

**5<sup>th</sup> INTERNATIONAL CONFERENCE  
ON FOOD CONTAMINANTS:  
CHALLENGES ON EXPOSURE ASSESSMENT,  
HEALTH IMPACT AND SUSTAINABILITY OF FOOD SYSTEMS**



# **BOOK OF ABSTRACTS**

**4-6 September, Campinas, Brazil**





4 – 6 SEPTEMBER 2023  
CAMPINAS, BRAZIL

**CONFERENCE THEME**  
CHALLENGES ON EXPOSURE ASSESSEMENT,  
HEALTH IMPACT AND SUSTAINABILITY  
OF FOOD SYSTEMS

**ICFC2023 BOOK OF ABSTRACTS**

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**OC7** – Exposure assessment of children to dietary mycotoxins: a pilot study conducted in Ribeirão Preto, Brazil

**OC8** – Exposure to the pyrethroid pesticide metabolite in pregnancy: association with maternal outcomes and newborn anthropometric measures

**OC9** – Infant’s exposure to ochratoxin A, zearalenone and deoxynivalenol from the consumption of milk formula and baby cereal in Chile

**OC10** – Bioaccessibility as a tool to assess the risk associated with trout consumption

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The Organizing Committee of this event expresses the profoundest gratitude to all those who contributed and supported this event. This event succeeds other events held in Lisbon (ICFCF2015), Braga (ICFC2017), Aveiro (ICFC2019) and Lisbon online (ICFC2021) under the seal of the National Institute of Health Doctor Ricardo Jorge (INSA).

## Institutional Supporters

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## Dear Participants,

On behalf of the National Institute of Health Doctor Ricardo Jorge (INSA), we are honored and delighted to welcome you to the 5<sup>th</sup> International Conference on Food Contaminants (ICFC2023) and to receive you in the beautiful city of Campinas/São Paulo (SP), Brazil.

The conference will be held, for the first time outside Portugal, at the Instituto Agronomico de Campinas (IAC) in Campinas/SP, Brazil, from 4 to 6 September, as a hybrid event (presential and online) to broaden the scope of this international conference and to contribute to a closer scientific cooperation between Portugal and Brazil. The ICFC2023 aims to promote contact with realities of different continents in the fields of exposure assessments of food contaminants, human health and sustainability of food systems.

Like many countries around the world, Brazil and surrounding neighbors from Latin America, reported relevant problems concerning food safety and security, difficulties related to the sustainability of food systems and their transformation and a scarcity of available data on human exposure to chemicals and associated health effects.

In addition, scientific knowledge on these topics needs to be shared between different regions and populations, and preferably, locally addressed, to provide effective support for policymaker's decisions that aim to protect human health and the environment. Therefore, ICFC2023 represents a unique opportunity to debate different ideas, methods, and approaches on food contaminants and human health, towards desirable and sustainable food futures.

We wish you an excellent Conference!

**Fernando de Almeida**

Chairman of the Executive Board of the  
National Institute of Health Doctor Ricardo Jorge, I.P.  
ICFC2023 Honorary President



## Conference Chairs

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**Carlos Oliveira**

(FZEA/USP, Brazil)

**Paula Alvito**

(INSA, Portugal)

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The Organizing Committee would like to welcome all of you and express sincere appreciation for choosing the city of Campinas, Brazil, as the venue for the 5<sup>th</sup> International Food Contaminants Conference (ICFC2023).

The ICFC2023 is organized by a joint effort of researchers from the National Institute of Health Doctor Ricardo Jorge (INSA), from Lisboa and the Centre for Environmental and Marine Studies (CESAM) of the University of Aveiro, both from Portugal and the Faculty of Animal Science and Food Engineering (FZEA/USP), from Brazil. The ICFC 2023 also has the support of the Brazilian Society of Science and Food Technology (SBCTA) and the São Paulo Research Foundation (FAPESP). This three days conference is an international forum to gather researchers from different continents, to share and discuss their findings regarding the broad and interdisciplinary field of food contaminants under the theme “Challenges in exposure assessment, health impact and sustainability of food systems”.

Following the rationality on the impact of food contaminants on human health of ICFC conferences (ICFC2015 <http://hdl.handle.net/10400.18/3214>, ICFC2017 <http://hdl.handle.net/10400.18/4882>, ICFC-2019 <http://hdl.handle.net/10400.18/7166> and ICFC2021 <http://hdl.handle.net/10400.18/7795>), ICFC2023 will address the challenges related to the i) occurrence of food contaminants worldwide; ii) exposure assessment and health impact; iii) health, environment and sustainability of food systems and iv) networking and societal concerns of food contaminants.

Participants at this conference are cordially invited to contribute with a full manuscript to the Special Issue on “Chemical Contaminants in Foods: Exposure Assessment, Health Impact & Sustainability of Food Systems” on Food Research International.

Throughout this conference, we hope to create an atmosphere where everyone, academia, professionals and students, can exchange ideas and establish collaborations.

We look forward to welcoming and hosting you in Campinas and hope this conference becomes an unforgettable moment.

**Carlos Oliveira and Paula Alvito**  
Chairs of ICFC2023

**4.09.2023 – Day 1**

08.00 - 10.30h	Registration
10.30 - 11.15h	Opening/welcome
11.15 - 12.00h	"ICFC2023: Lets Open the Horizons - Portugal meets Brazil" (Paula Alvito, INSA, Portugal   Carlos Oliveira, FZEA/USP, Brazil)

*Chair: Paula Alvito, INSA, Portugal*

12.00 - 12.30h	KL	"EFSA Strategy 2027 – Science, food and sustainability" (George Kass, EFSA, Italy)
12.30 - 14.30h		LUNCH & POSTER VIEWING AND NETWORKING

**14.30 - 17.00h    Session 1 - Occurrence of Food Contaminants Worldwide**

*Chair: Anderson Sant'Ana, UNICAMP, Brazil*

14.45 - 15.15h	L1	"Understanding fungi contamination across the food chain in Brazil: Challenges and opportunities" (Marta Taniwaki, ITAL, Brazil)
15.15 - 15.30h	OC1	"Assessment of emerging endocrine disruptor contaminants in seafood from Portuguese estuaries" (José Fernandes, REQUIMTE LAQV, Portugal)
15.30 - 15.45h	OC2	Investigation of toxic trace elements in spices commercialized in Morocco by inductively coupled plasma-mass spectrometry (Mourad El Youssfi, Mohammed V University in Rabat, Morocco)
15.45 - 16.15h		COFFEE BREAK & POSTER VIEWING AND NETWORKING
16.15 - 16.30h	OC3	"Halophytes in the diet: Health benefits and food safety issues" (Maria Lopes, Faculty of Pharmacy, University of Coimbra, Portugal)
16.30 - 16.45h	OC4	"Monitoring of mycotoxins in maize stored under simulated real conditions" (Bruna Carbas, CITAB-UTAD, Portugal)
16.45 - 17.00h	OC5	"Optimizing PAHs extraction for determination in seafood samples using new alternative solid phases" (Rodrigo Hoff, LFDA/RS, Brazil)

**5.09.2023 – Day 2****9.00 - 12.00h Session 2 - Exposure assessment and health impact***Chair: Carlos Oliveira, FZEA/USP, Brazil*

9.15 - 9.45h	L2	The dietary exposome: a challenge for toxicologist" (Isabelle Oswald, INRAE, France)
9.45 - 10.15h	L3	"European risk assessment of chemical mixtures" (Jacob van Klaveren, RIVM, The Netherlands)
10.15 - 10.30h	OC6	"Portuguese population's dietary exposure and intake to contaminants and nutrients: the first harmonised Total Diet Study" (Elsa Vasco, INSA, Portugal)
10.30 - 10.45h	OC7	"Exposure assessment of children to dietary mycotoxins: a pilot study conducted in Ribeirão Preto, Brazil" (Sher Ali, University of São Paulo, Brazil)
10.45 - 11.15		<b>COFFEE BREAK &amp; POSTER VIEWING AND NETWORKING</b>
11.15 - 11.45h	L4	"Current efforts on the characterization of Ciguatera Fish Poisoning as an emerging risk in several coastal areas of the European Atlantic Coast" (Ana Gago Martínez, University of Vigo, Spain)
11.45 - 12.00h	OC8	"Exposure to the pyrethroid pesticide metabolite in pregnancy: association with maternal outcomes and newborn anthropometric measures" (Juliana Guimaraes, University of Porto, Portugal)
12.00 - 12.15h	OC9	"Infant's exposure to ochratoxin A, zearalenone and deoxynivalenol from the consumption of milk formula and baby cereal in Chile" (Claudia Foerster, University of O'Higgins, Chile)
12.15 - 14.00h		<b>LUNCH &amp; POSTER VIEWING AND NETWORKING</b>

**14.00 - 17.00h Session 2 - Exposure assessment and health impact (cont.)***Chair: Isabelle Oswald, INRAE, France*

14.10 - 14.40h	L5	"Innovating in the exposure and risk assessment of chemical contaminants – exploring synergies with the European PARC Project" (Maria João Silva, INSA, Portugal)
14.40 - 15.10h	L6	"The Urban Water-Energy-Food Nexus: Social Practices and Adaptability in the Context of Contemporary Global Changes" (Leandro Giatti, Faculty of Public Health, University of São Paulo, Brazil)
15.10 - 15.25h	OC10	"Bioaccessibility as a tool to assess the risk associated with trout consumption" (Fabiola Santos, Embrapa Agroindústria de Alimentos, Brazil )
15.25 - 15.40h	OC11	"Tracking of dietary acrylamide exposure from childhood to adolescence" (Sofia Costa, University of Porto, Portugal)
15.40 - 16.10		<b>COFFEE BREAK &amp; POSTER VIEWING AND NETWORKING</b>
16.10 - 16.25h	OC12	"Evaluation of the nutritive profile and risk assessment of enteral and parenteral nutrition formulas administered to individuals in a critical state" (Iohanna Menezes, Federal University of Paraná, Brazil)
16.25 - 16.40h	OC13	"Toxicological interactions of regulated and emerging mycotoxins on human neuronal SH-SY5Y cells" (Sara Cunha, REQUIMTE LAQV, Portugal )
16.40 - 17.40h	RT1	"Brazil meets Europe – Exposure assessment and Health impact"

*Moderator: Adriana Pavesi, UNICAMP, Brazil*

RT1- OC1	"Risk management actions to reduce aflatoxin exposure" (Larissa Porto, ANVISA, Brasil )
RT1- OC2	"Occurrence, dietary exposure, and risk characterization of polycyclic aromatic hydrocarbons in grilled and fried meats in São Paulo, Brazil" (Alessandra Jager, University of São Paulo, Brazil)
RT1- OC3	"Control of prenatal exposure to mercury: Portuguese results from the HBM4EU-mom project" (Sónia Namorado, INSA, Portugal)

**6.09.2023 – Day 3****9.00 - 12.00h Session 3 - Health, environment and sustainability of food systems***Chair: Ana Gago Martinez, University of Vigo, Spain*

9.15 - 9.45h	L7	"Food processing, Public Health, Food and Sustainable Development" (Fernanda Martins, Unilever, Brazil)
9.45 - 10.15h	L8	"Environmental Circularity: Agriculture; Waste Management; Insects" (Diogo Cardoso, CESAM, University of Aveiro, Portugal)
10.15 - 10.30h	OC14	"Microbial quality and safety of ready-to-eat germinated pulses" (Carla Motta, INSA, Portugal)
10.30 - 10.45h	OC15	"Effect of bacteriophages against biofilms of Escherichia coli on food handling surfaces" (Marcia Braz, CESAM, University of Aveiro, Portugal)
10.45 - 11.15h		<b>COFFEE BREAK &amp; POSTER VIEWING AND NETWORKING</b>
11.15 - 11.30h	OC16	Investigating ingested nanomaterials' safety – the case of TiO <sub>2</sub> and innovative nanocelluloses. (Henriqueta Louro, INSA, Portugal)
11.30 - 11.45h	OC17	"Do mycotoxins in aquafeeds affect the nutritional quality of farmed fish?" (Flávia de Mello, FCIências.ID, Portugal)
11.45 - 13.45h		<b>LUNCH &amp; POSTER VIEWING AND NETWORKING</b>

**13.45 - 17.00h Session 4 - Networking and societal concern of food contaminants***Chair: Paula Alvito, INSA, Portugal*

14.00 - 14.30h	L9	"The Power of Global Networks for Capacity Building in Mycotoxin Research" (Sarah De Saeger, Ghent University, Belgium)
14.30 - 15.30h	RT2	"Networking and societal concern of food contaminants"

*Moderator: Paula Alvito, INSA, Portugal and Carlos Oliveira, FZEA/USP, Brazil*

	RT2- OC1	"Networking as a strategy to strengthen Public Policies on Food and National Security in Portuguese speaking African Countries" (Maria Rita Oliveira, São Paulo State University, MU-CONSAN-CPLP, Brasil)
	RT2- OC2	"Coffee Agroforestry and Sustainable Development: the case of Gorongosa, Mozambique" (Ana Ribeiro, ISA, Portugal)
	RT2- OC3	"Tackling societal concerns on food contaminants: a perspective of the European Partnership for the Assessment of Risks from Chemicals" (Maria João Silva, INSA, Portugal)
15.30 - 16.00h		<b>COFFEE BREAK &amp; POSTER VIEWING AND NETWORKING</b>
16.00 - 16.15h		Best Poster Award
16.15 - 16.45h		Portugal meets Brazil: Top Lessons Learned
16.45 - 17.00h		Closing Session

INSA – National Institute of Health Doctor Ricardo Jorge; FZEA/USP – Faculty of Animal Science and Food Engineering, University of São Paulo; EFSA – European Food Safety Authority; ITAL – Food Technology Institute; REQUIMTE LAQV – Associated Laboratory for Green Chemistry (LAQV) Network of Chemistry and Technology (REQUIMTE); CITAB-UTAD – Centre for the Research and Technology of Agro-Environmental – University of Trás-os-Montes and Alto Douro; LFDA/RS – Laboratórios Federais de Defesa Agropecuária; RIVM – National Institute for Public Health and the Environment; INRAE – Institut National de la Recherche Agronomique; ANVISA – Brazilian Health Regulatory Agency; CESAM – Centre for Environmental and Marine Studies; FCIências.ID – Associação para a Investigação e Desenvolvimento de Ciências; MU-CONSAN-CPLP – Mechanism for Facilitating University Participation in the Food and Nutritional Security Council of the Community of Portuguese Language Countries.



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# INVITED LECTURES

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## KL – EFSA Strategy 2027 – Science, food, and sustainability

**George E N Kass**

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The European Food Safety Authority (EFSA) carries out risk assessments in relation to food and feed safety. Following a recent in-depth scan and analysis of the internal and external environment in which it operates, EFSA published in 2021 its Strategy 2027. It sets out how EFSA intends to respond to a rapidly changing environment, particularly the newly amended General Food Law in the EU and to prepare for the challenges ahead, such as digitalisation, capacity to meet emerging needs, and coordinated support to the EC sustainability agenda. The Strategy centres around three Strategic Objectives, which are (i) to deliver trustworthy scientific advice and communication of risks from farm to fork, (ii) to ensure preparedness for future risk analysis needs and (iii) to empower people and ensure organisational agility. The presentation will provide an overview of the EFSA Strategy 2027 and will focus on Strategic Objective 2 and its activities in the development and regulatory application of new methodologies and tools, the so-called New Approach Methodologies or NAM and how EFSA will use them for regulatory assessment to minimise animal testing.



## L1 – Understanding mycotoxin contamination across the food chain in Brazil: challenges and opportunities

**Taniwaki, M.H.; Pitt, J.I.; Copetti, M.V.; Teixeira, A.A.; Iamanaka, B.T.**

Food Technology Institute – ITAL, Campinas, São Paulo, Brazil  
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Brazil is one of the largest food producers and exporters in the world. In the early 1990s, the European Union program for the harmonization of regulations for contaminants in food, including mycotoxins, brought about the necessity to examine mycotoxin contamination in foods at a global level. The rejection of food by the European Union and other countries became a Brazilian national priority because of economic and food safety aspects. Ochratoxin A in coffee and aflatoxins in Brazil nuts are examples of the impact of technical trade barriers on Brazilian foods. To overcome these threats, several strategies were undertaken by Brazilian and international organizations. In this context, the Codex Commission on Food Contaminants (CCCF) has emerged as a forum to discuss with more transparency issues related to mycotoxins, focusing on establishing maximum levels and codes of practices for some commodities and mycotoxins to ensure fair trade and food safety. Our experience in investigating and understanding mycotoxin contamination across the food chains in Brazil has contributed nationally and internationally to providing some answers to these issues.



## L2 – The dietary exposome: a challenge for toxicologist

Isabelle P. Oswald

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Food is contaminated by multiple contaminants, mycotoxins being the most frequently occurring natural ones. Mycotoxins are secondary fungal metabolites produced mainly by *Aspergillus*, *Penicillium* and *Fusarium*. Mycotoxins co-contamination is confirmed by the co-occurrence of these toxins in food and feed stuff and by co-exposure monitoring survey. The co-occurrence of mycotoxins in food is explained by three different reasons: (i) most fungi are able to simultaneously produce several mycotoxins, (ii) commodities can be contaminated by several fungi simultaneously or in quick succession, and (iii) the complete diet comprised different commodities. In practice, the co-occurrence of mycotoxins represents the rule and not the exception. Besides mycotoxins, food can be contaminated with other contaminant such as heavy metals.

Unfortunately, the toxicity of combinations of contaminant cannot always be predicted based upon their individual toxicities. The data on the combined toxic effects of mycotoxins are limited and therefore, the health risk from exposure to a combination of mycotoxins is incomplete. Most of the studies concerning the toxicological effect of contaminant have been carried out taking into account only one compound. A synergistic effect between mycotoxins of the trichothecene family mycotoxins was observed both for intestinal cytotoxicity and inflammatory response and the synergy was already seen at low doses. The combined exposure to the mycotoxin deoxynivalenol and Cadmium was also studied in several human cell lines and interactions were specific to the target organ. The interaction between deoxynivalenol and genotoxins, present either as food



contaminant or as toxins from our microbiota, was also investigated. We demonstrated that deoxynivalenol exacerbated the intestinal DNA damages induced these toxins, suggesting a role in colorectal cancer. Altogether, these data demonstrated that (i) mycotoxin cocktails can lead to synergistic interaction and that (ii) mycotoxins also interact with other food contaminants (heavy metal, genotoxins...) and with the intestinal microbiota. Thus, contaminations should be taken in the global context of the exposome.



## L3 – European risk assessment of chemical mixtures

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There is growing societal and political concern about the risk of combined exposure to multiple chemicals, also referred to as mixture risk assessment. The European Chemical Strategy for Sustainability has highlighted the importance of mixture risk assessment and the European Food Safety Authority (EFSA) is working on a Roadmap on the Assessment of the Risk of Combined Exposure to Multiple Chemicals (RACEMiC).

According to European Regulation 396/2005 and 1107/2009, EFSA is responsible for establishing methods for the risk assessment of combined exposure to multiple pesticides. The results of mixture risk assessment of the EFSA pilot studies are published. From 2022 onwards, EFSA, the European Commission and the Dutch Institute for Public Health and the Environment (RIVM) are progressing towards the implementation of these methods in the risk assessment and management of pesticides in Europe. The Monte Carlo Risk Assessment (MCRA) software and data will become accessible to stakeholders.

The European Commission and the 27 Member States are funding the Partnership for the Assessment of the Risk of Chemical (PARC) to address future regulatory needs as described in the Chemical Strategy for Sustainability such as the need to perform mixture risk assessment for all chemicals. PARC will collect new human biomonitoring data, will improve hazard data collection of untested chemicals and will deliver new methods for regulatory risk assessment.

