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Plastics and plastic additives as inducers of oxidative stress

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Abstract

Since plastics in the environment break down to smaller particles, contain additives, trap environmental pollutants and cross cell membranes, there is growing concern about the toxicological consequences for humans and vulnerable aquatic species. Recent studies have shown the ability of plastic additives to disrupt oxidative metabolism and cause damage to macromolecules as part of their mechanism of action. This article focuses on human and fish cell models because they serve to unravel the mechanisms of action of plastic additives and to predict the consequences of exposure. In addition, some in-vivo studies revealing the action of plastics and its additives on oxidative stress parameters in aquatic organisms are reviewed. The selected works highlight an alteration of the oxidative stress balance as the accepted mechanism of action of plastics and warn about the negative consequences on humans and wildlife.

Keywords: oxidative stress; plastic additives; human cells; fish; antioxidant enzymes

Introduction

Plastics have significantly improved human lifestyle due to their low cost, multiple uses, durability and lightweight. They contain complex mixtures of chemicals, including residual or unreacted monomers, and different plastic additives that confer flexibility and strength to polymers, and often make up a large proportion of the plastic product. The weak bond of plastic additives to the polymers facilitates their transfer to food, liquids and the environment, becoming global pollutants and causing significant human and environmental concern. Ingestion of contaminated food/beverages, inhalation and dermal absorption are the main pathways of human exposure to plastic additives [1, 2, 3]. In the aquatic environment, plastics break down into microplastics (< 5 mm) or nanoplastics (<100 nm) and exert their toxicity through disruption of cell membrane integrity. Exposure of aquatic organisms to plastic polymers of different sizes and their role as carriers of organic pollutants has been extensively documented [4, 5, 6]. However, the toxic effects associated with exposure have been less intensively investigated, with the exception perhaps of their properties as endocrine disruptors [7]. Recent studies show that plastics and their additives cause oxidative stress in both humans and aquatic species. This work compiles these evidences.

Toxicity of plasticizers as inducers of oxidative stress in human and fish models

Plasticizers are suspected of being toxic and modulating or altering the lipid and endocrine metabolism of exposed humans and wildlife. They can cross the human placenta and reach the fetus, and embryonic and fetal development are considered as particularly vulnerable periods. Prenatal exposure to plasticizers has been linked to preterm delivery; oxidative stress is a likely contributor to this event [8]. Ferguson et al. [9] reported that 8-isoprostane, a

biomarker of oxidative stress, and di(2-ethylhexyl)phthalate (DEHP) metabolites in urine of pregnant women were associated to preterm birth. One of the likely causes was oxidative stress, which induced inflammation at the fetus-mother interface, and this could lead to an early rupture of membranes and initiation of parturition. In another study, Liu et al. [10] showed that the levels of oxo-2'-deoxyguanosine (8-OHdG), an oxidative stress biomarker, were positively correlated with urinary levels of bisphenol A (BPA) and derivatives, and with body mass index, suggesting that high oxidative stress may be associated with BPA exposure and obesity.

While detecting biomarkers of oxidative stress associated with exposure to plastics represents a useful approach, discriminating whether oxidative stress is exclusively due to plastics exposure is a difficult task, as human populations are exposed to complex mixtures of compounds. However, by using cell based in-vitro assays, plastic additives such as phthalates, have been recognized as reprotoxic and inducers of oxidative stress. Table 1 shows an overview of oxidative stress effects caused by plastic additives in human and fish cells. An increase in the generation of reactive oxygen species (ROS) (up to 2.5-fold) was detected in embryonic kidney cells HEK-293 exposed to 100-400 μM DEHP [11], and an even greater response (4-fold ROS induction) was observed in pancreatic β cells INS-1 [12]. In contrast, exposure to dimethyl phthalate (DMP), benzylbutyl phthalate (BBP), dibutyl phthalate (DBP) or DEHP did not generate significant cytotoxicity nor increased the generation of ROS in human placental JEG-3 cells; the lack of response was partially attributed to the low availability of the hydrophobic phthalates in the culture medium [13]. Similarly, Gutierrez-García et al. [14] observed a low ROS induction (1.5-fold) in HepG2 cells exposed to the highly hydrophobic plasticizer di-isononyl phthalate (DiNP). Induction of antioxidant enzymes occurs as an adaptive mechanism to the increased levels of ROS.

Thus, after exposure to DEHP, superoxide dismutase (SOD) and catalase (CAT) activities increased significantly in embryonic kidney cells HEK-293, although this response was insufficient to neutralize the excess of ROS [11]. In contrast, SOD activity did not increase but diminished in INS-1 and mouse NE-4C neural stem cells exposed to DEHP [12, 15]. A bell-shaped response in SOD activity was observed in human erythrocytes exposed to DMP, and was attributed to overcompensation, free radical scavenging, and impaired immune function. Concomitantly, decreased CAT and glutathione peroxidase (GPX) activities coupled to an increase on glutathione (GSH) levels were evidenced [16]. GSH is an important ROS scavenger that was significantly depleted in INS-1 and NE-4C cells after exposure to DEHP [12, 15]. Additionally, GPX activity, which requires GSH to reduce hydrogen peroxide and lipid hydroperoxides, decreased in NE-4C cells [15].

