Effect-directed toxicity assessment of sediments from Bergen harbour (Norway) using luciferase reporter gene and cell-based bioassays

Master thesis in environmental toxicology

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Introduction

Picture from VeliHavn.no, 2023

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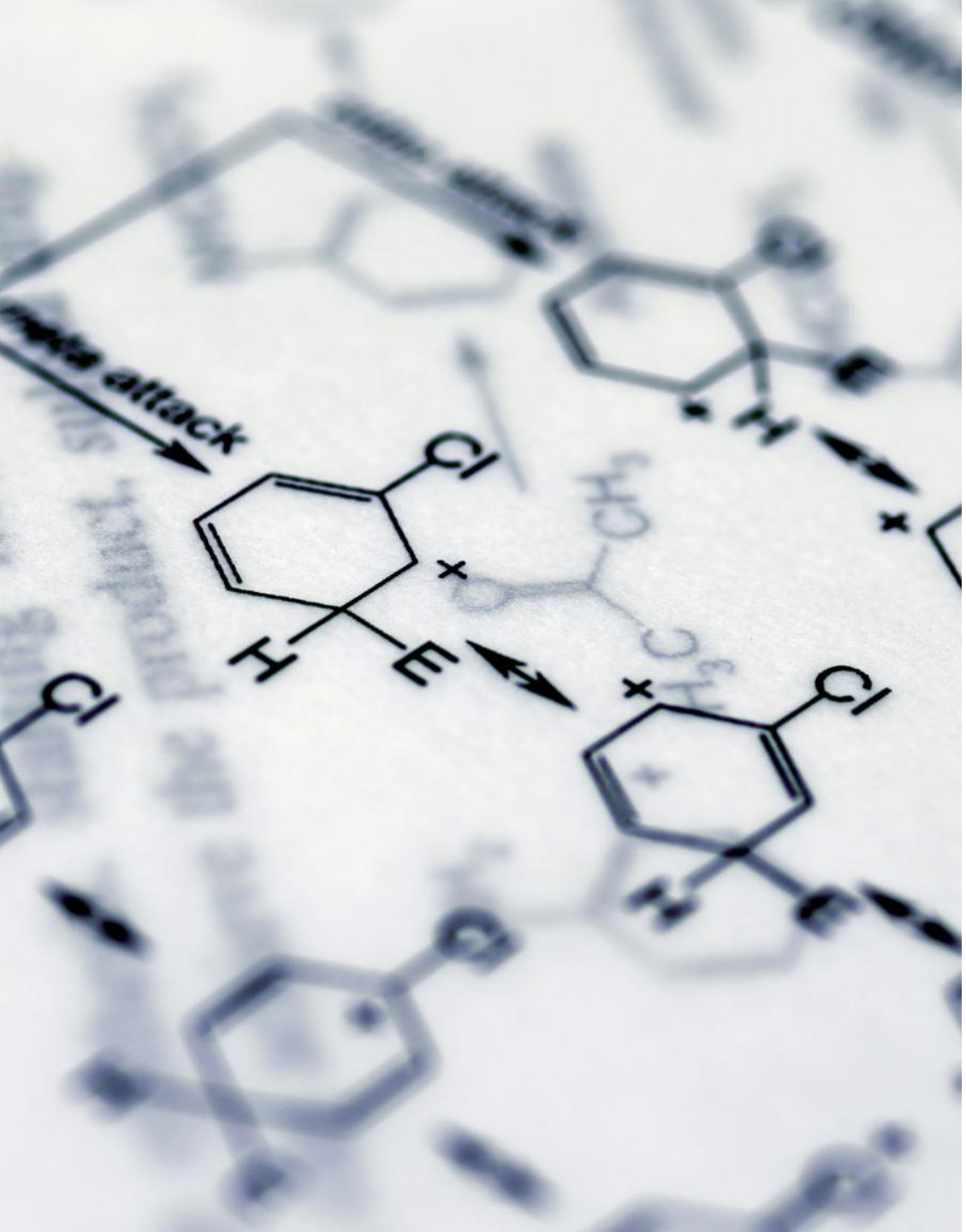

Aim of thesis

The aim of this master thesis was to study the toxicity of sediments from Vågen, Bergen and examine if effect-directed analysis can be used as an approach to identify the cause of the biological effects observed

