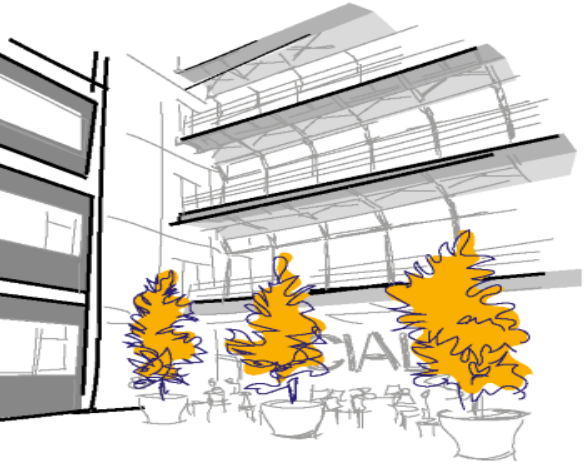


Dietary modulators of human & animal gut microbiome- One health approach

F. Javier Moreno
(javier.moreno@csic.es)

RESEARCH IN FOOD SAFETY

Administration luxembourgeoise vétérinaire et alimentaire (ALVA)
9th december 2022

Declaration of interests

Affiliations/ financial interests	Organisation
Employment	Spanish National Research Council (**CSIC**)
Grants/Research support	AGL2017-84614-C2-1-R / **Spanish State Research Agency** GA 818368 / **EU-Horizon 2020** GA 101060130 / **EU-Horizon Europe** GA 101060218 / **EU-Horizon Europe** GA GP/EFSA/ENCO/2020/02 – 1 / **EFSA**
Scientific advisory board/consultant	None
Government	None
Other	

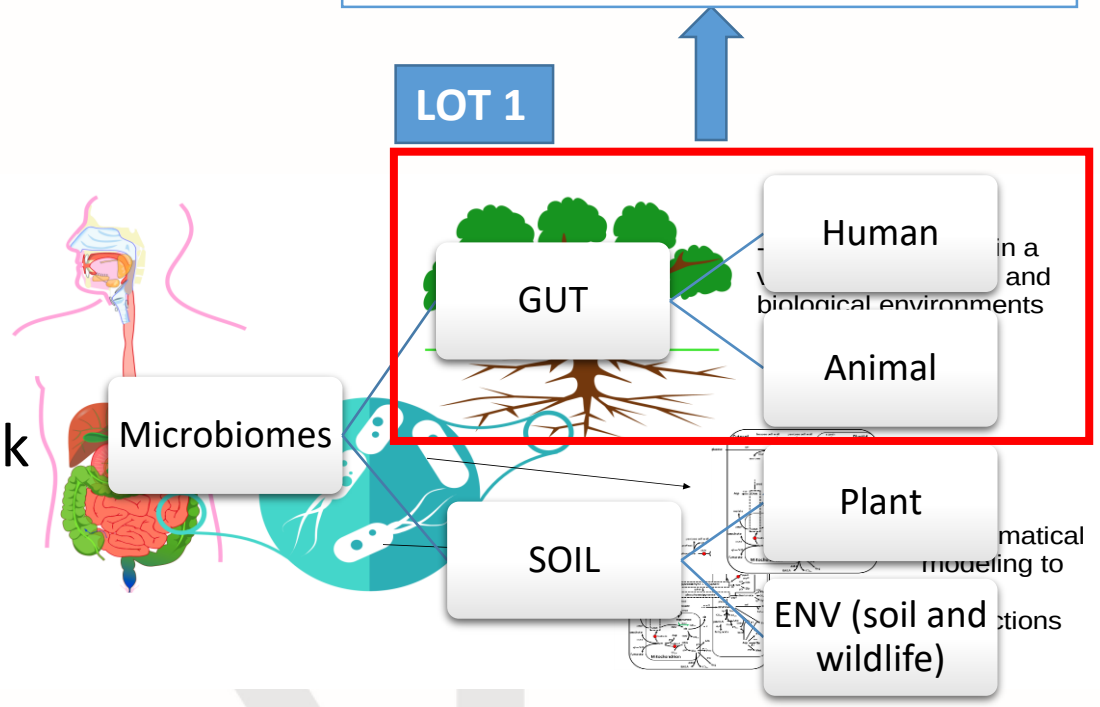
Thematic grants: Preparedness for future challenges in specific areas of EFSA`s work

The main objective is to **build capacity** for:

✓ evaluating the impact on gut/soil microbiomes by various modulators under EFSA's assessments

determine whether microbiomes can be included in risk assessments under EFSA's remit or not

Review **I**mpact
MICrobiome **I**n **A**ssesment
RIMICIA



Thematic grants: Preparedness for future challenges in specific areas of EFSA`s work

CIAL
INSTITUTO DE INVESTIGACIÓN EN CIENCIAS DE LA ALIMENTACIÓN

General objective of LOT 1

To focus on the possible exposure to modulators of the GI microbiome via dietary pathway and their effect on human/domestic animal health

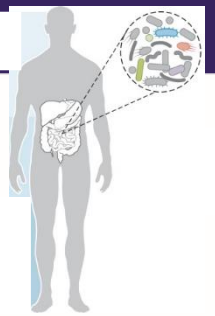
Scope of LOT 1

To cover for possible risk/benefit assessments relevant for EFSA's panels: FAF, CONTAM, BIOHAZ, FEEDAP, GMO, NDA, PPR, AHAW and SC

Multidisciplinary and international consortium with expertise in:
bibliometrics and science analysis, microbiology, food science and technology, analytical chemistry, bioinformatics, genomics, transcriptomics, metabolomics, systems biology, food allergy, immunology, food safety, animal health and welfare, human and animal gut microbiota, and risk assessment

