Do polystyrene microplastic particles modulate the toxicity of pesticides? Mortality and biochemical responses of the snail *Marisa cornuarietis* and the non-biting midge *Chironomus riparius*

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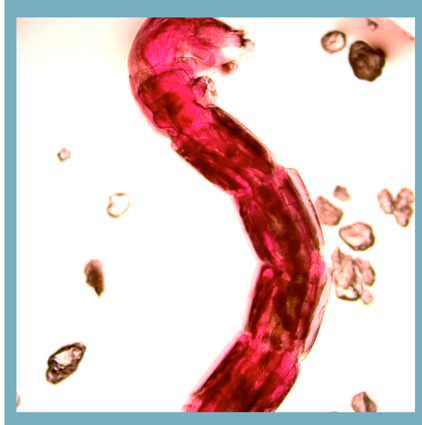
Microplastics are of particular interest in ecotoxicology because they can interfere with organic substances like pesticides or pharmaceuticals, modulate their toxicity and in addition, mechanically affect exposed organisms. Whereas in the past, most of the studies on microplastics have focused on the marine environment, there is still little knowledge about possible impacts on freshwater ecosystems.

The aim of this study is to examine whether polystyrene (PS) microparticles (cryogenically milled granules, fractionated to < 100 µm) in combination with different organic pesticides (methiocarb, thiacloprid) influence health parameters in the giant ramshorn snail (*Marisa cornuarietis*) and larvae of the midge *Chironomus riparius*. As endpoints of toxicity, oxidative stress (lipid peroxides, superoxide dismutase), proteotoxicity (stress protein level hsp70), neurotoxicity (inhibition of acetylcholinesterase), tissue integrity and mortality rates were studied.

Material and methods

Marisa cornuarietis

- Adult *M. cornuarietis* were exposed to PS particles (10,000 particles / L; < 100 µm) alone and in combination with methiocarb (MET) (1,000; 10,000 µg/L)
- Endpoints of toxicity:
  Neurotoxicity (acetylcholinesterase, carboxylesterase)
  Oxidative stress (lipid peroxidase, superoxide dismutase)
  Proteotoxicity (stress protein level hsp70)
  Histopathology

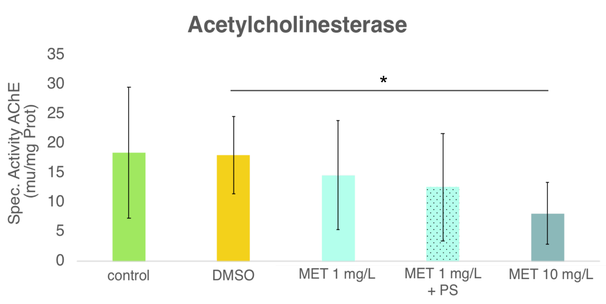


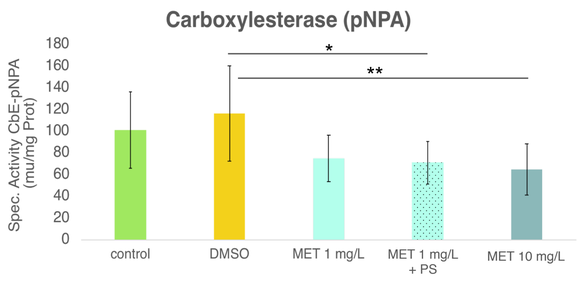
Chironomus riparius

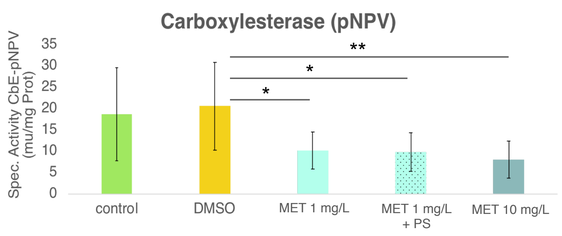
- *C. riparius* larvae (L3-4) were exposed for 96 h to PS particles (150,000 particles / L; < 50 µm) alone and in combination with thiacloprid (TH; 1 µg/L)
- Endpoints of toxicity:
  Mortality
  Neurotoxicity (acetylcholinesterase, carboxylesterase)