1. Extract and fractionate
2. Use bioassays to determine activation
3. Compare biological and chemical data

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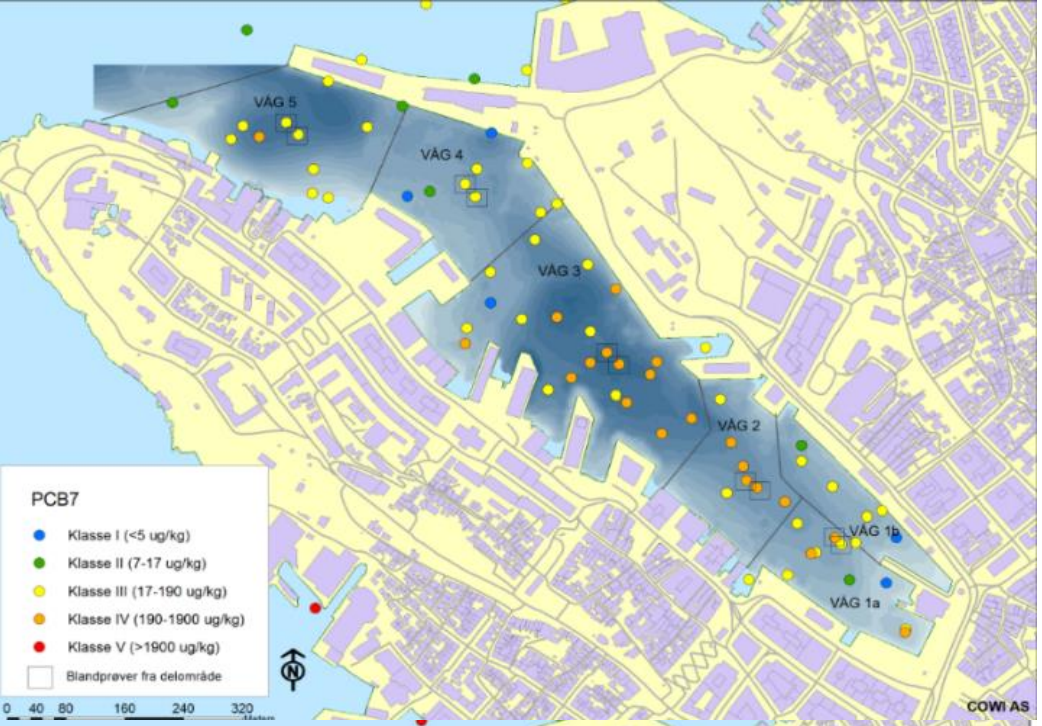

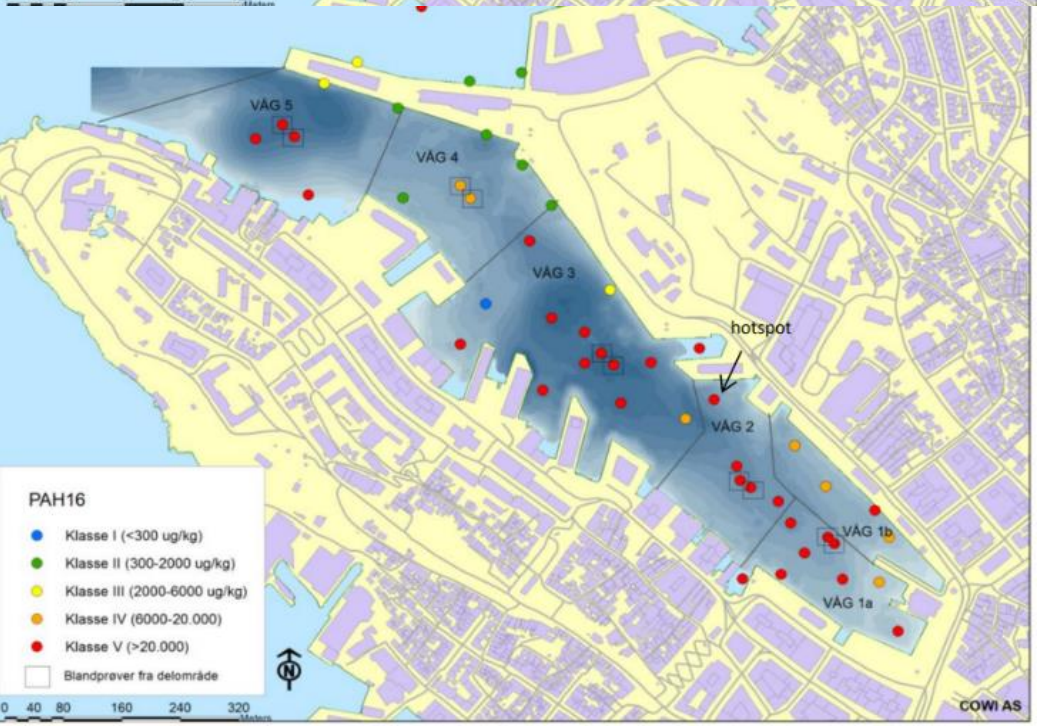

Sediments as sink for Pollutants

- Diverse habitat for a large and unique range of organisms

- Accumulation of pollutants – long reaching effects

- 1993-1996 study of 120 areas in Norwegian fjords show 90% of these heavily polluted

- Vågen, historically polluted area

- Examples of pollutants are polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs)

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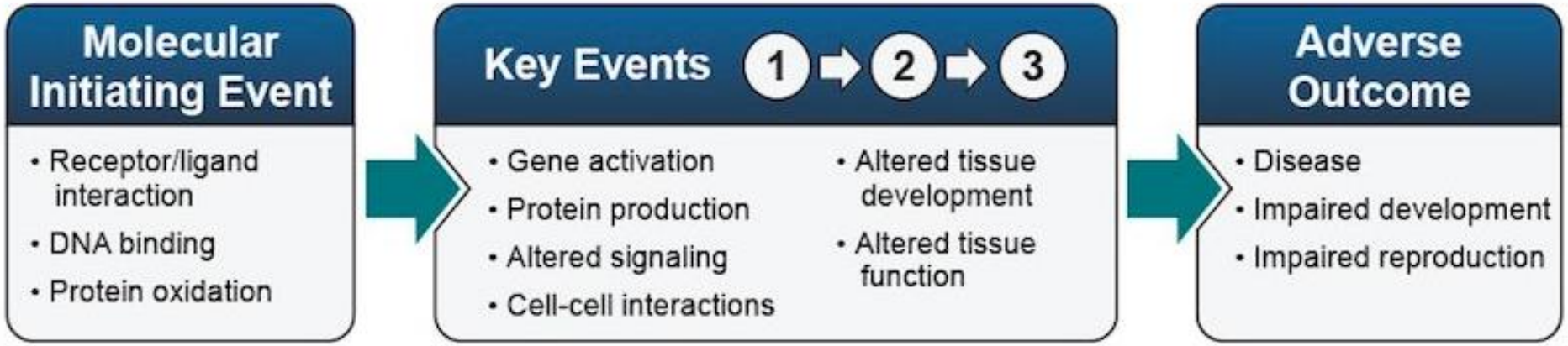
Adverse outcome pathway

