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

First draft statement on the potential risks from exposure to microplastics

Introduction

1. A scoping paper was presented to the Committee in October 2019 (TOX/2019/62)[1] on the potential risks from exposure to microplastics, given the interest in this area, the Committee concluded that a statement providing an overview of the sources, data gaps and research needs would be helpful in determining the potential risk from exposure to microplastics.

Existing evaluations

European Food Safety Authority (EFSA) opinion on microplastics in food, with particular focus on seafood

2. The EFSA Panel on Contaminants in the Food Chain (CONTAM) defined microplastics as a heterogeneous 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. 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) (EFSA, 2016). The EFSA opinion considered both microplastics and nanoplastics, as does this paper.

3. 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 EFSA 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.

4. Overall, the EFSA CONTAM Panel concluded that the risks of toxicity 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.

5. 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.

World Health Organisation (WHO) report on microplastics in drinking-water

6. Microplastics were described as ubiquitous in the environment and enter freshwater environments primarily from surface run-off water and wastewater 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.

7. 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.

8. No adverse health effects are expected from chemical contaminants present in microplastics for drinking-water based on margin of exposure (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.

9. 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.

10. 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).

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

12. 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 (WHO, 2019).

Narrative review of potential risks from inhalation exposure to micro and nanoplastics

13. Gasperi et al. (2018), 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.

14. 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.

15. 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.

16. 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[2], 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).

17. 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 77-83.

EU Science Advice for Policy by European Academies (SAPEA)

18. The EU SAPEA published a scientific perspective on microplastics in nature and society in January 2019[3]. The evidence report offers scientific perspective on knowledge about the implications of micro and nanoplastics in nature and society, further highlighting uncertainties and knowledge gaps in order to inform appropriate future actions (SAPEA, 2019).

19. The SAPEA concluded that there is a need for improved quality and international harmonisation of the methods used to assess exposure, fates and effects of nano- and microplastics on biota and humans.

20. The conclusion of the working group is that there was no evidence of widespread risk to human health from micro and nanoplastics at present, and that the absence of concrete evidence of microplastic risks at present did not allow us the working group to conclude with sufficient certainty either that risk is present or that it is absent in nature.

21. Adverse effects were observed (negative effect on food consumption, growth, reproduction and survival) once effect thresholds are exceeded. The concentrations utilised are higher than those reported in the environment. Furthermore, the utilisation of virgin or spherical particles are not

[2] 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.

representative, and often short exposure times are applied in laboratory studies in several aquatic organisms. As such, there is no evidence that these effects in nature. Therefore, these limit the reliability of the risk assessments for micro and nanoplastics.

22. Chemicals associated with microplastics can have additional human health effect(s) (which is deemed difficult to assess), *e.g.* reproductive toxicity and carcinogenicity, however, the relative contribution to chemical exposure of micro and nanoplastics among the mix of other chemicals probably represents a small proportion.

23. The SAPEA working group recommendations are listed in the following paragraphs. Firstly, it was recommended that there is a need to understand the potential modes of toxicity for different size-shape-type of micro and nanoplastics combinations in selected human models, before robust conclusions real human risks can be made.

24. Secondly, communicating transparently about the uncertainties in the scientific evidence is a safer approach than assuming a lack of risk, especially in sensitive domains such as food and human health. The authors conclude that there is consensus and momentum for action and no evidence of “plastic denial” phenomenon. Due to the lack of scientific understating, the precautionary principle has been part of the foundation for current regulations.

25. Close interdisciplinary collaboration between the natural, social and behavioural, and regulatory sciences was recommended as a way forward for addressing the complex issue of plastic waste and pollution.

26. The working group further concludes that it will be important to implement both agreements and legislation which focus on emission reduction and the use of less hazardous materials. Evidence suggests that focus should be on circular economy approaches, away from linear processes and end-of life clean-up.

EU Group of Chief Scientific Advisors; Scientific Advice Mechanism (SAM)

27. The EU Group of Chief Scientific Advisors, through SAM has published an opinion piece on the environmental and health risks of microplastic pollution in April. 2019[4]. The scientific evidence base and policy context were reviewed (SAM, 2019).

28. As an executive summary, the SAM advisors agreed that most laboratory studies to date does not reflect real-world exposure; and a better understanding is required of the effects of different concentrations, compositions, sizes and shapes of microplastics in ecosystems and humans before robust conclusions can be drawn about real risks.

29. Currently, available evidence suggests that microplastic pollution at present does not pose widespread risk to humans or the environment, however, there are significant grounds for concern and for precautionary measures to be taken.

30. A clear evidence-based communication of the uncertainties related to the environment, food and human health was also deemed necessary by the SAM advisors.

31. The SAM advisors provided three recommendations. Firstly, the broadening of policy cover to prevent and reduce microplastic pollution. Secondly, to address wider socio-economic and trade-off implications of microplastic pollution and policy actions. Lastly, to promote global cooperation, high-quality scientific exchange and policy coherence.

Literature search

32. 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 A, the years search was from 2016 – September 2019. 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.

Toxicokinetics

33. The size of particles is a determining key factor of uptake (*Fig. 1*). 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.

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

35. 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. 2-3), 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.

Nano-plastics (NPs)

Absorption, Distribution, Metabolism and Excretion (ADME)

Mammalian data

In vivo

36. 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.

37. 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.

38. 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%).

In vitro - Macrophage internalisation of nanoplastics

39. 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.

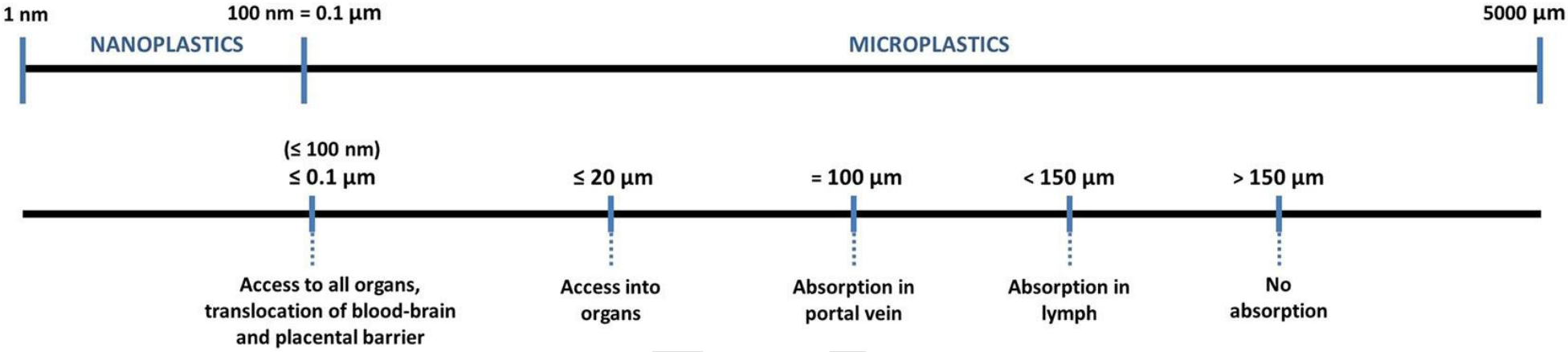

Figure. 1 - Diagram illustrating the fate of micro and nanoplastics in mammalian bodies. Microplastics particles > 150 µm are not absorbed, those that are smaller are able to absorb in the lymph (<150 µm), in the portal vein (=100 µm), and into organs (≤ 20 µm). Nanoplastic particles ranging from 1 –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

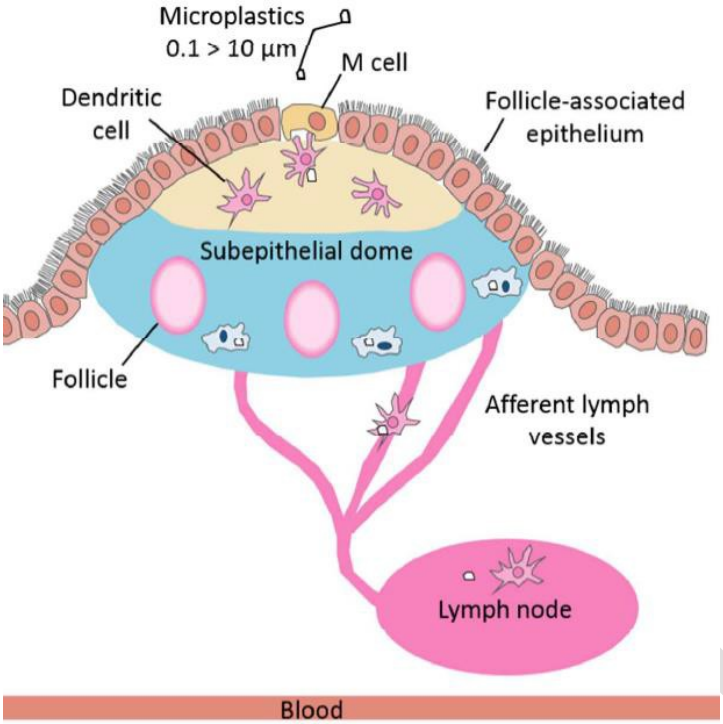

Figure. 2 - Diagram demonstrating the hypothesised microplastic uptake and clearance mechanisms in the GIT. Microplastic (0.1 > 10 µm) uptake from the GIT lumen via endocytosis by the M cells of the Peyer's patches. M cells sample and transport particles from the intestinal lumen to the mucosal lymphoid tissues (reproduced from Wright & Kelly, 2017).

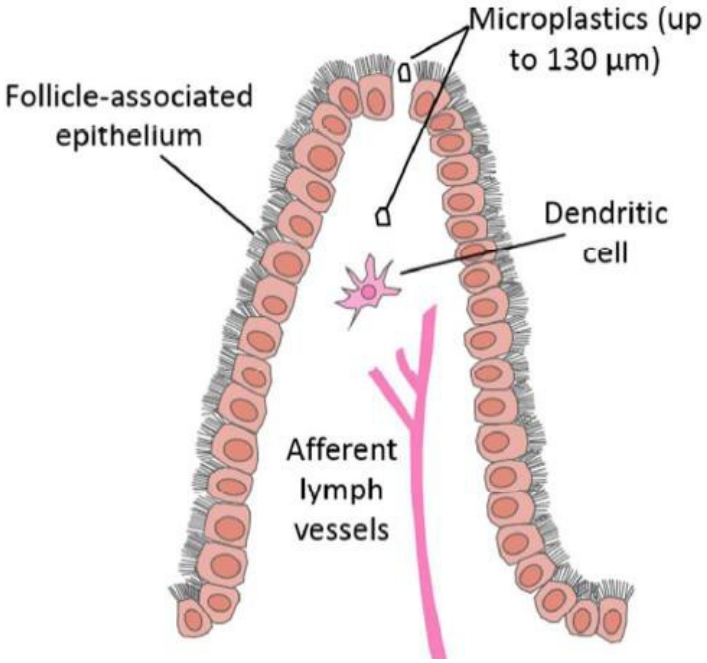

Figure. 3 - Diagram demonstrating the hypothesised microplastic uptake and clearance mechanisms in the GIT. Microplastic uptake from the GIT lumen via paracellular persorption. Non-degradable particles, such as microplastics, may be mechanically kneaded through loose junctions in the single-cell epithelial 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).

