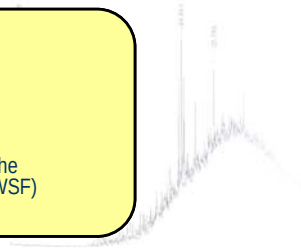
Toxicity of the unresolved complex mixture (UCM) of a petrogenic oil

Knut-Erik Tollefsen[1], Jorunn N. Hokstad[2], Alf G. Melbye[2], Odd G. Brakstad[2], Andy Booth[2], Steve Rowland[3], Inger K. Gregersen[1] and Bjørn Henrik Hansen[2*]

[1] NIVA, Oslo, Norway [2] SINTEF – Materials & Chemistry, Marine Environmental Technology, 7465 Trondheim, Norway, [3] University of Plymouth, Plymouth, UK. *Corresponding author, Mail to: bjorn.h.hansen@sintef.no

What are unresolved complex mixtures?

- ✓ Poorly characterized oil compounds
- ✓ Often referred to as "chromatographic humps"
- ✓ Comprise thousands of unidentified compounds
- ✓ In some oils UCMs may comprise over 50% of the organic material in the water soluble fractions (WSF)
- ✓ UCMs have shown to be toxic and persistent



Objectives of the study

- Fractionate WSF of a Norwegian Sea oil based on polarity by HPLC
- Determine toxicity in individual fractions by in vitro bioassays
- Generate synthetic mixtures of target analytes (from Norwegian regulatory monitoring) in selected fractions and test them for toxicity
- Evaluate the toxicity of the unknown oil compounds by comparing toxicity data of HPLC-fractions with those of synthetic mixtures

Chemical fractionation

Water soluble fractions (56 liters) of an evaporated North Sea oil (200°C+) was generated, extracted by DCM and concentrated to 1 ml. The extract was fractionated by HPLC with a polarity gradient using hexane, DCM and methanol. A total of 14 fractions were collected. The material was transferred to a low-toxic solvent (DMSO) and tested for toxicity by using rainbow trout hepatocyte in vitro bioassay.

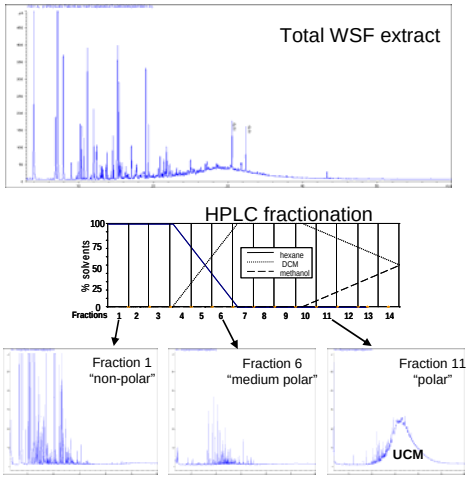


Fig. 1: GC chromatograms of Total WSF extract, non-polar fraction 1 (containing e.g. naphthalenes) medium-polar fraction 6 (containing e.g. alkylphenols) and polar fraction 11 (containing the UCM and trace amounts of dibenzothiophenes). The HPLC fractionation with DCM, hexane and methanol is given.

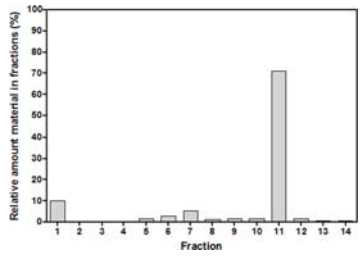


Fig. 2: Relative amount of material present in the 14 HPLC-separated fractions compared to total amount found in the fractions.

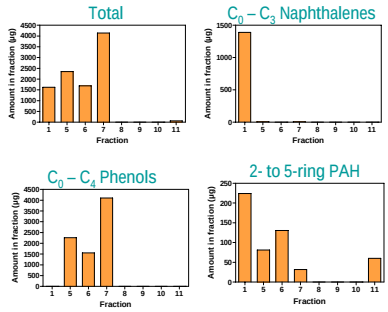
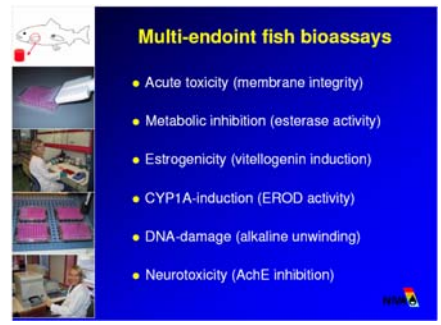


Fig. 3: Distribution of target analytes (~60 oil compounds regularly monitored in Norway) present in the WSF and selected HPLC-separated fractions.

