Chemosphere 231 (2019) 249–255

Contents lists available at ScienceDirect

Chemosphere

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

Review

Adverse outcome pathways potentially related to hazard identification of microplastics based on toxicity mechanisms

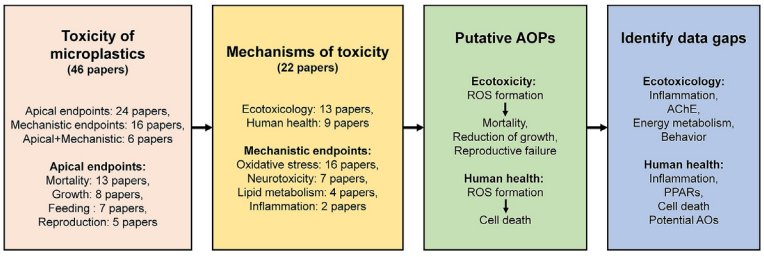
Jaeseong Jeong, Jinhee Choi*

School of Environmental Engineering, University of Seoul, 163 Seoulsiripdae-ro, Dongdaemun-gu, Seoul, 02504, South Korea

HIGHLIGHTS

- Microplastic toxicity mechanisms in terms of ecotoxicity and human health toxicity were reviewed.
- Microplastic toxicology research has focused on ecotoxicity using apical endpoints.
- Toxicity mechanisms matched with KE from the AOP Wiki and putative AOPs were proposed.
- MIE is ROS formation and AOs are increasing mortality, reduction of growth, and reproduction failure.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 25 February 2019
Received in revised form
30 April 2019
Accepted 1 May 2019
Available online 6 May 2019

Handling Editor: Tamara S. Galloway

Keywords:
Microplastics
Toxicity mechanism
Adverse outcome pathway
AOP Wiki
Oxidative stress

ABSTRACT

Increasing concern over microplastics has recently brought increased attention to studies on microplastic toxicity. Here, we conduct a systematic review on toxicity of microplastics that focuses on identifying data gaps in the mechanisms of microplastic toxicity. We observe that microplastic toxicology research thus far has focused on ecotoxicity using apical endpoints and only a few studies deal with toxicity mechanisms. Based on this review, we propose putative Adverse Outcome Pathways (AOPs) applicable to microplastic management to understand microplastic toxicity. We matched toxicity mechanisms and apical endpoints to a key event (KE) and adverse outcome (AO) information from the AOP Wiki. Overall, our results suggest that the molecular initiating event (MIE) was reactive oxygen species (ROS) formation and the AO was increased mortality, decreased growth and feeding, and reproduction failure. However, there are a limited number of studies on toxicity mechanisms of microplastics and, therefore, evidence concerning the relationship between KEs is not sufficient. Clearly, more studies on toxicity mechanisms are required to fill these gaps in data. This study also suggests that the AOP framework is a suitable tool to integrate existing data from various literature sources and can identify data gaps in microplastic toxicity mechanisms.

Contents

* Corresponding author.
E-mail address: jinhchoi@uos.ac.kr (J. Choi).

https://doi.org/10.1016/j.chemosphere.2019.05.003
0045-6535/

1. Introduction

The increasing production and consumption of plastics have led to a significant increase in the number of plastics in the environment. An estimated amount of 4.8–12.7 million tons of plastic wastes entered the marine environment in 2010 and are predicted to increase by as much as 100 to 250 million tons by 2025 (Jambeck et al., 2015). Plastic wastes are crushed to micro-sized particles in marine environments due to the influences of ultraviolet or mechanical abrasion (Barnes et al., 2009). Microplastics are plastic particles that are less than 5 mm and distribute throughout all oceans, including coastlines, seabed sediments, and beaches (Gallagher et al., 2016). Microplastic intake can lead to abrasions, ulcers, satiation, oxidative stress, reduction in the growth rate, and a reduced reproductive fitness (Fossi et al., 2016; Sutton et al., 2016).

Microplastics are easily ingested by a variety of organisms in marine environments (Cole et al., 2013; Ferreira et al., 2016; Lusher et al., 2015a, 2015b) due to their small size. Since microplastics are distributed in various trophic levels, microplastic concentrations in the body may increase via bioaccumulation at higher trophic levels. Humans are also exposed to microplastics via the food chain (Bouwmeester et al., 2015; Carbery et al., 2018; Farrell and Nelson, 2013; Nelms et al., 2018; Rochman et al., 2015). This suggests that humans may suffer from the most devastating effects of microplastic toxicity. In addition to microplastic toxicity itself, microplastics may also cause unknown toxic effects because they are known to absorb toxic chemicals (Li et al., 2016), such as polychlorinated biphenyls (PCBs) (Zarfl and Matthies, 2010), polycyclic aromatic hydrocarbons (PAHs) (Teuten et al., 2007), and organochlorine pesticides, such as DDT (Ivar Do Sul and Costa, 2014), from the surrounding environment and spread them throughout the food chain (Reisser et al., 2014). For this reason, it is urgent to assess the hazards of microplastics to human health.

In recent years, the number of studies on microplastic toxicity has rapidly increased. Microplastic toxicology research has used a wide range of model systems, including marine, freshwater, and terrestrial vertebrates and invertebrates (de Sá et al., 2018; Li et al., 2016). Since microplastics have a wide variety of their size, shape, and polymer type, as well as nanomaterials, the toxicity test results are very diverse and vary from species to species (Foley et al., 2018; Ziajahromi et al., 2018). Even some studies have reported no negative impact on some species in environmental concentrations of microplastics (Burns and Boxall, 2018; Weber et al., 2018). According to Weber et al. (2018), for example, chronic toxicity studies of PET on a freshwater amphipod *Gammarus pulex* did not show the toxicity at the environmental concentrations and even higher, contrary to previous studies, and it may be explained by differences in the exposure scenarios, plastic characteristics and species-specific traits. Because of the diversity of the microplastics, it is difficult to assess their toxicity, and there is a limit to testing the toxicity of various microplastics one at a time. To address these limitations, a toxicity mechanism based approach, such as an Adverse Outcome Pathway (AOP), is needed rather than a substance-based approach. The majority of microplastic toxicity studies, however, focus on ecotoxicity using apical endpoints and only a few studies deal with microplastic toxicity mechanisms.

