Brussels, 17 May 2024

COST 023/24

DECISION

Subject: Memorandum of Understanding for the implementation of the COST Action "International networking on in vitro colon models simulating gut microbiota mediated interactions" (INFOGUT) CA23110

The COST Member Countries will find attached the Memorandum of Understanding for the COST Action International networking on in vitro colon models simulating gut microbiota mediated interactions approved by the Committee of Senior Officials through written procedure on 17 May 2024.

MEMORANDUM OF UNDERSTANDING

For the implementation of a COST Action designated as

COST Action CA23110
INTERNATIONAL NETWORKING ON IN VITRO COLON MODELS SIMULATING GUT MICROBIOTA MEDIATED INTERACTIONS (INFOGUT)

The COST Members through the present Memorandum of Understanding (MoU) wish to undertake joint activities of mutual interest and declare their common intention to participate in the COST Action, referred to above and described in the Technical Annex of this MoU.

The Action will be carried out in accordance with the set of COST Implementation Rules approved by the Committee of Senior Officials (CSO), or any document amending or replacing them.

The main aim and objective of the Action is to gather most European expertise, including academic and industrial actors, related to the establishment of gut models for food/feed science and pre-clinical studies. This will be achieved through the specific objectives detailed in the Technical Annex.

The present MoU enters into force on the date of the approval of the COST Action by the CSO.

OVERVIEW

Summary
Scientific literature is shedding light on the centrality of GI for human health and wellbeing. Indeed, the physiologic effects of nutrients, bioactives and even toxic compounds (including foodborne pathogens) are mediated by their absorption rate in the intestine and by their interaction with gut microbiota and its host ecosystem. Testing food, feed, supplements or drugs in clinical studies gives rise to ethical issues, and the transferability of animal data across species is often problematic because of differences in physiology, metabolism and chemical susceptibilities. According to a recent survey of European Commission (EURL ECVAM, 2021), complex in vitro models (CIVMs) approaches should be adequate not only for regulatory use-contexts, but also for application in the research field provided that standardized CIVMs are developed, enabling a consensus on their use. A new COST Action would fill the knowledge gap on in vitro colon models providing consensus protocols and robust data sets to improve our knowledge of the events taking place in the intestinal milieu, including the complex interactions between the microbiota and the host. Moreover, innovative educational tools will be suggested to increase knowledge on gut models in young researchers and widen to society to avoid any unhealthy consumer choices coming from misleading messages. Bringing together different experts in Gastroenterology, Microbiology, Physiology, Nutrition, Food Science, Biochemistry, Bioinformatics, Biotechnology etc., the new COST Action could represent an effective strategy for the development of healthy food and for the counteraction of diseases.

Areas of Expertise Relevant for the Action	**Keywords**
● Health Sciences: Nutrition and dietetics ● Clinical medicine: Gastroenterology and hepatology ● Biological sciences: Biological systems analysis, modelling and simulation ● Clinical medicine: Bacteriology ● Biological sciences: Metabolomics	● in vitro gut microbiota colon models ● 3R principles ● personalised medicine ● omics ● bioinformatic tools

Specific Objectives
To achieve the main objective described in this MoU, the following specific objectives shall be accomplished:

Research Coordination
● to create a data management system to efficiently distribute and share results from many studies
● to integrate into strategic dissemination the gained expertise and new guidelines for experimentation with in vitro gut models
● to harmonize guidelines for in vitro testing of food/feed and diseases factors in order to spread such technologies in European laboratories and reduce animal testing
● to coordinate the research agenda between academic laboratories and industrial partners to ensure the commercialization of products relative to in vitro gut models and to digital tools

Capacity Building
● to build a large, world-leading but flexible interdisciplinary and intersectoral collaborative network in Europe
● to increase intersectoral exchange via the creation of an enriched relationship between academic and industrial structures of different expertise
● to encourage new young talents and educate the next generation of leaders (YRIs) in the field,

empowering them and increasing their competitiveness for participation in European projects and initiatives
● to create subdivision of present Scientific Societies or create new Scientific Society in the European sector of Gastrointestinal Research or food/feed science, dedicated to gut models
● to upscale the network with non-European scientists, clinicians and industrial companies to contribute to the dissemination of network outcomes worldwide
● to establish a stable interactive dialogue between clinician gastroenterologists and gut models' researchers to address new applications supporting new therapies
● to increase the cooperation between private research and the scientific community to fasten an increase of available commercial gut models in the market
● to stimulate YRIs toward ambitious careers and allow women to lead teams of the network or WGs by ensuring gender-balance
● to involve policy makers in the evaluation of feasibility of guidelines leading to a standardization of in vitro testing of microbiota-host interactions

TECHNICAL ANNEX

1. S&T EXCELLENCE

1.1. SOUNDNESS OF THE CHALLENGE

1.1.1. DESCRIPTION OF THE STATE OF THE ART

Gut microbiome in health and diseases. Scientific research is consistently demonstrating the central role of the gastrointestinal tract for animal and human health. A multitude of gastrointestinal processes are modulating the solubility, stability and bioactivity of ingested dietary substrates and chemical constituents and impact the viability of ingested microorganisms, including probiotic strains and enteric pathogens. The **gut microbiome**, present across the entire digestive tract but predominantly abundant in the colon, is an important determinant of physiological effects that are derived from the aforementioned gastrointestinal processes. The last decade has highlighted a strong increase in research on the gut microbiota, and this has led to an expansion of knowledge on the factors that shape the composition, community structure and functionality of microorganisms' resident in the animal and human intestine. Among the multiple endogenous and exogenous factors involved, diet (prebiotics, probiotics, supplements functionalized foods etc.) emerges as a fundamental determinant of the structure and function of the intestinal microbiota community. Dietary components are essential not only for animal and human health but also for the maintenance and contribution to functional niches for the trillions of microbes inhabiting within the animal and human intestines. Furthermore, alterations in the composition or functionality of the gut microbiome itself may underly or contribute to the etiopathology of gastrointestinal and even systemic disorders. Indeed, when the gut microbiota eubiosis is perturbed, the onset of **dysbiosis** is the ecological condition that underlies the occurrence of not only most human intestinal diseases (Frank et al., 2007), but also of systemic inflammation (Malezsa et al, 2021), metabolic syndromes (Qin et al., 2012) and others, shaping the individual's risk for disease (Perler et al, 2023). While association studies have provided important evidence on the link between the gut microbiome and health/disease states, such studies do not indicate causality, which is a prerequisite for transposition from bench to bedside. However, it is still unclear whether and how the gut microbiome is mechanistically linked to human and animal diseases.
The evidence-based associations between human and animal health status and the composition, ecosystem structure and functionality of the gut microbiota suggested to explore **causal relationships**:
a) between gut and **dietary factors** and b) gut and **diseases factors** (drugs, pathogens, contaminants, life-style, xenobiotics etc.). To answer those questions, *in vivo* approach is the gold standard, but it remains difficult, expensive, time consuming, and raises ethical issues even when animal models are used. The use of animal models as an *in vivo* alternative to clinical studies is complicated due to differences in physiology, metabolism, chemical susceptibility and microbiome colonization. In fact, the importance of replacing animal testing with *in vitro* tests is due both to the animal welfare (Khuituan et al., 2022) and the need to employ more sustainable and rapid methodologies. In addition, there is a growing awareness that well designed ***in vitro* human based models** are more reflective of real human conditions than the environmental conditions inherent to different animal models (Vasquez et al., 2022). A recent survey of the European Commission (EURL ECVAM, 2021) confirms that the use of complex *in vitro* models can be an adequate tool for addressing mode-of-action studies since human intervention studies are not suitable due to ethical constraints.
***In vitro* gut models in microbiome research**. Over the last 30 years, several *in vitro* models have been developed to study the human or animal gastrointestinal tract with different degree of complexities, advantages and limitations, and often lacking appropriate validation against human or animal *in vivo* data. This reduces the comparability of results between model systems and risks slowing down scientific progress or lower efficient use of research funding. The i*n vitro* gut models are first categorized as static and dynamic fermentation models. They are used to study the interaction of a given compound with the gut microbiota, i.e. to analyze the interplay of a given food matrix, food constituent, active pharmaceutical ingredient or any xenobiotic with the colon microbiota, **independently from interaction with the host cells**. Static gut microbiota ecosystem based on **batch fermentation models** is the simplest, most versatile and accessible approach (costly, logistically flexible and easy to use) because it is characterized by a closed anaerobic environment and short simulation time. This type of systems has the disadvantage of having limited resemblance to the *in vivo* condition. A higher resemblance to *in vivo* occurrence could be obtained with **dynamic fermentation models**. These models enable to assess gut microbiota response to food compounds over a prolonged time and spatially over a more complex ecosystem relative to the different

