

Micro plastics in the oceans and their effect on the marine fauna.

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Mikroplast i haven och dess effekt på den marina faunan.

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SAMMANFATTNING

Mikroplast är små plastpartiklar som släpps ut i havet antingen direkt som mikroplast i skönhetsprodukter, tandkräm och industriprodukter, men också via nedbrytning av större plastbitar, framförallt från nedskräpning på stränder. Djuren i havet förväxlar mikroplasterna med plankton eller får i sig dem via bytsdjur. Väl uppätna kan partiklarna ge effekter på djuren antingen på grund av deras fysiska egenskaper, kemiska byggstenar (plastpolymerer eller tillsatser) eller att persistenta organiska miljögifter (POPs) ansamlas på deras yta. Det senare då mikroplast har en stor och hydrofob yta, vilken kan samla på sig POPs i koncentrationer upp till 10^5 - 10^6 gånger mer än i det omgivande vattnet.

De huvudsakliga förmodade effekterna på grund av intag av mikroplasterna i sig är minskat födosöksbeteende, viktminskning, och ett starkt immunsvar, men det finns också flera studier som inte kunnat hitta några sådana samband. Tillsatser som bisfenol A och ftalater är i andra sammanhang kända för sina hormonstörande egenskaper, dock saknas studier gjorda med syftet att undersöka sambandet mellan intag av mikroplast och dessa tillsatser. POPs, som t.ex PCB, DDT, PBDE och PAH, på mikroplast utgör ett stort potentiellt hot eftersom upptag av dessa ökar vid intag av mikroplast. Det är inte heller helt klarlagt om mikroplaster bioackumuleras.

SUMMARY

Small pieces of plastic, termed "micro plastic" in the oceans derive mainly from degradation of big plastics such as beach littering, but also from sources of direct emission from e.g. beauty scrubbers and synthetic sand-blasting. These micro plastics are ingested by marine animals – mistaking them for plankton – or via prey. When ingested, the particles affect the animals due to their physical properties, their chemical properties (the plastic polymer itself and additives) and persistent organic pollutants (POPs) gathered on their surface. The latter because micro plastics have a large hydrophobic surface, which accumulate POPs to a great extent, leading to up to 10^5 - 10^6 times more POPs on micro plastics than in the surrounding water.

Main effects due to the particles themselves include reduced feeding activity, weight loss, and strong immune response, but studies concluding otherwise are also abundant. Additives such as bisphenol A and phthalates are otherwise known to be hormone disrupters and interfere with development and sexuality, but there is a lack of studies concerning exposure of additives via ingested micro plastics. POPs, including PCB, DDT, PBDE and PAH, on micro plastics are potentially a big threat with increased uptake of POPs because of ingested micro plastics. The question of bio-accumulation of micro plastics is not fully investigated.

LIST OF ABBREVIATIONS

POP – persistant organic pollutant. Examples are PCB, PAH, DDT and PBDE

PCB – polychlorinated biphenyl. A kind of POP.

PAH – polycyclic aromatic hydrocarbon. A kind of POP.

DDT – dichlorodiphenyltrichloroethane. A kind of POP. Can be metabolized to DDE, which also is a kind of POP.

PBDE – polybrominated diphenyl ether. A kind of POP

LD50 – Dose of any substance which is lethal for 50% of the study group or population.

AChE- Acetylcholinesterase. An enzyme which breaks down acetylcholine and thus ends the nervous transmission.

IDH – Isocitrate dehydrogenase. An enzyme which is part of, and is required for, the aerobic pathway of energy production.

INTRODUCTION

Plastics in the environment have been an increasing concern (Andrady et al. 2011) over the now more than a hundered years of usage by man (Andrady 2003). Production of plastics is an ever-growing industry, which in 2011 churned out 245 million tons of plastic (Andrady et al. 2011). This plastic does not always stay where we leave it, and we do not know how much of it that finds its way to the oceans (Andrady et al. 2011). 80% of the plastics we find in the oceans actually originate from littering on land (Andrady et al. 2011). With an increasing amount of micro plastics derived both from degradation of the large plastics and from the usage of micro plastics in consumer and industry products, large chunks of plastics are no longer the only problem.