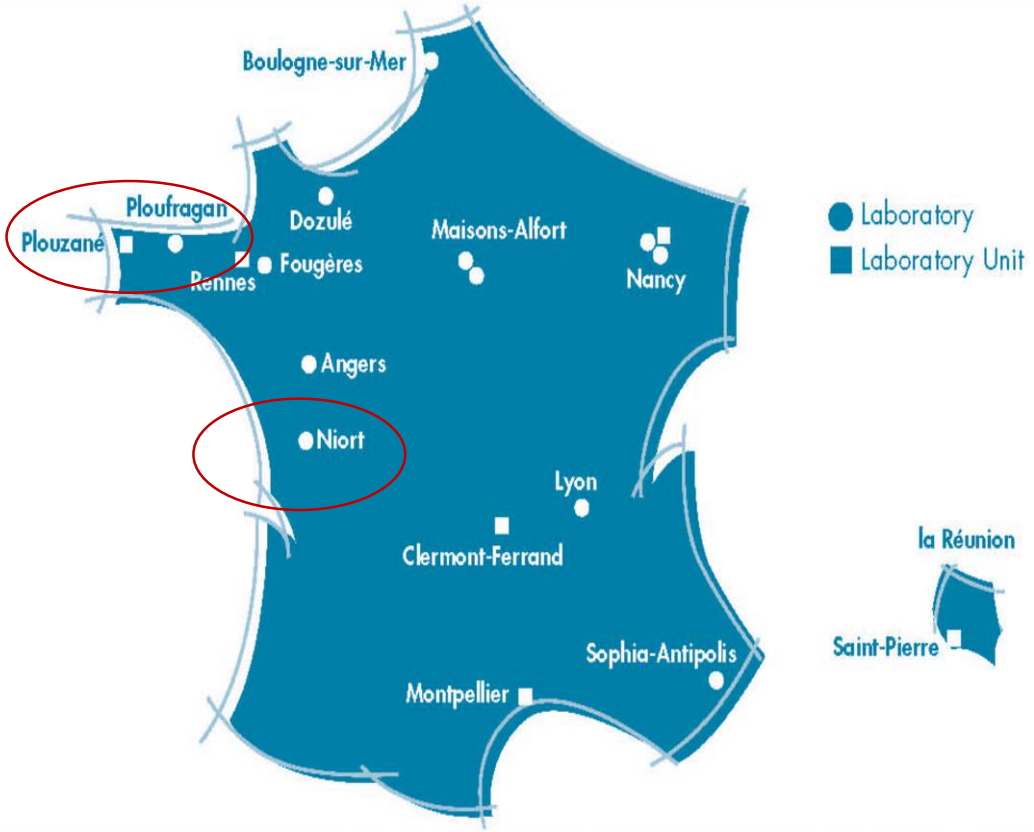
Our Team

CSIC
Consejo Superior de Investigaciones Científicas



CIAL

anses



Rationale followed for literature searches

disease

Microbiota-related microorganism or metabolite

Dietary modulators-related chemical compounds

(~160 different compounds)

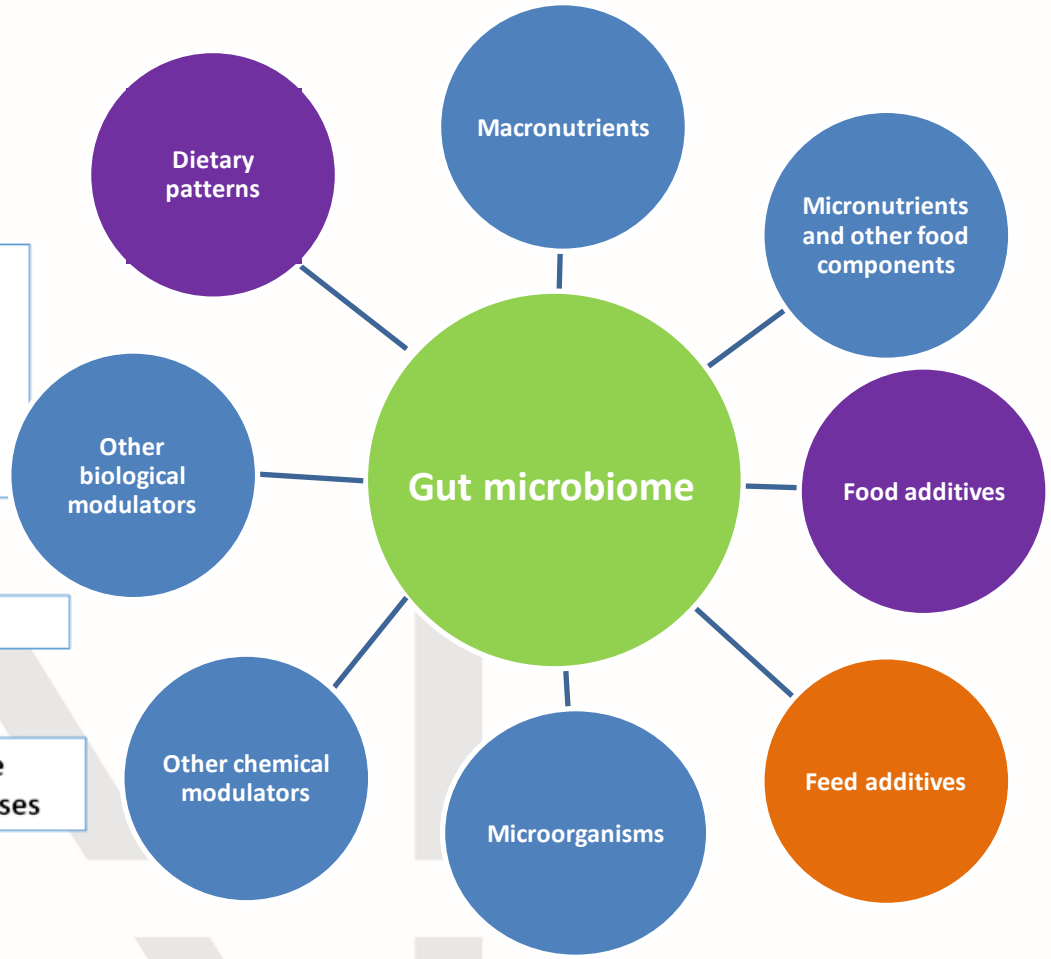
Literature searches performed according to different category dietary modulators and animal species by using controlled (MeSH) terms combined with expanded queries in title, abstract and author keywords

PubMed.gov · Clarivate Web of Science™ · Scopus · NIH U.S. National Library of Medicine ClinicalTrials.gov

Ad hoc web interface to process sets of articles (based on a single MeSH term, citation networks, etc.)

