Supporting a future with safe, nutritious, and sustainable seafood

SEAFOOD^{TOMORROW} Final Event, 15.04.2021

Enzymatic biosensors for the detection of environmental and processing contaminants in seafood

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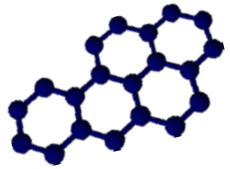
Target contaminants

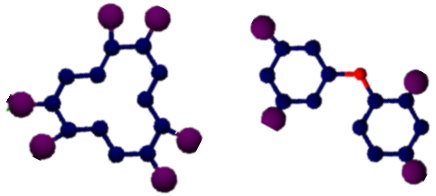
Environmental contaminants are organic compounds created during industrial processes or synthetized to use in several devices and objects.

Polycyclic aromatic compounds (PAHs)

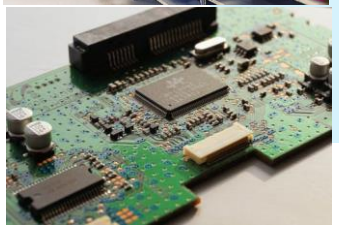


Generated during the combustion of organic material.

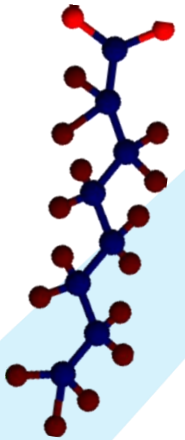
Brominated flame retardants (BFRs)



Used as flame retardants in different objects: electronical devices, textiles, plastics…



Perfluorinated compounds (PFCs)



Used in coatings to produce water resistant objects and also as part of fire-fighting floams.

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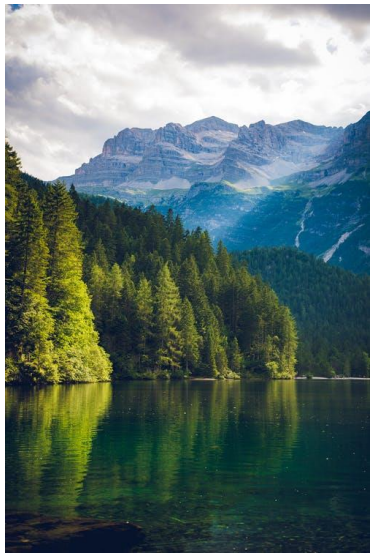
Contaminants in fish

From industrial and other human daily activities contaminants are released into the environment. During the trophic chain, they can accumulate and, consequently, critical concentrations have been found in plants and animals that are for human consumption.

HUMAN ACTIVITY

Contaminants



Processing

Contaminants

Through atmospheric deposition, rivers and direct waste, seas accumulate contaminants. As a result, they are bioaccumulated, endangering seafood safety. For this reason, authorities have established control methods and legal or recommended concentration limits in fish and seafood products.

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Why an enzymatic biosensor

- **Biosensors:**
  - → Time saving
  - → Cost effectiveness
  - → Unskilled labour
  - → Portable
- **Enzymatic biosensor:**
  - → Selectivity
  - → Multi-target
  - → Sensitivity
  - → Rapid

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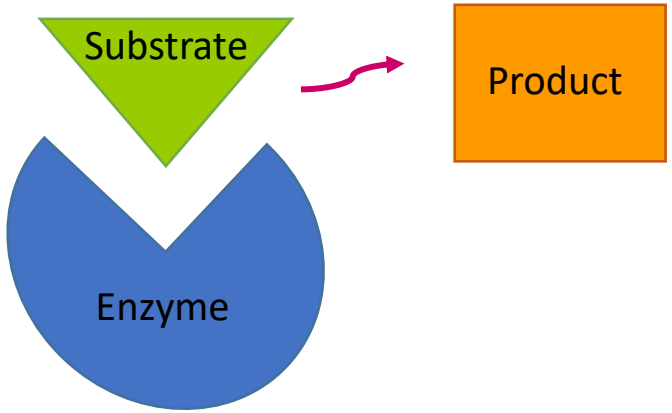
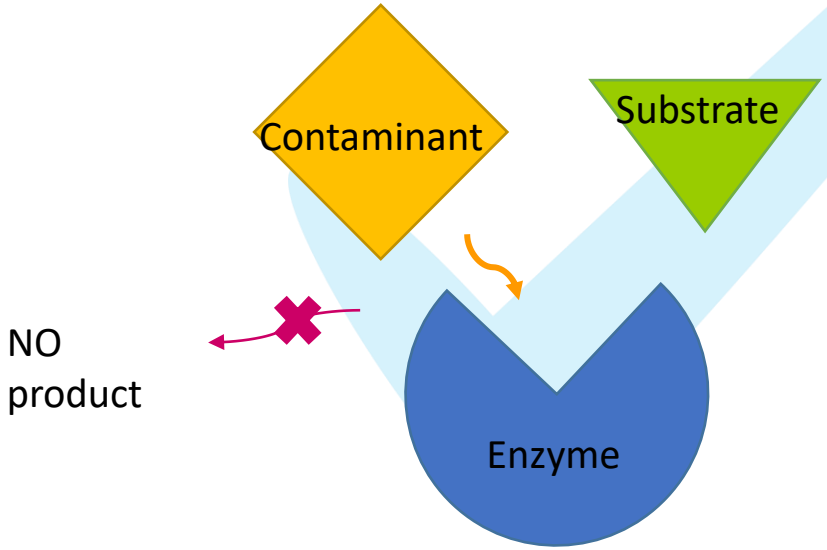
Why an enzymatic biosensor