This paper will examine literature regarding the possible effects of micro plastics in the oceans on the marine fauna, including birds because of possible bio-accumulation.

MATERIALS AND METHODS

The database Primo was primarily used, by access from SLU. The search words in the primary search were microplastic* AND (effect* OR impact* OR disease*) AND (animal* OR "marine mammal*" OR bird* OR fish*) and a secondary search was done using only microplastic* AND (effect* OR impact* OR disease*) as a title search. This provided 292 and 72 hits respectively, of which 9 and 4 of them were deemed relevant and more references were found using these articles' or reviews' reference list. A quick view of all articles showed that certain areas – like the prevalence of micro plastics in animals – had plenty of research, while other – like the area of effects of micro plastics were more scarce. Thus the most relevant studies of the former were chosen, while retrieving every study of the latter. Identical searches were run in the databases Web of Science, PubMed and Scopus. Only one of the studies found, in Web of Science, was deemed relevant.

LITERATURE OVERVIEW Plastic

"Plastic" is not a single entity, but a wide range of different types of hydrocarbons polymers, often combined with additives such as the plasticizers bisphenol A and phthalates (Andrady 2003). The most used plastic is polyethylene, but other kinds of common plastics are polystyrene, nylon and polyvinyl chloride (Andrady 2003). Petroleum and natural gas are often the raw material, even though coal and biomass can be used (Andrady 2003).

No matter what chemical components, all kinds of plastic can affect wildlife in a variety of ways because of their size, their similarity to food and they being situated in or in the vicinity of the ocean. Gregory et al. (1996) reviewed that plastics can block excretion of gastric enzymes, decrease feeding stimulus and thus feeding activity, lower the levels of steroid hormones, delay ovulation, and lead to problems with reproduction. The former issues are especially relevant with birds which cannot regurgitate food, like *Procellaritformes*, but also for those which can regurgitate and the possible transfer of the plastics to their younglings (Gregory 1996).

Toxic additives in plastics

The polymers of plastics are considered biologically inert (Andrady et al. 2011). In contrast, additives can leach and migrate from the polymers depending on size, temperature, pH and the properties of the additive (Teuten et al. 2009). For example, one kind of phthalate, dimethyl phthalate, is highly hydrophilic, which facilitates this leakage (Teuten et al. 2009). Bisphenol A and phthalates are often used examples of plasticizers since they are high volume chemicals with a production of over 1 million ton a year, and have been used for at least a hundred years (Koch et al. 2009). Besides being used in the making of different plastics, bisphenol A and phthalates have very different structures (Koch et al. 2009).

Bisphenol A

Bisphenol A is a single chemical used in the production of polycarbonate plastics and resin pellets, which are used in for example polyvinylchloride (or PVC) plastic and printer ink (Teuten et al. 2009). It can only be degraded in aerobic conditions and can accumulate and be preserved in sediment where it can be more anaerobic (Teuten et al. 2009).

Phthalates

In contrast to bisphenol A, phthalates are a group of several different dialkyl- and alkylarylesters made of 1,2-benzenedicarboxylic acid (Koch et al. 2009). They are very commonly used in PVC-plastic – where they can constitute around half of the entire plastic – and in clothes, packaging, toys and in the building industry (Koch et al. 2009). Since phthalates have such a widespread use and consist of several different chemicals, this leads to a higher exposure of them than of bisphenol A (Koch et al. 2009). Phthalates are not bound to the plastic polymers and can thus migrate directly from the plastic in to organisms which ingest them (Koch et al. 2009). Because of the readily migration, phthalates are ubiquitous

and very often found in low levels, even in laboratory equipment. This makes analyzing the result from samples with phthalates difficult (Koch et al. 2009).

