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Effects of oil pollution and persistent organic pollutants (POPs) on glycerophospholipids in liver and brain of male Atlantic cod (*Gadus morhua*)

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HIGHLIGHTS

- ▶ Atlantic cod were exposed to a combination of weathered crude oil and POPs.
- ▶ Only small changes in the membrane lipid composition in liver and brain were shown.
- ▶ Transcriptional effects in the liver were studied by microarray and RT-qPCR.
- ▶ A combination of oil and POPs increased the clearing rate of PAHs and POPs.
- ▶ The mixture of oil and POPs induced the CYP1a detoxification system.

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ABSTRACT

Fish in the North Sea are exposed to relatively high levels of halogenated compounds in addition to the pollutants released by oil production activities. In this study male Atlantic cod *(Gadus morhua)* were orally exposed to environmental realistic levels (low and high) of weathered crude oil and/or a mixture of POPs for 4 weeks. Lipid composition in brain and in liver extracts were analysed in order to assess the effects of the various pollutants on membrane lipid composition and fatty acid profiles. Transcriptional effects in the liver were studied by microarray and quantitative real-time RT-PCR. Chemical analyses confirmed uptake of polychlorinated biphenyls (PCBs) and chlorinated pesticides, polybrominated diphenyl ethers (PBDEs) and perfluorooctanesulfonate (PFOS) in the liver and excretion of metabolites of polyaromatic hydrocarbons (PAHs) in the bile. Treatment with POPs and/or crude oil did not induce significant changes in lipid composition in cod liver. Only a few minor changes were observed in the fatty acid profile of the brain and the lipid classes in the liver. The hypothesis that pollution from oil or POPs at environmental realistic levels alters the lipid composition in marine fish was therefore not confirmed in this study. However, the transcriptional data suggest that the fish were affected by the treatment at the mRNA level. This study suggests that a combination of oil and POPs induce the CYP1a detoxification system and gives an increase in the metabolism and clearing rate of PAHs and POPs, but with no effects on membrane lipids in male Atlantic cod.

Abbreviations: AGPAT, 1-acylglycerol-3-phosphate O-acyltransferase; CYP, cytochrome P450; DDT, dichlorodiphenyltrichloroethane; ELOVL, elongase of very long chain fatty acid; ESR1, estrogen receptor 1; FFA, free fatty acid; HCH, *gamma*-hexachlorocyclohexane (lindane); LPCAT, lyso-PC acyltransferase; MUFA, mono-unsaturated fatty acids; NL, neutral lipid; NPD, sum of naphthalene, phenanthrene, dibenzothiophene, and their C1–C3 alkylated homologs; PAH, polycyclic aromatic hydrocarbons; PBDE, polybromodiphenyl ethers; PC, phosphatidylcholine; PCB, polychlorinated biphenyl; PE, phosphatidylethanolamine; PEMT, Phosphatidylethanolamine N-methyltransferase; PFOS, Perfluorooctanesulfonate; PI, phosphatidylinositol; PISD, phosphatidylserine decarboxylase; PL, phospholipid; PLA, phospholipase; POPs, persistent organic pollutants; PS, phosphatidylserine; PUFA, poly-unsaturated fatty acids; SFA, saturated fatty acids; TNC, trans-nonachlor (chlordane); Vtg A, vitellogenin A; ZP3, Zona pellucida.

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1. Introduction

Fish in the North Sea and along the Norwegian coast are exposed to pollutants released into the environment from both offshore oil and land-based industries (Green and Knutzen, 2003; Grøsvik et al., 2009). While many studies have focused on the effects that oil pollution alone has on marine organisms, few have also taken into account the effects of background pollution from other persistent organic pollutants (POPs).

The POPs and many oil compounds are characterized by high lipophilicity and thus have the potential to bioaccumulate in aquatic organisms, mainly in the storage or membrane lipids (Endo et al., 2011). The incorporation of pollutants into the membrane lipids can induce changes in cell membrane fluidity and such changes in membrane structures may have severe effects on the cell homeostasis (Zepik et al., 2008; Tekpli et al., 2011).

In vitro experiments have shown effects on membrane lipids after exposure to hydrophobic and amphiphilic compounds. Decreased melting points have been reported for α- and ß-endosulfan (Videira et al., 1999), PAHs (Nelson, 1987; Engelke et al., 1996; Jimenez et al., 2002), DDTs (Antunes-Madeira and Madeira, 1990; Bonora et al., 2008) and PCBs (Bonora et al., 2003; Tan et al., 2004; Yilmaz et al., 2006; Campbell et al., 2008). Increased membrane fluidity has been reported after exposure to PAHs (Engelke et al., 1996; Gorria et al., 2006; Korchowiec et al., 2008), lindane (Suwalsky et al., 1998), and PFOS (Hu et al., 2003; Matyszewska and Bilewicz, 2008; Matyszewska et al., 2008). POPs and PAHs are also known to induce alterations in the cell ultrastructure e.g. increasing the numbers of lipid droplets (Hacking et al., 1978; Hinton et al., 1978a, 1978b; Hugla et al., 1996; Korchowiec et al., 2008; Tekpli et al., 2010).

