TOX/2021/38

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

Sub-statement on the potential risks from exposure to microplastics: Oral route (First draft)

Background

1. In 2019, as part of horizon scanning, the Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment (COT) identified the potential risks from microplastics as a topic it should consider (TOX/2019/08)[1]. In 2021, the COT published an overarching statement on the potential risks from exposure to microplastics (COT Statement 2021/02)[2]. This document provided a high-level overview of the current state of knowledge, data gaps and research requirements with regards to this topic.

2. The purpose of this sub-statement is to provide supplementary material to the overarching statement (COT Statement 2021/02) and consider in detail the potential toxicological risks of exposure from microplastics *via* the oral route (*i.e.* resulting from the presence of microplastics in food, drinking water and bottled drinks). It is based on current available literature and data from internal tools at the UK Food Standards Agency (FSA).

United Kingdom (UK) food safety alerts on the presence of plastic particles on food items

3. Computational tools currently being developed internally within the UK FSA provide up to date information on various facets of plastics including presence, hazards, and trends. The output of these tools will be monitored internally, reviewed and when necessary presented to the COT on an *ad hoc* basis.

4. Outputs from this internal tool show that in the past 12 months (June 2020 – June 2021), twelve incidents have been reported for the presence of plastic foreign bodies across several food categories in the UK. This included: cereals and bakery products, dietetic foods, poultry meat, beef and pork meat, and milk and milk products. Each of these incidents have had product recalls on the affected batches.

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[1] TOX/2019/08 is available on the COT website.
[2] COT Statement 2021/02 is available on the COT website. The lay summary is also available on the COT website.

5. It should be noted that the food incident alert often lacks granularity, where the basic properties (*e.g.* size and shape) of the plastic pieces are not recorded in full detail. Food incident alerts typically have the following reporting format: "[Company X] recalls [food product] because it (may) contain (small) pieces of plastic."

6. It is hypothesised that the presence of plastic in these food products are introduced during the later stage of the food manufacturing process, most likely during packaging. In UK legislation, the Food Safety Act 1990[3] and General Food Regulations 2004[4], state that manufacturers have due diligence and if foreign bodies (*e.g.* plastic) are present in the product, they are able to provide evidence that they have done as much as is reasonably practicable to prevent the contamination.

7. Although the number of food safety reports in the system were low, this value may be underreported due to late reporting, lack of post-market surveillance, and because not all food product types will be screened/monitored for the presence of micro- and nanoplastics on a regular basis.

Update on literature

8. A short update on the emerging literature is provided in the following paragraphs.

Reviews

9. The COT Members have been previously informed of the ongoing research efforts by the UK Food Standards Agency (FSA) on performing a critical literature review on the microbiological colonisation of nano- and microplastics (NMPs) and their significance to the food chain (FS307021)[5], which was contracted to the Centre for Environment Fisheries & Aquaculture Science. Preliminary outputs from this review were first presented by Bakir *et al*., (2021) during the European Food Safety Authority's (EFSA) Scientific Colloquium 25 titled, "A coordinated approach to assess the human health risks of micro- and nanoplastics in food" on May 2021[6].

10. The four research areas as part of this work package (WP) include: NMPs in the environment (WP1); pathways of colonised NMPs into food chains (WP2); interactions between NMPs and microorganisms (WP3); and NMP-specific microbial risks to consumers (WP4).

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[3] The Food Safety Act 1990 is available on the UK legislation website here.
[4] The General Food Regulations 2004 is available on the UK legislation website here.
[5] Further details concerning this research project (FS307021) are available on the FSA website.
[6] Further information on EFSA's scientific colloquium is available on the EFSA website.

11. The evidence gaps and recommendations for further work in each WP is shown in *Figures 1-2*.

12. An authoritative full synthesis report and special report document will be published later in the year to provide a collated and impartial summary of the scientific evidence on the impacts of microplastics and human health, utilizing the most relevant and contemporary scientific data available.

13. Paul *et al*., (2020) published a review on the current state of knowledge of micro- and nanoplastics with the focus on oral uptake and toxicity. They concluded that risk assessment of micro- and nanoplastics is still not possible, due to various data gaps in terms of exposure, biodistribution and related effects. Data from the literature suggests that, passage though the gastrointestinal barrier is possible for a low value of ingested particles (particularly those at the nanoscale). Cellular toxicity-related effects have been detected; however, these occur at high-doses and often without dose-response relationships. Overall, the authors considered that the number of available studies were still very limited.

WP1: Evidence gaps and recommendations for further work	WP2: Evidence gaps and recommendations for further work
1. There are currently a lack of standardised and universally-applied methods to collect data	1. There is a lack of standardised approaches to investigate the interactions between plastics and microorganisms. Isolating DNA resulting in different result depending on libraries compared to. Lack of characterisation of surface properties makes it difficult to identify key properties for colonisation.
2. The need for internationally accepted definition regarding NMPs.	2. There is a lack of inclusion of non-plastic substrates in controlled exposure experiments to better understand the factors influencing colonisation and to determine whether plastic substrate-driven selection is occurring.
3. A huge number of studies have looked at the fate of NMPs in the environment, in particular marine settings, however less emphasis on other environments.	3. Further work is needed to determine the role NMP-associated biofilms play in selecting for and/or transporting pathogenic bacteria. , Standardisation
4. Little is known about the transformations of plastics in seawater, including the time scales of degradation and its ultimate sinks.	4. Research needs to be expanded in the relatively new field of determining whether NMPs themselves, or sorbed antimicrobials exert a selective effect for antimicrobial resistance through species sorting or de novo acquisition of AMR through mutations or horizontal gene transfers.
5. A lack of standardised, laboratory-based data on long-term fate of NMPs.	
6. Although the presence of NMPs have been widely reported in certain food commodities, there are very little detailed studies on these at ecosystem-levels.	
7. There is a lack of data on microplastic contamination and the cycling of these contaminants between different environmental compartments.	

Figure 1 – Provides a list of the evidence gaps and recommendations for further work for Work Package 1 (WP1) and Work Package 2 (WP2); nano- and microplastics (NMPs) in the environment and pathways of colonised NMPs into food chains, respectively (reproduced from Bakir *et al*., 2021).