Oxidative stress can result in peroxidative damage to polyunsaturated fatty acids (PUFA) in constitutive cellular lipids (Fig. 1). Consequently, significant increased levels of malondialdehyde (MDA), a biomarker of lipid peroxidation, have been observed in NE-4C, INS-1 and HEK-293 cells exposed to DEHP [11, 12, 15]. DEHP caused a significant increase in DNA strand breaks and oxidative damage in pancreatic β cells INS-1. Moreover, mitochondrial dysfunction and damage of lysosomal membranes could lead to increased ROS formation after DEHP exposure [12]. Phthalate metabolites also triggered the generation of ROS, an up-regulation of oxidative stress-responsive genes and DNA damage [17, 18].

Comparatively, fish cell lines are more sensitive than human cell models to oxidative stress induced by phthalates. DBP and DEHP led to significant induction of ROS in *Poeciliopsis lucida* hepatoma (PLHC-1) and zebrafish liver (ZFL) cell lines (Pérez-Albaladejo et al.,

unpublished results). Similarly, *Dicentrarchus labrax* embryonic cells showed DNA strand breaks due to oxidative damage upon DEHP exposure [19].

In addition to phthalates, bisphenol A (BPA) has been reported to induce ROS in human erythrocytes, L-02 hepatocytes, placental and endometrial stromal cells [20, 13, 21, 22]. BPA exposure generated ROS with decreased expression of antioxidant enzymes and an up-regulation of inflammatory cytokine expression and estrogen receptor ER-α in endometrial stromal cells, suggesting a relationship between exposure to BPA and endometrial-related disorders. When cells were treated with an ER-α inhibitor, most of these responses were reversed, which is indicative of a key role of ER-α in the oxidative stress process [21]. Similarly, BPA inhibited P450 aromatase activity and induced ROS production in human placenta JEG-3 cells [13]. Regarding to BPA derivatives, recent studies showed a higher toxicity, endocrine and lipid disruption potential of bisphenol A diglycidyl ether (BADGE), and other derivatives in comparison to BPA. However, BADGE and derivatives weakly induced the generation of ROS in human placenta cells [23].

Due to the unwanted effects of BPA, the use of alternative analogs, presumably safer than BPA, has increased in the last years. Bisphenol F (BPF), bisphenol S (BPS) and bisphenol AF (BPAF) are among the most used BPA substitutes. Although, BPS has shown low oxidative potential in comparison with other bisphenols, there is growing evidence that BPF and BPAF are no safer than BPA [20, 24]. Thus, despite its lower cytotoxicity, BPF exhibited higher ability to generate ROS (up to 5-fold induction) than BPA (2-fold) in fish liver PLHC-1 cells, and lead to significant alterations in the cell's lipidomics profile, which consisted mainly in a significant depletion of different polyunsaturated lipids, presumably oxidized by ROS (Pérez-Albaladejo et al., unpublished results). Macrophages of the freshwater fish *Cyprinus carpio*

were also sensitive to BPS, which induced ROS production and a deregulation of genes involved in oxidative stress, although no lipid peroxidation occurred. Modulation of the PPARγ signaling pathway by rosiglitazone, reduced ROS formation in fish macrophages exposed to BPS, suggesting the involvement of PPARγ in the oxidative stress and inflammatory processes induced by BPS [25, 26]. In contrast, neither BPA, BPS, BPF nor BPAF dysregulated the expressions of antioxidant enzymes (*GPX, GSR, SOD1A, CAT*) in human HepG2 cells, which resulted less sensitive than fish cells [27]. Given that concentrations of BPA, BPS and BPF in the ng/L to mg/L range have been reported in aquatic environments [28], the potential risks to fish species chronically exposed to these chemicals can be significant.

Alkylphenols (APs) are another group of plastic additives often present in herbicides, cosmetics, resins and detergents, with estrogenic potential and ability to induce the generation of ROS. Octyl- (OP) and nonylphenol (NP) induced ROS generation up to 4-fold in human placental JEG-3 cells, while heptylphenol (HP) increased ROS production up to 40-fold [29]. Consequently, placental cells exposed to HP displayed a lipid signature reflecting oxidative stress (Figure 1). Oxidative stress and significant lipid peroxidation were common responses of fish liver cells and spermatozoa exposed to NP [30, 31]. As with the other plasticizers, the endocrine-disruptive action of NP in catfish occurred through ROS formation and oxidative damage [32].

Evidences of oxidative stress in the aquatic environment

Oxidative stress responses induced by plasticizers have been detected in aquatic systems along the trophic web, from microalgae and zooplankton to filter feeders and predators alongside the trophic web, the final consumers (e.g. humans) being susceptible to higher exposures [33, 34]. The consequences of exposure to plastic additives on key organisms in the marine trophic chain are of utmost importance from an ecological, economical and health perspective, since many of these additives can be transferred through the food chain [7].

