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Environmental OMICS: Current Status and Future Directions

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Abstract

Objectives: Applications of OMICS to high throughput studies of changes of genes, RNAs, proteins, metabolites, and their associated functions in cells or organisms exposed to environmental chemicals has led to the emergence of a very active research field: environmental OMICS. This developing field holds an important key for improving the scientific basis for understanding the potential impacts of environmental chemicals on both health and the environment. Here we describe the state of environmental OMICS with an emphasis on its recent accomplishments and its problems and potential solutions to facilitate the incorporation of OMICS into mainstream environmental and health research.

Data sources: We reviewed relevant and recently published studies on the applicability and usefulness of OMICS technologies to the identification of toxicity pathways, mechanisms, and biomarkers of environmental chemicals for environmental and health risk monitoring and assessment, including recent presentations and discussions on these issues at The First International Conference on Environmental OMICS (ICEO), held in Guangzhou, China during November 8-12, 2011. This paper summarizes our review.

Synthesis: Environmental OMICS aims to take advantage of powerful genomics, transcriptomics, proteomics, and metabolomics tools to identify novel toxicity pathways/signatures/biomarkers so as to better understand toxicity mechanisms/modes of action, to identify/categorize/prioritize/screen environmental chemicals, and to monitor and predict the risks associated with exposure to environmental chemicals on human health and the environment. To improve the field, some lessons learned from previous studies need to be summarized, a research agenda and guidelines for future studies need to be established, and a focus for the field needs to be developed.

Conclusions: OMICS technologies for identification of RNA, protein, and metabolic profiles and endpoints have already significantly improved our understanding of how environmental chemicals affect our ecosystem and human health. OMICS breakthroughs are empowering the fields of environmental toxicology, chemical toxicity characterization, and health risk assessment. However, environmental OMICS is still in the data generation and collection stage. Important data gaps in linking and/or integrating toxicity data with OMICS endpoints/profiles need to be filled to enable understanding of the potential impacts of chemicals on human health and the environment. It is expected that future environmental OMICS will focus more on real environmental issues and challenges such as the characterization of chemical mixture toxicity, the identification of environmental and health biomarkers, and the development of innovative environmental OMICS approaches and assays. These innovative approaches and assays will inform chemical toxicity testing and prediction, ecological and health risk monitoring and assessment, and natural resource utilization in ways that maintain human health and protects the environment in a sustainable manner.

Keywords: OMICS; Environmental; Health; Chemicals; Toxicology; Genomics; Proteomics; Metabolomics; Biomarkers; Metagenomics; Risk Assessment; Biomarker.

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1. Introduction

The suffix "-omics" was applied to describing heterogeneous networks of objects by physicists and computer scientists who produced novel papers on scale-free network properties in biological systems in the 1990s [1]. It later became a specific word describing the science that was comprehensively embraced by the four disciplines of genomics, transcriptomics, proteomics, and metabolomics in the early 2000s [2]. OMICS has developed rapidly in recent decades, triggered by the improvements in genome decoding techniques and high-throughput technologies enabling profiling of mRNA, proteins, and metabolites. OMICS-based data collectively provide a snapshot picture of gene expression, protein expression, and metabolite pattern, which altogether can enable much deeper insight into how tested organisms cope with external stressors. Applications of OMICS technologies to environmental toxicology and health research resulted in the emergence of a new research field: environmental OMICS. Environmental OMICS is the applications of OMICS technologies including genomics, transcriptomics, proteomics, and metabolomics to better understand the environmental and genetic factors, toxicity mechanisms, and modes of action in response to both acute and chronic exposure to environmental chemicals and, in the long-term, development of diseases caused or influenced by these exposures (Figure 1). Environmental OMICS is still in the early stage of OMICS data collection and validation of the molecular profiles for identifying toxic mechanisms, toxicity signatures, biomarkers, and pathways after exposure to environmental chemicals.

Environmental OMICS research can be roughly divided into three categories. The first category focuses mainly on chemical toxicity and environmental monitoring enabling risk assessment. The second category focuses on adverse human health outcomes and environmental impacts, while the last category focuses on ecological functions and environmental adaptation. The primary goal of these research fields is similar, namely to identify molecular changes, especially changes at expression levels of mRNA, proteins, and metabolites, in cells or tissues exposed to environmental toxicants and to relate these molecular changes to ecological and health outcomes.

Environmental OMICS has been used to study toxicity mechanisms and short- and long-term effects of environmental chemicals on human health outcomes, to define the acceptable levels and potential impacts of environmental toxicants on sensitive target species and ecosystems, to pro-