WP3: Evidence gaps and recommendations for further work	WP4: Evidence gaps and recommendations for further work
1. There has been a strong focus on microplastics in the environment. Far less has been done on the route between that environment and the consumer.	1. Data on the presence of viruses on NMPs is currently lacking.
2. Large amounts of data are available on seafood but not for the product that has passed through processing and packaging to points of sale and people's home.	2. Research into human disease risks and NMPs focuses on toxicological effects, but very little attention is focused on the potential role of associated microorganisms.
3. For most other types of food, there is no data for any part of the process. This may lead to an exaggeration of the importance of inhaled NMPs as many major parts of our diet (meats, vegetables) lack sufficient data.	3. Many studies regarding the presence of pathogens on NMPs in environmental settings are purely anecdotal and lack robust controls such as comparisons to other substrates.
4. There is also data missing for very small NMPs in all parts of the process from environment to plate while studies have found smaller particles to be far more abundant.	4. Studies on AMR tend to lack appropriate controls, making direct comparisons as well as ascertaining overall relevance to risk difficult to gauge.
5. Throughout the research, there is a lack of standardisation which inhibits comparisons between studies. Analysis methods and size classes investigated differ strongly, leading to widely different NMP concentrations in comparable food types.	5. There is a lack of published evidence regarding dysbiosis in humans, although there are studies in other model organisms (e.g. zebrafish, mice).
6. While there is little data on many typical types of food, information on toxic effects of NMPs and subsequent risks to human health are even rarer, making any risk assessment very tentative.	6. There is little current data regarding impacts of NMPs on pathogens and human health outcomes including a clear lack of epidemiological data.

Figure 2 - Provides a list of the evidence gaps and recommendations for further work for Work Package 3 (WP3) and Work Package 4 (WP4); interactions between nano- and microplastics (NMPs) and microorganisms, and NMP-specific microbial risks to consumers, respectively (reproduced from Bakir *et al*., 2021).

Toxicological data

14. A limited search (*i.e* non-systematic search) was carried out to identify any new literature since the publication of COT overarching statement on the potential risks from exposure to microplastics (COT Statement 2021/02) in February 2021 – current. Below provide brief summaries of toxicological studies which investigated the effects of micro- and nanoplastics following oral exposure.

Reproductive toxicity

In vivo (animal models)

15. Deng *et al*., (2021) investigated whether the distribution of virgin polyethylene microplastic (PE-MPs) spheres would affect the bioaccumulation of phthalate esters (PAEs), as well as investigating effects on reproductive toxicity from exposure to PAE-contaminated PE-MPs in 5-week old male CD-1 mice (n=120; 12/group). Four PAEs were tested, these were di-2-ethylhexyl phthalate (DEHP), dibutyl phthalate (DBP), diethyl phthalate (DEP) and dimethyl phthalate (DMP). The composition of the PE-MP spheres was confirmed by Fourier Transform Infrared Spectroscopy (FT-IR). Spheres were 40-50 µm in size, as confirmed by Scanning Electron Microscopy (SEM) and laser scattering particle size distribution analysis.

16. Mice were split into 10 treatment groups. One was treated as a control. Another was dosed with virgin PE-MPs. DEHP-contaminated PE-MPs were prepared by mixing 0.2 g/L of PE-MPs with either 5 or 50 µg/L DEHP. A mixture of all four PAEs was prepared in equimolar proportions to 5 or 50 µg/L and was mixed with 0.2 g/L of PE-MPs. The virgin PE-MPs and PAE-contaminated PE-MPs (100 mg/kg bw) were administered to mice *via* oral gavage for 30 days. The remaining four groups were exposed to DEHP and the PAE mixture alone; the concentration was based on the adsorption of PAEs on PE-MPs and the MPs administered for mice each day. The carrier was unclear; however, it is believed to be distilled water. Post-treatment all animals were anaesthetised. The epididymis, liver and gut were collected for histopathological and biochemical analyses. The testes were also collected for transcriptomic analyses.

17. Irrespective of whether the PAEs were carried by PE-MPs or not, the accumulation of PAE was gut > liver > testis. The maximum accumulation of DEHP and PAE mixture in the gut was ~470 ng/g dry weight, which was ~2 times higher than that in the liver and testis.

18. The number of sperm was significantly reduced in all PAE-contaminated PE-MPs treatment groups when compared with the PAE alone treatment groups. Acid phosphatase (ACP), succinate dehydrogenase (SDH) and lactate dehydrogenase (LDH) levels were used as biomarkers for disturbance in spermatogenesis. ACP and LDH were increased, and SDH was significantly decreased in in all PAE-MPs groups compared with PAEs

alone. When compared to the virgin PE-MPs alone, the same observation was found for the PAE-MPs groups at higher concentrations. Oxidative stress was also induced in the testis, as higher levels of superoxide dismutase (SOD) and malonaldehyde (MDA) were measured.

19. Transcriptomic analysis of the left testis showed that higher concentrations of DEHP-MPs and PAE mix-MPs induced transcriptomic changes (~180 upregulated and downregulated genes; and ~51 upregulated and 90 downregulated genes, respectively). Differentially expressed genes that were of statistical significance were involved with transport and catabolism, energy metabolism and amino acid metabolism.

20. The authors concluded that exposure to PAE contaminated microplastics increased the accumulation of PAEs in the gut and liver of mice, when compared to virgin PE-MPs and PAE alone. No statistically significant increase in accumulation of PAEs was observed in the testis. Although, PAE-contaminated PE-MPs were seen to enhance reproductive toxicity, as changes in sperm parameters and oxidative stress were observed.

21. Hou *et al*., (2021) evaluated the effect(s) of 5 µm polystyrene microplastics (PS-MPs) on spermatogenesis in 4-5-week-old male ICR mice (n=40; 10/group). The particle size (shape undescribed) was determined by Kurt particle size analysis. The PS-MPs concentrations were 100 µg/L, 1,000 µg/L, and 10 mg/L. The mice in the control group were given distilled water only. All solutions (tests and control) were provided as drinking water on an *ad libitum* basis. The average daily PS-MP exposure dose of each group was calculated to be 0.6–0.7 µg/day, 6–7 µg/day, and 60–70 µg/day, for each respective solution concentrations. Mice were exposed to PS-MPs for 35 days.

