

## Getting the chemicals right: Gaps and opportunities in addressing inorganics in life cycle assessment

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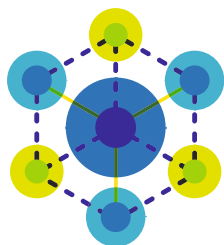
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microparticles on marine filter feeders, the present work, deploying the Mediterranean mussel as model, comprises three interlinked aims: i) estimating plastic microparticle clearance rate through filter-feeding; ii) tracing the presence of microparticles in organisms, and iii) determining effects of particle ingestion. Mussels were exposed to two polystyrene microparticles with different sizes (6  $\mu\text{m}$  and 10  $\mu\text{m}$ ), single and combined, at an environmentally-relevant concentration (1000  $\text{part}\cdot\text{ml}^{-1}$ ), in a series of short and mid-term bioassays. The biological effects of exposure were chiefly determined histopathologically. The findings indicate that the animals are able to rapidly remove both particles from the water column. Interestingly, the larger particles could be found in digestive tract just after 5 min of exposure, whereas the smaller particles required about 15 min. In either case, and in the mixed exposure, particles were only found in the lumen of gut (and faeces) and not in gills or the digestive gland. No traces of microplastics were found in the remaining visceral mass. Regardless of particle, singly or combined, no severe internal lesions were found, including abrasion of digestive epithelia, even after 21 days. However, focal haematocytic infiltration was observed in the same epithelia, without clear time-responsiveness. The results indicate that the animals are indeed able to rapidly remove the particles from the water column and transfer them into the digestive tract. Here, they accumulate and eventually generate moderate inflammatory-like responses, at least during the time of the bioassays and under ecologically-relevant concentrations of microplastics. In spite of the reduced internal damage, the fact that the animals are able to swiftly translocate plastic particles to the gut reveals that filter feeding organisms are indeed a target of concern. Altogether, the study raises further concerns on microplastic risk, with particular respect to realistically long exposures to micromaterials that even such specialised filter-feeders as bivalve molluscs are unable to discriminate.

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#### **Tissue alteration in blue mussels (*Mytilus galloprovincialis*) caused by the ingestion of polyethylene microplastic particles from toothpaste**

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Polyethylene (PE) microplastic particles (< 5 mm) found in certain consumer products, such as toothpaste, find their way into the marine environment via effluents from wastewater treatment works. There is serious concern that these particles may harm marine organisms, particularly filter feeders such as bivalves. In order to evaluate the effects of such particles on bivalves, blue mussels (*Mytilus galloprovincialis*) were exposed over 21 days to PE particles (10 mg/L; 50-570  $\mu\text{m}$ ) isolated from toothpaste. Both virgin and weathered PE particles (deployed in the Outer Oslofjord for 3 weeks) were used to best mimic environmentally realistic exposure scenarios in marine waters. The mussels ingested both virgin and weathered particles, but 26% more of the weathered particles. Particles < 383  $\mu\text{m}$  in size were ingested. To our knowledge, this is the first study that identifies the ingestion of primary microplastic from consumer products in bivalves. PE particle ingestion resulted in structural changes to the gills and digestive gland and necrosis in other tissues such as the mantle.

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#### **Gene expression in liver of European sea bass *Dicentrarchus labrax* experimentally exposed to PVC microplastics**

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Plastic debris, in particular microplastics (MPs; < 1 mm), can potentially affect a wide range of marine organisms. The effects on fish species, in particular species for human consumption, are still under debate. Few species have shown toxicological or physical impact, such as liver toxicity caused by the ingestion of virgin and/or contaminated MPs or the alteration of intestinal tissues. The European seabass (*Dicentrarchus labrax*) is one of the most consumed fish species in Europe and it is potentially exposed to the ingestion of MPs both in its natural habitat and in the aquaculture plants. We measured, by quantitative Real-Time PCR, the variation of four different early warning signals in the liver of the European sea bass exposed for 90 days to virgin (MPV) and marine polluted PVC (MPI) MPs supplemented with food. The selected genes are: the TNF receptor associated factor 3 (TRAF3), related to the activation of the immune and tumor-associated responses; the Peroxisome Proliferators Activator Receptors alpha and gamma (PPAR $\alpha/\gamma$ ), which mediate the responses to several chemicals including plasticizers; the Estrogen Receptor alpha (ER $\alpha$ ), a nuclear receptor involved in the activation of transcription of estrogen-related molecular pathways, included xenoestrogens. The mRNA levels were quantified on 42 fish sampled at time 0, after 30, 60 and 90 days of exposure to the two treatments (MPV and MPI) plus control (CTRL). The expression of TRAF3 appears to be down-regulated with increasing time of exposure. The ER $\alpha$  mRNA levels are higher in the control of each treatment in comparison to MPI and MPV