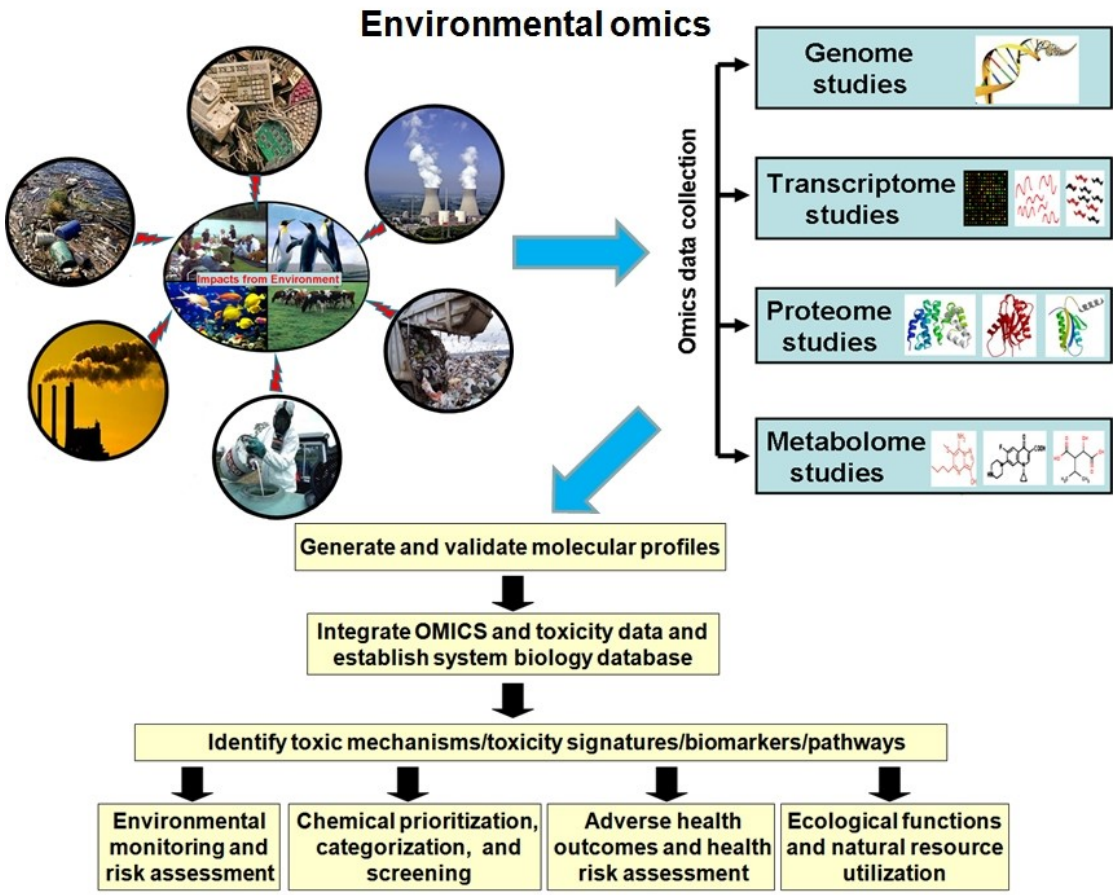

Figure 1. Environmental OMICS and its applications

vide insights into yet unsolved problems of environmental risk assessment such as chemical mixtures and combined effects of different environmental stressors, and to uncover unknown microbial communities and other natural resources. For example, genomic, proteomic, and metabolomic technologies have been used extensively to study the molecular mechanisms of how arsenic acts as a carcinogen [3, 4]. Also, the difference in gel electrophoresis (DIGE) proteomics technology has been used to decipher toxicity pathways and detoxification pathways of nanomaterials, protein-interacting network maps, biological response, potential toxicity, and detoxification pathways in titanium dioxide-treated BEAS-2B cells [5]. Proteomics was also applied to the investigation of differential proteomes of environmental bacteria for a better understanding of antibiotic and antibiotic-resistance mechanisms [6].

Genomic, proteomic, or metabolomic expression profiling is probably the most popular application of OMICS to environmental toxicology and health risk research. These and other OMICS studies help to reduce uncertainties associated with the ecological and health risk assessment process by deciphering toxicity mechanisms and modes of action [7-9], identifying biomarkers of exposure and toxicity [10], studying toxic effects and environmental diseases [11, 12], and facilitating cross-species extrapolation [13, 14]. A combination of genomic, proteomic, and bioinformatic approaches was also used to study toxic mechanisms of fungicides [15], and toxicity pathways of endocrine disrupting chemicals with different known or unknown toxic modes of action [16]. These integrated OMICS studies provided novel insights into toxic mechanisms and/or modes of action of environmental chemicals. Data from these experiments were used for human health risk assessment of the chemical, and established the basis of toxicity prediction approaches for species, endpoint, and chemical extrapolation.

In addition to the expression profiling studies, identification of modifications at the gene, protein, and metabolite levels is another important application of OMICS to environmental toxicology and human health research [17, 18, 19]. It is known that protein carbonylation and phosphorylation are among the major signal transduction pathways in cell biology. They are currently being studied to determine whether they are also the key to estimate toxicity pathways for environmental pollutants. For example, to understand the contributions of oxidative stress to toxicity, an integrated proteomics approach involving the identification of carbonylated proteins was utilized for the systematic measurement of protein oxidation in the livers of propiconazole-treated mice [17]. This study suggested a mode of propiconazole-induced toxicity in the mouse liver that primarily involves oxidative damage to cellular proteins. In another set of experiments, Enan and Matsumura [20] reported that the environmental chemical 2, 3, 7, 8-tetrachlorodibenzo-*p*-dioxin (TCDD) at very low concentrations was found to cause a rapid rise in protein phosphorylation activities in the extranuclear fraction of the adipose tissue from male guinea pigs. They are currently being investigated to determine whether they are also the key to estimate toxicity pathways for environmental pollutants, and have received comparatively more attention than other protein modifications in environmental toxicology research.