22. Post-treatment all animals were anaesthetised. The testes and epididymal tissues were collected and weighed prior to histopathological analyses. Gene expression of inflammatory (interleukins (IL-1β, IL-6)[7], TNFα[8], NF-$_K$β[9] and Nrf2/HO-1[10]) proteins were analysed by Western blot and

[7] Interleukin (IL)-1β and IL-6 are pro-inflammatory cytokines for host-defence responses.
[8] Tumour necrosis factor-α (TNF- α) is a mediator of inflammatory and immune functions.
[9] Nuclear factor kappa-light-chain-enhancer of activated B cells (NF-$_K$β) is a protein complex that controls transcription of DNA, cytokine production and cell survival.
[10] Nuclear factor erythroid 2-related factor 2 (Nrf2) regulates the expression of antioxidant proteins that protect against oxidative damage, of which, heme oxygenase (HO-1) is one. The Nrf2/HO-1 signalling pathway regulates anti-inflammation and antioxidation.

qPCR[11]. Apoptosis-related proteins (Bax and Bcl2) were also analysed by Western blot.

23. No deaths or significant changes in body weight were observed. Although, testicular weight decreased in the low and high treatment groups when compared to the control. The ratio of live sperm in the epididymis to the total number of sperm in the treatment groups was significantly lower when compared to the control group; however, no obvious dose-response relationship was observed. Sperm deformities were observed such as two-tailed, hookless or swollen neck, particularly in the mid and high dose ranges. With regards to the testicular tissue structure, when compared to the control, the treatment groups had decreased number of spermatids and were disorderly arranged in the testicular seminiferous tubules. A decrease in number of spermatozoa, as we all as; pyknosis, nucleus rupture and cell detachment were also observed. Outputs from the TUNEL[12] staining of the testis showed a positive correlation between the dose administered and the number of apoptotic cells.

24. The expression of all inflammatory factors (IL-1β and IL-6) were increased for all treatment groups, whereas expression of TNF-α was only significantly increased in the high dose group. When compared to the control and mid-dose group, the expression of Bcl2 and Bax in the high dose group was significantly decreased and increased, respectively. Results from the qPCR analyses showed that expression of NF-κB, IL-1β, and IL-6 increased significantly, whereas that of the anti-inflammatory molecule Nrf2/HO-1 decreased.

25. Based on the authors' observations, they considered that the abnormal sperm quality in ICR mice caused by PS-MP exposure is closely related to the Nrf2/ HO-1/NF-κB pathway.

26. Xie *et al*., (2020) investigated the impact of 5 - 5.9 μm PS-MPs on the reproductive system of 5-6-week-old male Balb-C mice (n=80; 10/group). They first investigated the effects of PS-MP exposure on testicular injury in mice and then attempted to confirm the effects of PS-MP exposure on oxidative stress and MAPK[13] signalling pathways, as well as its effects on triggering proinflammatory cytokine production. N-acetyl cysteine (NAS) was used as the antioxidant and SB203580 was used as the p38 MAPK inhibitor.

[11] Quantitative polymerase chain reaction (qPCR) is a method by which the amount of the PCR product can be determined, in real-time, and is very useful for investigating gene expression

[12] Terminal deoxynucleotidyl transferase dUTP nick end labelling (TUNEL) staining is an assay used to detect DNA breaks formed during the final phase of apoptosis.

[13] Mitogen-activated protein kinase (MAPK) cascades are key signalling pathways that regulate a wide variety of cellular processes including proliferation, differentiation, apoptosis, and stress responses.

27. The particle size and shape were not confirmed by separate analyses. The PS-MPs were mixed with ultrapure water. The mice were split into 8 groups: (1) saline group (control); (2) 100 mg/kg/d NAC; (3) 5 mg/kg SB203580; (4) 0.01 mg/d PS-MPs; (5) 0.1 mg/d PS-MPs; (6) 1 mg/d PS-MPs; (7) 1 mg/d PS-MPs + NAC; (8) 1 mg/d PS-MPs + SB203580. The mice in groups (4), (5), (6), (7), and (8) were given 0.25 mL of the different concentrations of PS-MPs by oral gavage, once a day for 42 days.

28. Post-treatment all mice were anesthetised. Blood, testes, and epididymis samples were collected for biochemistry and histopathology analyses (*e.g.* haematoxylin and eosin; H & E staining). Reactive oxygen species (ROS) content was determined by DCFH-DA method[14]. The concentrations of IL-1β, IL-6, TNF-α and Casp-3[15] in the supernatant of the tissue homogenate, and the concentration of testosterone in the serum were measured using commercial ELISA[16] kits.

29. No deaths were observed. Although, significant decrease in the body weight (post-6 weeks of treatment) was observed in PS-MP treatment groups when compared to the control group. H & E staining showed decrease in the number of spermatogenic cells which were loosely arranged, and some blank cavities were also observed in the testicular tissue of PS-MP treatment groups. The number of sperms in the PS-MP treatment groups was significantly lower than that in the control group and the sperm deformity rate gradually increased with increasing exposure concentrations of PS-MPs.

30. LDH and SDH enzyme activities, as well as testosterone concentration levels were significantly reduced in PS-MP treatment groups when compared to the control. Administration of NAC and SB203580 inhibitors significantly attenuated the decrease in testosterone levels and SDH enzyme activity in group 7 mice compared to group 6. Exposure to PS-MPs caused a significant increase in ROS and MDA levels compared to the saline group (group 1). These results indicate that exposure to PS-MPs adversely affects the sperm count and quality of mice.

31. To determine the effect of PS-MP exposure on the p38 MAPK signalling pathways, the authors measured the extent of p38 phosphorylation using ELISA. The intensity of the p38 MAPK immunofluorescence signals in group 6 and group 7, was significantly alleviated by NAC treatment. This indicates that MAPK activation occurs after oxidative stress is induced by PS-MP exposure. When compared with the control (group 1), levels of Casp-3, TNF-α, IL-1β and IL-6 were significantly increased in the PS-MP treatment

[14] Dichloro-dihydro-fluorescein diacetate (DCFH-DA) is a quantitative method for oxidative stress assessment. It detects levels of intracellular hydrogen peroxide (H_2O_2).
[15] Caspase-3 (Casp-3) is a lysosomal enzyme involved in the apoptotic pathway. It interacts with Casp-8 and Casp-9.
[16] Enzyme-linked immunosorbent assay (ELISA) is an immunological assay commonly used to measure antibodies, antigens,
proteins, and glycoproteins in biological samples.

groups. The levels of Casp-3 and IL-1β increased with increasing exposure concentrations of PS-MP.

32. Based on the authors' observations, they considered that exposure to PS-MPs induces oxidative stress and activates the p38 and JNK MAPK signalling pathways, resulting in poorer sperm quality in mice, decreased testosterone production, and decreased SDH and LDH enzyme activity.