- Mechanistic representation of the toxicological effects on different levels of biological organizations



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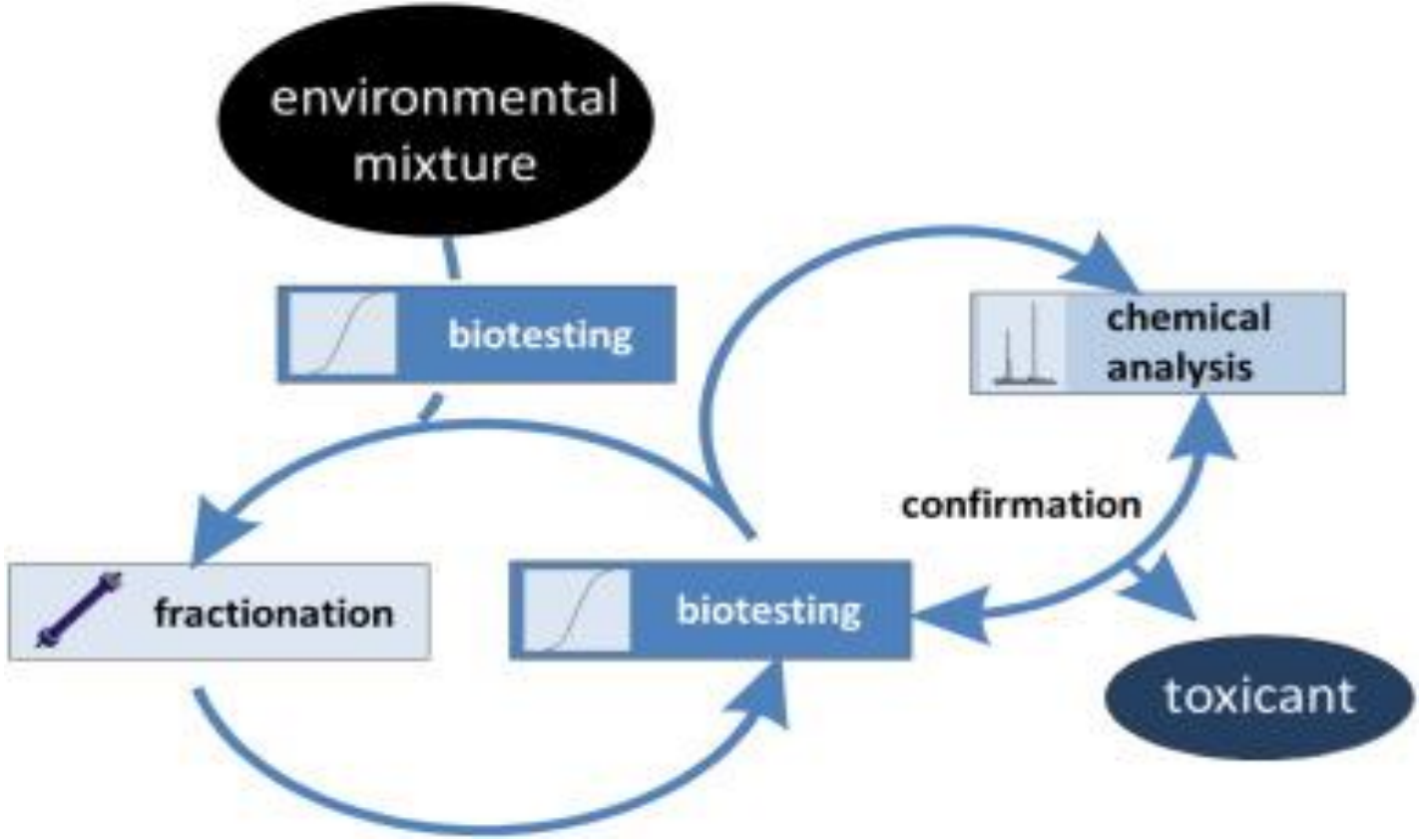
Receptors

The four receptors employed:

- **Pregnane x receptor** – NR1, regulation of cytochrome CYP3A and xenobiotic metabolism
- **Androgen receptor** – NR3, influencing transcription of androgen responsive genes
- **Estrogen receptor** – NR3, two mammalian subtypes; alpha and beta. Three in cod; Erα, Erβ-1 and Erβ-2
- **Aryl hydrocarbon receptor** – Ligand-dependent transcription factor, activated by dioxins, dioxin like compounds and related chemicals.