Micro plastic

Although "micro" technically means 1/1 000 000, the term "micro plastic" is not clearly defined and varies from a upper limit of 0,5 mm to 5 mm (more commonly used), and a lower limit of some µm often employed of practical reasons (Andrady et al. 2011). Consisting of countless shapes, they all have a large, hydrophobic, surface in relation to their mass, in common.

At least 180 marine animal species and birds have been found to ingest plastic (Teuten et al. 2009). Among fish there are several different studies showing a 35% prevalence of micro plastics (Lusher et al. 2013). Lusher et al. (2013) showed that there is no significant difference between content of micro plastic in pelagic and demersal fish, and that the plastics found was mostly polyamide (common in the fishing industry) and semi-synthetic cellulose-like material (found in clothes, furniture and feminine hygiene products).

Sources of micro plastics

Micro plastics in the oceans originate from two sources; those emitted directly as micro plastics, and those which are break-down products of bigger pieces of plastic (Andrady et al. 2003). The plastics directly emitted as micro plastics are found in products where they are used for exfoliation. One can find micro plastics, which are too small for the sewage plants to capture, in beauty products such as hand and facial cleansers, shaving products and different kind of "scrubbers", and in synthetic "sand" or air blasting media used in removing paint from metal (Gregory 1996).

The second and major part of the micro plastics in oceans is created by weathering of macro plastics (Andrady et al. 2011). Most of it derives from beach littering, but also from other sources like dumping or loss of plastic equipment from the fishing industry (Andrady et al. 2003). Plastics on the beach break down a lot faster than in water due to wind, higher temperature, more oxygen and solar UV-light (Andrady et al. 2011). Microorganisms which are able to degrade plastics do exist, although rare, but biodegrading is much slower than regular weathering (Andrady et al. 2011).

Pollutants on micro plastics

Due to the hydrophobic nature of the micro plastics, they attract persistent organic pollutants, known as POPs (Andrady et al. 2011; Teuten et al. 2009; Mato et al. 2001). The most commonly described POPs in literature in association with micro plastics are PCB, DDT, PBDE and PAH. These POPs are persistent, bio-accumulate, and have a low octanol-water coefficient which drives concentration equilibrium towards the micro plastics (Andrady et al. 2011). Because of this equilibrium, POPs accumulate on micro plastics to a large extent. Micro plastics have also been found to accumulate heavy metals like cadmium and lead, which could imply a greater bio-accessibility of metals when animals eat micro plastic (Aston

et al. 2010). Since neither POPs nor metals are bound irreversibly to the micro plastics, there is a risk they might bio-accumulate in animals upon ingestion (Andrady et al. 2011).

Mato et al. (2001) studied both POPs on existing micro plastics in the water, and the accumulation of POPs on "virgin" resin pellets put experimentally in the same water. On the virgin resins, elevated levels of PCB and DDE were detected for each day of the six-day experiment, which in the end led to 10^5 - 10^6 times more PCB on the micro plastics compared to the water surrounding them. The kinds of PCB found on the pellets and in the water were highly correlated. The micro plastics used in the experiment was of polypropylene, which is of relevance because different kinds of plastics accumulate different kinds of POPs and in different quantities (Teuten et al. 2009).

A study where collected small plastic pieces on Californian, Hawaiian and Mexican beaches were analyzed for PAH, PCB and DDT content, resulted in the following values: \sum PAH = 39–1200 ng/g, \sum PCB = 27–980 ng/g, \sum DDT = 22–7100 ng/g (Rios et al. 2007). Teuten et al. (2009) found lesser values were found for PBDE, ranging 0,9-57 ng/g outside the Japanese coastline and in the North Pacific central gyre.

Sources of POPs

DDT was introduced as an insecticide 1939 and used widely, but banned in several countries in the 70's when evidence of its toxic effects became clear (Toxipedia 2013). PCB was previously used in e.g. plastics and electronic equipment (Rios et al. 2007) up until the 70's, and can thus either be a part of old plastics still circulating or accumulate from sea water on the surface of micro plastics like other POPs (Mato et al. 2001). After PCB, the market started to use for example PBDE, a similar compound but brominated instead of chlorinated, which is also used as an additive in plastics and other materials to lessen their combustibility (Tanaka et al. 2013). PAH are a group of over a hundred chemicals with three different origins; natural processes such as diagenesis, incomplete burning of products like coal, gas but also meat and tobacco, and manufactured PAHs (Rios et al. 2007).