33. Amereh *et al*., (2020) investigated the potential endocrine disturbance(s) with particular emphasis to the reproductive toxicity of 25 and 50 nm virgin spherical PS-nanoplastics (PS-NPs) in ~8-week-old male Wistar rats (n=30; 6/group). The particles were dispersed in deionised water to make up stock solutions. Prior to dosing, stock solutions were made to the right concentration by the addition of distilled water. The particle size distribution and zeta potential were evaluated by laser diffraction, whilst the agglomeration state and deposition kinetics were quantified through dynamic light scattering. Animals were split into five groups: (1) control (distilled water only); (2) 1 mg/kg bw/day, (3) 3 mg/kg bw/day, (4) 6 mg/kg bw/day and (5) 10 mg/kg bw/day PS-NP treatment groups. Rats were dosed *via* oral gavage, once a day for 35 days. Note that at the 1 and 6 mg/kg bw/day doses, the PS-NPs were fluorescently labelled to investigate the bioavailability and biodistribution of PS-NPs.

34. Post-treatment all mice were anaesthetised. Blood, serum, left testis, and epididymis were collected. ELISA kits were used to analyse the levels of follicle-stimulating hormone (FSH), luteinizing hormone (LH) and testosterone. H & E staining was used for microscopic observation of the testis structure.

35. When compared to the control, concentration levels of testosterone, LH and FSH in the serum were statistically lower in the treatment groups; however, only LH showed this in a dose-dependent manner. In terms of sperm characteristics, the number of sperm declined by ~8, 16, 24 and 45% for each dose group (in ascending order), respectively, when compared to the control. Sperm motility also reduced by up to ~48% at the highest dose. At the highest dose, ~35% of sperm were abnormal (compared to the control at 6.8%). The most common deformities include coiled or bent tails, conjoined sperm with double heads and double tails, and no-hook head. Levels of DNA damage were up to 35%, at the highest dose.

36. Analyses of the H & E stain of testes from treatment groups exhibited seminiferous tubule degeneration (characterised by disorganised, shrunken tubules with irregular/buckled basement membranes), which suggested incomplete spermatogenesis. Severity of lesions and an increase in the reduced numbers of spermatogenic and Sertoli cells were proportional to the tested dose concentrations.

37. Through whole-animal image scanning, fluorescently labelled PS-NPs for the 1 and 6 mg/kg bw/day dose groups accumulated in the testes suggesting that these particles are able to cross the blood-testis barrier (*Figure 3*).

38. To conclude, severe histological lesions and alterations in the morphology of sperm, and varying concentrations of semen biomarkers were observed in testis of rats following exposure to PS-NPs. Although, the authors consider that it is still too early to draw conclusions regarding the potential health risks of plastic particles to other species (including humans), since exposure levels in humans are expected to be lower than those seen in/dosed in some studies in the literature.

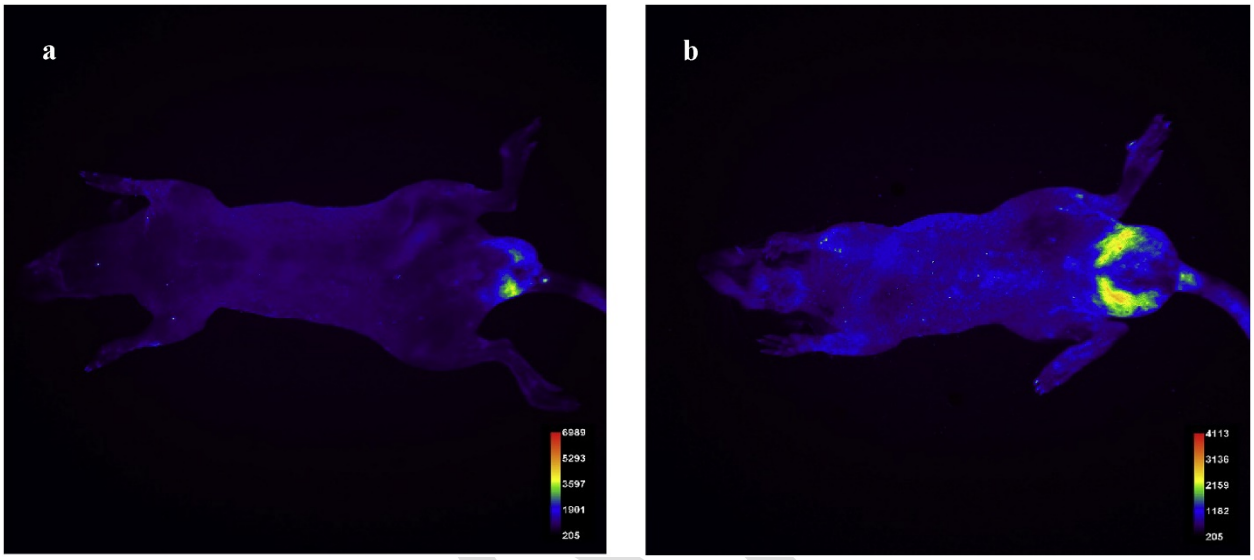

Figure 3 Tissue bioaccumulation of fluorescently labelled polystyrene nanoplastics (PS-NPs). Rats were exposure to (a) 1 and (b) 6 mg PS-NPs/kg bw/day for 35 days (reproduced from Amereh *et al*., 2021).

Other

In vivo (animal models)

39. Zheng *et al*., (2021) performed a comparative investigation between the response activity to 5 µm PS-MPs of 6-week-old C57 mice with acute colitis[17] and healthy mice (n=50; 10/group). The morphology of PS-MPs was analysed by SEM. Mice were divided into 5 groups; (1) treatment with 3% dextran sodium sulfate (DSS) through drinking water for 7 days to induce acute colitis; (2) healthy mice fed with distilled water only (negative control group); (3) healthy mice fed with distilled water containing PS-MPs at a concentration of 500 µg/L; (4) Mice with acute colitis– distilled water only (positive control group); (5) Mice with acute colitis fed with distilled water containing PS-MPs at a concentration of 500 µg/L. Animals were exposed to their treatment for 28 days (except group 1).

[17] Colitis is a chronic digestive disease characterised by inflammation of the inner lining of the colon.

