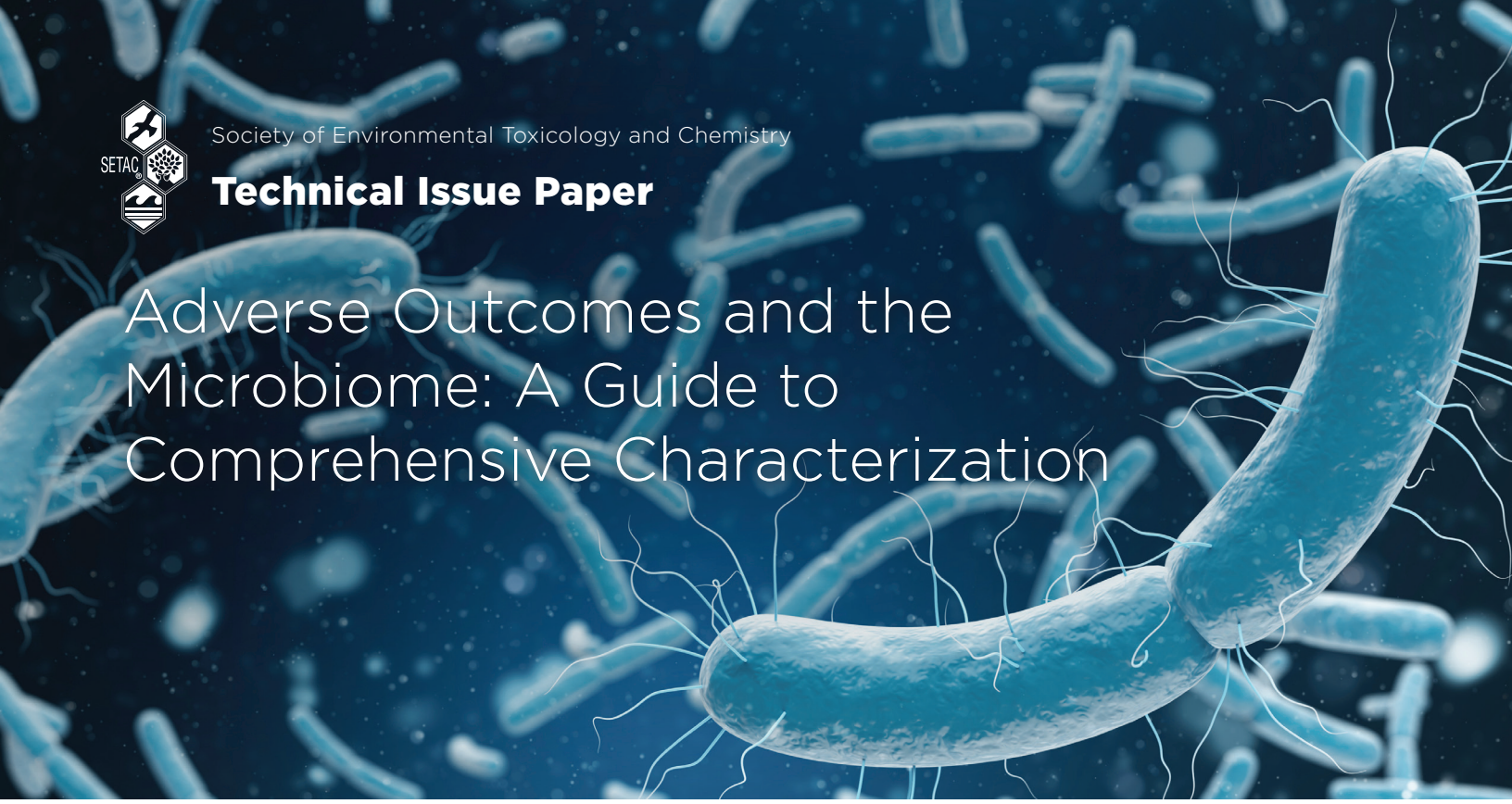

Society of Environmental Toxicology and Chemistry

Technical Issue Paper

Adverse Outcomes and the Microbiome: A Guide to Comprehensive Characterization

Introduction to the Microbiome

The term microbiome refers to the collection of microorganisms, including bacteria, archaea, fungi, protozoa and viruses, that reside in an environmental niche (e.g., water or soil) or that inhabit different intracellular or extracellular spaces or tissues of an organism (e.g., gut, lung or skin) in a mutual relationship with the host. Microorganisms, present on the surface or within an organism, form complex microbial communities. Microbial communities have been characterized in different tissues of aquatic and terrestrial organisms, including the gills, skin, gastrointestinal tract and swim bladder in fish; gastrointestinal tract and body surface in crustaceans; and skin, brain and gastrointestinal and reproductive tracts in mammals, among others.

