the aquatic ecosystem, biological effect monitoring has to be applied, in addition to chemical monitoring. Earlier research on fish biomarkers clearly demonstrated an added value to the risk assessment compared to traditional classification methods. Fish biomarkers, however, also have certain limitations, such as the site-specific impact of confounding factors. Current research is focussed on the development of more robust alternative methods to reliably assess potential environmental risks without sacrificing living animals. In the present study, an overview will be presented of promising combinations of passive sampling with laboratory-based effect monitoring techniques that will provide time-integrated information on both the bioavailability and the effects of the wide range of toxic substances present in aquatic ecosystems. Semi-permeable membrane devices (SPMDs) were used as passive samplers that accumulated hydrophobic organic compounds from the water phase during a four week exposure period. Extracts were tested chemically for polycyclic aromatic hydrocarbons (PAHs) levels as well as biologically for various toxic effects, e.g. acute toxicity (Microtox), dioxin-like toxicity (DR-Calux), endocrine disruption and genotoxicity (umu-C). Preliminary results demonstrate that combinations of passive sampling and bioassays can become valuable tools for environmental risk assessment.

Comparison of gene expression profile of atrazine, flusilazole, and tamoxifen in bedding yeast *Saacharomyces cerevisiae*

M. Minuzzo [b], M. Scandroglio [c], E. Bizzotto [c], T. Lettieri [a]
[a] *Laboratory of Molecular Ecotoxicology, Institute for Environment and Sustainability DG-European Commission Joint Research Centre, TP 300, I-21020 Ispra (Va), Italy*
[b] *Biotechnology Science, University "Degli Studi di Milano- Bicocca", Milano, Italy*
[c] *Environmental Science, University "Degli Studi di Milano- Bicocca", Milano, Italy*
E-mail address: teresa.lettieri@jrc.it

Abstract

The measurements of global gene expression levels upon exposure to a chemical can be used to provide information about both the mechanism of action of toxicants and to form a sort of "genetic signature" for the identification of specific genetic markers of exposure and response. We have used DNA microarray analysis to measure gene expression changes after exposure to three compounds, the herbicide atrazine, the fungicide flusilazole, and to the pharmaceutical compound tamoxifen in the budding yeast *Saacharomyces cerevisiae*. For every chemical we have tested three different concentrations, starting from very low and environmentally-relevant concentrations. Some of the most interesting changes in gene regulation have then been confirmed by Real Time PCR. Gene expression profiling upon exposure to flusilazole showed regulation in several genes involved in sterol and ergosterol metabolism, and lipid and fatty acid biosynthesis. A clear pattern of gene expression changes is apparent also at the lowest concentration tested of 10^{-2} nM (2ng/L). Atrazine exposure gives rise to a quite different gene expression pattern with only a limited number of expression changes in genes involved in DNA maintenance integrity and repair. These results suggest that it should be possible to use yeast DNA microarray to discriminate between these two classes of environmental pollutants using gene expression profiling. Preliminary data on tamoxifen will also be presented.

5-Aminolevulinate synthase (ALAS) gene expression in oyster *Crassostrea gigas*: A potential biomarker of domestic sewage exposure

Thiago Bruce Rodrigues, Igor Dias Medeiros, Afonso Celso Dias Bainy, Maria Risoleta Marques
Laboratório de Biomarcadores de Contaminação Aquática e Imunoquímica, Departamento de Bioquímica, CCB, Universidade Federal de Santa Catarina, Florianópolis, SC, Brasil
E-mail address: mrfmarques@hotmail.com

Abstract

All living organisms require tetrapyroles, primarily in the form of heme and/or chlorophyll. Heme serves as the prosthetic group in a number of important proteins, including hemoglobin, myoglobin, and cytochromes, whereas chlorophyll is used in photosynthesis. Formation of tetrapyroles involves the synthesis of 5-aminolevulinic acid (ALA). The enzyme 5-aminolevulinate synthase (*ALAS*), EC 2.3.1.37, catalyzes the condensation of succinyl-CoA and glycine to yield ALA in a mitochondrial step. In the present study, *Crassostrea gigas* oysters were exposed to crude domestic sewage (33% dilution) for 48 h in laboratory. RNA extract was obtained from gills and digestive gland samples and submitted to semi-quantitative RT-PCR experiments using specific primers. Although a significant increase in *ALAS* gene expression was not seen in the digestive gland of the exposed group, a 4-fold increase was observed in gills in the same group when compared to the control animals. These finding suggest that *ALAS* gene may be a potential candidate as a molecular biomarker of domestic sewage exposure. *Supported by* CNPq-Universal to ACDB.