Since the introduction of the AOP concept, a number of studies have applied AOP for chemical risk assessment due to its highly promising characteristics (Jeong and Choi, 2017; Kang et al., 2018; Martens et al., 2018). The AOP concept was originally intended as a tool to predict toxicity by analyzing the mechanisms that link a molecular event to an apical endpoint (Ankley et al., 2010; Villeneuve et al., 2014a, 2014b). AOP is defined as the concatenation of a series of events that begins with a molecular initiating event (MIE). Events that originate from the MIE proceed through a series of key events (KEs), which includes changes in cell status, metabolic pathways, signal transduction, or cellular function. Finally, events lead to an adverse outcome (AO), which is the classical apical endpoint traditionally used for hazard and risk assessment (Leist et al., 2017). AOPs span numerous levels of biological organization, from molecular to organism or even to population levels for ecotoxicology scenarios (Leist et al., 2017). Since AOP is an evidence-based framework, it continues to be revised in accordance with new evidence of toxicity mechanisms, increasing its reliability and utility (Villeneuve et al., 2014a). It also allows us to identify data gaps for the future research based on the current AOP. Thus, although the study on toxicity mechanisms of microplastics is limited, identifying the currently available molecular-level toxicity information and linking it with AO is a very important beginning for the risk assessment of microplastics.

In this context, we conduct a systematic review of microplastic toxicity studies to: 1) quantify the research completed on toxicity mechanisms, 2) identify data gaps in microplastic toxicity concerning human health implications, and finally 3) propose appropriate AOP to manage microplastics based on the current literature. We assign microplastic toxicity mechanisms to KEs and AOs that exist in the AOP Wiki database. In addition, we propose a putative AOP based on these KEs and AOs. This will provide an improved understanding of microplastic toxicity and allow us to assess the risks that derive from various microplastics.

2. Current status of microplastic toxicity studies

We identified 46 publications on microplastic toxicity using Google Scholar (January 2019). Twenty-four papers focus on apical endpoints, such as mortality, growth, feeding behavior, and reproduction, sixteen address mechanistic endpoints, and six studied both mechanisms and apical endpoints. Among these publications, six are applicable to both human health and ecotoxicity using model species that include zebrafish and *Caenorhabditis elegans*. Three publications discuss only human health implications using human cell lines and mice (Table 1).

Table 1
A summary of publications that discuss microplastic toxicity mechanisms (Google scholar, January 2019).

		Papers on apical endpoints		Papers on mechanisms		Papers on mechanisms and apical endpoints	
		Category	No. of papers	Category	No. of papers	Category	No. of papers
Endpoints		Mortality	13	Oxidative stress pathway	11	Oxidative Stress + growth/ reproduction	3
		Growth/ development	8	Neurotoxicity	5	Oxidative Stress + mortality/growth	2
		Feeding	7	Lipid metabolism	4	Neurotoxicity/oxidative Stress + behavior	2
		Reproduction/ fecundity	5	Inflammation	2	Biosynthesis + growth	1
Test organisms	Ecotoxicity	Marine organisms	13	Marine organisms	7	Marine organisms	2
		Freshwater organisms	5	Freshwater organisms	2	Freshwater organisms	2
	Ecotoxicity and human health implication			Zebrafish	4	*Caenorhabditis elegans*	2
						Zebrafish	1
	Human health implication			Mouse (*in vivo*)	2		
				Human cell lines (*in vitro*)	1		
Total number of papers		24		16		6	

2.1. MP toxicity mechanisms in marine, freshwater, and terrestrial organisms: implications for ecotoxicity

Next, to identify gaps in data on microplastic toxicity mechanisms, we conducted a systematic review on the publications that study toxicity with or without ecotoxicity mechanisms (Table 2; a full summary of microplastic toxicity mechanisms for ecotoxicity is found in Table S1). Thirteen out of the 22 (59%) toxicity publications, in fact, discussed microplastic mechanistic endpoints. These publications mainly focus on ecotoxicity using marine, freshwater, and terrestrial organisms. We found nine studies that use marine species, such as European seabass and copepod, and four studies that use freshwater species, such as *Daphnia magna* and green algae. Out of these publications, polystyrene (PS) was reported in

Table 2
Microplastic toxicity mechanisms: ecotoxicity in marine, freshwater, and terrestrial organisms.

