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Assessment Of The Bioaccumulation And The Effects Of Per- And Polyfluoroalkyl Substances On Blue Mussel (*Mytilus edulis*)

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Introduction

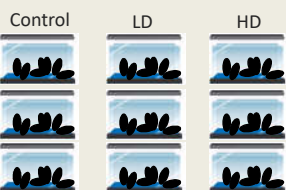
Per- and polyfluoroalkyl substances (PFAS) are a family of non-naturally occurring chemicals, regrouping more than 4000 non-naturally compounds. They have been in the spotlight for the last two decades over reports of their **ubiquitous presence in the environment** and especially in oceans. Combined evidence for their **persistence, bioaccumulative and toxic properties** have led to the ban of specific compounds (e.g. PFOS, long-chain PFCAs) but a large number of precursor compounds of unknown environmental behavior remains in use. Despite their properties, there is still a dramatic lack of knowledge on the **exposure of biota to PFAS** and on their subsequent bioaccumulation and potential toxic effects.

Experimental Design

Exposures (E- 8 days) + Depuration (D- 5 days)

- 20 L glass tanks, 20 mussels per tank
- Aerated artificial seawater (33 PSU, 15°C), Artificial light of 12:12 h
- Changes of water every 2 days
- Feeding with contaminated food every 2 days during exposure
- 3 tanks per condition: LD = 625 ng PFAS/g dw artificial food
HD = 6250 ng PFAS/g dw artificial food

Mix of 21 PFAS with different chain lengths:



- 5 perfluoroalkyl sulfonates (PFSA, C4-C12)
- 11 perfluoroalkyl carboxylic acids (PFCA, C4-C18)
- 2 fluorotelomer carboxylic acid (FTCA, C7; C9)
- 3 fluorotelomer sulfonate (FTS, C8-C12)

Sampling

Sacrifices using liquid N₂



Analyses in 3-4 mussels per tank => 10 per condition

Bioaccumulation and Effects

- Lipidomics, defense and damage biomarkers



Digestive glands, gills, mantle

- CAT (Catalase)
- GST (Glutation-S-transferase)
- SOD (Superoxide dismutase)
- AChE (Acetylcholinesterase)

Digestive glands

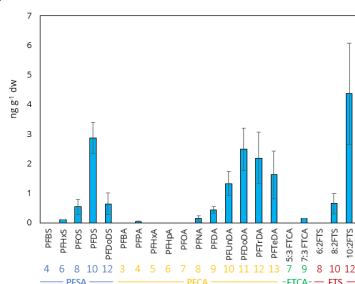
- Lipidomics after MeOH/CH₂Cl₂ extraction

- Bioaccumulation: PFAS on the whole organisms by LC-HRMS after extraction and purification on cartridges

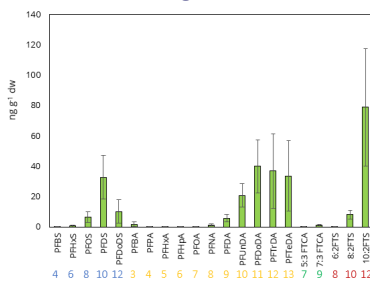
BIOACCUMULATION

PFAS profiles after 8 days of trophic contamination

Low Dose



High Dose



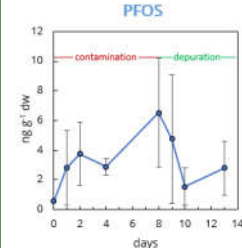
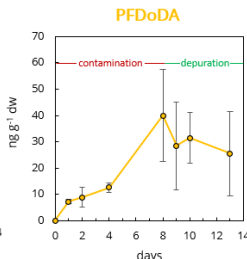
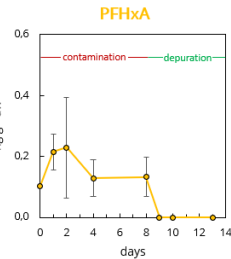
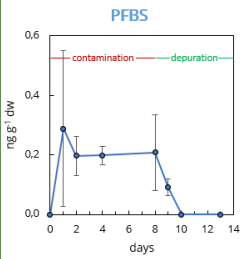
Same bioaccumulation profiles for mussels exposed to both low and high dose

Concentrations in tissues increasing with PFAS chain length

Limited/no accumulation for n_c < 8

For a given chain length: PFSA>PFCA>FTS (except for 10:2 FTS) and PFCA>FTCA

Kinetics



High intraspecific variability (up to 10 x)

For short-chain PFAS, equilibrium reached within 1-2 days in both accumulation and depuration phases

No steady state reached within 8 days for bioaccumulative PFAS (n_c > 8)

EFFECTS

Defense and Damage Biomarkers

Significant increases of SOD and GST activities in the digestive glands of mussels exposed to PFAS compared to controls, recovered after the depuration phase

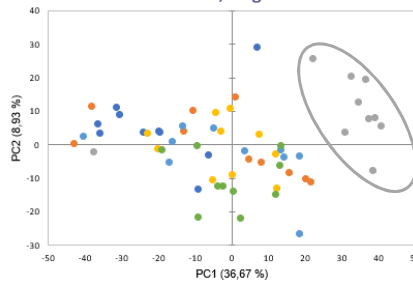
No effect on CAT and AChE activities

Lipidomics

Processing of FIA-HRMS data with MZmine2 to obtain a matrix of the intensity of the signal of all observed positive and negative ions of each sample, higher than 3xblanks

Principal Component Analysis (PCA, unsupervised analysis)

Observations, Negative ions

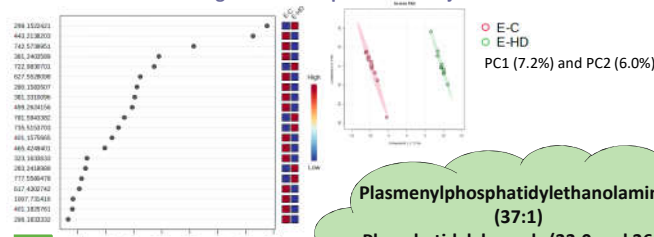


The mussels exposed to the highest dose during 8 days are grouped and separated from the others

Partial Least-Squares Discriminant Analysis (PLS-DA, supervised analysis)

Mussels from control (E-C) and tanks contaminated with the highest dose (E-HD)

First 20 negative ions important in Projection



Plasmenylphosphatidylethanolamines (37:1)
Phosphatidylglycerols (33:0 and 36:0)
Monogalactosyldiacylglycerols (36:4 and 24:0)

LipidBlast®

Conclusions

- Bioaccumulation of the PFAS in mussels after alimentary exposures depending on the dose and the PFAS chain length
- Bioaccumulation and depuration phases observed during the kinetic analysis
- The exposure of mussels to PFAS lead to oxidative stress (increase of GST and SOD activities in the digestive glands)
- Impairments of the lipidome of the mussels exposed to the highest dose of PFAS compared to controls and determination of some putative lipid structures by the non-targeted lipidomic approach applied to negative ions detected in FIA-HPLC/MS

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