Effect directed analysis



- Aim to reduce the complexity of a sample while limiting the possibility of overlooking significant contributors to risk and effect

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Methods

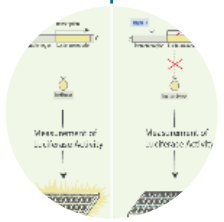
Sediment[1]

Accelerated Solvent Extraction (ASE)[2]

Fractionation[3]

EROD[4]

Chemical analysis[5]



Luciferase reporter gene assay[6]

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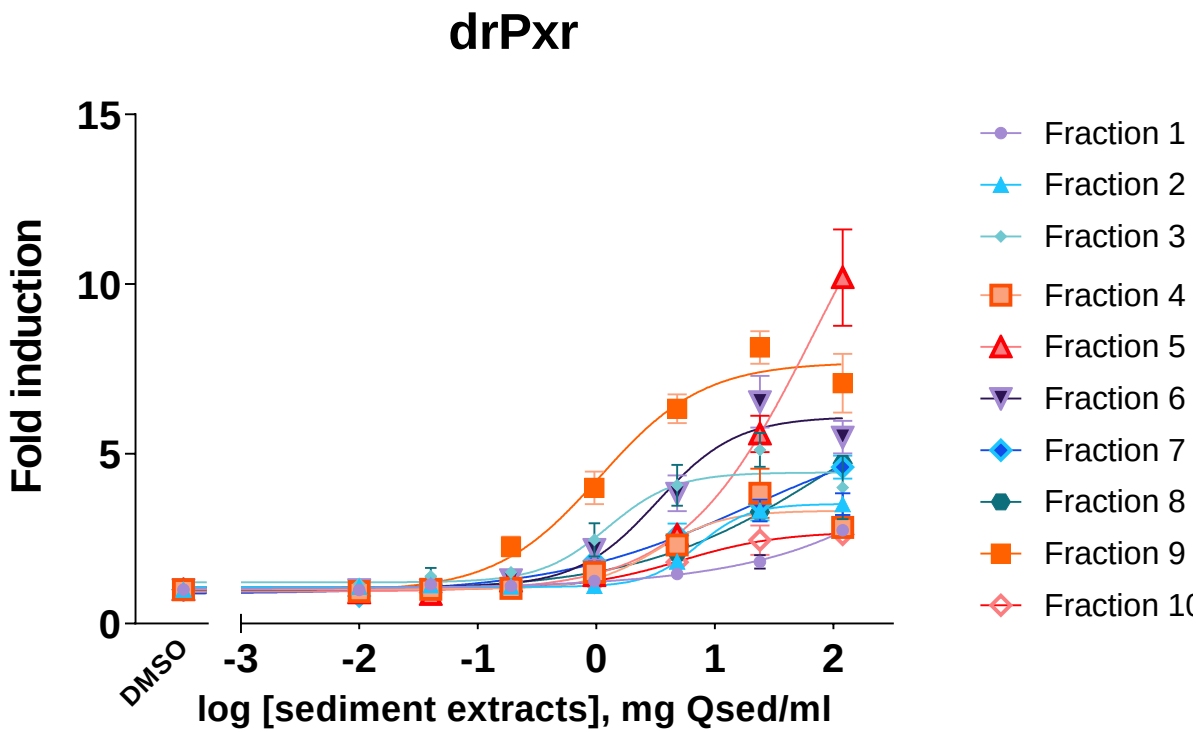
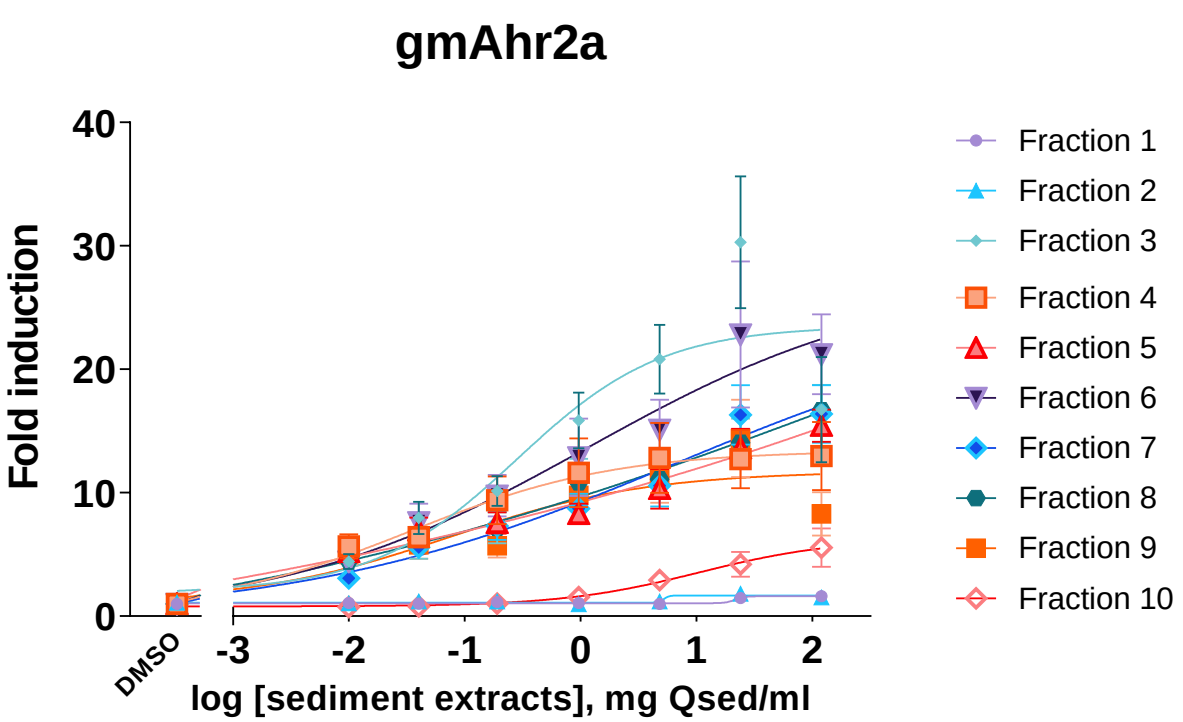
Results

