exposed to PFAS. The reductions in antigen-specific antibody responses in experimental rodent models occur when exposed animals are immunized with either T cell-dependent or T cell-independent antigens, suggesting that the deficiency in antibody production lies at the level of the B cell. However, earlier studies reporting antibody reduction did not report reductions in the overall number of B cells in the spleen. Thus, experiments were conducted to dive more deeply into B cell subsets in mice exposed to the legacy PFAS, perfluorooctanoic acid (PFOA) at a dose and duration known to suppress the T cell-dependent antibody response (TDAR). Male and female C57BL/6 mice were orally dosed with 0 or 7.5 mg/kg of PFOA for 15 days; one subset was immunized with a T cell-dependent antigen five days before dosing ended and another subset received no immunization. One day after dosing ended, animals were humanely euthanized, spleens were removed, and prepared into single cell suspensions. The subset of spleen cells from immunized animals were stained with markers to identify the following B cell subsets: naive, marginal zone, follicular, plasmablasts, and memory. From the subset of spleen cells that were not immunized, naive B cells were isolated, activated ex vivo with anti-CD40+ and IL-4. After a 24 hour incubation, activation was verified with flow cytometry and mitochondrial markers were evalauted with a mitochondrial stress test kit on a Seahorse XFe96. Alterations were found in numbers of follicular cells and plasmablasts in male animals and shifts in mitochondrial energy use were detected in B cells collected from both male and female animals. These data suggest that exosure to PFAS may inhibit the ability of B cells to differentiate and/or proliferate due to altered bioenergetics. Future work will continue to explore this potential mechanism of suppression of the antigen-specific antibody response across different PFAS structures.

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S04 | New developments in micro- and nanoplastics research

S04-01
Inflammation-related key events stimulated by micro- and nanoplastics

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The presence of micro- and nanoplastic particles (MNP) in our environment has raised increasing public and political concern. Increasing evidence suggests that humans are exposed to these MNP, mostly via inhalation or ingestion. The EC-Horizon project POLYRISK (https://polyrisk.science) aims to establish an IATA/AOP-based tiered approach to assess human health risks of MNP. In our research, we focus on assessing immunotoxicological effects of MNP.

We have tested a series of primary, secondary, environmentally aged (incubated in River Rine water) and chemically altered MNP for their potential to affect dendritic cells or macrophages. MNP used in this study include primary and weathered PS, secondary PVC, PA, PP (with or without talc) and PE. The secondary PE and PP (w/o talc) particles appeared to have oxidized groups on their surface.

Data shows that MNP can be engulfed by macrophages (PMA-stimulated human THP1 cells) and human blood-derived dendritic cells (DCs). The effects of virgin as well as secondary MNP (0, 10 or 100 µg/ml) on THP1 macrophages were limited to decrease of mitochondrial activity (Alamar blue), increase of cellular leakage (LDH), and stimulation of lysosomal activity. None of these MNP stimulated gene expression of NFκB or release of pro-inflammatory cytokines (IL-6, TNFα, IL-1β). On the other hand, secondary PP and PE with oxidized surface groups did increase NFκB gene expression and release of cytokines by THP1 macrophages. Virgin and weathered PS particles were tested using DCs, and only weathered PS did stimulate DC activity (increased costimulatory molecules CD83, CD86) and as consequence allogeneic T cells. DC activation appeared to result from environmental contaminants.

In conclusion, of all MNPs tested only those that contained active surface groups or environmental components appeared to be immunostimulatory, whereas primary and secondary MNP rather reduced macrophage activity and viability (at relatively high concentrations of 100 µg/ml). Further research is needed to reveal molecular mechanisms and to translate *in vitro* findings to real-world exposure scenarios.

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S04-02
Redox-mediated toxicity of micro- and nanoplastics in intestinal models

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Micro- and nanoplastics (MNPs) have been detected in various human tissues, including blood, lung, colon, placenta, liver, kidney and spleen, yet there is limited knowledge about their health effects. A primary route of MNP exposure for humans is through ingestion, with the intestine acting as the first line of defence. Similar to other (nano)particles, alterations in the cellular redox state represent a fundamental step in the toxicological pathways associated with MNPs.

In this talk, I will elaborate on the link between MNPs and redox responses within *in vitro* intestinal models (Caco-2, Caco-2/HT29-MTX-E12), and how physicochemical properties of MNPs are of importance. Because of the diversity in sizes, polymer types, and shapes of MNPs, we use a range of both commercially available and environmentally relevant particles to tackle the complexity of MNPs (e.g. polystyrene beads and fibres, polyvinyl chloride fragments, low density poly ethylene fragments). I will focus on the interplay between mitochondria and redox state by looking into the expression of redox-related genes, H_2O_2 levels, mitochondrial DNA content, footprint and mitochondrial network morphology.

Overall, our findings show that smaller MNPs (<1µm) are more easily taken up, and translocated across the intestinal barrier, than larger MNPs. None of the MNPs induce a cytotoxic response, but distinct redox responses (such as higher *HMOX1* gene expression) and mitochondrial stress responses (including higher mitochondrial DNA content) are observed depending on the size and shape of the MNPs. Weathering by artificial stomach acid changes the behaviour of the MNPs, and thus their underlying redox responses.

These results stress the importance to further explore the relationship between the physicochemical properties of MNPs, their uptake kinetics and redox responses to fully comprehend the hazard that MNPs may pose to the intestinal barrier.

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