Microbial communities can regulate physiological homeostases. More specifically, microbial communities modulate host physiology via metabolites produced by the microorganisms, many of which would not be present otherwise. These microbiomes and their metabolic products influence a wide range of biological functions, and can provide immune defence from pathogenic microorganisms, facilitate nutrient processing and uptake, and actively participate in the metabolism of toxicants. Disrupting the balance of a microbiome in harmony with its host can cause "dysbiosis," which often leads to disproportional metabolites and has been associated with the occurrence of diseases such as obesity, diabetes, cancer, neurodegeneration, metabolic syndrome, and multiple sclerosis in mammals. Microbial community imbalance is also associated with chronic inflammation, a hallmark of many diseases.

It is important to note that the composition and diversity of the microbiome is not conserved across all organisms or individuals. Organisms belonging to the same population or species typically share a "core microbiome," especially at the phylum level. This has been well documented in mammals where Firmicutes, Proteobacteria, Fusobacteria, Bacteroidetes and Verrucomicrobia represent the most dominant phyla in the gut. Evidence for a core microbiome in aquatic organisms are now being identified in model species, such as zebrafish (*Danio rerio*) and other species of economic and ecological importance. More specifically, the core microbiota of zebrafish has been found to be made up of β-Proteobacteria, β-Proteobacteria, Fusobacteria, Bacilli, Flavobacteria and Actinobacteria classes with *Aeromonas* and *Shewanella* appearing as the most frequent genera. Despite the presence of a core microbiome, important differences arise in microbiome diversity, and abundance and many factors, such as development, life history, sex, diet, disease status, geography and environment, confound identification of core

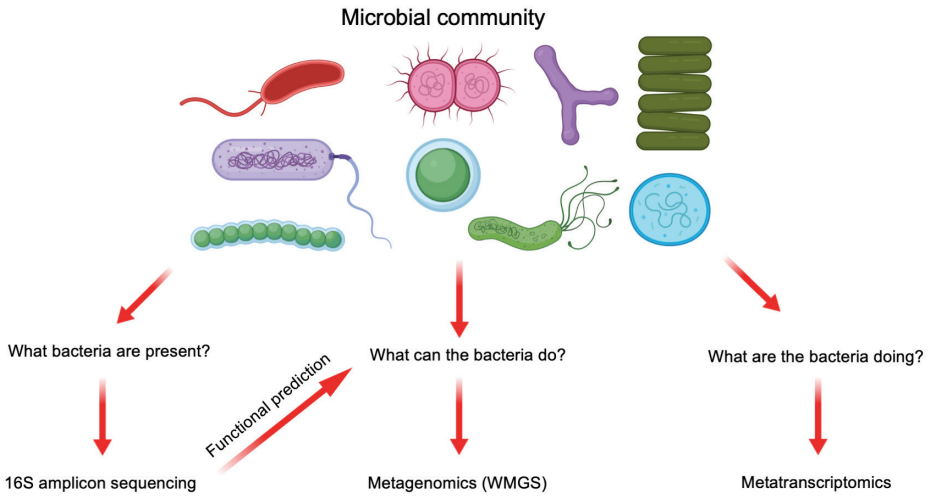

Figure 1: Overview of the different methodologies employed for the study of the microbiome.

microbiomes among species. Indeed, the microbiome of farmed and wild fish can differ as a consequence of diet and environment. Even within individuals of a given population, interindividual variability in the composition and function of the microbiome must be considered when developing intervention strategies. In addition, the functionality of the species within a phylum or a class may significantly differ from one another. Given these considerations, the microbiome is a complex ecosystem regulated by different factors that requires rigorous examination in order to be able to successfully describe the functional dynamics that modulate host physiology.

