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Toxicity and Elevation of 7-Ethoxyresorufin *O*-Deethylase Activity in Turbot (*Scophthalmus maximus* L.) Larvae Exposed to Contaminated Sea Surface Microlayer

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At the air-sea interface, a number of complex physical and chemical processes can cause the formation of microfilms. These microfilms contain a mixture of marine and/or terrestrial particulate and dissolved organic and inorganic matter of both natural and anthropogenic origin (Larsson *et al.*, 1974; Liss et al, 1997). The thickness and exact composition of this film is variable, however conceptual models propose that there is a lipid and fatty acid film supported by a polysaccharide-protein complex up to 0.1μm thick at the air-sea interface (Hardy 1997). Below this exists a diverse and abundant number of species, including some permanent inhabitants of the microlayer consisting of bacterioneuston, phytoneuston, zooneuston, and ichthyoneuston (Zaitsev 1997). The chemical identification and composition of the surface microlayer (SMIC) can be influenced by the method of sampling however high concentrations of contaminants e.g. aliphatic and polycyclic aromatic hydrocarbons (PAHs), polychlorobiphenyls (PCBs), organochlorine pesticides, synthetic surfactants, organotin antifouling agents, metals and radionuclides have been determined in SMIC from contaminated sites (Cross et al., 1987; Liss and Duce 1997; Word et al., 1987).

Recent studies demonstrate that the concentrations of contaminants in the SMIC can exceed water quality standards (Hardy and Cleary 1992) and exert adverse biological effects on aquatic species (Cleary et al., 1993; Hardy 1997). The early life stages of organisms are very sensitive to contaminant exposure (Peters et al., 1996; Rumbold and Snedaker 1997) and both mortality and abnormal development have been reported in vertebrate and invertebrate species (Cleary et al., 1993; Kocan et al, 1987; Rumbold and Snedaker 1997; Westernhagen et al., 1987) although little or nothing is understood concerning the mechanism of SMIC toxicity.

Cytochrome P4501A (CYP1A) is a component of the hepatic mixed function oxidase system that may be induced in fish following exposure to PAHs and other organic xenobiotics (Buhler and Wang-Buhler 1998). This induction may be measured by an activity associated with CYP1A, termed 7-ethoxyresorufin *O*-deethylase (EROD) activity. Induction results in an increased capacity to metabolise contaminants but can also result in the formation of highly reactive metabolites e.g. the bioactivation of procarcinogens into carcinogens (Buhler and

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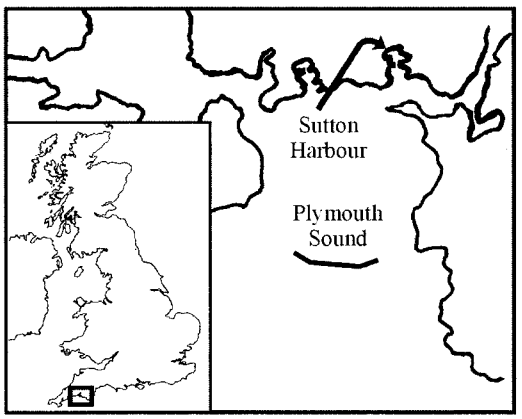

Figure 1. Location of sea surface microlayer sampling sites

Wang-Buhler 1998). Following exposure to PAHs, turbot *Scophthalmus maximus* larvae induce EROD activity (Peters and Livingstone 1995; Peters et al., 1996) and produce potentialh carcinogenic PAH metabolites (Peters et al., 1996). Thus, elevation of *S. maximus* larval EROD activity has the potential to indicate exposure to PAHs in SMIC and provide possible mechanisms of SMIC toxicity.

In this study, total PAHs were determined in SMICs collected from a contaminated site (Sutton Harbour) and a relatively clean site (Plymouth Sound) in the UK. To assess SMIC mortality and sub-lethal effects of exposure (indicated by levels of larval EROD activity), *S. maximus* larvae were exposed to; clean and contaminated SMICs, dichloromethane (DCM) extracts from the SMICs – containing the lipid/lipid soluble contaminants and artificial SMICs comprising of benzo[a]pyrene (BaP) and oleic acid.

