TOX/2020/58

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

Overarching statement on the potential risks from exposure to microplastics: Third draft

Background

1.   The potential risks from exposure to microplastics have previously been discussed at COT meetings from October 2019 – March 2020. The second draft of the overarching statement (Annex A of TOX/2020/40)[1] was presented to the COT in September 2020. It brought together previous discussions, setting out the current state of knowledge, data gaps, and research needs with regard to this topic and summarising the conclusions reached to date.

2.   A short update on the literature was also provided in TOX/2020/40[2].

3.   The Committee requested several changes to the draft as recorded in the September 2020 meeting minutes[3], these are addressed on the third draft of the overarching statement as presented in Annex A.

Update on literature

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

Authoritative reviews

5.   The Environment and Climate Change Canada (ECCC), and Health Canada (HC) recently finalised and published their report on science assessment of plastic pollution in October 2020[4]. The intended purpose of the report is to act as a guide for future scientific and regulatory activities; not to quantify the risks of plastic pollution on

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[1] Annex A of TOX/2020/40 is available on the COT website.
[2] TOX/2020/40 is available on the COT website.
[3] The final minutes of the meeting of the COT on 15th of September 2020 is available on the COT website.
[4] The ECCC and HC report on Science assessment of plastic pollution is available to on the Government of Canada website.

the environment or human health. A brief summary of the 'Impacts on human health' chapter is provided in the following paragraphs.

6.   The effects of macroplastic pollution on human health were not considered in the report "as it is not anticipated to be a concern". As for microplastics, the ECCC and HC reviewed the toxicity of microplastics *via* the ingestion and inhalation routes of exposure. A comprehensive review of *in vitro* studies on microplastics was not conducted "as their relevance to human health is unclear".

7.   In this report, it was stated that upon ingestion (of food, bottled water, and tap water) or inhalation (of indoor and outdoor air), microplastics may exert effects due either to their physical presence in the gut or lung or to the chemical composition of the plastic polymers themselves or their monomers, additives or sorbed[5] substances.

8.   Post oral exposure, it was noted that the degradation of microplastics to smaller polymers has been observed in environmental models (*e.g.* Antarctic krill; Dawson *et al*., 2018), however, it is uncertain whether this occurs in the human gastro-intestinal tract (GIT). Particle concentration can also influence toxicity, as biological clearance systems are expected to be overwhelmed at higher concentrations. It was concluded that, "at present, it is unclear how other properties, such as shape and surface chemistry, may affect the uptake, retention, and/or toxicity of ingested microplastics". It was noted that there are limited data regarding the toxicokinetic fate of orally ingested microplastics in mammalian species, and that they can either remain confined in the GIT, translocate from the GIT into organs or tissues (*via* endocytosis in 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.

9.   As for the specific effects on microplastics on the lung; the ECCC and HC concluded that there is a paucity of information on the physical hazards related to inhalation of microplastics. The relevance of occupational data on airborne microplastics to the general population was explored; it was concluded that this is unknown due to the extrapolation from high-dose occupational exposures to lower doses (as would be expected for the general population). It was also highlighted that this extrapolation may be difficult to perform since there is an absence of observed health effect data at lower concentrations. Most studies did not investigate the impact of dose-response on the health outcomes. Additionally, workers in these studies might have had co-exposures to other chemicals associated with adverse health effects (*e.g.* monomers, catalysts, additives, and other compounds used in the workplace). It was recommended that studies should focus on confirming and exploring the toxicological mechanisms of physical hazards associated with microplastics, including their effects on the lung and cardiovascular system, and their capacity to translocate to extra-pulmonary tissues (*e.g.*

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[5] Sorption is a physical and chemical process by which one substance becomes attached to another.

pleura, lymph nodes, abdomen, genitourinary tract, skin, joints, and bones, or meninges).

10.   The potential effects of sorbed chemicals and biofilms on plastic surfaces were also briefly reviewed. The extent of release for sorbed chemicals depend on various factors. For example, the properties of the receiving environment, the plastic particle, and the bound chemical. Based on recent international reviews (ESFA, 2016; Lusher *et al*, 2017; WHO, 2019), the ECCC and HC were in the view that there is likely a low health concern for human exposure to chemicals from ingestion of microplastics from food or drinking water. As for biofilms, it was concluded by the ECCC and HC that there is currently no indication that microplastic-associated biofilms would impact human health, and it is anticipated that drinking water treatment would inactivate biofilm-associated microorganisms.

11.   To conclude the following research were recommended (ECCC & HC, 2020):

   i).   Developing standardised methods for sampling, quantifying, characterising and evaluating the effects of macroplastics and microplastics;
   ii).   Furthering the understanding of human exposure to microplastics;
   iii).   Furthering the understanding of ecotoxicological effects of microplastics;
   iv).   Furthering the understanding of microplastics on human health and;
   v).   Expanding and developing consistent monitoring efforts to include poorly characterised environmental compartments such as soil.

