Perspective

Do Interactions Between Environmental Chemicals and the Human Microbiome Need to Be Considered in Risk Assessments?

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One of the most dynamic and fruitful areas of current health-related research concerns the various roles of the human microbiome in disease. Evidence is accumulating that interactions between substances in the environment and the microbiome can affect risks of disease, in both beneficial and adverse ways. Although most of the research has concerned the roles of diet and certain pharmaceutical agents, there is increasing interest in the possible roles of environmental chemicals. Chemical risk assessment has, to date, not included consideration of the influence of the microbiome. We suggest that failure to consider the possible roles of the microbiome could lead to significant error in risk assessment results. Our purpose in this commentary is to summarize some of the evidence supporting our hypothesis and to urge the risk assessment community to begin considering and influencing how results from microbiome-related research could be incorporated into chemical risk assessments. An additional emphasis in our commentary concerns the distinct possibility that research on chemical–microbiome interactions will also reduce some of the significant uncertainties that accompany current risk assessments. Of particular interest is evidence suggesting that the microbiome has an influence on variability in disease risk across populations and (of particular interest to chemical risk) in animal and human responses to chemical exposure. The possible explanatory power of the microbiome regarding sources of variability could reduce what might be the most significant source of uncertainty in chemical risk assessment.

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1. INTRODUCTION

In 2017, the National Academies of Sciences, Engineering, and Medicine (NASEM) convened a committee to review the available research literature on interactions between the human microbiome and environmental chemicals and to make recommendations for research that is needed to better understand the health risks that might arise because of such interactions.

The work of the committee was sponsored by the Environmental Protection Agency and the National Institute of Environmental Health Sciences and was published in 2018 (NASEM, 2018). The National

Academies report ("Environmental Chemicals, the Human Microbiome, and Health Risk: A Research Strategy") does not concern the well-established domain of microbial risk assessment but is entirely focused on the types of interactions that can occur between environmental chemicals and the human microbiome, the possible consequences for health of these interactions, and the challenges that arise in considering such interactions in chemical risk assessments. The committee was careful to emphasize that a structured research effort must be undertaken to understand whether such interactions have important health consequences and noted that available evidence supports the need for such an effort.

The potential importance of research on environmental chemicals and the microbiome is evident in the current scientific interest in the relationships between adverse health outcomes and perturbations in the microbiome. Before and since the National Academies (2018) report was published, workshops on the topic have brought diverse disciplines together to discuss the research that has begun (Health and Environmental Sciences Institute [HESI], 2018; National Academies of Sciences [NAS], 2016); professional societies have hosted symposia (International Society of Exposure Science [ISES], 2018; Society of Toxicology [SOT], 2018); and journals in multiple disciplines continue to publish research, much of which was discussed in the National Academies report.

The research strategies described in the National Academies (2018) report will, if implemented, clarify the importance to risk assessment and human health of understanding and quantifying chemical–microbiome interactions.

2. THE MICROBIOME IN HUMANS AND ANIMAL MODELS

The human body hosts great numbers of diverse microorganisms, as do all animals, including those used in research. The collection of microbes inhabiting a particular body site or niche is referred to as the *microbiota*, and significant variations in microbiota composition exist between and within organ systems. The most well-described microbiota are those representing body sites more readily sampled, such as the lower gastrointestinal tract, skin, and oral or nasal passages (Human Microbiome Project [HMP] Consortium, 2012). Extensive efforts to characterize human microbiota have been spurred by interests in how host-associated microbes shape states of health

or disease. Although current understanding of how microbiota specifically influence disease risk or disease heterogeneity is far from complete, the strength of associative evidence in many clinical contexts has motivated ongoing research to understand the microbial, metabolic, and pathophysiologic processes involved.

In contrast to the term microbiota, the term *microbiome* is more thorough, referring to "all microorganisms on or in the body, their genes, and surrounding environmental conditions" (NASEM, 2018). The term is often used in conjunction with a specific body site, such as the gut, skin, or respiratory microbiome, and captures the ecological contexts that shape microbial behaviors. For example, oxygen content, pH, and nutrient availability, among many other factors, all influence what microbes live where and explain the broad differences between the gut, skin, and oral microbiomes.

