlasticsEurope (2018). PlasticsEurope: Annual review _ The Facts 2017-2018.

Rochman C. M. 2015. The Complex Mixture, Fate and Toxicity of Chemicals Associated with Plastic Debris in the Marine Environment. In: Marine Anthropogenic Litter. Cham: Springer International Publishing. p. 117-140.

Rochman C. M., Hentschel B. T., Teh S. J. (2014). Long-Term Sorption of Metals Is Similar among Plastic Types: Implications for Plastic Debris in Aquatic Environments. PLOS ONE. 9, e85433

Rochman C. M., Hoh E., Kurobe T., Teh S. J. (2013). Ingested plastic transfers hazardous chemicals to fish and induces hepatic stress. Scientific Reports. 3, 3263

Rouchon A. M., Phillips N. E. (2017). Short larval exposure to low level of copper has long-lasting latent effects on juvenile performance in the sea urchin Evechinus chloroticus. Marine Ecology Progress Series. 579, 67-79

Saco-Álvarez L., Durán I., Ignacio Lorenzo J., Beiras R. (2010). Methodological basis for the optimization of a marine sea-urchin embryo test (SET) for the ecological assessment of coastal water quality. Ecotoxicology and Environmental Safety. 73, 491-499

Schönlau C., Larsson M., Lam M. M., Engwall M., Giesy J. P., Rochman C., Kärrman A. (2019). Aryl hydrocarbon receptor-mediated potencies in field-deployed plastics vary by type of polymer. Environmental Science and Pollution Research. 26, 9079-9088

Schymanski E. L., Jeon J., Gulde R., Fenner K., Ruff M., Singer H. P., Hollender J. (2014). Identifying Small Molecules via High Resolution Mass Spectrometry: Communicating Confidence. Environmental Science & Technology. 48, 2097-2098

Steer M., Cole M., Thompson R. C., Lindeque P. K. (2017). Microplastic ingestion in fish larvae in the western English Channel. Environmental Pollution. 226, 250-259

Teuten E. L., Saquing J. M., Knappe D. R. U., Barlaz M. A., Jonsson S., Björn A., Rowland S. J., Thompson R. C., Galloway T. S., Yamashita R., et al. (2009). Transport and release of chemicals from plastics to the environment and to wildlife. Philosophical Transactions of the Royal Society B: Biological Sciences. 364, 2027-2045

Titelman J., Hansson L. J. 2006. Feeding rates of the jellyfish Aurelia aurita on fish larvae. In: Marine Biology. Springer Berlin Heidelberg. p. 297-306.

Vignet C., Le Menach K., Mazurais D., Lucas J., Perrichon P., Le Bihanic F., Devier M.-H., Lyphout L., Frère L., Bégout M.-L., et al. (2014). Chronic dietary exposure to pyrolytic and petrogenic mixtures of PAHs causes physiological disruption in zebrafish - part I: Survival and growth. Environmental Science and Pollution Research. 21, 13804-13817

Westerfield M. 2000. The zebrafish book. A guide for the laboratory use of zebrafish (Danio rerio). 4th edition.: Univ. of Oregon Press, Eugene.

Supplementary material

Table 1, SM: Fold change comparison between PB and MG samples. Confidence level were selected according to Schymanski et al., 2014.

	m/z	rt	formula	Delta (ppm)	Mass fragments	Formula fragments	Delta (ppm)	Confidence level*	Higher in MG or PB?
	295.0668	11.3	C14H22O	-0.26	191.1429 175.1117 163.1117 147.0804 135.0805 109.0648 77.0386	C13H19O C12H15O C11H15O C10H11O C9H11O C7H9O C6H5	-0.73 -0.32 -0.28 -0.17 0.08 0.29 -0.29	4	MG
M+H of trichlorobenzene	**180.9372**	**13.98**	**C6H4Cl3**	**-0.72**				**2**	**MG**
	247.2055	14.28	C17H27O	-1.06	149.0959 135.0803 107.0491 91.0542	C10H13O C9H11O C7H7O C7H7	-1.13 -0.81 -0.51 -0.59	4	PB
	191.1427	14.35	C13H19O	-1.61	247.2053 149.0959 135.0803 121.0647 107.0490 91.0542	C17H27O C10H13O C9H11O C8H9O C7H7O C7H7	-1.46 -1.44 -1.03 -0.83 -0.95 -0.81	4	PB
	219.1377	14.85	C14H19O2	-1.07	191.1064 175.0751 159.0803	C12H15O2 C11H11O2 C11H11O	-1.4 -1.32 -1.1	4	MG
	135.0803	14.9	C9H11O	-0.8	149.0960 121.0648 107.0491 91.0542	C10H13O C8H9O C7H7O C7H7	-0.93 -0.29 -0.56 -0.49	4	PB
	175.148	15.07	$C_{13}H_{19}$	-0.93	161.1324 119.0855 91.0542 79.0542	$C_{12}H_{17}$ C_9H_{11} C_7H_7 C_6H_7	-0.73 -0.17 -0.32 -0.52	4	PB
	203.0854	16.23	$C_{16}H_{11}$	-0.52				4	PB
	189.0908	17.06	$C_{12}H_{13}O_2$	-1.2	245.1534 147.0803 123.1166	$C_{16}H_{21}O_2$ $C_{10}H_{11}O$ C_9H_{15}	-0.74 -0.87 -1.73	4	MG
	273.1484	21.78	$C_{17}H_{21}O_3$	-0.27				4	MG
	226.1714	21.88	$C_{17}H_{22}$	-0.37				4	MG
Bumetrizole (UV stabilizer)	**300.0897**	**26.4**	**$C_{16}H_{15}ON_3Cl$**	**-0.21**	**315.1133 272.0585**	**$C_{17}H_{18}ON_3Cl$ $C_{14}H_{11}ON_3Cl$**	**-0.07 -0.06**	**2**	**MG**
	323.2369	26.54	$C_{23}H_{31}O$	-0.56	366.2914 281.1898 199.1115 123.0803 85.1011 71.0854	$C_{26}H_{38}O$ $C_{20}H_{25}O$ $C_{14}H_{15}O$ $C_8H_{11}O$ C_6H_{13} C_5H_{11}	-0.78 -0.55 -0.97 -0.83 -1.07 -1.16	4	MG
	351.2683	27.17	$C_{25}H_{35}O$					4	MG
	185.096	27.44	$C_{13}H_{13}O$					4	MG
	323.2369	27.44	$C_{23}H_{31}O$					4	MG
	309.2212	27.44	$C_{22}H_{29}O$					4	MG
	120.057	27.47	C_8H_8O	0.24				4	PB
	281.19	27.85	$C_{20}H_{25}O$					4	MG
	185.096	27.85	$C_{13}H_{13}O$					4	MG
	309.2212	27.86	$C_{22}H_{29}O$					4	MG
	309.2212	28.14	$C_{22}H_{29}O$					4	MG
	219.1744	29.9	$C_{15}H_{23}O$	0.11	203.143	$C_{14}H_{19}O$	-0.11	4	MG
UV stabilizer	**326.1879**	**30.1**	**$C_{21}H_{26}O_3$**	**-0.63**	**213.0546 137.0234**	**$C_{13}H_9O_3$ $C_7H_5O_3$**	**-0.04 0.42**	**2**	**MG**
	229.2162	30.33	$C_{14}H_{29}O_2$	-0.15	155.143 123.0804	$C_{10}H_{19}O$ $C_8H_{11}O$	-0.36 -0.54	4	PB
	365.3202	32	$C_{27}H_{41}$	0.13	231.2109 159.1169 123.1169 79.0543	$C_{17}H_{27}$ $C_{12}H_{15}$ C_9H_{15} C_6H_7	0.69 0.38 0.88 1.03	4	MG

Article 5

Chronic exposure to environmental microplastics reduce growth and reproduction efficiency in F0 and F1 survival in zebrafish (*Danio rerio*)

Bettie Cormier, Jérôme Cachot, Mathieu Cabar, Sarah Zapata, Mélanie Blanc, Christelle Clérandeau, Florane Le Bihanic, Marie-Laure Bégout, Xavier Cousin

Chronic exposure to environmental microplastics reduce growth and reproduction efficiency in F0 and F1 survival in zebrafish, *Danio rerio*

Bettie Cormier[1,2], Jérôme Cachot[1], Mathieu Cabar[3], Sarah Zapata[1], Mélanie Blanc[2], Christelle Clérandeau[1], Marie-Laure Bégout[7, 8], Xavier Cousin[8,9], Jérôme Cachot[1*]

[1] Bordeaux University, EPOC, UMR CNRS 5805, Avenue des Facultés, 33400 Talence, France

[2] Man-Technology-Environment Research Centre, School of Science and Technology, Örebro University, Fakultetsgatan 1, 701 82 Örebro, Sweden

[3] L'HOUMEAU

[4] Race for Water Foundation, Lausanne, 1007, Switzerland

[5] UMR BOREA CNRS-7208, IRD-207, MNHN, UPMC, UCBN, Université des Antilles et de la Guyane, DYNECAR, Campus de Fouillole, BP 592, 97159, Pointe-à-Pitre, France

[6] Institut des Molécules et Matériaux du Mans (IMMM, UMR CNRS 6283), Université du Maine, Avenu Olivier Messiaen, Le Mans F-72085, France

[7] Ifremer, UMR MARBEC, 34250 Palavas, France.

[8] MARBEC, Univ. Montpellier, CNRS, Ifremer, IRD, , 34250 Palavas-les-flots, France

[9]Université Paris-Saclay, INRAE, AgroParisTech, GABI, 78350, Jouy-en-Josas, France

Abstract

Microplastics (MPs) are widely present in aquatic ecosystems, and can be ingested by several organisms. However, toxicity assessments focusing on thephysical and chemical effects of environmental MPs on aquatic organisms are relatively rare. The present study aims to investigate the toxicity of environmental microplastics over different life stages of zebrafish (*Danio rerio*). MP samples were collected from two beaches in the Guadeloupe archipelago, Marie Galante (MG) and Petit-Bourg (PB) located near the North Atlantic gyre. Both MPs samples had the same polymer composition, mainly polyethylene (> 70 %) and polypropylene (> 20 %), but different micropolluant profiles. First, early life stages of zebrafish were exposed either to organic extracts (1, 10 and 100 mg/L) or particles (1 and 10 mg/L). Toxicity assessment involved monitoring of mortality, developmental toxicity, EROD activity and larval swimming behavior. Juvenile and adult zebrafish were then exposed to MPs over 4.5 months, via 1 % of MPs being added to their food. The exposure of early life stages to particles did not induce toxic effects, while high concentrations of organic extracts induced EROD activity for PB samples and larval swimming hyperactivity for both MG and PB samples. Chronic dietary exposure led to an alteration of growth in females both MG and PB samples compared to controls, along with a decrease in the reproductive success for PB sample. A decrease in survival rate was observed for the F1 offspring generation from zebrafish exposed to MG sample compared to controls. Larval swimming activity was increased for the offspring of PB sample exposed fish. This study highlights the ecotoxicological consequences of both short and long-term exposure to environmental microplastics relevant to coastal marine areas, which represent essential habitats for a wide range of aquatic organisms, including teleost.

Introduction

Over the last 60 years, plastics have become an environmental concern, attracting interest from public, media and scientists (Law and Thompson, 2014). Total plastic production reached up almost 350 million tons in 2017, fueled by an ever more intense use of plastic products (PlasticsEurope, 2018). These huge quantities of plastic being produced have led to millions of tons of plastic waste accidently or intentionally being discharged into aquatic environment (Geyer et al., 2017; Jambeck et al., 2015). Plastics in the environment tend to degrade and fragment into smaller particles via chemical, physical or biological processes (Barnes et al., 2009). Manufactured plastic debris typically ends up in oceans, and will eventually show up as macroplastics (> 5 mm) or microplastics (MPs), i.e. particles between 1 µm and 5 mm in size (Arthur et al., 2009; Jambeck et al., 2015; Thompson et al., 2009). MPs consist of either plastic particles designed for the purposes of plastic production (de Sá et al., 2018; Ogata et al., 2009) or small plastic fragments derived from the breakdown of larger pieces (Anbumani and Kakkar, 2018; Derraik, 2002; Li et al., 2018; Thompson et al., 2004). MPs are now considered as a potential threat for aquatic ecosystems (Cole et al., 2011; Pannetier, Morin, et al., 2019; Pannetier et al., 2020).

Due to their slow degradation rate (Cole et al., 2011), plastic debris tend to accumulate in oceanic gyres but also in coastal areas (Eriksen et al., 2014; Lebreton et al., 2018). Beaches are hotspots for MPs and, more generally, from plastic pollution caused by the fragmentation of macro debris (Andrady, 2011). The presence of MPs on beaches has been reported in various areas around the world, including the Caribbean Sea (Acosta-Coley et al., 2019; Acosta-Coley and Olivero-Verbel, 2015; Bosker et al., 2017; Bosker et al., 2018).

Plastic production uses additives to improve specific characteristics. It has been demonstrated that these additives can be toxic (Barnes et al., 2009; Lithner et al., 2011). In addition, given their persistence in aquatic environments, MPs are globally distributed in various environments worldwide including some far from source areas (Bogdal et al., 2013; Corsolini, 2009; Rigét et al., 2016). Besides additives, MPs particles have been shown to sorb significant amounts of persistent organic pollutants from the surrounding environment (Barnes et al., 2009; Koelmans et al., 2016; Mato et al., 2001; Rios et al., 2007). Weathering processes, combined with fragmentation, can lead to an increase of the reactive specific surface area of particles and further facilitate the sorption of pollutants (Teuten et al., 2007). Chemicals adsorbed during the ageing of plastic are mostly hydrophobic organic compounds, including polychlorinated biphenyls (PCBs), polycyclic aromatic compounds (PAHs) and organochlorine pesticides

(Derraik, 2002; Karapanagioti and Klontza, 2008; Koelmans et al., 2016), but can also include trace metal elements (Rochman, Kurobe, et al., 2014). MPs are believed to act as a 'Trojan Horse', transporting a wide range of pollutants into the marine environment (Andrady, 2011; Koelmans et al., 2014).

Plastic contamination of marine ecosystem has been widely reported (Law and Thompson, 2014), but only a few studies have investigated deleterious effects of MPs on aquatic organisms. Toxicological effects induced by chemicals sorbed on MPs or included in MPs such as additives, e.g. bisphenol A, phthalates, UV stabilizer, etc. (Koelmans, 2015; Koelmans, Besseling and Foekema, 2014; Rochman, 2015) or pollutants (Pannetier, Cachot, et al., 2019; Pannetier, Morin, et al. 2019) have been documented.

Early life stages of fish are currently used in ecotoxicology, mainly due to their high sensitivity to diverse chemicals (Beiras et al., 2018; Embry et al., 2010; Lammer et al., 2009; Oliviero et al., 2019). Early life stages of zebrafish (*Danio rerio*) have been demonstrated to be as sensitive to chemical pollutants as adult fish (Belanger et al., 2013; Braunbeck et al., 2005; Braunbeck et al., 2015; Lammer et al., 2009; Nagel, 2002). The zebrafish model has been used to investigate dietary exposure to various pollutants (Alfonso et al., 2019; Horri et al., 2018). Indeed the different routes of exposure are direct waterbone exposure and trophic exposure via food. MPs sizes favor ingestion by organisms, and their transfer into food webs has been reported in laboratory studies (Farrell and Nelson, 2013; Pannetier et al., 2020; Setälä et al., 2014).

After ingestion, MPs can cause a variety of toxic effects to aquatic organisms, such as physical effects or chemical effects. Clogging of the digestive tract (Cole et al., 2011), ulcerative lesions or gastric rupture (Law and Thompson, 2014) or intestinal alterations (Pedà et al., 2016) are some of the physical alterations observed after MPs exposure. The ingestion of MPs may cause endocrine disruption and hepatic stress including CYP1A expression, oxidative stress, changes in metabolic parameters, decrease in enzyme activity, and cellular necrosis (Browne et al., 2013; Law and Thompson, 2014; Mazurais et al., 2015; Oliveira et al., 2013; Rochman, Hoh, Kurobe, et al., 2013; Rochman, Kurobe, et al., 2014; Teuten et al., 2009). The ingestion of MPs has been shown to induce other toxic effects, and may compromise growth and organism development, reproduction dynamics and the survival of a population. However, to date, studies investigating the toxicity of environmental MPs samples are scarce (Pannetier, Cachot et al., 2019; Pannetier, Morin, et al., 2019).

In 2017, a sampling campaign was carried out across all oceans (Race for Water Odyssey 2017-2021) by the Race for Water Foundation, to collect MPs from the water column, sediments, living organisms and beaches. Sampling sites were selected in the North Atlantic gyre, with a stopover in the Caribbean Islands, including two beaches in Basse-Terre and Marie-Galante located in the Guadeloupe archipelago. The purpose of this study was to investigate the toxicity of those environmental MPs samples on different life stages of zebrafish (*Danio rerio*) such as embryo-larval, juveniles and adults stages. Embryos were exposed to either organic extracts or MPs particles, and juveniles/adults were fed with food supplemented with environmental MPs as 100 µm particles for 4.5 months.

Materials and methods

Composition and chemical characterization of environmental MPs

MPs were collected as described in the previous article (Cormier et al., in-prep. 2020). During the Race for Water Odyssey 2017-2021, environmental samples of plastics were collected from two sandy beaches in the Guadeloupe archipelago (France); Capesterre in Marie-Galante Island (MG), and Viard-Petit Bourg (PB) in Basse-Terre islands; situated in the Caribbean (Table 1 and Figure 1), in October 2017. Samples were collected following the standardized NOAA protocol (Lippiatt et al., 2013).

Table 1: Characteristics and GPS coordinates of the sampled beaches in Guadeloupe (France).

Sample	GPS Coordinates	Place	Description
MG (Marie Galante)	15°53'28.6"N 61°13'15.5"W	Capesterre Marie-Galante beach	Beach opposite side of main island, in the city center
PB (Petit Bourg)	16°09'59.6"N 61°35'06.2"W	Viard beach (Petit Bourg) in Basse Terre	Beach close to the main industrial zone of Guadeloupe

Polymer composition was determined using Fourrier-transform infrared spectroscopy (FT-IR) with a spectrum Vertex 70v (Bruker), results are expressed in % mass. MPs were ground using a cryogenic mill (SPEX, 6770 Freezer-mill), and sieved for 12 hours through 100 and 53 µm sieves. The particle-size distribution was measured using a Malvern laser diffraction particle size analyzer. Chemical analysis of environmental samples were previously described (Cormier et al., in-prep. 2020) and summarized below. Chemical analysis was non-target, using a

liquid/solid extraction. Analysis was performed on gas chromatography (GC) coupled with an orbitrap (Q-Exactive, ThermoFisher). PAHs, hydrocarbons, and chlorinated and brominated compounds were analyzed in both MPs samples. Confidence levels were attributed to each identification molecule following Schymanski et al. (2014). Levels 1, 2, 3, 4 and 5 relate to a confirmed structure, a probable structure, tentative candidate(s), unequivocal molecular formula and exact mass (m/z) of the compound, respectively (Schymanski et al., 2014).



Figure 1: Map of Guadeloupe (Caribbean Island) with the two sampled beaches, Petit-Bourg (PB) on the main island and Marie-Galante (MG) on a secondary island

Organic extracts

Organic extracts were prepared according to Pannetier, Morin, et al. (2019), with modifications described below. MPs samples were sieved to obtain particles below 250 µm. Organic extracts were prepared by adding 400 mg of MPs to 4 mL of dimethyl sulfoxide (DMSO) in the dark, shaken for 16 h on an horizontal mixing table at 175 rpm (KS 501 Digital, IKA-Werke). After the shaking, extracts were centrifuged at 4000 rpm for 5 minutes. Then, 100 µL of the liquid fraction were sampled with extra-long sterile tip, and re-centrifuged to remove particles. Extracts were kept at 4 °C for 7 days maximum.

Husbandry and egg production

Zebrafish (*Danio rerio*), TU strain were bred in 10 L tanks at 27 ± 1 °C with a photoperiod of 14/10 day/night (7.8 ± 0.2, 480 ± 130 µS, for pH and conductivity, respectively). Fish were fed daily, morning and evening, *ad libitum* with SDS100-300 µM diet (Special Diet Services, SDS France) or InicioPlus (500 µm, Biomar SAS, France), supplemented with MPs. A third meal was given at mid-day and consisted of freshly hatched brine shrimp *Artemia* nauplii. Permanent

flow-through conditions and water aeration guaranteed that ammonia, nitrite, and nitrate were kept below detection limits (5, 0.1 and 140 mg/L, respectively). Zebrafish eggs were collected within hours following spawning induction by light on in the fish room.

Toxicological analysis using zebrafish (*Danio rerio*) embryos

Zebrafish embryo acute toxicity test

Both exposures, with particles or organic extracts, started at 3 hpf (hours post fertilization). Zebrafish embryos were exposed to samples for 4 days until the end of the eleutheroembryo phase (96 hpf). Embryos (N= 30 per replicate) were exposed to different equivalent concentrations of plastics of 1 and 10 mg/L, and a supplementary concentration of 100 mg/L was added for organic extracts only. The control condition for particles exposition consisted of embryos cultivated in E3 medium supplemented with 0.35 mM of methylene, while for organic extracts, 1% of DMSO was added. Positive control consisted of embryos cultivated in 4 mM of 3,4-dichloroaniline (DCA), for the test validation (OECD 236). Glass petri dishes (55 mm diameter) containing 20 mL of solution and 20 embryos were used in semi-static conditions with a daily renewal of exposure medium. Each treatment condition was replicated 3 times. To maintain a temperature of 26 ± 1 °C, experiments were performed in temperature-controlled chambers (Snijders Scientific, Tilburg, Nederlands) with a photoperiod set up on 14:10, light:dark. For the entire exposure period, dissolved oxygen was daily controlled (> 85 %) with a fiber-optic oxygen mini-sensor Fibox 3 (PreSens Precision Sensor, Regensburg, Germany).

Developmental toxicity

Individuals were examined following OECD 236 (OECD, 2013), and four apical endpoints were recorded as indicators of lethality: coagulation of fertilized eggs, lack of somite formation, lack of detachment of the tail-bud from the yolk sac and lack of heartbeat. Embryos were checked daily for survival. Dead embryos were counted and immediately removed to avoid contamination and modification of the medium. Developmental abnormalities and lesions were also recorded: edema; body (scoliosis, lordosis); craniofacial abnormalities and cardiovascular system malformation (blood circulation) following previously published protocols (Le Bihanic, Clérandeau, et al., 2014). Leica MZ7.5 stereomicroscope with Leica DFP420C CDD camera and Leica Microsystems software v3.8 were used to perform these measurements (Leica, France).

Biometry

At 96 hpf, six to ten embryos per replicate were anesthetized with 200 mg/L of benzocaine. Total length and head length, were measured using a Leica MZ7.5 stereomicroscope with Leica DFP420C CDD camera and Leica Microsystems software v3.8 (Leica, France), and the ratio of head/total length was calculated.

In vivo EROD assay

In vivo EROD activity was analyzed according to published methods with modifications (Jönsson et al., 2002; Le Bihanic et al., 2013). Measurements were conducted on 96 hpf embryos. Five embryos per replicate were placed into individual wells of a 48-well microplate containing 1 µM of 7-ethoxyresorufin (7-ER), and triplicates were analyzed. After 1 hour of incubation in the dark, the 7-ER solution was removed and replaced by freshly prepared 1 µM 7-ER. Fluorescence of 100 µL of the incubation media was measured at time 0 and after 4 hours of incubation in duplicate in 96 white well plates. Resorufin formed was measured with a BMG Labtech plate reader (Germany) excitation and emission wavelength of 560 and 590 nm respectively. Solution of 7-ER was added to the resorufin standard used to reach a final concentration of 1 nM. A batch of five embryos was exposed to benzo[a]pyrene (70 nM) for 1h before incubation in 7-ER to obtain a positive control.

Larval swimming behavior

The swimming behavior was investigated following published protocol (Le Bihanic, Clérandeau, et al., 2014) using the larval photomotor response test (LPMR) to record fish behavioral responses after a light stimuli. 96 hpf embryos were transferred into a 48-well plate filled with 500 µL of E3 medium without methylene blue and acclimated for 20 min in the dark. The LPMR consisted of 5 min light, 5 min dark and 5 min light. Swimming activity (velocity and position in the well) was recorded with a video tracking system in a Daniovision chamber (Noldus, Wageningen, The Netherlands). LPMR was recorded for of 10 to 12 randomly chosen embryos per replicate at 28 °C. Videos were analyzed with Ethovision software 12.0 (Noldus, Wageningen, The Netherlands).

Statistical analyses

Normality of data was checked using the Shapiro-Wilk test. Analysis of variance was performed followed by Fisher's test compared to control. ANOVA was used for normal data, and for data that did not fulfill normal distribution, the Kruskal-Wallis non-parametric test was performed. All statistical analyzes were carried out at a 95 % level of significance and values are represented as mean ± SD.

Trophic exposure of zebrafish to food supplemented with environmental MPs

Preparation of food supplemented with MPs for trophic exposure

Pellet food adapted to the size of fish, SDS 100-300 µm was purchased from DIETEX (France) and Inicio 500 µm from BioMar (France). To obtain a homogenous distribution of MPs in the food, MPs were put with food pellet at a final ratio of 1% ww in glass tubes and mixed with a 4 mm bottlebrush fixed on a drill for 3 minutes. Afterwards, to reinforce binding of MPs to pellets, flaxseed oil was added (5µL per gram of food) and mixed for additional 3 minutes.

Experimental design

Rearing and exposure were performed at the laboratory facilities at Ifremer Research Centre, L'Houmeau which have the authorization to use and breed laboratory animals (EEA 171901) and the project was performed under the approval of the Animal Care Committee of Poitou-Charentes # 84 COMETHEA (France) with project authorization number APAFIS 10883 and followed the recommendations of Directive 2010/63/EU. Rearing was performed as described previously (Alfonso 2019; Vignet 2014).

A total of 450 zebrafish embryos were placed into 9 Petri dishes (10 cm, with 20 mL of E3 medium), following an equal distribution of eggs from at least 5 different spawn to avoid parental bias. At 5 days post fertilization (dpf), larvae were transferred from Petri dishes to 1 L tanks and were randomly assigned to each treatment including 3 replicates. At 21 dpf, they were transferred from 1 L tanks to 3 L tubes located in 10 L tanks in the rearing system. At 26 dpf, tubes were gently poured in 10 L tanks (Figure 2). Embryos and larvae were monitored daily for any mortality. Fish at 30 dpf were exposed for 5 months (until 6 months age) to three different treatment diets: control (CTRL): food supplemented with Marie-Galante MPs (MG) or Petit-Bourg MPs (PB). At the end of the exposure, the offspring generation F1 was reared with plain pellets, i.e. no further exposure. During the exposure, fish were monitored for any

possible signs of impaired health status (i.e. fish condition, external lesions, modification of feeding behavior).

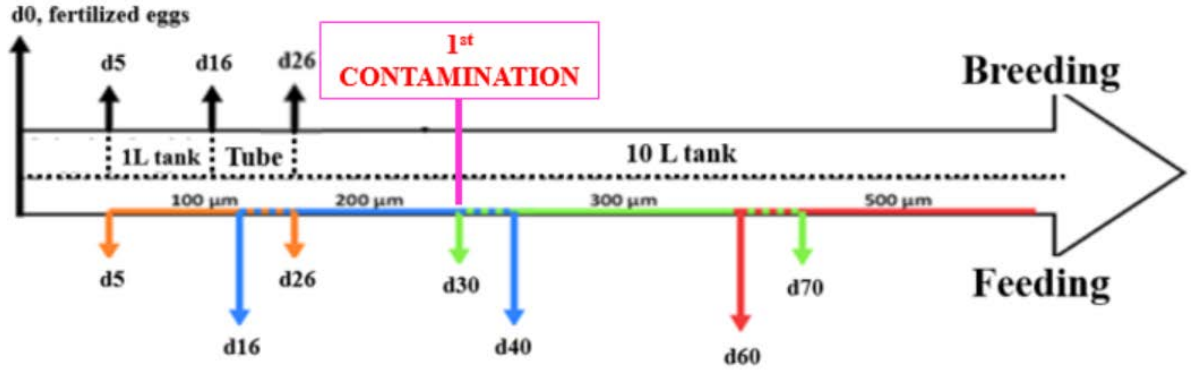


Figure 2: Experimental design for the feeding of zebrafish. The 1st contamination started at 30 days post fertilization, until the end of the exposure. Pellets size from 100 to 300 µm were SDS and 500 µm were InicioPlus.

Biomarkers of toxicity in the F0 generation

Survival and growth

Throughout the exposure, dead larvae or fish were monitored daily. Individual standard length (mm) and body weight (g) were measured at 2, 4 and 6 months of age (Vignet et al., 2014). At 2 and 4 months, fish were sampled and anesthetized, and euthanized at 6 months of age with 50 and 500 mg/L of benzocaine (MS222, Sigma Aldrich, France), respectively.