MATERIALS AND METHODS

SMIC samples were collected from Plymouth Sound and Sutton Harbour (Figure 1) using a floating, rotating glass-drum "skimmer" as described in Cleary et al. (1993). The "skimmer" was deployed from the lowered bow ramp of the Plymouth Marine Laboratory Research Vessel *Tamaris* and allowed to drift freely to a radius of 100 m from the vessel, for the sample site in Plymouth Sound. Samples were collected on two separate trips; the wind-speed during collecting trips was in the range 0 - 20 knots and the sea state ranged from calm to moderate. For the Sutton Pool marina sample, the "skimmer" was launched from the marina and allowed to drift, tethered to the pontoons with a 20 m line; the water surface was calm within the confines of the marina. Aliquots of the SMIC from each site was stored at -20°C. Further aliquots were acidified with concentrated hydrochloric acid, and

lipids/organic pollutants extracted with DCM. Total PAHs were estimated by the method of Law et al. (1988) and expressed as the sum of the PAHs estimated against chrysene and naphthalene standards. Artificial polluted SMIC samples were prepared using microlayers of 20 ppm oleic acid and 1 ppb or 1 ppm BaP which ranged the reported concentrations of SMIC BaP (Cross *et al.*, 1987).

S. maximus larvae (3 days post-hatch) were maintained at 15°C under a 12 hr light/12 hr dark cycle in either 250 mL glass beakers containing 200 mL water or SMIC for the mortality studies, or 500 mL glass beakers containing 400 mL water or SMIC for the enzyme determinations. The water volume to larvae density was set at approximately 1 mL per individual and at least 2 beakers per treatment were used. The larvae were exposed to: i) SMIC from a polluted site (Sutton Pool, Plymouth - total PAHs 10 mg/litre), ii) contaminant (DCM) extracts of polluted site SMIC (organic pollutants), iii) SMIC from a clean site (Plymouth Sound - total PAHs 10 μg/litre), iv) contaminant (DCM) extracts of clean site SMIC and v) artificial contaminated SMIC samples containing either 1 ppb or 1 ppm BaP in 20 ppb oleic acid. *S. maximus* larvae of this developmental stage have yolk-sacs and thus were not fed during the exposure period. After distribution of the larvae, the exposure time started with the addition of the toxicants to the appropriate beakers. Larvae were examined at 8 hr intervals and the mortalities (confirmed by absence of heart beat) counted and removed.

Larvae for enzyme analysis were filtered from the treatment sea-water, rinsed with clean artificial sea-water and excess water removed. They were then plunged into liquid nitrogen and stored up to 6 weeks at -70°C prior to biochemical analysis. EROD activity was measured fluorometrically as described in Peters and Livingstone (1995). Briefly, aliquots of 8 to 56 mg of larvae were weighed, sonicated in 1 mL of 0.15*M* KCl (pH adjusted to 7.5 by KOH) containing 1m*M* EDTA at 4°C and then centrifuged at 11,600g for 3 minutes at 4°C in a microcentrifuge. The resulting supernatants were used for EROD activity determinations. Incubations were made in duplicate and the reaction volume of 150 μL, contained 59 m*M* K_2HPO_4/KH_2PO_4 pH 7.6, 0.23 m*M* NADPH, 3.74 μ*M* 7-ethoxyresorufin and 90μL larval supernatant. Resorufin fluorescence was measured against an 85 n*M* resorufin standard and the EROD activity was expressed in pmoles resorufin produced per minute per gram wet weight of larval tissue. Statistical analysis was performed by Analysis of Variance ($P < 0.05$).