Notably, there is great variability between individuals in their microbiomes. This variability reflects a constellation of individual-specific factors, both endogenous (genetics, life stage, health status, immune cell functions) and exogenous (diet and inhaled or applied exposures) (Markle et al., 2013; Stein et al., 2016; Suez et al., 2014; Trompette et al., 2014), and will be important to consider in attempts to understand how the microbiome influences health. Moreover, there is growing recognition in murine model research that husbandry practices can greatly affect microbiota composition and potentially impact, even confound, measured effects of an intervention (Dickson et al., 2018; Ericsson et al., 2018; Servick, 2016). It is important to note that in the vast majority of studies to date, the most commonly used technique to characterize microbiota (sequence analysis for the conserved bacterial 16S ribosomal RNA gene) does not provide direct readouts of microbial gene functions or products. As methods to characterize microbial functions improve, these new insights will advance understanding of the complex functional interactions occurring in microbiomes. This foundation is needed to inform the basis of microbiome variability between individuals, of the effects of microbiome perturbations resulting from exposures and other interventions, and to explain known associations between microbiome perturbation and disease states.

A large body of evidence already exists showing that particular interventions or exposures can affect the assembly, maturation, and stability of human or mouse microbiomes. In one well-studied human context, exposures in early life—including

home environment (e.g., proximity to domesticated animals), antibiotic use, and source of nutritional support (breast milk vs. infant formula)—can affect the gut microbiome and its trajectory of maturation in the first years of life (Li, Wang, & Donovan, 2014). This is an important developmental period for the immune system (Chung et al., 2012), and differences in the intestinal microbiome are linked to future risk of allergic diseases (Li et al., 2014). Similarly, differences in the nasopharyngeal microbiome of infants have been linked to increased risk of childhood asthma (Teo et al., 2018). Among interventions, antibiotics most clearly and consistently alter microbiomes, most well-studied in the gut and in animal models (Dethlefsen & Relman, 2011; Morgun et al., 2015). However, the short-term vs. persistent effects of such models can vary and differ by study design or clinical context. Other studies that focus on the gut microbiome have shown that administration of nonantimicrobial agents (Suez et al., 2014; Wu et al., 2017) alters the intestinal microbiome. In some cases, mechanisms by which microbiota members process or transform a pharmacologic agent have been elucidated (Maurice, Haiser, & Turnbaugh, 2013; Spanogiannopoulos, Bess, Carmody, & Turnbaugh, 2016). Clearly, these lines of evidence easily extend to consideration of other important scenarios that have yet to be studied. It is plausible that exposure to certain environmental chemicals could perturb human microbiomes or, conversely, that chemicals could be processed and transformed by human microbiota, with downstream effects on risk of disease (NASEM, 2018).

3. CHEMICAL AND MICROBIOME INTERACTIONS RELEVANT TO RISK ASSESSMENT

Given this background, it is reasonable to postulate that the microbiome can play a role in the development of chemical toxicity. Two lines of evidence support such a role. First, as described above, it is well established that alterations in the microbiome can lead to adverse health outcomes. It is also reasonably well established that exposures to some chemicals can alter the microbiome. What remains to be established is whether various types of chemically induced microbiome perturbations can induce adverse health outcomes (toxicities) separate and apart from those induced through well-known toxicity mechanisms. Second, research has established that the chemical metabolism and uptake of at least

some chemicals can be altered by the microbiome. Questions remain about the magnitude of the effect of the microbiome on the production of toxic metabolites and the role the microbiome may have in the kinetics of absorption, distribution, and elimination of chemicals (Diaz-Bone & Van de Wiele, 2010).

It is not now possible to evaluate the significance of those possible pathways of microbiome-influenced toxicities. But there are good reasons to do the needed research. It is critically important to understand whether current methods for identifying chemical toxicities (hazards), dose–response relationships, and the most relevant measures of dose adequately reflect the influences of the microbiome. If they do not, and those influences are significant, then risk assessments based on data generated with the use of those methods will not provide adequate human risk characterizations (NASEM, 2018).