The PARC project real-life mixture will study the exposure to mixtures of PFAS, mycotoxins, heavy metals and/or pesticides sharing the same adverse outcome. The Human Bio Monitoring for Europe (HBM4EU)



project, which data collection will be continued in PARC, collected HBM data all over Europe. This HBM data will be studied for mixture patterns in several countries. The combined effect of the chemicals to which an individual is exposed, needs to be summed according to scientific criteria set by EFSA. Furthermore, the kinetic conversion of chemicals within the human body needs to be understood. Chemicals might be metabolised in the human body before reaching a targeted organ, some chemicals be excreted within hours while other (persistent) chemicals might remain for longer time-intervals within the human body. Mixtures of chemicals to which individual are most frequently exposed will be further tested to understand the real-life mixture effect. PARC will also provide innovative methods and capacities to monitor chemicals in appropriate human.



## L4 – Current efforts on the characterization of ciguatera fish poisoning as an emerging risk in several coastal areas of the European Atlantic coast

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Ciguatera fish poisoning (CFP), is a foodborne illness caused by eating reef fish contaminated with certain toxins of lipophilic character known as ciguatoxins which might affect human health causing symptoms that may include diarrhea, vomiting, numbness, itchiness, sensitivity to hot and cold, dizziness, and weakness. These toxins have been found mostly in tropical and subtropical areas of Pacific and Caribbean and even Indian oceans. Recently these toxins have emerged to subtropical areas of the Atlantic Coasts such as Madeira (Portugal and Canary Islands (Spain) archipelagos. The characterization of this Emerging risk is being a priority for the Health authorities in the areas affected, and in particular for the European Food Safety Authority (EFSA). This presentation focus on describing the current efforts that are being carried out under a multidisciplinary point of view to evaluate and characterize the ciguatera risk in the areas above mentioned under EFSA support. The efforts carried out on the development of confirmatory analytical methods, as well as in the preparation of reference materials from naturally contaminated fish allowed the confirmation of Caribbean ciguatoxins as the main responsible for the CFP toxicity in the geographical areas mentioned above. The application of the methods developed also allowed to progress on the identification of the CTXs analogues involved in the CFP contamination. An update on the current situation of the risk characterization of these emerging toxins will be presented and discussed.



## L5 – Innovating in the exposure and risk assessment of chemical contaminants – exploring synergies with the European PARC Project

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The prevalence of many human diseases can be drastically reduced through the identification and prevention of environmental risk factors. This fact constitutes a strong incentive to innovate in the exposure and hazard assessment of chemicals, through all available knowledge and technologies, towards a next-generation risk assessment (NGRA).

The European Partnership for the Assessment of Risks from Chemicals (PARC, 2022 – 2029) coordinated by the French Agency for Food, Environmental and Occupational Health & Safety (ANSES), brings together around 200 partners from all Europe and three European Agencies, with the common goal of strengthening research and increasing the capacity for innovation in risk assessment, to protect human health and the environment. PARC is organized in three main pillars; i) establishment of a high level network at the interface of science and policy, setting the science-policy agenda and developing synergies collaboration and awareness; ii) implementation of Research & Innovation towards NGRA , comprising the fields of exposure and monitoring, hazard assessment, innovation in regulatory risk



assessment and concepts and toolboxes; iii) development of capacities and platforms, to ensure FAIR data production and to develop infrastructures and human capacities.

PARC was built on lessons learned and knowledge acquired in other initiatives, e.g., the Human Biomonitoring Initiative for Europe (HBM4EU) but intends to go further and use a more holistic approach through the development of tools and methods that will enable the integration of all sources and routes of exposure to chemical contaminants and by targeting human and environmental health. Several substances groups have been prioritised for study, including heavy metals, bisphenol A analogues, pesticides, and mycotoxins. Taking advantage of established and innovative tools and technologies, PARC also expects to increase the efficiency of in vitro toxicity testing while reducing the use of animals. The development of Adverse Outcome Pathways will set the biological framework to enable the development of integrated approaches to testing and assessment for regulatory decision-making. This will support the implementation of preventive measures, interventions, or new policies, to better protect public health.

To boost its impact, a strategy for the communication and dissemination of PARC outcomes, targeted to different end-users, has been established. Furthermore, PARC offers a unique opportunity to foster synergies and establish effective collaborations with other relevant scientific and/or regulatory initiatives at a global level, which will be crucial to maximize the global impact and regulatory acceptance of NGRA.

**Acknowledgment:** PARC has received funding from the European Union's Horizon Europe research and innovation programme under Grant Agreement No 101057014.



## L6 – The urban water-energy-food nexus: social practices and adaptability in the context of contemporary global changes

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The water-energy-food nexus focuses on interdependent forms of scarcity among production chains that are essential for social inclusion, vulnerability reduction and food security. Despite the broad territorial perspective for the analysis of its components, the so-called nexus thinking has also contributed to the pursuit of sustainable development goals from urban vulnerable contexts. In this sense, I will present research on social practices that contemplate synergies regarding the urban nexus from vulnerable settlements in studies carried out in Brazil, Bulgaria, Uganda, United Kingdom and South Africa. Exploring plural urban contexts and the daily search for quality of life using nexus thinking allows us to reflect and learn about unimagined alternatives, allowing innovations and hybridizations on human adaptability to cope with the contemporary global changes.



## L7 – “Food processing, public health and sustainable food system”

### Fernanda Martins

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The current global food system is unbalanced and is inefficient. One billion people around the world are hungry while two billion are obese or overweight. On top of that, one-third of the total food produced globally is wasted. Urgent changes are needed to ensure we can feed the world in a more sustainable and healthy way.

Since 1980 dietary guidelines worldwide have included the recommendation to limit the consumption of sodium, sugars and saturated fat to maintain a healthy diet. More recently some guidelines indicate that dietary shifts towards more plant-based foods with fewer animal source foods confers improved health, as well as environmental benefits.

For decades, food processing has been used to improve the nutritional quality of food products by reducing sugars, sodium and (saturated) fats and increasing fiber and micronutrients levels; to improve food safety and reduce food waste. Processing can change the food matrix and impact how the food is metabolized. These changes can be positive (processing tomatoes make its lycopene more bioavailable) or negative (processing fruit into fruit juice releases more its sugars and removes its fibers). Emerging food processing technologies are allowing the development of plant-based alternatives to animal origin foods, with the potential to have an even bigger impact on sustainability. These food technologies include ingredient, packaging and process solutions. Despite all the benefits, food processing has received criticism over the last decade due to the lack of knowledge of the ingredients and techniques applied in food processing. Terminologies such as ‘ultra-processed foods’ have been used to pejoratively classify industrialized



foods and has generated confusion among health professionals and the general population. Although many junk foods would be classified as ultra-processed foods, not all ultra-processed foods are junk food. A non-negligible proportion of foods classified as UPF is considered as being of balanced nutritional composition and recommended for a healthy diet. Current excessive attention to the level of processing is shifting the attention from important elements (e.g. portion size) to improve population's health and the food system.

Food processing is a tool that can help in the transition to a more sustainable food system, with less loss and waste, more diverse and nutritious foods that last longer, are safe, affordable and accessible; being a significant contributor to food and nutrition security.

The solution to progress towards ending hunger, achieving food security and improving nutrition in a more sustainable way is through food processing.



## L8 – Environmental circularity: agriculture; waste management; insects

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Circular solutions with the reuse and valorisation of waste, contribute to the mitigating of climate change scenarios and reductions of greenhouse gases emission, contribute to a sustainable future. Based on circularity solutions, the valorization of wastewaters, from waste to valuable resources, and the promotion of sustainability in wastewater treatment are crucial for the future.

This presentation will address the role of insects in this circularity process, from their use as bioreactors, in bioconversion, as producers of fertilizers or as food and feed source. Ecotoxicology plays a crucial part as foundational science to tackle gaps in knowledge by evaluating the feasibility of insects' usage in circularity and as a sustainable solution.

Insects can digest wastes and transform them into fertilizers. During this digestion process, insects can ingest or absorb several contaminants, resulting in potential bioaccumulation and biomagnification within food webs. In this case, toxicokinetic and toxicodynamic studies are crucial to obtain data on uptake and elimination rate constants, which depend on time, the physiology of the organisms and the exposure routes. The uptake rate depends on exposure concentrations, while elimination ability depends on the concentration in the organisms, along with the internalization of the contaminant.

Mercury, PAHs and mycotoxins are some of the priority and emergent contaminants in food, feed and wastes that insects can ingest and accumulate. Toxicokinetic and toxicodynamic studies provide data on



the residence time of contaminants in insects or their ability to excrete/eliminate contaminants, which is crucial for their after-life use as food and feed. In addition, their excretion products include their feces and moulting products. This product is called frass and is rich in several nutrients essential for plants. At an industrial scale, it can be converted into an agriculture fertilizer, and replace other organic fertilizers usually associated with contaminants released into agriculture fields (e.g. pharmaceuticals from manure), or mineral fertilizers, related to nutrient leaching into ground waters.

Considering this, insects like the yellow mealworm or the larvae of the black soldier fly can solve a significant and actual problem by creating value, reducing gas emissions, or replacing food sources with high water demands, waste production, and gas emissions.

**Funding:** This study was supported by the project ENTOSAFE (PTDC/CTA-AMB/0730/2021), financially supported by national funds (OE), through FCT/MCTES, as well as for financial support to CESAM by FCT/MCTES (UIDP/50017/2020 + UIDB/50017/2020 + LA/P/0094/2020), through national funds. D. Cardoso was hired under the Scientific Employment Stimulus – Individual Call (CEECIND/01190/2018).



## L9 – The power of global networks for capacity building in mycotoxin research

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One of the most persistent food safety challenges worldwide are caused by mycotoxin producing fungi. Many factors including climate change, globalization, poverty, and conflicts worsen the impact of unsafe food on human and animal health and are an obstacle in reaching the sustainable development goal No 2. Capacity building is defined as the process of developing and strengthening the skills, instincts, abilities, processes and resources that organizations and communities need to survive, adapt, and thrive in a fast-changing world.

As scientists, it is very important to join global efforts and networks to work in a most efficient way with the limited available financial resources. Global networks such as the International Society for Mycotoxicology (ISM), MYTOX-SOUTH®, and other international societies play a key role in our mission towards a mycotoxin-safe world.

In the presentation key challenges for sustainable mycotoxin research will be proposed that can only be successful through concerted actions:

1. building of equitable partnerships with focus on sustainable bottom-up human capacity building through training and education of young & gender-inclusive innovators in the food safety area, including the low- and middle-income countries (LMICs).
2. investing in an harmonized global collection of accessible mycotoxin contamination data according to the FAIR principle, which means that data should be findable, accessible, interoperable and reusable. Open-access data for all stakeholders, from farmers to policy-makers to consumers is necessary.
3. prioritization of high-quality food safety research, linking food



safety-related exposure to disease outcomes, more specifically through combining large-scale epidemiological designs along with mechanistic insights. Breakthrough innovation is expected from the exposome concept in which we measure all environmental influences and associated biological responses throughout the entire lifespan of humans, which consists of the analysis and integration of big data sets.

4. linking natural sciences with social sciences. Despite our efforts for technological innovations, implementation of mycotoxin mitigation measures and legislation is still running behind in LMICs. It is important to map the science-policy-society system to better understand how different actors influence the food safety systems in LMICs. Examples on how global networks such as ISM and MYTOX-SOUTH® are contributing to these key challenges will be presented.



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# ORAL COMMUNICATIONS

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## OC1 – Assessment of emerging endocrine disruptor contaminants in seafood from Portuguese estuaries

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**Keywords:** Seafood, Endocrine disruptor Contaminants, Food Safety.

**Introduction:** Emerging Endocrine Disruptors Contaminants (eEDCs) comprise a large and diverse group of compounds that includes pharmaceuticals, personal care products, bisphenols and several other substances, well known by the adverse impacts to human and animal health. Nonetheless, there is a limited knowledge about the presence of eEDCs in seafood from estuarine food webs and consequently their potential risk for public health.

**Methodology:** This study deals with the determination of 21 pesticides, 4 polycyclic musk fragrances, 4 UV-filters, 7 bisphenols, 6 polybrominated diphenyl ethers (PBDEs) and 8 of their methoxylate metabolites (MeO-BDEs) in several estuarine species fish collected seasonally along one year in distinct areas of Douro River and Tagus River estuaries. A sample preparation approach combining QuEChERS and dispersive liquid-liquid microextraction (DLLME) techniques was employed for extracting the target compounds [1,2]. Gas chromatography-mass spectrometry (GC-MS) was used to determine the content of all contaminants [1], except for PBDEs and MeO-BDEs, which were analyzed using gas chromatography tandem mass spectrometry (GC-MS/MS) [2].



**Results:** Out of the 409 muscle samples analyzed from grey mullet (*Mugil cephalus*, n = 137), sea bass (*Dicentrarchus labrax*, n = 126), flounder (*Platichthys flesus*, n = 119), and barbus (*Barbus barbus*, n = 27), grey mullet exhibited the highest incidence of contaminants, particularly in spring. Bisphenol A and bisphenol F were the primary EDCs detected in all fish species analyzed. Galaxolide, tonalide, isoamyl 4-methoxycinnamate, and 2-ethylhexyl salicylate were also frequently found. Among the pesticides analyzed, alachlor, bifenthrin, p,p'-DDT, ethion, and prochloraz were the main compounds observed, with approximately 60% of the samples containing residues of at least one pesticide. Generally, lower-brominated PBDE congeners were more abundant than higher-brominated ones across all seasons, with BDE-47 being the predominant congener in all species. MeO-BDEs were present in lower levels compared to PBDEs.

**Conclusion:** Our findings confirm the necessity of continuous monitoring of EDCs in fish, considering the significant adverse effects they can have on the health of consumers.

**Topic relevance:** Evaluate the presence of EDCs in seafood is vital for safeguarding human health, ensuring food safety, protecting the environment, and promoting sustainable practices in the fishing industry.

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## OC2 – Investigation of toxic trace elements in spices commercialized in Morocco by inductively coupled plasma-mass spectrometry

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**Keywords:** Trace elements, Spices, Risk assessment, Regulations, Morocco.

**Introduction:** Trace elements (TEs) are common environmental contaminants, and their presence in food can cause harmful effects to human health. These elements are different from other pollutants because they cannot be chemically or biologically degraded like organic pollutants. In recent years, there has been a growing emphasis on monitoring the levels of trace elements TEs in spices. The aim of this study was to assess the levels of toxic TEs in commonly consumed spices in Morocco.

**Methodology:** A total of one hundred twenty-three (n=123) samples of spices commercialized in different markets in Rabat-Salé-Témara region in Morocco were investigated for their toxic metals (Pb, Cd, and As) levels using inductively coupled plasma-mass spectrometry (ICP-MS).

**Results:** The concentrations of toxic metals in spices were in the range of 0.017-4.330 mg/kg for Pb, 0.003-0.773 mg/kg for Cd, and 0.003-1.510 mg/kg for As. The obtained results were compared with the maximum limits (MLs) established by Moroccan legislative decree. The risk associated with TEs was evaluated based on health-based guide



values, such as the provisional tolerable weekly intake (PTWI) and the tolerable weekly intake (TWI). Most of the analyzed TEs in the samples were found to be within the MLs set by national regulatory agency. However, some samples exceeded the MLs set for Pb.

**Conclusions:** There is no health risk for consumers based on the available data. However, it should be noted that minimizing long-term exposure to these elements is vital to ensure food safety and alleviate potential risks.

**Relevance:** This study is of great interest to public health, as it would reveal the levels of these elements in the Moroccan diet and their potential role in consumer health through spices consumption.



## OC3 – Halophytes in the diet: Health benefits and food safety issues

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**Keywords:** Halophytes; food security; food safety; human health.

**Introduction:** Nearly 3 billion people around the world are currently food insecure. Indeed, availability, accessibility, utilisation and stability – the four main pillars of food security – are increasingly threatened by both anthropogenic (e.g. population growth) and climatic (e.g. rising salinisation of the planet) factors. Novel foods, such as halophytes, have the potential to significantly increase food yields, thereby contributing to the mitigation of the food crises. These extremophilic, salt-tolerant plant species not only exhibit remarkable nutritional value, but also high levels of bioactive compounds with health-promoting properties, which makes them ingredients of choice for our diet. Yet, as their full potential to sustainably feed nations is recognised and effectively exploited in a growing number of countries, issues related to the safety of their consumption, thus far little discussed, are becoming more pressing.

**Methodology:** Hence the present study, which aimed to investigate two halophytes, *Salicornia ramosissima* and *Sarcocornia perennis alpini*, both from the Mondego estuary, in Portugal, namely 1) the nutritional characterisation analysis, performed in accordance with the methodology of the Association of Official Analytical Chemists (AOAC); 2) the study of the phenolic profile, conducted by an ultra-high performance liquid chromatography (UHPLC) methodology; and 3) the assessment of the presence of mycotoxins and heavy metals,



performed resorting to a UHPLC methodology for the former, and to flame atomic absorption spectrometry or graphite furnace atomic absorption spectrometry for the latter.

**Results:** Overall, both species proved to be important sources of nutrients, particularly minerals and fibre, and of bioactive compounds, mainly phenolic acids and flavonoids, which renders them highly suitable for use in the agro-food and pharmaceutical sectors. Concerning the risk of contamination, aflatoxin B1 was found in *S. ramosissima* at 5.21 µg/kg dry weight, which may represent a health risk. Cd and Pb levels were low in both species, but the 0.01 mg/Kg Hg in *S. perennis* reached the legal limit for marine species.

**Conclusion:** In short, the implementation of specific regulations and well-devised cultivation strategies, ideally in controlled environments, are crucial to minimise the risk of contamination and to provide consumers with the maximum benefits from these species.



## OC4 – Monitoring of mycotoxins in maize stored under simulated real conditions

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**Keywords:** *Zea mays* L.; good practices; harvest; storage; fumonisins.

**Introduction:** Maize (*Zea mays* L.) is one of the most important cereals in food and feed of world, highly susceptible to fungal contamination and when mycotoxigenic fungi are present, maize also shows high susceptibility to mycotoxin contamination. Mycotoxins are secondary metabolites of fungus produced by the genera *Aspergillus*, *Penicillium*, *Claviceps*, *Alternaria*, and *Fusarium*, which appear as the fungal adaptation to stressful environmental conditions. The adoption of good pest management practices is a current challenge for grain quality assurance.

The objective of this work is to quantify the occurrence of mycotoxins in maize during ten months of storage.

**Methodology:** Six maize samples (M1, M2, M3, M4, M5, M6) collected in Tagus Valley Region, Portugal in 2019 were dried and 10 Kg were stored in barrels simulating the real 'in silo' conditions and tested at harvest (T0), after 3 months (T1), 6 months (T2) and 10 months (T3) of storage.

**Results:** Among all mycotoxins analyzed on the maize grains collected at harvested time and from the stored in barrels, fumonisins were only detected, although toxin T2, zearalenone, deoxynivalenol, ochratoxin



A and aflatoxins were screened and not found in any sample collected during the ten months of storage. At harvest, the levels of Fum B1 and Fum B2 were 330 µg/Kg and 156 µg/Kg for M1, and 256 µg/Kg and 125 µg/Kg for M2. However, for the stored moments T1, T2 and T3 no fumonisins were detected. The occurrence of fumonisins in M3 and M4 had fluctuations over storage time, but with a tendency to decrease after 10 months of storage in barrel. This tendency may occur due to the fungus inactivity and degradation of mycotoxins over the storage. Opposite tendency was verified in barrels with maize M5 and M6, the levels of fumonisins increased from T0 to T3. In M6, at harvest (T0) no levels of fumonisins, in T1 Fum B1 was 207 µg/Kg, T2 was 221 µg/Kg and in T3 was 1541 µg/Kg, while Fum B2 was not detected during the storage period.

**Conclusion:** Our research revealed the importance to apply the good pest management practices in pre- and post-harvest for occurrence of mycotoxins.



## OC5 – Optimizing PAHs extraction for determination in seafood samples using new alternative solid phases

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**Keywords:** PAHs, matrix solid phase extraction, pressurized liquid extraction, green analytical chemistry.