Text-mining studies → Expert's assessment and judgment ← Gut microbiome specialized databases

Evidence about associations between dietary moduladors - gut microbiome - diseases



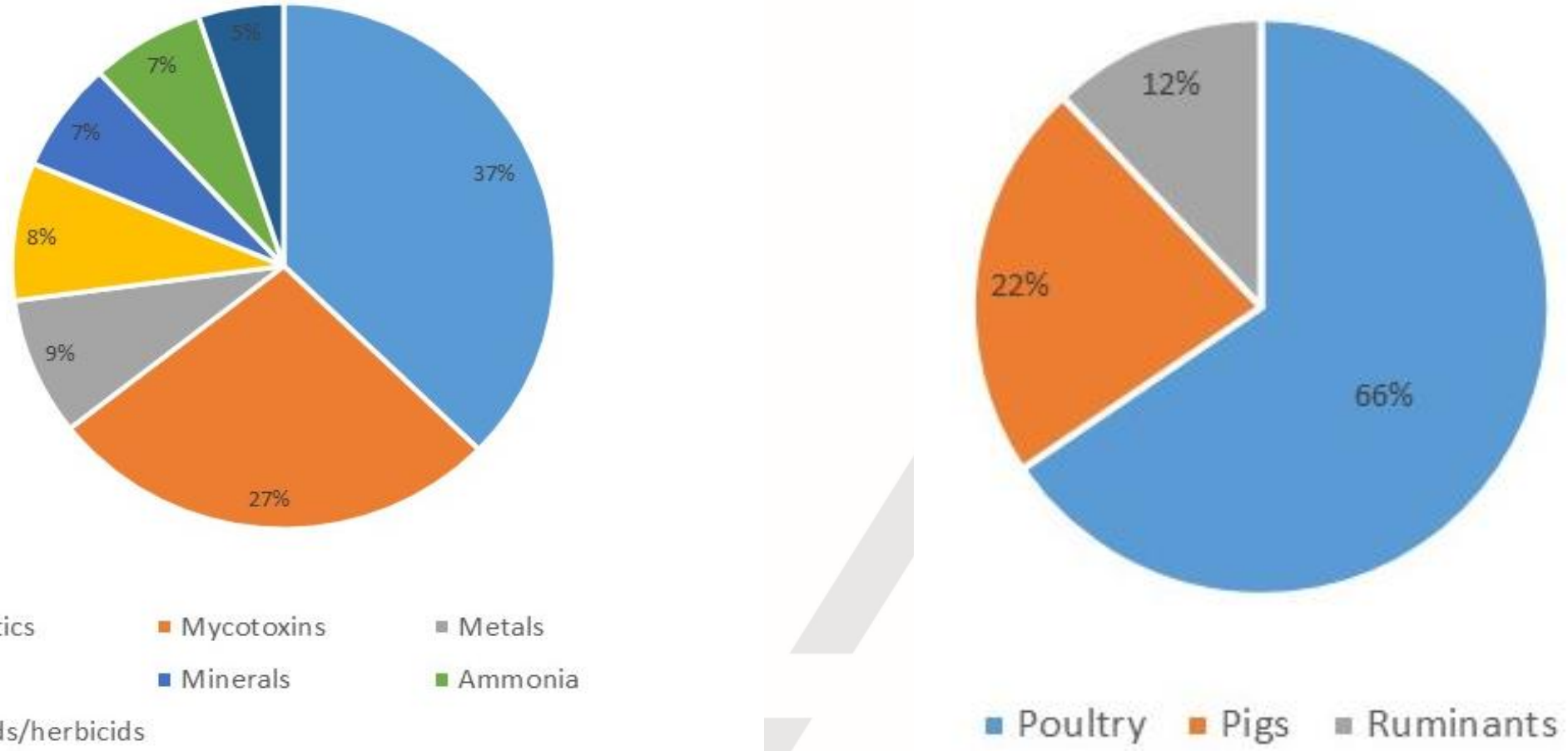
Summary of the outcome of the main literature searches

Topic	PubMed		clinicaltrials.gov		Scopus[1]	WoS CC[1]	To screen
	# records[2]	Clinical Trials	#records	en Pmed	Exclusive records[2]	Exclusive records[2]	
Macronutrients	3851	381	72	26	923	695	5469
Micronutrients and other food components	1582	110	34	19	458	289	2329
Food additives	700	28	29	4	214	165	1079
Feed additives	1888	N.A.			261	383	2532
Microorganism based	5225	559	45		1336	879	7440
Other chemical modulators	1223	22	0	0	62	265	1550
Other biological modulators	3547	227	30	9	540	974	5061
Dietary patterns	4274	338	27	34	2460	280	7014

Parameter	Inclusion criteria	Exclusion criteria
Population	Poultry, Pigs and Ruminants Male and/or Female	Other species
Intervention	Harmful modulator Orally administration	Other modulations
Comparison	Microbiota composition	No statistics
Outcomes	Quantitative study of the gut microbiota and/or pathogenic bacteria.	No study of the microbiota. Qualitative studies of the gut microbiota
Study design	In vivo studies	Review, in vitro or in silico studies, consensus papers, letters to editor, book chapters, thesis, controlled trials

