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Review

Aggregate exposure pathways for microplastics (mpAEP): An evidence-based framework to identify research and regulatory needs

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ABSTRACT

Microplastics as emerging contaminants have been detected from peaks to poles. High concerns on the risks of microplastic pollution to humans and ecosystems have therefore been raised in the past decade. While a large number of studies have been conducted to investigate the environmental levels and toxicity of microplastics, the information generated to support risk assessment is fragmented and the coherence between different types of study is largely lacking. Here we introduced the Aggregate Exposure Pathway (AEP), a conceptual framework originally proposed for chemical exposure assessment, to facilitate organization, visualization and evaluation of existing information generated from microplastic research, and to efficiently identify future knowledge and regulatory needs. A putative microplastic AEP network (mpAEP) was developed to demonstrate the concept and model development strategies. Two mpAEP case studies, with polyethylene (PE) as a prototype, were then presented based on existing environmental exposure data collected from the Changjiang Estuary and the East China Sea (Case I), and the Oslo Fjord (Case II), respectively. Weight of evidence (WoE) assessment of the mpAEPs were performed for evaluating the essentiality, theoretical plausibility, empirical evidence and quantitative understanding of the evidence and relationships in the AEPs. Both cases showed moderate/high WoE to support the strength of the models, whereas also displayed clear knowledge gaps, thus providing guidance for future investigations and regulations. The mpAEP framework introduced herein presents a novel strategy for organizing fragmented information from diverse types of microplastic research, enhancing mechanistic understanding of causal relationships and facilitating the development of quantitative prediction models for research and regulation in the future.

1. Introduction

Plastics mark the start of a new geological epoch, the Plastic Age, by its deposition in fossil records (Brandon et al., 2019). An estimated 710 million metric tons of plastic debris have ended up in the terrestrial and aquatic environments by 2016 (Lau et al., 2020). Once entering the environment, plastics can be fragmented into tiny pieces by physical, chemical and biological processes (Thompson et al., 2004; van Sebille et al., 2020; Zettler et al., 2013). The small plastic fragments and fibers less than 5 mm in size are referred to as microplastics. They are considered contaminants of emerging concern due to their ubiquitous distribution and persistency in the environment. Accumulating evidence in recent years has shown considerable levels of microplastics in various environmental compartments, including water, soil and atmosphere (Hale et al., 2020; Hurley et al., 2018; Lusher, 2015; Rillig and Lehmann, 2020).

Although a large number of studies have been conducted in the past decade to investigate the environmental fate and risks of microplastics, the knowledge generated was fragmented and several remaining issues have not been fully resolved. First, information related to environmental levels of microplastics is fragmented. The reported sizes of microplastics in the environment range from micro- and nanometre to 5 mm, spanning more than 6 orders of magnitude. There is normally no size specification associated with the reported abundance of microplastics, making the data incomparable across different studies. Second, there are no clear causal linkages of size and abundance data reported for sources,

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environmental media and biota. Integrated study on point and diffuse sources where microplastics are released into the environment is lacking. Microplastics with various shapes, density and polymer types behave differently in the environment, and its pathway into biota are different from that into various environmental media (Garcia et al., 2021). Third, there are large discrepancies between the measured exposure levels of microplastics in biota and the levels of microplastics used for (eco)toxicity testing, (Gouin et al., 2019; Phuong et al., 2016), making the hazard assessment of microplastics unreliable.

These issues call for more holistic and mechanism-based approaches for organizing disjointed streams of knowledge into a coherent information framework, with critical knowledge gaps clearly visualized. Such new approaches are key for next generation (NexGen) risk assessment of microplastic pollution. As one of the new approaches to integrate, organize, visualize and evaluate existing exposure information, the Aggregate Exposure Pathway (AEP) framework has been gradually gaining momentum in human and ecological risk assessment (Teeguarden et al., 2016). An AEP links a cascade of causally related key exposure states (KESs), such as source, exposure medium, external exposure, internal exposure and target site exposure (TSE) of a chemical by their key transitional relationships (KTRs) into a pathway or pathway network (Fig. 1A), thereby providing mechanistic information on how a chemical reaches an organism and mediates adverse effects of regulatory concern. A well-developed AEP can integrate exposure information from multiple types of studies and guide laboratory (eco)toxicity assessment. The AEP framework was proposed to mirror Adverse Outcome Pathway (AOP), a conceptual framework to organize (eco)toxicological information relevant for hazard and risk assessment (Ankley et al., 2010). Compared to the AOP framework, the AEP framework is still in its infancy and has only been demonstrated by a few cases (Clewell et al., 2020; Hines et al., 2019, 2018; Tan et al., 2018; Teeguarden et al., 2016). Nevertheless, it has great potential to improve the current microplastic research by offering a new way of organizing exposure data and identifying coherence between information generated from different studies. The present study aims to: 1) Critically review the existing knowledge and develop an AEP framework for microplastics (mpAEPs) to improve mechanistic and quantitative understanding of microplastic exposure; 2) Assess weight of evidence of the AEPs to identify critical knowledge gaps; 3) Demonstrate the usefulness of the mpAEPs by case studies; 4) Propose potential applications of the mpAEP framework in microplastic research and regulation.

2. mpAEP assembly and assessment

A conceptual mpAEP network was assembled first to provide a general overview on the knowledge status and to illustrate the AEP development strategies (Fig. 1B). This putative AEP network links point and diffuse sources to immune cells as the TSE, via 8 KESs that have been frequently reported as key exposure states of microplastics in the environment and biota. The selection and assessment of major nodes in this AEP network will be discussed in detail in the following sections.

2.1. *Stressor*

This putative mpAEP network is considered to cover all types of plastic particles at the micrometer scale (1 μm – 5 mm). Engineered nanoparticles (<100 nm, ENPs) or large plastic particles (>5 mm) are not applicable to this mpAEP network due to their relatively lower environmental relevance (Hüffer et al., 2017; Xu et al., 2020). In contrast, synthetic and cellulosic microplastic fibers are included due to their prevalence in the composition of microplastics (Athey et al., 2020). As transport and fate of microplastics may differ by polymer type, size, and shape, additional information on key characteristics (size, shape), sampling strategies and locations from published studies were also considered important information to support this AEP network.