Reproduction

Reproduction was evaluated according to Alfonso et al., (2019). To monitor reproduction, at 90 dpf, one female and one male were placed in a spawning box (AquaSchwarz, Germany) in early evening; five pairs were set up per attempt, to reach up to 60 attempts.

Reproductive success was calculated according to the percentage of total number of spawning events obtained relative to the number of attempts (equal to the number of inspected rearing tanks per treatment). Eggs were collected the next morning and sorted to count the total number of eggs obtained per attempt and the fertilization rate was assessed after the collection. Eggs were kept and placed in Petri dishes filled with 30 mL of the isotonic mixture E3 (1 L: 17.2 g NaCl, 0.76 g HCl, 2.9 g $CaCl_2.2H_2O$, 4.9 $MgSO_4.7\ H_2O$) and methylene blue (250 µL/L). The

hatching rate and survival of F1 generation without feeding were monitored to evaluate their available energy ressources, with a daily medium exchange.

EROD

Zebrafish liver tissue were homogenized in 0.1 M phosphate buffer (pH = 7.7; 0.1 M KCl). The homogenate was then centrifuged at 9000 g at 4 °C for 20 min. The supernatant (fraction S9) was then collected in a clean microtube and stored at −80 °C before being used for all enzymatic activity measurements. The protein concentration in the S9 fraction was measured using Bradford's (1976) method with bovine serum albumin (BSA) as standard. Spectrophotometric measurements were performed in a Biotek Synergy HT microplate reader, and expressed as mg/mL. EROD activity was determined using the Burke and Mayer method (1974). The reaction mixture consisted of a NaH_2PO_4/K_2HPO_4 buffer (0.1 M, pH 7.5), 7-ethoxyresorufin (0.15 mM) and NADPH (5.57 mM). The change in fluorescence was recorded (excitation wavelength 530 nm, emission wavelength 585 nm) and enzyme activity was calculated as pmol/min/mg protein using a resorufin standard. In total, 6 fish per treatment were analyzed, composed of 3 males and 3 females.

TBARs

The amount of reactive oxygen species (ROS) to evaluate the oxidative stress is difficult to measure due to a short half-life, so the measure of thiobarbituric acid reactive substances (TBARS), an oxidative product (Buege and Aust, 1978), was studied. TBARs were measured in zebrafish muscles sampled after 4.5 months of exposure, and following the colorimetric protocol developed by Buege and Aust (1978) and adapted to a microplate reader as described in Weeks Santos et al. (2019). Results are expressed as nM of TBARs equivalents per mg of protein. Pools of 3 males or 3 females were used to obtain 3 measures per treatment.

AChE

AChE was performed on S9 fraction of zebrafish brain (4.5 months), following Ellman et al. (1961), adapted to a microplate reader. Extraction was performed following Lowry et al. (1951), modified as in Weeks Santos et al., 2019. Each well contained 10 µL of S9, 180 µL of 5,5'-dithiobis 0.5 mM in 0.05 M of Tris Buffer (pH 7.4) and 10 µL of acetylcholine iodide. The rate of increase of optical density was measured using a spectrophotometer (Synergy HT, BioTek) at 412 nm. Results are expressed as nmol/min/mg protein. Per condition, pooled of 3 males or 3 females have been used to obtain 3 measures per treatment.

Biomarkers of toxicity on the F1 generation

Larval behavior

Larval behavior was monitored at 120 hpf using the larval photomotor response (LPMR) test as described in Vignet et al. (2014). Larvae were individually transferred into one well of a 24-well plate (Krystal 24, opaque wall and clear bottom microplate, Dutscher) and plates were transferred to an enlighten incubator at 28°C in the behavior room. Plates were then successively placed into DanioVisionTM (Noldus, NL) in the dark for 10 minutes of acclimation before the LPMR test which included the following 5-min steps: Light on–1 (LON1, 70 lux), Light off (LOFF, <1 lux) and Light on–2 (LON2, 70 lux) with constant infra-red light maintained during video recording. Distance travelled (cm) over the 5 min of each period were then calculated using Ethovision (Noldus, NL). Larvae with tracking issues were removed resulting in 16 to 24 larvae analyzed per treatment.

Larval survival

For the F1 generation, a survival experiment was performed using unfed offspring from exposed fish. This aimed to determined whether the exposure of parents to MPs could alter the survival of their offspring larvae (F1). The monitoring of F1 larvae survival has been performed using eggs collected along the 60 attempts, from spawns above 60 % of fertilized eggs. Thirty eggs were collected from each clutch. They were kept in a Petri dishes in 30 mL of E3 medium at 28 °C, dead embryos were daily counted and removed for 14 days.

Statistical analyses

Each exposure treatment was repeated three times, with approximately 50 fish per replicate. Normality of the data was checked using the Shapiro-Wilk test. Analysis of variance was performed, followed by Fisher's test compared to control. ANOVA was used for normal data, and for data that did not fulfill normal distribution, the Kruskal-Wallis non-parametric test was performed. All statistical analyzes were carried out at a 95 % level of significance and values are represented as mean ± SD.

Results

Characterization of environmental samples

The predominant shape of MPs grinded macroplastic collected was fragments (> 98 %) for both sites, composed primarly of polyethylene (PE) and polypropylene (PP) (Table 2). The

granulometry analysis of MG sample demonstrated that the median particle size was 33.74 µm and the mean particle size was 87.1 µm.

Table 2: Polymer composition of MPs samples from two different locations in Guadeloupe, % based on the mass.

Polymers	Marie-Galante	Petit-Bourg
Polyethylene	78.3	74.6
Polypropylene	21.2	24.8
Polystyrene	0.1	0.4
Polyvinyl acetate	0	0.2
Other	0.4	0

Chromatogram superposition showed that the two sites contained peaks similarities and dissimilarities (Figure 3). Non-target chemical analyses revealed some halogenated compounds in both samples. For example, brominated flame retardant such as tribromophenol ($C_6H_3Br_3O$) and its metabolite tribromoanisole ($C_7H_5Br_3O$) were found in both samples. There was also a homologous series of pesticides from pentachlorobenzene (C_6HCl_5) and dichlorobenzene ($C_6H_4Cl_2$).

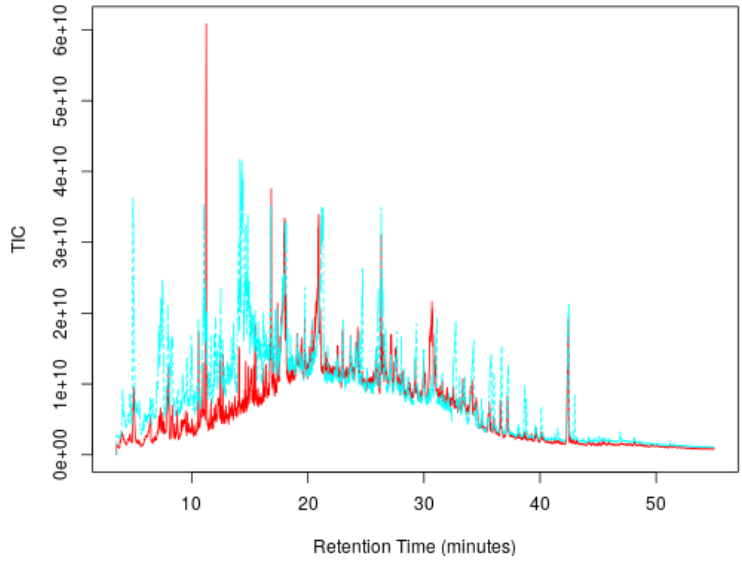


Figure 3: GC chromatograms expressed as relative abundance with the retention time (RT, minute) for MG (red chromatogram) and PB (blue chromatogram).

Overall, MG sample was characterized by higher concentrations of halogenated compounds, as well as trichlorobenzene, bumetrizole and octabenzone. The presence of phthalates was investigated, and some were only identified in MG sample as diisobutylphtalate, dibutylphtalate, diethylphtalate, ether di-(2-ethylhexyl) phthalate and di-n-octyl phthalate. However, the PB sample was contaminated with more hydrocarbons than in MG MPs. These compounds are mainly $C_xH_yO_z$. Peaks specific to PB have been identified and corresponded mainly to alkanes, corresponding to $C_{11}H_{18}O_3^{+\cdot}$ (confidence level 4), $C_{20}H_{34}O^{+\cdot}$ (confidence level 4) which seemed to correspond to a phenol C_7H_8O with an alkane chain and an isomer of octadecanoic acid $C_{18}H_{36}O_2^{+\cdot}$ (Schymanski et al., 2014). All compounds presented have a confidence level of 4, corresponding to an unequivocal molecular formula. More detailed results are presented in Cormier et al., (in-prep. 2020).

Early life stages of zebrafish

Exposure to MPs particles

In parallel to the organic extract exposures, embryos were exposed to particles at 1 and 10 mg/L. Direct exposure of zebrafish embryos to MG and PB MPs at both concentrations did not induce any embryonic toxicity compared to the controls. Survival was not altered by exposure to particles, nor was there any modification of hatching time and growth alteration. EROD activity as well as the swimming activity, were not significantly altered compared to control larvae.

Organic extracts

The embryo-larval survival rate in DMSO control was 96.7 ± 0.0 %. Hatching rate was 97.78 ± 1.6 %. Average head length of control larvae at 96 hpf was 0.70 ± 0.04 mm, total length was 3.82 ± 0.04 mm, ratio head/total lengths was 0.20 ± 0.01. Exposure to organic extracts of environmental MPs up to 100 mg/L equivalent MPs did not induce lethal toxicity, nor developmental toxicity (data not shown).

Larvae exposed to the equivalent of 10 mg/L of PB MPs extract exhibited a significant but moderate induction of EROD activity ($p=0.0078$). However, no significant *in vivo* EROD activity induction compared to control was observed in larvae exposed to 1 and 100 mg/L of PB MPs extracts and 1, 10 and 100 mg/L of MG MPs extracts. EROD activity induced by BaP positive control was twice that in control larvae (Figure 4).

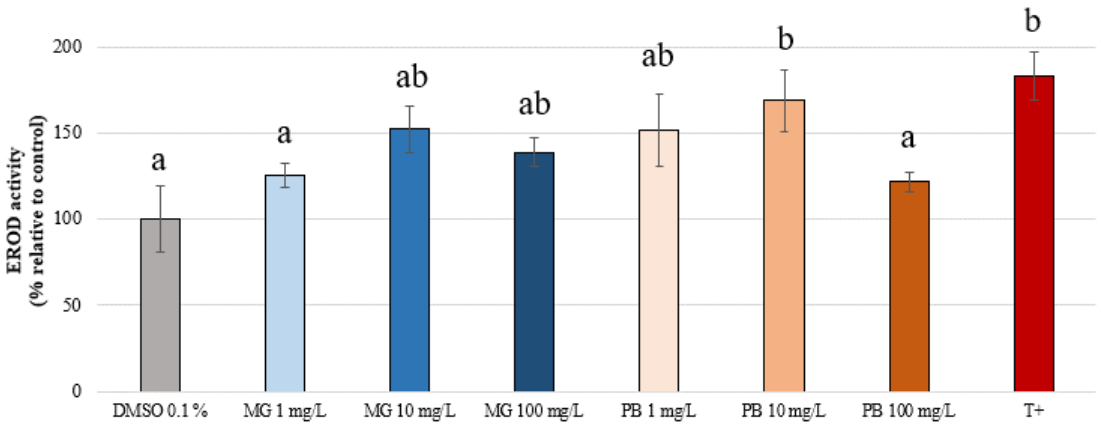


Figure 4: EROD activity in zebrafish larvae exposed to the equivalent of 1, 10 or 100 mg/L of MPs from MG or PB location. EROD activity is given relative to the control. Mean ± SD; n=3; ANOVA, p<0.05. Different lowercase letters indicate a significant difference between treatments.

Exposure to DMSO extracts of MG and PB MPs, at 1 and 10 mg/L did not significantly alter swimming activity in fish larvae in the LPMR test, compared to control larvae for all light periods (LON1, LOFF and LON2). However, at the highest concentrations tested, (100 mg/L equivalent MPs), MG and PB MPs extracts induced a significant reduction in swimming speed during LOFF (Figure 5), without any significant differences observed in the position of larvae in the well.

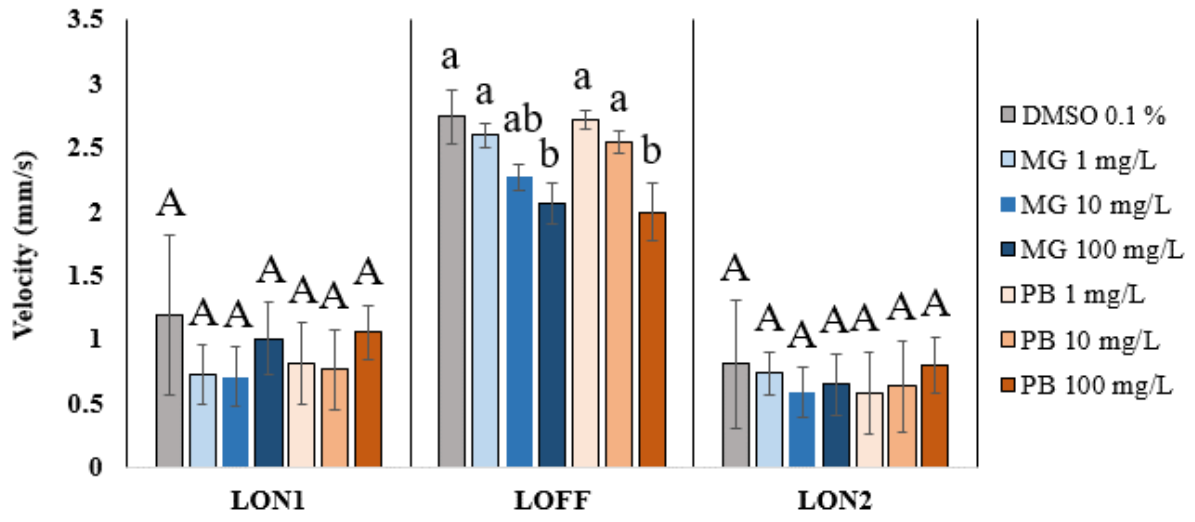


Figure 5: Larval photomotor response in 5 days-old zebrafishafter exposure to organic extracts of MG or PB MPs at an equivalent concentration of 1, 10 or 100 mg/L of MPs. Distance travelled over 5 min periods including two light-on periods (LON1, LON2) and one light off period (LOFF). Data are given as means ± SD (n = 20 larvae per treatment; repeated–measure ANOVA). Different letters indicate significant differences between treatments within the same light condition(p<0.05).

Dietary exposure to environmental MPs

F0 generation

Some sporadic mortality was observed, but there was no significant difference between conditions including control.

Fish growth was recorded firstly at 2 months of age, then at 4.5 months of age with the distinction of male and female phenotypic sex. At 2 months, no significant differences between fish exposed to MPs and controls were observed, neither on total length nor on weight. Fish total length at 4.5 months was identical across all treatments. However, significant reductions were observed for female weight exposed both to either MG or PB MPs compared to controls (ANOVA $F_{(6, 130)}$=28.3, p<0.01). Male weight did not differ between treatments (Figure 6).

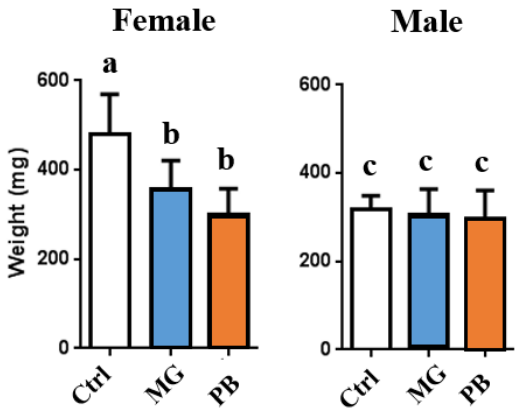


Figure 6: Zebrafish body weight (mg) after feeding for 4.5 months with diet containing 1% of MG or PB MPs. Treatments correspond to plain diet (ctrl), MG and PB MPs spiked diet at a ratio of 1%. (Mean +/- SD, n=18), Kruskall Wallis test. Different letters indicate significant differences between treatments (p<0.05).

Reproduction was evaluated based on three criteria. In comparison with the control, MG MPs exposure did not modify reproductive success (p=0.275), while PB MPs exposure induced a decrease in reproductive success (-70%; p<0.0001) (Figure 7). On the contrary no difference was observed for spawn characteristics, including the total number of eggs collected, as well as the number of fertilized eggs or the fertilization rate (Figure 8).

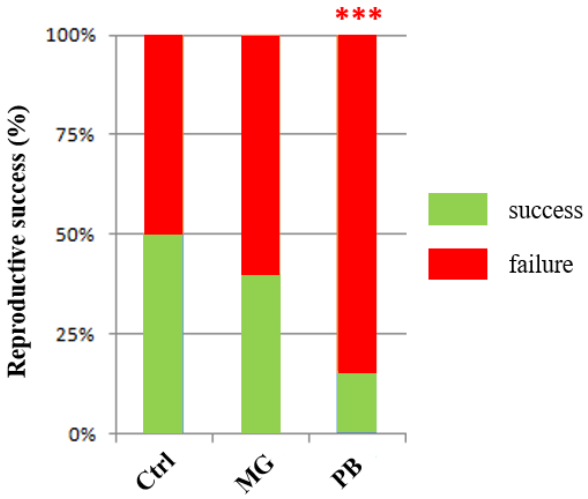


Figure 7: Zebrafish reproductive success after feeding for 4.5 months with diet containing 1% of MG or PB MPs. The reproductive success is the total number of spawning events obtained, relative to attempts number. In red are represented the failure ratio against success (green). Fisher test.

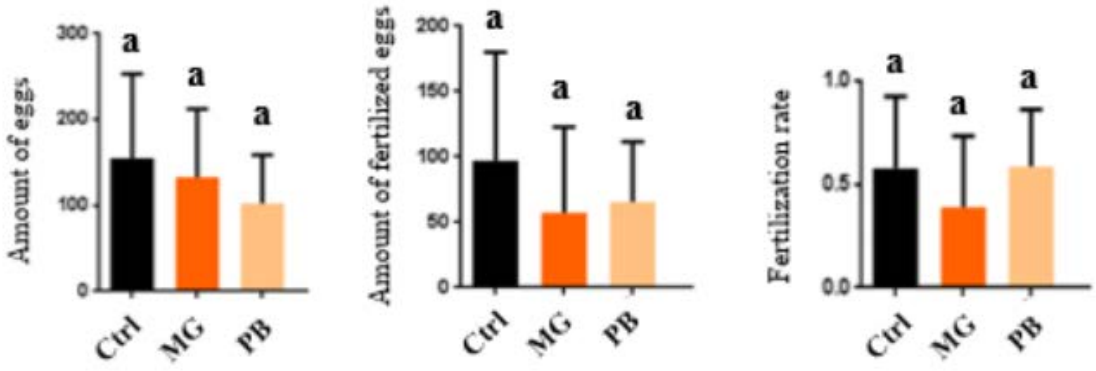


Figure 8: Characteristics of spawns obtained after feeding for 4.5 months with diet containing 1% of MG or PB MPs. (Mean +/- SD, n=63 attempts), Kruskall Wallis. Different letters indicate significant differences between treatments (p=0.05).

Biomarkers monitored in the present study e.g. EROD activity, AChE and TBARs, did not reveal any significant differences between treatments (results are not presented).

F1 generation

The analysis of individual larval standard length, measured at 5 dpf, did not reveal any significant differences between treatments (ANOVA ($F_{12, 135}$) =16.47, p=0.170). In comparison with controls, MG MPs parental exposure induced significant premature mortality in F1 larvae (Chi^2 value=5.823, p=0.016; Figure 9). The decrease in the age at which 50% of mortality was approximately of 14h and reached at 10.78 days [95% CI: 10.52-11.12] compared to the Control larvae [11.37, 95% CI:11.35-11.39]. On the contrary, no difference was observed for PB MPs for larvae survival (Chi^2 value=0.966, p=0.3257).

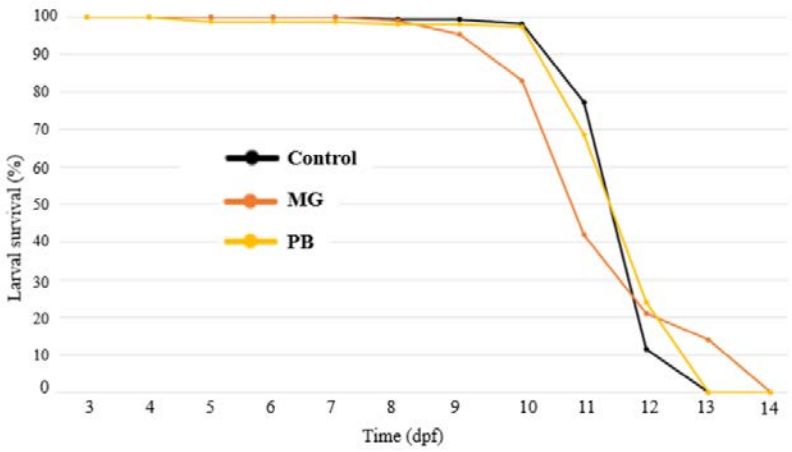


Figure 9: Larval survival (%) of the F1 generation, monitored for 14 days following feeding of parents for 4.5 months with diet containing 1% of MG or PB MPs MG.

The LPMR test was performed at 5 dpf to monitor early behavioral disruption. Independent of the treatment, LPMR showed a clear increase in the distance travelled during the light-off (LOFF) period compared to light-on (LON) periods (ANOVA, $p < 0.001$). Parental PB MPs resulted in exposure altered the swimming behavior of F1 larvae by inducing hyperactivity (repeated–measure ANOVA, $F_{(6, 438)}=2.9$, $p < 0.01$) during both LOFF et LON2 periods (Figure 10).

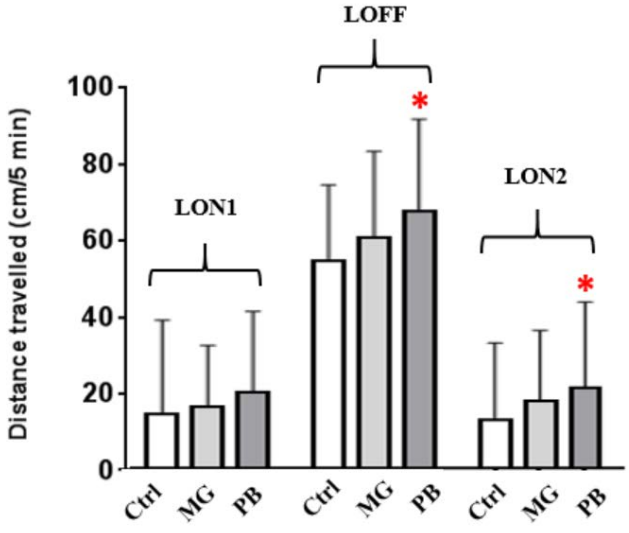


Figure 10: Larval photomotor response in F1 generation, 5 d old zebrafish (Danio rerio) after exposure of F0 generation to control (Ctrl) and MPs treatments (MG and PB). Distance travelled over 5 min periods including two light-on periods (LON1, LON2) and one light off period (LOFF). Data are given as means ± SD (n = 50 to 60 larvae per treatment; (repeated–measure ANOVA, $F_{(6, 438)}=2.9$). * indicates significant differences between treatments within the same observation period ($p<0.01$).

Discussion

MPs collected from beaches in Guadeloupe shared common characteristics with MPs collected from other beaches (Pannetier, Cachot et al., 2019) which are a major proportion of PE and presence of HOCs. Detailed characteristics have already been discussed (Cormier et al., in-prep 2020).

Due to their high sensitivity to a wide range of chemical substances, fish early life stages (ELS) are currently used in ecotoxicology and as screening tests (Embry et al., 2010; Lammer et al., 2009). In the present study, organic extracts and particle toxicity were tested on fish ELS. As reported in a previous study (Beiras et al 2018, Cormier 2019), particle exposure did not induce toxic effects. That being said, organic extracts of PB MPs revealed a moderate induction of EROD activity at the equivalent concentration of 10 mg/L. While chorion can limit the penetration of chemical compounds into the embryo and may protect against external contamination, due to its limited permeability (Strähle et al., 2012). It has been shown that some PAHs can be transferred across the chorion of fish (González-Doncel et al., 2008; Le Bihanic, Clérandeau, et al., 2014; Le Bihanic, Morin, et al., 2014; Perrichon et al., 2014).

The presence of PAHs in PB MPs may be responsible for the induction of EROD activity (Carlson et al., 2004; Wessel et al., 2007). However, many other organic compounds are known to activate AhR pathway, inducing modified EROD activity in fish, including alkylated PAHs, PCBs, dioxin like compounds, PFOS and PBDE (Cormier et al., 2019; Koenig and Solé, 2012; Lyons et al., 2011; Wessel et al., 2010). Because some of these compounds have been detected in PB MPs, we cannot assign with certainty the EROD activity to PAHs exposure only.

At the highest concentration tested, both MG and PB MPs induced hypo-activity in zebrafish larvae. It has been recently reported that environmental MPs sampled from different islands situated in oceanic gyres can impact the swimming behavior after light/dark stimulation of Japanese medaka larvae (Pannetier, Morin et al., 2019). Some PAHs mixtures, including pyrene or BaP have also been shown to modify fish swimming behavior (Le Bihanic, Clérandeau, et al., 2014; Le Bihanic, Morin, et al., 2014; Oliveira et al., 2013; Vignet et al., 2014). Reduced swimming activity was reported after fish embryo exposures to a wide range of organic pollutants, including PAHs, pesticides and psychotropic drugs (Chiffre et al., 2016; Faria et al., 2014; Le Bihanic, Clérandeau, et al., 2014).

Exposure to environmental MPs samples from two Guadeloupe beaches led to significant growth alteration in zebrafish revealed after 3.5 months of chronic exposure. In particular, a

significant decrease in the weight of female exposed to both MG and PB MPs samples appeared, compared to the control. Previous long term dietary exposure to organic pollutants have been shown to produce growth alterations: either a decrease in length and weight after exposure to PAHs mixtures (Vignet et al., 2014) or an increase after exposure to a mixture of PCBs and PBDEs (Horri et al., 2018). A decrease in weight without changes in fish length has been observed in dietary exposure of zebrafish to environmentally realistic freshwater mixtures of POPs, including PCBs and PBDEs as part of the major contaminants identified (Nourizadeh-Lillabadi et al., 2009). Such growth deregulation can be linked, at least in part, to a disruption of endogenous hormone functions, demonstrated by analysis of gene expression (Lyche et al., 2010; Lyche et al., 2011; Nourizadeh-Lillabadi et al., 2009). Previous studies have suggested that chemicals can increase or decrease growth, depending on make-up of chemical mixtures. Environmental MPs are potential vectors of mixtures of pollutants with various physico-chemical properties and modes of action, leading to complex molecular interactions and toxic effects. Because decreases in weight occurred for females exposed to both samples, this suggests the involvement of similar chemicals or chemicals with similar mode of action in both MPs. It is noteworthy that of the chemicals found in both MPs, tribromophenol and its metabolite tribromoanisole, as well as pentachlorobenzene, have been shown to adversely affect fish physiology. However, tribromophenol seemed to promote weight, and increase the condition factor of zebrafish of both sex (Deng et al., 2010) while pentachlorobenzene decreased growth without indication on sex (Chaisuksant et al., 1998). Another explanation may be related to the disruption of energy budget due to decreased food consumption or an increase in metabolic costs reported for numerous organisms upon exposure to MPs (Besseling et al., 2013; Green et al., 2016; Rist et al., 2016; Sussarellu et al., 2016; Watts et al., 2015; Yin et al., 2018). In several fish species, mature females have higher food consumption and/or metabolic costs compared with males as this has been shown in e.g. northern pike, walleye, yellow perch, catfish or zebrafish (Beaudouin et al., 2015; Diana, 1983; Rennie et al., 2008; Xie et al., 1998), a disruption in energy budget would have greater consequences for females.

Different aspects of reproduction were evaluated in the present study, and the reproductive success decreased for zebrafish exposed to PB MPs. Various studies have demonstrated that chemicals may act as endocrine disruptors by altering hormone pathways responsible for the regulation of reproductive functions (Horri et al., 2018; Mills and Chichester, 2005). Chemicals such as PBDEs have been shown to induce significant reduction in mature sperm in fathead minnows (Muirhead et al., 2006). Delays in follicle maturation have also been identified after

exposure to PCBs or PAHs (Vignet et al., 2016). In addition to chemicals, pristine microplastics can affect the reproduction of aquatic organisms. For examples, polystyrene MPs have been shown to induce alterations in marine medaka reproduction in a sex-dependent manner (Wang et al., 2019; Zhu et al., 2020) as well as in the reproduction of the bivalve *Crassostrea gigas* (Sussarellu et al., 2016).