Uptake, bioavailability and accumulation throughout the food chain

Micro plastics are the perfect size (Andrady et al. 2011) to be mistaken for planktons and could thus enter the very foundation of the food chain, should they be able to migrate from the gastrointestinal area to other bodily tissues (Andrady et al. 2011). Lungworms which were allowed to reside in clean sediment after 28 days in micro plastic-polluted sediment all got rid of the micro plastics overnight (Besseling et al. 2013), and both zooplankton and sea urchin larvae have been seen to eat micro plastics and excrete them straight afterwards (Cole et al. 2012; Kaposi et al. 2013). There is also some evidence of bio-accumulating plastics. Experiments on the Norway lobster, *Nephros norvegicus*, an important catch for Scotlands fishing industry, showed that 100% of the lobsters fed with micro plastics-containing fish carried themselves micro plastics 24 hours later (Murray et al. 2011). The same study also found plastics in 83% of sampled wild lobsters, plastics of the same kind used in the fishing industry.

From studies on the lungworm *Arenicola marina* it has been showed that their gut fluids can detach PAH from man-made micro particles like tire treads, diesel soot and urban particulates, but not from carbon dust and fly ashes (Voparil et al. 2004). This indicates that more PAH is made bioavailable than with only equilibrium (Voparil et al. 2004). Teuten et al. (2007) were able to show that PAH on micro plastics were transferable, with a significant increase in PAH in *A. marina* which had resided in sediment with only 1 µg of PAH-contaminated micro plastics mixed per gram sediment.

The first evidence of PCB from ingested plastics accumulating in animals was a 1988-study of the bird *Puffinus gravis*, which found a positive correlation between PCB-concentration and plastic content in the birds (Ryan et al. 1988). The study also found a negative correlation between DDE and plastic content, which the authors interpreted as evidence that PCB-concentrations came from plastics and not from individual variations. Findings of PBDE in fat tissue from the bird *Puffinus tenuirostris* also indicated that POPs can gather on micro plastics and bio-accumulate in animals (Tanaka et al. 2013). The kind of highly brominated PBDEs found in the birds were not found in their prey from the same area, yet found in plastics in their stomachs. Three out of the twelve examined birds had these highly brominated PBDEs, and the other nine only had the lower brominated kind found in their prey. Since the highly brominated PBDEs are only found in plastics, this is more reliable evidence of POPs accumulation due to plastic than PCB which is also found in prey to a great extent (Tanaka et al. 2013).

Effects of micro plastics on marine animals

Andrady et al. (2011) concluded that the potentially toxic effects from ingestion of micro plastics is due to either 1) the stress of micro plastic ingestion itself, 2) leakage from the plastics (i.e additives), 3) pollutants associated with the plastic (i.e POPs and heavy metals), or 4) toxic products from changed, for example burned, plastic (not to be discussed in this paper). It is not fully investigated whether 1) is due to the physical or chemical properties of the micro plastic.

Effects of ingested micro plastics

There are several studies examining the effects of ingested micro plastics. At the bottom of the food chain, the crustacean *Centropages typicus* normally eats phytoplankton (Cole et al. 2012). When exposed to micro plastics mixed with the phytoplankton, they ate significantly less than in an environment with only plankton (Cole et al. 2012). The correlation was only found for micro plastics the size of 7.3 µm and not for those sized 20.6 µm. Larvae from the sea urchin *Tripneustes gratilla*, was also found to eat micro plastics but only a small, nondose dependent correlation with growth was found, and none for survival (Kapossi et al. 2013). Accordingly, the same, non-existing relationship was found in the bigger crustacean *Nephros norwegicus* (Murray et al. 2011). In experiment with the lungworm *Arenicola marina*, a benthic deposit feeder, exposed to different concentrations of polystyrene micro plastics in the sediment, decreased feeding activity was seen when the micro plastics

constituted 7.4% of the sediment (Besseling et al. 2013). The study found no correlation was found between concentration of the micro plastics and mortality.

