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Published in:
Current Opinion in Toxicology

Link to article, DOI:
10.1016/j.cotox.2018.09.004

Publication date:
2019

Document Version
Publisher's PDF, also known as Version of record

Link back to DTU Orbit

Citation (APA):
Licht, T. R., & Bahl, M. I. (2019). Impact of the gut microbiota on chemical risk assessment. *Current Opinion in Toxicology*, *15*, 109-113. https://doi.org/10.1016/j.cotox.2018.09.004

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Current Opinion in Toxicology

Impact of the gut microbiota on chemical risk assessment

Tine Rask Licht and Martin Iain Bahl

Abstract

It is well established that the multitude of microbes residing in the human intestine play a key role for health. Recently, it has become apparent that ingested chemicals affect the composition of the human gut microbiota. Additionally, the gut microbes affect the uptake and metabolism of chemicals in multiple ways. Here, we outline the current knowledge about the complex interplay between gut microbes, ingested xenobiotics and toxicological effects. We propose that the intestinal microbiota plays a key role in chemical toxicity, which is typically overlooked in existing approaches for risk assessment. This means that factors such as animal provider, batch/litter differences, and co-caging may significantly influence the outcome of toxicity evaluations based on rodent experiments.

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Current Opinion in Toxicology 2019, **15:**109–113

This review comes from a themed issue on **Risk Assessment in Toxicology**

Edited by **Richard Judson** and **Anne Marie Vinggaard**

Available online 3 October 2018

For a complete overview see the Issue and the Editorial

https://doi.org/10.1016/j.cotox.2018.09.004

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Keywords

Risk assessment, Intestinal microbiota, Gut permeability, Microbial metabolism of chemicals.

1. Introduction

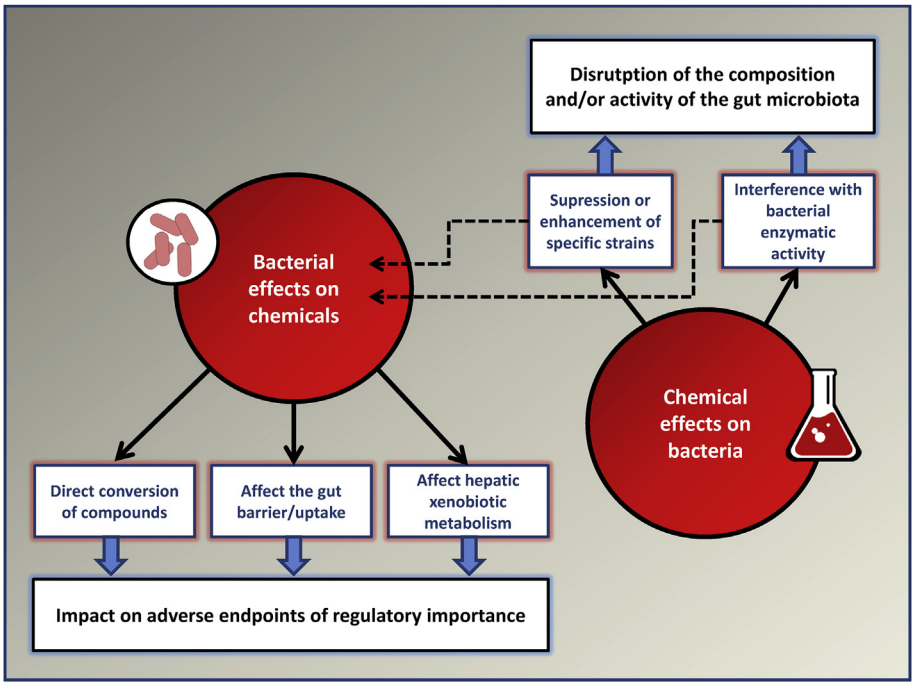
The human intestinal tract harbours between 10^{13} and 10^{14} bacterial cells [1], which engage in a complex interplay with each other as well as with the human host [2]. The role of this interplay for human health is well established [3]. It has recently been proposed that he gut microbiota may also play a key role in the toxicity of environmental chemicals [4], as the intestinal microbes putatively interact with ingested xenobiotic chemicals in multiple ways (Figure 1).

Ingested xenobiotics may directly affect the host microbiota, and thereby potentially the host health, by selective suppression or enhancement of specific bacterial species within the complex community [5,6]. As the microbiota is reported to be involved in the metabolism of several drugs and pollutants [7], inter-individual variation in bacterial community composition may result in a personalized response to given xenobiotic compounds. Furthermore, the composition of the microbial community of the gut influences the permeability of the intestinal barrier as well as the uptake of nutrients [8], and it can thus be anticipated that the microbiota influences the uptake of xenobiotic compounds.

In summary, it is highly likely that the toxic effects of ingested xenobiotics are significantly enhanced or reduced by the gut microbiota. Nevertheless, the role of the microbiota in animal models applied for chemical risk assessment is very rarely taken into account. We propose that a great deal of the variation in dose–response output observed in such assessments can be attributed to differences in the intestinal microbial populations of the experimental animals applied.

