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Hazard/Risk Assessment

RELATING BIOMARKERS TO WHOLE-ORGANISM EFFECTS USING SPECIES SENSITIVITY DISTRIBUTIONS: A PILOT STUDY FOR MARINE SPECIES EXPOSED TO OIL

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Abstract—Biomarkers are widely used to measure environmental impacts on marine species. For many biomarkers, it is not clear how the signal levels relate to effects on the whole organism. This paper shows how species sensitivity distributions (SSDs) can be applied to evaluate multiple biomarker responses in species assemblages. To our knowledge, the present study compared for the first time SSDs based on biomarker response levels for marine species to a SSD for whole-organism responses. The comparison indicates that for exposure to dispersed oil in the marine environment, the selected biomarkers were, on average, 35- to 50-fold more sensitive than the whole-organism effect. At the 5% hazardous concentration derived from the SSD for whole-organism effects, which is a conservative threshold level, the potentially affected fraction of species showing biomarker response corresponds to approximately 80%. Variation in species sensitivity, expressed either as biomarker or as whole-organism response levels, were similar. Although uncertainties exist, the link between biomarkers and risk assessment presented here provides a preliminary guideline for deciding when biomarker responses likely are hazardous and, therefore, require further investigation.

Keywords—Biomarkers Whole-organism effects Species sensitivity distribution Ecological risk assessment Offshore oil/gas

INTRODUCTION

A range of changes occurs in organisms exposed to chemicals. Depending on exposure levels and duration, these changes include increases in tissue concentrations; changes in metabolic, molecular, and biochemical responses; and effects on the whole organism (behavior, reproduction, growth, and survival). Biomarkers are defined as detectable biochemical and tissue responses that represent changes in organisms after exposure to pollutants [1]. They often are proposed as tools for measuring effects on individuals [2–4]. During the last few decades, biomarker responses have been measured in marine organisms [2]. Biomarkers in exposed fish and mussels are presumed to represent the extent of biological effects as a result of discharges from offshore oil and gas platforms (see, e.g., Hylland et al. [5]) and are used in recommendations made by the International Council for the Exploration of the Sea [6] (http://www.ices.dk/products/cmdocs/cm-2007/mhc/wgbec07.pdf).

Despite their increased application, the efficacy of biomarkers in assessment of ecosystem health is widely debated [7]. Because of the transient nature of the response functions and natural fluctuations in responses, single biomarker measurements cannot easily be translated to a generic health indicator for the ecosystem under consideration [8,9]. Therefore, the need exists for a better understanding of variations in biomarker responses before application in environmental risk assessment [4]. Several authors suggest that application of suites of biomarkers, responding at suborganismal levels in several species with different habitats and feeding strategies, should reduce the uncertainty in the interpretation of biomarker responses [3,4,9]. These suites may be used for establishing relationships between environmental stressors and ecological effects [3] and may minimize the influence of natural variation and random errors [9]. For many biomarkers, however, it remains unclear which signal levels are acceptable in the sense of environmental impacts.

In regulatory environmental risk assessment, standard methods have been derived, and these methods have been condensed in guidance documents from, for example, the European Union [10] (http://ecb.jrc.it/DOCUMENTS/TECHNICAL_GUIDANCE_DOCUMENT/EDITION_2/tgdpart3_2ed.pdf) to derive quality criteria for toxic compounds, with the aim of being sufficiently protective of the structure and function of ecosystems [11]. Within that context, risk refers to the potential occurrence of adverse, whole-organism effects to individual species (effects on growth, reproduction, and mortality). Linking environmental risk limits, based on whole-organism effects, to responses of suites of biomarkers could facilitate the definition of acceptable biomarker responses.