intestinal niches. This more complex systems have a limited reproducibility due to possible fluctuations on parameters settings, but the advantages of being cost accessible (especially when self-assembled), customizable and operator friendly. They include the more recent models such as the Reading Model, the micro-Matrix platform, SIMGI, MiCoMo, MiGut, Robogut, TIM-2, MICODE, PolyFermS, ARCOL and SHIME models, among others (Nissen et al. 2020; Isenring et al., 2023). To mimic the **host-microbiome interactions** more complex models were proposed and categorized accordingly. **Transwell systems** enable the study of host-microbe interactions thanks to a physical barrier mimicking the gut epithelium. However, may oversimplify the complex in vivo environment with many limits for studying host cells and microbes' interactions. Moreover, **human Gut- on-a-Chip** which are based on a microfluidic system mimicking the mechanical colon ecosystem, they enable the study of host-microbe but it is complex to set up and maintain and limitedly replicate all aspects of the colon environment. **Co-culture models** allow the co-culture of host cells (e.g., Caco-2 cells) with gut microbiota and enable the study of host-microbe interactions at the cellular level but may not represent the complexity of the entire colon environment and are limited to short-term experiments. To increase complexity, **multilayered cell culture models** were suggested**.** They include multiple cell layers to simulate the gut barrier offering a more complex representation of host-microbe interactions. However they ca describe just the interactions at the epithelial barrier without capturing the dynamics of the entire colon. More recently it has been proposed **organoids,** based on three-dimensional structures derived from human tissue enabling the study of host-microbe interactions in a more physiologically relevant context. However they are suitable just for short-term experiments, complex to set up and maintain.
Finally, ***in silico* models** are cost-effective computational models allowing for rapid testing of hypotheses. However, they depend on the quality of input data and model assumptions and may oversimplify the real scenario.
Up to now, most *in vitro* models integrating gut microbiome have been set-up to reproduce digestive conditions from healthy adult individuals, both in human and animal. There are scarce models reproducing human diseased conditions, in addition very few of them were accurately validated by *in vitro*/*in vivo* comparisons. A further consideration may be the development of **disease models** for microbiome research, integrating the presence of host components which enable the monitoring and evaluation of how alterations in human or animal-derived microbial communities – either due to pathology, or due to intervention – may result in different readouts of health-related biomarkers.

The different *in vitro* gut models achieve associated advantages and drawbacks, but due to the absence of standardization, it is very difficult to compare and interpret observations from different studies and to support *in vivo* trials.

Data management to fasten human applications. An efficient use of *in vitro* gut models relates to data management. Data are frequently dispersed and phenotypically diverse, which constitutes a challenge and a great opportunity for future data integration for an integrative gut model considering host-microbiome interactions. Furthermore, the field of *in silico* modelling of the gut and its interactions with the microbiome has seen significant advancements through various approaches. These include machine learning and predictive modelling, agent-based modelling, metabolic modelling, integrated systems biology, and the use of simulation platforms. Therefore, there is a need to develop a **comprehensive database of gastrointestinal methodologies from set up and multiparametric control of experiments to data output and create a consensus on what model features are required to address specific research questions in the field. Precision medicine and nutrition** are trending aspects for the near future and omic technology is the foundation.

The COST Action INFOGUT will activate a transversal bottom-up discussion among top scientists and early researchers in **microbiology, gastroenterology, nutrition, bioinformatics and bioengineering** on the opportunities of *in vitro* studies of gut microbiota-host interactions that may result in **translational research** predictive for human and animal applications, where new nutritional, preventive or therapeutic strategies can be explored and effectively tested. Moreover, *in vitro* models significantly contribute to replacing and **reducing the use of animal models**, a fair request from European legislation (Directive 2010/63/EU).

1.1.2. DESCRIPTION OF THE CHALLENGE (MAIN AIM)

Several limitations still exist in *in vitro* modeling the effects of diet, drug therapies or other external factors on the complexity of host–microbiome interactions and their ability to recapitulate the key factors in human and animal health and diseases. The main challenge of INFOGUT is **to reach a consensus**

on the validation and standardization of main aspects from a set of protocols to delineate, regulate and substantiate the study of those aspects with human or animal *in vitro* gut models. A further challenge is the scaling up to more complex models including microbiota and cellular interactions, with a focus on the lower digestive tract.

The main purpose of INFOGUT is the validation and standardization of a series of protocols to outline, regulate and substantiate the study of food digestion/absorption/colonic fermentation with complex *in vitro* models capable of simulating the entire gastro-intestinal tract, including the microbiota and cellular interactions, with particular attention to the lower digestive tract.