Table 1 is a summary of the environmental OMICS articles that were published from June 2011 to June 2012. In the past year, 611 genomic, 231 proteomic, and 84 metabolomics studies have been published based on a search of PubMed. Publications based on the chemicals, subjects, purposes, experimental systems, and goals that were used in these studies were also categorized to get an overview of the developing

Table 1. Summary of environmental OMICS publications from June, 2011 to June 2012

		Transcriptomics	Proteomics	Metabolomics	Systems Biology
No. of publications		611	231	84	598
% of publications	*Single chemicals*	70	98	86	96
	Chemical mixtures	30	2	14	4
	Health	72	52	46	38
	Ecosystem	28	48	54	62
	Mechanisms	30	34	8	8
	Pathway/biomarkers	70	66	92	92
	In vitro	56	42	40	48
	In vivo	44	58	60	52
	Environmental monitoring	80	52	54	58
	Health risk assessment	20	48	46	42

trends in environmental OMICS. The criteria to categorize the published literature were whether known contaminants were studied either as single chemicals or mixtures; whether risk factors for human health or environmental damage should be clarified further, and whether mechanisms (modes of action) and pathways were studied that are beyond the parameters for approval of hazardous properties. As shown in Table 1, genomics (611 publications) and proteomics (231 publications) are still the major "work horses" in environmental OMICS. Surprisingly, the number of environmental OMICS studies using a systems biology approach was ranked the second (598 publications), suggesting a systems biology approach is becoming increasingly important to environmental research. In addition, environmental OMICS studies focusing on toxicity pathways, biomarkers, and adverse health outcomes received more attention than studies focusing on either toxicity mechanisms or ecosystem toxicity. Interestingly, *in vitro* and *in vivo* experimental systems are almost equally used in the OMICS studies. However, OMICS studies focusing on chemical mixtures were much less numerous than studies focusing on individual chemicals. As for the techniques used in OMICS studies, it is worth mentioning that the percentage of the environmental proteomic studies using mass spectrometry-based approaches was almost the same as those using 2-D gel based approaches. This suggests that the previous dominance of the 2-D gel electrophoresis as a proteomic tool is waning.

While substantial progress has been made in certain areas of environmental OMICS, further improvements are still needed to facilitate the incorporation of OMICS into environmental toxicology and health research. There are a number of experimental design, sample collection, technical implementation, and data interpretation issues that impede the use of OMICS approaches in environmental and health research. In environmental OMICS research, many biological samples come from outbred individuals, populations, and communities. The field of environmental OMICS is thus a diverse and heterogeneous discipline, often involving a complex array of organisms and multifactorial experiments that can have an extremely large number of measurable parameters. Therefore, environmental OMICS strategies and approaches for the selection

and control of measurable parameters, as well as the identification and capture of essential information associated with environmental toxicity and health risks, should be established. Technical guidelines for standardization of OMICS research procedures are crucial, which need to cover sampling, technical data analysis, and interpretation of results, as well as the definition of cut off criteria, reference points, and normal values.

This paper provides an overview of recent environmental OMICS research including the presentations at The First International Conference on Environmental OMICS (ICEO) with an emphasis on the applications of genomics and proteomics technologies and methodologies to environmental science and health research. In the following discussions, we do not attempt to be exhaustive, but rather focus on some representative and traditional genomics and proteomics research topics such as OMICS characterizations of chemical toxicity, oxidative stress, and protein post-translational modifications. We also discuss some emerging research topics such as OMICS characterization of chemical mixtures and OMICS-based environmental monitoring and adaptation. Some comments on the challenges, potential solutions, and future directions in these research fields are also provided in this review. Our aim in writing this review is to stimulate interest in a bold, new, scientifically rigorous, and comprehensive environmental OMICS strategy for environmental toxicology and health research.

2. Environmental OMICS research issues

2.1 *OMICS-based vs. traditional environmental toxicology*

Traditional environmental toxicology and environmental health research mainly focus on the effects of environmental chemicals on the functions of various organ systems or toxicity phenotypes and modes of actions of the chemicals at cellular levels, usually relying on animal models for these studies [5, 21]. In addition to being labor-, time- and resource-intensive, this approach is primarily descriptive in nature and is low throughput and unable to characterize the full spectrum of targets and toxicity mechanisms for chemicals that affect multiple systems. To understand the toxicity mechanisms and/or modes of action, there is a need to understand the toxic processes at the molecular level. This will involve integrating genomic, proteomic, and metabolomic technologies for toxicology research.

Genomics was probably the first OMICS approach to be applied to mechanistic studies of toxicity, toxicity pathways, tumor biomarkers, and carcinogenicity. These studies involved environmental chemicals and their mixtures and mainly focused on characterization of the levels of gene expression that is reflected by the abundance of specific mRNA transcripts in a biological sample [22]. Gene expression profiling was used to compare the expression profiles of groups of genes with and without exposure to environmental chemicals. Although gene expression profiling experiments, especially those involving microarray technology, revolutionized numerous aspects of biological research and enabled thousands of gene transcripts to be monitored simultaneously, transcriptional responses often do not accurately reflect important toxicologically relevant biological responses. This could be due to the fact that there are important changes in proteins and metabolites within cells that are not detectable by just studying the levels of mRNAs. Therefore, proteomics and metabolomics are often used to complement genomics for toxicological and health studies [16, 3].

Proteomics is the large-scale study of protein expression and related biological functions. Proteins are ultimately functional molecules involved in most cellular processes. Toxic responses are driven by interactions between chemi-

cals and biomolecular targets, many of which are proteins. Most toxicological endpoints are preceded by changes of protein expression. Therefore, proteomic-based toxicity studies and biomarkers are highly-relevant to biological functions, adverse health outcomes, and health risk assessment.