- Ligand activation of Pxr and Ahr2a using LRA





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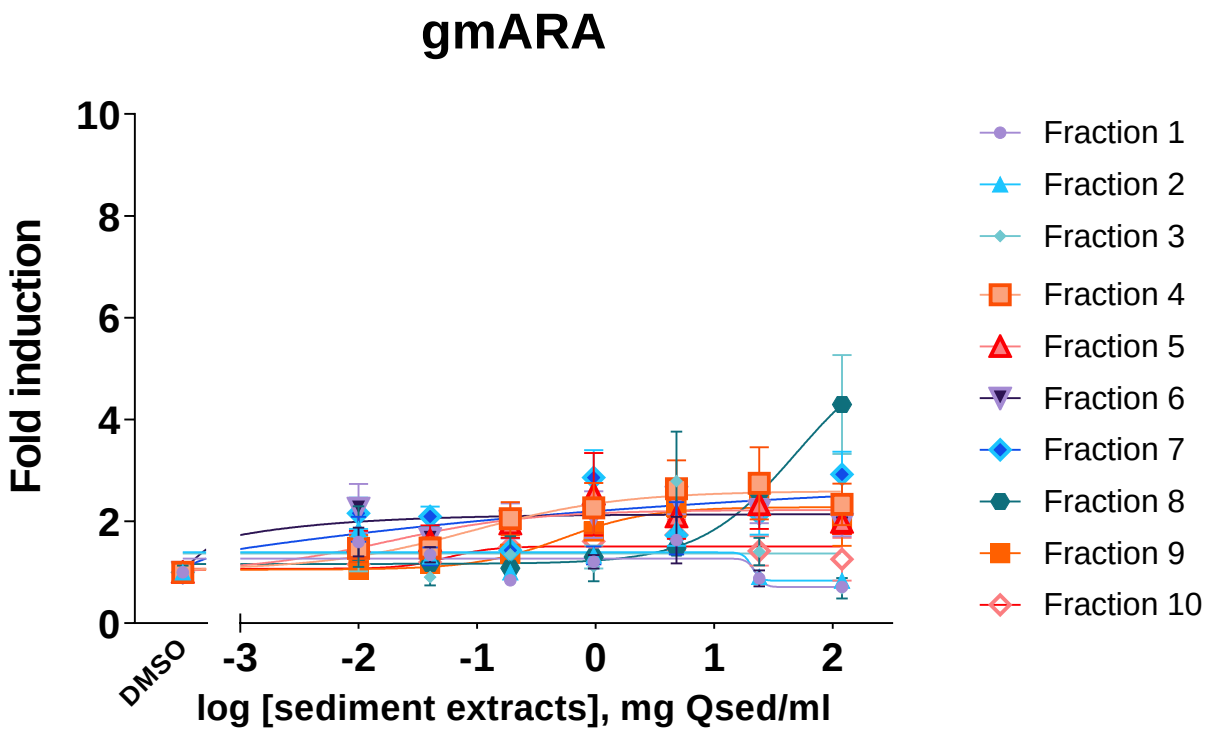
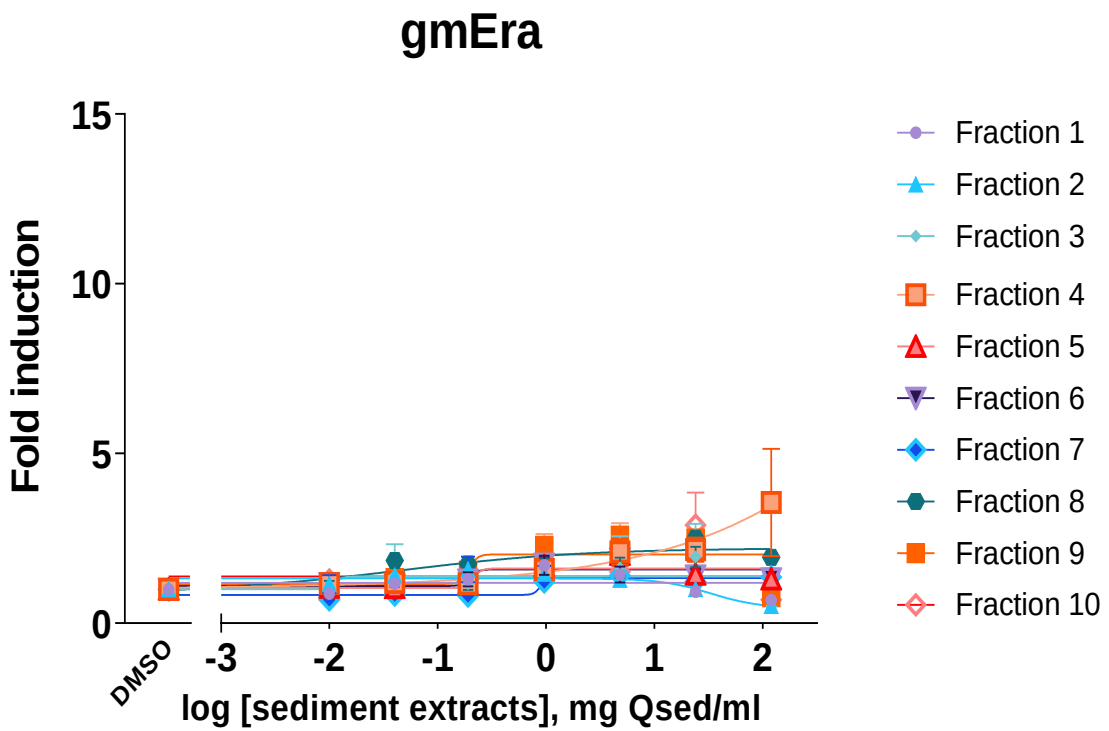
Results