Alterations in lipid composition have been reported in wild fish caught in areas near oil installations in the North Sea (Grøsvik et al., 2009; Balk et al., 2011). A reduction in the ratio of $(n-3)$/$(n-6)$-polyunsaturated fatty acids (PUFAs) in the muscle, and an elevation in the concentration of arachidonic acid (20:4$(n-6)$) in the liver in both Atlantic cod (*Gadus morhua*) and haddock (*Melanogrammus aeglefinus*) collected near the oil installations in the Tampen field in the North Sea compared to a reference area at the Egersund bank (Balk et al., 2011). Similar findings have been reported for haddock liver from the same area (Grøsvik et al., 2009). Haddock from the Tampen field were in general in lower condition than haddock from reference areas, with both relatively small livers and low hepatic lipid levels, and had approximately 50% of the energy reserve compared with fish from the other areas (Grøsvik et al., 2009). However, it is important to perform more controlled laboratory experiments with fish to establish if oil pollution can indeed induce changes in the lipid composition similar to those previously reported in fish from field studies. In an earlier study from our group, Atlantic cod experimentally exposed to short-chained alkylphenols showed increased levels of saturated fatty acids (SFAs) and decreased levels of $(n-3)$-PUFA in the liver phospholipids (PLs), in addition to a similar reduction in the $(n-3)$/$(n-6)$-PUFA ratio to that reported from field samples (Meier et al., 2007). Alterations of membrane lipids after exposure to xenobiotic organic compounds have also been shown for other animals; a decrease in PUFA was observed in mink adipose tissue after exposure to PCB (Kakela and Hyvarinen, 1999). Kudo et al. (2011) reported that hepatic PL in mice that had been exposed to various perfluorinated fatty acids, had increased proportions of monounsaturated fatty acids (MUFA) (16:1$(n-7)$ and 18:1$(n-9)$), and 20:3$(n-6)$, and decreased proportions of 20:4$(n-6)$, 18:2$(n-6)$ and 18:0. The increase in MUFA was correlated with stearoyl-CoA desaturase (SCD) expression. The PC and PE fractions also had decreased proportions of 22:6$(n-3)$. The bacterium *Bacillus stearothermophilus* increased straight chain FA and decreased branched chain FA in the membrane lipids when exposed to DDT (Donato et al., 1997). An increase in SFA and/or a decrease in PUFA in the membrane lipids might be a mechanism of homeoviscous adaptation (Sinensky, 1974) to the higher membrane fluidity caused by interference with POPs.

Alterations of the lipid composition towards more saturation often occur when fish acclimatizes from colder to warmer water (Farkas and Csengeri, 1976; Hazel and Williams, 1990). Cod, like other poikilothermic animals, have the ability to remodel the membrane structure to maintain optimal membrane fluidity when environmental temperature is changed (Williams and Hazel, 1994), so-called homeoviscous adaptation (Sinensky, 1974). This can be done by changing the acyl chain structure, changing the head group structure, or reshuffling of acyl chains to form new molecular species (Henderson and Tocher, 1987; Tocher, 1995; Rilfors and Lindblom, 2002). An altered lipid composition after exposure to POPs could therefore be a mechanism of homeoviscous adaptation to correct for altered membrane fluidity after exposure to organic pollutants. POPs and oil compounds are also known to induce oxidative nstress2x1xstress which can affect the lipid composition by increasing the turnover of PUFA in the membrane (Kelly et al., 1998; Valavanidis et al., 2006; Catala, 2012).

The aim of the present investigation was to compare the effects of weathered crude oil and a mixture of different POPs on liver cell and brain cell membrane composition, and on genome-wide transcription, in male Atlantic cod.

Two hypotheses for mechanisms behind changes in membrane lipid composition were considered.

1. Oil and POP compounds have direct effects on the gene expression of lipogenic enzymes. To test this hypothesis, we have studied gene transcription of a numbers of key enzymes such as: Acyl-CoA-desaturases and elongases involved in the synthesis of long-chain PUFA; glycerol-3-phosphate acyltransferase, lysophosphatidate acyltransferase, choline phosphotransferase, ethanolamine phosphotransferase involved in the synthesis of the two major phospholipid classes (PC and PE); and phosphatidylserine decarboxylase (converting PS to PE); and phosphatidylethanolamine methyltransferase (converting PE to PC).
2. Oil compounds affect the lipid composition by inducing oxidative stress and this leads to higher turnover of PUFA in the membrane. To test this hypothesis, we studied gene transcription of phospholipase A1 and A2 and genes that are induced by oxidative stress. These include superoxide dismutases (Cu/Zn SOD and Mn SOD), catalase, glutathione peroxidises, heat shock proteins, and cytochrome P450 genes.

The exposure doses were chosen to represent realistic background levels of POPs (Low group); a 50 times higher dose was included as a positive control (High group). An overview of reported levels (Falandysz et al., 1994; Green and Knutzen, 2003; Kallenborn et al., 2004) of selected POPs in cod liver from the North Sea and along the Norwegian coast is given in the Supplementary material, Table S1. PAH levels after an acute spill of crude oil at the Statfjord A field in the North Sea (Grøsvik et al., 2008) are also shown. The oil exposure was calculated to represent low oil exposure from a minor oil spill (or chronical exposure from operational discharges of produced water) and high oil exposure from a major oil spill.

Cod were tube-fed with a food-paste containing weathered crude Troll oil and/or a mixture of POPs, for 4 weeks. The lipid composition and fatty acid profile were analyzed by High Performance Thin Layer Chromatography (HPTLC) and gas chromatography (GC-FID). The POPs mixture contained chlorinated (PCBs, pesticides), brominated (polybrominated biphenyl ethers, PBDE) and fluorinated (PFOS) compounds. The uptake and accumulation of POPs