Parental transfer of chemicals, e.g. heavy metals, pesticides, PAHS, PCBS, to offspring has been documented in numerous species, including birds (Ackerman et al., 2016; Bargar et al., 2001), amphibians (Metts et al., 2013), and reptiles (Rauschenberger et al., 2007). Over generations, persistency of chemicals can differ according to the mechanisms involved. In fish, different studies have demonstrated the maternal transfer of pollutants to eggs (Miller, 1993; Niimi, 1983; Nyholm et al., 2008; Yu et al., 2011). However, to date, no previous study has investigated the parental transfer of chemicals linked to environmental MPs exposure to the offspring. In the present study, exposure to MG sample led to a significant premature mortality of unfed larvae offspring. Exposures to different POPs induced elevated mortality among unfed larvae progeny (Foekema et al., 2012; Foekema et al., 2014; Horri et al., 2018; Wang et al., 2019; Yu et al., 2011). Besides changes to larvae survival, swimming activity was increased for the offspring of fish exposed to PB, without any changes to the behavior of F0 generation. The MG sample induced embryotoxicity that can be linked to the presence of phthalates, while PB tends to alter the swimming activity of larvae perhaps due to the presence of PAHs inducing neurotoxicity. To date, the investigation of the toxicity of environmental MPs on the F1 generation has never been studied. However, environmental MPs from island beaches increased the swimming activity of Japanese medaka (*Oryzias latipes*) (Pannetier et al., 2020). The presence of PAHs in PB sample may explain the hyperactivity of offspring larvae as PAHs are known to alter fish behavior in response to light stimulation (Le Bihanic, Clérandeau, et al., 2014; Vignet et al., 2014).

Conclusions

This study demonstrates that chronic dietary exposure of zebrafish to environmental microplastics has significant deleterious effects on adult zebrafish, as well as on their unfed F1 offspring (survival, behavior). For both MPs samples, growth was decreased in adult females, and reproduction was altered. Deleterious effects observed on F1 offspring larvae could be induced by either the parental transfer of chemicals desorbed from MPs and/or as an indirect

effect of the parental physiological status leading to poorer egg quality. In addition, direct exposure of fish larvae to organic extracts of MPs led a reduction in their swimming speed. This can be linked to the presence of plastic additives and/or sorbed pollutants in organic extracts. The present study revealed that acute regulatory tests are not efficient in predicting MPs toxicity. Polymer compositions were similar between the two sampled sites, however, the adsorbed chemical composition was different and may explain the different toxicity effects observed. This study raises the question of the environmental risk of environmental microplastics, weathered at sea, on aquatic organisms and in particular after ingestion and on progeny of fish.

Author Contributions

BC, JC, MLB and XC designed the experiment; BC, and SZ conducted the early life stages experiment; MC, MLB and XC performed the long term exposure; BC, MC, MB, MLB and XC performed all the toxicological analyses. BC wrote the manuscript and all authors corrected the manuscript.

Funding

This work was developed under the EPHEMARE project (Ecotoxicological effects of microplastics in marine ecosystems), supported by national funding agencies within the framework of JPI Oceans (FCT JPIOCEANS/0005/2015; FORMAS, 2015-01865; ANR-15-JOCE-0002-01; German BMBF 03F0735A). Bettie Cormier was directly supported by an IdEx grant from the University of Bordeaux.

References

Ackerman J. T., Eagles-Smith C. A., Herzog M. P., Hartman C. A. (2016). Maternal transfer of contaminants in birds: Mercury and selenium concentrations in parents and their eggs. Environmental Pollution. 210, 145-154

Acosta-Coley I., Mendez-Cuadro D., Rodriguez-Cavallo E., de la Rosa J., Olivero-Verbel J. (2019). Trace elements in microplastics in Cartagena: A hotspot for plastic pollution at the Caribbean. Marine Pollution Bulletin. 139, 402-411

Acosta-Coley I., Olivero-Verbel J. (2015). Microplastic resin pellets on an urban tropical beach in Colombia. Environmental Monitoring and Assessment. 187, 435

Alfonso S., Blanc M., Joassard L., Keiter S. H., Munschy C., Loizeau V., Bégout M.-L., Cousin X. (2019). Examining multi- and transgenerational behavioral and molecular alterations resulting from parental exposure to an environmental PCB and PBDE mixture. Aquatic Toxicology. 208, 29-38

Anbumani S., Kakkar P. (2018). Ecotoxicological effects of microplastics on biota: a review. Environmental Science and Pollution Research. 25, 14373-14396

Andrady A. L. (2011). Microplastics in the marine environment. Marine Pollution Bulletin. 62, 1596-1605

Arthur C., Baker J. E., Bamford H. A. (2009). Proceedings of the International Research Workshop on the Occurrence, Effects, and Fate of Microplastic Marine Debris, September 9-11, 2008, University of Washington Tacoma, Tacoma, WA, USA.

Bargar T. A., Scott G. I., Cobb G. P. (2001). Maternal transfer of contaminants: Case study of the excretion of three polychlorinated biphenyl congeners and technical-grade endosulfan into eggs by white leghorn chickens (Gallus domesticus). Environmental Toxicology and Chemistry. 20, 61-67

Barnes D. K. A., Galgani F., Thompson R. C., Barlaz M. (2009). Accumulation and fragmentation of plastic debris in global environments. Philosophical Transactions of the Royal Society B: Biological Sciences. 364, 1985-1998

Beaudouin R., Goussen B., Piccini B., Augustine S., Devillers J., Brion F., Péry A. R. R. (2015). An Individual-Based Model of Zebrafish Population Dynamics Accounting for Energy Dynamics. PLOS ONE. 10, e0125841

Beiras R., Bellas J., Cachot J., Cormier B., Cousin X., Engwall M., Gambardella C., Garaventa F., Keiter S., Le Bihanic F., et al. (2018). Ingestion and contact with polyethylene microplastics does not cause acute toxicity on marine zooplankton. Journal of Hazardous Materials. 360, 452-460

Belanger S. E., Rawlings J. M., Carr G. J. (2013). Use of fish embryo toxicity tests for the prediction of acute fish toxicity to chemicals. Environmental Toxicology and Chemistry. 32, 1768-1783

Besseling E., Wegner A., Foekema E. M., van den Heuvel-Greve M. J., Koelmans A. A. (2013). Effects of Microplastic on Fitness and PCB Bioaccumulation by the Lugworm Arenicola marina (L.). Environmental Science & Technology. 47, 593-600

Bogdal C., Scheringer M., Abad E., Abalos M., van Bavel B., Hagberg J., Fiedler H. (2013). Worldwide distribution of persistent organic pollutants in air, including results of air monitoring by passive air sampling in five continents. TrAC Trends in Analytical Chemistry. 46, 150-161

Bosker T., Behrens P., Vijver M. G. (2017). Determining global distribution of microplastics by combining citizen science and in-depth case studies. Integrated Environmental Assessment and Management. 13, 536-541

Bosker T., Guaita L., Behrens P. (2018). Microplastic pollution on Caribbean beaches in the Lesser Antilles. Marine Pollution Bulletin. 133, 442-447

Bradford M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Analytical Biochemistry. 72, 248-254

Braunbeck T., Böttcher M., Holler H., Rudolf T. K. L. L., Seitz N. (2005). Towards an alternative for the acute fish LC(50) test in chemical assessment: the fish embryo toxicity test goes multi-species -- an update. ALTEX. 22, 87-102

Braunbeck T., Kais B., Lammer E., Otte J., Schneider K., Stengel D., Strecker R. (2015). The fish embryo test (FET): origin, applications, and future. Environmental Science and Pollution Research. 22, 16247-16261

Browne Mark A., Niven Stewart J., Galloway Tamara S., Rowland Steve J., Thompson Richard C. (2013). Microplastic Moves Pollutants and Additives to Worms, Reducing Functions Linked to Health and Biodiversity. Current Biology. 23, 2388-2392

Buege J. A., Aust S. D. 1978. [30] Microsomal lipid peroxidation. In: Methods in Enzymology. Academic Press. p. 302-310.

Burke M. D., Mayer R. T. (1974). Ethoxyresorufin: direct fluorimetric assay of a microsomal O-dealkylation which is preferentially inducible by 3-methylcholanthrene. Drug Metabolism and Disposition. 2, 583

Carlson E. A., Li Y., Zelikoff J. T. (2004). Suppressive effects of benzo[a]pyrene upon fish immune function: Evolutionarily conserved cellular mechanisms of immunotoxicity. Marine Environmental Research. 58, 731-734

Chaisuksant Y., Yu Q., Connell D. W. (1998). Effects of Halobenzenes on Growth Rate of Fish (Gambusia affinis). Ecotoxicology and Environmental Safety. 39, 120-130

Chiffre A., Clérandeau C., Dwoinikoff C., Le Bihanic F., Budzinski H., Geret F., Cachot J. (2016). Psychotropic drugs in mixture alter swimming behaviour of Japanese medaka (Oryzias latipes) larvae above environmental concentrations. Environmental Science and Pollution Research. 23, 4964-4977

Cole M., Lindeque P., Halsband C., Galloway T. S. (2011). Microplastics as contaminants in the marine environment: A review. Marine Pollution Bulletin. 62, 2588-2597

Cormier B., Batel A., Cachot J., Bégout M.-L., Braunbeck T., Cousin X., Keiter S. H. (2019). Multi-Laboratory Hazard Assessment of Contaminated Microplastic Particles by Means of Enhanced Fish Embryo Test With the Zebrafish (Danio rerio). 7,

Cormier B., Perdriat Q., Tato T., Gambardella C., Costa E., Dubocq F., Karmann A., Van Arkel K., Lemoine S., Lagarde F., et al. (in-prep. 2020). Toxicity assessment of environmental microplastics on early life stages of three aquatic organisms.

Corsolini S. (2009). Industrial contaminants in Antarctic biota. Journal of Chromatography A. 1216, 598-612

de Sá L. C., Oliveira M., Ribeiro F., Rocha T. L., Futter M. N. (2018). Studies of the effects of microplastics on aquatic organisms: What do we know and where should we focus our efforts in the future? Science of The Total Environment. 645, 1029-1039

Deng J., Liu C., Yu L., Zhou B. (2010). Chronic exposure to environmental levels of tribromophenol impairs zebrafish reproduction. Toxicology and Applied Pharmacology. 243, 87-95

Derraik J. G. B. (2002). The pollution of the marine environment by plastic debris: a review. Marine Pollution Bulletin. 44, 842-852

Diana J. S. (1983). An energy budget for northern pike (Esox lucius). Canadian Journal of Zoology. 61, 1968-1975

Ellman G. L., Courtney K. D., Andres V., Featherstone R. M. (1961). A new and rapid colorimetric determination of acetylcholinesterase activity. Biochemical Pharmacology. 7, 88-95

Embry M. R., Belanger S. E., Braunbeck T. A., Galay-Burgos M., Halder M., Hinton D. E., Léonard M. A., Lillicrap A., Norberg-King T., Whale G. (2010). The fish embryo toxicity test as an animal alternative method in hazard and risk assessment and scientific research. Aquat Toxicol. 97, 79-87

Eriksen M., Lebreton L. C. M., Carson H. S., Thiel M., Moore C. J., Borerro J. C., Galgani F., Ryan P. G., Reisser J. (2014). Plastic Pollution in the World's Oceans: More than 5 Trillion Plastic Pieces Weighing over 250,000 Tons Afloat at Sea. PLOS ONE. 9, e111913

Faria A. M., Borges R., Gonçalves E. J. (2014). Critical swimming speeds of wild-caught sand-smelt Atherina presbyter larvae. Journal of Fish Biology. 85, 953-959

Farrell P., Nelson K. (2013). Trophic level transfer of microplastic: Mytilus edulis (L.) to Carcinus maenas (L.). Environmental Pollution. 177, 1-3

Foekema E. M., Fischer A., Parron M. L., Kwadijk C., de Vries P., Murk A. J. (2012). Toxic concentrations in fish early life stages peak at a critical moment. Environmental Toxicology and Chemistry. 31, 1381-1390

Foekema E. M., Lopez Parron M., Mergia M. T., Carolus E. R. M., vd Berg J. H. J., Kwadijk C., Dao Q., Murk A. J. (2014). Internal effect concentrations of organic substances for early life development of egg-exposed fish. Ecotoxicology and Environmental Safety. 101, 14-22

Geyer R., Jambeck J. R., Law K. L. (2017). Production, use, and fate of all plastics ever made. Science Advances. 3, e1700782

González-Doncel M., González L., Fernández-Torija C., Navas J. M., Tarazona J. V. (2008). Toxic effects of an oil spill on fish early life stages may not be exclusively associated to PAHs: Studies with Prestige oil and medaka (Oryzias latipes). Aquatic Toxicology. 87, 280-288

Green D. S., Boots B., Sigwart J., Jiang S., Rocha C. (2016). Effects of conventional and biodegradable microplastics on a marine ecosystem engineer (Arenicola marina) and sediment nutrient cycling. Environmental Pollution. 208, 426-434

Horri K., Alfonso S., Cousin X., Munschy C., Loizeau V., Aroua S., Bégout M.-L., Ernande B. (2018). Fish life-history traits are affected after chronic dietary exposure to an environmentally realistic marine mixture of PCBs and PBDEs. Science of The Total Environment. 610-611, 531-545

Jambeck J. R., Geyer R., Wilcox C., Siegler T. R., Perryman M., Andrady A., Narayan R., Law K. L. (2015). Plastic waste inputs from land into the ocean. Science. 347, 768

Jönsson E. M., Brandt I., Brunström B. (2002). Gill Filament-Based EROD Assay for Monitoring Waterborne Dioxin-like Pollutants in Fish. Environmental Science & Technology. 36, 3340-3344

Karapanagioti H. K., Klontza I. (2008). Testing phenanthrene distribution properties of virgin plastic pellets and plastic eroded pellets found on Lesvos island beaches (Greece). Marine Environmental Research. 65, 283-290

Koelmans A. A. 2015. Modeling the role of microplastics in bioaccumulation of organic chemicals to marine aquatic organisms. A critical review. In: Marine Anthropogenic Litter. p. 309-324.

Koelmans A. A., Bakir A., Burton G. A., Janssen C. R. (2016). Microplastic as a Vector for Chemicals in the Aquatic Environment: Critical Review and Model-Supported Reinterpretation of Empirical Studies. Environmental Science and Technology. 50, 3315-3326

Koelmans A. A., Besseling E., Foekema E. M. (2014). Leaching of plastic additives to marine organisms. Environmental Pollution. 187, 49-54

Koenig S., Solé M. (2012). Natural variability of hepatic biomarkers in Mediterranean deep-sea organisms. Marine Environmental Research. 79, 122-131

Lammer E., Carr G. J., Wendler K., Rawlings J. M., Belanger S. E., Braunbeck T. (2009). Is the fish embryo toxicity test (FET) with the zebrafish (Danio rerio) a potential alternative for the fish acute toxicity test? Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology. 149, 196-209

Law K. L., Thompson R. C. (2014). Microplastics in the seas. Science. 345, 144

Le Bihanic F., Clérandeau C., Le Menach K., Morin B., Budzinski H., Cousin X., Cachot J. (2014). Developmental toxicity of PAH mixtures in fish early life stages. Part II: adverse effects in Japanese medaka. Environmental Science and Pollution Research. 21, 13732-13743

Le Bihanic F., Couillard C. M., Rigaud C., Légaré B. (2013). A simple and reliable in vivo EROD activity measurement in single Fundulus heteroclitus embryo and larva. Marine Environmental Research. 84, 17-23

Le Bihanic F., Morin B., Cousin X., Le Menach K., Budzinski H., Cachot J. (2014). Developmental toxicity of PAH mixtures in fish early life stages. Part I: adverse effects in rainbow trout. Environmental Science and Pollution Research. 21, 13720-13731

Lebreton L., Slat B., Ferrari F., Sainte-Rose B., Aitken J., Marthouse R., Hajbane S., Cunsolo S., Schwarz A., Levivier A., et al. (2018). Evidence that the Great Pacific Garbage Patch is rapidly accumulating plastic. Scientific Reports. 8, 4666

Li J., Liu H., Paul Chen J. (2018). Microplastics in freshwater systems: A review on occurrence, environmental effects, and methods for microplastics detection. Water Research. 137, 362-374

Lippiatt S., Opfer S., Arthur C. (2013). Marine Debris Monitoring and Assessment. NOAA Technical Memorandum. NOS-OR&R-46,

Lithner D., Larsson Å., Dave G. (2011). Environmental and health hazard ranking and assessment of plastic polymers based on chemical composition. Science of The Total Environment. 409, 3309-3324

Lowry O. H., Rosebrough N. J., Farr A. L., Randall R. J. (1951). Protein measurement with the folin phenol reagent. 193, 265-275

Lyche J. L., Nourizadeh-Lillabadi R., Almaas C., Stavik B., Berg V., Skåre J. U., Alestrøm P., Ropstad E. (2010). Natural Mixtures of Persistent Organic Pollutants (POP) Increase Weight Gain, Advance Puberty, and Induce Changes in Gene Expression Associated with Steroid Hormones and Obesity in Female Zebrafish. Journal of Toxicology and Environmental Health, Part A. 73, 1032-1057

Lyche J. L., Nourizadeh-Lillabadi R., Karlsson C., Stavik B., Berg V., Skåre J. U., Alestrøm P., Ropstad E. (2011). Natural mixtures of POPs affected body weight gain and induced transcription of genes involved in weight regulation and insulin signaling. Aquatic Toxicology. 102, 197-204

Lyons M. C., Wong D. K. H., Mulder I., Lee K., Burridge L. E. (2011). The influence of water temperature on induced liver EROD activity in Atlantic cod (Gadus morhua) exposed to crude oil and oil dispersants. Ecotoxicology and Environmental Safety. 74, 904-910

Mato Y., Isobe T., Takada H., Kanehiro H., Ohtake C., Kaminuma T. (2001). Plastic Resin Pellets as a Transport Medium for Toxic Chemicals in the Marine Environment. Environmental Science & Technology. 35, 318-324

Mazurais D., Ernande B., Quazuguel P., Severe A., Huelvan C., Madec L., Mouchel O., Soudant P., Robbens J., Huvet A., et al. (2015). Evaluation of the impact of polyethylene microbeads ingestion in European sea bass (Dicentrarchus labrax) larvae. Marine Environmental Research. 112, 78-85

Metts B. S., Buhlmann K. A., Tuberville T. D., Scott D. E., Hopkins W. A. (2013). Maternal Transfer of Contaminants and Reduced Reproductive Success of Southern Toads (Bufo [Anaxyrus] terrestris) Exposed to Coal Combustion Waste. Environmental Science & Technology. 47, 2846-2853

Miller M. A. (1993). Maternal Transfer of Organochlorine Compounds in Salmonines to Their Eggs. Canadian Journal of Fisheries and Aquatic Sciences. 50, 1405-1413

Mills L. J., Chichester C. (2005). Review of evidence: Are endocrine-disrupting chemicals in the aquatic environment impacting fish populations? Science of The Total Environment. 343, 1-34

Muirhead E. K., Skillman A. D., Hook S. E., Schultz I. R. (2006). Oral Exposure of PBDE-47 in Fish: Toxicokinetics and Reproductive Effects in Japanese Medaka (Oryzias latipes) and Fathead Minnows (Pimephales promelas). Environmental Science & Technology. 40, 523-528

Nagel R. (2002). DarT: The embryo test with the zebrafish Danio rerio - A general model in ecotoxicology and toxicology. ALTEX. 19, 38-48

Niimi A. J. (1983). Biological and Toxicologtcal Effects of Environmental Contaminants in Fish and Their Eggs. Canadian Journal of Fisheries and Aquatic Sciences. 40, 306-312

Nourizadeh-Lillabadi R., Lyche J. L., Almaas C., Stavik B., Moe S. J., Aleksandersen M., Berg V., Jakobsen K. S., Stenseth N. C., Utne Skåre J., et al. (2009). Transcriptional Regulation in Liver and Testis Associated with Developmental and Reproductive Effects in Male Zebrafish Exposed to Natural Mixtures of Persistent Organic Pollutants (POP). Journal of Toxicology and Environmental Health, Part A. 72, 112-130

Nyholm J. R., Norman A., Norrgren L., Haglund P., Andersson P. L. (2008). Maternal transfer of brominated flame retardants in zebrafish (Danio rerio). Chemosphere. 73, 203-208

OECD. 2013. Test No. 236: Fish Embryo Acute Toxicity (FET) Test.

Ogata Y., Takada H., Mizukawa K., Hirai H., Iwasa S., Endo S., Mato Y., Saha M., Okuda K., Nakashima A., et al. (2009). International Pellet Watch: Global monitoring of persistent organic pollutants (POPs) in coastal waters. 1. Initial phase data on PCBs, DDTs, and HCHs. Marine Pollution Bulletin. 58, 1437-1446

Oliveira M., Ribeiro A., Hylland K., Guilhermino L. (2013). Single and combined effects of microplastics and pyrene on juveniles (0+ group) of the common goby Pomatoschistus microps (Teleostei, Gobiidae). Ecological Indicators. 34, 641-647

Oliviero M., Tato T., Schiavo S., Fernández V., Manzo S., Beiras R. (2019). Leachates of micronized plastic toys provoke embryotoxic effects upon sea urchin Paracentrotus lividus. Environmental Pollution. 247, 706-715

Pannetier P., Cachot J., Clérandeau C., Faure F., Van Arkel K., de Alencastro L. F., Levasseur C., Sciacca F., Bourgeois J.-P., Morin B. (2019). Toxicity assessment of pollutants sorbed on environmental sample microplastics collected on beaches: Part I-adverse effects on fish cell line. Environmental Pollution. 248, 1088-1097

Pannetier P., Morin B., Clérandeau C., Laurent J., Chapelle C., Cachot J. (2019). Toxicity assessment of pollutants sorbed on environmental microplastics collected on beaches: Part II-adverse effects on Japanese medaka early life stages. Environmental Pollution. 248, 1098-1107

Pannetier P., Morin B., Le Bihanic F., Dubreil L., Clérandeau C., Chouvellon F., Van Arkel K., Danion M., Cachot J. (2020). Environmental samples of microplastics induce significant toxic effects in fish larvae. Environment International. 134, 105047

Pedà C., Caccamo L., Fossi M. C., Gai F., Andaloro F., Genovese L., Perdichizzi A., Romeo T., Maricchiolo G. (2016). Intestinal alterations in European sea bass Dicentrarchus labrax (Linnaeus, 1758) exposed to microplastics: Preliminary results. Environmental Pollution. 212, 251-256

Perrichon P., Le Bihanic F., Bustamante P., Le Menach K., Budzinski H., Cachot J., Cousin X. (2014). Influence of sediment composition on PAH toxicity using zebrafish (Danio rerio) and Japanese medaka (Oryzias latipes) embryo-larval assays. Environmental Science and Pollution Research. 21, 13703-13719

PlasticsEurope (2018). PlasticsEurope: Annual review _ The Facts 2017-2018.

Rauschenberger R. H., Wiebe J. J., Sepúlveda M. S., Scarborough J. E., Gross T. S. (2007). Parental Exposure to Pesticides and Poor Clutch Viability in American Alligators. Environmental Science & Technology. 41, 5559-5563

Rennie M. D., Purchase C. F., Lester N., Collins N. C., Shuter B. J., Abrams P. A. (2008). Lazy males? Bioenergetic differences in energy acquisition and metabolism help to explain sexual size dimorphism in percids. Journal of Animal Ecology. 77, 916-926

Rigét F., Vorkamp K., Bossi R., Sonne C., Letcher R. J., Dietz R. (2016). Twenty years of monitoring of persistent organic pollutants in Greenland biota. A review. Environmental Pollution. 217, 114-123

Rios L. M., Moore C., Jones P. R. (2007). Persistent organic pollutants carried by synthetic polymers in the ocean environment. Marine Pollution Bulletin. 54, 1230-1237

Rist S. E., Assidqi K., Zamani N. P., Appel D., Perschke M., Huhn M., Lenz M. (2016). Suspended micro-sized PVC particles impair the performance and decrease survival in the Asian green mussel Perna viridis. Marine Pollution Bulletin. 111, 213-220

Rochman C. M. 2015. The Complex Mixture, Fate and Toxicity of Chemicals Associated with Plastic Debris in the Marine Environment. In: Marine Anthropogenic Litter. Cham: Springer International Publishing. p. 117-140.

Rochman C. M., Hoh E., Kurobe T., Teh S. J. (2013). Ingested plastic transfers hazardous chemicals to fish and induces hepatic stress. Scientific Reports. 3, 3263

Rochman C. M., Kurobe T., Flores I., Teh S. J. (2014). Early warning signs of endocrine disruption in adult fish from the ingestion of polyethylene with and without sorbed chemical pollutants from the marine environment. Science of The Total Environment. 493, 656-661

Schymanski E. L., Jeon J., Gulde R., Fenner K., Ruff M., Singer H. P., Hollender J. (2014). Identifying Small Molecules via High Resolution Mass Spectrometry: Communicating Confidence. Environmental Science & Technology. 48, 2097-2098

Setälä O., Fleming-Lehtinen V., Lehtiniemi M. (2014). Ingestion and transfer of microplastics in the planktonic food web. Environmental Pollution. 185, 77-83

Strähle U., Scholz S., Geisler R., Greiner P., Hollert H., Rastegar S., Schumacher A., Selderslaghs I., Weiss C., Witters H., et al. (2012). Zebrafish embryos as an alternative to animal experiments—A commentary on the definition of the onset of protected life stages in animal welfare regulations. Reproductive Toxicology. 33, 128-132

Sussarellu R., Suquet M., Thomas Y., Lambert C., Fabioux C., Pernet M. E. J., Le Goïc N., Quillien V., Mingant C., Epelboin Y., et al. (2016). Oyster reproduction is affected by exposure to polystyrene microplastics. Proceedings of the National Academy of Sciences. 113, 2430

Teuten E. L., Rowland S. J., Galloway T. S., Thompson R. C. (2007). Potential for Plastics to Transport Hydrophobic Contaminants. Environmental Science & Technology. 41, 7759-7764

Teuten E. L., Saquing J. M., Knappe D. R. U., Barlaz M. A., Jonsson S., Björn A., Rowland S. J., Thompson R. C., Galloway T. S., Yamashita R., et al. (2009). Transport and release of chemicals from plastics to the environment and to wildlife. Philosophical Transactions of the Royal Society B: Biological Sciences. 364, 2027-2045

Thompson R. C., Moore C. J., vom Saal F. S., Swan S. H. (2009). Plastics, the environment and human health: current consensus and future trends. Philos Trans R Soc Lond B Biol Sci. 364, 2153-2166

Thompson R. C., Olsen Y., Mitchell R. P., Davis A., Rowland S. J., John A. W. G., McGonigle D., Russell A. E. (2004). Lost at Sea: Where Is All the Plastic? Science. 304, 838

Vignet C., Larcher T., Davail B., Joassard L., Le Menach K., Guionnet T., Lyphout L., Ledevin M., Goubeau M., Budzinski H., et al. (2016). Fish Reproduction Is Disrupted upon Lifelong Exposure to Environmental PAHs Fractions Revealing Different Modes of Action. Toxics. 4,

Vignet C., Le Menach K., Mazurais D., Lucas J., Perrichon P., Le Bihanic F., Devier M.-H., Lyphout L., Frère L., Bégout M.-L., et al. (2014). Chronic dietary exposure to pyrolytic and petrogenic mixtures of PAHs causes physiological disruption in zebrafish - part I: Survival and growth. Environmental Science and Pollution Research. 21, 13804-13817

Wang J., Liu X., Liu G., Zhang Z., Wu H., Cui B., Bai J., Zhang W. (2019). Size effect of polystyrene microplastics on sorption of phenanthrene and nitrobenzene. Ecotoxicology and Environmental Safety. 173, 331-338

Watts A. J. R., Urbina M. A., Corr S., Lewis C., Galloway T. S. (2015). Ingestion of Plastic Microfibers by the Crab Carcinus maenas and Its Effect on Food Consumption and Energy Balance. Environmental Science & Technology. 49, 14597-14604

Weeks-Santos S., Cachot J., Gourves P.-Y., Clérandeau C., Morin B., Gonzalez P. (2019). Sub-lethal effects of waterborne copper in early developmental stages of rainbow trout (Oncorhynchus mykiss). Ecotoxicology and Environmental Safety. 170, 778-788

Wessel N., Rousseau S., Caisey X., Quiniou F., Akcha F. (2007). Investigating the relationship between embryotoxic and genotoxic effects of benzo[a]pyrene, 17α-ethinylestradiol and endosulfan on Crassostrea gigas embryos. Aquatic Toxicology. 85, 133-142

Wessel N., Santos R., Menard D., Le Menach K., Buchet V., Lebayon N., Loizeau V., Burgeot T., Budzinski H., Akcha F. (2010). Relationship between PAH biotransformation as measured by biliary metabolites and EROD activity, and genotoxicity in juveniles of sole (Solea solea). Marine Environmental Research. 69, S71-S73

Xie X., Long T., Zhang Y., Cao Z. (1998). Reproductive investment in the Silurus meridionalis. Journal of Fish Biology. 53, 259-271

Yin L., Chen B., Xia B., Shi X., Qu K. (2018). Polystyrene microplastics alter the behavior, energy reserve and nutritional composition of marine jacopever (Sebastes schlegelii). Journal of Hazardous Materials. 360, 97-105

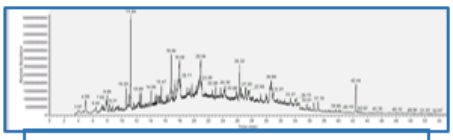
Yu H., Huang G.-h., An C.-j., Wei J. (2011). Combined effects of DOM extracted from site soil/compost and biosurfactant on the sorption and desorption of PAHs in a soil–water system. Journal of Hazardous Materials. 190, 883-890

Zhu M., Chernick M., Rittschof D., Hinton D. E. (2020). Chronic dietary exposure to polystyrene microplastics in maturing Japanese medaka (Oryzias latipes). Aquatic Toxicology. 220, 105396

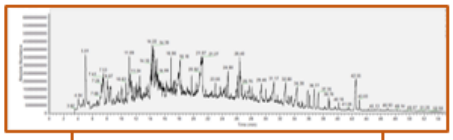
Chapter 5 – Conclusion

Results

Chemical composition



MG
- More halogenated compounds
- Phthalates



PB
- More hydrocarbons

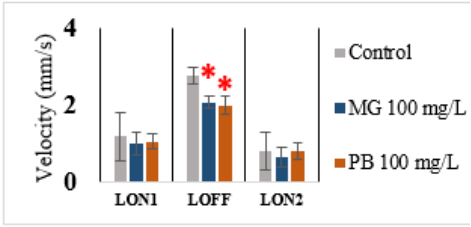
Early life stages

- **Particles** & **leachates**: no toxicity on ELS

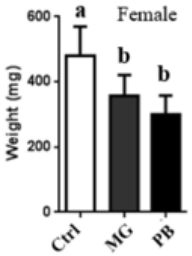
- **Organic extracts** (DMSO)

PB (10 mg/L): ↑ EROD

MG, PB (100 mg/L): **hyperactivity**

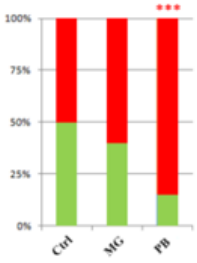


Chronic exposure

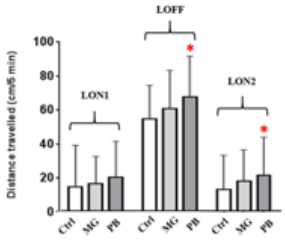


F0 generation

- Growth alteration MG and PB
- ↓ Reproduction PB



F1 generation ➔ PB: **hyperactivity**



Key points

- **Polymer composition** of both samples were **similar** and composed of **PE** (>70 %) and **PP** (> 20%).
- Chemical composition of both samples were analyzed and dissimilarities were observed as the presence of **phthalates** in **MG** MPs samples while **PB** MPs were mainly contaminated with **hydrocarbons**, probably due to the **location of PB** close to an important **industrial zone**.
- **Particles** and **leachates** of MG and PB MPs did **not induce toxicity** to **ELS** of zebrafish
- However, **DMSO-extracts** of MPs revealed an **induction of EROD activity** for fish exposed to 10 mg/L of **PB**, and at the **highest concentration** (100 mg/L), both samples induced **hyperactivity** of the larvae
- **Chronic dietary exposure** revealed that the exposure to **PB** MPs sample **decreased** the **reproductive success** of zebrafish and induced **hyperactivity** of **unexposed F1**.

