TOX/2019/62

COMMITTEE ON TOXICITY OF CHEMICALS IN FOOD, CONSUMER PRODUCTS AND THE ENVIRONMENT

Scoping paper on the potential risks from exposure to microplastics

Introduction

1. As part of horizon scanning, two COT Members raised the potential risks from microplastics as a topic the COT should consider.

2. Public Health England (PHE) has further expressed an interest in this topic especially with regards to microplastics in the air.

3. The European Food Safety Authority (EFSA) Panel on Contaminants in the Food Chain (CONTAM) published a statement on microplastics in food in 2016, focusing on their presence in seafood, which is attached at Annex A. The World Health Organisation (WHO) published a report on microplastics in drinking-water in 2019, which is attached at Annex B.

4. This paper summarises the EFSA statement, the WHO report, and describes developments in the literature since the EFSA statement in 2016. Literature searches were performed to identify papers reporting on exposure to microplastics via food and water, and its toxicity, since 2016. In addition to dietary exposure, an emerging area of concern is inhalation exposure to microplastics that may be present in indoor or outdoor air (Gasperi *et al*., 2018). No evaluations have been made of risks from inhaling microplastics, but a 2018 narrative review of the emerging evidence is also summarised below and is attached at Annex C.

EFSA opinion on microplastics in food, with particular focus on seafood

5. The EFSA opinion is attached at Annex A. The EFSA CONTAM panel defined microplastics as a heterogenous mixture of differently shaped materials referred to as fragments, fibres, spheroids, granules, pellets, flakes or beads in the size range 0.1-5,000 µm (EFSA, 2016). They can be primary microplastics, *i.e*., deliberately manufactured at that size, or secondary, *i.e*. from fragmentation of larger debris. The panel separately defined nanoplastics as plastic particles with any external dimension in the nanoscale or having internal structure or surface structure in the nanoscale (0.001-0.1 µm). The statement considered both microplastics and nanoplastics, as does this paper.

6. The occurrence of microplastics has been reported in seafood, honey, beer and salt, with most of the data being on occurrence in seafood. However, for fish, only data on microplastics in the digestive tract were available and the digestive tract is usually discarded and not consumed. The CONTAM Panel

considered that the quantity of microplastics in the edible tissue of fish is likely to be negligible. Occurrence data were also available on organic contaminants such as dioxins and phthalates, adhered to microplastics in the marine environment, and on additives such as plasticisers in microplastics in the marine environment. Nanoplastics are expected to be present as a result of weathering and fragmentation of microplastics, the use of engineered nanoplastics in industrial processes and possibly microbial degradation. However, to date analytical methods have not been sufficiently developed and therefore no data are available on the occurrence of nanoplastics.

EFSA review of toxicity data of microplastics and nanoplastics

7. Regarding the toxicity of microplastics and nanoplastics themselves, the CONTAM Panel noted that only plastic particles smaller than 150 µm (nanoplastics and the smaller sized microplastics) may translocate across the gut epithelium, leading to systemic exposure. Therefore, for particles >150 µm potential effects are limited to local effects on the immune system and inflammation of the gut. In general, following oral exposure >90% of the particles will be excreted in the faeces.

8. For the microplastics, 0.04-0.3% of 2 µm latex particles were absorbed in rodents (Carr *et al*., 2012). Limited absorption (0.2%) of 3 µm polylactide-co-glycolide was measured according to an *in vitro* study using human mucosal colon tissue (Schmidt *et al*., 2013). Limited information was available on the distribution of absorbed particles, but microparticles >0.2 µm that are in lymph will be excreted through the splenic filtration system into the gut whereas microparticles in blood will be removed by the liver and excreted in bile. Particles >1.5 µm are not expected to enter the capillaries of organs and therefore are not expected to penetrate deeply into organs.

9. For the nanoplastics, highly variable uptakes of polystyrene nanoparticles measuring between 0.05-0.5 µm were reported in *in vitro* gastrointestinal models, ranging 0.2-10%, while a study of 0.05 µm and 0.1 µm polystyrene particles in rats indicated oral bioavailabilities of 7% and 4%, respectively. Whole body distribution has been shown for gold nanoparticles (De Jong *et al*., 2008). Following the intravenous administration of nano gold particles (0.01-0.25 µm) to rats the smaller particles (0.01 µm) were found in the liver, spleen, heart, lungs, thymus, reproductive organs, kidney and brain (crossing the blood-brain barrier). The larger particles (>0.01 µm) were mainly present in the liver and spleen. With regards specifically to nanoplastics, using an *ex vivo* human placental perfusion model, polystyrene particles with diameters ranging 0.05-0.24 µm were found to be taken up by the placenta, with the transfer being greater for the smaller particles. Particles sized 0.5 µm (so at the low end of the microplastics scale) were mainly retained in the maternal circulation or the placenta.

10. Toxicology studies of microplastics and nanoplastics are limited. In mice administered nanoplastics and small microplastics (up to 12.5 µm in diameter in one study and up to 1 µm in another) by intraperitoneal injection or inhalation, T-cells were activated, and particles were phagocytosed and

transported to the lymph nodes. There were some distribution differences between polymethylmethacrylate and polystyrene particles regardless of particle size. A study in mussels (*Mytilus galloprovincialis*) described decreased phagocytic activity caused by polystyrene nanoparticles (Canesi *et al*., 2015).

11. The effects of plastic nanoparticles on iron absorption was studied in an *in vivo* chicken model (Mahler *et al*., 2012). A single dose of 2 mg/kg bw 0.05 µm carboxylated polystyrene particles resulted in three-fold lower gastrointestinal iron absorption, whereas repeated daily dosing (at 2 mg/kg bw) for two weeks resulted in increased iron absorption and an increase in the overall volume of villi in the duodenum. The authors suggested that this observation was a compensatory response to impaired nutrient absorption which was then increasing the iron absorption. An *in vitro* study in various human cell lines suggests that positively charged polystyrene nanoparticles (0.05 or 0.2 µm) can disrupt intestinal iron intake (Mahler *et al*., 2012).

12. Polystyrene microspheres (diameter 3 µm or 9.6 µm) ingested by mussels (*Mytilus edulis*) were translocated from the gut to the circulatory system but did not cause adverse effects. However, in another study in the same species granulocytoma formation, increased number of haemocytes and decreased lysosomal stability were observed 48 hours following uptake of 1-80 µm microplastic (unspecified). In a two-month study in Japanese medaka fish exposed to polyethylene pellets ground to <500 µm diameters, female fish exposed to the microparticles showed less choriogenin H gene (Chg H)[1] expression than controls (Rochman *et al*., 2014).

13. Della Torre *et al*., (2014) carried out a study in sea urchin embryos to investigate disposition and toxicity of either 0.04 µm carboxylated polystyrene particles or 0.05 µm amino-modified polystyrene particles. The carboxylated polystyrene particles accumulating in the digestive tracts of the developing embryos whereas the amino-modified polystyrene particles were more widely distributed. Exposure to the amino-modified polystyrene particles was reported to be more embryotoxic than to the carboxylated polystyrene particles, which the authors suggested may be due to a difference in the surface charge of the particles.

14. In a study in pacific oyster larvae, no adverse effects were observed on larval growth or feeding following exposure for 8 days to carboxylated polystyrene particles of amino-modified polystyrene particles (1 and 10 µm) (Cole & Galloway, 2015).

15. The EFSA CONTAM Panel noted that, in contrast to nanoplastics, the toxicity of engineered nano-scale metals and metal oxides has been more widely studies and reported effects have included reactive oxygen species production and associated inflammation, liver and kidney damage, secondary genotoxic effects and immune system effects. However, caution should be

[1] Choriogenin H (Chg H): precursor proteins of the egg envelope of Medaka species, regarded as sensitive biomarkers for oestrogenic pollutants.

applied in extrapolating these substances to nanoplastics as toxicity depends on the chemical nature of the material in addition to factors such as the size, shape, charge and surface chemistry.

EFSA review of additives and adsorbed chemicals

16. It has been postulated that microplastics could act as a vehicle for metal (*e.g.* aluminium, chromium, cobalt, iron, manganese, nickel, zinc, cadmium and lead) transport, however, the EFSA CONTAM Panel could not identify a study that assessed the contribution of metals adsorbed to microplastics in food.

17. The EFSA CONTAM Panel also considered the microbial contamination of microplastics and its relevance to food and consequence(s) to human health, however, due to data limitations it was not possible to perform risk assessments.

18. The EFSA CONTAM Panel conducted risk assessments for exposure to contaminants and additives in microplastics based on the highest concentration of microplastics reported in bivalve molluscs, for which the digestive tract is consumed, in contrast to fish. A study of Chinese mussels reported the highest concentration of microplastic particles, a median value of 4 particles/g. Considering an average portion of mussels to weigh about 225 g (without shells), this equated to 900 particles ingested per portion. Assuming spherical particles with an average diameter of 25 µm, based on a different study of microplastics in mussels, and using the density of low-density polyethylene (LDPE) of 0.9 g/cm^3, since this is the most common polymer that has been reported in microplastics (Bouwmeester *et al*., 2015), this would equate to ingestion of about 7 µg of plastics.

19. The EFSA CONTAM Panel made use of the highest concentrations of polyaromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) that had been reported in microplastics deposited at beaches, 24,000 ng/g and 2,750 ng/g, respectively. In this conservative scenario the estimated exposures were 170 pg of PAHs and 19 pg of PCBs. EFSA had previously estimated the median dietary exposure to PAHs by the European population to be 3.8 µg/day and exposure to PCBs to be 0.3-1.8 µg/day. The increases in these dietary exposures from consuming a portion of mussels per day would be 0.001-0.006% for the PAHs and 0.004% for the PCBs.

20. Concerning additives in microplastics, EFSA concluded that on average plastics consist of ~ 4% w/w additives. EFSA estimated that if a portion of mussels contains 7 µg of microplastics, this would equate to 0.28 µg of additives. They utilised bisphenol A (BPA) as an example for a conservative exposure estimate. EFSA previously estimated an average BPA exposure for adults from dietary and non-dietary sources these are 0.19-0.20 µg/kg bw/day. For a 70 kg adult, 14 µg BPA would be typically consumed per day, BPA originating from microplastics in mussels was calculated to contribute 2% to this value. The EFSA CONTAM Panel concluded that the

exposure to other additives from microplastics is not expected to be substantially different.

21. Exposures from reported numbers of microplastics in honey, beer and salt would be lower. For example, in 24 samples of German pilsner beer, mean concentrations of 0.025 fibres/mL, 0.033 fragments/mL and 0.017 granules/mL were reported determined by Rose Bengal staining (the sizes were not reported but the particles were retained by a 0.8 µm sieve) (Liebezeit and Liebezeit, 2014). Thus, the total number of microplastic particles consumed in a litre of pilsner beer would be 75, which is lower than the estimated 900 particles ingested in a sample of mussels. In five samples of German wheat beer, the mean concentrations were 0.026 fibres/mL, 0.031 fragments/mL and 0.03 granules/mL, totalling 0.087/mL, and in nine samples of German alcohol-free beer the mean concentrations were 0.017 fibres/mL, 0.047 fragments/mL and 0.022 granules/mL, totalling 0.086/mL.

22. For German honey (n=19), the mean concentrations of 0.17 fibres/g and 0.009 fragments/g were reported (Liebezeit and Liebezeit, 2013). The mean consumption of honey in UK adults is 0.11 g/kg/d, for a 70 kg this would be 7.7 g/d. If an adult were to consume 7.7 g of honey this would result in exposure of 1.3 fibers/day (Bates *et al*., 2014, 2016; Roberts *et al*., 2018).

23. There are limitations with the references discussed above, these include limited number of samples and limitations with the analytical method not allowing for polymer identification, as well as difficulties in establishing a clear overall dominance of one microplastic fraction.

24. Analysis of Chinese table salts by Yang *et al*., (2015), showed microplastic content of between 0.007 and 0.68 particles/g from different sources (rock/well, lake and sea salts).

Summary of the EFSA opinion on microplastics in food, with particular focus on seafood

25. Overall, the EFSA CONTAM Panel concluded that the risks of toxicity from micro- and nanoplastics themselves from oral exposure could not be assessed due to the lack of data, especially with regards to metabolism and excretion. Concerning the presence of additives or contaminants in microplastics in seafood, conservative estimates would have a small effect on the overall exposure to additives or contaminants.

26. The EFSA CONTAM Panel recommended that analytical methods should be further developed and standardised for micro and nanoplastics. Additionally, quality assurance should be in place and is demonstrated. Research on the effects of food processing, toxicokinetics and toxicity; including local effects on the gastrointestinal tract (GIT) and microbiota are needed. Lastly, research on the degradation of microplastics and potential formation of nanoplastics in the human GIT is required.

WHO report on microplastics in drinking-water

27. The World Health Organisation (WHO) has recently published a report on the presence of microplastics in drinking-water specifically on bottled and tap water. Nanoplastics were also considered in the report, although at the time of assessment there was insufficient information available for an in-depth evaluation (WHO, 2019).

28. The WHO Panel identified that there was a lack of uniform definition of microplastics since it is widely described as plastic particles <5 mm in length, however, the upper bound size range is not expected to occur in treated drinking-water. Since plastics can be further modified with additives, colourants and other processing aids, the WHO Panel observed that there is no standard specification for the composition of microplastics.

29. In the context of drinking-water, various plastics are involved from the water treatment systems to the mains. Polyethylene (PE) and polyvinyl chloride (PVC) are used in water distribution mains and epoxy resins, polyurethane (PUR) is utilised to reline existing mains, polypropylene (PP) for other components and polyamide (PA) as a coagulant aid in water treatment. For bottled waters, polyethylene terephthalate (PET) is commonly utilised with PP or PE as the bottle caps.

30. Estimated global quantities of plastics produced were reported to be 407 metric tons in 2017, a figure that incorporated plastic fibres and additives. This value is expected to double by 2025 and more than triple by 2050, considering the estimated worldwide population growth rate, current consumption and waste habits. Of total non-fibre plastic production, PE (36%), PP (21%), PVC (12%), PET (<10%), PUR (<10%), PS (<10%) and PA (<10%) were described to account for 92% of all plastics ever made. Whilst intentional primary microplastic production represents <0.1% of total plastic production.

31. The sources and transport of microplastics into water is not yet fully understood, however, the WHO Panel considers that the inherent properties of the microplastics (*i.e.* density, size and shape) are key factors in this process. The presence of biofilms will also affect the density of the microplastic and thus where it is present in the water column. The WHO Panel did not regard modelling predictions on inputs of microplastics into the aquatic environment as a reliable source of estimation, since there are insufficient data to validate the predictions.

32. The sources of microplastics into freshwater were discussed, these included: run-off from land-based sources (*e.g.* road surface run-off from the breakdown of road-marking paints and tyre wear debris, city dust, microplastic fibres from textiles due to wear-and-tear and washing, agricultural run-off from the use of sludge), wastewater effluents (*e.g.* synthetic textile fibres from

clothes washing, cosmetic microbeads, combined sewer overflows[2] and erosion or degradation of some treatment-plant plastic components) and mishandled plastic wastes.

33. Other sources of microplastic exposure, were also briefly mentioned including direct exposure of microplastics as a result of abrasion from everyday household objects such as cutlery, toothbrush, and cups. The WHO Panel hypothesised that small children may have increased exposure given the number of plastic items a child can be exposed to during oral exploration as part of a normal stage of child development. In both scenarios, the WHO Panel acknowledged that there are no data on such exposures.

34. In fresh water sources, the frequency of microplastics particles by polymer type directly correlates with plastic production volumes and plastic densities. Fragments and fibres were the most predominant shapes reported, with PET and PP being the most frequent polymers detected.

35. In terms of drinking-water from bottled sources, the WHO reviewed four studies, which are further described in order of decreasing quality in Table.1. The ordering was based on Koelmans *et al*., (2019) quality assessment criterion, which is further discussed in paragraph 289.

36. The WHO Panel acknowledged that there is currently no standard method for sampling and analysis of microplastics in the environment. This process involves three steps: sampling, sample extraction and isolation, and identification, characterisation and quantification.

37. Important factors to consider when conducting sampling and analyses were recommended (refer to pp. 15 on Annex B).

38. The smallest particles determined in both freshwater and drinking-water studies was often determined by the size of the mesh used in sampling. In freshwater, particle count range was 0 – 10^3 particles/L, this was lower for drinking-water at 0 – 10^4 where smaller mesh sizes were typically applied. Due to analytical constraints the smallest detected size was 1 µm. Since there is currently no standardised methodology for the sampling and detection of microplastics from different water sources, no direct comparisons of the generated data from freshwater and drinking-water studies can be made.

39. The WHO Panel therefore identified the following research needs to clarify the occurrence of microplastics in drinking-water and freshwater sources: more studies (which have been conducted using quality assured methods) are needed on the occurrence of microplastics in drinking-water to assess human exposure from drinking-water adequately.

[2] Combined sewer overflows: sewers that are designed to collect rainwater run-off, domestic sewage, and industrial wastewater in the same pipe, which is then transported to a sewage treatment plant, where it is typically treated, however, if there is an increased overflow, untreated wastewater enters water bodies.

Table. 1 Studies reporting presence of microplastics in drinking-water from bottled sources (modified from Pivonsky *et al*., 2018).

Sample source	Sample size	Method of detection	Size (µm)	Microplastic abundance (mpp/L)		Detected polymers (%)	Common morphology	Reference
				Mean	Range			
Mineral water from single-use bottles	259	Nile Red[a] and Fourier-transform infrared spectroscopy	> 100 6.5-100	10.4 325	0-66 0-10,000	PP (54), nylon (16), PS (11), PE (10), PEST (6), others (3)	Fragment	Mason *et al*., (2008)
Mineral water from returnable drinking bottles	15	Micro-Raman spectroscopy	> 5	118 ± 88	28-241	PEST (84), PP (7), PE (5), PA (2)	Fragment	Schymanski *et al*., (2018)
Mineral water from single-use bottles	10		> 5	14 ± 14	2-44	PEST (59), PE (9), PP (1), PA (1)		
Mineral water from glass bottles	9		> 5	50 ± 52	4-156	PEST (41), PE (35), PP (8), PA (12)		
Mineral water from beverage cartons	3		> 5	11 ± 8	5-20	PE (38), PEST (32), PP (26)		
Mineral water from single-use PET bottles	10	Micro-Raman spectroscopy	> 1	2,649 ± 2857	90-9,311	PET (78), PET + olefin (11), PP (10), PE (0.7)	Particles (not further detailed)	Oβmann *et al*., (2018)
Mineral water from reusable PET bottles	12		> 1	4,889 ± 5432	0-11,301	PET (74), PP (10), PET + olefin (7.7), PE (5.4)		
Mineral water from glass bottles	10		> 1	6,292 ± 10,521	813-35,462	PE (46), PP (23), Styrene-butadiene-copolymer (14), Others (13), PET (3.6)		
Mineral water from single-use bottles	3	Rose Bengal[b]	< 5	3.57	1.78-3.57	Uncharacterised[c]	Fibres	Kosuth *et al*., (2018)

Abbreviations: PA; Polyacrylamide, PE; Polyethylene, PEST; Polyethylene terephthalate, PP; Polypropylene, mpp/L; microplastic particles/litre.
a: Nile Red – fluorescent dye, utilised for detection of synthetic materials. b: Rose Bengal – fluorescent dye, utilised for detection of synthetic materials. c: No confirmatory spectroscopic analyses were conducted; unstained particles were described as anthropogenic particles rather than microplastic.

WHO review of the potential human health risks from microplastics in drinking-water

40. The WHO reviewed the potential human health risk from microplastics in drinking-water. They identified the following potential hazards associated with microplastics: physical (unbound monomers, additives, and sorbed chemicals form the environment), and the presence of biofilms (attachment and colonisation of microorganisms) on the microplastics.

41. No epidemiological data or human studies on ingested microplastics were identified by the WHO Panel, most toxicological studies have focused on aquatic organisms or ecotoxicology. Data from rat and mice studies were found to be inadequate to inform human health risk assessment of microplastic ingestion.