Harmful modulators of the intestinal microbiota – animal part

Antibiotics deeply disrupt and deleteriously affect the intestinal microbiota



Harmful modulators of the intestinal microbiota – animal part

MYCOTOXINS

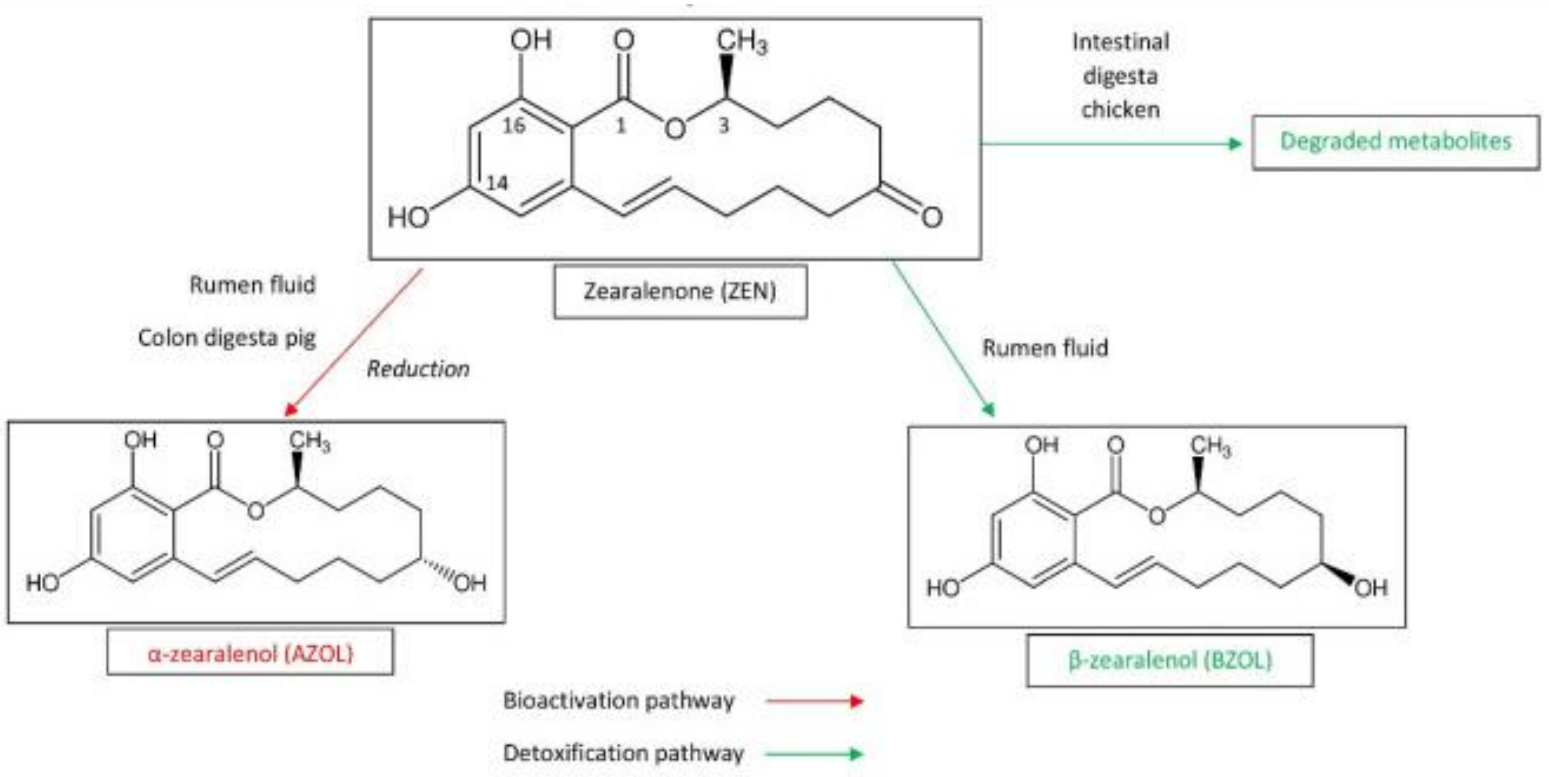
	Poultry	Pigs	Ruminants
Aflotoxin B1	↓Lactobacillus ↑E.coli	No data	↓ Butyrivibrio
Ochratoxin A	↓Fimicutes ↑Bacteroidetes ↓ SCFA and lactic acid producing bacteria	No data	No data
Deoxynivalenol	No microbiota modifications	↓ Lactic acid producing bacteria ↑E.coli	No data
Zearalenone	No data	↓ Lactic acid producing bacteria ↑E.coli ↑Prevotella	No data

Role of animal gut microbiota seems to be essential in mycotoxins risk assessment

Harmful modulators of the intestinal microbiota – animal part

MYCOTOXINS

Gut microbiota may affect internal exposure levels to mycotoxins by reducing or increasing exposure to the toxic form of the mycotoxin by either detoxification or reactivating a masked form