Toxicological data

In vivo (animal models)

Reproductive toxicity

12.   Jin *et al*., (2021) investigated the reproductive toxicity of fluorescent polystyrene microplastics (PS-MPs) in male reproduction of BALB/C mice (n=12; 3/group). Raman spectra was utilised to confirm that the monomer was PS. PS-MPs were of different diameters: 0.5, 4 and 10 µm as confirmed by field emission scanning electron microscopy (SEM).

13.   The control group (n=3) was administered with 100 µL of ultra-pure and sterile water, whilst each of the three PS-MP size was administered once a day to one group (n=3) at 10 mg/mL *via* oral gavage for 28 days. The Secretariat wishes to highlight the small number of groups sizes, as the recommended number for each group is 10 males according to the Organisation for Economic Co-operation and Development (OECD) Test Guideline (TG) No. 421 (OECD, 2016).

14.   During the period of exposure, the body weights of mice, food and water intake were monitored once a week. The tissues (gastric, testis, and epididymis) and blood were collected and were analysed using fluorescence, Western blotting and enzyme-linked immunosorbent assays (ELISA), respectively. Sperm morphology was assessed as described by Linder *et al*., (1992). Haematoxylin and Eosin (H & E) staining was conducted to assess the effects of PS-MPs exposure on testicular structure.

15.   A significant decrease in food consumption was reported for groups exposed to 4 and 10 µm PS-MPs, as measured once a week by weighing food intake (g). The body weights in these groups decreased after treatment for 2 and 3 weeks, respectively. No significant changes were induced by 0.5 µm PS-MPs for these parameters. Moreover, there were no significant changes on consumption of water or paired testis/body weights post-treatment of all sizes of PS-MPs. Based on H & E staining; PS-MPs caused shedding and disordered arrangement of spermatogenic cells. Occurrence of multinucleated gonocytes[6] within the seminiferous tubule was also observed.

16.   Testosterone levels in the serum significantly decreased at ~2.1, 1.9, 1.8 and 1.6 ng/mL for the control, 0.5, 4 and 10 µm PS-MPs treatment groups, respectively. Furthermore, a significant decrease in sperm motility, whilst an increase in sperm abnormalities (acrosome loss, cephalic (small head), acephalia (no head), cervical folding and tailless) were observed for all treated groups. For the described endpoints above, there was no statistical difference between the tested sizes of PS-MPs. Based on the results of ELISA and real-time quantitative reverse transcription polymerase chain reaction analyses, an increase in tumour necrosis factor alpha, interleukin-6, monocyte chemoattractant protein-1, chemokine, and ligand 10 was detected, indicating that exposure to PS-MPS (for all sizes) induces testicular inflammation.

17.   PS-MPs were detected in the gastric tissue for all PS-MP sizes based on e*x vivo* fluorescence images of excised gastric tissue. As for the testis, fluorescence intensity was only observed in 4 and 10 µm treated groups; with "barely no fluorescence intensity" in the 0.5 µm treated group. Disassembly of the blood-testis barrier was recorded across all PS-MP treatment groups. In addition, the expression of blood-testis barrier-associated junction proteins (*e.g.* tight junction proteins) were downregulated.

18.   Based on the results, the authors concluded that PS-MPs exposure could induce male reproductive toxicity in mice, and that future studies should focus on the molecular mechanisms involved in this process.

Maternal and F1 toxicity

19.   Luo *et al*., (2019) exposed pregnant ICR mice to 0.5 and 5 µm pristine PS-MPs at 100 and 1,000 µg/L (in drinking water; *ad libitum*) to investigate maternal MPs

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[6] Gonocytes are long-lived precursor germ cells responsible for the production of spermatogonial stem cells.

exposure during gestation and evaluate the potential effects on the offspring. Pregnant mice were divided into five groups (n=5-6; randomly divided to ensure there were no significant differences in average body weights). The control group received normal drinking water. Physiological observation, gene expression and metabolite analyses were carried out to record effects. The Secretariat wishes to highlight the small number of groups sizes, as the OECD recommends that each test and control group should contain at least 20 pregnant females per dose group, in accordance with OECD TG No. 443 (OECD, 2018).

20.   Body weights of F1 female and male were assessed, and no significant differences were observed. The serum triglyceride levels and hepatic total cholesterol, triglyceride levels of F1 male offspring displayed a particle size dependent effect, although the statistical significance was only observed for the 5 μm PS-MPs treated group. In female F1 offspring, amino acids (arginine, leucine, methionine and valine) tended to decrease whilst most amino acids (alanine, citrulline, tyrosine, methionine, and proline) for the male F1 offspring tended to increase after maternal exposure to PS-MPs. The authors noted that the specific mechanism(s) for this difference deserves further study. Free carnitine and acyl-carnitine were also changed, where concentrations of free carnitine increased significantly in the serum of female F1 offspring; while the long chain acyl-carnitines were decreased significantly for male F1 offspring. Gene expression involving β-oxidation (Ppar-α, Acox, Cptα and Mcad) were mostly suppressed after maternal exposure to 5 μm PS-MPs.