Chemical contamination and ageing might explain the different toxicities of both MPs samples.

Chapter 6: General discussion

All results presented in the present PhD manuscript were already discussed; consequently, this chapter will provide a brief recap of the main results obtained, in view of the initial objectives of this thesis and present knowledge on microplastics. As a reminder, the objectives were: (1) to develop and implement protocols to artificially sorb model pollutants, PFOS, BaP and BP3 to different size ranges of HDPE and PVC MPs as well as the study of sorption processes of PFOS onto HDPE, (2) to investigate the ability of MPs to act as a vector of pollutants by using industrial particles spiked with model pollutants (PFOS, BaP and BP3) using early life stages, juveniles and adults of zebrafish and (3) to study the toxicity of environmental MPs (sandy beaches, Guadeloupe, France) on different life stages of zebrafish.

The ability of MPs to sorb pollutants will be discussed, followed by the capacity of MPs to act as a threat to aquatic organisms by playing a role of vector for pollutants, either using ELS or adult zebrafish. Furthermore, some perspectives will be mentioned in the present chapter.

1) Sorption of organic pollutants on plastics

Artificial microplastics have been used since the 90's as passive sampler in semipermeable polymeric membrane devices, made of PE tubing containing lipids (Huckins et al., 1990). Since then, passive samplers have been modified and implemented, but sheets made of PE and pristine PE remain widely used (Adams et al., 2007; Lohmann, 2017). The use of PE for passive samplers led to the interest for the study of PE as one of the reference polymers to investigate chemical sorption to MPs (Hüffer and Hofmann, 2016; Lee et al., 2014; Li et al., 2018; Xu et al., 2018b). MPs provide a surface onto which many waterborne pollutants can be adsorbed, including aqueous metals (Rochman, Hentschel, et al., 2014) and POPs (Rios et al., 2007; Rochman, Hoh, Hentschel et al., 2013). In the aquatic environment such chemicals are commonly found at their highest concentrations in the surface microlayer, where low-density MPs, such as PE are most abundant as well (Ng and Obbard, 2006; Rios et al., 2007; Teuten et al., 2007). As a consequence, MPs have been hypothesized to act as vectors and carriers for a wide range of pollutants in the marine environment (Andrady, 2011; Barnes et al., 2009; Hammer et al., 2012; Koelmans et al., 2014; Rios et al., 2010; Thompson et al., 2004).

The processes of sorption and desorption of organic pollutants onto MPs are complex and poorly understood. The equilibrium kinetics of contaminants on MPs depends on the inherent properties of the chemicals, but also the size, density, polymer type, aging of plastic particles and quality of the MPs (Wang, Shih, et al., 2015; Wang et al., 2019). The present study

investigated the artificial sorption of model pollutants (PFOS, BaP and BP3) onto industrial MPs, as well as the chemical characterization of environmental samples of MPs. We demonstrated that artificial MPs are able to adsorb chemicals at different sorption rates. For PFAAs, it is assumed that hydrophobic and/or electrostatic interactions are the two main sorption processes onto organic material (Higgins and Luthy, 2006). Differences in sorption efficiency can be attributed to differences in functional groups. For example, the sulfonate group of PFOS bears a stronger polarity than the carboxylic group of PFOA. Therefore, the resulting stronger electrostatic repulsion suggests a lower sorption affinity of PFOS to PE compared to PFOA (Bakir et al., 2014). PFASs are amphiphilic substances and might therefore show a highly substance-specific sorption behavior. The sorption kinetics are also a relevant insight into the mechanisms involved in the vectorization of chemicals by sorbents (Azizian, 2004). In general, sorption kinetics using MPs are performed on a small scale for a limited number of hours (Zhan et al., 2016; Zhang, Wang, Shih, et al., 2018; Zhang, Zheng, et al., 2018). However, in the present study, sorption kinetics were investigated over 180 days and the linear sorption observed is in accordance with a previous study on PFOS sorption conducted by Wang, Shih, et al. (2015), on PE, PS and PVC. The limited sorption of PFOS onto PE could be due to physical properties of the compound. Nevertheless, sorption rates increased over time, and after 180 days, significant variability was observed between replicates. The high variation of sorption after 180 days could be due to the aging of particles remaining in solution for 6 months and leading to a degradation of MPs surfaces resulting in the modification of the sorption sites.

The properties of plastics can modify the sorption of pollutants, such as surface charge, surface area, functional groups present (Fotopoulou and Karapanagioti, 2015). For example, the measurement of the surface area of polymers used by Seidensticker et al. (2018), demonstrated that PE is non-porous while PS is mesoporous. This difference resulted in a higher sorption rate of contaminants to PS than PE, which is confirmed by non-linear sorption isotherms in the study. In equal conditions, PVC particles demonstrated higher adsorption capacity compared to PE for different synthetic musk (Dong et al., 2019). However, other studies reported that PE microplastics had higher sorption capacities for HOCs than PS and PVC particles (Wang and Wang, 2018a; Zuo et al., 2019), attributed to the arrangement of the molecular chain of plastic. Sorption efficiency also depends on the molecular structure and composition of MPs and pollutants (Wang, Shih, et al., 2015). Lee et al. (2014), observed that the concentrations of PCBs and PAHs as well as the partitioning coefficients between the different types of MPs followed

the order of PS > PE > PP. The high sorption capacity of PS has been already demonstrated by using PAHs, and PS had 8–200 times order of magnitude sorption than PET, PVC, PE and PP (Rochman, Hoh, Hentschel et al., 2013). The sorption capacity of PS was related to its amorphous structure, in comparison with other polymer types. In fact, sorption processes are linked to the contaminant as well as the polymer types.

The aging of MPs may play a role in the sorption/desorption processes of chemicals. Aged MPs were characterized with rough surfaces and large surface areas, which enhanced the sorption of pollutants (Liu, Zhu, et al., 2019). Environmental MPs are subjected to aging by UV radiation, oxidation and thermal radiation, resulting in the properties changes of MPs (Celina, 2013). It has previously been demonstrated that the sorption of aged MPs differs from non-aged primary MPs (Kokalj et al., 2019). To investigate the aging of MPs, artificial aging of plastic was studied. For example, the artificial aging of polystyrene significantly modified the adsorption mechanisms of pharmaceuticals onto PS particles (Liu et al., 2020). Specifically, artificially aged MPs using UV radiation demonstrated that aged MPs had a higher adsorption capacity for heavy metals than virgin MPs (Wang et al., 2020). Changes to the surface properties of MPs may change their adsorption properties (Liu, Qian, et al., 2019). The aging of MPs influences not only the sorption of chemicals but also their polymer properties, with changes in density, leading to modification of sinking properties (Kokalj et al., 2019).

In the present study, a decrease of the amount of BaP adsorbed on MPs was observed, whereas for a similar compound, pyrene, sorption was achieved after 48 hours of exposure to either polyethylene, polystyrene and polyvinyl chloride microparticles (Wang and Wang, 2018a). The decrease in BaP adsorbed onto PE over time can be due to the degradation of the compound, e.g. photodegradation or biodegradation in the water (Akyüz and Cabuk 2010). To avoid biodegradation, another kinetic experiment was carried out with the addition of azide (NaN3). Unfortunately, due to technical problems linked to PhD mobility, all samples of water and MPs were analyzed, but no BaP contamination was identified. The absence of BaP in all samples could be due to failure to observe the cold chain while the samples were in transit. In the present study we cannot conclude on the potential degradation of BaP during its sorption. In fact, degradation of BaP is enhanced by different degradation processes in natural water, and half-lives can vary from 27.5 to 266.6 days, in pond water and seawater, respectively (Kot-Wasik et al., 2004). Degradation is not the only process that can modify the sorption of BaP; according to the literature, around 30 to 40% of BaP can be adsorbed on glassware (Bowman et al., 2002).

Sorption of PAHs onto MPs has been studied very little under laboratory conditions. Inconsistent data, or a general lack of reliable data from laboratory studies (Llorca et al., 2018; Wang, Shih, et al., 2015) would appear to highlight the need for further research on the interaction behavior between chemicals and MPs particles. However, on environmental samples of MPs, PAHs are commonly detected and quantified (Pannetier et al., 2020; Schönlau et al., 2019).

In the environment, MPs have a tendency to accumulate in oceanic gyres but also in coastal areas due to hydrodynamics and human activities (Eriksen et al., 2014; Law and Thompson, 2014; Lebreton et al., 2018). Beaches are known to be common sites for marine litter and a hotspot for plastic fragmentation because of shallow water and exposure to UV (Andrady, 2011). MPs have already been widely reported in the sea and on beaches in the Caribbean (Acosta-Coley et al., 2019; Acosta-Coley and Olivero-Verbel, 2015; Bosker et al., 2017; Bosker et al., 2018). In the present study, MPs collected from Guadeloupe's beaches were mainly composed of PE and PP, representing more than 70 % and 20 % of the particles, respectively. This is consistent with the fact that PE is the most abundant polymer found in plastic litter (Cheang et al., 2018; Fossi et al., 2017; Hidalgo-Ruz et al., 2012; Karthik et al., 2018), followed by PP and PS.

The present thesis evaluated the sorption and toxicity of PE particles, both for primary microplastics and environmental samples. Low-density MPs are the predominant polymers reported in MPs collected on beaches. A predominance of PE and PP has previously been reported in MPs collected from several beaches around the world (Frias et al., 2010; Pannetier et al., 2020). Also, accumulation of biofilm at the surface of MPs tends to increase the sinking behavior of MPs (Kaiser et al., 2017; Kooi et al., 2017; Kowalski et al., 2016).

Chemical analysis of environmental samples from beaches on the island of Guadeloupe revealed multi-contamination of MPs with diverse organic pollutants, demonstrating the ability of MPs to sorb pollutants into the aquatic environment. Halogenated compounds including brominated flame retardant, PCBs, chlorinated pesticides and chlorinated PAHs (chloroacenatphthylene) were found on both sites, while no unsubstituted PAHs were detected. Phthalates (diisobutylphtalate, dibutylphtalate, diethylphtalate, ether di-(2-ethylhexyl) phthalate and di-n-octyl phthalate) were only detected in MPs from Marie-Galante Island. On the other hand, MPs from the Petit-Bourg site (Basse-Terre island) contained a high number of hydrocarbons. This could be explained by the proximity of industrial areas and harbor of Pointe-à-Pitre. Environmental samples of MPs often contained PAHs, PCBs and pesticides (Pannetier,

Cachot, et al., 2019, Schönlau et al., 2019). The profile and concentration of hydrophobic organic chemicals (HOCs) are linked to the chemical exposure of particles throughout their drift history (Rochman et al., 2015). Koelmans et al. (2016) estimated that plastics need 2 to 4 years to reach the sorption equilibrium for all HOCs in the marine environment, including additives and plasticizers. However, this sampling period took place in the aftermath of Hurricane Maria (October 2017) and some of the plastic debris collected on beaches may have come from further inland. It is impossible to estimate the residence time of MPs in the water, thus making it equally difficult to evaluate the sorption equilibrium for HOCs.

Trace metals were found in plastic debris from the North Atlantic subtropical gyre (Prunier et al., 2019) and it was demonstrated that plastics debris are able to bind and accumulate metals (Acosta-Coley, Mendez-Cuadro, Rodriguez-Cavallo, de la Rosa and Olivero-Verbel, 2019; Davranche et al., 2019). To complete the present PhD thesis, chemical analysis are still in progress to characterize the metallic contamination of environmental MPs.

2) **Toxicity of microplastics using early life stages of zebrafish**

In the present study, different life stages of zebrafish were exposed to industrial or environmental MPs. Embryo-larval exposure involved direct contact with different MPs.

Embryos, along with the larvae of fish and invertebrates play a fundamental role in the marine structure and functioning of marine ecosystems. The long-term sustainability of healthy populations depends on the good health and survival of larvae (Steer et al., 2017). In addition, early life stages are particularly sensitive to pollutants (Embry et al., 2010; Lammer et al., 2009). A wide array of bioassays has been developed to assess the toxicity of chemicals or environmental samples. ELS of vertebrates that depend on the use of yolk are not protected by the current European legislation on the protection of animals used for scientific purposes (EU, 2010; Strähle et al., 2012). The FET test has been recommended as an alternative to conventional fish acute toxicity testing (e.g. OECD TG 203) (OECD, 1992) and is approved as Test Guideline 236 (OECD, 2013). Based on this guideline, FET has successfully been applied to a wide range of substances exhibiting diverse modes of action, solubilities, volatilities, and hydrophobicities. However, given the particulate nature of MPs, the question arises as to whether the FET is suited to the assessment of MP toxicity. Indeed, there are major differences between soluble pollutants and discrete objects such as MPs including, e.g., dispersion, homogeneity, localized desorption events of additives or adsorbed pollutants. Since Batel et al. (2018) already documented that the transfer of BaP was not sufficient to cause acute embryo

toxicity, additional endpoints to reveal sublethal effects were incorporated into the FET protocol (OECD 2013) to enhance its sensitivity. The purposes of the present work presented in this thesis was therefore to evaluate the suitability of FET for MP toxicity assessment and further document the release of pollutants from MPs, their transfer across the zebrafish chorion and their effects.

Primary virgin PE particles did not induce acute toxicity on zebrafish early life stages, and similar results were observed after exposure of embryos and larvae of marine medaka (*Oryzias melastigma*) to the same PE particles (Beiras et al., 2018). On the contrary, PE particles artificially contaminated with model pollutants (PFOS, BaP and BP3) led to moderate but significant induction of *cyp1a* transcription and/or EROD activity, involved in phase I of xenobiotic metabolism and often used as a biomarker of exposure. Albeit with some differences in response due to chemical used with respect to exposure duration − which may reflect the need for translation or enzyme accumulation − an overall triggering of CYP1A (either as EROD activities and/or as *cyp1a* transcription) was observed after exposure to MPs spiked with pollutants, whereas virgin MPs failed to do so. Results indicate that pollutants spiked on MPs are able to desorb and reach concentrations sufficient to activate AhR pathway and induce CYP1A protein synthesis.

An increasing number of studies uses behavioral responses to monitor sublethal effects of pollutants such as PFOS, BaP or BP3 on aquatic organisms (Huang et al., 2010; Le Bihanic, Morin, et al., 2014; Le Bihanic, Clérandeau, et al., 2014; Le Bihanic et al., 2015; Wang, Wang, et al., 2015), and changes in, e.g., the photomotor response are commonly interpreted as evidence of potential neurotoxicity (Legradi et al., 2018). Larvae exposed to industrial MPs did not reveal any change in swimming behavior using LPMR. However, previous studies have shown that MPs may reduce other behavioral parameters, such as predatory performance of fish depending on the shape of plastic particles (Choi et al., 2018; de Sá et al., 2015). To complement the present study, different behavioral experiments might be used on exposed adults, such as the anxiety or the learning ability analyses. For example, the learning ability of zebrafish was altered after an exposure to copper (Pilehvar et al., 2020). Additional behavioral parameters should be investigated with the aim of being able to identify with greater certainty the presence or absence of toxic effects of MPs on fish behavior.

Beyond the different parameters studied, the particular species of fish is important. Indeed, the same experiments were carried out using Marine medaka, and demonstrated acute toxicity for embryos and larvae (Beiras et al., 2018). The difference of sensitivity between these two fish

species could be explained by direct contact of MPs with villi at the surface of the marine medaka chorion, which may facilitate the uptake of toxicants. While villi or similar structures do not exist on the zebrafish chorion, Batel et al. (2018) showed that small MPs (1 - 5 µm) were able to adhere to the outer surface of the zebrafish chorion and transfer BaP to embryos across the chorion. Besides the difference in egg surface (Hart and Donovan, 1983; Hart et al., 1984) which may interfere with MP contact, additional factors, such as exposure time, may also account for the different responses observed between marine medaka and zebrafish. Indeed, different sensitivities for lethal and sublethal endpoints have been demonstrated between early life stages of Japanese medaka and zebrafish exposed to sediments spiked with two model PAHs (Perrichon et al., 2014). For the FET test, one major difference between these two species is the duration of the embryonic stage. For Japanese medaka, the embryonic stage lasts until days 10 to 11 dpf at 26 °C (Padilla et al., 2009), whereas this period is restricted to 48 to 72 hpf in zebrafish reared at 26-28 °C (Kimmel et al., 1995). Similar to Japanese medaka, mean hatching time for marine medaka is 11 dpf at 28 °C (Beiras et al., 2018). Future investigations into MP effects on zebrafish could consider prolonged exposure, using direct exposure via the water medium, or the use of different fish species, such as the Japanese medaka or the marine medaka.

In addition, for all biomarkers studied, DNA damage can also be used to evaluate the potential toxic of MPs associated with chemicals. Genotoxicity was observed in medaka larvae exposed to extracts of environmental MPs (Pannetier, Morin, et al., 2019). The presence of oxidized bases in DNA can be identified using the comet assay, supplemented with the Fpg enzyme (Kienzler et al., 2012). Oxidative damage has previously been reported in marine lugworms *Arenicola marina* exposed to MPs (Browne et al., 2013). In the present study, genotoxicity was investigated on entire zebrafish larvae, yet negative control levels were similar to positive control. Average tail length was around 50 %. The method was improved by optimizing some parameters such as cell dissociation, lysis solution, and duration of the lysis and electrophoresis. However, negative controls were still around 30 % of DNA breaks. The method still needs to be improved to obtain a percentage of DNA breaks lower than 10% for the negative control. In view of this not all results were suitable for presentation in the present study.

To conclude, toxicity of environmental samples was investigated by exposing zebrafish embryos to either particles, organic extracts or leachates. Contact with particles and leachates did not reveal toxic effects, while organic extracts at the same concentration induced EROD activity and hyperactivity of larvae. Previous studies have demonstrated toxic effects caused by

the exposure of Japanese medaka to MP extracts (Pannetier, Morin, et al., 2019). Organic extraction of MPs facilitates the desorption of contaminants and additives adsorbed at the surface of the particles, meaning that organisms are directly exposed to contaminants (Pannetier, Morin, et al., 2019). The results observed highlighted the fact that toxicity of MPs in the environment without ingestion of particles could be explained by the desorption of contaminants or leakage of additives more than by the particles themselves.

3) Toxicity following ingestion of microplastics

The ingestion of MPs can lead to the desorption of environmental contaminants, additives or pollutants, as demonstrated in numerous previous studies (Fossi et al., 2012; Mato et al., 2001; Oliveira et al., 2013; Teuten et al., 2007, 2009). We first, we investigated the artificial desorption of PFOS from industrial MPs particles. For all quantities of exposed PFOS-spiked particles, desorption ranged between 70 and 80 %. The high desorption rate (between 70 and 80 %) is most likely down to molecular properties and chemical interactions between the bile salt sodium taurocholate and PFOS, both composed of a hydrophobic and a polar functional group. The bioavailability of PFOS may be enhanced by their inclusion in bile salt micelles or into their micellar layer, has been shown for lecithin (Mazer et al., 1980). Enhanced desorbing effects mitigated through bile have been already demonstrated in previous studies for different compounds and matrices. For example, a mixture of PCBs and lindane was desorbed from soil after *in vitro* digestion with chicken bile (Oomen et al., 2000). Another study investigated the digestion of soil contaminated with PAHs and PCBs, revealing a mobilization of these substances after gastro-intestinal digestion up to two times higher than that without bile (Hack and Selenka 1996). This emphasizes the important role played by bile in the assimilation of toxicants at the same time as nutrients.

However, there are only a few investigations into the desorption behavior of MPs sorbed to chemicals under physiological conditions, and these are mainly focused on common HOCs (Teuten et al., 2009; Ziccardi et al., 2016). Despite differences in particular affinities between different HOCs and MPs, Bakir et al. (2014) demonstrated a significant increase in desorption rates in the presence of gut surfactant between 2 to 20 times higher compared to sea water. Similar results were presented by Teuten et al. (2007) for phenanthrene (Phe) in combination with PE and PP. While desorption rate constants of Phe from natural sediment were significantly higher than those determined from PE and PP, the sediment's sorption capacities were remarkably lower. It is proposed that even though contaminants desorb more slowly from

plastics than from sediment, the relative amount desorbing from plastics under physiological conditions might exceed the quantities desorbing from natural sediment particles (Bakir et al., 2014). Among teleost fish, approximately 60 % of species produce only C24 bile salts for their digestive juices (Hofmann et al., 2010). In addition, taurine conjugates, such as sodium taurocholate, are the most dominant C24 bile salts among carnivore species (Moschetta and Kliewer, 2005). This promotes sodium taurocholate as a suitable model bile salt to assess the desorption behavior of MPs toxicants in a broad range of species.

This lends weight to the hypothesis that MPs may deliver HOCs in biota. In that sense, chronic dietary exposures were performed with a variety of MPs as virgin MPs, MPs artificially spiked with model pollutants and environmental MPs. The range of MPs used was designed to better understand the mechanisms involved in the toxicity of MPs after ingestion, and to apply the investigation to environmental MPs.

Exposure to pristine MPs for 5 months induced slight effects on the F0 generation, with a decrease of weight in females exposed to PE and PVC, while PVC tends to alter the reproduction with an increase of the failure ratio of the reproductive success. Pristine MPs can affect the reproduction of aquatic organisms. For example, polystyrene MPs in contact with aquatic organisms induced an alteration in marine medaka reproduction in a sex-dependent manner (Wang et al., 2019) as well as the reproduction of the bivalve *Crassostrea gigas* (Sussarellu et al., 2016). The fact that the F1 generation, corresponding to unfed larvae, was affected by the parents exposure to pristine MPs, with an alteration of the larval survival, may indicate that toxic effects could be linked to chemical components of MPs, such as additives. Indeed, plastics are made of polymers containing numerous additives used to improve specific characteristics, and it has been demonstrated that additives can be toxic (Barnes et al., 2009; Lithner et al., 2011) and can negatively alter the health of living organisms (Koelmans et al, 2015; Lusher et al, 2015; Oehlmann et al., 2009; Rochman et al., 2015). Results showed that MPs made of PVC induced higher toxicity compared to PE particles. The production of PVC includes numerous additives (Fred-Ahmadu et al., 2020) which may cause the higher toxicity observed after ingestion compared to PE.

The potential contribution of MPs to act as vector of organic pollutants to biota has for long been subject to controversy (Bakir et al., 2016; Koelmans et al., 2016; Lohmann, 2017). However, in our study we confirmed that MPs can play an important role in the potential toxicity of MPs (Batel et al., 2016; O'Donovan et al., 2018, Rainieri et al., 2018). To better understand the role of MPs as vector of pollutants, zebrafish were fed with PE or PVC spiked

with model pollutants such as PFOS, BaP and BP3. These artificial situations allow a clearer understanding of toxicity relating to polymers, chemicals and combinations of both. In parallel, environmental samples of MPs were added to food to simulate realistic environmental contamination, including a mixture of pollutants and polymers. Exposures to the different model pollutants spiked to either PE or PVC induced diverse effects, such as alteration of female growth and decrease in weight (all MPs tested). Similar results were observed for zebrafish exposed to both environmental samples of MPs collected on beaches, MG and PB. Previous long-term dietary exposure to POPs produced growth alteration, either a decrease in length and weight (PAHs, Vignet et al., 2014). Decreased weight without changes in fish length was observed in a dietary exposure of zebrafish to environmentally realistic freshwater mixtures of POPs, including PCBs and PBDEs as part of the major contaminants identified (Nourizadeh-Lillabadi et al., 2009). This kind of growth regulation can be linked to a disruption of endogenous hormone synthesis or functioning, demonstrated by analysis of gene expression (Lyche et al., 2011, Lyche et al., 2010, Nourizadeh-Lillabadi et al., 2009). In the present study, growth alteration was not linked to a specific contaminant. Had that been the case, it would have been interesting to investigate genes involved in the growth. It is noteworthy that growth decrease occurred after at least 4 months of exposure and only in females. This would tend to suggest a modification of the energy balance between growth and reproduction which is expensive in terms of energy budget for females. It would have been interesting to monitor energy reserves in females of all conditions.

Different aspects of reproduction were evaluated in the present study. Reproductive success decreased for zebrafish exposed to PE-BP3 and PVC-BP3. BP3 is a UV-filter commonly used in cosmetics such as sunscreens but also as an additive in plastics, to protect against UV degradation. BP3 is known to act as an endocrine disrupting chemical interfering with reproduction and sex hormone signaling, inducing reproductive pathologies and altering reproductive fitness (Bluthgen et al., 2012; Coronado et al., 2008; Kunz et al., 2006). At higher concentrations, reduced egg production and hatching have been demonstrated, along with an increase of the vitellogenin level in males, causing gender shifts (Coronado et al., 2008; Nimrod and Benson, 1998). It is noteworthy that the effects of PVC-BP3 appeared to be much higher than PE-BP3. PB particles also altered the reproductive success and decreased female condition. This may be an explanation for defects in sexual maturation, thus leading to a decrease in spawning probability in fish (Grift et al., 2007; Mollet et al., 2007; Whiteley et al., 2011; Wright et al., 2013). Various studies have demonstrated that chemicals may act as endocrine disruptors

by altering hormone pathways responsible for the regulation of reproductive functions (Horri et al., 2018; Mills and Chichester, 2005). Chemicals such as PBDEs have been demonstrated to induce significant reduction in mature sperm in fathead minnows (Muirhead et al., 2006). Delay in follicle maturations were shown after exposure to PCBs or PAHs (Vignet et al., 2016).

