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FAO TECHNICAL MEETING ON THE GUT MICROBIOME IN FOOD SAFETY CHEMICAL RISK ASSESSMENT

ROME, 12–14 DECEMBER 2023
MEETING REPORT

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ABBREVIATIONS AND ACRONYMS

ADI	acceptable daily intake
ADME	absorption, distribution, metabolism and excretion
ARfD	acute reference dose
DNA	deoxyribonucleic acid
FAO	Food and Agriculture Organization of the United Nations
GIT	gastrointestinal tract
JECFA	Joint FAO/WHO Expert Committee on Food Additives
JMPR	Joint FAO/WHO Meeting on Pesticide Residues
mADI	microbiological acceptable daily intake
mARfD	microbiological acute reference dose
MIC	minimal inhibitory concentration
VICH	Veterinary International Conference on Harmonization
WHO	World Health Organization

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DECLARATIONS OF INTEREST

All participants completed a Declaration of interest form in advance of the meeting. The following participants indicated interests in relation to the subject of this meeting:

- Mark Feeley and Stephen Brooks **declared having paid employment**.
- Mark Feeley **declared consulting, including serving as a technical or other advisor**.
- Javier Moreno, Yolanda Sanz and Stephen Brooks **declared having received research support**.
- Stephen Brooks **declared having received non-monetary support for travel to meetings with an interest related to the subject of this meeting**.
- Yolanda Sanz **declared having patents, trademarks or copyrights**.
- Alan Boobis and Yolanda Sanz **provided expert opinion or testimony as part of a regulatory, legislative, judicial or other governmental process**.
- Stephen Brooks and Sangeeta Khare **reported participating in expert committees or scientific advisory groups**.
- Stephen Brooks **declared that to his knowledge, the outcome of the meeting or work would benefit or adversely affect interests of others with whom he has substantial common personal, financial or professional interests**.
- Jaime Aguilera **declared, excluding FAO, an entity paid or contributed towards his travel costs in connection with this meeting**.
- Alan Boobis and Andrew Holmes **reported participating in expert panels, committees and scientific advisory groups in subjects not directly related to the matter of the meeting**.
- Daniel Ramón **declared he works in the private sector on the study of the microbiome and the development of microbiome modulators, but his work is not related to the topic of the meeting**.

Following the FAO guidance document for declaration of interests, the declarations noted above were assessed as to the extent to which each interest could be reasonably expected to affect and exercise influence on the experts' judgment.

The declared interests of Alan Boobis, Andrew Holmes, Jaime Aguilera, Javier Moreno, Mark Feeley, Sangeeta Khare, Stephen Brooks and Yolanda Sanz were considered unlikely to potentially impair the individual's objectivity or cause a significant perception of influencing the impartiality, neutrality and integrity of the work. They participated in the meeting fully as experts. Daniel Ramón Vidal participated in the meeting as a resource person, and did not take part in the last session.

The participation by these individuals in the meeting is not reasonably expected to create unfair competitive advantages nor are the meeting outcomes reasonably foreseen to affect the individuals' declared interests. The declared interests of all participants were disclosed and made available at the beginning of the meeting to all meeting attendees.

FAO determined that none of the remaining experts had declared any interests that could be perceived as potentially conflicting with the meeting objectives.

All the experts participated in their own individual capacities and not as representatives of their countries, governments or organizations.

EXECUTIVE SUMMARY

The FAO Technical Meeting: Gut Microbiome in food safety chemical risk assessment was held in Rome from 12 to 14 December 2023. Seventeen participants, representing diverse disciplines, attended the meeting: 11 experts and 1 resource person (including food safety risk assessors and microbiome ecology experts) and 5 FAO team members. The objective of the meeting was to explore challenges and needs related to applying microbiome data in future food safety chemical risk assessment. The meeting resulted in the identification of a series of steps required to facilitate further considerations and integration of microbiome data into the risk assessment of regulated substances.

Initial discussions led to the identification of current challenges limiting the usability of available microbiome data for risk assessment purposes. These challenges include the need for microbiome-related definitions, improved and fit-for-purpose study designs based on realistic exposure scenarios, suitable and predictable biomarkers and endpoints, a better understanding of microbiome-chemical and microbiome-host interactions, and support for interpreting microbiome study results and linking the results to adverse effects. Further discussions addressed technical questions related to microbiome science (specifically sampling, models and omics technologies) and to new developments with more significant and relevant potential to improve the field. The experts identified the advantages, shortcomings and potential improvements of various methodological approaches, models and omics methods. They also highlighted the methods most suitable for addressing specific research questions related to chemical exposure, such as interactions between chemicals and the microbiome and related adverse health effects. They highlighted the critical need for guidelines covering several research aspects, including the reporting of findings, as well as the need for international standardization and harmonization of different aspects of microbiome methodologies.

The experts also identified several critical aspects where the inadequacy of available data currently hampers the systematic inclusion of microbiome data in the risk assessment of regulated substances. These inadequacies can be roughly grouped into three categories: definitions, research needs, and standardization and standard harmonization. This initial exploratory meeting paved the way for follow-up meetings to address these categories, which will likely require the involvement of a broader group of experts and disciplines.

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INTRODUCTION

Food can contain substances that are added intentionally (such as food additives[1] and processing aids) as well as substances that are present unintentionally or inadvertently, either through their upstream use (as is the case with pesticide residues[2] and veterinary drug residues[3]) or via their natural or anthropogenic origins (including certain heavy metals and dioxins). In most countries, such substances are subject to a risk assessment[4] that aims to establish health-based guidance values (such as the acceptable daily intake, or ADI), followed by the development of regulatory limits.

Triggered by our increased understanding of the importance of the microbiome on the health of consumers, a question has arisen in the field of food-safety risk assessment as to whether it may be necessary to consider possible additional effects of substances present in food on the microbiome and potential consequent contribution to adverse effects[5]. Such an inclusion, however, is hampered by several concurrent challenges, including knowledge gaps, methodological and analytical limitations, and a lack of translatability of data obtained in different research models to the human context.

FAO has conducted preliminary analyses of scientific methodologies, interpretations and conclusions from publications reporting on the effects of pesticide residues,[6]

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1 Food additive: In the Codex Alimentarius Commission context, any substance not normally consumed as a food by itself and not normally used as a typical ingredient of the food, whether or not it has nutritive value, the intentional addition of which to food for a technological (including organoleptic) purpose in the manufacture, processing, preparation, treatment, packing, packaging, transport or holding of such food results, or may be reasonably expected to result, (directly or indirectly) in it or its by-products becoming a component of or otherwise affecting the characteristics of such foods. The term does not include contaminants or substances added to food for maintaining or improving nutritional qualities (FAO and WHO, 2009).

2 Pesticide residue: Any specified substances in or on food, agricultural commodities or animal feed resulting from the use of a pesticide. The term includes any derivatives of a pesticide, such as conversion products, metabolites, reaction products and impurities considered to be of toxicological significance. The term "pesticide residue" includes residues from unknown or unavoidable sources (e.g. environmental) as well as known uses of the chemical (FAO and WHO, 2009).

3 Veterinary drug residues: The parent compounds and/or their metabolites in any edible portion of the animal product. They include residues of associated impurities of the veterinary drug concerned (FAO and WHO, 2009)

4 Risk assessment: A process intended to calculate or estimate the risk to a given target organism, system or (sub)population, including the identification of attendant uncertainties, following exposure to a particular agent, taking into account the inherent characteristics of the agent of concern as well as the characteristics of the specific target system. The risk assessment process includes four steps: hazard identification, hazard characterization, exposure assessment and risk characterization. It is the first component in a risk analysis process (FAO and WHO, 2009).

5 Adverse effect: Change in the morphology, physiology, growth, development, reproduction or lifespan of an organism, system or (sub)population that results in an impairment of functional capacity, an impairment of the capacity to compensate for additional stress or an increase in susceptibility to other influences (FAO and WHO, 2009).

6 See: The impact of pesticide residues on the gut microbiome and human health. A food safety perspective https://www.fao.org/documents/card/en/c/cc5306en

veterinary drug residues,[7] microplastics[8] and food additives[9] on the gut microbiome and the resulting health impact. As a follow-up activity, FAO engaged a multidisciplinary group of experts (including food safety risk assessors and microbiome ecologists) in a dialogue to discuss the limitations and challenges in understanding microbiome science for future applicability of microbiome data in risk assessments. The objective of the meeting was to identify a set of actions needed to integrate microbiome data in the risk assessment of regulated chemicals.

To set the context for the discussions, several meeting participants introduced the following topics:

> regulatory science and risk assessment and the role of the gut microbiome (see Annex I);

> microbial risk assessment of veterinary drug residues (see Annex II);

> decision frameworks to be considered for incorporating microbiome data in risk assessment strategies (see Annex III).

> gut microbiome concepts important for risk assessment (see Annex IV);

> models and omics technologies used to investigate the gut microbiome (see Annex V);

> chemical exposure and the gut microbiome – a food safety perspective (see Annex VI).

The discussions were organized around three main tasks (see Annex VII):

> Task 1: Experts were asked to identify microbiome-related data gaps and needs for risk assessment.

> Task 2: Experts were invited to evaluate the suitability (maturity and relevance) of different microbiome data for risk assessment. This activity aimed to identify (1) the benefits and limitations of research models and analytical technologies, (2) the potential of microbiome endpoints and biomarkers of adverse alterations, and (3) considerations to evaluate the biological relevance of microbiome changes.

> Task 3: Experts were invited to develop a data maturity ranking (if existing knowledge allows) and identify the conditions or developments needed to integrate the different gut microbiome data into risk assessment.

A set of supporting questions was developed to facilitate and enrich the discussions for each task. These questions were designed to stimulate dialogue and ensure that each topic was explored from multiple perspectives (see Annex VII).

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7 See: The impact of veterinary drug residues on the gut microbiome and human health. A food safety perspective https://www.fao.org/documents/card/en/c/cc5301en

8 See: The impact of microplastics on the gut microbiome and health. A food safety perspective https://www.fao.org/documents/card/en/c/cc5294en

9 Report in preparation.

CHAPTER 1
INTEGRATING MICROBIOME DATA IN RISK ASSESSMENT: CONSIDERATIONS AND CHALLENGES

The increasing recognition of the human gut microbiome as a player in chemical biotransformation, health and disease is leading regulatory organizations to consider the gut microbiome in chemical risk assessment procedures. This chapter explores the current position of regulatory bodies and organizations in this regard, current gaps and limitations hindering the integration of microbiome data in risk assessment, and relevant interactions between exogenous chemicals and the gut microbiome. Additional discussions include microbiome metrics, endpoints and biomarkers, to address a key question: When do gut microbiome changes transition from normal variation to a cause for concern?

CURRENT POSITION OF REGULATORY AGENCIES AND OTHER ORGANIZATIONS ON THE INTEGRATION OF MICROBIOME DATA IN RISK ASSESSMENT

The experts discussed the consideration of microbiome data by international organizations and regulatory bodies in the risk assessment of chemicals.

