Paper V

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AQUATIC TOXICOLOGY

Effects of alkylphenols on redox status in first spawning Atlantic cod (*Gadus morhua*)

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Abstract

Offshore oil production releases large amounts of lipophilic compounds in produced water and into the ocean. The discharge of produced water from the Norwegian petroleum sector has increased from 26 million m^3 in 1993 to 120 million m^3 in 2001, and it continues to increase. Produced water contains significant amounts of alkylphenols, which have been reported to be estrogenic, causing endocrine disruption in fish. In year 2000, approximately 44 tons of alkylphenols were released on the Norwegian continental shelf in connection with discharge of produced water. Except from being estrogenic, relatively little is known about the effects of alkylphenols when released in the marine environment. Our objective was to study how alkylphenols affect the redox status in first spawning Atlantic cod (*Gadus morhua*) of both sexes. Model compounds tested included 4-*tert*-butylphenol (C_4), 4-n-pentylphenol (C_5), 4-n-hexylphenol (C_6) and 4-n-heptylphenol (C_7), all found in produced water. First spawning Atlantic cod were force-fed a mixture of these four alkylphenols, ranging between 0.02 and 80 ppm or 5 ppm 17β-estradiol (E_2), for 1 or 4 weeks. Increased hepatic total glutathione concentration in response to alkylphenol exposure was detected in female fish compared to control group after 1-week exposure, an effect not seen after 4 weeks. Furthermore, hepatic total glutathione concentration was sex dependent, where male fish sampled after 4 weeks had higher levels of glutathione than female fish. Increased glutathione reductase catalytic activities in both male and female fish were seen after exposure to 0.02 ppm alkylphenol mixture in 4 weeks. The glutathione *S*-transferase activity was only affected in male fish exposed to 0.02 ppm alkylphenols, and glucose-6-phosphate dehydrogenase activity increased in female fish exposed to 0.02 ppm alkylphenol mixture for 1 week. The increase of hepatic total glutathione content as well as the effects on glutathione reductase activities suggests that alkylphenol exposure affects the redox status in Atlantic cod.
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Keywords: Produced water; Alkylphenols; Oxidative stress; Glutathione; Fish

1. Introduction

Oil production in the Norwegian sector of the North Sea results in large disposal of produced water into the ocean. The amount of produced water is increasing rapidly with the age of the oil field, and the discharge

of produced water from the Norwegian sector reached a level of more than 120 million m^3 in year 2001 (SFT; OLF). The produced water is contaminated with a wide spectrum of organic and inorganic compounds originating from the oil, formation water or production additives. Alkylated phenols with an alkyl chain length ranging from C_4 to C_7 have been reported in concentrations of 2–237 ppb in produced water from platforms outside the coast of Norway (Brendehaug et al., 1992).

The acute toxicity of produced water to marine organisms is low. The dilution factor is high in offshore discharge, and thus the concentration of alkylphenols in the water column will be low. However, relatively little is known about the fate and long-term effects of alkylphenols in the marine environment (Røe, 1998). The biodegradation rate of phenols dramatically decreases with increasing length of alkyl chain, and their bioavailability increases due to their lipophilic nature. The bioconcentration factors in fish range from 118 to 578 for butyl- to heptylphenol (McLeese et al., 1981; Freitag et al., 1985; Tollefsen et al., 1998).

The estrogenic activity of alkylphenols has been well established both in vitro and in vivo (Nimrod and Benson, 1996; Arukwe et al., 2000, 2001). The alkylphenols are shown to interact with the estrogen receptor, although weaker than 17β-estradiol. The estrogenicity of the alkylphenols depends on position (*para>meta > ortho*) and branching (tertiary > secondary = primary) of the alkyl chain. The highest estrogenic activity is found in C_6–C_8 para substituted tertiary alkylphenols (1000- to 6000-fold less potent than E_2), but also C_5, C_4 and C_3 phenols are estrogenic (100 000- to 20 000 000-fold less potent than E_2) (Routledge and Sumpter, 1997).