40. Post-treatment, all animals were anaesthetised. Blood was collected for ELISA analysis of IL-1β, TNF-α and IFN-γ[18], the livers were also collected for biochemistry (triglyceride, MDA and PPAR-γ levels) and histopathology analyses (H & E staining). Metabolomic analyses to record changes in the liver were also carried out using proton nuclear magnetic resonance (^{1}H NMR) analysis.

41. The results from the H & E stain showed formation of fatty vacuoles in healthy mice exposed to PS-MPs (group 3); these were observed with mild severity. On the other hand, moderate formation of fatty vacuoles and inflammatory cell infiltration were observed in mice with acute colitis and exposed to PS-MPs (group 5).

42. PS-MP exposure induced inflammatory effects; the levels of IFN- γ in treatment groups 1, 3 and 5 was significantly higher than in the control group (group 2). Exposure to PS-MPs exaggerated DSS-induced acute colitis, as well as lipid disorders, which were verified by the increased expression of inflammatory factors (IL-1β, TNF-α and IFN-γ) and triglyceride accumulation in the gut. ^{1}H NMR analyses indicated that PS-MPs had a greater impact on the metabolic level (lower concentrations of metabolites involved in amino acid, energy, and lipid metabolism) in treatment group 5.

43. The authors hypothesised that the increased intestinal permeability of mice with acute colitis caused by exposure to PS-MPs may be responsible for the upregulated adverse effects, and that populations with chronic diseases might be more sensitive to environmental contamination (such as microplastics).

Cytotoxicity

In vitro (human cell line)

44. Stock *et al*., (2021) investigated the uptake of sedimenting and buoyant polyethylene (PE) and polypropylene (PP) microplastic particles in human intestinal Caco-2 cells. Cytotoxicity was investigated in the following human cell lines: Caco-2, HepG2 and HepaRG using MTT assay[19]. Undigested PE particles were between 1–4 µm, 10–20 µm. Polydisperse PE, PP, PET and PVC powders were characterised by SEM. In terms of size, the mean diameters were 2.2 µm (PE 1–4 µm), 16.5 µm (PE 10–20 µm), 90.1 µm (PE), 67.1 µm (PP), 60 µm (PET) and 136.5 µm (PVC). For shape, PE-MPs were

[18] Interferon-gamma (IFN-γ) is a cytokine that induces and modulates an array of immune responses.

[19] The 3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide (MTT) assay is a colourimetric assay for assessing cell metabolic activity. Actively respiring cells convert the water-soluble MTT to an insoluble purple formazan. The formazan is then solubilised, and its concentration determined by optical density.

porous roundish, PP-MPs were shred-shaped, whilst PVC and PET-MPs were smooth and roundish.

45. All cell lines were exposed to varying concentrations of PE (1–4 µm), PE (10–20 µm), PE, PP, PET and PVC (*Table 1*). Cells were incubated for 24 hours.

Table 1 - shows the varying dose concentrations of each polymer type: polyethylene (PE), polypropylene (PP), polyethylene terepthalate (PET) and polyvinychloride (PVC) in the tested human cell lines; intestinal (Caco-2), and liver (HepaRG and HepG2). Note that controls were also tested: medium and 0.01% Triton-X-100. Conc.; concentration. (adapted from Stock *et al*., 2021).

	Polymer type					
	PE (1–4 µm)	PE (10–20 µm)	PE	PP	PET	PVC
Cell line	**Conc. (mg/mL)**					
Caco-2	1, 5, 10, 25, 50	1, 5, 10, 25	1, 5, 10, 25, 50, 75, 100	1, 5, 10, 25, 50	1, 5, 10, 25, 50, 75, 100	1, 5, 10, 25, 50, 75, 100
HepaRG	1, 5, 10, 25, 50	1, 5, 10, 25	1, 5, 10, 25, 50, 75, 100	1, 5, 10, 25, 50	1, 5, 10, 25, 50, 75, 100	1, 5, 10, 25, 50, 75, 100
HepG2	1, 5, 10, 25, 50	1, 5, 10, 25	1, 5, 10, 25, 50, 75, 100	1, 5, 10, 25, 50	1, 5, 10, 25, 50, 75, 100	1, 5, 10, 25, 50, 75, 100

46. MTT cytotoxicity assays revealed a decrease in viability only for PE (HepG2) and PVC (all cell lines) at extremely high particle concentrations, whereas smaller 1–4 µm and 10–20 µm PE particles as well as PP and PET were non-toxic. This effect was associated with the high extracellular overload of large PE and PVC-MP particles rather than intracellular damage when considering the size limits for cellular uptake. No cytotoxic effects due to specific particle material or shapes could be detected. Caspase activity assays in HepaRG and Caco-2 cells revealed mild effects of PVC and PE on both proteins involved in the extrinsic (Casp-8) and intrinsic (Casp-3 and -9) apoptosis pathway, suggesting unspecific effects of toxicity.

47. The measurement of cellular contact yielded values below 1% for all particles. Little to no contact was found for the PVC-MPs, whilst cell surface contact was found for the other tested plastic particles. The larger particles were observed to be mostly embedded in the cell monolayer, the authors were of the opinion that these particles are likely to be rapidly eliminated *in vivo*, as intestinal cells renew (approximately every 72 hours).

48. In terms of observations for the intracellular uptake of plastic particles, a small fraction (0.42%) of PE-MPs in the 1–4 µm (mean diameter 2.2 µm) size range was transported through the cell monolayer. Absorbed particles of samples treatment with PE-MPs in the 10–20 µm size range were all ~5 µm in size. The authors postulated that the smaller PE-MP particles were already transported to the basolateral compartment after 24 hours, whereas the larger PE-MPs were still detectable intracellularly.

49. To conclude none of the tested particles induced acute toxic effects, regardless of their shape and size. The authors recommended future studies which investigate milder cellular reactions that are not necessarily associate with cell death (*e.g.* inflammatory responses, lysosomal changes, or xenobiotic metabolism) and cellular uptake and transport of plastic particles in the low micrometre and nanometre range.

Questions on which the views of the Committee are sought

50. Members are invited to consider the following questions regarding the first draft of the sub-statement and to raise any other matters that arise from the newly submitted data:

i). Do Members have any comments on the additional information presented in this cover paper?

ii). Regarding paragraph 14; do Members prefer an annexed reference to all of the discussion papers previously presented by the Secretariat (as part of this work stream) or to tabulate all the toxicological papers reviewed?

iii). Do the Members wish to see any other information that they would like to be included in the sub-statement?

iv). Do the Members have any other comments?

