Session III Assessing the impact microbiome

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Intestinal organoids as *in vitro* models for assessing host-microbiome interactions and safety of intervention

109. **Dr Tamas Korcsmaros (Imperial College London)** introduced his talk, which focused on intestinal organoids, and their use as *in vitro* models to assess interactions between the host and the microbiome.

110. A chart was shown comparing the number of procedures carried out for animal research in Great Britain in 2020. Imperial College London was in the top 10 with 63,670 procedures, however it was commented that it was hoped that this value would decrease in coming years.

111. The speaker explained that organoids are ex *vivo* primary cultures capable of self-renewal and self-organisation and exhibited similar three-dimensional structure and functionality as the tissue of origin. The concept of this is that structures could be grown from adult cells with the functionality of organ tissues.

112. Organoids allow for the modelling of different organs, including those that cannot be obtained from biopsy such as the brain. Furthermore, organoids allow for personalised modelling including the interactions between different cells; genetically modified organoids can also be used to examine specific diseases and for omics analysis.

113. Although organoid technology for screening is still estimated to be another 3-5 years away, there are currently some established screening approaches.

114. The first of the two main approaches is the many to one approach, which uses a fixed genotype organoid exposed to various metabolites, microbial species, or other molecular libraries. The second is the one-to-many approach, in which organoids with different genetic backgrounds are exposed to the same molecular or microbial species.

115. An example of this in practice is the human Autophagy Reporter Colon Organoid (hARCO) line. It was noted that organoid technology is very expensive for both the standardisation and optimisation aspects. An example of how this technology would be relevant using gut organoids was illustrated.

116. Problems with traditional approaches were raised. It was explained that in the epithelium layer in the colon there are many cell types, however these are not included in traditional cell cultures. Additionally, a lot of knowledge currently comes from mouse models, yet the microbiomes of mice and humans are not comparable and could work differently. Organoids would eliminate these problems as all cells would be included and these would be grown from humans.

117. Issues arise, however, when growing intestinal organoids as the luminal side, which would be the outside layer in the gut, becomes the inside layer in the organoid model. Although this still allows for the study of epithelial homeostasis, regeneration, cell-cell interactions and intracellular processes, it is not appropriate to study cross microbe interactions.

118. To overcome the difficulties in this technique, micro-injections can be used to administer microbes to the middle of the organoid to interact with the lumen, or an easier but less efficient method would be to flip the organoid so that the basolateral side is inside and the lumen outside. Work is also undergoing to create a 2D system with both a luminal and basolateral side.

119. It was explained that there were multiple models currently available, all varying in complexity, yet the aim was to be able to produce a 'Gut-On-A-Chip' microfluidics systems. Work towards this is now being set up in Imperial College London in collaboration with a number of companies. These chips would work by combining organoid cells, patient metadata, and microbiota and nutrients, which would be characterised and undergo multi-omics analysis to allow use of these systems for screening.

120. It is hoped over time these systems will allow for the study of cell-cell and cell-microbe interactions and be useful diagnostic and prognostic tools in combination with omics approaches.

Analytical strategies to study the gut microbiome in toxicology

121.**Professor Michael Antoniou (Kings College London)** introduced the topic by outlining the parameters required for microbiota compositional and metabolic function investigations. These included:

- Determination of both bacterial and fungal populations.
- Gut omics analysis (transcriptomics/proteomics/metabolomics).
- Gut integrity measures.
- Correlations with internal organ/system analysis.
- Functional studies *in vitro* that can complement *in vivo* investigations.

122. The speaker then presented the results of three studies that highlighted the effects of glyphosate-based herbicides on gut structure and function. These herbicides are non-selective (*i.e.* broad spectrum), are the most heavily applied globally and their worldwide spread of use and usage continues to rise.

123. It was highlighted that commercial glyphosate-based herbicide formulations contain many additives (co-formulants/adjuvants) in addition to glyphosate with the co-formulants shown to be toxic in their own right. Thus, it was emphasised that toxicity studies whenever possible need to compare glyphosate with typical commercial formulations, since the latter can be far more toxic than glyphosate alone.

124. The mechanism of action of glyphosate was introduced. In brief, it interferes with the shikimate pathway in plants and thus inhibits formation of aromatic amino acids. It was once thought that this pathway was exclusive to plants, however, it is also present in some bacteria and fungi, including those in the gut of animals and humans.

125. The first results presented were from a comparative toxicogenomics study of glyphosate and typical EU glyphosate-based herbicides using an *in vitro* murine embryonic stem-cell based genotoxicity assay and *in vivo* molecular profiling (omics) in Sprague-Dawley rats (Mesnage R et al., 2021). Marked metabolic disturbances in the gut in both treatment groups were observed even though there was little change in the rat's gut microbiome composition. The metabolic changes were reflective of the treatments inducing oxidative stress.

126. The second results presented were from the Global Glyphosate Study, focusing on the effects of prenatal exposure to glyphosate, 2,4-D and dicamba, when in the formulation on gut function and integrity in Wistar rats. Both treatment groups decreased bacterial diversity and increased fungal diversity in the gut.

127. Data from unpublished work was also presented where Wistar rats starting at prenatal stage of development were treated with either glyphosate alone or as a mixture with two other highly used herbicides in the USA, 2,4-D and dicamba. Glyphosate at the UK/EU no-observed adverse effect level dose and more so the mixture of glyphosate/2,4-D/dicamba at each at the UK/EU acceptable daily intakes caused alterations in gut bacterial and fungal composition, inflammation, redox imbalance and compromised integrity ("leaky gut").

128. Also presented was a computational study drawing on data from the human gut microbiome database. Among the 44 subspecies reference genomes, (72% of the total assigned microbial abundance in 2144 human faecal metagenomes), 35 species are predicted to be sensitive to glyphosate. Thus, it was shown that glyphosate can potentially affect the human gut microbiome (Mesnage R & Antoniou MN, 2020).

129. The final results presented were from a study conducted to evaluate the effects of glyphosate and a typical US Roundup commercial formulation on the gut microbiota of a healthy 3-year-old child using the SHIME® (Simulator of the Human Intestinal Microbial Ecosystem) technology (Mesnage R et al., 2022). It was observed that Roundup and to a lesser extent glyphosate caused changes in fermentation and metabolic activity: i) increased lactate and acetate caused acidification of the microbiological environment; ii) decreased short chain fatty acids and iii) increased long chain polyunsaturated fatty acids. It was also found that Roundup increased ammonium production reflecting increased proteolytic activity.

130. To conclude, these studies showed that several analytical methods are required to holistically evaluate the potential health effects of glyphosate and its commercial formulations on the human microbiome. This included omics, biochemical/gene expression and histological measures, molecular profiling analyses and the SHIME® system.

131.Several regulatory recommendations were put forward for consideration.

These were:

- Multi-omics analyses should become an integral part of chemical toxicity evaluation.
- Chemical administration *in vivo* should begin pre-natally and preferably continue life-long to more accurately reflect real world exposure scenarios.
- Gut and internal organs/systems need to be assessed in parallel.
- Long-term toxicity testing of commercial pesticide formulations as well as active ingredients is needed.
- Pesticide risk assessment and acceptable daily intake values need to be established based on tests of chemical mixtures.