Alternative pathways of microplastic exposure – microplastics and associated POPs on zebrafish gills (*Danio rerio*) and zebrafish eggs

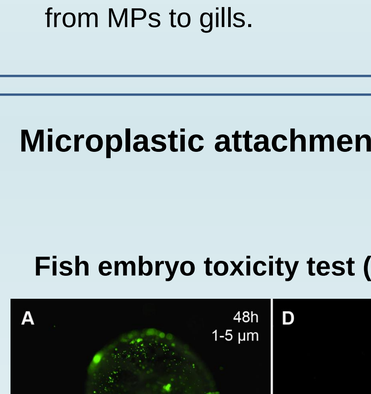
Annika Batel *, Flora Borchert, Hannes Reinwald, Thomas Braunbeck

Aquatic Ecology and Toxicology, Centre for Organismal Studies, University of Heidelberg *corresponding author: annika.batel@cos.uni-heidelberg.de

Introduction

This study analyzes the accumulation pattern and potential transfer of toxic substances on zebrafish (*Danio rerio*) gills and embryos *via* microplastic exposure. Until now, the main focus in microplastic research was set on microplastic abundance, behavior and ingestion by a wide range of aquatic organisms, as well as the absorbance and potential transfer of associated persistent organic pollutants (POPs) along with microplastic ingestion. Only very few studies included the accumulation and effects of microplastics other than by ingestion (Lu et al. 2016, Watts et al. 2014). In this study, two different sizes of fluorescently labelled polymers (1-5 and 10-20 µm) and the model substance benzo[a]pyrene (BaP) as representative for POPs were used to analyze the behavior, accumulation and POP transfer of microplastics on zebrafish (*Danio rerio*) gills and embryos with histological analyses, a Gill EROD assay (Jönsson et al. 2009) and fluorescence tracking (Batel et al. 2016).

Microplastic accumulation and POP transfer on zebrafish gills

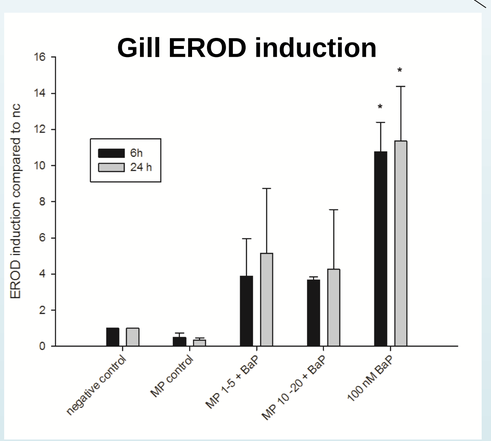


Accumulation

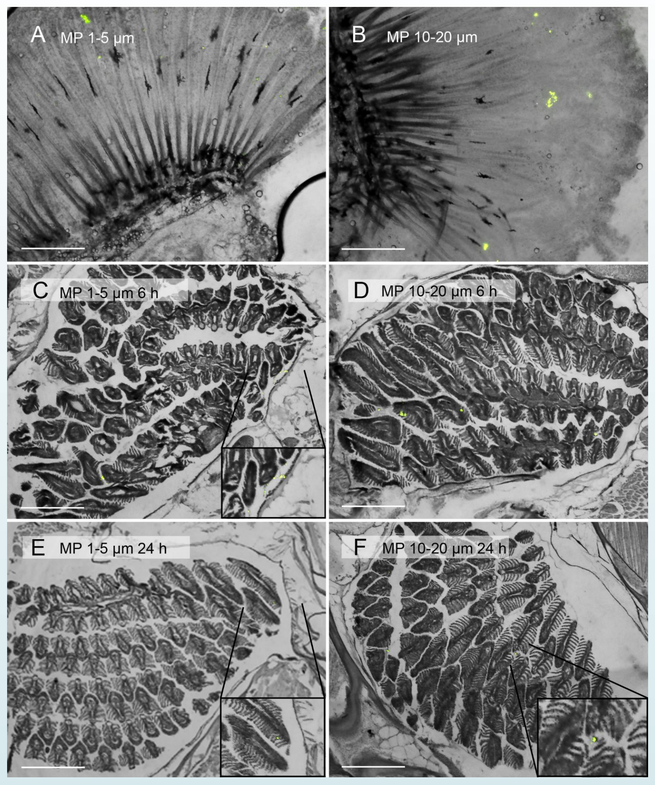
- MPs attached to gill filaments and mucus, not in surrounding tissues.
- Mucus and particles were excreted permanently.
- No difference between particles size and incubation time.