Parental transfer of chemicals, e.g. heavy metals, pesticides, PAHs, PCBs, to offspring has been documented in numerous species, including birds (Ackerman et al., 2016; Bargar et al., 2001), amphibians (Metts et al., 2013), and reptiles (Rauschenberger et al., 2007). In fish, different studies have demonstrated the maternal transfer of pollutants to eggs (Alfonso et al., 2019; Miller, 1993; Niimi, 1983; Nyholm et al., 2008). However, no study has yet investigated the transfer of effects from parents to the offspring of MPs contaminated with model pollutants or environmental MPs. In the present study, besides the effects observed on F0 generation, F1 larvae from F0 exposed to PVC-BP3 and PB exhibited increased swimming activity. Hyperactivity was observed in offspring of fish exposed either to PVC spiked with BP3 or to environmental samples from PB site. To date, the investigation of the toxicity of environmental MPs on F1 has never been studied in fish. However, the chemical contamination of PB sample, such as aliphatic hydrocarbons, could explained the hyperactivity of offspring larvae as hydrocarbons are known to alter fish behavior in response to light stimulation (Le Bihanic, Clérandeau, et al., 2014; Vignet et al., 2014). Effects on F1 generation revealed a transfer of effects, either from the alteration of physiology of parents, leading to the production of defective eggs or from the transfer of chemicals causing direct effects. In the present study, the detection and quantification of chemicals in F1 larvae was not performed, but it may have added information on a potential transgenerational transfer. Only the condition of MPs-PFOS resulted in the bioaccumulation of the compound in adult fish. In the present study, we decided that chemical analyses of F1 generation will not be investigated due to the quantity of larvae required and the larvae available.

While all biomarkers were investigated, physical damages was not studied in the present thesis. Physical effects have been demonstrated, leading to a blockage of the digestive tract or a feeling of fullness (Cole et al., 2011; Rochman, Hoh, Kurobe et al., 2013) along with ulceration or perforation of the digestive tract wall or the gill epithelium (Peda et al., 2016). These physical alterations could be linked to toxic effects observed, such as changes in swimming activity. Specific toxicological effects induced by plastic components such as plastic monomers or plastic additives e.g. phthalates, bisphenol A, flame retardant, UV stabilizer and dyes were demonstrated (Koelmans, 2015; Koelmans et al., 2014; Rochman et al., 2015), but in the present

study the effects of additives were not separated from those of other chemicals. Further studies should focus on the toxic effects of additives on zebrafish.

The PhD studies investigated the sorption and toxic effects of MPs on zebrafish, however, fragmentation of MPs can lead to nano-sized plastic particles (NPs). Although the theoretical formation mechanism of MPs and NPs has been explained in previous works (Cooper and Corcoran, 2010) only one experiment has demonstrated the formation of NPs from MPs fragmentation (Enfrin et al., 2020). However, the accumulation of NPs was demonstrated in different tissues of zebrafish (Chen, Yin, et al., 2017). Instead of MPs, beads of PS NPs were detected inside the gut and have been demonstrated to migrate outside the intestinal epithelium (Parenti et al., 2019). Besides accumulation of NPs via direct exposure, passive diffusion of NPs through the skin was reported in zebrafish exposed to MPs fragmented into NPs (Enfrin et al., 2020). Also, the diffusion of NPs in zebrafish tissues was showed by the accumulation of NPs in adult zebrafish livers after ingestion (Lu et al., 2016). In zebrafish embryos, NPs crossed the chorion and accumulated in the yolk sac and migrated to other tissues: gastrointestinal tract, gallbladder, liver, pancreas, heart and brain, throughout embryos development (Pitt, Kozal, et al., 2018). These exposures to NPs induced oxidative stress, inhibition of larvae locomotion, reduction of larvae body length, modification of AChE activity and decreased heart rate (Chen, Gundlach, et al., 2017; Parenti et al., 2019; Pitt, Kozal, et al., 2018). Dietary exposure of zebrafish to PS NPs led to a maternal transfer of PS NPs to the F1 generation with the presence of NPs in the yolk sac, the gastrointestinal tract, the liver, and the pancreas (Pitt, Trevisan, et al., 2018). Exposure to PS NPs modified the antioxidant system responses in tissues of exposed adults and in unexposed F1 larvae (Pitt, Trevisan, et al., 2018). The scientific community should therefore focus not only on MPs toxicity but also on NPs, since it has been shown that NPs are able to migrate into tissues of organisms through passive diffusion.

Chapter 7: Conclusion and Perspectives

The present work aimed to study the potential role of MPs as vectors of pollutants for aquatic organisms. It investigates the kinetics of sorption of model pollutants onto PE MPs, as well as their toxicity for zebrafish, and the chemical impregnation and toxicity of environmental MPs.

Model pollutants were successfully sorbed to industrial PE and PVC MPs, with the observation of different sorption patterns linked to the chemical used and the polymer. However, due to a lack of time, long-term sorption experiments were limited, and the kinetics sorption kinetics of BaP and BP3 were not investigated. These should however be studied in order to evaluate generalization or not of sorption mechanisms. We demonstrated that over a long time period of 180 days, PFOS concentration continuously increasing for MP particles. Simulated desorption was carried out using PFOS spiked on PE, and we showed that the bile salt desorbs PFOS from PE within 4 days during artificial digestion. Desorption accounted for 70 to 80 %. Sodium taurocholate enhanced the bioavailability of PFOS associated to PE MPs. To further investigate the desorption efficiency of sodium taurocholate, desorbed quantities of PFOS should be measured at more time points to determine desorption kinetics. The method developed could be used as an approach to better understand desorption of toxicants from MPs during digestion. This study is limited in using one single polymer type, with irrelevant environmental concentration of MPs and relatively high contaminant concentrations. Further studies should be conducted by using different types of polymers (e.g. PS, PP) and environmentally relevant concentrations of MPs as well as relevant mixture and concentrations of contaminants. Investigations on emerging pollutants and further PFASs, in combination with other plastic types are needed since adsorption and desorption behavior depends both on the plastic type and the considered pollutant. Future projects should also make use of higher tier studies to evaluate the burden of MPs as vectors for toxic substances as well as the reliability of artificial digestion experiments.

To investigate the toxicity of MPs, early life stages of zebrafish were exposed to industrial MPs spiked with model pollutants or to environmental MPs. The present work demonstrated that exposures to HDPE particles from different size ranges did not induce acute toxic or sublethal effects in zebrafish embryos, following the standardized protocol of OECD TG 236 (fish embryo test). However, additional endpoints may be used to record further sublethal effects such as biotransformation at the molecular and biochemical levels. Therefore, the FET demonstrated that there is no acute toxicity associated with MPs. LPMR was not altered under any artificial MPs tested, while organic extracts of environmental samples revealed hypoactivity in larvae. The chemical composition of MPs seems to explain the toxicity

observed, since PB samples were characterized by numerous hydrocarbons detected. Results indicate that sublethal toxic effects observed using early life stages of zebrafish exposed to leachates are most likely caused by pollutants associated with MPs rather than by the MPs themselves. Overall, for the identification of adverse effects using embryos and larvae due to exposures to MPs and potentially associated pollutants, more sensitive biomarkers are needed. In addition, the use of another fish model with a longer embryonic stage (i.e. Japanese or Marine medaka) might be necessary to better characterize developmental toxicity of MPs.

Finally, chronic dietary exposure of zebrafish to MPs had significant deleterious effects on adult zebrafish as well as on their unfed F1 offspring (survival, behavior). Growth decreased in adult females and reproductive success was also altered. This can be linked to some plastic additives and/or sorbed pollutants, directly bioavailable to the fish after ingestion of MPs. Deleterious effects observed on the F1 offspring larvae could be induced by either the parental transfer of chemicals desorbed from MPs and/or be an indirect effect on the parental physiological state leading to poorer egg quality. The chemical composition of MPs might explain the different toxicity effects observed. This study raises the question of the environmental risk of environmental MPs, on aquatic organisms and in particular on fish recruitment.

In general, the objectives presented in the introduction were fulfilled, even if some aspects were not been fully solved. In fact, the artificial sorption of model pollutants onto MPs demonstrated that MPs are able to sorb pollutants at high concentrations. The use of zebrafish ELS seems insufficient in term of sensitivity to conclude on the toxicity of different MPs, however, results obtained from exposure of ELS to organic extracts of MPs tended to confirm the fact that MPs might play a role in the vectorization of pollutants. Finally, chronic dietary exposure of adults to MPs induced deleterious effects in exposed fish and their progeny and confirmed the role of MPs as vector for pollutants. In conclusion, the standardized protocol using zebrafish embryos (OECD TG236) might not be suitable to detect the potential effects of MP exposure. More sensitive endpoints, such as swimming activity of larvae or biochemical biomarkers including EROD activity for example should be investigated to test the toxicity of MPs using fish ELS. Chronic dietary exposure provided clear indications for existing or non-existing MPs specific effects on aquatic organisms.

References

Abdel-Shafy H. I., Mansour M. S. M. (2016). A review on polycyclic aromatic hydrocarbons: Source, environmental impact, effect on human health and remediation. Egyptian Journal of Petroleum. 25, 107-123

Acampora H., Lyashevska O., Van Franeker J. A., O'Connor I. (2016). The use of beached bird surveys for marine plastic litter monitoring in Ireland. Marine Environmental Research. 120, 122-129

Ackerman J. T., Eagles-Smith C. A., Herzog M. P., Hartman C. A. (2016). Maternal transfer of contaminants in birds: Mercury and selenium concentrations in parents and their eggs. Environmental Pollution. 210, 145-154

Acosta-Coley I., Mendez-Cuadro D., Rodriguez-Cavallo E., de la Rosa J., Olivero-Verbel J. (2019). Trace elements in microplastics in Cartagena: A hotspot for plastic pollution at the Caribbean. Marine Pollution Bulletin. 139, 402-411

Acosta-Coley I., Olivero-Verbel J. (2015). Microplastic resin pellets on an urban tropical beach in Colombia. Environmental Monitoring and Assessment. 187, 435

Adams R. G., Lohmann R., Fernandez L. A., MacFarlane J. K. (2007). Polyethylene Devices: Passive Samplers for Measuring Dissolved Hydrophobic Organic Compounds in Aquatic Environments. Environmental Science & Technology. 41, 1317-1323

Ahrens L., Bundschuh M. (2014). Fate and effects of poly- and perfluoroalkyl substances in the aquatic environment: A review. Environmental Toxicology and Chemistry. 33, 1921-1929

Ahrens L., Felizeter S., Ebinghaus R. (2009). Spatial distribution of polyfluoroalkyl compounds in seawater of the German Bight. Chemosphere. 76, 179-184.

Akyüz M., Çabuk H. (2010). Gas–particle partitioning and seasonal variation of polycyclic aromatic hydrocarbons in the atmosphere of Zonguldak, Turkey. Science of The Total Environment. 408, 5550-5558

Alfonso S., Blanc M., Joassard L., Keiter S. H., Munschy C., Loizeau V., Bégout M.-L., Cousin X. (2019). Examining multi- and transgenerational behavioral and molecular alterations resulting from parental exposure to an environmental PCB and PBDE mixture. Aquatic Toxicology. 208, 29-38

Ali S., Champagne D. L., Richardson M. K. (2012). Behavioral profiling of zebrafish embryos exposed to a panel of 60 water-soluble compounds. Behavioural Brain Research. 228, 272-283

Alomar C., Sureda A., Capó X., Guijarro B., Tejada S., Deudero S. (2017). Microplastic ingestion by *Mullus surmuletus Linnaeus*, 1758 fish and its potential for causing oxidative stress. Environmental Research. 159, 135-142

Anbumani S., Kakkar P. (2018). Ecotoxicological effects of microplastics on biota: a review. Environmental Science and Pollution Research. 25, 14373-14396

Andrade-Eiroa A., Canle L. M., Leroy-Cancellieri V., Victor C. (2015). Solid phase extraction of organic compounds: a critical review - Part I. 80, 641-654

Andrady A. L. (2011). Microplastics in the marine environment. Marine Pollution Bulletin. 62, 1596-1605

Andrady A. L., Hamid H. S., Torikai A. (2003). Effects of climate change and UV-B on materials. Photochemical & Photobiological Sciences. 2, 68-72

Andrady A. L., Neal M. A. (2009). Applications and societal benefits of plastics. Philosophical Transactions of the Royal Society B: Biological Sciences. 364, 1977-1984

Antunes J. C., Frias J. G. L., Micaelo A. C., Sobral P. (2013). Resin pellets from beaches of the Portuguese coast and adsorbed persistent organic pollutants. Estuarine, Coastal and Shelf Science. 130, 62-69

Arthur C., Baker J. E., Bamford H. A. (2009). Proceedings of the International Research Workshop on the Occurrence, Effects, and Fate of Microplastic Marine Debris, September 9-11, 2008, University of Washington Tacoma, Tacoma, WA, USA.

Avio C. G., Gorbi S., Milan M., Benedetti M., Fattorini D., d'Errico G., Pauletto M., Bargelloni L., Regoli F. (2015). Pollutants bioavailability and toxicological risk from microplastics to marine mussels. Environmental Pollution. 198, 211-222

Azizian S. (2004). Kinetic models of sorption: a theoretical analysis. Journal of Colloid and Interface Science. 276, 47-52

Baduel C., Paxman C. J., Mueller J. F. (2015). Perfluoroalkyl substances in a firefighting training ground (FTG), distribution and potential future release. Journal of Hazardous Materials. 296, 46-53

Bakir A., O'Connor I. A., Rowland S. J., Hendriks A. J., Thompson R. C. (2016). Relative importance of microplastics as a pathway for the transfer of hydrophobic organic chemicals to marine life. Environmental Pollution. 219, 56-65

Bakir A., Rowland S. J., Thompson R. C. (2012). Competitive sorption of persistent organic pollutants onto microplastics in the marine environment. Mar Pollut Bull. 64, 2782-2789

Balmer M. E., Buser H.-R., Müller M. D., Poiger T. (2005). Occurrence of Some Organic UV Filters in Wastewater, in Surface Waters, and in Fish from Swiss Lakes. Environmental Science & Technology. 39, 953-962

Bargar T. A., Scott G. I., Cobb G. P. (2001). Maternal transfer of contaminants: Case study of the excretion of three polychlorinated biphenyl congeners and technical-grade endosulfan into eggs by white leghorn chickens (*Gallus domesticus*). Environmental Toxicology and Chemistry. 20, 61-67

Barhoumi T., Briet M., Kasal D. A., Fraulob-Aquino J. C., Idris-Khodja N., Laurant P., Paradis P., Schiffrin E. L. (2014). Erythropoietin-induced hypertension and vascular injury in mice overexpressing human endothelin-1: exercise attenuated hypertension, oxidative stress, inflammation and immune response. 32, 784-794

Barnes D. K. A., Galgani F., Thompson R. C., Barlaz M. (2009). Accumulation and fragmentation of plastic debris in global environments. Philosophical Transactions of the Royal Society B: Biological Sciences. 364, 1985-1998

Barnes D. K. A., Walters A., Gonçalves L. (2010). Macroplastics at sea around Antarctica. Marine Environmental Research. 70, 250-252

Barse A. V., Chakrabarti T., Ghosh T. K., Pal A. K., Jadhao S. B. (2007). Endocrine disruption and metabolic changes following exposure of *Cyprinus carpio* to diethyl phthalate. Pesticide Biochemistry and Physiology. 88, 36-42

Batel A., Borchert F., Reinwald H., Erdinger L., Braunbeck T. (2018). Microplastic accumulation patterns and transfer of benzo[a]pyrene to adult zebrafish (*Danio rerio*) gills and zebrafish embryos. Environmental Pollution. 235, 918-930

Batel A., Linti F., Scherer M., Erdinger L., Braunbeck T. (2016). Transfer of benzo[a]pyrene from microplastics to Artemia nauplii and further to zebrafish via a trophic food web experiment: CYP1A induction and visual tracking of persistent organic pollutants. Environmental Toxicology and Chemistry. 35, 1656-1666

Beaudouin R., Goussen B., Piccini B., Augustine S., Devillers J., Brion F., Péry A. R. R. (2015). An Individual-Based Model of Zebrafish Population Dynamics Accounting for Energy Dynamics. PLOS ONE. 10, e0125841

Becker A. M., Gerstmann S., Frank H. (2008). Perfluorooctane surfactants in waste waters, the major source of river pollution. Chemosphere. 72, 115-121

Beiras R., Bellas J., Cachot J., Cormier B., Cousin X., Engwall M., Gambardella C., Garaventa F., Keiter S., Le Bihanic F., et al. (2018). Ingestion and contact with polyethylene microplastics does not cause acute toxicity on marine zooplankton. Journal of Hazardous Materials. 360, 452-460

Beiras R., Tato T., López-Ibáñez S. (2019). A 2-Tier standard method to test the toxicity of microplastics in marine water using *Paracentrotus lividus* and *Acartia clausi* larvae. Environmental Toxicology and Chemistry. 38, 630-637

Bejgarn S., MacLeod M., Bogdal C., Breitholtz M. (2015). Toxicity of leachate from weathering plastics: An exploratory screening study with *Nitocra spinipes*. Chemosphere. 132, 114-119

Belanger S. E., Rawlings J. M., Carr G. J. (2013). Use of fish embryo toxicity tests for the prediction of acute fish toxicity to chemicals. Environmental Toxicology and Chemistry. 32, 1768-1783

Bellas J., Granmo Å., Beiras R. (2005). Embryotoxicity of the antifouling biocide zinc pyrithione to sea urchin (*Paracentrotus lividus*) and mussel (*Mytilus edulis*). Marine Pollution Bulletin. 50, 1382-1385

Bellas J., Martínez-Armental J., Martínez-Cámara A., Besada V., Martínez-Gómez C. (2016). Ingestion of microplastics by demersal fish from the Spanish Atlantic and Mediterranean coasts. Marine Pollution Bulletin. 109, 55-60

Bessa F., Barría P., Neto J. M., Frias J. P. G. L., Otero V., Sobral P., Marques J. C. (2018). Occurrence of microplastics in commercial fish from a natural estuarine environment. Marine Pollution Bulletin. 128, 575-584

Besseling E., Wegner A., Foekema E. M., van den Heuvel-Greve M. J., Koelmans A. A. (2013). Effects of Microplastic on Fitness and PCB Bioaccumulation by the Lugworm *Arenicola marina* (L.). Environmental Science & Technology. 47, 593-600

Biesinger M. C., Payne B. P., Grosvenor A. P., Lau L. W. M., Gerson A. R., Smart R. S. C. (2011). Resolving surface chemical states in XPS analysis of first row transition metals, oxides and hydroxides: Cr, Mn, Fe, Co and Ni. Applied Surface Science. 257, 2717-2730

Blüthgen N., Zucchi S., Fent K. (2012). Effects of the UV filter benzophenone-3 (oxybenzone) at low concentrations in zebrafish (*Danio rerio*). Toxicology and Applied Pharmacology. 263, 184-194

Bocquené G., Roig A., Fournier D. (1997). Cholinesterases from the common oyster (*Crassostrea gigas*). FEBS Letters. 407, 261-266

Boehler S., Lörracher A.-K., Schubert J., Braunbeck T. (2018). Comparative live-imaging of in vivo EROD (ethoxyresorufin-O-deethylase) induction in zebrafish (*Danio rerio*) and fathead minnow (*Pimephales promelas*) embryos after exposure to PAHs and river sediment extracts. Science of The Total Environment. 621, 827-838

Boerger C. M., Lattin G. L., Moore S. L., Moore C. J. (2010). Plastic ingestion by planktivorous fishes in the North Pacific Central Gyre. Marine Pollution Bulletin. 60, 2275-2278

Bogdal C., Scheringer M., Abad E., Abalos M., van Bavel B., Hagberg J., Fiedler H. (2013). Worldwide distribution of persistent organic pollutants in air, including results of air monitoring by passive air sampling in five continents. TrAC Trends in Analytical Chemistry. 46, 150-161

Bosker T., Behrens P., Vijver M. G. (2017). Determining global distribution of microplastics by combining citizen science and in-depth case studies. Integrated Environmental Assessment and Management. 13, 536-541

Bosker T., Guaita L., Behrens P. (2018). Microplastic pollution on Caribbean beaches in the Lesser Antilles. Marine Pollution Bulletin. 133, 442-447

Bour A., Haarr A., Keiter S., Hylland K. (2018). Environmentally relevant microplastic exposure affects sediment-dwelling bivalves. Environmental Pollution. 236, 652-660

Bouraoui Z., Banni M., Ghedira J., Clerandeau C., Narbonne J. F., Boussetta H. (2009). Evaluation of enzymatic biomarkers and lipoperoxidation level in *Hediste diversicolor* exposed to copper and benzo[a]pyrene. Ecotoxicology and Environmental Safety. 72, 1893-1898

Bowman J. C., Zhou J. L., Readman J. W. (2002). Sorption and desorption of benzo(a)pyrene in aquatic systems. Journal of Environmental Monitoring. 4, 761-766

Bradford M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Analytical Biochemistry. 72, 248-254

Bratkovics S., Sapozhnikova Y. (2011). Determination of seven commonly used organic UV filters in fresh and saline waters by liquid chromatography-tandem mass spectrometry. Analytical Methods. 3, 2943-2950

Bratkovics S., Wirth E., Sapozhnikova Y., Pennington P., Sanger D. (2015). Baseline monitoring of organic sunscreen compounds along South Carolina's coastal marine environment. Marine Pollution Bulletin. 101, 370-377

Braunbeck T., Boettcher M., Hollert H., Kosmehl T., Lammer E., Leist E., Rudolf M., Seitz N. (2005). Towards an alternative for the acute fish LC_{50} test in chemical assessment: The zebrafish (*Danio rerio*) embryo toxicity test - an update. ALTEX. 22, 87-102.

Braunbeck T., Kais B., Lammer E., Otte J., Schneider K., Stengel D., Strecker R. (2015). The fish embryo test (FET): origin, applications, and future. Environmental Science and Pollution Research. 22, 16247-16261

Bringer A., Thomas H., Prunier G., Dubillot E., Bossut N., Churlaud C., Clérandeau C., Le Bihanic F., Cachot J. (2020). High density polyethylene (HDPE) microplastics impair development and swimming activity of Pacific oyster D-larvae, *Crassostrea gigas*, depending on particle size. Environmental Pollution. 260, 113978

Browne M. A., Crump P., Niven S. J., Teuten E., Tonkin A., Galloway T., Thompson R. (2011). Accumulation of Microplastic on Shorelines Woldwide: Sources and Sinks. Environmental Science & Technology. 45, 9175-9179

Browne Mark A., Niven Stewart J., Galloway Tamara S., Rowland Steve J., Thompson Richard C. (2013). Microplastic Moves Pollutants and Additives to Worms, Reducing Functions Linked to Health and Biodiversity. Current Biology. 23, 2388-2392

Brydson J. A. 1999. Plastics Materials (Butterworth-Heinemann).

Bucheli T. D., Fent K. (1995). Induction of cytochrome P450 as a biomarker for environmental contamination in aquatic ecosystems. Critical Reviews in Environmental Science and Technology. 25, 201-268

Buege J. A., Aust S. D. 1978. [30] Microsomal lipid peroxidation. In: Methods in Enzymology. Academic Press. p. 302-310.

Burke M. D., Mayer R. T. (1974). Ethoxyresorufin: direct fluorimetric assay of a microsomal O-dealkylation which is preferentially inducible by 3-methylcholanthrene. Drug Metabolism and Disposition. 2, 583

Butterworth A., Clegg I., Bass C. 2012. Marine debris: a global picture of the impact on animal welfare and of animal-focused solutions WSPA International, 222 Grays Inn Road, London, WC1X 8HB: WSPA International, 222 Grays Inn Road, London, WC1X 8HB.

Campani T., Baini M., Giannetti M., Cancelli F., Mancusi C., Serena F., Marsili L., Casini S., Fossi M. C. (2013). Presence of plastic debris in loggerhead turtle stranded along the Tuscany coasts of the Pelagos Sanctuary for Mediterranean Marine Mammals (Italy). Marine Pollution Bulletin. 74, 225-230

Cardona L., Álvarez de Quevedo I., Borrell A., Aguilar A. (2012). Massive Consumption of Gelatinous Plankton by Mediterranean Apex Predators. PLOS ONE. 7, e31329

Carlson E. A., Li Y., Zelikoff J. T. (2004). Suppressive effects of benzo[a]pyrene upon fish immune function: Evolutionarily conserved cellular mechanisms of immunotoxicity. Marine Environmental Research. 58, 731-734

Carpenter E. J., Smith K. L. (1972). Plastics on the Sargasso Sea Surface. Science. 175, 1240

Casale P., Freggi D., Paduano V., Oliverio M. (2016). Biases and best approaches for assessing debris ingestion in sea turtles, with a case study in the Mediterranean. Marine Pollution Bulletin. 110, 238-249

Castaño A., Bols N., Braunbeck T., Dierickx P., Halder M., Isomaa B., Kawahara K., Lee L. E. J., Mothersill C., Pärt P., et al. (2003). The use of Fish Cells in Ecotoxicology: The Report and Recommendations of ECVAM Workshop 47. Alternatives to Laboratory Animals. 31, 317-351

Celina M. C. (2013). Review of polymer oxidation and its relationship with materials performance and lifetime prediction. Polymer Degradation and Stability. 98, 2419-2429

Chaisuksant Y., Yu Q., Connell D. W. (1998). Effects of Halobenzenes on Growth Rate of Fish (*Gambusia affinis*). Ecotoxicology and Environmental Safety. 39, 120-130

Chatel A., Bruneau M., Lievre C., Goupil A., Mouneyrac C. (2017). Spermatozoa: A relevant biological target for genotoxicity assessment of contaminants in the estuarine bivalve *Scrobicularia plana*. Marine Pollut Bull. 116, 488-490.

Cheang C. C., Ma Y., Fok L. (2018). Occurrence and Composition of Microplastics in the Seabed Sediments of the Coral Communities in Proximity of a Metropolitan Area. Int J Environ Res Public Health. 15, 2270

Chen H., Reinhard M., Nguyen V. T., Gin K. Y.-H. (2016). Reversible and irreversible sorption of perfluorinated compounds (PFCs) by sediments of an urban reservoir. Chemosphere. 144, 1747-1753

Chen Q., Gundlach M., Yang S., Jiang J., Velki M., Yin D., Hollert H. (2017). Quantitative investigation of the mechanisms of microplastics and nanoplastics toward zebrafish larvae locomotor activity. Sci Total Environ. 584-585, 1022-1031.

Chen Q., Yin D., Jia Y., Schiwy S., Legradi J., Yang S., Hollert H. (2017). Enhanced uptake of BPA in the presence of nanoplastics can lead to neurotoxic effects in adult zebrafish. Science of The Total Environment. 609, 1312-1321

Chen T.-H., Wang Y.-H., Wu Y.-H. (2011). Developmental exposures to ethanol or dimethylsulfoxide at low concentrations alter locomotor activity in larval zebrafish: Implications for behavioral toxicity bioassays. Aquatic Toxicology. 102, 162-166

Chiffre A., Clérandeau C., Dwoinikoff C., Le Bihanic F., Budzinski H., Geret F., Cachot J. (2016). Psychotropic drugs in mixture alter swimming behaviour of Japanese medaka (*Oryzias latipes*) larvae above environmental concentrations. Environmental Science and Pollution Research. 23, 4964-4977

Chiou M.-S., Li H.-Y. (2002). Equilibrium and kinetic modeling of adsorption of reactive dye on cross-linked chitosan beads. Journal of Hazardous Materials. 93, 233-248

Choi J. S., Jung Y.-J., Hong N.-H., Hong S. H., Park J.-W. (2018). Toxicological effects of irregularly shaped and spherical microplastics in a marine teleost, the sheepshead minnow (*Cyprinodon variegatus*). Marine Pollution Bulletin. 129, 231-240

Cole M., Lindeque P., Fileman E., Halsband C., Goodhead R., Moger J., Galloway T. S. (2013). Microplastic Ingestion by Zooplankton. Environmental Science & Technology. 47, 6646-6655

Cole M., Lindeque P., Halsband C., Galloway T. S. (2011). Microplastics as contaminants in the marine environment: A review. Marine Pollution Bulletin. 62, 2588-2597

Collins J. F., Brown J. P., Dawson S. V., Marty M. A. (1991). Risk assessment for benzo[a]pyrene. Regulatory Toxicology and Pharmacology. 13, 170-184

Cooper D. A., Corcoran P. L. (2010). Effects of mechanical and chemical processes on the degradation of plastic beach debris on the island of Kauai, Hawaii. Marine Pollution Bulletin. 60, 650-654

Corcoran P. L., Biesinger M. C., Grifi M. (2009). Plastics and beaches: A degrading relationship. Marine Pollution Bulletin. 58, 80-84

Cormier B., Batel A., Cachot J., Bégout M.-L., Braunbeck T., Cousin X., Keiter S. H. (2019). Multi-Laboratory Hazard Assessment of Contaminated Microplastic Particles by Means of Enhanced Fish Embryo Test With the Zebrafish (*Danio rerio*). Front. Mar. Sci. 7.