42. The rat study assessed was an OECD-compliant 90-day dietary study by Merski *et al*., (2008). PET powder was mixed into the diet of Sprague-Dawley rats (n=10/sex) and was dosed at 0, 0.5, 2.5 or 5% PET in the diet. The size and count of the PET particles was not determined/reported, however, it was deemed likely to be in the range of 1-50 µm. No treatment-related adverse health effects on blood parameters, organ weights or histopathology, as well as mutagenicity were observed. A no observed adverse effect level (NOAEL) was not reported by the authors, however, the NOAEL can be considered the highest dose ~2,500 mg/kg bw/day (at the highest 5% inclusion in the diet).

43. A more recent study by Deng *et al*., (2017) administered 5 or 20 µm polystyrene microplastic particles (PS-MPs) in mice at 0.01, 0.1 and 0.5 mg/day *via* oral gavage per mice for 28 days. Adverse liver effects were observed; however, the reported dataset has been critiqued by Braeuning (2019). The number of animals per dose (n=5) were considered insufficient, and the number of observed detected particles in histopathological analyses exceeded the administered dose.

44. Another rat study by Deng *et al*., (2018) which assessed the co-exposure study with PE or PS microplastics with organophosphorus flame retardants for 90-days was not further reviewed by the WHO Panel due to the high microplastic exposure and the inability to assess the individual contributions of PE, PS or the flame retardants to the observed effects.

45. Many of the i*n vitro* studies were not further reviewed by the Panel due to the limited relevance to the human health effects assessment. Although, a study by Schirinzi *et al*., (2017) whom exposed PS (10 µm) and PE (3-16 µm) microplastics in two human cell lines was reviewed. Exposure to PS showed oxidative stress at the highest concentration of 10 mg/mL, and no effects were seen at 0.05, 0.1 or 1 mg/mL.

46. With regards to nanoplastic oral exposure, one study by Rafiee *et al*., (2018) was reviewed. Adult male Wistar rats were administered PS nanoplastics (mixture of 25 and 50 nm, average hydrodynamic diameter of

38.92 nm) *via* gavage at 0, 1, 3, 6, or 10 mg/kg bw/day for 5 weeks (particle count not reported). No effects on body weight or on a battery of neurobehavioral tests were observed.

47. Note that all studies above have been further detailed in the toxicity section of this discussion paper.

48. Release of plastic particles from surgical materials (*e.g.* prostheses) were considered to provide limited utility to inform possible health effects of microplastics since it represents a different exposure scenario from that of ingested microplastics in drinking-water and the relevance of adverse findings (*e.g.* changes in gene expression, oxidative stress, cellular proliferation, tissue necrosis, DNA damage, tissue necrosis and inflammation) is uncertain. Studies regarding the inhalation of microplastics were not further reviewed since this exposure scenario have unclear relevance to exposure through drinking-water.

49. Summaries of micro and nanoplastic uptake and kinetics were derived from existing evaluations of the EFSA CONTAM Panel for the presence of microplastics in seafood as described in the last section and a Food and Agriculture Organisation (FAO) report on microplastics in fisheries and aquaculture (FAO, 2017). Briefly, microplastics >150 µm were not likely to be absorbed and that uptake of smaller microplastics was expected to be limited (≤0.3%). The absorption and distribution may be more significant for nanoplastics (up to 7% for <0.1µm particles) than microplastics.

50. The potential effects in the gut were discussed. Oral exposure to particles at high levels, has been associated with mild intestinal irritation and inflammation. The accumulation of plastic particles in the phagocytes of the gut tissue and resulting immunotoxicity have also been speculated based on observations from other particles (*e.g.* titanium dioxide and aluminosilicates), although, this remains to be established. Significant inter-species variations in the microbiome were reported within the literature, however, the WHO Panel concluded that the relevance of these findings to humans needs to be investigated.

51. It was acknowledged by the WHO Panel that microplastics degrade faster than larger particles, however, it is unclear whether the conditions within the human GI tract are amenable to plastic degradation.

52. The relative potential for persistent organic pollutants (POPs) to leachg from microplastics is dependent on a variety of factors including the relative size of the particle, mass of the chemical accumulated, the relative level of contamination within the gut and the residence time of the particle within the GIT. Since POPs are associated with potentially adverse effects such as neurotoxicological effects, and immune system suppression, much like the additives, health-based guidance values have been established by international agencies.

53. An upper-bound daily intake estimates of chemicals from microplastics, maximum levels of contaminants and corresponding margin of exposure values are presented in Table 2.

54. To assess the potential risks of microplastics in drinking-water to humans a conservative exposure scenario was carried out by the WHO Panel. Several parameters were assumed prior to the calculation. These were the shape (sphere), size in diameter (150 µm), density (2.3 g/cm^3) and the number of particles in water (10.4 particles/L). Considering the above assumptions on particle characteristics and a default consumption of drinking-water of 2 L/day; an intake of 85 µg of microplastics/day was estimated, which corresponds to 1.4 µg of microplastics/kg bw/day for a 60 kg adult, although, realistic estimates based on reported data ranged from 0.01 – 8.7 µg of microplastics/ kg bw/day.

Table. 2 – Estimated maximum daily intake of chemicals present in microplastics (reproduced from WHO, 2019).

Chemical	Upper bound concentration in microplastic (µg/g)	Maximum daily intake (ng/kg bw/day)	Point of departure (µg/kg bw/day)
Bisphenol A	0.7297	0.001	609
Cadmium	3,390	5.0	0.8
Chlordane	0.0144	0.00002	50
Di(2-ethylhexyl)phthalate	0.0699	0.0001	2,500
Dichlorodiphenyltrichloroethane	7.1	0.0001	1,000
Hexachlorobenzene	0.0587	0.00002	50
Polyaromatic hydrocarbons	119	0.06	100
PBDEs	9.9	0.01	100
PCBs	18.7	0.03	5

Abbreviations: PBDEs; Polybrominated diphenyl ethers, PCBs; Polychlorinated biphenyls.

55. To provide perspective, the WHO Panel compared the estimated worst-case intake from seafood alone carried out by EFSA based on a 225 g portion of mussels – leading to an exposure of 7 µg of microplastics.

56. Margin of Exposure (MOE) assessments were also performed. For this, assumptions on the following parameters have been made: chemical concentrations were the highest reported and the leaching/bioavailability of the chemical contaminant in the body was assumed to be at 100%. The chemicals assessed included; BPA, cadmium, chlordane, di(2-ethylhexyl)phthalate, cichlorodiphenyltrichloroethane, hexachlorobenzene, PAHs, PBDEs and PCBs. MOE values were generally much greater than 100, and for the two carcinogens (PAHs; benzo(a)pyrene and hexachlorobenzene), the MOE values were greater than 10,000. Therefore, the WHO Panel concluded that this risk assessment was adequately protective and indicated low health concern for exposure to chemicals in microplastics through ingestion of drinking-water.

WHO review of treatment technologies for removing microplastics from water

57. The WHO reviewed the treatment technologies for removing microplastics from water since they have been found in fresh water (primarily surface waters). It was highlighted that limited empirical data exist on the ability of different treatment systems to remove microplastics, however, there is data available on the removal of particles similar to microplastics.

58. The mechanism for removing particles includes adsorption, enmeshment in coagulation floc aggregates, flotation, sedimentation, filtration and straining by size exclusion. Additional membrane processes such as microfiltration, ultrafiltration, nanofiltration and reverse osmosis aims to remove and or reduce the number of particles from waste water.

59. A review of 18 studies on the occurrence of microplastics in wastewater found that wastewater treatment plants (WWTPs) effluent typically has a lower median concentration (~1-7 10^{-3}/L) of microplastic particles compared to the influent (~5-6 10^{-3}/L). The WHO Panel proposed that the observed range was due to a non-standardised treatment practices across WWTPs and or are not designed for optimal removal of microplastics.

60. The discharge from WWTPs pose as a route for microplastics to enter into fresh waters and subsequently into drinking-water. It is expected that the contribution of microplastics into water bodies of low-and-middle income countries is higher due to the lack of sewer connection infrastructures.

61. The WHO Panel reviewed 9 studies that assessed the efficacy of microplastic removal in WWTPs of these; one Scottish study was reported which is briefly detailed in the next paragraph.

62. The recorded average microplastic concentration was 15.7 mpp/L (size 598 ± 0.89) in wastewater influent. Treatment processes removed 98.4% of microplastic particles, with much of the removal taking place during the grease removal process.

63. Based on the available data, it was concluded that the removal of microplastics is influenced by their surface characteristics (such as roughness, hydrophobicity and surface charge) as well as their size.

64. Drinking-water treatment processes were also reviewed for their effectiveness in removing microplastics. As before, limited empirical data exist on the presence of microplastics in drinking-water treatment processes, as such no firm conclusions could be drawn by the WHO Panel, however, drinking-water treatment has proven effective in removing particles of smaller size (<1 µm) and at higher concentrations than those of microplastics.

65. The particle removal processes used in drinking-water treatment were then discussed. These include two processes; clarification and membrane.

Clarification processes use combinations of coagulation, flocculation, sedimentation/flotation and filtration. During coagulation, small particles and colloids are destabilised with a chemical coagulant, aggregates known as microflocs are then grown during flocculation. Large aggregates are settled or float in the water, and residual particles that remain are then removed by granular media filtration processes, which typically involve sand grains. Particles between 80-100 µm are unable to pass through the spaces between the filter media and are strained from the water. Particles that are smaller can be removed when they attract to media grains, however, this mechanism is dependent on their size, shape and charge.

66. Nanoplastics may be harder to filter during the solid-liquid separation process in the presence of organic matter since they are hydrophobic, they are able to adsorb organic matter and adopt to its characteristics.

67. Membrane processes are divided into diffusional and porous membranes, where particles above the pore size will be rejected. Typically, microfiltration rejects particles >1 µm, ultrafiltration rejects particles >0.01 µm, and nanofiltration >0.001 µm.

68. Pivonsky *et al*., (2018) carried out comparative assessments on three urban water treatment plants (WTPs) whom utilise surface water for drinking-water production in the Czech Republic. Microplastics (MPs) were found in all water samples (n=54 L for raw and 27 L for treated water). 1,473-3,605 mpp/L in raw water and 338-628 mpp/L in treated water were the average abundance reported. Raman spectroscopy was utilised to determine the proportion of MPs <10 µm in size (range of 1-10 µm), they were found to account up to 95% of MPs present in both raw and treated water. Fragments were the most common morphology, followed by fibres and spheres. MPs > 50 µm were observed to be almost completely removed from the water at treatment plants. In total 12 polymer types were identified, however, PET (> 70%), PP, and PE were the most common.

Summary of WHO report on microplastics in drinking-water

69. Microplastics are ubiquitous in the environment and enter freshwater environments primarily from surface run-off water and waste water effluent, degraded plastic waste and atmospheric deposition. Yet, there are limited data to quantify the contribution of each of the different inputs with their upstream sources. Additionally, there is limited evidence indicating that some microplastics found in drinking-water may come from treatment and distribution systems for tap water and/or bottling of bottled water.

70. The WHO Panel concluded that based on the limited evidence available, chemicals and microbial pathogens associated with microplastics in drinking-water pose a low concern for human health, stating that humans have ingested microplastics and other particles in the environment for decades with no related indication of adverse health effects. Furthermore, drinking-water treatment is effective at removing particles, especially with advanced membrane filtration techniques which is expected to achieve 100%

removal of plastic particles > 0.001 µm for nanofiltration, >0.01 µm for ultrafiltration and >1 µm for microfiltration.

71. No adverse health effects are expected from chemical contaminants present in microplastics for drinking-water based on MOE calculations. As for pathogens in microplastic associated biofilms, the risks were considered to be lower than the risk posed by the high concentrations and diversity of pathogens present in human and livestock waste resulting from inadequate water treatment. Drinking-water treatment processes are designed to remove particles present in the water and the use of disinfection will reduce the potential for any pathogens to be present in drinking-water.

72. With regards to nanoplastics, there was insufficient information available at the time of review for the WHO Panel to be able to draw conclusions on their toxicity, although, no reliable information suggests it is of concern to humans.

73. The WHO Panel recommended that at this time, there is no need to routinely monitor the presence of microplastics in drinking-water, as there is no evidence to indicate human health concern. Water suppliers and regulators should not divert their attention and resources from other high impact issues associated with untreated water, which is a source of microbial pathogens and this remains the most significant risk to human health from drinking-water, although more research is required to better understand the occurrence of microplastics in the environment that may eventually result in human exposure (*e.g.* return of microplastics to agricultural land *via* sludge biosolids).

74. The WHO Panel further recommends improving management of plastics and reducing the use of plastics where feasible, to minimalise the numbers of plastics released into the environment.

75. Regarding knowledge gaps and research needs, the WHO Panel recommended the need to improve, standardise and harmonise microplastic sampling and analysis in water since most studies conducted to date were not considered fully reliable. More data on the return and significance of treatment waste streams were also recommended due to the biopersistant properties of plastics. Further data is required to understand the toxicological effects of microplastics following ingestion, and data on its uptake and fate in the GIT tract. Lastly, a better understanding of overall microplastic exposures in the environment was recommended in order to consider the relative exposure to microplastics in drinking-water to that of microplastics in the air and in food.

Search strategies

76. The following search strategies were combined to identify literature relevant to the exposure and toxicity of microplastics to humans. Pubmed, Science Direct and Google Scholar databases were searched using single words or combinations of terms as described in Annex D. Reports from authoritative bodies that have reviewed the toxicity and human health effects

of exposure to microplastics were appraised and relevant literature cited within these reports were identified.

Potential risks from inhalation exposure to micro and nanoplastics

77. A narrative review of this emerging topic was published in 2018 Gasperi *et al*. (2018) and this is included in Annex C.

78. The authors described the potential sources of airborne microplastics. These include synthetic fibres used in clothing, which may be released as the clothing wears or during washing and drying, and may undergo photo-oxidative degradation in the environment together with wind shear and/or abrasion against other particles, resulting in fragmentation into fine particles.

79. Two studies had demonstrated the presence of fibrous microplastics in the atmosphere. One of these studies (Dris *et al*., 2016), investigated the presence of fibrous microplastic in total atmospheric fallout (TAF) at two sites in Paris, one urban and one suburban. TAF was between 2 and 355 fibres/m^2/day, with 29% of fibres being synthetic or a mix of natural and synthetic materials. The lengths of fibres were predominantly in the range 200-600 µm, while the diameters were mainly between 7 and 15 µm. TAF was higher at the urban site than at the suburban one, furthermore it was observed that TAF during wet weather periods were substantially higher than dry weather periods.

80. The second study (Dris *et al*., 2017) investigated fibres in indoor and outdoor air and in indoor settled dust. Three indoor sites in urban Paris were studied, two apartments and one office; outdoor air was sampled close to the office. The air sampling used a pump which extracted 8 L/minute onto quartz fibre filters (1.6 µm). Indoor concentrations ranged 1-60 fibres/m^3 and outdoor concentrations ranged 0.3-1.5 fibres/m^3, 67% of indoor fibres were natural material and the remaining 33% were petrochemical based. Settled indoor dust contained a concentration of fibres ranging from 190-670 fibres/mg from collected vacuum samples. The length of fibres found in indoor dust fall was 4,650-4,680 µm, whilst the longest observed length in indoor air was 3,250 µm and 1,650 µm in outdoor air.

81. Gasperi *et al*., (2018) noted the importance of distinguishing inhalable and respirable particles or fibres. Inhalable particles or fibres can enter the nose and mouth and deposit in the upper airway, which are them likely to be subjected to mucociliary clearance[3], thus leading to gastro-intestinal exposure. Whereas respirable materials can deposit deeper in the lung tissue and is likely to persist depending on the biopersistence properties of the material (*e.g.* length of fibres).

[3] Mucociliary clearance: is the removal of particles from the airways as the result of the movement of the mucus coating due to the beating of the underlying cilia, it is therefore considered as a protective process; preventing settlement of inhaled particles in the lungs.

82. Occupational exposure data from workers in the textile industry (n=7) were reported to have foreign-body-containing granulomatous lesions in their respiratory tract resulting from inhalation of the synthetic/natural textile dust matter. Asthma-like syndromes, and chronic bronchitis were some of the clinical symptoms observed (Pimentel *et al*., 1975). Further discussed in paragraphs 91-93.

Factors affecting deposition of inhaled particles in the lung

83. One of the factors affecting deposition of inhaled particles in the lung is the aerodynamic diameter, which is a function of geometric size, shape, and density, primarily dictates where in the human airway a particle deposits out of the inhaled airstream due to inertial impaction, sedimentation, diffusion, interception and electrostatic precipitation (Carvalho *et al*., 2011).

84. Particles <10 µm aerodynamic diameter are described to be of interest with respect to potential health effects; whilst those <2 µm aerodynamic diameter may reach the deep lung and potentially taken up by macrophages and epithelial cells (*Fig. 1*). Once in the airway, potential effects are described below.

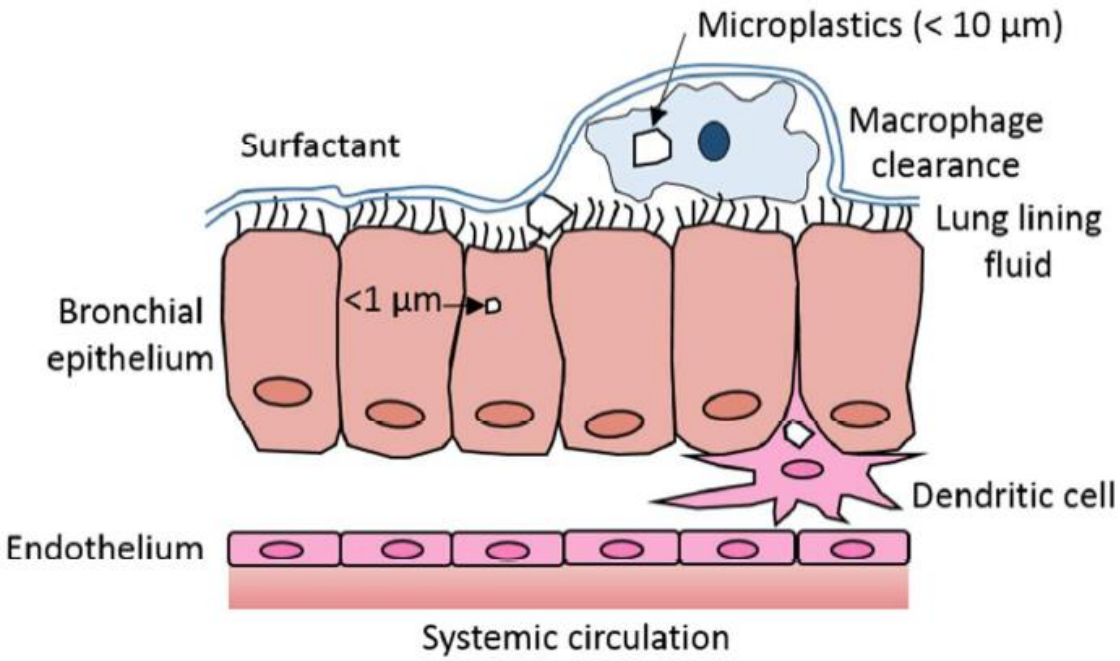

Figure. 1 Diagram of hypothesised microplastic uptake and clearance mechanisms in the lung. If the aerodynamic diameter of a microplastic permits deposition deeper in the lung, it may penetrate the thinner lung lining fluid and contact the epithelium, translocating via diffusion or active cellular uptake (reproduced from Wright & Kelly, 2017).

Potential mechanisms of inhalation toxicity

85. Gasperi *et al*., (2018) postulated that synthetic fibres and asbestos may share toxicological effects due to the similarity of their shapes (*i.e.* fibres).

Physical particle effects

86. The generation and release of intracellular messengers and cytotoxic factors are observed due to direct cellular contact of cells with fibres. This may then lead to lung inflammation, and potentially cause secondary genotoxicity resulting from the continued and excessive production of reactive oxygen species. Longer fibres which cannot be effectively phagocytosed stimulate cells to continue releasing inflammatory mediators that can lead to the progression of pulmonary fibrosis (Gasperi *et al*., 2018).