21.   The authors offered two reasons for the metabolic disorder of the offspring. Firstly, maternal metabolism was changed as a result of PS-MP exposure, which induced intergenerational effects on the F1 offspring. Secondly, it was hypothesised that small-sized MPs could pass through the placental-barrier, and even transfer to the next generation. The authors concluded that the observed consequences induced by maternal PS-MP treatment seemed to have a close relationship with particle size, as metabolic changes (as evidenced by gene expression analyses) were more pronounced in the 5 μm PS-MP treated group.

Cardiotoxicity

22.   Li *et al*., (2020a) investigated the impact of PS-MP exposure on the cardiovascular system and any underlying toxicological mechanism in male Wister rats (n=32; 8/group). Rats in the experimental groups were exposed to 0.5 μm PS-MPs at 0.5, 5 and 50 mg/L *via* drinking water for 90 days (*ad libitum*). Rats in the control group drank deionised water. The shape and composition of the particles were confirmed by SEM and Fourier-transform infrared spectroscopy (FT-IR).

23.   The health and weight of the animals were monitored regularly throughout the treatment. Post-sacrifice, blood and heart tissues were collected. A third of heart samples were immediately fixed with 4% paraformaldehyde, another third was fixed in 2.5% glutaraldehyde, and the remaining third was frozen for storage. Histological

analyses included transmission electron microscopy, H & E and immunohistochemical staining. Oxidative stress in the heart samples (n=6) was evaluated by measuring levels of antioxidant components (*e.g.* superoxide dismutase, catalase, glutathione peroxidase) and malondialdehyde (MDA) assay kits. ELISA was used to detect the levels of creatine kinase and troponin-1 in the heart samples (n=6). Western blotting was also carried out to measure Wnt/β-catenin[7] signalling protein expression levels including: Wnt, β-catenin, p-β-catenin, TGF-β, α-SMA, collagen I, collagen III, fibronectin, and β-actin. No explanation for the discrepancy between the number of samples (n=6) and group size (n=8/group) was provided.

24.   For the mid dose group (5 mg/L) the following observations were recorded: PS-MPs were observed in cardiomyocytes and the mitochondria; significant increase in MDA level and a decrease in antioxidant components; significant increased expressions of Wnt, TGF-β, p-β-catenin, α-SMA, collagen I and fibronectin; and increased vascular congestion.

25.   For the high dose group (50 mg/L) marked interstitial hyperplasia of myocardial tissue (with some thinning and rupturing); and a significant increase in expressions of β-catenin and collagen III were further observed.

26.   Based on the results, the authors concluded that PS-MPs can translocate to the heart *via* the circulatory system, and that they have the potential to induce apoptosis of cardiomyocytes and activation of the Wnt/β-catenin signalling pathways through oxidative stress.

Other

27.   Deng *et al*., (2020) investigated whether virgin polyethylene microplastic (PE-MPs) spheres can act as vectors and release phthalate esters (PAEs) in guts of male CD-1 mice (n=72), and its observed toxic effects. 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 FT-IR. Spheres were 45-53 μm in size, as confirmed by SEM and laser scattering particle size distribution analysis.

28.   Mice were split into twelve treatment groups (n=6/group). PAE-contaminated PE-MPs were prepared by mixing 0.2 g/L of PE-MPs with either 5 or 50 μg/L of each PAE. Two cages of mice were exposed to pure water and virgin PE-MPs. Eight cages of mice were exposed to PAE contaminated PE-MPs (at both concentrations). The two remaining groups were exposed to DEHP alone as the positive control. The

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[7] Wnt/β-catenin is a signalling pathway that causes an accumulation of β-catenin in the cytoplasm and its eventual translocation into the nucleus to act as a translational co-activator of transcription factors belonging to the TCF/LEF family.

concentration of DEHP was selected according to the adsorption of DEHP on PE-MPs and the MPs administered for mice each day. The animals were exposed for 30 days *via* oral gavage. Blood chemical, gut transcriptomics and gut microbiota analyses were performed to investigate the effects of PAE-contaminated PE-MPs.

29.   The maximum absorption of PAEs on PE-MPs was ~70, ~60, ~40 and ~30 µg/g for DEHP, DBP, DEP and DMP, respectively. The intestinal accumulation of individual PAE released by contaminated MPs followed the order of DEHP > DBP > DEP > DMP; their respective maximum accumulation in the gut was ~180, 140, ~120 and ~100 ng/g dry weight. To summarise, the doses varied ~2-fold between the adsorption of PAEs on PE-MPs and the intestinal accumulation varied with the dose. Using polarised light microscopy, the authors observed the presence of virgin PE-MPs and DEHP-contaminated PE-MPs in mice gut for each respective treatment group suggesting that PE-MPs localised in the small intestine.