Organisms		Microplastics	Endpoint		Reference
Habitat	Species	Type	Molecular endpoint (MIE/KE)	Apical endpoint (AO)	
Marine	*Mullus surmuletus*	Various (PET, Cellophane, PAK, PAN, etc.)	Oxidative stress (GST)	NA	Alomar et al. (2017)
	Pomatoschistus microps	PE	Neurotoxicity (AChE inhibition)	NA	Oliveira et al. (2013)
	Paracyclopina nana	PS	ROS, MAPK (p-ERK, p-p38, Nrf2), Oxidative stress (GR, GPx, GST, SOD)	Development, fecundity	Jeong et al. (2017)
	Sparus aurata	PVC, PE	Phagocytic ability, respiratory burst activity, oxidative stress	NA	Espinosa et al. (2018)
	Dicentrarchus labrax	Fluorescence red polymer	Phagocytic capacity, respiratory burst activity Neurotoxicity (AChE inhibition), Lipid peroxidation (LPO), Energy production (LDH, IDH)	NA	Barboza et al. (2018)
	Eriocheir sinensis	PS	Liver damage (GPT, GOT), Neurotoxicity (AChE), Oxidative stress (SOD, CAT, GPx, GST, GSH, MDA), MAPK (p38, JNK, ERK, AKT, MEK)	NA	Yu et al. (2018)
	Cyprinodon variegatus	PE	Oxidative stress (CAT, SOD3), Chemokine (CXCR5), Apoptosis (CASP3, Tp53)	Slight decrease behavioral activity	Choi et al. (2018)
	Mytilus galloprovincialis	PE	Immunological responses (granulocytes/hyalinocytes ratio), Neurotoxicity (AChE inhibition)	NA	Avio et al. (2015)
		PS	Immunological responses (haemocytes lysosomal membrane stability), Neurotoxicity (AChE inhibition), Sntioxidant defenses (Se-dependent glutathione peroxidases)		
	Mytilus spp.	PS	Hemocyte mortality, ROS production, Antioxidant (CAT, SOD, catalase, glutathione reductase), Lipid peroxidation, Detoxification (PGP), Digestion (PK), Immunity (LYS)	NA	Paul-Pont et al. (2016)
Freshwater	*Daphnia magna*	MixA (PA, PCB, PET, PVC)	General stress (HSP60, HSP70)	NA	Imhof et al. (2017)
		MixB (ABS, PVC-P, POMH, SAN)	Oxidative stress (GST)		
	Brachionus koreanus	PS	ROS, MAPK and Oxidative stress (p-JNK, p-p38, GPx, GR, GST, SOD)	Growth rate, fecundity, lifespan	Jeong et al. (2016)
	Chlamydomonas reinhardtii	PP	Sugar biosynthesis (UGD, UGE, UGLD)	Growth (PP)	Lagarde et al. (2016)
		HDPE	Photosynthetic (rbcL), Sugar biosynthesis (UGD, UGE, UGLD)	NA	
	Chlorella scenedesmus	PS	ROS production	NA	Bhattacharya et al. (2010)

six studies (37.5% of total), four studies on polyethylene (PE; 25%), and one study each for polyvinylchloride (PVC), polypropylene (PP), high density polyethylene (HDPE), fluorescence red polymer, and a laboratory- and field-mixture sample (6.25%). These publications report various toxic effects but the most frequently mentioned endpoints were oxidative stress-related endpoints, which include reactive oxygen species (ROS) generation, oxidative stress, and a mitogen-activated protein kinase (MAPK) signaling pathway. Ten out of the thirteen studies measured oxidative stress and its related pathway and concluded that these are the main microplastic toxicity mechanisms (Alomar et al., 2017; Avio et al., 2015; Bhattacharya et al., 2010; Choi et al., 2018; Espinosa et al., 2018; Imhof et al., 2017; Jeong et al., 2017, 2016; Paul-Pont et al., 2016; Yu et al., 2018). Based on these studies, microplastics generate ROS, increase glutathione S-transferase (GST) activity, and activate antioxidant-related enzymes and MAPK signaling pathways. After oxidative stress pathways, the most frequently measured endpoints were neurotoxicity with experiments performed in four studies (Avio et al., 2015; Barboza et al., 2018; Oliveira et al., 2013; Yu et al., 2018). According to these studies, microplastics cause neurotoxicity via acetylcholinesterase (AChE) inhibition. Some studies also addressed the relationship between toxicity mechanisms and apical endpoints. Studies on marine species addressed oxidative stress with apical endpoints, such as development, growth, behavioral activity, lifespan, and fecundity (Choi et al., 2018; Jeong et al., 2017, 2016).

2.2. MP toxicity mechanisms in model organisms and in-vitro models: human health implications

Next, we reviewed publications discussing toxicity with or without toxicity mechanisms related to the implications for human health (Table 3; a full summary of microplastic toxicity mechanisms for human health is found in Table S2). Nine out of 22 (41%) publications analyzed microplastic toxicity mechanisms in human health-related models. One publication used an *in-vitro* system with human T98G (glioblastoma multiforme) and HeLa (cervical carcinoma) cell lines. On the other hand, the majority of the studies conducted *in-vivo* experiments using toxicity model species on human health, such as mice, zebrafish, and *C. elegans*. Zebrafish and *C. elegans* are useable as test model organisms for both human health toxicology and ecotoxicology studies due to both have high human genome similarity, as well as ecotoxicity relevance (Choi et al., 2014).

All studies used PS, while one study also used PE and another study used PE, polyamide (PA), PP, and PVC, together with PS. Compared with ecotoxicity studies, human health toxicity studies have investigated relatively different endpoints but, nonetheless, six studies have examined the effects of oxidative stress-related endpoints (Chen et al., 2017; Deng et al., 2017; Lei et al., 2018b, 2018a; Lu et al., 2016; Schirinzi et al., 2017). In addition to oxidative stress, other toxic effects include lipid metabolism, microbiota, neurotoxicity, and inflammation (Jin et al., 2018; Lei et al., 2018a; Lu et al., 2018). Two studies addressed the relationship between toxicity mechanisms and apical endpoints. Lei et al. (Lei et al., 2018b, 2018a) confirmed the effects that microplastics have on mortality and histopathological changes in zebrafish, as well as survival, growth, reproduction, and behavior in *C. elegans*.