The effects of alkylphenols on redox status and detoxification enzymes in Atlantic cod are unclear. Estrogenic environmental compounds such as *para*-nonylphenol and bisphenol A were found to stimulate hydroxyl radical formation in the rat striatum (Obata and Kubota, 2000). Another study concluded that phenols showed a double action, acting as antioxidants at lower doses, but acting as pro-oxidants at higher doses under certain circumstances (Fujisawa et al., 2002). Nonylphenol is also found to inhibit cell growth and cellular oxygen consumption in *Saccharomyces cerevisiae*, suggesting nonylphenol-induced oxygen radical generation in yeast mitochondria. Nonylphenol-induced cell growth inhibition was efficiently protected by the lipophilic antioxidants α-tocopherol and β-carotene (Okai et al., 2000).

The phenol group makes the alkylphenols directly available to phase II enzymes, and thus the alkylphenols are relatively rapid metabolized. In fish, organic compounds usually are conjugated to either glutathione or glucuronic acid (Ankley and Agosin, 1987). Glutathione *S*-transferase (GST) is a family of enzymes catalysing conjugation of a large variety of foreign compounds to glutathione. Glutathione (GSH) also functions as an antioxidant (DeLeve and Kaplowitz, 1991). For example, hydrogen peroxide can be reduced by GSH in the presence of selenium-dependent glutathione peroxidase. As a consequence, GSH is oxidized to GSSG, which rapidly is reduced back to GSH by glutathione reductase (GR) at the expense of NADPH (DeLeve and Kaplowitz, 1991). NADPH is regenerated by oxidation of glucose-6-phosphate to 6-phosphogluconate within the pentose phosphate pathway. The key enzyme for this pathway is glucose-6-phosphate dehydrogenase (G6PDH) (Eggleston and Krebs, 1974).

The aim of this investigation was to study the effects of alkylphenols on the redox status in first spawning Atlantic cod. A computer simulation estimated the discharge of alkylphenols in produced water from the Halten Bank area outside the Norwegian West coast, and the probable uptake by pelagic fish species was calculated (Rye et al., 1996). The calculations estimated the body burden of alkylphenols in fish to be in the 0–10 ppb range and the lowest exposure dose was chosen on the basis of this calculation and the bioconcentration factor 600 for the alkylphenol mixture. Oxidative stress response was determined by measuring total and reduced amount of glutathione as well as GR and G6PDH activities in the liver. In addition, the activity of GST was determined. Our results, together with a study of CYP1A and CYP3A expression and activities (Hasselberg et al., 2004) and a study on endocrinology changes (Meier et al., 2002), will hopefully facilitate the evaluation in environmental risk assessment studies of alkylphenols.

2. Materials and methods

2.1. Chemicals

Bakers yeast glutathione reductase, 1-chloro-2,4-dinitrobenzene (CDNB), 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB), dimethyl sulfoxide (DMSO), glucose-6-phosphate, glutathione (reduced and oxidized form), $NADP^+$ and NADPH were all purchased from Sigma–Aldrich Sweden AB (Stockholm, Sweden). 4-tert-Butylphenol (C_4) and 4-n-hexylphenol (C_6) were purchased from Sigma–Aldrich Norway AS (Oslo, Norway). 4n-Pentylphenol (C_5) was obtained from Acros (Gell, Belgium) and 4-n-heptylphenol (C_7) from Avocado Research Chemicals LTD (Lancashire, U.K.). Monobromobimane was from Calbiochem–Behring Diagnostics (La Jolla, CA, USA). BCA Protein Assay Kit and Coomassie® Plus Assay Reagent were obtained from Pierce (Rockford, IL, USA). All other chemicals used were of analytical grade available in Sweden and Norway from Sigma–Aldrich, Fluka Chemie AG or VWR international.