The two principal sources of data for chemical risk assessments are observational epidemiology studies in selected human populations and studies in experimental animals (EPA, 2014). Investigations into the role of the microbiome in the production of observed toxicities have been rarely undertaken in either study type. Some might argue that, if the microbiome were somehow involved in the production of observed toxicities, its role would have been inherently captured in whatever outcomes were observed. Others might posit that determining whether and how the microbiome was involved, while of academic interest, is not essential for developing relevant and reliable risk assessments. This argument would have merit were it not for the variability of the microbiome. For that reason, observations of microbiome-influenced toxicities in one population (e.g., an agricultural or worker population) might have little or no relevance to other populations (e.g., children, the aged, people with chronic diseases) that have substantially different microbiome features and functions. The extent to which observations lack relevance and the direction of the difference (i.e., are unstudied populations more or less susceptible or might they experience completely different types of toxicity?) cannot be ascertained without investigation of the role played by the microbiome and the specific characteristics of microbiome functions that were involved.

Most of the toxicity data used in risk assessment are developed in studies in animal models, and the compositional and functional differences in microbiomes between animals and humans have not been

well studied (HMP Consortium, 2012). Observations of microbiome-mediated toxicities in standard animal models are, therefore, of uncertain relevance to humans.

In addition to questions of microbiome-mediated toxicities or hazards, there are major unknowns about dose–response relationships and relevant measures of dose, both essential for risk characterization (Dietert & Silbergeld, 2015). At present, scientists do not know how the microbiome is altered as chemical dose changes, and they do not know how the dose–response relationship of microbiome perturbations is related to the dose relationship of the ultimate manifestation of toxicity. That knowledge will be necessary to determine "effect" and "no effect" doses for risk assessment. The added problem of identifying the measure of dose most relevant to the microbiome perturbation of interest will be difficult to solve. Is it, for example, only the dose that reaches the relevant site of microbiome perturbation and, if so, how are the extrapolations necessary to identify comparable human doses to be made?

Until there is better understanding of whether, and through what mechanisms, the microbiome influences chemical toxicity, and until there are relevant data on dose–response relationships and measures of dose specific to the toxic effects, risk assessments cannot with any confidence reflect influences of the microbiome and might mischaracterize human risks to unknown degrees.

Published research in chemical–microbiome interactions suggests that data are being developed that even now could help characterize the influence of the microbiome and, in the future, will be important to incorporate into risk assessments.

4. USING EMERGING DATA IN RISK ASSESSMENTS

Data on microbiome–chemical interactions that better define pharmacokinetics of exposure for a few chemicals might already be available for use in risk assessments. When the action of the microbiome on a chemical before absorption into cells can be quantified, the amount of bioavailable chemical can be better estimated, and previous exposure estimates might be increased or decreased accordingly. In addition, current research indicates that data will become available on the chemical metabolites produced by microbiomes. Laboratory animal data on the metals mercury and arsenic suggest that exposure es-

timates could shift to new parent and metabolite ratios and that toxicity evaluations could shift to different chemical metabolites (Diaz-Bone & Van de Wiele 2010; Van de Wiele et al., 2010). However, research is needed to characterize and compare the animal microbiomes with the human microbiome. Efforts to develop physiologically based pharmacokinetic models that incorporate the role of the microbiome could contribute to improved understanding of dose response and the relevance of animal studies to humans.

Risk assessors will find it more difficult to characterize and use emerging data on chemical-induced change or harm to the microbiome as a health effect. Comprehensive data on how changes to the microbiome alter host health are not currently available. In addition, current studies are not designed to separate a chemical's direct effect on the host from the chemical's effect on the microbiome, although the concept has been tested by examining the effect of a chemically altered microbiome transplanted in a new host (Suez et al., 2014). A chemical that alters the community composition and function of a microbiome might lead to a direct health effect, but also might alter chemical exposures by damaging the metabolic capacity of the microbiome or changing the environment that supports microbiome-induced chemical metabolism. For example, the gut microbiome has a regulatory effect on the host liver's production of bile acids (Wahlstrom et al., 2017). Research is needed to examine the potential that change in the microbiome might result in a change in the gut environment that could both alter optimal function and alter the microbiome's effect on the chemical. To use emerging data on a chemical's effect on the microbiome, the role of the microbiome in supporting a healthy organism needs to be well characterized and adverse human health effects (in the absence of chemical exposure) from an impaired microbiome need to be defined for both function and composition. Indeed, the very concept of an "impaired microbiome" requires clarification.

One of the most exciting possibilities in chemical–microbiome research is the potential for new interpretations of variation in responses to chemicals between and within species. A wide range of environmental, developmental, and physiological determinants of microbiome variation might be responsible for between-study differences in exposure, health effects, and dose response. A new risk assessment activity will likely involve relating emerging data on pharmacokinetics and health

consequences of chemical–microbiome relationships to an understanding of variation of animal and human microbiomes. A new field of inquiry for risk assessors who work with any specific risk assessment and target population will be to evaluate the extent to which epidemiology and toxicity studies, particularly standardized studies used repeatedly in the past for regulatory purposes, sufficiently evaluate the similarity of microbiomes of studied populations to the target population (e.g., the U.S. general population that includes infants). A key question for risk assessors is which populations (e.g., infants with limited diversity in microbiomes, persons with compromised microbiomes from illness) are more or less susceptible to the adverse effects of chemical–microbiome interactions.

The same environmental factors that contribute to chemical exposure might also alter microbiome composition and function (e.g., quality of ambient air, water, and soil; household and workplace environments; an individual's diet; use of personal care products; nutrition; and use of health supplements and pharmaceuticals). Risk assessors need to be aware that while a chemical might not be present in a particular medium, product, or location, microbiomes shaped by environmental factors might explain an individual's susceptibility to a chemical exposure.

Environmental chemical risk assessors typically have only data from toxicity studies conducted using highly controlled exposure conditions and well-defined laboratory animals. Historically, such studies do not describe the composition and function of the microbiome in the animals. However, comparing results of existing studies (such as studies of gut or lung health) across species or exposure paradigms might suggest differences in microbiome-related factors. Environmental epidemiology studies often produce disparate or conflicting results, which prevent them from being used in risk assessment. Understanding the microbiome variability in the observed human populations might help to explain the study differences and allow greater weight to be applied to epidemiology data or allow risk assessors to select among studies those that are most relevant based on knowledge of chemical–microbiome interactions.

5. CHALLENGES AND OPPORTUNITIES FOR RISK ASSESSORS

Risk assessors can have a role today in guiding chemical–microbiome research to focus on en-vironmentally relevant exposures, dose–response relationships, and salient health effects. As in much nascent exposure, toxicity, and epidemiology research, high levels of chemical exposure or microbiome disruption might be used to demonstrate chemical–microbiome interactions. How the effects from high exposures scale to environmentally relevant exposures will need to be explored, especially as high-throughput research is conducted. Similarly, risk assessors will need to grapple with defining what is adverse for microbiome disruption and will need to pay attention to research that describes microbiome dysfunction and key events (including upstream events that may be identified through –omics research) that might lead to microbiome disruption. Risk assessors must be involved in reaching agreement on composition and functional measures of microbiome disruption that will be considered an adverse health effect. In addition, measurement parameters and interpretation need to be agreed upon for such concepts as redundancy and conservation of function, functional recovery of the microbiome, local vs. distal effects, and acute vs. long-term effects.

Risk assessors should advocate for measures of microbiome composition and function in current exposure, toxicity, and epidemiology studies. They should also advocate for developing and implementing high-throughput testing that could implicate or rule out microbiome–chemical interactions. As high-throughput data are developed, it is likely that risk assessors will need to advocate for additional data on the nature and magnitude of the chemical–microbiome interaction to extrapolate study results to target populations. Additionally, they will be able to identify candidate chemicals for further testing based on what is now understood about the role of the microbiome. As described in the National Academies (2018) report, risk assessors, for example, might want testing to include chemicals for which large intraspecies variability has been found in epidemiology or toxicity studies, or chemicals with health end points that have been linked to adverse effects that are known to be mediated by microbiomes.

6. CONCLUSION

Important discoveries are anticipated from this nascent field that could profoundly affect risk assessment. Risk assessors and managers should monitor new findings but should also be involved in the

research so that the research will yield useful information for risk assessment.

As described above, risk assessors can contribute to identifying chemicals of interest and can identify those species and strains of laboratory animals whose microbiomes might be most important to characterize and contrast with humans. In addition, risk assessors have experience in understanding the challenges of defining adverse effects and can help determine how to quantitate harm from changes in microbiomes. They can also help to interpret quantitative data on chemical–microbiome interactions to improve dose–response estimates. Finally, risk assessors can begin today to educate risk managers on the importance of microbiome research for improving risk assessments.

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