3. Relevance to AOP development

To apply microplastic toxicity to an AOP framework, we matched information on microplastic toxicity mechanisms with existing KEs and AOs based on the AOP Wiki (https://aopwiki.org/). First, we assigned toxicity mechanisms using KEs (Table 4; a full list of KEs is found in Table S3). The KE ontology used in this study is presented in Table S4. As a result, we confirmed 43 studies with 21 kEs. The most common KE was “Oxidative Stress” (KE1392; 30.2%), followed by “ROS formation” (KE1278; 14.0%), “Inhibition, Acetylcholinesterase” (KE12; 11.6%), “Increase, Inflammation”

Table 3
Mechanisms of microplastic toxicity: applicable in both human health toxicity and ecotoxicity for *in-vitro* and *in-vivo* studies.

Organisms		Microplastics	Endpoint		Reference
Test system	Cell lines or species	Type	Molecular endpoint (MIE/KE)	Apical endpoint (AO)	
In vitro	T98G cells HeLa cells	PE PS	ROS generations	NA	Schirinzi et al. (2017)
In vivo	*Mus musculus*	PS	Energy metabolism (ATP, LDH, creatine, 2-oxoglutarate, citrate), Lipid metabolism (T-CHO, TG), Oxidative stress (GSH-Px, SOD, CAT, threonine, pyruvate, lysine), Neurotoxicity (AChE, threonine, aspartate, taurine), Lipid disturbance (taurine, ethanol, lipid, choline), Lipid droplets, Inflammation	NA	Deng et al. (2017)
			Triglyceride, Total cholesterol, Pyruvate, Glucose (Pk, Chrebp), β-oxidation (PPARα), FA transporters (Fat, Fatp2), Krebs cycle (Cs), FA synthesis (PPARγ, Fas, Acc, Acl), TG synthesis (Dgat1, Dgat2, Gpat), Changes in Microbiota (*Firmicutes*, *Bacteriodetes*, *Actinobacteria*, *α-proteobacteria*)	NA	Lu et al. (2018)
Human health implication	*Danio rerio*	PS	Inflammation (IL1α, IL1β, Ifn, IL6, IL8), Changes in Microbiota (*Firmicutes*, *Bacteroidetes*, *γ-Proteobacteria*, *β-Proteobacteria*)	NA	Jin et al. (2018)
			Oxidative stress (SOD, CAT), Necrosis, Infiltration, Fat droplets, Fatty acids, Amino acids	NA	Lu et al. (2016)
			Complement, Ion binding/transport, Transporters, Apoptotic process, Oxidoreductase activity, Immune system process, Endopeptidase activity	NA	Veneman et al. (2017)
			Oxidative stress (GSH), Visual system (zfrho)	NA	Chen et al. (2017)
		PA, PE, PP, PVC, PS	NA	Mortality (PP), histopathological change (PA, PE, PP, PVC)	Lei et al. (2018b)
	Caenorhabditis elegans		Calcium level, Oxidative damage	Mortality, growth, reproductive toxicity	
		PS	Neurotoxicity (unc-17, unc-47), Oxidative damage (gst-4)	Mortality, body length, lifespan, behavior	Lei et al. (2018a)

Table 4
Summary of the assigned KEs based on the mechanism of microplastic toxicity.

Category	Sub-category	Number of studies	% of studies
Key Event	Oxidative Stress (KE1392)	13	30.2
	ROS formation (KE1278)	6	14.0
	Inhibition, Acetylcholinesterase (AchE) (KE12)	5	11.6
	Increase, Inflammation (KE149)	2	4.7
	Lipid Peroxidation (KE1511)	2	4.7
Type of microplastics	PS	34	79.1
	PE	7	16.3
	PVC	2	4.7
	Fluorescence red polymer	2	4.7
	Mixture	2	4.7
	PP	1	2.3
	PA	1	2.3
	HDPE	1	2.3
Test species	*M. musculus* (human health implication)	11	25.6
	D. rerio (human health implication)	6	14.0
	P. nana (marine)	4	9.3
	C. elegans (human health implication)	3	7.0

(KE149; 4.7%), and "Lipid Peroxidation" (KE1511; 4.7%). These results also indicate that oxidative stress pathways are the most frequent KE for microplastic toxicity mechanisms in both ecotoxicity and human health toxicity. The most commonly used type of microplastic in these studies was PS (79.1%), followed by PE (16.3%). For organisms used in the studies, 25.6% used mice, followed by zebrafish (14.0%), which indicates that KEs have a greater diversity in human health toxicity studies than ecotoxicity studies.

Next, we assigned toxicity information on the AOs (Table 5; a full list of AOs is found in Table S5). The most abundant AO was "Increased, Mortality" (KE351; 34.1%), followed by "Growth, reduction" (KE1467; 22.7%), "Inhibition, Feeding" (KE1016; 15.9%), and "Reproductive failure" (KE1277; 11.4%). These studies used six types of microplastics, which include PS (41.0%), PE (27.9%), PP (14.8%), and PVC (9.8%). *D. magna* was the most commonly used test species, i.e., reported in 22.2% of the studies. Most studies used aquatic organisms to identify adverse outcomes and very few studies investigated adverse outcomes using model species for human health toxicity (13.3%).

Overall, our results indicate that microplastics cause toxicity through mainly oxidative stress pathways, which ultimately leads to mortality, growth reduction, and reproductive failure. By combining these results, we propose putative AOPs that are applicable to microplastics in ecotoxicity, as well as contexts for the implications of human health (Fig. 1). The formation of ROS leads to both oxidative stress and an inflammation response that is applicable to both ecotoxicity and human health perspectives. Acetylcholinestrase inhibition and alteration of the energy metabolism lead to mortality and growth and reproduction failure via behavior alteration that is possibly an appropriate AOP for microplastic ecotoxicity. Alteration of lipid metabolism controlled by oxidative stress pathways and PPAR activation leads to cell death that is an AOP in the human health context.