- Ligand activation of androgen and estrogen receptors using LRA





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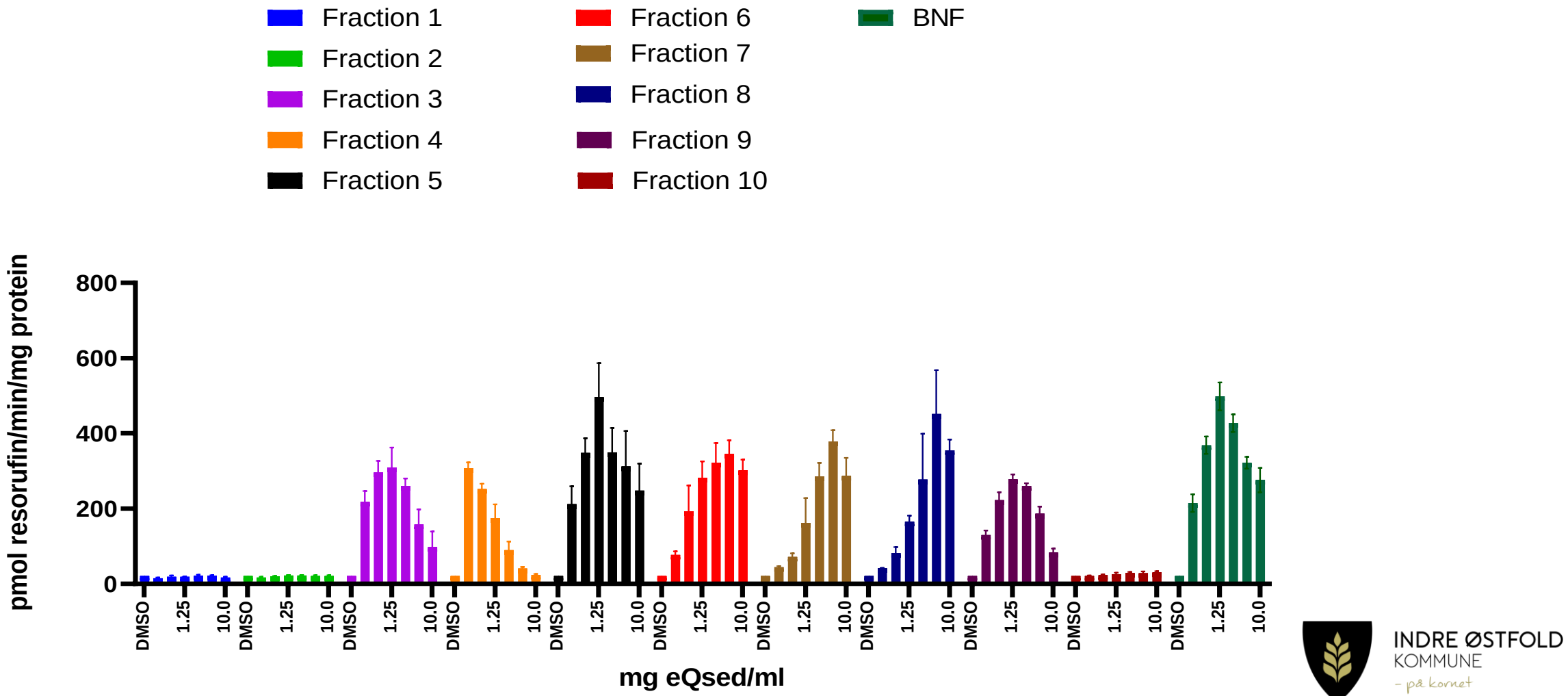
Results