Secretariat
July 2021

References

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

Sub-statement on the potential risks from exposure to microplastics: Oral route (First draft)

First draft sub-statement

The attached document is a draft. It should not be cited and does not necessarily represent the views of the Committee. The final version of the statement will be published in due course on the COT website: https://cot.food.gov.uk/

Secretariat
July 2021

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

Sub-statement on the potential risks from exposure to microplastics: Oral route (First draft)

Background

1. In 2019, as part of horizon scanning, the Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment (COT) identified the potential risks from microplastics as a topic it should consider (TOX/2019/08)[20]. In 2021, the COT published an overarching statement on the potential risks from exposure to microplastics (COT Statement 2021/02)[21]. This document provided a high-level overview of the current state of knowledge, data gaps and research requirements with regards to this topic.

Scope and purpose

2. Data on the presence of plastic particles on some foodstuffs (Touissant *et al*., 2019) within the literature is available and the number of such articles is constantly increasing with developments in analytical detection methodologies and growing consumer interest.

3. The purpose of this sub-statement is to provide supplementary material to the overarching statement (COT Statement 2021/02); as referenced in paragraph 1) and consider in detail the potential toxicological risks of exposure from microplastics ingested *via* the oral route (*i.e.* resulting from the presence of microplastics in food, drinking water and bottled drinks). It is based on current available literature and data from internal tools at the UK Food Standards Agency (FSA).

Toxicokinetics

4. Oral ingestion involves a number of processes that influence the physicochemical properties of particles (*e.g.* pH levels of the saliva/digestive fluids may change their surface charge and zeta potential, formation of protein

[20] TOX/2019/08 is available on the COT website.
[21] COT Statement 2021/02 is available on the COT website. The lay summary is also available on the COT website.

coronas) and therefore, their interaction with cells/organs and any subsequent observed health effect.

5. The size of particles is one of the determining key factors of uptake in the gastrointestinal tract (GIT). Particles within the nanoscale (*i.e.* 1 to 100 nm) can distribute to all organs and can be translocated across the blood-brain and placental barriers. The extent of such absorption is poorly described. It is generally accepted that large particles > 150 µm will not be absorbed and thus does not lead to systemic exposure.

6. Two uptake pathways of microplastics (0.1 > 10 µm) from the GIT lumen have been described in the literature.

7. Firstly, *via* endocytosis[22] by the microfold (M) cells of the Peyer's patches, where the M cells sample and transport particles from the intestinal lumen to the mucosal lymphoid tissues. It should be noted that the Peyer's patches are located in the ileum of the small intestine, which represents a small fraction of the total GIT surface area.

8. Secondly, *via* paracellular persorption[23], where 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[24] such particles, subsequently transporting them to underlying lymphatic vessels and veins. Potential distribution to secondary tissues including the liver, muscle and brain may occur (Wright & Kelly, 2017).

9. 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.

10. The COT has previously reviewed relevant animal toxicokinetic studies as a result of exposure from microplastics *via* the oral route (presented in *Annex A*).

11. At the time of review, only one study was found to describe the potential fate of microplastic particles in human GIT. Schwabl *et al*., (2018) assessed the microplastic concentrations in human stool (n=8). Stool samples were tested for 10 types of plastics using Fourier-transform infrared spectroscopy. Up to 9 types were detected ranging from 50 to 500 µm, with polypropylene (62.8%) and polyethylene terephthalate (PET) (17.0%) being

[22] Endocytosis is a cellular process by which cells take in substances from outside of the cell by engulfing them in a vesicle.
[23] Paracellular transport refers to the transfer of substance across an epithelium by passing through the intracellular space between the cells (often referred to as tight junctions).
[24] Phagocytosis is a cellular process by which a cell uses its plasma membra to engulf a particle, giving rise to an internal compartment called a phagosome.

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). Several limitations were identified by the authors including: the low number of participants, and each provided only 1 stool sample. The origin and other fates of microplastics in the GIT (*i.e* absorption, distribution, and metabolism) were not investigated (Schwabl *et al*., 2019).

12. Available data for maternal transfer of nano- and microplastics (NMPs) to embryo/fetus are limited. Ragusa *et al*., (2021) reported the evidence of microplastics in human placenta (analysed by Raman microscopy). In total, 12 microplastic particles ranging from 5 to 10 mm in size (spheric or irregular in shape) in 4/6 placentas from women whom had non-caesarean birth; however, the presence of microplastics in the placenta could not be attributed to an exposure route nor its source.

13. Based on the available information, the COT concluded that there are limited data regarding the toxicokinetic fate of orally ingested microplastics in mammalian species, and that microplastic particles can either remain confined in the GIT, translocate from the GIT into organs or tissues (*via* endocytosis by M cells and paracellular persorption), and/or be excreted (~>90%). There is lack of information on possible metabolism. Furthermore, no epidemiological or controlled dose studies that evaluated the effects of orally ingested microplastics in humans were identified.

Toxicity

14. At the time of review, the COT observed that there is a plethora of literature regarding the presence and toxicity of micro- and nanoplastics in the marine environment, whilst there is limited data that are of direct relevance to humans. The background papers (which includes summaries of literature) previously reviewed by the COT is presented in Annex B.

15. Due to the uncertainties previously mentioned, the toxic effects are often hypothesised. These hypotheses are hazard based and are driven by the physicochemical properties of micro- and nanoplastics. These are:

i). Physical (*e.g*. bulk, which could lead to gut blockage, as observed in aquatic and avian species);
ii). Chemical composition (unbound monomers, additives, sorbed chemicals from the environment e.g. persistent organic pollutants and metals);
iii). Metabolism or degradation to form monomers or other derivatives, some of which could be chemically reactive (*e.g*. isocyanates from polyurethane) and;
iv). The presence of biofilms (attachment and colonisation of microorganisms on the plastics).

Physical hazard

16. A common hypothesis is the local irritation of the intestinal tissues caused by physical mechanical disruption of the intestinal epithelium cells (IEC) membrane layer by retained plastic particles in the lumen. However, intestinal crypts undergo constant cycles of IEC replenishment and renewal, and under normal homeostatic conditions it is estimated that an entire crypt is replaced every 4-5 days (van der Flier & Clevers, 2009).