4. Conclusions

In this study, we reviewed microplastic toxicity mechanisms in terms of ecotoxicity and human health toxicity. In addition, we matched the toxicity mechanisms with KE and AO information from the AOP Wiki and we proposed putative AOPs for microplastics. Overall, our results suggest that the MIE was ROS formation and the AOs were increasing mortality, decreasing rates of growth, and reproduction failure. However, there are a limited number of studies on microplastic toxicity mechanisms and evidence on the relationship between KEs are not sufficient. To fill gaps in data, clearly need more studies on toxicity mechanisms and, accordingly, the putative AOPs proposed in this study can revise. More research on toxicity mechanisms in humans is also needed to expand the taxonomic

Table 5
Summary of the assigned AOs for toxicity of microplastics.

Category	Sub-category	Number of studies	% of studies
Adverse Outcome	Increased, Mortality (KE351)	15	34.1
	Growth, reduction (KE1467)	10	22.7
	Inhibition, Feeding (KE1016)	7	15.9
	Reproductive failure (KE1277)	5	11.4
	Decrease, Fecundity (KE328)	4	9.1
	Altered, Larval development (KE339)	2	4.5
	Decreased, Body Weight (KE864)	1	2.3
Type of microplastics	PS	25	41.0
	PE	17	27.9
	PP	9	14.8
	PVC	6	9.8
	PA	2	3.3
	PES	1	1.6
	PET	1	1.6
Test species	*D. magna* (freshwater)	10	22.2
	H. azteca (freshwater)	4	8.9
	C. elegans (human health implication)	4	8.9
	H. attenuata (freshwater)	3	6.7
	P. lividus (marine)	3	6.7
	D. rerio (human health implication)	2	4.4

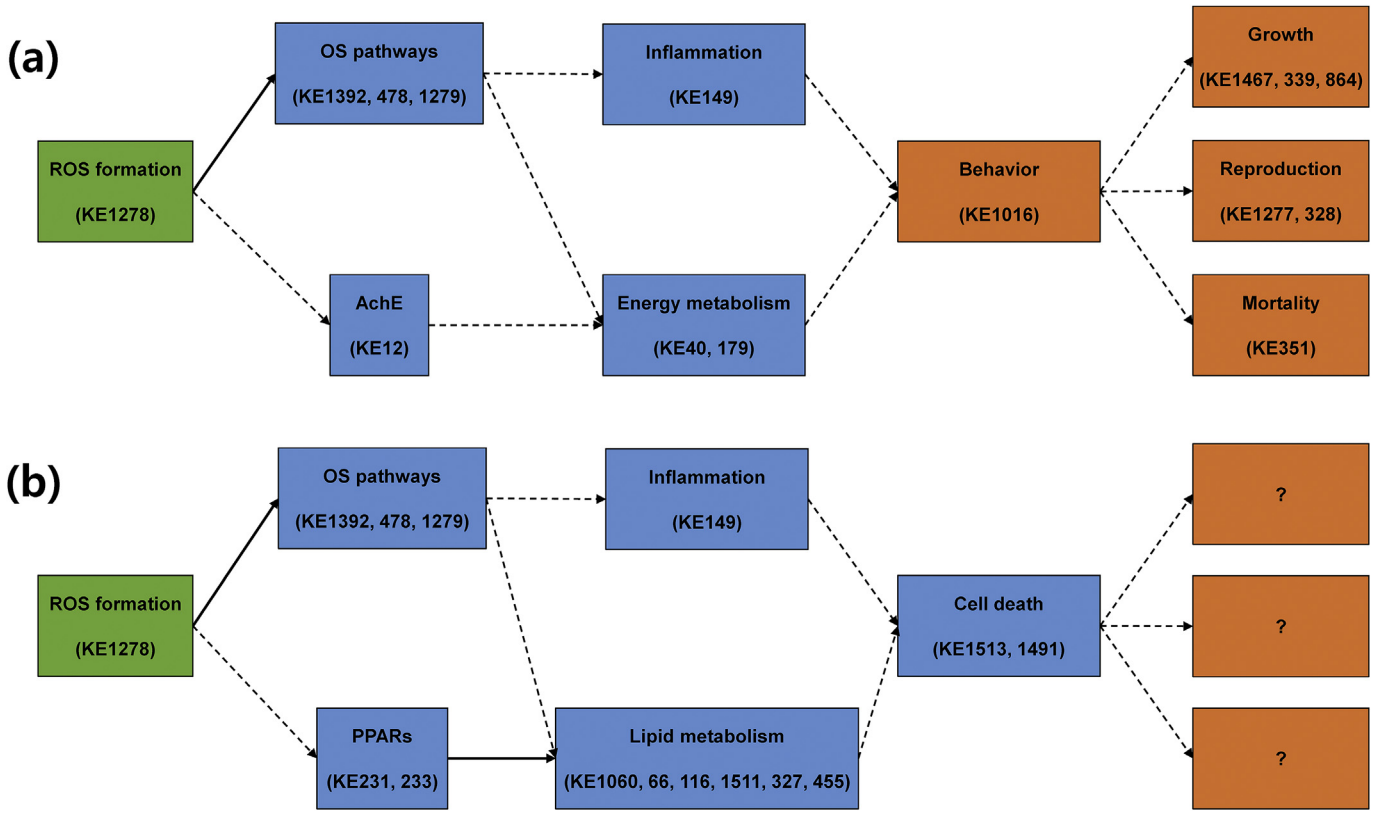


Fig. 1. Schematic diagram of the putative AOPs for microplastics. The AOPs for ecotoxicity (a) and human health implications (b). Green boxes: molecular initiating events, Blue boxes: key events, Orange boxes: adverse outcomes, Solid lines: strong evidence, Dashed lines: weak evidence (ROS: reactive oxygen species; OS: oxidative stress; AchE: acetylcholinesterase; PPARs: peroxisome proliferator-activated receptors). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

applicability of the AOP and assess the hazards of microplastics on human health. As the reliability of the AOP improves by filling the data gaps in the future, the AO can be predicted through the AOP, even if the shape, size, and type of the microplastics vary. Overall, this study suggests that the AOP framework is a suitable tool to integrate existing data from various literature sources and can identify data gaps in microplastic toxicity mechanisms.