Enzymatic activity can be affected by contaminants. The most common effect is inhibition of activity, obstructing production of the product compound. Measurement of changes in product concentrations can alert to the presence of contaminants.

Enzymatic activity



Inhibition of enzymatic activity



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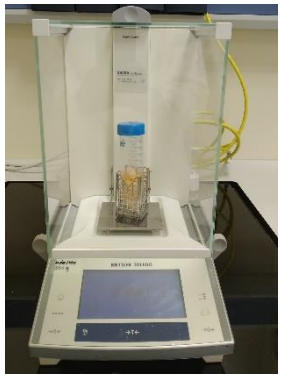
Sample preparation

1 Weigh homogenized fish fillets

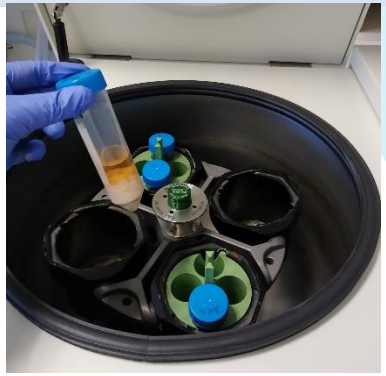


2 - For PAHs and BFRs → Extract with organic solvent + QuEChERS (mixture of NaCl and $MgSO_4$).

- For PFCs → Extract with a basified organic solvent.

Vortex (2 min)

3 Centrifugate and take the supernatant



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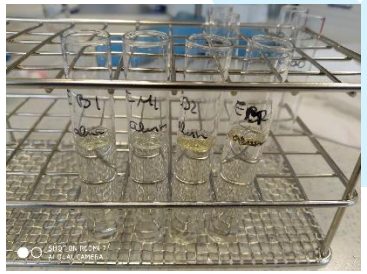
Sample preparation

4 Cleaning steps:
For PAHs and BFRs → freeze-out + C18 cartridge + aluminium oxide-PSA cartridge
For PFCs → C18 cartridge + SAX cartridge

5 Evaporate the organic solvent, as necessary



6 Reconstitute in buffer solution.

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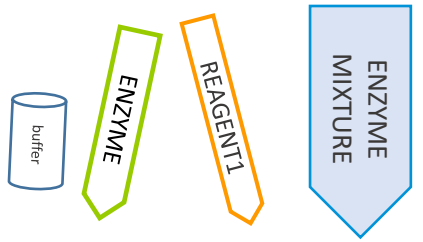
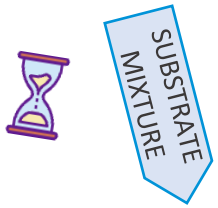
Assay protocol

7 Prepare the enzyme mixture.

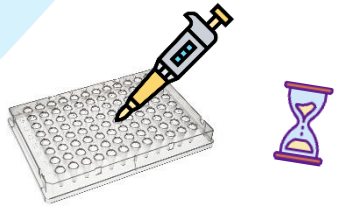


8 Add to wells sample solution and enzyme mixture.



9 Incubate 10 minutes at 37˚C and prepare substrate mixture.

10 Add substrate to wells, incubate 60 minutes at 37˚C and read the signal with a reader.



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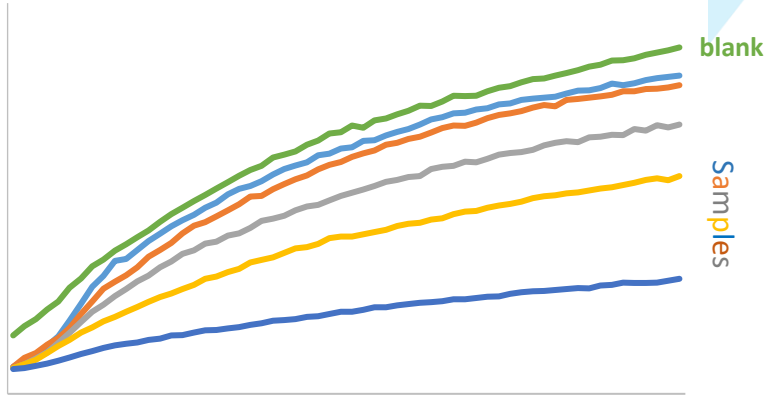
Analysis of results

Measured signal is proportional to product generation.