Metabolomics, the study of metabolic profiles consisting of small metabolites, is the latest addition to the OMICS family. Metabolites can be collected from urine, saliva, and plasma. The formation of metabolites is probably the final manifestation of gene expression alterations. Therefore, metabolomics is a potentially useful tool for characterizing phenotypes under normal physiological and pathological conditions. Metabolic profiling supports and confirms the mechanisms derived from genomics and proteomics [23].

Although genomics, proteomics, and metabolomics target different molecules that may regulate and control biochemical pathways, as well as biological activities and events at different levels, the informative OMICS data of mRNA, proteins, and metabolites need to be integrated to achieve an effective and comprehensive understanding of modes of action and mechanisms of chemical-induced toxic responses and disease processes.

Implementation of OMICS technologies into environmental toxicology and environmental health research has been catalyzed by the report of the U.S. National Research Council (NRC) on Toxicity Testing in the 21st Century [24]. This report described how OMICS technologies could dramatically increase the efficiency and accuracy in evaluating both chemical toxicity and adverse human health outcomes through the development of novel OMICS toxicological endpoints, toxicity pathways, and biomarkers for chemical toxicity testing. Researchers working on environmental toxicology and health are increasingly turning to the application of OMICS technologies to answer fundamental questions in environmental sciences [15, 25].

The major objectives of environmental OMICS include elucidation of molecular mechanisms of toxicity, xenobiotic interactions with biological systems, and identification of mRNA, protein, and/or metabolite signatures or biomarkers for chemical toxicity testing, environmental monitoring, and human health risk assessment. Human health risk assessment evaluates long term effects of exposure to contaminants, frequently giving special attention to individual sensitivity such as preexisting disorders. For ecotoxicology, the focus is on monitoring of exposure and effect, which depends on the strengths of biomarkers and signatures for the stability of local and regional ecosystems. Additionally, environmental OMICS technologies have been widely used to address a variety of environmental and human health issues, such as effects of climate changes on different species [26], plant and animal responses to complex environmental stressors in soil and air [23, 27], effects of marine stressors such as acidification and hypoxia on sentinel organisms [28], chronic or acute exposures to metals in aquatic organisms and humans [29, 30], effects of exposure to novel pollutants such as nanomaterials [5, 31] and environmental microorganisms [32]. OMICS can often detect molecular changes before the appearance of visible morphological or physiological changes and thus can predict toxicity and reduce the time needed for more traditional toxicity testing.

Current applications of OMICS to environmental toxicology mainly focus on identifying and relating changes at RNA, protein, and metabolite expression levels, and how these factors reflect changes in networks or pathways in cells or tissues after exposure to toxicants with known adverse health outcomes. The ability of OMICS to efficiently identify the molecular changes within biological samples provides valuable information for understanding toxic processes, pathways, and mechanisms, which are the major focuses of traditional environmental toxicology.

OMICS approaches can also help to address many of the challenges that cannot be easily tested using traditional environmental toxicology approaches, e.g. toxicity data extrapolation to determine whether chemicals will affect human health. To date, many chemical toxicity testing approaches are primarily based on endpoints derived from animal toxicity studies. OMICS could be the solution for toxicity data extrapolation across species and doses. Among various species and different types of cells and tissues, it is remarkable that a common subset of genes involved in a set of conserved signaling pathways are conserved through evolution, Therefore, an extrapolation of potential toxic responses and toxicity outcomes across species and doses could be done at the molecular level through the comparison of OMICS data sets that reflect mRNA, protein, and metabolite levels. Combination of OMICS data from mRNA, proteins, and metabolites, cells, tissues, individuals, and ultimately populations will help to develop a much-improved predictive capacity for toxicity data extrapolation and health risk assessment.

2.2 *Environmental OMICS of single chemicals vs. chemical mixtures*

2.2.1 Single environmental chemicals

Much of current environmental OMICS research focuses on the toxicity of single chemicals. One reason for this is that the experimental methodologies, approaches, and technologies for studying the toxicity of single chemicals and their risks to human health are well-established. Arsenic is probably one of the most common and well-studied single environmental chemicals [33]. It is found ubiquitously in our environment including drinking water, foods, soil, and airborne particles, and there is a generally well-accepted dose-response relationship between arsenic ingestion and cancer incidence [34, 35]. Microarray-based expression profiling of the livers of zebrafish exposed to arsenic revealed global transcriptional changes and suggested that DNA and protein damages due to arsenic metabolism and the arsenic-induced oxidative stress are the major causes of cellular injuries observed in the liver [36]. Many genes encoding proteins in-

volved in DNA damage/repair, antioxidant activity, hypoxia induction, iron homeostasis, arsenic metabolism, heat shock proteins, and ubiquitin-dependent protein degradation were found to be differentially expressed [36]. cDNA microarray and enzyme-linked immunosorbent assays were also used to identify genes involved in arsenic-associated atherosclerosis [37]. Arsenic was also found to activate stress gene expression [38, 39] and to induce cell proliferation and apoptosis pathways [40, 3]. Using 2-D gel electrophoresis and MALDI-TOF-based proteomic approaches, several up-regulated proteins, including α-enolase, HSP90β, pyruvate kinase, aldolase reductase, GAPDH, phosphoglyceratemutase B, Cu-Zn SOD, and thioredoxin were identified in LEC transformed cells. Several proteins including intermediate filament proteins such as peripherin, cytokeratin 14, and cytokeratin 8 were down-regulated [41, 42]. Recently, RNA sequencing was used to acquire global transcriptome alterations and miRNA regulation in rice under As (III) treatment at different times and dosages to investigate the metabolic and regulatory network and their interactions in the plant [43]. Additionally, proteomics in conjunction with morphological, physiological, and biochemical analyses have been employed to unravel for the first time survival strategies of the diazotrophic cyanobacterium anabaena sp. PCC7120 under arsenic stress [44]. These studies together with other published studies on arsenic toxicity typically reflect many common interests, approaches, and potential problems that were found in the OMICS analysis of single environmental chemicals.