Laboratory exposures to plasticizers have mostly focused in freshwater models. The cladoceran *Daphnia magna* and zebrafish (*Danio rerio*) were selected to evaluate the oxidative stress consequences of exposure to DEHP and leached 3D printed plastic [35, 36]. Adult carps (*C. carpio*) exposed to 0.1-1000 µg/L BPA for 30-days suffered an increase of lipid peroxidation, while antioxidant defenses decreased in a dose dependent manner [37]. A similar study, but lasting for 60-days, revealed a comparable oxidative stress response when fish were exposed to the BPA analog, BPF [38]. The red tilapia, *Oreochromis niloticus*, was used to show interactive effects of polystyrene (PS) microplastics and other pollutants. Thus, microplastics increased the bioaccumulation of the antibiotic roxithromycin (ROX) in tilapia, but mitigated the neurotoxicity and oxidative damage caused by ROX [39]. In the aquatic realm, the trophic chain transfer of plastics and their associated additives, and the consequent generation of an oxidative stress condition, can alter the ecosystem's balance and pose a threat to final consumers. Table 2 lists a selection of studies showing oxidative stress effects on fish after exposure to microplastics and plastic additives.

In the marine environment, oxidative stress responses to plastics and/or plasticizers, individually or as a mixture, have been documented in primary producers as microalgae [40], but also in zooplankton constituents [41]. Coral ecosystems are of particular concern due to

their fragility to anthropogenic stressors [42]. Larger invertebrate species, such as the crustacean *Artemia franciscana*, were selected to test PS toxicity over oxidative stress parameters after short (48 h) and long term (14 day) exposures [43]. Filter feeding bivalves, such as the mussel *Mytilus galloprovincialis*, were the sentinel species selected to assess ROS formation in hemocytes after exposure to the organophosphate flame retardant tris-(1-chloro-2-propyl) phosphate (TCPP) used as plastic additive [44]. Among marine vertebrates, the toxicity of microplastics alone and combined with Hg was investigated in sea bass -*Dicentrarchus labrax*- juveniles. Microplastics likely adsorbed mercury from the water and influenced its bioaccumulation, and both, mercury and microplastics caused neurotoxicity, oxidative stress and lipid peroxidation [45]. Another fish of commercial interest, the sea bream -*Sparus aurata*- was considered for assessing the toxicity of DiNP (a substitute of the toxic phthalate, DEHP) in a 21-day diet exposure, which revealed an upregulation of oxidative stress related genes [46]. Under a more realistic scenario, microplastics occurrence in the digestive tract and oxidative stress parameters in the liver were reported in the striped red mullet *Mullus surmuletus* from Mediterranean fishing grounds [47]. The fish *Pomatoschistus microps* was the model selected to predict the consequences of microplastics and antibiotics co-exposure and temperature rise [48]. Alkylphenols (OP and NP) have been described to cause an oxidative stress condition in a wide range of exposed biota, from microalgae to fish [49, 50].

Conclusion

Studies conducted in the past two years show that some of the currently used plastic additives induce significant oxidative stress responses in human cells at concentrations that are likely

to occur in daily exposures. Although the use of human cohorts for the study of oxidative effects due to exposure to plastics is a useful approach, alternative in vitro methods with human cells models provide evidence of the mechanism of action of individual compounds. Similarly, the use of fish cell lines, which are considered even more sensitive to plastic additives than human ones, represents a valuable tool for detecting oxidative stress and predicting the risk of these compounds to aquatic fauna. However, in vitro systems still have some limitations to overcome, mainly related to the low solubility and availability of hydrophobic chemicals in the culture medium, the lower prevalence of cell to cell communication, the lower differentiation and the higher proliferation rate of cultured cells compared to cells in tissues and organs. Thus, in vivo exposures to aquatic organisms are still needed to corroborate whether microplastics and/or plastic additives could pose a threat from an ecological and human health perspective.

Conflict of interest statement

Nothing declared.

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References

1. Kashyap, D., Agarwal, T., 2018. Concentration and factors affecting the distribution of phthalates in the air and dust: A global scenario. Sci. Total Environ. 635, 817–827.

2. Martínez, M.A., Rovira, J., Prasad Sharma, R., Nadal, M., Schuhmacher, M., Kumar, V., 2018. Comparing dietary and non-dietary source contribution of BPA and DEHP to prenatal exposure: A Catalonia (Spain) case study. Environ. Res. 166, 25–34.

****This paper shows as indoor environments, and especially dust ingestion, are the main non-dietary contributors to the total exposure of BPA and DEHP. Diet is the highest contributor to BPA exposure.*

3. Russo, G., Barbato, F., Mita, D.G., Grumetto, L., 2019. Occurrence of Bisphenol A and its analogues in some foodstuff marketed in Europe. Food Chem. Toxicol. 131, 110575.

4. Avio, C.G., Gorbi, S., Regoli, F., 2017. Plastics and microplastics in the oceans: From emerging pollutants to emerged threat. Mar. Environ. Res. 128, 2-11.

****Interesting overview on plastics occurrence, types, geographical distribution in marine regions, plastics as vectors of pollutants and toxicity on aquatic organisms.*

5. Guzzetti, E., Sureda, A., Tejada, S., Faggio, C., 2018. Microplastic in marine organism: Environmental and toxicological effects. Environ. Toxicol. Phar. 64, 164-171.

6. Prokic, M.D., Radovanovic, T.B., Gavric, J.P., Faggio, C., 2019. Ecotoxicological effects of microplastics: Examination of biomarkers, current state and future perspectives. Trends in Analytical Chemistry 111, 37-46.