In the present study, we explore the link between whole-organism effects applied in environmental risk assessment and individual biomarker response levels (which can be measured in situ) by applying a species sensitivity distribution (SSD) approach. For marine species exposed to dispersed oil, two SSDs based on biomarker response levels (DNA damage and oxidative stress) are compared to a SSD based on chronic no-observed-effect concentrations (NOECs) for whole-organism

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effects (growth, reproduction, and survival). Because only few studies report both biomarker responses and whole-organism responses from the same exposures, different data sources were used to collect biomarker data and whole-organism response levels. This use of secondary sources adds some uncertainty to the conclusions, which should be regarded as giving a first indication of the likely whole-organism effects.

MATERIALS AND METHODS

Species sensitivity distribution

Species sensitivity distributions are statistical distributions that represent the cumulative fraction of species affected as a function of the exposure to the stressor. They often are applied to extrapolate single-species laboratory toxicity test results to predicted impact levels for species assemblages, looking at direct responses only and assuming that the test species are representative of natural species assemblages and that their response distribution is not biased by other environmental factors. The average sensitivity is represented by the 50% hazardous concentration (HC50). The standard deviation of the distribution reflects the slope of the SSD, which is a measure of the variation in sensitivity among species. In the present study, SSDs were derived following the method described by Aldenberg et al. [11] using the log-normal distribution. Whether collected effect data should be represented by a log-normal distribution was tested with the Anderson–Darling goodness-of-fit test [11]. Estimates and confidence intervals for the HC50 and the 5% hazardous concentration (HC5), corresponding to a potentially affected fraction of species of 50 and 5%, respectively, were derived following the procedure described by Aldenberg and Jaworska [12]. For environmental risk assessment, SSDs usually are based on effect concentrations for whole-organism responses (survival, reproduction, or growth). In the present study, biomarker response levels (DNA damage and oxidative stress) also were used to construct SSDs.

Chronic NOECs for growth, reproduction, and survival

Scholten et al. [13] collected NOECs for 26 marine organisms for exposures to several types of oil. All test exposures focused on whole-organism effects: Reproduction, growth, and survival. Description of the test protocol in a peer-reviewed publication, facilitating the quality assurance of the data, was a prerequisite for including the NOEC in the dataset. For the present study, NOECs from the Scholten et al. dataset were selected, with exposure times exceeding 7 d representing chronic exposure. If more than one NOEC was available per species, the geometric mean of the values was taken to represent the sensitivity of the species. A SSD for oil was constructed based on the common logarithm (base 10) of these chronic NOECs.

Biomarker data

Results of biomarker measurements in different marine organisms exposed to different types of dispersed oil were selected from an internal database available at the Biomiljø Department of the International Research Institute of Stavanger (Norway). Two sets of biomarker data were selected. One set represented responses indicating DNA damage; the other represented responses indicating oxidative stress.

The biomarkers for DNA damage included in the database are the alkaline unwinding assay, comet assay, DNA adducts, and micronuclei frequency. Both the alkaline unwinding assay, described by Batel et al. [14], and the comet assay, described by Singh et al. [15], aim to quantify the number of single-strand breaks. The DNA adducts are covalent bonds between the DNA molecule and organic contaminants, such as polycyclic aromatic hydrocarbons, which can be quantified following the protocol of Reichert and French [16] (http://www.nwfsc.noaa.gov/publications/techmemos/tm14/tecmem14.html). Micronuclei are chromatin-containing cell structures with no detectable link to the cell nucleus that are formed as a result of cytogenic damage [17].

The biomarkers for oxidative stress included in the database are glutathione-*S*-transferase activity, catalase activity, and total oxygen radical scavenging capacity. Catalase and glutathione-*S*-transferase activity are enzymatic biomarkers applied in numerous aquatic organisms (see, e.g., Lee et al. [18]). The total oxygen radical scavenging capacity assay measures the capability of the organisms' antioxidant system to neutralize oxyradicals [19]. All biomarker measurements for DNA damage and oxidative stress included in the database are based on the protocols mentioned above. Further details regarding the experimental test setup, biomarker measurements, and oil analyses can be found in the original test reports [20–25] (http://www.iris.no/internet/bestakva.nsf).