According to the experts, most regulatory agencies are considering the possibility of incorporating microbiome data into risk assessment practices, but no consensus regarding how to do that in practice has yet emerged. For this reason, there are no specific actions, guidelines or methodologies in place to request and use microbiome-related data in risk assessment. The United States Food and Drug Administration is working to establish standardized methodologies but has not yet finalized any specific guidance for chemical risk assessment, and the European Food Safety Authority is considering a roadmap to incorporate the gut microbiome in food and feed risk

assessments. In addition, the Joint FAO/WHO Expert Committee on Food Additives (JECFA) has data requirements for deriving a microbiological acceptable daily intake (mADI) and a microbiological acute reference dose (mARfD) for veterinary drug residues in foods (see Annex II). Data requirements include specific endpoints, which may be based on *in vitro* effects, including disruption of the colonization barrier and increase of antimicrobial-resistant bacteria. For its part, the Joint FAO/WHO Meeting on Pesticide Residues (JMPR) has recommended this approach for the assessment of pesticide residues in food, while the Organisation for Economic Co-operation and Development has not yet started any formal microbiome-related developments, although a working group on omics has been established.

At this time, the scientific understanding of the gut microbiome and its effects on human health does not appear to be robust enough or adequately suited to address safety concerns and regulatory requirements accurately and effectively, including its integration in risk assessment. This is due to limitations in analytical approaches for addressing the complex and multifactorial causality underpinning health outcomes. Consequently, regulators face obstacles in formulating practical questions and defining regulatory criteria for submitting microbiome data for risk assessment purposes.

GAPS AND LIMITATIONS HINDERING THE INTEGRATION OF MICROBIOME DATA IN RISK ASSESSMENT

The experts discussed whether there is a need to incorporate gut microbiome data in risk assessment. They also identified relevant knowledge gaps and the limitations in understanding the impact of chemicals on the gut microbiome and the potential of this microbial community to influence health outcomes. Furthermore, the experts identified the type of research and data that would be considered suitable for such assessments. The following are the main points raised in the discussions:

- **Definitions of pertinent questions:** There is a need to define specific questions regarding whether and which uncertainties in chemical risk assessment can be effectively reduced through the integration of gut microbiome data.
- **Chemical–gut microbiome interactions:** A deeper and more comprehensive understanding of the interactions between chemicals and the gut microbiome is essential; specifically, how different chemicals can modulate the microbiome and, in turn, how the microbiome can influence the bioavailability and toxicity of chemicals.
- **Improved modelling:** There is a need for models that better mimic the gut microbiome and its interaction with the host. These models should provide valid and fit-for-purpose data that can be directly utilized in risk assessment processes.
- **Robust data:** A significant challenge lies in the lack of reproducible and comparable data obtained using standardized methods and technologies.
- **Realistic exposure scenarios:** There is a need for research designed to mimic realistic exposure scenarios that accurately reflect the conditions (for example, doses and exposure periods) under which chemicals and the gut microbiome interact.

- **Dose-response:** A deficiency exists in which dose-responses are not routinely assessed.
- **Longitudinal studies:** More information is needed to understand how the gut microbiome changes over time and how such changes relate to host-health outcomes.
- **Full microbiota spectrum:** Studies are biased primarily towards evaluating the bacterial community, especially the most abundant bacteria and those associated with health benefits or diseases. However, it would be important not to disregard low-biomass microbes, such as viruses (including phages), archaea or eukaryotes (such as yeasts and filamentous fungi), which may modulate the activity of the microbial population and participate directly or indirectly in the interactions with chemicals and with the host.
- **Functional insights:** Many existing microbiome studies, especially those investigating the impact of regulated substances and environmental contaminants, rely strongly on phylogeny. There is a need for more information on net metabolic activity and exchange of microbe cell components and metabolite outputs with the host system.
- **Endpoints:**[10] There is a need to identify, evaluate and potentially redefine endpoints beyond those traditionally used in the assessment of veterinary drug residues to determine the microbiological ADI.
- **Identification and definition of biomarkers:**[11] Identifying suitable and predictive biomarkers to measure relevant microbiome changes or microbiome-related host alterations is an essential need for using microbiome data in risk assessment.

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10 Endpoint: Qualitative or quantitative expression of a specific factor with which a risk may be associated as determined through an appropriate risk assessment (FAO and WHO, 2009).

11 Biomarkers: Indicators of changes or events in human biological systems. Biomarkers of exposure refer to cellular, biochemical or molecular measures that are obtained from biological media such as human tissues, cells or fluids and are indicative of exposure to a substance. Biomarkers of effect refer to biological changes that represent an alteration in endogenous body constituents (e.g. depression of cholinesterase levels as an indicator of exposure to pesticides) (FAO and WHO, 2009).

- **Magnitude of changes:** It is necessary to elucidate the magnitude of the change that is biologically relevant for the ecosystem and its relation to host-health outcomes.
- **Defining normality vs changes of concern:** A critical challenge is differentiating between what constitutes a normal or temporary variation in the gut microbiome and changes that lead to adverse effects or health concerns.
- **Correlation and causal links between microbiome changes and adverse effects:** Currently, there is a lack of robust evidence correlating and causally linking microbiome changes with adverse effects. There is a need to formulate strategies to clarify this connection by designing research studies using appropriate models and analytical methodologies.
- **Interpretation of microbiome changes:** For the assessor, it is challenging to understand the meaning of disturbances caused by acute or chronic chemical exposures, especially those identified using omics technologies, and to link such changes to potential host-health outcomes or adverse effects. Therefore, the development and availability of guidelines to support the interpretation of reported findings would be very useful.
- **Understanding mechanisms of action:** In most cases, the mechanisms underlying microbiome-mediated effects on the host physiology are not fully defined, emphasizing the need for research in this area.
- **Definitions:** Several definitions are needed that will serve the peculiarities of risk assessment. These include the identification of features of a healthy microbiome and of dysbiosis. Other concepts needing clarification are microbiome elasticity and resilience, which relate to feedback cycles of microbiome variations along with host responses (for instance, inflammation, followed by recovery).

Defining a healthy gut microbiome baseline: challenges and approaches in chemical risk assessment

The experts highlighted that a fundamental aspect of understanding the impact of chemicals on the gut microbiome and on health is to define a baseline for a healthy gut microbiome. Many factors have been considered and questions raised when searching for approaches to define a healthy gut microbiome; for example, age, dietary habits, environmental factors, lifestyles and health status will all influence a microbiome's configuration and function. Given the number of factors influencing intra- and inter-individual microbial variability, how many human subpopulations would be needed to define a healthy gut microbiome? It does seem clear that the gut microbiome is stable in healthy individuals. Under this notion, the experts indicated that the definition should encompass the host's health status. One possible approach to address this complex question could be to find shared gut microbiome features linked to healthy status in large population studies or microbiome interventions that contribute in one way or another to healthy outcomes. These could be defined as bacterial taxa, gene expression patterns, functions, metabolites or other end-products known to participate in a healthy microbiome ecosystem. Some microbiome

components could play a passive role and be functionally redundant, while others may be more essential in defining a healthy microbiome. Therefore, understanding or identifying the core gut microbiome or specific keystone bacterial strains would be a remarkable advancement to bridge the gap between gut microbiome science and chemical risk assessment.

Given the many factors that influence the normal dynamics of the gut microbiome of a healthy individual (including diet, age and gender), one of the main challenges is how to distinguish the actual effects of a chemical being assessed from background changes induced by such factors. Diet is a contextual element that is very relevant for the gut microbiome and is not always considered in the models used in assessments. It would be useful to identify associations between specific diets and gut microbiome patterns and include these diets as reference in research guidelines. The challenge is to integrate the diet as an element into data analysis, which would require further discussions and investigations. To address this complexity, the experts suggested shifting from the notion of a "healthy" population, which lacks clarity, to the concept of "reference populations", which is less dependent on an individual's health status while incorporating the contextual factors mentioned, such as diet. Different types of reference populations could be instrumental in addressing diverse research questions, providing a more targeted and contextually relevant perspective for studying the impacts of chemicals on the gut microbiome.

There is also a need to define gut dysbiosis. There are different definitions of the term used in the scientific literature, but the definition needs to be updated, ideally based on consensus. Dysbiosis is linked with an undesirable state of the holobiome[12], either dysfunctional or disease, and self-sustained. Dysbiosis can be transient but adverse (as when it is related to antibiotic effects), while stable dysbiosis is more related to chronic diseases.

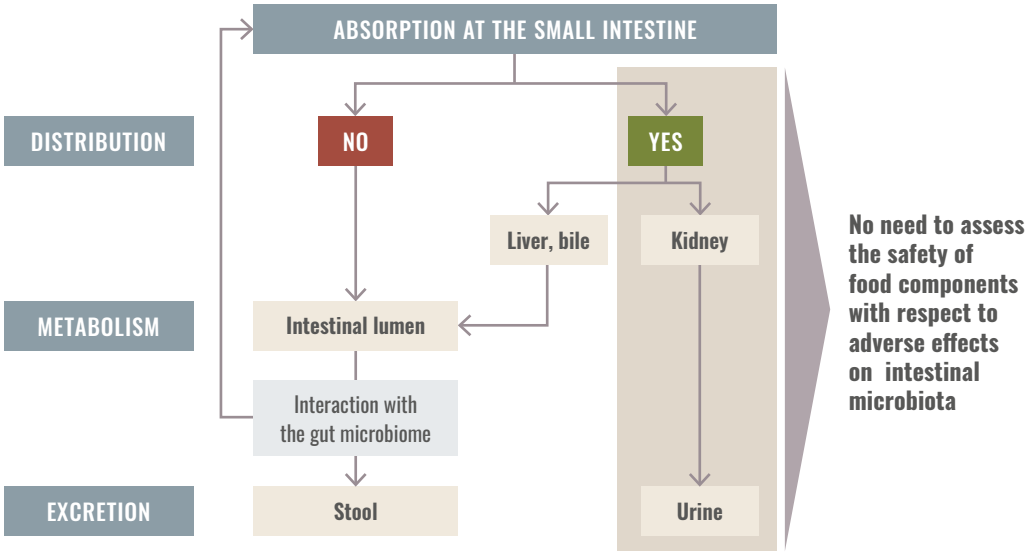
INTERACTIONS BETWEEN EXOGENOUS CHEMICALS AND THE GUT MICROBIOME

After ingestion, exogenous chemicals can interact with the gut microbiome at various stages along the gastrointestinal tract (GIT). The time and nature of these interactions depend on factors such as the chemical properties and transit time. It is also important to understand the toxicokinetics and the absorption (at the small intestine level), distribution, metabolism and excretion (ADME) of the ingested chemical to better understand if it can interact with the gut microbiome (Figure 1).

A priority for risk assessment identified by the experts is to fully understand and characterize the interactions of the exogenous chemical with the gut microbiome. This interaction can occur in two directions: microbiome effects on chemicals and chemical effects on the gut microbiome.

[12] Holobiome: sum total of the component genomes in a eukaryotic organism; it comprises the genome of an individual member of a given taxon (the host genome) and the microbiome (the genomes of the symbiotic microbiota) (Guerrero, Margulis and Berlanga, 2013).

FIGURE 1. **SCHEMATIC REPRESENTATION OF ADME OF INGESTED CHEMICALS**



Source: Authors' own elaboration.

