THE INTEGRATED USE OF BIOMARKERS TO ASSESS PAH EXPOSURE

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Introduction

The polycyclic aromatic hydrocarbons (PAH) are a major group of contaminants present in the estuarine and coastal environment. The current study was initiated to provide an integrated suite of biomarkers as predictive tools for PAH exposure. The studies described are part of a larger programme of work investigating causative links between chronic PAH exposure, biomarker responses and histopathological endpoints.

Materials and Methods

Fish were exposed to PAHs for 4 months via their food. Natural uptake of contaminants in the food is a pathway for the entry of contaminants into an organism that may have an associated toxicological risk (McElroy and Sissons, 1989). A mix of four PAHs were selected for use in this study based on their common occurrence in the estuarine environment (Woodhead, *et al*., 1999) and on the basis of their toxicity (phenanthrene and pyrene) and carcinogenicity (benzo[a]pyrene (BaP), benzanthracene (BaA)), to aquatic organisms. The nominal dosing ranges of PAH were 100 and 500mg kg^{-1} (food weight) and a control group receiving the feed spiked with the solvent carrier (hexane) only. In this study selected biomarkers were applied to flounder (*Platichthys flesus*) after chronic exposure to PAH.

Flounder was chosen as a suitable experimental species as it is widely distributed in European coastal and estuarine waters and can be found in both contaminated and unpolluted environments. Furthermore, flounder have recently been identified as a monitoring species under the UK National Marine Monitoring Programme (NMMP).

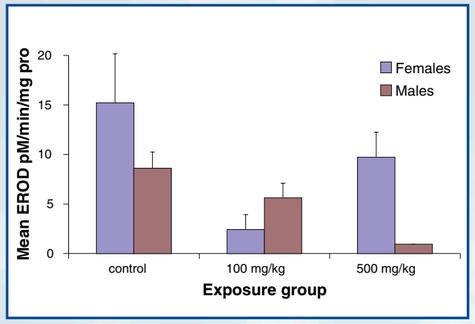
*Flounder (*Platichthys flesus*).*

Biomarkers

The suite of biomarkers included hepatic EROD activity, bile metabolites and hepatic DNA adducts, which are recommended biomarkers for PAH exposure under the Joint Assessment Monitoring Plan (JAMP) guidelines (Stagg, 1998). Also included for evaluation were two non-destructive biomarkers for genotoxic exposure in peripheral erythrocytes, the comet assay and the induction of micronuclei. Sampling was carried out when spawning influences were at a minimum, so as not to affect the assay result.

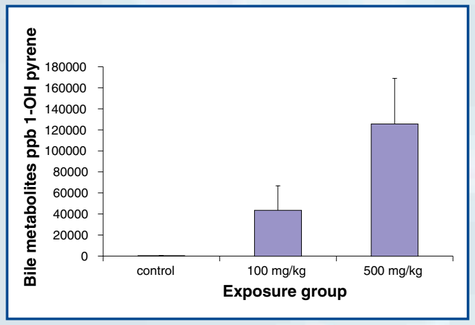
EROD

EROD analysis was performed using a modification of the method described in (Stagg *et al*., 1995).



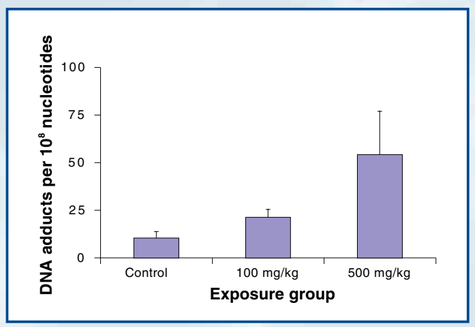
Bile Metabolites

Bile metabolites were analysed using synchronous fluorescence spectrometry (SFS) to detect conjugated 1-hydroxy pyrene metabolites (Ariese *et al*., 1993).



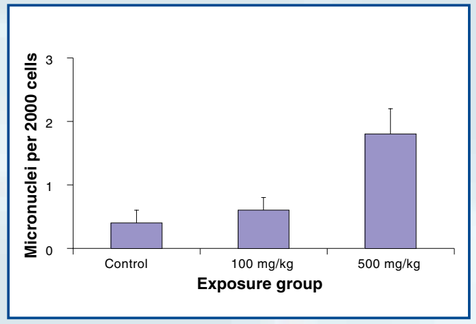
DNA adducts

Hepatic DNA adducts were analysed by the ^{32}P-postlabelling assay using nuclease-P1 enrichment and TLC resolution (Jones *et al*., 1991).



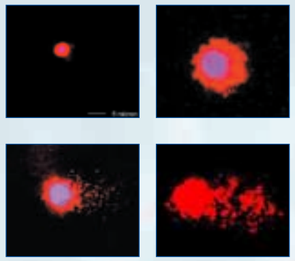
Micronucleus assay

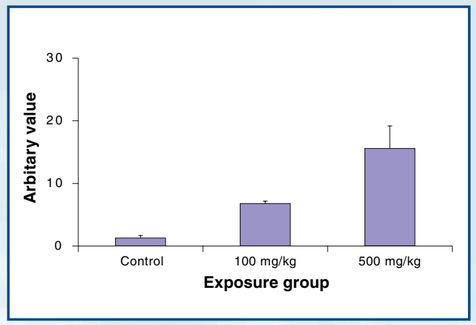
Peripheral erythrocytes were stained with giemsa and scored for micronuclei according to the criteria of (Al-Sabti and Metcalfe, 1995).



Comet Assay

The alkaline comet assay was used to detect strand breaks, open repair sites, cross-links, and alkali labile sites. The method employed was essentially that as described by (Singh *et al*., 1988).





Conclusions and future work

- All biomarkers, with the exception of EROD activity, demonstrated a clear dose response relationship with PAH exposure.
- An integrated suite of biomarkers as employed in this study provides environmental managers with greater power to determine toxicant exposure and effect
- Histological examination of target tissues is currently being carried out to assess toxicant related effects.
- Further data analysis and additional sampling dates from this study are being carried out to assess the impact of confounding factors.

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