The focuses on single chemical OMICS has been on the identification of toxic pathways, mechanisms, and biomarkers of the chemicals. Previous studies on the changes of various genes, proteins, metabolites and their underlying toxicity pathways and toxic mechanisms in various tissues, organs, or in whole organisms in response to exposure to arsenic or other single chemicals have demonstrated the importance of incorporating OMICS data into the regulation frameworks for environmental chemicals. However, with few exceptions, to date most of these OMICS studies on arsenic and other single chemicals have been limited to a qualitative description of alteration in gene and protein levels from *in vitro* cells and animal tissues exposed to the chemicals at doses that are much higher than environmental exposure doses, with minimal reporting regarding the biological outcomes and with little correlation to toxicity or the contribution to human health risk assessment. To solve these problems, innovative approaches on how to differentiate between specific and unspecific changes in the genome, proteome, and metabolome, and how to decide the relevance of such changes for further toxicological research and biomarker identification, need to be developed. The appropriate use of controls including negative and positive controls, time points, and treatment dosages of single chemicals for OMICS studies is important for the evaluation and interpretation of the research results. Typically, toxicity is a persistent and easily identified endpoint. However, genomics, proteomic, and metabolomic responses are dynamic and only capture expression of mRNAs, proteins, and metabolites at a certain time point. Therefore, comparison of OMICS data requires sampling at multiple time points. The OMICS profiles collected at different time points also can help with the identification of true toxicity-specific changes. Analyzing samples treated with different dosages of toxicants is also very important in identifying toxicity-specific changes. Higher doses might provide additional sensitivity that could help in the initial identification of significant effects, while low dosage sample analysis could help establish thresholds that need to be exceeded prior to the initiation of the cascade of molecular responses leading to an adverse or toxic effect. Changes that are consistently presented in the OMICS expression profiles obtained from samples treated with different dosages of toxicants would indicate a toxicity-specific change. In addition, computational toxicology, bioinformatics, and system biology tools are also needed to integrate and model the complex OMICS data sets in order to understand the biological activities and toxic processes of other chemicals that lead to environmental toxicity and risks to human health.

2.2.2 Chemical mixtures

Humans are exposed to multiple chemicals. This may occur in the form of mixtures of chemicals, where multiple chemicals occur in a given environmental medium, or as a cumulative exposure, where multiple chemicals are encountered from multiple environmental media via multiple exposure routes [45]. For example, chemical disinfectants react with naturally occurring organic and inorganic matter in water to produce a wide variety of disinfection byproducts (DBPs), and more than 500 DBPs have been identified. Beyond environmental exposure scenarios, tobacco smoking is one of the most prominent drivers for damage to human health. Tobacco smoke contains more than 4000 chemicals, and at least 200 of them are toxic to humans and over 50 are recognized as known or probable human carcinogens [46]. Tobacco smoking remains a major public health problem, threatening the lives of over one billion people during this century, and tobacco use is estimated to kill more than 5 million people each year worldwide [47]. Some intrinsically complex mixtures such as diesel exhausts, welding fumes, coke oven emissions, and metal working fluids are routinely encountered in occupational settings. Chemical mixture effects are therefore a major issue in the environmental and health risk assessment of chemicals [48] and studying the environmental toxicity of chemical mixture and risks of chemical mixtures is more important than of individual chemicals.

However, to date very few biological systems and technologies have been available and suitable to address the toxic mechanisms and toxicity pathways of exposures to chemical mixtures. Such biological systems and technologies are needed to decipher all of the interactions among complex mix-

tures at molecular, cellular, and organ levels, which are critical to the toxicity characterization and risk assessment of chemical mixtures. The dilemma of the lack of biological systems, technologies, and scientific information versus the perception of the high risk from exposures to chemical mixtures poses an enormous challenge for the public health and risk assessment community. Therefore, there is an important need to develop novel and biology-based methodologies and approaches for both efficient analysis of environment toxicity pathways, biomarkers, and toxic mechanisms associated with exposure to chemical mixtures and accurate distinction of the chemicals in the mixture that present little or no concern from those with the greatest likelihood of causing an adverse effect in the target species. With support of OMICS-based technologies, this information gap can be filled.