RESULTS AND DISCUSSION

Mortalities were observed in all treatments between 8 and 96 hours (Figure 2), with the Plymouth Sound SMIC, the lipid (DCM) extract from Plymouth Sound, and the artificial SMIC models reporting low levels of mortalities, similar to those

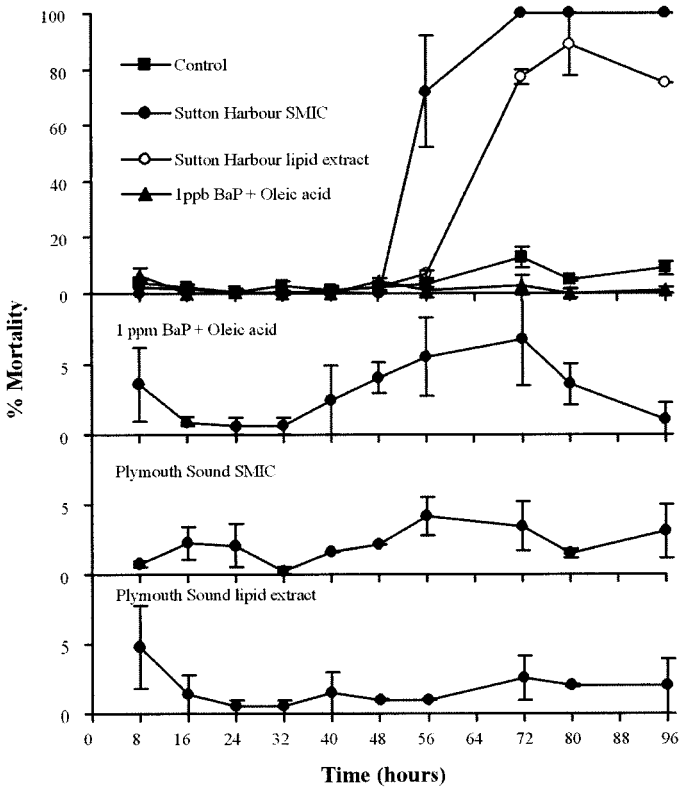

Figure 2. Time-dependent percentage mortality of turbot (*Scophthalmus maximus* L.) larvae exposed to Sutton Harbour sea surface microlayer (SMIC) (10 mg/L total polycyclic aromatic hydrocarbons (PAHs), Plymouth Sound SMIC (10 μg/L total PAHs), dichloromethane extracts containing lipids and lipid-soluble contaminants and artifical SMICs containing of 1 ppb or 1 ppm benzo[a]pyrene (BaP) in 20 ppb oleic acid (mean ± range; n = 2).

observed in the control treatment. In contrast, between 48 and 56 hours exposure to Sutton Harbour SMIC, high levels of mortality occurred where 72% of the larvae died compared with c.5% mortalities in control treatments. The mortalities continued during the subsequent 8 hours resulting in 100% mortality in the exposure Sutton Harbour SMIC condition. Between 56 and 72 hours, a similar but delayed-onset increase in mortality was observed in treatments containing Sutton Harbour DCM extracts. In contrast to the total SMIC sampled from Sutton harbour which contained both organic and inorganic components, the levels of mortalities did not exceed 88% for the duration of the experiment when the larvae were exposed to only the DCM soluble components. Since both the onset of mortality was slower and the total observed mortality was lower, it may be concluded that although Sutton Harbour SMIC was toxic to *S. maximus* larvae, the