7. Hermabessiere, L., Dehaut, A., Paul-Pont, I., Lacroix, C., Jezequel, R., Soudant, P., Duflos, G., 2017. Occurrence and effects of plastic additives on marine environments and organisms: A review. Chemosphere 182, 781-793.

****Overview stressing the importance of plastic additives, their occurrence, transfer and toxicity in marine organism derived from laboratory and field studies. Practical experiments on toxicity due to plastic leaching included.*

8. Huang, S., Li, J., Xu, S., Zhao, H., Li, Y., Zhou, Y., Fang, J., Liao, J., Cai, Z., Xia, W., 2019. Bisphenol A and bisphenol S exposures during pregnancy and gestational age – A longitudinal study in China. Chemosphere 237, 124426.

9. Ferguson, K.K., Chen, Y.H., Vanderweele, T.J., McElrath, T.F., Meeker, J.D., Mukherjee, B., 2017. Mediation of the relationship between maternal phthalate exposure and preterm birth by oxidative stress with repeated measurements across pregnancy. Environ. Health Perspect. 125, 488–494.

***This paper shows the relationship between phthalate exposure and preterm birth caused by oxidative stress*

10. Liu, M., Jia, S., Dong, T., Han, Y., Xue, J., Wanjaya, E.R., Fang, M., 2019. The occurrence of bisphenol plasticizers in paired dust and urine samples and its association with oxidative stress. Chemosphere 216, 472–478.

11. Amara, I., Timoumi, R., Graiet, I., Ben Salem, I., Adelou, K., Abid-Essefi, S., 2019. Di (2-ethylhexyl) phthalate induces cytotoxicity in HEK-293 cell line, implication of the Nrf-2/HO-1 antioxidant pathway. Environ. Toxicol. 34, 1039-1042.

12. She, Y., Jiang, Liping, Zheng, L., Zuo, H., Chen, M., Sun, X., Li, Q., Geng, C., Yang, G., Jiang, Lijie, Liu, X., 2017. The role of oxidative stress in DNA damage in pancreatic β cells induced by di-(2-ethylhexyl) phthalate. Chem. Biol. Interact. 265, 8–15.

13. Pérez-Albaladejo, E., Fernandes, D., Lacorte, S., Porte, C., 2017. Comparative toxicity, oxidative stress and endocrine disruption potential of plasticizers in JEG-3 human placental cells. Toxicol. In Vitro 38, 41–48.

14. Gutiérrez-García, A.K., Choudhury, M., De Leon-Rodriguez, A., 2019. Diisononyl phthalate differentially affects sirtuin expression in the HepG2 cell line. Chem. Res. Toxicol. 32, 1863-1870.

15. Wu, M., Xu, L., Teng, C., Xiao, X., Hu, W., Chen, J., Tu, W., 2019. Involvement of oxidative stress in di-2-ethylhexyl phthalate (DEHP)-induced apoptosis of mouse NE-4C neural stem cells. Neurotoxicology 70, 41–47.

16. Li, S., Chi, Z., Li, W., 2019. In vitro toxicity of dimethyl phthalate to human erythrocytes: From the aspects of antioxidant and immune functions. Environ. Pollut. 253, 239–245.

17. Meruvu, S., Zhang, J., Choudhury, M., 2016. Mono-(2-ethylhexyl) phthalate increases oxidative stress responsive miRNAs in first trimester placental cell line HTR8/SVneo. Chem. Res. Toxicol. 29, 430–435.

18. Liu, N., Jiang, L., Sun, X., Yao, X., Zhai, X., Liu, X., Wu, X., Bai, Y., Wang, S., Yang, G., 2017. Mono-(2-ethylhexyl) phthalate induced ROS-dependent autophagic cell death in human vascular endothelial cells. Toxicol. In Vitro. 44, 49–56.

19. Molino, C., Filippi, S., Stoppiello, G.A., Meschini, R., Angeletti, D., 2019. In vitro evaluation of cytotoxic and genotoxic effects of di(2-ethylhexyl)-phthalate (DEHP) on European sea bass (*Dicentrarchus labrax*) embryonic cell line. Toxicol. In Vitro 56, 118–125.

20. Maćczak, A., Cyrkler, M., Bukowska, B., Michałowicz, J., 2017. Bisphenol A, bisphenol S, bisphenol F and bisphenol AF induce different oxidative stress and damage in human red blood cells (in vitro study). Toxicol. In Vitro 41, 143–149.

**The paper investigates the mechanism of oxidative action of BPA and its analogs BPS, BPF and BPAF in human erythrocytes and the potential effects during occupational exposure.*

21. Cho, Y.J., Park, S. Bin, Park, J.W., Oh, S.R., Han, M., 2018. Bisphenol A modulates inflammation and proliferation pathway in human endometrial stromal cells by inducing oxidative stress. Reprod. Toxicol. 81, 41–49.

22. Piao, X., Liu, Z., Li, Y., Yao, D., Sun, L., Wang, B., Ma, Y., Wang, L., Zhang, Y., 2019. Investigation of the effect for bisphenol A on oxidative stress in human hepatocytes and its interaction with catalase. Spectrochim. Acta - Part A Mol. Biomol. Spectrosc. 221, 117-149.

23. Marqueño, A., Pérez-Albaladejo, E., Flores, C., Moyano, E., Porte, C., 2019. Toxic effects of bisphenol A diglycidyl ether and derivatives in human placental cells. Environ. Pollut. 244, 513–521.