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MICROBIOME EFFECTS ON CHEMICALS OR MICROBIOME MODULATION OF TOXICITY

The enzymatic repertoire of the gut microbiome constitutes a diverse collection of enzymes that can participate in the biotransformation of exogenous chemicals, therefore contributing to the complex interplay between the gut microbiome and the host in response to different types of substances. From the toxicological point of view, several types of transformations can lead to changes in the toxicity and bioavailability of the chemical:

> **Inactivation of active chemicals or activation of inactive chemicals** to a desirable or undesirable state, or transformation of a chemical in a way that results in products with higher or lower toxicity than the parent compounds.

> **Deconjugation and reactivation of chemicals by the gut microbiota,** facilitating their entrance into enterohepatic circulation. This means that compounds absorbed from the GIT, conjugated and detoxified in the liver and then excreted back to the intestine can be deconjugated by the local intestinal microbiota and reabsorbed back to the liver. This metabolic cycle prolongs the chemical exposure, although it may not necessarily impact the chemical toxicity.

There is a need to better understand the microbial processes that activate or inactivate toxicants. The presence or absence of microbiome members with such metabolic

activities may explain some of the differences observed in individual susceptibilities to specific chemicals, for instance responders vs. non-responders and variations in sensitivities to different exposure levels. In this sense, the microbiome could be a predictor, guiding us in identifying susceptible subjects.

Being able to predict the activation and reactivation of toxicants by the gut microbiome is a key priority. These effects can be picked up by predictive toxicological studies, which are useful in investigating the chemical's pharmacokinetics. For example, *in vivo* toxicokinetic studies used to evaluate metabolic profile can identify excretion delay, which indicates the chemical entering enterohepatic circulation. *In vitro* models are also informative as predictors of metabolic profiles. Current microbiome modulation of toxicity focuses on researching chemical metabolites, and the involvement of the gut microbiome can be picked up by these studies. Still, how the microbial community participates in the chemical biotransformation remains underexplored. However, the role of the gut microbiome in chemical transformation could be included implicitly in the toxicological analysis.

As a first step, investigating the bioavailability of chemicals can help predict their potential to interact with the gut microbiome. Compounds with limited bioavailability (such as those with low absorption) are more likely to pass through the GIT and lead to a higher exposure of the microbiome to this agent (Figure 1). The potential biotransformation of compounds can also be evaluated *in vitro*. An example was given of the risk assessment of an arsenic-based feed additive (roxarsone) for poultry, carried out by the United States Food and Drug Administration[13] (Stolz *et al.*, 2007), which used faecal material fermentations to demonstrate the biotransformation of this compound by the gut microbiome. The detection of microbial enzymes – or their genes – could also indicate the microbiome's potential to metabolize the chemical. However, this requires the previous identification of the relevant enzymes or genes.

CHEMICAL EFFECTS ON THE GUT MICROBIOME OR TOXICANT MODULATION OF THE MICROBIOME

Chemicals can affect microbiome populations in different ways, including:

- disrupting the colonization barrier, which is the ability of the gut microbiome to prevent the invasion of pathogens or opportunistic microbes;
- increasing gut microbiome subpopulations resistant to antimicrobials, potentially reducing the effectiveness of these and other drugs.
- changing the gut microbiome from a desirable to an undesirable state and leading to alterations in microbial composition, gene expression and function (depending on the extent of chemical exposure – dose, time – and the type, duration and degree of alteration, the microbial disturbance could eventually impact host health).

[13] See: Withdrawal of Approval of New Animal Drug Applications; Roxarsone https://www.federalregister.gov/documents/2013/12/27/2013-30837/withdrawal-of-approval-of-new-animal-drug-applications-roxarsone (accessed 26 March 2024)

These interactions between the chemical and the gut microbiome are not always easy to detect by classical toxicology using animal studies. Therefore, research studies are required to target the microbiome specifically.

Although the gut microbiota, especially populations of distal intestinal segments or stools, have been the focus of most research, there is nascent evidence about the role of the oral microbiome (preliminary results at this point) in the interaction with food compounds.

MICROBIOME METRICS, ENDPOINTS AND BIOMARKERS

The experts agreed that there is a need to revise current assessments. It would involve the evaluaton of existing gut microbiome-related endpoints and discussed the need for additional ones. It was emphasized that this exercise would not be intended to change the principles of toxicology, but to expand or complement assessments with additional information.

The experts acknowledged that, currently, only two gut microbiome-related endpoints are being routinely considered by JECFA in the risk assessment of veterinary drug residues, mainly limited to those compounds with an antimicrobial mode of action (Annex II). The two endpoints are defined in the guideline developed by the International Cooperation on Harmonisation of Technical Requirements for Registration of Veterinary Medicinal Products, or VICH (2019), which also provides a framework for the establishment of the mADI. The endpoints are:

> **Disruption of the colonization barrier:** The colonization barrier is a function of the normal intestinal flora that limits colonization of the colon by exogenous microorganisms, as well as overgrowth of indigenous, potentially pathogenic microorganisms. This endpoint focuses on inhibiting certain bacterial strains associated with gut-barrier impairment, which is not directly measured.

> **Increase of the population(s) of resistant bacteria:** Resistance is defined as the increase of the population(s) of bacteria in the intestinal tract that is (are) insensitive to the test drug or other antimicrobial drugs. This effect may be due either to the acquisition of resistance by previously sensitive organisms or to a relative increase in the proportion of organisms that are already less sensitive to the drug.

While the two endpoint assessments and decision tree framework established by JECFA and VICH are currently limited to the evaluation of veterinary drug residues, those working on efforts to harmonize risk assessment procedures have recently proposed expanding their application to the evaluation of pesticides by the JMPR.

The experts agreed that the identification and inclusion of additional endpoints and biomarkers related to the gut microbiome and its relationship with the host could add information for the risk assessment of certain chemicals. They also indicated that relying solely on microbiome endpoints or biomarkers to establish a direct link to human adverse effects may be insufficient and suggested that it would be more relevant to integrate these gut microbiome indicators with host-specific markers. These could involve, for example, host endpoints and biomarkers influenced by the

gut microbiome, such as gut-barrier function, inflammatory status or inflammatory markers. Gut microbiome markers could involve specific microbial metabolites or taxonomic signatures. It should be considered that a single endpoint may not be universally applicable to all situations. Therefore, endpoints should be adapted on a case-by-case basis and, ideally, be predictable, validated, reproducible and comparable.

Currently, there is a lack of definitions for gut microbiome biomarkers suitable for the risk assessment of chemicals, and the experts identified several characteristics that such biomarkers should have. Biomarkers should be consistent, reproducible and predictive of adverse effects. Additionally, the information provided by biomarkers (and endpoints) from model studies should be extrapolatable to the human context. For example, the depletion of filamentous segmented bacteria – a keystone bacteria in mice – is not translatable to adult humans. In situations where chemical exposure leads to changes in the gut microbiome, a key factor is the ability of the biomarker to quantify and qualify whether these changes are harmful, particularly if they are responsible for adverse effects such as inflammation. Ideally, the biomarker should be involved in the biological plausibility of a mode of action, serving as a key indicator linking specific biological processes or changes to the underlying mechanisms of action leading to an adverse effect.

Existing gut microbiome metrics rely heavily on observed changes in the metrics of community structure in response to chemical exposure, although it was indicated that these indices may change between individuals and are, therefore, of limited value. Such community metrics are typically based on taxonomic markers, but they may be also based on functional genes that are potentially informative in predicting chemical biotransformation (genes coding relevant enzymes) or changes in antimicrobial resistance (antimicrobial resistance genes). Community outputs may also serve as functional markers, such as microbial metabolites. These should also be considered as they play vital roles in ecosystem maintenance, therefore moving away from relying solely on community structure. When looking for suitable biomarkers, the experts also recommended paying attention not only to the species membership (taxonomic markers) and phenotypic traits (functional markers) but also to the activity of the bacterial community and the influence of non-bacterial members of the gut microbiome. For example, phages can act as regulators of bacterial populations, and fungi can exert effects on antibiotics.

However, in the complex biological ecosystem formed by the microbial community, the host and their functional interactivity, most biomarkers are likely to interact with others. As such, the experts concluded that, depending on the context, it may make sense to rely on several biomarkers (composite biomarkers or a matrix of biomarkers) – considering both the gut microbiome and the host – that are related functionally and contribute to specific adverse effects or endpoints.

The experts highlighted that a general understanding of gut microbiome characteristics can be achieved, but it is not yet possible to identify specific endpoints for risk assessment on a universal scale. This means there will be no universal marker, but it could be feasible to identify changes in certain keystone species, metabolites

or co-abundant groups. In addition, the concepts of elasticity, resilience and stability of the microbiome were identified as potentially relevant markers. However, these concepts must be better defined, as it is not currently possible to infer if a gut microbiome is resilient or stable and to what degree during chemical exposure(s).

Other challenges and current limitations that hinder the identification of suitable and informative gut microbiome-related biomarkers include the need to define populations of reference based on, for example, health status (for instance, non-obese/lean vs. obese), age and dietary groups (such as, vegan vs. vegetarian vs. omnivores; Western diet). Finally, it is necessary to clarify what gut microbiome fluctuations mean for health, as discussed in the next section.

RELEVANCE OF GUT MICROBIOME CHANGES: FROM NORMALITY TO CONCERN

Understanding the biological relevance of microbiome changes in response to chemical exposure is essential to differentiate normal and transient microbiome fluctuations from alterations linked to host-adverse health effects. Investigating the biological meaning and relevance of changes in the gut microbiome is a complex task due to the intricate and multifactorial relationship between the host microbiome and the host.

In this context, the experts identified the need to define "biological relevance". This would involve determining whether the magnitude and nature of observed changes in the gut microbiome due to chemical exposure have significant implications for host health. Despite the challenges of finding and establishing the causal link between the gut microbiome and adverse health effects, it is vital to assess the risk posed by such changes.

A first step would be distinguishing between normal gut microbiome variability and alterations of concern. A related question pertains to a better understanding of the magnitude of changes that would need to be exceeded to be considered adverse. Thus, the discussions focused on possible thresholds between normality and alterations of concern (or adverse effects) in the gut microbiome and host following chemical exposure, which resulted in several key considerations.

In the context of chemical exposure, a universal understanding of the microbiome may not be necessary. Instead, the focus should be on discerning the nature and extent of changes elicited by chemical exposure compared to a **baseline**. This approach prioritizes understanding the specific impact of chemicals on the gut microbiome rather than a comprehensive knowledge of the microbiome itself. Therefore, understanding these baselines is crucial for identifying deviations that might be caused by chemical exposure. The experts noted that studies involving large cohorts have reported **normality ranges** or **thresholds** for human gut microbiota. These ranges are typically associated with specific phenotypes indicative of health or disease states (Asnicar *et al.*, 2021; Wang *et al.*, 2021). However, as responses to chemicals may differ across ages and may depend on other factors, such as

population susceptibilities, so do thresholds. This situation highlights once again the need to identify and define reference populations.