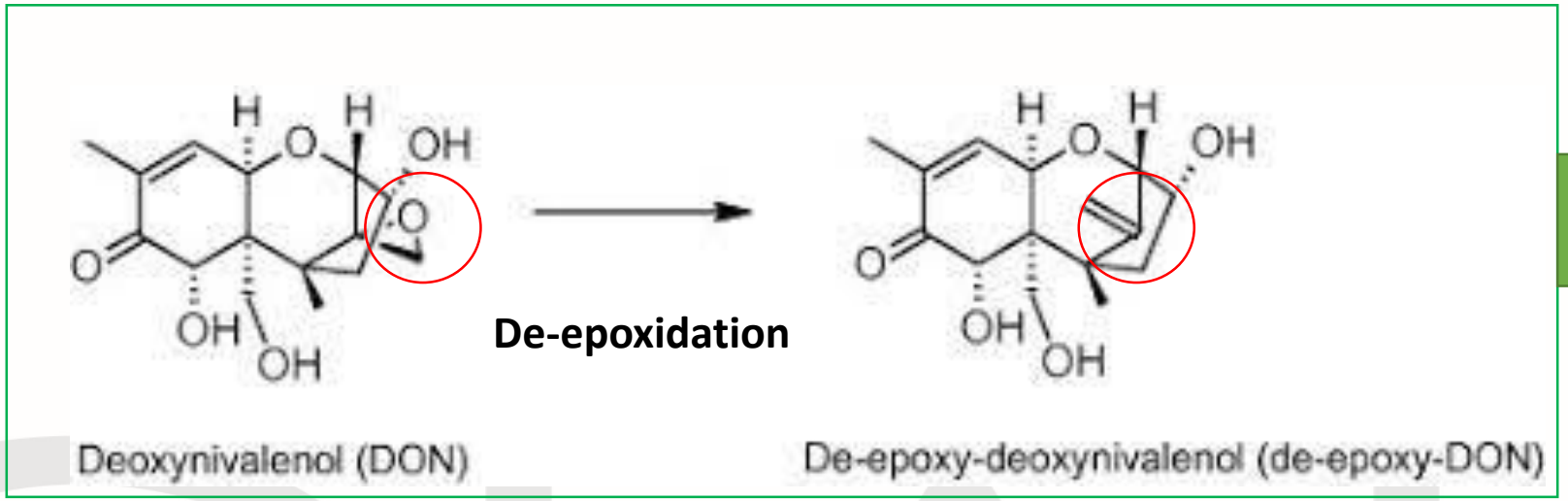
Guerre, Toxins 12, 769 (2020)

Harmful modulators of the intestinal microbiota – animal part

MYCOTOXINS

De-epoxydeoxynivalenol (Dom-1) is a non toxic metabolite

Deoxynivalenol (DON) is frequently found in animal feed



Detoxification pathway

Clostridium sp. WJ06 allow to decrease Don excretion and increase Dom-1 excretion

Li et al. Toxins 9, 383 (2017)

Some conclusions – animal part

- Antibiotics and mycotoxins are the most studied harmful modulators of the gut microbiota in farm animals.

- The administration of antibiotics induces deep changes in the composition of the gastrointestinal microbiota regardless of the species studied. The most found modification is the increase in the abundance of *Proteobacteria* and decrease in the abundance of *Firmicutes*.

- The administration of antibiotics to farm animals leads to an increase in the abundance of antibiotic resistance genes.

- The sensitivity of the intestinal microbiota of farm animals to mycotoxins depends on the type of mycotoxins but also on the animal species studied.

- Some bacteria naturally present in the gastrointestinal microbiota are capable of metabolizing mycotoxins into non-toxic metabolites.

Summary of the outcome of the main literature searches

Topic	PubMed		clinicaltrials.gov		Scopus[1]	WoS CC[1]	To screen
	# records[2]	Clinical Trials	#records	en Pmed	Exclusive records[2]	Exclusive records[2]	
Macronutrients	3851	381	72	26	923	695	5469
Micronutrients and other food components	1582	110	34	19	458	289	2329
Food additives	700	28	29	4	214	165	1079
Feed additives	1888	N.A.			261	383	2532
Microorganism based	5225	559	45		1336	879	7440
Other chemical modulators	1223	22	0	0	62	265	1550
Other biological modulators	3547	227	30	9	540	974	5061
Dietary patterns	4274	338	27	34	2460	280	7014

Literature searches largely associated with potentially beneficial dietary modulators (i.e., fiber (prebiotics), HMOs, ω-3 PUFAS, probiotics, phenolics and phytochemicals, vitamins)

Clinical trials are mainly focused on the study of modulators providing potential health benefits

Literature searches that have allowed the identification of potentially harmful dietary compounds for the human gut microbiome but with a low number of clinical trials

Harmful modulators of the intestinal microbiota – human part

INSTITUTO DE INVESTIGACIÓN EN CIENCIAS DE LA ALIMENTACIÓN

Food additives

	Compound
Colorants (azo dyes)	Allura red AC (E129)
	Yellow 6 (E110)
	Tartrazine (E102)
	Azorubine (E122)
	Amaranth (E123)
	Ponceau 4R (E124)
Emulsifiers	Sodium carboxymethyl cellulose (E466)
	Polysorbate 80 /Tween 80 (E433)
	Carrageenan (E407)
	Locust bean gum (E410)
	Hydroxypropyl methyl cellulose (E464)
	Docusate sodium (E480)
	Sorbitan monostearate (E491)
	Glycerol Monolaurate (E471)
	Sorbitan monostearate (E491)
	Sophorolipids
	Rhamnolipids
Non-nutritive sweeteners	Saccharin (E954)
	Sucralose (E955)
	Acesulfame-K (E950)
	Sodium cyclamate (E952)
	Aspartame (E951)
	Neotame (E961)
	Advantame (E969)
Nanoparticles	Titanium dioxide (TiO_2) (E171)
	Iron oxides and hydroxides (E172)
	Silver (E174)
	Gold (E175)
	Silicon dioxide (SiO_2) (E551)
	Cationic liposomes