- **Ethoxyresorufin-O-deethylase (EROD)**



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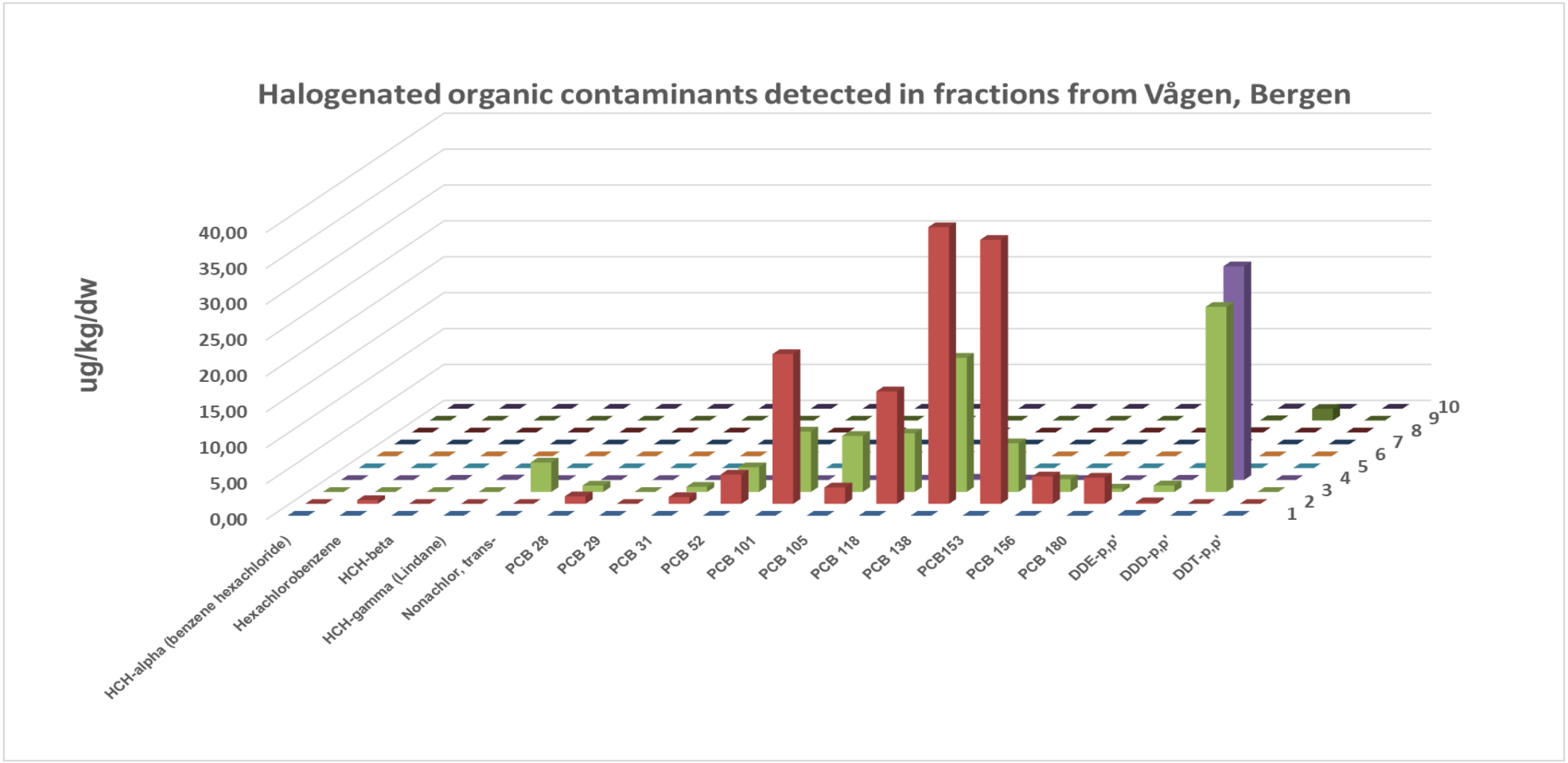
Results – chemical analysis



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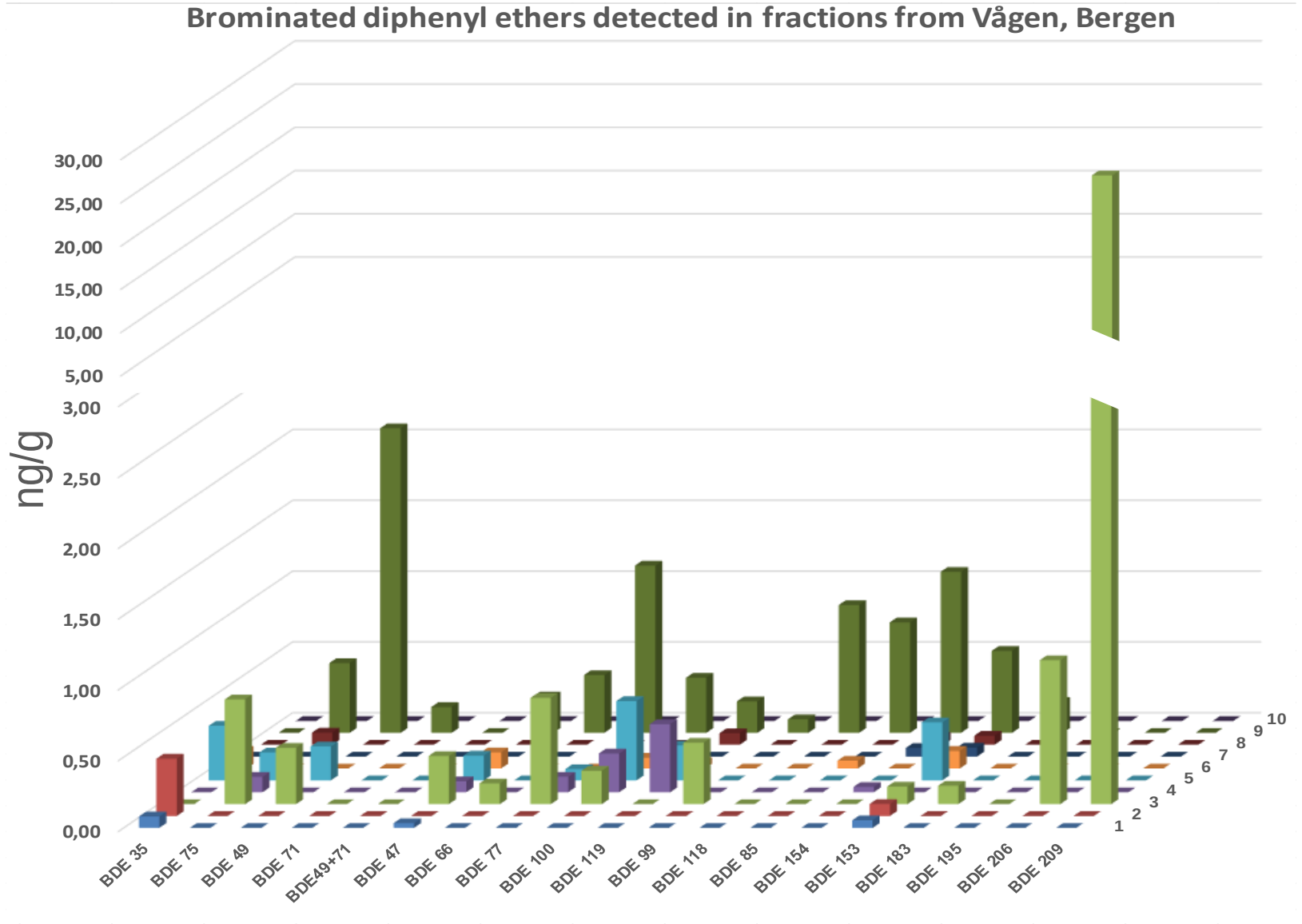
Results – chemical analysis