Cormier B., Perdriat Q., Tato T., Gambardella C., Costa E., Dubocq F., Karmann A., Van Arkel K., Lemoine S., Lagarde F., et al. (in-prep. 2020). Toxicity assessment of environmental microplastics on early life stages of three aquatic organisms.

Coronado M., De Haro H., Deng X., Rempel M. A., Lavado R., Schlenk D. (2008). Estrogenic activity and reproductive effects of the UV-filter oxybenzone (2-hydroxy-4-methoxyphenyl-methanone) in fish. Aquatic Toxicology. 90, 182-187

Corsolini S. (2009). Industrial contaminants in Antarctic biota. Journal of Chromatography A. 1216, 598-612

Costa E., Gambardella C., Piazza V., Greco G., Lavorano S., Beltrandi M., Bongiovanni E., Gnone G., Faimali M., Garaventa F. (2015). Effect of neurotoxic compounds on ephyrae of *Aurelia aurita* jellyfish. Hydrobiologia. 759, 75-84

Costa M. F., Ivar do Sul J. A., Silva-Cavalcanti J. S., Araújo M. C. B., Spengler Â., Tourinho P. S. (2010). On the importance of size of plastic fragments and pellets on the strandline: a snapshot of a Brazilian beach. Environmental Monitoring and Assessment. 168, 299-304

Cózar A., Echevarría F., González-Gordillo J. I., Irigoien X., Úbeda B., Hernández-León S., Palma Á. T., Navarro S., García-de-Lomas J., Ruiz A., et al. (2014). Plastic debris in the open ocean. Proceedings of the National Academy of Sciences. 111, 10239

Daan N. (1973). A quantitative analysis of the food intake of North Sea cod, Gadus Morhua. Netherlands Journal of Sea Research. 6, 479-517

Davranche M., Veclin C., Pierson-Wickmann A.-C., El Hadri H., Grassl B., Rowenczyk L., Dia A., Ter Halle A., Blancho F., Reynaud S., et al. (2019). Are nanoplastics able to bind significant amount of metals? The lead example. Environmental Pollution. 249, 940-948

D'Costa A., Shepherd I. T. (2009). Zebrafish Development and Genetics: Introducing Undergraduates to Developmental Biology and Genetics in a Large Introductory Laboratory Class. Zebrafish. 6, 169-177

de Sá L. C., Luís L. G., Guilhermino L. (2015). Effects of microplastics on juveniles of the common goby (*Pomatoschistus microps*): Confusion with prey, reduction of the predatory performance and efficiency, and possible influence of developmental conditions. Environmental Pollution. 196, 359-362

de Sá L. C., Oliveira M., Ribeiro F., Rocha T. L., Futter M. N. (2018). Studies of the effects of microplastics on aquatic organisms: What do we know and where should we focus our efforts in the future? Science of The Total Environment. 645, 1029-1039

Della Torre C., Bergami E., Salvati A., Faleri C., Cirino P., Dawson K. A., Corsi I. (2014). Accumulation and Embryotoxicity of Polystyrene Nanoparticles at Early Stage of Development of Sea Urchin Embryos *Paracentrotus lividus*. Environmental Science & Technology. 48, 12302-12311

Deng J., Liu C., Yu L., Zhou B. (2010). Chronic exposure to environmental levels of tribromophenol impairs zebrafish reproduction. Toxicology and Applied Pharmacology. 243, 87-95

Deng S., Niu L., Bei Y., Wang B., Huang J., Yu G. (2013). Adsorption of perfluorinated compounds on aminated rice husk prepared by atom transfer radical polymerization. Chemosphere. 91, 124-130

Derraik J. G. B. (2002). The pollution of the marine environment by plastic debris: a review. Marine Pollution Bulletin. 44, 842-852

Dévier M.-H., Le Menach K., Viglino L., Di Gioia L., Lachassagne P., Budzinski H. (2013). Ultra-trace analysis of hormones, pharmaceutical substances, alkylphenols and phthalates in two French natural mineral waters. Science of The Total Environment. 443, 621-632

Diana J. S. (1983). An energy budget for northern pike (*Esox lucius*). Canadian Journal of Zoology. 61, 1968-1975

Díaz-Cruz S. M., Llorca M., Barceló D., Barceló D. (2008). Organic UV filters and their photodegradates, metabolites and disinfection by-products in the aquatic environment. TrAC Trends in Analytical Chemistry. 27, 873-887

Dong X., Zheng M., Qu L., Shi L., Wang L., Zhang Y., Liu X., Qiu Y., Zhu H. (2019). Sorption of Tonalide, Musk Xylene, Galaxolide, and Musk Ketone by microplastics of polyethylene and polyvinyl chloride. Marine Pollution Bulletin. 144, 129-133

Dris R., Gasperi J., Rocher V., Saad M., Renault N., Tassin B. (2015). Microplastic contamination in an urban area: a case study in Greater Paris. Environmental Chemistry. 12, 592-599

Duan X., Liu J., Zhang D., Yin P., Li Y., Li X. (2015). An assessment of human influences on sources of polycyclic aromatic hydrocarbons in the estuarine and coastal sediments of China. Marine Pollution Bulletin. 97, 309-318

Egan R. J., Bergner C. L., Hart P. C., Cachat J. M., Canavello P. R., Elegante M. F., Elkhayat S. I., Bartels B. K., Tien A. K., Tien D. H., et al. (2009). Understanding behavioral and physiological phenotypes of stress and anxiety in zebrafish. Behavioural Brain Research. 205, 38-44

El Nemr A., Abd-Allah A. M. A. (2003). Contamination of polycyclic aromatic hydrocarbons (PAHs) in microlayer and subsurface waters along Alexandria coast, Egypt. Chemosphere. 52, 1711-1716

Ellman G. L., Courtney K. D., Andres V., Featherstone R. M. (1961). A new and rapid colorimetric determination of acetylcholinesterase activity. Biochemical Pharmacology. 7, 88-95

Embry M. R., Belanger S. E., Braunbeck T. A., Galay-Burgos M., Halder M., Hinton D. E., Léonard M. A., Lillicrap A., Norberg-King T., Whale G. (2010). The fish embryo toxicity test as an animal alternative method in hazard and risk assessment and scientific research. Aquat Toxicol. 97, 79-87

Emran F., Rihel J., Dowling J. E. (2008). A Behavioral Assay to Measure Responsiveness of Zebrafish to Changes in Light Intensities. JoVE. e923

Endo S., Koelmans A. A. 2016. Sorption of hydrophobic organic compounds to plastics in marine environments: Equilibrium. In: Hazardous Chemicals Associated with Plastics in the Marine Environment. Springer International Publishing Switzerland. p. 185-204.

Engler R. E. (2012). The Complex Interaction between Marine Debris and Toxic Chemicals in the Ocean. Environmental Science & Technology. 46, 12302-12315

Eriksen M., Lebreton L. C. M., Carson H. S., Thiel M., Moore C. J., Borerro J. C., Galgani F., Ryan P. G., Reisser J. (2014). Plastic Pollution in the World's Oceans: More than 5 Trillion Plastic Pieces Weighing over 250,000 Tons Afloat at Sea. PLOS ONE. 9, e111913

Eriksson U., Roos A., Lind Y., Hope K., Ekblad A., Kärrman A. (2016). Comparison of PFASs contamination in the freshwater and terrestrial environments by analysis of eggs from osprey (*Pandion haliaetus*), tawny owl (*Strix aluco*), and common kestrel (*Falco tinnunculus*). Environmental Research. 149, 40-47

Erni-Cassola G., Zadjelovic V., Gibson M.I., Christie-Oleza J.A. (2019). Distribution of plastic polymer types in the marine environment; A meta-analysis. Journal of Hazardous Materials. 369, 691-698

Espinosa C., García Beltrán J. M., Esteban M. A., Cuesta A. (2018). In vitro effects of virgin microplastics on fish head-kidney leucocyte activities. Environmental Pollution. 235, 30-38

EU (2010). Directive 2010/63/EU of the European parliament and of the council of 22 September 2010 on the protection of animals used for scientific purposes. Off J EU. L, 33-79.

Faimali M., Garaventa F., Piazza V., Costa E., Greco G., Mazzola V., Beltrandi M., Bongiovanni E., Lavorano S., Gnone G. (2014). Ephyra jellyfish as a new model for ecotoxicological bioassays. Marine Environmental Research. 93, 93-101

Fang C., Wu X., Huang Q., Liao Y., Liu L., Qiu L., Shen H., Dong S. (2012). PFOS elicits transcriptional responses of the ER, AHR and PPAR pathways in *Oryzias melastigma* in a stage-specific manner. Aquat Toxicol. 106-107, 9-19.

Faria A. M., Borges R., Gonçalves E. J. (2014). Critical swimming speeds of wild-caught sand-smelt *Atherina presbyter* larvae. Journal of Fish Biology. 85, 953-959

Farrell P., Nelson K. (2013). Trophic level transfer of microplastic: *Mytilus edulis* (L.) to *Carcinus maenas* (L.). Environmental Pollution. 177, 1-3

Fendall L. S., Sewell M. A. (2009). Contributing to marine pollution by washing your face: Microplastics in facial cleansers. Marine Pollution Bulletin. 58, 1225-1228

Fent K., Kunz P. Y., Zenker A., Rapp M. (2010). A tentative environmental risk assessment of the UV-filters 3-(4-methylbenzylidene-camphor), 2-ethyl-hexyl-4-trimethoxycinnamate, benzophenone-3, benzophenone-4 and 3-benzylidene camphor. Marine Environmental Research. 69, S4-S6

Foekema E. M., Fischer A., Parron M. L., Kwadijk C., de Vries P., Murk A. J. (2012). Toxic concentrations in fish early life stages peak at a critical moment. Environmental Toxicology and Chemistry. 31, 1381-1390

Foekema E. M., Lopez Parron M., Mergia M. T., Carolus E. R. M., vd Berg J. H. J., Kwadijk C., Dao Q., Murk A. J. (2014). Internal effect concentrations of organic substances for early life development of egg-exposed fish. Ecotoxicology and Environmental Safety. 101, 14-22

Fossi M. C., Panti C., Guerranti C., Coppola D., Giannetti M., Marsili L., Minutoli R. (2012). Are baleen whales exposed to the threat of microplastics? A case study of the Mediterranean fin whale (*Balaenoptera physalus*). Marine Pollution Bulletin. 64, 2374-2379

Fossi M. C., Romeo T., Baini M., Panti C., Marsili L., Campani T., Canese S., Galgani F., Druon J.-N., Airoldi S., et al. (2017). Plastic Debris Occurrence, Convergence Areas and Fin Whales Feeding Ground in the Mediterranean Marine Protected Area Pelagos Sanctuary: A Modeling Approach. 4,

Fotopoulou K. N., Karapanagioti H. K. (2015). Surface properties of beached plastics. Environmental Science and Pollution Research. 22, 11022-11032

Frantzen M., Falk-Petersen I.-B., Nahrgang J., Smith T. J., Olsen G. H., Hangstad T. A., Camus L. (2012). Toxicity of crude oil and pyrene to the embryos of beach spawning capelin (*Mallotus villosus*). Aquat Toxicol. 108, 42-52

Fred-Ahmadu O. H., Bhagwat G., Oluyoye I., Benson N. U., Ayejuyo O. O., Palanisami T. (2020). Interaction of chemical contaminants with microplastics: Principles and perspectives. Science of The Total Environment. 706, 135978

Frias J. P. G. L., Sobral P., Ferreira A. M. (2010). Organic pollutants in microplastics from two beaches of the Portuguese coast. Marine Pollution Bulletin. 60, 1988-1992

Fries E., Zarfl C. (2012). Sorption of polycyclic aromatic hydrocarbons (PAHs) to low and high density polyethylene (PE). Environmental Science and Pollution Research. 19, 1296-1304

Gago-Ferrero P., Díaz-Cruz M. S., Barceló D. (2011). Occurrence of multiclass UV filters in treated sewage sludge from wastewater treatment plants. Chemosphere. 84, 1158-1165

Galgani F., Hanke G., Maes T. 2015. Global Distribution, Composition and Abundance of Marine Litter. In: Marine Anthropogenic Litter. Cham: Springer International Publishing. p. 29-56.

Gall S. C., Thompson R. C. (2015). The impact of debris on marine life. Marine Pollution Bulletin. 92, 170-179

Galloway T. S., Cole M., Lewis C. (2017). Interactions of microplastic debris throughout the marine ecosystem. Nature Ecology & Evolution. 1, 0116

Galloway T. S., Lewis C. N. (2016). Marine microplastics spell big problems for future generations. Proceedings of the National Academy of Sciences. 113, 2331

Gandara e Silva P. P., Nobre C. R., Resaffe P., Pereira C. D. S., Gusmão F. (2016). Leachate from microplastics impairs larval development in brown mussels. Water Research. 106, 364-370

Gardon T., Reisser C., Soyez C., Quillien V., Le Moullac G. (2018). Microplastics Affect Energy Balance and Gametogenesis in the Pearl Oyster *Pinctada margaritifera*. Environmental Science & Technology. 52, 5277-5286

Gerger C. J., Weber L. P. (2015). Comparison of the acute effects of benzo-a-pyrene on adult zebrafish (*Danio rerio*) cardiorespiratory function following intraperitoneal injection versus aqueous exposure. Aquatic Toxicology. 165, 19-30

Geyer R., Jambeck J. R., Law K. L. (2017). Production, use, and fate of all plastics ever made. Science Advances. 3, e1700782

Giesy J. P., Kannan K. (2001). Global Distribution of Perfluorooctane Sulfonate in Wildlife. Environmental Science & Technology. 35, 1339-1342

Giesy J. P., Kannan K. (2002). Peer Reviewed: Perfluorochemical Surfactants in the Environment. Environmental Science & Technology. 36, 146A-152A

Gigault J., Halle A. t., Baudrimont M., Pascal P.-Y., Gauffre F., Phi T.-L., El Hadri H., Grassl B., Reynaud S. (2018). Current opinion: What is a nanoplastic? Environmental Pollution. 235, 1030-1034

González-Doncel M., González L., Fernández-Torija C., Navas J. M., Tarazona J. V. (2008). Toxic effects of an oil spill on fish early life stages may not be exclusively associated to PAHs: Studies with Prestige oil and medaka (*Oryzias latipes*). Aquatic Toxicology. 87, 280-288

González-Soto N., Hatfield J., Katsumiti A., Duroudier N., Lacave J. M., Bilbao E., Orbea A., Navarro E., Cajaraville M. P. (2019). Impacts of dietary exposure to different sized polystyrene microplastics alone and with sorbed benzo[a]pyrene on biomarkers and whole organism responses in mussels *Mytilus galloprovincialis*. Science of The Total Environment. 684, 548-566

Green D. S., Boots B., Sigwart J., Jiang S., Rocha C. (2016). Effects of conventional and biodegradable microplastics on a marine ecosystem engineer (*Arenicola marina*) and sediment nutrient cycling. Environmental Pollution. 208, 426-434

Gregory M. R. (2009). Environmental implications of plastic debris in marine settings—entanglement, ingestion, smothering, hangers-on, hitch-hiking and alien invasions. Philosophical Transactions of the Royal Society B: Biological Sciences. 364, 2013-2025

Grift R. E., Heino M., Rijnsdorp A. D., Kraak S. B. M., Dieckmann U. (2007). Three-dimensional maturation reaction norms for North Sea plaice. Marine Ecology Progress Series. 334, 213-224

Grosell M., Laliberte C. N., Wood S., Jensen F. B., Wood C. M. (2001). Intestinal HCO3− secretion in marine teleost fish: evidence for an apical rather than a basolateral Cl−/HCO3− exchanger. Fish Physiology and Biochemistry. 24, 81-95

Gudger E. W., Hoffmann W. H. The Whale Shark off Havana Harbor; 1931.

Guney M., Zagury G. J. (2012). Heavy Metals in Toys and Low-Cost Jewelry: Critical Review of U.S. and Canadian Legislations and Recommendations for Testing. Environmental Science & Technology. 46, 4265-4274

Guo L., Zhang B., Xiao K., Zhang Q., Zheng M. (2009). Levels and distributions of polychlorinated biphenyls in sewage sludge of urban wastewater treatment plants. Journal of Environmental Sciences. 21, 468-473

Hack A., Selenka F. (1996). Mobilization of PAH and PCB from contaminated soil using a digestive tract model. Toxicology Letters. 88, 199-210

Hahladakis J. N., Velis C. A., Weber R., Iacovidou E., Purnell P. (2018). An overview of chemical additives present in plastics: Migration, release, fate and environmental impact during their use, disposal and recycling. Journal of Hazardous Materials. 344, 179-199

Hansen E., Nilsson N. H., Lithner D., Lassen C. 2013. Hazardous substances in plastic materials.

Hart N. H., Donovan M. (1983). Fine structure of the chorion and site of sperm entry in the egg of *Brachydanio*. Journal of Experimental Zoology. 227, 277-296

Hart N. H., Pietri R., Donovan M. (1984). The structure of the chorion and associated surface filaments in *Oryzias*—evidence for the presence of extracellular tubules. Journal of Experimental Zoology. 230, 273-296

Hartmann N. B., Rist S., Bodin J., Jensen L. H. S., Schmidt S. N., Mayer P., Meibom A., Baun A. (2017). Microplastics as vectors for environmental contaminants: Exploring sorption,

desorption, and transfer to biota. Integrated Environmental Assessment and Management. 13, 488-493

Haslewood G. A., Tökés L. (1969). Comparative studies of bile salts. Bile salts of the lamprey *Petromyzon marinus* L. Biochem J. 114, 179-184

Heneweer M., Muusse M., Berg M. v. d., Sanderson J. T. (2005). Additive estrogenic effects of mixtures of frequently used UV filters on pS2-gene transcription in MCF-7 cells. Toxicology and Applied Pharmacology. 208, 170-177

Heo N. W., Hong S. H., Han G. M., Hong S., Lee J., Song Y. K., Jang M., Shim W. J. (2013). Distribution of small plastic debris in cross-section and high strandline on Heungnam beach, South Korea. Ocean Science Journal. 48, 225-233

Hidalgo-Ruz V., Gutow L., Thompson R. C., Thiel M. (2012). Microplastics in the Marine Environment: A Review of the Methods Used for Identification and Quantification. Environmental Science & Technology. 46, 3060-3075

Higgins C. P., Luthy R. G. (2006). Sorption of Perfluorinated Surfactants on Sediments. Environmental Science & Technology. 40, 7251-7256

Hofmann A. F., Hagey L. R., Krasowski M. D. (2010). Bile salts of vertebrates: structural variation and possible evolutionary significance. J Lipid Res. 51, 226-246. Epub 2009/07/28

Hong L., Luthy R. G. (2008). Uptake of PAHs into polyoxymethylene and application to oil-soot (lampblack)-impacted soil samples. Chemosphere. 72, 272-281

Hong W.-J., Jia H., Li Y.-F., Sun Y., Liu X., Wang L. (2016). Polycyclic aromatic hydrocarbons (PAHs) and alkylated PAHs in the coastal seawater, surface sediment and oyster from Dalian, Northeast China. Ecotoxicol Environ Saf. 128, 11-20.

Hornung M. W., Cook P. M., Fitzsimmons P. N., Kuehl D. W., Nichols J. W. (2007). Tissue Distribution and Metabolism of Benzo[a]pyrene in Embryonic and Larval Medaka (*Oryzias latipes*). Toxicological Sciences. 100, 393-405

Horri K., Alfonso S., Cousin X., Munschy C., Loizeau V., Aroua S., Bégout M.-L., Ernande B. (2018). Fish life-history traits are affected after chronic dietary exposure to an environmentally realistic marine mixture of PCBs and PBDEs. Science of The Total Environment. 610-611, 531-545

Houde M., Czub G., Small J. M., Backus S., Wang X., Alaee M., Muir D. C. G. (2008). Fractionation and Bioaccumulation of Perfluorooctane Sulfonate (PFOS) Isomers in a Lake Ontario Food Web. Environmental Science & Technology. 42, 9397-9403

Houde M., De Silva A. O., Muir D. C. G., Letcher R. J. (2011). Monitoring of Perfluorinated Compounds in Aquatic Biota: An Updated Review. Environmental Science & Technology. 45, 7962-7973

Howe K., Clark M. D., Torroja C. F., Torrance J., Berthelot C., Muffato M., Collins J. E., Humphray S., McLaren K., Matthews L., et al. (2013). The zebrafish reference genome sequence and its relationship to the human genome. Nature. 496, 498-503

Huang H., Huang C., Wang L., Ye X., Bai C., Simonich M. T., Tanguay R. L., Dong Q. (2010). Toxicity, uptake kinetics and behavior assessment in zebrafish embryos following exposure to perfluorooctanesulphonicacid (PFOS). Aquatic Toxicology. 98, 139-147

Huckins J. N., Tubergen M. W., Manuweera G. K. (1990). Semipermeable membrane devices containing model lipid: A new approach to monitoring the bioavaiiability of lipophilic contaminants and estimating their bioconcentration potential. Chemosphere. 20, 533-552

Hüffer T., Hofmann T. (2016). Sorption of non-polar organic compounds by micro-sized plastic particles in aqueous solution. Environmental Pollution. 214, 194-201

Incardona J. P., Day H. L., Collier T. K., Scholz N. L. (2006). Developmental toxicity of 4-ring polycyclic aromatic hydrocarbons in zebrafish is differentially dependent on AH receptor isoforms and hepatic cytochrome P4501A metabolism. Toxicology and applied pharmacology. 217, 308-321

Incardona J. P., Linbo T. L., Scholz N. L. (2011). Cardiac toxicity of 5-ring polycyclic aromatic hydrocarbons is differentially dependent on the aryl hydrocarbon receptor 2 isoform during zebrafish development. Toxicology and Applied Pharmacology. 257, 242-249

Iversen T.-G., Skotland T., Sandvig K. (2011). Endocytosis and intracellular transport of nanoparticles: Present knowledge and need for future studies. Nano Today. 6, 176-185

Jabeen K., Su L., Li J., Yang D., Tong C., Mu J., Shi H. (2017). Microplastics and mesoplastics in fish from coastal and fresh waters of China. Environmental Pollution. 221, 141-149

Jambeck J. R., Geyer R., Wilcox C., Siegler T. R., Perryman M., Andrady A., Narayan R., Law K. L. (2015). Plastic waste inputs from land into the ocean. Science. 347, 768

Jantzen C. E., Annunziato K. A., Bugel S. M., Cooper K. R. (2016). PFOS, PFNA, and PFOA sub-lethal exposure to embryonic zebrafish have different toxicity profiles in terms of morphometrics, behavior and gene expression. Aquat Toxicol. 175, 160-170.

Jeong C.-B., Kang H.-M., Lee M.-C., Kim D.-H., Han J., Hwang D.-S., Souissi S., Lee S.-J., Shin K.-H., Park H. G., et al. (2017). Adverse effects of microplastics and oxidative stress-induced MAPK/Nrf2 pathway-mediated defense mechanisms in the marine copepod *Paracyclopina nana*. Scientific Reports. 7, 41323

Jönsson E. M., Brandt I., Brunström B. (2002). Gill Filament-Based EROD Assay for Monitoring Waterborne Dioxin-like Pollutants in Fish. Environmental Science & Technology. 36, 3340-3344

Jönsson M. E., Brunström B., Brandt I. (2009). The zebrafish gill model: induction of CYP1A, EROD and PAH adduct formation. Aquat Toxicol. 91, 62-70.

Jovanović B., Gökdağ K., Güven O., Emre Y., Whitley E. M., Kideys A. E. (2018). Virgin microplastics are not causing imminent harm to fish after dietary exposure. Marine Pollution Bulletin. 130, 123-131

Kais B., Ottermanns R., Scheller F., Braunbeck T. (2018). Modification and quantification of in vivo EROD live-imaging with zebrafish (*Danio rerio*) embryos to detect both induction and inhibition of CYP1A. Science of The Total Environment. 615, 330-347

Kais B., Schiwy S., Hollert H., Keiter S. H., Braunbeck T. (2017). In vivo EROD assays with the zebrafish (*Danio rerio*) as rapid screening tools for the detection of dioxin-like activity. Sci Tot Environ. 590-591, 269-280.

Kaiser D., Kowalski N., Waniek J. J. (2017). Effects of biofouling on the sinking behavior of microplastics. Environmental Research Letters. 12, 124003

Kameda Y., Kimura K., Miyazaki M. (2011). Occurrence and profiles of organic sun-blocking agents in surface waters and sediments in Japanese rivers and lakes. Environmental Pollution. 159, 1570-1576

Kannan K., Corsolini S., Falandysz J., Fillmann G., Kumar K. S., Loganathan B. G., Mohd M. A., Olivero J., Wouwe N. V., Yang J. H., et al. (2004). Perfluorooctanesulfonate and Related Fluorochemicals in Human Blood from Several Countries. Environmental Science & Technology. 38, 4489-4495

Karami A., Groman D. B., Wilson S. P., Ismail P., Neela V. K. (2017). Biomarker responses in zebrafish (*Danio rerio*) larvae exposed to pristine low-density polyethylene fragments. Environ Pollut. 223, 466-475.

Karapanagioti H. K., Klontza I. (2008). Testing phenanthrene distribution properties of virgin plastic pellets and plastic eroded pellets found on Lesvos island beaches (Greece). Marine Environmental Research. 65, 283-290

Kärrman A., van Bavel B., Järnberg U., Hardell L., Lindström G. (2006). Perfluorinated chemicals in relation to other persistent organic pollutants in human blood. Chemosphere. 64, 1582-1591

Karthik R., Robin R. S., Purvaja R., Ganguly D., Anandavelu I., Raghuraman R., Hariharan G., Ramakrishna A., Ramesh R. (2018). Microplastics along the beaches of southeast coast of India. Science of The Total Environment. 645, 1388-1399

Keiter S., Burkhardt-Medicke K., Wellner P., Kais B., Färber H., Skutlarek D., Engwall M., Braunbeck T., Keiter S. H., Luckenbach T. (2016). Does perfluorooctane sulfonate (PFOS) act as chemosensitizer in zebrafish embryos? Science of The Total Environment. 548-549, 317-324

Kienzler A., Tronchère X., Devaux A., Bony S. (2012). Assessment of RTG-W1, RTL-W1, and PLHC-1 fish cell lines for genotoxicity testing of environmental pollutants by means of a Fpg-modified comet assay. Toxicology in Vitro. 26, 500-510

Kiessling I. 2003. Derelict fishing gear and other marine debris in Northern Australia.

Kim D., Chae Y., An Y.-J. (2017). Mixture Toxicity of Nickel and Microplastics with Different Functional Groups on Daphnia magna. Environmental Science & Technology. 51, 12852-12858

Kim I.-Y., Hyun C.-K. (2006). Comparative evaluation of the alkaline comet assay with the micronucleus test for genotoxicity monitoring using aquatic organisms. Ecotoxicology and Environmental Safety. 64, 288-297

Kim S., Choi K. (2014). Occurrences, toxicities, and ecological risks of benzophenone-3, a common component of organic sunscreen products: A mini-review. Environment International. 70, 143-157

Kime D. E. (1995). The effects of pollution on reproduction in fish. Reviews in Fish Biology and Fisheries. 5, 52-95

Kimmel C. B., Ballard W. W., Kimmel S. R., Ullmann B., Schilling T. F. (1995). Stages of embryonic development of the zebrafish. Developmental Dynamics. 203, 253-310

Kinnberg K. L., Petersen G. I., Albrektsen M., Minghlani M., Awad S. M., Holbech B. F., Green J. W., Bjerregaard P., Holbech H. (2015). Endocrine-disrupting effect of the ultraviolet filter benzophenone-3 in zebrafish, *Danio rerio*. Environ Toxicol Chem. 34, 2833-2840.

Klumpp D. W., Humphrey C., Huasheng H., Tao F. (2002). Toxic contaminants and their biological effects in coastal waters of Xiamen, China.: II. Biomarkers and embryo malformation rates as indicators of pollution stress in fish. Marine Pollution Bulletin. 44, 761-769

Knecht A. L., Truong L., Simonich M. T., Tanguay R. L. (2017). Developmental benzo[a]pyrene (B[a]P) exposure impacts larval behavior and impairs adult learning in zebrafish. Neurotoxicol and Teratol. 59, 27-34.

Koelmans A. A. (2015). Modeling the role of microplastics in bioaccumulation of organic chemicals to marine aquatic organisms. A critical review. In: Marine Anthropogenic Litter. p. 309-324.

Koelmans A. A., Bakir A., Burton G. A., Janssen C. R. (2016). Microplastic as a Vector for Chemicals in the Aquatic Environment: Critical Review and Model-Supported Reinterpretation of Empirical Studies. Environmental Science and Technology. 50, 3315-3326

Koelmans A. A., Besseling E., Foekema E. M. (2014). Leaching of plastic additives to marine organisms. Environmental Pollution. 187, 49-54

Koelmans A. A., Besseling E., Wegner A., Foekema E. M. (2013). Plastic as a Carrier of POPs to Aquatic Organisms: A Model Analysis. Environ Sci Technol. 47, 7812-7820.