2.2. Animals, treatment and sampling

First-time spawning Atlantic cod (*Gadus morhua*) of both sexes, body weight 600–800 g, were supplied by Austevoll Aquaculture Research Station, Institute of Marine Research, Bergen, Norway. The fish were divided into seven groups with 40 fish in each group and transferred to 10 m^3 indoor tanks, provided with continuously flowing seawater at a temperature of $9 \pm 1\,°C$ with a salinity of 34.5‰ and subjected to a natural photoperiod (August to December, Bergen, 60°N). From August until the experiment started, fish were fed commercial fish feed (dry pellets from Felleskjøpet AS, 10% lipid). During the experimental period (November to December), fish were force-fed once a week with 0.02, 2, 20, 40 and 80 ppm alkylphenol mixture relative to body weight. The alkylphenol mixture consisted of 4-tert-butylphenol (C_4), 4-n-pentylphenol (C_5), 4-n-hexylphenol (C_6) and 4-n-heptylphenol (C_7) in a 1:1:1:1 ratio. A positive estrogenic control group also was included (5 ppm 17β-estradiol) together with a control group that received vehicle (1,2-propanediol). The alkylphenols and estradiol were dissolved in 1,2-propanediol

and mixed into a paste consisting of ground dry pellets, water and fish oil (paste composition: 50.5% dry pellets, 40.5% water, 5.0% fish oil and 4.0% 1,2-propanediol/alkylphenol solution). The lipid content and fatty acid profile of the paste were identical to the original composition of the dry pellets. The paste was aspirated into a plastic tube using a piston and then inserted directly into the stomach of the anaesthetized fish. Both fish and paste were weighed immediately before the alkylphenol mixture was administered. One week after the first exposure, 20 fish from each group were removed for analysis. The remaining fish were given three additional doses and were sacrificed 1 week after the final dose. The Atlantic cod were anaesthetized with benzocaine (20 mg/l) and killed by a blow to the head. Body weights and fish lengths were measured, and livers were rapidly removed using a scalpel and snap frozen in liquid nitrogen. Only female fish livers were sampled after 1 week of exposure. After 4-week-exposure, livers were sampled from fish of both sexes.

2.3. Total and reduced glutathione

Total free glutathione (tGSH) and reduced glutathione (GSH) concentrations were determined according to a method of Svardal et al. (1990), whereby 50–100 mg aliquots of frozen liver were homogenized in ice-cold 5% sulphosalicylic acid containing 50 μM dithioerythriol (1:40 w/v) and centrifuged at 10 000 × g for 5 min at 5 °C. tGSH (reduced form + disulphide + dissolved mixed disulphide) and GSH were analysed in the supernatant. To reduce oxidized glutathione and for derivatization of free –SH groups, we used $NaBH_4$ and monobromobimane, respectively. After derivatization, the thiolbimane adducts were quantified using reverse-phase ion-pair liquid chromatography and fluorescence detection.

2.4. Glutathione reductase activity

Frozen livers were weighed and homogenized in 0.1 M sodium phosphate, pH 7.5, containing 1 mM ethylenediamine tetra-acetic acid (EDTA) (1:4 w/v), by a Potter–Elvehjem glass–teflon homogenizer. Homogenates were centrifuged at 12 000 × g for 20 min at 4 °C, and S9-fractions were stored at −80 °C. Total protein content was measured using the BCA Protein

Assay Kit from Pierce and bovine serum albumin as standard. The reaction mixture for GR activity consisted of 150 μl of 0.1 mM DTNB, 20 μl of 12 mM NADPH and 20 μl of sample. The reaction was initiated by addition of 20 μl of 3.25 mM GSSG (Cribb et al., 1989). All reagents were dissolved in 0.1 M sodium phosphate buffer, pH 7.5, containing 1 mM EDTA. Formation of product was monitored at A_{405} at room temperature. The increase in absorbance at A_{405} is proportional to GR activity.

2.5. Glucose-6-phosphate dehydrogenase activity

Frozen livers were weighed and homogenized in 0.1 M sodium phosphate, pH 7.4, 0.15 M KCl, 1 mM EDTA, 1 mM dithiothreitol and 10% glycerol (1:4 w/v) by a Potter–Elvehjem glass–teflon homogenizer. Homogenates were centrifuged at $12\,000 \times g$ for 20 min at 4 °C, and supernatants were stored at −80 °C. Total protein content was measured by the Coomassie® Plus Assay Reagent from Pierce with bovine gamma globulin as standard. The reducing capacity was determined in S9-fractions as the enzyme activity of G6PDH and 6-phosphogluconate dehydrogenase (6PGDH) (Deutsch, 1987). The rate of increase in absorbance at 340 nm was a measure of enzyme activity in the presence of glucose-6-phosphate and NADP$^+$. All reagents were dissolved in 100 mM Tris–HCl with 10 mM MgCl$_2$, pH 7.5.