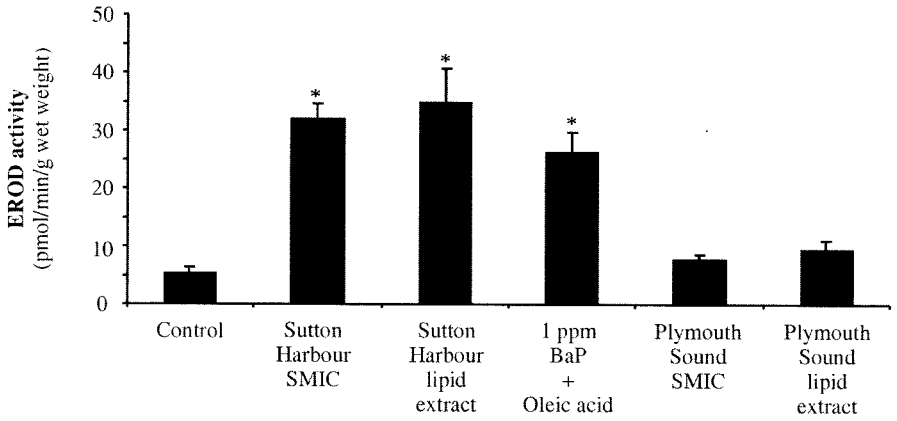

Figure 3. Elevation of turbot (*Scophthalmus maximus* L.) larval 7-ethoxyresorufin *O*-deethylase activity following 48 hour exposure to Sutton Harbour sea surface microlayer (SMIC) (10 mg/L total polycyclic aromatic hydrocarbons (PAHs), Plymouth Sound SMIC (10 μg/L total PAHs), dichloromethane extracts containing lipids and lipid-soluble contaminants and artifical SMIC containing 1 ppm benzo[a]pyrene (BaP) in 20 ppb oleic acid.* = $P < 0.05$ vs control by analysis of variance (mean ± se; n = 3)

lipid extract from the SMIC demonstrated an overall lower level of toxicity. Both exposure conditions were clearly more toxic than the artificial SMIC, Plymouth Sound SMIC and the artificial sea-water control.

All treatments reported low levels of mortality between 8 and 48 hours exposure (Figure 2) and indicated that 48 hours was an appropriate duration for monitoring the sub-lethal effects of the pollutants. Larval EROD activity was determined in all samples assayed and the results plotted in Figure 3. Larvae exposed to Sutton Harbour SMIC and Sutton Harbour DCM extract reported respectively, a 6-fold and 6.5-fold elevation of EROD activity compared to control conditions ($P<0.05$). A 4.9-fold elevation in activity ($P<0.05$) was also detected in samples from larvae exposed to 1 ppm BaP dissolved in 20 ppb oleic acid. This contrasted the toxicity data where this treatment reported no significant difference in toxicity compared to control conditions. No significant differences were observed between control and Plymouth Sound samples.

In this study, the circa 5- to 6-fold elevation in EROD activity following 48 hour exposure to SMIC were comparable to elevations of *S. maximus* larval EROD activity following exposure to 5 ppb BaP for 24 hours or 1 ppb γ-hexachlorocyclohexane for 48 hours, which resulted in a respective 3-fold and 6-fold elevation in EROD activity (Peters and Livingstone 1995). *S. maximus* larvae have the potential to bioactivate PAHs to carcinogens (Peters et al., 1996) which

in juveniles can form deleterious hydrophobic adducts with liver DNA (Peters et al., 1997). Although little is known of the fate of these adducts in larvae, it is noteworthy that numerous aberrations in fish embryo and larval development have been reported following embryo and larval exposure to SMIC (Hardy et al., 1987; Kocan et al., 1987; Westernhagen et al., 1987).

In this study, contaminated SMIC, collected from the polluted environment of a marina was highly toxic to *S. maximus* larvae. The hydrophobic component extracted from the contaminated SMIC, containing both lipids and lipophilic contaminants, demonstrated an apparent lower toxicity, where both the onset and potency of mortality were respectively slower and lower than the SMIC containing lipophilic, hydrophilic and metal contaminants. Contaminated and model SMICs elevated *S. maximus* larval EROD activity, indicating the presence of PAHs, planar PCBs or dioxin-like molecules. Since the elevation in larval EROD activity was prior to larval mortalities, the enzyme activity may be a useful sensitive, sub-lethal biomarker of exposure, however elevation of EROD activity was not a pre-disposition to mortality.

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