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II. Species sensitivity distributions based on biomarkers and whole organism responses for integrated impact and risk assessment criteria

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ABSTRACT

The aim of this paper is to bridge gaps between biomarker and whole organism responses related to oil based offshore discharges. These biomarker bridges will facilitate acceptance criteria for biomarker data linked to environmental risk assessment and translate biomarker results to higher order effects.

Biomarker based species sensitivity distributions ($SSD_{biomarkers}$) have been constructed for relevant groups of biomarkers based on laboratory data from oil exposures. SSD curves express the fraction of species responding to different types of biomarkers. They have been connected to SSDs for whole organism responses (WORs) constructed in order to relate the $SSD_{biomarkers}$ to animal fitness parameters that are commonly used in environmental risk assessment.

The resulting SSD curves show that biomarkers and WORs can be linked through their potentially affected fraction of species (PAF) distributions, enhancing the capability to monitor field parameters with better correlation to impact and risk assessment criteria and providing improved chemical/biological integration.

1. Introduction

Biomarkers of pollution are defined as detectable biological changes in organisms after exposure to pollutants, and can be measured at various levels of biological organisation (from molecules to the whole organism) (Depledge, 1994). Despite their increased application in effect and monitoring studies, the efficacy of biomarkers in assessment of ecosystem health has been debated (Forbes et al., 2006). Due to the lack of mechanistic understanding of some links in reaction chains from biomarker signals to whole organsim responses (WORs), the natural variations and the transient nature of responses, single biomarker measurements are not easily translated to a generic health indicator for the ecosystem. However, improved statistical relationships of biomarker responses providing links to higher order effects and other endpoints at different levels of biological organization will improve the basis for applying biomarkers in monitoring activity related to environmental risk assessment (ERA). This may also strengthen weight of evidence based approaches in the application of suites of biomarkers.

Species sensitivity distributions (SSDs) are models of the variation in sensitivity of species to a particular stressor (Posthuma et al., 2002; Del Signore et al., 2016). The biomarker bridge concept, that represents links between SSDs based on WOR endpoints applied in ERA and biomarker response endpoints applied in corresponding environmental monitoring, was published by Smit et al. (2009). For marine species exposed to dispersed oil, two SSDs based on biomarker response levels (i.e. DNA damage and oxidative stress) were compared to a SSD based on chronic no observed effect concentrations (NOECs) for whole organisms.

Herein, the biomarker bridge concept builds further on the application of suites of biomarkers. These suites, responding at sub-organism levels in several species with different habitats and feeding strategies, should reduce the uncertainty in the interpretation of biomarker responses (Depledge and Galloway, 2005; Hagger et al., 2006). The objective of this study is, with newly emerged data, to develop the biomarker bridges from a conceptual stage to an applied one, integrating assessments of prognostic environmental risk with biomarker based diagnostic field monitoring. Linking threshold levels defined by environmental risk

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procedures based on WORs to responses of suites of biomarkers can facilitate the definition of acceptable biomarker responses. Biomarker bridges can help to determine which response levels may result in environmental impacts, and when follow up actions are required. This is particularly enhanced through the linked whole organism and biomarker SSDs, since the biomarker signal levels can then be related to the acceptance limits provided in ERA. Another aim of the present work is to enhance the early warning capabilities for the assessment methods established to safe-guard marine populations and communities, in particular in relation to oil based offshore discharges (i.e. produced water and acute releases of oil from exploration and production activities). Heavier oils, e.g. bunker oil released from ship accidents, are not represented with the present data.

2. Materials and methods

2.1. SSD concept and method

SSDs are statistical distributions representing the cumulative fraction of species affected (Potentially Affected Fraction of species; PAF) as a function of the exposure to a stressor (Aldenberg et al., 2002). The SSD concept was originally developed with the mortality of each species as the parameter expressing sensitivity (van Straalen and Denneman, 1989), and the predominant use of SSDs in present ERA procedures is based on this (European Commission, 2003). In our study, we have introduced different endpoints or groups of endpoints (effects/toxicities) for the SSDs, and denoted them accordingly by $SSD_{effect/toxicity}$. All SSD curves are based on the mean (*Xm*) and standard deviation (*Sm*) values, and can be reconstructed according to Aldenberg et al. (2002). $SSD_{effect/toxicity}$ curves show the increasing fraction (or percentage) of species that are affected in their specific effect/toxicities on the y-axis as a function of increasing stressor concentration on the x-axis. The y-axis parameter is commonly called the PAF.

