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# Specificity of the peroxisome proliferation response in mussels exposed to environmental pollutants

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**Abstract**

Peroxisome proliferation has been proposed as novel biomarker of exposure to organic pollutants in aquatic organisms. Peroxisome proliferator compounds comprise a heterogeneous group of substances known for their ability to cause massive proliferation of peroxisomes and liver carcinogenesis in sensitive species such as rodents. Recently, several marine organisms (mussels and fish) have been shown as target species of peroxisome proliferators. In the present work, we aimed to investigate the specificity of the peroxisome proliferation response in mussels. For this purpose, mussels (*Mytilus edulis*) were exposed for three weeks to North Sea crude oil (NSO), a mixture of NSO, alkylphenols and extra PAHs (MIX), diallylphthalate (DAP), bisphenol-A (BPA) and tetrabromodiphenylether (TBDE), or transplanted for three weeks to four stations showing different copper concentrations in a copper mine. Peroxisome proliferation was assessed by measuring the activity of the peroxisomal β-oxidation enzyme acyl-CoA oxidase (AOX) and the volume density occupied by peroxisomes ($V_{VP}$) in the digestive gland. Mussels exposed to NSO and MIX showed significantly increased AOX activities and $V_{VP}$ compared to control animals. Significantly higher $V_{VP}$ was also found in DAP and TBDE exposed mussels. $V_{VP}$ did not vary in mussels transplanted into a copper concentration gradient. Our results confirm the usefulness and specificity of peroxisome proliferation as a suitable biomarker of exposure to organic contaminants such as oil derived hydrocarbons, phthalate plasticizers and polybrominated flame retardants in mussels.
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*Keywords:* Peroxisome proliferation; Mussels; Biomarkers; Aquatic pollution

## 1. Introduction

Peroxisomes acquired relevance with the discovery that fibrates, used as hypolipidemic drugs to lower the lipid levels in serum of patients with hypercholesterolemia, caused a massive proliferation of peroxisomes and liver carcinogenesis in rodents (Hess et al., 1965; Reddy and Lalwani, 1983). Peroxisomes are ubiquitous single membrane-bound cellular organelles. Their size, abundance and enzyme composition vary considerably depending on species and cell type considered (Cancio and Cajaraville, 2000). Peroxisomes are found in all eukaryotic cells ranging from yeast to plants and animals (Singh, 1997; Cancio and Cajaraville, 2000). These cytoplasmic microbodies are characterized by the presence of $H_2O_2$-generating flavin oxidases and $H_2O_2$-scavenging catalase. Enzymes present in mammalian liver peroxisomes are associated with lipid metabolism, namely with β-oxidation of very long-chain fatty acids and biologically important lipid derivatives such as prostaglandins and leukotrienes, and in the synthesis of plasmalogens and cholesterol, the precursor of steroid hormones. Peroxisomes also play an important role in the degradation of steroids (Markus et al., 1995; Keller et al., 2000). Finally, peroxisomes are key organelles in the homeostasis of oxygen free radicals (Singh, 1997; Orbea et al., 2000; Cajaraville et al., 2003b; Schrader and Fahimi, 2004).

Peroxisome proliferation is a pleiotropic cellular response which involves a drastic increase in peroxisomal volume and/or induction of β-oxidation enzymes, caused by several factors including exposure to certain organic environmental pollutants (Cancio and Cajaraville, 2000; Keller et al., 2000; Cajaraville et al., 2003a). Peroxisome proliferation is regulated in vertebrates by peroxisome proliferator-activated receptors (PPARs), which belong to the family of nuclear hormone receptors. After ligand binding, PPARs heterodimerize with another nuclear receptor, the retinoid-X-receptor (RXR) and bind to peroxisome proliferator response elements (PPREs) in the promoter region of target genes (Lemberger et al., 1996; Keller et al., 2000). PPARs have been identified in several fish species (Andersen et al., 2000;

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Ibabe et al., 2002, 2004; Leaver et al., 2005; Raingeard et al., in press) but not yet in invertebrates. The dimerization partner RXR and other nuclear receptors have been recently found in molluscs (Thornton et al., 2003; Nishikawa et al., 2004; Bouton et al., 2005).