2.6. Glutathione-S-transferase activity

Glutathione-S-transferase activity was determined in S9-fractions (prepared as described in Section 2.5), using 2 mM CDNB dissolved in DMSO and 1 mM GSH (Habig et al., 1974). All reagents were dissolved in 0.1 M sodium phosphate buffer, pH 7.4. The reaction was started by adding the sample, and glutathione conjugation was monitored at A_{340} over time.

2.7. Statistical analyses

Data were analysed using Kruskal–Wallis followed by two-tailed Mann–Whitney *U*-tests. Data are presented as means ± standard error (S.E.). Significance levels are $^*P = 0.05$. Statistical analyses were performed using SPSS 11.0 software from SPSS Sweden AB (Sundbyberg, Sweden).

3. Results

3.1. Glutathione

Both tGSH and GSH increased in female Atlantic cod liver after 1 week of exposure to alkylphenols compared to controls (Fig. 1A). The ratio of tGSH versus GSH was relatively constant in all treatment groups (Fig. 1A–C). Control fish had close to 100% of all glutathione in its reduced form after 1 week. The corresponding levels for female and male fish after 4 weeks were 95 and 91%, respectively. After 4 weeks of exposure to alkylphenols, there was a decrease in tGSH levels (nearly 50%) in the 2 ppm dose but not in the higher doses in female fish (Fig. 1B). No effects on tGSH levels in male Atlantic cod were seen after 4 weeks of exposure (Fig. 1C). Male Atlantic cod displayed significantly higher hepatic glutathione concentrations than females, 512 nmol GSH per mg wet weight, compared to the female average of 360 nmol GSH per mg wet weight. Furthermore, 17β-estradiol had no effect on tGSH levels in either male or female Atlantic cod.

3.2. Glutathione reductase

Hepatic GR activity was affected in a biphasic mode. Thus, the GR activity increased at lower concentrations of alkylphenols and decreased at higher concentrations in female fish after 4 week of exposure to alkylphenols compared to controls (Fig. 2A). Hepatic GR activities also were significantly increased (about 80%) in fish of both sexes exposed for 4 weeks to the lowest dose of alkylphenols. However, increasing the dose of alkylphenols had no significant effects on GR activities in these fish (Fig. 2B and C). This was evident for both male and female Atlantic cod. 17β-Estradiol did not affect GR activity in these fish (Fig. 2A–C).