Chemicals affect the gut microbiota. Several different classes of xenobiotic chemicals have been reported to interfere with the biochemical and enzymatic activity of gut microbes affecting bacterial community composition and overall gut microbiome homeostasis, with possible harmful consequences to the host [4]. Specifically, effects of pesticides on bacterial communities has recently attracted much attention and consequently various fungicides [9], insecticides [10] and herbicides [11] have been shown to affect the gut microbiota. The observed changes in bacterial composition are a kin to those observed following oral administration of antibiotics, but are typically much more subtle. The molecular mechanisms involved in the chemical–microbe interactions are mostly unknown. An exception to this is the herbicide glyphosate which is known to specifically block the synthesis of the three essential aromatic amino acids tyrosine, phenylalanine and tryptophan by inhibiting the 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS), in the shikimate pathway of some bacterial species as well as in plants [12,13].

Figure 1



The intestinal microbes putatively interact with ingested xenobiotic chemicals in multiple ways.

The gut microbiota is dependent on nutrients provided either by ingested feed or by indigenous secretion in the gut environment. Because different bacterial species vary in nutritional requirements and also in their sensitivity to xenobiotic compounds, statistically significant changes in the microbiota, albeit slight, are very likely to occur in animal exposure studies involving chemicals, provided that such studies are well controlled for confounding effects and that a sufficient number of animals are included. Therefore, the research question to be asked should be extended to whether induced changes in the microbiota have any biologically relevant effect in the host species. We propose the term *microbiota disrupting chemicals* to describe substances that fulfill the following two criteria; (i) the substance alters the composition and/or activity of the intestinal microbiota and (ii) these alterations mediate an adverse health effect in the host species. This is in line with the WHO criteria used to define endocrine disrupters [14], and requires that there is a causal relationship between microbiota changes and observed adverse health effects. Establishing such causal relationships can be achieved by established protocols for fecal transplantation to germ-free animal models [15] and further molecular mechanisms may be elucidated. For endocrine disruptors, it has been debated whether the definition needs to include the mediation of an adverse effect in the host, or could be limited to any aspect of hormone action [16]. However, in the context of microbiota disruption, we find it necessary to include the second criterion given above, since many types of food additives alter the gut microbial composition and/or activity, and are sometimes even added with this purpose, since such changes may also be beneficial. Without the second criterion, these additives would fall under the definition of microbiota disrupting chemicals.

Lost in translation? Both *in vitro* and *in vivo* study designs have routinely been used to investigate effects of xenobiotic compounds on microbial communities [10,17,18]. The advantages of *in vitro* fermentation based studies include the possibility to rigidly control for confounding factors and the possibility for high-throughput analysis of many different compounds or exposure levels in parallel. Both continuously fed and batch fermentation systems may be employed, of which the first provides a more realistic set of conditions but is more difficult to multiplex. The most obvious drawback of *in vitro* systems is the complete separation from the host organism of the bacterial community, making host-dependent effects impossible to study. Another challenge is to simulate the growth conditions to reflect the *in vivo* conditions. Several different bacterial growth media have been developed to mimic the conditions of the colon and may include meat or yeast extract, bile acids, short chain fatty acids, amino acids and oligosaccharides, which combined with anaerobic conditions sustain the growth requirements of a large diversity of

different gut bacteria [19]. A recent study from our lab highlights the importance of growth conditions for the evaluation of the effect of the herbicide glyphosate on the intestinal community [20]. In this study we find only very limited effects of glyphosate on the microbial community composition *in vivo* as compared to previous *in vitro* studies [11,18] and demonstrate that this is due to the presence of sufficient levels of intrinsic aromatic amino acids in the gut to alleviate the growth inhibitory effect of glyphosate *in vivo*. This shows that careful consideration should be given to bacterial growth conditions when designing *in vitro* studies and further points to the continued importance of employing animal models to test chemicals for microbiota disrupting properties.

In vivo models that may be applied in studies of interactions between microbes and a host factors relevant for chemical risk assessment include mice [21], rats [22], zebrafish [23], the nematode *Caenorhabditis elegans* [24] and the insect *Drosophila melanogaster* [25]. Additionally, originally germ-free mice colonized with microbial communities derived from humans [26] represent a useful model for investigations of the causal effects of chemically induced microbiota changes on host health. In risk assessment, the choice of animal model will primarily rely on the feasibility of the model for assessment of the adverse outcome, which is expected to result from chemical exposure, such as e.g. the measurement of anogenital distance in rat models applied for investigation of putative endocrine disruptors [27]. With all animal models, translation to the human situation should however be done with great caution. In studies of microbiota disrupting chemicals, it is worth noticing that the commensal rodent microbiota is very different from that of humans. For example, mice often lack genera such as bifidobacteria, which are considered important beneficial bacteria in humans [28], while these genera are more abundant in rats [29]. The commensal microbiota of fish, rats and nematodes is however even more distant from the human microbiota than that of rodents (and other mammals) [30]. In models where a human microbiota is introduced into an animal host, it should be noted that due to the absence of co-evolution between host and microbes, the 'foreign' microbes will typically not have as much impact on the host response as those indigenously present in the given host species [31]. Furthermore, not all microbes derived from a human gut will be able to colonize e.g. in a mouse [26]. In spite of these reservations, animal models still constitute an important tool in the assessment of potentially adverse effects of chemicals, including also microbiota disrupting effects.