The range of chemicals inducing peroxisome proliferation is diverse and no evident structural similarities can be observed among them, with the exception of a common acidic group, generally a carboxyl group (Lake, 1995; Cancio and Cajaraville, 2000). The peroxisome proliferation response varies greatly among different species, being rodents the most sensitive species (Keller et al., 2000). In bivalve molluscs, peroxisome proliferation has been demonstrated in both laboratory and field experiments (Cajaraville et al., 2003a). Mussels show peroxisome proliferation after exposure to organic pollutants such as the water accommodated fraction of crude and lubricant oils (Fahimi and Cajaraville, 1995; Cajaraville et al., 1997), polycyclic aromatic hydrocarbons (PAHs; Krishnakumar et al., 1997; Cancio et al., 1998; Orbea et al., 2002b), polychlorinated biphenyls (PCBs; Krishnakumar et al., 1997) and phthalate plasticizers (Cancio et al., 1998; Orbea et al., 2002a). Field studies have also shown that peroxisome proliferation occurs in mussels inhabiting environments with organic pollution (Krishnakumar et al., 1995; Orbea et al., 1999, 2002b; Porte et al., 2001; Bilbao et al., in press). Mussel response to peroxisome proliferators appears to be reversible, since mussels transplanted from polluted to clean sites show a reduction in peroxisomal volume density and acyl-CoA oxidase (AOX) activity concomitant with the loss of the PAHs and PCBs (Cajaraville et al., 2003a).

The aim of the present work was to investigate the specificity of peroxisome proliferation as biomarker of exposure to organic pollutants in mussels. With this purpose, two laboratory experiments and one field study were carried out. In the first laboratory experiment, mussels were exposed for three weeks to North Sea oil, and to a mixture of North Sea oil, alkylphenols and extra PAHs during early winter. The second laboratory experiment was performed in late winter–early spring and mussels were exposed to bisphenol-A, the phthalate plasticizer diallylphthalate and the flame retardant tetrabromodiphenylether congener-47. In the field study, mussels were transplanted for three weeks into a copper concentration gradient in the area of a copper mine in Visnes (Norway). Peroxisome proliferation was assessed in the digestive gland, which is the key metabolic organ in bivalves (Moore and Allen, 2002; Dimitriadis et al., 2004; Luedeking and Koehler, 2004).

## 2. Materials and methods

### 2.1. Laboratory experiments

Adult blue mussels (*Mytilus edulis*), 5.5–6.5 cm long, from Førlandsfjorden (59°17′N, 5°27′E) in Norway, were exposed in two complementary laboratory experiments to different environmental pollutants. Experiments were carried out in the facility of Akvamiljø a/s (Randaberg, Norway). Exposures were done using a continuous flow-through system with water aeration and mussels were fed every second day with a mix of *Isochrysis* and *Rhodomonas* algae.

The first laboratory experiment (experiment 1) was carried out during November–December 2002. Mussels were exposed during three weeks to 0.5 ppm Statfjord B North Sea oil (NSO) and a mixture of 0.5 ppm NSO + 0.1 ppm alkylphenol mix (mainly $C_1$–$C_5$ phenols) + 0.1 ppm extra PAHs found in produced water (MIX). Selected composition and doses were typical of produced water obtained from off-shore oil extraction platforms (Larsen et al., 2003) and are detailed in Sundt et al. (this issue). Filtered seawater (10–12 °C, 34‰) was used as reference.

The second laboratory experiment (experiment 2) was performed during March–April 2003. Mussels were exposed to three compounds at sub-lethal concentrations: 50 ppb bisphenol-A (BPA, purchased from Merck, Darmstadt, Germany), 50 ppb diallylphthalate (DAP, purchased from Fluka, Buchs, Switzerland) and 5 ppb tetrabromodiphenylether congener-47 (TBDE, obtained from Chiron AS, Emeryville, CA, USA). Exposure concentrations were selected based on previously reported LC50 values that were divided with a factor of 100 (Sundt et al., 2003, this issue) and are close to concentrations described in the environment (Tyler et al., 1998; Jeannot et al., 2002; Vethaak et al., 2002). Acetone (grade > 99.5) was used as carrier for all compounds at concentrations lower than 2 ppb (PNEC 25 ppb for acetone). The experiment lasted three weeks and the conditions were the same described for experiment 1.

At the end of each experiment digestive gland samples from 20 animals were obtained from each experimental group. Digestive glands were dissected out, quickly frozen in liquid nitrogen and stored at −80 °C until subsequent analysis. Studied endpoints included acyl-CoA oxidase activity of digestive gland and volume density of peroxisomes in digestive gland cells.

### 2.2. Field study

Mussels from a farm in Førlandsfjorden were transplanted into a copper concentration gradient in Visnes (59°22′N, 5°13′E), Norway, for a period of three weeks in November 2003. Mussels were located in four stations, where mussels from Station 4 were in the copper mine and the rest of stations were in a decreasing Cu gradient, considering Station 1 as reference site. The copper gradient was confirmed by chemical analysis performed in the whole soft tissue of mussels (Andersen et al., 2003) and in the digestive gland of mussels (Zorita et al., this issue). Ten mussels were collected per station and digestive glands were excised, frozen in liquid nitrogen and stored at −80 °C until subsequent analysis.

### 2.3. Acyl-CoA oxidase (AOX) activity measurements

Samples of digestive gland were processed for spectrophotometrical measurement of the activity of the peroxisomal enzyme acyl-CoA oxidase (AOX). Five digestive gland pools (each composed of two digestive glands) per each exposure

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