**Introduction:** Polycyclic aromatic hydrocarbons (PAHs) are a group of chemical compounds that can be found in various environmental sources, including seafood. Seafood, particularly fish and shellfish, can accumulate PAHs from contaminated water and sediments, making them a potential source of human exposure to these compounds. Some PAHs have been classified as carcinogens, which makes their monitoring a public health concern. Determining PAHs in seafood is often complex and time-consuming due to extensive extraction and purification protocols, as well as the trace levels of substances. Analytical methods typically involve liquid or gas chromatography coupled with mass spectrometry. In this study, we evaluated alternative solid-phase materials for sample preparation using matrix solid phase dispersion (MSPD) technique followed by pressurized liquid extraction (PLE).

**Methodology:** The use of alternative phases was compared with a high-cost imported reference material (Q-Matrix). The tested alternative materials included microcrystalline cellulose, vermiculite, sweet manioc starch, sour manioc starch, shell powder, carbonized rice husk, diatomaceous earth, perlite, barley malt hulls peat mosses and rice bran. After the MSPD-PLE procedure, samples were analyzed using LC-MS/MS and GC-MS/MS. Performance of each solid phase was assessed and compared in terms of analyte signal, matrix effects, signal-to-noise



ratio, and interference signals intensity. The three solid phases with better performance were selected for further validation.

**Results:** The evaluation criteria were organized in terms of numeric score for each parameter. Alternative solid phases perlite, sweet manioc starch and barley showed the higher scores. Thus, these three solid phases will be used for further full validation. The findings are promising and demonstrate a fast, inexpensive sample preparation method capable of detecting PAH levels in fish at  $0.75 \mu\text{g kg}^{-1}$ . This ensures monitoring of these carcinogenic substances in fish-derived food products at regulatory concentrations.

**Conclusion:** The developed extraction method proves highly useful for PAHs determination in seafood samples, achieving quantification limits of  $0.75 \mu\text{g kg}^{-1}$ .

**Topic relevance:** Seafood security.



## OC6 – Portuguese population's dietary exposure and intake to contaminants and nutrients: the first harmonised Total Diet Study

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**Keywords:** MCRA software, semi-probabilistic approach, FoodEx2 food classification system, risk assessment, nitrate, arsenic, cadmium, lead, mercury, copper, manganese, selenium, zinc.

**Introduction:** The first Total Diet Study (TDS) was performed in Portugal using harmonised procedures developed under the TDS-Exposure project to assess the population's baseline dietary exposure and intake of contaminants and nutrients.

**Methodology:** Occurrence of nitrate, arsenic, cadmium, lead, mercury, copper, manganese, selenium and zinc was determined by different analytical methodologies in 164 TDS samples pooled based on the 20 food groups of FoodEx2 classification system and prepared as consumed. The TDS samples were representative of 18 to 74 years old population's diet. The exposure/intake was estimated using a semi-probabilistic approach with the MCRA software by combining food occurrence and individual consumption data. Risk characterization was assessed by comparing estimated exposures to contaminants with health-based guidance values (HBGV) or lower benchmark dose limits (BMDL). The nutrient estimated intakes were compared with dietary reference values (DRVs). The percentage of individuals exceeding the HBGV or BMDL was evaluated.

**Results:** The estimated mean exposure was 31.5 % of the acceptable daily intake for nitrate, 96% of the tolerable weekly intake (TWI) for methylmercury, 9.2% of TWI for inorganic mercury and 54.3% of the TWI for cadmium. Exposure to methylmercury and cadmium exceeded the TWI at the 95<sup>th</sup> percentile (P95). Inorganic arsenic and lead were



below the BMDL at mean exposure and exceeded it at P95. The percentage of the population exceeding the HBGV or the BMDL was 3.2% for nitrate, 28% for methylmercury, 3.5% for inorganic mercury, 5.4% for cadmium, 28.4% for inorganic arsenic and 0.47% for lead. Copper, manganese, selenium and zinc, mean and P95 exposures did not exceed the tolerable upper intake level. DRVs achievement by the population were as follows: zinc, 33% of population reference intake for males and 43% for females; manganese, 28% of adequate intake (AI); copper, 12.8% of AI for males and 10.8% for females; and selenium, 73% of AI.

**Conclusion:** The baseline exposure/intake estimated for the different elements highlights the need for further attention regarding the contaminants, cadmium, inorganic arsenic, lead and methyl mercury, as well as the nutrients, copper, manganese, selenium and zinc.

**Topic relevance:** The use of harmonised procedures allows comparison of exposure/intake assessment with other countries and evaluation of exposure trends.

**Acknowledgements:** This research was partially funded by the European Commission's Seventh Framework Programme [FP7/2012–2016] as a part of TDSExposure project [grant agreement number 289108]. The authors thank the Sociedade Portuguesa de Ciências da Nutrição (SPCNA) for providing the raw food consumption dataset used in this work from the study Portuguese Population's Food Habits and Lifestyles (Alimentação e Estilos de Vida da População Portuguesa) conducted by SPCNA with the support of Nestlé, within a protocol of scientific sponsorship between the two institutions, and the ACPP – Portuguese Association of Professional Cooks for providing the kitchen facilities.



## OC7 – Exposure assessment of children to dietary mycotoxins: a pilot study conducted in Ribeirão Preto, Brazil

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**Keywords:** Children, foods, fungi, multi-mycotoxins, contamination, LC-MS/MS.

**Introduction:** Exposure to mycotoxins, fungal toxic secondary metabolites, through food is a major health concern, especially for youngsters.

**Methodology:** This study aimed to conduct a preliminary investigation on the exposure of children to dietary mycotoxins in Ribeirão Preto, Brazil. Sampling procedures were conducted in August 2022 (1<sup>st</sup> sampling–SP1) and December 2022 (SP2), to collect foods ( $N = 243$ ) available for consumption in the households of children ( $N = 67$ ) cared in the Vila Lobato Community Social Medical Center of Ribeirão Preto, including preschoolers (aged  $\geq 2$  to  $< 6$ ,  $n = 13$ ), schoolchildren (aged  $\geq 6$  to  $< 10$ ,  $n = 18$ ), and adolescents (aged  $\geq 10$  to  $< 19$ ,  $n = 36$ ). Liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) was utilized to determine the concentrations of mycotoxins in food samples.

**Results:** The median levels of total aflatoxins ( $B_1 + B_2 + G_1 + G_2$ ), fumonisins ( $FB_1 + FB_2$ ), zearalenone (ZEN), T-2 toxin, deoxynivalenol (DON) and ochratoxin A (OTA) in all food samples were 1.45, 13.37, 2.74, 7.20, 240.88 and 0.43  $\mu\text{g}/\text{kg}$ , respectively. The mean probable



daily intake (PDI) for total aflatoxins (AFs) in the corn products, bean, and rice ranged from  $0.002 \pm 0.002$ ,  $0.004 \pm 0.001$  to  $0.005 \pm 0.000$   $\mu\text{g}/\text{kg}$  body weight (bw)/day, respectively. The mean PDI for fumonisins among food products varied from  $0.001 \pm 0.000$  to  $0.086 \pm 0.237$   $\mu\text{g}/\text{kg}$  bw/day. Bean, corn and wheat products, cascava, chocolate, and rice contributed to the PDI for ZEN at mean levels ranging from  $0.002 \pm 0.002$  to  $0.071 \pm 0.243$   $\mu\text{g}/\text{kg}$  bw/day. Rice and chocolate provided lower PDI levels for OTA, between  $0.001 \pm 0.000$  and  $0.001 \pm 0.001$   $\mu\text{g}/\text{kg}$  bw/day.

**Conclusion:** The mean PDI for DON ranged from  $0.004 \pm 0.000$  to  $2.189 \pm 5.341$   $\mu\text{g}/\text{kg}$  bw/day. However, wheat-based products had higher levels of DON, hence contributing for a mean PDI of  $2.189 \pm 5.341$   $\mu\text{g}/\text{kg}$  bw/day that exceeded the tolerable daily intake (TDI) of  $1.0$   $\mu\text{g}/\text{kg}$  bw/day recommended by the World Health Organization. These findings indicate that wheat-based foods may pose a potential health risk, particularly to the young children, thus emphasizing the need for further preventive measures to avoid DON contamination in these products.

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## OC8 – Exposure to the pyrethroid pesticide metabolite in pregnancy: association with maternal outcomes and newborn anthropometric measures

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**Keywords:** 3-PBA; pyrethroid pesticides; foods; pregnancy; newborn; anthropometry.

**Introduction:** The widespread detection of pyrethroid pesticides in agricultural soils stresses the relevance of extending monitoring programs to assess their health impacts. Exposure to 3-Phenoxybenzoic acid (3-PBA), a metabolite that results from environmental or endogenous enzymatic degradation of many pyrethroid pesticides, has been associated with poor pregnancy outcomes. To characterize pregnant women exposure to 3-PBA and to investigate whether this exposure is associated with maternal and neonatal outcomes.

**Methodology:** This was a prospective observational study based on



the IoMum pregnancy cohort. Women attending their first trimester ultrasound scan from April 2018 to April 2019 at Centro Hospitalar Universitário S. João, Porto, Portugal, were invited to participate. Women with gestational age between 10 and 13 weeks, confirmed fetal vitality, and who signed an informed consent were included. 3-PBA was measured in spot urine samples by gas chromatography with mass spectrometry (GC-MS).

**Results:** Median urinary 3-PBA concentration was 0.263 (0.167; 0.458)  $\mu\text{g/g}$  creatinine ( $n = 145$ ). 3-PBA excretion is associated negatively with maternal pre-pregnancy body mass index (BMI) ( $p = 0.049$ ). 3-PBA excretion was increased during the summer when compared to winter ( $p < 0.001$ ) and it was higher for higher frequencies of fish or yogurt consumption, particularly during the winter ( $p = 0.002$  and  $p = 0.015$ , respectively), when a lower environmental exposure is expected. No association was found between frequency of cow's milk, or cheese consumption. Moreover, 3-PBA excretion was increased in women reporting levothyroxine use, a proxy for hypothyroidism, when compared to women free of thyroid disease ( $p = 0.01$ ), which could be due to a putative thyroid hormone antagonistic effect of 3-PBA. 3-PBA levels were not associated with the anthropometric measures of the newborn.

**Conclusion:** Pregnant women living in Portugal are exposed to 3-PBA, particularly during the summer and food such as fish and yogurt may be a source of 3-PBA dietary exposure. 3-PBA exposure during pregnancy may be associated with maternal clinical characteristics, an issue which deserves future research.



## OC9 - Infant's exposure to ochratoxin A, zearalenone and deoxynivalenol from the consumption of milk formula and baby cereal in Chile.

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**Keywords:** Exposure assessment, mycotoxins, infants, baby food, Chile.

**Introduction:** Mycotoxins are metabolites of filamentous fungi that contaminate food, and their exposure is associated with various adverse health effects, including cancer and estrogenic, immunosuppressive, gastrointestinal, and renal disorders. Infants (< 2 years), are the most vulnerable group, representing a unique combination of restricted food consumption types, low body weight, lower ability to eliminate toxins, and more future years to accumulate toxins.

**Methodology:** We aimed to estimate the infants' exposure to ochratoxin A (OTA), zearalenone (ZEN), and deoxynivalenol (DON) because of milk formula and baby cereal consumption.

We sampled n=41 batches of 5 brands of milk formula and 4 types of the most consumed brand of baby cereal (n=30). We analyzed the samples with a commercial ELISA. Exposure was assessed by the Estimated Daily Intake (EDI) approach (mean and worst case, WCS) with levels in a Medium Bound (MB) and Upper Bound (UB); consumption (< 6m, 7-12m, and 13-24m); adjusted by the weight of each group. The risk was estimated by the comparison of the EDI with a reference tolerable daily intake or by the margin of exposure (MOE) in the case of OTA.

**Results:** DON and OTA in infant's milk formula were 34% and 41%, respectively. The co-occurrence between these mycotoxins was 22%.



No samples were over the limit of quantification (LOQ) of the method, and no milk formulas were contaminated with ZEN. In the case of baby cereals, the occurrences were 17% for OTA, 30% for DON, and 7% for ZEN, all of them <LOQ. Co-occurrence was seen in two samples between ZEN and OTA. According to exposure calculations, the MOE for OTA was less than 10,000 in all models for milk formula and in WCS for cereal consumption. Risk was observed for DON in the mean levels of both models in < 6 months and in the WCS for all ages for milk consumption.

**Conclusion:** Ochratoxin A and DON must be considered a priority for public health. Co-occurrence assessments are needed to evaluate the additive effects of these mycotoxins. Special regulation for infants is highly recommended in Chile, based on local risk assessments.

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## OC10 – Bioaccessibility as a tool to assess the risk associated with trout consumption

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**Keywords:** fatty acids, in vitro digestion, rainbow trout.

**Introduction:** In nutritional terms, seafood is rich in polyunsaturated fatty acids, important in fetal development, in the prevention of cardiovascular and cancer diseases. However, seafood can be a source of environmental contaminants such as mercury, considered an endocrine disruptor because, in its organic form, it can cause male infertility and change in the sexual behavior of animals. In recent years, researchers have used the bioaccessibility tool to assess, through in vitro assays, the amount of food or contaminant released into the gastrointestinal fluid after digestion, which becomes available for absorption by the intestinal mucosa, potentially causing risks to human health.

**Methodology:** Our research used the in vitro digestion model to determine the bioaccessibility of methylmercury in samples of rainbow trout (*Onchorhynchus mykiss*) produced and marketed in Lisbon, Portugal. The simulated gastrointestinal digestion was performed in three phases: mouth, stomach and small intestine. Saliva (pH 7.0), gastric juice (pH 2.0), duodenal juice and bile (pH 7.0) were used. The digestion efficiency was measured by the N content before and after the simulation. Fatty acid methyl esters of total lipids were determined by acid-catalyzed transesterification. The lipid profile was determined



by gas chromatography. Methyl-Hg was determined by atomic absorption spectrometry using an automated Hg analyzer. Risk-benefit assessment was performed by determining intake of methyl-Hg and PUFA by the associated probability of exceeding tolerable weekly intakes (TWI, per methyl-Hg) or dietary reference intakes.

**Results:** Trout showed concentrations of 0.105 mg Hg/kg sample and 0.081 mg Methyl-Hg/kg sample. Methyl-Hg represented 76.98% of the total Hg. The bioaccessibility of Hg was 46.68%. EPA was 0.15 mg/kg of sample and DHA was 1.03 mg/kg. All samples showed values lower than the legal limit of 0.5 mg/kg of Hg in fish muscle. No risk was found for Methyl-Hg related to the consumption of trout for an adult weighing 60 kg with a weekly intake of 300 g of this fish.

**Conclusion:** The results showed that trout remains an excellent source of PUFA.

**Funding:** Embrapa



## OC11 – Tracking of dietary acrylamide exposure from childhood to adolescence

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**Keywords:** food contaminants, longitudinal analysis, risk assessment.

**Introduction:** Acrylamide is a compound formed during the Maillard reaction and has been associated with a higher risk of health outcomes due to its probable genotoxic effects. This study aims to estimate the acrylamide dietary exposure from childhood to adolescence.

**Methodology:** Data from the 4 waves of Portuguese population-based birth cohort Generation XXI (G21) were used ( $n_{4\text{years}} = 2488$ ;  $n_{7\text{years}} = 3569$ ;  $n_{10\text{years}} = 2853$ ;  $n_{13\text{years}} = 2829$ ). Dietary data were collected by 3-day food diaries (2 weekdays and 1 weekend day). Occurrence data of acrylamide in food were extracted from European Food Safety Authorization. The margins of exposure (MOE) were analyzed using the benchmark dose lower confidence limit for a 10% extra risk for peripheral neuropathy and neoplastic effects (BMDL10). MOE <125 and <10,000 indicates human health concern for peripheral neuropathy and neoplastic effects, respectively.

**Results:** In the G21 cohort sample, the median (IQR) dietary exposure to acrylamide ranged between 0.79 $\mu\text{g}/\text{kg}/\text{d}$  (0.53-1.17) at 4 years of age to 0.43 $\mu\text{g}/\text{kg}/\text{d}$  (0.28-0.63) at 13 years. Males compared to females presented higher exposure levels in all waves. For neuropathic effects, the MOE for median exposure was 544, whereas at 13 years was 1000. For neoplastic effects, at 4 years, the MOE for median acrylamide exposure was 215, and at 13 years was 395. The MOE calculated for the



95<sup>th</sup> percentile of exposure was 223 and 413 for neuropathic effects at 4 and 13 years, respectively, and 88 and 163 for neoplastic effects.

**Conclusions:** From childhood to adolescence, dietary exposure to acrylamide is of concern, especially regarding neoplastic effects. Our results point to the need to reduce exposure to acrylamide in the early stages of life.

**Funding:** FOCAcCla-POCI-01-0145-FEDER-031949; UI/BD/150785/2020.



## OC12 – Evaluation of the nutritive profile and risk assessment of enteral and parenteral nutrition formulas administered to individuals in a critical state

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**Keywords:** Bioaccessibility; *in vitro* digestion; enteral nutrition; trace elements.

**Introduction:** Nutritional therapy is used to treat malnutrition and in the care of patients in critical condition. Enteral and parenteral nutrition formulas have adequate fortification for micro and macronutrients, nevertheless literature report the presence of potentially toxic elements at trace levels in these formulations. It should also be mentioned that patients under nutritional therapy may have impairment of the body's ability to control the absorption of chemical elements, which may lead to the deposition of contaminants in the human body or inefficiency in the treatment of the malnutrition condition.

**Methodology:** The studied samples were evaluated and the essential and potentially toxic elements contents were determined by GFAAS or ICP OES. A statistic *in vitro* simulation model of gastrointestinal food digestion (INFOGEST) was applied to obtain the bioaccessible fractions of inorganic elements.

**Results:** The risk assessment of the 30 evaluated enteral formulas, 23 parenteral solution components and 3 parenteral solutions showed that some of the raw materials used in the manufacture of parenteral solutions are potential sources of Al and Mn contamination. In addition, some of the enteral samples presented risk quotient >1 for the elements Cr, Mn and/or Pb, indicating risk to the consumer. The enteral samples presented higher bioaccessible fractions in relation to conventional foods, as expected, since these formulas are prepared



specifically for patients with gastrointestinal tract problems and often present problems associated with malnutrition.

**Conclusion:** It is possible to establish that the samples of enteral nutrition for adults are mostly safe for consumption by the Brazilian population regarding the levels of Al, As and Cd, according to ANVISA. Two samples had lead levels ( $>0.25 \mu\text{g kg}\cdot\text{day}^{-1}$ ) too high for safe consumption, showing the need to monitor this contaminant. Others presented risk in relation to the consumption of Cr and Mn ( $> 250 \mu\text{g day}^{-1}$  and  $11\text{mg day}^{-1}$ , respectively).

It is essential to ensure the safety of enteral and parenteral nutritional formulas since patients with problems associated with the gastrointestinal tract usually depends exclusively on this source of nutrient intake. The *in vitro* digestion simulation studies is an effective tool for this purpose.



## OC13 – Toxicological interactions of regulated and emerging mycotoxins on human neuronal SH-SY5Y cells

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**Keywords:** combination index, cereals, cytotoxicity, SH-SY5Y cells, interactions.

**Introduction:** Cereal-based products are extensively consumed worldwide, making them the primary source of exposure to numerous well-known mycotoxins, such as enniatin B (ENNB), sterigmatocystin (STG), aflatoxin B1 (AFB1), cyclopiazonic acid (CPZ), citrinin (CIT), and ochratoxin A (OTA).

**Methodology:** The study assessed the cytotoxicity of single compounds, binary and ternary combinations, in human SH-SY5Y neuronal cells, following the median-effect equation of the mass-action law described by Chou and Talalay (Chou 2006).