Synthetic mixtures

Three fractions (non- medium and polar fractions 1, 6 and 11, respectively) were selected for determination of the toxicity of target analytes. Synthetic mixtures of decalines, naphthalenes, PAHs and alkylated phenols were prepared to mimic the concentrations of these compounds within each fraction.

In vitro fish bioassay



In vitro bioassay toxicity testing of fractions

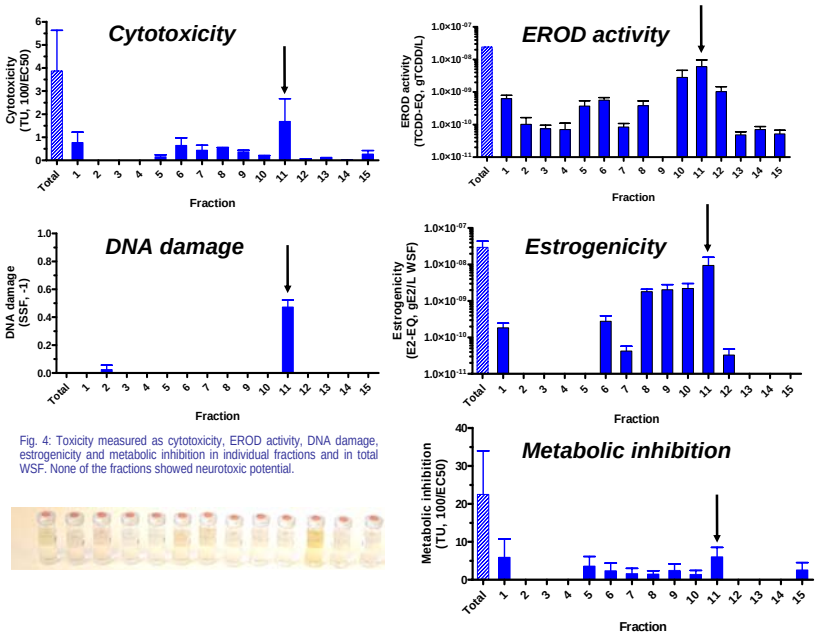


Fig. 4: Toxicity measured as cytotoxicity, EROD activity, DNA damage, estrogenicity and metabolic inhibition in individual fractions and in total WSF. None of the fractions showed neurotoxic potential.

In vitro bioassay toxicity testing of synthetic mixtures

Several of the HPLC-fractions exhibited toxic responses to rainbow trout hepatocytes, however, most pronounced was fraction 11 containing polar UCMs.

From the synthetic mixtures, toxicity (metabolic inhibition and estrogenicity) was only detected in the medium polar mixture 6, rich in alkylated phenols. The results of these studies strongly indicated that the polar UCMs are important contributors to the toxicity of WSFs of petrogenic oils, and that this toxicity was not associated with target analytes such as decalines, naphthalenes and PAHs.

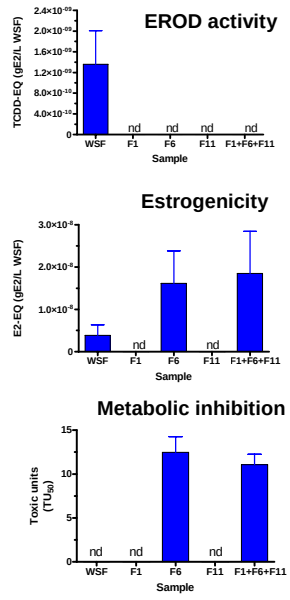


Fig. 5: Toxicity measured as EROD activity, estrogenicity and metabolic inhibition in synthetic mixtures of fractions 1, 6 and 11 and in total WSF.

Conclusions: Chemical fractionation

- ✓More than 50% of the WSF compounds were present in the polar fraction 11
- ✓Polar fractions represented typical "humps" in GC-FID chromatograms
- ✓Content of known and regularly monitored target analytes were negligible in this fraction

Conclusions: Toxic response to HPLC-separated fractions

- ✓ Higher toxic responses to polar than to non-polar and medium-polar HPLC-fractions
- ✓ Only the synthetic mixture of target analytes from fraction 6 exhibited toxic effects (estrogenicity and metabolic inhibition)
- ✓ Toxic responses to polar fraction are thus caused by other compounds than target analytes, proving that UCM is toxic and should be included in regulatory monitoring.