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TREND EDITORIAL

Biomarkers as tools for monitoring within the Water Framework Directive context: concept, opinions and advancement of expertise

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Abstract
The Water Framework Directive (WFD) currently supports chemical and ecological monitoring programmes in order to achieve the good water surface status. Although chemical and ecological assessments are necessary, they have some limitations. Chemical approaches focus on certain substances identified as priorities, but they do not take into account other potentially harmful substances and also ignore the hazards related to contaminant cocktails. On the other hand, while ecological approaches provide holistic information on the impairment of biological communities in ecosystems, they do not distinguish the role of contaminants in these alterations, and consequently do not allow the establishment of contaminant impact reduction plans. Consequently, ecotoxicologists suggest the use of effect-based tools such as biomarkers. Biomarkers highlight the effect of potentially harmful substances (or a cocktail), and their specificity towards the chemicals makes it possible to properly discriminate the role of toxicants within biological community impairments. Thus, the integration of such tools (besides existing chemical and ecological tools) in the WFD could considerably improve its biomonitoring strategy. The B n' B project (Biomarkers and Biodiversity) exposes key objectives that will allow to (i) establish an inventory of the biomarkers developed by French laboratories; (ii) determine their methodological advancement and limits and, on this basis, formulate recommendations for biomonitoring use and future research needs; (iii) discuss the biomarkers' ecological significance, specificity to contaminants and interpretation capacity; (iv) establish, *in fine*, a selection of valuable biomarkers to enter the WFD; and (iv) propose integrative tools to facilitate the decision-taking by stakeholders.

Keywords Biomarkers · Ecotoxicology · Water Framework Directive · Biomonitoring strategy · Contaminants

Limitations of ecological and chemical approaches as a plea for an integration of effect-based tools in the Water Framework Directive

The Water Framework Directive (WFD) (WFD 2000/06/EC) was established in order to achieve a good status of continental, transitional and coastal waters. The WFD promotes the establishment of biomonitoring programmes aiming at evaluating and, if necessary, improving the chemical and ecological status of water bodies. Briefly, the chemical status is considered good if concentrations of priority substances in biota and the water column do not exceed calculated environmental quality standards. The ecological status is evaluated using ecological

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indicators (for example the diversity and abundance of plant and animal groups). Birk et al. (2012) presented an overview of 297 methods used in 27 European countries that could implement the assessment of ecological status within the WFD context. Although these chemical and ecological analyses were of great interest for the first WFD management plan (achieved in 2015), the scientific community and decision makers highlighted several bottlenecks in these approaches. Specifically, the chemical approaches focused on select priority compounds that are highly suspected to pose a threat to ecological communities and/or human health, screenings of all contaminants present in the environment being impossible for technical and economic reasons (as noted by Amiard and Amiard-Triquet 2008). Thereby, chemical assessments could ignore a corpus of potentially harmful substances. Additionally, the chemical approach provides insight into the environmental quality of water bodies by considering each compound (and their toxic profile) separately. Thus, such an approach ignores "cocktail" effects and, consequently, the toxicity of contaminant mixtures formed in the field to which organisms are exposed (Beyer et al. 2014). In parallel to these chemical analyses, ecological evaluation was proposed as a tool of interest to assess the impact of chemical and physical stress on ecosystems. However, some authors consider that such an *a posteriori* approach, i.e. determining the decline of a population and/or community, will not provide an early enough indication of toxicity effect (Amiard et al. 2015). Therefore, in some cases, only remediation plans could be set up afterwards, even though prevention plans are more cost-effective. Moreover, using an ecological approach, discrimination of the effects of contaminants (or cocktails of contaminant) from the effects of other abiotic factors (e.g. temperature, salinity, food resources, eutrophication and/or habitat degradation) is complex since all these factors are likely to alter ecological parameters (e.g. Schiedek et al. 2007; Thrush et al. 2008; Moe et al. 2013).