In the mussel *Mytilus edulis*, von Moos et al. (2012) showed that polyethylene micro plastics sized <80 µm migrated to the mussels digestives glands and gathered in the lysozymes. The study showed that they generated histopathological changes with a strong immune response of eosinophilic granulocytomas containing plastics. The micro plastics also destabilized the membranes of the lysozymes. No oxygen stress, lipid peroxidation or effects on nutrition state was evident (von Moos et al. 2012). In addition to these effects, another similar study with *M. edulis* saw a significant increase in DNA breakage with a factor of 1.8 (Mamaca et al. 2005). Micro plastics could in the same species migrate to the circulatory system and persist there for at least 48 days after a single exposure (Browne et al. 2008). A study on Japanse medaka contributed liver cancer to the exposure of micro plastics (Rochman et al. 2013).

Ryan (1988) fed chickens with polyethylene pellets and ordinary feed. The results showed that they ate 14.5% less than the controls, and subsequently weighed significantly less when the 18 days of the experiment were over. A similar relationship was found in the red phalarope, *Phalaropus fulicarius*, where fat was negatively correlated with the amount of plastics in their stomachs (Connors et al. 1982). The same did not apply for short-tailed shearwaters, *Puffinus tenuirostris*, where ingested plastics did not correlate with weight, although a correlation was found for lower-chlorinated PCBs in fat and ingested plastics (Yamashita et al. 2011).

Effects of additives in ingested micro plastics

Fossi et al. (2012) showed that phthalates in micro plastics can migrate to, and accumulate, in the blubber of the fin whale *Balaenoptera physalus*, and can thus act as a marker of micro plastic ingestion. The study compared the amount of micro plastics found associated with plankton (the fin whales staple), the amount of phthalates in the micro plastics and the amount of phthalates in the blubber of five stranded fin whales. Relevant concentrations of phthalates were found in four of the five whales, which correlated with the phthalates on the micro plastics.

In marine animals like annelids, mollusks, crustaceans, insects, fish and amphibians, phthalates and bisphenol A can effect reproduction and hormone function (Oehlmann et al. 2009). Amphibians, crustaceans and mollusks seem to be the most sensitive, with effects following as low concentrations as ng-µg/l (Oehlmann et al. 2009). Being an estrogen agonist (Koch et al. 2009), bisphenol A has corresponding effects (although it can also act on androgen synthesis) but is not as estrogenic as estradiol in vertebrates (Oehlmann et al. 2009). Levels of ug/l have induced acute toxicity in crustaceans and insects (Oehlmann et al. 2009). Fish's gonads, gamets and spermatogenesis are affected when exposed to environmentally relevant levels (Oehlmann et al. 2009). Koch et al. (2009) reviewed that exposure stimulated female rats sexuality and that exposure during gestation and/or lactation in rats resulted in higher mortality rates, lower birth weight and delayed sexual maturity.

Phthalates can also act on the production of endogen hormones, but on testosterone instead of estrogen. This is evident during the fetal development and can result in structural and functional abnormalities in males (Koch et al. 2009). In annelids and mollusks phthalates can cause inhibition of mitosis, chromosomal abbreviations and, as previously mentioned, effects on development, but have low acute toxicity (Oehlmann et al. 2009). There is evidence of altered behavior in fish exposed to phthalates, and of reduced sperm motility due to oxidative stress (Oehlmann et al. 2009). Effects on amphibians include altered sexual behavior, teratogenic effects and effects on thyroid hormones (Oehlmann et al. 2009).

Effects of POPs on ingested micro plastics

The effects of pollutants on ingested micro plastics are not only depending on the amount of pollutants on the micro plastics and how much plastics the animals eat, but also how long retention time they have in the gastro intestines and the kinetics of the POPs versus the tissues (Andrady et al. 2011).