Data on aquatic organisms

In vivo

40. 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[5], 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.

41. 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 the 250 nm PS-NPs, with uptake values of 0.5 and 0.2 Bq/h[6]. 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

42. 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.

43. 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

[5] Chorion: the outermost membrane surrounding an embryo of a reptile, bird, or mammal.
[6] 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.

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).

44. 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.

45. 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[7] 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.

46. 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

47. 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.

[7] Bradycardia: is a slower than normal heart rate, what's considered too slow is dependent on age and physical condition.

48. Stool samples were tested for 10 types of plastics utilising Fourier-transform infrared spectroscopy (FTIR) 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).

49. 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

50. 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.

51. Cell viability of Caco-2 cells was measured by the cell titer blue and MTT[8] 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.

52. 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 monoculture. For 10 µm PS-MPs, lesser extent of recovery was observed of up to 0.07% in the mucus co-culture.

53. 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[9], 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.

54. 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.

[8] MTT assay: dimethylthiazole-2-yl]-2,5-diphenyltetrazolium bromide (MTT) is a colourimetric assay for assessing cell metabolic activity.
[9] THP-1: A human monocytic cell line derived from an acute monocytic leukaemia patient.

55. The impact on intestinal immune cells was analysed by Western blot and quantitative reverse transcription polymerase chain reaction (qRT-PCR). Western blot analysis showed that STAT-1 and STAT-6 proteins[10] 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

56. 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.

57. 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.

58. 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.

59. 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 (MTC) 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 MTC for the same tissues were 0.76, 0.78, and 0.78 mg/g. MTC 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

[10] 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.

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.

60. Although adverse effects were observed the study by Deng *et al*., (2017) 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.

61. 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.

62. 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).

63. 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.

64. 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.

65. 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.

66. In summary, age appears to be more important factor in determining the extent of uptake than sex or species.

Bioaccumulation and generational transfer

67. Al-Jaibachi *et al*., (2018) investigated the possible ontogenic transference of fluorescent MPs using mosquitos (*Culex pipiens*). 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.

68. 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[11].

69. The authors suggest that their results have implications for 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.

70. 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

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

72. 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

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

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.

73. Maternal transfer of nanoplastics has 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.

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

Toxicity

75. 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).

76. A study by Stock *et al*., (2019) (as discussed in paragraph 50 and further mentioned in paragraph 85) 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 vivo

77. 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.

78. 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.

79. 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.

80. 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).

81. 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.

82. 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.

83. 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.

In vitro studies

84. The Committee reviewed studies from Hwang *et al*., (2019), Wu *et al*., (2019) and Schrinzi *et al*., (2017). 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[12] function which may lead to cellular apoptosis.

Mammalian data

In vivo

85. Stock *et al*., (2019) conducted a study in male Hmox1[13] 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.

86. 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.

87. With reference to the toxicokinetic study by Deng *et* al., (2017), as summarised in paragraphs 70-73, adverse effects were also observed. Differences in final body and liver weight were not observed between the control and treated groups. No significant changes in daily food consumption between these two groups were 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 adenosine trisphosphate (ATP) levels and an increase in lactate dehydrogenase activity in a dose-dependent manner.

88. Metabolomic analyses determined a total of 37 differential metabolites to be significantly different across the exposure groups when compared to the control (*Fig. 4*). 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.

[12] ABC transporter: ATP-binding cassette (ABC) transporters are membrane proteins that are involved in the uptake and expulsion of a variety of substrates, including ions and small molecules such as sugars, amino acids, xenobiotics and vitamins, as well as larger molecules such as peptides, proteins and polysaccharides.
[13] Haeme oxygenase 1 (Hmox1): Hmox1 is a cytoprotective enzyme with anti-inflammatory and antioxidant properties, which induced in response to multiple environmental stimuli and disease states.

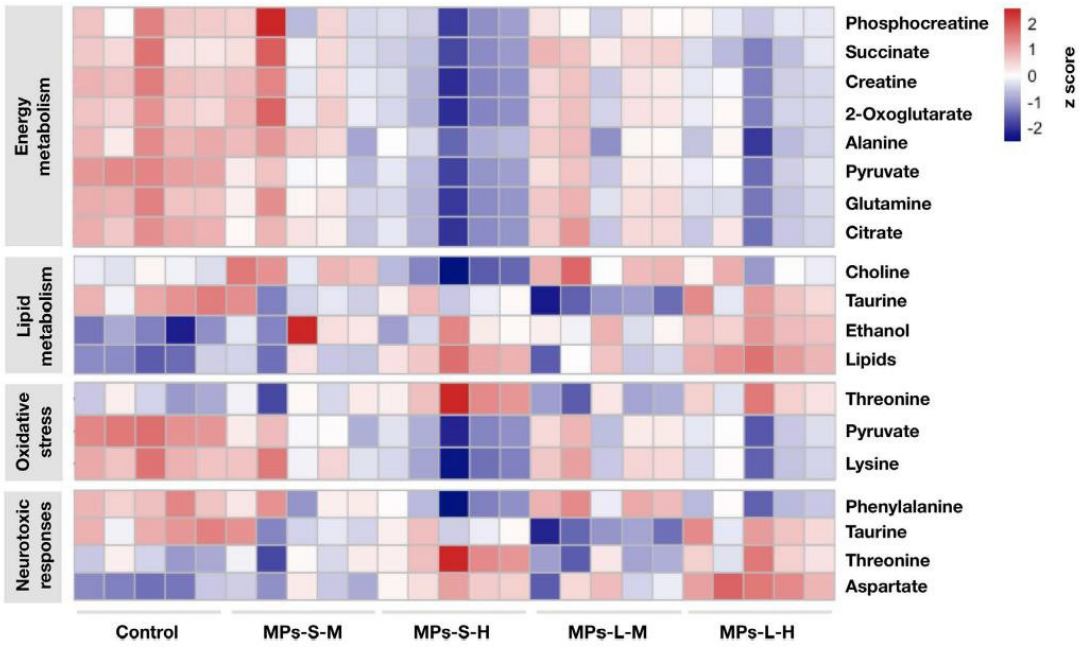

Figure. 4 - A heat map to depict the affected differential metabolites identified in different treatment groups calculated by z-scores[14]. M; 0.1 mg/day and H; 0.5 mg/day. S; small 5 µm and L large 20 µm. Red; increased activity and blue decreased activity (reproduced from Deng *et al*., 2017).

89. With reference to the study by Yang *et al*., (2019), as summarised in paragraphs 74-75, toxicity thresholds were estimated. 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.

90. 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).

Effects on the gut microbiota

91. 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

[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.

(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.

92. 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).

93. 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.

94. 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.

95. 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[15] serum levels increased at the highest treated group (*Table. 1*).

Table. 1 - 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.

96. Transcription analysis of genes related to ion transport were down-regulated in the colons (*Cftr*, *nkcc1*, *Nhem*, and *SLC26A*) and ileums (*Ano1*,

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

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. KEGG analysis[16] 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

97. 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). The doses used 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.

98. 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).

99. 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.

100. 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).

101. 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.

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

Co-exposure of microplastics with other contaminants

102. 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 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 x 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).

103. 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.

104. 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

105. 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.

106. 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

107. A brief overview above has been provided for the toxic effects of micro and nanoplastics. *Fig. 5* 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).

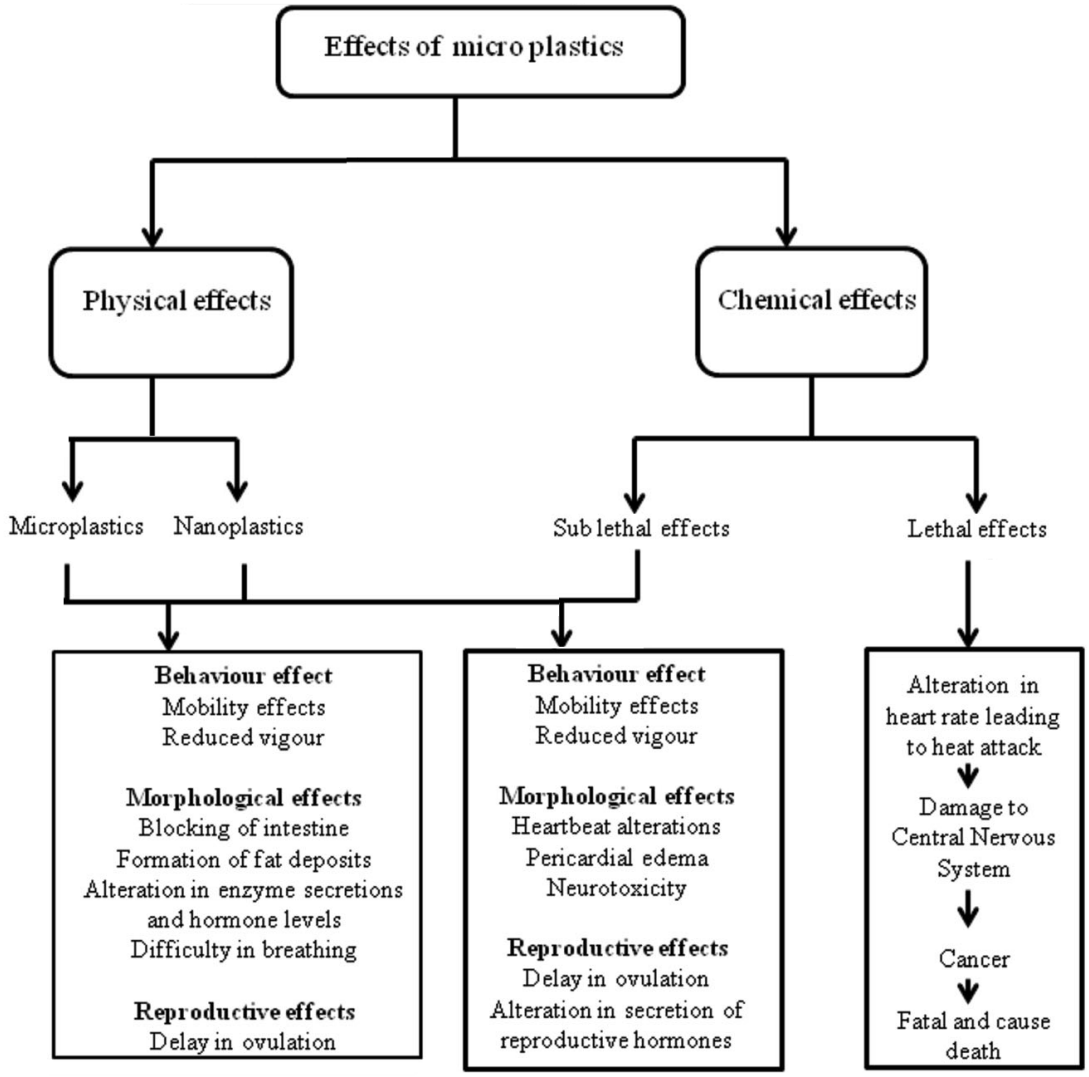

Figure. 5 - Flow diagram illustrating the adverse effects of microplastics on animal health (adapted from Sharma & Chatterjee, 2017).

108. 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.