3.3. Glucose-6-phosphate dehydrogenase and glutathione S-transferase

G6PDH and GST activities also were determined. Exposure to the lowest dose of alkylphenols (0.02 ppm) resulted in an increased G6PDH activity in female Atlantic cod after 1 week (Table 1). Higher doses did not affect the G6PDH activity in these fish
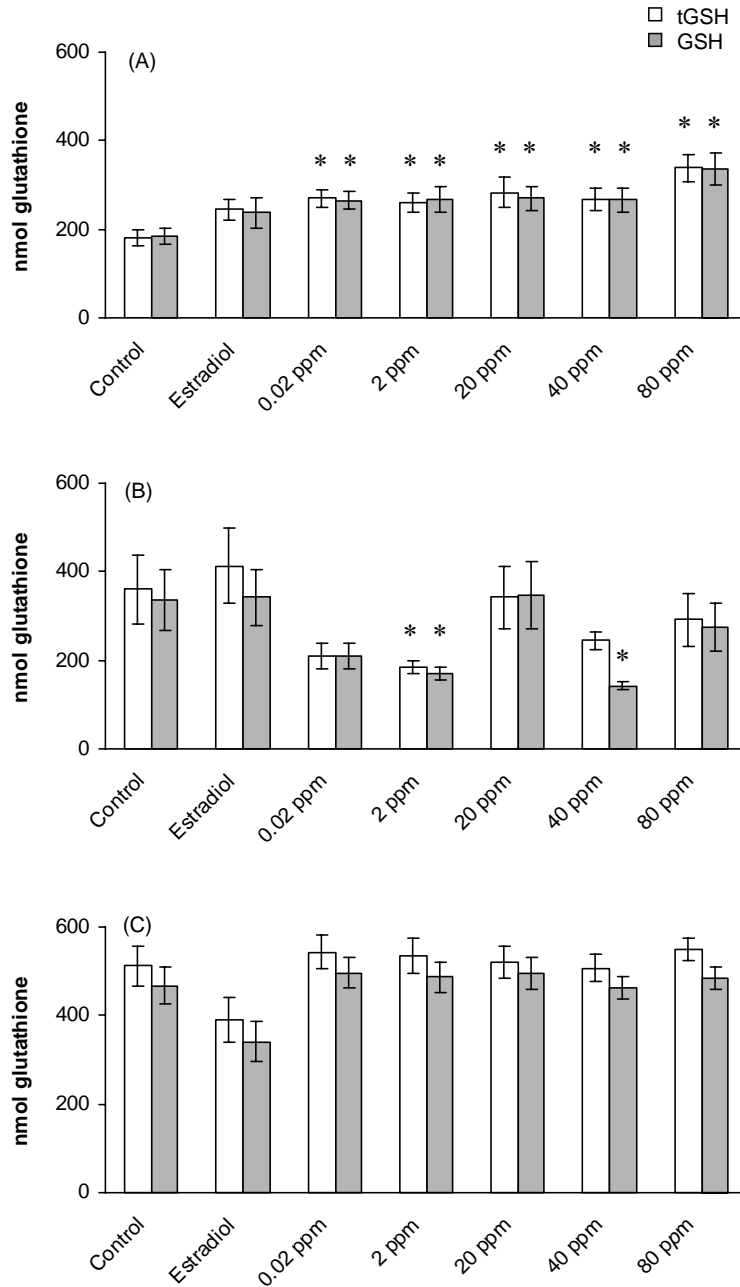
Fig. 1. Total (tGSH) and reduced hepatic glutathione (GSH) concentrations in (A) female Atlantic cod after 1 week (B) female Atlantic cod after 4 weeks and (C) male Atlantic cod after 4 weeks of oral exposure to 17β-estradiol or various doses of the alkylphenol mixture (C_4:C_5:C_6:C_7 ratio 1:1:1:1). Data represent the means ± S.E. of 5–15 individuals in each treatment group. Asterisk indicates statistically difference from control (*$P \leq 0.05$). Open bars represent tGSH, and filled bars represent GSH. Concentrations expressed in nmol glutathione per mg wet weight.
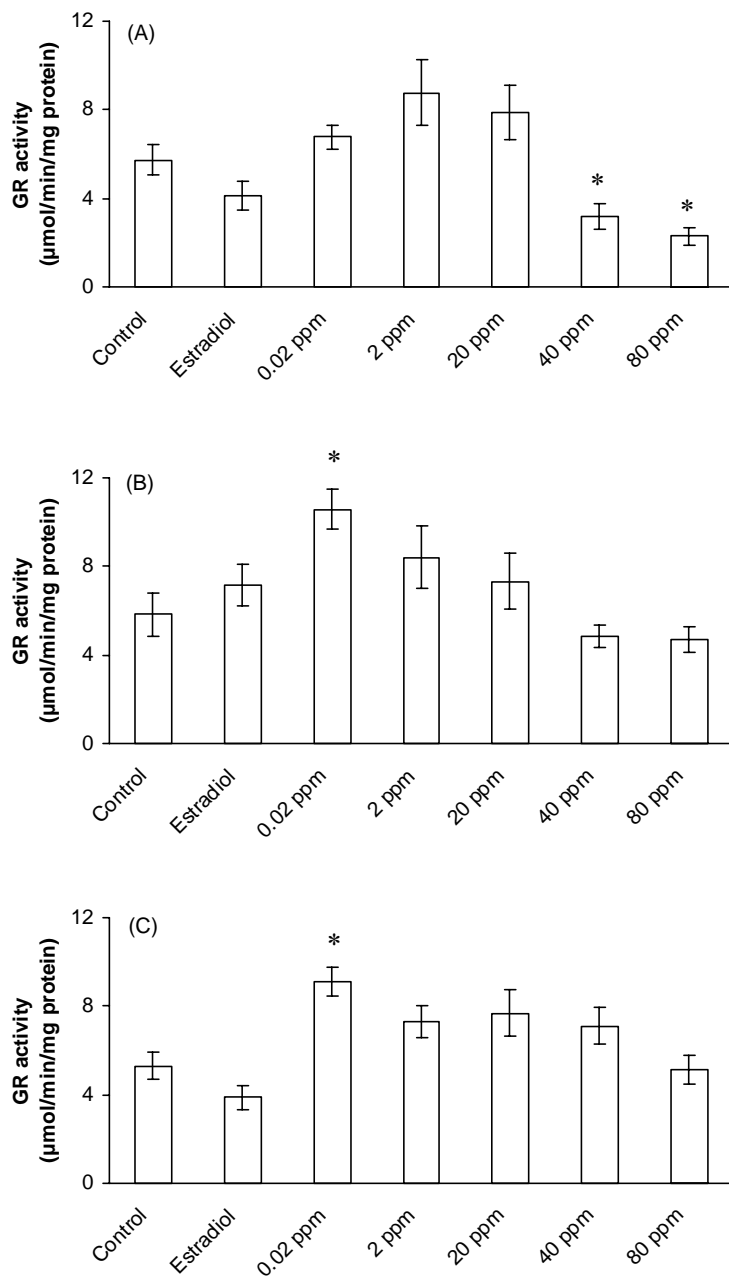
Fig. 2. Hepatic glutathione reductase activities (GR) in (A) female Atlantic cod after 1 week (B) female Atlantic cod after 4 weeks and (C) male Atlantic cod after 4 weeks oral exposure to 17β-estradiol or various doses of the alkylphenol mixture (C_4:C_5:C_6:C_7 ratio 1:1:1:1). Data represent the means ± S.E. of 5–15 individuals in each treatment group. Asterisk indicates statistically difference from control (*$P \leq 0.05$). GR activity expressed as μmol/min/mg protein.