Koelmans A. A., Kooi M., Law K. L., Van Sebille E. (2017). All is not lost: Deriving a top-down mass budget of plastic at sea. Environmental Research Letters. 12,

Koenig S., Solé M. (2012). Natural variability of hepatic biomarkers in Mediterranean deep-sea organisms. Marine Environmental Research. 79, 122-131

Kokalj A. J., Kuehnel D., Puntar B., Žgajnar Gotvajn A., Kalčikova G. (2019). An exploratory ecotoxicity study of primary microplastics versus aged in natural waters and wastewaters. Environmental Pollution. 254, 112980

Kooi M., Nes E. H. v., Scheffer M., Koelmans A. A. (2017). Ups and Downs in the Ocean: Effects of Biofouling on Vertical Transport of Microplastics. Environmental Science & Technology. 51, 7963-7971

Kot-Wasik A., Dąbrowska D., Namieśnik J. (2004). Photodegradation and biodegradation study of benzo(a)pyrene in different liquid media. Journal of Photochemistry and Photobiology A: Chemistry. 168, 109-115

Kowalski N., Reichardt A. M., Waniek J. J. (2016). Sinking rates of microplastics and potential implications of their alteration by physical, biological, and chemical factors. Marine Pollution Bulletin. 109, 310-319

Kunisue T., Chen Z., Buck Louis G. M., Sundaram R., Hediger M. L., Sun L., Kannan K. (2012). Urinary Concentrations of Benzophenone-type UV Filters in U.S. Women and Their Association with Endometriosis. Environmental Science & Technology. 46, 4624-4632

Lagadic L., Caquet T., Amiard J. (1997). Biomarqueurs en écotoxicologie : principes et définitions. Biomarqueurs en écotoxicologie Aspects fondamentaux. 1-9

Lahnsteiner F., Berger B., Kletzl M., Weismann T. (2005). Effect of bisphenol A on maturation and quality of semen and eggs in the brown trout, *Salmo trutta f. fario*. Aquatic Toxicology. 75, 213-224

Laist D. W. 1997. Impacts of Marine Debris: Entanglement of Marine Life in Marine Debris Including a Comprehensive List of Species with Entanglement and Ingestion Records. In: Marine Debris: Sources, Impacts, and Solutions. New York, NY: Springer New York. p. 99-139.

Lammer E., Carr G. J., Wendler K., Rawlings J. M., Belanger S. E., Braunbeck T. (2009). Is the fish embryo toxicity test (FET) with the zebrafish (*Danio rerio*) a potential alternative for the fish acute toxicity test? Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology. 149, 196-209

Langford K. H., Thomas K. V. (2008). Inputs of chemicals from recreational activities into the Norwegian coastal zone. Journal of Environmental Monitoring. 10, 894-898

Larcher, T., Perrichon, P., Vignet, C., Ledevin, M., Le Menach, K., Lyphout, L., Landi, L., Clerandeau, C., Lebihanic, F., Ménard, D., Burgeot, T., Budzinski, H., Akcha, F., Cachot, J., Cousin X. (2014). Chronic dietary exposure of zebrafish to PAH mixtures results in carcinogenic but not genotoxic effects. Environ Sci Pollut Res 21: 13833.

Larsson M., Hagberg J., Rotander A., van Bavel B., Engwall M. (2013). Chemical and bioanalytical characterisation of PAHs in risk assessment of remediated PAH-contaminated soils. Environmental Science and Pollution Research. 20, 8511-8520

Law K. L., Morét-Ferguson S., Maximenko N. A., Proskurowski G., Peacock E. E., Hafner J., Reddy C. M. (2010). Plastic Accumulation in the North Atlantic Subtropical Gyre. Science. 329, 1185

Law K. L., Thompson R. C. (2014). Microplastics in the seas. Science. 345, 144

Le Bihanic F., Clérandeau C., Le Menach K., Morin B., Budzinski H., Cousin X., Cachot J. (2014). Developmental toxicity of PAH mixtures in fish early life stages. Part II: adverse effects in Japanese medaka. Environmental Science and Pollution Research. 21, 13732-13743

Le Bihanic F., Couillard C. M., Rigaud C., Légaré B. (2013). A simple and reliable in vivo EROD activity measurement in single *Fundulus heteroclitus* embryo and larva. Marine Environmental Research. 84, 17-23

Le Bihanic F., Morin B., Cousin X., Le Menach K., Budzinski H., Cachot J. (2014). Developmental toxicity of PAH mixtures in fish early life stages. Part I: adverse effects in rainbow trout. Environmental Science and Pollution Research. 21, 13720-13731

Le Bihanic F., Sommard V., Perrine d. L., Pichon A., Grasset J., Berrada S., Budzinski H., Cousin X., Morin B., Cachot J. (2015). Environmental concentrations of benz[a]anthracene induce developmental defects and DNA damage and impair photomotor response in Japanese medaka larvae. Ecotoxicology and Environmental Safety. 113, 321-328

Le Dû-Lacoste M., Akcha F., Dévier M.-H., Morin B., Burgeot T., Budzinski H. (2013). Comparative study of different exposure routes on the biotransformation and genotoxicity of PAHs in the flatfish species, *Scophthalmus maximus*. Environmental Science and Pollution Research. 20, 690-707

Lebreton L., Slat B., Ferrari F., Sainte-Rose B., Aitken J., Marthouse R., Hajbane S., Cunsolo S., Schwarz A., Levivier A., et al. (2018). Evidence that the Great Pacific Garbage Patch is rapidly accumulating plastic. Scientific Reports. 8, 4666

Lee H., Shim W. J., Kwon J.-H. (2014). Sorption capacity of plastic debris for hydrophobic organic chemicals. Science of The Total Environment. 470-471, 1545-1552

Lee J., Hong S., Song Y. K., Hong S. H., Jang Y. C., Jang M., Heo N. W., Han G. M., Lee M. J., Kang D., et al. (2013). Relationships among the abundances of plastic debris in different size classes on beaches in South Korea. Marine Pollution Bulletin. 77, 349-354

Lee K.-W., Shim W. J., Kwon O. Y., Kang J.-H. (2013). Size-Dependent Effects of Micro Polystyrene Particles in the Marine Copepod *Tigriopus japonicus*. Environmental Science & Technology. 47, 11278-11283

Lee W. M. G., Tong H. C., Yeh S. Y. (1993). Partitioning model of PAHs between gaseous and particulate phases with consideration of reactivity of PAHs in an urban atmosphere. Journal of Environmental Science and Health Part A: Environmental Science and Engineering and Toxicology. 28, 563-583

Legradi J. B., Di Paolo C., Kraak M. H. S., van der Geest H. G., Schymanski E. L., Williams A. J., Dingemans M. M. L., Massei R., Brack W., Cousin X., et al. (2018). An ecotoxicological view on neurotoxicity assessment. Environmental Sciences Europe. 30, 46

Lei L., Wu S., Lu S., Liu M., Song Y., Fu Z., Shi H., Raley-Susman K. M., He D. (2018). Microplastic particles cause intestinal damage and other adverse effects in zebrafish *Danio rerio* and nematode Caenorhabditis elegans. Science of The Total Environment. 619-620, 1-8

Lemaire-Gony S., Lemaire P., Pulsford A. L. (1995). Effects of cadmium and benzo(a)pyrene on the immune system, gill ATPase and EROD activity of European sea bass *Dicentrarchus labrax*. Aquatic Toxicology. 31, 297-313

Li J., Liu H., Paul Chen J. (2018). Microplastics in freshwater systems: A review on occurrence, environmental effects, and methods for microplastics detection. Water Research. 137, 362-374

Lippiatt S., Opfer S., Arthur C. (2013). Marine Debris Monitoring and Assessment. NOAA Technical Memorandum. NOS-OR&R-46,

Lithner D., Damberg J., Dave G., Larsson Å. (2009). Leachates from plastic consumer products – Screening for toxicity with *Daphnia magna*. Chemosphere. 74, 1195-1200

Lithner D., Larsson Å., Dave G. (2011). Environmental and health hazard ranking and assessment of plastic polymers based on chemical composition. Science of The Total Environment. 409, 3309-3324

Liu G., Zhu Z., Yang Y., Sun Y., Yu F., Ma J. (2019). Sorption behavior and mechanism of hydrophilic organic chemicals to virgin and aged microplastics in freshwater and seawater. Environmental Pollution. 246, 26-33

Liu P., Lu K., Li J., Wu X., Qian L., Wang M., Gao S. (2020). Effect of aging on adsorption behavior of polystyrene microplastics for pharmaceuticals: Adsorption mechanism and role of aging intermediates. Journal of Hazardous Materials. 384, 121193

Liu P., Qian L., Wang H., Zhan X., Lu K., Gu C., Gao S. (2019). New Insights into the Aging Behavior of Microplastics Accelerated by Advanced Oxidation Processes. Environmental Science & Technology. 53, 3579-3588

Liu T., Pan L., Jin Q., Cai Y. (2015). Differential gene expression analysis of benzo(a)pyrene toxicity in the clam, *Ruditapes philippinarum*. Ecotoxicology and Environmental Safety. 115, 126-136

Llorca M., Schirinzi G., Martínez M., Barceló D., Farré M. (2018). Adsorption of perfluoroalkyl substances on microplastics under environmental conditions. Environmental Pollution. 235, 680-691

Lobelle D., Cunliffe M. (2011). Early microbial biofilm formation on marine plastic debris. Marine Pollution Bulletin. 62, 197-200

Loganathan B. G., Sajwan K. S., Sinclair E., Senthil Kumar K., Kannan K. (2007). Perfluoroalkyl sulfonates and perfluorocarboxylates in two wastewater treatment facilities in Kentucky and Georgia. Water Research. 41, 4611-4620

Lohmann R. (2017). Microplastics are not important for the cycling and bioaccumulation of organic pollutants in the oceans—but should microplastics be considered POPs themselves? Integrated Environmental Assessment and Management. 13, 460-465

Loraine G. A., Pettigrove M. E. (2006). Seasonal Variations in Concentrations of Pharmaceuticals and Personal Care Products in Drinking Water and Reclaimed Wastewater in Southern California. Environmental Science & Technology. 40, 687-695

Lorenzo J. I., Nieto O., Beiras R. (2002). Effect of humic acids on speciation and toxicity of copper to *Paracentrotus lividus* larvae in seawater. Aquatic Toxicology. 58, 27-41

Lowry O. H., Rosebrough N. J., Farr A. L., Randall R. J. (1951). Protein measurement with the folin phenol reagent. 193, 265-275

Lu Y., Zhang Y., Deng Y., Jiang W., Zhao Y., Geng J., Ding L., Ren H. (2016). Uptake and Accumulation of Polystyrene Microplastics in Zebrafish (*Danio rerio*) and Toxic Effects in Liver. Environmental Science & Technology. 50, 4054-4060

Lusher A. L., McHugh M., Thompson R. C. (2013). Occurrence of microplastics in the gastrointestinal tract of pelagic and demersal fish from the English Channel. Marine Pollution Bulletin. 67, 94-99

Lusher A. L., Tirelli V., O'Connor I., Officer R. (2015). Microplastics in Arctic polar waters: the first reported values of particles in surface and sub-surface samples. Scientific Reports. 5, 14947

Lyche J. L., Nourizadeh-Lillabadi R., Almaas C., Stavik B., Berg V., Skåre J. U., Alestrøm P., Ropstad E. (2010). Natural Mixtures of Persistent Organic Pollutants (POP) Increase Weight Gain, Advance Puberty, and Induce Changes in Gene Expression Associated with Steroid Hormones and Obesity in Female Zebrafish. Journal of Toxicology and Environmental Health, Part A. 73, 1032-1057

Lyche J. L., Nourizadeh-Lillabadi R., Karlsson C., Stavik B., Berg V., Skåre J. U., Alestrøm P., Ropstad E. (2011). Natural mixtures of POPs affected body weight gain and induced transcription of genes involved in weight regulation and insulin signaling. Aquatic Toxicology. 102, 197-204

Lyons M. C., Wong D. K. H., Mulder I., Lee K., Burridge L. E. (2011). The influence of water temperature on induced liver EROD activity in Atlantic cod (*Gadus morhua*) exposed to crude oil and oil dispersants. Ecotoxicology and Environmental Safety. 74, 904-910

Mani T., Hauk A., Walter U., Burkhardt-Holm P. (2015). Microplastics profile along the Rhine River. Scientific Reports. 5, 17988

Marine-Mammal U. 1996. Annual Report of the Marine Mammal Commission: A Report to Congress: The Commission.

Maršić-Lučić J., Lušić J., Tutman P., Bojanić Varezić D., Šiljić J., Pribudić J. (2018). Levels of trace metals on microplastic particles in beach sediments of the island of Vis, Adriatic Sea, Croatia. Marine Pollution Bulletin. 137, 231-236

Martin J. W., Mabury S. A., Solomon K. R., Muir D. C. G. (2003). Dietary accumulation of perfluorinated acids in juvenile rainbow trout (*Oncorhynchus mykiss*). Environmental Toxicology and Chemistry. 22, 189-195

Martins J., Sobral P. (2011). Plastic marine debris on the Portuguese coastline: A matter of size? Marine Pollution Bulletin. 62, 2649-2653

Marty G. D., Hinton D. E., Short J. W., Heintz R. A., Rice S. D., Dambach D. M., Willits N. H., Stegeman J. J. (1997). Ascites, premature emergence, increased gonadal cell apoptosis, and cytochrome P4501A induction in pink salmon larvae continuously exposed to oil-contaminated gravel during development. Canadian Journal of Zoology. 75, 989-1007

Masih J., Singhvi R., Kumar K., Jain V. K., Taneja A. (2012). Seasonal Variation and Sources of Polycyclic Aromatic Hydrocarbons (PAHs) in Indoor and Outdoor Air in a Semi Arid Tract of Northern India. Aerosol and Air Quality Research. 12, 515-525

Mato Y., Isobe T., Takada H., Kanehiro H., Ohtake C., Kaminuma T. (2001). Plastic Resin Pellets as a Transport Medium for Toxic Chemicals in the Marine Environment. Environmental Science & Technology. 35, 318-324

Matsumura S., Nasu K. 1997. Distribution of Floating Debris in the North Pacific Ocean: Sighting Surveys 1986–1991. In: Marine Debris: Sources, Impacts, and Solutions. New York, NY: Springer New York. p. 15-24.

Mazer N. A., Benedek G. B., Carey M. C. (1980). Quasielastic light-scattering studies of aqueous biliary lipid systems. Mixed micelle formation in bile salt-lecithin solutions. Biochemistry. 19, 601-615

Mazurais D., Ernande B., Quazuguel P., Severe A., Huelvan C., Madec L., Mouchel O., Soudant P., Robbens J., Huvet A., et al. (2015). Evaluation of the impact of polyethylene microbeads ingestion in European sea bass (*Dicentrarchus labrax*) larvae. Marine Environmental Research. 112, 78-85

McKay G., Blair H. S., Gardner J. R. (1982). Adsorption of dyes on chitin. I. Equilibrium studies. Journal of Applied Polymer Science. 27, 3043-3057

Metts B. S., Buhlmann K. A., Tuberville T. D., Scott D. E., Hopkins W. A. (2013). Maternal Transfer of Contaminants and Reduced Reproductive Success of Southern Toads (*Bufo [Anaxyrus] terrestris*) Exposed to Coal Combustion Waste. Environmental Science & Technology. 47, 2846-2853

Milinkovitch T., Ndiaye A., Sanchez W., Le Floch S., Thomas-Guyon H. (2011). Liver antioxidant and plasma immune responses in juvenile golden grey mullet (*Liza aurata*) exposed to dispersed crude oil. Aquatic Toxicology. 101, 155-164

Miller M. A. (1993). Maternal Transfer of Organochlorine Compounds in Salmonines to Their Eggs. Canadian Journal of Fisheries and Aquatic Sciences. 50, 1405-1413

Mills L. J., Chichester C. (2005). Review of evidence: Are endocrine-disrupting chemicals in the aquatic environment impacting fish populations? Science of The Total Environment. 343, 1-34

Mineau P. 1991. Cholinesterase-inhibiting insecticides: their impact on wildlife and the environment: Elsevier.

Miranda A. L., Roche H., Randi M. A. F., Menezes M. L., Ribeiro C. A. O. (2008). Bioaccumulation of chlorinated pesticides and PCBs in the tropical freshwater fish *Hoplias malabaricus*: Histopathological, physiological, and immunological findings. Environment International. 34, 939-949

Mithani S. D., Bakatselou V., TenHoor C. N., Dressman J. B. (1996). Estimation of the Increase in Solubility of Drugs as a Function of Bile Salt Concentration. Pharmaceutical Research. 13, 163-167

MITI. 1992. Biodegration and bioaccumulation data of existing chemicals based on the CSCL Japan.

Molina-Molina J.-M., Escande A., Pillon A., Gomez E., Pakdel F., Cavaillès V., Olea N., Aït-Aïssa S., Balaguer P. (2008). Profiling of benzophenone derivatives using fish and human estrogen receptor-specific in vitro bioassays. Toxicology and Applied Pharmacology. 232, 384-395

Mollet F. M., Kraak S. B. M., Rijnsdorp A. D. (2007). Fisheries-induced evolutionary changes in maturation reaction norms in North Sea sole *Solea solea*. Marine Ecology Progress Series. 351, 189-199

Monteverdi G. H., Di Giulio R. T. (2000). Vitellogenin Association and Oocytic Accumulation of Thyroxine and 3,5,3′-Triiodothyronine in Gravid *Fundulus heteroclitus*. General and Comparative Endocrinology. 120, 198-211

Moore C. J., Moore S. L., Leecaster M. K., Weisberg S. B. (2001). A Comparison of Plastic and Plankton in the North Pacific Central Gyre. Marine Pollution Bulletin. 42, 1297-1300

Morgana S., Ghigliotti L., Estévez-Calvar N., Stifanese R., Wieckzorek A., Doyle T., Christiansen J. S., Faimali M., Garaventa F. (2018). Microplastics in the Arctic: A case study with sub-surface water and fish samples off Northeast Greenland. Environmental Pollution. 242, 1078-1086

Moschetta A., Kliewer S. A. (2005). Weaving betaKlotho into bile acid metabolism. J Clin Invest. 115, 2075-2077

Mrosovsky N., Ryan G. D., James M. C. (2009). Leatherback turtles: The menace of plastic. Marine Pollution Bulletin. 58, 287-289

Muirhead E. K., Skillman A. D., Hook S. E., Schultz I. R. (2006). Oral Exposure of PBDE-47 in Fish: Toxicokinetics and Reproductive Effects in Japanese Medaka (Oryzias latipes) and Fathead Minnows (*Pimephales promelas*). Environmental Science & Technology. 40, 523-528

Nagel R. (2002). DarT: The embryo test with the zebrafish *Danio rerio* - A general model in ecotoxicology and toxicology. ALTEX. 19, 38-48.

Nakashima E., Isobe A., Kako S. i., Itai T., Takahashi S. (2012). Quantification of Toxic Metals Derived from Macroplastic Litter on Ookushi Beach, Japan. Environmental Science & Technology. 46, 10099-10105

Nehls S., Segner H. (2001). Detection of DNA damage in two cell lines from rainbow trout, RTG-2 and RTL-W1, using the comet assay. Environmental Toxicology. 16, 321-329

Nerland I. L., Halsband C., Allan I., Thomas K. V. 2014. Microplastics in Marine Environments: Occurrence, Distribution and Effects. Oslo, Norway.

Ng K. L., Obbard J. P. (2006). Prevalence of microplastics in Singapore's coastal marine environment. Marine Pollution Bulletin. 52, 761-767

Nicolau L., Marçalo A., Ferreira M., Sá S., Vingada J., Eira C. (2016). Ingestion of marine litter by loggerhead sea turtles, *Caretta caretta*, in Portuguese continental waters. Marine Pollution Bulletin. 103, 179-185

Niimi A. J. (1983). Biological and Toxicologtcal Effects of Environmental Contaminants in Fish and Their Eggs. Canadian Journal of Fisheries and Aquatic Sciences. 40, 306-312

Nimrod A. C., Benson W. H. (1998). Reproduction and development of Japanese medaka following an early life stage exposure to xenoestrogens (R823450). Aquatic Toxicology. 44, 141-156

Norén F., Naustvoll L. 2010. Survey of microscopic anthropogenic particles in skagerrak.

Nourizadeh-Lillabadi R., Lyche J. L., Almaas C., Stavik B., Moe S. J., Aleksandersen M., Berg V., Jakobsen K. S., Stenseth N. C., Utne Skåre J., et al. (2009). Transcriptional Regulation in Liver and Testis Associated with Developmental and Reproductive Effects in Male Zebrafish Exposed to Natural Mixtures of Persistent Organic Pollutants (POP). Journal of Toxicology and Environmental Health, Part A. 72, 112-130

Nüsslein-Volhard C., Dahm R. 2002. Zebrafish: a practical approach: New York: Oxford University Press.

Nyholm J. R., Norman A., Norrgren L., Haglund P., Andersson P. L. (2008). Maternal transfer of brominated flame retardants in zebrafish (*Danio rerio*). Chemosphere. 73, 203-208

Obbard R. W., Sadri S., Wong Y. Q., Khitun A. A., Baker I., Thompson R. C. (2014). Global warming releases microplastic legacy frozen in Arctic Sea ice. Earth's Future. 2, 315-320

Oberbeckmann S., Kreikemeyer B., Labrenz M. (2018). Environmental Factors Support the Formation of Specific Bacterial Assemblages on Microplastics. Front Microbiol. 8, 2709-2709

O'Donovan S., Mestre N. C., Abel S., Fonseca T. G., Carteny C. C., Cormier B., Keiter S. H., Bebianno M. J. (2018). Ecotoxicological Effects of Chemical Contaminants Adsorbed to Microplastics in the Clam *Scrobicularia plana*. Front. Mar. Sci. 5.

OECD. 1992. OECD Guidelines for the Testing of Chemicals. In: Section 2: Effects on biotic systems OECD Test Guideline 203: Fish, Acute toxicity test: Organization for Economic Cooperation and Development, Paris, France.

OECD. 1998. Test No. 212: Fish, Short-term Toxicity Test on Embryo and Sac-Fry Stages.

OECD. 2013. OECD Guidelines for the Testing of Chemicals. In: Section 2: Effects on Biotic Systems Test No 236: Fish Embryo Acute Toxicity Test: Organization for Economic Cooperation and Development, Paris, France.

OECD. 2019. Test No. 203: Fish, Acute Toxicity Test.

Oehlmann J., Schulte-Oehlmann U., Kloas W., Jagnytsch O., Lutz I., Kusk K. O., Wollenberger L., Santos E. M., Paull G. C., Van Look K. J. W., et al. (2009). A critical analysis of the biological impacts of plasticizers on wildlife. Philosophical Transactions of the Royal Society B: Biological Sciences. 364, 2047-2062

Ogata Y., Takada H., Mizukawa K., Hirai H., Iwasa S., Endo S., Mato Y., Saha M., Okuda K., Nakashima A., et al. (2009). International Pellet Watch: Global monitoring of persistent organic pollutants (POPs) in coastal waters. 1. Initial phase data on PCBs, DDTs, and HCHs. Marine Pollution Bulletin. 58, 1437-1446

Oliveira M., Ribeiro A., Hylland K., Guilhermino L. (2013). Single and combined effects of microplastics and pyrene on juveniles (0+ group) of the common goby *Pomatoschistus microps* (Teleostei, Gobiidae). Ecological Indicators. 34, 641-647

Oliviero M., Tato T., Schiavo S., Fernández V., Manzo S., Beiras R. (2019). Leachates of micronized plastic toys provoke embryotoxic effects upon sea urchin *Paracentrotus lividus*. Environmental Pollution. 247, 706-715

Olsen G., W., Huang H.-Y., Helzlsouer Kathy J., Hansen Kristen J., Butenhoff John L., Mandel Jeffrey H. (2005). Historical Comparison of Perfluorooctanesulfonate, Perfluorooctanoate, and Other Fluorochemicals in Human Blood. Environmental Health Perspectives. 113, 539-545

Omolaoye J. A., Uzairu A., Gimba C. E. Heavy metal assessment of some soft plastic toys imported into Nigeria from China; 2010.

Oomen A. G., Sips A. J. A. M., Groten J. P., Sijm D. T. H. M., Tolls J. (2000). Mobilization of PCBs and Lindane from Soil during in Vitro Digestion and Their Distribution among Bile Salt Micelles and Proteins of Human Digestive Fluid and the Soil. Environmental Science & Technology. 34, 297-303

Oros D. R., Hoover D., Rodigari F., Crane D., Sericano J. (2005). Levels and Distribution of Polybrominated Diphenyl Ethers in Water, Surface Sediments, and Bivalves from the San Francisco Estuary. Environmental Science & Technology. 39, 33-41

Ory N. C., Gallardo C., Lenz M., Thiel M. (2018). Capture, swallowing, and egestion of microplastics by a planktivorous juvenile fish. Environmental Pollution. 240, 566-573

Padilla S., Cowden J., Hinton D. E., Yuen B., Law S., Kullman S. W., Johnson R., Hardman R. C., Flynn K., Au D. W. T. (2009). Use of Medaka in Toxicity Testing. Current Protocols in Toxicology. 39, 1.10.11-11.10.36.

Pannetier P., Cachot J., Clérandeau C., Faure F., Van Arkel K., de Alencastro L. F., Levasseur C., Sciacca F., Bourgeois J.-P., Morin B. (2019). Toxicity assessment of pollutants sorbed on environmental sample microplastics collected on beaches: Part I-adverse effects on fish cell line. Environmental Pollution. 248, 1088-1097

Pannetier P., Morin B., Clérandeau C., Laurent J., Chapelle C., Cachot J. (2019). Toxicity assessment of pollutants sorbed on environmental microplastics collected on beaches: Part II-adverse effects on Japanese medaka early life stages. Environmental Pollution. 248, 1098-1107

Pannetier P., Morin B., Le Bihanic F., Dubreil L., Clérandeau C., Chouvellon F., Van Arkel K., Danion M., Cachot J. (2020). Environmental samples of microplastics induce significant toxic effects in fish larvae. Environment International. 134, 105047

Paredes E., Perez S., Rodil R., Quintana J. B., Beiras R. (2014). Ecotoxicological evaluation of four UV filters using marine organisms from different trophic levels *Isochrysis galbana*, *Mytilus galloprovincialis*, *Paracentrotus lividus*, and *Siriella armata*. Chemosphere. 104, 44-50

Parenti C. C., Ghilardi A., Della Torre C., Magni S., Del Giacco L., Binelli A. (2019). Evaluation of the infiltration of polystyrene nanobeads in zebrafish embryo tissues after short-term exposure and the related biochemical and behavioural effects. Environmental Pollution. 254, 112947

Pascall M. A., Zabik M. E., Zabik M. J., Hernandez R. J. (2005). Uptake of Polychlorinated Biphenyls (PCBs) from an Aqueous Medium by Polyethylene, Polyvinyl Chloride, and Polystyrene Films. Journal of Agricultural and Food Chemistry. 53, 164-169

Patrolecco L., Ademollo N., Capri S., Pagnotta R., Polesello S. (2010). Occurrence of priority hazardous PAHs in water, suspended particulate matter, sediment and common eels (*Anguilla anguilla*) in the urban stretch of the River Tiber (Italy). Chemosphere. 81, 1386-1392

Paul A. G., Jones K. C., Sweetman A. J. (2009). A First Global Production, Emission, And Environmental Inventory For Perfluorooctane Sulfonate. Environmental Science & Technology. 43, 386-392

Pazos R. S., Maiztegui T., Colautti D. C., Paracampo A. H., Gómez N. (2017). Microplastics in gut contents of coastal freshwater fish from Río de la Plata estuary. Marine Pollution Bulletin. 122, 85-90

Pedà C., Caccamo L., Fossi M. C., Gai F., Andaloro F., Genovese L., Perdichizzi A., Romeo T., Maricchiolo G. (2016). Intestinal alterations in European sea bass *Dicentrarchus labrax* (Linnaeus, 1758) exposed to microplastics: Preliminary results. Environmental Pollution. 212, 251-256

Peng J., Wang J., Cai L. (2017). Current understanding of microplastics in the environment: Occurrence, fate, risks, and what we should do. Integr Environ Asses. 13, 476-482.

Perrichon P., Le Bihanic F., Bustamante P., Le Menach K., Budzinski H., Cachot J., Cousin X. (2014). Influence of sediment composition on PAH toxicity using zebrafish (Danio rerio) and Japanese medaka (*Oryzias latipes*) embryo-larval assays. Environmental Science and Pollution Research. 21, 13703-13719

Pfaffl M. W. (2001). A new mathematical model for relative quantification in real-time RT–PCR. Nucleic Acids Research. 29, e45-e45

Pfaffl M. W., Horgan G. W., Dempfle L. (2002). Relative expression software tool (REST©) for group-wise comparison and statistical analysis of relative expression results in real-time PCR. Nucleic Acids Research. 30, e36-e36

Pfaffl M. W., Tichopad A., Prgomet C., Neuvians T. P. (2004). Determination of stable housekeeping genes, differentially regulated target genes and sample integrity: BestKeeper - Excel-based tool using pair-wise correlations. Biotechnol Lett. 26, 509-515.