In order to deal with these issues, scientists promote the use of diversified effect-based tools (e.g. Brack et al. 2017; Vethaak et al. 2017; Altenburger et al. 2019). Wernersson et al. (2014) described two categories of effect-based tools besides ecological indicators: (i) bioassays that measure the toxicity, at cellular and individual levels, of environmental samples under defined laboratory conditions; (ii) biomarkers, i.e. the biological responses at the sub-individual or individual level observed in field exposed organisms. If biomarkers are judiciously used, they could adequately supplement chemical and ecological approaches in order to solve the main issues cited above. Indeed, the use of a set of biomarkers with a selective responsiveness for certain families of contaminants will inform on the presence of harm in the ecosystem and thus serve as initial non-targeted chemical screening steps before defining the orientation of further necessary chemical measurements (Depledge et al. 1995). Moreover, pollutant-responsive biomarkers allow scientists to evaluate the biological effects of chemical mixtures and discriminate the effect of contaminants from the effects of other factors such as temperature, oxygen levels and seasonality. On this basis, biomarkers could help identify which toxicological substances are responsible for the biological impacts (observed through ecological status evaluation). Finally, since biomarkers are measured at the sub-individual or individual levels, they are claimed to be more precocious than ecological indicators (Amiard et al. 2015), which are measured at the population or community level. They could therefore provide initial insight into the future potential alteration of an ecosystem's health status and thus allow establishment of early cost-effective prevention plans by decision makers instead of expensive remediation plans.

However, in response to these different stakes, there are still several issues with regard to the use of biomarkers for biomonitoring programmes. Forbes et al. (2006) note that biomarker modulation could present false alarm rather than early warning. The authors argue that certain biomarker responses observed in the field could be the results of confounding factors rather than chemical exposure. To address this criticism, several authors recently investigated, integrated and/or corrected confounding factors in their methodologies. They defined reference and statistical threshold values that integrate the confounding (biotic and abiotic) factors and ensure that the observed biomarker modulations are due to contaminants (e.g. Coulaud et al. 2011; Charron et al. 2013; Erraud et al. 2018). Another criticism for the use of biomarker is their "complicated time or dose-dependent responses" (Forbes et al. 2006) since these indicators already exhibit some transient responses that may be greatly reduced even if the chemical stress is still present or even more intense. In this line, biomarker kinetics and dose-responses were studied in order to improve the interpretation of the biomarkers' modulations observed on the field (e.g. Zhang et al. 2008; Nahrgang et al. 2009; Pathiratne and Hemachandra 2010). Finally, since biomarkers are measured at the sub-individual or individual level, Forbes et al. (2006) raise the question as to how such modulation could affect population, then community and finally the whole ecosystem structure. Initially, the main goal of multi-biomarker studies—those that use biomarkers of effects and biomarkers of exposure—was to provide an early warning of a potential ecosystem health status alteration, without quantifying the link between the modification observed at the sub-individual level and repercussions at higher levels. More recently, researcher observed propagations of biological effects from the individual or sub-individual level (e.g. oxidative stress, immunotoxicity, neurotoxicity, genotoxicity, apoptosis, abnormalities of development and/or behavioural alterations) up to higher organisation levels (e.g. Lacaze et al. 2011; Santos et al. 2013). Mechanistic pathways related to adverse outcome pathways (AOP) approach, as initially defined by

Ankley et al. (2010), were investigated in order to highlight and quantify these propagations of effects (e.g. Durou et al. 2008; Xuereb et al. 2009; Lacaze et al. 2011; Coulaud et al. 2015). AOPs permitted to obtain better information about the impact on ecosystem health and were consequently the main focus of the Environmental, Health and Safety Programme recently launched by the OECD (Organisation for Economic Cooperation and Development).