**Results:** ENNB was the mycotoxin with the most potent neurotoxicity ( $IC_{50}=3.72 \mu M$ ), followed by OTA ( $IC_{50}=9.10 \mu M$ ) and STG ( $IC_{50}=9.99 \mu M$ ). Meanwhile, OTA+STG ( $IC_{50}=3.77 \mu M$ ) was the most toxic mixture, and CPZ+CIT was the least harmful. Almost 70% of the binary combinations/levels tested showed a synergic/additive effect, although some, ENNB+STG, ENNB+AFB1, and CPZ+CIT, had antagonistic effects. The ternary combination exhibited a moderate antagonism at the lowest fraction affected and moderate synergism in all other doses. Particularly noteworthy were the interactions involving OTA, as they demonstrated nearly 90% synergism. These findings hold significant relevance given OTA's prevalence as one of the most commonly reported mycotoxins



in foods. Conversely, interactions involving ENNB predominantly displayed antagonistic effects. Among the mixtures tested, the most synergistic combination was STG+CIT at a low exposure level. This remarkable synergy enabled a dose reduction of approximately 50 times for CIT to produce an equivalent toxic effect when combined, compared to its isolated effect.

**Conclusions:** Identifying robust synergistic effects, particularly among mycotoxins such as STG and CIT that commonly co-occur in food, holds substantial biological significance. These findings emphasize the necessity of considering such interactions for an accurately evaluation of human exposure risks.

**Topic relevance:** Given the frequent co-occurrence of these toxicants in food sources, their combined toxicity can give rise to synergistic, additive, or antagonistic effects. Consequently, evaluate these interactions becomes crucial in enhancing toxicity assessment methodologies.

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## OC14 – Microbial quality and safety of ready-to-eat germinated pulses

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**Keywords:** microbiological indicators; beans; chickpea; lentils; sprouts.

**Introduction:** Germination of pulses has gained popularity because it is claimed to improve seed nutritional value. However, sprouting is marketed as a simple practice, and consumers can be unaware of major microbial risks related to home sprouting. As the consumption of ready-to-eat raw sprouts has been linked to foodborne outbreaks, risks should be studied and disseminated.

**Methodology:** Organic pulses were purchased from retail stores. Seeds were washed and soaked to germinate during 24 and 48 hours at 18°C or 25°C under aseptic conditions. A temperature treatment (80°C, 20 seconds) was applied to the sprouts. Aerobic mesophilic (CAM), *Enterobacteriaceae*, *Escherichia coli*, yeasts and moulds were quantified with conventional methods (ISO standards) in the soaked seeds and sprouts to evaluate microbiological quality. *Salmonella* spp. and *Listeria monocytogenes* were detected by the VIDAS® method on ready-to-eat sprouts.

**Results:** In all studied microorganisms, from the soaked seeds to the first 24h sprouts (18°C; 25°C), an increase of at least one Log UFC/g was observed, except for moulds. According to the National Guidelines (INSA, 2019), levels of *Enterobacteriaceae* in cowpea seeds and sprouts, all mung beans, chickpeas, and lentils sprouts after 24h and 48h (25°C) presented unsatisfactory levels. For CAM, cowpea and chickpea sprouts after 48h at 18°C and 24h/48h at 25°C, and mung bean sprouts after 24h at 25°C also present unsatisfactory levels



for consumption. Temperature treatments were not effective in decreasing microorganism quantification. No *E. coli*, *Salmonella* spp. or *Listeria monocytogenes* were detected. Considering that seeds were germinated under aseptic and hygienic conditions and high levels of CAM and *Enterobacteriaceae* were found, we can predict that practising germination in a domestic environment, without aseptic conditions, could produce sprouts with high microbiological risk.

**Conclusion:** The work intends to address if homemade sprouting can become a consumer risk. Results indicate that sprouting without hygienic practices in a homemade environment can be a practice that, instead of promoting healthy habits through nutritional value, becomes a hazard. Some published practices, such as temperature treatments, used to minimize the microbiological risk can, in some cases, not be effective in microbial decrease.



## OC15 – Effect of bacteriophages against biofilms of *Escherichia coli* on food handling surfaces

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**Keywords:** *Escherichia coli* biofilm; Phage treatment; Surface; Food safety.

**Introduction:** Food safety is one of the most important public health issues. The ability of bacteria to adhere to food contact surfaces by biofilm formation is a threat to human health, as these surfaces can serve as a reservoir of contamination with pathogenic bacteria. *Escherichia coli* is one of the biofilm-forming bacteria and one of the most involved in surface contamination. Despite the correct application of all disinfection protocols, bacterial contamination on surfaces continues to occur. In this sense, the implementation of new effective and sustainable approaches to minimize the risk of contamination is crucial. Bacteriophages (or simply phages), viruses that only infect bacteria, are an example of an eco-friendly approach that has proven to be effective in reducing biofilms.

**Methodology:** In this study, phage phT4A was used to inactivate *E. coli* biofilm on food handling surfaces (plastic and stainless steel). The assays were carried out in Tryptic Soy Broth (TSB), at 25 °C and with a multiplicity of infection (MOI, the ratio between the number of phages and bacteria) of 10. In all assays a bacterial control containing only the bacterium *E. coli*, a phage control containing only the phage phT4A and a sample containing the bacterium and the phage.

**Results:** On plastic, the maximum reduction of *E. coli* biofilm was about 5 log colony forming units (CFU)/cm<sup>2</sup>, after 6 h of phage application. On stainless steel, the maximum reduction of *E. coli* biofilm was approximately 4 log CFU/cm<sup>2</sup>, after 9 h of incubation.



**Conclusions/Topic relevance:** Phages have been shown to be effective in reducing *E. coli* biofilms, proving to be a promising approach for decontaminating food handling surfaces, and thus ensuring food safety.

**Acknowledgment:** The authors acknowledge financial support to CESAM by FCT/MCTES (UIDP/50017/2020 + UIDB/50017/2020 + LA/P/0094/2020). Márcia Braz thanks FCT for the PhD grant (2020.06571. BD). Carla Pereira is supported by a Junior Research contract (CEEC Individual/03974/2017). Thanks, are also due to Biology Department and University of Aveiro, where this research work was performed.



## OC16 – Investigating ingested nanomaterials' safety – the case of TiO<sub>2</sub> and innovative nanocelluloses.

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**Keywords:** nanocellulose, titanium dioxide, nanomaterials, human digestion, safety

**Introduction:** The development of nanomaterials(NMs)-based technologies led to their increased use in key sectors and products related to food, food contact materials and feed. Many available products have NMs, as intentional constituents or contaminants from process or food packaging release, such as silicon or titanium dioxide(TiO<sub>2</sub>) NMs. Others are being developed, like nanocelluloses(CNMs; doi:10.3390/nano12193375). However, it is recognised that the NMs' specific physicochemical properties, conferring them unique beneficial characteristics, can also elicit nano-bio interactions leading to toxic potential. Also, their dynamic behaviour in the surrounding matrix, may lead to secondary features determining the toxicological outcomes. Recognizing that processes like intake or digestion may modify the NMs' characteristics leading to unexpected toxicity in human cells, EFSA included the use of in vitro digestion models in their specific guidelines concerning risk assessment of nanomaterials for food and feed(DOI: 10.2903/j.efsa.2021.6768).

**Methodology:** With the aim to contribute to the safety assessment of NMs, intestinal cell models (Caco-2 and HT29-MTX-E12 cells) were exposed to TiO<sub>2</sub> NMs or innovative CNMs. Additionally, samples submitted previously to in vitro simulation of human digestion were



used, and the genotoxicity (comet and micronucleus assays) was investigated with and without the digestion process.

**Results:** After TiO<sub>2</sub> NMs' exposure, the micronucleus assay, an indicator of cancer risk, suggested effects on the chromosomal integrity in the HT29-MTX-E12 cells, for all the tested TiO<sub>2</sub> NMs, especially after the in vitro digestion. Upon exposure to the two CNMs, no chromosomal damage was observed in the micronucleus assay, but the comet assay revealed DNA damage in the same cells, after 3h and 24h exposure, an effect slightly more relevant after the digestion of the cellulose nanofibril.

**Conclusion:** Overall, the results show different outcomes when using different NMs, and with/without digestion. Thus, it is important to consider the primary and secondary NMs' characteristics determining the adverse effects, taking into account the human digestion for nanosafety assessment.

**Funding:** Funded by FCT/MCTES through 2020.07168.BD (NV), PTDC/SAUPUB/29481/2017, and ToxOmics (UIDB/00009/2020;UIDP/00009/2020). Thanks to P. Ferreira, J. Pedrosa (University of Coimbra) and A.F. Lourenço (RAIZ) for the CMNs; to P. Alvito, R. Assunção, M. Silva (Food Safety Department, INSA) for in vitro digestion.



## OC17 – Do mycotoxins in aquafeeds affect the nutritional quality of farmed fish?

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**Keywords:** Seabream; Fatty acids; Protein; Essential elements; *In vivo* assay; Mycotoxins

**Introduction:** Mycotoxin contamination is a current issue in aquaculture sector due to the increase use of plant-based ingredients in the feed production. Despite the toxic effects on animals and humans, little is known about the effects of these contaminants on the nutritional quality of farmed fish and consequently, remain a serious food safety concern. For this reason, this study aimed to investigate the potential changes on nutritional quality of juvenile gilthead seabream (*Sparus aurata*) fed with contaminated feed by mycotoxins.

**Methodology:** During 28 days, the equivalent to 1.5% of the fish average body weight of two control (CTR; CTR-solvent) and three contaminated



aquafeeds (fumonisin, FB1; enniatin B, ENNB; FB1+ENNB) were daily provided to fish. Before and after the exposure period, three specimens per treatment were randomly collected. Biometric data were obtained, and muscle were sampled, freeze-dried and kept at -80 °C until further analyses. Moisture, ash, free fat, including the fatty acid profile by GC-FID, crude protein and gross energy were determined. Essential elements (potassium, K; sodium, Na; magnesium, Mg; iron, Fe; copper, Cu; zinc, Zn; manganese, Mn) were also quantified according to the procedures described by Jorhem (2000) using flame atomic absorption spectrometry (FAAS). In addition, the animal fitness and growth performance were also assessed.

**Results:** Preliminary results showed effects on the fish condition, such as a decrease of final fish body weight, weight gain and specific growth rate in seabream after consumption of aquafeeds contaminated with mycotoxins, mainly the mixture of FB1+ENNB. In relation to essential elements, no significant differences were observed in the fish muscle after ingestion of the contaminated diets. In contrast, total protein and fatty acid composition were affected, particularly the EPA+DHA levels and the  $\Sigma\omega3/\Sigma\omega6$  ratio, which may compromise the nutritional quality of this species.

**Conclusion:** Overall, this study provides new insights to understand and foretell the mycotoxins impacts on nutritional quality of seafood and highlights the importance to perform a robust risk-benefit analysis of fish consumption in order to ensure maximum protection for farmed fish consumers.

**Acknowledgement:** This work was supported by the Project MycoFish – Occurrence, bioavailability and mitigation strategies for mycotoxins in farmed fish and associated feed ingredients: Gilthead seabream as case study (PTDC/CVT-CVT/2660/2021).



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# POSTERS

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## P1 – A green and simple procedure based on deep eutectic solvents for the extraction of contaminants of emerging concern from water

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**Keywords:** Contaminants, deep eutectic solvents, microextraction

**Introduction:** Contaminants of emerging concern (CECs) such as UV-filters, musks, bisphenols and pesticide residues in waters are associated with the increasing urbanization and limited removal efficiency of CECs by Wastewater Treatment Plants. These contaminants represent a great threat to the environment and human health.

**Methodology:** The study aimed to assess the effectiveness of deep eutectic solvents (DES) as extractive solvent in dispersive liquid-liquid microextraction (DLLME) to isolate and concentrate various pesticide residues, bisphenols, musks, and UV filters. The determination of these CECs was accomplished using gas chromatography coupled with tandem mass spectrometry.

**Results:** Initially, main factors affecting the extraction such as type and volume of DES, pH, and ionic strength, were optimised. Once established, the final protocol was thoroughly validated in terms of linearity, extraction efficiency and sensitivity according to the SANTE/11312/2021 guidelines.

**Conclusions:** In this work, a green, inexpensive, simple and fast DES-based DLLME procedure was evaluated, for the first time, for the extraction of several CECs from water. The developed method offers a suitable alternative for the simultaneous determination of CECs in water, enabling the use of small volumes of biodegradable and environmentally friendly extraction agents. This approach aligns with the principles of Green Analytical Chemistry.

**Topic relevance:** Water, as the primary nutrient, is vital for human



existence. Therefore, monitoring the levels and potential health effects of CECs in water is crucial for guaranteeing the safety of drinking water supplies and safeguarding public health.

**Acknowledgement:** This work received financial support from PT national funds (FCT/MCTES, Fundação para a Ciência e Tecnologia and Ministério da Ciência, Tecnologia e Ensino Superior) through the project UIDB/50006/2020. Sara C. Cunha acknowledges FCT for the 2022.07841.CEECIND/CP1724/CT0014 contract. The authors thanks FCT Fundação para a Ciência e a Tecnologia through national funds to the project CleanH2O 2022.03009.PTDC.



## P2 – Mycotoxins in aquaculture

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**Keywords:** alternative feeds, protein sources, FB1, ENNB, quality control

**Introduction:** Aquaculture is an important food source worldwide. For economic and environmental sustainability reasons plant-based proteins are commonly used as partial replacement of animal-based proteins, which can result in higher exposure to contaminants, such as naturally occurring mycotoxins. The exposure of fish to these contaminants can result in health problems, stunted growth or even death, consequently leading to economic losses for the producers and health concerns for the consumers.

**Methodology:** In the present work, the occurrence of 22 mycotoxins (13 regulated and 9 emerging) was evaluated using a methodology based on QuEChERS (Quick, Easy, Cheap, Effective, Rugged, and Safe) extraction, with detection and quantification using LC-MSMS. A total of 87 samples were tested, out of which 50 were complete fishfeeds, and 37 were feed ingredients (21 cereal-based, 7 algae-based, and 9 other vegetables).

**Results:** Six samples of cereal-based ingredients were positive for 6



regulated mycotoxins (218 – 5324 µg/kg), and 4 emerging mycotoxins (66 – 4286 µg/kg). The most prevalent metabolites were AFG2 and ENNB. Algae-based ingredients had a lower degree of contamination with only one positive sample (324 µg/kg AFB2, 163 µg/kg AFG1 and 293 µg/kg ENNB). Similarly, only one sample from the “other vegetable”-based group was contaminated with AFG1 (150 µg/kg), ENNB (265 µg/kg), ENNA1 (303 µg/kg) and ENNA (290 µg/kg). Twelve complete fishfeed samples were contaminated with 4 regulated mycotoxins (135-7348 µg/kg) and 2 emerging mycotoxins (132-3154 µg/kg). In this group of samples, FB2 and ENNB were the most detected compounds.

**Conclusion:** Feed ingredients and complete fishfeed were contaminated with high levels of mycotoxins, with aflatoxins, fumonisins and enniatins the most predominant ones. Rising temperatures, crop selectivity, agricultural practices and subpar storage conditions contribute to higher rates of fungal growth, even non-native species. The presence of various mycotoxins, both regulated and emerging, underlines the need for tighter control measures in feed production.

**Topic relevance:** Worldwide distribution of mycotoxins allied to consistent growth of aquaculture production reinforces the necessity of complete feed and feed products quality assessment, during harvest, production and in the final product.

**Acknowledgement:** Sara C. Cunha thanks FCT for the 2022.07841. CEECIND/CP1724/CT0014 contract. All authors acknowledges FCT through the Project MycoFish – PTDC/CVT-CVT/2660/2021.

**Funding:** This work was supported from PT national funds (FCT/MCTES, Fundação para a Ciência e Tecnologia and Ministério da Ciência, Tecnologia e Ensino Superior) through the project UIDB/50006/2020.



## P3 – Removal of paralytic shellfish poisoning toxins from microalgae and mussels

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**Keywords:** saxitoxins, bivalves, adsorption, food security

**Introduction:** The presence of biotoxins in marine systems and their ingestion by organisms constitute a serious threat to the food security of humans who consume them. Additionally, the consequent interdiction of harvesting and closure of shellfish production facilities has a huge economic impact and compromises the availability of protein-based food. Paralytic shellfish toxins (PST) account for the highest percentage (46%) of toxicity events associated with seafood and cause the most serious health consequences in humans, including death. Several studies have been carried out aiming to develop effective solutions for shellfish detoxification, but the methodologies proposed have shown limited effectiveness.

**Methodology:** Our group has been devoted to the development of chemical methodologies to remove PST from marine aquatic systems. Cation-exchange resins were prepared and tested for their ability to adsorb biotoxins, over different times. Experiments were conducted using cultures of microalgae *Gymnodinium catenatum*, and mussels *Mytilus edulis*. Toxins were quantified by high-performance liquid chromatography with fluorescence detector (HPLC-FLD).

**Results:** Studies using microalgae cultures revealed a decrease of around 80% in overall toxicity after 48h and a differentiated adsorption by the resin, depending on the molecular structure of the toxins. In live mussels, preferential adsorption was again observed, although



the reduction in overall toxicity was not as great as in cultures. Nevertheless, a significant decrease in toxicity was observed, compared to normal clearance. Some factors may explain the lower effectiveness of cation-exchange resins in removing toxins from mussels, such as the absorption capacity of the materials by mussels and/or greater competition for the same binding sites in the presence of salts and natural organic matter. Further studies are ongoing to optimize the materials.

**Conclusion:** The methodology based on materials that competitively adsorb PST afforded promising results in detoxification. Our study draw attention to the importance of testing the methodologies *in vivo* and revealed some aspects that may affect the experimental results, namely the pH-effect. Follow-up studies are planned for optimization of materials and conditions.

**Topic relevance:** Our results represent an important step towards the optimization of solutions to food security and to mitigate the socioeconomic effects caused by PST poisoning of marine shellfish.

**Acknowledgement:** This study received funds from the project MAR-01.03.01-FEAMP-0049 co-financed by the Operational Program Mar 2020, Portugal 2020, and the European Union (EU), through the European Maritime Affairs and Fisheries Fund (EMFF), and from FCT – Foundation for Science and Technology, through projects UIDB/04326/2020, UIDP/04326/2020 and LA/P/0101/2020 (CCMAR). Joana F. Leal also thanks FCT-Portugal for her research contract (2022.08181.CEECIND).



## P4 – Recent insights into the occurrence and implications of mycotoxins in foods: A Review

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**Keywords:** contaminated food; emerging mycotoxins; occurrence studies.

**Introduction:** The occurrence of mycotoxins in food presents a significant concern for human and animal health, as they can lead to acute and chronic conditions.

**Methodology:** This review summarizes recent findings on mycotoxin occurrence in food. Twelve studies published in 2023 were selected from the “ScienceDirect” and “PubMed” databases, covering 16 food categories collected between 2017 and 2022, from 30 countries.

**Results:** A total of 10,694 samples, including cereals, fruits, vegetables, plant-based meat, bee pollen, and medicinal plants, were analyzed using mostly LC-MS/MS. Among the 46 mycotoxins investigated, 38 were detected, encompassing both regulated and non-regulated emerging mycotoxins. The occurrence of positive samples varied, with notable occurrences of aflatoxin B<sub>1</sub> (AFB<sub>1</sub>), fumonisin B<sub>1</sub> (FB<sub>1</sub>), ochratoxin A (OTA) beauvericin, enniatin, and alternariol monomethyl ether. While the average concentrations rarely exceeded European legislation limits, maximum amounts often surpassed the permitted thresholds. For instance, the studies found maximum amounts of 10,091 µg/kg of AFB<sub>1</sub> and 60,276 µg/kg of fumonisins in animal feed (China), along with 1875 µg/kg of FB<sub>1</sub> in coix seed (China) and 38.1 µg/kg of OTA in agri-foods (Chile). Highly contaminated foods were still prevalent worldwide.

**Conclusion:** These occurrence studies lay the groundwork for further research into the toxic potential of emerging mycotoxins, decontamination methods and revisions to the current maximum tolerated levels of mycotoxins in food and feed.