Although approaches for determining the mechanisms by which a single chemical induces toxicity or carcinogenicity have been relatively well established, these approaches cannot be readily extended to the study of chemical mixtures. High-throughput and high-content OMICS technologies and methods applied to predictive toxicology provide opportunities to address these challenges. First of all, OMICS tools have the potential to improve our understanding and predictability of combined effects. The interplays between environmental stresses and the dynamic responses of organisms at the levels of genes, RNAs, proteins, and metabolites can be efficiently determined using OMICS technologies. Second, OMICS enables high throughput analysis and can identify multiple molecular targets, pathways, and environmental responses to exposed organisms simultaneously, which is critical to understanding the modes of actions of chemicals and determining the components that actually cause toxicity in a mixture. Third, OMICS offers great potential to identify novel molecular toxicity endpoints for identification, categorization, and prioritization of chemical mixtures [25, 49]. In a study of gene expression profiles of rainbow trout exposed to a simple mixture of chromium, 2,2,4,4-tetrabromodiphenyl ether, and 17b-ethynylestradiol, no single compound dominated gene expression profiles, and the toxicity of the mixture was not simply the sum of the toxicities of the individual chemicals [50]. However, combined effects of polyfluorinated and perfluorinated compounds on primary cultured hepatocytes from rain minnows were observed in a genomic study [51]. Thus, the relationships among the expression patterns, chemical interactions, and ultimate mixture toxicity are very complicated. Exposure to mixtures can result in common response [52], synergistic and antagonistic interactions depending on the genes [52, 53], and/or unpredicted combined effects with unique transcriptional signatures [54].

Proteomics and metabolomics have also been used to characterize chemical interactions and mixture toxicity [55-57]. These studies focused on the identification of protein or metabolite signatures associated with the toxicity of both individual chemicals and mixtures. This approach attempted to distinguish the exposure signatures of individual chemicals in the mixtures so as to identify those chemicals in the mixture that present major concern and the greatest likelihood of causing an adverse effect. The information generated from such studies both improves the certainty about the assumptions in predictive models used to quantify the environmental toxicity of chemical mixtures and helps to refine our current exposure monitoring and assessment of chemical mixtures. In addition, the integration of genomic, proteomic, and metabolomic approaches is important for improving our understanding and predictive capability of the combined effects of chemical mixtures. Presently there is an urgent need to develop well-accepted conceptual frameworks and standards, including experimental design, data interpretation, and modeling for the use of OMICS tools to study chemical mixture toxicity and associated risks to our environment and human health.

2.3 *Molecular modifications of genes, RNAs, proteins, and metabolites*

Genes, mRNA, proteins, and metabolites are frequently modified in response to environmental stresses. It has been widely recognized that knowledge of a gene, mRNA, or protein and its sequence is just a prelude to understanding the role of that molecule and its product within the cell and is only a starting point for a full description of its function. While the modified form of the molecule is essential to the understanding molecular functions and mechanisms, it is expected that more and detailed studies on the modified forms of the genes and functional molecules under a variety of environmental conditions will be carried out.

Oxidative stress and protein oxidation are examples that stress the importance, challenges, and directions of OMICS applications to the identification of gene and protein modifications. Environmental stressors frequently result in formation of ROS, and proteins absorb about 70% of the ROS [58]. Therefore, using proteomic technologies to detect protein changes and modifications in response to environmental pollutant-mediated oxidative stresses has been an important research topic. Redox proteomics is probably one of the most extensively studied areas for characterization of protein modifications under environmental stresses. Sheehan et al. [59] developed a "toolkit" of redox proteomic approaches that can detect quite low levels of redox lesions in two dimensional electrophoresis separations. An example of this approach is the use of activated thiol sepharose to select for protein thiols or redox variants, such as disulphides, using both 2-D gel electrophoresis and gel-free shotgun proteomics [60]. Oxidation of cysteine residues may not always result in an effect on protein function, and sometimes is a reversible process analogous to protein phosphorylation [61]. Protein thiol modification is an important signal transduction mechanism regulating oxidative and antioxidative processes and/or events [62]. In addition to protein thiol modifications, protein carbonylation in response to environmental exposures and stresses has also been studied exten-

sively [63, 64].

Carbonyls are relatively difficult to induce as compared to cysteinyl derivatives and may reflect more severe oxidative stress [59]. Protein carbonylation is generally associated with permanent loss of protein function and has been used as a marker for assessing oxidative damage [65]. Although technologies for the detection of oxidized proteins have been advanced recently and offer some advantages for identifying oxidative stress biomarkers, studies on the identification of oxidized protein biomarkers to determine chemically-induced oxidative stress and injury are still severely lacking. As proteomics studies progress, the goal will not only be to identify proteins in mixtures, but also to derive more information about protein modifications from the samples analyzed. The ability to identify oxidized proteins will yield in-depth information on protein structure and function, which, in turn, can facilitate the pace of studies to understand toxic processes, pathways, and mechanisms of environmental chemicals. In addition to protein modifications, RNA and metabolite modifications could also function as mechanistic linkages between environmental exposures and outcomes and serve as important biomarkers of environmental exposure, toxicity, and effects. However, little is known about the RNA and metabolite modifications.

2.4 *Environmental monitoring and health risk assessment*

Pollution is one of the most important environmental challenges we are currently facing. Some environmental chemicals, like hormones or drugs, can act at very low doses to disrupt ecological balance and threaten growth and development of precious species with knock-in effects on biodiversity and ecological services [66]. Environmental chemicals can also damage human health directly or indirectly through food chains. Monitoring of chemical toxicity and risk in ecological systems is important to natural resource protection, environmental sustainability, and human health. Assessments of ecosystem health play essential roles in the development of effective strategies for not only protecting the environment but human health as well. In aquatic ecosystems from oceans to river basins, water assessment has focused on providing specific information regarding the dynamics of pollutants and their effects on the health of different species.