The gut microbiota affects uptake and metabolism of ingested compounds. Xenobiotic suppression or enhancement of proliferation of specific groups of intestinal microbes may lead to alterations of the composition of the intestinal bacterial population, which in turn is known to affect the permeability of the intestinal epithelium. For example, specific bacterial species are known to upregulate genes responsible for epithelial expression of tight junctions and mucins *in vitro* [32,33], while faecal water from elderly, who have a different gut microbiota as compared to younger people, apparently causes a decrease in the integrity of epithelial cell layers *in vitro* [34]. Moreover, studies from our lab reveal that antibiotic treatment impacts gut permeability in rodent models [29]. It is thus highly likely that the integrity of the gut barrier can be affected by the changes induced by *microbiota-disrupting chemicals*. This may in turn lead to alteration in the uptake of the given chemical, as well as of other toxic and/or beneficial components present in the gut.

Additionally, microbes known to reside in the gut harbour a different pool of enzymes than their mammalian host, and many of these microbial enzymes can metabolise xenobiotic compounds directly [35,36]. Examples include the direct bioactivation of polycyclic aromatic hydrocarbons by human colonic microbial communities, leading to formation of estrogenic metabolites [37], and the recently reported modulation of the toxicity of organophosphate insecticides mediated by specific strains of *Lactobacillus* [25]. Furthermore, the intestinal microbes regulate xenobiotic metabolism in the liver as monitored by differential expression of genes connected to xenobiotic metabolism in conventional and germ-free animals, respectively [38]. The microbes may also de-conjugate conjugated xenobiotics recycled from the liver [39], leading to regeneration of the original toxin, or to formation of new toxic agents.

In vivo variation may be partly attributed to differences in microbiota. The efficacy and toxicity of chemotherapeutic agents is affected by the gut microbiota [40], however the mechanisms behind such individual responses are currently not understood. We find it likely that the individual response of experimental animals to chemical exposure is similarly highly dependent on their microbiota. We thus suggest that a significant part of the inter-individual variation as well as the study-to-study differences currently observed in risk assessment of ingested chemicals based on experimental rodents may be explained by differences in the microbiota of the animals.

Complete gene catalogues of the intestinal microbiota of mice and guinea-pigs including comparisons to that of humans are available [28,41]. For experimental mice, it is additionally well described that the composition of the microbiota is highly dependent on the animal provider. In fact, this factor dominates the microbial gut profile more than factors such as diet, mouse strain or housing lab [28]. In both animals and humans, littermates/siblings harbour more similar microbiotas than unrelated individuals [42]. As rodents practice

coprophagy, co-housing (e.g. living in the same cage) is known to affect the microbiota-derived metabolic traits of the mouse host [15]. Moreover, a recent study from our lab reveals that even the transfer of microbes occurring between animals not in direct contact, e.g. as airborne spreading or through handling, is sufficient to create distinct microbial patters as well as to affect the metabolic responses in mice [26]. These factors are thus crucial to take into account in the planning of animal experiments. For example, if animals challenged with two different doses of a chemical are co-housed in two separate groups receiving one dose per group, it can be speculated that the sharing of microbiota between co-housed animals significantly affect the toxicological endpoints. Potentially, observations interpreted as results of different doses may in this case in fact result from different microbiotas.

Consideration of the gut microbiota in risk assessment. As discussed above, it is becoming increasingly evident that the gut microbiota plays an important role in toxicology studies and may constitute a substantial confounding effect. In order to minimize this effect and thus some of the variability observed in animal exposure studies we propose the following considerations: (i) When possible, choose animal models with a high bacterial diversity - preferably comparable to wild animals. The absence of a 'natural' complex microbiota is likely to affect the outcome parameters as explained above. (ii) Request animals with standardized microbiota from vendors that offer this [43]. If standardized animal models are not available, make sure to obtain information about the origin of the animals (litter, breeding barrier), which should be applied for randomization. (iii) Analyze intestinal bacterial diversity and composition routinely in animal studies addressing the effect of oral exposure to xenobiotic substances, and compare this to observed end-point measurements in the individual animal hosts. (iv) Carefully consider how animals are co-caged, as microbiota spread between co-caged animals due to coprophagia and environmental contact. (v) Consider supplementation of animal experiments with studies of the effect of given toxic substrates on bacterial strains and communities, and with studies of bacterial metabolism and conversion of these substrates.

Acknowledgements

The authors thank Lene Nørby Nielsen and Anne-Marie Vinggaard for inspiring input and discussions. This review was funded by the Ministry of Environment and Food of Denmark.

Conflict of interest

The authors declare no conflict of interest.

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