Chemical effects

87. Airborne microplastics can act as vectors for other pollutants due to their hydrophobic surface. Polyaromatic hydrocarbons and transition metals may be carried by airborne microplastics in urban environments (Gasperi *et al*., 2018).

Intrinsic contaminants

88. Unreacted monomers, additives and other plastic modifiers may exert potential adverse toxicological effects should they leach or volatilise and accumulate from the microplastic (Gasperi *et al*., 2018).

Inhalation toxicity data

89. The following data presented for the inhalation of microplastics in humans mainly relate to the occupational exposure of workers involved in synthetic fibre manufacturing/processing.

90. Pimentel *et al*., (1975) first described respiratory disease caused by synthetic fibres as a new occupational disease. Seven patients exposed to the inhalation of synthetic fibres were found to present bronchopulmonary diseases such as asthma, allergic alveolitis, chronic bronchitis, spontaneous pneumothorax and chronic pneumonia. Histochemical and histophysical methods were employed for the identification of textile fibres. These included solubility and staining techniques, the birefringent properties of the fibres were also assessed.

91. All cases reported had their own unique characteristics; the authors suggested that the different manifestations of bronchopulmonary disease were partly due to the dose and concentration to which the patient was exposed to; different length of working careers within the industry. Precise diagnosis could only be made *via* pathological examination of lung tissue

obtained by needle or biopsy, because of the non-specific nature of the lesions when routine histological techniques were used.

92. *In vivo* animal data were also reported (Pimentel *et al*., 1975). Guinea pigs (sex undetermined) were exposed to nylon dust (n=18) and acrylic fibres (n=10) of 2 g three times a day, in poorly ventilated cages for 325 days. Six mortalities were observed at days 48, 88, 127, 192, 210 and 230 days; animals were reported to have died with no apparent cause. Histopathological results determined the presence of lesion in the lungs of 14/18 guinea pigs exposed to nylon dust and 10/10 exposed to acrylic fibres. No appreciable difference between the lesions were observed between the two fibres.

93. Hillerdal *et al*., (1988) further reported three patients (women; aged 47, 52 and 66 years) whom worked with synthetic textiles (measuring and cutting). Across all three multiple foreign bodies were observed in fibrotic areas of the lungs, it was suspected that the inhaled fibres caused inflammation, however, no further analyses could be performed due to their miniscule size (size range undefined).

94. Pauly *et al*., (1998) reported findings of inhaled cellulosic and plastic fibres in human lung tissue, and its correlation with cancer. In this study, a fibre was recognised as having a length: diameter ratio of ≥ 3 and a length of ≥ 5 µm. The presence of fibres was detected with polarised light; cellulosic and plastic fibres were recognised by their morphology and birefringence. Near-term foetal bovine lungs and non-lung tumour human tumours were utilised as the controls.

95. Inhaled fibres were seen in 83% of non-neoplastic lung specimens (n=67/81) of these 26/31 specimens from patients with squamous cell carcinoma contained inhaled fibres. Furthermore, 97% of malignant lung specimens (n=32/33) were also observed to contain inhaled fibres. It should be noted that tissue specimens were obtained from different pulmonary sites, as such inhaled fibres were distributed throughout the lung and were not confined to large air spaces.

96. Therefore, fibres were present in 87% of all collected samples (n=99/114), in three of these samples, some fibres were present as clusters (>10, >25 and >60 fibres/cluster), however, the fibres in these bundles could not be counted accurately. Inhaled fibres of > 250 µm in length and width of ~50 µm were also observed, and some were distressed (*e.g.* frayed and discoloured). Inhaled fibres were heterogenous in terms of length, width, surface morphology, birefringence, and colour. The authors concluded that these bio-resistant and bio-persistent cellulosic and plastic fibres are candidate agents contributing to the risk of lung cancer.

Phagosome

In vitro data for macrophage internalisation of nanoplastics

97. Yacobi *et al.,* (2008) exposed rat alveolar epithelial cell monolayers to 176 µg/mL amidine-modified (positively charged 20 or 120 nm polystyrene nanoplastic particles; PS-NPs) or carboxylate-modified (negatively charged 20 or 100 nm PS-NPs) for 2 or 24 hours, to investigate trafficking of PS-NPs. Uptake of NPs was determined using confocal microscopy. Positively charged PS-NPs were trafficked 20-40 times faster than negatively charged PS-NPs of comparable size. Trafficking rates decreased with increasing PS-NP diameter. Confocal microscopy revealed nanoparticles localised to cell cytoplasm, whereas cell junctions and nuclei appeared free of PS-NPs. The authors concluded that more research is required to understand the underlying mechanisms of PS-NP trafficking across the investigated cell line, however, trafficking rates are dependent on net surface charge density and size.

Other sources of microplastics in the air

98. Wright *et al*., (2019) developed a filter-based sampling method compatible with both air quality monitoring and Raman spectral imaging (RSI). Clean and ambient particulate matter contaminated filters of various composition were screened. Polymeric microbeads were used as a reference (Table. 3).

99. A total of 36 RSIs were taken with an average of 7,563 spectra for RSI (range 3,325-23,652). The RSI area average was 56,960 µm^2 (range 23,510-166,037 µm^2. Results revealed that the greatest intensities for microplastics were observed against the silver membrane filter, and inhalable microplastics were still detectable in the particulate matter sample for 4 (PS; 10 µm and PE; 20 µm) and 24 hours (PS; 10 µm, PET; 14 µm wide and 1,000 µm long, and copper phthalocyanine[4] ~5 wide and 6 µm long). Therefore, the study appears to confirm presence of microplastics in ambient particulate matter.

Table. 3 provides the different types of filters utilised to capture air samples and the polymeric microbeads for reference values (adapted from Wright *et al*., 2019).

Material	Type	Pore size (µm)
Filter	Quartz microfibre	2.2
	Polytetrafluoroethylene	2.0
	Silver membrane	1.2
	Mixed cellulose ester membrane	0.8
	Alumina-based membrane	0.2
Polymeric microbeads	Polymethylmecrylate	5-27
	Polyethylene	10-27
	Polyamide	5-50
	Polystyrene	4 and 10

100. Zapata (2018) estimated microplastic emissions from composting facilities as a novel source for contributing to air pollution. Both bulk compost

[4] Copper phthalocyanine: a synthetic organic pigment associated with dyeing fabrics.

and air samples were collected, screening were performed for microplastics of 10 µm and 30 µm in size by Nile Red, quantification and classification by size and shape was performed by using a fluorescence microscope. Fourier-transform infrared spectroscopy (FTIR) was utilised for chemical identification.

101. Microplastics were detected in all stages of the composting process, however, the highest concentrations were detected at the end of the process; 10 µm; 2,954 mpp/kg and 30 µm 2,640 mpp/kg of dry compost. The most predominant types of plastics in all stages were PP and PS. Further breakdown is shown in *Fig. 2*.

102. For the air samples, higher concentrations were observed when compared with down or upwind samples measuring at 30-45 mpp/m^3, 5 and 4 mpp/m^3, respectively. The types of polymers detected in the downwind air samples correlated with those present on-site. The author concludes that the results indicate that airborne microplastics are emitted during the composting activities (shredding, turning and screening).

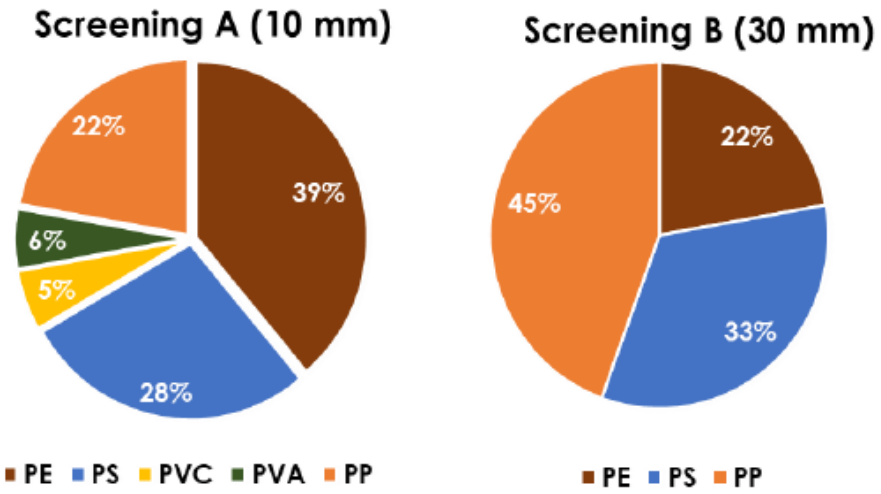

Figure. 2 Pie chart showing the proportion of detected microplastic types in screened 10 µm and 30 µm bulk compost samples. For 10 µm five plastic polymer types were characterised: PE; 39%, PS; 28%, PVC; 22%, PVA; 6% and PVC; 5%. For 30 µm only three were identified: PP; 45%, PS; 33% and PE; 22% (reproduced from Zapata, 2018).

103. The Air Quality Expert Group (AQEG) has prepared a report on non-exhaust emissions (NEE) from road traffic for the Department of Environment, Food and Rural Affairs (Defra), the Scottish and Welsh Government, as well as the Department of Environment in Northern Ireland in 2019 (AQEG, 2019).

104. In this report, it was identified that there is no legislation currently in place specifically to limit or reduce NEE particles. Data from the UK National Atmospheric Emission Inventory (1970-2017) indicate that tyre wear and road surface wear constitute 73% (by mass).

105. NEE PM arise from a range of vehicle-related sources, in which tyre wear has been described as a main contributor. Tyre abrasion results in the release of large quantities of rubber particles of various sizes. Larger particles typically remain on the road surface until they are washed off from the road surface. Smaller particle sizes < 10 µm are attributed to likely become airborne, which contributes to non-exhaust particles in the atmosphere.

106. There is some debate as to whether rubber tyre particles are considered microplastics. Within the AQEG report, the term tyre wear was utilised without any implication as to whether they are also considered microplastic particles, however, if they are – tyre wear would constitute an important source of microplastics in the environment for both road surface wash-off and the airborne route.

107. It is estimated that tyre wear could be adding 5-28% of the releases of primary microplastics to the world's oceans. Average PM_{10} emission factors for tyre wear for different vehicles at typical, urban, and rural and motorway speeds are provided in Table. 4.

Table. 4 Table showing the average PM_{10} emissions factors for tyre and brake wear for different vehicles in the UK at typical urban, rural and motorway speeds (reproduced from AQEG, 2019).

mg PM_{10}/km	Speed	Tyre
Cars	Urban	8.7
	Rural	6.8
	Motorway	5.8
LGVs	Urban	13.8
	Rural	10.7
	Motorway	9.2
Rigid HGVs	Urban	20.7
	Rural	17.4
	Motorway	14.0
Artic HGVs	Urban	47.1
	Rural	38.2
	Motorway	31.5
Buses	Urban	21.2
	Rural	17.4
	Motorway	14.0
Motorcycles	Urban	37.
	Rural	2.9
	Motorway	2.5

Abbreviations: LGVs; Large goods vehicles, HGVs; Heavy goods vehicles.

Summary of the potential risks of inhalation of micro and nanoplastics

108. Environmental exposure to airborne microplastics is dependent on the wide distribution of their sources. Synthetic textiles, erosion of synthetic rubber tyres, and city dust are the most reported sources of airborne microplastics within the literature. Wind transfer is estimated to be responsible for 7% of the ocean's contamination.

109. There is still little information regarding the concentrations of airborne microplastics, however, the Dris *et al*., (2016, 2017) studies carried out in Greater Paris provides indoor concentrations of 1-60 fibres/m^3 and outdoor concentrations of 0.3-1.5 fibres/m^3. Although, these numbers are affected by climate conditions, and seasonality, but also of the sampling methodology.

110. The fate and dispersion of microplastics in indoor and outdoor environments are dependent on several factors, that ultimately influences human exposure. These factors include; vertical pollution concentration gradient (higher concentrations near the ground), wind speed, land topography, wind speed and direction, precipitation and temperature. Exposure to low concentrations of airborne microplastics is expected in outdoor air due to dilution. Whilst indoor behaviour of airborne microplastics behaviour is dependent factors like; dependent on room partition, ventilation and airflow, resulting in higher concentrations in rooms downwind.

111. Occupational inhaled MPs result in toxicity after inhalation of plastic particles or their leachates. The response in humans depends on differences on individual metabolism and susceptibility. It is not yet known whether synthetic fibres may have similar or lower toxicities when compared to organic/natural fibres.

112. The deposition of inhaled microplastics is dependent on particle properties, and the patient's physiology and lung anatomy. Deposition in the upper airways occurs by impaction, while in the small airways it occurs by sedimentation. Fibres have higher potential for penetration (Donaldson & Tran, 2002). Clearance relies on mechanical methods (mucous progression towards the pharynx caused by the beating of cilia), alveolar macrophage phagocytosis and latter migration and by lymphatic transport.

113. In general, the mechanisms of inhaled particle injury include dust overload (high surface particles induce high chemotactic gradients that prevent macrophage migration), oxidative stress (production of reactive oxygen species, which induces cell injury and release of inflammatory mediators), cytotoxicity (free intracellular particles may damage cellular structures), and translocation (injury of secondary sites and vascular occlusion by particles or increased coagulability). Cancer, whilst not a mechanism in itself, can be developed as a result from chronic inflammation or from gene mutation cause by oxidative stress.

Potential risks from ingestion of micro and nanoplastics from drinking-water

114. The following section below presents literature data that was deemed relevant by the Secretariat, which was not discussed by the WHO drinking-water report. It also provides UK specific data where available.

Bottled drinking-water

115. Zucarello *et al*., (2019) published the first quantitative study of exposure to microplastics (<10 µm) associated to plastic bottle mineral water consumption. The extraction and analytical methods are under patent by the Italian Ministry of Economic Department, however, qualitative and quantitative detection analysis were carried out by scanning electron microscopy (SEM) coupled with an energy dispersive detector (SEM-EDX). The authors reported an estimated daily intake of 1, 531, 524 p/kg bw/day corresponding to 40.1 µg/kg bw/day in adults and 3,350,208 p/kg bw/day corresponding to 87.8 µg/kg bw/day in children.

116. The estimated mean microplastic particle concentration was 656.8 µg/L of water, whilst the mean diameter of detected was 2.44 µm. ANOVA[5] analyses showed that microplastic contamination in bottled mineral waters was correlated to the water pH and the density of the bottle. Additionally, it was found that the plastic thickness also played a contributing factor, the most contaminated sample was from a bottle made from poor quality plastic. The authors further acknowledged that the presence of microplastics in bottled waters may not be exclusively derived from the packaging since contamination could occur further upstream prior to the bottling process.

117. Further reported studies reporting presence of MPs in drinking-water from bottled sources are presented in Table 1, which the WHO Panel has reviewed.

Summary of the presence of microplastics in bottled water

118. Based on the available literature, microplastics have been detected in drinking-water from bottled sources. In general, the major polymer type detected is PET, which is the most common polymer utilised in bottle manufacturing.

119. Varied quantities and morphology are reported, depending on material type. The source of microplastic either stems from the packaging itself or through manufacturing processes.

UK specific data

[5] Analysis of Variance (ANOVA) analysis: used to analyse the differences among group means in a sample.

120. Defra are currently funding a research project on the removal of microplastics by drinking-water treatment processes (research code: WT2217), which is expected to be completed in November 2020 (Defra, 2018). Correspondence with the project lead has been commenced by the Secretariat, however, insight can be gained from historic Parliamentary discussions on microplastic prevention and solutions.

121. In 2016, it was mentioned that WWTPs in England are not designed to retain microplastics, and the resulting sewage effluent can carry fibres and microbeads out to rivers, lakes and estuaries and the sea. According to a Eunomia report for the European Commission, the percentage of microplastic particles captured in waste water treatment sludge ranges from 65-100%.

122. It was further highlighted that sludge forms a vital biosolid product that is recycled to agricultural land, and should the concentration of microplastics increase, it raises concerns about the quality of the product and may therefore put at risk a valuable source of nutrients for the agricultural sector.

123. Representatives from various water companies (Veolia, Wessex Water, United Utilities, Northumbrian Water, Thames Water, Yorkshire Water and Water UK) commented that there was no agreed methodology for taking plastic pollution measurements, and that there are no specifically designed sewage treatment processes to capture very small particles. Each water company have different filtration ranges (*i.e.* United Utilities commented that particles over 0.5 µm were filtered out through general surface water treatment processes, whilst Southern Water captures plastics >6 mm). Furthermore, the water industry has no current experience or technologies to separate out microplastics and its related treatment by the water industry has never been explored.

124. The discussion concluded that prevention at source is the most viable option for reducing the number of microplastics flushed into the oceans, however, there are also opportunities to capture microplastics through effective waste and water sewage treatment processes, which currently do not require the monitoring of microplastics (Parliament, 2016).

UK Water Industry Research 2019 report

125. Defra have recently funded a research project titled, "*Sink to River – River to Tap: A review of potential risks from nanoparticles & microplastics*." (research code: WT2219)[6]. The contractor was UK Water Industry Research (UKWIR) which finished in July. 2019 (UKWIR, 2019).

126. The aim of the study was to inform the UK and Irish water companies on the levels of microplastics present in raw and treated water, wastewater and treated effluent, as well as the sludges produced by their treatment works. Furthermore, the study aimed to develop a robust sampling and detection

[6] Full report available at: https://ukwir.org/view/$NvDnwfm!

methodology for the quantification of microplastic particles at different points within the water industry's infrastructure. In this project, microplastics were defined as particles >25 µm that had been captured on 10 µm filters.

127. Briefly, samples were taken from 8 water treatment works (WTWs) and 8 WWTPs from different companies across the UK during the summer and winter months of 2018-2019. Raw water, potable water, and waste sludge were collected from WTWs. Whilst influent, effluent and sludge cake were collected from WWTPs.

128. Different field sampling methods were carried out depending on the sample type. For the collection of raw and potable water, *in situ* filtration was employed where a large volume of water (>100 L) at the WTW sites was passed through a stainless steel 10 µm pore size filter. For WWTPs, a 24 hours composite sampling method was used for influent and effluent collection to obtain a representative sample. 4.8 L of influent (n=16) were collected whilst 7.5 L of effluent (n=18) were collected. Sludge collection was collected in 1 L glass jars with aluminium foil between the jar and the lid, to prevent contact with the rubber coating inside the jar lid.

129. In terms of processing, this again was dependent on the sample type (Table. 5).

130. The approach taken for processing the field-filtered samples (raw, potable and wastewater effluent) involved washing of the particles from the filter taken from the field rig and dispersion in <1 L of water, then the original dispersed sample was divided for storage or processing, following this Fenton's reaction was performed to oxidise general organic materials (skipped for potable water samples), this was followed with enzymatic digestion to remove protein and complex carbohydrates. Finally, the sample was preserved in 50% ethanol prior to analysis by FTIR.

131. The general approach to processing sludge samples from WTWs and WWTPs involved drying of the sludge to obtain a dry sample for subsequent processing. 1 g of the sludge in dry weight (dw) was then sub-sampled following breaking up of the aggregated material and sieving to <1 mm, Fenton's reaction was then performed to oxidise organic material, heavier residuals were then separated through floatation in concentrated zinc chloride, enzymatic digestion was also carried out, lastly filtering to coarse (>178 µm) and fine (<178 µm) fractions prior to dispersion in 50% ethanol for storage and analysis by FTIR.

132. For particle quantification and polymer characterisation, FTIR was selected and was paired with MPhunter (an analysis software package). The FTIR method was reported to identify particles ≥ 6.5 µm, however, due to time constraints the scanning resolution was carried out at 25 µm. The generated spectra files were matched against a custom polymer database, using a threshold of 0.65 (where 1.0 was considered a perfect match and 0 is a complete mismatch). This threshold was decided on by the authors as a

compromise to allow for spectral modifications that occur when microplastics weather in the environment.