2.2. *Key exposure states*

A key exposure state (KES) is an essential element of an AEP. A KES refers to the level of a stressor at a critical state relevant for exposure (Teeguarden et al., 2016; Tan et al., 2018), such as source, exposure medium, external exposure, internal exposure and target site exposure.

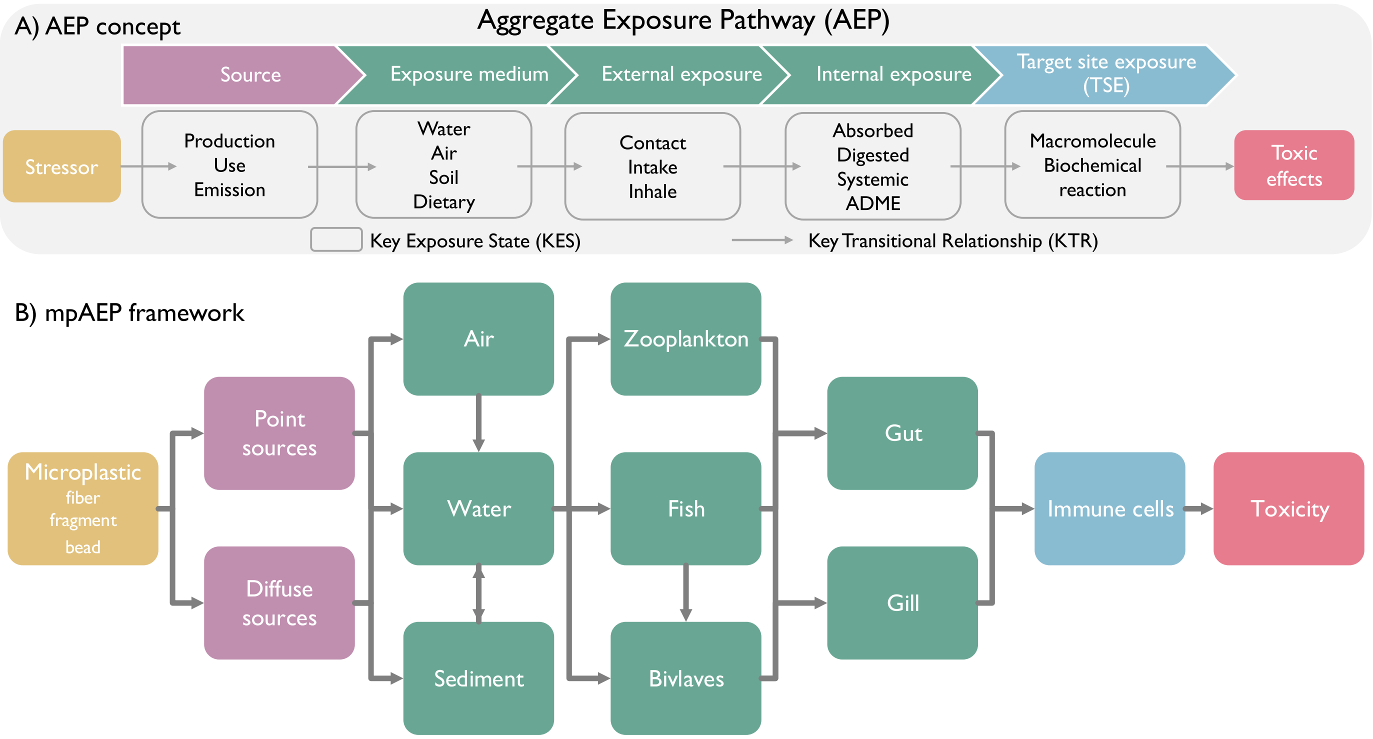

Fig. 1. (A) The Aggregate Exposure Pathway (AEP) framework and (B) a conceptual AEP network for microplastics (mpAEP).

2.2.1. Source

Source refers to the levels and rates of release of a pollutant at the site of its origin (Tan et al., 2018). The starting points could be the initial creation of the substance, or it could be based on an arbitrary point that is convenient for measurement and control. Both point and diffuse sources are considered major sources of microplastics in this AEP network. Although the sources of microplastics are not as clearly defined as other chemical pollutants, an increasing number of studies have started to investigate the sources of microplastics such as atmospheric transport, wastewater treatment plants (WWTP), dust and food (Habib et al., 2020; Zhang et al., 2020b, 2020c).

2.2.2. Exposure medium

Exposure medium refers to the level of a pollutant in a key environmental compartment that is directly associated with the exposure of biota, such as water, air or soil (Clewell et al., 2020). In the proposed AEP network, air, water and soil are considered the main exposure media. While most reports on microplastics levels focus on the marine environment, some studies showed significantly higher levels of microplastics in the freshwater environment, especially in the vicinity of populated urban areas (Wang et al., 2017). Moreover, atmospheric and terrestrial transport of microplastics has recently been recognized (Allen et al., 2019; Rillig and Lehmann, 2020). For microplastic abundance, the reported units can be n L^{-1}, n m^{-3} and n km^{-2} for water samples and n kg^{-1} wet weight or n kg^{-1} dry weight for sediment samples due to different sampling strategies.

2.2.3. External exposure

External exposure refers to the level of a pollutant adsorbed, inhaled or ingested by an organism (e.g., dermal exposure). In the putative AEP network, the amount of microplastics (particles individual^{-1} or g^{-1} wet weight) in digestive tracts and gills are therefore considered as external exposure levels based on the reported data.

2.2.4. Internal exposure

Internal exposure refers to the body burden (internal level) of a pollutant absorbed in an organism. Key processes such as adsorption, distribution, metabolism and elimination (ADME), or toxicokinetics of pollutants are considered driving factors determining the internal exposure level. Compared to organic chemicals, large particles such as microplastics are considered to have much simpler toxicokinetics in organisms. The frequently reported internal exposure level of a pollutant is normally the measured concentration in tissue, organ or body fluid (e.g., blood or haemolymph). Microplastics are normally not considered readily absorbed by organisms due to large sizes and difficulties in penetrating biological membranes.