for all the exposures, suggesting an upregulation of the gene related to contaminated food pellets. On the contrary, the PPAR $\alpha$  gene expression increases over the time from 60 to 90 days of exposure. The PPAR $\gamma$  does not show a temporal trend, but seems to be mostly affected by the MPV exposure, suggesting an effect due to leaching of plastic additives from PVC. The present study represents one of the first investigation on the effects of the exposure to virgin and marine polluted PVC MPs on an edible species, the European seabass. These results represent an early warning signal on the chemical and physical hepatic stress on this species. Further investigations are needed to better understand the role of the partitioning of chemicals from and to MPs, the physical effects of MP ingestion and the related effects on fish and, potentially, on human health.

#### **Increasing the relevance of toxicity assessment in LCA: in the need for a cross fertilization between RA and LCA (I)**

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#### **Un-characterized elementary flows: to which extent is the quantification of the toxicity impact categories in LCA complete?**

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The total number of elementary flows contributing to aquatic freshwater and human toxicity are in a range of 1500 to 2000, but the most common methods used for impact assessment associate a characterisation factor (CF) only a small portion of them. This means that only a small fraction of the substances in the inventory, and usually a small mass, contributes to the overall impact score. A deep analysis of the life cycle inventory of 17 food products, taken as a case study, showed that the proportion of un-characterized flow can be very important: 55% of ELFLs to air compartment (66% as mass), 32% of ELFLs to soil (12% as mass) and 83% of ELFLs to water (24% as mass). This high number of un-characterized flows may lead to high underestimation of the toxicity impact categories and wrong identification of the hotspots. A check of the inventoried elementary flows that are not characterized at the LCIA phase should always be done to support interpretation of results. Indeed, the characterisation of the inventory into potential environmental impacts may not occur if some of the elementary flows are not covered by the chosen characterization models. Toxicity impact categories in LCA and in the Product Environmental Footprint (PEF) are often the dominating impact (after normalization) and metals are often identified as the most contributing elementary flows (ELFLs).

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#### **Getting the chemicals right: Gaps and opportunities in addressing inorganics in life cycle assessment**

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Life cycle assessment (LCA) is used to compare products and product systems in terms of their environmental sustainability and for that LCA needs to include all potential impacts on humans and the environment. Currently, quantifying the toxicity potential of several thousand organic substances and certain cationic metals is included in existing characterization models within life cycle impact assessment (LCIA). However, a variety of additional inorganic substances used e.g. in the textile, personal care, and building and construction industry are included neither in current life cycle inventory databases, nor current LCIA methods. Without the integration of the various economically relevant and potentially human toxic and/or ecotoxic inorganic substances such as inorganic salts, acids, bases and elements, however, no satisfying conclusions regarding the environmental sustainability of any technology containing any of these substances can be drawn. We provide an overview of different substance groups already incorporated in LCIA toxicity characterization modeling, the economic and environmental relevance of inorganic chemicals, and an outline of possible ways towards incorporating inorganic chemicals in LCIA toxicity characterization. The analysis of existing LCIA approaches of specific organic and inorganic chemical groups including PFASs, nanoparticles, salts causing salinization, and common ionic liquids show that the fate, exposure and effect modeling have to be adapted at various levels for the characterization of inorganic substances other than cationic metals. Differences in physicochemical properties and environmental fate and transformation processes of these specific substance groups compared to inorganic substance groups show that the existing LCIA model USEtox cannot be applied to inorganic substances without further modification towards including specific reaction- and process-kinetics. Possibly relevant chemical reaction pathways will be outlined as a necessary step toward improving the environmental fate and (human and ecosystem) exposure assessment of various inorganic substances. Also, we present an overview of the availability of ecotoxicity and human toxicity effect data for elements and inorganic compounds in state-of-the-art databases. An overview of how conventional toxicity effect data can be adapted and used for estimating toxicity-related effects of inorganic substances on humans and ecosystems will be provided.

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