109. 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

Potential risks of inhalation of micro and nanoplastics

Factors affecting deposition of inhaled particles in the lung

110. 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, it 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).

111. 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. Once in the airway, potential effects are described in the following section.

Potential mechanisms of inhalation toxicity

112. 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

113. 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

114. 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

115. 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).

Other sources of microplastics in the air

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

117. 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 polyethylene (PE); 20 µm) and 24 hours (PS; 10 µm, polyethylene terephthalate (PET); 14 µm wide and 1,000 µm long, and copper phthalocyanine[17] ~5 wide and 6 µm long). Therefore, the study appears to confirm presence of microplastics in ambient particulate matter.

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

119. 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 polypropylene (PP) and PS. For 10 µm five plastic polymer types were characterised: PE; 39%, PS; 28%, polyvinyl chloride (PVC); 22%, polyvinyl alcohol (PVA); 6% and PVC; 5%. For 30 µm only three were identified: PP; 45%, PS; 33% and PE; 22%.

120. 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).

121. 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).

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

122. 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).

123. 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.

124. 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.

125. It is estimated that tyre wear could be adding 5-28% of the releases of primary microplastics to the world's oceans.

Summary of the potential risks of inhalation of micro and nanoplastics

126. 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.

127. 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.

128. 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.

129. Occupational inhaled microplastics (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.

130. 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.

131. 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). Such mechanisms can lead to endpoints such as cancer, which can develop as a result of chronic inflammation or from gene mutation cause by oxidative stress.

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

132. 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

133. 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.

134. 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

135. 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).

136. In 2016 Parliamentary discussions[18], it was mentioned by water utility companies (Veolia, United Utilities, Northumbrian Water, Thames Water, Yorkshire Water and Water UK) that wastewater treatment plants (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 wastewater treatment sludge ranges from 65-100%.

137. 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.

138. 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.

139. 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

140. 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)[19]. The contractor was UK Water Industry Research (UKWIR) which finished in July. 2019 (UKWIR, 2019).

141. 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 methodology for the quantification of microplastic particles at different points

[18] Full record of the Parliamentary discussion can be found here: https://publications.parliament.uk/pa/cm201617/cmselect/cmenvaud/179/17907.htm.
[19] Full report available at: https://ukwir.org/view/$NvDnwfm!

within the water industry's infrastructure. In this project, microplastics were defined as particles >25 µm that had been captured on 10 µm filters.

142. 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.

143. 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.

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

145. The most common polymer type found in raw water were PE, PET and PP. For potable water, the polymers detected above the limit of quantification were acrylonitrile butadiene styrene (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.

146. 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.

147. 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)

148. 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

149. 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.

150. 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%).

151. 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. 6*).

152. As discussed previously, water treatment plants 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).

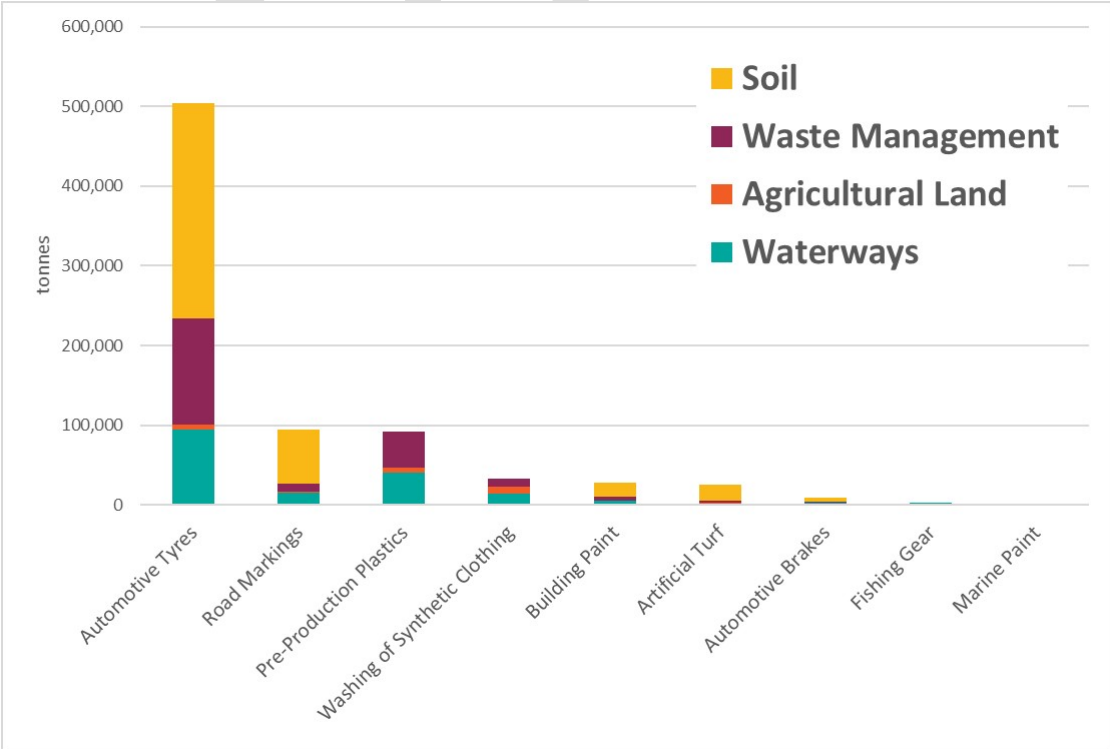

Figure. 6 - Bar graph to show the source generation and fate of microplastics from wear and tear in the EU (midpoint estimate), calculations were based on Eunomia modelling (reproduced from Hann *et al*., 2018).

153. 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.

154. 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).

155. 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

156. 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).

157. 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 low-density polyethylene (LDPE) (Huerta Lwanga *et al*., 2018), and *Enterobacter asburiae* YT1 and *Bacillus sp*. from the guts of Indian meal moth (*Plodia interpunctella*) have been shown to be capable of chewing and eating PE films (Yang *et al*., 2014) (abstract only). Caterpillars of the Honeycomb wax moth (*Galleria mellonella*) were also reported to degrade PE at an average of 0.23 mg cm^2/hour (Bombelli *et al*., 2017).

158. The specific organisms described above may not be always available in each agrosystem, and it has been hypothesised that co-metabolism[20] may be a more appropriate and/or realistic process for the bioremediation of micro and nanoplastics present in the soil.

159. 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 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.

160. 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 polyamide (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

161. 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.

162. 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.

163. 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.

164. 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).

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

Uptake of plastics by plants

165. 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).

166. 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 100 nm beads were excluded (Bandmann *et al*., 2012).

167. 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 Rough cocklebur (*Xanthium strumarium*) and Asiatic dayflower (*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).

168. 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.

169. 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.

170. 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

171. 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.

172. 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)

173. 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. 7).

174. 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.

175. 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.

176. 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.

177. 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.

178. 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.

179. 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.

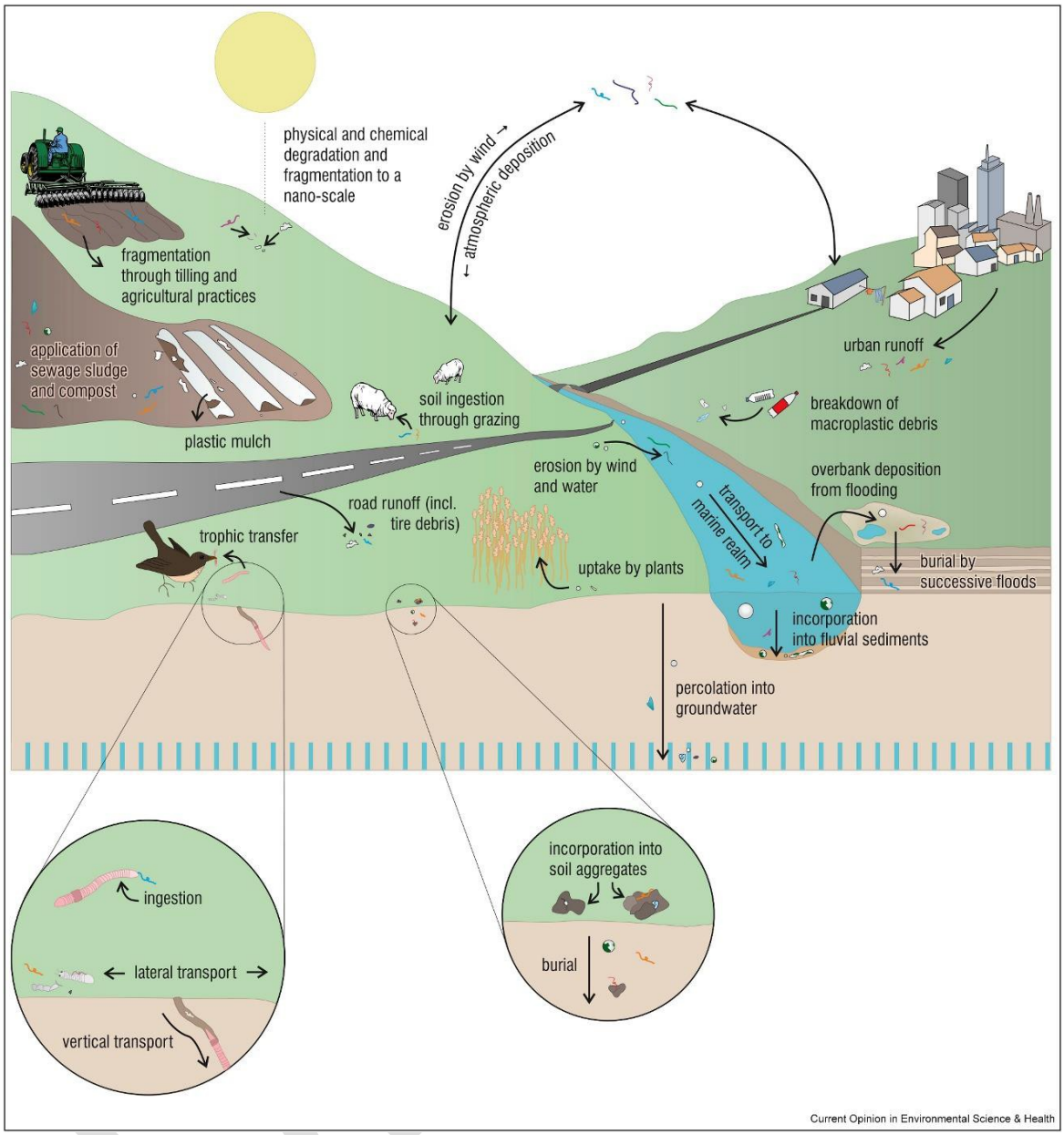

Figure. 7 - Diagram to demonstrate the processes that potentially affect the concentration of micro(nano)plastics in soil systems, including sources and fate processes (reproduced from Hurley & Nizetto, 2018).

Adsorption of chemical/microbiological agents on microplastic fragments

180. 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.

181. 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

182. 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 benzo[a]pyrene (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.

183. 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.

184. 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.

185. 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.

186. 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.

187. 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

188. 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. Scanning electron microscopy and X-ray diffractometer analysis revealed different surface characteristics and various degree of crystallinity.

189. 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

190. 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).

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

192. 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).

193. 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 < 5%.