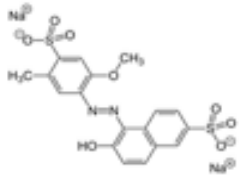
Food chemical contaminants

	Compound
Environmental contaminants	Dioxins
	Polychlorinated Biphenyls (PCBs)
	Heavy metals
	Chlorinated paraffins
	Microplastics
	Brominated Flame Retardants (BFRs)
	Organotins
Processing/manufacturing contaminants	Polycyclic aromatic hydrocarbons (PAHs)
	Heterocyclic aromatic amines (HCAs)
Agricultural contaminants	Pesticides
	Nitrates
	Mycotoxins

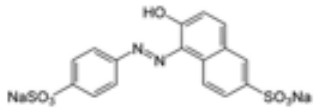
Potential mechanisms: Microbiome-mediated enzymatic metabolism

CIAL
INSTITUTO DE INVESTIGACIÓN EN CIENCIAS DE LA ALIMENTACIÓN

Food additives

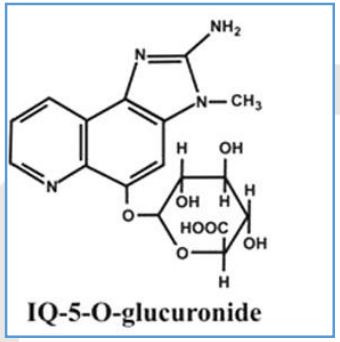


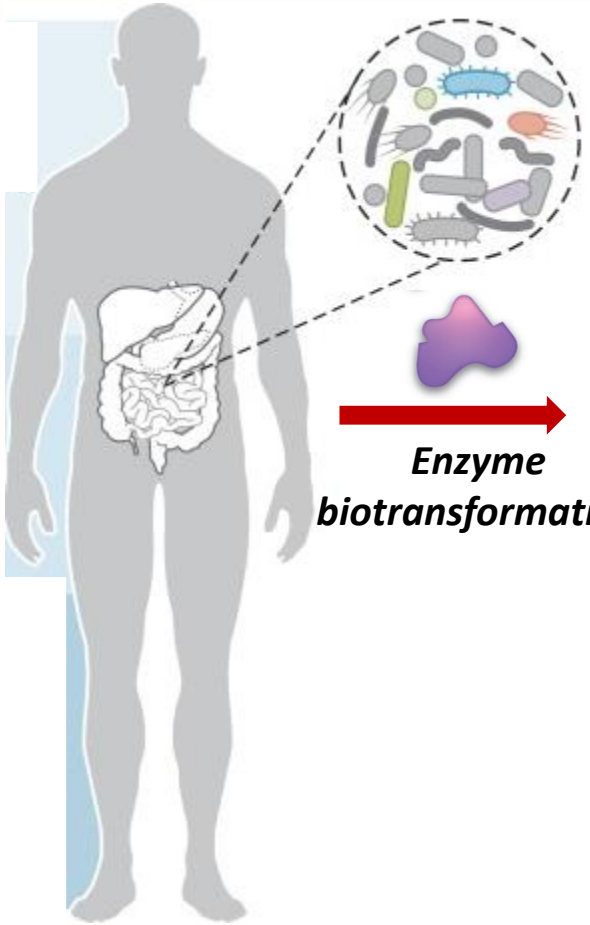
Red 40 colorant
(allura red)
E129



Yellow 6
(sunset yellow)
E110

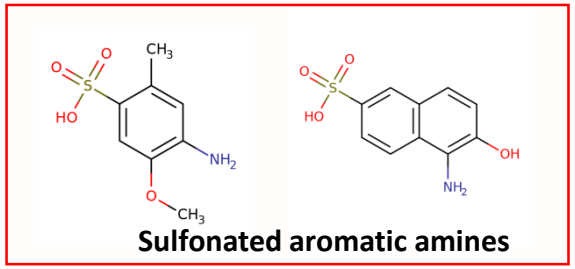
Food chemical contaminants



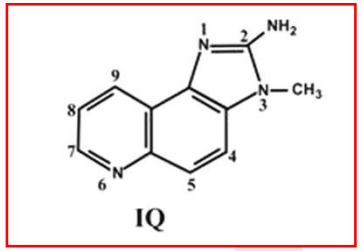


Enzyme biotransformation

Hazardous metabolites



Azo-reductases



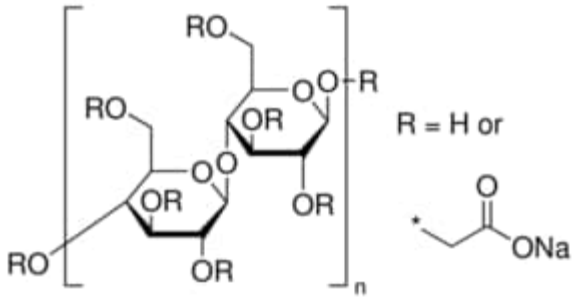
β-glucuronidases

Other involved enzyme families : nitro- and nitrate reductases, β-glycosidases, sulfatases, β-lyases and organophosphorus hydrolases

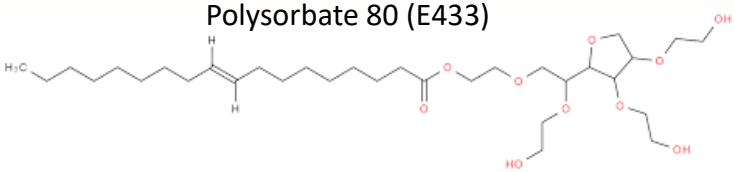
Potential mechanism: Alteration of the gut microbiota

Food additives

Sodium carboxymethyl cellulose (E466)

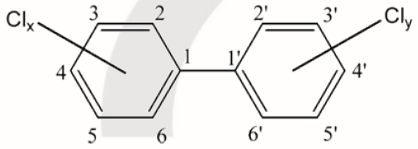


Polysorbate 80 (E433)

