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Are we closer to the vision? A proposed framework for incorporating omics into environmental assessments

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ABSTRACT

Environmental science has benefited a great deal from omics-based technologies. High-throughput toxicology has defined adverse outcome pathways (AOPs), prioritized chemicals of concern, and identified novel actions of environmental chemicals. While many of these approaches are conducted under rigorous laboratory conditions, a significant challenge has been the interpretation of omics data in "real-world" exposure scenarios. Clarity in the interpretation of these data limits their use in environmental monitoring programs. In recent years, one overarching objective of many has been to address fundamental questions concerning experimental design and the robustness of data collected under the broad umbrella of environmental genomics. These questions include: (1) the likelihood that molecular profiles return to a predefined baseline level following remediation efforts, (2) how reference site selection in an urban environment influences interpretation of omics data and (3) what is the most appropriate species to monitor in the environment from an omics point of view. In addition, inter-genomics studies have been conducted to assess transcriptome reproducibility in toxicology studies. One lesson learned from inter-genomics studies is that there are core molecular networks that can be identified by multiple laboratories using the same platform. This supports the idea that "omics-networks" defined *a priori* may be a viable approach moving forward for evaluating environmental impacts over time. Both spatial and temporal variability in ecosystem structure is expected to influence molecular responses to environmental stressors, and it is important to recognize how these variables, as well as individual factor (i.e. sex, age, maturation), may confound interpretation of network responses to chemicals. This mini-review synthesizes the progress made towards adopting these tools into environmental monitoring and identifies future challenges to be addressed, as we move into the next era of high throughput sequencing. A conceptual framework for validating and incorporating molecular networks into environmental monitoring programs is proposed. As AOPs become more defined and their potential in environmental monitoring assessments becomes more recognized, the AOP framework may prove to be the conduit between omics and penultimate ecological responses for environmental risk assessments.

1. Environmental genomics: will we reach the vision?

Omics as an approach in environmental toxicology is now firmly established, and all sectors (governments, industry, and academic research programs) utilize high-throughput technologies (e.g. transcriptomics, proteomics, metabolomics, epigenetics, miRNA profiling, and lipidomics) in some capacity to address mechanistic questions about chemicals. As anthropogenic pressures continue to increase on the environment, so does the need for new technological and computational approaches to address these global issues. Omics approaches have been applied to address these global challenges, including the impacts of climate change (Parkinson et al., 2018), toxicity screening and prioritization (Haswell et al., 2018), and invasive species (Mahon and Jerde, 2016). However, while these tools are used heavily by the scientific community, the debate about whether omics can be useful (or is needed) in environmental risk assessment has lasted for more than a decade. Fent and Sumpter (Fent and Sumpter, 2011) posed several years ago the question about whether the technology was driving innovation; the authors pointed out that there were a lack of clear links between molecular responses to meaningful endpoints indicating adverse consequences. Around the same time, Pina and Barata (2011) pointed out that careful attention was needed in interpretation of such data, and omics profiles at different developmental and reproductive stages needed to be further elucidated and defined for ecologically-relevant non-model species. The community in many ways has responded positively to these challenges over the past several years. Adverse outcome pathways (AOPs) now provide a strong framework for linking molecular responses (molecular initiating and key events) to adverse

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biological outcomes in organisms. There has been a strong focus to synthesize molecular responses into topographical interpretations that describe variability in a phenotype of interest (Huang et al., 2017). Retrospective analyses prior to an experiment is now possible, and may be limited more by inexperience in computational biology rather than lack of publicly available information. These approaches are conducted more routinely in medicine compared to ecotoxicology, but nonetheless, provide examples for experimental approaches moving forward.

The long-term vision of integrating omics into environmental monitoring programs and risk assessment has been recognized for some time, perhaps as early as some of the first cDNA-based microarray applications in environmental science at the turn of the millennium (Hogstrand et al., 2002; Larkin et al., 2002; Miracle et al., 2003; Neumann and Galvez, 2002; Snell et al., 2003). Estrogenic pharmaceuticals in the environment were a primary concern, and efforts were directed towards identifying estrogen-responsive genes in fish using this new technology (Larkin et al., 2003; Larkin et al., 2002). The goal was to develop molecular biomarkers for estrogens and endocrine disrupting compounds. There now exists more than 100 peer-reviewed studies that report on transcriptional profiles in fish and aquatic invertebrates following estrogenic treatments – an impressive dataset that can be leveraged with other databases to identify estrogen-responsive gene networks (Feswick et al., 2017b). Simply put, the objective for omics in environmental science is to identify biologically meaningful molecular clusters that *predict* adverse outcomes which lead to negative impacts on individual fitness.