Results

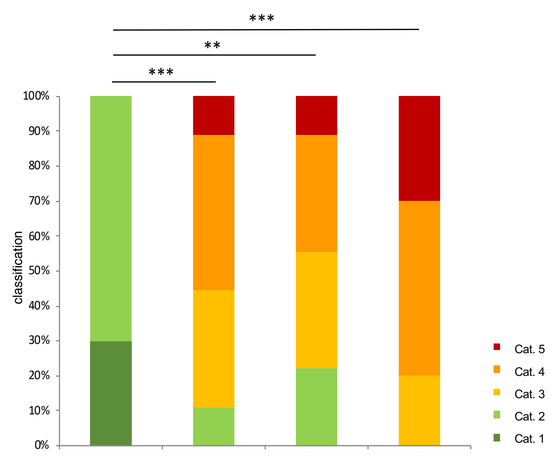
Analysis of carboxylesterase activity



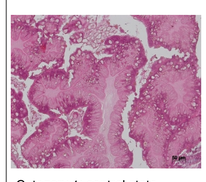




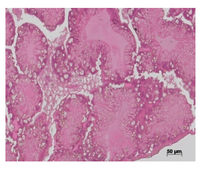
Histopathological assessment
(Hepatopancreas)



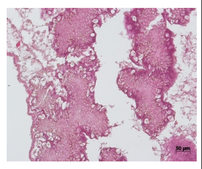
Semi-quantitative assessment of hepatopancreas. Likelihood Ratio χ^2 Test, followed by Bonferroni-Holm correction: DMSO vs. MET 1: df=4, n=19, χ^2=20,259, p=0.0001; DMSO vs. MET 1+PS: df=4, n=19, χ^2=16,752, p=0.0004; DMSO vs. MET 10: df=4, n=20, χ^2=27,726, p=0.0022.



Category 1: control state



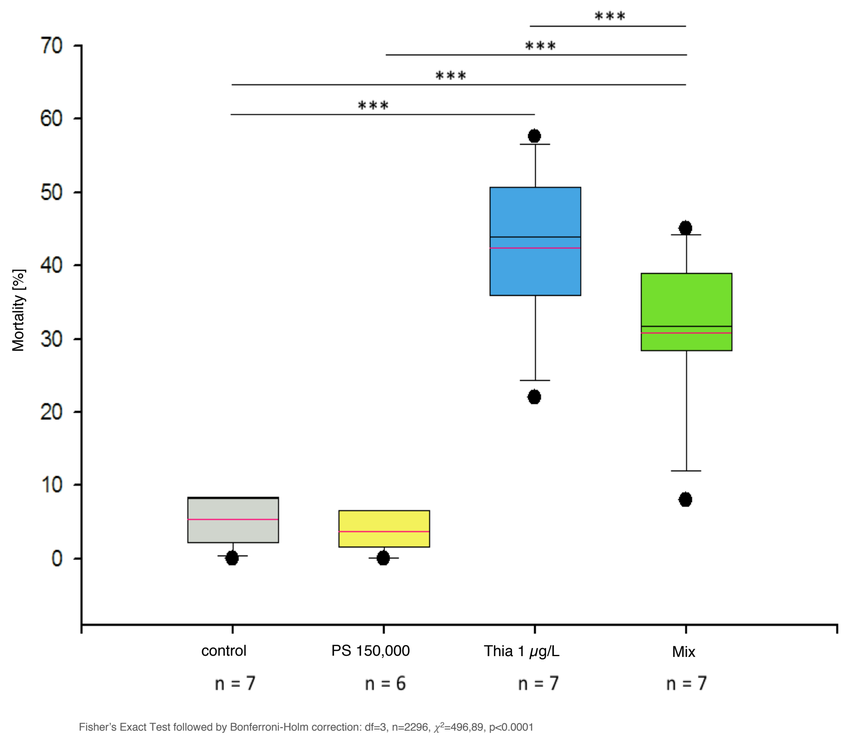
Category 3: state of reaction



Category 5: state of destruction

Histopathological assessment was conducted qualitatively and semi-quantitatively (blinded evaluation) by categorizing the samples in five different categories depending on the condition of the tissue.
The analysis showed no significant difference between the treatments MET 1 and MET 1 + PS. All effects on the tissue integrity are caused by methiocarb.

Acute toxicity test (96 h)



Fisher's Exact Test followed by Bonferroni-Holm correction: df=3, n=2296, χ^2=496,89, p<0.0001

The results showed a significantly lower mortality rate in the Mix treatment compared to the thiacloprid treatment, indicating that PS particles are able to reduce the toxicity of thiacloprid. This effect has been shown in six out of seven runs of the experiment that have been performed. However, the underlying mechanism is not clear up to now, and therefore, further investigations are necessary.

- **Methiocarb (> 1 mg/L) inhibits carboxylesterases**
- **Methiocarb causes effects on hepatopancreatic tissue**
- **PS particles do not modulate the toxicity of methiocarb** ✗

- **Thiacloprid increases mortality of *C. riparius***
- **PS particles (150,000 P./L) reduce the toxicity of thiacloprid** ✓

Endpoint	Result
Lipid peroxidation	No effect
Superoxide dismutase	No effect
Stress protein level hsp70	No effect

Endpoint	Result
Acetylcholinesterase	No effect
Carboxylesterase	No effect

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