Microbiological agents

194. In June 2019, the FSA put out a tender to commission a critical literature review on the microbiological colonisation of micro and nanoplastics and their significance to the food chain (FS307021)[21]. 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

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

196. 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.

Review papers

197. 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

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

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.

198. 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).

199. Some data gaps identified by Touissant *et al*., (2019) 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.

200. 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.

201. 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).

202. Cox *et al*., (2019) evaluated the number of 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.

203. 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 FTIR and Nile Red 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).

204. 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.

205. 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).

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

Risk assessment

207. 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).

208. 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.

209. 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.

210. In general, 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 a no observed adverse effect level (NOAEL), for each polymer type. The COT, however, postulated that an initial risk assessment that can be completed utilising exposure to tyre wear. The following section will present available risk assessment data on the potential risks from exposure to tyre wear.

Preliminary risk assessment for ingestion of microplastics utilising information from exposure to tyre wear particles

211. For ease of flow, the background on tyre wear and literature review data are presented in Annex B. The following paragraphs will provide summaries on literature directly associated with risk assessments following exposure from tyre wear particles.

212. The ECHA evaluated the possible health risks of recycled rubber granules used as infill in synthetic turf sports field in 2017 (ECHA, 2017), further detailed in paragraphs 102-127 in Annex B. In brief, ECHA investigated the risks to children playing football (and other sports) on

synthetic sports fields (including goalkeepers), adults playing professional sports and workers installing/maintaining the fields. The considered exposure routes to rubber granules were skin contact, ingestion and inhalation (of substances evaporating from the granules, as well as the dust generated by the granules themselves).

213. In terms of oral exposure, the accidental swallow of rubber granulate for children and adults were 0.05 and 0.01 g, in one event respectively.

214. The exposure to EU-8 PAHs[22] was evaluated for cancer risk. The lowest benchmark dose level that corresponds to 10% extra risk ($BMDL_{10}$) was 0.49 mg/kg bw/day for the mixture of 8 PAHs derived by EFSA from a 2-year dietary carcinogenicity study in female mice with coal tar mixtures by Culp *et al*, (1998). It is worth noting that the EFSA 8 PAHs replaced BeP and BjFa with benzo[ghi]perylene and indeno(123-cd)pyrene, however, ECHA assumed that to toxicological potency of the 8 PAHs doesn't cause a significant change.

215. The excess lifetime cancer risk, for EU-8 PAHs was calculated and was below one in a million for players, goalkeepers and workers. The full set of calculations are available in Annex VII[23] of the ECHA 2017 report. It was therefore concluded that there was a very low level of concern from exposure to PAHs from recycled rubber granules utilised as infill for synthetic turfs.

Preliminary risk assessment for inhalation of microplastics utilising information from exposure to tyre wear particles

216. Kreider *et al*., (2019) published an industry supported (Tyre Industry Project) article on the human health risk assessment of tyre and road wear particles (TRWP) present in the air. The risk assessment addresses potential human health impacts of exposure to TRWP. A literature review was carried out and the authors developed a screening value for TRWP reliant on the available hazard data and appropriate dosimetric adjustments. The few relevant studies identified by the authors that addressed potential hazards associated with tyres and their wear products are presented in *Table. 2*, the authors noted that of these studies, none evaluate the effects of TRWP as a composite mixture, and that extracts of tread rubber may not accurately represent what may or/is extracted in the lung following inhalation.

[22] The EU-8 PAHs are as follows: Benzo(a)pyrene, benzo(e)pyrene, benzo(a)anthracene, chrysene, benzo(b)fluoranthene, benzo(j)fluoranthene, Benzo(k)fluoranthene, and Dibenz(a,h)anthracene.
[23] Annex VII available at: https://echa.europa.eu/documents/10162/13563/annexes_to_axv_report_rubber+granules_en.pdf/f3cc9f58-8ab3-8e4a-0258-51466817f0fd (pp.82).

Table. 2 - Provides the relevant studies identified and reviewed by Kreider *et al*., (2019). Summaries are provided in the relevant paragraph numbers in Annex B.

Reference	Paragraphs
Karlsson et al., (2011)	152-153
Gualtieri et al., (2008)	154-157
Beretta et al., (2007)	165-166
Lindbom et al., (2006)	167-169
Karlsson et al., (2006)	170-172
Gualtieri et al., (2005)	173-174
Gerlofs-Nijland et al., (2019)	183-185
Mantecca et al., (2010)	193-199
Mantecca et al., (2009)	200-205
Gottipolu et al., (2008)	206-209
Poma et al., (2019)	210-212
Lindbom et al., (2007)	213-214
He et al., (2011)	221-223
Zhang et al., (2002)	224-226

217. A previous study by the same group (Kreider *et al*., (2012), as summarised in paragraphs 186-192 in Annex B, was identified to have evaluated human health hazards associated with TRWP (so are therefore considered to be more realistic since the particles contained both tread rubber and road wear particles). Additionally, it was the only study that addressed the inhalation route.

218. As a brief summary, the Kreider et al., (2012) study exposed TRWP in male and female rats at 0, 10, 40 and 100 µg/m3 for 6 hours/day over 28 days. No adverse effects of TRWP were detected at any concentration for any marker and a no-observed adverse effect concentration (NOAEC) of 112 µg/m3 was identified.

219. The species and time adjusted NOAEC in humans ($NOAEC_{HEC}$) for respirable TRWP was 55 µg/m^3. The $NOAEC_{HEC}$ was then compared to exposure estimates for respirable TRWP for both typical and worst-case scenarios based on age specific activity patterns to determine MOE for TRWP (*Table. 3*).

220. The estimated daily exposure was based on a 95th upper confidence limit of mean measured TRWP concentrations from sampling campaigns based on Panko *et al*., (2013).

221. Briefly, Panko *et al*., (2013) evaluated TRWP concentrations in locations representative of potential human exposure (France, the United States and Japan). Air samples were size selective (≤10 µm). The frequency of detection of TRWPs was 74%. Contributions of PM_{10} were <1%. As a follow-up study, Panko *et al*., (2019) evaluated TRWP in $PM_{2.5}$ which accounted for <0.3% in ambient air. The estimated daily exposure of TRWP ranged from 0.079-0.147 µg/m^3, resulting in an MOE of range of 700-400, respectively.

Table. 3 - Daily tyre and road wear particle exposure concentrations by population group and microenvironment including; indoor residential and institutional, outdoor and transport (reproduced from Kreider *et al*., 2019).

Exposure scenario (years)	Exposure duration (minutes)	Average daily exposure (µg/m^3)	Margin of Exposure
Infant (<1)	1440	0.079	695
Child (1-16)		0.092	597
Teenager (16-21)		0.090	611
Adult (21-64)		0.100	546
Retired adult (>64)		0.106	514
Outdoor worker		0.147	372

222. The authors highlighted the following uncertainties associated in the hazard assessment. Firstly, the utilisation of the NOAEC of 112 based on the Kreider *et al*., (2012) study may not have been appropriate since the true threshold for a response is likely to be higher, but uncertain. Secondly, the study may also not be representative of the entire distribution of particles generated during abrasion. Additionally, it must be considered that the duration of exposure was sub-acute (28 days) *i.e.* there is a potential for adverse effects to emerge with longer exposure scenarios. Thirdly, the risk assessment focused on endpoints that are believed to be the most common and important outcomes associated with exposure to particulate matter *e.g.* cardiovascular outcomes, proposed carcinogenic and reprotoxic substances. Lastly, the TRWP evaluated was generated in a laboratory; variations in the particles are possible depending on specific driving conditions, road surfaces, tyre types and individual driving behaviours. It is currently unknown how much these variations may affect TRWP particles.

223. The following uncertainties regarding the exposure assessment including: representatives of the exposure estimate to other environments, assumptions regarding the exposure scenarios (*i.e.* time spent in microenvironments, time with open versus closed windows), and assumptions regarding the markers (vinylcyclohexane[24]) that estimated the TRWP concentrations in the Panko *et al*., (2013) study were also highlighted by the authors.

224. Considering the above uncertainties, the authors concluded that the current weight of evidence suggests that TRWP presents as a low risk to human health. The risk assessment is very likely to be conservative and thus tending to overestimate the risk. Furthermore, PM_{10} in ambient air have an annual average guideline of 20 µg/m^3, as set by the WHO in 2005 (WHO, 2005); in comparison to this the average concentration of TRWP in air is 0.275 µg/m^3, as determined by Panko *et al*., (2013).

[24] Vinylcycohexane: a pyrolysis product from styrene-butadiene and butadiene rubbers.

225. Wik & Dave (2009) reviewed the existing knowledge on the occurrence of tyre wear particles (TWP) in the environment and their ecotoxicological effects. Results from their meta-analysis confirmed the presence of TWP in all environmental components including (air, water, soil/sediments and biota). The results from their previous studies with water fleas (*Ceriodaphnia dubia*) and microalga (*Pseudokirchneriella subcapitata*) were used to derive Predicted No Effect Concentrations (PNECs). The upper ranges for PEC/PNEC ratios in water and sediment were >1, which indicate the TWP present as a potential risk for aquatic organisms. The maximum PECs of TWP in surface water ranges from 0.03-56 mg/mL, whilst the maximum PECs in sediments range from 0.3-155 g/kg_{dw}.

226. In this preliminary risk assessment, the authors conferred that there were uncertainties and variabilities. This included the variability associated with measurements of environmental concentrations, which may be related to the use of different analytical methods and chemical markers to estimate tyre concentrations, and the variability associated with the estimates of toxic effect concentrations related to different tyres (rubber formulations) tested, interspecies variability, and to the use of different procedures for leachate extraction.

Challenges and limitations

Microplastics

227. There is no internationally agreed definition of what a microplastic is. In the views of the Committee, tyre wear and wear from other rubber materials are considered to be part of the definition of a plastic.

228. 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.

229. 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, and therefore suitable reference materials are also required. Contamination with airborne microplastics or cross-contamination of samples pose as an issue, control samples may be difficult to ascertain.

230. 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).

231. 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.

232. 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*.

233. 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.

234. The Committee suggested to evaluate as to what extent absorbed substances on to microplastics are already taken into account in current analytical methods. For example, when a foodstuff is analysed for PAHs, will this also include any adsorbed particles present. This will be further completed in the following draft statement.

235. 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).

236. Furthermore, it may be possible to review the relevance of historic data from research studies on microbeads.

237. 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.

Tyre and road wear particles

238. Despite their importance, the current scientific knowledge on non-exhaust emission (NEE) particulates is scarce. Furthermore, it is difficult to convincingly evaluate population exposure to traffic-related components in large cities due to the significant variation of such components on a smaller scale. One factor that is known to contribute to the uncertainty, is the lack of a reliable emission estimates for NEE. Additionally, emissions vary from location to location due to the impact of several factors including; climate, road surface characteristics and traffic conditions (Amato et al., 2011).

239. Data gaps identified in the literature include the following:

Human toxicology

- The scarcity of human epidemiological studies.