The experts proposed lines of research that could help to advance the investigation of baselines (or ranges of normalities) and thresholds. The group emphasized the importance of **dose-response** studies to better understand the relationship between the level of chemical exposure and the resulting impact on the gut microbiome and the development of adverse effects. Such studies are fundamental in establishing health-based guidance values and thresholds for safe exposure levels, while estimating risks.

For a more meaningful understanding of biological relevance, research should be based on **realistic exposure scenarios**. These include the consideration of the structure and form of the chemical, doses resembling typical levels of exposure in the human population, different exposure durations (acute, chronic, seasonal) and contexts (co-exposure scenarios, transgenerational – maternal – exposure). In addition, such studies should also consider **gender**, by including both male and female subjects in animal studies. This approach accounts for gender-based physiological differences (such as hormone status), which can be particularly informative in understanding the host-gut microbiome dynamics and their health implications.

Another important research consideration relates to the intestinal epithelium, which serves as a physical barrier that separates the host from the microbial environment in the gut and mediates the interactions between the two. Therefore, changes in the integrity of the intestinal epithelium and markers of inflammation, among others, may reflect microbiome alterations following oral exposure to chemicals.

The experts also identified another critical research aspect, which aims to provide information about gut microbiome resilience, that is, the potential for the gut microbiome and host effects to **revert** to their pre-exposure state (baseline) once chemical exposure stops. This aspect is investigated by extending studies beyond the treatment period – incorporating a wash-off period or recovery phase and comparing the results obtained at the end of this period with the baseline. This research aspect, often disregarded in the design of studies investigating the impact of regulated substances on the gut microbiome, is key to assessing delayed effects and the long-term impact of chemicals on the microbial community and the host.

A key goal and fundamental aspect in understanding the biological relevance derived from gut microbiome-host interactions is to determine the **causal relationship** between the two. In the context of chemical exposure, this would involve the confirmation of (1) the microbiome participation in the adverse effect(s) on the host or, on the contrary, (2) changes in the gut microbial population as a consequence of adverse effects to the host caused by chemical exposure. In this process, it is essential to differentiate between changes directly attributable to the chemical exposure and those arising from other confounding sources, such as diet, lifestyle or environmental influences.

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CHAPTER 2
STUDY DESIGN STRATEGIES AND CONSIDERATIONS

The experts addressed some critical aspects of study design, specifically focusing on sampling strategies, method selection and analytical tools, which are relevant not only to providing additional insights into the gut microbiome interactions with chemicals and the host but are also important from the risk-assessment perspective. Following a comprehensive overview of models and omics technologies, including their advantages and limitations, provided by Qixiao Zhai (Annex V), the experts discussed the specific aspects of study design and considerations described in this section.

SAMPLING STRATEGY

The foundation of robust microbiome research lies in a well-thought-out sampling strategy, since this will determine the quality of the data obtained, provide a contextual scenario, and facilitate the evaluation of the dynamics between the chemical, the gut microbiome, and the host.

One of the first decisions about sampling is to determine the **type of sample** to collect. In gut microbiome analysis, stool samples are the most common type of sample as they are easily accessible. However, questions have been raised regarding whether such samples are sufficiently representative of the microbial populations from other gut sites, especially proximal intestinal segments, or the mucosa-associated microbiota. For this purpose, samples of the intestinal mucosa are the most indicated, although they are difficult to obtain and require invasive techniques. The experts indicated that, from a metabolic perspective, faecal samples represent the overall end-product of microbial metabolism and are useful for studying the pharmacokinetics, pharmacodynamics and biotransformation of chemicals. In addition to investigating metabolites derived from gut microbiome function in faecal samples, blood also provides insights into the microbiome's functional output. Taking into account the difficulties in obtaining intestinal samples, faecal and blood samples are today considered sufficient for most studies.

Consistent **sampling methods** and protocols minimize variability and enable more accurate comparisons between different studies or groups within a study. This is crucial for identifying and interpreting true gut microbiome changes due to specific factors such as chemical exposure, as opposed to changes due to methodological inconsistencies. The sampling method, whether invasive (such as biopsies) or non-invasive (such as stool samples), can affect research outcomes and, therefore, the interpretability of data. Each method has biases that must be acknowledged in data analysis and reporting.

The emergence of advanced sampling technologies, such as "smart capsules", can potentially revolutionize gut microbiome research. These devices are swallowed and transit the gut naturally (non-invasively), allowing the device to collect samples from the various regions of the GIT, including those of difficult access, such as the small intestine (Rehan *et al.*, 2024). Although such devices are still in their early stages of development and not yet ready for routine use, the experts suggested investing more research resources in these technologies due to their expected impact in the field.

Time series sampling is another element to consider in the design of longitudinal studies. Implementing multiple sampling and determining its frequency depends on the study duration (short- or long-term) and the scientific question to be addressed. Time series sampling enables, for example, the evaluation of community dynamics, changes in the presence and abundance of chemical resistance genes, pharmacokinetics, pharmacodynamics, and the evolution of host parameters. The experts also indicated that samples collected in longitudinal studies should be independent of each other. To effectively achieve this, researchers should determine the optimal interval between each collection. This interval should consider completing a full digestive cycle (the time from chemical ingestion to faecal excretion). In humans, for example, this cycle typically spans an average of about three days.

The experts recognized that processes involved in **handling samples**, from collection to storage, are important sources of variability. If these activities are not planned with care and carried out consistently, they can lead to inaccurate analysis and interpretation. For example, poorly collected, handled, or stored samples can lead to degradation, post-collection metabolism, or contamination of the microbial deoxyribonucleic acid (DNA) and metabolites. Standardizing these processes (including, for example, control times from collection to storage, use of preservatives and use of optimal storage temperature) is a step forward for maintaining sample integrity and ensuring reliable results.

The experts also highlighted the need to consider, determine or control several other factors that are not assessed regularly but can influence the analytical output and interpretation of findings. These factors include the timing of food intake, gut transit time, intestinal volume, water content in stool samples, and cell density.

The experts recommended the preparation of guidance covering sampling, sample handling and storage to maintain sample integrity and ensure the accurate representation of the population under study. It is important to note that the sampling strategy will depend on the specific research question to be addressed. In human

studies, it would be essential to advise researchers and study participants about providing samples and minimizing the effect of external factors (such as drugs).

MODEL SELECTION

The choice of model – whether *in vitro*, *in vivo*, or *ex vivo* – for studying the gut microbiome in relation to chemical exposure should be a deliberative process, guided by the specific research question and by a thorough understanding of the capabilities and limitations of each model. This careful selection is essential for producing accurate and relevant data for a practical risk assessment.

Method selection should start by defining the research question, which should be formulated based on existing knowledge of the potential acute or chronic adverse effects of the chemical under assessment. The experts indicated that the model selected should be capable of replicating the conditions under which the microbiome interacts with the chemical under investigation and should facilitate the translation of findings to real-world situations. In addition, they identified the following criteria to assist in the selection of the most suitable model:

- bioavailability of the chemical;
- potential of the chemical to influence the gut microbiome;
- potential of the chemical to be metabolized by the gut microbiome;
- response sensitivity – the ability of the model to detect subtle changes in the gut microbiome (and in the host, in animal models) in response to chemical exposure;
- whether controlled exposure of humans is possible (with the important consideration of distinguishing between intentional exposure (such as food additives) or unintentional or inadvertent exposures (as in the case of veterinary drug residues or other chemical contaminants in food);
- ethical considerations (testing only substances permitted in food in human trials, while using surrogate models to test other substances).

Considering these aspects of model selection, the experts discussed the strengths, limitations and applicability of the different models available to produce data suitable for risk assessment, including *in vitro* models; advanced, technology-based models (organoids, biopsies, multi-cell cultures and gut on a chip); animal models; and human interventional studies.

IN VITRO MODELS

Static batch fermentation: These models can provide a fast screening of the microbiome and its activity and are suitable for high throughput. These features make them valuable, simple and flexible systems for the initial screening of multiple compounds or to study the interindividual variation in individual gut microbiome responses to a given factor. However, they are far from mimicking the normal *in vivo* physiological state and tend to oversimplify the actual complexity of the processes occurring in the colon.

Multicompartmental (dynamic) continuous systems: Among other advantages, these models can provide a continuous flow into several vessels, mimicking conditions found in portions of the human GIT and can enable mechanistic research by multiparametric control of dietary and digestive parameters on human microbiota from the same biological background. However, they have a low capability to test different compounds.

In summary, *in vitro* models allow for the direct investigation of the human gut or faecal microbiome. They can be helpful to control variables, elucidate mechanisms, and to understand the physiological role of chemicals and their interactions with the gut microbiome. They can also respond to some questions regarding microbial dynamics, but are limited due to the absence of the host component.

ORGANOIDS, BIOPSIES, MULTICELL CULTURES AND GUT-ON-A-CHIP MODELS

These advanced models are promising for mimicking the human gut ecosystem and studying host–microbiome interactions. While organoids, multicell cultures and gut-on-a-chip technologies offer significant advancements over traditional *in vitro* models, they still require further developments, particularly in sustaining long-term microbial cultures. Cellular models are not suitable for studying a complex community. Gut-on-a-chip, a microfluidic device that simulates the physical and mechanical environment of the GIT, has high potential in microbiome research. Nevertheless, challenges, such as maintaining the long-term stability of microbial communities, present relevant limitations in utilizing this system. Current model development also includes intestinal biopsies, which include the gut microbiota. However, similar to the gut-on-a-chip model, one of the main challenges is the medium or long-term maintenance of the model system.

ANIMAL MODELS

Animal models play a crucial role in studying the effects of chemical exposure on the gut microbiome, particularly where human studies are not feasible. Unlike previously mentioned models, animals provide a unique opportunity to study the implications of the intricate interplay between host physiology, the gut microbiome and chemicals at the local intestinal level and systemically. Animal models are instrumental in exploring transgenerational and longitudinal effects that cannot be investigated in humans. However, there are inherent challenges in translating findings from animal models to human scenarios. In this respect, it is crucial to consider not only the anatomical and physiological parallels between the chosen animal species and humans, but also to understand the similarities and differences in their respective gut microbiomes. Such considerations ensure a more accurate interpretation of how findings might translate to the human context. Exposure studies in animals involving the gut microbiome will need the application of an uncertainty factor for translating outcomes to the human scenario, as is common in classical toxicology studies.

Rodents are widely used in gut microbiome research and are well-established models in toxicology for both short- and long-term exposure studies. Their versatility – characterized by the availability of different genetic backgrounds of inbred or outbred strains, ease of genetic manipulation, short lifespan and cost-effectiveness – allows for different experimental design approaches. Although humanized mice were mentioned as a possibility to produce data that are more relevant to the human context, experts indicated that they do not seem to offer significant benefits over the use of well-established and less-costly conventional mice. With small size animals, it is possible to use a reasonably high number of animals in very controlled environmental conditions. However, husbandry and handling activities need to be carefully implemented as they are sources of microbial variability (such as diet, temperature and light cycles, co-caging and coprophagy). Rodent models are highly accessible for sampling and can be used effectively to understand physiological effects and mechanisms, and for hypothesis generation. The experts acknowledged that, while there is a discrepancy in the gut microbial composition and abundance between rodents and humans, the functional and metabolomic aspects of rodents' gut microbiomes more closely resemble those of humans.