A kinetic approach to the PAH detoxification system in a marine invertebrate species, the crab *Carcinus maenas*

Carmen Morales-Caselles [a,c], M. Laura Martín-Díaz [a,c], Inmaculada Riba [a,c], Carmen Sarasquete [a], T. Ángel DelValls [b,c]
[a] *Instituto de Ciencias Marinas de Andalucía (ICMAN-CSIC), Avda. República Saharaui s/n, Puerto Real 11510 Cádiz, Spain*
[b] *UNESCO UNITWIN/UNICOP, Facultad de Ciencias del Mar y Ambientales, Universidad de Cádiz, Polígono Río San Pedro s/n, Puerto Real 11510 Cádiz, Spain*
[c] *Unidad Asociada de Calidad Ambiental y Patología (CSIC & UCA), Avda. República Saharaui s/n, Puerto Real 11510 Cádiz, Spain*
E-mail address: carmen.morales@uca.es

Abstract

To test the hypothesis that invertebrates possess a significant PAH-detoxification system, the induction of ethoxyresorufin O-deethylase (EROD) activity and gluthatione-*S*-transferase (GST) was studied in the crab *Carcinus maenas*. A bioassay was performed by exposing the organisms to bulk sediment contaminated by PAHs under laboratory and field conditions. Sediments were collected and transferred to the laboratory where they were subsampled for chemical analysis and toxicity tests. Crabs were kept during 28 days in tanks with the collected sediment samples and caged crabs were placed in the selected field study sites during the same period. Sampling was performed weekly. Hepatopancreas samples were homogenized and centrifuged to obtain

fractions for the biomarker determination. Results obtained show a relationship between the change in levels of the biomarkers measured in the crabs and the chemical characteristic of the sediment. Assuming that EROD in crab represents a PAH-oxidizing capacity, the results suggest a the capability for the detoxification of PAH in *C. maenas*.

Analysis of the effects of algal PSP toxins in the SAF-1 cell line from sea bream (*Sparus aurata*) using a cDNA microarray and DIGE

Amer Diab [a], Catherine Mackenzie [a], Lesley Stobo [b], Ian Davies [b], Moshe Tom [c], Stephen George [a]
[a] *Institute of Aquaculture, University of Stirling, Stirling, FK9 4LA, UK*
[b] *FRS Marine laboratory, Aberdeen, UK*
[c] *IOLR, Haifa, Israel*
E-mail address: sgg1@stir.ac.uk

Abstract

The primary mode of toxicity of heterocyclic guanidine toxins (saxatoxins, STXs) produced by marine gonyaulacoid and gymnodinioid dinoflagellate species is through binding to site 1 of the voltage dependent sodium channel in nerve cell membranes thus causing blockage of conduction and resulting in fatal paralysis. Little is known of other interactions or metabolism of these compounds and in this study we have used 'omic techniques to investigate other potential effects of STX's on non-neuronal cells. SAF-1 cells established from sea bream were exposed to a mixture of STX's (10 nM STX, NeoSTX, GTX's and others) for 48 h and then harvested for extraction of proteins and RNA. Protein expression profiling was performed using 2-D DIGE using a pH 4–7 gradient and 10% SDS PAGE. Analysis was performed with DeCyder (GE healthcare) software. Of 1300 matched protein spots, expression of 11 spots were decreased >2-fold with significance at $p < 0.1$ (by ANOVA) and only 3 were significant $p < 0.05$. None were elevated. Exposure to 25 nM STX alone resulted in down regulation of three spots of the less significant set and overexpression of 2 proteins ($p < 0.1$). Microarray analysis using the GENIPOL striped sea bream liver microarray showed upregulation of immune response genes and glutathione *S*-transferases by STX and the mixture. (*Supported by* EU GENIPOL Grant ENV-2001-0057 and NERC Grant NE/C507688/1).

