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# PCB77 (3,3′,4,4′-tetrachlorobiphenyl) co-exposure prolongs CYP1A induction, and sustains oxidative stress in B(*a*)P-exposed turbot, *Scophthalmus maximus*, in a long-term study

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ABSTRACT

Cytochrome P4501A (CYP1A), benzo(*a*)pyrene (B(*a*)P) activation and biliary elimination, phase II activities, and peroxisomal and antioxidant activities of turbot (*Scophthalmus maximus*) were studied in a long-term controlled experiment. Fish were serially exposed in water on day 1 and on completion of months 3, 6 and 9 to 0.1, 0.2, 0.1 and 0.1 mg B(*a*)P/l, respectively, while another group was identically treated with additional PCB77 (3,3′,4,4′-tetrachlorobiphenyl) at 1% of concomitant B(*a*)P (w/w). Temporally persistent responses were obtained by sampling on week 3 and 3 months from each latest exposure. Serial exposure to B(*a*)P + PCB77 progressively induced liver 7-ethoxyresorufin *O*-deethylase (EROD) activity and CYP1A protein levels (ELISA, western blotting) towards months 9, 12 and gill EROD activity on month 12. It associated with an apparent increase in liver benzo(*a*)pyrene diol epoxide (BPDE)–DNA adduct levels (ultrasensitive enzyme radioimmunoassay), and elevated bile B(*a*)P metabolite levels on month 9 females as compared to males. In contrast, B(*a*)P alone did not cause ($p > 0.05$) comparable effects on liver EROD, CYP1A, adducts nor on bile metabolites. Both exposed groups demonstrated evidence for lasting oxidative stress as hepatic superoxide dismutase, catalase and glutathione peroxidase activities were significantly altered ($p < 0.05$) with symptomatic pro-oxidant associations among them. Both treatments affected liver somatic index similarly (increase on month 3, decrease on month 9 in males). Continued exposure on month 18 (0.2 mg B(*a*)P/l, 1% PCB77) followed by sampling 6 months later showed sustained induction ($p < 0.001$) of hepatic EROD in B(*a*)P + PCB77 group, which was not seen in B(*a*)P alone treatment. Thus, PCB77 co-exposure prolonged CYP1A induction and contributed to a persistent oxidative challenge in B(*a*)P-exposed turbot. The results indicate synergistic effects of polycyclic aromatic hydrocarbon (PAH) and polychlorinated biphenyl (PCB) exposure in the aquatic environment.



## 1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) are chemical pollutants of marine and other aquatic systems and their pervasive biological impact represent a widespread environmental problem. This awareness has largely emerged as toxicant-induced changes at various levels of biological organization. However, how individual responses develop upon co-exposure to toxicants, mutual influence of changes and subsequent re-exposures remains an open question.

Fish CYP1A gene expression is induced by the pro-carcinogenic PAH, B(*a*)P (Boleas et al., 1998) and by the persistent co-planar PCB congener 77 (Schlezinger and Stegeman, 2000) in addition to many other PAHs and PCBs. Both pollutants exert biological toxicities and occur in aquatic environments. The toxicity of those lipophilic chemicals is centered on their ability to act as fitting ligands to cytosolic aryl hydrocarbon receptor (AhR) which consequently mediates CYP1A induction in the nucleus. Basal or induced CYP1A can be followed as 7-ethoxyresorufin *O*-deethylase (EROD) activity and by immunodetection of CYP1A protein (Goksøyr, 1995; Goksøyr and Husøy, 1998).

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Hepatic CYP1A-mediated B(*a*)P metabolism has been shown in teleost models such as rainbow trout (Williams and Buhler, 1984) and zebrafish (Miranda et al., 2006). In the process, the parent compound is bio-activated by CYP-dependent epoxidation and epoxide hydrolases into carcinogenic, benzo(*a*)pyrene-7,8-diol-9,10-epoxide (BPDE) (Stegeman and Lech, 1991; Kelly et al., 1993; Peltonen and Dipple, 1995). BPDE covalently binds to guanines and form BPDE–DNA adducts (Varanasi et al., 1989; Sikka et al., 1990; Telli-Karakoç et al., 2002). B(*a*)P and its metabolites are predominantly excreted via the hepatobiliary route in fish (Steward et al., 1991; Seubert and Kennedy, 2000) and positive correlations between hepatic CYP1A and bile metabolite levels have been shown (Miller et al., 1999).

Microsomal B(*a*)P metabolism also generates reactive oxygen species (ROS) (Peters et al., 1996) and redox cycling quinones (Williams and Buhler, 1984). In fish liver, B(*a*)P was shown to have induced proliferation of peroxisomes (Au et al., 1999) that harbour acyl-CoA oxidase (AOX) activity (Yang et al., 1990). Being a marker of peroxisome proliferation, AOX generates $H_2O_2$ during its activity in peroxisomal lipid β-oxidation (Singh et al., 1997). In fish, PCB77-stimulated CYP1A-mediated ROS release (Schlezinger et al., 1999, 2000; Schlezinger and Stegeman, 2001) and altered antioxidant enzymatic defense (Otto and Moon, 1995). A solitary PCB exposure was shown to have sustained the increased microsomal ROS release for more than 3 months in teleost liver (Livingstone et al., 2000).