2.2.5. Target site exposure

Target site exposure (TSE) refers to the level of a pollutant at its biological site of toxic action, such as a specific type of cell (e.g., immune cell) or macromolecules (e.g., DNA, protein, lipid). TSE is tightly coupled with the molecular initiating event (MIE) of an AOP, which refers to the initial biochemical interaction that leads to an adverse outcome. The suspected target sites of microplastics include lysosome cells, enterocyte, hepatopancreas and phagocyte (Brennecke et al., 2015; Frydkjær et al., 2017; Gambino et al., 2020; Jeong et al., 2016; Park et al., 2020; Sucharitakul et al., 2020; von Moos et al., 2012; Xu et al., 2020). The immune system in aquatic organisms is regarded as a sensitive target for effects induced by particulate pollutant at nano-scales (Barmo et al., 2013). Therefore, immune cells are determined as TSE in the conceptual mpAEP.

2.3. Key transitional relationships

A key transitional relationship (KTR) describes the relationship between two adjacent KESs. AEPs have two types of KTRs, which are movement and conversion of stressors between KESs (Tan et al., 2018). In the conceptual mpAEP network (Fig. 1B), the KTRs are proposed based on the theoretical plausibility suggested by relevant literature. For particulate microplastics, movement KTRs are dominant, albeit conversion KTRs are possible due to abrasion or loss of microplastics by agglomeration and sedimentation. However, the selection of sources for mpAEPs indicates that conversion KTRs may be less important (e.g., WWTPs in the case-studies). Microplastics are known to be discharged into the air, water and sediments from both point and diffuse sources. Water is an import medium for microplastic precipitation from the air, deposition into the sediment and exposure of aquatic organisms such as zooplankton, fish and bivalves. The frequently documented target organs of exposure are gill and gut. Very few studies have reported the exact molecular/cellular targets of microplastics. There is however limited evidence showing that the immune cells might be the initial target of exposure in these organisms (Park et al., 2020).

2.4. Weight of evidence assessment

Weight of evidence (WoE) indicates the strength of scientific evidence supporting an AEP. As there are currently no WoE assessment criteria developed for the AEP framework (Clewell et al., 2020), we have adapted a set of criteria (Table 1) based on OECD's guidance on AOP development and assessment (OECD, 2018). The criteria include: (1) essentiality of the KES, (2) theoretical plausibility of the KTR, (3) empirical support for the KTR, and (4) quantitative understanding of the KTR. The confidence level is scored as High, Moderate or Low according to the quality of evidence support. In a formal AEP document (similar as AOPs), the AEP developers are expected to not only provide final WoE scores, but also provide detailed justifications underlying the scores and point out critical data gap and knowledge needs. It consists of data gap where knowledge is well defined but empirical data hasn't been reported in literature, and a data gap where there it is not clear if there is a transition, a model description or data indicating two KESs are connected.

3. Demonstrative cases

We will then use two practical case-studies to demonstrate the development and assessment of AEPs for microplastics. Two distinct types of aquatic environments in China (Case I: Changjiang/Yangtze Estuary-East China Sea) and Norway (Case II: Oslo Fjord) were selected because considerable amount of data in the study areas was available to support the development and assessment of the AEPs. Both cases focus on polyethylene (PE) microplastics as a prototype, as PE is the most produced polymer in the world (Plastics Europe, 2020) and ubiquitously identified in the aquatic environment. The PE polymer has a density range of 0.91–0.94 g cm^{-3} (low density polyethylene, LDPE) or 0.93–0.97 g cm^{-3} (high density polyethylene, HDPE). In the two case-studies, only investigations that successfully identified PE using chemical techniques (Fourier-transform infrared spectrometry, Raman spectroscopy or Mass spectrometry) were considered valid. In addition, the reported microplastic concentrations displayed for the two cases were the total concentrations of all types of polymers in different environmental compartments.

3.1. Case I: discharge of microplastics from wastewater treatment plants leading to fish exposure in China

3.1.1. Overview of the study region

The Changjiang River is the largest river in China with a basin area of 1.8×10^6 km^2 (Gao and Wang, 2008). Located in a monsoon climate, the freshwater discharge to the East China Sea forms a strong Changjiang River plume with sediment concentration of 0.005 kg m^{-3} that reaches 200 km from the estuary (Wu et al., 2011). Being the largest estuary in China, the Changjiang Estuary (CE) has active material exchange and

Table 1
Proposed criteria for weight of evidence (WoE) assessment of Aggregate Exposure Pathway (AEP) adapted from OECD Guidance Document on Developing and Assessing Adverse Outcome Pathways (OECD, 2018).

Category	Criterion	Confidence level		
		High	**Moderate**	**Low**
Essentiality of the KES	$KES_{downstream}$ will be reduced/ will not take place if $KES_{upstream}$ is reduced/stopped.	Multiple lines of direct evidence, with no inconsistencies or contradictions.	Some direct evidence or multiple lines of indirect evidence, or limited number of inconsistencies or contradictions.	No direct evidence or considerable inconsistencies or contradictions.
Theoretical plausibility of the KTR	Theoretical knowledge supporting dependent and sequential change of two adjacent KESs.	Widely accepted and in-depth mechanistic understanding supporting the causal relationship between $KES_{upstream}$ and $KES_{downstream}$.	Partial mechanistic understanding with known knowledge gaps.	No or limited theoretical understanding.
Empirical support for the KTR	Empirical data supporting dependent and sequential change of two adjacent KESs, with temporal, spatial and incidence concordance.	Multiple lines of evidence with high temporal, spatial and incidence concordance, no or few data gaps or conflicting data.	Some direct evidence or multiple lines of indirect evidence, with some temporal, spatial and incidence concordance, and a limited number of inconsistencies or contradictions.	No or very limited evidence.
Quantitative understanding of the KTR	Quantitative model describing dependent and sequential change of two adjacent KESs.	Precise prediction of $KES_{downstream}$ from $KES_{upstream}$ with a low uncertainty. Key modulating factors and feedback/ feedforward are fully captured in the model. The model is generalized across the applicability domains of the AEP.	Precise prediction of $KES_{downstream}$ from $KES_{upstream}$ with a high uncertainty. Key modulating factors and feedback/ feedforward are not fully captured in the model. The model is only valid for a limited number of cases in the applicability domains of the AEP.	Only qualitative or semi-quantitative understanding. Known modulating factors and/or known feedback/feedforward mechanisms are not captured.

huge material fluxes, which forms the largest fishing ground in China, the Zhoushan Fishing Ground (Gao and Wang, 2008). Shanghai, the economic center and the world's largest port, is situated in the Changjiang Estuary with a population over 24 million, posing pressure to the coastal environments. We have therefore developed an AEP for assessing microplastic exposure in this region, with special focus on how PE microplastics discharged from urban wastewater can lead to exposure of marine fish (Fig. 2).