Application of a biomarker protocol in the sea bass *Dicentrarchus labrax* to assess biological effects of diethylene glycol (DEG) and produced waters of Adriatic offshore platforms

Stefania Gorbi [a], Claudia Virno Lamberti [b], Fulvio Onorati [b], Giacomo Martuccio [a], Ginevra Moltedo [b], Adriana Canapa [a], Marco Barucca [a], Rossella Stefani [a], Francesco Regoli [a]
[a] *Istituto di Biologia e Genetica, Università Politecnica delle Marche, Ancona, Italy*
[b] *ICRAM, Istituto Centrale per la Ricerca Scientifica e Tecnologica Applicata al Mare (Roma)*
E-mail address: f.regoli@univpm.it

Abstract

Diethylene glycol (DEG) is used during oil and gas exploitation on offshore platforms, and a maximum concentration of 3500 ppm is allowed in produced waters by the Italian of Ministry of Environment. The aim of this work was to investigate whether environmental levels of DEG induce molecular or cellular responses in marine organisms, or synergistically modulate the effects of produced waters. Juveniles sea bass (*Dicentrarchus labrax*) were exposed to DEG alone (concentration range 50–5000 ppm) or to various mixtures of DEG and produced waters from three Adriatic platforms. Levels of cytochrome P450, bile metabolites and acetylcolinesterase activity were measured as exposure biomarkers. Oxidative stress measures included the main antioxidant defences (catalase, glutathione *S*-transferases, glutathione reductase, glutathione peroxidases, levels of total glutathione and accumulation of malondialdehyde), integrated with the measurement of total oxyradical scavenging capacity (TOSC) toward peroxyl and hydroxyl radicals. The loss of DNA integrity (single strand breaks and frequency of micronuclei) was analyzed as an index of cellular damage, and vitellogenin gene expression was selected as a marker of estrogenic effects. Results did not reveal marked effects in organisms exposed to DEG alone. On the other hand, significant differences were observed between the produced waters from the three platforms in modulating the biotransformation system, the oxidative stress indices and the onset of DNA damage. Co-exposure experiments revealed synergistic effects of DEG only during some experimental conditions, further confirming the chemical complexity and the different biological reactivity of produced waters from various off-shore platforms.

Assessing PAH detoxification in the clam *Ruditapes philippinarum*

Carmen Morales-Caselles [a,c], M. Laura Martín-Díaz [a,c], Rocío Antón-Martín [b,c], Inmaculada Riba [a,c], Carmen Sarasquete [a], T. Ángel DelValls [b,c]
[a] *Instituto de Ciencias Marinas de Andalucía (ICMAN-CSIC), Avda. República Saharaui s/n, Puerto Real 11510 Cádiz, Spain*
[b] *UNESCO UNITWIN/UNICOP, Facultad de Ciencias del Mar y Ambientales, Universidad de Cádiz, Polígono Río San Pedro s/n, Puerto Real 11510 Cádiz, Spain*
[c] Unidad Asociada de Calidad Ambiental y Patología (CSIC & UCA), Avda. República Saharaui s/n, Puerto Real 11510 Cádiz, Spain
E-mail address: carmen.morales@uca.es

Abstract

The PAH detoxification system in the clam *Ruditapes philippinarum* was studied by analyzing the kinetics of phase I and phase II detoxification enzymes in digestive gland. Levels of phase I cytochrome P450A (CYP1A)-like enzymes were measured as ethoxyresorufin O-deethylase (EROD) activity and phase II by gluthatione-*S*-transferase. Analyses were performed on clams exposed to PAH-contaminated sediments after 7, 14, 21 and 28 days of exposure. Contaminated sediments were collected from two areas of the Spanish coast affected acutely (Galician Coast) and chronically (Bay of Algeciras) by oil spills; bioassays were performed under both laboratory and field conditions. Sediments from the selected sites were chemically characterized and the data obtained were compared with the biomarker results. A kinetic approach to the induction of these biomarkers potentially related to PAH detoxification in clam was performed.