## P5 – Influence of Good Management Practices on the occurrence of Aflatoxins in Brazil nuts from a Brazilian Amazon Region

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**Keywords:** mycotoxin; *Bertholletia excelsa*; moisture content

**Introduction:** Brazil nut (*Bertholletia excelsa*) is an Amazonian seed of economic importance, collected in the forest by traditional populations or indigenous communities. The activity takes place in a context with rudimentary activities and without strict technological controls, requiring the adoption of good management practices (GMP), such as prevention against aflatoxin (AFL). After collected, the seed goes through storage stages in which the ambient temperature and relative humidity in which some collectors use GMP. Therefore, the objective of this work was to evaluate, for three consecutive harvests (2019 to 2021), the influence of adopt GMP on the AFL content of Brazil nut from a region of the State of Amazonas-Brazil.

**Methodology:** Samples of unshelled Brazil nuts ( $N=60$ ) were collected directly from collectors in a region of the state of Amazonas-Brazil. The samples were composed of increments of samples of 300g, calculated according to the batch size. After being packed, they were sent for immediate laboratory analysis within 24 h. Samples were evaluated by High performance liquid chromatography.

**Results:** Total Limit of Detection (LOD) for total AFL was  $0.772 \mu\text{g}/\text{kg}$ . In 2019 and 2020 harvests there was at least one sample with AFTotal values  $> 4\text{ppb}$  with GMP. Only in the year 2021 was the difference between samples with and without management statistically significant, when the mean for the unmanaged samples was  $3.4 \text{ p.p.b}$  with values of up to  $9.4 \text{ ppb}$ . In that year, even the unmanaged samples had an average contamination value greater than 2019 and 2020. A possible



explanation lies in the occurrence of the Covid-19 pandemic (in 2020) in that many collectors stopped collecting in the forest, resuming activity only in 2021.

**Conclusion:** The adoption of GMP seems to be an important tool for the sanitary quality of Brazil nuts, with the length of time the nuts remain in the forest being the variable that stood out as a determinant in increasing the AFL content in samples without management. We suggest that other GMP continue to be included for comparison in future works, such as sorting and silo aeration to better understand the ambient condition to prevent aflatoxin.

**Topic relevance:** Occurrence of chemical contaminants in food products.



## P6 – Occurrence of aflatoxins in Brazil nut oil from discarded seeds

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**Keywords:** mycotoxin; *Bertholletia excelsa*; edible oil

**Introduction:** there are reports of mycotoxin contamination in edible oils in several countries, generally related to the possibility that the raw material collected in the Amazon Forest become contaminated by improper storage for long periods in conditions that can promote fungal growth and mycotoxin production. In this context, the objective of this work was to evaluate, the occurrence of ochratoxin A in Brazil nut oil samples obtained from discarded seeds from the Brazil nut factories in Brazilian Amazon.

**Methodology:** Samples of Brazil nut oil ( $n=15$ ) were collected directly from Brazilian facilities in 2022-2023 harvests, from discarded seeds (seeds that do not meet the standard) to obtain the oil by manual pressing. The samples were about 1L in plastic bottles and were evaluated by High performance liquid chromatography. The total Limit of Detection (LOD) for total AFL was 2.32  $\mu\text{g}/\text{kg}$ .

**Results:** from the samples, 20% were positive for AFL and the average was 7.78  $\mu\text{g}/\text{kg}$  (5.6 to 8.4  $\mu\text{g}/\text{kg}$ ). There is no legal limit for AFL in Brazil nut oil in Brazil, but all samples were above the maximum limit 4.0  $\mu\text{g}/\text{kg}$  of European Union. Other edible vegetable oils such as hazelnut and peanut have shown AFL contamination and it seems that most of the AFL contamination remains in the “cake” fraction after pressing to obtain the oil fraction.

**Conclusion:** there were positive Brazil nut oils for total AFL in the evaluated samples obtained from discarded seeds from Brazil nut factories. In this sense we suggest actions of good management



procedures in the Amazon Forest, to collect, storage and transport the seeds to the factories, in a safe way, to prevent the contamination in the raw material.

**Topic relevance:** Occurrence of chemical contaminants in food products.



## P7 – Cadmium in commercial chocolates bars with milk and plant-based extracts

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**Keywords:** plant-based foods; inorganic contaminants; public health; ICP-MS

**Introduction:** The growing demand for plant-based foods has expanded to the confectionery market, which includes chocolate bars that are usually formulated with sugars and fats from different plant-based sources, plant-based extracts to replace milk, and cocoa liquor. Studies have reported that plant-based foods can have significant levels of health-damaging contaminants from air pollution, contaminated soil and groundwater, and intensive use of pesticides, among other sources. Cadmium (Cd), a toxic element that can be harmful to health even at trace levels, is one of the inorganic contaminants found in chocolate.

**Methodology:** Thus, the objective of the study was to evaluate the Cd content in commercial chocolate bars (50-60% cocoa) formulated with oat, coconut, coconut with nuts and sweet potato extracts and a traditional milk chocolate bar for comparison. Chocolate bars were ground, stored with light protection and frozen until the time of analysis. The samples were prepared by acid digestion (50 % nitric acid solution and 30 % hydrogen peroxide) in analytical microwave (power: 1800 mW; time: 35 minutes; temperature: 80-200 °C) and the determination of Cd was performed by inductively coupled plasma mass spectrometry (ICP-MS). The limit of quantification (LOQ) was 5.0



$\mu\text{g}/\text{kg}$ , while the Cd content was 29.0; 33.0; 68.0 and 93.0  $\mu\text{g}/\text{kg}$  for the chocolate bars formulated with oat, coconut with nuts, sweet potato, and coconut extracts, respectively.

**Results:** The Cd content for traditional milk chocolate bar was 8.0  $\mu\text{g}/\text{kg}$ . These results indicated that plant-based chocolates bars have higher Cd contents than traditional milk chocolate bar. However, comparing the levels obtained with the Normative Instruction IN No. 88/2021, which establishes the maximum tolerated limits (MTL) of inorganic contaminants in chocolate with more than 40% cocoa in Brazil, all samples evaluated showed Cd contents below 300  $\mu\text{g}/\text{kg}$ .

**Conclusion:** Although Cd is in low contents in these new chocolates, its effect is cumulative in the body, and may cause long-term negative impacts on human health. Therefore, it is necessary to invest in good agricultural and manufacturing practices to ensure the quality and safety of the raw materials used in the formulation of chocolates, and thus prevent their consumption from becoming a public health problem.



## P8 – Evaluation of total mercury content in fish and seafood available in Portuguese market

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**Keywords:** mercury, fish, seafood, food safety, Portugal

**Introduction:** In Portugal, there is a tradition of high consumption of fish and seafood, higher than in the European Union (EU) countries, and above both EU and world averages. Although fish and seafood are important sources of high biological value proteins, omega 3 fatty acids and essential minerals, it can contain environmental contaminants, such as mercury compounds, which undergo bioaccumulation in the aquatic food chain. Long-lived predatory fish species, such as tuna or swordfish, are an important human exposure source. Methylmercury, the most toxic mercury form, mainly targets the central nervous system. The most susceptible population to the toxic effects of methylmercury are pregnant women's and children and the prenatal period represents a period of greatest vulnerability regarding neurodevelopmental effects on the fetus.

However, due to the fact that it is not possible to eliminate mercury from environment or fish, and that fish remains an important food in the Portuguese diet, recommendations for fish consumption for Portuguese population were prepared. The aim of this research was to determine mercury contamination in fish and seafood available in Portuguese markets.

**Methodology:** For this study, a total of 24 different species of fish and fishery and aquaculture products, representative of Portuguese consumption, were selected and acquired on the market during 2014 and 2015. Total mercury (THg) content was determined in the samples by thermal decomposition and amalgamation atomic absorption



spectrophotometry (TDA/AAS), in compliance with ISO/IEC 17025. All the analyzed samples have revealed THg levels above the limit of quantification of the method ( $LoQ = 1.1 \mu\text{g}/\text{kg}$ ), but within the legislated limit. The values ranged between  $4.2 \mu\text{g}/\text{Kg}$  (catfish) and  $574 \mu\text{g}/\text{Kg}$  (scabbard fish).

**Results:** These results provide information regarding analytical data of chemical substances of interest and are a contribution to food risk assessment. It also reinforces the importance of disseminating the recommendations for fish consumption for the Portuguese population as a risk communication strategy, in order to either protect susceptible populations from exposure to this chemical, and simultaneously promoting the important health benefits associated with fish consumption.



## P9 – Assessing 165 Emerging Contaminants in Water, Sediments, and Fish through LC-MS/MS Target Screening Approach

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**Keywords:** medicines; antibiotics; illicit drugs; quantification; fish.

**Introduction:** Artisanal fisheries play a crucial role in sustaining the livelihoods of coastal communities by providing food security and nutrition. However, anthropic activities that impact coastal ecosystems can have detrimental effects on both fishing practices and the well-being of the population reliant on fish from these areas. Therefore, this study aimed to identify contaminants of emerging concern (CECs) in fish (n = 22), water (n = 27), and sediment samples (n = 27) collected in the Lagoa da Conceição region in Florianópolis, Santa Catarina.

**Methodology:** The target screening of 165 CECs was conducted using liquid chromatography coupled with mass spectrometry (LC MS/MS).

**Results:** In the qualitative stage, 35 CECs were detected and identified, with diclofenac, sertraline, nitrazepam, benzoylecgonine, nimesulide, caffeine, clindamycin, piroxicam, azeperol and temazepam being the most frequent compounds among the samples. The LEvap point had the highest incidence of CECs in the water, with 23 occurrences, evidencing the accumulation of CECs at the exit of the region's effluent treatment station. In fish samples, the most frequently detected CECs, present were sertraline (22/22), diclofenac (21/22) and nitrazepam (15/22). The detection of CECs in sediment samples emphasized the



occurrence of piroxicam, benzoylecgonine and caffeine. Some of the analytes detected in the qualitative assessment could not be confirmed with their respective analytical standards. Samples from sediments showed the highest incidence of CECs, with caffeine reaching the highest concentration ( $55.89 \mu\text{g.Kg}^{-1}$ ). Ciprofloxacin was the only CEC quantified in fish above the limit of quantification, with concentrations between  $2.94$  and  $4.18 \mu\text{g.Kg}^{-1}$ . The quantitative results corroborated, to some extent, with the target screening assessment.

**Conclusions:** The screening method used was considered satisfactory, as in addition to confirming the presence of CECs, it allowed observing their occurrence in different environmental matrices.

**Topic Relevance:** The frequent detection of several CECs may indicate a significant risk to the estuarine biota. In addition, the accumulation of these compounds in food that is widely consumed by the local population must be constantly evaluated, in order to obtain data for risk assessment and prevent possible harm to human health.

**Acknowledgment:** Authors thank FAPESC for their financial support of this project under grant number 2022TR001403.



## P10 – Investigating the etiology of Haff disease: optimization of a palytoxin extraction method using micro-scale oxidation and analysis of fish and crustacean samples associated with the disease triggering in Brazil

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**Keywords:** foodborne disease; dark brown urine disease; North of Brazil; Northeast of Brazil.

**Introduction:** Haff disease is typically developed after eating contaminated sea and freshwater fish, leading to rhabdomyolysis and myoglobinuria. First reported in 1924, in Germany, it has since spread to other countries, including Brazil. Despite analyzing related food and clinical samples, its etiology remains unknown, thus being a priority research scope. Therefore, the objective of the present work was to contribute to this investigation, taking as the main etiological hypothesis the palytoxins, an emerging group of aquatic biotoxins whose main symptoms of intoxication are similar to those found in Haff disease.

**Methodology:** An experimental design was employed in order to optimize the palytoxins extraction. The method includes a solid-phase extraction (Strata-X, 500mg/6ml) containing a periodic acid micro-scale oxidation. The protocol was conducted using fish samples fortified with known concentrations of palytoxin standard. The optimized method was validated and further applied in 5 fish and crustacean samples (*in natura* and leftover meals) associated with the Haff disease triggering in Brazil. The analysis of the oxidation products of palytoxins was performed in a LC-MS/MS instrument.



**Results:** Inspection of the response surface of the experimental design indicated that the optimum volume of initial extraction solvent was 15 mL, while the total volume of water and the percentage of methanol used in the solid-phase extraction steps were 4.5 mL and 65%, respectively. The optimized protocol significantly increased the area of the analyte peaks (approximately 20%), as well as detect concentrations as low as  $10 \mu\text{g}\cdot\text{kg}^{-1}$  of palytoxin in fish samples. The common oxidation product of both palytoxin and its analogues was detected in all 5 samples related to Haff disease. Regarding the specific oxidation products for each analogue, it was identified a signal related to homopalytoxin in 4 of the 5 samples.

**Conclusions:** The improved protocol for palytoxin extraction serves as an excellent approach for screening suspicious samples. There is a strong indication of palytoxins presence in samples associated with Haff disease in Brazil, but further high-resolution mass spectrometry analysis will make it possible to confirm the presence of these toxins.

**Topic relevance:** Public health problem, emerging disease.



## P11 – Assessment of Cadmium Content in Chocolates from Pichincha, Ecuador: Implications for Consumer Health and Regulatory Compliance

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**Keywords:** Cadmium, Chocolate, Atomic absorption spectrometry, Graphite furnace.

**Introduction:** Due to the global increase in the consumption of cocoa-derived products and the emergence of companies producing chocolate for both domestic consumption and export in Pichincha, Ecuador, it has become necessary to determine the concentration of cadmium (Cd) in chocolates manufactured in the province. This research aims to protect consumers' health and ensure compliance with the limits established in Regulation 488/2014 of the European Union.

**Methodology:** A non-probabilistic convenience sampling method was employed in the cities of Quito and Cayambe (Pichincha) to collect 15 samples, with 3 samples belonging to category 2 (chocolate with a total dry matter content of cocoa <50%; milk chocolate with a total cocoa dry matter content  $\geq 30\%$ ) and 12 samples belonging to category 3 (chocolate with a total cocoa dry matter content  $\geq 50\%$ ). Subsequently, the samples were ground and treated with nitric acid (68%) and hydrogen peroxide (30%) using microwave digestion. The Cd content was quantified using Graphite Furnace Atomic Absorption Spectrometry (GFAAS) and a calibration curve with standards of 1.0, 1.5, 3.0, and 5.0  $\mu\text{g/l}$  of Cd.



**Results:** The Cd content in category 2 chocolates was found to be  $0.0565 \pm 0.0240$  mg/kg, while category 3 products exhibited a Cd content of  $0.3830 \pm 0.1837$  mg/kg. These values were found to be below the limits established in Regulation 488/2014. Additionally, a significant positive correlation (Pearson's  $r = 0.908$ ) was observed between the percentage of cocoa solids and the Cd concentration in category 2 products. However, no correlation was found in category 3 products (Pearson's  $r = 0.394$ ).

**Conclusion:** To date, no studies have been conducted in Ecuador regarding the Cd content in chocolates. Therefore, it is crucial to possess this information to safeguard the health of the Ecuadorian population. The detrimental effects of Cd ingestion are widely recognized, and the absence of Ecuadorian regulations establishing maximum limits for this contaminant in food underscores the significance of this research. The obtained values are expected to contribute towards the formulation of future regulations in Ecuador.



## P12 – Seafood: organic contaminants and legislation aspects for food safety in baixada santista (sp)

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**Keywords:** public health, fish, shellfish, bivalve, environmental sustainability, persistent contaminants

**Introduction:** More than 157 million tons of global fish production is for human consumption and represents a source of income for more than 58 million workers directly involved in fishing and aquaculture activities. However, its safety for consumption may be compromised because several economic activities include numerous combustion and thermal processes, potentially generating organic contaminants. The study area is under the influence of the Port of Santos and the Cubatão Industrial Complex, making the region a potential focus of pollution.

**Methodology:** Given the growing consumption of fish in the country and the need to provide information about the levels of environmental contaminants in species of artisanal fishery of commercial interest in the Baixada Santista Region, the organic contaminants (PAHs, dioxins, furans and PCBs) were analyzed by gas chromatography coupled to mass spectrometry. The legislation and its protective impact was also evaluated.

**Results:** The results obtained did not show any contamination of edible portions of the six analyzed species: Fat snook (*C. parallelus*), Common snook (*C. undecimalis*), White mullet (*M. curema*), Clam (*A. flexuosa*), Uçá crab (*U. cordatus*) and Blue crab (*C. danae*), considering the maximum



tolerable limits of dioxins, furans and PCBs which are provided by current regulation, ANVISA's IN 160/22.

**Conclusion:** The national legal framework is broad and has been advancing, however, many environmental contaminants still do not have limits established, such as PAHs in food, focusing on fresh and frozen fish, being necessary to resort to international regulations and other matrices. These gaps hinder the applicability of updated public health protection measures, and may expose seafood consumers to obsolete and/or inadequate food safety standards and to substances that are deleterious to the maintenance of human and environmental health. This lack of regulation affects the governance of the productive sector, as well as the safety of fish-based food products.

The “one health” approach can be employed throughout the productive chain and favor the interlocution between production and application of knowledge, in shared actions and public policies that contribute to the health of the environment, the people, and the seafood, contributing to the safety of consumption and risk mitigation due to contaminatin exposure through food.

**Funding:** This study was financed by São Paulo (MPSP) and Federal (MPF) Public Ministries – Third Amendment to the Term of Commitment and Conduct Adjustment of civil inquiries: 14.0703.0000028/2015-1 (MPSP) e 1.34.012.000220/2015—55 (MPF).



## P13 – Development of a reliable method for the quantification of tropane alkaloids in buckwheat using LC-UHPLC-MS/MS

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**Keywords:** *Solanaceae*, scopolamine, hyoscyamine, mass spectrometry

**Introduction:** Tropane alkaloids (TAs) are secondary metabolites from different species of the *Solanaceae* family and are known for their anticholinergic effects. The presence of these contaminants in food has raised significant concerns following reported cases of widespread contamination in various regions. This problem became more pressing due to the mechanization of the harvests, especially in the last decades, since some *Solanaceae* plants can grow in crops such as millet, buckwheat, sorghum, flax, soybean, and sunflower. When parts of the foreign plant are mixed with the crop and are not separated through traditional cleaning procedures, the resulting product may contain TAs in harmful levels. Therefore, reliable quantification methods for TAs in food are crucial but remain challenging due to their low maximum concentration levels and complex matrix composition.

This study addresses the challenges associated with monitoring TAs, particularly (±)-hyoscyamine and (-)-scopolamine in buckwheat matrices, including raw grains, flours, and mixed flours.

**Methodology:** We propose a novel method for extracting (±)-hyoscyamine and (-)-scopolamine using solid-liquid extraction at low temperature (SLE-LTP), followed by quantification using bidimensional chromatography coupled to tandem mass spectrometry (LC-UHPLC-MS/MS).

**Results:** The utilization of LC-UHPLC-MS/MS offers several advantages, including the use of two chromatographic columns, enabling the injection of a larger volume, efficient matrix clean-up, and improved



chromatographic separation of analytes. However, it is essential to optimize the developed method for better results. Different conditions were evaluated, such as chromatographic columns, loading volume and mobile phase, additives, and sample injection volumes. Mass spectrometry detection's inherent selectivity and sensitivity further enhance the quantification process, providing essential parameters for accurate TA analysis.

For sample preparation, SLE-LTP was the method of choice since it allows an extraction efficiency around 50% and acceptable matrix effect (less than 26%). The developed method was validated, demonstrating a linear response in the concentration range of 2.5 to 15  $\mu\text{g kg}^{-1}$  ( $r > 0.99$ ). Precision and accuracy were within the ranges of 0.8 to 11.0% and 96 to 103%, respectively. The limit of quantification (LOQ) was determined to be 2.5  $\mu\text{g kg}^{-1}$ . Eighteen commercial samples were analyzed, and they did not exhibit contamination levels above the LOQ.



## P14 – Occurrence of highly toxic pesticides in grape

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**Keywords:** Pesticide residues, grapes, mass spectrometry.