Methods to Investigate the Microbiome

The standard approach for the evaluation of the effects of a stressor on the microbial community includes measuring microbial diversity via diversity indexes. Alpha diversity indexes are based on the number of detected species within the sample, and beta diversity indexes are focused on the differences among samples. Both indexes provide insight into the taxonomic diversity and serve as a quantitative measure of a specific change in microbial composition. For example, identifying the dominant bacterial taxa may indicate specific stressors or adverse outcomes, specifically if there is an increased abundance of pathogenic bacteria.

While taxonomic profiling allows for identification and quantification of the bacterial components present down to the genus level, it does not provide information on how changes in the numbers and species present will influence toxicity from a mechanistic and functional standpoint. This is where collaboration between metabolomics and microbiome research can significantly advance the field of toxicology and the microbiome. Recently developed computational tools, such as PICRUSt, Tax4Fun, and Paprika, predict functional composition of the metagenome i.e., the metabolic and biosynthetic pathways involved in the production of bioactive metabolites. To achieve deeper understanding of the effects of a chemical stressor on the production of microbial metabolites, scientists can employ other computational tools to examine host–microbiome interactions such as those that predict the relative changes in the production or consumption of metabolites to model microbiota metabolome alterations. This allows toxicologists to make linkages between changes in the composition of the microbiome and impacts on the health of an organism.

Microbial profiling by 16S sequencing remains one of the most employed methodologies; however, reduced cost and recent advances in sequencing technology have enabled the advancement of both whole metagenome shotgun sequencing (WMGS) and metatranscriptomics. WMGS, also known as community genomics, targets all DNA material in the sample and provides detailed insight into the community composition, their genes and associated functions. Although both 16S and WMGS methodologies are dependent upon strong databases (16S rRNA or other marker genes) to assign taxonomy, WMGS has an advantage over 16S sequencing as it can analyze all the DNA in a sample and, thus,

uncover true metabolic potential. Nevertheless, we can use 16S data to predict functional content of the community using bioinformatics approaches, such as PICRUST or other tools mentioned above, as a first step towards understanding potential changes in microbial function. In addition, 16S sequencing may be more powerful in capturing bacterial diversity. Metatranscriptomics is whole-gene expression profiling of complex microbial communities and provides insight into the expressed transcripts within the microbial community. This approach is based on RNA sequencing that measures relative mRNA abundance of genes from microbiota and identifies the effect of stressors on microbial metabolic pathways and microbial enzymes, as two examples. The relative differences in the abundance of genes are predictive of functional changes, such as changes in detoxification and degradation capacities, antibiotic resistance, or production of proteins and metabolites. Although metatranscriptomics is a valuable tool to analyze the activity of the microbiome, the transcriptome reference databases are still limited in their coverage and in many cases the transcript does not always correspond with the respective enzyme or metabolite. An overview of the different methodologies employed for the study of the microbiome and the questions addressed is provided in Figure 1.

Applications in Environmental Toxicology

The microbiome has emerged as an important endpoint in the field of environmental toxicology as we seek to understand mechanisms of toxicity. As the role of the microbiome in overall homeostasis and disease becomes more apparent from the fields of medicine and public health, toxicologists have sought to study the impacts of the microbiome of chemical metabolism as well as the reciprocal influence of chemicals on the microbiome. For example, studies have shown that contaminants, such as methylmercury, triclosan and phthalates, disrupt the composition of the gastrointestinal microbiome. Moreover, crude oil exposure has been shown to cause dysbiosis of the gill microbiome in fish. Examples from invertebrates include impacts of glyphosate on gut microbiome dysbiosis in Chinese mitten crabs. In addition, studies indicate that, while chemicals can alter the microbiome, the microbiome is also capable of altering the metabolism of chemicals before they are taken up into the host. This can influence the toxicity of chemicals to non-target organisms and is an area of research that is in its infancy.

Role in Risk Assessment and Environmental Monitoring

Moving forward, it will become increasingly important to identify the unique roles that the microbiome has in chemical risk assessment and for monitoring of species in polluted environments. Similar to other -omics technologies, microbiome data can yield important information about the health status of individuals. Previous studies have identified microbial species that are more often associated with adverse health outcomes compared to those that are beneficial for health; as mentioned above, inflammation and dysbiosis of the gastrointestinal tract are often associated with an increased abundance of pathogenic bacteria.