In total, biomarker data for six marine species were available. From the available data for each species, the lowest oil concentrations giving significantly different biomarker responses from the controls were selected (i.e., lowest-observed-effect concentrations [LOECs]). For biomarker responses, LOECs were selected instead of NOECs, because this resulted in a larger dataset containing more species. As usual in the SSDs approach, sensitivity was assumed to be log-normally distributed. The common logarithmic values of the LOECs for the selected biomarker responses in the different species were plotted in a SSD. If a species was tested with more than one oil type, resulting in more than one LOEC per species, the geometric mean of the values was taken to represent the sensitivity of the species.

RESULTS

The set of NOECs for whole-organism responses with exposure times exceeding 7 d included 30 NOECs for 17 marine species from five taxonomic groups (Table 1). For biomarker responses, the final dataset contained nine LOECs for DNA damage in six marine species from four taxonomic groups. For oxidative stress, seven LOECs were available for five marine species from four taxonomic groups (Table 2). For both whole-organism effects and DNA damage, the filter-feeding bivalve *Mytilus edulis* is the most sensitive species to oil. Oxidative stress in *M. edulis* is relatively insensitive, but another filter-feeding bivalve (*Chlamys islandica*) turned out to be the most sensitive. In all three datasets, fish were among the least sensitive species.

Figure 1 presents three SSDs based on the data presented in Tables 1 and 2. As expected, DNA damage and oxidative stress were, on average, more sensitive measurement endpoints than effects on growth, reproduction, and survival. The difference between DNA damage and oxidative stress was not statistically significant (analysis of variance; significance level, 5%). For DNA damage, the HC50 and HC5 corresponded to a total hydrocarbon concentration (THC) of 16 (5.1–50) and 1.4 (0.09–4.7) μg/L, respectively (median value [5–95% confidence interval]). For oxidative stress, these values were 23 (6.1–85) and 2.0 (0.07–7.4) μg/L of THC, respectively. The HC50 and HC5 based on NOECs for growth, reproduction,

Table 1. Overview of selected chronic no-observed-effect concentrations (NOECs) for whole-organism responses expressed as total hydrocarbon concentration (THC) for 17 marine species exposed to different oil types selected from Scholten et al. [13]

Species	Group	No. of NOECs	Duration (d)	Geometric mean NOEC (μg THC/L)	Effect	Reference
Acanthopleura haddoni	Mollusk	1	7	2,514	Mortality	[32]
Calcinus latens	Crustacean	1	7	2,541	Mortality	[32]
Cancer magistrus	Crustacean	1	40	130	Mortality	[33]
Ctenodrilus serratus	Polychaete	1	28	870	Reproduction	[33]
Cyprinodon variegatus	Fish	1	8	870	Reproduction	[34]
Evasterias troschelli	Echinoderm	1	12	240	Growth	[35]
Fundulus heteroclitus	Fish	1	8	2,175	Reproduction	[34]
Fundulus similis	Fish	1	8	9,900	Reproduction	[34]
Limulus polythemus	Crustacean	1	100	435	Mortality	[33]
Macoma balthica	Mollusk	1	183	300	Mortality	[36]
Mytilus edulis	Mollusk	1	33	30	Reproduction	[37]
Nerita forskali	Mollusk	1	7	8,470	Mortality	[32]
Ophryotrottus sp.	Polychaete	1	28	870	Reproduction	[33]
Palaemon pacificus	Crustacean	2	7	1,523	Mortality	[32]
Pontogammarus maeoticus	Crustacean	10	10–50	648	Mortality	[38]
Rhitropanopeus harrisii	Crustacean	3	183	320	Growth	[39]
Siganus rivulatus	Fish	2	7	834	Mortality	[32]

and survival were 804 (435–1,487) and 70.5 (21.8–153) μg/L of THC, respectively. Normality of the effect data was accepted for all datasets with the Anderson–Darling test for normality ($p > 0.05$). The variation in species sensitivity represented by the standard deviation of the log-normal distributions for DNA damage, oxidative stress, and NOECs for whole-organism effects was 0.60, 0.60, and 0.63, respectively.