Declarations of interest

None.

Acknowledgement

This work was supported by the Korean Ministry of Environment under the 'Environmental Health R&D Program' (2017001370001) and was a part of the project titled 'Environmental risk assessment of microplastics in the marine environment' (20150248) funded by the Ministry of Oceans and Fisheries, KOREA.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.chemosphere.2019.05.003.

References

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. Environ. Res. 159, 135–142. https://doi.org/10.1016/j.envres.2017.07.043.

Ankley, G.T., Bennett, R.S., Erickson, R.J., Hoff, D.J., Hornung, M.W., Johnson, R.D., Mount, D.R., Nichols, J.W., Russom, C.L., Schmieder, P.K., Serrrano, J.A., Tietge, J.E., Villeneuve, D.L., 2010. Adverse outcome pathways: a conceptual framework to support ecotoxicology research and risk assessment. Environ. Toxicol. Chem. 29, 730–741. https://doi.org/10.1002/etc.34.

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. Environ. Pollut. 198, 211–222. https://doi.org/10.1016/j.envpol.2014.12.021.

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. https://doi.org/10.1016/j.aquatox.2017.12.008.

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

Bhattacharya, P., Lin, S., Turner, J.P., Ke, P.C., 2010. Physical adsorption of charged plastic nanoparticles affects algal photosynthesis. J. Phys. Chem. 114, 16556–16561. https://doi.org/10.1021/jp1054759.

Bouwmeester, H., Hollman, P.C.H., Peters, R.J.B., 2015. Potential health impact of environmentally released micro- and nanoplastics in the human food production chain: experiences from nanotoxicology. Environ. Sci. Technol. 49, 8932–8947. https://doi.org/10.1021/acs.est.5b01090.

Burns, E.E., Boxall, A.B.A., 2018. Microplastics in the aquatic environment: evidence for or against adverse impacts and major knowledge gaps. Environ. Toxicol. Chem. 37, 2776–2796. https://doi.org/10.1002/etc.4268.

Carbery, M., O'Connor, W., Palanisami, T., 2018. Trophic transfer of microplastics and mixed contaminants in the marine food web and implications for human health. Environ. Int. 115, 400–409. https://doi.org/10.1016/j.envint.2018.03.007.

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. https://doi.org/10.1016/j.scitotenv.2017.01.156.

Choi, J., Tsyusko, O.V., Unrine, J.M., Chatterjee, N., Ahn, J.M., Yang, X., Thornton, B.L., Ryde, I.T., Starnes, D., Meyer, J.N., 2014. A micro-sized model for the in vivo study of nanoparticle toxicity: what has Caenorhabditis elegans taught us?