The INFOGUT challenge is the ideal follow-up to the former INFOGEST (COST ACTION FA1005 – 2011-15 which involved 746 researchers from 57 countries and still very active) which standardized method of *in vitro* upper digestion (Minekus et al., 2014), related to oral, gastric and duodenal (pancreatic) compartments simulation. In fact, the INFOGUT *in vitro* colon models' protocols will be sequenced with new harmonized ones that simulates; a) the mucosal phase of gastrointestinal digestion, not included in the INFOGEST protocol; b) the colonic fermentation phase including the incorporation of *ex vivo* human or animal colonic microbiota *via* fecal donation.

Indeed, despite the unquestionable dynamism of the field, important progress in the design, implementation and harmonization of relevant *in vitro* colon models is still lacking because **the following issues have not yet been overcome**:

- **Lack of a comprehensive overview of lower gut model systems**, the specific gastrointestinal features they are mimicking and insight into which model systems have been properly validated against human or animal *in vivo* data.
- **Lack of clear panel of parameters and selection criteria** that enable the assessment of which model systems are useful for addressing specific research questions and which one can be used across the different stages of product development (high-throughput to highly specific)
- **Substantial necessity of interdisciplinarity into model development**, validation and use. This ranges from clinical and biomedical expertise to microbial ecology, cell biology and biomedical engineering, *in silico* research and multi-omics analyses.
- **Coordination of interdisciplinary efforts** of available expertise for developing new technical approaches to create standout, sophisticated, but easy to run, *in vitro* colon models enabling the coupling to multi-omics analytical methodologies (metabolomics, proteomics, transcriptomics, and microbiomics amongst others). Indeed, *in vitro* intestinal models are generally developed and used by food scientists, and to a lesser extent by physiologists and nutritionists.
- **Implementation of more complex models** which include not only microbiota from other compartments than the colonic one, but also the interactions with other human or animal organs. Occasionally, the gut model effluents are administered to cultured cells. Although the big expectation of clinical research towards robust and representative human gut models to study gut microbiota -host interactions, a certain reluctance to consider as a predictive tool is associated with the uncertainty on how to integrate the complexity and unpredictability of the colon microbiome into PK/PD or into models for studying the nutrition etiopathology of specific disorders.
- **Limited cooperation between academic and industrial partners** to elaborate, validate and standardize *in vitro* complex gut models suitable for pre-clinical tests, scientifically robust and cost effective. More cooperation between biologists and bioinformaticians is required to interpret and validate the results of omic applications.

The **strategic relevance of INFOGUT** is based on the need to fill the gaps on four pillars of the current international scientific orientation:

- **the reduction of animal tests** and their replacement with *in vitro* methods.
- **the characterization of the impact of foods/feed** on the ecology of the human/animal colon to obtain functional food products that are sustainable and modulated on the specific needs of population subgroups (precision nutrition).
- **the adaptation of *in vitro* gut models** to diseased situations and their subsequent validation with *in vivo* data allowing to gather useful mechanistic insights into the role of microbiota in disease etiology in human and animal.
- **modelling the interactions** between the microbiome and host tissues by integrating metagenomics with host epigenomics/transcriptomics data would allow the creation of the first

complete gut framework for further *in silico* investigations.

INFOGUT aims to fill the scientific gap related to completing the simulation of the whole digestion/colonic fermentation process with an optimized standardized simple system. **The overall goal of the INFOGUT Action will be carried out through the constitution of an interdisciplinary and intersectoral network dedicated to cutting-edge research in both basic and translational sciences, and their interfaces. The network shall share the latest technical advances in *in vitro* complex gut model development within the scientific community, with the aim to improve and develop standard protocols and generate from results clear messages to disseminate to all stakeholders**.

1.2. PROGRESS BEYOND THE STATE OF THE ART

1.2.1. APPROACH TO THE CHALLENGE AND PROGRESS BEYOND THE STATE OF THE ART

INFOGUT will gather most European expertise, including academic and industrial actors, related to the establishment of gut models for food/feed science and pre-clinical studies to maintain Europe's leadership position in the field, generating a database relative to the effects of dietary and environmental factors on colon ecology in order to drive the industry and the Institutional bodies in directing strategic choices. Specifically, INFOGUT advancements will concretely provide to:

- **Review and harmonization of guidelines for using and analyzing *in vitro* gut models**
  INFOGUT is expected to regulate and validate the many different protocols adopted by different research groups in the scientific community leading into harmonized guidelines, to substantiate the research activity, improve reproducibility, precision and accuracy of results, and reduce animal testing in food/feed science and technology. The guidelines are necessary to all comparative studies but also importantly to harmonize the protocols to run *in vitro* colonic fermentation (e.g. procedures for chemical preparation, media preparation, fecal collection and colonic model inoculation), and subsequent analytical protocols (e.g. microbiomics and metabolomics). This harmonization will ultimately give a precise, full, and robust description of colonic microbiota perturbations by any effectors.
- **Implementation of *in vitro* gut models for studies on other gut compartments**
  An important advancement is based on the extension/coupling of the gut models to encompass other digestive compartments harboring microbes (oral cavity, stomach, small intestine). The other idea is to better understand how to integrate the host compartment, at first with a mucus and epithelial barrier, but also in later stage with other cell types (such as lung, liver, brain, etc.) along several gut-organ axes. This would allow to create comprehensive and physiologically representative *in vitro* models that faithfully recapitulate *in vivo* situations.
- **Extension gut models' application to disease situations**
  INFOGUT should also contribute to the implementation of these technologies in studies of diseased conditions associated to enteric infection, contamination by pollutants but also non-communicable diseases. This will help to clarify the main knowledge and technological gaps in approaching diseased physiology in the human or animal gastrointestinal tract but also the interactions between extra-intestinal tissues and microbes.
- **Data management of omics resources**
  Another progress expected over the state of the art concerns the procedures to extrapolate results from the suggested recognized analytical systems. These protocols are also to be regulated and the datasets generated from omic technologies are big data matrices to be analyzed by multivariate statistics. Also, multi omic and inter-omic combinations, as correlations or networks, are necessary to give a precise message. The generated data will serve to populate a database and educate artificial intelligence (AI), with the aim to run digital tools able to easily predict the impact of a food/feed ingredient or diseased factors towards the colon ecology and the host-related effects and produce friendly reports. It will be possible to plan to generate a catalogue of gut omics data resources, hosted in an accessible platform such as ExperimentHub to improve data accessibility and reusability, following the FAIR (Findable, Accessible, Interoperable, Reusable) principles.

Ultimately, the expected advancements will create:

- **Comprehensive and physiologically representative *in vitro* models of gut microbiota - host interactions that faithfully recapitulate healthy and diseased *in vivo* situations.** Advancements in these areas can significantly revolutionize our understanding of gut

physiology, drug metabolism, nutrient absorption, and the role of the microbiota in both gut-related and systemic diseases. As such, these developments in challenges faced in translating data from laboratory animals to the human situation pave the way for personalized nutrition and medicine.