While rodent models offer convenience and cost-effectiveness, non-human primates and piglets can provide more physiologically relevant insights into the human gut microbiome, due to similar anatomy, metabolic functions and gut microbiome composition. However, there are several limitations to the use of these models. The use of non-rodent mammals, especially non-human primates, is subject to stringent ethical considerations, therefore limiting their use in research settings. Also, they require specialized facilities and care, making them less cost-efficient than smaller models such as rodents.

HUMAN INTERVENTIONAL STUDIES

For obvious ethical reasons, human interventional studies can be conducted to evaluate already authorized substances or substances with expected low toxicity (such as food additives), but not for potentially dangerous compounds. The experts also indicated that despite being influenced by confounding factors, epidemiological (observational) studies can be helpful in retrieving data on exposure to specific chemicals and associated health effects from different populations.

ANALYTICAL TECHNOLOGIES

With regard to analytical technologies, the discussions focused primarily on the use and purpose of different omics technologies. The main points discussed by the experts are described in this section.

DNA-based sequencing methods (such as prokaryotic 16S rRNA gene sequencing and shotgun metagenomics) are widely accepted and provide detailed insights into the taxonomical composition of the microbiome and the functional potential (shotgun metagenomics). Although the level of adoption of shotgun metagenomics is not yet as high as that of 16S rRNA gene sequencing due to cost and technical challenges, it is expected that its use will increase in the upcoming years. However, in chemical exposure and for risk-assessment purposes, it is key to better understand functional aspects, such as the characterization of the interactions between the microbiome and the chemical, and to identify the final products resulting from this interaction. Such activities can be determined using metabolomics approaches.

The experts identified several considerations that require attention and some aspects of metagenomics analysis that require improvement. These include improving taxonomic reference databases for assigning sequence read data to well-defined species (or strains), quality of genome-based species definitions, functional annotations of those genomes, identification of core genes suitable as species biomarkers, and the inclusion of less-abundant species or strains – most notably those that occur in less-studied populations (considering geography or diets). There is also a need to define the use of sequence reads as surrogates for taxon metrics (operational taxonomic units). Both 16S amplicon (which uses amplicon sequence variants) and shotgun metagenomics (which matches sequence reads to diagnostic core genes) ultimately ascribe one sequence read to a taxon for community metrics. Caution should be exerted not to drive analysis only by short reads, because of the potential for false identification and imperfect correlations between read abundance and cell abundance. Another aspect to consider is the sequencing depth. Higher sequencing depth can facilitate the identification of rare microbial species, which can potentially influence the interactions with chemicals and the host. A crude generalization is that amplicon sequencing favours sequencing depth (within limits of primer targets) and metagenomics favours taxonomic resolution (and breadth of microbiome sampling, for instance, for viruses and fungi).

The experts acknowledged the need to integrate omics data into exposure research (the combination of metagenomics and metabolomics in particular), and to develop toolkits to perform these analyses. Such toolkits can be complemented with more classical analysis, such as traditional microbiological techniques (including, for instance, culture-based methods), which can be used to set simplified environments and confirm the roles and capabilities of specific microbial species or to validate the functionality of microbial species identified through omics approaches. Also relevant is the need to elucidate the mechanisms underlying omics findings.

STANDARDIZATION AND HARMONIZATION

The experts agreed on the need for standardization in microbiome research methodologies encompassing different aspects of study design (for instance, sample collection and handling and, in animal husbandry, caging, bedding and experimental diets), omics analysis (for instance, DNA and metabolite extraction, sequencing, mass spectrometry and bioinformatic pipelines) and results reporting. Standardized protocols for experimental procedures would enable more reliable comparisons and conclusions across different studies, and data-standardized reporting would ensure that results are interpreted properly and consistently. In addition, the experts emphasized the need to carry out standardization activities through international consensus and in a harmonized manner.

The experts acknowledged the limitations and challenges of standardization activities and suggested a few practical approaches to limit the effect of bottlenecks. One approach would be establishing minimal requirements for research methodologies and reporting. Some such requirements are available in other research fields and can be adapted to consider the specific needs of microbiome studies — for example, standardization of husbandry and animal handling or sample collection and storage. The second approach recognizes that full standardization might be unfeasible and unnecessary if proper and robust internal controls are implemented in the studies. Standardization should also consider the acceptability and accessibility to technologies to study the gut microbiome, especially the omics.

Engaging multidisciplinary experts and seeking the collaboration of standard development organizations (such as the European Committee for Standardization and the International Organization for Standardization) could facilitate the development and application of different standards in gut microbiome research. The expert group also highlighted the need to create or update guidance documents to assist researchers and facilitate the implementation of standardization. For example, they suggested engaging bodies like the Organisation for Economic Co-operation and Development to integrate gut microbiome assessment aspects into existing guidelines, such as those for toxicological studies involving animals.


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CHAPTER 3
CONCLUSIONS

DATA MATURITY AND ASSESSMENT FRAMEWORKS

One of the objectives of this meeting was to discuss the level of data maturity (data robustness); that is, the degree of readiness of the data for use in food-safety chemical risk assessment. Currently, only two gut microbiome-related endpoints are included in the evaluation of veterinary-drug residues. The experts concluded that it is impossible to define an exhaustive data maturity ranking at this point. In addition, overall, it is currently challenging to determine the weight of evidence provided by gut microbiome research. As discussed in this report, the reasons include (1) gaps in the knowledge of the gut microbiome itself and the lack of clear understanding of the biological relevance of microbial changes, (2) the limited data sets obtained in a reproducible manner using realistic scenarios and standardized methodologies, and (3) different degrees of development of the different analytical tools. It is expected that improvements in technology and the expansion of our knowledge about the gut microbiome and its influences on the host will enhance data accuracy and overall data quality. The challenge will be to determine at which point the evidence is robust enough to draw conclusions that meet the risk assessment needs.

The experts briefly discussed the need for frameworks to assist in assessing gut microbiome data. They pointed to the possibility of adapting an existing program and referred to the document on the mode of action framework developed by the International Program on Chemical Safety,[14] as well as other documents endorsed by international safety authorities describing decision trees, which could be helpful to evaluate the data maturity ranking.

NEEDS FOR APPLYING MICROBIOME DATA IN FUTURE FOOD SAFETY CHEMICAL RISK ASSESSMENT

The experts identified conditions and developments that are needed in order to be able to integrate gut microbiome data into risk assessment. They prioritized them in a series of actions grouped into three distinct categories (definitions, research and guidelines/harmonization). One key area of focus was promoting collaboration

[14] See the IPCS mode of action framework, IPCS harmonization project document no. 4, at https://www.who.int/publications/i/item/9789241563499.

between risk assessment bodies and academia. This collaboration is vital to ensure that research is aligned with regulatory needs, and to share expertise effectively. These needs, which have been mentioned throughout this report, are summarized in the following points:

DEFINITIONS

Definitions of the following aspects should be developed or improved:

> to define the gut population of reference microbiomes and microbiome fluctuations that are meaningful for health (including concepts such as microbiome-disrupting, modulatory or disturbing chemicals);

> to better define gut microbiome resilience, elasticity and stability.

RESEARCH

Advance knowledge on:

> methods and models to relate gut microbiome changes and adverse health effects;

> development of promising non-animal models (such as organoids, multi-cell and gut-on-a-chip models);

> real-time sampling approaches (such as smart capsules);

> omics techniques in gut microbiome research, including their application;

> host markers, together with gut microbiome biomarkers;

> activation, inactivation and reactivation of toxicants by the gut microbiota (individual susceptibilities to specific chemicals);

> gut microbiome resilience, elasticity, and stability (considering the need for methods and metrics to assess these features);

> non-bacterial gut microbiome members;

> gut microbiome populations of reference and what fluctuations mean for health;

> new endpoints for risk assessment based on functions to be protected (such as those related to the gut barrier function, anti-inflammatory status, specific metabolites or taxonomic signatures).

GUIDELINES AND HARMONIZATION

The following aspects require the development of guidelines or harmonization:

> ranking the quality of data/evidence;

> need for standardized methodologies and approaches to identify and assess relevant gut microbiome perturbations and qualify them as healthy, transient or adverse;

- need to internationally harmonize standards (that is, minimum methodological requirements and reference materials, data analysis and reporting) to help interpret results in the right direction;
- guidelines on sampling, storage and obtaining representative samples from a population, depending on the data analysis, interpretation and reporting;
- guidelines for study design, which should cover considerations of dependencies, including influential and contextual aspects (such as the identification of diets) for use in research studies, and should indicate the need to use exposure levels relevant for realistic scenarios and representative populations;
- the role of the gut microbiome in chemical transformation to be implicitly included in toxicological analysis;
- methodological strategies, awareness of hazards, chemical exposure in diets and gut microbiome considerations in exposure studies.

NEXT STEPS

This technical meeting has paved the way for forthcoming expert discussions involving a broader multidisciplinary group to address specific actions identified during this initial exploratory meeting.

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ANNEX I
REGULATORY SCIENCE AND RISK ASSESSMENT AND THE ROLE OF THE GUT MICROBIOME

By Mark Feeley

The standard safety evaluation of food-borne chemicals, be they intentionally or non-intentionally added substances, involves four stages: hazard identification,[15] toxicity assessment, exposure assessment[16] and risk characterization[17], the latter being the final step in the process, which integrates all the relevant data. The toxicity assessment, in particular, involves generating data covering all major aspects of chemical toxicology, typically using various experimental models, the study protocols of which have been standardized and harmonized by various international organizations, such as the Organisation for Economic Co-operation and Development. The key priority in food-safety chemical risk assessment is to identify the highest exposure level (dose or concentration) that does not cause treatment-related effects that could be considered relevant to human health.

Ingested chemicals and their interactions with the gut microbiome can be described as either chemicals having direct effects on or modulating the microbiome or the microbiome affecting or modulating ingested chemicals. Classic microbiome endpoints which can be impacted by direct action of ingested chemicals (such as antibacterial agents), include the disruption of the colonization barrier and the potential increase of microbiome subpopulations with resistance to antimicrobials. These two endpoints are currently assessed as part of a standard veterinary drug-safety evaluation. Less well defined is the action of chemicals on the gut microbiome, which results in the loss of the microbiome equilibrium state. This, in turn, can lead to alterations in the microbial population composition or dynamics, gene expression changes, changes in function endpoints (such as metabolite generation), and possible

[15] Hazard identification: The identification of the type and nature of adverse effects that an agent has an inherent capacity to cause in an organism, system or (sub)population. Hazard identification is the first stage in hazard assessment and the first step in the process of risk assessment (FAO and WHO, 2009).

[16] Exposure assessment: Evaluation of the exposure of an organism, system or (sub)population to an agent (and its derivatives). Exposure assessment is one of the steps in the process of risk assessment (FAO and WHO, 2009).

[17] Risk characterization: The qualitative and, wherever possible, quantitative determination, including attendant uncertainties, of the probability of occurrence of known and potential adverse effects of an agent in a given organism, system or (sub)population, under defined exposure conditions. Risk characterization is the fourth step in the risk assessment process (FAO and WHO, 2009).

host health effects. It is considered that standard toxicology testing protocols, without modifications, would be less likely to detect subtle changes in the gut microbiome caused by ingested chemicals, leading to a possible state of dysbiosis.