Environ. Chem. 11, 227–246. https://doi.org/10.1071/EN13187.
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. https://doi.org/10.1016/j.marpolbul.2018.02.039.
Cole, M., Lindeque, P., Fileman, E., Halsband, C., Goodhead, R., Moger, J., Galloway, T.S., 2013. Microplastic ingestion by zooplankton. Environ. Sci. Technol. 47, 6646–6655. https://doi.org/10.1021/es400663f.
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? Sci. Total Environ. 645, 1029–1039. https://doi.org/10.1016/j.scitotenv.2018.07.207.
Deng, Y., Zhang, Y., Lemos, B., Ren, H., 2017. Tissue accumulation of microplastics in mice and biomarker responses suggest widespread health risks of exposure. Sci. Rep. 7, 1–10. https://doi.org/10.1038/srep46687.
Espinosa, C., García Beltrán, J.M., Esteban, M.A., Cuesta, A., 2018. In vitro effects of virgin microplastics on fish head-kidney leucocyte activities. Environ. Pollut. 235, 30–38. https://doi.org/10.1016/j.envpol.2017.12.054.
Farrell, P., Nelson, K., 2013. Trophic level transfer of microplastic: Mytilus edulis (L.) to Carcinus maenas (L.). Environ. Pollut. 177, 1–3. https://doi.org/10.1016/j.envpol.2013.01.046.
Ferreira, P., Elsa, F., Elisa, S, M., Carvalho, F., Guilhermino, L., 2016. Effects of multi-stressors on juveniles of the marine fishPomatoschistus microps: gold nanoparticles, microplastics andtemperature. Aquat. Toxicol. 170, 89–103. https://doi.org/10.1016/j.aquatox.2015.11.011.
Foley, C.J., Feiner, Z.S., Malinich, T.D., Höök, T.O., 2018. A meta-analysis of the effects of exposure to microplastics on fish and aquatic invertebrates. Sci. Total Environ. 631–632, 550–559. https://doi.org/10.1016/j.scitotenv.2018.03.046.
Fossi, M.C., Marsili, L., Baini, M., Giannetti, M., Coppola, D., Guerranti, C., Caliani, I., Minutoli, R., Lauriano, G., Finoia, M.G., Rubegni, F., Panigada, S., Bérubé, M., Urbán Ramírez, J., Panti, C., 2016. Fin whales and microplastics: the mediterranean sea and the sea of cortez scenarios. Environ. Pollut. 209, 68–78. https://doi.org/10.1016/j.envpol.2015.11.022.
Gallagher, A., Rees, A., Rowe, R., Stevens, J., Wright, P., 2016. Microplastics in the Solent estuarine complex, UK: an initial assessment. Mar. Pollut. Bull. 102, 243–249. https://doi.org/10.1016/j.marpolbul.2015.04.002.
Imhof, H.K., Rusek, J., Thiel, M., Wolinska, J., Laforsch, C., 2017. Do microplastic particles affect Daphnia magna at the morphological, life history and molecular level? PLoS One 12, 1–20. https://doi.org/10.1371/journal.pone.0187590.
Ivar Do Sul, J.A., Costa, M.F., 2014. The present and future of microplastic pollution in the marine environment. Environ. Pollut. 185, 352–364. https://doi.org/10.1016/j.envpol.2013.10.036.
Jambeck, J.R., Geyer, R., Wilcox, C., Siegler, T.R., Perryman, M., Andrady, A., Narayan, R., Law, K.L., 2015. The ocean. Am. Assoc. Adv. Sci. 347, 768–771. https://doi.org/https://doi.org/10.1126/science.1260352.
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., Lee, J.S., 2017. Adverse effects of microplastics and oxidative stress-induced MAPK/Nrf2 pathway-mediated defense mechanisms in the marine copepod Paracyclopina nana. Sci. Rep. 7, 1–11. https://doi.org/10.1038/srep41323.
Jeong, C.B., Won, E.J., Kang, H.M., Lee, M.C., Hwang, D.S., Hwang, U.K., Zhou, B., Souissi, S., Lee, S.J., Lee, J.S., 2016. Microplastic size-dependent toxicity, oxidative stress induction, and p-JNK and p-p38 activation in the monogonont rotifer (Brachionus koreanus). Environ. Sci. Technol. 50, 8849–8857. https://doi.org/10.1021/acs.est.6b01441.
Jeong, J., Choi, J., 2017. Use of adverse outcome pathways in chemical toxicity testing: potential advantages and limitations. Environ. Health Toxicol 33, e2018002. https://doi.org/10.5620/eht.e2018002.
Jin, Y., Xia, J., Pan, Z., Yang, J., Wang, W., Fu, Z., 2018. Polystyrene microplastics induce microbiota dysbiosis and inflammation in the gut of adult zebrafish. Environ. Pollut. 235, 322–329. https://doi.org/10.1016/j.envpol.2017.12.088.
Kang, D.S., Yang, J.H., Kim, H.S., Koo, B.K., Lee, C.M., Ahn, Y.-S., Jung, J.-H., Seo, Y.R., 2018. Application of the adverse outcome pathway framework to risk assessment for predicting carcinogenicity of chemicals. J. Cancer Prev. 23, 126–133. https://doi.org/10.15430/jcp.2018.23.3.126.
Lagarde, F., Olivier, O., Zanella, M., Daniel, P., Hiard, S., Caruso, A., 2016. Microplastic interactions with freshwater microalgae: hetero-aggregation and changes in plastic density appear strongly dependent on polymer type. Environ. Pollut. 215, 331–339. https://doi.org/10.1016/j.envpol.2016.05.006.
Lei, L., Liu, M., Song, Y., Lu, S., Hu, J., Cao, C., Xie, B., Shi, H., He, D., 2018a. Polystyrene (nano)microplastics cause size-dependent neurotoxicity, oxidative damage and other adverse effects in Caenorhabditis elegans. Environ. Sci. Nano 5. https://doi.org/10.1039/c8en00412a, 2009–2020.
Lei, L., Wu, S., Lu, S., Liu, M., Song, Y., Fu, Z., Shi, H., Raley-Susman, K.M., He, D., 2018b. Microplastic particles cause intestinal damage and other adverse effects in zebrafish Danio rerio and nematode Caenorhabditis elegans. Sci. Total Environ. 619–620, 1–8. https://doi.org/10.1016/j.scitotenv.2017.11.103.
Leist, M., Ghallab, A., Graepel, R., Marchan, R., Hassan, R., Bennekou, S.H., Limonciel, A., Vinken, M., Schildknecht, S., Waldmann, T., Danen, E., van Ravenzwaay, B., Kamp, H., Gardner, I., Godoy, P., Bois, F.Y., Braeuning, A., Reif, R., Oesch, F., Drasdo, D., Höhme, S., Schwarz, M., Hartung, T., Braunbeck, T., Beltman, J., Vrieling, H., Sanz, F., Forsby, A., Gadaleta, D., Fisher, C., Kelm, J., Fluri, D., Ecker, G., Zdrazil, B., Terron, A., Jennings, P., van der Burg, B., Dooley, S., Meijer, A.H., Willighagen, E., Martens, M., Evelo, C., Mombelli, E., Taboureau, O., Mantovani, A., Hardy, B., Koch, B., Escher, S., van Thriel, C., Cadenas, C., Kroese, D., van de Water, B., Hengstler, J.G., 2017. Adverse outcome pathways: opportunities, limitations and open questions. Arch. Toxicol. 91, 3477–3505. https://doi.org/10.1007/s00204-017-2045-3.