**Introduction:** Grapes are one of the most consumed fruits in the world, as well as their by-products. They are sources of vitamins, polyphenols, essential amino acids and rich in pigments (e.g. anthocyanins), in addition bring some benefits to human health, such as for reducing the risk of developing cardiovascular diseases and cancer prevention. In grape production, pesticides are used to control pests aiming to increase harvest. In this study, pesticide residues were determined in grape samples commercialized in markets of São Paulo State – Brazil, in 2019.

**Methodology:** The analysis of 120 active ingredients (a.i.) was performed by modified QuEChERS and followed by Gas Chromatography coupled to a Tandem Mass Spectrometry (GC-MS/MS) and Ultra High-Performance Liquid Chromatography coupled to a High-Resolution Mass Spectrometry (UHPLC-HRMS) for identification and quantification. The limits of detection (LOD) and quantification (LOQ) were 0.005 and 0.010 mg kg<sup>-1</sup>, respectively and blank samples showed no interference in the studied compounds. Validation results complied with criteria established by the European Community guide SANTE 11813/2017.

**Results:** A total of 17 samples were analyzed and detected the presence of 10 different a.i.. Acephate was detected in three samples and dimethoate as well, one sample was contaminated with both, furthermore in these seven samples were detected other a.i.. Dichlorvos was found in one sample at the LOQ level. Although the most detected pesticide was procymidone found in 10 samples



below Maximum Residue Level ( $5.0 \text{ mg kg}^{-1}$ ). Only two samples were not found a.i.. Among the pesticides detected in grape samples, acephate, dimethoate, dichlorvos (organophosphates, not authorized) and procymidone (dichlorophenyl dicarboximide) are substances considered highly toxic to humans and other animals, as well as to the environment.

**Conclusion:** The high prevalence of these compounds indicates the importance of permanent pesticide residues monitoring programs in Brazil, as well as the adoption of Good Agricultural Practices (GAP) and increased inspection of farming.



## P15 – Occurrence of ochratoxin A in Brazilian artisanal cheese

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**Keywords:** Extraction, Mycotoxin, Chromatography

**Introduction:** Brazilian artisanal cheese is produced following protocols and traditions of each producing region, and can be made with fresh, raw milk, without heat treatment, with the addition of natural yeast or starter cultures. Some cheeses are matured to allow the growth of filamentous fungi (flowering rind) to develop a special flavour and aroma. In addition, they can be matured in different environments, such as maturation rooms and caves, to promote growth of distinct microbiota. Because it is produced in different ways, artisanal cheese is characterized as a product with a high risk of contamination by microorganisms, including fungi that produce mycotoxins. In Brazil, there is no legislation that regulates and determines the acceptable levels of ochratoxin A (OTA) in cheese. This work aimed to investigate the occurrence of OTA in artisanal cheeses from the Serra da Canastra region (Minas Gerais) and São Paulo.

**Methodology:** A total of 124 samples were collected from different producers. Toxin extraction was performed with 100 mL of solvent (acetonitrile: sodium bicarbonate 0.13 M v/v). A 10 mL aliquot was passed on through an immunoaffinity column (Ochra Test – Vicam) specific for OTA and eluted using methanol:acetic acid (98:02) as solvent. The detection and quantification of OTA were performed in HPLC-FLD (Agilent-USA).

**Results:** OTA concentrations were found from not detected to 9.4 µg/kg for cheeses evaluated in a single part and < 0.2 µg/kg to >1000 µg/kg for cheese analyzed in 2 parts separately (cheese rind and inner part). The data suggest that the occurrence of OTA in Brazilian artisanal cheese may be of concern, especially in cave cheese.



**Conclusion:** More studies are needed for a better diagnosis of the presence of this toxin in these products. In addition, the present work may help the producers to minimize the occurrence of OTA during cheese manufacturing to ensure consumer safety.



## P16 – Development of chromatography methodology to detect Aflatoxin M<sub>1</sub> in cheese

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**Keywords:** AFM<sub>1</sub>, Artisanal cheese, Mycotoxin.

**Introduction:** Brazil is an important world producer of cheese, with Minas Gerais, the state where Serra da Canastra is located, one of the main artisanal cheese production regions in the country. Canastra cheese is manufactured without thermal treatment of milk, which makes the product prone to be contaminated by microorganisms, including fungi. *Aspergillus* and *Penicillium* are two of the most spoilage fungi in food and some species are potentially toxigenic. Aflatoxins (AFs) are among the most frequently found mycotoxins in food and feed. They can occur in cheese due to direct contamination of fungi or through the milk from dairy animals fed with feed contaminated by aflatoxin M<sub>1</sub>. This mycotoxin is metabolized in the animal organism, resulting in aflatoxin M<sub>1</sub>, which is released into the milk. Therefore, this study aimed to develop an effective methodology for aflatoxin M<sub>1</sub> in order to investigate its occurrence in artisanal cheese samples.

**Methodology:** Ten grams of cheese sample were weighed and 50 mL of pepsin solution added. The samples were homogenized, centrifuged (10 min), vacuum filtered and passed through an immunoaffinity column for aflatoxin M<sub>1</sub> (Vicam – USA) to retain the toxin. Subsequently, the toxin was eluted with acetonitrile and dried under nitrogen. The toxin was resuspended in water:acetonitrile:methanol (57:17:26). The extracts were analyzed by high performance liquid chromatography (HPLC, Agilent). The toxin quantification was performed by comparing the retention times with the standard, according to the area of the integrated peak.



**Results:** The developed method showed a recovery of 94.77% with limit of detection of 0.09  $\mu\text{g}/\text{kg}$  and limit of quantification of 0.27  $\mu\text{g}/\text{kg}$ . Analysis of aflatoxin  $M_1$  was carried out from a total of 124 cheese samples collected from different producers from Minas Gerais and São Paulo states.

**Conclusion:** The maximum tolerated limit for aflatoxin  $M_1$  in cheese is 2.5  $\mu\text{g}/\text{kg}$ , which shows the importance of this study to ensure food safety for consumer protection.



## P17 – Micotoxins in summer crops in Uruguay

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**Keywords:** deoxynivalenol, zearalenone, nivalenol, aflatoxins, fumonisins, soybean, sorghum, maize.

**Introduction:** Contamination of grains by mycotoxigenic fungi causes damage to human and animal health, as well as economic losses by affecting crop yield and quality.

**Methodology:** In Uruguay, summer crops destined for animal consumption were sampled for mycotoxins detection. Sorghum grains were sampled in 2016/17 (n=95) and 2017/18 (n=72); whereas maize and soybean grains in 2018/19 (n=92 and n=100 respectively) and 2019/20 (n=58 and n=100 respectively). *Fusarium* species in the three crops and *Aspergillus* in maize and soybean were the main fungal species detected. Deoxynivalenol (DON), zearalenone (ZEA) and nivalenol (NIV) content was analysed in all grains during both harvests. Fumonisins (FB) were quantified in corn and sorghum samples, and aflatoxins (AF) in corn (2018/19) and soybean (2019/20). All analyses were performed by high performance liquid chromatography (HPLC). Results of detection and quantification of mycotoxins are presented below and compared with some of the current regulations of animal food.



**Results:** In sorghum (2016/17 and 2017/18) and corn (2018/19), less than 10% of the samples of the different grains presented mycotoxins concentration above current regulations of animal food. 64-63% of sorghum samples showed ZEA concentration above 250 µg/kg, in 2016/17 and 2017/18 respectively (Uruguayan recommendation); while 51% and 35% of samples in these respective harvests exceeded 500 µg/kg (limit established for corn, as there is no value for sorghum in Chinese regulations). In 2018/19, 22% of corn samples showed concentrations above 250 µg/kg, and 14% exceeded 500 µg/kg (China). Likewise, all medians were below the acceptable limits.

**Conclusion:** To sum up, most of the mycotoxins did not exceed the regulatory limits for animal consumption. However, several samples did exceed maximum values established in some regulations of cereals/grains destined for human consumption. Therefore measures should be taken to reduce fungal infection in crops and mitigate the indirect impact on human health.

This work provides relevant information that could serve as a basis for defining and adopting agricultural management measures to avoid negative impacts of mycotoxins and to establish or update current regulations of each toxin.



## P18 – Potato inorganic composition and its bioaccessible fractions, nutrition or toxicity?

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**Keywords:** Bioaccessibility, nutrition, toxicity, inorganic elements, *in vitro* digestion.

**Introduction:** Food is essential in obtaining nutrients by humans however, it is also considered one of the principal routes of exposure to potentially toxic elements. This is because products such as agrochemicals and fertilizers used in the cultivation of different crops can contain various inorganic contaminants. Being necessary the evaluation of food matrices in relation to their chemical composition. However, it is important to emphasize that not all the ingested substance becomes accessible to be absorbed by the human body, so that for there are nutritional or toxic effects, the elements need to be present in the bioaccessible fraction and indeed be absorbed.

**Methodology:** Thus, this study evaluated the elements in relation to the total contents and their bioaccessible fractions in different potato cultivars, through *in vitro* experiments (based on the INFOGEST protocol) and spectrometric techniques.

**Results:** The highest total contents were observed for the macroelements ( $\text{mg g}^{-1}$ ), while the bioaccessible fractions ( $\% \text{ w w}^{-1}$ ) varied considerably: K (10.27 – 31.98, 57 – 72%), P (1.18 – 2.61, 59 – 76%), Mg (0.42 – 1.38, 83 – 103%) and Ca (0.15 – 0.79, 30 – 123%) and the smallest ( $\mu\text{g g}^{-1}$ ) to Cu (5.16 – 10.35, 27 – 74%) and Mn (4.22 – 12.02, 60 – 119%). Regarding the potentially toxic elements, Cd and Pb were present in 75% of the samples, being below the maximum level allowed by the National Health Surveillance Agency of Brazil ( $0.10 \mu\text{g g}^{-1}$ ), while Cr (0.21 and  $0.80 \mu\text{g g}^{-1}$ ) was only found in two potato skins. However, these elements



were not present in the bioaccessible fractions quantified by GFAAS (LOQ:  $0.063 \mu\text{g L}^{-1}$  Cd,  $0.65 \mu\text{g L}^{-1}$  Cr e  $0.44 \mu\text{g L}^{-1}$  Pb).

**Conclusion:** Since the potentially toxic elements did not present in the bioaccessible fractions, it is observed that, the potato samples studied have shown good nutritional values taking into account the daily recommendation of these nutrients by regulatory agencies. The relevance of these studies is because potatoes are one of the main foods consumed in Brazil, and knowledge about their effective nutritional contribution helps to guarantee the food security for the population of that country.



## P19 – Pesticide control plan in Madeira Island, Portugal

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**Keywords:** pesticide; contaminant; foodstuff; sampling; control

**Introduction:** Chemical contaminants are substances that can be harmful to human and animal health. People are daily exposed to a wide range of chemical contaminants that are unintentionally present in food and water, with negative impacts on health that can be irreversible. Due to the short- and long-term impact on health, legislation has been published in Europe and adopted by European member states. In the Autonomous Region of Madeira (Portugal), the Regional Authority for Economic Activities (ARAE) is responsible to supervise the compliance with legislation in all economic sectors, including the food sector.

**Methodology:** In 2017, ARAE established a protocol with the Regional Directorate of Agriculture and Rural Development, which is responsible for coordinating the control plan of pesticides in this region. Due to this protocol, ARAE performs sampling of foodstuffs for laboratory analysis. The detection of a prohibited active substance or an authorized one in concentrations above the maximum residue level established in European legislation results in the arrest of the entire foodstuff batch and its destruction. In any of these situations, the national phytosanitary and veterinary sanitary authority carries out an assessment of the acute risk to the health of the final consumer, which helps to determine the infraction as an administrative or criminal offense.

**Results:** Between 2017-2021, 646 samples were collected to test for the presence of pesticide residues, 418 (64,7%) of which were positives. The major positivity ratio was detected in 2021 (80,4%). Considering the positive samples obtained, 70 administrative offenses and 12 criminal



offenses were established. Rocha pear, grapefruit, and grapes were the foodstuffs in which the highest number of different pesticides were detected ( $\geq$  eight). Pesticides were detected in 100% of Rocha pears tested.

**Conclusions:** Although a large proportion of the samples did not result in an infraction, pesticides are still present, possibly causing long-term effects. This protocol was an important step forward in the control of these contaminants. It is important in the future to establish protocols for the control of other contaminants.

**Topic relevance:** Exposure to pesticides is associated with short- and long-term negative impact on the health of final consumers.



## P20 – Aflatoxin M<sub>1</sub> in blood plasma and milk of lactating dairy cows fed AFB<sub>1</sub>

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**Keywords:** Mycotoxins; LC/MS/MS; biomarkers.

**Introduction:** Aflatoxins like mycotoxins are toxic secondary metabolites produced by fungi of the genus *Aspergillus* that grow naturally in several agricultural commodities and contaminate food before and after processing, which causes risks to human health and economic losses in animal production. When a dairy cow eats a contaminated feed with AFB<sub>1</sub>, part of this toxin is destroyed in the rumen, whereas the absorbed AFB<sub>1</sub> quickly undertakes a metabolic process in the liver to this toxin be biotransformed in AFM<sub>1</sub>, which is a monohydroxylated derivative of AFB<sub>1</sub>, which is secreted in the milk and may be subsequently transferred into other dairy products.

**Methodology:** The objective of the present study was evaluating the concentrations of aflatoxin M<sub>1</sub> (AFM<sub>1</sub>) in blood plasma and milk from dairy cows receiving 480 µg aflatoxin B<sub>1</sub> (AFB<sub>1</sub>) per day. Furthermore, the effect of different sources of *Saccharomyces cerevisiae* (SC) biomass (20.0 g/d) obtained from sugarcane (cell wall, CW; dried sugarcane yeast, DSY and autolyzed yeast, AY) was evaluated in plasma and milk AFM<sub>1</sub> concentration. A completely randomized design was used with 2 lactating cows assigned to each dietary treatments, as follows: negative controls (no AFB<sub>1</sub> or SC-based biomass), positive controls (AFB<sub>1</sub>), CW, CW + AFB<sub>1</sub>, DSY, DSY + AFB<sub>1</sub>, AY, AY + AFB<sub>1</sub>. The cows in the aflatoxin treatment group received AFB<sub>1</sub> from d 1 to 6, while the SC biomass was administered with the AFB<sub>1</sub> bolus from d 4 to 6. Blood plasma and milk were collected on days 1, 5, and 10 to assess the percentage of AFM<sub>1</sub> in milk and plasma analyzed by LC/MS-MS for AFM<sub>1</sub> quantification.



**Results:** On the first day all treatments showed aflatoxin in milk, while in plasma AFM<sub>1</sub> was detected some treatments: AY and DSY+ AFB<sub>1</sub>. On the 5th day there was no detection AFM<sub>1</sub> in plasma or milk for cows fed without AFB<sub>1</sub>, while AFM<sub>1</sub> was detected in plasma and milk in all animals received AFB<sub>1</sub>. On the 10th day there was no detection of AFM<sub>1</sub> in plasma in either group and a very similar percentage of AFM<sub>1</sub> in milk in both groups, with and without AFB<sub>1</sub> (0.007 – 0.49 µg/kg). This occurred due to the effect of the different sources of to bind AFB<sub>1</sub>.

**Conclusion:** Results suggest that AFM<sub>1</sub> reduction in plasma and milk was proportional in both treatments, with and without AFB<sub>1</sub>, throughout the experiment. The results also show that when cows are subjected to medium levels of dietary AFB<sub>1</sub> contamination, as in the present study, AFM<sub>1</sub> determination in milk was more common than determination in blood plasma.

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## P21 – Comparison of ELISA, LC-MS/MS and Lateral Flow Immunoassay methods for determination of total aflatoxins, fumonisins and zearalenone in corn

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**Keywords:** mycotoxin; corn; LC-MS/MS; immunoassays

**Introduction:** Mycotoxins are toxic secondary metabolites produced by filamentous fungi that occur naturally in agricultural commodities worldwide. Aflatoxins, fumonisins, and zearalenone are among the most important ones for food and feed safety. These compounds can be carcinogenic, mutagenic, teratogenic, neurotoxic, and nephrotoxic. Analytical techniques for detecting mycotoxins have been developed to monitor their concentration in food and feed. Conventional analytical approaches for mycotoxin identification include high-performance liquid chromatography (HPLC), especially coupled to mass spectrometry (LC-MS/MS). However, rapid methods for mycotoxin analysis are also becoming increasingly relevant, as the enzyme-linked immunosorbent assay (ELISA) and Lateral Flow Immunoassay (LFI). The current study aimed to compare LC-MS/MS, ELISA, and LFI for the quantification of total aflatoxins (B<sub>1</sub>, B<sub>2</sub>, G<sub>1</sub> and G<sub>2</sub>), fumonisins (B<sub>1</sub> and B<sub>2</sub>) and zearalenone in Brazilian corn samples after grading from the states of Minas Gerais and Goias (n=100).

**Methodology:** Samples were milled and performed liquid extractions for Aflatoxins, Fumonisins and Zearalenone were performed by ELISA kit from Romerlabs and LFI was from Neogen. LC-MS/MS used was high-performance liquid chromatography coupled to mass spectrometry (LC-MS/MS using an Infinity 1200 Series HPLC, coupled to a 5500 QTRAP mass spectrometer).



**Results:** For aflatoxins and zearalenone, based on statistical results, ELISA and LFI methods are not comparable, ELISA presented highest variability, for LC-MS/MS all samples presented value below 1ppb and 20 ppb, it was not possible to compare them. Fumonisin, based on statistical results, methods are comparable in 95% confidence and ELISA presented highest variability.

**Conclusion:** ELISA, LFI and LC-MS/MS for aflatoxins and zearalenone were not statistically comparable, and ELISA presented high variability, in the other hand fumonisin methods are statistically comparable. ELISA was the method that presented high variability among LFI and LC-MS/MS. Further investigation is required to understand the high variability for ELISA for aflatoxin and zearalenone analysis, for fumonisins the ELISA, IFL and LC-MS/MS techniques can be used for control or regulatory purposes, having in mind their inherent advantages and limitations.



## P22 – Determination of citrinin in red yeast rice and food supplements by ultra high liquid chromatography tandem mass spectrometry

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**Keywords:** citrinin; red dye rice; food supplements; QuEChERS; liquid chromatography tandem mass spectrometry

**Introduction:** Citrinin, a mycotoxin that can cause serious adverse health effects in mammalian species, was identified in many species of *Aspergillus*, *Fusarium*, *Penicillium*, and *Monascus* strains, commonly employed in food manufacturing, being red yeast rice and derived food supplements the main sources of exposure [1].

**Methodology:** Citrinin is extracted from red yeast rice and food supplements by a Quick, Easy, Cheap, Effective, Rugged and Safe (QuEChERS) methodology and determined by ultra-high performance liquid chromatography system coupled to a triple quadrupole mass spectrometer (UHPLC-MS/MS), using isotopically labeled citrinin as internal standard. The method has been validated in accordance with the criteria required by the European Commission Regulation [2].

**Results:** A total of 4 red yeast rice and 5 complements samples were analyzed by the proposed QuEChERS UHPLC-MS/MS methodology, being the citrinin content in all cases lower than maximum limits (100 µg/kg) established by the European Commission [3].

**Conclusions:** Citrinin was detected in all samples analyzed, showing a certain grade of exposure in the population. However, the levels found, far from the legal limits, demonstrate the strength of the food system in Europe.



## P23 – Development of an analytical methodology based on infrared microscopy for microplastic quantification and identification in bivalve mollusks

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**Keywords:** microplastics; bivalve mollusks; infrared microscopy

**Introduction:** The European Food Safety Authority (EFSA) has defined microplastics as “a heterogeneous mixture of particles ranging from 0.1 to 5000 µm in diameter” [1]. Apart from the environmental concerns of microplastics, there is also growing uncertainty about their impact on human health [2]. This work aimed to develop a methodology for quantifying and identifying microplastics in bivalve mollusks, which can accumulate significant amounts of these contaminants and serve as a potential source of exposure for humans.

**Methodology:** The method includes sample preparation consisting of chemical digestion with KOH, protease and density modifiers such as surfactant Tween 80 (Polysorbate 80) for several days, vacuum filtration through silica filters of 1 µm pore size and determination of microplastics by infrared microscopy.