3.1.2. *General description of the AEP*

Twenty peer-reviewed publications were selected to develop a location-specific AEP for PE microplastic in the Changjiang Estuary and the East China Sea (SI Table S1). The literature was selected by searching keywords including "microplastic", "Changjiang Estuary/Yangtze River Estuary" and "East China Sea" using Google Scholar (scholar.google.com) for publications in English, and Baidu Scholar (xueshu.baidu.com) for publications in Chinese. Only environmental data on the aqueous phases was included, as the target exposure organ in this case-study is

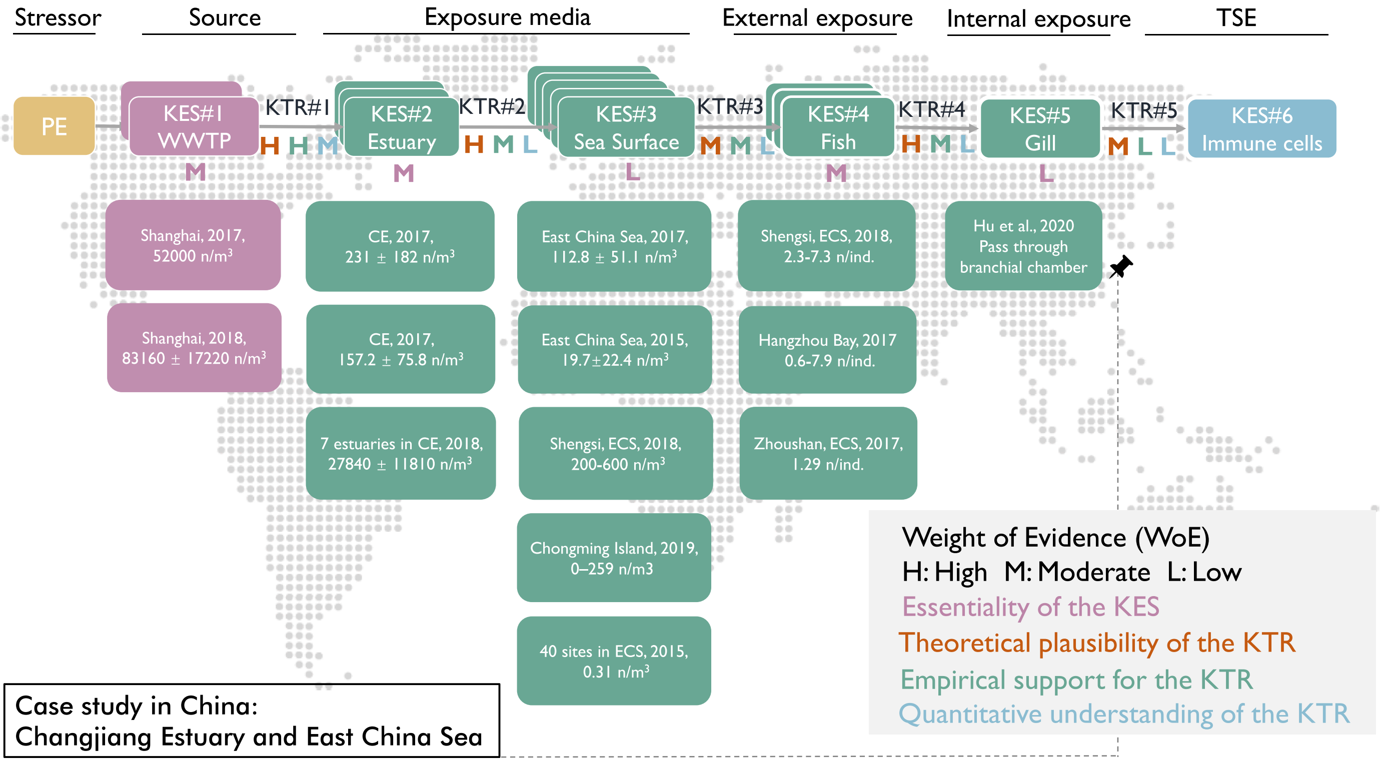

Fig. 2. The Aggregate Exposure Pathway for microplastics (mpAEP) linking discharge of polyethylene (PE) from a wastewater treatment plant (WWTP) to target exposure site in immune cells in the Changjiang Estuary and the East China Sea. Rectangles of stack boxes represent Key Event States (KESs) and arrows represent Key Transitional Relationships (KTRs). Coloured letters represent weight of evidence (WoE) assessment results for the essentiality of Key Exposure States (KESs) and theoretical plausibility, empirical support and quantitative understanding of Key Transitional Relationships (KTRs). Confidence levels: H - High, M - Moderate, L - Low.

gills of fish. Quantification methods for KES#1–4 require spectroscopic methods with detection limit down to 20 μm, i.e., micro Fourier transform infrared spectroscopy or micro Raman spectroscopy.

An AEP with six KESs was assembled to describe the movement pathways of PE in the Changjiang Estuary-East China Sea region (Fig. 2). The source (KES#1) was considered to be the WWTP on the east coast of Shanghai where PE microplastic was discharged into the South Passage of the Changjiang Estuary (Bai et al., 2018; Jia et al., 2019). Surface water in the estuary (KES#2: Exposure Medium 1) was considered to be the initial exposure medium (KES#2), as supported by multiple studies reporting the microplastic abundance in the Changjiang Estuary in 2017 and 2018 (Xu et al., 2018; Zhang et al., 2019; Zhao et al., 2019). The surface water in the East China Sea was considered as the secondary exposure medium (KES#3), as evidenced by five studies reporting measured PE concentrations (Li et al., 2020; Sun et al., 2018; Zhang et al., 2020; Zhao et al., 2019). The external exposure level (KES#4) was estimated based on the whole-body concentrations measured in wild fish, whereas the internal exposure (KES#5) was supported by the measured levels of PE in fish gills and microplastic fibers can pass through branchial chamber of fish gills (Su et al., 2019; Zhang et al., 2020; Zhang et al., 2019; Hu et al., 2020). In this case, immune cells in fish were considered the target site of exposure (KES#6), because quantification method using imaging flow cytometry proved phagocytosis of microplastics by fish immune cells (Park et al., 2020). It should be noted that there are also several other biological targets of PE, e.g., liver, muscle (Barboza et al., 2020; Collard et al., 2017). However, we will only focus on the immune cells as the TSE for this AEP as relatively more evidence support could be obtained.