POP transfer via MPs

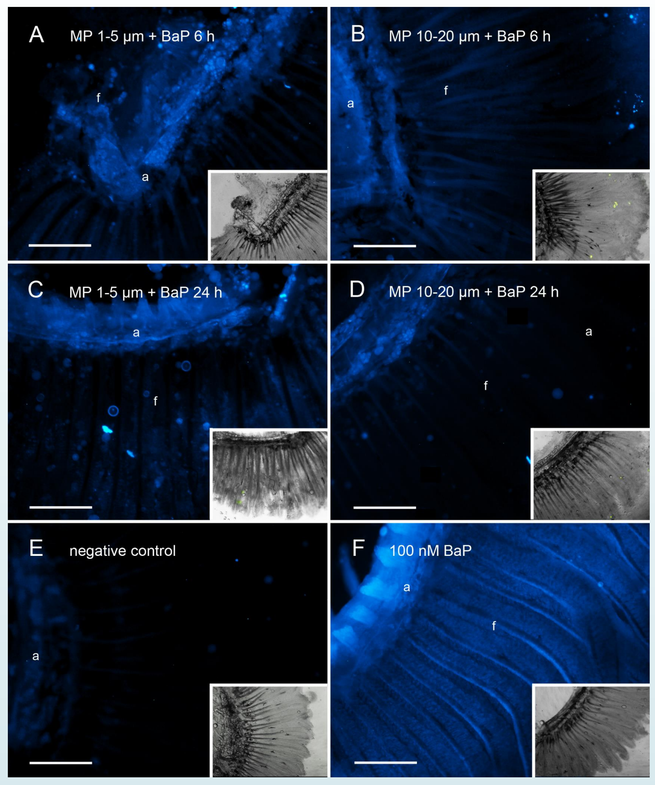
- There was a tendency in Gill EROD induction compared to negative control groups for BaP coated MPs.
- The fluorescence tracking of BaP also revealed a transfer of BaP from MPs to gills.