As we measure a blank (buffer solution) together with samples, the differences between signals can give the percentage of enzymatic inhibition.

$$Inhibition\ (\%) = \left(1 - \frac{S_{sample}}{S_{blank}}\right) x\ 100$$



A Cut-off value is calculated for each contaminant and correlated with a concentration.

A positive sample (contaminant present above a certain concentration level) will provide an inhibition higher than the cut-off.

A negative sample (contaminants below a certain concentration level) will provide an inhibition lower than the cut-off.

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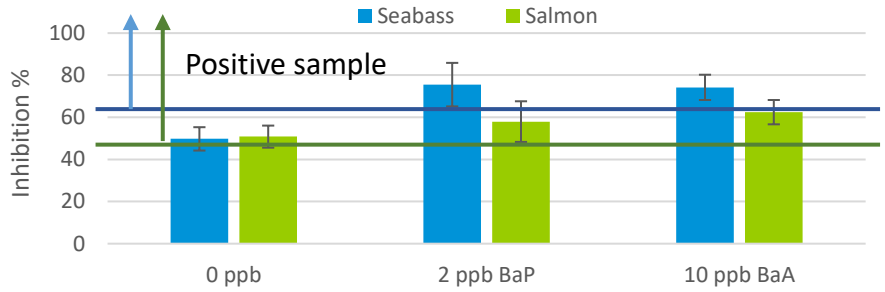
Sensitivity

PAHs

LOD: 2 ppb BaP
10 ppb ∑4PAH

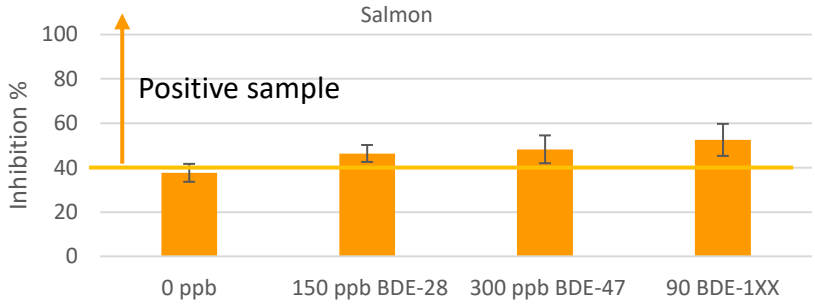
Cut-off value ≈ 50% (for salmon)
Cut-off value ≈ 65% (for seabass)



BDEs

LOD: 90 ppb BDE-100/BDE-153/BDE-154
150 ppb BDE-28/BDE-99
300 ppb BDE-47

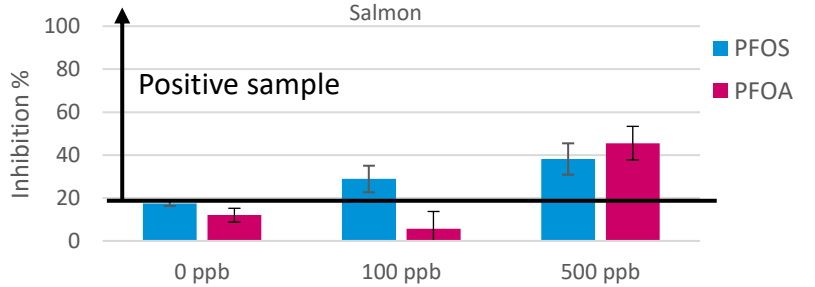
Cut-off value ≈ 40%



PFCs

LOD: 100 ppb PFOS
500 ppb PFOA

Cut-off value ≈ 20%



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Conclusions

- **Qualitative** assays.
  POSITIVE sample (contaminant present above a certain concentration level)
  NEGATIVE sample (contaminants below a certain concentration level)
- Analysis (extraction and enzymatic assay) can be performed in less than **90 min** (3 samples in parallel)
- Validated for two fish species. Less than **5% false negatives**
- **Good sensitivity**. For PAHs: 2 ppb for BaP; 10 ppb for PAH4

- **Easy to use** - no need for expensive trained labour
- These kits can be **easily implemented** in an industrial setting or in the field
- **Cost effective** – no need for expensive reagents or equipment
- When a sample is a positive, this can be **confirmed by chromatography**, meaning **only positive** samples undergo expensive analysis

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Thank You

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