Ryan et al. (1988) reviewed that PCB cause reproduction malfunction, hormone changes, increased risk of different diseases and even death, in animals. Bio-accumulation of PCB is increased with a factor of 1,1-3,6 when exposed of micro plastics compared to exposure of PCB in the living environment (Besseling et al. 2013). DDT is commonly known to cause eggshell thinning in birds, but it also affect the reproduction ability in fish and is suspected to be cancerogenic, endocrine disruptive and toxic during development (Toxipedia 2013). Although not very acute toxic, it is very persistent and bio-accumulates (Toxipedia 2013).

Oliveira et al. (2012) studied the acute toxic effects of pyrene, a kind of PAH, on the common goby, *Pomatoschistus microps*. The dosage was between 0,125 mg/L and 1 mg/L and they found significant changes in the fish's ability to swim, decreased AChE and IDH levels, and increased lipid peroxidation in liver and gills. LD50 was found to be 0.871 mg/L, which correlated with other studies on other kinds of fish (Oliveira et al., 2012). Another study examined the combined effects of pyrene (0.2 mg/L) and micro plastics on *P. microps*, conducted by Oliveira et al. (2013) found a toxicological interaction between pyrene and polyethylene micro plastic. Combined exposure delayed pyrene-induced death (60 hours versus 40 hours with only pyrene) and increased pyrene metabolites in the fish's bile. Micro plastics and pyrene, combined or separately, decreased AChE activity, by 22% in average. Combined exposure did not increase the inhibition, which indicated that they work in different ways. The final effect recorded in the study was a decreased IDH with 52% after combined, but not separate, exposure. This results in less available energy through aerobic pathways of production. No significant effects were found on glutathione S-transferase or lipid peroxidation.

Rochman et al. (2013) conducted a study focusing on the effects of polyethylene micro plastics which were allowed to accumulate POPs in a marine environment for a few months. The plastics were then fed to Japanese medakas, *Oryzias latipes*, for two months. After one

month, no significant differences in POP concentration were found between the group exposed to marine plastics, virgin plastics and the control group. After two months both groups fed with plastics had increased levels of PAHs, PCB and PBDE compared to the control group (with slightly more in the fish fed with marine plastics). Liver toxicity and stress (glycogen depletion, fatty vacuolation and cell necrosis) was found in both groups fed with plastics but with a higher prevalence in those fed with marine plastics. Difference in mortality was not statistically significant.

DISCUSSION

A first conclusion – the foundation of further discussion – is that marine animals and birds do ingest micro plastics. All examined studies and reviews come to this conclusion, which leads me to conclude that further study is not required in this area. In some cases, studies of bio-accumulation show immediate excretion upon ingestion of micro plastics (Cole et al. 2012; Kaposi et al. 2013; Besseling et al. 2013), while others show a retention of micro plastics (Murray et al. 2011) or an uptake in tissues and the circulatory system (Browne et al. 2008; von Moos et al. 2012). Considering the size of micro plastics, potential problems for conclusive research might be detection or the size of the plastic in relation to the size of the studied organism. It is also possible that different levels, different kinds of micro plastics, and different feeding technics affect the excretion and uptake, although animals with similar feeding technics like the lungworm (eating sediment) and the mussel (filtrating) were excreting and accumulating, respectively.

Regarding the dangers of micro plastic in itself, research is inconclusive with some studies indicating a relationship between the amount of plastics ingested and weight, growth or feeding behavior (Besseling et al. 2013; Ryan 1988; Connors et al. 1982; Cole et al. 2012), and some finding no correlation at all (Kaposi et al. 2013; Murray et al. 2011; Yamshita et al. 2011). The very different results are puzzling, but could be an effect of excretion rate, evolutionary adaptation, different coping strategies in different species or the experimental method itself. The study by Connors et al. (1982) had a very small group of study animals, and it is thus hard to draw any real conclusions, however, the other studies mentioned are more extensive and also well conducted.