Table. 5 Table summarising of the different processing steps required for each of the five sample types analysed (adopted from UKWIR, 2019).

	Sample type				
Processing type	Potable water	Raw water	WWTP influent	WWTP effluent	Sludge
Sub-sampling	✓	✓	✓	✓	✓
Fenton's reaction	×	✓	✓	✓	✓
Density separation	×	×	×	×	✓
Enzyme digestion	✓	✓	✓	✓	✓
Coarse and fine fractions	×	×	×	×	✓
Storage in ethanol	✓	✓	✓	✓	✓

Abbreviations: WWTP; wastewater treatment plant.

133. As for the types of polymers, the following nine were selected based on their ubiquity and presence in water, as reported in the literature: acrylonitrile butadiene styrene (ABS), PA, PE, PET, poly(methyl methacrylate) (PMMA), PP, PS, PVC and PU.

134. In the study, the limit of detection (LOD) was defined as the mean of the blank samples plus 3.3 x the standard deviation of the blank. The limit of quantification (LOQ) was expressed as the mean of the blank plus 10 x the standard deviation of the blank (refer to Appendix D on the UKWIR report).

135. The results for the WTWs revealed that >99.99% of microplastic particles are removed through the treatment process, with raw water (n=30) having an average of 4.9 mpp/L and for potable water (n=39) an average of 0.00011 mpp/L. Four out of eight WTWs had quantifiable amounts of microplastics from 15-113 PE mpp/L, it should be mentioned that these four plants obtained their water source from lowland rivers.

136. The most commonly detected microplastic polymers in raw water were PE, PET and PP. Other polymer types (PMMA, PS and PU and PVC) were occasionally detectable, however, these were below the LOQ.

137. In comparison, polymers detected above the LOQ in the potable water were PS and ABS. It was noted that polymers were more commonly detected below the LOQ but above the LOD. It was also further observed that the type of polymer found in the potable water were not necessarily found in the raw water samples collected on the same day. The authors postulated that the microplastics found in potable water may have been, on occasion, generated within the WTW itself.

138. From the WWTPs results, treatment processes were able to remove 99.9% of the microplastics particles with average levels of 5,611 mpp/L in the influent (n=16) and 5.1 mpp/L detected in the final effluent (n=18).

139. The polymers that were detected in the influent were PE, PET, and PP at levels of 1,000-17,000 mpp/L, however, it must be noted that due to the high quantity of obscuring material present in the original processed sample; only 0.6-2.3% could be put onto the silver filter disc for FTIR analysis and quantification. The same polymers were detected in the effluent with microplastic levels of 2-50 mppp/L.

140. Due to the efficiency of the removal rates of microplastic particles through both water and wastewater treatment processes, a higher level of microplastics were detected in sludge at an average of 2,000-4,000 mpp/g_{dw}. The detected microplastic polymers were PE and PP, however, it must be noted that 0.2-3.7% of the 1g original processed sample was analysed by FTIR.

141. Size distribution analysis by the MPhunter software implies that a large proportion of microplastics were less than 25 µm, which have not been quantified. Examination of particles <25 µm was not performed in this study due to time constraints. Furthermore, the FTIR methodology would only be capable of identifying and enumerating to 6.5 µm.

142. The analytical procedure was also not able to specifically distinguish microfibres from other non-microfibres plastic particles. Therefore, it is possible that thin fibres were under-represented in the final dataset if they had a width below 25 µm.

143. Further insight was provided by the senior project manager; Amanda Fletton at UKWIR and one of the project steering group members; John Hayley (personal communication, 2019). It was highlighted that the presence of black particles such as tyres fragments were difficult to quantify utilising the FTIR methodology, and as such their presence was not accounted for within the report. There is a general acknowledgement that black plastic is not well detected utilising available analytical methods and is an issue in recycling plants.

144. In this communication, it was advised that the reported average number of mpp/L in samples are considered as concentrations (within context to the amount of water that was filtered; >100 L) and not as a mass.

Summary of UKWIR 2019 Report

145. To summarise, the UK water industry has been found to be successful at removing microplastics >25 µm in size from raw water or crude sewage, >99.99%. Raw water was detected with an average of 4.9 mpp/L and potable water having an average of 0.00011 mpp/L, whilst the average was 5.1 mpp/L for wastewater effluent samples. Sludge samples were found to have levels of

2,000 – 4,000 mpp/g_{dw}, due to the high removal rates of microplastic particles through both water and wastewater treatment processes.

146. Smaller particles were not analysed and as such the report could not comment on how effective water treatment processes are at filtering these materials.

147. The most common polymer type found in raw water were PE, PET and PP. For potable water, the polymers detected above the LOQ were ABS and PS, it was hypothesised that these polymers were generated within the WTW. Polymers that were detected in wastewater influent and effluent samples were PE, PET and PP.

148. The authors recommended that further research is required to examine whether the presence of microplastics is comparable or greater than those within the potable water sampled directly from the treatment plant, as well as to observe the differences in polymer types detected. It was observed that at some locations and at differing sampling dates raw water had higher microplastic content. Therefore, further research would be beneficial to review the source of microplastics.

149. In terms of further research for WWTPs, the authors postulated whether aerobic and or anaerobic digestion as a wastewater treatment process is effective at eliminating microplastics. Furthermore, research in the relative concentrations in sludge amended and non-amended soils within the context of agriculture would be valuable to understand any significant differences.

Potential risks from ingestion of micro and nanoplastics from ground soil exposure (*via* possible transfer to food crops)

150. The occurrence of micro and nanoplastics in soil have been largely unexplored when compared to aquatic environments. The plastic particle loading in agroecosystems could be high due to inputs of some recycled organic waste, plastic film mulching, and aerial depositing of plastic particles.

Sources of micro and nanoplastics in agroecosystems

151. Plastic mulch films, greenhouse materials and soil conditioners are direct sources of micro and nanoplastics in agriculture. Indirect sources include; general litter and the use of treated wastewater and biosolids. To a lesser extent, composts derived from residential or municipal solid waste and garden organic waste are additional sources of plastic pollution in agroecosystems.

152. Boucher & Friot (2017) provided a global evaluation of sources of microplastics in the ocean with an estimate between 0.8 – 2.5 million tonnes/year. A value close to two-thirds (63.1%) of the releases are due to the laundry of synthetic textiles (34.7%) and erosion of synthetic rubber tyres

while driving (28.3%). City dust was the third most important contributor at 24.2%, other sources were road markings (7%), marine coatings (3.7%), personal care products (2%) and plastic pellets (0.3%).

153. An updated report by Hann *et al*., (2018) reveals that automotive tyres are the main source of microplastics in the EU, followed by road markings, pre-production plastics and washing of synthetic clothing (*Fig. 3*).

154. As discussed previously, WTPs have the capacity to filter out microplastic particles, the captured percentage ranges from 65-100% depending on the filtration method employed. In Europe, 63,000 – 430, 000 tonnes of microplastics enter agroecosystems annually through biosolids alone (Nizzetto *et al*., 2016).

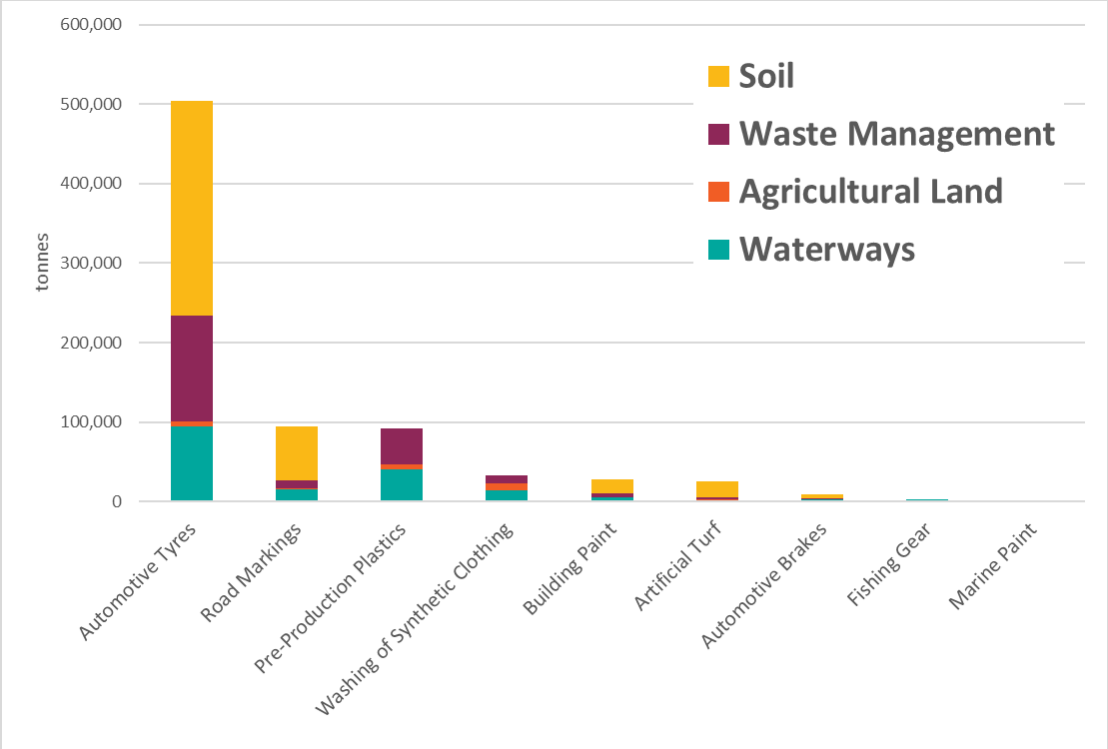

Figure. 3 Bar graph to show the source generation and fate of microplastics from wear and tear in the EU (midpoint estimate), calculations were based on Eunomia modelling (reproduced from Hann *et al*., 2018).

155. Ng *et al*., (2018) further provided biosolid application rates of microplastics based on the EU Directive 86/278/EEC ranging from 0.045 to 0.63 tonnes/hectare/year.

156. Sludge by-products of WWTPs utilised on agricultural land have been found to contain synthetic clothing fibres (4 fibres/gram in dewatered sludge), which have been found to persist up to 5 years post-application. Fibres that were detected along preferential flow paths and/or in horizons largely below the mixed layer suggests some potential for translocation. Furthermore,

synthetic fibres have been detected in field site soils 15 years post-application (Zubris & Richards, 2005).

157. When considering the concentrations of nanoplastics in aquatic sediment, Koelmans *et al*., (2009) has estimated that the prevalence of black carbon and natural carbonaceous nanoparticles (BCNPs) in soil would be more than manufactured carbon-based nanoparticles (MCNPs) based on modelling calculations. These calculations accounted for sedimentation fluxes, removal rates due to aggregation or degradation and burial in deeper sediment layers. MCNPs worst case concentrations were 2,000 - 40 µg/kg dry sediment, and the MCNP to BCNP weight ratio was $4 \times 10^{-4} – 8 \times 10^{-6}$. The authors concluded that the exposure and toxic effects of MCNPs in sediments and soils will be negligible compared to that of BCNPs.

Behaviour of plastic particles on land

158. PE, PP, PS and PVC are the most common polymers found to contaminate the environment. These molecules possess a carbon backbone that is resistant to degradation, both hydrolytic and enzymatic processes, however, these polymers can be degraded *via* oxidation. This process is triggered by free radicals generated when materials are exposed to ultraviolet light (UV) or other sources of thermal energy under aerobic conditions, therefore the degradation process will only occur when the plastic is at or very near to the soil surface. The oxidative degradation process is influenced by various environmental conditions (*e.g.* temperature, soil composition, UV exposure, moisture and the presence of oxygen); as well as the physicochemical properties of the plastic, specifically its chemical structure and crystallinity (Shah *et al*., 2008).

159. Various bacterial strains have been reported to have the capability to degrade plastic polymers, these include; *Ideonella sakaiensis* 201-F6, which produces two enzymes that hydrolyze PET (Yoshida *et al*., 2016) (abstract only), strains of the *Actinobacteria* and *Firmicutes* phylum isolated from the earthworm *Lumbricus terrestris* and its ability to decay LDPE (Huerta Lwanga *et al*., 2018), and *Enterobacter asburiae* YT1 and *Bacillus sp*. from the guts of *Plodia interpunctella* (Indianmeal moth) have been shown to be capable of chewing and eating PE films (Yang *et al*., 2014) (abstract only). Caterpillars of the wax moth *Galleria mellonella* (Honeycomb moth) were also reported to degrade PE at an average of 0.23 mg cm^2/hour (Bombelli *et al*., 2017).

160. The specific organisms described above may not be always available in each agrosystem, and it has been hypothesised that co-metabolism[7] may be a more appropriate and/or realistic process for the bioremediation of micro and nanoplastics present in the soil.

161. Huffer *et al*., (2019) investigated the influence of PE-MPs (<250 µm) on the transport of atrazine and 4-(2,4-dicholorophenoxy) butyric acid in soil

[7] Co-metabolism: The degradation of the compound in the presence of another compound that is used as a carbon source.

under different aqueous conditions; soil, soil with 10% PE-MPs w/w, and PE-MPs alone. The presence of PE-MPs in soil reduced the sorption of the two chemicals investigated, which suggests that PE-MP contamination may increase the mobility of organic contaminants in soil by reducing its natural retention capacity.

162. Machado *et al*., (2018) explored the potential of microplastics to disturb soil aggregation and water retention for ~5 weeks. Loamy sand soil was exposed to PA fibres (average length; 3,756 µm and diameter 18 µm) and beads (15-20 µm), and PE fibres (average length: 5,000 µm and diameter 8 µm) and fragments (largest dimension of 643 µm) of up to 2% w/w. Microplastics were shown to affect bulk density, water holding capacity and the relationship between microbial activity and water stable aggregates.

Particle interactions with the soil interface

163. Anionic or polar surface groups are likely to be introduced on plastic particles during the oxidative degradation process, this provides further additional surfaces for interaction with soil components.

164. Ramos *et al*., (2015) evaluated endosulfan (organo chlorine pesticide) recovery from LDPE plastic mulch films (25 and 100 µm), results have shown that endosulfan and various pesticides (chlorpyrifos, procymidone and trifluralin) accumulate and/or become more stabilised on the surface of plastic mulch film with a range of 584 – 2, 284 µg pesticide/g of plastic; when compared to the soil at 13 – 32 µg pesticide/g of soil.

165. Huerta Lwanga *et al*., (2016) (abstract only) found fragmented microplastics (<50 µm) released in the casts of the earthworm *L. terrestris* are encapsulated in eco-coronas or biofilms that are composed of soil biota, and soil derived organic and inorganic macromolecules.

166. These eco-coronas have also been described in marine microplastics, which have been described to increase density and surface charge of particles and thus changes their mobility and degradation, as well as its bioavailability and toxicity (Galloway *et al*., 2017).

Uptake of plastics by plants

167. To date the uptake of microplastics in plants has not been reported. Based on the literature, this observation is not expected due to the high molecular weight or the large size of the microplastic particles. This physicochemical property prevents their penetration through the plant cell wall (Teuten *et al*., 2009).

168. In contrast, 20 and 40 nm PS-NPs have been shown to enter plant cells *in vitro* by tobacco BY-2 cells *via* clathrin-dependent and clathrin-independent endocytosis, whilst 100nm beads were excluded (Bandmann *et al*., 2012).

169. Carpita *et al*., (1979) found that molecules sized 4.5-5.2 nm were able to freely pass through isolated palisade parenchyma cells of the leaves of *Xanthium strumarium* and *Commelina communis* by using a solute exclusion technique. Therefore, they estimated that particles <6 nm in one dimension may be able to permeate the cell wall, however, it is noted that the characteristics and permeability of the plant cell wall will vary. Plant species will also vary in their uptake, translocation and accumulation of contaminants due to anatomical and physiological differences (*e.g.* it is plausible that some cell types possess specialised channels of secretion that may be blocked to the entry of external large solutes by the ongoing process of secretion, or that a small number of larger accessible pores exist that would allow very slow rates of permeation of large solutes).

170. No studies have investigated the uptake of nanoplastics in whole plant specimens, however, MCNPs which have been developed to study cell plant biology, or as delivery vectors for agrochemicals and biomolecules have been documented in whole plants. Their activities may aid in providing an idea on the possible modes of nanoplastic interaction with plants and bioavailability due to their similarity in size, shape, and surface functional groups.

171. Zhao *et al*., (2017) studied the uptake of ^{14}C labelled multi-wall carbon nanotubes (MWCNTs) in rice, maize, soybean and *Arabidopsis*. The ^{14}C labelled MWCNTs content in different plant tissues ranged from 0.53 (in maize sheath) to 76.6 (in soybean root) mg/kg. The highest content was observed in *Arabidopsis* leaves at 13.0 mg/kg.

172. Maize and soybean samples accumulated high amounts of MWCNTs in their roots, when compared with the aboveground tissues. No significant differences were evident between the stem/sheath and leaf tissues. The authors were unable to describe CNTs accumulation in the stems, which they attributed to the rapid movement of MWCNTs through the stems to the leaves.

Pathways for nanoplastic uptake in plants

173. The absorption of carbon nanoparticles (CNPs) depends on its interaction with suspended organic materials, its colloidal nature and the homo-heterogenous media which allows its flow into the plant system.

174. The proposed pathways for entry of CNPs into plants include; endocytosis *via* the plasmodesmata, passage *via* ion transport channels, carrier proteins or aquaporins, and additionally soil carbon or root exudate mediated entry (Ng *et al*., 2018).

Summary of the potential risks from ingestion of micro and nanoplastics from ground soil exposure (via possible transfer to food crops)

175. The plastic particle loading in agroecosystems could be high due to inputs of some recycled organic waste, plastic film mulching, and aerial depositing of plastic particles (*Fig. 4*).

176. On the soil surface, plastics degrade *via* the oxidative degradation process which is influenced by various environmental conditions. Plastic particles are reported to form eco-coronas with organic and in-organic soil biota, which may affect its bioavailability and toxicity.

177. Information about the bioavailability and bioaccumulation of microplastics in soil organisms is generally lacking. Results from studies in earthworms reveal that they either survive and disperse micro and nanoplastics with them *via* defecation or cast shedding or they die from high exposures.

178. Nanoplastic uptake has been shown in edible food crops and there is concern that plant metabolic processes may produce novel compounds within the food chain.

179. Future research in the analytical and methodological aspects of sampling and quantification are required to perform an accurate assessment of the presence of micro and nanoplastics in soil.

180. Baseline studies on soil exposure, will provide an establishment of the scale of contamination and can potentially allow the determination of sources *e.g.* micro and/or nanoplastics fibres and microbeads as indicators of sludge application for agriculture or tyre dust as an indicator for road runoff.

181. Additional studies are required to assess and better understand microplastic transfer from soil to humans through uptake in food webs and through leaching to the groundwater.