**Results:** Results evidenced the need of a standardized way to interpret the results of the reagent blank. In addition, this work highlights the necessity of harmonized methodologies, including sample preparation and determination, to obtain comparable results. The method was applied to the analysis of 9 samples of bivalve mollusks. The amount of microplastics ranged from 2 to 16 per gram, with polypropylene being the most abundant type of plastic found.



**Conclusion:** This fact represents a serious threat to marine ecosystems and may raise concerns about human health. Reducing the production and consumption of plastics, as well as implementing proper waste management practices, are crucial measures to address this problem and protect both environment and health.



## P24 – Incidence, bioavailability and mitigation strategies for mycotoxins in farmed fish and feeds

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**Keywords:** mycotoxins, aquaculture, food safety, bioavailability

In the last years, the profitability and sustainability of the aquaculture sector has been addressed, with strong efforts being made to replace ingredients from animal sources (fish meals and oils) traditionally used in aquafeeds, by vegetable sources (e.g., cereals). However, this strategy may raise negative implications, as cereals (e.g., corn, wheat) commonly used in aquafeeds often contain high levels of toxic metabolites produced by fungi, i.e., mycotoxins. Consequently, a higher incidence of mycotoxicosis in fish is expected to occur, which can ultimately cause adverse health effects in animals and humans. Little is known about the effects of ingestion of contaminated feeds with mycotoxins by fish, as well as the effects on their gastrointestinal tract (GIT) and absorption efficiency in the gut, thus remaining a serious food safety concern.



Within this context, MycoFish project (PTDC/CVT-CVT/2660/2021) addresses this topic with the main goals: i) assess regulated and emerging mycotoxins' incidence in feed ingredients and aquafeeds; ii) evaluate *in vivo* bioaccumulation and toxicity of the most relevant mycotoxins to farmed fish; iii) develop and optimize models allowing the *in vitro* simulation of nutrients/contaminants bioaccessibility and bioavailability in fish GIT, with subsequent *in vivo* validation; and iv) assess the potential human health risks from mycotoxins exposure and propose mitigation strategies to reduce mycotoxins toxicity through the use of natural detoxifying antioxidants. This innovative project joint efforts of 3 renowned research organizations (IPMA, MARE-FCUL and REQUIMTE) with expertise in aquaculture, food safety and optimization/validation of analytical methods. Overall, MycoFish will evaluate the risk of mycotoxins contamination in aquafeeds, in order to implement robust risk assessment, ensure maximum protection for farmed fish consumers and farmers, with the delivery of safe and high quality products for consumers, and ultimately ensure and improve the sustainable development of marine resources and the aquaculture sector.

**Acknowledgement:** This work was supported by the Project MycoFish – Occurrence, bioavailability and mitigation strategies for mycotoxins in farmed fish and associated feed ingredients: Gilthead seabream as case study (PTDC/CVT-CVT/2660/2021).



## P25 – Assessing Contaminant Uptake and Elimination in Insects: Implications for Sustainable Food Production and Waste Management.

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**Introduction:** Considering the global food crisis and the pressing issue of waste generation, sustainable solutions for food demand are crucial. Insects have emerged as a promising protein source for food and feed due to their efficient bioconversion capacity and ability to utilize non-valuable products. However, ensuring the safety of insects for food and feed is essential. This study aims to comprehensively evaluate the uptake and elimination of contaminants by insects, going beyond conventional measurements of contamination levels.

**Methodology:** The study employed two-phase bioaccumulation studies involving an uptake phase with a contaminated substrate followed by an elimination phase in a clean substrate. Data from these studies were used in toxicokinetic modelling. The objective was to assess the bioaccumulation capacity of mercury (Hg), cadmium (Cd), lead (Pb), arsenic (As), and various polycyclic aromatic compounds (benzo[a]pyrene [B[a]P], benz[a]anthracene [B[a]A], benzo[b]fluoranthene [B[a]F], and crysene [Cry]) in black soldier fly (BSF) larvae (*Hermetia illucens*). Although high metal bioaccumulation potential (e.g., for Hg, Cd, Pb,



As) in edible insects has already been identified as risk, there is a gap regarding hazard assessment of the organic contaminants (e.g., B[a]P, B[a]A, B[a]F, Cry).

**Results:** The findings indicate that when insects were exposed to the maximum allowed levels of metals according to European Union feed regulations, they accumulated contaminant levels unsuitable for feeding other animals. However, after a depuration period, the insects could reduce the contaminant concentrations to comply with the existing legislation. For instance, a 4 to 5 days depuration period was found sufficient for safer consumption of insects exposed to Hg, while a longer period was required for Cd elimination. Interestingly, no significant accumulation of polycyclic aromatic compounds was observed when insects were fed at the maximum allowed levels.

**Conclusion:** These findings have significant implications, particularly when insects are employed as waste converters, where concerns arise regarding contaminants. This study supports the implementation of a depuration period after exposure to waste that may vary depending on the classes of contaminants involved. Toxicokinetics is an adequate tool for the safety assessment of insects as feed and food, but it also defines the conditions that will assure their sustainable use in waste management.

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## P26 – Determination of Four Polycyclic Aromatic Hydrocarbons in Smoked Sausages from Brazil

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**Keywords:** Sausages; smoking food; PAHs; Ultra-high performance liquid chromatography (UHPLC).

**Introduction:** Polycyclic aromatic hydrocarbons (PAHs) are contaminants with two or more aromatic rings. They are carcinogenic agents that can be generated during smoking process, that involves the incomplete combustion of organic matter, such as wood or coal, and form these compounds that can deposit on the surface of foods. The Regulation N<sup>o</sup>. 835/2011 of European Community establishes limits for four priority PAHs (PAH4) in smoked meats, including benz(a) anthracene, chrysene (Chr), benzo(b)fluoranthene and benzo(a) pyrene (BaP), and the values are 2.0 and 12.0 µg/kg, for BaP and PAH4, respectively. The objective of this work was to evaluate the contamination of PAH4 in commercial smoked sausages from Brazil.

**Methodology:** Ten samples of commercial smoked sausages from São Paulo city, Brazil, were evaluated and PAH4 were analyzed. The method included saponification, liquid-liquid extraction with n-hexane, purification with silica solid phase extraction (SPE) cartridges, and quantification by ultra-performance liquid chromatography with fluorescence detection.

**Results:** The concentration ranges found for BaP and PAH4 were <0.50-2.44 µg/kg and <2.50-22.54 µg/kg, respectively. Chr was the most frequent hydrocarbon and with the highest concentrations (<0.50-



13.78 µg/kg). The component with the lowest average concentration was BaP. About the Regulation No. 835/2011, three samples were considered unsatisfactory, one for BaP and two for PAH4.

**Conclusion:** The results indicated that some products were unsatisfactory to the permitted levels in the European Union, 10% (1/10) of samples for BaP, and 20% (2/10) for PAH4, respectively. Some products showed unsatisfactory results, indicating that the monitoring of PAHs in processed foods should be frequent since some PAHs are widely referred to as carcinogens.

**Relevance of the topic:** Some factors may contribute to the formation of PAHs in smoking, including type of smoking (direct or indirect way), type of product wrapping, smoking conditions (humidity, temperature, time and air flow), type of fuel used, food composition, among others. Industries and manufacturers of smoked products must follow good practice codes to control these variables, such as the Codex Alimentarius document CAC/RCP 68/2009.



## P27 – The presence of polycyclic aromatic hydrocarbons (PAHs) in commercial breads from Brazil

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**Keywords:** Bread; PAHs; High performance liquid chromatography-fluorescence detector.

**Introduction:** Bread is among the most consumed foods by the Brazilian population, with an average daily consumption of approximately 51.2g per person. The baking process favors the formation of food contaminants, such as polycyclic aromatic hydrocarbons (PAHs). These contaminants can lead to the development of cancer, with benz(a)anthracene, chrysene, benzo(b)fluoranthene, and benzo(a)pyrene (BaP) being the most frequently found in food. Therefore, there are maximum limits in food (1.00 µg/kg for BaP and ΣPAH4) established by the European Community, according to Regulation N<sup>o</sup>. 835 of 2011, due to their mutagenic potential. The quantification of PAHs in this type of food is fundamental considering the volume of these products consumed by the population.

**Methodology:** The study was conducted using convenience sampling, where thirty commercial breads samples (loaf bread, whole wheat bread, white bread and australian bread) from the city of São Paulo, Brazil, were analyzed for PAH4. The methodology for extracting the compounds involved liquid-liquid extraction using n-hexane, purification with silica cartridges for solid-phase extraction, and quantification using high-performance liquid chromatography with fluorescence detection.



**Results:** Among the thirty commercial breads analyzed, 27 samples detected at least one of the monitored PAHs, and the most frequently identified were BaA and Cri. Concentrations between  $<0.25$  and  $5.96$   $\mu\text{g}/\text{kg}$  of BaP were detected among 27 samples, and values above the limit of  $1.0$   $\mu\text{g}/\text{kg}$  were found in seven samples ( $1.16$  to  $5.96$   $\mu\text{g}/\text{kg}$ ). Regarding the sum of PAH4, 21 samples showed unsatisfactory values ( $1.26$  to  $19.00$   $\mu\text{g}/\text{kg}$ ).

**Conclusion:** The results reveal that some commercial breads have concentrations of BaP and sum PAH4 above the satisfactory levels, which may contribute to an increase human exposure to these carcinogenic agents.

**Relevance of the topic:** The type of thermal processing and the ingredients used in bread composition play a crucial role in the formation of PAHs in bread. Knowledge the origin of ingredients used in breadproduction (wheat flour, grains, and spices) and the constant monitoring of raw materials are important steps in reducing contamination throughout the food production chain and considering the population's exposure to the carcinogenic agents.



## P28 – Occurrence of contaminants and food additives in the Portuguese food chain: data collection and transmission of official control samples to EFSA from 2017 to 2023

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**Keywords:** Data, Harmonisation, Transmission, EFSA, SSD2, Contaminants, Additives

**Introduction:** The collection of accurate, reliable and comparable data on food and feed is essential to monitor their safety and to support informed dietary exposure and risk assessments and management, contributing to higher consumer safety and health.

The National Institute of Health Doctor Ricardo Jorge (INSA) is responsible for gathering, harmonising and electronically submitting to the European Food Safety Authority (EFSA), according to their reporting requirements, the information on food and feed contaminants and food additives from the official control plans in Portugal and from studies performed in INSA laboratories. We aim to present the process of data collection and transmission to EFSA and the main results during the period 2017-2023 (samples collected in 2016-2022).

**Methodology:** Annually, INSA gathers information on contaminants and additives from the official control plans of the Portuguese authorities and from INSA studies. This information was reported according to the EFSA Standard Sample Description for Food and Feed (SSD2), complemented by FoodEx2, a standardised system for classifying and describing food, to ensure data harmonisation, standardisation and quality. It were used EFSA's Excel Tools and Catalogue Browser to facilitate the process.

**Results:** Between 2017 and 2023, 62963 analytical results were gathered and transmitted to EFSA according to SSD2, almost entirely



on contaminants (61054). These results correspond to 12214 food samples collected. During this period, of all the reported results evaluated (59021) only 0.2% (129) were non-compliant (6.3% (3942) were not evaluated because they corresponded to studies). Animal feed data were reported in 2023 but can only be accounted for next month.

**Conclusions:** The use of the SSD2 and FoodEx2 data model and system and all other tools made available by EFSA ensure data harmonisation, standardisation, and overall quality, consistency, and reliability in the information reported.

During the period under review, there was a high level of compliance (99.8%) in the results of samples collected under the Portuguese official control plans.

**Topic relevance:** Up-to-date, reliable and comparable information across Member States is crucial to both scientists carrying out risk assessments and decision-makers ensuring food and feed safety, ultimately aiming to protect and promote consumer health in Europe.



## P29 – Total mercury in marine bivalves, gastropods and echinoderms from the Portuguese continental coast

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**Keywords:** Bivalves, marine gastropods, echinoderms, mercury.

**Introduction:** Bivalves, marine gastropods and some echinoderms, as sea urchin, can represent a good source of protein, being often caught in Portugal for human consumption. However, these species can concentrate contaminants in their organism, namely mercury that can have a telluric origin. Thus, the objective of this work was to obtain more data on these species, especially on marine gastropods and echinoderms from the Portuguese continental coast, with regard to the concentration of this metal, and, at the same time compare its contents among the various studied species.

**Methodology:** About 340 samples between 2020 and 2022 were analysed. All body were considered edible for bivalves and marine gastropods, except for knobbed triton where viscera were removed. In what concerns sea urchin only gonads were evaluated. Total mercury (THg) quantification was performed by atomic absorption spectrometry, according to standard method.

**Results:** Median concentrations of THg levels in all samples of bivalves (0.013 mg/kg), marine gastropods (0.011 mg/kg) and echinoderms (< 0.011 mg/kg) were lower than the EU limits. The highest levels were obtained in marine gastropods with a maximum value of 0.33 mg/kg obtained in knobbed triton (*Charonia rubicunda*), surpassing the actual EU limit of 0,30 mg/kg. Bivalves presented values between < 0.004 and 0,031 mg/kg, much lower than the EU limit of 0,50 mg/kg. Sea urchin



were the species that showed the lowest THg contents, only attaining a value of 0,020 mg/kg.

**Conclusion:** The high levels of THg in knobbed triton may probably be associated with the fact that they are predators, as they eat bivalves after chipping or drilling their shells

**Acknowledgments:** This work was funded by project SNMB-Monitor IV.



## P30 – Contaminant metals in marine macroalgae from the Portuguese continental coast

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**Keywords:** cadmium, total mercury, lead

**Introduction:** The use of edible seaweed is currently marketed in Europe as it is a natural food with high nutritional value but low in calories. They are low in fat and have polysaccharides that behave, in general, as fibers without caloric value, having minerals (iron and calcium), proteins (with all the essential amino acids) and vitamins, essentials for the proper functioning of the human body. There are several applications of macroalgae in food products such as pasta, bread and snacks, as well as direct consumption in salads or soups. However, it is also known that algae can retain pollutants from the habitat, namely contaminant metals. Thus, the objective of this work was to evaluate the contamination by cadmium, total mercury and lead in several macroalgae species existing on the Portuguese continental coast, such as *Undaria pinnatifida*, *Porphyra sp.*, *Osmunda pinnatifida*, *Gracilaria sp.*, *Saccharina latissima* and *Saccorhiza polychides*.

**Methodology:** The analysis was carried out by atomic absorption spectrometry on freeze-dried fresh samples according to reference methods and the results are expressed in dry weight.

**Results:** Total mercury concentrations obtained in the studied macroalgae were always below the limits indicated by French and European legislation (considering the dry seaweed as a food supplement). As for the results attained for lead, these were also always lower than the limit of 5 mg/kg dw. With regard to the levels found for



cadmium, these varied between 0.14 mg/kg dw (*Saccharina latissima*) and 1.3 mg/kg dw (*Porphyra umbilicalis*). This concentration is higher than the 0.5 mg/kg, dw, value indicated by French legislation. However, for food supplements consisting exclusively or mainly of dried seaweed or of products derived from seaweed, the maximum allowed level of cadmium is set at 3.0 mg/kg in the European Regulation 2023/915.

**Conclusion:** A more detailed study on other seaweed contaminants such as total and inorganic arsenic and also iodine are needed in order to allow a risk/benefit approach related to the consumption of seaweed and seaweed products.

**Acknowledgment:** This work was funded by project I9+ PROALGA (Ref.: 16-01-03-FMP-0011). The authors are grateful to Professor Leonel Pereira and João Cotas (University of Coimbra) for the collection and identification of seaweeds and to Margarida Muro for her technical assistance on analytical methods.



## P31 – Overview of *in vitro* approaches to investigate chemical contaminants intestinal transport

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**Keywords:** food, chemical contaminants, intestinal transport

**Introduction:** The occurrence of chemical contaminants in food products is a serious threat to public health and can lead from mild gastroenteritis to fatal liver, kidney and neurological syndromes. The main route of exposure to chemical contaminants is ingestion, with the intestine being the first organ to come into contact with these compounds. *In vitro* intestinal cell assays may constitute a useful tool to predict the impact of chemical contaminants on the intestinal barrier. Differentiated Caco-2 cells cultivated in porous Transwell inserts, as an *in vitro* model to evaluate the intestinal transport of food chemical contaminants, is recurrent. These toxicants can be transported via transcellular (transport that occurs through the epithelial cell) or paracellular (transport that occurs in between cells) pathway.

**Methodology:** This study aims to make an overview of *in vitro* approaches to investigate chemical contaminants intestinal transport and associated pathways. A bibliographical search was carried out in two databases of scientific publications, "PUBMED" and "WEB OF SCIENCE", using the keywords "food", "chemical contaminants", "cell transport", in scientific publications from 1999 to 2021. Forty publications were used for data extraction.



**Results:** In sum, Caco-2 cells were the most frequently used model for the evaluation of the food contaminants intestinal transport. In a few works, this cell line was combined with the human HT-29 cells. In other studies, specifically evaluating the implication of membrane transporters, Madin-Darby canine kidney (MDCK) cells were used. HTC-8 intestinal organoids were also reported and gastric absorption of food toxicants was described in a single work using NCI-N87 cells. The use intestinal mucosa explants mounted in Ussing chambers, in studies of food contaminants intestinal transport in farmed animals, was also reported, although in much less extension.

**Conclusion:** The transcellular route was the most reported pathway for chemical contaminants, with paracellular being less referred (mycotoxins and chloropropanols). Further studies concerning absorption and transport of chemical contaminants are needed to better characterize its permeability across epithelial membranes which will contribute to a comprehensive understanding of its impact on human health. In addition, their frequent co-occurrence raises an additional number of questions that can only be answered using high-throughput *in vitro* methods.

**Acknowledgement:** Thanks are due to the Early Myco project (PTDC/MED-TOX/28762/2017), and to CESAM (UIDP/ 50017/2020+UIDB/50017/2020+LA/P/0094/2020), funded by FCT/MCTES through national funds.



## P32 – Levels of manganese, iron, zinc and mercury in vegetarian foods

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**Keywords:** manganese, iron, zinc, mercury, vegetarian foods

**Introduction:** The increasing popularity of vegetarian diet has induced science to better study these foods. As long as it includes the necessary intake of nutrients, such as minerals, can be beneficial to health. In addition, concern about food safety has increased in recent years, leading to studies to evaluate food contamination by toxic metals, regarding food poisoning prevention and public health improvement. The purpose of this work was to quantify manganese, iron, zinc and mercury contents in different foods for a plant-based diet.

**Methodology:** Mineral levels were determined by Inductive Plasma Coupled Optical Emission Spectrometry (ICP-OES). Total mercury content was determined by a thermal decomposition and amalgamation atomic absorption spectrophotometry (TDA/AAS).

**Results:** Manganese level ranged from 0.10 mg/100g (<Limit of Quantification (LoQ)) in mushrooms, vegetable drinks and margarines to 22.6 mg/100g in seaweed Nori. Content of iron ranged from 0.22 mg/100g (<LoQ) in bamboo shoots mushrooms, brown mushrooms vegetable drinks, rice vegetable drink and margarines to 25.8 mg/100g in seaweed Nori. Level of zinc ranged from 0.22 mg/100g (<LoQ) in shoots, vegetable drinks and vegetable yogurt to 2.98 mg/100g in seaweed Nori. Regarding mercury content, all samples presented levels below LoQ (1.1 µg/kg), with exception of pleurotus mushrooms, that presented a low but quantified value (8.9 µg/kg).