- **A comprehensive catalog of accessible and user-friendly gut omics data resources**, design a benchmarking protocol for *in silico* modeling of the gut and microbiome, and promote community-driven open-source data integration frameworks.
- Besides scientific, clinical, and ethical achievements, INFOGUT, by promoting public–private links, will contribute to **sustaining the economic growth of our industrial partners within Europe,** i.e. by boosting their research and developments (R&D), saving costs at the levels of nutritional screening, accelerating product development and enhancing efficacy of clinical trials, thanks to pre-clinical *in vitro* gut models.

1.2.2. OBJECTIVES

1.2.2.1. *Research Coordination Objectives*

The ambitious objective of the Action is to optimally transfer technologies, materials, and knowledge across Action participants. Indeed, INFOGUT represents an ideal instrument which will bring together many groups and academic and industrial experts from diverse fields of investigative food/feed science, nutrition, gastroenterology and beyond. **Only an extensive and well-coordinated network of tenured senior and junior academic researchers in microbiology, nutrition, engineering and clinicians in association with industrial R&D departments can take up the challenge to (1) rapidly standardize and identify specific protocols for precise gut models at the international stage and (2) bring Inclusiveness Target Countries (ITCs) actively into the field.**
Thus, the objectives of INFOGUT are manifolds and their impact will be exploited according to four main axes: scientific, educational, environmental and commercial.

1. Scientific: A data management system will be created at the start of the Action to efficiently distribute and share results from many studies and to accelerate data dissemination to every network participant. INFOGUT will be a reservoir for innovation and will rigorously develop and share protocols *via* **an Atlas** including all **validated protocols in healthy and diseases gut microbiota-interaction *in vitro* studies**, descriptions of **gut models and computational models developed by COST partners**. The Atlas will be publicly accessible. INFOGUT partners will contribute to the creation of an **initial database** that will gather all publications and information on existing gut models, experimental and data management protocols. Furthermore, the COST Action will also give **guidelines for validation** of gut models and analytical procedures, after revisions of interdisciplinary and intersectoral synergies within the network. INFOGUT innovations (models, protocols, technologies, digital tools) will be assessed to set up future strategies necessary for their improvement. INFOGUT partners will contribute to data curation and the Working Group (WG) relative to data management will indicate guidelines for statistical computation and AI preparation. This is a prerequisite for delivering high-quality models and providing the scientific community with relevant and reproducible data and protocols that can be additionally used for pre-clinical research, or for commercial purposes.

2. Educational: The gained expertise and the new guidelines for experimentation with *in vitro* gut models will be integrated into **strategic dissemination** (extensive, sustained and strong towards ITCs) and **educational programs** (including academic courses and PhD summer schools), aimed at expanding a highly cooperative European network for *in vitro* gut models. Next-generation scientists, Young Researchers and Innovators (YRIs) will be trained within the network for the long-term dissemination and development of gut models that offer powerful alternatives to animal experiments. Short-Term Scientific Missions (**STSMs**), practical **training schools** and the elaboration of a **mentoring program** will be fundamental INFOGUT educational tools. Dissemination of research results to the general public and relevant stakeholders (e.g. policy-makers, governmental and non-governmental (NGO) organizations, public associations) will be ensured *via* participation to Action activities and invitations to Action conferences and *via* general media (e.g. journals, streaming channels, social media), interviews, website and industrial partners, hence increasing the general awareness to scientific advances and the importance of *in vitro* gut models.

3. Environmental: INFOGUT aims to harmonize guidelines for *in vitro* testing of food/feed and diseases factors in order to spread such technologies in European laboratories and reduce animal testing. Also, the Action wishes indirectly reduce the toxic waste from animal trials and husbandry. So far, high-performance computational models have a significant environmental and ethical impact by being an

alternative to animal experiments. Thus, **the Action timely responds to the 3Rs on animal and waste management.**

3. Commercial: A coordinated research agenda between academic laboratories and industrial partners including SMEs of the Action will ensure the **commercialization** of products relative to *in vitro* gut models and to digital tools. **Patents** may be applied, and **spin-offs** created, expanded or reinforced. Moreover, INFOGUT will provide a framework that facilitates the development of new softwares.

1.2.2.2. Capacity-building Objectives

INFOGUT will have the required critical mass of expertise and of interdisciplinary and intersectoral participants, hence ensuring long-lasting European leadership in the research area. Indeed, INFOGUT will build form a network of members from COST Full/Cooperating Members (countries), among them ITCs, focus will be given to keep the gender balance and involve as much as possible YRIs. INFOGUT will likely arouse the interest of many academic and industrial partners firstly in Europe and potentially worldwide.

INFOGUT Action will eventually:

- Construct a large, world-leading but flexible interdisciplinary and intersectoral collaborative network in Europe, directed by brilliant scientists and outstanding academics to stimulate a bottom-up dynamic regarding *in vitro* gut models and greater advances. This will make Europe the core of exceptional research in the field.
- Increase intersectoral exchange via the creation of an enriched relationship between academic and industrial structures of different expertise.
- Allow women to lead teams of the network or WGs by ensuring gender-balance to the Action.
- Reveal and encourage new young talents and educate the next generation of leaders (YRIs) in the field, empowering them and increasing their competitiveness for participation in European projects and initiatives.
- Create subdivision of present Scientific Societies or create new Scientific Society in the European sector of Gastrointestinal Research or food/feed science, dedicated to gut models to extend the longevity of the INFOGUT network.
- Upscale the network with non- European scientists, clinicians and industrial companies to contribute to the dissemination of network outcomes worldwide. Guarantee a gender balance in the leadership positions of the network.
- Establish a stable interactive dialogue between clinician gastroenterologists and gut models' researchers to address new applications supporting new therapies such as fecal microbiota transplantation or other gut mediated therapies.
- Increase the cooperation between private research and the scientific community to fasten an increase of available commercial gut models in the market.
- Stimulate YRIs toward ambitious careers by increasing their ability to participate in different collaborative projects.
- Involve policy makers (EFSA, JRC for example) in the evaluation of feasibility of guidelines leading to a standardization of *in vitro* testing of microbiota-host interactions.