24. Mokra, K., Woźniak, K., Bukowska, B., Sicińska, P., Michałowicz, J., 2018. Low-concentration exposure to BPA, BPF and BPAF induces oxidative DNA bases lesions in human peripheral blood mononuclear cells. Chemosphere 201, 119–126.

25. Qiu, W., Yang, M., Liu, S., Lei, P., Hu, L., Chen, B., Wu, M., Wang, K.J., 2018. Toxic effects of bisphenol S showing immunomodulation in fish macrophages. Environ. Sci. Technol. 52, 831–838.

26. Qiu, W., Yang, M., Liu, J., Xu, H., Luo, S., Wong, M., Zheng, C., 2019. Bisphenol S-induced chronic inflammatory stress in liver via peroxisome proliferator-activated receptor γ using fish in vivo and in vitro models. Environ. Pollut. 246, 963–971.

27. Hercog, K., Maisanaba, S., Filipič, M., Sollner-Dolenc, M., Kač, L., Žegura, B., 2019. Genotoxic activity of bisphenol A and its analogues bisphenol S, bisphenol F and bisphenol AF and their mixtures in human hepatocellular carcinoma (HepG2) cells. Sci. Total Environ. 687, 267–276.

28. Chen, D., Kannan, K., Tan, H., Zheng, Z., Feng, Y.L., Wu, Y., Widelka, M., 2016. Bisphenol Analogues Other Than BPA: Environmental occurrence, human exposure, and toxicity - A Review. Environ. Sci. Technol. 50, 5438–5453.

***Comprehensive review on the occurrence of BPA and analogues in the environment, consumer products, foodstuffs, human exposure and toxicity.*

29. Pérez-Albaladejo, E., Lacorte, S., Porte, C., 2018. Differential toxicity of alkylphenols in JEG-3 human placental cells: alteration of P450 aromatase and cell lipid composition. Toxicol. Sci. 167, 336-346.

30. Derakhshesh, N., Movahedinia, A.A., Salamat, N., Hashemitabar, M., Bayati, V., 2017. Using a liver cell culture from *Epinephelus coioides* as a model to evaluate the nonylphenol-induced oxidative stress. Mar. Pollut. Bull. 122, 243–252.

31. Shaliutina, O., Shaliutina-Kolešová, A., Lebeda, I., Rodina, M., Gazo, I., 2017. The in vitro effect of nonylphenol, propranolol, and diethylstilbestrol on quality parameters and oxidative stress in sterlet (*Acipenser ruthenus*) spermatozoa. Toxicol. In Vitro 43, 9–15.

32. Sayed, A.E.D.H., Ismail, R.F.K., 2017. Endocrine disruption, oxidative stress, and testicular damage induced by 4-nonylphenol in *Clarias gariepinus*: the protective role of *Cydonia oblonga*. Fish Physiol. Biochem. 43, 1095–1104.

33. Prata, J.C., da Costa, J.P., Lopes, I., Duarte, A.C., Rocha-Santos, T., 2019. Effects of microplastics on microalgae populations: A critical review. Sci. Total Environ. 665, 400-405.

34. Jeong, J., Choi, J., 2019. Adverse outcome pathways potentially related to hazard identification of microplastics based on toxicity mechanisms. Chemosphere 231, 249-255.

35. Wang, Y., Wang, T., Ban, Y., Shen, C., Shen, Q., Chai, X., Zhao, W., Wei, J., 2018. Di-(2-ethylhexyl) phthalate exposure modulates antioxidant enzyme activity and gene expression in juvenile and adult *Daphnia magna*. Arch. Environ. Contam. Toxicol. 75, 145-156.

36. Walpitagama, M., Carve, M., Douek, A.M., Trestrail, C., Bai, Y., Kaslin, J., Wlodkowic, D., 2019. Additives migrating from 3D-printed plastic induce developmental toxicity

and neuro-behavioural alterations in early life zebrafish (*Danio rerio*) Aquat. Toxicol. 213,105227.

37. Qiu, W., Chen, J., Li, Y., Chen, Z., Jiang, L., Yang, M., Wu, M., 2016. Oxidative stress and immune disturbance after long-term exposure to bisphenol A in juvenile common carp (*Cyprinus carpio*). Ecotox. Environ. Saf.. 130, 93-102.

38. Qiu, W., Zhan, H., Tian, Y., Zhang, T., He, X., Luo, S., Xu, H., Zheng, C., 2018. The in vivo action of chronic bisphenol F showing potential immune disturbance in juvenile common carp (*Cyprinus carpio*). Chemosphere 205, 506-513.

39. Zhang, S., Ding, J., Razanajatovo, R.M., Jiang, H., Zou, H., Zhu, W., 2019. Interactive effects of polystyrene microplastics and roxithromycin on bioaccumulation and biochemical status in the freshwater fish red tilapia (*Oreochromis niloticus*). Sci. Total Environ. 648, 1431-1439.

40. Zhu, Z.-l., Wang, S.-c., Zhao, F.-f., Wang, S.-g., Liu, F.-f., Liu, G.-z., 2019. Joint toxicity of microplastics with triclosan to marine microalgae *Skeletonema costatum*. Environ. Pollut. 246, 509-517.