3.1.3. *Essentiality of the KES*

Essentiality evaluates whether the occurrence of a downstream event is dependent on an upstream event. The essentiality of **KES#1 (source-WWTP**) is considered **Moderate,** with some supporting evidence showing massive and direct discharge of microplastic from the WWTPs in Shanghai to the Changjiang Estuary, resulting in high concentrations of microplastics in the Changjiang Estuary (Peng et al., 2017; Sun et al., 2019). The essentiality of **KES#2 (external exposure medium--estuary**) is considered **Moderate**, as the estuary is the main geophysical and hydrological feature in the study region. Studies on seasonal variations of microplastic concentration in the Changjiang Estuary and the East China Sea (CE and ECS) also showed consistent relationship of microplastic levels between CE and ECS (Zhao et al., 2019). Reduced discharge from CE also leads to a decrease in microplastic concentration in ECS. The essentiality of **KES#3 (external exposure medium-sea surface water**) is considered **Low** due to a lack of evidence to support that a change in microplastic concentration in surface water leads to a change in microplastic concentration in fish. **KES#4 (external exposure-fish**) is considered to have a **Moderate** essentiality, as the importance of fishery in the ECS has led to abundant investigation on food chain composition, and higher level fish species are found to be sentinels for indicating microplastic pollution (Zhang et al., 2019). These facts collectively support that fish is a critical node in the AEP of PE microplastic. **KES#5 (internal exposure-gill**) *per se* was supported by the measured concentrations of microplastics, suggesting bioaccumulation of PE in these organs (Su et al., 2019), albeit quantification methods for small microplastics (<50 μm) have not been widely applied to field studies. However, there is no study reporting the correlation between KES#5 and KES#6 (target site exposure-immune cells). Therefore, the essentiality of KES#5 is considered **Low**.

3.1.4. *Theoretical plausibility of the KTR*

Physicochemical processes account for most factors affecting the behavior of microplastics in the aquatic environments (Table 1). The theoretical plausibility of **KTR#1** (WWTP to estuary) is considered **High** (Fig. 2), as an in-depth literature review has shown consistent relationships between WWTPs removal of microplastics and adjacent waters (Iyare et al., 2020; Kay et al., 2018; Woodward et al., 2021). The theoretical plausibility of **KTR#2** (CE to ECS) is considered **High**, with several evidence showing riverine plastic discharge influenced by the development index of coastal countries, including the outflow from the CE to the ocean (Mai et al., 2020). Physicochemical plausibility of **KTR#3** (sea surface to fish) is considered **Moderate**, as this causal relationship has only been partially established for microplastic particles. The theoretical plausibility of **KTR#4** (fish to gill) is considered **High**, as such causality has been widely recognized, with a well-understood mechanism for gill epithelium functioning as the forefront for external pollutants (Evans, 1987). The theoretical plausibility of **KTR#5** is considered **Moderate**, as immune cells have been considered the primary targets of xenobiotics in fish gills (Alzaidan, 2013; Hayton and Barron, 1990; Tort et al., 2003), albeit not much work has been done to elucidate how exactly microplastics affect immune cells.

3.1.5. *Empirical support for the KTR*

Empirical support evaluates the evidence-related relationships of the two events, including time, space and quantity (concentration) etc. The empirical support for **KTR#1** is considered **High** (Fig. 2), with multiple publications consistently reporting the causal relationships between the PE levels at the WWTPs and in the downstream estuary (Bai et al., 2018; Jia et al., 2019; Sun et al., 2019). **KTR#2** also has a **Moderate** empirical support, with strong evidence from field investigations indicating that the concentration of microplastics in the CE and the ECS causally fluctuated across seasons (Xu et al., 2018; Zhao et al., 2019). The empirical support for **KTR#3** is considered **Moderate** with a study reporting more microplastic accumulation in higher trophic level fish species that may function as sentinel species for microplastic pollution (Zhang et al., 2019a). The empirical support for **KTR#4** is also **Moderate** with only one study reporting the distribution of microplastics in fish organs, with gills and gut being the ones with the highest PE levels (Su et al., 2019). Currently there is no empirical evidence directly supporting the relationships of PE levels between gills to immune cells). Therefore, the empirical support for KTR#5 is considered **Low**.

3.1.6. *Quantitative understanding*

There is currently no literature providing quantitative understanding of the KTRs in this AEP. Therefore, we have attempted to de novo build the quantitative relationships based on available data from various sources, in order to demonstrate how basic quantitative understanding can be established based on limited empirical data. Log-normal distribution was assumed for all microplastic abundance data as suggested by Beiras and Schönemann (2020) and Monte Carlo simulation was conducted for 100,000 runs for uncertainty analysis. Results showed that given a hypothetical level of 50,000 ± 20,000 n m^{-3} microplastics at the source (WWTP), the resulting cumulative exposure to fish in CE was estimated to be 0.49±1.85 (range: 0.07 – 3.50 with 95% confidence interval) n m^{-3}. The most uncertainty comes from the processes of uptake from water to gill and gut (Fig. 3), pointing out a need for more investigations on this part of the AEP. It should also be noted that due to a lack of valid data and sufficient knowledge of the underlying mechanisms, the quantitative understanding of the AEP described herein is preliminary, with a high level of uncertainty. In summary, the quantitative understanding of **KTR#1** is considered **Moderate**, whereas that of the **KTR#2–5** in this AEP are considered **Low**.