Pham C. K., Ramirez-Llodra E., Alt C. H. S., Amaro T., Bergmann M., Canals M., Company J. B., Davies J., Duineveld G., Galgani F., et al. (2014). Marine Litter Distribution and Density in European Seas, from the Shelves to Deep Basins. PLOS ONE. 9, e95839

Pilehvar A., Town R. M., Blust R. (2020). The effect of copper on behaviour, memory, and associative learning ability of zebrafish (*Danio rerio*). Ecotoxicology and Environmental Safety. 188, 109900

Pitt J. A., Kozal J. S., Jayasundara N., Massarsky A., Trevisan R., Geitner N., Wiesner M., Levin E. D., Di Giulio R. T. (2018). Uptake, tissue distribution, and toxicity of polystyrene nanoparticles in developing zebrafish (*Danio rerio*). Aquatic Toxicology. 194, 185-194

Pitt J. A., Trevisan R., Massarsky A., Kozal J. S., Levin E. D., Di Giulio R. T. (2018). Maternal transfer of nanoplastics to offspring in zebrafish (*Danio rerio*): A case study with nanopolystyrene. Science of The Total Environment. 643, 324-334

Pittura L., Avio C. G., Giuliani M. E., d'Errico G., Keiter S. H., Cormier B., Gorbi S., Regoli F. (2018). Microplastics as Vehicles of Environmental PAHs to Marine Organisms: Combined Chemical and Physical Hazards to the Mediterranean Mussels, *Mytilus galloprovincialis*. Front. Mar.Sci. 5.

PlasticsEurope (2015). Plastics - the Facts 2015, An analysis of European latest plastics production, demand and waste data.

PlasticsEurope (2018). PlasticsEurope: Annual review _ The Facts 2017-2018.

Poiger T., Buser H.-R., Balmer M. E., Bergqvist P.-A., Müller M. D. (2004). Occurrence of UV filter compounds from sunscreens in surface waters: regional mass balance in two Swiss lakes. Chemosphere. 55, 951-963

Powley C. R., George S. W., Russell M. H., Hoke R. A., Buck R. C. (2008). Polyfluorinated chemicals in a spatially and temporally integrated food web in the Western Arctic. Chemosphere. 70, 664-672

Pravettoni R. 2018. How microplastics are generated. In.

Prunier J., Maurice L., Perez E., Gigault J., Pierson Wickmann A.-C., Davranche M., Halle A. t. (2019). Trace metals in polyethylene debris from the North Atlantic subtropical gyre. Environmental Pollution. 245, 371-379

Rainieri S., Conlledo N., Larsen B. K., Granby K., Barranco A. (2018). Combined effects of microplastics and chemical contaminants on the organ toxicity of zebrafish (*Danio rerio*). Environmental Research. 162, 135-143

Rani M., Shim W. J., Han G. M., Jang M., Song Y. K., Hong S. H. (2017). Benzotriazole-type ultraviolet stabilizers and antioxidants in plastic marine debris and their new products. Science of The Total Environment. 579, 745-754

Rauschenberger R. H., Wiebe J. J., Sepúlveda M. S., Scarborough J. E., Gross T. S. (2007). Parental Exposure to Pesticides and Poor Clutch Viability in American Alligators. Environmental Science & Technology. 41, 5559-5563

Reisser J., Shaw J., Hallegraeff G., Proietti M., Barnes D. K. A., Thums M., Wilcox C., Hardesty B. D., Pattiaratchi C. (2014). Millimeter-Sized Marine Plastics: A New Pelagic Habitat for Microorganisms and Invertebrates. PLOS ONE. 9, e100289

Rennie M. D., Purchase C. F., Lester N., Collins N. C., Shuter B. J., Abrams P. A. (2008). Lazy males? Bioenergetic differences in energy acquisition and metabolism help to explain sexual size dimorphism in percids. Journal of Animal Ecology. 77, 916-926

Rigét F., Vorkamp K., Bossi R., Sonne C., Letcher R. J., Dietz R. (2016). Twenty years of monitoring of persistent organic pollutants in Greenland biota. A review. Environmental Pollution. 217, 114-123

Rillig M. C. (2012). Microplastic in Terrestrial Ecosystems and the Soil? Environmental Science & Technology. 46, 6453-6454

Rios L. M., Jones P. R., Moore C., Narayan U. V. (2010). Quantitation of persistent organic pollutants adsorbed on plastic debris from the Northern Pacific Gyre's "eastern garbage patch". Journal of Environmental Monitoring. 12, 2226-2236

Rios L. M., Moore C., Jones P. R. (2007). Persistent organic pollutants carried by synthetic polymers in the ocean environment. Marine Pollution Bulletin. 54, 1230-1237

Rist S. E., Assidqi K., Zamani N. P., Appel D., Perschke M., Huhn M., Lenz M. (2016). Suspended micro-sized PVC particles impair the performance and decrease survival in the Asian green mussel Perna viridis. Marine Pollution Bulletin. 111, 213-220

Robards M. D., P.J. G., J.F. P. 1997. The Highest Global Concentrations and Increased Abundance of Oceanic Plastic Debris in the North Pacific: Evidence from Seabirds. In: Marine Debris. Springer, New York, NY.

Rochman C. M. (2015). The Complex Mixture, Fate and Toxicity of Chemicals Associated with Plastic Debris in the Marine Environment. In: Marine Anthropogenic Litter. Cham: Springer International Publishing. p. 117-140.

Rochman C. M., Browne M. A., Halpern B. S., Hentschel B. T., Hoh E., Karapanagioti H. K., Rios-Mendoza L. M., Takada H., Teh S., Thompson R. C. (2013). Classify plastic waste as hazardous. Nature. 494, 169-171

Rochman C. M., Hentschel B. T., Teh S. J. (2014). Long-Term Sorption of Metals Is Similar among Plastic Types: Implications for Plastic Debris in Aquatic Environments. PLOS ONE. 9, e85433

Rochman C. M., Hoh E., Hentschel B. T., Kaye S. (2013). Long-Term Field Measurement of Sorption of Organic Contaminants to Five Types of Plastic Pellets: Implications for Plastic Marine Debris. Environmental Science & Technology. 47, 1646-1654

Rochman C. M., Hoh E., Kurobe T., Teh S. J. (2013). Ingested plastic transfers hazardous chemicals to fish and induces hepatic stress. Scientific Reports. 3, 3263

Rochman C. M., Kurobe T., Flores I., Teh S. J. (2014). Early warning signs of endocrine disruption in adult fish from the ingestion of polyethylene with and without sorbed chemical pollutants from the marine environment. Science of The Total Environment. 493, 656-661

Rochman C. M., Lewison R. L., Eriksen M., Allen H., Cook A.-M., Teh S. J. (2014). Polybrominated diphenyl ethers (PBDEs) in fish tissue may be an indicator of plastic contamination in marine habitats. Science of The Total Environment. 476-477, 622-633

Rodil R., Moeder M., Altenburger R., Schmitt-Jansen M. (2009). Photostability and phytotoxicity of selected sunscreen agents and their degradation mixtures in water. Analytical and Bioanalytical Chemistry. 395, 1513

Rodríguez-Fuentes G., Sandoval-Gío J. J., Arroyo-Silva A., Noreña-Barroso E., Escalante-Herrera K. S., Olvera-Espinosa F. (2015). Evaluation of the estrogenic and oxidative stress

effects of the UV filter 3-benzophenone in zebrafish (*Danio rerio*) eleuthero-embryos. Ecotoxicol Environ Saf. 115, 14-18.

Romeo T., Pietro B., Pedà C., Consoli P., Andaloro F., Fossi M. C. (2015). First evidence of presence of plastic debris in stomach of large pelagic fish in the Mediterranean Sea. Marine Pollution Bulletin. 95, 358-361

Rossi G., Barnoud J., Monticelli L. (2014). Polystyrene Nanoparticles Perturb Lipid Membranes. The Journal of Physical Chemistry Letters. 5, 241-246

Rouchon A. M., Phillips N. E. (2017). Short larval exposure to low level of copper has long-lasting latent effects on juvenile performance in the sea urchin *Evechinus chloroticus*. Marine Ecology Progress Series. 579, 67-79

Rusina T. P., Smedes F., Klanova J., Booij K., Holoubek I. (2007). Polymer selection for passive sampling: A comparison of critical properties. Chemosphere. 68, 1344-1351

Russell W. M. S., Burch R. L. (1960). The Principles of Humane Experimental Technique. Medical Journal of Australia. 1, 500-500

Sackerman J., Donegan J. J., Cunningham C. S., Nguyen N. N., Lawless K., Long A., Benno R. H., Gould G. G. (2010). Zebrafish Behavior in Novel Environments: Effects of Acute Exposure to Anxiolytic Compounds and Choice of *Danio rerio* Line. Int J Comp Psychol. 23, 43-61

Saco-Álvarez L., Durán I., Ignacio Lorenzo J., Beiras R. (2010). Methodological basis for the optimization of a marine sea-urchin embryo test (SET) for the ecological assessment of coastal water quality. Ecotoxicology and Environmental Safety. 73, 491-499

Schirmer K., Chan A. G. J., Greenberg B. M., Dixon D. G., Bols N. C. (1998). Ability of 16 priority PAHs to be photocytotoxic to a cell line from the rainbow trout gill. Toxicology. 127, 143-155

Schneider C. A., Rasband W. S., Eliceiri K. W. (2012). NIH Image to ImageJ: 25 years of image analysis. Nature Methods. 9, 671-675

Schneider J. E., Brozek J. M., Keen-Rhinehart E. (2014). Our stolen figures: The interface of sexual differentiation, endocrine disruptors, maternal programming, and energy balance. Hormones and Behavior. 66, 104-119

Schönlau C., Larsson M., Lam M. M., Engwall M., Giesy J. P., Rochman C., Kärrman A. (2019). Aryl hydrocarbon receptor-mediated potencies in field-deployed plastics vary by type of polymer. Environmental Science and Pollution Research. 26, 9079-9088

Schreurs R., Lanser P., Seinen W., van der Burg B. (2002). Estrogenic activity of UV filters determined by an in vitro reporter gene assay and an in vivo transgenic zebrafish assay. Archives of Toxicology. 76, 257-261

Schrey E., Vauk G. J. M. (1987). Records of entangled gannets (*Sula bassana*) at Helgoland, German Bight. Marine Pollution Bulletin. 18, 350-352

Schwarz A. E., Ligthart T. N., Boukris E., van Harmelen T. (2019). Sources, transport, and accumulation of different types of plastic litter in aquatic environments: A review study. Marine Pollution Bulletin. 143, 92-100

Schymanski E. L., Jeon J., Gulde R., Fenner K., Ruff M., Singer H. P., Hollender J. (2014). Identifying Small Molecules via High Resolution Mass Spectrometry: Communicating Confidence. Environmental Science & Technology. 48, 2097-2098

Seidensticker S., Grathwohl P., Lamprecht J., Zarfl C. (2018). A combined experimental and modeling study to evaluate pH-dependent sorption of polar and non-polar compounds to polyethylene and polystyrene microplastics. Environmental Sciences Europe. 30, 30

Setälä O., Fleming-Lehtinen V., Lehtiniemi M. (2014). Ingestion and transfer of microplastics in the planktonic food web. Environmental Pollution. 185, 77-83

Shah A. A., Hasan F., Hameed A., Ahmed S. (2008). Biological degradation of plastics: A comprehensive review. Biotechnology Advances. 26, 246-265

Shi X., Du Y., Lam P. K., Wu R. S., Zhou B. (2008). Developmental toxicity and alteration of gene expression in zebrafish embryos exposed to PFOS. Toxicol. Appl. Pharmacol. 230, 23-32.

Shugart L. R., McCarthy J. F., Halbrook R. S. (1992). Biological Markers of Environmental and Ecological Contamination: An Overview. Risk Analysis. 12, 353-360

Sieratowicz A., Kaiser D., Behr M., Oetken M., Oehlmann J. (2011). Acute and chronic toxicity of four frequently used UV filter substances for *Desmodesmus subspicatus* and *Daphnia magna*. Journal of Environmental Science and Health, Part A. 46, 1311-1319

So M. K., Taniyasu S., Lam P. K. S., Zheng G. J., Giesy J. P., Yamashita N. (2006). Alkaline Digestion and Solid Phase Extraction Method for Perfluorinated Compounds in Mussels and Oysters from South China and Japan. Archives of Environmental Contamination and Toxicology. 50, 240-248

STAP. 2011. Marine Debris as a Global Environmental Problem Introducing a solutions based framework focused on plastic. Global Environment Facility, Washington, DC ed: ASI Document.

Staples C. A., Peterson D. R., Parkerton T. F., Adams W. J. (1997). The environmental fate of phthalate esters: A literature review. Chemosphere. 35, 667-749

Steer M., Cole M., Thompson R. C., Lindeque P. K. (2017). Microplastic ingestion in fish larvae in the western English Channel. Environmental Pollution. 226, 250-259

Strähle U., Scholz S., Geisler R., Greiner P., Hollert H., Rastegar S., Schumacher A., Selderslaghs I., Weiss C., Witters H., et al. (2012). Zebrafish embryos as an alternative to animal experiments—A commentary on the definition of the onset of protected life stages in animal welfare regulations. Reproductive Toxicology. 33, 128-132

Suaria G., Avio C. G., Mineo A., Lattin G. L., Magaldi M. G., Belmonte G., Moore C. J., Regoli F., Aliani S. (2016). The Mediterranean Plastic Soup: synthetic polymers in Mediterranean surface waters. Scientific Reports. 6, 37551

Sussarellu R., Suquet M., Thomas Y., Lambert C., Fabioux C., Pernet M. E. J., Le Goïc N., Quillien V., Mingant C., Epelboin Y., et al. (2016). Oyster reproduction is affected by exposure to polystyrene microplastics. Proceedings of the National Academy of Sciences. 113, 2430

Tallec K., Huvet A., Di Poi C., González-Fernández C., Lambert C., Petton B., Le Goïc N., Berchel M., Soudant P., Paul-Pont I. (2018). Nanoplastics impaired oyster free living stages, gametes and embryos. Environmental Pollution. 242, 1226-1235

Tang J., Ni X., Zhou Z., Wang L., Lin S. (2018). Acute microplastic exposure raises stress response and suppresses detoxification and immune capacities in the scleractinian coral *Pocillopora damicornis*. Environmental Pollution. 243, 66-74

Tang L., Eaton J. W. (1995). Inflammatory Responses to Biomaterials. American Journal of Clinical Pathology. 103, 466-471

Tao L., Kannan K., Kajiwara N., Costa M. M., Fillmann G., Takahashi S., Tanabe S. (2006). Perfluorooctanesulfonate and Related Fluorochemicals in Albatrosses, Elephant Seals, Penguins, and Polar Skuas from the Southern Ocean. Environmental Science & Technology. 40, 7642-7648

Ter Halle A., Jeanneau L., Martignac M., Jardé E., Pedrono B., Brach L., Gigault J. (2017). Nanoplastic in the North Atlantic Subtropical Gyre. Environmental Science & Technology. 51, 13689-13697

Teuten E. L., Rowland S. J., Galloway T. S., Thompson R. C. (2007). Potential for Plastics to Transport Hydrophobic Contaminants. Environmental Science & Technology. 41, 7759-7764

Teuten E. L., Saquing J. M., Knappe D. R. U., Barlaz M. A., Jonsson S., Björn A., Rowland S. J., Thompson R. C., Galloway T. S., Yamashita R., et al. (2009). Transport and release of chemicals from plastics to the environment and to wildlife. Philosophical Transactions of the Royal Society B: Biological Sciences. 364, 2027-2045

Thompson R. C., Moore C. J., vom Saal F. S., Swan S. H. (2009). Plastics, the environment and human health: current consensus and future trends. Philos Trans R Soc Lond B Biol Sci. 364, 2153-2166

Thompson R. C., Olsen Y., Mitchell R. P., Davis A., Rowland S. J., John A. W. G., McGonigle D., Russell A. E. (2004). Lost at Sea: Where Is All the Plastic? Science. 304, 838

Titelman J., Hansson L. J. 2006. Feeding rates of the jellyfish *Aurelia aurita* on fish larvae. In: Marine Biology. Springer Berlin Heidelberg. p. 297-306.

Tovar-Sánchez A., Sánchez-Quiles D., Basterretxea G., Benedé J. L., Chisvert A., Salvador A., Moreno-Garrido I., Blasco J. (2013). Sunscreen Products as Emerging Pollutants to Coastal Waters. PLOS ONE. 8, e65451

Tremblay L., Kohl S. D., Rice J. A., Gagné J.-P. (2005). Effects of temperature, salinity, and dissolved humic substances on the sorption of polycyclic aromatic hydrocarbons to estuarine particles. Marine Chemistry. 96, 21-34

Triebskorn R., Braunbeck T., Grummt T., Hanslik L., Huppertsberg S., Jekel M., Knepper T. P., Krais S., Müller Y. K., Pittroff M., et al. (2019). Relevance of nano- and microplastics for freshwater ecosystems: A critical review. TrAC. 110, 375-392.

Turner A. (2018). Black plastics: Linear and circular economies, hazardous additives and marine pollution. Environment International. 117, 308-318

UNECE. 2014. The Aarhus Convention.

UNEP. 2010. Annual Report 2010.

van der Oost J., Voorhorst W. G. B., Wittenhorst V., de Vos W. M. 1996. Genetic and biochemical characterization of two distinct alcohol dehydrogenases from Pyrococcus furiosus.

Vicquelin L., Leray-Forget J., Peluhet L., LeMenach K., Deflandre B., Anschutz P., Etcheber H., Morin B., Budzinski H., Cachot J. (2011). A new spiked sediment assay using embryos of the Japanese medaka specifically designed for a reliable toxicity assessment of hydrophobic chemicals. Aquatic Toxicology. 105, 235-245

Vignet C., Larcher T., Davail B., Joassard L., Le Menach K., Guionnet T., Lyphout L., Ledevin M., Goubeau M., Budzinski H., et al. (2016). Fish Reproduction Is Disrupted upon Lifelong Exposure to Environmental PAHs Fractions Revealing Different Modes of Action. Toxics. 4,

Vignet C., Le Menach K., Mazurais D., Lucas J., Perrichon P., Le Bihanic F., Devier M.-H., Lyphout L., Frère L., Bégout M.-L., et al. (2014). Chronic dietary exposure to pyrolytic and petrogenic mixtures of PAHs causes physiological disruption in zebrafish - part I: Survival and growth. Environmental Science and Pollution Research. 21, 13804-13817

Vione D., Caringella R., De Laurentiis E., Pazzi M., Minero C. (2013). Phototransformation of the sunlight filter benzophenone-3 (2-hydroxy-4-methoxybenzophenone) under conditions relevant to surface waters. Science of The Total Environment. 463-464, 243-251

von Moos N., Burkhardt-Holm P., Köhler A. (2012). Uptake and Effects of Microplastics on Cells and Tissue of the Blue Mussel *Mytilus edulis* L. after an Experimental Exposure. Environmental Science & Technology. 46, 11327-11335

Voparil I. M., Mayer L. M. (2004). Commercially Available Chemicals That Mimic a Deposit Feeder's (*Arenicola marina*) Digestive Solubilization of Lipids. Environmental Science & Technology. 38, 4334-4339

Votier S. C., Archibald K., Morgan G., Morgan L. (2011). The use of plastic debris as nesting material by a colonial seabird and associated entanglement mortality. Marine Pollution Bulletin. 62, 168-172

Vrana B., Mills G. A., Dominiak E., Greenwood R. (2006). Calibration of the Chemcatcher passive sampler for the monitoring of priority organic pollutants in water. Environmental Pollution. 142, 333-343

Wang F., Shih K. M., Li X. Y. (2015). The partition behavior of perfluorooctanesulfonate (PFOS) and perfluorooctanesulfonamide (FOSA) on microplastics. Chemosphere. 119, 841-847

Wang J., Liu X., Liu G., Zhang Z., Wu H., Cui B., Bai J., Zhang W. (2019). Size effect of polystyrene microplastics on sorption of phenanthrene and nitrobenzene. Ecotoxicology and Environmental Safety. 173, 331-338

Wang J., Wang X., Xiong C., Liu J., Hu B., Zheng L. (2015). Chronic bisphenol A exposure alters behaviors of zebrafish (*Danio rerio*). Environmental Pollution. 206, 275-281

Wang M., Wang X., Luo X., Zheng H. (2017). Short-term toxicity of polystryrene microplastics on mysid shrimps *Neomysis japonica*. IOP Conference Series: Earth and Environmental Science. 61, 012136

Wang W., Wang J. (2018b). Comparative evaluation of sorption kinetics and isotherms of pyrene onto microplastics. Chemosphere. 193, 567-573

Wang W., Wang J. (2018a). Different partition of polycyclic aromatic hydrocarbon on environmental particulates in freshwater: Microplastics in comparison to natural sediment. Ecotoxicology and Environmental Safety. 147, 648-655

Wang X.-C., Zhang Y.-X., Chen R. F. (2001). Distribution and Partitioning of Polycyclic Aromatic Hydrocarbons (PAHs) in Different Size Fractions in Sediments from Boston Harbor, United States. Marine Pollution Bulletin. 42, 1139-1149

Wardrop P., Shimeta J., Nugegoda D., Morrison P. D., Miranda A., Tang M., Clarke B. O. (2016). Chemical Pollutants Sorbed to Ingested Microbeads from Personal Care Products Accumulate in Fish. Environmental Science & Technology. 50, 4037-4044

Watts A. J. R., Urbina M. A., Corr S., Lewis C., Galloway T. S. (2015). Ingestion of Plastic Microfibers by the Crab *Carcinus maenas* and Its Effect on Food Consumption and Energy Balance. Environmental Science & Technology. 49, 14597-14604

Waychunas G. A., Kim C. S., Banfield J. F. (2005). Nanoparticulate Iron Oxide Minerals in Soils and Sediments: Unique Properties and Contaminant Scavenging Mechanisms. Journal of Nanoparticle Research. 7, 409-433

Weeks-Santos S., Cachot J., Gourves P.-Y., Clérandeau C., Morin B., Gonzalez P. (2019). Sub-lethal effects of waterborne copper in early developmental stages of rainbow trout (*Oncorhynchus mykiss*). Ecotoxicology and Environmental Safety. 170, 778-788

Weigt S., Huebler N., Strecker R., Braunbeck T., Broschard T. H. (2011). Zebrafish (*Danio rerio)* embryos as a model for testing proteratogens. Toxicology. 281, 25-36

Welden N. A. C., Lusher A. L. (2017). Impacts of changing ocean circulation on the distribution of marine microplastic litter. Integrated Environmental Assessment and Management. 13, 483-487

Wessel N., Rousseau S., Caisey X., Quiniou F., Akcha F. (2007). Investigating the relationship between embryotoxic and genotoxic effects of benzo[a]pyrene, 17α-ethinylestradiol and endosulfan on *Crassostrea gigas* embryos. Aquatic Toxicology. 85, 133-142

Wessel N., Santos R., Menard D., Le Menach K., Buchet V., Lebayon N., Loizeau V., Burgeot T., Budzinski H., Akcha F. (2010). Relationship between PAH biotransformation as measured by biliary metabolites and EROD activity, and genotoxicity in juveniles of sole (*Solea solea*). Marine Environmental Research. 69, S71-S73

Westerfield M. 2000. The zebrafish book. A guide for the laboratory use of zebrafish (Danio rerio). 4th edition.: Univ. of Oregon Press, Eugene.

Whiteley A. R., Bhat A., Martins E. P., Mayden R. L., Arunachalam M., Uusi-HeikkilÄ S., Ahmed A. T. A., Shrestha J., Clark M., Stemple D., et al. (2011). Population genomics of wild and laboratory zebrafish (*Danio rerio*). Molecular Ecology. 20, 4259-4276

Whyte J. J., Jung R. E., Schmitt C. J., Tillitt D. E. (2000). Ethoxyresorufin-O-deethylase (EROD) Activity in Fish as a Biomarker of Chemical Exposure. Critical Reviews in Toxicology. 30, 347-570

Whyte J. J., Tillitt D. E. (1995). Erod activity. Signal Transduct.

Wilks S. (2006). The Plastic Baggers: An Evaluation of a Boutique Cause in Targeted Environmentalism. AQ: Australian Quarterly. 78, 11-40

Wolff S., Kerpen J., Prediger J., Barkmann L., Müller L. (2019). Determination of the microplastics emission in the effluent of a municipal waste water treatment plant using Raman microspectroscopy. Water Research X. 2, 100014

Wright S. L., Rowe D., Thompson R. C., Galloway T. S. (2013). Microplastic ingestion decreases energy reserves in marine worms. Current Biology. 23, R1031-R1033

Xie X., Long T., Zhang Y., Cao Z. (1998). Reproductive investment in the *Silurus meridionalis*. Journal of Fish Biology. 53, 259-271

Xu B., Liu F., Brookes P. C., Xu J. (2018a). Microplastics play a minor role in tetracycline sorption in the presence of dissolved organic matter. Environmental Pollution. 240, 87-94

Xu B., Liu F., Brookes P. C., Xu J. (2018b). The sorption kinetics and isotherms of sulfamethoxazole with polyethylene microplastics. Marine Pollution Bulletin. 131, 191-196

Yamashita N., Kannan K., Taniyasu S., Horii Y., Petrick G., Gamo T. (2005). A global survey of perfluorinated acids in oceans. Marine Pollution Bulletin. 51, 658-668

Ye L., Wu L., Jiang Y., Zhang C., Chen L. (2009). Toxicological study of PFOS/PFOA to zebrafish (*Danio rerio*) embryos. Chin J Environ Sci. 30, 1727-1732

Yin L., Chen B., Xia B., Shi X., Qu K. (2018). Polystyrene microplastics alter the behavior, energy reserve and nutritional composition of marine jacopever (*Sebastes schlegelii*). Journal of Hazardous Materials. 360, 97-105

Yoo H., Washington J. W., Jenkins T. M., Laurence Libelo E. (2009). Analysis of perfluorinated chemicals in sludge: Method development and initial results. Journal of Chromatography A. 1216, 7831-7839

Yu H., Huang G.-h., An C.-j., Wei J. (2011). Combined effects of DOM extracted from site soil/compost and biosurfactant on the sorption and desorption of PAHs in a soil–water system. Journal of Hazardous Materials. 190, 883-890

Zareitalabad P., Siemens J., Hamer M., Amelung W. (2013). Perfluorooctanoic acid (PFOA) and perfluorooctanesulfonic acid (PFOS) in surface waters, sediments, soils and wastewater – A review on concentrations and distribution coefficients. Chemosphere. 91, 725-732

Zarfl C., Matthies M. (2010). Are marine plastic particles transport vectors for organic pollutants to the Arctic? Marine Pollution Bulletin. 60, 1810-1814

Zhan Z., Wang J., Peng J., Xie Q., Huang Y., Gao Y. (2016). Sorption of 3,3′,4,4′-tetrachlorobiphenyl by microplastics: A case study of polypropylene. Marine Pollution Bulletin. 110, 559-563

Zhang C., Chen X., Wang J., Tan L. (2017). Toxic effects of microplastic on marine microalgae *Skeletonema costatum*: Interactions between microplastic and algae. Environmental Pollution. 220, 1282-1288

Zhang D., Moore W. R. (1999). Wheat bran particle size effects on bread baking performance and quality. Journal of the Science of Food and Agriculture. 79, 805-809

Zhang H., Wang J., Zhou B., Zhou Y., Dai Z., Zhou Q., Chriestie P., Luo Y. (2018). Enhanced adsorption of oxytetracycline to weathered microplastic polystyrene: Kinetics, isotherms and influencing factors. Environmental Pollution. 243, 1550-1557

Zhang X., Zheng M., Wang L., Lou Y., Shi L., Jiang S. (2018). Sorption of three synthetic musks by microplastics. Marine Pollution Bulletin. 126, 606-609

Zhou Q., Deng S., Zhang Q., Fan Q., Huang J., Yu G. (2010). Sorption of perfluorooctane sulfonate and perfluorooctanoate on activated sludge. Chemosphere. 81, 453-458

Zhu M., Chernick M., Rittschof D., Hinton D. E. (2020). Chronic dietary exposure to polystyrene microplastics in maturing Japanese medaka (*Oryzias latipes*). Aquatic Toxicology. 220, 105396

Ziccardi L. M., Edgington A., Hentz K., Kulacki K. J., Driscoll S. K. (2016). Microplastics as vectors for bioaccumulation of hydrophobic organic chemicals in the marine environment: A state-of-the-science review. Environ Toxicol Chem. 35, 1667-1676.