41. Park, J.C., Lee, M.-C., Yoon, D.-S., Han, J., Kim, M., Hwang, U.-K., Jung, J.-H., Lee, J.-S., 2018. Effects of bisphenol A and its analogs bisphenol F and S on life parameters, antioxidant system, and response of defensome in the marine rotifer *Brachionus koreanus*. Aquat. Toxicol. 199, 21-29.

**Study on the consequences of in vivo exposure to the plasticizer BPA and its analogs over the detoxification pathways and oxidative parameters on a planktonic marine rotifer.*

42. 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*. Environ. Pollut. 243, 66-74.

43. Varo, I., Perini, A., Torreblanca, A., Garcia, Y., Bergami, E., Vannuccini, M.L., Corsi, I., 2019. Time-dependent effects of polystyrene nanoparticles in brine shrimp *Artemia franciscana* at physiological, biochemical and molecular levels. Sci. Total Environ. 675, 570-580.

44. Wu, H., Zhong, M., Lu, Z., Shan, X., Li, F., Ji, C., Cong, M., 2018. Biological effects of tris (1-chloro-2-propyl) phosphate (TCPP) on immunity in mussel *Mytilus galloprovincialis*. Environ. Toxicol. Phar. 61, 102-106.

45. Barboza, L.G.A, Vieira, L.R., Branco, V., Figueiredo, N., Carvalho, F., Carvalho, C., Guilhermino, L., 2018. Microplastics cause neurotoxicity, oxidative damage and energy-related changes and interact with the bioaccumulation of mercury in the European seabass, *Dicentrarchus labrax* (Linnaeus, 1758). Aquat. Toxicol. 195, 49-57.

46. Carnevali, O., Santobuono, M., Forner-Piquer, I., Randazzo, B., Mylonas, C.C., Ancillai, D., Giorgini, E., Maradonna, F., 2019. Dietary diisononylphthalate contamination induces hepatic stress: a multidisciplinary investigation in gilthead seabream (*Sparus aurata*) liver. Arch. Toxicol. 93, 2361-2373.

47. Alomar, C., Sureda, A., Capo, X., Guijarro, B., Tejada, S., Deudero, S., 2017. Microplastic ingestion by *Mullus surmuletus* Linnaeus, 1758 fish and its potential for causing oxidative stress. Environ. Res. 159, 135-142.

48. Fonte, E., Ferreira, P., Guilhermino, L., 2016. Temperature rise and microplastics interact with the toxicity of the antibiotic cefalexin to juveniles of the common goby (*Pomatoschistus microps*): Post-exposure predatory behaviour, acetylcholinesterase activity and lipid peroxidation. Aquat. Toxicol. 180, 173-185.

49. Gao, Q.T., Wong, Y.S., Tam, N.F.Y., 2017. Antioxidant responses of different microalgal species to nonylphenol-induced oxidative stress. J. Appl. Phycol. 29, 1317-1329.

50. Asmonaite, G., Larsson, K., Undeland, I., Sturve, J., Almroth, B.C., 2018. Size Matters: Ingestion of relatively large microplastics contaminated with environmental pollutants posed little risk for fish health and fillet quality. Environ. Sci. Technol. 52, 14381-14391.

51. 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*). Mar. Pollut. Bull. 129, 231–240.

52. Qiao, R., Sheng, C., Lu, Y., Zhang, Y., Ren, H., Lemos, B., 2019. Microplastics induce intestinal inflammation, oxidative stress, and disorders of metabolome and microbiome in zebrafish. Sci. Total Environ. 662, 246–253.

53. Yang, W.K., Chiang, L.F., Tan, S.W., Chen, P.J., 2018. Environmentally relevant concentrations of di(2-ethylhexyl)phthalate exposure alter larval growth and locomotion in medaka fish via multiple pathways. Sci. Total Environ. 640–641, 512–522.

54. Hamed, H.S., Abdel-Tawwab, M., 2017. Ameliorative effect of propolis supplementation on alleviating bisphenol-A toxicity: Growth performance, biochemical variables, and oxidative stress biomarkers of Nile tilapia, *Oreochromis niloticus* (L.) fingerlings. Comp. Biochem. Phys. C 202, 63-69.

55. Dong, X., Zhang, Z., Meng, S., Pan, C., Yang, M., Wu, X., Yang, L., Xu, H., 2018. Parental exposure to bisphenol A and its analogs influences zebrafish offspring immunity. Sci. Total Environ. 610, 291-297.

56. Mohamed, W.A.M., El-Houseiny, W., Ibrahim, R.E., Abd-Elhakim, Y.M., 2019. Palliative effects of zinc sulfate against the immunosuppressive, hepato- and nephrotoxic impacts of nonylphenol in Nile tilapia (*Oreochromis niloticus*). Aquaculture 504, 227-238.

57. Qiu, W., Shao, H., Lei, P., Zheng, C., Qiu, C., Yang, M., Zheng, Y., 2018. Immunotoxicity of bisphenol S and F are similar to that of bisphenol A during zebrafish early development. Chemosphere 194, 1-8.

Table 1. Overview of oxidative stress effects caused by plastic additives in human and fish cell models.