Table 1
Hepatic glucose-6-phosphate dehydrogenase (G6PDH) activity in Atlantic cod after oral exposure to 5 ppm 17β-estradiol or various doses of the alkylphenol mixture (C_4:C_5:C_6:C_7 ratio 1:1:1:1)

	Control	Estradiol	0.02 ppm	2 ppm	20 ppm	40 ppm	80 ppm
Female, 1 week	32.1 ± 8.4	32.3 ± 6.7	49.4** ± 5.9	41.1 ± 13.0	34.4 ± 6.7	33.3 ± 8.8	31.9 ± 7.8
Female, 4 weeks	25.4 ± 6.9	24.5 ± 7.4	29.7 ± 9.7	25.3 ± 4.4	25.0 ± 6.3	31.6 ± 4.6	31.3 ± 7.6
Male, 4 weeks	36.6 ± 12.7	24.5* ± 4.7	34.2 ± 12.5	34.6 ± 7.1	32.0 ± 7.0	30.8 ± 5.8	34.1 ± 7.8

Data represent the means ± S.E. of measurements on 5–15 individuals in each treatment group. Activity presented as μmol/min/mg protein.
* $P \leq 0.05$, indicates statistically difference from control.
** $P \leq 0.01$, indicates statistically difference from control.

and no effects were seen in the other groups treated with alkylphenols. However, 17β-estradiol treatment lowered G6PDH activity in male fish after 4 weeks of exposure to the same level as seen in the female control group. The GST activity was decreased in male Atlantic cod exposed to 0.02 ppm alkylphenols compared to control (Table 2).