The role of the microbiome in risk assessment has been recently proposed by different scientists. One area that could be enhanced for risk assessment and the microbiome is a better understanding of the dose–response relationships between chemicals and the individual (i.e., pharmacodynamics and absorption, distribution, metabolism and excretion). Microbes can facilitate biotransformation or metabolism of chemicals, producing metabolites that are more (or less) available for uptake through the gastrointestinal lumen (Figure 2). Again, it is important to recognize that species differ in the

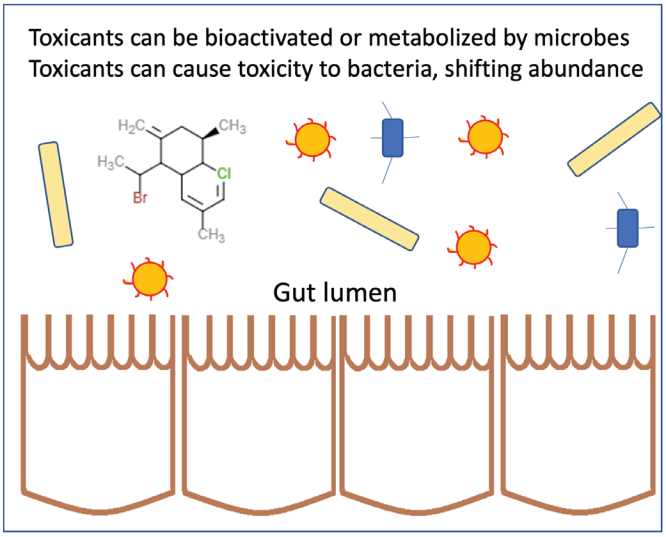

Figure 2: Chemicals are metabolized by microorganisms in the lumen of the gut. This can significantly affect bioavailability and toxicity of chemicals.

composition of microbial communities, and this could explain why there are broad responses across taxa in terms of chemical sensitivity. Functional assessments of microbiota will also shed light into the biological transformation of chemicals as outlined above.

In the context of environmental monitoring of species and conservation, studies have employed non-invasive techniques to sample the microbiome in animals in relation to environmental contaminants and endocrine disruptors. Non-invasive samples have been collected from skin, feces, nasal cavity, and vagina of both terrestrial and aquatic large animals in an attempt to determine whether specific microbial communities are predictive of impaired reproduction, immunosuppression, or stress. Identifying bacterial species that correlate to apical endpoints in adverse outcome pathways can inform on population-level effects. One can perhaps imagine a scenario where a specific bacterial species can be used as a bio-indicator for a population inhabiting polluted environments. The ultimate goal would be to collect a microbial sample from the skin, mouth, or nasal cavity, which could then be used as a diagnostic tool to predict animal health. However, while there is great excitement about the resurgence of microbiome research, there remains significant challenges. Baseline data on microbial communities over a diverse range of species are required to discern whether or not specific alterations in structure and abundance are indeed related to adverse health outcomes. What is most important is the dynamic interaction among microbial communities and the host, and the scientific community will need to characterize this relationship more completely before adopting such data into chemical and environmental assessments. Only through robust experimentation will we strengthen conclusions regarding microbiome-host responses to a specific stressor.

Future Directions

Moving forward, we envision a role for microbial data in adverse outcome pathways to more accurately predict higher level effects. Chemicals are ingested and can be metabolized in the gut, leading to epithelial inflammation, leakiness of the gut, impaired nutrient transport and, eventually, long-term health issues impacting reproduction, growth and longevity. The field of microbiome analysis is moving quickly, and as next-generation sequencing becomes less expensive and more accessible, we expect toxicologists to move towards shotgun metagenomics or metatranscriptomics-based approaches to study chemical contaminants. These methods will allow toxicologists to test hypotheses related to impacts on microbial function within a given microbiome. Just as RNAseq has become a valuable tool for exploring whole transcriptomes for key molecular initiating events related to chemical toxicity, shotgun-based approaches will allow for quantification of genomic and transcriptomic alterations that can link microbiome impacts to changes in host health. Using such data, we can use axenic cultures or gene editing, followed by inoculation of specific bacterial species, to test hypotheses related to the relationships between chemical exposure, dysbiosis of the microbiome, and adverse effects on the host.

Acknowledgments

SETAC is grateful for the efforts and contributions of everyone involved in the advancement of this topic: Ondrej Adamovsky, Danilo Basili, Joseph H. Bisesi, Christopher Martyniuk, and Stanislav Smatana.

Copyright