2. NETWORKING EXCELLENCE

2.1. ADDED VALUE OF NETWORKING IN S&T EXCELLENCE

2.1.1. ADDED VALUE IN RELATION TO EXISTING EFFORTS AT EUROPEAN AND/OR INTERNATIONAL LEVEL

Expertise from network will be used to develop next-generation *in vitro* gut models and next-generation food/feed products. Reciprocally, the network will empower them by offering outstanding educational programs and leading positions. Moreover, the full exploitation of COST networking activities will enable optimal transfer of knowledge towards ITCs, allowing them to develop state-of-the-science technologies in their laboratories, gain in visibility and recognition, draw strong collaborations with leading academic institutions and meet top-tier industrial partners, which is indispensable for a long-lasting and upward transformation. The Action appropriately covers criteria of scientific excellence, clinical relevance, technological advancement, ethical commitment, economic impact, fair geographical distribution, and educational requirements to ensure European leadership in the field of gut models to study gut microbiota -host interactions. The added value generated from the INFOGUT Action is about the

regulation and validation of the many different protocols adopted by different research groups in the scientific community into harmonized guidelines, to substantiate the research
activity, improve reproducibility, improve precision of results, and reduce animal testing in food/feed science and technology. So far, there are no other international consortia trying to harmonize the protocols and procedures to run *in vitro* gut models. **WG participants will contact coordinators or members for the European projects related to gut and food massive data management and analysis, including:**

- INFOGEST: previous COST Action on *in vitro* models of the upper digestive tract (without microbiota)
- Enterprise Ireland and industry funded Food for Health Ireland (FHI) project 2019-2023 investigating the impact of dairy fermented on the gut microbiota using *ex vivo* colon models.
- FNS-Cloud: federating existing and emerging datasets, tools and services to support European research.
- ELIXIR FOOD AND NUTRITION COMMUNITY: Community focused in gathering, standardizing, making FAIRer food and nutritional massive data around the EU.
- ELIXIR SYSTEMS BIOLOGY COMMUNITY: Community focused on fostering efforts in Systems Biology, both related to microbes and human health, including the modeling of microbiomes and other microbial communities.
- COST Action ML4Microbiome: Collaboration among groups developing machine learning methods for the study of the microbiome.
- NFDI4microbiota: An initiative to standardize all aspects of microbiome research.
- IHEC: the International Human Epigenome Consortium hosts plenty of gut & intestine epigenomic datasets, which might be employed for integration in a future gut model.

2.2. ADDED VALUE OF NETWORKING IN IMPACT

2.2.1. SECURING THE CRITICAL MASS, EXPERTISE AND GEOGRAPHICAL BALANCE WITHIN THE COST MEMBERS AND BEYOND

The NETWORK of INFOGUT will contribute to:

- **Create a transversal and interactive scientific community** of microbiologists, gastroenterologists, nutritionists, bioinformatics and bioengineers for a bottom-up discussion targeted to define the potentials and limits of *in vitro* studies of gut microbiota-host interactions and how to fill those gaps.
- **Systematization of concepts** substantiating the most relevant gut models around Europe and possibly world, by providing a data set discriminating the different experimental approaches according to: constructive set up, protocols and bioprocesses parameters, specific applications (food, feed, nutraceuticals, supplements, toxicants, drugs, prebiotics, probiotics etc.) and targets (humans, animals, healthy, diseased etc.).
- **Facilitate technology transfer** by providing to the manufacturing sector (producer of facilities and their users) a decision tool as market strategy to select among the several potential opportunities coming from the implementations of models for specific applications.
- **Disseminate** 1) in scientific community consensus conclusions on the most important aspects of experimental methods mainly towards ITC countries to generate an increase of research on gut models and to facilitate network creation and cooperative project(s); 2) to a general audience to support the EU efforts in pushing the increase of *in vitro* tests alternative to animal ones.
- **Train young researchers** by creating different interactive activities to work with top experts in gut models and expanding their contacts and collaboration around Europe for future joint research projects (STSMs, training schools, in presence and virtual conferences).
- **Increase the number of ITC gut model labs.**
- **Involve policy makers in the evaluation of feasibility of suggested guidelines** leading to a standardization of *in vitro* testing of microbiota-host interactions.

2.2.2. INVOLVEMENT OF STAKEHOLDERS

Due to the limited number of gut model manufacturers, there will be **the main 3 European manufacturers which subscribed a Letter of support**, involved. Action members and industrial supporters will concomitantly promote the Action advances and disseminate the results by workshops dedicated to industries mainly related to INFOGUT impact on technology advancement (at least 1

workshop/year by the second year). On the other hand, **10 European companies, working in functional foods, feed, prebiotics, prebiotics, nutraceuticals, pharma foods, health supplements, etc.,** will be involved as users of gut models in pre-clinical tests. Their interest toward standardized methods to assay their innovative products perfectly
fit the aim of this Action. Moreover UEG (United European Gastroenterology) will participate mainly in dissemination activities thanks to their platform to share scientific contents, engage clinicians organize training schools, virtual seminars and conferences. These institutions will be part of the Consortium. Also, TEAGASC and other agencies like EFSA and JRC will be involved in the COST Action.

3. IMPACT

3.1. IMPACT TO SCIENCE, SOCIETY AND COMPETITIVENESS, AND POTENTIAL FOR INNOVATION/BREAKTHROUGHS

3.1.1. SCIENTIFIC, TECHNOLOGICAL, AND/OR SOCIOECONOMIC IMPACTS (INCLUDING POTENTIAL INNOVATIONS AND/OR BREAKTHROUGHS)

The development of harmonized *in vitro* gut models will advance the field of microbiology, food/feed science, nutrition, and translational medicine and likely beyond, by delivering the academic and research sectors with models that could be translated into novel research strategies, therapeutic approaches, and pre-clinical screening, in order to expedite results and speed up innovations, and also to reduce animal testing in line with the 3R's principles and the European Regulation (EU2010/63). This impact can be observed in short or long-term timeframe.

Scientific impact A wide number of researchers will benefit from the generation of a unified and benchmarked resources list, in addition to publications and training activities. In human health, the standardization and speciation of *in vitro* experimental methods opens up new possibilities for treating patients and gaining novel insights into gut mediated beneficial properties and role of gut microbiota in specific diseases (short term). The participation of female young scientists at leading positions will contribute to equilibrating the gender balance in the headquarters roles of research in universities and companies (short term). Moreover, gut models' protocols generated within INFOGUT will increase the quality, significance and soundness of the research and assist clinical therapies for different applications including a preliminary risk assessment of transplant rejection of microbiota provided by healthy donors (long term). The tailoring of *in vitro* gut models will also help to formulate the food, nutrients and pharmaceutics for specific consumers' categories (e.g. celiacs, lactose intolerant, vegans, allergies) (long term.)
Technological impact. Industrial partners of INFOGUT will benefit from the network to develop their product portfolio, broaden their customer reservoir and find new market opportunities (short term). The development of standardized gut models will generate new families of fermentation systems potentially subjected to patenting will strengthen R&D and help the economic development of industrial partners, including in ITCs (short term/long term). Furthermore, INFOGUT participants will be stimulated to create spin offs/start-ups in partnership with local companies or Universities (short term/long term).
Socioeconomic impacts. Users of gut models are on all continents and the market is enormous considering functional foods and ingredients, feed, nutraceuticals, pharmafoods, drugs, etc. The standardization of gut models including bioinformatic tools will reduce the use of experimental animals and shorten the production time of food/feed and supplements. In addition, it will make a breakthrough in the use of personalized medicine and nutrition (long term). In socioeconomic terms, these models may also reduce healthcare costs for public stakeholders and increase benefits of companies by providing new intellectual properties and patent licenses for further exploitation.