Except the effects on feeding, research have been done on *M. edulis* and found several effects on cell or sub-cell levels, including strong immune response (von Moos et al. 2012) and DNA-breakage (Mamaca et al. 2005). Although these effects have only been found in this particular mussel, they could be assumed to be true in other animals as well since these are functions and molecules found in all animals. One study contributed two cases of liver cancer to micro plastics, although no statistically significant relationship was found (Rochman et al. 2013). It has not – in my findings – been examined whether the effects are due to the physical or chemical properties (e.g. additives) of the micro plastics.

The additives bisphenol A and phthalates can leach from the plastics, but if they do so before being ingested, their effects cannot be associated with micro plastics but more so with plastic

as a product. Because of the leakage, one can assume that animals are not exposed to as much additives through the micro plastics as potentially possible as if fed virgin resin pellets. The only study found on the matter of additive accumulation in animals as a result of micro plastics ingestion, was done on a small sample of fin whales (Fossi et al. 2012). The sample of only five animals, with only four found to correlate between amount of ingested micro plastic and phthalates in blubber, makes it difficult to make viable conclusions. Even so, the results indicate that phthalates do bio-accumulate from micro plastics, and if so the effects of phthalates such as teratogenic toxicity, reduced sperm motility and problems in male fetal development (Koch et al. 2009; Oehlmann et al. 2009), could apply to micro plasticsingesting animals. Research in this area is really scarce and no actual conclusions can be done. No study on any relationship between bisphenol A and micro plastics was found, and more research are needed. For now, it seems wise to assume that effects of general exposure of bisphenol A could be a reality for micro plastics-ingesting animals as well, in accordance to the precautionary principle.

What actually have been shown is that micro plastics do accumulate POPs on their surface, up to a factor of five or six (Mato et al. 2001), making irrelevant concentrations of POPs in oceans possibly relevant to animals which ingest micro plastics. Oliveira et al. (2012) discusses the relevance of their results because of the higher concentration of pyrene used in the study compared to the concentration in the marine environment, but Rios et al. (2007) found micro plastics with even higher concentration of PAHs then used in the previous study.

Uptake of POPs from micro plastics is an area studied by Voparil et al. (2004), concluding that PAHs are dissolved from the surface of different anthropogenic particles by gut fluid, and thus available to be taken up by the animal. The study did not cover plastics as a group of study but the more diffuse "urban particles". Even though the first "evidence" of bio-accumulation of PCB via micro plastics by Ryan et al. (1988) were not statistically significant, other studies on animals *in vivo* showed that bio-accumulation of both PCB (Besseling et al. 2013; Rochman et al. 2013), PBDE (Tanaka et al. 2013; Rochman et al. 2013), and PAH (Rochman et al. 2013) was larger in animals containing more micro plastics. These studies are important since animals (especially high up in the food chain), also accumulate POPs through their feed. Some methods of separating exposure of POPs from micro plastic and from feed do exist, like lower- and higher brominated PBDEs (Tanaka et al. 2013), but still it is often difficult to interpret results.

The results in the study done by Oliveira et al. (2013), with simultaneous exposure of pyrene and micro plastics, is the only one examining a toxicological interaction. The effects on AChE could be quite alarming, indicating a high impact on the nervous system which potentially could lead to both functional and behavioral problems. IDH-levels were diminished by over 50%, but in contrast to AChE the body has double systems regarding energy production and may be able to make up for at least the loss in aerobic production with the anaerobic pathways. This study exposed the fish to micro plastics and pyrene simultaneously but separately, and includes no evidence of pyrene uptake from micro plastics.

From this evidence, I conclude that POPs on ingested micro plastics are dissolved and accumulated in animals. When inside the animals, I find no indication why they should not act and affect the animals as they do when bio-accumulated from prey or from exposure from other sources. On the opposite, these effects would come easier and would be greater because of the accumulation on micro plastics. The pollution of micro plastics in our oceans could be a way to make our pollution of POPs worse.

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