Since the early 2000s, there has been a moderate increase in the number of peer-reviewed studies that focus on environmental applications of omics. Leung, Leung (2018) conducted an examination of the number of omics studies in risk assessment, environmental risk assessment, and environmental management and points out that applications for environmental management is lagging behind other disciplines using omics, for example medicine. Reasons highlighted by Leung (2018) and echoed by many in the field include lack of expertise with the technology, lack of clear case studies demonstrating benefits of omics in regulation, and debate about how best to standardize technical and bioinformatics pipelines. Furthermore, monitoring programs rely on endpoints that are closely linked to population-level consequences, which is difficult to do with molecular data. Endpoints used for monitoring are biologically relevant and straightforward to collect in the field; interpretation of the data must be clear. A persistent challenge with omics is to identify the most optimal approach to determine which changes are not meaningful (i.e. transient, indirect to the chemical itself) compared to those molecular response indicative of the exposure. There can exist both general responses to toxicants (i.e. general and oxidative stress) and responses that are specific to the chemical class (e.g. receptor-mediated or enzyme dependent). AOPs are now defining these responses more clearly into a framework that facilitates testable hypotheses. When omics data are incorporated into adverse outcome pathways (AOPs), predictive ability is strengthened and knowledge as to the cause or trigger of a change is increased (i.e. identifying the molecular initiating event that reveals potential chemical initiators). Data suggest that transcript levels can indicate perturbations in higher level biological responses (i.e. histopathology), even when expression level changes are transient or slight (Rossi et al., 2016). Thus, integrating different omics methodology with morphological analysis can be meaningful for monitoring biological effects in contaminated environments.

Although the vision may be relatively defined in terms of the overarching objective, an accepted framework to achieve this vision is still lacking. Reasons for this centers around the requirements for the science to be reproducible, reliable and consistent. To date, studies that quantify variability and sensitivity in the technology are not often done for ecotoxicology, although significant efforts have been made to address this (Biales et al., 2007; Feswick et al., 2017a; Flick et al., 2014; Simmons et al., 2015; Vidal-Dorsch et al., 2016). Studies are still needed to address intra- and inter-laboratory reproducibility in the omes within the context of ecotoxicology before any implementation can occur into a monitoring program. Despite previous attention and calls for action to consider extraneous variables in chemical assessments using omics (Simmons et al., 2015), the priorities continue to remain on evaluating the vast chemical space (emerging chemicals of concern) driven by the latest omics methods, rather than addressing fundamental questions about reproducibility and reliability of data within and across laboratories. This lack of rigorous evaluation acts to delay the use of these method for environmental monitoring and hampers any acceptance by stakeholders and the legal community.

This review highlights some of the pressing challenges for environmental omics and proposes a general framework for advancing omics in applications for environmental monitoring. Some current needs to advance the science include the following; (1) Baseline data in individuals within a site or the use of multiple reference sites to reduce confounding interpretation of environmental omics datasets; (2) Quantitation of variability in omics responses under various laboratory and environmental conditions; (3) Increased confidence that methods are repeatable and reproducible within and among laboratories; (4) Discussions on what a compensatory response is at the level of the transcriptome, proteome, or metabolome; (5) Discussions about how to define and measure recovery in terms of an omics response (or whether this is possible); (6) How to quantify the magnitude of an “omics” response and what the change means from both a stakeholder and researcher point of view.

2. The omics-chemical interface: challenges ahead

Wildlife and humans are continuously exposed to low concentrations of thousands of chemicals on a daily basis. The number of iterations in terms of exposure risk is exponential due to the vast chemical space, and biological responses can be unpredictable and dependent upon an individual’s physiology and health status. While novel, high throughput strategies are sought to quantify risk associated with the overwhelming number of chemicals, there is also high value in a retrospective approach, one that carefully examines where we have been and what steps we have made towards an ultimate goal of predictive toxicology.

It has become apparent through programs such as the Tox21, ToxCast, and EU ToxRisk initiatives that chemicals do not have a single mode of action; rather chemicals can be promiscuous, binding multiple membrane and nuclear receptors, antagonizing enzymes, and acting through diverse non-receptor mediated pathways. Therefore, single endpoints may not always be optimal in some cases when measuring biological impacts of chemical exposures in the environment. “Big data” approaches are able to capture a broad range of biological responses and can be integrated with other anchoring endpoints at different levels of biological organization. Moreover, omics data can be used to refine adverse outcome pathways (AOP), and are important for unveiling novel molecular initiating events for adverse outcomes by defining linkages to ecologically relevant phenotypes (e.g. reproduction, growth, energy, development, behavior). It is important to recognize that these pathways are not linear, but are rather integrated and complex–-perturbations at one point along the AOP can have consequences for other integrated pathways (Conolly et al., 2017). These intersecting AOPs can be organized into networks to better understand how the system responds at different points from chemical perturbations. The AOP framework, coupled to adaptive monitoring, has been suggested as a viable approach in environmental monitoring assessments (Arciszewski et al., 2017) and AOPs may act as the conduit between molecular responses and penultimate ecological responses. Other helpful approaches moving forward include knowledge assembly models (KAMs) to integrate biological and chemical information into environmental impact assessments (Schroeder et al., 2017).