Classical biomonitoring programs directly quantify the bioaccumulation of pollutants in tissues of exposed organisms or analyze different effects or responses to the xenobiotic in those exposure organisms. Bivalves have been extensively utilized as sentinel organisms due to their sessile nature, filter feeder habits, and high capacity to accumulate contaminants, providing therefore temporally and spatially integrated levels of contamination [67]. In the last decade, proteomics in bivalves has greatly contributed to the identification of more specific and sensitive markers of pollution, thereby providing an accurate estimation of ecosystem health [68, 69]. Historically, mussels have been utilized to evaluate a broad range of hazardous compounds such as metals [70], polycyclic aromatic hydrocarbons [71], anthropogenic pollution [71], and the biological consequences of oil spills in marine environments, such as after the accidents of the Exxon Valdez in Alaska [72] and Prestige in NW Spain [73]. The main reason that hinders the application of bivalve proteomics for large biomonitoring programs is the absence of an assembled and annotated genome sequence from bivalves. MS–proteomics approaches require a fairly complete genome annotation in publicly available databases, and unfortunately sequences from genes and proteins from bivalves are still scarce. Greater information could be obtained from mtDNA [74, 75]. Alternately, the development of assays for multiple reaction monitoring (MRM) or selected ion monitoring (SIM) could be explored. These approaches could improve environmental assessment and biomonitoring. In a more restricted way, this strategy has been used to analyze the toxin profile of *M. galloprovincialis* collected from an area with remarkable concentration of *Alexandrium ostenfeldii* cells in seawater [75].

Although the analysis of the effects of environmental stressors utilizing bivalves as sentinel organisms could be considered a pioneer area of research in environmental proteomics, it lags behind the achievement of proteomics in biomedicine. Important aspects of proteomics that were discussed in the First International Conference on Environmental OMICS included strategies to join efforts for the inter-laboratory validation of protein expression patterns (PES), developing proteomics methodologies for the verification or validation of selected target candidates, evaluating pollutant mixtures for possible synergies and modes of action, and systematically exploring protein translation modifications. Finally, the next generation of sequencing technologies has started to develop for environmental issues and applications.

The advantages of bivalves should not be forgotten for marine pollution assessment. The research in bivalves has been historically one of strongest research areas in biomonitoring, because the sessile nature of bivalves could provide a better correlation between analyzed stressors and topographic information [76]. The integration of genomics and proteomics data and the application of EST data for protein identification could hopefully improve biomonitoring programs and define the mechanisms underlying pollutant toxicity.

Environmental monitoring and health risk assessment require different and specific biomarkers, because environmental contaminants induce multiple responses in organisms that are not necessarily correlated. OMICS technologies offer the promise of fast, cost-effective, and broad-scale data gathering capacity, all of which are necessary to be able to identify environmental biomarkers and establish critical links between exposure, response, and disease. By comparing OMICS profiles of environmentally-exposed cells, tissues, and organisms to a database containing profiles induced by known toxicants, environmental biomarkers of exposure and toxicity were identified [77]. OMICS technologies are very

efficient and powerful in identifying environmental toxicity biomarkers. This approach is a popular application in ecotoxicology research. Although several candidate biomarkers for environmental monitoring have been reported [78], they must be validated for their potential usage in risk assessment, since these biomarkers could be the result of artifacts of specimen collection, bioinformatics bias, and/or experimental variations rather than truly toxicological responses.

In addition to the generation of large and information-rich datasets on the changes at expression levels of genes, proteins, and metabolites for the purpose of identifying environmental biomarkers of exposure, toxicity and effects of environmental chemicals, establishment of suitable experimental model systems for monitoring of environmental and health risks under different pollution situations or scenarios is also very important. The zebrafish is a well established experimental model for environmental monitoring and risk assessment, because it offers a combination of the advantages of *in vitro* and *in vivo* systems and many well-developed genetic tools including transgenesis. In recent years, the zebrafish has also been used to model human diseases, because the zebrafish is in the vertebrate family and shares many fundamental similarities in body plan, organ systems, physiology, pathology, and diseases with other vertebrates [79].

Two general approaches have been used to develop zebrafish-based environmental monitoring tools. The first approach is to employ selective, inducible promoters to generate transgenic fish that respond to environmental pollutants by expressing visible fluorescent colors [80]. The second approach is to employ zebrafish DNA chips or next generation sequencing to identify biomarkers from fish exposed to different environmental chemicals. In this way, these fish biomarkers can be used to identify environmental pollutants and to infer associated toxicity effects and risks [81], In two independent genomic studies [82, 83], zebrafish were exposed to polycyclic aromatic hydrocarbons, organic nitrogen compounds, organochlorine pesticides, endocrine disrupters, and metallo-compounds, and gene expression changes in exposed zebrafish were characterized. Both studies reported that chemically treated samples could be correctly grouped base

d on the hierarchical clustering of transcriptomic data, and subsets of genes could be linked to specific exposures [83, 82]. A recent proteomic study of zebrafish revealed that chronic toxicity of microcyetin is different from acute toxicity, and the reactive oxygen species pathway is the main toxic pathway [84]. Moreover, it has been determined that microcystin causes neurotoxicity in zebrafish at the proteomic level.

In addition to zebrafish, application of genomics to several other organisms such as fathead minnow (*Pimephales promelas*), medaka (*Oryzias latipes*) and several invertebrates such as water fleas (*Daphnia magna*) and soil nematode (*Caenorhabditis elegans*), have also been employed as tools for environmental monitoring [85, 86]. With the application of next generation sequencing technology in ecologically relevant organisms, ecotoxicogenomics and environmental risk assessment have been shown to be more feasible and productive [87].