Compound	Exposure	In-vitro model	Response	References
Phthalates				
DEHP	5-500 µM	Placental choriocarcinoma (JEG-3) Embryonic kidney cells (HEK-293) Pancreatic β cells (INS-1) *Dicentrarchus labrax* embryonic cell line (DLEC) *Poeciliopsis lucida* hepatome (PLHC-1) Zebrafish liver cell line (ZFL)	Induction of ROS in HEK-293, INS-1, PLHC-1 and ZFL, but not in JEG-3 cells Alteration of SOD activity and lipid peroxidation (HEK-293, INS-1) Increase of CAT activity (HEK-293) Depletion of GSH levels, lysosomal membrane alteration and mitochondrial dysfunction (INS-1) DNA oxidative damage (INS-1, DLEC)	[11, 12, 13, 19]
DMP	5-500 µM	JEG-3 Erythrocytes	Increase of SOD activity and GSH levels, decrease of GPX and CAT activities, and lipid peroxidation (erythrocytes) No significant oxidative responses in JEG-3 cells at 500 µM	[16, 29]
BBP DBP	500 µM	JEG-3 PLHC-1 ZFL	Induction of ROS by DBP in fish cells (PLHC-1, ZFL) No significant ROS generation in JEG-3 cells	[29]
DiNP	238 µM	Hepatocytes HepG2	Induction of ROS	[14]
Bisphenols				
BPA	0.004-2000 µM	Erythrocytes Peripheral blood mononuclear cells (PBMC) JEG-3 Endometrial stromal cells (ESCells) HepG2 Hepatocytes L-02 Cortical neurons (hCN) PLHC-1 ZFL	Induction of ROS Lipid peroxidation, decreased GSH levels and CAT, SOD and GPX activities (erythrocytes) Strong oxidative damage to DNA pyrimidines and purines (PBMC) Down-regulation of *SOD* and *HO*, but not *GPX* (ESCells) No significant differences in the expression of *GPX1, GSR, SOD1A*, but up-regulation of *GCLC* (HepG2) Deregulation of CAT activity (L-02 cells) Deregulation of the expression of *GSS* and *CAT* genes (hCN)	[20, 21, 22, 24, 27, 29, 35]
BPF BPAF	0.004-2000 µM	Erythrocytes PBMC HepG2 PLHC-1 ZFL	Induction of ROS Lipid peroxidation and decreased GSH levels and SOD activity (erythrocytes) Decreased CAT and GPX by BPF, but BPAF did not (erythrocytes) DNA oxidation (PBMC) No significant differences in the expression of *GPX1, GSR, SOD1A* and *GCLC* (HepG2)	[20, 24, 27]
BPS	0.004-2000 µM	Erythrocytes PBMC HepG2 Macrophages of *Cyprinus carpio*	Induction of ROS Decreased GPX (erythrocytes) DNA oxidation (PBMC) No significant differences in the expression of *GPX1, GSR, SOD1A* and *GCLC* (HepG2) No lipid peroxidation, but deregulation of oxidative stress involved genes in fish macrophages	[20, 24, 25, 26, 27]

BADGE BADGE·2H2O	5-500 µM	JEG-3 PLHC-1 ZFL	Low or no induction of ROS, except in PLHC-1 (2-fold induction)	[23]
Alkylphenols				
HP OP NP	1-40 µM	JEG-3 HepG2 Fish hepatocytes (*Epinephelus coioides)* Fish spermatozoa *(Acipenser ruthenus*)	OP and NP induced ROS generation (JEG-3, HepG2) HP induced ROS up to 43-fold, which led to lipidic changes in JEG-3 NP induced lipid peroxidation, alteration of antioxidant enzymes and/or loss of membrane integrity in fish cells	[13, 29, 29, 30, 31]

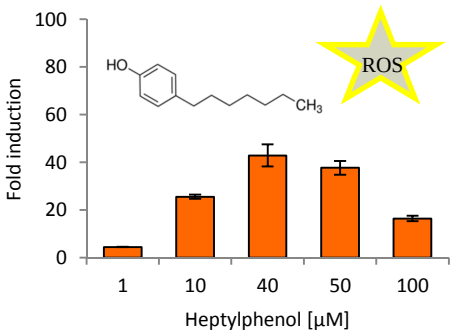
Table 2. Selection of studies showing oxidative stress effects in fish after exposure to microplastics and plastic additives.