Gut microbiome interactions with ingested chemicals can occur as follows: Well-adsorbed compounds are transported from the gut to the liver through the portal vein and are metabolized through the action of liver enzymes. Certain phase II metabolic processes in the liver involve conjugation reactions in order to increase chemical elimination through the faeces. Conjugated or detoxified chemicals which are released back into the intestine within the bile can subsequently be deconjugated by various gut microbes, resulting in the reabsorption of the parent compound to the liver. This process is known as enterohepatic circulation and can result in prolonged exposure to the parent compound and possible increased toxicity. The gut microbial community can also directly metabolize non-adsorbed chemicals (activation or inactivation) or directly bind chemicals, reducing their bioavailability.

ANNEX II
MICROBIAL RISK ASSESSMENT OF VETERINARY DRUG RESIDUES

By Alan Boobis

The Joint FAO/WHO Expert Committee on Food Additives (JECFA), which addresses the safety of residues of veterinary drugs in food, developed a decision-tree approach for the evaluation of the potential impact of residues of antimicrobial veterinary drugs on the gut microbiome. The approach was first introduced in 1995 and was adopted for systematic use in 1999. In the interest of harmonization, the VICH agreed on a refinement of the JECFA approach, and since 2006, JECFA has utilized the VICH guideline in its assessments. The guideline, entitled Studies to evaluate the safety of residues of veterinary drugs in human food: General approach to establish a microbiological ADI, guideline number VICH GL36 (R2) (VICH, 2019), is now in its second revision. The approach is described in Principles and methods for the risk assessment of chemicals in food Environmental health criteria (WHO, 2009), although some updating is necessary.

The approach utilizes a decision tree to assess two endpoints of potential concern. The first of these is disruption of the colonization barrier, which can result in colonization of the colon by exogenous microorganisms, or overgrowth of indigenous, potentially pathogenic microorganisms. The second is an increase in the population of antimicrobial-resistant bacteria. This may be due either to the acquisition of resistance by organisms which were previously sensitive or to an increase in the proportion of organisms already resistant to the drug.

The approach involves a series of sequential steps, which comprise the decision tree: Step 1: Are residues of the drug, and (or) its metabolites, microbiologically active against representatives of the human intestinal flora? It is recommended that this be assessed from minimal inhibitory concentration (MIC) data, obtained by standard test methods, from a specified series of relevant genera of the intestinal bacteria. Step 2: Do residues enter the human colon? Ideally this would be based on human ADME data, but if not available, animal data could be used. Step 3: Do the residues entering the human colon remain microbiologically active? Information on this can be obtained either from *in vitro* studies, where the drug is incubated with faeces, or *in vivo*. If the answer to any of the first three steps is "No", then no microbiological ADI is required. Step 4: Is there any scientific justification to eliminate the need for testing either one or both endpoints of concern? Step 5: Determine the no-observed-adverse-effects concentrations/no-observed-adverse-effects levels for the endpoint(s) of concern as established in Step 4.

Test systems for assessing disruption of the colonization barrier include a number of *in vitro* models: MIC data, faecal slurries, semicontinuous, continuous, fed-batch culture test systems, as well as *in vivo* models, including human flora-associated animals. It is recognized that the use of MIC data obtained in *in vitro* studies would result in a conservative estimate of a no-observed-adverse-effects concentrations for disruption of the colonization barrier, but, in the absence of other data, they would be a suitable basis for a microbiological ADI. Test systems for assessing changes in resistant populations include semicontinuous, continuous and fed-batch culture test systems, *in vitro* and *in vivo* models, including human flora-associated animals.

If necessary, a microbiological ADI is determined by calculating the amount of drug present in the colon without apparent effect: Point of departure (either MIC_{calc} or no-observed-adverse-effects concentrations, as appropriate) × colon volume × any necessary adjustment factor, corrected for fraction of dose available to microorganisms and for body weight. Default factors are suggested for colon volume: 500 ml based on most recent data, adjustment factor of 1, body weight of 60 kg.

In the last few years, JECFA has extended this approach to consider potential acute effects, with the microbiological acute reference dose (mARfD) as a counterpart to the well-established toxicological acute reference dose (ARfD). The approach is very similar to that used in addressing the need and if necessary, establishment, of a mADI. In considering the two endpoints of concern, JECFA concluded that it was unlikely that a single exposure to residues of veterinary drugs would provide sufficient selective pressure to enable the emergence of a resistant bacterial population and, hence, in the absence of evidence to the contrary, only disruption of the colonization barrier need be considered. The mARfD is calculated in a similar way to that used for the mADI, with the inclusion of an additional adjustment factor of 3, to allow for temporal dilution during gastrointestinal transit and for dilution by consumption of additional meals during the day.

JECFA has recently extended these considerations beyond antimicrobial drugs, and now systematically assesses the possible need for a mADI and a mARfD for all veterinary drugs. To that end, JECFA expects to receive relevant information and data to assess the relevant endpoints for all drugs, as appropriate. While approaches to assessing the two endpoints considered to be of potential concern are now well established, knowledge of the gut microbiome and its possible role in human health is rapidly expanding. Hence, in 2022, JECFA recommended that a microbiome expert working group be convened to explore developments in the evolving area of the microbiological effects of residues of veterinary drugs, which should include consideration of: whether assessments should be expanded beyond bacteria; which endpoints are of concern, beyond those currently considered; and other relevant issues (such as test methods, extrapolation model and read-across).

With the recognition that not only antimicrobials can exert an effect on the microbiome, the JMPR (also in 2022) concluded that there was a need to consider how concerns over possible effects of pesticide residues on the gastrointestinal microbiome could be addressed, and that a good starting point would be VICH GL36 (R2), which may be sufficient for this purpose. The JMPR also recommended that a microbiome expert working group be convened to consider these issues.

ANNEX III
DECISION FRAMEWORKS TO BE CONSIDERED FOR INCORPORATING GUT MICROBIOME DATA IN RISK ASSESSMENT STRATEGIES

By Sangeeta Khare

The contribution of the gut microbiome in maintaining human health has been widely studied over the past decade. However, there is a need to further understand the roles of the gut microbiome that influence exposures to, and risks posed by, chemicals in food. Federal authorities regulate many products that interact directly or indirectly with human and animal microbiomes. The GIT plays a major role in maintaining homeostasis and harbours chemical and biological diversity (commensal microbiota) along the length of the intestine. Moreover, it is also the largest compartment of the immune system, with significant amounts of organized lymphoid tissue and huge populations of immune cells. A balance among the interaction of food chemicals with microbiota, gut-mucosa-associated responses, and metabolites is key to human health.

Some of the current approaches that could provide science-based regulatory decisions for risk assessment of the GIT may include: i) no adverse effects are observed (identify gut-microbiota-disrupting chemicals); ii) determining an acceptable human exposure for hazards in foods (absence of microbial dysbiosis); iii) potential GIT toxicity (maintenance of epithelial permeability and gut-mucosa-associated immune responses); iv) identifying potential developmental and reproductive toxicity (transgenerational toxicity); v) differential responses by subpopulations (new alternative methods). A step-by-step decision tree was presented to show risk assessment of the GIT.

ANNEX IV
GUT MICROBIOME CONCEPTS IMPORTANT FOR RISK ASSESSMENT

By Andrew Holmes

Chemicals that might be ingested intentionally or incidentally can impact human health in a variety of ways (for instance, toxicity and as risk factors for chronic disease). The gut microbiome is relevant to food-safety chemical risk assessment in two broad ways: a) Toxic effects of food chemicals on the gut microbe may result in the loss of beneficial effects that impact health, and b) the exposure of human cells and tissues to toxic effects of chemicals is potentially modified by metabolic activities of the gut microbiome. A challenge for risk assessment with respect to the gut microbiome is that prevailing paradigms do not effectively account for the complex "emergent causality" of microbiome effects on human health. This is especially relevant to assessing the risk of food-borne chemicals for the development of nutrition-related chronic diseases such as atopic disorders and diabetes.

The current state-of-the-art to develop predictive models is the application of machine learning to big datasets, that include multiomics, biometrics and diet. This requires the identification of "features" to describe the system, and there are a number of ecological concepts that are especially useful. For gut microbiome data we can simplify these as being functional (genes) or taxonomic (species) features. It is apparent that the type of feature which is most useful depends on the question being asked – for metabolic health, classifiers trained on taxonomic features appear to be more informative than those trained on functional ones. It is also necessary to consider how microbe taxa are defined, how they may interact with each other, and how they interact with environmental factors.

There are currently two broad approaches to assigning sequence data to microbe taxa – exact sequence variants for 16S amplicon datasets (amplicon sequence variants) and metagenome-assembled genomes for shotgun sequencing. Although differences in resolution, ease-of-use and cost exist, these appear to be broadly consistent and mature platforms for describing community composition. In contrast, approaches to assessing how taxa interact with each other, or with environmental factors, are still developing. Data from multiple sources, including human cohort studies, animal models and *in silico* models, all show that accounting for differences in "habitat type" (staple diet or local environment) and baseline community are necessary for predicting outcomes to interventions. The challenge is to simplify the categorization

of habitat descriptors or baseline community types sufficiently for them to be feasible to measure at public-health scales, while still being sufficiently informative to add value to risk assessment. There is reason to be optimistic that simplification of diet to a limited number of macronutrient dimensions, and microbiome to a few enterotypes, may be useful in predictive modelling.

ANNEX V
MODELS AND OMICS TECHNOLOGIES USED TO INVESTIGATE THE GUT MICROBIOME

By Qixiao Zhai

Models used to investigate the gut microbiome include *in vitro* models, *ex vivo* models (cellular and organoid models), *in vivo* models (animal models), human studies, and *in silico* models.

In vitro and ***ex vivo*** **models** offer pros due to their rapid and cost-effective nature. They facilitate precise control over experimental conditions and enhance reproducibility and efficient throughput. However, these models are constrained by their limited capacity to emulate the intricate environments and interactions observed *in vivo*. **Animal models and human studies** are instrumental in enabling whole-host level studies, which is essential for investigating the *in vivo* environment of the organism, including interactions among the immune system, metabolism and physiological processes. Human studies, in particular, offer the most directly relevant data due to their ability to observe effects within the actual human system. However, an important aspect of *in vivo* research that needs to be considered is ethical issues, especially when the subjects are humans as well as non-primate species. Furthermore, human studies grapple with the challenge of controlling confounding factors such as diet, age, lifestyle and genetic variables, which can muddy the clarity of the research outcomes. ***In silico*** **models** stand at the forefront of biological research, offering an in-depth summation of biological rules through the analysis of extensive datasets. Yet, the prowess of computational models is fundamentally tied to the quality and availability of the underlying data. Additionally, the complexity of these models often leads to interpretability issues, making it challenging for researchers to understand and convey the intricacies of their findings.