3.1.7. *Critical knowledge gaps and uncertainties*

The WoE assessment of the AEP pointed out critical knowledge gaps that may hamper mechanistic and quantitative understanding of the microplastic exposure pathways, even in an area where a high number of investigations have been conducted. There have been few studies reporting trophic levels of the studied organism and microplastic transfer in the natural environments (Goswami et al., 2020), and mpAEP doesn't follow the same principle as biomagnification of chemical pollutants. The parameterization of mpAEP should therefore be based on

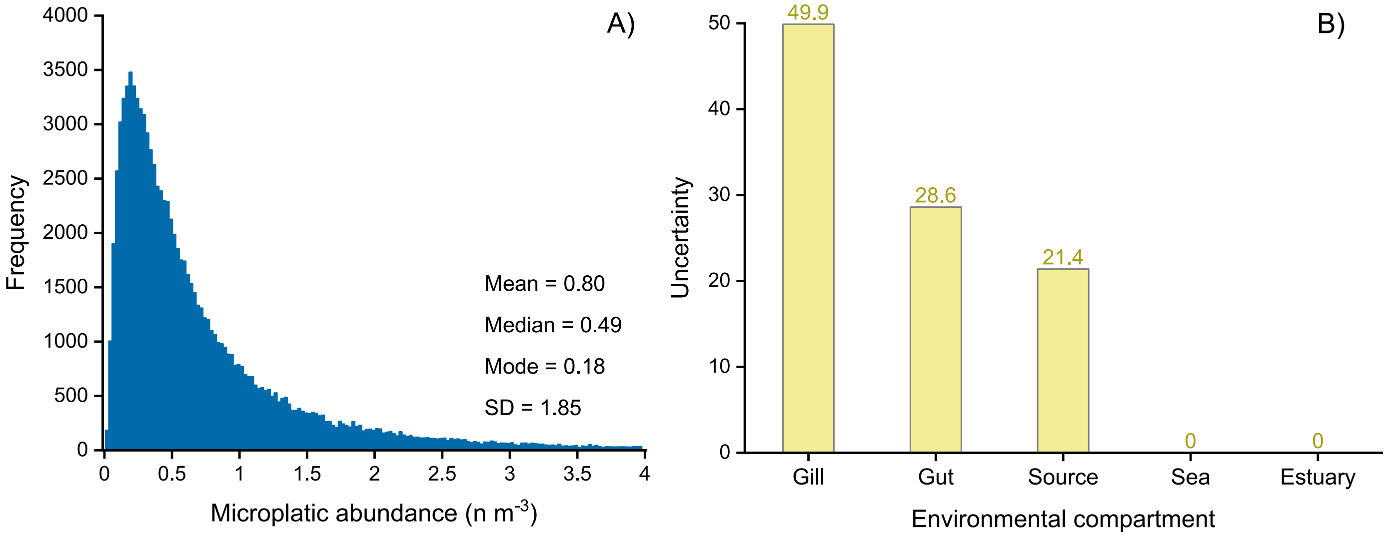

Fig. 3. Prediction results (A) and uncertainty analysis (B) for Case I.

the feeding strategy for particles rather than trophic levels (Birnstiel et al., 2019). As shown by Case I, the microplastic (PE) abundance in the sea surface has the most evidence support, whereas an apparent lack of data to support the external and internal exposure of microplastics, possibly due to immature quantification methods with biological samples (Stanton et al., 2019). The strength of the causal relationships in general decreases when walking down the AEP from the fate of microplastics in the environment to biota, suggesting strong needs for measuring both environmental and biological levels of microplastics in the same investigation (i.e., same spatial and temporal scales, and same analytical conditions). The current data collected from the published reports could not provide sufficient data to construct a reliable quantitative AEP (qAEP) model, as the empirical relationships in the AEP were not able to be parameterized in an appropriate manner, albeit the model construction approach presented herein can still be applied in the future when more data accumulates. Note that the exposure pathway (external and internal) are intentionally simplified, and more efforts on the external exposure pathways through water filtration (targeting gill) and food intake through food web (targeting gut) should be made. In summary, Case I has displayed a good example to illustrate how an mpAEP could efficiently organize existing data and identify critical knowledge gaps in the microplastic research on the Changjiang Estuary and East China Sea regions. A trial quantitative assessment has also revealed uncertainties of the empirical evidence supporting this AEP and highlighted research needs for better assessment of microplastics in biota and simultaneous measurement of at least two key exposure states in the same study.

3.2. *Case II: Discharge of microplastics from wastewater treatment plants leading to mussel gill exposure in Norway*

3.2.1. *Overview of the study region*

The Norwegian coastlines are characterized by deep-silled fjords, especially in the western Norway, where low-salinity Norwegian Coastal Current overlaps heavier water with Atlantic origin and flows northward under the influence of coastal wind (Erga and Heimdal, 1984), contributing to microplastic pollution in the Arctic (Ross et al., 2021). Oslo, the capital of Norway with a population of ca. 0.7 million, is located in the inner Oslo Fjord. The Aker river (Akerselva in Norwegian) with a drainage area of 250 km^2 runs through the city of Oslo and reaches inner Oslo Fjord. The study area is under eutrophication since the population growth in the 1900s with industrial development, and the establishment of WWTPs and improvement of sewage treatment (e.g., Bekkelaget in 1963 located to the southeast of Oslo) significantly affects cultural eutrophication in the Oslo Fjord (Dale et al., 1999). Although both Case I and Case II focus on microplastic pollution from urban wastewater to coastal environments, there are fundamental differences between the two cases in terms of hydrographic features (Case I – estuary versus Case II – fjord).

3.2.2. *General description of the AEP*

For literature review, we searched Google Scholar using the keywords “microplastic” “Norway”. A total of 21 published literature including peer-reviewed research articles, master theses and technical reports were used for Case II (SI Table S2), of which six publications directly reported the levels of PE in different environmental compartments. Investigation on microplastic pollution in Norway mainly focused on the Oslo Fjord and its vicinity, but most data failed to report spatiotemporal variation of microplastic concentrations in a specific area but random sampling efforts at some spots. A lack of causality of empirical data made it unlikely to construct an mpAEP in the region even though considerable efforts have been dedicated to microplastic research. Sundt (2014) summarized sources of microplastics (PE being the dominant type) in Norwegian waters, including consumer products, production spill, abrasion (ship paint, laundry), wear and tear (dust, road paint, tire dust) and waste shredding (recycling, landfill) that enters the environment through runoff, drain, air, sludge, soil, sea and water. Two sources were identified considering the geographic location. Bekkelaget is a WWTP (KES#7) that discharges wastewater into the inner Oslo Fjord, but no data on its microplastic removal efficiency or discharge rate has been collected (Fig. 4). A report investigated only microplastics in sludge from Bekkelaget and a master thesis investigated microplastic concentration in WWTPs in Telemark County, but no chemical analysis was performed to identify PE (Lusher et al., 2017; Lage, 2019). Another source is the Aker river (KES#8) that flows into the inner Oslo Fjord without water exchange with the WWTP. A project funded by the Norwegian Environment Agency investigated three rivers in Oslo in 2019, with PE detected in two rivers (Lorenz et al., 2020). Olsen et al. (2020) investigated microplastic in surface water (KES#9) in the outflow of the Aker river and near WWTP sewage. Two studies investigated microplastic exposure in blue mussels (KES#10) (Bråte et al., 2018). No study investigated internal exposure (KES#11) and target site (e.g., immune cells KES#12) of microplastic accumulation in mussels from this region (Avio et al., 2015).