Measurements and source contributions;

- Lack of harmonised methods that implement source apportionment methods (for different size fractions and types of NEE particulates);
- Further research is necessary to better separate the individual contributions from road dust resuspension, brake, tyre and road wear given that the relative toxicity and mitigation measures are different;
- There is not enough knowledge about which pavement properties are the most important for particle formation (*e.g.* asphalt pavement with granite, pavement with quartzite, *etc*);
- Comprehensive inventory of tyres and brake composition in Europe is needed to serve as an emission source profile so future source apportionment analysis would provide more reliable outputs and;
- Overall, there is a need for new measurement studies aimed at understanding the interaction between road surface texture, moisture, chemistry, dust load and dust emission.

240. Due to the limitations described above, the development of reliable description of the particles in atmospheric dispersion models are hampered.

241. Additionally, the data available from the emission inventory presents as a major source of uncertainty for the modelling of NEEs. To further explain, EU Member States are required to report their emission inventories as a commitment to the Convention on Long-range Transboundary Air Pollution. Current nomenclature for reporting source categorisations includes tyre wear with brake wear as one source category (NFR 1.A.3.b.vi). Major uncertainties are presented below as an opinion of Amato *et al*., (2014):

- Resuspension is not included in the emission inventory, which dominates PM emissions in some countries. Base emission factors are still lacking for many countries and their spatial and temporal variations are generally unknown;
- Improving resuspension emission models has also been considered a priority;
- Some countries do not report on wear emissions, and values that are reported are likely to be affected by incompleteness and inconsistency in approaches. Uncertainties are likely to be influenced by the use of inadequate tracers, uncertainties in measurements, variability in brake and tyre composition (manufacturer, vehicle type, service-life history, and the effects of ageing);
- There is a need for harmonised and consistent bottom-up inventories for tracers (in addition to metal tracers like copper, zinc, lead, barium and antimony), to enable valuable input for modelling and exposure calculations in order to deeper understand the relationship between emissions, concentrations, exposure and human health impact(s).

242. The recommendations for mitigation and policy for the possible strategies in reducing NEE can be categorised as those aimed at minimising the sources by:

- Innovation – specifically improving the wear properties of material and reducing the wear potential of traffic;
- Minimising suspension to air by removing/immobilising dust from road surfaces (road cleaning), binding dust to road surface and;
- The development of better road design for traffic efficiency *i.e.* adjusting traffic (less traffic, lower speed, less heavy vehicles.

243. The optimum strategy is likely to involve a combination of the three categories mentioned.

European Tyre and Road Wear Particles Platform (ETRWP) – Way Forward Report 2019

244. The "Way Forward Report" aims to outline the main learnings and best practices to reduce TRWP generation and improve their capture and removal[25]. Some of the key knowledge gaps include: the lack of shared methodology to quantify TRWP and the lack of methodology to identity and count TRWP in a complex sample; the lack of field work data, the lack of agreement on the definition of microplastics; the lack of a standards tests for tyre tread and road abrasion; the lack of knowledge on the impact of road pavements or the road drainage system and the lack of indicators on microplastics in the WWTPs.

245. The European TRWP platform agreed that TRWP generation, transportation and capture is an issue that must be addressed, however, the existing data gaps (as discussed above) hinders any progress.

246. The potential solutions presented by the Platform are visualised in *Figs .8-9*. The report emphasised the importance of collaborative work, and measures were prioritised for both TRWP generation, and transportation and capture. Measures for TRWP generation included work on methodologies, incentives for positive driving behaviour, and addressing knowledge gaps. As for measures for TRWP transportation and capture; sharing and dissemination of knowledge, identification of hotspots and creation of awareness campaigns were deemed important.

[25] Available at: https://www.tyreandroadwear.com/wp-content/uploads/2019/10/FINAL-Way-Forward-Report.pdf

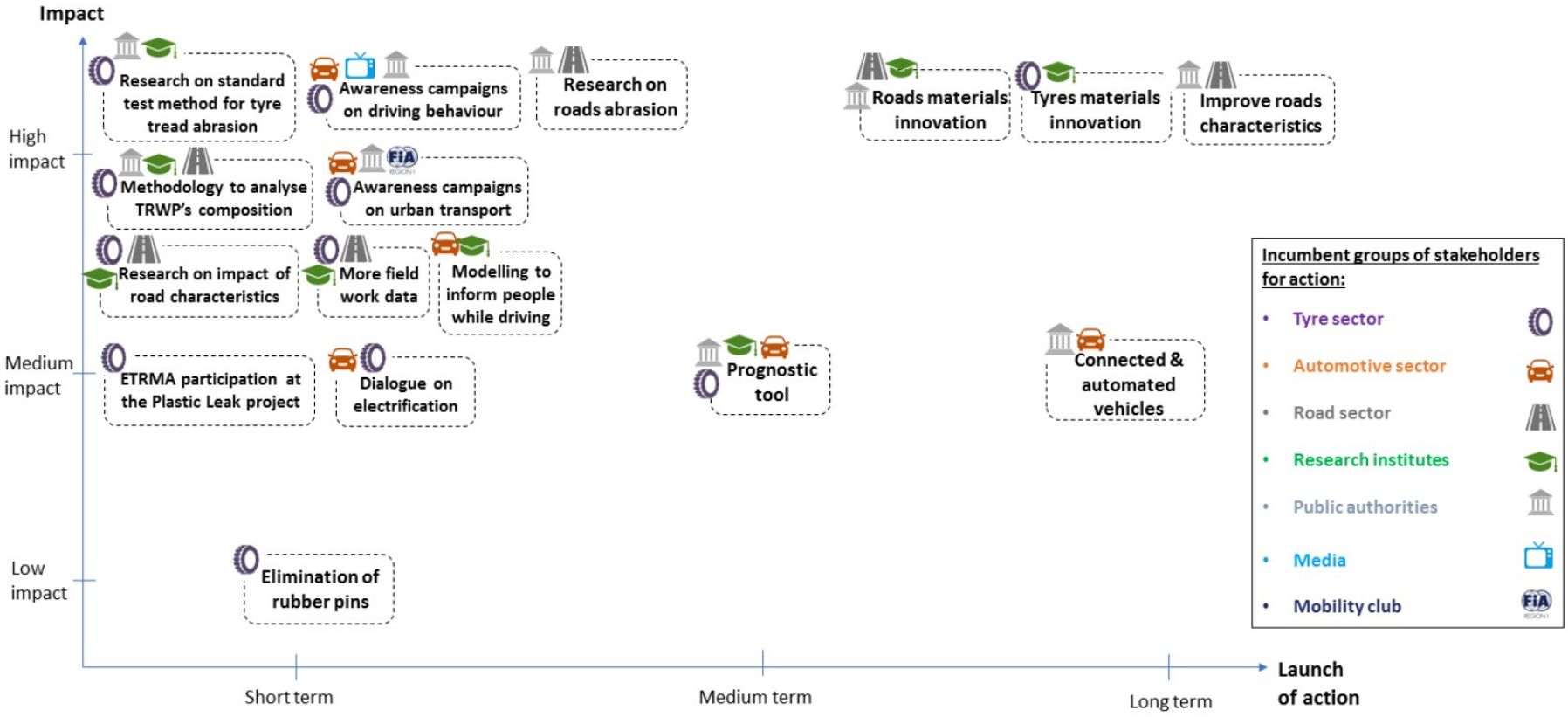

Figure. 8 – presents an impact graph (low, medium, or high) and its course of action (short, medium or long term) on the various solutions to address tyre and road wear particle generation (reproduced from ETRWP Platform Way Forward Report, 2019).

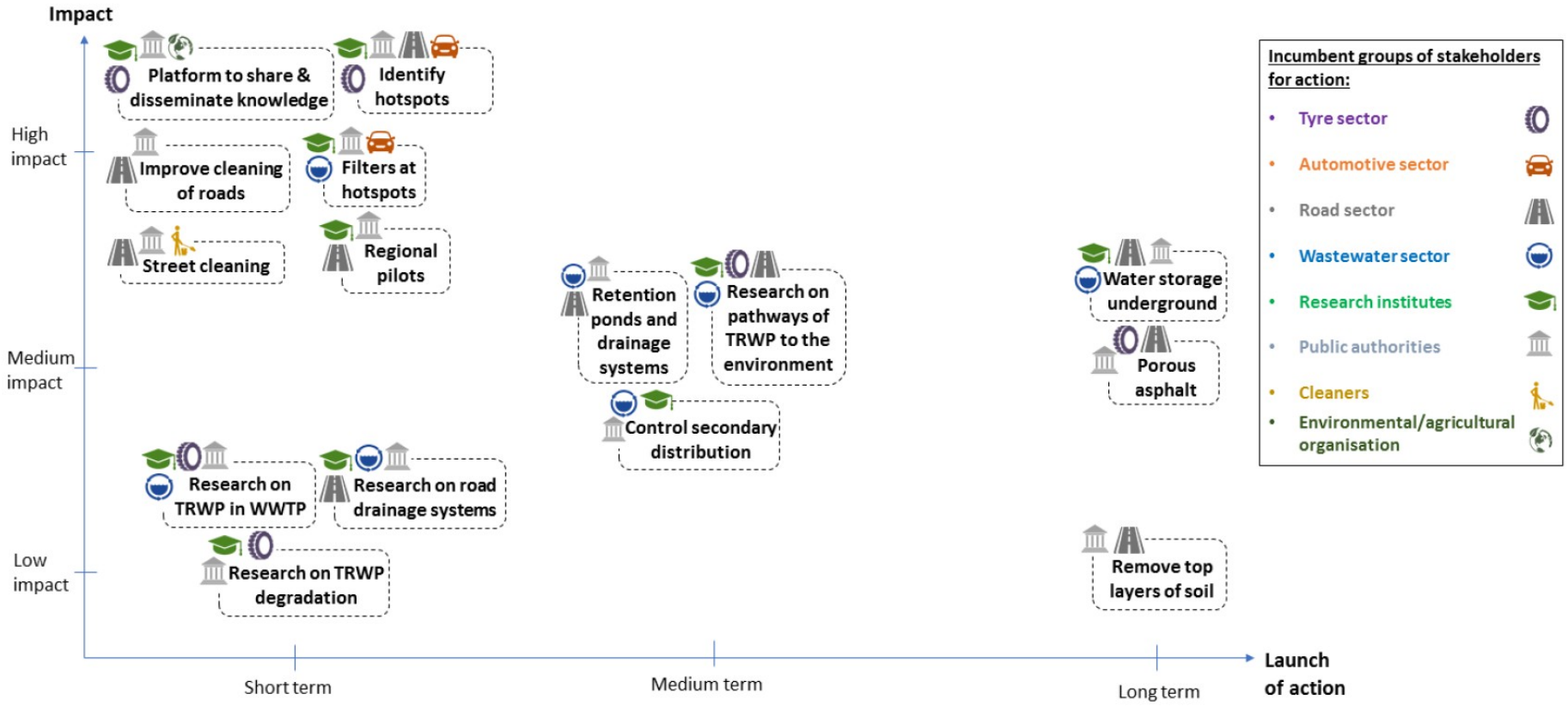

Figure. 9– presents an impact graph (low, medium, or high) and its course of action (short, medium or long term) on the various solutions to address tyre and road wear particle capture and removal (reproduced from ETRWP Platform Way Forward Report, 2019).

Summary

Microplastics

247. 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.

248. The routes for which humans can be exposed to microplastics 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.

249. 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.

250. 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.

251. 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.

252. 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.

253. 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.

254. 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.

255. 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. Fibres (up to 250 μm) have been shown to have higher potential for penetration deep in the lung tissue.