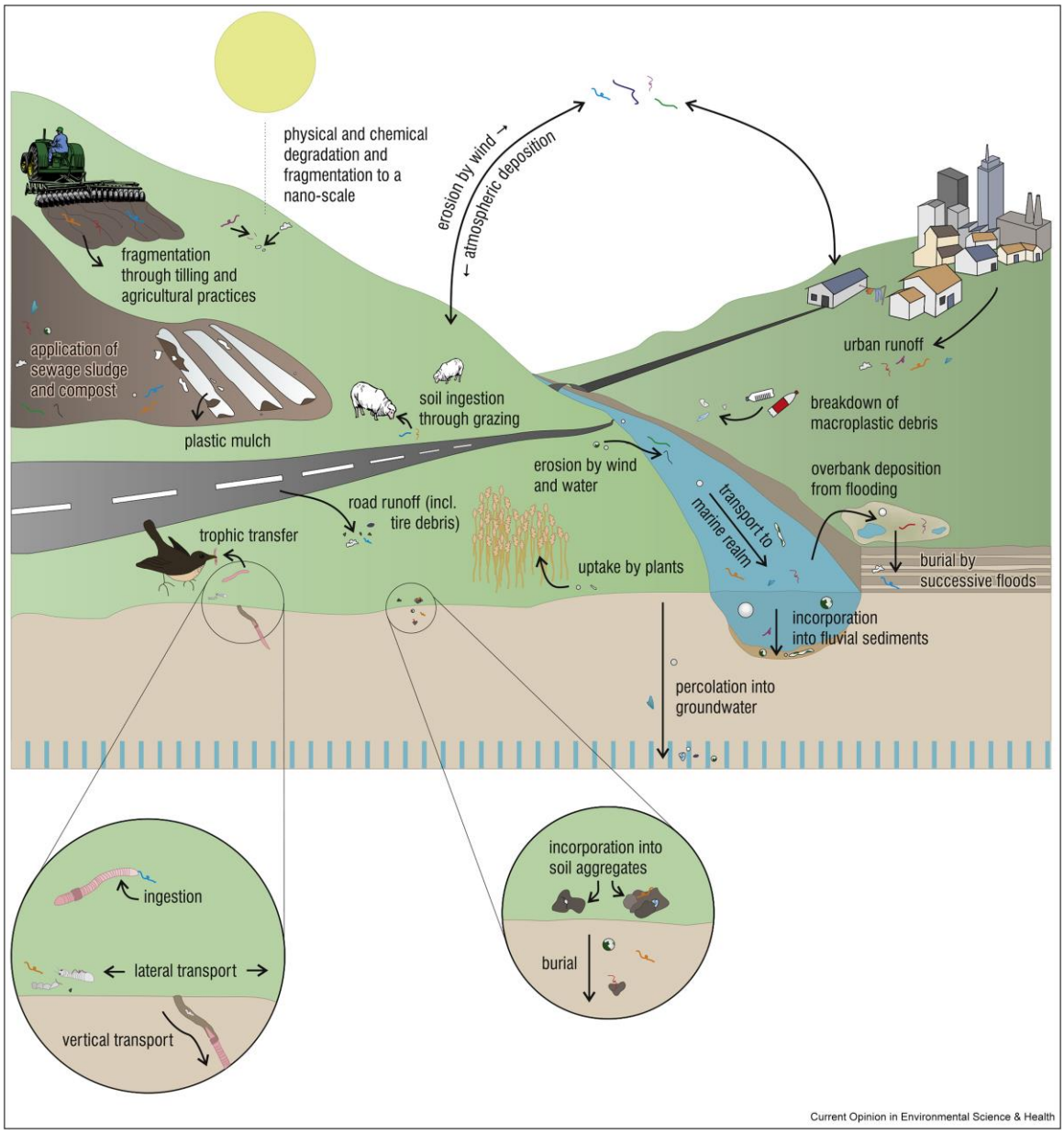

Figure. 4 Diagram to demonstrate the processes that potentially affect the concentration of micro(nano)plastics in soil systems, including sources and fate processes (reproduced from Hurley & Nizetto, 2018).

Adsorption of chemical/microbiological agents on microplastic fragments

182. There is a concern for microplastics to act as vectors for chemical and microbiological agents due to their high surface area to volume ratio, but also due to their size they might introduce adsorbed chemicals into new environments.

183. This section will provide any further relevant data within the literature which has not been reported or considered in the EFSA or WHO reports.

Chemical agents

Polyaromatic hydrocarbons

184. Batel *et al*., (2018) analysed the accumulation pattern and transfer of benzo[a]pyrene (BaP) in adult zebrafish (*Danio rerio*) and embryos. Two fluorescent microplastic particles (MPPs) with sizes of 1-5 µm (undisclosed; proprietary polymer) and 10-20 µm PE-MPs were loaded with BaP; 3 mg of both groups of MPPs were pre-incubated with 20 µL of 12.6 mg/mL BaP in 10mL Aqua bidest in a 50 mL glass bottle at 26°C.

185. Four fish per group (n=4) were exposed in 1L tanks under static conditions to either pure water, with MPPs with BaP dissolved in water, or with waterborne BaP for 6-24 hours, under constant airflow to ensure mixing of microplastics – to study the accumulation and transfer to fish gills.

186. A modified fish embryo toxicity test according to OECD test guideline 236, was utilised. Both MPPs size groups were incubated in 10 µm BaP solution for 24 hours prior to exposure experiments. Twenty embryos per group (n=5) were exposed to either MPPs with BAP dissolved in water, MPPs only, and only BaP.

187. Both sizes of MPPs were found to not permanently adhere to zebrafish gill filaments, which may be due to constant irrigation of the gills and permanent mucus secretion. Transfer of BaP was much higher than expected based on the number of MPPs observed during histopathological analyses. The authors hypothesised that the mucus changed the lipophilic milieu, and that BaP re-dissolved from the MPPs into the water column and was then taken up by the gill tissues. Almost no BaP re-dissolved in pure water after 24 hours of incubation.

188. Results from the embryo study revealed that the smaller sized MPPs (1-5 µM) with higher density properties accumulated at higher numbers on the outer surface of the fish egg chorion than the lighter 20 µm PE-MPs. The transfer of BaP from the MPPs or the water column were not observed to cause morphological, physiological nor developmental adverse effects to the embryo. Fluorescence tracking revealed that there was increased BaP accumulation in fatty tissues of the embryo. The 20 µm PE-MPs, induced a

stronger BaP signal in embryos despite its lower adherence to the chorion, when compared to the smaller MPP.

189. The authors recognised that their study was carried out at very high concentrations of MPPs (~5 million and 1.2 million MPPs/L for the gill analyses and ~5 million and 1.5 million MPPs/L for the fish embryo analyses) and BaP (25.2 µg/L and 2.5 mg/L). It was also observed that BaP bound less firmly to the larger PE-MPs, when compared with the proprietary polymer, which suggests that different types of polymers are likely to release adsorbed chemicals at different rates.

Antibiotics

190. Li *et al*., (2018) investigated the adsorption of 5 antibiotics; sulfadiazine (SDZ), amoxicillin (AMX), tetracycline (TC), ciproflaxin (CIP) and trimethoprim (TMP) on 5 types of microplastics PE, PS, PP, PA and PVC in the freshwater and seawater systems. The since range of the polymers were between 75 – 180 µm. Distribution coefficient (k_d) values were calculated utilising a linear adsorption model. SEM and X-ray diffractometer analysis revealed different surface characteristics and various degree of crystallinity.

191. PA was shown to have the strongest adsorption capacity for antibiotics with estimated k_d values ranging from 7.36 to 756 L/kg for SDZ and AMX in freshwater systems, respectively. This observation was attributed to the porous structure and high capability of forming hydrogen bonds with the antibiotics. Relatively low adsorption capacity was seen for the other four microplastics, the adsorption amounts of the 5 antibiotics on PS, PE, PP, and PVC decreased in the following order: CIP > AMX > TMP > SDZ > TC. Adsorption of CIP and AMX did not occur in the seawater system; sorption capacities of the other antibiotics decreased compared with the freshwater system. Differences in ionic strength and pH values may be used to explain difference, since the pH of the seawater system was higher than the freshwater system.

Metals

192. It has been previously mentioned that EFSA were unable to identify a study that assessed the contribution of metals adsorbed to microplastics in food (EFSA, 2016).

193. No further information could be identified from the literature.

194. In the WHO microplastics in drinking water report, the presence of lead was not considered in the risk assessment because the WHO concluded that it was not appropriate to set a health-based guidance value for this metal. Although, a provisional guidance value of 0.01 mg/L is based on practical achievability, where lead may be used in plumbing materials in buildings, including fittings, solders and pipes, as well as service connections to buildings. A highly conservative maximum intake estimate for a child would be

0.025 µg/kg bw; equating to ~2% of the intake resulting from the provisional guidance value for water and was therefore considered of low concern (WHO, 2019).

195. An upper bound concentration of cadmium in microplastic was estimated to be 3, 390 µg/g of microplastic, which corresponds to a maximum daily intake of 5.0 ng/kg bw/day. The contribution of cadmium to the WHO guideline value is less than 5%.

Microbiological agents

196. The FSA has called a tender in June. 2019 to carry out a critical literature review on the microbiological colonisation of micro and nanoplastics and their significance to the food chain (FS307021)[8]. The critical review is expected to present critical evidence concerning the diversity of microorganism(s) that colonise micro and nanoplastics (including agglomerates), they key pathways that these microbiologically contaminated materials could enter the food chain from environmental sources (*e.g.* water, soil, and air), and the risks these pose to the consumer. The review will also consider antimicrobial resistance and virulence genes and the formation of biofilms and dysbiosis in the environmental media (*e.g.* soil or sediment) and in organisms (FSA, 2019).

Summary of adsorption of chemicals/microbiological agents on microplastic fragments

197. Adsorption of various chemical agents have been studied, these include antibiotics, polycyclic aromatic hydrocarbons (*e.g* BaP), dioxins, metals and microbiological agents.

198. Based on the EFSA and WHO worst-case scenario exposure calculations, adverse effects are not expected from chemicals present in microplastic fragments *i.e* additives and/or adsorbed compounds, since their addition to health-based guidance values represent a small proportion. Furthermore, food and/or water sources for some chemicals represent as a non-major source of exposure.

Toxicokinetics

199. The size of particles is a determining key factor of uptake (*Fig. 5*). Particles within the nanoscale (1 – 100 nm) can gain access to all organs and are able to be translocated in the blood-brain and placental barriers. It is generally accepted that particles > 150 µm will not be absorbed.

200. The uptake pathway is dependent on the property of both the cell type and the target particle; including its surface chemistry and size. Surface

[8] Tender available at: https://food.bravosolution.co.uk/esop/toolkit/opportunity/opportunityDetail.do?opportunityId=43766&oppList=PAST

charge, hydrophobicity also influences the adsorption of proteins to the particle surface – each particle could have its own unique protein corona (Lundqvist *et al*., 2008). The digestive environment (*i.e.* the pH) of the gut will affect the microplastic surface chemistry. The action of digestive enzymes will also likely alter the chemical characteristics of microplastics as they progress through the GIT.

201. Throughout evolution, it is likely that both the lungs and GIT have been exposed to non-degradable exogenous micro and nanoparticles, subsequently the human body has evolved coping mechanisms (*Figs. 6-7*), however, the biological response to microplastics in comparison to other non-degradable microparticles may differ to their unique physicochemical properties. They are resistant to chemical degradation *in vivo*, and once internalised they may also resist mechanical clearance. Retention time influenced by physicochemical properties of particle (*e.g*. size, shape, solubility and surface chemistry), its anatomical site of deposition, and its interaction with different biological structures.

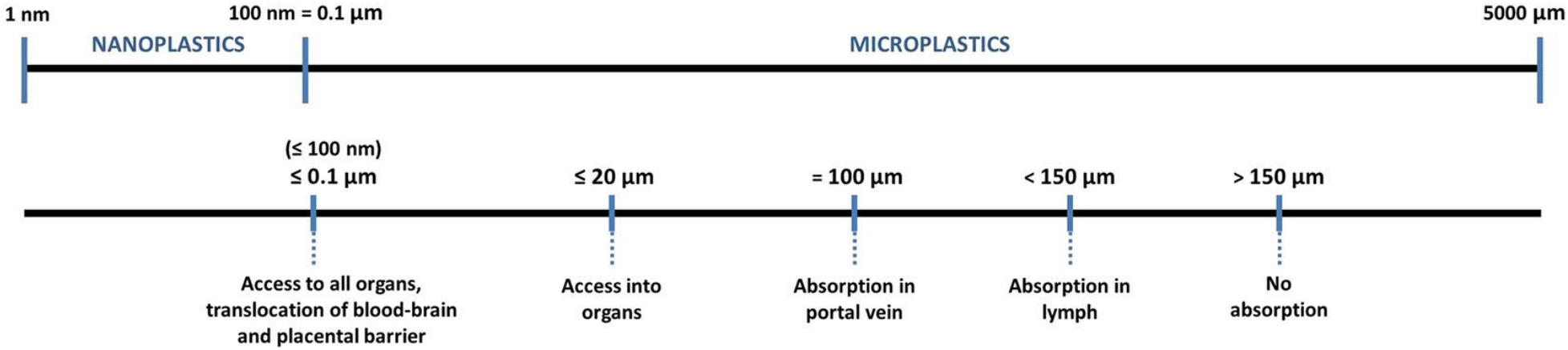

Figure. 5 Diagram illustrating the fate of micro and nanoplastics in mammalian bodies. Microplastics particles > 150 µm are not absorbed, those that are smaller are able to absorb in the lymph (<150 µm), in the portal vein (=100 µm), and into organs (≤ 20 µm). Nanoplastic particles ranging from 1 – 100 nm are able to access all organs, and translocate to the blood-brain and placental barriers (reproduced from Barboza *et al*., 2018).

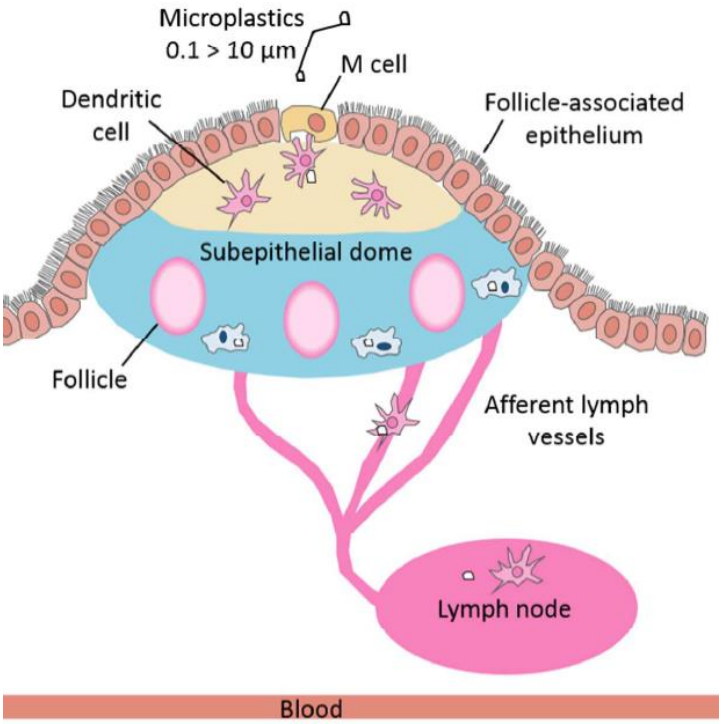

Figure. 6 Diagram demonstrating the hypothesised microplastic uptake and clearance mechanisms in the GIT. Microplastic (0.1 > 10 µm) uptake from the GIT lumen via endocytosis by the M cells of the Peyer's patches. M cells sample and transport particles from the intestinal lumen to the mucosal lymphoid tissues (reproduced from Wright & Kelly, 2017).

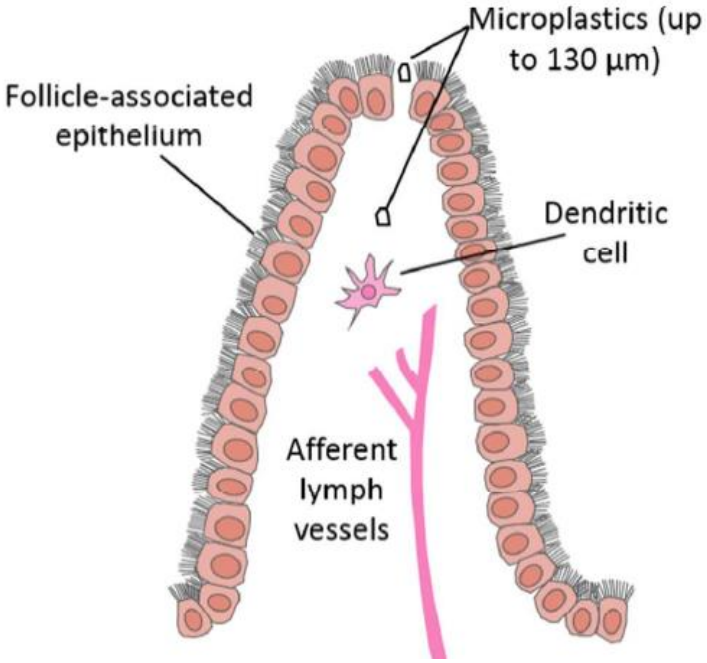

Figure. 7 Diagram demonstrating the hypothesised microplastic uptake and clearance mechanisms in the GIT. Microplastic uptake from the GIT lumen via paracellular persorption. Non-degradable particles, such as microplastics, may be mechanically kneaded through loose junctions in the single-cell epithelial layer into the tissue below. Dendritic cells can phagocytose such particles, transporting them to the underlying lymphatic vessels and veins. Distribution to secondary tissues including the liver, muscle and brain could occur (reproduced from Wright & Kelly,2017).

Nano-plastics (NPs)

ADME

Mammalian data

In vivo

202. A study by Walczak *et al*., (2015) assessed the bioavailability and biodistribution of differently charged PS-NPs upon single exposure in rats. This study has been reviewed by the EFSA CONTAM Panel in their 2016 evaluation, however, it is presented here in further detail.

203. Male Fisher 344 rats (n=25/5 per group) were administered 125 mg/kg bw of 50 nm fluorescent PS-NPs of different charges (neutral, positive and negative) *via* oral gavage; 6 hours post-administration rats were sacrificed under anaesthesia. Negatively charged PS-NPs were taken up more than the other charges.

204. Histopathological results showed that the highest amounts were found in the stomach wall (98.3 µg/g tissue), small intestinal wall (94.4 µg/g tissue), heart (52.8 µg/g tissue) and kidney (37.4 µg/g tissue). Neither neutral or positively charged PS-NPs were detected in the liver. The estimated bioavailability of different types of NPs ranged from 0.2-1.7% *in vivo*, which was reported to be lower based on the groups previous *in vitro* study (1.6-12.3%).

Data on aquatic organisms

In vivo

205. Pitt *et al*., (2018a) further characterised the uptake and distribution of polystyrene PS-NPs in developing zebrafish (*Danio rerio*). PS-NPs were shown to penetrate the chorion[9], and initially accumulate in the yolk sac as early as 18 hours post exposure, these then migrated to the GIT, gallbladder, liver, pancreas, heart and brain throughout development. Accumulation decreased during the depuration phase in all organs, however, this occurred at a slower rate in the pancreas and GIT, suggesting that the GIT is an important site for PS-NPs excretion, or alternatively, the clearance rate of PS-NPs adsorbed within the intestinal tract is slow, or potentially impeding gut function.

206. Al-Sid-Cheikh *et al*., (2019) assessed the uptake and whole-body distribution of 24 or 250 nm spherical PS-NPs in English scallops (*Pecten maximus*) (n=108) at environmentally realistic concentrations of 15 µg/L for 6 hours. An uptake of 30% of 24 and 15% of 250 nm PS-NPS from the available NP burden in the medium. 24 nm PS-NPs were taken up 2.5 times faster than

[9] Chorion: the outermost membrane surrounding an embryo of a reptile, bird, or mammal.

the 250 nm PS-NPs, with uptake values of 0.5 and 0.2 Bq/h[10]. The authors calculated that at these rates, the accumulation capacity (defined by the authors as 95% of the scallop capacity) would be reached after 11 and 30 hours of continued exposure for the 24 and 250 nm PS-NPs, respectively. Through quantitative whole-body autoradiography, the smaller PS-NPs was distributed in the hepatopancreas (1,579 ng), gills (I1,385 ng), gonad (913 ng), muscle (863 ng), kidney (328 ng), intestine (226 ng) and anus (163 ng). Scallops exposed to the larger PS-NPs was significantly lower and was only detectable as a single spot of activity in the intestine. Activity for the other organs were below the limit of detection (0.08 Bq/mL). Furthermore, the authors modelled the bioaccumulation of PS-NPs, during chronic exposures (>100 days) 250 nm PS-NPs would become more bioconcentrated in scallops than 24 nm PS-NPs. The predicted concentrations after a year would be 1.8 and 2.7 mg/g (wet weight) for 24 and 250 nm PS-NPs, respectively (if there is a constant environmental concentration of 15 µg/L).

Bioaccumulation and generational transfer

207. Pitt *et al*., (2018b) examined whether dietary exposure of adult zebrafish to PS-NPs (nominal diameter of 42 nm) could lead to transfer of NPs to the offspring, and whether it would affect zebrafish physiology.