Compound	Dose	Fish model	Response	References
Microplastics				
Commercial microplastics	0.26 & 0.69 mg/L	Juvenile *Dicentrarchus labrax*	Increase muscle and brain LPO at high dose	[45]
Environmental microplastics	-	Wild *Mullus surmuletus* from Balearic coast (Spain).	27% of 417 collected fish contained microplastics in their stomach. No evidences of oxidative stress in liver, induction of glutathione S-transferase (GST)	[47]
Polystyrene microplastics	100–400 µm	rainbow trout (*Oncorhynchus mykiss*)	Small changes on gene expression and activities of antioxidant enzymes and muscle LPO	[50]
Polyethylene microspheres 150-180 µm	50 – 250 mg/L	Sheepshead minnow (*Cyprinodon variegatus*)	Alteration of ROS key related genes *Cat* and *Sod3*	[51]
Polystyrene microspheres 5 µm	0.050 – 0.5 mg/L	Zebrafish (*Danio rerio*)	Increase SOD and CAT activities	[52]
Phthalates				
DiNP	15 & 1500 µg/kg bw/day	gilthead seabream (*Sparus aurata*)	Upregulation of mRNA g6pdh, glut red, gpx1 and CAT activity at higher dose	[46]
DEHP	20-200 µg/L	Medaka fish larva (*Oryzias latipes*)	Suppression of the expression of SOD, CAT, GPX, GST, PPARα, RXRα Alteration of fish growth and locomotion partially through oxidative stress pathway	[53]
Bisphenols				
BPA	1-1000 µg/L	Juvenile common carp (*Cyprinus carpio*) Nile tilapia (*Oreochromis nilotocus*) Zebrafish embryos (*D. rerio)*	Lipid peroxidation Inhibition of SOD and CAT in *C. carpio*, but increased in *O. nilotocus* Decrease of GPX and GST in *O. nilotocus* Increase of SOD, ROS levels and expression of related genes in zebrafish embryos	[38, 54,57]
BPF	1-1000 µg/L	Juvenile common carp (*C. carpio*) Zebrafish embryos (*D. rerio)*	Lipid peroxidation No alterations in SOD and CAT activities were detected in *C. carpio,* but up-	[38,57]

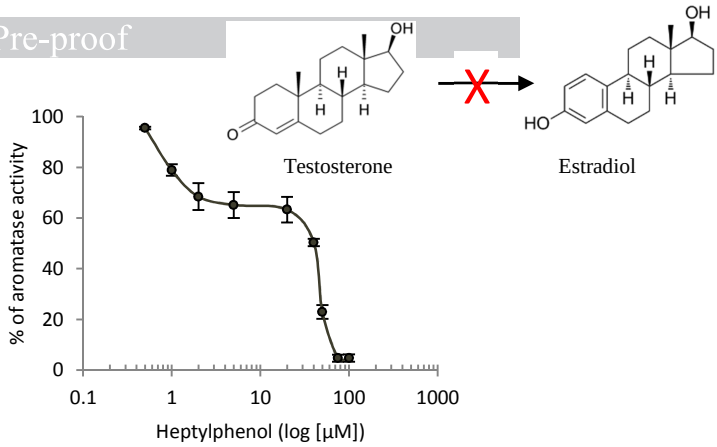
			regulation of OE-related genes Increase of SOD, CAT, ROS levels and expression of related genes in zebrafish embryos	
BPS	100-1000 µg/L	Zebrafish embryos (*D. rerio)*	ROS production Lipid peroxidation Increase of SOD and CAT Up-regulation of OE-related genes	[57]
BPA, BPF, BPS	0.1-10 µg/L	Zebrafish (*D. rerio*) F1 larvae, after parental exposure	GPX was not changed Decrease CAT (BPA and BPS) and SOD activities	[55]
Alkylphenols				
NP	50 -100 µg/L	Nile tilapia (*O. nilotocus*) Catfish (*Clarias gariepinus*)	Lipid peroxidation Disruption of CAT, SOD, GPX Increase of E2 (estrogenic) and decrease of T in catfish	[32] [56]

Fig. 1. (A) Heptylphenol significantly induced the generation of reactive oxygen species (up to 43-fold at 40 µM) in human placenta JEG-3 cells, and consequently, (B) an inhibition of P450 aromatase activity was observed, together with (C) a significant depletion of polyunsaturated phospholipids (phosphatidylcholines (PC), phosphatidylethanolamines (PE), plasmanyl and plasmenyl -PCs and -PEs (PC-P, PC-O, PE-P)), and triacylglycerols (TG). TGs act as cell depots of polyunsaturated fatty acids; the hydrolysis of TGs will provide fatty acids to replace damaged/oxidized lipids. The significant depletion of highly unsaturated phospholipids may result in a decrease of cell membrane fluidity, imperfect packing of the lipid bilayer and alter ion transport, signal transduction and cell integrity. Adapted from Pérez-Albaladejo et al. [29].

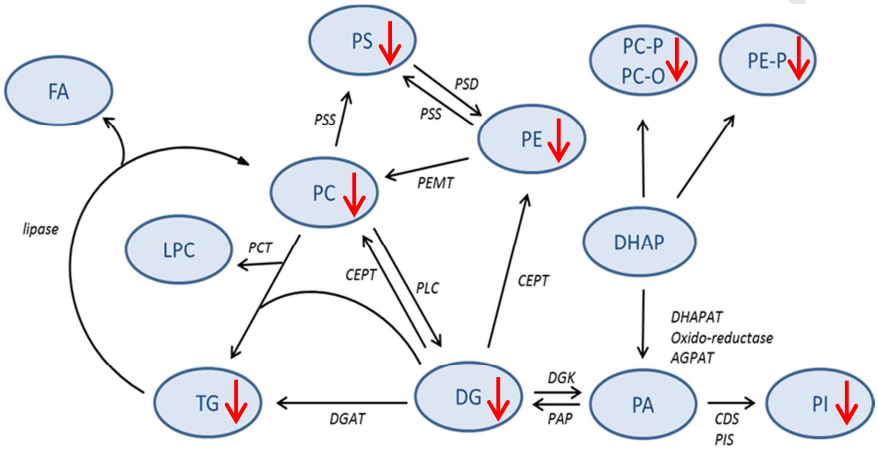
A



B



C



Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: