

FORUM ARTICLE

The Gut Microbiome and Xenobiotics: Identifying Knowledge Gaps

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ABSTRACT

There is an increasing awareness that the gut microbiome plays a critical role in human health and disease, but mechanistic insights are often lacking. In June 2018, the Health and Environmental Sciences Institute (HESI) held a workshop, “The Gut Microbiome: Markers of Human Health, Drug Efficacy and Xenobiotic Toxicity” (<https://hesiglobal.org/event/the-gut-microbiome-workshop>) to identify data gaps in determining how gut microbiome alterations may affect human health. Speakers and stakeholders from academia, government, and industry addressed multiple topics including the current science on the gut microbiome, endogenous and exogenous metabolites, biomarkers, and model systems. The

workshop presentations and breakout group discussions formed the basis for identifying data gaps and research needs. Two critical issues that emerged were defining the microbial composition and function related to health and developing standards for models, methods and analysis in order to increase the ability to compare and replicate studies. A series of key recommendations were formulated to focus efforts to further understand host-microbiome interactions and the consequences of exposure to xenobiotics as well as identifying biomarkers of microbiome-associated disease and toxicity.

Key words: microbiome; gut; health; xenobiotics; environmental; biotransformation; biomarkers; animal models.

The gastrointestinal (GI) microbiota play an underlying role in health. Alterations in stability and functional capabilities of microbiota are associated with disease although it is unclear whether this is a cause or a result of the disease. Although there is emerging research regarding the physiological functions of intestinal microbes, less is known about the consequences of chemically induced alterations to intestinal microbiome composition and toxicological effects to the host. The interactions of xenobiotics with the microbiota as a result of drug therapy or environmental exposures are of increasing interest to public health.

Health and Environmental Sciences Institute (HESI) assembled a cross-disciplinary group of experts (Table 1) to examine gut microbial-host dynamics in order to better understand what is currently known about the effect of chemical exposures on the microbiome and to identify key knowledge gaps related to such exposures (Figure 1).

KEY ISSUE NO. 1: DEFINING GUT MICROBIOMES ASSOCIATED WITH HEALTH

The intestinal microbiome is made up of between 500 and 1000 bacterial species as well as viruses, archaea, and eukaryotic microorganisms, many of which play a role in human health and disease (Backhed et al., 2012; Gilbert et al., 2018; Hanson and Weinstock, 2016; Ogilvie and Jones, 2015; Qin et al., 2010; Tuddenham and Sears, 2015; Virgin, 2014; Ward et al., 2018). There is great variation in species diversity, taxonomic

composition, and population density of microbiota in the gut of humans and animal species. For those taxa that contribute to human health little is known about the part that each microbial species is filling, how well they perform that function, their interactions with other microorganisms and with the host, and where in the host they are acting (Backhed et al., 2012; Barratt et al., 2017; Cho and Blaser, 2012; Gilbert et al., 2018). Understanding the functions, features and normal ranges of the microbial communities that support health will be essential in addressing impacts of environmental agents and potentially identifying microbial configurations that result in disease (Lloyd-Price et al., 2016, 2019). The inclusion of functional measurements may lead to different interpretations of microbiota diversity, compared with taxonomic classification alone, leading to greater analytical challenges (Fu et al., 2016; Zhu et al., 2015). However, there is a growing consensus that functional characterizations are necessary for understanding and modeling the physiology of the microbiome (Heintz-Buschart and Wilmes, 2018; Moya and Ferrer, 2016; Zhu et al., 2015).

Before beneficial or adverse effects of chemical exposure can be determined, it is necessary to have a baseline for the bacterial species diversity as well as an understanding of the functional capacities associated with health. Given the considerable variation across human populations, among individuals and even within an individual, this will not be a single microbiome but a group of features, capabilities and characteristics of microbial communities that contribute to health. In defining such microbiomes, temporal variability in these communities, such as short-term perturbations associated with diet, lifestyle traits,

Table 1. Workshop Topics and Presenters

Workshop Topics	Expert Presenters
Drugging gut microbial enzymes for treatment of CVD Biotransformation	<ul style="list-style-type: none"> • Mark Brown, Cleveland Clinic • Julia Cui, University of Washington • Gary Perdew, Penn State University
Biomarkers of adverse effects	<ul style="list-style-type: none"> • Andrew Patterson, Penn State University • Rod Dietert, Cornell University • Carrie Brodmerkel, Janssen • Carl Cerniglia, US FDA-NCTR
Biomarkers of toxicity and disease	<ul style="list-style-type: none"> • Joseph Petrosino, Baylor College of Medicine • Kun Lu, University of North Carolina Chapel Hill • Emily Hollister, Diversigen
Human susceptibility	<ul style="list-style-type: none"> • Elaine Richards Sumners, University of Florida • Nathan Price, Institute for Systems Biology • Eugene Chang, University of Chicago
Animal models	<ul style="list-style-type: none"> • Marijke Faas, University of Groningen • Aaron Ericsson, University of Missouri • Barbara Rehmann, NIH/NIDDK • Tamara Tal, U.S. EPA

Experts from all sectors addressed state of the science and need for identification of biomarkers to advance understanding and decision making on efficacy and safety of xenobiotics. Presentations can be found on the HESI website here (<https://hesiglobal.org/event/the-gut-microbiome-workshop/>).

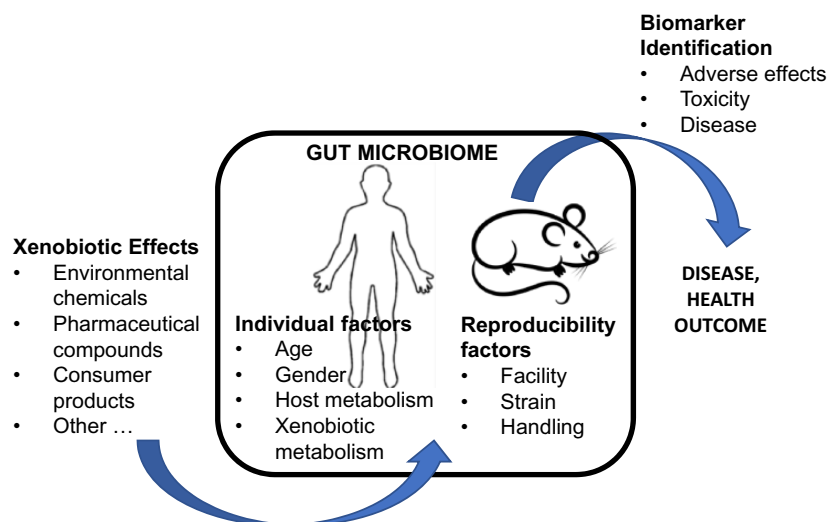


Figure 1. Key data gaps in the microbiome research field identified at the 2018 HESI workshop. HESI's June 2018 workshop identified key data gaps in the gut microbiome research field. The results identify needs for easily accessible biomarkers and improved human and animal experimental model studies for testing and validation of the impact of changes in the gut microbiome on human health outcomes.

antibiotic exposure, or acute illness, as well as more long-term impacts that may lead to deleterious chronic health outcomes must be considered (Kundu *et al.*, 2017). In addition, factors such as geographic location, ethnicity, age, physical activity, genetics and gender, resistance (the ability to withstand stress or perturbations), and resilience (the capacity to return to a healthy state) influence an individual's microbiome (Backhed *et al.*, 2012). For example, diet is associated with significant differences in microbiota both across populations and with longitudinal studies, yet there may not be apparent adverse impacts to human health (Clemente *et al.*, 2015; Yatsunenko *et al.*, 2012). Such observations suggest that there may be functional redundancy in biochemical pathways in the microbiome (Baumler and Sperandio, 2016). Large cohort studies may be needed in defining normal variations within a microbiome, especially if the studies include longitudinal sampling of the microbiome (Faust *et al.*, 2015; Fettweis *et al.*, 2019; Flores *et al.*, 2014; Lloyd-Price *et al.*, 2019; Proctor *et al.*, 2019; Vandeputte *et al.*, 2017a,b; Yassour *et al.*, 2016; Zhou *et al.*, 2019). Measurement of blood metabolome is useful in predicting gut α -diversity in such studies (Price *et al.*, 2017; Wilmsanski *et al.*, 2019).

For most healthy adults, there appears to be a window of microbiome normalcy whose bounds are not typically exceeded, even though there may be minor transitory variation in composition observed with dietary changes or exposures to certain xenobiotics (Kundu *et al.*, 2017; Moya and Ferrer, 2016). However, changes in microbial communities can be associated with adverse health consequences. In one model, this may occur if a person's microbiome leaves their respective window of normalcy reaching a "tipping point" where their health is affected. Alternatively, rather than a distinct shift there can be stochastic changes in the microbiome leading to dispersion of the microbiome composition as well as increased functions that are related to disease (Armour *et al.*, 2019; Zaneveld *et al.*, 2017).

RESEARCH NEED NO. 1

In order to understand, adverse health effects of the microbiome resulting from disease or xenobiotic exposure, there is a need to better understand the essential components,

capabilities, and range of microbial variation linked to a person's health. This requires comprehension of both the structure and the function of the microbial community. Identification of key conserved functional, metabolic, and biochemical pathways present in healthy individuals may provide a strong starting point.

It is evident that multiple factors contribute to the composition, diversity and function of the gut microbiome with regard to health, drug efficacy, and xenobiotic toxicity. The list includes genetics, diet, age, obesity, concomitant diseases, drugs, and gender to name a few. There is a need to not only understand the impact of a single factor on the dynamics between gut microbiome and the host but also to determine contributions of combinations of these factors. However, defining what constitutes microbial diversity and functionally of both healthy and unhealthy individuals will help to elucidate the relative contributions of host factors.

Large cohort studies are valuable sources of data for defining microbiomes associated with health, but comparison and reproducibility across such studies is hindered by differences in experimental design and data analysis. Variation and bias in sampling procedures, stabilization conditions, shipment and storage practices, methods for analyte extraction and purification, sample preparation and analysis, bioinformatic analysis algorithms as well as the reference databases used are contributing factors (Backhed *et al.*, 2012; Gilbert *et al.*, 2018; Sinha *et al.*, 2017; Stulberg *et al.*, 2016). Standardization of methods and analytical approaches is needed to develop a well-characterized measurement pipeline. However, procedures may need to remain somewhat fluid to account for continuing technological developments and evolving understanding of the biology involved.

KEY ISSUE NO. 2: DEFINING CAUSES AND EFFECTS OF ALTERING THE GUT MICROBIOME

GI microbiota are essential in maintaining health and wellness by digesting food nutrients, producing endogenous metabolites and biotransforming xenobiotics (Dietert and Silbergeld, 2015b). Furthermore, in a healthy individual the gut microbiome,

together with the intestinal mucosa, is necessary to maintain gut homeostasis and provide an epithelial barrier between the lumen and the rest of the body (Hiippala et al., 2018; Rogers, 2015).

Loss, disruption or dispersion of a functional microbiome can be associated with acute adverse health effects or chronic diseases. For example, antibiotics can alter a healthy individual's microbiome resulting in diarrhea, leaving the body susceptible to the development of *Clostridium difficile* infection (Backhed et al., 2012; Schaffler and Breitruck, 2018; Schubert et al., 2014). Changes in gut microbiota may be linked to the development of inflammatory bowel disease, colorectal cancer, and fatty liver disease (Miyoshi et al., 2017; Sharpton et al., 2019; Wang et al., 2017).

Many of these chronic diseases have inflammatory or altered immune system characteristics. Critical immune functions related to epithelial signaling, inflammatory responses, production of antimicrobial factors, and induction of Immunoglobulin A antibodies are propagated locally in the GI tract and associated secondary lymphoid tissues (Peyer's Patches; Baumler and Sperandio, 2016; Dietert and Dietert, 2015a; Hiippala et al., 2018). The microbiome contributes to the development and maintenance of the immune system (Agace and McCoy, 2017). Early life is characterized by a period of microbial flux and assembly which is affected by multiple factors such as manner of delivery (vaginal or c-section), nutrition (breast milk or formula) and antibiotic exposure. Although dynamic in nature, in adults the majority of the composition and function of the microbiome of a healthy person is fairly stable, but this decreases with age due to the loss of key species and a progressive gain of pathobiotic bacteria (Buford, 2017).

The systemic influence of the GI microbiota on human health can be altered by exogenous compounds during drug therapy or environmental exposures. Antibiotics are used to remove a pathogen from the host, but an unintended consequence can be alteration of the composition and function of the gut microbiota (Miyoshi et al., 2017). Exposure to nicotine, arsenic, or polybrominated diphenyl ethers change the gut microbiome diversity (Chi et al., 2017, 2018; Li et al., 2018).

Exposure to xenobiotics can have effects in multiple ways. These include those that are transitory, established, or developmentally programmed (Dietert and Dietert, 2015a; Faust et al., 2015; Fofanova et al., 2016; Moya and Ferrer, 2016; Norman et al., 2015; Pascal et al., 2017; Sanz et al., 2015; Stenman et al., 2016). The transitory outcomes, such as changes after eating, are typically easily correctable and the gut microbiome return to the composition and functionality exhibited before the insult. Established outcomes occur when there is the alteration of a key-stone species within the microbiota which would require significant host and microbiota changes to recover the original microbiota (eg, after *C. difficile* infection). The developmentally programmed outcomes are those that occur when the adverse impact is the result of alteration during a critical window of the microbiome development and can lead to later-in-life problems associated with the microbiome (eg, antibiotics during early childhood).

RESEARCH NEED NO. 2

The gut microbiome plays an essential role in maintaining health and modification of the microbial communities can have negative effects in the body. Defining what changes in the microbiota are associated with acute or chronic adverse effects, as well as the magnitude of change necessary, is needed. Adverse effects can range from changes to the intestinal barrier integrity to alterations or dispersion of the normal microbiota of

the host population of microbiota leading to a diseased state. Identification of such adverse effects may provide biomarkers of disease progression. To begin to identify causes and effects of changes in the microbial population, 2 approaches are possible. One is to start from a healthy individual's baseline microbiome then determine deviations associated with a disease state. The second is determining what is a diseased state and ascertaining if there are key nodes, drivers, or contributing factors that move individuals from health to illness.

Despite the improvements in analytical, genomic, and bioinformatic techniques related to structure and function of the microbiome in health and disease, there is still a knowledge gap in the translation of high-throughput data from genotype to phenotype and microbiome composition stability/instability versus core function of the microbial community.

KEY ISSUE NO. 3: ACCOUNTING FOR BIOTRANSFORMATION

GI microbiota have the capacity to metabolize endogenous and exogenous compounds. The metabolites can have positive, neutral, or negative effects on the host. Endogenous microbial metabolites can exert physiological functions. Gut microbe-derived metabolites can signal via receptors at the epithelium interface and communicate with the host. For example, microbial metabolism of tryptophan generates aryl hydrocarbon receptor (AHR) ligands, such as indole, indirubin, and indigo (Hubbard et al., 2015). These ligands activate AHR and promote intestinal homeostasis through regulation of innate cytokine or chemokine gene expression, regulation of enterocyte differentiation, as well as regulation and development of intraepithelial lymphocytes and innate lymphoid cells. Gut microbial metabolism can result in products associated with adverse health and disease progression. The microbiome converts dietary lipid phosphatidylcholine to trimethylamine (TMA), which is then metabolized by hepatic flavin monooxygenases to TMA-N-oxide (TMAO). TMAO is a proatherogenic factor associated with cardiovascular disease (CVD; Brown and Hazen, 2015). Such findings suggest that endogenous microbial metabolites could be used as potential biomarkers of health or disease.

GI microbiota express biotransformation enzymes that metabolize a variety of therapeutic drugs and environmental compounds that can result in changes in efficacy and toxicity (Klaassen and Kui, 2015; Spanogiannopoulos et al., 2016). Gut bacteria can convert a prodrug to an active drug or detoxify the parent compound leading to modified drug availability, as well as changes in pharmacokinetics-pharmacodynamics (PKPDs). Sulfasalazine, a prodrug for ulcerative colitis, is directly metabolized by the gut bacteria at the azo bond to generate 2 bioactive metabolites, sulfapyridine (antimicrobial) and 5-aminosalicylate (anti-inflammatory; Peppercorn and Goldman, 1972). The cardiac drug glycoside digoxin undergoes reduction to an inactive metabolite, dihydrodigoxin, which is significantly decreased if antibiotics are preadministered (Haiser et al., 2013; Lindenbaum et al., 1981). Microbial metabolites of a drug can also increase toxicity. The chemotherapeutic drug irinotecan is metabolized to SN-38 which is both the active and toxic metabolite. SN-38 is formed and glucuronidated by the liver and then transported to the gut where it undergoes deconjugation by gut bacteria. This results in longer gastrointestinal (GI) exposure, reabsorption, and greater bioavailability of SN-38 (Wallace et al., 2010). The gut microbiome is also implicated in the interindividual variability in metabolism of the analgesic and antipyretic

acetaminophen. The endogenous microbial metabolite p-cresol and acetaminophen are substrates for sulfotransferases. Individuals with high levels of p-cresol generated by bacteria may have decreased acetaminophen metabolism through competition for hepatic sulfonation, which could result in an elevated risk for acetaminophen-induced hepatotoxicity (Clayton et al., 2009).

Nontherapeutic drugs and environmental xenobiotics can induce compositional changes and functional changes in gut microbiota. In turn, these compounds may also be biotransformed by the microbiome. Arsenic is metabolized by gut bacteria and also alters the abundance and profile of the gut microbiome (Chi et al., 2018; Gokulan et al., 2018; Lu et al., 2014). Polychlorinated biphenyl or polybrominated diphenyl ether exposed mice show altered bile acid homeostasis resulting in part from changes in gut microbiota bile acid metabolism (Cheng et al., 2018; Li et al., 2018). Gut anaerobes are capable of transforming Hg_2^+ to highly toxic and permeable methylmercury and significantly contributing to its body burden and poisoning (Edwards and McBride, 1975).

In addition to directly biotransforming chemicals, the gut microbiome can influence the host xenobiotic metabolizing capability. Comparison of hepatic drug metabolizing genes in conventional and germ-free (GF) mice show that 34 genes, including Cyp3a, decrease, whereas 21 genes, including Cyp4a, increase (Selwyn et al., 2015). A cluster of 112 hepatic genes linked to xenobiotic metabolism and retinoid X receptor-inhibiting pathways are differentially expressed in conventional and GF mice. This results in more efficient pentobarbital metabolism and shorter anesthesia time in GF mice (Bjorkholm et al., 2009).

RESEARCH NEED NO. 3

Current experimental approaches to evaluate microbe-mediated biotransformation primarily include: (1) *in vitro* incubation of individual bacterial strains, mixed cultures in bioreactors, or purified enzymes with compounds, (2) *ex vivo* incubation of a fecal microbiome community (or other GI regions of interest) with compounds, and (3) *in vivo* administration of compounds into animals such as rodents. These approaches provide evidence in support of microbial metabolism of many approved drugs (Sousa et al., 2008; Zimmermann et al., 2019). However, more caution is required when extrapolating these results into humans than with traditional PKPD approaches, given the high diversity and interindividual variability of the human gut microbiome. Comprehensive and accurate modeling approaches for analyzing microbial metabolism information and incorporating into host PKPD models are necessary.

As more information on biotransformation of xenobiotics by the microbiome is published, a public database would be valuable. Such a database would be a repository centralizing comprehensive data that identifies compounds, responsible bacteria and/or enzymes, metabolites generated and metabolic pathways. This requires a collaborative effort involving researchers from academia, government and industry, working together with the regulatory agencies to promote a better understanding of microbiome-mediated biotransformation.

KEY ISSUE NO. 4: DETERMINING BIOMARKERS OF DISEASE AND TOXICITY

Biomarkers of disease and toxicity resulting from perturbations of the gut microbiota composition and function, from changes

in microbial metabolites, or as a consequence of microbial biotransformation of xenobiotics would be useful. There are a number of characteristics for an ideal biomarker. It should be able to differentiate between disease progression and/or response to treatment. Stool samples or accessible tissues and/or biofluids should be used and analysis be affordable to facilitate acceptance and use for large scale screening. The biomarker needs to work for high-risk populations and distinguish between the disease and other potentially microbiome-mediated effects.

There are some examples of microbiome biomarkers of disease that are promising. Alterations in gut microbiota are associated with hypertension. Exposure of hypertensive rats to the antibiotic minocycline attenuates blood pressure (Yang et al., 2015). In animals and humans systolic blood pressure is correlated with microbiota composition and metabolites, as well as alterations in gut structure (Kim et al., 2018). Zonulin, a bacterial product and a marker of intestinal permeability, is elevated with high systolic blood pressure but is not an ideal biomarker since it is also associated with celiac disease (Fasano et al., 2000; Kim et al., 2018). A more specific indicator may be the negative correlation of butyrate producing bacteria which alter systolic blood pressure and plasma butyrate levels (Kim et al., 2018).

TMAO blood levels are of interest as a biomarker for CVD, insulin resistance, and type 2 diabetes (Miao, 2015). The production of TMAO in mammals primarily occurs via gut bacteria. Studies in humans and animals show circulating levels of TMAO are associated with risk and progression of CVD (Tang et al., 2014). Sensitive assays, particularly those amenable to the clinical setting, are being developed which will facilitate use of TMAO as a biomarker (Garcia et al., 2017). The initial association of TMAO with CVD resulted from using untargeted metabolomics in cardiac patients (Wang et al., 2011). This approach can be expanded to use multiple data streams (clinical tests, metabolomes, proteomes, genome sequence, and microbiome) to develop correlation networks in order to identify bacterial analytes associated with normal physiology or disease (Price et al., 2017). For example, there is an association between phenylacetylglutamine and the *Coriobacteriaceae* and *Mogibacteriaceae* families. Phenylacetylglutamine is a microbial metabolite and is a risk factor for CVD in those with chronic kidney disease (Poesen et al., 2016).

Microbial biomarkers may also prove useful for identifying xenobiotic toxicity. Low dose exposure of mice to polychlorinated biphenyls alters microbiota composition leading to a greater abundance of species that generate secondary bile acids causing increases in serum bile acids (Cheng et al., 2018). At higher doses serum bile acids were not affected due to an increase in hepatic efflux transporters (Cheng et al., 2018).

Although *in vivo* studies are useful for discovering biomarkers, there is also a need for *in vitro* methods. In mice, tempol acts as an antioxidant by detoxifying reactive oxygen species and modulates the gut microbiome host signaling axis resulting in prevention of weight gain (Cai et al., 2016; Li et al., 2013). Incubation of mouse cecal content with tempol and analysis of samples using flow cytometry as well as high-throughput mass spectrometry and nuclear magnetic resonance-based metabolomics show that tempol disrupted microbiota membrane physiology and metabolic activity consistent with the *in vivo* data (Cai et al., 2016). Such a multi-prong *in vitro* approach holds promise for screening xenobiotics for gut microbial toxicity and identifying potential biomarkers.

RESEARCH NEED NO. 4

Case studies provide examples of potential biomarkers that could serve as the basis for noninvasive diagnostic tests to

identify disease and track its progression. When an association between a disease and gut microbiota is recognized, additional work will be needed to distinguish between microbial changes that are a cause or a result of the disease. Biomarkers should also be applicable beyond their discovery cohorts and be able to distinguish between disease states of interest and nontarget diseases, as well as having a reasonable degree of specificity.

Discovery and qualification of biomarkers will also be advanced by the adoption of standard protocols which will generate robust and reproducible data that facilitates comparisons of studies. Detailed methods and procedures for analysis of gut microbiota structure and function that will be useful for toxicologic studies are available (Nichols et al., 2018).

KEY ISSUE NO. 5: OPTIMIZING ANIMAL MODELS

Although animal species have limitations in completely replicating humans, they provide an opportunity to manipulate host genetics and the environment to allow insight into the relationship between the microbiota, xenobiotics, toxicity, and disease. Although species differences must be recognized in extrapolating animal studies to humans, such data are valuable.

Rodents are widely used in studying the microbiome. The compositions of gut microbiota vary among genetically distinct strains of mice (Friswell et al., 2010). Differences also occur in laboratory strains compared with genetically similar wild populations (Rosshart et al., 2017). GF mice and gene-knockout mice are also useful models. GF mice can be colonized with a single (mono-associated) agent, a defined bacterial combination such as altered Schardler flora or human fecal samples (De Palma et al., 2017; Franklin and Ericsson, 2017; Orcutt et al., 1987; Ridaura et al., 2013). Interestingly, introducing microbiota from wild mice into laboratory mouse strains increases resistance to influenza infection, decreases inflammation and increases resistance to certain types of cancer (Rosshart et al., 2017). The wild mouse microbiome develops as a result of evolutionary pressures due to continuous exposure to natural toxins and pathogens, thus it may offer a better model for human disease (Rosshart et al., 2017).

Disparities in gut microbiota of the same mouse strain can be attributed to vendor or institutional location (Friswell et al., 2010). Within a facility, the room, cage housing, food, water, and bedding all have an effect (Ericsson et al., 2018; Friswell et al., 2010; Hildebrand et al., 2013; Hugenholtz and de Vos, 2018; Robertson et al., 2019).

Although rodents are the most commonly used models in microbiome studies, zebrafish are also proving to be useful. Zebrafish develop rapidly and externally to the mother, thereby enabling direct embryo exposures. The GI tract is homologous to that in mammals, although there are differences in the immune system. Zebrafish are kept at a lower temperature than mammals which may compromise the ability to study human-relevant microorganisms. Xenobiotic-microbiome interactions can be evaluated in the zebrafish model. For example, exposure of zebrafish larvae to the antimicrobial compound triclosan results in changes in the community structure of the microbiome. There is an increase in triclosan resistant species which results in a greater ability to biotransform triclosan (Weitekemp et al., 2019). Gut microbiota in zebrafish also alter metabolism and mediate the neurodevelopmental toxicity of 17- β -estradiol (Catron et al., 2019).

RESEARCH NEED NO. 5

Study reproducibility in the biosciences is an ongoing concern (Mullane and Williams, 2017). Variations in experimental design, methods, and statistical analysis can contribute to an inability to replicate results and conclusions. Complicating this further is the diversity and function of the gut microbiota (Franklin and Ericsson, 2017; Turner, 2018).

Reproducibility in studies of gut microbiome host dynamics can be increased by recognizing the sources of variation, prioritizing them; then, identifying ways to address them (Ericsson and Franklin, 2015; Franklin and Ericsson, 2017). To limit variation in mouse models, numerous approaches have been developed to standardize gut microbiota profiles including cohousing, cross fostering, and GF derivation, although each has advantages and disadvantages (Ericsson and Franklin, 2015; Franklin and Ericsson, 2017). Maintaining a stable microbiome composition across animals and experiments is a problem that is not easily solved. Banking fecal samples annually and defining the microbiome at the initiation of a study would allow institutional drift to be monitored (Franklin and Ericsson, 2017). In some instances, repeating studies in the second generation addresses the need for exposure to microbiota in early development (Franklin and Ericsson, 2017). For zebrafish, protocols for GF derivation and gnotobiotic husbandry provide useful guidance for standardization (Melancon et al., 2017).

Often the discrepancies in reported results can be attributed to variations in study design. Unfortunately, key details are often missing from publications. Omissions can be reduced by adoption of Animal Research: Reporting of Experiments guidelines (Kilkenny et al., 2012). This checklist of 20 items covers species, strain and number of animals, husbandry, experimental design, methods, and statistical analysis.

Determining the optimal microbiota to facilitate translating results from mice to humans depends on the question being asked. The current model of colonizing GF mice with microbiota derived from inbred, wild murine, or human sources has great potential. This approach is currently limited by the availability of the number of defined combinations and characterized mouse or human material. The development of a wider array of standardized samples would be useful.

Several *in vitro* models of host-microbiome interactions have been described that complement *in vivo* studies (Arnold et al., 2016; von Martels et al., 2017). Although there are anaerobic bacteria gut epithelial cell cocultures showing feasibility for experimental use, further refinements and standardization are still necessary (von Martels et al., 2017). It is also recognized that no one model is perfect, the best one to use will depend on the question being addressed.

RECOMMENDATIONS

The gut microbiome plays a pivotal part in health, but understanding its multiple roles still needs to be fully elucidated. HESI's gut microbiome workshop was convened to identify research areas critical to determining how gut microbiome alterations may influence human health. Data gaps and research needs to address specific issues have been identified based on workshop presentations and breakout group discussions (Table 2).

There are 2 topics that cut across the key issues. The first is the lack of clarity in what constitutes gut microbiomes in healthy individuals. It is recognized that there are many factors that contribute to variation across and within human populations. Consequently, there is not a single microbiome but rather

Table 2. Key Issues and Research Needs

Key Issue	Research Needs
Defining gut microbiomes associated with health	<ul style="list-style-type: none"> • Identify composition and functional requirements of the microbiota community • Develop guidelines for the normal range of variation • Determine the effects of host factors • Standardize samples collection procedures
Defining causes and effects altering the gut microbiome	<ul style="list-style-type: none"> • Identify changes in species composition and function associated with disease • Define the magnitude of changes required • Determine the effects on host organ systems • Understand mechanisms affecting host susceptibility to diseases and adverse effects correlated with microbiota <ul style="list-style-type: none"> • Factors impacting the microbiome • Factors impacting the host
Accounting for biotransformation	<ul style="list-style-type: none"> • Characterize biotransformation enzymes in microbiota species • Identify endogenous and exogenous substrates of microbiota enzymes • Develop and maintain a public database of compounds, microbiota, and metabolites generated
Determining biomarkers of disease and toxicity	<ul style="list-style-type: none"> • Identify and characterize microbiota metabolites associated with disease and toxicity • Characterize developed biomarkers <ul style="list-style-type: none"> • Specificity • Applicability to individuals and populations • Develop reliable and inexpensive analytical methods for these biomarkers to facilitate translation from the research laboratory to a clinical setting
Optimizing animal models	<ul style="list-style-type: none"> • Standardize husbandry practices that influence microbiome composition and function • Adopt practices that facilitate comparison and reproducibility of studies • Identify the impact of the source of the microbiome in mouse models <ul style="list-style-type: none"> • Wild mice • Laboratory mice • Human • Refine <i>in vivo</i> and <i>in vitro</i> models of host-microbiome interactions

Key research areas and needs, as identified by workshop speakers and participants who participated in breakout group discussions.

a suite of microbiomes that are associated with health. As yet, these have not yet been adequately defined. Having information on which species comprise such microbiomes and the normal range of variation is critical in order to define disease and adverse effects. The second is the need to adopt standards for methods, models and data analysis. This will facilitate comparisons across studies and reproducibility of data.

Moving forward the key recommendations are to focus efforts on the following important areas:

- Defining the range of gut microbiota composition and function gut microbiomes associated with health and/or disease;
- Identifying microbiome changes linked to disease and adverse health effects;
- Characterizing the formation and function of microbiota metabolism of endogenous products on health and disease;
- Determining the effects of xenobiotics on microbiota composition and function;
- Increasing understanding of the impact of microbiota biotransformation of xenobiotics on efficacy and toxicity;
- Identifying a suite of biomarkers to monitor health, disease and adverse effects resulting from microbiota-host interactions;
- Standardizing variables such as husbandry, study design, sample collection, analysis, and statistical methods

Addressing these issues will provide further insight into the role of the microbiome in human health, disease and toxicity.

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DECLARATION OF CONFLICTING INTERESTS

The authors of the article volunteered to serve on the HESI Microbiome committee and were involved in the planning and execution of the workshop. The views expressed in this

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