208. Adult female and male zebrafish (F0 generation) were exposed to fluorescent or non-fluorescent PS-NPs in the diet (10% of the food by mass; assuming that animals consumed 100% of the food, each individual was exposed to ~0.3mg per feeding; about 1 mg of PS-NPs per gram of fish) for one week and bred to produce the F1 generation. Four F1 groups were generated: control (unexposed females and males), maternal (exposed females), paternal (exposed males), and co-parental (exposed males and females).

209. Co-parental PS-NP exposure did not significantly affect reproductive success. Histopathological assessment of tissues from F0 fish revealed that PS-NP exposure significantly reduced glutathione reductase activity in brain, muscle and testes, but did not affect mitochondrial function parameters in the heart or gonads.

210. Assessment of F1 embryos and larvae revealed that PS-NPs were present in the yolk sac, GIT, liver and pancreas of the maternally and co-paternally exposed F1 embryos/larvae. Bradycardia[11] was also observed in embryos from maternal and co-paternal exposure groups. Furthermore, activity of glutathione reductase and the thiols were reduced in F1 embryos/larvae from maternal and/or co-parental exposure groups. Mitochondrial function and locomotor activity were not affected in F1 larvae.

[10] Bq/h: Bq stands for becquerel which is the International System of Units derived unit of radioactivity. One becquerel is defined as the activity of a quantity of radioactive material in which one nucleus decays per second.
[11] Bradycardia: is a slower than normal heart rate, what's considered too slow is dependent on age and physical condition.

211. The authors concluded that PS-NPs are transferred from mothers to offspring and exposure to PS-NPs modifies the antioxidant system in adult tissues and F1 larvae, and that PS-NPs could bioaccumulate and be passed on to the offspring, however, this does not lead to major physiological changes.

Microplastics (MPs)

ADME

Human data

In vivo

212. Schwabl *et al*., (2018) presented the preliminary results of a prospective study assessing the microplastic concentrations in human stool during the United European Gastroenterology Week. The pilot study was conducted with 8 participants (n=3 males; n=5 females: aged 33-65 years) across the globe (Finland, Netherlands, Poland, Austria, United Kingdom, Italy, Russia and Japan). Food diaries were recorded in the week leading up to the stool sampling, from this all participants were found to be exposed to plastics by consuming plastic wrapped foods or drinking from plastic PET-water bottles (average of 750 mL/day). None of the participants were vegetarians, six of which consumed seafood during the observation period, and two were daily chewing-gum users.

213. Stool samples were tested for 10 types of plastics utilising FT-IR micro-spectroscopy and up to 9 were detected ranging from 50-500 µm, with PP (62.8%) and PET (17.0%) being the most common (and were detected in all eight samples). On average, 20 microplastic particles/10 g of stool were detected (range of 18-172 particles).

214. The full paper has been published recently in September. 2019. Schwabl *et al*., (2019) (abstract only) acknowledged the limitations of the data presented, these included; the low number of participants, and each provided only 1 stool sample. The origin and fate of microplastics in the gastrointestinal tract were also not investigated. The authors concluded that results suggest inadvertent ingestion from different sources and that further research on the extent of microplastic intake and the potential effect on human health is needed.

In vitro

215. Stock *et al*., (2019) analysed the uptake and effects of 1 or 4 (1 x 10^8/mL) or 10 µm (3 x 10^6/mL) pristine spherical fluorescent PS-MPs in three different human Caco-2 based models (mono-culture, mucus co-culture and M-cell model) incubated for 24 and 48 hours.

216. Cell viability of Caco-2 cells was measured by the cell titer blue and MTT[12] assays. Pronounced loss of cell viability occurred only in the presence of very high concentrations (1 x 10^8/mL) of the 1 µm particles. No pronounced cytotoxicity was observed with the larger particles.

217. At 1 µm, up to 0.8% particle recovery was observed, whilst for 4 µm PS-MPs particle uptake was seen up to 3.8% across all three cell lines. Both 1 and 4 µm PS-MPs recovered significantly higher rates in the co-culture models, when compared to the mono-culture. For 10 µm PS-MPs, lesser extent of recovery was observed of up to 0.07% in the mucus co-culture.

218. The authors further studied the effects of 1 (100,000 particles/mL), 4 (250,000 particles/mL) or 10 (60,000 particles/mL) µm PS-MPs on macrophage polarisation in human THP-1 cells, to detect a possible impact on intestinal immune cells, after an incubation period of 24 hours. PS-MP uptake was quantified at 24 and 72 hours after the induction of polarisation.

219. Preferential size uptake was observed; 4 > 1 > 10 µm. The quantity of particle uptake in macrophages was larger when compared with the intestinal cells. Overall fractions of the macrophages had taken up 40-80% of the 4 µm sized PS-MPs and 10-20% for the smaller and bigger particles. This observation could be explained based on the results from the cell viability assays. Particles of 1 µm were shown to be more cytotoxic than the other tested sizes at the highest concentration.

220. The impact on intestinal immune cells was analysed by Western blot and quantitative qRT-PCR. Western blot analysis showed that STAT-1 and STAT-6 proteins[13] were phosphorylated for M1 and M2 macrophages, respectively and at the same phosphorylation levels in cells not exposed to PS-MPs. This suggests that the presence of PS-MPs had no influence on the phosphorylation of the above-mentioned proteins. qRT-PCR tested the levels of CD209 and CD206 surface receptors, as well as CXCL10 and CCL22 expression levels. Results showed that levels of protein expression did not differ from control samples. To conclude, although uptake is evident, evidence for effects on macrophage polarisation and/or chemokine release were not produced.

Mammalian data

In vivo

221. To date, there are two published studies reporting toxicokinetic data in mice. The latter Doyle- McCullough *et al*., (2007) reports age as a determining factor that influences the uptake of microplastics.

[12] MTT assay: dimethylthiazole-2-yl]-2,5-diphenyltetrazolium bromide (MTT) is a colourimetric assay for assessing cell metabolic activity.
[13] STAT-1 and STAT-6 proteins: Signal transducer and activator of transcription (STAT) 1 & 6. These proteins play a central role in exerting Interleukin 4 mediated biological responses.

222. Deng *et al*., (2017) quantified the distribution and accumulation of PS-MPs in male mice (n=75/5 per group). Five were utilised as a negative control (treated with MP free water), thirty-five were administered with 0.1 mg/L of 5 µm fluorescent PS-MPs and the remaining thirty-five were treated with 0.1 mg/L of 20 µm fluorescent PS-MPs *via* oral gavage for 28 days. Five mice from each group were sacrificed at 1, 2, 4, 7, 14, 21- and 28-days post exposure. An additional 10 mice (n=5/group) were administered with 0.1 mg/L of 5 or 20 µm fluorescent PS-MPs *via* oral gavage to assess the retention of MPs in mice for 28 days, one week after this exposure period the mice were sacrificed.

223. A further 40 (n=5/group) male mice were utilised for the toxicological experiment. Similar to the exposure regime above; five mice were utilised as negative control and fifteen were exposed to 5 µm fluorescent PS-MPs at 0.01, 0.1 or 0.5 mg/day. The remaining fifteen mice were exposed to 20 µm fluorescent PS-MPs *via* oral gavage at the same concentrations as above for 28 days.

224. Both PS-MP sizes displayed tissue accumulation over time and steady-state was reached in the liver, kidney and gut within 14 days post exposure. The maximal tissue concentrations (MCT) of 5 µm PS-MPs in the liver, kidney and gut were 0.30, 0.95 and 1.39 mg/g, respectively. For the 20 PS-µm MPs, the MCT for the same tissues were 0.76, 0.78, and 0.78 mg/g. MCT concentrations of 5 µm PS-MPs accumulated in the kidney and gut were significantly higher than that of 20 µm PS-MPs. Although, significantly fewer 5 µm PS-MPs were retained in the liver when compared to the 20 µm PS-MPs after 28 days of exposure. Both PS-MP sizes were still observed to be present within the three tissues one week after the termination of the exposure. Accumulations of 20 µm PS-MPs appeared consistently distributed among all tissues, whilst 5 µm PS-MPs were observed to have higher accumulation in the gut.

225. Differences in final body and liver weight were not observed between the control and treated groups. No significant changes for daily food consumption between these two groups were also observed. Additional histopathological analyses observed inflammation and lipid droplets in the livers of the PS-MPs treated mice. Further biological parameter observations in the liver included decrease in ATP levels and an increase in lactate dehydrogenase activity in a dose-dependent manner.

226. Metabolomic analyses determined a total of 37 differential metabolites to be significantly different across the exposure groups when compared to the control (*Fig. 8*). Phosphocreatine, succinate, creatine, 2-oxoglutarate, alanine, pyruvate, glutamine, citrate, choline, lysine and phenylalanine significantly decreased, while taurine, threonine, lipids and aspartate significantly increased with increasing doses of PS-MPs.

227. Yang *et al*., (2019) assessed PS-MPs mice system based on toxicity-based toxicokinetic/toxicodynamic modelling to quantify organ bioaccumulation and biomarker responses with data published by Deng *et al*.,

(2017), as detailed above. Based on calculations the gut had the highest bioaccumulation factor (BCF) of ~8 for 5 µm PS-MPs with a mean residence time (MRT) of 16 days. The BCF for 20 µm PS-MPs in the gut was ~5, with an MRT of 16 days. Predictive threshold concentrations causing 50% inhibition or increment of biomarkers in mice liver were also estimated; at 5 µm PS-MPs values for each biomarker are reported in the following order; triglyceride (TG) > superoxide dismutase (SOD) > catalase (CAT) > ATP at 40, 13, 11 and 8 µg/g, respectively. For 20 µm PS-MPs the order of was; CAT > TG > ATP > SOD at 91, 88, 2, and 0.70 µg/g, respectively.

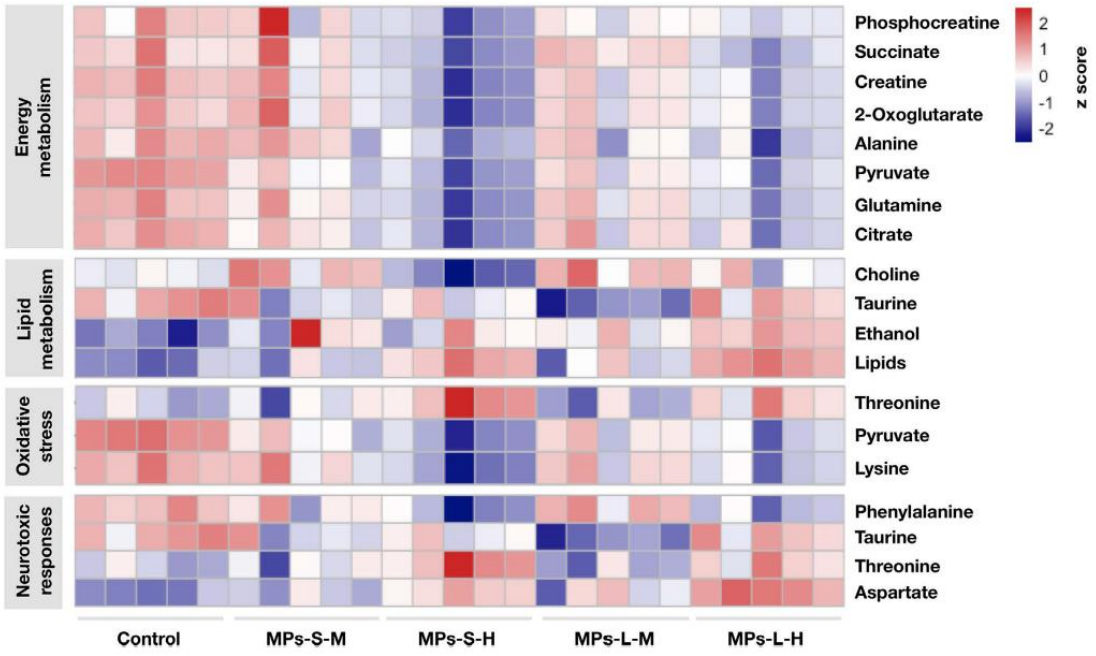

Figure. 8 A heat map to depict the affected differential metabolites identified in different treatment groups calculated by z-scores[14]. M; 0.1 mg/day and H; 0.5 mg/day. S; small 5 µm and L large 20 µm. Red; increased activity and blue decreased activity (reproduced from Deng *et al*., 2017).

228. The authors proposed a four-step extrapolation algorithm for extrapolating the results from a mice system to humans. First, threshold concentrations are determined by applying the Weibull threshold model. Second, the threshold concentrations are converted to human equivalent doses, a safety factor is then applied and lastly, the algorithm could be applied in risk assessment frameworks. Limitations were also highlighted, mainly relating to the lack of data from other exposure routes (*e.g.* inhalation).

229. No further data could be identified for the metabolism of microplastics. This process is not expected since they are resistant to degradation and will therefore persist unless eliminated (Wright & Kelly, 2017).

[14] Z scores: calculated based on the following formula (abundance of individual metabolite in treatment group – mean abundance of metabolite in control) / standard deviation of metabolite abundance in control.

230. Doyle-McCullough *et al*., (2007) compared the microparticle uptake of 2 µm fluorescent PS latex microspheres (2.5% solid latex in distilled water) in animals of different ages (3-52 weeks), gender (male/female) and species (mice, rats and guinea pigs) and at different time points (5, 30- and 90-minutes post administration). The dose for rats and guinea pigs was 1.42-1.95 x 10^9 particles in 0.25 mL, whilst for mice the dose was 6.84 x10^8 in 0.1 mL. Administration was through oral gavage for mice and rats but administration to guinea pigs was through a tube to the pharynx.

231. The proportion of uptake was almost entirely villous, rather than associated with the mesenteric lymphoid tissues, across all small intestine sections age groups (3,7, 17, and 52 weeks) in male and female rats (n=6/gender). Although, the male young adult group (7 weeks) showed significantly greater total uptake (2.52 x 10^6) and percentage uptake (0.13%) when compared to the other age groups. No substantial variation in particle uptake was observed between genders, although there was a higher trend for the uptake in females 30 minutes post-administration. The authors hypothesised that the hormone status of the female rats may have caused this observation.

232. Species differences did not substantially affect tissue uptake or the percentage of uptake in the young adult group (range of 0.12-0.32%), however, the age of rats does affect the extent of the uptake. Tissue uptake was highest in 3 weeks old rats, whilst the percentage of administered dose taken up was higher in the 7-week-old age group.

233. To summarise, age seems to be more of an important factor in determining the extent of uptake than gender or species.

Bioaccumulation and generational transfer

234. Al-Jaibachi *et al*., (2018) investigated the possible ontogenic transference of fluorescent MPs using *Culex pipiens* mosquitos. Four treatments with five replicates (n=10 larvae) were used: a control with no MPs, a treatment of 8 x 10^5 2 µm PS-MPs/mL, a treatment of 8 x 10^2 15 µm PS-MPs/mL, and a 1:1 mixture of both treatments. One random individual was removed from each beaker when every mosquito had moulted into the fourth instar, and again when they pupated or emerged as adults.

235. No MPs were found in control groups of any mosquito life stage. The number of MPs decreased between successive ontogenic levels from the larval stage at 3,047 to 40 of 2 µm PS-MPs in the adults, and for 5 µm PS-MPs the detected number of particles were 279 and 0 at the larval and adult stage, respectively. For the mixed exposure scenario, 2 µm PS-MPs were again significantly observed in the larval stage at 3,952 PS-MPs decreasing to 16 in the adult stage. Within the adult stage, the PS-MPs were detected in the adult abdomen, specifically inside the Malpighian tubules[15].

[15] Malpighian tubules: A type of excretory and osmoregulatory system found in some insects, myriapods, arachnids, and tardigrades.

236. The authors suggest that their results have implications on ecological systems since any aquatic life stage that is able to consume MPs and transfer them to their terrestrial life stage is a potential vector of MPs onto novel aerial and terrestrial habitats. Adult mosquitos are also predated by various species including; flies, spiders, birds and bats.

237. Nelms *et al*., (2019) analysed sub-samples of scat from captive grey seals (*Halichoerus grypus*) and whole digestive tracts of the wild-caught Atlantic mackerel (*Scomber scombrus*) that they are fed upon to investigate microplastics trophic transfer. Polymer types was confirmed using FTIR spectroscopy. Approximately, half of scat sub-samples (48%; n=15) and a third of fish (32%; n=10) contained 1-4 microplastic fragments. Particles were mainly black, clear, red and blue in colour. Mean lengths were 1.5 mm and 2 mm in scats and fish, respectively. Ethylene propylene was the most frequently detected polymer type in both (n=12). The authors concluded that the trophic transfer represents an indirect, yet potentially major, pathway of microplastic ingestion for any species whose feeding ecology involves the consumption of whole prey.

Summary of toxicokinetics

238. Many factors can influence the uptake of micro and nanoplastics; size is the most important.

239. Within context of the EFSA report, the CONTAM Panel noted that only plastic particles smaller than 150 µm (nanoplastics and the smaller sized microplastics) may translocate across the gut epithelium, leading to systemic exposure. Therefore, for particles >150 µm potential effects are limited to local effects on the immune system and inflammation of the gut. In general, following oral exposure >90% of the particles will be excreted in the faeces. The metabolism of microplastics is not expected due to their biopersistent nature.

240. Maternal transfer of nanoplastics have been observed in zebrafish, whilst microplastics have been observed to occur ontogenically and *via* trophic transfer, however, there is still a lack of evidence for bioaccumulation of micro and nanoplastics in animal models.

241. Available human data shows that microplastics have been detected in the stool, eluding that uptake is possible, however, the dataset has its limitations.

Toxicity

242. There is a plethora of literature regarding the toxicity of micro and nanoplastics in the marine environment, whilst there is limited data that are of direct relevance to humans (Touissant *et al*., 2019).

243. A study by Stock *et al*., (2019) (as discussed in paragraph 215 and further mentioned in paragraph 257) has been reviewed by the German Federal Institute for Risk Assessment (BfR). From this, they observed that there was no evidence of intestinal damage from pristine polystyrene microplastics in mice, however, there are still large gaps in the data regarding the size and material of microplastics. Furthermore, no conclusions can be drawn from the generated data on the effects in the intestine of microplastics made out of other polymer types (BfR, 2019).

Human data

In vitro studies

244. Hwang *et al*., (2019) investigated the cellular responses of human dermal fibroblasts (HDFs), murine macrophage (Raw 264.7) and human mast cell line -1 (HMC-1) upon different exposure scenarios to PP-MPs. The mean diameters were ~20 µm and 25-100 µm.

245. 20 mg PP-MPs were dispersed in 200 µL of dimethyl sulfoxide (DMSO), with either 9.8 mL of phosphate-buffered saline (PBS) or Dulbecco's Modified Eagle Medium (DMEM) to obtain a final concentration of 2 mg/mL. Secondly, PP-MP powder was unmodified and used directly at concentrations of 0.1, 0.3, 1.5, 3.0, or 4.5 mg/well.

246. For cytotoxicity assessments, Raw 264.7 cells were treated with different concentrations of either ~20 and 25-200 µm PP-MPs at 0, 50, 250 and 500 µg/mL, whilst HDF cells were treated at concentrations of 0, 10, 50, 100, 500, and 1,000 µg/mL for 48 hours.

247. For histamine profiling tests, HMC-1 cells were treated with ~20 or 25-200 µM PP in DMSO at a concentration of 500 µg/mL. Cells were also treated with ~20 or 250 µM at 0, 100 and 500 µg/mL for 48 hours.

248. PP particles showed low toxicity effects in a size and concentration dependent manner, however, at high concentrations at the ~20 µm size and under DMSO conditions, were shown to stimulate the immune system and enhance potential hypersensitivity to PP-MPs *via* an increase in the levels of cytokines and histamines in Raw 264.7 and HMC-1 cells.