Following the recommendations by Van Straalen and Denneman [26], the median estimate of the HC5 from the SSD based on whole-organism responses (70.5 μg/L of THC) can be regarded as a maximum allowable exposure level for oil. According to the SSDs presented in Figure 1, this level of exposure corresponds to DNA damage in approximately 86% of the species and to oxidative stress in approximately 79% of the species.

DISCUSSION

Reliability of results

The present study has shown that the HC50s for biomarkers (representing DNA damage and oxidative stress) and for whole-organism effects differ by a factor of 35 to 50. The accuracy in the difference in species sensitivity for biomarker responses and whole-organism effects depends on the accuracy of the constructed SSDs, which relies mainly on the accuracy of the data. Uncertainties in the accuracy of the biomarker SSDs result, in part, because of the limited availability of data and also because the biomarker response data and whole-organism effect data come from different experiments. Furthermore, studies with different oil types were included. These uncertainties are discussed below.

Uncertainty in the SSD is associated with the number of input data [12]. The SSD based on whole-organism responses is based on threefold as many data points as the SSDs based on biomarker response levels. By choosing LOECs instead of NOECs for biomarker responses, the number of data points increased. Also, short-term exposure data instead of chronic data for *Gadus morhua* were included in the SSD for oxidative stress, because no other data regarding oxidative stress for fish were available. More information on the time dependency of biomarker expressions is needed to enable the quantification

Table 2. Overview of lowest-observed-effect concentrations (LOECs) for biomarkers expressed as total hydrocarbon concentration (THC) obtained from the internal database of the International Research Institute in Stavanger, Biomiljø Department, indicating DNA damage (alkaline unwinding, comet assay, and DNA adducts) and oxidative stress (glutathione-*S*-transferase activity, catalase activity, and total oxygen radical scavenging capacity) in different marine species exposed to oil

Species	Group	No. of LOECs	Biomarker	Duration (d)	Geometric mean LOEC (μg THC/L)	Reference
DNA damage						
Pandalus borealis	Crustacean	2	Alkaline unwinding	30–90	21.2	[24]
Mytilus edulis	Mollusk	1	Comet assay	210	2.8	[22]
Chlamys islandica	Mollusk	1	Comet assay	30	14.4	[22]
Strongylocentrotus droebachiensis	Echinoderm	1	Comet assay	210	4	[23]
Gadus morhua L.	Fish	3	DNA adducts	24–31	46.9	[20,25]
Cyprinodon variegatus	Fish	1	DNA adducts	21	100	[40]
Oxidative stress						
Mytilus edulis	Mollusk	1	Glutathione-*S*-transferase activity	30	63.4	[22]
Chlamys islandica	Mollusk	1	Glutathione-*S*-transferase activity	30	2.3	[22]
Pandalus borealis	Crustacean	2	Total oxygen radical scavenging capacity/ catalase activity	30–150	21.2	[24]
Strongylocentrotus droebachiensis	Echinoderm	1	Catalase activity	210	29	[23]
Gadus morhua	Fish	2	Glutathione-*S*-transferase activity	3	69.4	[25]