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

257. 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 could be high due to inputs of some recycled organic waste, plastic film mulching, and aerial depositing of plastic particles (*Fig. 7*).

258. 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 carbon nanoparticles 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.

259. 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.

260. 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.

261. Physical effects include behavioural, morphological and reproductive effects (*Fig. 5*). It is hypothesised that the physical presence of microplastics 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.

262. 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 microplastics, and it has been observed that phagocytosis by macrophages in the intestinal epithelium may occur with particles >0.5 µm.

263. 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.

264. From the available toxicokinetic data, distribution of Microplastics 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 (*Fig. 1*).

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

266. 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.

267. 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.

268. 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 in rats (Merski *et al*., 2008).

Tyre and road wear particles

269. In terms of TRWP, tyres contain a wide range of chemicals, the bulk of tyre tread is composed of a variety of rubbers, including natural rubber co-polymers, poly-butadiene rubber, styrene-butadiene rubber, nitride rubber, neoprene rubber, isoprene rubber, and polysulphide rubber. The interaction of tyres and pavement alters both the chemical composition and characteristics of particles generated compared to the original tyre tread due to heat and friction, as well as incorporation

of materials such as environmental "*dust*", brakes, fuels and the atmosphere, as well as roadway particles.

270. Human exposure to chemicals leached from tyres, shredded tyres, and tyre wear material can occur by dermal exposure from environmental sources and ingestion of contaminated materials, as well as inhalation of airborne particulate matter derived from tyre wear material.

271. The initial risk assessments carried out by various assessment groups (European Tyre and Road Wear Platform; Tyre Industry Project, Joint Research Centre, DEFRA, Health and Safety Executive, Committee on Medical Effects of Air Pollution, WHO, RIVM, and ECHA), showed variation.

272. The ETRWP TIP subgroup concluded that tyre wear has a significant share in general microplastics emissions (20-60%), however, the current data available does not allow for direct comparison due to differences in assumptions and target points in the environment.

273. A JRC NEE 2014 report concluded that exhaust and non-exhaust sources approximately contribute to total traffic related PM_{10} emissions. In terms of human adverse health effects, TRWP contains particles from all fractions involved in respiratory function. It was acknowledged that some constituents of TWPs have been recognised as hazardous (*e.g.* PAHs) or potentially dangerous for humans (*e.g.* presence of zinc and natural rubber latex), however, there were no comprehensive studies linking TWPs with adverse effects on human health, and the available *in vitro* studies were contradictory.

274. In addition to the report summarised above, the JRC further published a technical report on the migration of PAHs from plastic and rubber particles in 2018, none of the plastic polymeric materials led to detectable levels of the 8 target PAHs listed under Regulation (EU) No. 1272/2013.

275. Defra called for evidence on brake, tyre wear and road surface wear in July. 2018, in response to this the AQEG published a NEE report in 2019. AQEG identified that particles from brake wear, tyre wear and road surface wear currently constitute 60% and 73% (by mass), respectively, of primary $PM_{2.5}$ and PM_{10} emissions from road transport. It was stated that these emissions contribute to the total ambient particulate matter burden associated with human ill-health and premature mortality.

276. The HSE reviewed the recent epidemiological data concerning the exposure to beta-naphthylamine in the tyre manufacturing, as it has been historically associated with the increased risk of bladder cancer. The review concluded that these increased risks are no longer present in the tyre industry, however, observations of multiple myeloma cases were present in the general rubber goods sector only (currently under investigation).

277. The current WEL 6 mg/m^3 for rubber process dust and 0.6 mg/m^3 for rubber fume in an 8-hour time weighted average, however, it must be noted that these limits

apply to the dust produced from rubber manufacturing and does not include dusts arising from the abrasion of cured rubber.

278. The COMEAP highlighted that it is unlikely that all components of particulate matter have the same potency in causing adverse human health effects, and that the available evidence during their review in 2015 were insufficient to draw reliable conclusions regarding the most health-damaging components and/or sources of ambient particulate matter. Furthermore, the COMEAP were not able to recommend differential coefficients for quantification.

279. It was therefore concluded that, since there is evidence to suggest that both primary and secondary (particularly sulphate) particulate matter are detrimental to health, it's reduction as a source in the environment are likely to be beneficial to health.

280. THE WHO REVIHAPP project (2013) highlighted that there was a limited number of studies to suggest that traffic-generated dust, including road, brake and tyre wear, also contribute to human adverse health effects, however, they may become relatively more important with progressive reductions in exhaust emissions. Therefore, toxicological research increasingly indicates that such non-exhaust pollutants could be responsible for some of the observed effects on health.

281. RIVM estimated that the contribution of traffic-related NEE wear to total PM_{10} emissions in the Netherlands to be ~10%, an estimated 35 of which is caused by tyre wear, 20% by brake wear and the remaining 45% by road wear. TRWP were described to have a size range of 10-400 µm. The estimated distribution of car tyre wear amongst environmental compartments were; road residue (43%), soil (36%), surface water *via* sewerage (8%), surface water direct (3%), air (5%), sludge (6%).

282. The dermal, accidental swallowing and inhalation of fumes from rubber crumb material from synthetic turfs sports field were reviewed by ECHA. The substances that were identified to be commonly present in recycled rubber granules identified through a literature review included; PAHs, metals, phthalates, VOCs and SVOCs.

283. In terms of dermal contact, the migration factors of PAHs to artificial sweat have been detected from 0.007-0.02%, absorption of 100% was used in the risk characterisation, which takes into account the effects of any abrasion of the skin.

284. As for oral exposure, the accidental swallowing of rubber granulate for children and adults were 0.05 and 0.01 g, in one event respectively.

285. The highest measured value for inhalable dust has been 3.1 mg/m^3, whilst for respirable dust it was 1.4 mg/m^3. The maximum concentration for PM_{10} was 40 mg/m^3.

286. The excess lifetime cancer risk, for EU-8 PAHs was calculated and was below one in a million for players, goalkeepers and workers. The $BMDL_{10}$ was derived from a 2-year carcinogenicity study in female mice (Culp *et al*., 1998).

287. ECHA concluded that there was a very low level of concern from exposure to PAHs from recycled rubber granules since the concentrations of PAHs in recycled rubber granules have normally been below the limit values in the REACH restriction.

288. Furthermore, the data regarding migration of metals, showed negligible concern to those typically exposed (players and workers), since the levels are below the limits in accordance with the EU toys legislation (Directive 2009/48/EC), when compared with limit values for dry powder or pliable toy materials.

289. The concentrations of phthalates, benzothiazole, and methyl isobutyl ketone in rubber granules were found to be of no concern to players and workers, since these were below the concentrations that would lead to adverse health effects.

290. Lastly, ECHA acknowledged that reports that VOCs emitted from rubber granules in indoor halls may cause irritation to the respiratory track, eyes and skin.

291. Challenges associated with evaluating risk from exposure becomes complex when considering other factors such as the effects of weathering and ageing of tyre materials, the effects of temperature, pavement types and driving style. All in all, this will result in the generation of various chemicals with significantly different biological and toxicological effects and potencies.

COT Conclusions

292. The COT concludes that based on the available data, it is not yet possible to perform a complete risk assessment, however, they concur with the conclusions reached by other authoritative bodies.

293. In the views of the Committee, tyre wear and wear from other rubber materials are considered to be part of the definition of a plastic, for which a specific dataset may be required from that of typical plastics.

294. The most significant data gaps appear to be the lack of appropriate and harmonised analytical methods for the detection of micro and nanoplastics (together with suitable reference standards), as well as their toxicokinetic and toxicity profiles.

295. The Committee highlighted that additional information will be needed from all exposure sources, which include indoor and outdoor air, dust and soil. The presence of MPs in seafood and water may need to be put into perspective with other sources of MPs such as atmospheric fallout.

296. 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.

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). Furthermore, it may be possible to review the relevance of historic data from research studies on microbeads.

COT
March 2020

Abbreviations

ABC	Adenosine triphosphate binding cassette transporter
ABS	Acrylonitrile butadiene styrene
ADME	Absorption, Distribution, Metabolism and Excretion
AMX	Amoxicillin
ANOVA	Analysis of variance
AQEG	Air Quality Expert Group
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
CIP	Ciproflaxin
CMC	Carboxymethyl cellulose
CNPs	Carbon nanoparticles
CNTs	Carbon nanotubes
COMEAP	Committee on Medical Effects of Air Pollution
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
ECHA	European Chemicals Agency
EFSA	European Food Safety Authority
ENPs	Engineered nanoparticles
ETWRP	European Tyre and Road Wear Platform
EU	European Union
FAH	Fumarylacetoacetate
FTIR	Fourier-transform infrared spectroscopy
GIT	Gastrointestinal tract
Hmox1	Haeme oxygenase 1
HSE	Health and Safety Executive
JRC	Joint Research Centre
kd	Distribution coefficient
KEGG	Kyoto Encyclopaedia of Genes and Genomes
KEGG	Kyoto Encyclopaedia of Genes and Genomes
LDPE	Low-density polyethylene
MCNPs	Manufactured carbon-based nanoparticles
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 residence time
MTC	Maximal tissue concentration

MWCNTs	Multi-wall carbon nanotubes
NEE	Non-exhaust emissions
NOAEL	No observed adverse effect level
NOEAC	No observed effect adverse concentration
NPs	Nanoparticles
OECD	Organisation for Economic Co-operation and Development
OPFRs	organophosphorus flame retardants
PA	Polyamide
PAHs	Polyaromatic hydrocarbons
PE	Polyethylene
PEC	Predicted effect concentration
PE-MPs	Polyethylene microplastic particles
PET	Polyethylene terephthalate
PNEC	Predicted no effect concentration
PP	Polypropylene
PS	Polystyrene
PS-MPs	Polystyrene microplastic particles
PS-NPs	Polystyrene nanoplastic particles
PUR	Polyurethane
PVC	Polyvinyl chloride
RIVM	Netherlands National Institute for Public Health and the Environment
SAM	EU Group of Scientific Advisors; Scientific Advice Mechanism
SAPEA	EU Science Advice for Policy by European Academies
SDZ	Sulfadiazine
SOD	Superoxide dismutase
TAF	Total atmospheric fallout
TC	Tetracycline
TCEP	Tris(2-chloroethy) phosphate
TDCPP	tris(1,3-dichloro-2-propyl) phosphate
TG	Triglyceride
TIP	Tyre Industry Project
TMP	Trimethoprim
TRWP	Tyre and road wear particles
TWP	Tyre wear particles
UK	United Kingdom
UKWIR	UK Water Industry Research
UV	Ultraviolet light
WHO	World Health Organisation
WTWs	Water treatment works
WWTPs	Wastewater treatment plants

References

Al-Jaibachi, R., Cuthbert, R. N., Callaghan, A. (2018) Up and away: ontogenic transference as a pathway for aerial dispersal of microplastics. Biology Letters 14, Article number: 20180479.

Al-Sid-Cheikh, M., Rowland, S. J., Stevenson, K., Rouleau, C., Henry, T. B., Thompson, R. C. (2018) Uptake, whole-body distribution, and depuration of nanoplastics by the scallop *Pecten maximus* at environmentally realistic concentrations. Environmental Science and Technology 52, pp. 14480-14486.