3.2. MEASURES TO MAXIMISE IMPACT

3.2.1. KNOWLEDGE CREATION, TRANSFER OF KNOWLEDGE AND CAREER DEVELOPMENT

Knowledge creation. INFOGUT will allow the creation of *in vitro* gut models by harnessing domain experts, motivated young researchers and industrial partners. The establishment of new gut models will tremendously extend the knowledge in the field by facilitating a better understanding of healthy and gut-related diseased process, including metabolic syndromes and allergies, but also the role of non-colonic microbiota. Moreover, the process of *in vitro* gut model's development will generate many pitfalls and challenges to be solved before reaching completion and this itself will create novel knowledge at various levels.

Transfer of knowledge. A foundation of INFOGUT is the transfer of knowledge between participants, the scientific community, the industrial sector, public associations, scientific society, and policy-makers, particularly importantly, towards ITCs (strengthening or initiating firm collaborations) and young researchers. Interactions between academia and industrial partners/SMEs will create a bidirectional transfer of knowledge, favorable for the development of industrial R&D and of spin-offs and start-ups. Indeed, the success of the Action is based on the competence of the network to efficiently diffuse knowledge. Social media, website, live and e-meetings, conferences, workshops, hackathons, STSMs, and training schools will contribute to knowledge diffusion. Exchange program (STSMs) will enable YRIs to visit network laboratories and academics and confirm strong relationships between senior and junior scientists in order to create job opportunities and leadership qualities in early stages of their careers.

Career development. INFOGUT will offer excellent training, networking opportunities, support and an accompanying framework to develop their own projects and own groups. Moreover, this large network will generate cooperations and smaller clusters potentially enriched with women and members from ITCs, with capacity to initiate new joint research projects, hence boosting participant careers. INFOGUT will comprise many ITCs with high involvement of women professionals, and will serve as incentives towards YRIs, especially women, for mobility and leadership.

3.2.2. PLAN FOR DISSEMINATION AND/OR EXPLOITATION AND DIALOGUE WITH THE GENERAL PUBLIC OR POLICY

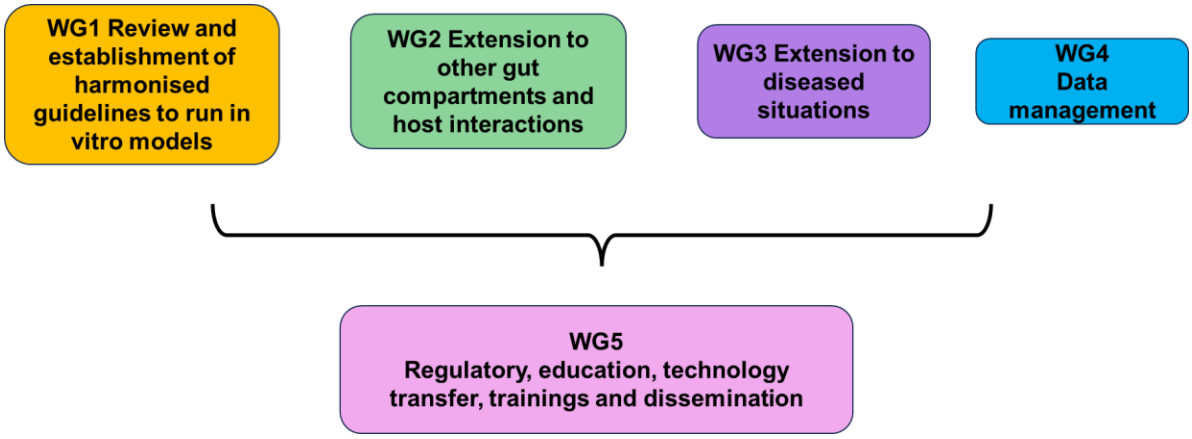
Because prompt dissemination of INFOGUT breakthroughs is central for optimal exploitation and acceptance of the results, the Action will establish a detailed dissemination plan in coordination with all network participants. INFOGUT will deliver webinars, masterclasses and hands-on training sessions for end-users promoted by associations of digestive health professionals including clinicians and translational scientists, and patients, which will contribute to effectively disseminate and to facilitate the adoption of our tools. INFOGUT Consortium will contribute with its expertise on the impact of technologies in healthcare practice, both from the clinicians' and patients' perspective. Dissemination activities, will facilitate INFOGUT engagement with healthcare professionals throughout the Action, also serving as an 'ambassador' of the tool at the international level, while considering the broader clinician and patients' perspective, ensuring that in the long-term, the solution becomes standard in clinical research practice.
The dissemination activities will be organized within links to:

- **Scientific outcomes:** production of an Atlas compiling validated protocols but also negative results, pitfalls, troubleshooting, drawbacks. Production of databases with all published data related to the field.
- **Educational programs:** WG meetings, conferences, training schools, seminars, workshops, tutorials, mentoring program. Videos and interviews will be included.
- **Internal and external partners' websites:** industry, SMEs, NGOs and patient groups and nature and animal defense advocates that will have accepted to be associated to INFOGUT.
- **Social media:** (e.g. Threads, X, Instagram, ResearchGate, LinkedIn, Facebook), videos (e.g. YouTube and JoVE), and **general media** (e.g. TV and radio interviews, newspapers, documentaries) showing network activities or outcomes. INFOGUT will communicate by partners platforms of communication channels on social media and via newsletters (approx. 60,000 newsletters' recipients, +14K followers on Twitter, +6K on LinkedIn).
- **Annual meetings:** research and medical networks related to the fields, at European and international levels.
- **Hackathon events, Tutorials:** events of bioinformaticians to evolve AI solutions and tutorials for data management and digital tools coding.
- **Contact details of all network experts:** A detailed list of contacts will be included to promote rapid communication and enable quick answers to urgent questions; Action industrial partners, professional organizations, NGOs, participant institutions (e.g. websites) and conferences attended by Action participants will be important for the dissemination of network outcomes via their websites and product portfolios.

4. IMPLEMENTATION

4.1. COHERENCE AND EFFECTIVENESS OF THE WORK PLAN

4.1.1. DESCRIPTION OF WORKING GROUPS, TASKS AND ACTIVITIES



The Action will be managed by the Management Committee, supported by coordinators and WG leaders to address challenges and facilitate decision-making. Three WGs (WG 1 to 3) are dedicated to scientific innovations, one WG (4) to data management and AI applications, and one WG (5) deals with regulatory aspects, including IP and ethics, along with dissemination, and educational programs (i.e. towards ITCs and YRIs). The Action Management Committee will adopt the following policies to support the Action objectives: (1) fair regional contribution and involvement of members from ITCs at different levels of Action management, (2) balanced generational representation with an enrichment in YRIs at leading positions (3) gender and diversity equity, involvement of female members in decision-making bodies. The interrelations between all WGs will promote information sharing during WG meetings and the annual Action conference. Overall, INFOGUT aims at advancing the state-of-the-art on *in vitro* and *in silico* gut models and generating guidelines to reproduce protocols in different laboratories.