4. Discussion

4.1. Glutathione

Intracellular glutathione is normally in its reduced form, and in mammals, oxidized glutathione constitute only a few percent or less of the total glutathione pool (Meister and Anderson, 1983). Thus, measuring total hepatic glutathione often reflects the reduced levels. In fish, the GSSG portion often is higher than in mammals, although the relationship between GSH and GSSG varies with species and tissue. In our study, we measured the level of tGSH and GSH and the ratio was calculated. The tGSH/GSH ratios were similar in controls, 17β-estradiol- and alkylphenols- exposed groups. In female fish, GSH constituted 95% and in male fish 91% of tGSH. Other studies have shown

similar levels. For example, eels (*Anguilla anguilla*) had 88% of all hepatic glutathione in its reduced form (Peña et al., 2000), and in rainbow trout (*O mykiss*), the hepatic glutathione pool consisted of 92 % GSH and 4% GSSG (Otto et al., 1997).

Hepatic tGSH increased in female fish after 1 week of exposure to alkylphenols. However, tGSH levels decreased in females exposed to 2 ppm alkylphenols after 4 weeks compared to controls, whereas no effect was observed in the other groups. After 4 weeks of exposure, there were no differences in tGSH levels in male fish. Studies by others have shown that many fish species show increased hepatic GSH concentration in response to exposure to environmental pollutants (Di Giulio et al., 1993; Hasspieler et al., 1994). Increased GSH biosynthesis has been observed in liver, gills and posterior kidney of channel catfish (*Ictalurus punctatus*) exposed to chlorothalonil (Gallagher et al., 1992). Polychlorinated biphenyls (PCB) are known to bioaccumulate and also to induce cytochrome P450-dependent monooxygenases activities (Kleinow et al., 1987). In addition, PCB induced enzymes associated with antioxidant defences such as GR, G6PDH and glutathione peroxidase activities in a number of tissues of rainbow trout. Thus, hepatic GSH concentrations as well as GSSG levels were increased, even

Table 2
Hepatic glutathione *S*-transferase (GST) activity in Atlantic cod after oral exposure to 5 ppm 17β-estradiol or various doses of the alkylphenol mixture (C_4:C_5:C_6:C_7 ratio 1:1:1:1)

	Control	Estradiol	0.02 ppm	2 ppm	20 ppm	40 ppm	80 ppm
Female 1 week	0.975 ± 0.125	1.078 ± 0.116	0.873 ± 0.101	1.101 ± 0.158	0.966 ± 0.144	1.081 ± 0.204	0.999 ± 0.131
Female 4 weeks	1.035 ± 0.185	0.963 ± 0.108	0.985 ± 0.066	1.028 ± 0.085	0.945 ± 0.112	1.061 ± 0.111	1.103 ± 0.128
Male 4 weeks	0.952 ± 0.153	0.899 ± 0.125	0.766** ± 0.088	0.945 ± 0.240	0.973 ± 0.211	0.961 ± 0.111	0.923 ± 0.124

Data represent the means ± S.D. of measurements on 5–15 individuals in each treatment group. Activity presented as μmol/min/mg protein.
** $P \leq 0.01$ indicates statistically difference from control.

though the GSSG/GSH ratio remained unchanged (Otto and Moon, 1995). Freshwater catfish (*Channa punctatus* Bloch) exposed to paper mill effluents showed an increase in hepatic GSH concentration, while the levels decreased in kidney and gill (Ahmad et al., 2000). However, channel catfish exposed to bleached kraft mill effluent showed decreased GSH level, even though the level recovered after long-term exposure to the effluent (Mather-Mihaich and Di Giulio, 1986). Otto and Moon (1996) found significantly lower tGSH levels in liver, kidney and white muscle of brown bullheads (*Ameiurus nebulosus*) from a PCB polluted area compared to bullheads from a reference site. In addition, hepatic GST activity was three-fold higher in fish from the polluted river.

The GSH biosynthesis is regulated by feedback inhibition, and increased GSH consumption will lead to an increase in synthesis to keep the glutathione homeostasis (Meister and Anderson, 1983). The contradictory results discussed here may be the cause of this feedback inhibition (van der Oost et al., 1996). Elevated GSH levels follow an initial decrease in GSH levels, and time for sampling can be of crucial importance. We found elevated tGSH levels after 1 week of alkylphenol exposure, whereas after 4 weeks, the tGSH level recovered.