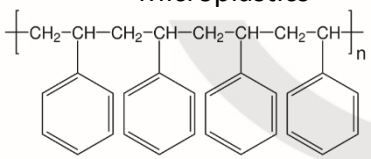


Food chemical contaminants

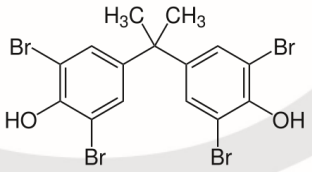
PCBs

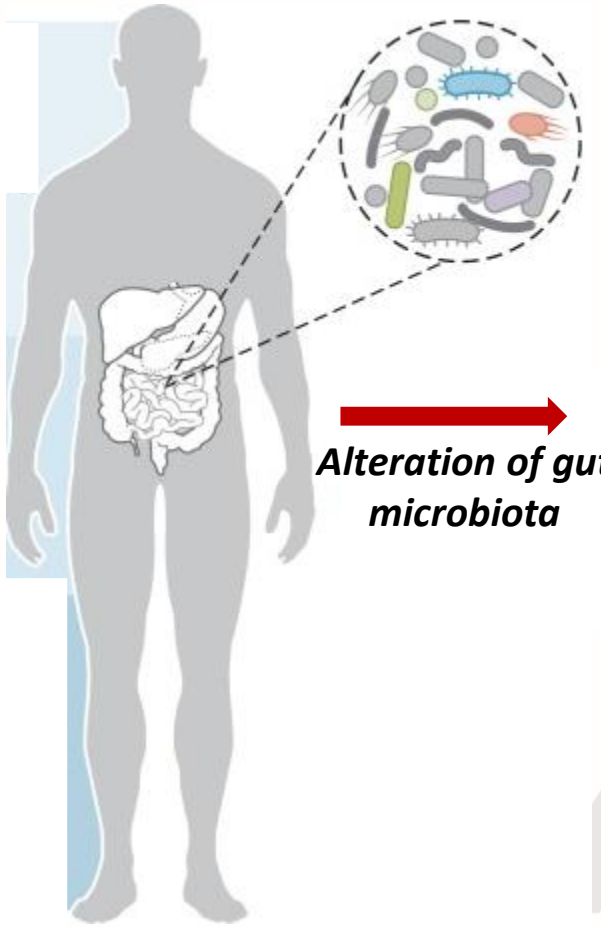


Microplastics



BFRs

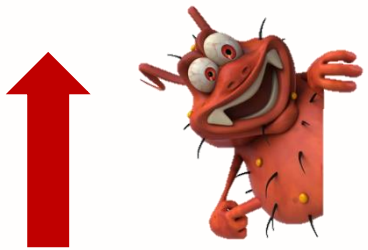




Alteration of gut microbiota

Large-scale turnover (similar to antibiotic treatments)

Enrichment of potentially specific detrimental taxa

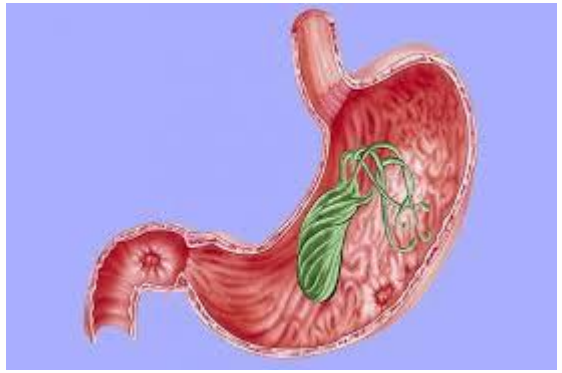


Depletion of potentially specific beneficial taxa

What does “detrimental/beneficial taxa” mean in a risk assessment context?

CIAL
INSTITUTO DE INVESTIGACIÓN EN CIENCIAS DE LA ALIMENTACIÓN

- ✓ Reducing/increasing numbers **of pathogenic or toxigenic microorganisms or their toxins** in the gastrointestinal ecosystem **could be straightforwardly linked to a beneficial/detrimental physiological effect.**



***Helicobacter pylori* and gastric diseases**

***Clostridioides difficile* infection and associated diarrhea**

- ✓ Most of studies describe increasing/decreasing numbers of commensal microorganisms (e.g., enterobacteria, clostridia, bacteroides) and these changes are related to “dysbiosis”, “imbalance of microbiota”, etc…

- ✓ **This cannot be the only evidence to claim a beneficial/detrimental physiological effect**.

MICROBIOTA CHANGES SHOULD BE LINKED TO PHYSIOLOGICAL AND/OR CLINICAL OUTCOMES AND SUPPORTED BY MOLECULAR MECHANISMS

Why is so difficult to establish causality for the gut microbiome?

INSTITUTO DE INVESTIGACIÓN EN CIENCIAS DE LA ALIMENTACIÓN

- Inherent limitations of the clinical studies and animal studies (extrapolation of data, observational studies, poor patient adherence to dietary regimes, etc.).
- Inter-individual variability.
- Many factors, apart from diet, such as age, sex, medications and ethnicity may also play a role in shaping the human gut microbiome.
- Multi-causal nature of many disorders.
- Incomplete biochemical profiling of food components which adds uncertainty to explore the health implications of our diet and its interaction with the gut microbiome.
- Difficulty of understanding the role of individual bacterial species in the complex gut ecosystem and its interaction with the host.