249. Wu *et al*., (2019) compared the cytotoxicity and efflux pump inhibition ability of 0.1 and 5 µm spherical PS-MPs in human Caco-2 cells. Both sizes exhibited low toxicity on cell viability and oxidative stress at the tested concentrations (1, 10, 40, 80 or 200 µg/mL) after an incubation time of 12 hours. Furthermore, low toxicity was also observed for both sizes for membrane integrity and fluidity at the concentrations of 1, 20, 50, or 80 µg/mL for the same time of exposure. Although, at low levels both sizes of PS-MPs induced mitochondrial depolarisation and inhibited plasma membrane

adenosine triphosphate (ATP) binding cassette (ABC) transporters[16] at low levels.

250. 0.1 µm PS-MPs (>20 µg/mL) were found to accumulate in lysosomes, the authors hypothesised that accumulation of PS-MPs can damage lysosomes over a longer incubation period that may result in apoptosis. The 0.1 µm PS-MPs size was found to exhibit a greater potency effect on the inhibition of plasma membrane ABC transporter activity compared to 5 µm. This effect was postulated to be caused by PSC833 and MK571[17] increasing the accumulation of 0.1 µm PS-MPs in Caco-2 cells, indicating that it might act as substrates of the ABC transporters.

251. 5 µm PS-MPs (>80 µg/mL) inhibited plasma membrane ABC transporter activity *via* mitochondrial depolarisation and potential depletion of ATP.

252. The authors further explored how the inhibition of ABC transporters could influence the accumulation and toxicity of other substances. For this, arsenic (As) at 75 or 150 µg/mL was chosen. The exposure concentrations were 20 µg/mL for 0.1 µm PS-MPs and 80 µg/mL for 5 µm PS-MPs, with an incubation period of 12 hours.

253. For 0.1 µm PS-MPs increased intracellular As concentrations in Caco-2 cells was observed, suggesting that at this size, the PS-MP decreased As efflux pump activity by inhibiting ABC transporter activity.

254. Whilst 5 µm PS-MPs did not obviously increase As accumulation in Caco-2 cells, it did however, cause a synergistic effect in causing cytotoxicity. The authors suggested that this might be attributed to the interactive effects between intracellular reactive oxygen (ROS) generation and mitochondrial depolarisation induced by both chemicals.

255. Schirinzi *et al*., (2017) studied the cytotoxic effects of 3-16 µm PE and 10 µm PS-MPs on cerebral (T98G) and HeLA epithelial cells[18] at 0.05, 0.1, 1 or 10 mg/mL for 24 hours. Exposure to both MP types at all tested concentrations was not shown to significantly reduce cell viability, however, in the case of PE-MPs; ROS generation was observed on T98G cells. Although, in both cell cultures PS-MPs produced higher ROS generation levels. The observation was thought to be size related by the authors. Effective concentration 50 (EC_{50}) values[19] were reported (Table. 6).

[16] ABC transporter: ATP binding-cassette transporter is responsible for the transport of solutes against a chemical gradient, resulting in alternating access from inside and outside of the cell for unidirectional transport across the lipid bilayer.
[17] PSC833 and MK751: are compounds that are known to block P-gp and MRP transporter activity, respectively.
[18] HeLa epithelial cells: immortal cell line derived from cervical cancer cells.
[19] EC_{50} (Effective concentration 50) values: are the concentrations of drugs or compounds that provide half-maximal response to a host organism.

Table. 6 EC_{50} values for the exposure of PE and PS-MPs on cerebral (T98G) and epithelial (HeLa) cells for 24 hours, as determined by high-content assay (reproduced from Schirinzi *et al*., 2017).

	EC_{50} (mg/L) (95% confidence intervals)	
	T98G	**HeLa**
Polyethylene	41.22 (12.8 – 133)	40.96 (17.8 – 178.8)
Polystyrene	9.62 (3.9 – 23.8)	13.56 (2-96)

Summary of human in vitro data

256. From the limited amount of data, it can be concluded that gut uptake of microplastic particles is possible, however, this is size and concentration dependent. Suggested adverse effects include, disruption of the ABC transporter function which may lead to cellular apoptosis.

Mammalian data

In vivo

257. Stock *et al*., (2019) conducted a study in male Hmox1[20] reporter mice (HOTT mouse (McMahon *et al*., 2018)) (n=20) to analyse transport at the intestinal epithelium and oxidative stress response, as a potential consequence of microplastic exposure. Mice were administered orally with either 1, 4 or 10 µm PS-MPs in 0.5% carboxymethyl cellulose (CMC) (w/v) at a volume of 10 mL/kg bw three times per week for 28 days. The control group only received 0.5% CMC (w/v) at the same volume previously stated.

258. Mortality was not observed at any of the doses tested; animals appeared healthy and no clinical signs of distress were observed. Furthermore, histopathological examination of intestinal tissue revealed normal tissue morphology. β-galactosidase reporter analyses did not reveal evidence for occurrence of inflammation and/or oxidative stress as a cause of PS-MPs exposure. No particles were found in other organs (*e.g.* liver, spleen and kidney). It is important to note that the authors did not carry out quantitative analysis of particle uptake, due to the very low numbers of particles detected in the intestinal tissue.

Effects on the gut microbiota

259. Lu *et al*., (2018) studied the effects of PS-MPs on the gut microbiota of male mice (n=40). Mice were split into four treatment and one control group (n=8/group) and were administered with 0.5 or 50 µm PS-MPs at 100 or 1,000 µg/mL via the drinking-water for 5 weeks. Control group were administered water without any PS-MPs.

[20] Haeme oxygenase 1 (Hmox1): Hmox1 is a cytoprotective enzyme with anti-inflammatory and anti-oxidant properties, which induced in response to multiple environmental stimuli and disease states.

260. At 1,000 µg/mL, both sizes of PS-MPs caused decreased body, liver and lipid weights. Serum levels of hepatic triglyceride and total cholesterol decreased in both sizes of PS-MPs for the higher treated dose, which corresponded to the transcription levels of genes related to glucose (*Cherp* and *Pk*) and lipid metabolism (*Fatp2*, *Fat*, *Cs*, *Pparα*, *Pparγ*, and *Fas*).

261. The secretion of mucin was shown to have decreased significantly for all treated mice when compared to the control. Changes in the gut microbiota were also reported. At the phylum level, *Firmicutes* and *α-proteobacteria* decreased in abundance for both sizes of PS-MPs at the highest dose and at 50 µg/mL of 50 µm PS-MPs. At the genus level; a total of 6 and 8 types of bacteria changed in the 0.5 µm and 50 µm PS-MPs treated groups, respectively.

262. A follow up study by the same group, explored the potential metabolic effects caused by the altered composition of gut microbiota (Jin *et al*., 2019). Twenty-four male ICR mice were split into 3 groups (n=8/group). Treated groups were administered 5 µm PS-MPs at 100 or 1,000 µg/mL *via* the drinking-water. Control groups received water without any PS-MPs. A further two groups of male ICR mice (n=5/group) were administered orally with 5 µm fluorescent PS-MPs at 0 and 1,000 µg/mL for histopathological findings.

263. Several result parameters were reported. Fluorescent signal was visible in the guts of mice that were treated with 1,000 µg/mL fluorescent PS-MPs for 6 weeks. Sera analysis revealed that serum pyruvate levels increased, whereas triglyceride and total cholesterol levels decreased. Arginine and fumarylacetoacetate[21] serum levels increased at the highest treated group (Table. 7).

Table. 7 Effects of PS-MPs exposure on the serum indexes; values expressed as mean ± standard error mean (reproduced from Jin *et al*., 2019).

	Control	100 µg/mL	1,00 µg/mL
Triglyceride (mmol/L)	1.31 ± 0.12	0.78 ± 0.13*	1.12 ± 0.18
Total cholesterol (mmol/L)	6.17 ± 0.41	5.88 ± 0.20	5.34 ± 0.48
Pyruvate (mmol/L)	0.54 ± 0.08	0.73 ± 0.20	0.63 ± 0.07
Arginine (U/L)	13.17 ± 0.54	13.53 ± 0.67	15.12 ± 0.55*
FAH (U/L)	57.5 ± 3.04	59.79 ± 2.98	66.08 ± 2.26*

Abbreviations FAH: fumarylacetoacetate; * p <0.05 versus control.

264. Transcription analysis of genes related to ion transport were down-regulated in the colons (*Cftr*, *nkcc1*, *Nhem*, and *SLC26A*) and ileums (*Ano1*, *Cftr*, *nkcc1*, *Nhem*, and *SLC26A*) of treated mice at the highest dose. Altered structure of the gut microbiota were also observed. The relative abundance of *Firmicutes* and *β-proteobacteria* in the 100 µg PS-MPs/mL treated group and *α-proteobacteria* and *γ-proteobacteria* in the 1,000 µg PS-MPs/mL treated group, were significantly decreased when compared to the control group.

[21] Fumarylacetoacetate: a metabolic enzyme that catalyses the last step of tyrosine catabolism.

KEGG analysis[22] was performed to understand the differences in the metabolic pathways of functional genes in the microbial community between treated and non-treated groups. From this analysis, pyruvate and tyrosine metabolism, fatty acid biosynthesis and bacterial invasion of epithelial cells were predicted.

Neurobehavioural effects

265. Rafiee *et al*., (2018) analysed the potential neurobehavioural effects of PS-NPs (~38.92 nm in diameter) after 5 weeks of oral dosing on male Wistar rats (n=6/dose group). Treated doses were 1, 3, 6, and 10 mg PS-NPs/kg bw/day and two control groups were also set-up, one with sterile deionised water and the other with surrounding medium.

266. Behavioural tests performed included locomotor activity in the open field, Y maze test (to assess spatial working memory), elevated plus maze (to assess total motor activity), rotarod test (to test co-ordination), and passive avoidance (to test memory retention).

267. The increase in body weights of rats did not differ among groups during exposure to PS-NPs. None of the rats showed any clinical sign of toxicity, and only one death was recorded in the 1 mg PS-NPs dose group (death not further explained). Abnormal behaviour was seen in the 6 mg PS-NPs dose group, animals were observed to have fought each other that resulted in injuries. The authors therefore, excluded this dose group in further data analysis.

268. No statistically significant behavioural effects were observed in all tests performed, however, in the elevated plus maze, PS-NPs exposed rats showed greater number of entries into open arms when compared to control rats. Additionally, in the time spent on the rotarod test for the 3 mg PS-NP dose group was shorter (~160 seconds) when compared to baseline values (~220-240 seconds).

269. The authors concluded that uptake of PS-NPs did not affect the behaviour of adult male Wistar rats. Although, no treatment related difference was observed, subtle and transient nature of neurobehavioural effects were observed, however, these could not be attributed to PS-NP exposure due to the lack of statistical power. The authors recognised that a follow-up study with a greater cohort number would be required.

Co-exposure of microplastics with other contaminants

270. Deng *et al*., (2018) co-exposed mice to PE and PS-MPs (size range of 0.5-1.0 µm) with either tris(2-chloroethy) phosphate (TCEP) or tris(1,3-dichloro-2-propyl) phosphate (TDCPP) for 90 days. A total of 65 five-week-old

[22] Kyoto Encyclopaedia of Genes and Genomes (KEGG) analysis: a database for systematic analysis of gene functions, linking genomic information with higher order functional information.

male mice were utilised (n=5/group). Five served as a control group; treated with water only, twenty were separately dosed with TCEP or TDCPP at concentrations of 10 and 100 µg/L, twenty were separately dosed with 2 mg/L PS-MPs (3.7×10^8 particles/L) and TCEP or TDCPP at 10 and 100 µg/L, the same was carried out for PE-MPs. Biochemical markers and metabolomics were used to determine whether MPs could enhance the toxicity of the organophosphorus flame retardants (OPFRs).

271. Results for biomarker analysis are hereby presented. Superoxide dismutase and catalase activity increased by 21% and 26% respectively in the 10 µg/L TDCPP and PE-MPs treated group compared to the TDCPP group. Lactate dehydrogenase activity in TDCPP and both MP groups were higher (18-30%) than those in the TDCPP groups. Acetylcholinesterase activity in TCEP and PE groups (both doses) were lower (10-19%) than those in TCEP treated group.

272. Metabolomic results are hereby presented. Forty-one metabolites in both TCEP MP treated groups were significantly changed (>1.2 fold-change), whilst for TDCPP PS and PE groups 40 and 37 metabolites were also significantly changed, respectively. Most of these metabolites related to pathways of amino acid (*e.g.* valine, leucine and isoleucine biosynthesis) and energy metabolism (*e.g.* glycolysis and gluconeogenesis). Based on the data, the authors concluded that MPs aggravate the toxicity of the OPFRs.

Data on aquatic organisms

In vivo data

Cardiotoxicity

273. Pitt *et al*., (2018a) studied the toxic effects of PS-NPs in developing zebrafish (*Danio rerio*). Embryos (n=8; 2/group, 6 hours post fertilisation) were exposed to 0, 0.1, 1 or 10 ppm of PS-NPS ranging from 20-100 nm for 114 hours.

274. Exposure to PS-NPs were not found to significantly induce mortality, deformities, or changes to mitochondrial functionality, however, all treated groups exhibited significant bradycardia when compared to the control group.

Summary of adverse effects of micro and nanoplastics in animal health

275. A brief overview above has been provided for the toxic effects of micro and nanoplastics. *Fig. 9* provides a general overview of the reported adverse effects within literature, which also includes reported effects in environmental animal models (*e.g.* mobility effects, reduced vigour, blocking of intestine).

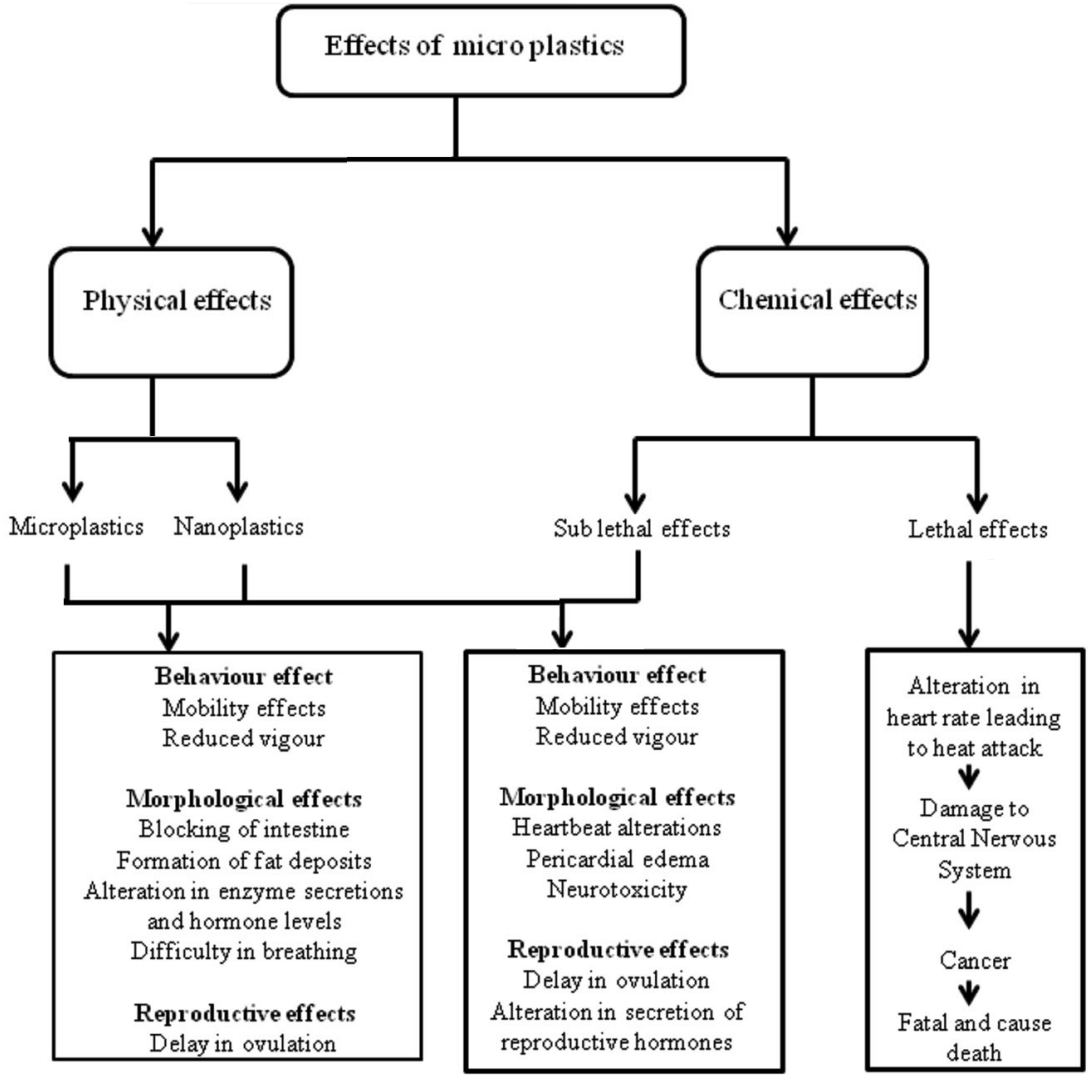

Figure. 9 Flow diagram illustrating the adverse effects of microplastics on animal health (adapted from Sharma & Chatterjee, 2017).

276. To refer back to the EFSA evaluation, the CONTAM Panel concluded that the risks of toxicity from micro- and nanoplastics themselves from oral exposure could not be assessed due to the lack of data, especially with regards to metabolism and excretion. The biomagnification of substances (additives or contaminants) in microplastics in seafood was low based on conservative exposure estimates, and it would have a small effect on the overall exposure to additives or contaminants.

277. The WHO Panel concluded that based on the limited evidence available, chemicals and microbial pathogens associated with microplastics in drinking-water pose a low concern for human health. No adverse health effects are expected from chemical contaminants present in microplastics for drinking-water based on MOE calculations. With regards to nanoplastics, the WHO panel concluded that no reliable information suggests it is of concern to humans.

Exposure data

278. Touissant *et al*., (2019) provided a review of micro and nanoplastic contamination in the food chain, which aimed to understand human exposure. They analysed peer-reviewed publications since 2010 that documented the presence of micro and nanoplastics in edible animal species (201 species; 164 sea fish, 23 molluscs, 7 crustaceans, 2 birds, 2 sweet water fish, 2 turtles and chicken) and some food products (canned sardines and sprats, sea salt sugar, honey, beer and water) that are part of the human food chain, which may or may not contribute directly or indirectly to the uptake of micro and nanoplastics in the human diet. The authors identified ~200 papers that were utilised for the review.

279. Micro and nanoplastics contamination is possible across all compartments of the environment (air, water and soil). Different sources of microplastics particles include domestic, industrial, agricultural and fishing use/production/waste of products containing plastic particles. Primary or intentionally added microplastics poses as an additional source that contaminates the environment and thus re-enters the food chain, resulting in undeliberate exposure to humans. The European Chemicals Agency (ECHA) has submitted a restriction proposal for microplastic particles that are intentionally added to mixtures used by consumers or professionals. Should the restriction be adopted, it is estimated that there will be a reduction of the amount of microplastics released to the environment in the EU by ~400,000 tonnes over 20 years (ECHA, 2019).

280. Some data gaps identified included the uncertainties of different intermediate food processing/treatment/ distribution steps and how it could potentially cause contamination by microplastics, the absence of data on farm animals' contamination through feeding and its potential effect on animal health or on meat quality for human consumption.

281. Comparability of results was deemed a challenge, as studies utilise different analytical methods (some with no blank/control analysis), and the expression of detected levels (*e.g.* in mussel studies results are expressed as items/g of mussels (as an average) or items/individual mussel). The standard deviation, in some cases, is larger than the average, which might reflect either a large scatter of the results and/or insufficient sample number, not representative of the population. Furthermore, it must be noted that some studies utilise concentrations and/or pristine particles that are not representative of what is found in the environment.

282. From the analysis the authors identified two challenges that hindered an accurate estimate the global human exposure to microplastic through the diet. These were the need for a detailed and agreed definition of micro and nanoplastics and the need for standardised methods and quality assurance. The authors further commented that it is important to address the variety of food product consumption around the world (and so different ethnicities).

