BIOLOGICAL EFFECTS OF EXPOSURE TO WATER SOLUBLE FRACTIONS (WSFs) OF OIL

BJØRN HENRIK HANSEN^{a,c,*}, KNUT-ERIK TOLLEFSEN^b, AUGUSTINE ARUKWE^c, ODD GUNNAR BRAKSTAD^a, DAG ALTIN^d, JORUNN HOKSTAD^a, TROND NORDTUG^a, ANDERS OLSEN^c, ALF G. MELBYE^a

^a SINTEF – Materials & Chemistry, Marine Environmental Technology, 7465 Trondheim, Norway

- ^b Norwegian Institute for Water Research, Gaustadallèen 21, N-0349 Oslo, Norway
- ^c Department of Biology, Norwegian University of Science and Technology, N-7491 Trondheim, Norway
- ^d BioTrix, N-7022 Trondheim, Norway
- * Corresponding author, Mail to: bjorn.h.hansen@sintef.no



Abstract

Water soluble fractions of oils (WSFs) constitute a wide range of potentially toxic compounds. A major problem has been to characterize the constituents of WSF, but naphthalenes, phenanthrenes and phenols are present. In addition, in some oils up to 60% of WSFs are present as unresolved complex mixture (UCM), and studies have shown both that some monoaromatic UCM-constituents are both accumulating in and causing toxic effects to marine organisms. In the present work Total WSF was generated and tested for toxicity on both Zebrafish (*Danio rerio*) and the copepod *Calanus finmarchicus*. Further fractionation of WSF was conducted, and 14 different fractions were tested for toxicity in a rainbow trout (*Oncorhynchus mykiss*) hepatocyte cultures.



WSF were prepared in closed, low-energy systems consisting of seawater with an oil film on top and a defined headspace. The preparation system is in accordance with recommendations set forth by CROSERF (Singer et al., 2000).

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Fractionation of the WSFs was performed on a semipreparative scale, using an Agilient LC1100 HPLC with a setup of two columns in series (Zorbax NH2 – 9.4 mm x 25 cm and Zorbax Si – 9.4 mm x 25 cm). Elution was performed using a gradient of hexane, dichloromethane and methanol.







The GeneChip® Zebrafish Genome Array was used to quantify expression of 14 900 transcripts in Zebrafish liver samples after exposure to 10% and 50% WSF solution for 24 and 72 hours. Marked responses on the AhR-CYP1Asystem was determined and verified by use of qPCR..

Calanus finmarchicus - WSF



A suppression subtractive hybridization library was constructed from control and WSF-exposed (50%) *Calanus* specimen, and ca 1000 clones were sequenced revealing 189 unique ESTs. After BLAST annotation, putative names and functions were determined. Primers were designed and qPCR used to determine expression of transcripts. Induction of transcripts involved in the ubiquitin-proteasome system were found along with a decrease in a P450 enzyme (CYP2C7) and an antioxidant (TrxR) (Hansen et al., in press).

Bio-assisted toxicity testing of water soluble fractions



Primary hepatocytes were isolated from male rainbow trout, seeded



References

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