3.2.3. *Essentiality of the KES*

No studies have investigated the relationships between upstream KESs and the downstream KESs, suggesting the essentiality of all the

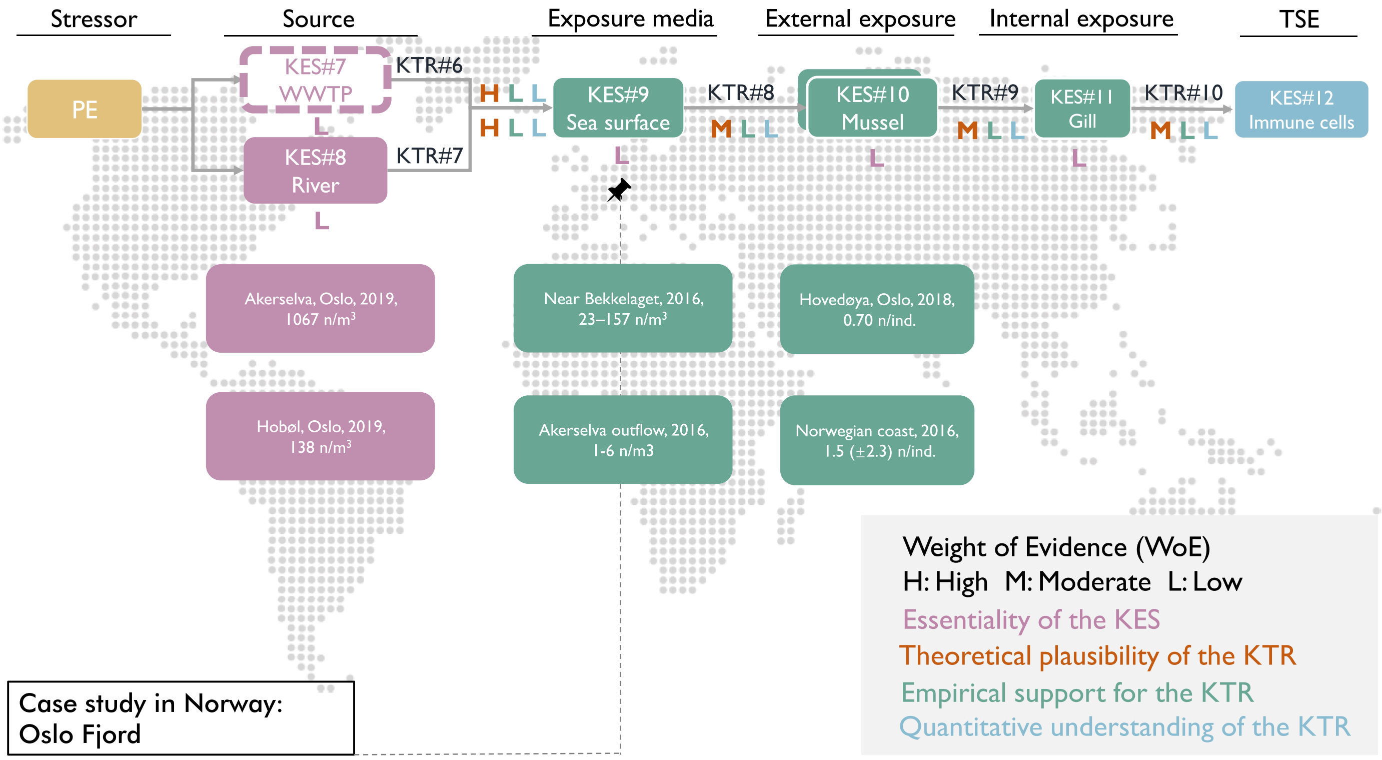

Fig. 4. The Aggregate Exposure Pathway for microplastics (mpAEP) linking discharge of PE from WWTP and rivers to target exposure site in immune cells in the Oslo Fjord in Norway. Rectangles of stack boxes represent Key Event States (KESs) and arrows represent Key Transitional Relationships (KTRs). Dotted box indicates no empirical data reported in the study region. Coloured letters represent WoE assessment results for the essentiality of Key Exposure States (KESs) and theoretical plausibility, empirical support and quantitative understanding of Key Transitional Relationships (KTRs). H: High, M: Moderate, L: Low.

KES#7–12 in the AEP are **Low**.

3.2.4. *Theoretical plausibility of the KTR*

The theoretical plausibility of **KTR#6** (WWTP to Sea surface) and **KTR#7** (River to Sea surface) is considered **High**, as many reviews and reports suggested that WWTPs and rivers are the major input sources of microplastic in the marine environment (Iyare et al., 2020; Lebreton et al., 2017; Mai et al., 2020). The theoretical plausibility of **KTR#8** (Sea surface to mussel) is considered **Moderate** with a review suggesting accumulation of microplastics in mussels living in coastal waters (Li et al., 2019). The theoretical plausibility of **KTR#9** (Mussel to Gill) and **KTR#10** (Gill-Immune cells) is considered **Moderate**. Although there are reviews on immune responses exposed to nanoparticles in bivalves (Barmo et al., 2013; Canesi et al., 2012; Park et al., 2020), nanoplastics are not all included in the current assessment.

3.2.5. *Empirical support for the KTR*

The empirical support for **KTR#6–10** is scored as **Low**, as no study has directly investigated the causal relationships between any of the adjacent KESs in this AEP.