Amato, F., Pandolfi, M., Moreno, T., Furger, M., Pey, J., Alastuey, A., Bukowiecki, N., Prevot, A. S. H., Baltensperger, U., Querol, X. (2011) Sources and variability of inhalable road dust particles in three European cities. Atmospheric Environment 45, pp. 6777-6787.

Amato, F., Cassee, F. R., Denier van der Gon, H. A. C., Gehrig, R., Gustaffson, M., Hafner, W., Harrison, R. M., Jozwicka, M., Kelly, F. J., Moreno, T., Prevot, A. S. H., Schaap, M., Sunyer, J., Querol, X. (2014) Journal of Hazardous Materials 275, pp. 31-36.

AQEG. (2019) Non-exhaust emissions from road traffic. Available at: https://uk-air.defra.gov.uk/assets/documents/reports/cat09/1907101151_20190709_Non_Exhaust_Emissions_typeset_Final.pdf. Accessed: 28/08/2019.

Bandmann, V., Müller, J. D., Köhler, T., Homann, U. (2012) Uptake of fluorescent nano beads into BY2-cells involves clathrin-dependent and clathrin-independent endocytosis. FEBS Letters 586, 3626-3632.

Barboza, L. G. A., Vethaak, A. D., Lavorante, B. R. B. O., Lundebye, A. K., Guilhermino, L. (2018) Marine microplastic debris: An emerging issue for food security, food safety and human health. Marine Pollution Bulletin 133, pp. 336-348.

Batel, A., Borchert, F., Reinwald, H., Erdinger, L., Braunbeck, T. (2018) Microplastic accumulation patterns and transfer of benzo[a]pyrene to adult zebrafish (*Danio rerio*) gills and zebrafish embryos. Environmental Pollution 235, pp. 918-930.

BfR. (2019) No evidence of intestinal damage from polystyrene microplastic in the laboratory. Available at: https://www.bfr.bund.de/cm/349/no-evidence-of-intestinal-damage-from-polystyrene-microplastic-in-laboratory.pdf. Accessed: 20/08/2019.

Bombelli, P., Howe, C. J., Bertocchini, F. (2017) Polyethylene bio-degradation by caterpillars of the wax moth *Galleria mellonella*. Current Biology 27, R283 – R293.

Boucher, J., Friot, D. (2017) Primary microplastics in the oceans: a global evaluation of sources. International Union for Conservation of Nature (IUCN), Gland, Switzerland. Lundin, C. G., Matos de Sousa, J. (eds). Available at: https://portals.iucn.org/library/sites/library/files/documents/2017-002.pdf. Accessed: 19/08/2019.

Braeuning, A. (2019) Uptake of microplastics and related health effects: a critical discussion of Deng et al., Scientific reports 7:46687, 2017. Archives of Toxicology 93, pp. 219-220.

Carpita, N., Sabularse, D., Montezinos, D., Delmer, D. P. (1979) Determination of the pore size of cell walls of living plant cells. Science 205, pp. 1144-1147.

Carvalho, T. C., Peters, J. I., Williams, R. O. (2011) Influence of particle size on regional lung deposition – What evidence is there? International Journal of Pharmaceutics 406, pp. 1-10.

Catarino, A. I., Macchia, V., Sanderson, W. G., Thompson, R. C., Henry, T. B. (2018) Low levels of microplastics (MP) in wild mussels indicate the MP ingestion by humans is minimal compared to exposure via household fibres fallout during a meal. Environmental Pollution 237, pp. 675-684.

Cox, K. D., Covernton, G. A., Davies, H. L., Dower, J. F., Juanes, F., Dudas, S. E. (2019) Human consumption of microplastics. Environmental Science & Technology 53, pp. 7068-7074.

Culp, S. J., Gaylor, D. W., Sheldon, W. G., Golstein, L. S., Beland, F. A. (1998) A comparison of the tumors induced by coal tar and benzo[a]pyrene in a 2-year bioassay. Carcinogenesis 19, pp 117–124.

Defra. (2018) Removal of microplastics by drinking-water treatment processes – WT2217. Available at: http://sciencesearch.defra.gov.uk/Default.aspx?Menu=Menu&Module=More&Location=None&ProjectID=20121&FromSearch=Y&Publisher=1&SearchText=microplastic&SortString=ProjectCode&SortOrder=Asc&Paging=10#Description. Accessed: 30/07/2019.

Deng, Y., Zhang, Y., Lemos, B., Ren, H. (2017) Tissue accumulation of microplastics in mice and biomarker responses suggest widespread health risks of exposure. Scientific Reports 7, Article number: 46687.

Deng, Y., Zhang, Y., Qiao, R., Bonilla, M. M., Yang, X., Ren, H., Lemos, B. (2018) Evidence that microplastics aggravate the toxicity of organophosphorus flame retardants in mice (*Mus musculus*). Journal of Hazardous Materials 357, pp. 348-354.

Donaldson, K., Lang Tran, C. (2002) Inflammation caused by particles and fibres. Inhalation Toxicology 14, pp. 5-27.

Doyle-McCullough, M., Smyth, S. H., Moyes, S. M., Carr, K. E. (2007) Factors influencing intestinal microparticle uptake *in vivo*. International Journal of Pharmaceutics 335, pp.79-89.

Dris, R., Gasperi, J., Saad, M., Mirande, C., Tassin, B. (2016) Synthetic fibres in atmospheric fallout: A source of microplastics in the environment? Marine Pollution Bulletin 104, pp. 290-293.

Dris, R., Gasperi, J., Mirande, C., Mandin, C., Guerrouache, M., Langlois, V., Tassin, B. (2017) A first overview of textile fibres, including microplastics, in indoor and outdoor environments. Environmental Pollution 221, pp. 453-458.

ECHA. (2017) Annex XV Report. An evaluation of the possible health risks of recycled rubber granules used as infill in synthetic turf sports fields. Available at: https://echa.europa.eu/documents/10162/13563/annex-xv_report_rubber_granules_en.pdf/dbcb4ee6-1c65-af35-7a18-f6ac1ac29fe4. Accessed: 14/01/2020.

ECHA. (2019) ECHA proposes to restrict intentionally added microplastics. Available at: https://echa.europa.eu/-/echa-proposes-to-restrict-intentionally-added-microplastics. Accessed: 07/08/2019.

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. Accessed: 10/06/2019.

EFSA. (2018) Guidance on risk assessment of the application of nanoscience and nanotechnologies in the food and feed chain: Part 1, human and animal health. Available at: https://www.efsa.europa.eu/en/efsajournal/pub/5327. Accessed: 20/09/2019.

European TRWP Platform. (2019) Way Forward Report. Available at: https://www.etrma.org/wp-content/uploads/2019/10/final-way-forward-report.pdf. Accessed: 19/12/2019.

FSA. (2019) A critical review of microbiological colonisation of nano- and microplastics (NMPs) and their significance to the food chain (FS307021). Unpublished report. Tender available at: https://food.bravosolution.co.uk/esop/toolkit/opportunity/opportunityDetail.do?opportunityId=43766&oppList=PAST. Accessed: 02/07/2019.

Galloway, T. S., Cole, M., Lewis, C. (2017) Interactions of microplastic debris throughout the marine ecosystem. Nature Ecology & Evolution 1, Article number: 0116.

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.

Hann, S., Sherrington, C., Jamieson, O., Hickman, M., Kershaw, P., Bapasola, A., Cole, G. (2018) Investigating options for reducing releases in the aquatic environment of microplastics emitted by (but not intentionally added in) products. Eunomia Research and Consulting and ICF. Available at: https://ec.europa.eu/environment/marine/good-environmental-status/descriptor-10/pdf/microplastics_final_report_v5_full.pdf. Accessed: 19/08/2019.

Hillerdal, G., Steinholtz, Rosenhall, L., Lindgren, A. (1988) Pulmonary fibrosis caused by synthetic textile fibres? VII[th] International Pneumoconioses Conference, pp. 1405-1407. Available at: https://www.cdc.gov/niosh/docs/90-108/pdfs/90-108-2.pdf?id=10.26616/NIOSHPUB90108. Accessed: 15/08/2019.

Huerta Lwanga, E., Gertsen, H., Gooren, H., Peters, P., Salánki, T., van der Ploeg, M., Besselingm E., Koelmans, A. A., Geissen, V. (2016) Microplastics in the terrestrial ecosystem: Implications for *Lumbricus terrestris* (Oligochaeta, Lumbricidae). Environmental Science & Technology 50, pp. 2685-2691 (abstract only).

Huerta Lwanga, E., Thapa, B., Yang, X., Gertsen, H., Salánki, T., Geissen, V., Garbeva, P. (2018) Decay of low-density polyethylene by bacteria extracted from earthworm's guts: A potential for soil restoration. Science of the Total Environment 624, pp. 753-757.

Huffer, T., Metzelder, F., Sigmund, G., Slawek, S., Schmidt, T. C., Hofmann, T. (2019) Polyethylene microplastics influence the transport of organic contaminants in soil. Science of the Total Environment 657, pp. 242-247.

Hurley, R. R., Nizzetto, L. (2018) Fate and occurrence of micro(nano) plastics in soils: Knowledge gaps and possible risks. Current Opinion in Environmental Science and Health 1, pp. 6-11.

Hwang, J., Choi, D., Han, S., Choi, J., Hong, J. (2019) An assessment of the toxicity of polypropylene microplastics in human derived cells. Science of the Total Environment 684, pp. 657-669.

Jin, Y., Liang, L., Tu, W., Luo, T., Fu, Z. (2019) Impacts of polystyrene microplastic on the gut barrier, microbiota and metabolism in mice. Science of the Total Environment 649, pp. 308-317.

Koelmans, A. A., Nowack, B., Wiesner, M. R. (2009) Comparison of manufactured and black carbon nanoparticle concentrations in aquatic sediments. Environmental Pollution 157, 1110-1116.

Koelmans, A. A., Nor, N. H. M., Hermsen, E., Kooi, M., Mintenig, S. M., De France, J. (2019) Microplastics in freshwaters and drinking water: Critical review and assessment of data quality. Water Research 155, pp. 410-422.

Kreider, M. L., Doyle-Eisele, M., Russell, R. G., McDonald, J. D., Panko, J. M. (2012) Evaluation of potential for toxicity from subacute inhalation of tire and road wear particles in rats. Inhalation Toxicology 24, pp. 907-917.

Kreider, M., L., Unice, K. M., Panko, J. M. (2019) Human health risk assessment of tire and road wear particles (TRWP) in air. Human and Ecological Risk assessment: An International Journal. DOI: https://doi.org/10.1080/10807039.2019.1674633.

Li, J., Zhang, K., Zhang, H. (2018) Adsorption of antibiotics on microplastics. Environmental Pollution 237, pp. 460-467.

Lu, L., Wan, Z., Luo, T., Fu, Z., Jin, Y. (2018) Polystyrene microplastics induce gut microbiota dysbiosis and hepatic lipid metabolism disorder in mice. Science of the Total Environment 631-632, pp. 449-458.

Lundqvist, M., Stigler, J., Elia, G., Lynch, I., Cedervall, T., Dawson, K. A. (2008) Nanoparticle size and surface properties determine the protein corona with possible implications for biological impacts. Proceedings of the National Academy of Sciences of the United States of America 108, pp. 14265-14270.