It might be most productive to put efforts on:

i) specific gut microbiome components, such as metabolic end-products, that could represent potential, steady and translatable markers of the gut microbiome function

ii) experimental tools with specific surrogate endpoints that could measure gut microbiome perturbations in a food safety context

Identification of potential key events and biomarkers following exposure to harmful diet-derived components

Gut epithelium inflammation

Disturbance of gut barrier

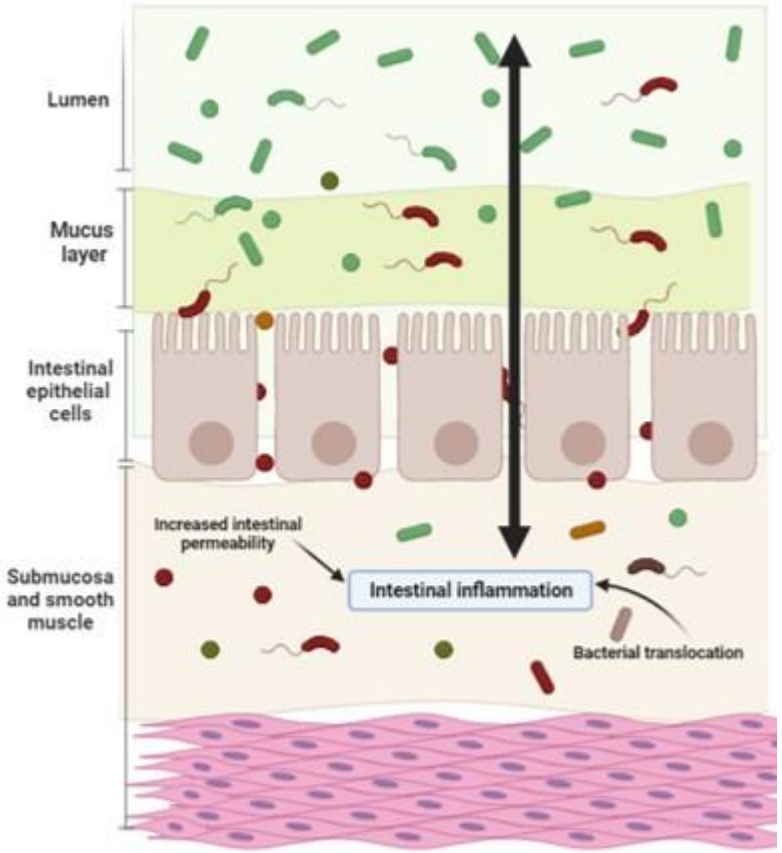
Dysregulation of immune responses

Mucus layer damage (↓ thickness)

↑ permeability

Bacterial translocation

Systemic effects (e.g., hepatic metabolism)



Pro/anti-inflammatory cytokines (IL-6, and IL-1b), lipocalins, flagellin, lipopolysaccharide, calprotectin, lipocalin 2

Tight junction proteins (claudin, occludin and ZO-1), *Akkermansia muciniphila*, mucins and mucin-like glycoproteins

Transepithelial electrical resistance, paracellular flux

Hazardous bacteria-dependant metabolites

Some conclusions – human part

- RIMICIA Project has compiled information on two main type of xenobiotics (i.e., food chemical contaminants and additives) requiring further attention based on the current evidence about their interaction with the gut microbiome. These identified compounds are clinically understudied since most studies were conducted in vitro, ex vivo or in rodents.

- Gut microbiota changes should be linked to physiological and/or clinical outcomes and supported by molecular mechanisms to provide helpful information in risk assessment.

- A shift from statistical associations to causal relationships with diseases is needed to reinforce the utility of the gut microbiome science for risk assessment purposes.

- Scientific evidence points out that human gut microbial enzymes can modify many classes of dietary compounds, including complex polysaccharides (dietary fiber), lipids, proteins, and phytochemicals (mainly polyphenols), exerting protective effects in some cases. However, gut microbiome can also play a significant role in certain xenobiotics-induced toxicity and proinflammatory states affecting gut mucosal integrity.

Why to apply for EFSA research grants?

Preparation of the proposal

- Clear instructions (administrative and scientific) on how to prepare the proposal.
- Efficient communication with EFSA to solve doubts/problems during the preparation stage.
- National EFSA Focal Points act as connecting hubs for information exchange, networking and engagement.

Development of the proposal

- Close collaboration with a multidisciplinary EFSA team with great knowledge in risk assessment.
- Facilitate a scientific and international cooperation framework.

Impact of the results

- EFSA is the keystone of EU risk assessment regarding food and feed safety and animal health.
- EFSA provides independent scientific advice and clear communication on existing and emerging risks.
- Results derived from EFSA grants may have a direct scientific and societal impact.

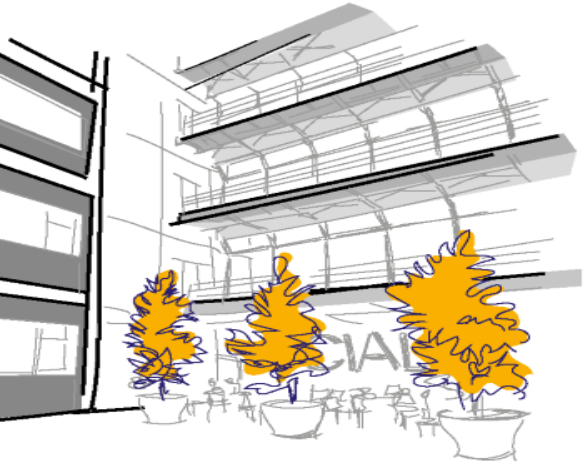
Acknowledgements

ANSES TEAM: Marianne Chemaly and her team (Cyrielle Payen…)

CSIC TEAM: Carlos Benito, Rodrigo Jiménez, Javier Martin, Florencio Pazos, Patricia Ruas and their respective teams

EFSA TEAM: Caroline Merten, Georgia Gkrintzali, Jaime Aguilera, Beatriz Guerra Roman, Irene Muñoz Guajardo, Elisa Pettenati, Nicola Smith, Melina Steinbach

External Advisory Board: Academic researchers and representatives from FDA, FSANZ and Health Canada



Thank you!

F. Javier Moreno
(javier.moreno@csic.es)

Administration luxembourgeoise vétérinaire et alimentaire (ALVA)
9th december 2022