3.2.6. *Quantitative understanding*

Blue mussels used in this case-study are common in temperate seas all around the globe and they are widely used both as seafood and as sentinel organisms in monitoring of anthropogenic pollution in coastal environment. The uptake and depuration mechanism of a blue mussel is well described for chemical pollutants (Björk and Gilek, 1997; Endicott et al., 1998), however, this model needs to be parameterized for microplastic bioaccumulation. Microplastics as particles behave differently compared to other chemical pollutants, and polymer types with varying densities should be taken into consideration when developing an mpAEP (Wang et al., 2016). Following the same method as defined in Case I, results showed that given a hypothetical WWTP source which contains 1000 ± 500 n m^{-3} plastic particles, the resulting cumulative exposure to mussels living in the Oslo Fjord was estimated to be 0.45±2.46 n m^{-3} (range: 0.07 – 5.1 with 95% confidence interval). The most uncertainty comes from the data collected from the biota, suggesting the importance of collecting relevant data to establish the quantitative links between microplastic exposure in the environmental and biological compartments in this region. As the AEP proposed for this case is simplified due to lack of data, future studies should make more efforts on the external exposure pathways through water filtration and link the information to the levels in biota. In summary, the quantitative understanding of **KTR#6–10** is considered **Low**.

3.2.7. *Critical knowledge gaps and uncertainties*

In Case II, the WoE assessment pointed out insufficient data for establishing confident causal relationships of PE microplastics from the source to target site of exposure (Fig. 4). A lack of causality in study design directly hampers the understanding of the spatiotemporal distribution of PE microplastics and its pathway into target sites in biota in the study region, and impedes the development of quantitative prediction models. While concentrations in rivers have been frequently reported, information on WWTPs, sea surface water and biota (external and internal exposure) are scarce. Although the proposed AEP is supported by good theoretical plausibility, empirical and quantitative understanding are lacking. In summary, lack of causality in exposure data from source to TSE in Oslo Fjord are still in great demand from each environmental and biological compartment to generate an mpAEP for regulatory needs.

4. Potential applications of the mpAEP framework

The mpAEP framework can facilitate the unification of the microplastic data reporting and repository systems. Since the majority of the current microplastic data are fragmented, a highly structured data framework such as AEP can help better organize existing information in an expert-curated common format, allowing direct comparison of data across different studies and reuse of information for more comprehensive analysis (e.g., meta-analysis) at a later stage. The mpAEP framework forms the basis for future construction of an exposure database for microplastics. The mpAEP framework can also promote the development of standard operation protocols (SOPs) and improve the data quality assurance criteria in microplastic research. A well-developed AEP normally contains a description of methods for measuring the KESs (SI, Table S1 and S2), thereby demanding highly standardized and repeatable operation protocols. Data quality is a critical factor influencing the weight of evidence assessment and quantitative understanding of the AEPs. This issue is not covered by the present study but has been discussed in detail elsewhere (Isobe et al., 2019; van Mourik et al., 2021).

Organization of existing knowledge in a structured and coherent manner such as AEP can aid to efficient identification of critical knowledge gaps and research needs. As illustrated by our case-studies, the lack of sufficient empirical data for propagating different exposure states of microplastics was clearly visualized by organizing information into mpAEPs. The WoE assessment of the AEPs further revealed a lack of coherence between the supporting data, emphasizing on the importance of measuring and correlating at least two adjacent exposure states in the same investigation to establish a causal relationship. In addition to empirical support, the AEPs also showed the need for better mechanistic understanding of the behavior of microplastics in both environment and organisms. When developing the mechanistic relationships of the mpAEP, potential quantitative models should be adjusted accordingly recognizing unique features of microplastics due to the difference in bioaccumulation, biomagnification, polymer types and internal transport pathways compared to chemical pollutants.

Aggregate exposure pathway in combination with AOP forms a Source-To-Outcome Pathway framework as part of the next generation risk assessment suite for organizing exposure and toxicological information (Hines et al., 2019). Such holistic approach is particularly important for microplastics, not only to re-unify fragmented efforts in the current microplastic research, but also to increase the cost-efficiency of microplastic risk assessment. A high number of (eco)toxicological studies have been performed to assess the hazards of microplastics in various organisms. Several AOPs have even emerged to describe the effects of microplastic across levels of biological organization (Hu and Palić, 2020; Jeong and Choi, 2020). However, the majority of the toxicological studies were not associated with environmentally relevant exposure levels (Botterell et al., 2019; Xu et al., 2019). The mpAEP framework can assemble and evaluate exposure data, extrapolate to different types of microplastics and species groups, thus providing useful guidance for AOP development and associated (eco)toxicity tests as well as ensuring environmental relevancy. A well-developed mpAEP-AOP framework with high quality supporting data can aid to the development of quantitative models for predicting the risk of microplastics based on the source information, thereby greatly reduce the tedious work in conventional risk assessment as well as laboratory animal tests.

For regulatory purposes, the AEPs can efficiently identify critical monitoring and controlling points to stop/reduce microplastic pollution, providing an effective tool for translating scientific data into comprehensible languages for policy-makers during life cycle analysis of plastic waste and microplastics. Designed for decision-makers, the mpAEP framework can be effectively leveraged in the collection of scientific evidence needed for risk assessment and targeted remediation measures across environmental compartments.

5. Conclusion

By adapting the novel concept of aggregate exposure pathway as a holistic approach for exposure assessment, this study has critically reviewed the current knowledge on microplastic exposure in the aquatic environment and assembled available information into the world's first microplastic AEP models. By conducting two practical case studies in China and Norway, the capabilities of AEPs for efficient organization of fragmented information and identification of critical knowledge gaps have been successfully demonstrated. High uncertainties associated with microplastic exposure in biota have been revealed by quantitative analysis in one of the demonstrative cases, highlighting the need for better linking the environmental monitoring data and biological exposure information in future investigations. Furthermore, new weight of evidence assessment criteria for AEPs have been proposed, not only to advance the development this conceptual framework, but also to emphasize on the importance of establishing causalities between data collected from different environment compartments in future microplastic research.

CRediT authorship contribution statement

Guyu Peng: Writing – review & editing, Visualization, Writing – original draft. **Yan Lin:** Formal analysis. **Bert van Bavel:** Writing – review & editing. **Daoji Li:** Writing – review & editing. **Jinren Ni:** Writing – review & editing. **You Song:** Conceptualization, Writing – original draft.

Declaration of Competing Interest

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.

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Supplementary materials

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