17. On the other hand, shedding of IECs from the epithelial monolayer may cause transient gaps or micro-erosions in the epithelial barrier, thus resulting in either: increased intestinal permeability or malabsorption to micro- and nanoplastics but also to other chemicals and solutes present in the intestinal tract. There is limited knowledge on the rate and effect of this process on the absorption and the resulting toxicity of micro- and nanoplastics is not known.

18. In certain disease states (*e.g.* individuals with gastrointestinal issues) the integrity of the intestinal barrier may be weaker and thereby affect the crossing of particles (including plastic particles), their systemic bioavailability and subsequent toxicity. The behaviour of micro- and nanoplastics in normal gut condition and in certain disease states warrants further investigation.

Chemical hazard

19. The majority of toxicological studies of micro- and nanoplastics investigate the toxicity of additives (*e.g.* phthalates), unbound monomers (*e.g.* styrene) and sorbed chemicals (*e.g.* persistent organic pollutants and metals).

20. Based on the available information, chemical leachates and adsorbed substances from microplastics are not expected to cause greater adverse health effects in humans due to their small contribution to the overall exposure from other sources of the same chemical as evidenced by the EFSA, 2016 review and the WHO, 2019 margin of exposure calculations.

Metabolism or degradation products

21. Particles >150 µm usually do not translocate across the gut epithelium, whilst smaller particles especially those within the nanoscale (1 nm to 0.1 µm) have the potential for uptake by organs (as mentioned previously). Microplastics may be taken up into cells but there is a lack of information on possible metabolism in humans, therefore this hazard is yet to be fully characterised.

22. Even so, the following question remains: whether large plastic particles (*e.g.* 5 µm) breakdown into smaller sizes in the GIT. If so, do these smaller plastic particles release a higher level of leachates/sorbed chemicals or produce new degradation products from the polymer itself.

Microbiological hazard

23. The UK FSA is currently performing a critical literature review on the microbiological colonisation of nano- and microplastics (NMPs) and their significance to the food chain (FS307021)[25], which was contracted to the Centre for Environment Fisheries & Aquaculture Science. Preliminary outputs from this review were first presented by Bakir *et al*., (2021) during the European Food Safety Authority's (EFSA) Scientific Colloquium 25 titled, "A coordinated approach to assess the human health risks of micro- and nanoplastics in food" on May 2021[26].

24. The four research areas as part of this work package (WP) include: NMPs in the environment (WP1); pathways of colonised NMPs into food chains (WP2); interactions between NMPs and microorganisms (WP3); and NMP-specific microbial risks to consumers (WP4).

25. The evidence gaps and recommendations for further work in - WP4 are summarised in depth here. It was found that data on the presence of viruses on NMPs is currently lacking, and in general very little attention is focused on the potential role of plastic associated microorganisms. Available studies on reporting the presence of pathogens on NMPs in environmental settings were found to be anecdotal and lack robust controls (*e.g.* comparison to other substrates). Additionally, published studies on possible presence of antimicrobial resistant organisms in NMPs were found to be of low quality (*e.g.* lack of appropriate controls), which made data comparison challenging when attempting to ascertain the overall relevance of this hazard to the risk. No human specific study on dysbiosis was found, although studies in other model organisms were available (*e.g.* mice and zebrafish).

26. Overall, for WP4, the authors considered that there is little data regarding the impacts of NMPs on pathogens and human health outcomes. Furthermore, there is a clear lack of available epidemiological data.

27. An authoritative full synthesis report and special report document will be published later in the year to provide a collated and impartial summary of the scientific evidence on the impacts of microplastics and human health, utilizing the most relevant and contemporary scientific data available.

[25] Further details concerning this research project (FS307021) are available on the FSA website.
[26] Further information on EFSA's scientific colloquium is available on the EFSA website.

COT evaluation

28. Presently, a full risk assessment on the potential toxic effect(s) of micro and/or nanoplastics could not be carried out due to several data gaps including:

- The unavailability of harmonised methodologies to characterise, quantify and identify NMPs;
- The lack of toxicokinetic and toxicity data in general. There is no identified no-observed-adverse-effect level (NOAEL) for the different polymer types except possibly for PET powder at 2,500 mg/kg bw/day in rats (Merski *et al*., 2008), which had a number of limitations (*e.g*. particle size and count were not determined/reported);
- The paucity of currently available data for microplastics in different food types and matrices and;
- The difficulty of performing an accurate exposure assessment.

29. For the reasons above, a case-by-case approach to risk assessments may need to be considered.

Research priorities for risk assessment

30. The COT recommends the following research priorities for addressing the data gaps in the potential toxicity of micro- and nanoplastics in humans.

- Comprehensive assessment of MPs and associated contaminant concentrations in different food types (*e.g.* seafood, edible meat tissue and offal, vegetables, fruit, drinks) and matrices (*i.e.* air, soil, food and water) and the impact of the effect of cooking on the desorption and subsequent bioavailability of contaminants/leachates.

- Assessment of the degradation of novel/emerging plastic-based materials on the market such as biobased plastics (*e.g.* bamboo ware, polylactic acid, chitin) and other advanced polymer matrix composite materials during their use and end-of-life for their possible contribution to NMPs. It is unclear whether and by how much they already contribute to the burden of NMPs.

- Studies (*in silico*, *in vitro* and/or *in vivo*) to explore the effect(s) of the same type of NMP on different tissues (*e.g.* heart, brain, liver, stomach, intestines), and of different types of NMP (*e.g*. polymer type, size, shape) on the same target tissue.

- Studies on the persistence and potential accumulation of NMPs in the human body, and on the extent to which NMPs are digestible.

- Investigation of the extent to which NMPs with a range of sizes and compositions are assimilated into human tissues and the development of techniques capable of identifying the presence of microplastics in the

human body (*e.g.* in biopsies, samples from tissue banks, if possible, histopathology sections).

31. The most significant data gaps hindering a robust risk assessment for exposure *via* the oral route include the lack of:

- Appropriate and harmonised analytical methods for the detection of different NMPs in various food matrices;
- Understanding of human exposure and;
- Human-relevant information on the absorption, distribution, metabolism and excretion (*i.e.* the toxicokinetic profile) and on the toxicity profiles of NMPs.

32. Microplastic concentrations are expected to increase in the future. In addition, an increase and widespread use of single-use plastic personal protective equipment (*e.g.* face masks and gloves) due to the COVID-19 pandemic may also be a major contributing source of plastic pollution (Silva *et al.*, 2021). Hence, there will be a need to regularly assess the levels of microplastics in relevant food stuffs, water and the air, such as by establishing a monitoring programme. This would best be achieved by collaboration among academia, researchers, and government bodies at a national and international level.