WG 1: Review of existing *in vitro* human colon models and establishment of harmonized guidelines to enable better comparison of data from different models.

This WG is dedicated to the development of harmonized procedures and *in vitro* gut models.

Task 1.1. Harmonization of fermentation protocols (batch, continuous, multi-assays)
Review and map of the current *in vitro* model systems with the objective of identifying key parameters that require standardization across all models.
Task 1.2. Inoculum (faeces, mock communities), fermentation medium and control
Apply the standardized parameters across the range of *in vitro* colonic models available within the INFOGUT network to establish the consistency and repeatability of gut microbiota readouts under standardized conditions.
Task 1.3. Non-human gut models
Based on the same approach adopted in Tasks 1.1 and 1.2, review the available protocols and experimental conditions to propose standardized parameters for *in vitro* animal gut models
Task 1.4. Prepare a peer review publication on the agreed standardized parameters and their application across the available *in vitro* colonic models, highlighting the key benefit of their application.

WG2: Extension to other gut compartments and host interactions

This WG is dedicated to the expansion of current *in vitro* models to include other body compartments.

Task 2.1. Connection to upper GI tract: Establish an overview of the current state-of-the art of model systems that regionalize the functionality and composition of the gut microbiota along the digestive tract, but also integrate the mucosal environment.
Task 2.2. Coupling with host cells: identify *in vitro* gut models that can be (in)directly coupled to host cells (intestinal, liver, pulmonary, brain cells) or 3D cultures to provide a more accurate representation of the intestinal and extra-intestinal environments and their interactions with the host.
Task 2.3. Miniaturization: analyze the potential of miniaturizing *in vitro* gut models to enable high-throughput screening of dietary or diseased factors or individual fecal samples.
Task 2.4. *In silico* gut models: discuss the application of *in silico* models for the exploration of human microbiomes in terms of functionality, growth kinetics, engraftment success and safety. In this context,

tools such as AGORA based prediction of growth kinetics, metabolic pathway prediction and screening of microbial genomes and metagenomes against publicly available databases can be further explored.
Task 2.5. *In vitro-in vivo* correlations: Gathering an overview of *in vitro/in vivo* correlation (IVIVC) studies which are pivotal to assess the relevance and predictive capabilities of new *in vitro* gut models associated or not with cells, to accurately mimic *in vivo* (animal or human) systems. IVIVC will serve to bridge the gap between lab-based experiments and real-world applications.

WG3 Extension to diseased situations

This WG aims to extend the potential of *in vitro* gut models to disease situations in human and animal by considering not only the dysbiotic microbiota (provided by faecal or oral samples) but also all the parameters shaping gut diseased environment.

Task 3.1. IBD/IBS and colon cancer diseases: Provide an overview on *in vitro* models' systems that have been adapted to reproduce gut microbiota dysbiosis associated to most common digestive pathologies: IBD, IBS and colon cancer gut models. Discuss the current limitations of these models in comparison to *in vivo* situation.
Task 3.2. Metabolic and non-communicable diseases: extend the potential of *in vitro* gut models to metabolic and non-communicable diseases (including metabolic syndrome, obesity, diabetes, steatosis hepatis, coeliac disease, etc.). The main objective is to maintain *in vitro* microbiota dysbiosis considered as a typical feature of these pathologies *in vivo*. ~~T~~he associated gut parameters should also be adapted to the specific diseased conditions (pH, transit time, bile acids profiles, nutrient availability, etc.).
Task 3.3. Enteric pathogens diseases: *in vitro* gut models can be used to simulate some facets of enteric infection by studying following pathogen inoculation, survival, virulence gene expression and its bilateral interactions with gut microbiota in both upper and lower tract *in vitro* models.
Task 3.4. Xenobiotics and food pollutants: *in vitro* models can be used to evaluate the impact of acute or chronic exposure to food pollutants (chemical, microplastics, etc.) on gut microbiota composition and activity but also reversely the ability of gut microbes to metabolize compounds and modify their toxicity.
Task 3.5. Pharma therapies and non-antibiotic strategies: evaluate the potential of different restauration strategies (of gut microbiota and metabolic perturbations), such as drugs (under various formulations), but also next generation restoration strategies including prebiotics, probiotics, postbiotics, live biotherapeutic products and fecal microbiota transplantation.

WG4 Data science and data management

The aim is to identify, organize and catalogue relevant open microbiome data sources, analytical techniques, and tools related with multi-omics data from the gut and evaluate the state of the art of *in silico* efforts for modelling gut function, with a focus on microbiome.

Task 4.1. Create FAIR catalogues for the gut omics data and bioinformatics tools: identify and organize relevant omics data repositories and studies for open gut microbiome data.
Task 4.2. Evaluate *in silico* approaches for gut modelling: gather and benchmark the most used AI/ML and bioinformatics tools for gut analysis.
Task 4.3. Develop training materials: design, organize, and implement training material for gut data analysis to be used by WG5.

WG5 Regulatory, education, technology transfer, trainings and dissemination

INFOGUT aims to become a think tank and a business incubator for technology related to reference *in vitro* gut models and related protocols. This requires a strict coordination of all channels of the Action WGs for permanent information flow throughout scientific community and between academia and industries also taking into consideration the guidance of ethical and regulatory bodies which will be involved. Moreover, the development capacity of INFOGUT lies in the opportunity for academic laboratories and industrial partners to develop an educational program together with a dissemination strategy as follows:

Task 5.1. Support to translational studies of food/feed, ingredients, supplements, drug therapies
Task 5.2. Educational tools (YRIs, consumers, academics, students)
Task 5.3. Transfer to industries and training to professionals.