MP accumulation in freshly dissected gills and histological section

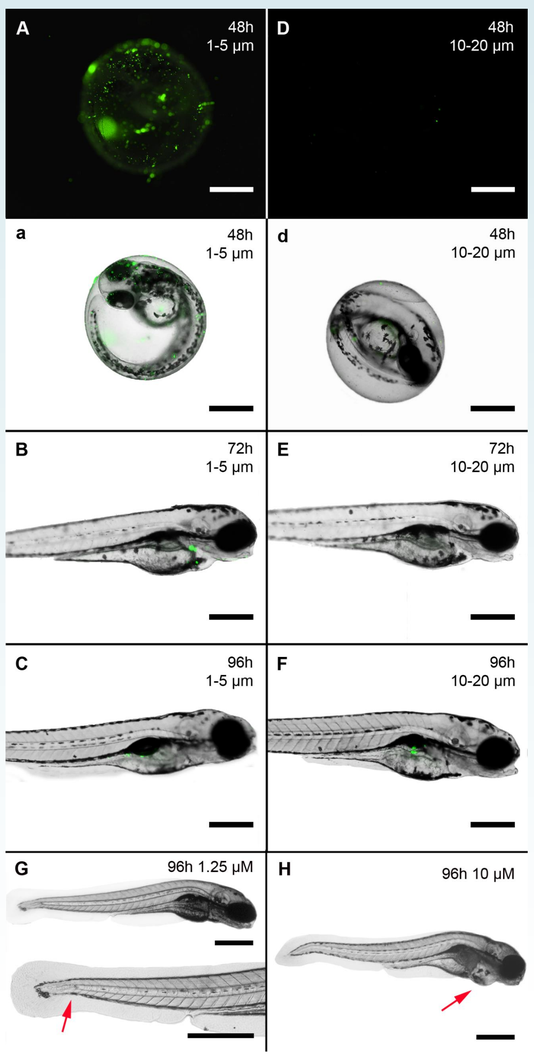


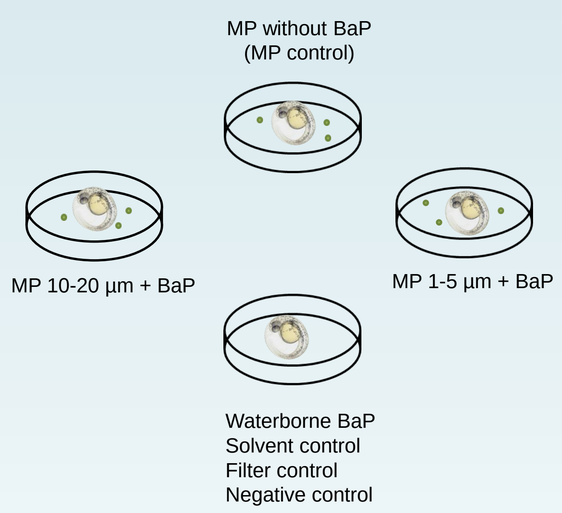
Benzo[a]pyrene (BaP) fluorescence tracking (BaP fluorescence peaks 405 and 435 nm)



Microplastic attachment and POP transfer on zebrafish eggs

Fish embryo toxicity test (OECD 236)