COT Conclusions

33. The COT noted that there are limited data regarding the toxicokinetic fate of orally ingested microplastics in mammalian species, and that microplastic particles can either remain confined in the GIT, translocate from the GIT into organs or tissues (*via* endocytosis by M cells and paracellular persorption), and/or be excreted (~>90%). No epidemiological or controlled dose studies that evaluated the effects of orally ingested microplastics in humans were identified.

34. As such, the COT concludes that based on the available data, it is not yet possible to perform a complete assessment for the potential risks from exposure to micro and nanoplastics to humans *via* the oral route; however, they concur with the conclusions reached by other authoritative bodies (EFSA, 2016; WHO, 2019; ECCC and HC, 2020; SAPEA, 2019; SAM, 2019, as described in the COT overarching statement on the potential risks from exposure to microplastics; COT Statement 2021/02[27]).

35. The COT concluded that the literature data on exposure to particles from tyre wear would need separate consideration from microplastic exposure from food, since the particles were chemically quite different in their polymeric nature. Risk assessment of such material was considered potentially outside the scope of the current exercise.

[27] COT Statement 2021/02 is available on the COT website. The summaries of each evaluation by these authoritative bodies are from paragraphs 101-129.

36. The most significant data gaps are the lack of appropriate and harmonised analytical methods for the detection and characterisation of micro- and nanoplastics (together with suitable reference standards), as well as information on their toxicokinetic and toxicity profiles in/relevant for humans.

37. The COT highlighted that additional information will be needed from all exposure sources, which include indoor and outdoor air, dust and soil before a holistic risk assessment can be completed. The presence of MPs in (sea)food and water needs to be put into perspective with other sources of MPs such as atmospheric fallout.

38. Comprehensive assessment of microplastics and contaminant concentrations in different foods and the impact of cooking on the desorption and subsequent bioavailability of contaminants/leachates, need to be further investigated to better understand the implications for human health.

39. Current studies typically focus on only one type of particle/tissue interaction, as such, further research is necessary to explore the effects of the range of particle types in different tissues *in vitro* and/or *in vivo*. These range of particle types should also take account of emerging/novel plastic-based materials such as bioplastics.

COT
July 2021
Statement Number 2021/XX

Abbreviations

COT	Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment
ECCC	Environment and Climate Change Canada
EFSA	European Food Safety Authority
FSA	Food Standards Agency
GIT	Gastrointestinal tract
HC	Health Canada
NMPs	Nano- and microplastics
NOAEL	No-observed-adverse-effect level
PET	Polyethylene terephthalate
SAM	EU Group of Chief Scientific Advisors; Scientific Advice Mechanism
SAPEA	EU Science Advice for Policy by European Academies
UK	United Kingdom
WHO	World Health Organisation

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Annex A

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

Sub-statement on the potential risks from exposure to microplastics: Oral route (First draft)

The COT has previously reviewed relevant animal toxicokinetic studies as a result of exposure from microplastics *via* the oral route. Table 1 below presents brief summaries of each reviewed article.

References

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Table 2 - Summaries of reviewed *in vivo* animal toxicokinetic studies.

Polymer type and size	**Animal Model**	**Dose**	**Exposure route and duration**	**Results**	**Reference**
Polystyrene (50 nm)	Male Fisher 344 rats	125 mg/kg bw *via* oral gavage	6 hours	High amounts were found in the stomach and intestinal wall, heart, and kidney. Estimated bioavailability was 0.2 to 1.7%.	Walczak *et al*., (2015)
Polystyrene (nominal diameter of 42 nm)	Zebrafish	~1 mg/g of fish	7 days	F1 fish – embryos and larvae of PS-NP exposed F0; PS-NPs were found in the yolk sac, GIT, liver, and pancreas.	Pitt *et al*., (2018)
Polystyrene (spherical; 24 or 250 nm)	English scallops	15 µg/L	6 hours	An uptake of 30% of 24 and 15% of 250 nm PS-NPs from the available NP burden in the medium.	Al-Sid-Cheikh *et al*., (2019)
Polystyrene latex (spheres; 2 µm)	Mice (both sexes)	6.84 x10^8 particles in 0.1 mL distilled water	Oral gavage 5, 30 and 90 minutes	Proportion of uptake was almost entirely villous rather than associated with mesenteric lymphoid tissues. Highest percentage of uptake in the intestine was 0.32% in mice, whilst in rats and guinea pigs this was 0.13% and 0.12%, respectively.	Doyle-McCollough *et al*., (2007)
	Rats (both sexes)	1.42 to 1.95 x 10^9 particles in 0.25 mL distilled water			
	Guinea pigs (both sexes)		Tube to the pharynx 5, 30 and 90 minutes		

Polystyrene (5 µm)			Water; *ad libitum* 28 days	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. MCT of 5 µm PS-MPs in the liver, kidney and gut were 0.30, 0.95 and 1.39 mg/g, respectively. For the 20 PS-µm MPs, the MCT for the same tissues were 0.76, 0.78, and 0.78 mg/g, respectively.	Deng *et al*., (2017)

Abbreviations: GT; PS-NPs – polystyrene nanoplastics; GIT – gastrointestinal tract; NP – nanoplastic; PS-MP - polystyrene microplastic; MCT – Multi

Annex B

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

Sub-statement on the potential risks from exposure to microplastics: Oral route (First draft)

Discussion papers presented to the COT on the potential risks from exposure to microplastics

TOX/2019/62 (22/10/2019)	Paper 1: Scoping paper on the potential risks from exposure to microplastics
TOX/2020/15 (11/03/2020)	Paper 2: Potential risks from exposure to microplastics: First draft overarching statement (Cover page)
Annex A	First draft overarching statement on the potential risks from exposure to microplastics
Annex B	Paper for information: Background on tyre wear
Annex C	Paper for information: Update on literature
TOX/2020/40 (15/09/2020)	Follow-up to Paper 2: Overarching statement on the potential risks from exposure to microplastics (Cover page)
Annex A	Second draft overarching statement on the potential risks from exposure to microplastics

TOX/2020/58 (01/12/2020)	Follow-up to September 2020 meeting: Overarching statement on the potential risks from exposure to microplastics: Third draft (Cover page)
Annex A	Third draft overarching statement on the potential risks to microplastics