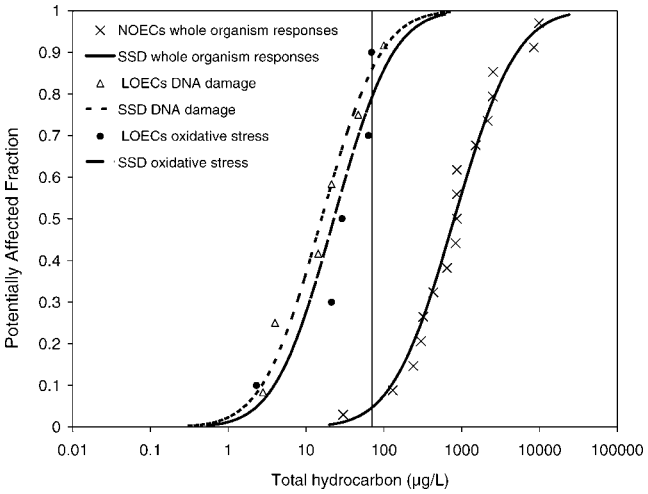

Fig. 1. Potentially affected fraction versus oil concentration (expressed as total hydrocarbon concentration) for chronic no-observed-effect concentrations (NOECs) for whole-organism responses (growth, reproduction, and survival), lowest-observed-effect concentrations (LOECs) for DNA damage, and LOECs for oxidative stress. Individual data (markers) and fitted log-normal species sensitivity distributions (SSDs) are shown. The vertical line at a total hydrocarbon concentration of 70.5 μg/L indicates the median estimate of the 5% hazardous concentration (HC5-50% confidence) of the SSD for whole-organism responses.

of the variation in biomarker response data caused by different exposure times [27].

The observed difference by a factor of 35 to 50 in HC50s for biomarkers and whole-organism effects, as determined in the present study, is not always reflected in data for the same species. Based on the data for *Cyprinodon variegatus* and *M. edulis*, with LOECs for DNA damage of 100 and 2.8 μg/L of THC, respectively, and NOECs for reproduction of 870 and 30 μg/L of THC, respectively, biomarker response levels are no more than a factor of 9 to 11 lower than response levels for whole-organism effects. Additionally, The biomarker LOEC for oxidative stress in the bivalve *M. edulis* (63.4 μg/L of THC) is twice as high as the NOEC for reproduction (30 μg/L of THC). It also should be noted that only in a few experiments have whole-organism effects and biomarker responses been measured simultaneously. From a single experiment with oil, Baussant [22] reported concentrations resulting in DNA damage in the bivalve *M. edulis* that were comparable to concentrations causing effects on the development of offspring of exposed parents (2.0 μg/L of THC). Also, in other experiments with some chemicals, biomarkers were observed to be equally or even less sensitive than whole organisms [7]. Although, on average, the data in the present study indicate that filter-feeding mollusks are most sensitive and fish least sensitive to exposure to oil, independent of the endpoint used, more effort should be devoted to studying the difference in biomarker and whole-organism response levels for specific taxonomic groups from single experiments. This also will reduce other sources of variability, such as differences in test conditions, potentially leading to different sensitivities of test organisms.

Oil consists of many different components, and variation in the composition of oil might cause additional variation in effect concentrations. Because the present study was intended to be a preliminary assessment, based on a straightforward SSD approach, we did not study the effects of the changing oil composition as a result of weathering and aging of oil on the experimental results. This would require a more detailed comparison including actual values for individual constituents, which were not available for the data regarding whole-organism effects. Based on an analysis of the variation in aquatic toxicity data of seven types of crude oils (including North Sea oils) and five types of oil products, Scholten et al. [13] showed that the type of oil was responsible for only 11% of the total variance in oil toxicity data. Because the variation of oil types in the biomarker experiments is much less (all North Sea oils), it is assumed that variance in the biomarker response data caused by the oil type is the same or less than that for whole-organism effects.

Harbers et al. [28] presented a generic slope value of 0.65 for SSDs based on whole-organism effects for nonpolar narcosis, which is the main toxic mode of action by oil constituents. With standard deviations of between 0.60 and 0.63 derived in the present study, the variation in species sensitivity for all endpoints is comparable and close to the generic value for nonpolar narcosis. This suggests that the nonspecificity of the nonpolar narcosis mode of action is expressed both in whole-organism responses and at the biochemical endpoints. Further study addressing the routes of uptake and metabolic activation of polycyclic aromatic hydrocarbons and other oil components inducing oxidative stress and DNA damage should be performed to evaluate possible nonspecificity of DNA damage and oxidative stress.