2.2. Data (WOR and biomarkers)

In order to develop a biomarker bridge concept for oil based exposures, SSDs were derived using concentrations for WORs (i.e. survival, reproduction or growth) and biomarker responses (i.e. DNA damage and oxidative stress; Smit et al., 2009). In the present study, these data have been augmented with new results on various vertebrate and invertebrate species, with more endpoints ranging from molecular responses to WORs. WOR data were divided into adult mortality and larval effects with twelve and thirteen species respectively. WOR SSDs were constructed for each category and also joined into a single combined SSD.

The available published data used to establish the different biomarker based SSDs are from fourteen different species, where eight overlap with the twenty-five species included in the WOR SSDs. The total of thirty-one species includes amphipods, copepods, shrimps, crabs, sea urchins, mussels, scallops, snails and fish (both pelagic and demersal species). The suite of biomarkers is composed of methods known to respond to oil based exposure, and some of them are specific to hydrocarbons. For the biomarker SSDs, they are divided into groups according to response/toxicity type (Table 1).

2.3. Analysis

Genotoxic effects are known to be exerted by chemical constituents of oil, and herein they are represented by four different biomarkers: DNA adducts (Gupta et al., 1982; Aas et al., 2002), comet assay (Collins, 2004; Akcha et al., 2003), alkaline unwinding assay (Birnboim and Jevcak, 1981; Bechmann et al., 2010), and erythrocytic nuclear abnormalities (Heddle et al., 1983; Baršienė et al., 2013). Possible enzymatic precursors of genotoxicity are biomarkers related to bio-transformed chemical constituents (i.e. PAH metabolites; Collier and Varanasi, 1991; Beyer et al., 2010) and detoxification system I (Goksøyr and Förlin, 1992; Aas et al., 2002). The latter two groups are biomarkers of exposure that are widely used in investigations of oil exposures and their mode of action is relatively well known. Biliary PAH metabolites are biomarkers of exposure with high sensitivity to oil based discharges (Aas et al., 2000; Beyer et al., 2010). A simple method based on fluorescence at three different wavelength pairs (fixed wavelength fluorescence; FF) is generally considered a screening method, but in oil based exposures good dose:response results are usually obtained (Skadsheim et al., 2009; Sanni et al., 2016a) Therefore, it is of interest to include data from this method together with data obtained by the gas chromatography/mass spectrometry (GC/MS) method used for quantitative measurements of petrogenic PAH metabolites (Jonsson et al., 2003). Petrogenic PAHs are usually dominated by small PAHs, including their alkylated forms.

Table 1
Overview of biomarker groups, methods and organisms included in the establishment of biomarker based species sensitivity distributions. PAH = polycyclic aromatic hydrocarbons, GC = gas chromatography, MS = mass spectrometry, FF = fixed wavelength fluorescence, LMS = lysosomal membrane stability.

Biomarker group	Method	Organisms	Abbreviation
PAH metabolites	GC/MS-Total PAH metabolites	Fish	TPAH met
	FF-Naphthalene type metabolites	Fish	FF-Nph
	FF-Pyrene type metabolites	Fish	FF-Pyr
	FF-benzo[a]pyrene type metabolites	Fish	FF-BaP
General stress	LMS - histochemical method	Fish	LMS-HC
	Neutral red retention time - microscopy method	Shrimp, sea urchin, scallop, fish	NRRT
Oxidative stress	Glutathione-S-transferase	Mussel, fish	GST
	Catalase	Sea urchin, scallop	Cat
	Total Oxyradical Scavenging Capacity	Shrimp	TOSC
Detoxification system I	Cytochrome P4501A ELISA assay	Fish	Cyp1A
	Cytochrome P4501A gene expression	Fish	qPCR *cyp1a*
	Ethoxyresorufin-O-deethylase activity	Fish	EROD
Genotoxicity	DNA adducts	Fish	DNA add
	Comet assay	Sea urchin, mussel, scallop, fish	CA
	Alkaline unwinding assay	Shrimp	AU
Immunotoxicity	Differential white blood cell counts	Fish	DWBC
	Respiratory burst	Fish	RB
Histopathology	Histopathological change analyses	Fish	Gill HP
			Liver HP
		Krill	Hep. HP

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