In the light of biomarker advantages as a monitoring strategy, scientists promote their insertion in the WFD revision text (soon to be revised, in 2019), in addition to ecological and chemical indicators (Lepom et al. 2009; Wernersson et al. 2014; Capela et al. 2016). However, taking into account biomarker issues as well as recent advances in this field to solve these issues, a qualitative selection of valuable biomarkers seems necessary. To do so, the Biomarkers and Biodiversity (B n' B) project will expose an inventory of the biomarkers used by the French laboratories and will propose a panel of relevant biomarkers likely to enter the WFD. Such an exhaustive inventory, conducted at the national scale, will permit to cover a wide range of the biomarkers that are actually used at the European Union scale.

B n' B project: participants, objectives and implementation

The B n' B project is funded by the French Biodiversity Agency (AFB) and led by the Rovaltain Foundation. It began in January 2018 and will end in November 2019. As a first step, the project identified members for the working group according to their expertise in the field of biomarker use for biomonitoring purposes. In order to cope with the issues presented above and to permit their entry in the revised WFD text, B n' B project has several objectives:

(i) To establish an inventory of the expert laboratories in the French territory and their competence in terms of the use of biomarkers and sentinel species for the biomonitoring of continental (e.g. rivers, lakes, underground waters), transitional (e.g. estuaries) and marine coastal waters
(ii) To evaluate the maturity of these biomarkers in terms of methodological development for use in the field, that is, to determine which biomarkers could be used for large-scale biomonitoring programmes and which one need further research development
(iii) To discuss the ecological significance of the biomarkers and their specificity to contaminants
(iv) To consider the limits and the potentials of the biomarkers as tools of interest to fill the gap between ecological and chemical status evaluations in the WFD framework
(v) To establish a critical inventory of the methodology (available at the international level) that convert multi-marker results into an easily understandable evaluation of ecosystem health for decision makers
(vi) To formulate recommendations and research needs for a better use of biomarkers in biomonitoring programmes and for the development of innovative tools

To achieve these objectives, members of the consortium will firstly identify French laboratory experts in the field and an inventory will establish the know-how of each laboratory in terms of biomarkers and species use.

Second, methodological maturity and the condition of application will be established for each previously identified biomarker/species couple thanks to a survey addressed to these laboratories. More precisely, the methodological maturity will be evaluated regarding the acquired knowledge of the laboratory concerning (i) the biomarker response profile (e.g. inhibition or activation), (ii) the biomarker kinetics and dose-response parameters (biomarker modulation at several time points and contamination intensities) and (iii) the existence of reference and statistical threshold values that integrate the confounding factors and ensure that the biomarker modulation is due to contaminants.

Regarding application, laboratories will provide information on the acquisition time necessary to obtain results, the type of biomonitoring (active or passive) already performed with each biomarker/species couple, the level of expertise required and the ethical issues linked with the use of biomarkers.

On the basis of these methodological criteria, the consortium will select valuable biomarkers to be used in large-scale biomonitoring as well as biomarkers that require slight refinement.

Third, the biomarkers' ecological significance and specificity to contaminants will be determined by the consortium according to their expertise and the literature.

Finally, the consortium will highlight the limits and the potential of the biomarkers inventoried in French territory in order to formulate recommendations for their use and improvement in a regulatory monitoring context.

After completing these tasks, a bibliographic analysis will be conducted in order to generate an inventory of the methodologies used to integrate multi-markers into an easily understandable tool for decision making. After categorising these tools according to the computational method used to integrate biomarkers, the relevance of these methodologies will be evaluated by characterising the pros and the cons of each method, by estimating their operability (needs for specific software, feasibility of the calculations, etc.) and discussing their significance (e.g. demonstration of statistical significance, numbers of species that could be integrated, etc.).

The B n' B project will highlight French expertise on biomarkers in ecotoxicology in order to evaluate the possibility of integrating these tools in the WFD. Results of this project will be associated with the deliverables of an activity conducted within the context of the WFD common implementation strategy work programme. This activity aims to define valuable bioassays that could enter the WFD. The results of our project and of this activity will contribute to integrate effect-based tools within the European directives.

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