4.1.2. DESCRIPTION OF DELIVERABLES AND TIMEFRAME

WG	Deliverable		Month
WG1	D1.1	Report mapping the current i*n vitro* colon models, their key process parameters, standardizations and quality control procedures across the range of *in vitro* colonic models available within the COST network	15
	D1.2	Non-human gut models: Review and map of currently *in vitro* model systems and identification of standardized parameters	18
	D1.3	Submitted manuscript on the agreed standardized parameters and their application across the available *in vitro* colonic models	36
WG2	D2.1	Map of the current *in vitro* models integrating microbiota in the upper gastrointestinal tract and associated peer-reviewed publication	18
	D2.2	Guidelines to implement microbiota in *in vitro* models of the upper gastrointestinal tract based on *in vivo* data	33
	D2.3	Report on guidelines to couple models of the upper and lower gastrointestinal tract and/or host cells based on *in vivo* data	33
	D2.4	Final report on symposia and/or training for young scientists on WG2 activities	42
WG3	D3.1	Submitted a literature review paper to identify available diseased *in vitro* gut models, their limitations and challenges that have to be faced	20
	D3.2	Guidelines on which parameters should be considered when developing a new diseased *in vitro* gut model	32
	D3.3	Report on training schools on the way to set-up diseased *in vitro* gut models for YRs and Industrials.	36
	D3.4	Report on a public event to communicate to the scientific community newly available *in vitro* gut models adapted to diseased situation and present associated results on their development, validation and potential use to test remediation strategies	36
WG4	D4.1	Open access publication (Action webpage) of the catalog of open data and bioinformatics resources;	18
	D4.2	Report on state-of-the-art data integration methods for gut microbiome data	24
	D4.3	Publication with the findings and summarizing the results from D4.1-D4.2	36
	D4.4	Final delivery of training materials for WG5 activities	38
WG5	D5.1	Atlas of support to translational studies to be implemented in the website	24
	D5.2	Plan of Training Event for Academics, YRIs and Professionals	18
	D5.3	Final report of dissemination activities for the General Public and Scientific Community	48

WG	Milestones		Month
WG1	M1.1	Comprehensive peer-reviewed data collection suitable for the publication on standardized parameters and/or hybrid comprehensive Methods optimization publication relating to human *in vitro* colon models.	12
	M1.2	Comprehensive peer-reviewed publication on standardized parameters relating to animal *in vitro* gut models	15
WG2	M2.1	Data collection for peer-reviewed publication ion models integrating microbiota in the upper gastrointestinal tract	15
	M2.2	Symposia / training for young researchers	33
WG3	M3.1	Data collection for a preliminary evaluation on eventual peer-reviewed publication on each *in vitro* gut disease model including those focused on metabolic disease	18
	M3.2	Data collection to support guidelines to implement technologies related to microbiome-host interactions in disease models	24
	M3.3	Organization of symposium/training for young researchers	36
WG4	M4.1	Generate an initial catalog	12
	M4.2	Definition of training materials	18
	M4.3	Draft of a road map for the final publications	30
WG5*	M5.1	Website	8
	M5.2	First workshop for industries and general public	18
	M5.3	Second workshop for industries and general public	28

*: all the reporting activities and General Conferences are reported in Gantt diagram

4.1.3. RISK ANALYSIS AND CONTINGENCY PLANS

WG	Objective	Risk	Contingency
WG1	Harmonization of parameters relating to *in vitro* colon models	Insufficient researchers working on these models within this timeframe	Regular communication, liaising and sharing of preliminary data and research outputs within the WG
	Collation of data for a Methods optimization publication of *in vitro* models	Insufficient funds available especially in non-medical Institutes for whole genome sequencing approaches	Regular communication and sharing of any sequencing data in public repositories. Also, inclusion of data from species-specific qPCR.
WG2	Integration of microbiota in the models of the upper gut	Paucity of *in vivo* data on microbiota in the human upper gut and/or inconsistencies between studies	Use of new microbiota sampling technics (i.e. smart pills).
		Lack of *in vivo* data on mechanical, physico-chemical and nutritional parameters of host microbiota interaction	Active collaboration with gastroenterologists (from the COST consortium)
	Coupling of upper and lower gut models with host cells	Loss of information due to cytotoxicity of gut samples and needs for less microbial dilutions.	Regular updates and collaboration effort with experts in the field to find technical solutions
		Oversimplified view of host-microbe interactions due to common practice to use immortalized cell models	Use of more complex cellular models such as organoids
WG3	Extend the potential of *in vitro* gut models to metabolic and NC diseases	Paucity of *in vivo* data from diseased patients on nutritional and microbial parameter	Use of new microbiota sampling technics (smart pills) and COSTs gastroenterologists' collaborations
		Difficulties to reproduce non-communicable diseases *in vitro* due to the influence of systemic or extra-digestive factors	Coupling with host cells to integrate host-microbe interactions and/or simulation of biological mediators (inflammation)
WG4	Identify, organize, catalogue databases, techniques and tools related with omics data from the gut models	Missing relevant resources	Regular updates and collaboration effort with experts in the field
		Varying level of data quality, reliability and availability	Provide extensive metadata on data source reliability and quality, and indicate the accessibility status
		Inadequate documentation and lack of standardizations	Use the established terminology and standardized categorization
	Suggestions on evaluation methods on the state of the art of in silico efforts for gut modelling	Bias due to limited understanding on gold standard benchmark datasets, evaluation metrics and comparison methods	Standardizing baseline methods in diverse benchmark datasets and comparisons amid state-of-the-art methods and the baseline ones
WG5	Disseminate, educate, train of students, scientists and professionals.	Limited knowledge and non-implementation of EU Directive 2010/63/EU	Improve general knowledge and bring the topic to the largest audience possible

4.1.4 GANTT DIAGRAM

ACTIVITY	YEAR 1				YEAR 2				YEAR 3				YEAR 4			
	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4
Kick off meeting	x															
MC meeting	x				x				x			x				x
WG1 meeting	x			x			x		x			x				
T1.1 Fermentation Protocols																
T1.2 Inoculum, media and controls				*M1.1*	**D1.1**											
T1.3 Non-human gut models					*M1.2*	**D1.2**										
T1.4 Standardized parameters												**D1.3**				
WG2 meeting	x			x			x			x		x				x
T2.1 Connection upper GI tract					*M2.1*	**D 2.1**						**D2.2**				
T2.2 Coupling with host cell models												**D2.3**				
T2.3 Miniaturization																
T2.4 *In silico* gut models																
T2.5 *in vitro - in vivo* correlations												*M2.2*		**D2.4**		
WG3 meeting	x						x		x			x				x
T3.1 IBD/IBS and colon cancer diseases						*M3.1*										
T3.2 Metabolic and non-communicable diseases							**D3.1**									
T3.3 Enteric pathogens diseases									*M3.2*							
T3.4 Xenobiotics and food pollutants												**D3.2**		**D3.3**		
T3.5 Pharma therapies and non-antibiotic strategies												*M3.3*		**D3.4**		
WG4 Data Management	x				x				x			x				x
T4.1 FAIR catalogues					*M4.1*	**D4.1**										
T4.2 Evaluate in silico approaches for gut modelling							*M4.2*	**D4.2**				**D4.3**				
T4.3 Develop training materials										*M4.3*				**D4.4**		
WG5 Regulatory, Education, Dissemination	x				x				x				x			
T5.1 Support to translational studies			*M5.1*				**D5.1**						**D5.1**			
T5.2 Educational tools							**D5.2**							**D5.2**		
T5.3 Transfer to industries and training to professional						*M5.2*				*M5.3*						**D5.3**
Web portal			x													
COST conference					x					x						x
Annual report					x					x						
Final report																x

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