Li, W.C., Tse, H.F., Fok, L., 2016. Plastic waste in the marine environment: a review of sources, occurrence and effects. Sci. Total Environ. 566–567, 333–349. https://doi.org/10.1016/j.scitotenv.2016.05.084.
Lu, L., Wan, Z., Luo, T., Fu, Z., Jin, Y., 2018. Polystyrene microplastics induce gut microbiota dysbiosis and hepatic lipid metabolism disorder in mice. Sci. Total Environ. 631–632, 449–458. https://doi.org/10.1016/j.scitotenv.2018.03.051.
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. Environ. Sci. Technol. 50, 4054–4060. https://doi.org/10.1021/acs.est.6b00183.
Lusher, A.L., Hernandez-Milian, G., O'Brien, J., Berrow, S., O'Connor, I., Officer, R., 2015a. Microplastic and macroplastic ingestion by a deep diving, oceanic cetacean: the True's beaked whale Mesoplodon mirus. Environ. Pollut. 199, 185–191. https://doi.org/10.1016/j.envpol.2015.01.023.
Lusher, A.L., Tirelli, V., O'Connor, I., Officer, R., 2015b. Microplastics in Arctic polar waters: the first reported values of particles in surface and sub-surface samples. Sci. Rep. 5, 1–9. https://doi.org/10.1038/srep14947.
Martens, M., Verbruggen, T., Nymark, P., Grafström, R., Burgoon, L.D., Aladjov, H., Torres Andón, F., Evelo, C.T., Willighagen, E.L., 2018. Introducing WikiPathways as a data-source to support adverse outcome pathways for regulatory risk assessment of chemicals and nanomaterials. Front. Genet. 9, 1–9. https://doi.org/10.3389/fgene.2018.00661.
Nelms, S.E., Galloway, T.S., Godley, B.J., Jarvis, D.S., Lindeque, P.K., 2018. Investigating microplastic trophic transfer in marine top predators. Environ. Pollut. 238, 999–1007. https://doi.org/10.1016/j.envpol.2018.02.016.
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). Ecol. Indicat. 34, 641–647. https://doi.org/10.1016/j.ecolind.2013.06.019.
Paul-Pont, I., Lacroix, C., González Fernández, C., Hégaret, H., Lambert, C., Le Goïc, N., Frère, L., Cassone, A.L., Sussarellu, R., Fabioux, C., Guyomarch, J., Albentosa, M., Huvet, A., Soudant, P., 2016. Exposure of marine mussels Mytilus spp. to polystyrene microplastics: toxicity and influence on fluoranthene bioaccumulation. Environ. Pollut. 216, 724–737. https://doi.org/10.1016/j.envpol.2016.06.039.
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. https://doi.org/10.1371/journal.pone.0100289.
Rochman, C.M., Tahir, A., Williams, S.L., Baxa, D.V., Lam, R., Miller, J.T., Teh, F.C., Werorilangi, S., Teh, S.J., 2015. Anthropogenic debris in seafood: plastic debris and fibers from textiles in fish and bivalves sold for human consumption. Sci. Rep. 5, 1–10. https://doi.org/10.1038/srep14340.
Schirinzi, G.F., Pérez-Pomeda, I., Sanchís, J., Rossini, C., Farré, M., Barceló, D., 2017. Cytotoxic effects of commonly used nanomaterials and microplastics on cerebral and epithelial human cells. Environ. Res. 159, 579–587. https://doi.org/10.1016/j.envres.2017.08.043.
Sutton, R., Mason, S.A., Stanek, S.K., Willis-Norton, E., Wren, I.F., Box, C., 2016. Microplastic contamination in the san francisco bay, California, USA. Mar. Pollut. Bull. 109, 230–235. https://doi.org/10.1016/j.marpolbul.2016.05.077.
Teuten, E.L., Rowland, S.J., Galloway, T.S., Thompson, R.C., 2007. Potential for plastics to transport hydrophobic contaminants. Environ. Sci. Technol. 41, 7759–7764. https://doi.org/10.1021/es071737s.
Veneman, W.J., Spaink, H.P., Brun, N.R., Bosker, T., Vijver, M.G., 2017. Pathway analysis of systemic transcriptome responses to injected polystyrene particles in zebrafish larvae. Aquat. Toxicol. 190, 112–120. https://doi.org/10.1016/j.aquatox.2017.06.014.
Villeneuve, D.L., Crump, D., Garcia-Reyero, N., Hecker, M., Hutchinson, T.H., LaLone, C.A., Landesmann, B., Lettieri, T., Munn, S., Nepelska, M., Ottinger, M.A., Vergauwen, L., Whelan, M., 2014a. Adverse outcome pathway (AOP) development I: strategies and principles. Toxicol. Sci. 142, 312–320. https://doi.org/10.1093/toxsci/kfu199.
Villeneuve, D.L., Crump, D., Garcia-Reyero, N., Hecker, M., Hutchinson, T.H., LaLone, C.A., Landesmann, B., Lettieri, T., Munn, S., Nepelska, M., Ottinger, M.A., Vergauwen, L., Whelan, M., 2014b. Adverse outcome pathway development II: best practices. Toxicol. Sci. 142, 321–330. https://doi.org/10.1093/toxsci/kfu200.
Weber, A., Scherer, C., Brennholt, N., Reifferscheid, G., Wagner, M., 2018. PET microplastics do not negatively affect the survival, development, metabolism and feeding activity of the freshwater invertebrate Gammarus pulex. Environ. Pollut. 234, 181–189. https://doi.org/10.1016/j.envpol.2017.11.014.
Yu, P., Liu, Z., Wu, D., Chen, M., Lv, W., Zhao, Y., 2018. Accumulation of polystyrene microplastics in juvenile Eriocheir sinensis and oxidative stress effects in the liver. Aquat. Toxicol. 200, 28–36. https://doi.org/10.1016/j.aquatox.2018.04.015.
Zarfl, C., Matthies, M., 2010. Are marine plastic particles transport vectors for organic pollutants to the Arctic? Mar. Pollut. Bull. 60, 1810–1814. https://doi.org/10.1016/j.marpolbul.2010.05.026.
Ziajahromi, S., Kumar, A., Neale, P.A., Leusch, F.D.L., 2018. Environmentally relevant concentrations of polyethylene microplastics negatively impact the survival, growth and emergence of sediment-dwelling invertebrates. Environ. Pollut. 236, 425–431. https://doi.org/10.1016/j.envpol.2018.01.094.