Although significant accomplishments have been achieved in the OMICS-based environmental monitoring, there are still many important questions to be addressed and challenges to be overcome in the field of environmental monitoring. These include how to deal with the lack of sequencing data in environmental model organisms, how to validate the environmental monitoring biomarkers, and what validation criteria should be used.

2.5 *Meta-omics: a high throughput tool to study microbial community in ecosystems*

To solve environmental challenges and to make our natural environment sustainable, there is an urgent need to develop reliable and practical experimental technologies, systems, and methods for evaluating the large variety of unknown microbes in nature. Application of OMICS to systematic analysis of environmental microbial communities has been used to identify novel catalysts that can degrade environmental pollutants, to purify contaminated natural resources, to produce novel bioproducts, and to identify novel biomarkers for environmental risk monitoring. This has resulted in the emergence of new research fields: metagenomics, metaproteomics and metametabolomics. Since the publications in metagenomics have been previously reviewed [88, 89] and very few studies on metabolomics have been published, here we provide a brief review on some recent publications in metaproteomics and provide some recommendations for future meta-omics research.

Tringe et al. [90] investigated the collective environmental signature obtained from different microbial ecosystems using a gene-based bioinformatic approach and found that the predicted protein complement of a community is influenced by its environment. Since then, there have been a few studies describing metaproteomics, including the examination of protein expression profiles from activated sludge [91, 92], freshwater samples following exposure to heavy metals [93], contaminated soil and groundwater [94], endosymbiont [95], lake water [96], and extracellular proteins in activated sludge [97]. Notably, an intensive proteomic study of acid mine biofilms has been performed, in which approximately 2200 proteins were identified with one novel protein as a key component of energy conservation in that environment [98].

Despite the limited number of investigations, the metaproteomic approach has already highlighted its potential for providing functional insight into overall microbial ecosystem function. Kan et al. [99] introduced the metaproteomics approach to study a microbial community collected from the Chesapeake Bay. Power et al. [100] investigated dissolved proteins in seawater. Following these studies, metaproteomic studies on various marine environmental samples were conducted, and many metabolic and physiological activities including nutrient utilization and environmental adaptation,

were revealed [32]. Metaproteomics were also applied to the examination of protein expression in complex marine environmental samples, thereby opening a new window for marine microbial oceanography and microbial biogeochemistry [101]. Recently, it has been reported that new species of microbes feeding on the Deepwater Horizon oil spill may exist based on the oil spill degradation rate [102]. However, the analysis of microbial community responses to the oil spill has suffered from the lack of a reliable and comprehensive microbial database and analytical techniques and approaches. Meta-omics could potentially fill this scientific gap and generate the scientific data and basis for assessing ecological risks associated with oil spills and other environmental disasters. It could provide novel insights into the molecular bases and mechanisms through which microbes respond to environmental perturbations and produce microbial enzymes for the biodegradations of the spilled oils in oceans and other aquatic environments.

Our natural environment contains a large community of microbes that are already adapted to the background supply of environmental pollutants. Eventually, the microbes will "take care" of the pollution problem by consuming the pollutants that are biodegradable. Environmental OMICS provides a high-throughput and high-resolution tool to glean a more complete picture of microbial community composition, and to identify novel catalysts and microbes that can biodegrade environmental pollutants. Therefore, meta-omics research offers the potential to provide a solid scientific foundation for the understanding and management of environmental pollution and for the utilization of natural microbial resources to maintain environmental sustainability.

3. Conclusions

Environmental OMICS approaches have already had a big impact in helping to identify toxicity pathways, toxicity mechanisms, and environmental biomarkers for health risk assessment. Additionally, OMICS approaches have impacted environmental monitoring and sustainability through high throughput and simultaneous measurement of multiple genomic, proteomic, and metabolomic profiles and parameters in a given system under defined environmental conditions. Environmental OMICS is still in the early stage of OMICS data collection, and the field is transitioning from "profiling OMICS" to "functional OMICS."

Beyond providing a list of molecular changes, OMICS approaches emphasize the biological significances of the identified molecular changes, which is key to the success of environmental OMICS development. In the future, environmental functional OMICS approaches may help to solve environmental challenges, such as the elucidation of chemical mixture toxicity, protein modifications, environmental and health monitoring, and meta-omics.

The development of research standards, guidelines, and frameworks for sample collection and OMICS data generation, collection, validation, interpretation, and presentation will need to be made in the near future. Other needs identified for environmental OMICS are the combined analysis of multiple OMICS data sets, including genomics, proteomics, and metabolomics, and the integration of OMICS data with toxicity data to define the links between OMICS data and particular toxic processes or environmental diseases under investigation. Integration of OMICS data with classical toxicology endpoints and clinical observation will allow more sensitive and earlier detection of adverse health effects, precise identification of toxicity signatures and biomarkers, and development of predictive environmental toxicology for more effective environmental biomonitoring and human health risk assessment.

To accomplish these goals, international collaborations among environmental OMICS scientists worldwide is needed. These collaborations should also include partnerships between governmental agencies and nongovernmental research groups in both academia and industry.

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During the conference, it was proposed to establish an International Society for Environment OMICS (ISEO) to consolidate different environmental OMICS research laboratories, groups, and organizations worldwide, to engage in scientific and educational activities that promote environmental OMICS research and technologies, and to assist in coordinating shared public environmental OMICS initiatives.

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