The enzymatic protection against ROS is provided by superoxide dismutase (SOD) that scavenges $O_2^{\bullet -}$, and by glutathione peroxidase (GPX) and catalase (CAT) which split $H_2O_2$. These antioxidant activities in fish (Filho et al., 1993; Förlin et al., 1995; Di Giulio et al., 1995; Nagai et al., 1999) and their protective role upon exposure to PAH (Lemaire et al., 1996; Jifa et al., 2006) and PCB (Otto and Moon, 1995; Schlezinger and Stegeman, 2001) have been established. Generation of ROS beyond the local removal capacity may cause oxidative damage to DNA (Kasai and Nishimura, 1986; Nishimoto et al., 1991; Breen and Murphy, 1995; Machella et al., 2004).

The present study aims to investigate the long-term interactive effects of the widely found AhR agonists, B(*a*)P and PCB77. In a 2-year continuous experiment, we selectively followed temporally persistent responses using delayed sampling points over serial exposure, and investigated cytochrome P4501A (CYP1A) induction, B(*a*)P activation and excretion, phase II activities, acyl-CoA oxidase activity and antioxidant enzymatic defense of turbot (*Scophthalmus maximus*). The turbot, a bottom dwelling marine flatfish living in close association with potentially contaminant laden sediments, provides a realistic model for studying long-term recurrent exposure scenarios in the marine environment.

## 2. Materials and methods

### 2.1. Exposure experiment

A monomorphic group of hatchery bread juvenile turbot (age, 9 months from hatching) was obtained from Austevoll Aquaculture Research Station, Institute of Marine Research, Norway, in May 1996, and acclimatized for 2 weeks to the prospective experimental conditions (identical fiberglass tanks with 88 random fish each, 1 $m^3$ flow-through sea water, flow rate = 6.3 l/min, 16 °C, salinity = 3.5%; temperature and salinity kept constant throughout the study). In a controlled laboratory experiment, turbot of one tank were exposed to B(*a*)P while those of another were exposed to B(*a*)P with PCB77. The toxicants were dissolved in equal volumes of acetone and added to tank water that was kept static for 18 h before flow was resumed. Fish were exposed on day 1 and on completion of months 3, 6, 9 and 18 where B(*a*)P doses of 0.1, 0.2, 0.1, 0.1 and 0.2 mg/l were used, respectively. This corresponded to biomass dosages of 10 mg B(*a*)P/kg (day 0, months 3 and 6), 5.8 mg/kg (month 9) and 3 mg/kg (month 18). The PCB77 co-exposures were kept at 1% of the concurrent B(*a*)P (w/w). The control was treated only with acetone. As fish grew in size, each group was redistributed equally into two tanks in month 5. Sampling was done on week 3, before month 3, 6 and 9 exposures and on completion of months 12 and 24. The final exposure (month 18) and sampling (month 24) were undertaken for further verification of CYP1A temporal trends. The natural photoperiod at 60°N was simulated throughout the experiment using the computer control system LYSSTYR. Fish were fed daily with pellet food (Ultra Red, Felleskjøpet, Norway), which was suspended on dosing and sampling days. The experiment was approved by the National Animal Research Authority of Norway.

### 2.2. Sampling

Randomly caught fish were measured for length and weight and sacrificed to obtain liver, gill and bile. The tissues were immediately homogenized in ice-cold 0.1 M Na–phosphate buffer (w/4v) containing 0.15 M KCl, 1 mM EDTA, 1 mM DTT, and 10% glycerol (pH 7.4) using a Potter–Elvehjem glass–teflon tissue homogenizer. The homogenate was centrifuged at 12,000 × *g* (20 min, 4 °C) to obtain the post-mitochondrial supernatant (PMS). The liver and gill PMS, a chunk of liver and bile from each fish were stored at −80 °C. For measurements of phase II activities, aliquots of PMS were pooled group-wise and centrifuged at 100,000 × *g* for 60 min at 4 °C to obtain the cytosolic fraction as the supernatant. The pellet, containing the microsomes, was resuspended (initial liver w/v) in 0.1 M Na–phosphate buffer containing 0.15 M KCl, 1 mM EDTA, 1 mM DTT, and 20% glycerol (pH 7.4).

### 2.3. EROD activity and CYP1A protein

CYP1A expression levels were determined as described before (Nilsen et al., 1998) in PMS. The gill and liver EROD activities were determined by cuvette-based fluorescence assay. Liver CYP1A protein level was semi-quantified complementarily by color ELISA and western blotting using C-10-7 monoclonal mouse anti-rainbow trout CYP1A-peptide IgG (Rice et al., 1998; Biosense Laboratories AS, Bergen, Norway). The immunoreactive areas were visualized by enhanced chemiluminescence (ECL™, Amersham International plc, England) in western blotting.

### 2.4. B(a)P-derived bile fluorescence

Bile fluorescence was measured by direct fluorimetry at excitation and emission wavelengths, 379/425 nm specific for B(*a*)P metabolites (Beyer et al., 1996). The whole bile, optimally diluted 1:1600 in 48% ethanol, was assayed.

### 2.5. BPDE–DNA adducts

An ultrasensitive enzyme radioimmunoassay (USERIA) was performed in general agreement with Shamsuddin et al. (1985) to determine BPDE–DNA adduct levels of turbot liver. DNA was extracted using DNAZOL™ Reagent (Life technologies Inc., USA) and quantified by absorbance measurement at 260 and 280 nm before storing at −80 °C until analyses. Highly modified BPDE–DNA (3.0 BPDE adducts/$10^3$ bp) kindly provided by Dr. Steinar Øvrebø was used as standards and in coating while unmodified calf thymus DNA was used as the carrier. The competition mixture was