30.   No deaths were observed, however, a significant decrease in final body weight was recorded in the PE-MP and high DEHP treated group. Through transcriptomic analysis, DEHP-contaminated PE-MPs significantly altered the expression of genes involved in oxidative response (*e.g.* Cyp1a2 and Cyp2a5), lipid metabolism, immune response (*e.g.* H2-DMb2, H2-Eb and Gm8909) and hormone metabolism (*e.g.* Aldh8a1, Scarb1 and Rdh16). Gut microbiota analyses showed at the phylum level; *Firmicutes* and *Bacteroidetes* dominated in both control and treatment groups, and the relative abundance of *Actinobacteria* significantly increased due to virgin PE-MPs and DEHP-contaminated PE-MPs exposure. At the genus level, *Lactobacillus* dominated in both virgin PE-MPs and DEHP-contaminated PE-MPs treatment groups.

31.   The authors concluded that exposure of DEHP-contaminated PE-MPs caused inflammation and metabolic disorders in mice gut manifested by increased intestinal permeability, enhanced inflammation, differentially regulated genes and altered gut microbiota.

Exposure data

Oral

32.   Li *et al*., (2020b) investigated the potential exposure of infants to microplastics from consuming formula prepared in polypropylene (PP) infant feeding bottles (n=20 each/three products; bottle body, accessories (round disk and straw) and gravity ball). The authors tested the quantity of microplastic release from the bottles during standard formula preparation steps as recommended by the World Health Organisation (WHO); cleaning (rinsing using 25°C deionised water; repeated three times), sterilising (soaking in 95°C deionised water for 5 minutes) and mixing techniques (pour in 70°C deionised water and shaking for 60 seconds).

33.   Samples were filtered (pore size 0.8 µm), the quantity and topography of the released PP microplastics were determined by Raman spectroscopy and atomic force spectroscopy. The quantity and size of the PP microplastics were analysed using ImageJ. The confirmation of reliability for the methodology protocol was conducted using a recovery test using standard polystyrene microplastic samples of similar size and concentration to the microplastics released from the PP products.

34.   The release characteristics of the representative PP products were measured. It was found that depending on the product tested, the measured microplastic levels were three to five orders of magnitude higher than the background level collected using the control sample (~170 particles/L). The highest release was recorded at 16,200,000 particles/L, and the release was shown to be dependent on water temperature. The majority of microplastics were <20 µm in size.

35.   Exposure estimates were calculated in 48 regions (including the UK, US, Brazil, China and India) by using the release rates from PP infant feeding bottles from the results of this study, the market share of PP infant feeding bottles, the non-breastfeeding rates and the infant daily milk intake volume relevant per region. UK infants were estimated to be exposed to >3 million particles/day, in comparison to estimated levels for the US, Brazil, China and India at 2 -3 million, 1-2 million, <100 thousand, and 100 thousand -1 million particles/day, respectively.

36.   The authors concluded that other unaccounted sources of MP release from other PP products (*e.g.* kettles, breast pumps) should be taken into account in the total exposure and that potential risks can be mitigated by changing local practices.

Questions on which the views of the Committee are sought

37.   Members are invited to consider the following questions regarding the third draft of the overarching 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).   Are there Members happy with the changes made to the draft statement?
   iii).   Do the Members have any other comments on the structure and content of the statement?
   iv).   Would the Members be happy for the overarching statement to be cleared *via* Chair's action?

Secretariat
December 2020

Abbreviations

COMEAP	Committee on the Medical Effects of Air Pollutants
COT	Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment
DBP	Dibutyl phthalate
DEHP	Di-2-ethylhexyl phthalate
DEP	Diethyl phthalate
DMP	Dimethyl phthalate
ECCC	Environment and Climate Change Canada
EFSA	European Food Safety Authority
ELISA	Enzyme-linked immunosorbent assay
FAO	Food and Agriculture Organisation
FT-IR	Fourier-transform infrared spectroscopy
GIT	Gastrointestinal tract
H & E	Haematoxylin and Eosin
HC	Health Canada
MDA	Malondialdehyde
OECD	Organisation for Economic Co-operation and Development
PAE	Phthalate esters
PC	Polycarbonate
PE	Polyethylene
PET	Polyethylene terephthalate
PP	Polypropylene
PS-MPs	Polystyrene microplastics
PVC	Polyvinyl chloride
SEM	Scanning electron microscopy
TG	Test guideline
WHO	World Health Organisation

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Overarching statement on the potential risks from exposure to microplastics: Third draft

Third draft 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
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