Implications for risk assessment

Despite remaining uncertainties in the basic assumptions underlying the SSD technique [29], SSDs based on chronic NOECs for whole-organism effects currently are applied in ecological risk assessment [11]. The HC5 of a SSD based on chronic NOECs serves as a threshold level for the protection of ecological structure. It is assumed that ecosystem structure is more sensitive than ecosystem functioning and that by protecting structure, functioning also is protected. By indicating no effects at the HC5 level, several experimental studies and modeling exercises showed the validity of this assumption (see, e.g., Van den Brink et al. [30] and De Laender et al. [31]). For the present study, this would imply that at levels where effects on species assemblages do occur, the fraction of species having biomarker responses would exceed 80%. Although this has not been tested for oil, it indicates the early warning character of the biomarkers included in the present study and the need to include other measurement endpoints if the objective is to link those endpoints to actual effects. Uncertainties in the evaluation of the measured fraction of species showing biomarker responses may be reduced by applying suites of biomarkers [9]. If the SSD concept, as applied in the present study to DNA damage and oxidative stress, is extended to include more species and more measurable early warning signals (e.g., physiological and behavioral responses), as suggested by, for example, Hagger et al. [9], then a biomarker toolbox could be developed for tiered assessment, as illustrated conceptually in Figure 2.

The preliminary finding that whole-organism effects may be a factor of 35 to 50 less sensitive than single biomarker indicators is approximate and might change somewhat if more and more accurate data regarding the effects of oil in the marine environment become available. Nevertheless, the SSDs developed here provide a tentative link between the fraction of species with certain biomarker expression, which can be mea-

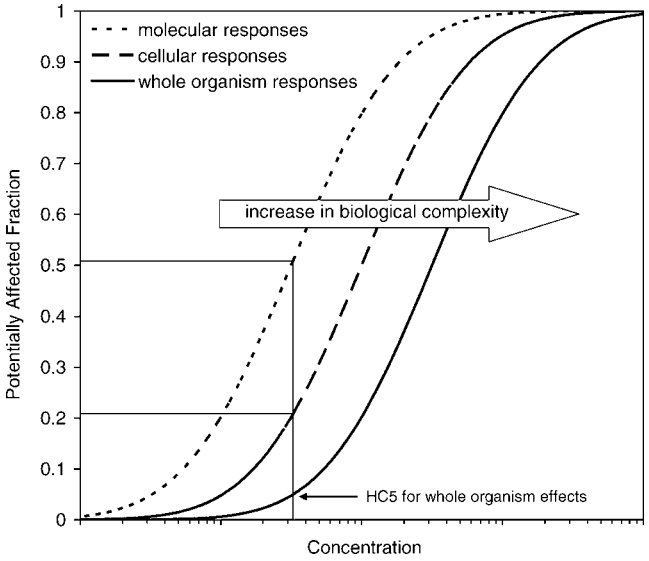

Fig. 2. Conceptual figure on use of the species sensitivity distribution (SSD) principle to evaluate suites of biomarkers and relate the fraction of species with observed biomarker responses at different levels of biological complexity to whole-organism responses. In the figure, the 5% hazardous concentration (HC5) for whole-organism effects is presented together with the corresponding fractions of species with molecular and cellular biomarker responses.

sured in the field, to individual whole-organism effects, which are used for risk characterization and definition of thresholds. Although uncertainties clearly exist and further analysis of the SSD concept to biomarker responses is required, the link to risk assessment, presented in the present paper, provides a preliminary guide about when biomarker responses should be followed up by further investigations and eventual correcting measures. This biomarker-bridge approach seems to be a useful way to link field monitoring with risk prognosis. In follow-up programs, data will be collected for more biomarkers in more species to make the toolbox ready for practical use (e.g., in relation to discharges of produced water from the oil and gas industry offshore).

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