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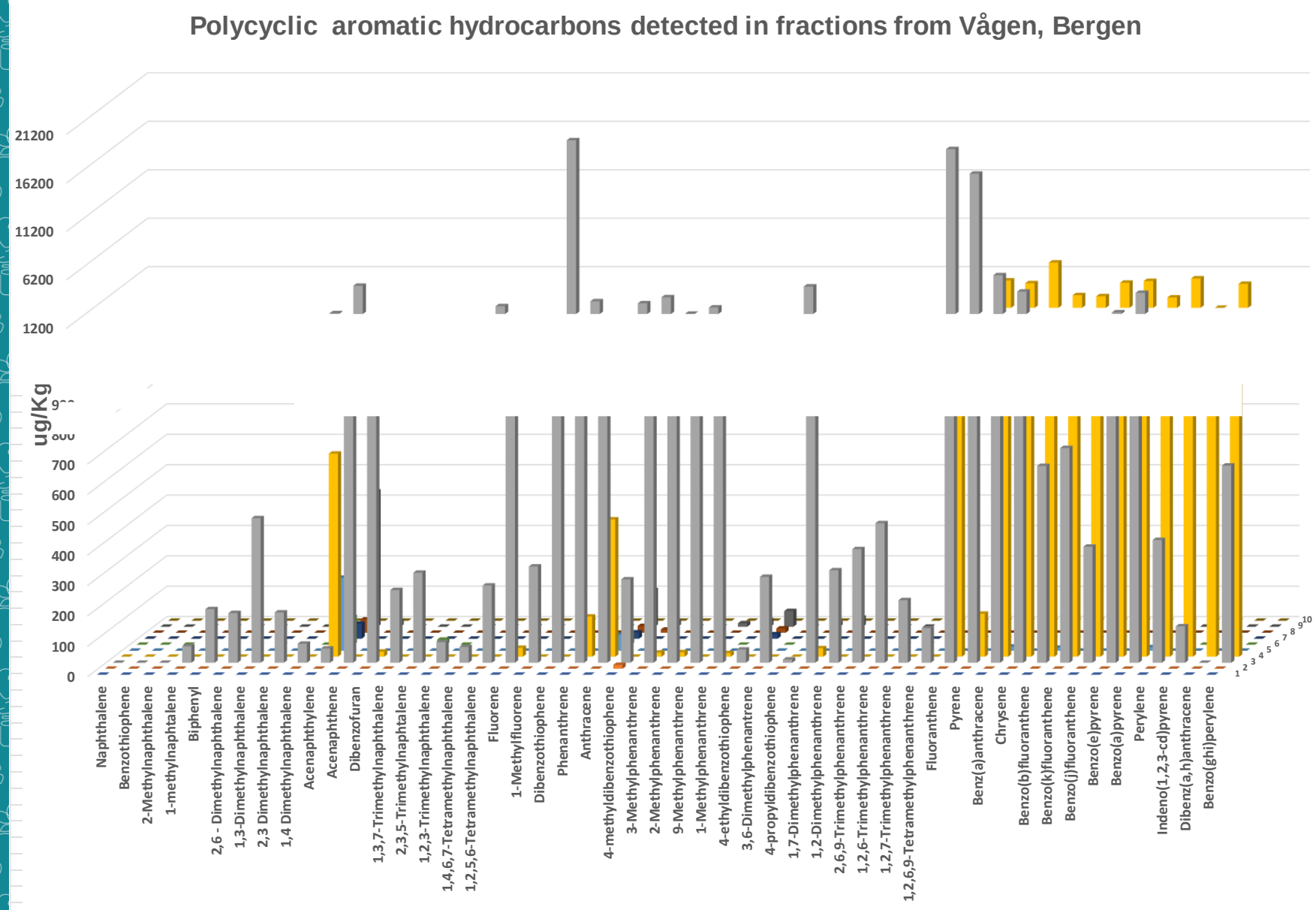
Results – chemical analysis



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Discussion - Pxr

The Pxr is a promiscuous receptor

High activation in fractions 5, 6 and 9

Some correlation between chemical analysis and biological data

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Discussion – Ahr2a and EROD

Clear similarities between Ahr2a and EROD activation

Earlier experiments showed no EROD assay activation – Proposed cause was inhibitor – Could have been removed by fractionation in this thesis

Decreasing Cyp1a induction in higher concentrations – possibly toxic to PLHC-1 cells or enzyme inhibition

Some correlation between the chemical analysis and the LRA results with fractions 3 and 4 strong activators and fraction 1,2 and 10 low activation

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Discussion – Ar and Er

Generally low activation in both receptors

A chemical not screened for could be behind the slight variation in activation and maybe inhibiting

Further fractionation and biotesting is needed as there is a number of contaminants that could affect these receptors

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Conclusion and further studies

- Complex mixture – activation of most fractions for the Ahr2a and Pxr with little activation of the Ar and Er
- Future work – further fractionation and testing

Thank you for listening

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