Machado, A. A. D. S., Lau, C. W., Till, J., Kloas, W., Lehmann, A., Becker, R., Rillig, M. C. (2018) Impacts of microplastics on the soil biophysical environment. Environmental Science and Technology 52, pp. 9656-9665.

McMahon, M., Ding, S., Acosta-Jimenez, L. P., Frangova, T. G., Henderson, C. J., Wolf, C. R. (2018) Measuring *in vivo* responses to endogenous and exogenous oxidative stress using a novel haem oxygenase 1 reporter mouse. The Journal of Physiology 596, pp. 105-127.

Merski, J. A., Johnson, W. D., Muzzio, M., Lyang, N. L., Gaworski, C. L. (2008) Oral toxicity and bacterial mutagenicity studies with a spunbound polyethylene and polyethylene terephthalate polymer fabric. International Journal of Toxicology 27, pp. 387-395.

MILC. (2016) Mothers' information on lactation and collection (MILC) study. Available at: http://www.milcstudy.com/. Accessed: 14/08/2019.

Nelms, S. E., Galloway, T. S., Godley, B. J., Jarvis, D. S., Lindeque, P. K. (2019) Investigating microplastics trophic transfer in marine top predators. Environmental Pollution 238, pp. 999-1007.

Nizzetto, L., Futter, M., Langaas, S. (2016) Are Agricultural Soils Dumps for Microplastics of Urban Origin? Environmental Science & Technology 50, pp. 10777-10779.

Ng, E-L., Huerta Lwanga, E., Eldridge, S. M., Johnston, P., Hu, H-W., Geissen, V., Chen. D. (2018) An overview of microplastic and nanoplastic pollution in agroecosystems. Science of the Total Environment 627, pp. 1377-1388.

Panko, J. M., Chu, J., Kreider, M. L., Unice, K. M. (2013) Measurement of airborne concentrations of tire and road wear particles in urban and rural areas of France, Japan, and the United States. Atmospheric Environment 72, pp. 192-199.

Panko, J. M., Hitchcock, K. M., Fuller, G. W., Green, D. (2019) Evaluation of tire wear contribution to $PM_{2.5}$ in urban environments. Atmosphere 10 (2), 99. https://doi.org/10.3390/atmos10020099

Parliament. (2016) Environmental Audit; Environmental impact of microplastics. Item 4: Microplastic prevention and solutions. Available at: https://publications.parliament.uk/pa/cm201617/cmselect/cmenvaud/179/17907.htm. Accesssed: 05/09/2019.

Pauly, J. L., Stegmeier, S. J., Allaart, H. A., Cheney, R. T., Zhang, P. J., Mayer, A. G., Streck, R. J. (1998) Inhaled cellulosic and plastic fibres found in human lung tissue. Cancer Epidemiology, Biomarkers and Prevention 7, pp. 419-428.

Pimentel, J. C., Avila, R., Lourenço, A. G. (1975) Respiratory disease caused by synthetic fibres: a new occupational disease. Thorax 30, pp. 204-219.

Pitt, J. A., Kozal, J. S., Jayasundara, N., Massarsky, A., Trevisan, R., Geitner, N., Wiesner, M., Levin, E. D., Di Giulio, R. T. (2018a) Uptake, tissue distribution and, and toxicity of polystyrene nanoparticles in developing zebrafish (*Danio rerio*). Aquatic Toxicology 194, pp.185-194.

Pitt, J. A., Trevisan, R., Massarsky, A., Kozal, J. S., Levin, E. D., Di Giulio, R. T. (2018b) Maternal transfer of nanoplastics to offspring in zebrafish (*Danio rerio*): A case study with nanopolystyrene. Science of the Total Environment 643, pp. 324-334.

Rafiee, M., Dargahi, L., Eslami, A., Beirami, E., Jahangiri-rad, M., Sabour, S., Amereh, F. (2018) Neurobehavioral assessment of rats exposed to pristine polystyrene nanoplastics upon oral exposure. Chemosphere 193, pp. 745-753.

Ramos, L., Berenstein, G., Hughes, E., A., Zalts, A., Montserrat. (2015) Polyethylene film incorporation into the horticultural soil of small periurban production units in Argentina. Science of the Total Environment 523, pp. 74-81.

SAM. (2019) European Commission Group of Chief Scientific Advisors – Scientific Advice Mechanism (SAM) Environmental and Health Risks of Microplastics Pollution. Available at: https://ec.europa.eu/info/sites/info/files/research_and_innovation/groups/sam/ec_rtd_sam-mnp-opinion_042019.pdf. Accessed: 17/10/2019.

SAPEA. (2019) Scientific Advice for Policy by European Academies – A Scientific Perspective on Microplastics in Nature and Society. Available at: https://www.sapea.info/wp-content/uploads/report.pdf. Accessed: 17/10/2019.

Schirinzi, G. F., Pérez-Pomeda, I., Sanchís, J., Rossini, C., Farré, M., Barceló, D. (2017) Cytotoxic effects of commonly used nanomaterials and microplastics on cerebral and epithelial human cells. Environmental Research 159, pp. 579-587.

Schwabl, P., Liebmann, B., Köppel, S., Königshofer, P., Bucsics, T., Trauner, M., Reiberger, T. (2018) Assessment of microplastic concentrations in human stool – Preliminary results of a prospective study. Available at: https://www.umweltbundesamt.at/fileadmin/site/presse/news_2018/UEG_Week_2018_-_Philipp_Schwabl_Microplastics_Web.pdf. Accessed: 13/08/2019.

Schwabl, P., Köppel, S., Königshofer, P., Bucsics, T., Trauner, M., Reiberger, T., Liebmann, B. (2019) Detection of various microplastics in human stool: A prospective case series. Annals of Internal Medicine; doi: 10.7326/M19-0618 (abstract only).

Shah, A. A., Hasan, F., Hameed, A., Ahmed, S. (2008) Biological degradation of plastics: A comprehensive review. Biotechnology Advances 26, pp. 246-265.

Sharma, S., Chatterjee, S. (2017) Microplastic pollution, a threat to marine ecosystem and human health: a short review. Environmental Science and Pollution Research 24, pp. 21530-21547.

Stock, V., Böhmert, L., Lisicki, E., Block, R., Cara-Carmona, J., Pack, K. P., Selb, R., Lichtenstein, D., Voss, L., Henderson, C. J., Zabinksy, E., Sieg, H., Braeuning, A., Lampen, A. (2019) Uptake and effects of orally ingested polystyrene microplastic particles *in vitro* and *in vivo*. Archives of Toxicology 93(7), pp. 1817-1833.

Teuten, E. L., Saquing, J. M., Knappe, D. R. U., Barlaz, M. A., Jonsson, S., Björn, A., Rowland, S. J., Thompson, R. C., Galloway, T. S., Yamashita, R., Ochi, D., Watanuki, Y., Moore, C., Viet, P. H., Tana, T. S., Prudente, M. P., Boonyatumanond, R., Zakaria, M. P., Akkhavong, K., Ogata, Y., Hirai, H., Iwasa, S., Mizukawa, K., Hagino, Y., Imamura, A., Saha, M., Takada, H. (2009) Transport and release of chemicals from plastics to the environment and to wildlife. Philosophical Transactions of the Royal Society B 364. Article ID: 20080284.

Touissant, B., Raffael, B., Angers-Loustau, A., Gilliland, D., Kestens, V., Petrillo, M., Rio-Echevarria, I. M., Van den Eede, G. (2019) Review of micro- and nanoplastic contamination in the food chain. Food Additives & Contaminants: Part A 36, pp. 639-673.

UKWIR. (2019) Sink to river – River to tap: A review of potential risks from nanoparticles and microplastics. Available at: https://ukwir.org/view/$NvDnwfm!. Accessed: 10/09/2019.

Walczak, A. P., Hendriksen, P. J. M., Woutersen, R. A., van der Zande, M., Undas, A. K., Helsdingen, R., van der Berg, H. H. J., Rietjens, I. M. C. M., Bouwmeester, H. (2015) Bioavailability and biodistribution of differently charged polystyrene nanoparticles upon oral exposure to rats. Journal of Nanoparticle Research 17:231.

Wik, A., Dave, G. (2009) Occurrence and effects of tire wear particles in the environment – A critical review and an initial risk assessment. Environmental Pollution 157, pp. 1-11.

WHO. (2005) WHO Air quality guidelines for particulate matter, ozone, nitrogen dioxide and sulfur dioxide. Global update 2005; Summary of risk assessment. Available at: https://www.who.int/phe/health_topics/outdoorair/outdoorair_aqg/en/. Accessed: 31/12/2019.

WHO. (2019) World Health Organisation Microplastics in drinking-water. Available at: https://www.who.int/water_sanitation_health/publications/microplastics-in-drinking-water/en/. Accessed: 22/08/2019.

Wright, S. L., Kelly, F. (2017) Plastic and human health: a micro issue? Environmental Science and Technology 51, pp. 6634-6647.

Wright, S. L., Levermore, J. M., Kelly, F. J. (2019) Raman spectral imaging for the detection of inhalable microplastics in ambient particulate matter samples. Environmental Science and Technology 53, pp. 8947-8956.

Wu, B., Wu, X., Liu, S., Wang, Z., Chen, L. (2019) Size-dependent effects of polystyrene microplastics on cytotoxicity and efflux pump inhibition in human Caco-2 cells. Chemosphere 221, pp. 333-341.

Yacobi, N. R., DeMaio, l., Xie, J., Hamm-Alvarez, S. F., Borok, Z., Kim, K., Crandall, E. D. (2008) Polystyrene nanoparticle trafficking across alveolar epithelium. Nanomedicine: Nanotechnology, Biology and Medicine 4, pp. 139-145.

Yang, J., Yang, Y., Wu, W-M., Zhao, J., Jiang, L. (2014) Evidence of polyethylene degradation by bacterial strains from the guts of plastic-eating waxworms. Environmental Science & Technology 48, pp. 13776-13784 (abstract only).

Yang, Y., Chen, C., Lu, T., Liao, C. (2019) Toxicity-based toxicokinetic/toxicodynamic assessment for bioaccumulation of polystyrene microplastics in mice. Journal of Hazardous Materials 366, pp. 703-713.

Yoshida, S., Hiraga, K., Takehana, T., Taniguchi, I., Yamaji, H., Maeda, Y., Toyohara, K., Miyamoto, K., Kimura, Y., Oda, K. (2016) A bacterium that degrades a assimilates poly(ethylene terephthalate). Science 351, pp. 1196-1199.

Zapata, R. (2018) Novel Air Pollution from Composting Facilities: Estimating Microplastic Emissions. MSc Thesis, Centre for Environmental Policy, Faculty of Natural Sciences, Imperial College London.

Zhao, Q., Ma, C., White, J. C., Dhankher, O. P., Zhang, X., Zhang, S., Xing, B. (2017) Quantitative evaluation of multi-wall carbon nanotube uptake by terrestrial plants. Carbon 114, pp. 661-670.

Zubris, K. A. V., Richards, B. K. (2005) Synthetic fibres as an indicator of land application of sludge. Environmental Pollution 138, pp. 201-2011.

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

First draft statement 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 31 of the statement 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 – September 2019.

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