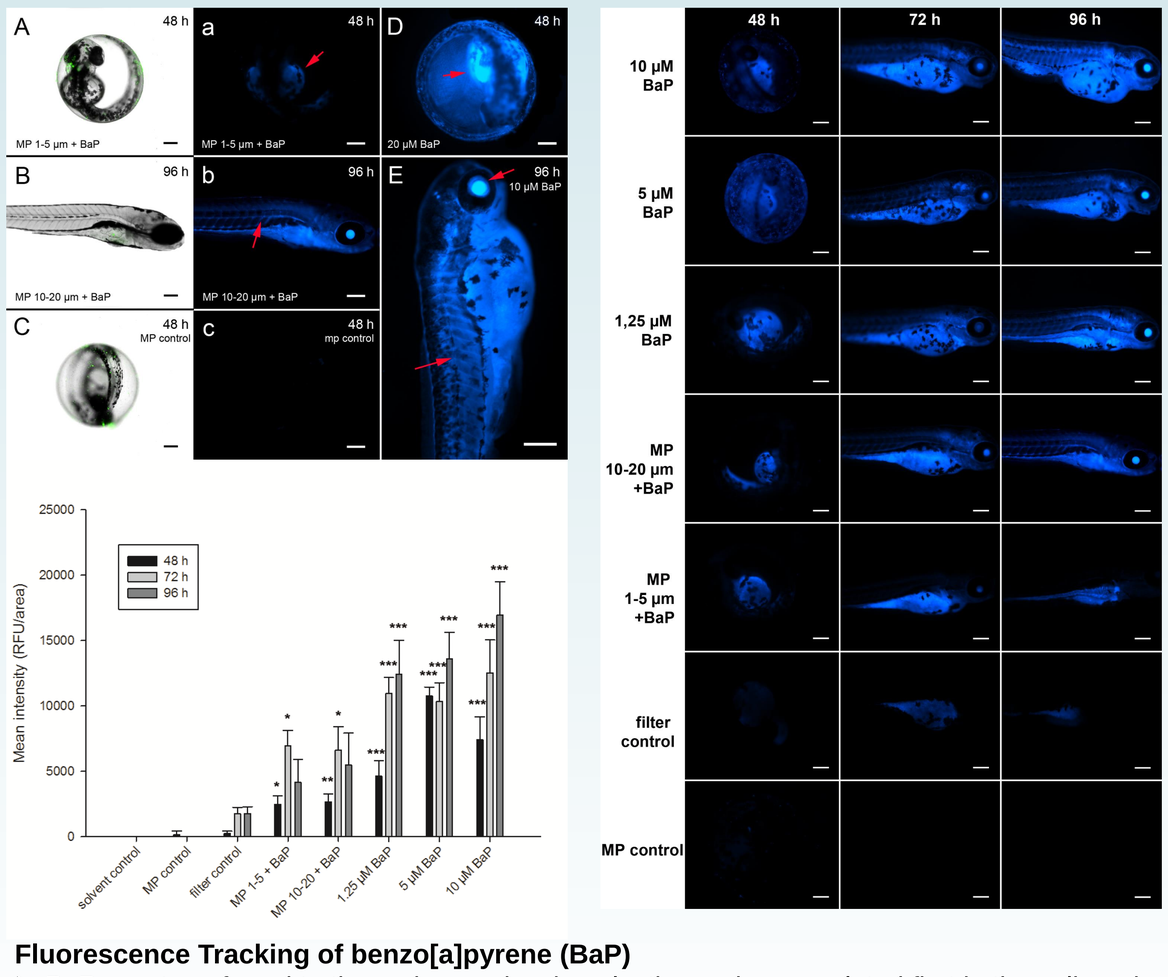


Exposure from 0 h to 96 h post fertilization at 26 ° C on a shaker under semi-static conditions

Fish Embryo Toxicity Test (OECD TG 236)

- There were no morphological effects visible in embryos exposed to MPs coated with BaP from 0- 96 hpf (semistatic).
- The 1-5 µm particles (proprietary polymer, 1.3 g/ccm) adhered more in number to the chorion compared to the 10-20 µm (polyethylene, 0.99 g/ccm).
- When embryos hatched, no particles were visible on outer epithelia
- Waterborne BaP positive exposure groups showed severe effects, such as edema and tail deformations

Benzo[a]pyrene (BaP) fluorescence tracking (BaP fluorescence peaks 405 and 435 nm)



Fluorescence Tracking of benzo[a]pyrene (BaP)

- BaP was transferred to the embryos *via* microplastics and accumulated first in the yolk and with higher waterborne concentrations in fatty tissue in the whole organisms (red arrows)
- MP exposure groups revealed lower fluorescence intensities than lowest waterborne positive exposure group (1.25 µM), but higher than filter and microplastic control groups
- Fluorescence intensities per area (RFU/area) for MP groups were partly significant higher than solvent control (1 % DMSO, representative also for negative control)

Conclusion

In this study, we analyzed the potential of microplastics to interfere with aquatic organisms other than by ingestion. We showed that microplastics are filtered in adult zebrafish gills and accumulate on zebrafish eggs. Furthermore, microplastics transferred associated benzo[a]pyrene (BaP) to zebrafish gills and eggs via simple attachment. BaP accumulated in gill archs and fatty tissues of zebrafish embryos upon exposure with BaP coated microplastics. Both gill EROD assay in adult zebrafish and fluorescence tracking in zebrafish embryos revealed a higher signal in groups exposed to BaP coated microplastics compared to control groups, but always lower compared to waterborne BaP exposures. Further research should be conducted on exact MP and substance concentrations. However, this study shows potential ‘modes of action’ and useful methods to detect effects of microplastics and associated POPs on fish gills and embryos.

Acknowledgments and Literature

The authors would like to thank the whole ‚Aquatic Ecology and Toxicology' group from the University of Heidelberg. Special thanks to the Hygiene Institute of the University of Heidelberg for their support in chemical analyses.

- Watts et al. 2014. Uptake and retention of microplastics by the shore crab Carcinus maenas. Environ Sci Techno.
- Lu et al. 2016. Uptake and accumulation of polystyrene microplastics in zebrafish (Danio rerio) and toxic effects in liver. Environ Sci Technol.l
- Batel et al. 2016. The transfer of benzo[a]pyrene from microplastics to Artemia nauplii and further to zebrafish via a trophic food web experiment - CYP1A induction and visual tracking of persistent organic pollutants. Environmental toxicology and chemistry / SETAC.
- Jonsson et al. 2009. The zebrafish gill model: induction of CYP1A, EROD and PAH adduct formation. Aquatic toxicology 91:62-70.