Omics technologies used to investigate the gut microbiome include metagenomics, metatranscriptomics, metaproteomics, metabolomics and culturomics. The limitations of omics technologies are rooted in their complexity and the vast scope of data they aim to integrate. Achieving coherence among disparate omics datasets is a notable challenge. Additionally, there is a gap in cross-intersectoral integration. The demands for high data quality in multiomics are stringent. Analytical methods

such as metagenomic sequencing require depth, precision and sensitivity, alongside consensus standards for data analysis. The quality of the sample itself, influenced by collection, processing, preservation and the extraction methods for DNA, RNA, metabolites or proteins, is equally critical to ensure reliable results. Another significant limitation is the heavy reliance on reference databases. Lastly, the sheer volume and complexity of omics data pose accessibility challenges.

ANNEX VI
CHEMICAL EXPOSURE AND THE GUT MICROBIOME: A FOOD SAFETY PERSPECTIVE

By Carmen Diaz-Amigo

FAO is carrying out a project to assess the current state of research evaluating the impact of pesticide residues, veterinary drug residues, microplastics and food additives on the gut microbiome and its implications for host health. To do so, researchers are evaluating the amount, quality and reliability of relevant scientific information in order to identify limitations, gaps and research needs, and to explore the applicability of microbiome data in food-safety risk assessments.

Research evaluating the exposure of the gut microbiome to regulated chemicals and some environmental pollutants has been diverse – addressing different research questions and conducted following diverse study designs, models, methodologies and statistical approaches. Gut microbiota assessment varies among studies, mainly targeting bacterial diversity and taxonomical effects relying on sequencing of different regions of the 16S rRNA gene, while the microbial function is not always investigated. A significant deficit identified was the need to consider realistic experimental exposure scenarios, including doses, form of administration and exposure periods. There is a need to better understand the representativeness of gut microbiota samples, such as faecal samples, which may not be indicative of the actual composition and function of the different intestinal sites. Most studies are cross-sectional, assessing endpoints at a single time point, often lacking information about the baseline microbiota and a recovery period after treatment, which would indicate delayed and long-term effects of chemical exposure. Although studies report the influence of microbial changes in the host (such as intestinal and metabolic function and inflammation), this connection is primarily observational, with limited investigation of causal links or underlying mechanisms.

Several conclusions can be drawn from this which are relevant to food-safety chemical risk assessment. First, there is a need to improve the quality of research by enhancing scientific rigour and the reporting of research results, as well as designing studies that reflect realistic exposure scenarios. It is also necessary to better understand gut microbiome perturbances of biological relevance. This includes identifying suitable gut microbiome-related biomarkers and endpoints, as well as establishing a clear cause-and-effect relationship between changes in the gut microbiome and adverse health outcomes. Finally, the use of gut microbiome data in the risk assessment of chemicals is currently very limited, and a full integration will require overcoming knowledge gaps and addressing existing methodological and technical challenges.

ANNEX VII
TASKS AND SUPPORTING QUESTIONS

TASK 1. Risk assessment context, gaps and needs

Objective: Identification of gut microbiome-related data gaps and needs for risk assessment. (This activity will frame the microbiome discussions of Task 2.)

Supporting questions:

> Can gut microbiomes provide evidence of adverse effects of chemicals that cannot be predicted by other means (such as toxicokinetics)? How is the gut microbiome relevant to risk assessment?

> Are there initiatives or organizations considering or implementing the integration of gut microbiome data into risk assessment?

> What type of gut microbiome evidence/endpoints is currently used in risk assessment? Are there limitations that need to be considered?

> What type of (gut microbiome-related) data gaps and needs does food-safety risk assessment face? How could measurements within the gut microbiome fill gaps left by current approaches to risk assessment? How could the identified gut microbiome data gaps be tackled and filled?

> What are the main reasons preventing the use of gut microbiome data in risk assessment?

> Which key gut microbiome-related definitions need to be established, refined or customized for risk assessment?

TASK 2. Suitability of gut microbiome data for risk assessment

Objectives: (1) Evaluation of the maturity and relevance of different gut microbiome data for risk assessment, and (2) definition of ranking criteria.

TASK 2.1. Type of gut microbiome data, existing models and analytical methodologies

Objectives: (1) Identification of the benefits and limitations of research models and analytical technologies. (2) Identification of criteria relevant to the topic of this subtask to develop an approach to assess data maturity and reliability.

Supporting questions:

> Human studies, models and analytical technologies (pros and cons): Which criteria should be used to assess the suitability of different models and analytical technologies for gut microbiome risk assessment? What are the limitations and the degree of translatability of information from the different models to the human context?

> Microbiota sampling: sampling methods, frequency and sampling site: How representative and reliable is the faecal microbiota compared to intestinal samples?

> Which criteria and strategy should we use to develop a data-maturity ranking based on the type, relevance and maturity of models/human studies and analytical technologies?

> Is it feasible to establish minimum requirements to define standards and methodologies universally applicable to gut microbiome data for risk assessment purposes?

TASK 2.2. Identification of microbiome metrics, endpoints and biomarkers

Objectives: Identification of potential gut microbiome endpoints and biomarkers of adverse alterations. Identifications of criteria relevant to the topic of this subtask to develop an approach to assess data maturity.

Supporting questions:

> Function, taxonomy and community networks: Which existing (considering specificity, sensitivity and validation) and promising gut microbiome-related metrics, biomarkers and endpoints could be helpful in risk assessment? Consider non-bacterial members of the gut microbiome.

> Healthy gut microbiome vs alterations of concern (definitions): For any of the markers or endpoints identified, do normality ranges/thresholds exist to help us distinguish a healthy gut microbiome or normal transient fluctuations from microbiome-related adverse effects?

> Which criteria should we use to rank the relevance of gut microbiome-related metrics, biomarkers and endpoints relevant for risk assessment?

TASK 2.3. Microbiome-host interactions and biological relevance

Objectives: Identification of the considerations to evaluate the biological relevance of gut microbiome changes. Identification of criteria relevant to the topic of this subtask to develop an approach to assess data maturity.

Supporting questions:

> Which key aspects should be considered to assess the biological relevance of gut microbiome changes and microbial chemical transformations in the context of risk assessment?

> Which aspects should be considered when evaluating the reliability of causal inference (including causal direction) in gut microbiome-related risk assessments?

> Which criteria should we use to develop a data-maturity ranking based on gut microbiome-host interactions?

TASK 3. Develop data-maturity ranking

Objective: Application of all the information obtained in previous tasks to (1) rank the maturity of gut microbiome data and – if time permits – (2) identify the conditions or developments that need to take place to integrate the different gut microbiome data into risk assessment.

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GLOSSARY

The concepts included in this glossary are defined in *Principles and methods for the risk assessment of chemicals in food (Environmental health criteria 240)* (FAO and WHO, 2009) or in *the Codex Alimentarius Commission Procedural Manual, 28th Edition* (FAO and WHO, 2023).

Acceptable daily intake (ADI): The estimate of the amount of a chemical in food or drinking-water, expressed on a body weight basis, that can be ingested daily over a lifetime without appreciable health risk to the consumer. It is derived on the basis of all the known facts at the time of the evaluation. The ADI is expressed in milligrams of the chemical per kilogram of body weight (a standard adult person weighs 60 kg). It is applied to food additives, residues of pesticides and residues of veterinary drugs in food.

Adverse effect: Change in the morphology, physiology, growth, development, reproduction or lifespan of an organism, system or (sub)population that results in an impairment of functional capacity, an impairment of the capacity to compensate for additional stress or an increase in susceptibility to other influences.

Bioavailability: For food additives, contaminants and pesticide residues, a term referring to the proportion of a substance that reaches the systemic circulation unchanged after a particular route of administration. For veterinary drug residues in food, it is used to reflect the fraction that can be released from the food matrix and is available for absorption.

Biomarkers: Indicators of changes or events in human biological systems. Biomarkers of exposure refer to cellular, biochemical or molecular measures that are obtained from biological media such as human tissues, cells or fluids and are indicative of exposure to a substance. Biomarkers of effect refer to biological changes that represent an alteration in endogenous body constituents (e.g. depression of cholinesterase levels as an indicator of exposure to pesticides).

Codex Alimentarius Commission (CAC): CAC was formed in 1962 to implement the Joint FAO/WHO Food Standards Programme. It is an intergovernmental body made up of more than 170 member nations, the delegates of which represent their own countries. CAC's work of harmonizing food standards is carried out through various committees, such as the Codex Committee on Food Additives (CCFA), the Codex Committee on Contaminants in Food (CCCF), the Codex Committee on Residues of Veterinary Drugs in Foods (CCRVDF) and the Codex Committee on Pesticide Residues (CCPR). The Joint FAO/WHO Expert Committee on Food Additives serves as the advisory body to CAC on all scientific matters concerning food additives, food contaminants, naturally occurring toxicants and residues of veterinary drugs in food.

The Joint FAO/WHO Meeting on Pesticide Residues serves as the advisory body to CAC on all scientific matters concerning pesticide residues.

Contaminant: Any substance not intentionally added to food or feed for food-producing animals, which is present in such food or feed as a result of the production (including operations carried out in crop husbandry, animal husbandry and veterinary medicine), manufacture, processing, preparation, treatment, packing, packaging, transport or holding of such food or feed, or as a result of environmental contamination. The term does not include insect fragments, rodent hairs, and other extraneous matter.

Dietary exposure assessment: The qualitative and/or quantitative evaluation of the likely intake of chemicals (including nutrients) via food, beverages, drinking-water and food supplements.

Dose: Total amount of an agent administered to, taken up by or absorbed by an organism, system or (sub)population.

Dose–response: Relationship between the amount of an agent administered to, taken up by or absorbed by an organism, system or (sub)population and the change developed in that organism, system or (sub)population in reaction to the agent.

Elimination: The expelling of a substance or other material from the body (or a defined part thereof), usually by a process of extrusion or exclusion, but sometimes through metabolic transformation.

Endpoint: Qualitative or quantitative expression of a specific factor with which a risk may be associated as determined through an appropriate risk assessment.

Enterohepatic circulation: Intestinal reabsorption of material that has been excreted through the bile followed by transfer back to the liver, making it available for biliary excretion again.

Exposure: Concentration or amount of a particular agent that reaches a target organism, system or (sub)population in a specific frequency for a defined duration.

Exposure assessment: Evaluation of the exposure of an organism, system or (sub) population to an agent (and its derivatives). Exposure assessment is one of the steps in the process of risk assessment.

Exposure scenario: A set of conditions or assumptions about sources, exposure pathways, amounts or concentrations of agents involved and exposed organisms, systems or (sub)populations (i.e. numbers, characteristics, habits) used to aid in the evaluation and quantification of exposures in a given situation.

Food additive: In the Codex Alimentarius Commission context, any substance not normally consumed as a food by itself and not normally used as a typical ingredient of the food, whether or not it has nutritive value, the intentional addition of which to food for a technological (including organoleptic) purpose in the manufacture, processing, preparation, treatment, packing, packaging, transport or holding of such food results, or may be reasonably expected to result, (directly or indirectly) in it or its byproducts becoming a component of or

otherwise affecting the characteristics of such foods. The term does not include "contaminants" or substances added to food for maintaining or improving nutritional qualities.

Hazard: Inherent property of an agent or situation having the potential to cause adverse effects when an organism, system or (sub)population is exposed to that agent.