283. Cox *et al*., (2019) evaluated the number of microplastic particles (MPPs) in commonly consumed foods in relation to their recommended daily intake for the American population. 402 data points from 26 studies representing over 3,600 processed samples were utilised for the analysis. Male adults were found to be exposed to 142 MPPs from the diet and 170 MPPs *via* inhalation, daily; this results in a total annual exposure to ~120,00 MPPs annually, for female adults the value was ~98,000 MPPs. Annual exposure combining both exposures in children were also estimated at ~81,000 for males and ~74,000 in females. The authors further noted, that their study did not consider the number of MPPs that enter the human digestive system by atmospheric fallout settling onto food during meals or the increases of MPPs content that occur during food preparation.

284. Catarino *et al*., (2018) further supports the hypothesis above. They compared the potential exposure to humans to household dust fibres during a meal to compare with amounts of MPPs present in edible mussels from Scottish waters collected throughout 2015. The mean number of MPPs in *M. modiolus* was 0.086/g ww (n=6). In *Mytilus* spp. the mean number of MPPs/g ww was 3.0 (n=36). Fibres were the most common shape morphology of detected fibres utilising FT-IR and NR staining techniques. PET was estimated to be the most common plastic type. The authors estimated that MPPs ingestion by humans via consumption of mussels is 123 MPPs/y/capita in the UK, however, the risk of plastic ingestion *via* mussel consumption was minimal when compared to fibre exposure during an evening meal via dust fallout in a household at ~14,000-68,000 MPPs/y/person. This range value was based on the following assumptions; 1 particle per 20 minutes for an area of 4.32 cm^2, extrapolate this value for a 12.5 cm radius plate, resulting in 114 particles, equating to ~42,000 MPPs consumption/year/person, for 20 minutes during consumption of an evening meal. During a cooking period of 20 minutes, 5 MPPs per 4.32 cm^2 was estimated, leading to the potential of ingesting a further ~207,000 MPPs/year/person. These values were then corrected by 33% which was reported to be the amount of petrochemical based fibres found in dust by Dris *et al*., (2017).

285. The potential exposure of microplastics from breast milk to infants were considered, specifically relating to its storage in plastic bottles. The potential risks of ingesting microplastics from bottled water as a source has been discussed. Available data suggest that the presence of MPPs in bottled water are due to the manufacturing process, however, the quality of the plastic and lid cracking have also been found to contribute to the overall number. Thus, it could be hypothesised that mothers storing breast milk for later personal use or for donation to hospitals or milk banks in plastic containers; may be a potential source of microplastic exposure to infants.

286. No data has yet been reported to prove this hypothesis, however, an ongoing study; titled Mothers' information on lactation and collection (MILC) study carried out by Bradman and his colleagues at UC Berkeley are assessing breastmilk collection and storage materials to determine whether inappropriate handling and storage increases chemical contamination in

breastmilk, however, it is not clear whether the presence of microplastics is within the scope of this research (MILC, 2016).

287. Due to the uncertainties as described in the Touissant *et al*., (2019) paper, an exposure assessment could not be performed at this time.

Risk assessment

288. The EFSA Scientific Committee have published a guidance on the risk assessment of the application of nanoscience and nanotechnologies in the food and feed chain. They acknowledged that waste nanoplastics are generated and that exposure of humans and animals occurs through the food chain, however, they considered the topic to be outside the scope of this working group and therefore was not addressed in the Guidance (EFSA, 2018).

289. Koelmans *et al*., (2019) proposed a quality assessment criterion to rank the reliability of published results in literature with the aim to better understand the potential exposure and to inform human health risk assessments. There are nine criteria based on reproducibility, precision, accuracy and sensitivity; these are sampling method, sample size, sample processing and storage, laboratory preparation, clean air conditions, negative controls, positive controls, core sample treatment and polymer identification. For each criterion, a value of 2 (reliable), 1 (reliable to a limited extent) or 0 (unreliable) is assigned. Therefore, the “Total Accumulated Score” is calculated by adding scores for individual criteria (maximum 18 points). For data to be considered reliable, a study should preferably have no ‘zero’ values for any of the individual scores.

290. At this stage, a full risk assessment on the potential toxic effects of micro and/or nanoplastics could not be carried out due to the lack of comparative data available for baseline levels of both compounds. Furthermore, there is no established NOAEL, for each polymer type.

291. From the perspective of both EFSA and WHO Panels, the risk of chemical leachants and adsorbed substances from microplastics is not expected to cause adverse health effects in humans due to their small contribution to the overall exposure from other sources of the chemical.

Further considerations

292. There is no internationally agreed definition of what a microplastic is. Analytical methodology processes are limited to FTIR, Nile Red, Micro-Raman spectroscopy and mass-spectroscopy. Additionally, there are no standardised testing methods for different matrices (*i.e.* air, soil, food and water), and the available methods have their own associated limitations. Furthermore, no single technique is suitable for all plastic types and for all particle sizes or shapes. Using a suite or generation of techniques may be necessary.

293. Comparison of studies can be difficult due to differences in sampling, extraction, purification and analytical methods for enumerating and characterising microplastics are not yet standardised. Contamination with airborne microplastics or cross-contamination of samples pose as an issue, control samples may be difficult to ascertain.

294. Most studies have performed tests on pristine particles, therefore it is important to consider inter-variability of samples and batches and how this may not be representative of what is present in the environment (*i.e.* particles have not undergone degradative processes in the environment).

295. Comprehensive assessment of MPs and contaminant concentrations in seafood species and the impact of what cooking may have on the desorption and subsequent bioavailability of contaminants/leachants, needs to be further investigated to better understand the implications for human health.

296. The presence of MPs in seafood and water may need to be put into perspective with other sources of MPs such as atmospheric fallout.

297. Current studies typically only deal with one type of particle/tissue interaction, as such, further research is necessary to explore the effects of the range of particle types *in vitro* and/or *in vivo*.

298. Since microplastic concentrations are expected to increase in the future, it will be important to establish a monitoring programme to regularly assess the levels of microplastics in food, water and the air.

299. There is also a need to study the assimilation of a range of microplastic sizes and compositions into human tissues and in the development of techniques capable of identifying the presence of microplastics in the human body (*e.g.* biopsies and tissue banks).

300. The most significant data gaps appear to be the lack of appropriate and harmonised analytical methods for the detection of micro and nanoplastics, as well as their toxicokinetic and toxicity profiles.

Summary and conclusions

301. Microplastics are omnipresent, they are either intentionally added to products or are fragmented down into smaller sizes by natural processes (*e.g.* weathering, corrosion *etc*.). There is no internationally agreed definition of what a microplastic is, however, there is a general acceptance that the size range is from 0.1-5,000 µm.

302. The routes for which humans can be exposed to MPs include oral *via* the food chain from marine products, drinking-water, and other food products such as beer, honey and salt. Airborne MPPs can also be inhaled.

303. The EFSA CONTAM Panel concluded that the risks of toxicity from micro- and nanoplastics themselves from oral exposure could not be assessed due to the lack of data, especially with regards to metabolism and excretion. A worst-case consumption of 7 µg microplastics in a 225 g portion of mussels was calculated. Concerning the presence of additives or contaminants in microplastics in seafood, conservative estimates would have a small effect on the overall exposure to additives or contaminants.

304. The WHO Panel estimated an intake 1.4 µg of microplastics/kg bw/day for a 60 kg adult, although realistic estimates based on reported data ranged from 0.01-8.7 µg of microplastics/kg bw/day. The WHO Panel concluded that based on the limited evidence available, chemicals and microbial pathogens associated with microplastics in drinking-water pose a low concern for human health and no adverse health effects are expected from chemical contaminants present in microplastics for drinking-water based on MOE calculations. Furthermore, the routine monitoring of the presence of microplastics in drinking water is not advised at this time; as there is no evidence to indicate human health concern. Additionally, drinking-water treatment processes have been shown to be efficient at removing particles including the nanoscale. With regards to nanoplastics, there was insufficient information available at the time of review for the WHO Panel to be able to draw conclusions on their toxicity, although, no reliable information suggests it is of concern to humans.

305. UK specific data for the presence of microplastics in drinking-water was available from the UKWIR report. To summarise, the UK water industry has been found to be successful at removing microplastics >25 µm in size from raw water or crude sewage, >99.99%. Raw water was detected with an average of 4.9 mpp/L and potable water having an average of 0.00011 mpp/L, whilst the average was 5.1 mpp/L for wastewater effluent samples. Due to technical constraints, smaller particles were not analysed and as such the report could not comment on how effective water treatment processes are at filtering these materials.

306. In terms of microplastics in the air, their fate and dispersion in indoor and outdoor environments are dependent on several factors. For fate these include; vertical pollution concentration (higher concentrations near the ground), wind direction and speed, precipitation (affecting particles >2.5 µm) and temperature. For dispersion these factors include; wind modulation caused by topography, local meteorology and thermal circulation. Particle residence time in the atmosphere is influenced by rainfall, wind, local conditions and the particle size. Polymers of lower densities are lighter and can therefore be carried by wind, whilst those of larger densities are found in sediments.

307. Exposure to low concentrations of airborne microplastics is expected in outdoor air due to dilution. There is still little information regarding the concentrations of airborne microplastics, however, the Dris *et al*., (2016, 2017) studies carried out in Greater Paris provides indoor concentrations of 1-60 fibres/m^3 and outdoor concentrations of 0.3-1.5 fibres/m^3.

308. The indoor behaviour of airborne microplastics is dependent on room partition, ventilation, airflow, resulting in higher concentrations in rooms downwind. Catarino *et al*., (2018) calculate fibre exposure during an evening meal *via* dust fallout in a household at ~14,000-68,000 MPPs/y/person.

309. Occupational diseases described seem to result from the toxicity after inhalation of plastic particles or their leachates. In humans, the response to inhaled particles are dependent on differences on individual metabolism and susceptibility, as well as, the patient's physiology and lung anatomy which is a factor of microplastic deposition. Clearance relies on mechanical methods, mucous progression towards the pharynx by persistent beating cilia, alveolar macrophage phagocytosis and latter migration and lymphatic transport (*Fig. 1*). Fibres (up to 250 µm) have been shown to have higher potential for penetration deep in the lung tissue.

310. In general, the mechanisms of inhaled particle injury include dust overload, oxidative stress, cytotoxicity, translocation and cancer.

311. In terms of the potential risks from plastics from ground soil exposure *via* transfer to food crops, there is evidence to suggest that the plastic particle loading in agroecosystems is could be high due to inputs of some recycled organic waste, plastic film mulching, and aerial depositing of plastic particles (*Fig. 4*).

312. Nanoplastic uptake has been shown in edible food crops and there is concern that plant xenobiotic processes may produce novel compounds within the food chain. The proposed pathways for entry of CNPs into plants include; endocytosis *via* the plasmodesmata, passage *via* ion transport channels, carrier proteins or aquaporins, and additionally soil carbon or root exudate mediated entry.

313. To date, no study has reported the uptake of microplastics in plants, however, it is recognised that additional studies are required to assess and better understand microplastic transfer from soil to humans through uptake in food webs and through leaching to the groundwater.

314. Toxic effects of microplastics in humans have been observed in terms of inhalation of synthetic microfibres in an occupational setting. Other observed toxic effects in animal models stem from either a direct physical or indirect chemical effect.

315. Physical effects include behavioural, morphological and reproductive effects (*Fig. 9*). It is hypothesised that the physical presence of MPs may be toxic due to their inherent ability to induce intestinal blockage or tissue abrasion, which has been observed in some animal models, however, intestinal blockage is not expected in humans.

316. Available *in vitro* data on human Caco-2 cells showed preferential size uptake was observed; 4 > 1 > 10 µm, with a recovery range of 0.8-3.8%.

Phagocytosis or endocytosis could be the preferred route of intake for MPs, and it has been observed that phagocytosis by macrophages in the intestinal epithelium may occur with particles >0.5 µm.

317. Inherent (additives, colourants) or adsorbed compounds are not expected to cause adverse effects since they contribute as a small percentage to the overall exposure of the compound.

318. From the available toxicokinetic data, distribution of MPs in tissues is partially determined by particle size. Particles > 150 µm are not absorbed, smaller particles especially those within the nanoscale (1 – 100 nm) are able to absorb into all organs.

319. The metabolism of microplastics is not expected due to their persistent nature.

320. Microplastic fibres have been detected in human stool samples, however, this dataset has limitations, such as low number of participants whom only provided one stool sample. Furthermore, the origin and fate of microplastics in the GIT were also not investigated.

321. In terms of exposure assessment, an American study (Cox *et al*., 2019) has proposed an estimated daily consumption and inhalation of 142 MPPs and 170 MPPs, respectively; this results in a total annual exposure to ~120,00 MPPs annually in males, and for female adults the value was ~98,000 MPPs. Although, this calculation did not include values for the atmospheric deposition of microplastics during food preparation and consumption. Should this factor be considered, an estimated additional microplastic fibre exposure of ~14,000-68,000 MPPs/y/person has been calculated during an evening meal *via* dust fallout in a household.

322. A full risk assessment on the potential toxic effects of micro and/or nanoplastics could not be carried out due to the lack of comparative data available for baseline levels of both compounds. Furthermore, there is no established NOAEL for each polymer type, however, data from the WHO report presents a NOAEL of ~2,500 mg/kg bw/day (at the highest 5% inclusion in the diet) for PET powder (paragraph 42).

Questions on which the views of the Committee are sought

323. Members are invited to consider the following questions:

i). From the information above, do the Committee agree that available data are not sufficient to perform a risk assessment?

ii). If yes, which data gap(s) do the Committee consider to be more important, so that a risk assessment may be performed?

iii). What future research would the Committee recommend?

iv). What are the Committees view on the quality of the exposure assessment data?

v). Does the Committee wish to see UK specific exposure data (utilising UK consumption and UK occurrence data, where available) that estimates overall exposure to microplastics?

vi). Do the Members consider a statement on microplastics would be useful?

vii). Does the Committee have any other comments?

Secretariat
October 2019

Abbreviations

ABC	Adenosine triphosphate binding cassette transporter
ABS	Acrylonitrile butadiene styrene
AMX	Amoxicillin
ANOVA	Analysis of variance
AQEG	Air Quality Expert Group
As	Arsenic
ATP	Adenosine triphosphate
BaP	Benzo[a]pyrene
BCF	Bioaccumulation factor
BCNPs	Black carbon nanoparticles
BfR	The German Federal Institute for Risk Assessment
BPA	Bisphenol A
CAT	Catalase
Chg H	Choriogenin H gene
CIP	Ciproflaxin
CMC	Carboxymethyl cellulose
CNPs	Carbon nanoparticles
CNTs	Carbon nanotubes
CONTAM	Contaminants in the Food Chain
COT	Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment
Defra	Department for Environment Food and Rural Affairs
DMEM	Dulbecco's Modified Eagle Medium
DMSO	Dimethyl sulfoxide
dw	Dry weight
EC_{50}	Effective concentration 50
ECHA	European Chemicals Agency
EFSA	European Food Safety Authority
EU	European Union
FAH	Fumarylacetoacetate
FAO	Food and Agriculture Organisation
FTIR	Fourier-transform infrared spectroscopy
GIT	Gastrointestinal tract
HDFs	Human dermal fibroblasts
HGVs	Heavy goods vehicles
HMC-1	Human mast cell line-1
Hmox	Haeme oxygenase 1
kd	Distribution coefficient
KEGG	Kyoto Encyclopaedia of Genes and Genomes
LDPE	Low-density polyethylene
LGVs	Large goods vehicles
LOD	Limit of detection
LOQ	Limit of quantification
MCNPs	Manufactured carbon-based nanoparticles
MCT	Maximal tissue concentration
MILC	Mother's information on lactation and collection
MOE	Margin of exposure
mpp/L	Microplastic particles per litre

MPPs	Microplastic particles
MPs	Microplastics
MRT	Maximum resident time
MWCNTs	Multi-wall carbon nanotubes
NEE	Non-exhaust emissions
NOAEL	No observed adverse effect level
NR	Nile Red
OECD	Organisation for Economic Co-operation and Development
OPFRs	organophosphorus flame retardants
PA	Polyamide
PAHs	Polyaromatic hydrocarbons
PBDEs	Polybrominated diphenyl ethers
PCBs	Polychlorinated biphenyls
PE	Polyethylene
PE-MPs	Polyethylene microplastic particles
PEST	Polyester with polyethylene terephthalate
PET	Polyethylene terephthalate
PHE	Public Health England
PMMA	Poly(methyl methacrylate)
POPs	Persistant organic pollutants
PP	Polypropylene
PS	Polystyrene
PS-MPs	Polystyrene microplastic particles
PS-NPs	Polystyrene nanoplastic particles
PUR	Polyurethane
PVC	Polyvinyl chloride
ROS	Reactive oxygen species
RSI	Raman spectral imaging
SDZ	Sulfadiazine
SEM	Scanning electron microscopy
SEM-EDX	Scanning electron microscopy coupled with an energy dispersive detector
SOD	Superoxide dismutase
TAF	Total atmospheric fallout
TCEP	tris(2-chloroethy) phosphate
TC	Tetracycline
TDCPP	Tris(1,3-dichloro-2-propyl) phosphate
TG	Triglyceride
TMP	Trimethoprim
UKWIR	UK Water Industry Research
UV	Ultraviolet light
WHO	World Health Organisation
WTPs	Water treatment plants
WTWs	Water treatment works
WWTPs	Waste water treatment plants

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COMMITTEE ON TOXICITY OF CHEMICALS IN FOOD, CONSUMER PRODUCTS AND THE ENVIRONMENT

Scoping paper on the potential risks from exposure to microplastics

EFSA. (2016) Presence of microplastics and nanoplastics in food, with particular focus on seafood. Available at: https://efsa.onlinelibrary.wiley.com/doi/epdf/10.2903/j.efsa.2016.4501.

Secretariat
October 2019

Annex B to TOX/2019/62

COMMITTEE ON TOXICITY OF CHEMICALS IN FOOD, CONSUMER PRODUCTS AND THE ENVIRONMENT

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WHO. (2019) World Health Organisation Microplastics in drinking-water. Available at: https://www.who.int/water_sanitation_health/publications/microplastics-in-drinking-water/en/.

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October 2019

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Scoping paper on the potential risks from exposure to microplastics

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Gasperi, J., Wright, S., L., Dris, R., Collard, F., Mandin, C., Guerrouache, M., Langlois, V., Kelly, F. J., Tassin, B. (2018) Microplastics in air: Are we breathing it in? Current Opinion in Environmental Science & Health 1, pp. 1-5.

Secretariat
October 2019

COMMITTEE ON TOXICITY OF CHEMICALS IN FOOD, CONSUMER PRODUCTS AND THE ENVIRONMENT

Scoping paper on the potential risks from exposure to microplastics

Details of literature search carried out by the Secretariat at the Food Standard (FSA)

Relevant literature was obtained from reviews published by authoritative bodies, as described in paragraph 4 of the main scoping paper discussion paper. In addition, searches for further literature relating to the toxicity of micro and nanoplastics were carried out utilising the search terms below. The literature searches were performed by the Secretariat at the FSA, with a limit of publication date ranging from 2016-current.

Search terms

“Microplastics OR Nanoplastics &”

Toxicity
Toxicokinetics
Bioavailability
Absorption
Distribution
Metabolism
Excretion
Acute Toxicity - oral
Sub(chronic)tox/ carcinogenicity
Human exposure
Human health effects
Risk assessment
Genotoxicity
Reprotoxicity
Reproductive toxicity
Development
Developmental toxicity
Immunology
Immune system
Immunotoxicity
Neurotoxicity
Brain
Neurological effects
Neurology
Respiratory effects
Inhalation
Inhalation toxicity

Endocrine
Endocrine effects
Food
Soil
Water - drinking-water, bottled water, water treatment plants
Air/aerial – atmospheric fallout
Bioaccumulation/retention
Europe
United Kingdom - UK
Sorption of environmental chemicals - metals, POPs, pharmaceuticals, microbes
Leachates
Biomonitoring
Biofilm
Ageing
Degradation
Effect of tides, season, temperature