In our study, hepatic tGSH level was higher in males than in females. Sex differences in tGSH concentration have been reported in a field study on brown bullhead. In that study, tGSH, as well as other biomarkers including superoxide dismutase and GST activities, displayed sexual dimorphic expression in fish (McFarland et al., 1999). These results also show the importance of sex determination in biomonitoring programmes. Furthermore, our results showed that the level of hepatic tGSH in controls was higher in female fish after 4 weeks than after 1 week of exposure. This may be due to seasonal variations.

4.2. Enzyme activities

The GR activity in Atlantic cod exposed to alkylphenols for 1 week was induced in a biphasic fashion. Although the GR activity was reduced in the highest alkylphenol doses, GSH was retained in its reduced form. However, the observed increase in GR activities in both female and male fish after 4 weeks of exposure to 0.02 ppm alkylphenols, conceivably, is involved in GSH homeostasis and increased GR activity indicates exposure to oxidative stress. Shorthorn sculpin (*Myoxocephalus scorpius*) from a polluted harbour showed significantly higher GR activities than fish from a clean harbour (Stephensen et al., 2000). Furthermore, the GR activity was the most responsive enzyme in rainbow trout injected with the redox cycling compounds paraquat and menadione (Stephensen et al., 2002). GST activities and tGSH contents were also affected by these chemicals. These results in fish indicate that GR activity as well as tGSH levels may be used for detecting changes in redox status and could be good biomarkers used to assess exposure to oxidative stress (Peña-Llopis et al., 2001; Stephensen et al., 2002).

Hepatic G6PDH activities were induced in female Atlantic cod after 1 week of 0.02 ppm alkylphenol exposure. Increasing the dose and exposure times did not affect G6PDH activities, which suggests that the level of NADPH was constant. In another study, the G6PDH activity in erythrocytes from Nile tilapia (*Oreochromis niloticus*) from a polluted site was increased, although tGSH content decreased (Bainy et al., 1996). Interestingly, 17β-estradiol decreased the G6PDH activity in male cod. Winzer et al. (2002) have shown that 17β-estradiol inhibit G6PDH activity in isolated hepatocytes of immature female and male European flounder (*Platichtys flesus*) and that hepatocytes from males showed higher G6PDH activity than those from females (Winzer et al., 2002).

The lowest alkylphenol dose resulted in a decreased GST activity in male Atlantic cod, while other doses did not. GST activities in female Atlantic cod were not affected at all. Induction of cytosolic GST activity, using CDNB as substrate, might be less relevant than the induction of certain GST isoenzymes (Sole et al., 2000). It has been suggested that glucuronidation (i.e. conjugation to glucuronic acid by UDP-glucuronosyltransferase) may be the major pathway in phase II detoxification in fish (Clarke et al., 1991; Otto and Moon, 1995). It also is known that glucuronidation is of particular importance in phase II metabolism of alkylphenols (Lewis and Lech, 1996; Meldahl et al., 1996; Thibaut et al.,

1998; Arukwe et al., 2000; Ferreira-Leach and Hill, 2001).

4.3. Conclusions

Our results suggest that alkylphenol exposure may cause oxidative stress in Atlantic cod. We found elevated tGSH levels as well as increased GR activity in alkylphenol-treated Atlantic cod and these observed changes in GSH status and GR activities suggest involvement of several factors. Alkylphenols, therefore, are likely to increase oxidative stress in Atlantic cod as well as stimulate GSH dependent detoxification. Interactions between pro-oxidant factors and antioxidant defences are very complex and many factors have to be taken into account. Oxidative stress may be associated with either induced or reduced enzyme activities or a combination of both. Species, sex, time of exposure, dose as well as properties of the chemicals tested can influence levels of tGSH and enzyme activities and needs to be carefully taken under consideration when results are interpreted. Diet is another important factor that can affect the results. Recent studies have revealed that food-deprived fish had lower GSH levels (Pascual et al., 2003) and that the hepatic GSH level decreased in fasted fish (Geter et al., 2003). Therefore, it is important to consider normal feeding during acute exposure in order to maintain the integrity of xenobiotic defences. Nevertheless, dietary vitamin E may enhance the hepatic antioxidant defence and conceal oxidative stress response caused by tested chemicals. To circumvent this, exposed groups should always be compared to an equally treated control group.

Acknowledgements

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