Hazard assessment: A process designed to determine the possible adverse effects of an agent or situation to which an organism, system or (sub)population could be exposed. The process includes hazard identification and hazard characterization. The process focuses on the hazard, in contrast to risk assessment, where exposure assessment is a distinct additional step.

Hazard characterization: The qualitative and, wherever possible, quantitative description of the inherent properties of an agent or situation having the potential to cause adverse effects. This should, where possible, include a dose–response assessment and its attendant uncertainties. Hazard characterization is the second stage in the process of hazard assessment and the second step in risk assessment.

Hazard identification: The identification of the type and nature of adverse effects that an agent has an inherent capacity to cause in an organism, system or (sub) population. Hazard identification is the first stage in hazard assessment and the first step in the process of risk assessment.

Health-based guidance value: A numerical value derived by dividing a point of departure (a no-observed-adverse-effect level, benchmark dose or benchmark dose lower confidence limit) by a composite uncertainty factor to determine a level that can be ingested over a defined time period (e.g. lifetime or 24 h) without appreciable health risk. Related terms: Acceptable daily intake, Provisional maximum tolerable daily intake, Provisional tolerable monthly intake, Provisional tolerable weekly intake, Tolerable daily intake.

Intake: For the purposes of food and feed risk assessment, the amount of a substance (including nutrients) ingested by a person or an animal as part of its diet (via food, beverages, drinking water and food supplements). This term does not refer to whole foods. The "intake" of whole foods is termed "food consumption".

Joint FAO/WHO Expert Committee on Food Additives (JECFA): An expert committee that has been meeting since 1956. JECFA has been engaged in collecting and evaluating scientific data on food additives and making recommendations on safe levels of use. This has been accomplished 1) by elaborating specifications for the identity and purity of individual food additives that have been toxicologically tested and are in commerce and 2) by evaluating toxicological data on these food additives and estimating acceptable intakes by humans. In 1972, the scope of the evaluations was extended to include contaminants in food, whereas in 1987, the scope was extended even further to include residues of veterinary drugs in food. When evaluating the latter compounds, maximum residue limits are recommended based upon acceptable intakes estimated by the Committee and data relating to Good Practice in the Use of Veterinary Drugs.

JECFA is a technical committee of specialists acting in their individual capacities. Each JECFA is a separately constituted committee. When the term "JECFA" or "the Committee" is used without reference to a specific meeting, it is meant to imply the common policy or combined output of the separate meetings over the years.

Joint FAO/WHO Meeting on Pesticide Residues (JMPR): The abbreviated title for the Joint Meeting of the FAO Panel of Experts on Pesticide Residues in Food and the Environment and the WHO Core Assessment Group on Pesticide Residues, which has been meeting since 1963. The meetings are normally convened annually. The FAO Panel of Experts is responsible for reviewing residue and analytical aspects of the pesticides considered, including data on their metabolism, fate in the environment and use patterns, and for estimating the maximum residue levels and supervised trials median residue levels that might occur as a result of the use of the pesticide according to Good Agricultural Practice. The WHO Core Assessment Group on Pesticide Residues is responsible for reviewing toxicological and related data on the pesticides and, when possible, for estimating acceptable daily intakes and long-term dietary intakes of residues. As necessary, acute reference doses for pesticides are estimated along with appropriate estimates of short-term dietary intake.

JMPR is a technical committee of specialists acting in their individual capacities. Each is a separately constituted committee. When the term "JMPR" or "the Meeting" is used without reference to a specific meeting, it is meant to imply the common policy or combined output of the separate meetings over the years.

Lowest-observed-adverse-effect level (LOAEL): Lowest concentration or amount of a substance, found by experiment or observation, that causes an adverse alteration of morphology, functional capacity, growth, development or lifespan of the target organism distinguishable from normal (control) organisms of the same species and strain under the same defined conditions of exposure.

Lowest-observed-effect level (LOEL): Lowest concentration or amount of a substance, found by experiment or observation, that causes any alteration of morphology, functional capacity, growth, development or lifespan of the target organism distinguishable from normal (control) organisms of the same species and strain under the same defined conditions of exposure.

Mechanism of action: The specific biochemical interaction through which a substance produces an effect on a living organism or in a biochemical system. Related term: Mode of action.

Model: A set of constraints restricting the possible joint values of several quantities; a hypothesis or system of beliefs regarding how a system works or responds to changes in its inputs. The purpose of a model is to represent as accurately and precisely as necessary with respect to particular decision objectives a particular system of interest.

Mode of action: A biologically plausible sequence of key events leading to an observed effect supported by robust experimental observations and mechanistic data. A mode of action describes key cytological and biochemical events—that is, those that are both measurable and necessary to the observed effect—in a logical framework. Related term: Mechanism of action.

No-observed-adverse-effect level (NOAEL): Greatest concentration or amount of a substance, found by experiment or observation, that causes no adverse alteration of morphology, functional capacity, growth, development or lifespan of the target organism distinguishable from those observed in normal (control) organisms of the same species and strain under the same defined conditions of exposure.

No-observed-effect level (NOEL): Greatest concentration or amount of a substance, found by experiment or observation, that causes no alteration of morphology, functional capacity, growth, development or lifespan of the target organism distinguishable from those observed in normal (control) organisms of the same species and strain under the same defined conditions of exposure.

Pesticide residue: Any specified substance in food, agricultural commodities, or animal feed resulting from the use of a pesticide. The term includes any derivatives of a pesticide, such as conversion products, metabolites, reaction products, and impurities considered to be of toxicological significance.

Pharmacodynamics: The study of the physiological effects of drugs on the body or on microorganisms or parasites within or on the body, the mechanisms of drug action and the relationship between drug concentration and effect. Related term: Toxicodynamics.

Pharmacokinetics: Description of the fate of drugs in the body, including a mathematical account of their absorption, distribution, metabolism and excretion. Related term: Toxicokinetics.

Risk: The probability of an adverse effect in an organism, system or (sub)population caused under specified circumstances by exposure to an agent.

Risk analysis: A process for controlling situations where an organism, system or (sub)population could be exposed to a hazard. The risk analysis pro-process consists of three components: risk assessment, risk management and risk communication.

Risk assessment: A process intended to calculate or estimate the risk to a given target organism, system or (sub)population, including the identification of attendant uncertainties, following exposure to a particular agent, taking into account the inherent characteristics of the agent of concern as well as the characteristics of the specific target system. The risk assessment process includes four steps: hazard identification, hazard characterization, exposure assessment and risk characterization. It is the first component in a risk analysis process.

Risk characterization: The qualitative and, wherever possible, quantitative determination, including attendant uncertainties, of the probability of occurrence of known and potential adverse effects of an agent in a given organism, system or (sub)population, under defined exposure conditions. Risk characterization is the fourth step in the risk assessment process.

Risk communication: Interactive exchange of information about (health or environmental) risks among risk assessors, managers, news media, interested groups and the general public.

Risk management: Decision-making process involving considerations of political, social, economic and technical factors with relevant risk assessment information relating to a hazard so as to develop, analyse and compare regulatory and non-regulatory options and to select and implement appropriate regulatory response to that hazard.

Toxicodynamics: The process of interaction of chemical substances with target sites and the subsequent reactions leading to adverse effects. Related term: Pharmacodynamics.

Toxicokinetics: The process of the uptake of potentially toxic substances by the body, the biotransformation they undergo, the distribution of the substances and their metabolites in the tissues, and the elimination of the substances and their metabolites from the body. Both the amounts and the concentrations of the substances and their metabolites are studied. The term has essentially the same meaning as pharmacokinetics, but the latter term should be restricted to the study of pharmaceutical substances. Related term: Pharmacokinetics.

Uncertainty factor: Reductive factor by which an observed or estimated no-observed-adverse-effect level or other reference point, such as the benchmark dose or benchmark dose lower confidence limit, is divided to arrive at a reference dose or standard that is considered safe or without appreciable risk.

Variability: Heterogeneity of values over time, space or different members of a population. Variability implies real differences among members of that population. For example, in exposure assessment, different individuals have different intakes and susceptibilities. In relation to human exposure assessment, differences over time for a given individual are referred to as intraindividual variability; differences over members of a population at a given time are referred to as interindividual variability.

Veterinary drugs residues: The parent compounds and/or their metabolites in any edible portion of the animal product. They include residues of associated impurities of the veterinary drug concerned.

Weight of evidence: A process in which all of the evidence considered relevant to a decision is evaluated and weighted.

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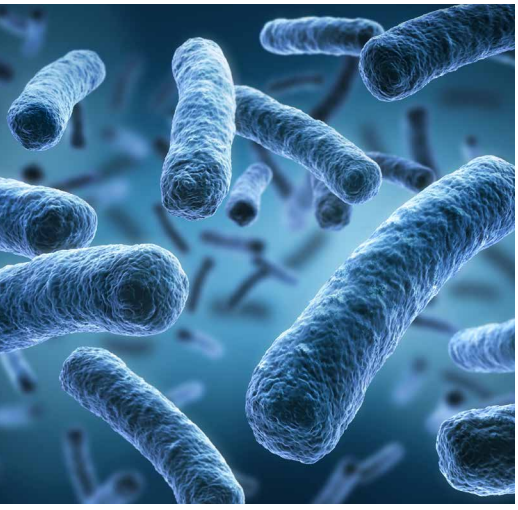

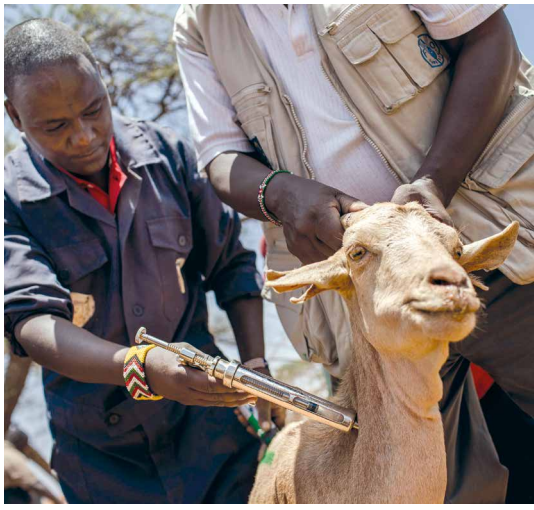

FAO TECHNICAL MEETING ON THE GUT MICROBIOME IN FOOD SAFETY CHEMICAL RISK ASSESSMENT

ROME, 12–14 DECEMBER 2023
MEETING REPORT

FAO hosted the Technical Meeting: Gut microbiome in food safety chemical risk assessment which was held from 12 – 14 December 2023. A multiregional and multidisciplinary group of experts – spanning from toxicologists to microbial ecology specialists and involved in chemical risk assessments and microbiome research programmes – discussed the identification of microbiome-related data suitability, gaps and needs for chemical risk assessment, as well as the microbiome-host interactions and its biological relevance.

The experts also identified the conditions and developments that need to take place in order to integrate the different gut microbiome data into chemical risk assessment which were categorized in three main areas, including definitions, research, and methodological and analytical standardization.

AGRIFOOD SYSTEMS AND FOOD SAFETY DIVISION – ECONOMIC AND SOCIAL DEVELOPMENT

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