**Conclusion:** It was found that, in general, the analyzed foods presented



mineral values below the Recommended Dietary Allowance (RDA) per 100 g of food, with exception of seaweed Nori, that exceed the RDA of manganese and iron, and chickpea that surpasses the RDA of manganese. This study provides support for future studies regarding mineral content in vegetarian foods and supply the Portuguese Food Composition Table. As expected, vegetarian food samples presented low levels of studied minerals, with the exception of grain and seaweed. Since mercury is a contaminant, pleurotus mushrooms may require attention to its presence, despite there is no legislation for mercury levels in mushrooms. It is concluded, therefore, that the vegetarian dietary pattern must be well planned, taking into account the importance of a varied, balanced and complete diet, including adequate food choices, in order to supply the deficit and reduce exposure to toxic substances.



### P33 – Elemental profiling and health risk of Serbian orange wines

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**Keywords:** Food safety; Lifetime Cancer Risk; Non-carcinogenic risk

**Introduction:** Orange wine is a type of wine made from white wine grapes where the grape skins stay in contact with the juice and supply coloring pigment, in contrast with conventional white wine production. The aim of the present study was to obtain elemental profiles of orange wines and to assess associated health risk.

**Methodology:** Elemental profiles (Be, B, Al, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Se, Sr, Mo, Cd, Sn, Sb, Te, Ba, Hg, Tl, Pb) of 24 bottled orange wines were obtained using ICP-MS. Adult men and women exposure was estimated taking into account wine consumption on population average, regular drinkers only and chronic heavy drinkers, according to the World Health Organization data, as well as Serbian food consumption survey.

**Results:** The study revealed rather versatile elemental profiles, but not one indicating potential for non-carcinogenic risk (all samples had hazard indices below 1, with maximum at 0.44) in any of the consumption scenarios. Both mean and high (last quartile mean) level of margin of exposure (MOE) associated to lead nephrotoxicity were over 10, for men, women and both sexes together. The lowest MOE was obtained for highly exposed males (consumers only – national consumption data, MOE 11). With regard to carcinogenic risk of arsenic, MOE approach indicted no risk (minimum MOE 20). Conversely, lifetime cancer risk approach showed that tolerable risk of 1 extra lifetime cancer case per 100,000 persons was exceeded across the exposure scenarios, by up



to 9 (men), 6 (women) and 8 (both sexes) wine samples. However, risk corresponding to mean exposure was acceptable, while high level of exposure resulted with increased risk for chronic heavy drinkers and consumers only, both men and women.

**Conclusion:** Orange wines are still relatively rare in Serbia and there is a lack of data related to their quality and safety. Elemental composition is influenced by endogenous sources, conditions of grape growth and winemakers' interventions and multi-element profiling could contribute to wine differentiation in terms of geographical origin. No less important, certain elements influence sensory properties of wine.



## P34 – Ethyl carbamate content and health risk associated to Serbian orange wines

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**Keywords:** Food safety; Lifetime Cancer Risk; Ethyl carbamate; Ochratoxin A

**Introduction:** Orange wine is a type of white wine made by leaving the grape skins and seeds in contact with the juice, creating a deep orange-hued product. Wine may contain various toxic compounds, including ethyl carbamate (EC) and mycotoxin ochratoxin A (OTA), probable and possible human carcinogen, respectively. EC can be formed from different precursors (urea, citrulline, cyanides) which could be produced during the process of fermentation, while OTA is produced by different *Aspergillus* and *Penicillium* fungus.

**Methodology:** A total of 24 Serbian orange wines were subjected to GC-MS analysis of EC and HPLC-FLD analysis of OTA. EC and OTA content were evaluated in terms of associated health risk, taking into account wine consumption on population average, regular drinkers only and chronic heavy drinkers, according to the World Health Organization data, as well as Serbian food consumption survey.

**Results:** Presence of OTA was not detected in any of the samples, hence the position 'no exposure, no risk' was taken. On the other hand, EC was quantified in half of the samples, in concentrations up to 16.8 µg/L. Resulting margins of exposure (MOE) were in the safe zone, above 10,000, at both mean and high exposure levels. Lifetime cancer risk (LCR) approach based on EC oral slope factor showed that tolerable risk of 1 extra lifetime cancer case per 100,000 persons was



exceeded in chronic heavy drinking and consumers only consumption scenarios at mean exposure of men and high exposure of women, while high exposure of men indicated risk concerns across all exposure scenarios. However, LCR based on EC virtually safe dose was negligible at both mean and high exposure levels, for both men and women, in every exposure scenario.

**Conclusion:** According to the World Health Organization, three million deaths every year result from the harmful use of alcohol. However, the well known connection between moderate wine consumption and substantial health benefit should not be ignored – it is necessary to secure that the benefit outweighs the risk, in case of orange or any other wine.



## P35 – Mycotoxins in infant foods: bioavailable fraction through Caco-2 cells system

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**Introduction:** Mycotoxins are secondary metabolites produced by fungi that occur naturally in foodstuffs, which can cause a large variety of toxic effects on vertebrates, including humans. The fact that some mycotoxins are carcinogenic (e.g., aflatoxins) and could be detected in breast milk, food and feedstuffs constitutes a serious human and animal health threat, mainly to children and young animals due to their high physiological vulnerability. In addition, bioavailability data are important items included in modern exposure assessment modelling approaches for mycotoxin mixtures in infant foods.

**Methodology:** As part of an ongoing Thematic Project supported by FAPESP, a set of *in vitro* methodologies using caco-2 cells (Assunção et al, 2014; 2016) that simulates the transport across the intestinal membrane, from apical to basolateral sides of intestinal membrane, will allow to elucidate the bioavailable portion that arrives to the intestinal and that is able to cause toxic effects on cells. Caco-2 cells derived from the American Tissue Culture Collection (ATCC) will be maintained at 37 °C in a humidified atmosphere containing 5 % CO<sub>2</sub> in Dulbecco's modified Eagle medium (DMEM) supplemented with 25 mM HEPES buffer, 4.5 g/L glucose, L-glutamine, and phenol red and without pyruvate.

**Expected results:** Cell viability will be also assessed. Briefly, Caco-2 cells will be grown to 100% confluence in 96 well microplates and then treated with AFB<sub>1</sub>, FB<sub>1</sub>, OTA, ZEN and DON alone or in combination for up to 12 h. The absorbance of each well will be read at 570 nm using a plate reader.



**Conclusion:** This task represents a potential challenge of the project, considering the diversity of the worldwide regulated mycotoxins found in contaminated foods, alone or in combinations. However, the outcomes will greatly contribute for a more accurate determination of the *in vitro* bioavailability of regulated mycotoxins in Brazil and the risk assessment of mycotoxins in foods generally consumed by young children.

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## P36 – Toxic inorganic elements in plant-based beverages: total concentration and dietary exposure

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**Keywords:** ICP-MS; inorganic contaminants; food analysis; intake estimation; plant-based food

**Introduction:** The environmental and the ethical benefits of plant-based beverages are well established. However, there have been concerns about their nutritional and safety profile due to the lack of information regarding the inorganic element contents in these foods.

**Methodology:** This study evaluated by ICP-MS the presence of elements with toxic potential (Al, Cr, Co, Ni, As, Mo, Cd, Ba, Sb, Pb, and Hg) in six cashew nut-based beverages marketed in Brazil and assessed the dietary exposure. The sample preparation was performed using a simple and rapid method assisted by ultrasound: 80°C for 35 min, using HNO<sub>3</sub> and H<sub>2</sub>O<sub>2</sub>. Standard body weights of 60 kg (adults) and 15 kg (children) as well as daily consumption of 200 mL of the beverage were used to estimate the daily intake (DI). To characterize the risk associated with exposure, the DI values were compared to the available health-based guidance values established in the literature.

**Results:** The results showed that the total contents of the inorganic elements As, Cd, Pb, Sb and Hg were below the limit of quantification (LOQ = 4 µg kg<sup>-1</sup>) for all samples, while total contents (µg kg<sup>-1</sup>) for Al (<200-15345), Cr (<4-43), Co (<4-22), Ni (74-437), Mo (6.2-18) and Ba (29-16113) were found above the LOQ for some samples. High levels of



Ni (437 and 433  $\mu\text{g kg}^{-1}$ ) and Ba (241 and 16113  $\mu\text{g kg}^{-1}$ ) were observed for beverages containing cashew nut + cocoa and cashew nut + Brazil nut. A highlight was Ba level in the sample containing Brazil nuts, as it presented a result more than 100 times higher than the other samples.

**Conclusion:** The exposure assessment demonstrated that risks to health are unlikely for most of the elements studied. However, higher levels of Ni and Ba observed in some samples, indicate a need for monitoring these elements, especially to protect the health of children. The present results are unprecedented and can be a relevant tool in providing data on inorganic contaminants in plant-based beverages, contributing to future regulations for these foods.

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## P37 – ARTEMIS: evaluation of the exposure of Portuguese mothers and children to environmental chemicals

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**Keywords:** Chemical exposure; Children; Women of childbearing age; Human Biomonitoring; Portugal.

**Introduction:** Nowadays we are exposed to numerous hazardous chemicals that are present in the environment that surrounds us. For some of these chemicals an important route of exposure is through diet, through the consumption of contaminated food or water. Some chemicals can naturally occur in certain foods, such as mercury in fish due to environmental contamination, while others may contaminate food during various stages, including storage, preparation, cooking, or handling. For example, bisphenols, commonly found in plastic containers, can leach into food and contribute to contamination. The project ARTEMIS aims to assess the current environmental exposure of Portuguese children and women of childbearing age, vulnerable groups of the population, to selected chemicals, particularly metals and endocrine disruptors.

**Methodology:** One rural and one urban area will be selected in the regions of Lisbon and Tagus Valley and Alentejo. Recruitment of children aged 6-11 years old and their mothers aged 45 years or less residing in



the selected areas will be done through schools. Hair, urine and saliva samples will be collected for the analysis of mercury (hair), other metals (including cadmium and arsenic) and bisphenols (urine) and salivary proteome (saliva). Information on socio-demographic characteristics, nutrition, lifestyles, health, and specific information related to the exposure to the chemicals to be analysed will be collected through CATI (Computer-Assisted Telephone Interviewing). The remanescence of the biological samples will be stored under appropriate conditions to allow the future analysis of additional chemicals in the samples.

**Expected results:** ARTEMIS will be an innovative project that will not only produce much needed data on the current exposure of the Portuguese population to chemicals, but will also allow the evaluation of time trends of exposure to some chemicals (by comparison with the results from previous projects) and the study of the associations between exposure levels and biomarkers of effect. Ultimately, ARTEMIS's results may support the development of policy measures aimed at minimizing exposure and improving the health of the population.

**Funding:** ARTEMIS is funded by the Comprehensive Health Research Center (CHRC) Research Grants 2022.



## P38–Potential effects and toxicity of exposure to Tetrabromobisphenol A (TBBPA) on mussels (*Mytilus galloprovincialis*)

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**Keywords:** TBBPA; *Mytilus galloprovincialis*; Biomarkers; Oxidative stress.

**Introduction:** Tetrabromobisphenol A (TBBPA) is a brominated flame retardant widely used in several industrial products. TBBPA has been identified as a potential environmental contaminant, causing endocrine disruption, neurotoxicity, immunotoxicity, nephrotoxicity, and hepatotoxicity. Bivalves are often used as biological models to study xenobiotic exposure due to their wide distribution, exposure environment and ability to accumulate contaminants in their tissues. Biomarkers, such as enzymes involved in the biotransformation process of xenobiotics (phase I and II) and antioxidant enzymes, are used in environmental toxicology studies.

**Methodology:** The study evaluated the effects of exposure to the compound TBBPA on *Mytilus galloprovincialis* (n=32), using different concentrations (0, 1, 10 and 100 µg/L) over 28 days. Biomarkers related to oxidative stress, Glutathione S - transferase (GST), Superoxide dismutase (SOD), Catalase (CAT), Lipid peroxidation (LPO), total antioxidant capacity (TAC), signalling of proteins to be degraded, ubiquitin (UBI), cell apoptosis - caspase-3 (CASP) and nerve impulse propagation function, acetylcholinesterase (AChE) were investigated.

**Results:** For GST, CAT, LPO, TAC and CASP, the statistical analysis revealed significant differences ( $p < 0.05$ ) between the control group



and the animals exposed to 100  $\mu\text{g/L}^{-1}$  of TBBPA of ( $p = 0.0139$ ), ( $p = 0.0033$ ), ( $p = 0.0009$ ), ( $p = 0.0100$ ) and ( $p = 0.0022$ ), respectively. In the case of AChE, the statistical analysis revealed significant differences between the control group and the animals exposed to 10  $\mu\text{g/L}^{-1}$  of TBBPA ( $p = 0.0031$ ) and animals exposed to 100  $\mu\text{g/L}^{-1}$  ( $p = 0.0047$ ). For SOD and UBI, the statistical analysis did not find significant differences ( $p > 0.05$ ) in SOD enzyme activity and ubiquitin concentration.

**Conclusions:** These correlations provide insights into the interplay between various biomarkers and suggest potential relationships between oxidative stress, apoptosis, and the defense mechanisms activated in mussels exposed to TBBPA. The most pronounced exposure concentration to observe the toxic effects of TBBPA on *M. galloprovincialis* was 100  $\mu\text{g/L}^{-1}$ .

**Topic relevance:** These results may be relevant to assess the risks associated with exposure to TBBPA in aquatic environments and to take appropriate mitigation measures to protect the marine ecosystem's health.



## P39 – Phage treatment for Salmonella Enteritidis reduction in liquid whole eggs

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**Keywords:** Salmonella Enteritidis; Phage treatment; Liquid whole egg; Food safety.

**Introduction:** Bacterial foodborne pathogens are a huge health threat as they are responsible for foodborne diseases. Food contamination by these bacteria can occur at any stage of the food production, transport and consumption chain. Salmonella enterica has become a foodborne pathogen of serious concern to the food industry being a major cause of foodborne illness worldwide. Outbreaks of salmonellosis have been reported in different food matrices, with eggs as the most implicated food in human salmonellosis. The most prevalent serovar isolated from egg contents is Salmonella enterica serovar Enteritidis. Despite the available decontamination methods, bacterial contamination of food continues to occur. Therefore, new strategies to overcome this problem are needed. For instance, phages, viruses that only infect bacteria, proved to be an effective strategy to inactivate foodborne bacteria.

**Methodology:** In this study, phage phSE-P1 was used to inactivate *S. Enteritidis* in liquid whole egg, at 25 °C. The assays were previously performed in Tryptic Soy Broth (TSB) with a multiplicity of infection (MOI, the ratio between the number of phages and bacteria) of 1, 10, 100 and 1000. All assays included a bacterial control containing only the *S. Enteritidis* bacterium, a phage control containing only the phage phSE-P1, and a sample containing both the bacterium and the phage.



**Results:** In TSB the maximum reduction of *S. Enteritidis* was around 4 log colony-forming units (CFU)/mL for the different values of MOI tested. In liquid whole egg, phage phSE-P1 led to a maximum reduction of *S. Enteritidis* of about 6 log CFU/mL for a MOI of 1000, after 12 h. After

24 h of incubation, phage phSE-P1 was able to maintain a bacterial reduction of around 5 log CFU/mL, which was not observed for the lower MOI values tested.

**Conclusions/Topic relevance:** Overall, the use of phages as an eco-friendly procedure for foodborne pathogens inactivation seems to be a promising approach for food decontamination in order to improve food safety.

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## P40 – Emerging contaminants in aquatic environments and bioaccumulation in food webs with potential impact in human health

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**Keywords:** aquatic pollution, emerging contaminants, bioaccumulation, food webs, human health.

**Introduction:** Humans are daily exposed to complex mixture of chemical substances. Environmental pollution by emerging contaminants (industrial compounds, pesticides, metals, plastics, pharmaceuticals, psychoactive substances, among others) is a worldwide public health problem since they are continuously introduced in aquatic and terrestrial ecosystems. Their presence in water bodies, high persistence and liposolubility increase their potential toxic effects. In this way, these compounds can bioaccumulated transferring through the food chain reaching human body causing several health problems mainly related with chronic exposure. Humans can be affected also via drinking water. This mini-review is related with aquatic emerging contaminants bioaccumulation in food webs and their impacts on human health.

**Methodology:** Scientific literature search was performed using *ScienceDirect* and *PubMed* data bases, looking for: water pollution, emerging contaminants, food webs, human health, food contamination.

**Results:** This mini-review confirm that the presence of emerging contaminants in water bodies is an increasing global issue. Among



the different forms of human exposure of toxics, food ingestion is the main potential route of entry of toxicants into the human body. Seafood from industrialized areas or top predator aquatic animals have been recognised as a main source of contaminants in human diet. For example, filter-feeding bivalve molluscs, benthonic and top predator fish are able to bioaccumulate organic and inorganic pollutants (presents in water, sediments, or prey fish) and transfer them to humans through consumption. Thus, chronic ingestion of contaminated food can cause serious illness, including food poisoning, liver and kidney damage, cardiovascular disorders, neurotoxicity, teratogenicity, endocrine disruption, and, more seriously, death.

**Conclusion:** In conclusion, it is essential to reduce the entrance of toxics (mainly persistent and high *Kow*) in the environment, air, soil, and aquatic ecosystems since they may be difficult to remove. Thus, compounds enter in food chains affecting humans, principally pregnant women or may become pregnant and early childhood. Additionally, is important to assess the presence of this toxics in food to address better advice mainly for these sensitive groups.

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## P41 – Emerging contaminants in soil, food contamination and human health impacts

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**Keywords:** soil pollution, emerging contaminants, food chain, human health.

**Introduction:** Soil supports terrestrial life and give vital ecosystem functions and services. In addition, soil can act as an “universal sink” for contaminants such as heavy metals, pesticides, industrial compounds, plastics, pharmaceuticals, psychoactive substances, PCBs, among others. Consequently, also serves as a source of pollutants that contaminated other environmental compartments like water bodies. Moreover, the soil is the basis of the agro-food system producing about 95% of the food we eat, being essential for the production of safe/healthy feed and food to sustain human populations (food security). Thus, the accumulation of toxic chemicals in soil can result in increased of human exposure through inhalation, dermal contact, or soil ingestion, but mainly through indirect exposure through food or water ingestion. This work aimed to make a mini-review related with soil pollution causing food contamination and human health consequences under chronic exposure.

**Methodology:** The scientific literature was performed using *ScienceDirect* and *PubMed* search engines, looking for: soil pollution, emerging contaminants, food contamination, and human health.



**Results:** This mini-review shows that land pollution reduces the ability of the soil to produce feed and food, and increases the contamination of feed, food crops and food chains. The pollutants that enter to human body through the food chain can cause various diseases after long exposure time, such as cholera, cancer, cardiovascular diseases, teratology, among others, and thus, the absorption of these substances in fruits, vegetables, meats becomes a serious problem. The European Commission, and the EU Action Plan for 2050: *A Healthy Planet for All* have determined that soil pollution must be reduced to levels that do not cause harm to human or ecosystem health.

**Conclusion:** Healthy soils are critical for supporting wildlife and, human population and health. Thus, is important to evaluate the impacts of emerging compounds in soil, food chains and human health. Additionally, soil must be protected and pollution must be reduced as much as possible, mainly in agricultural soils.

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## P42 – Effects of deoxynivalenol on oxidative stress and inflammatory profile of prepubertal and adult mice reproductive organs

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**Keywords:** Fusariotoxins, testicles, toxicity and inflammation

**Introduction:** Deoxynivalenol (DON) is a *Fusarium* mycotoxin widely distributed around the world. In recent years, the relationship between exposure to DON and impairment on the masculine reproductive system fertility has been suggested. However, the effect of DON on the male reproductive organs of prepubertal animals has been overlooked in mycotoxicological studies. Thus, the objective of this study was to evaluate the influence of age in DON's reproductive toxicity.

**Methodology:** Swiss mice prepubertal (21 days postnatal; n=30) and adults (65 days postnatal; n=30) were divided into two experimental groups (15 animals/group): a control group (free of mycotoxins) and a DON group (10 mg DON/kg of feed). After 15 days and 28 days receiving the treatments, prepubertal and adult animals were euthanized, respectively. The percentage of abnormal seminiferous tubules was evaluated by light microscopy. The antioxidant potential of the samples was determined by FRAP and ABTS assays. The production of superoxide and lipid peroxidation were evaluated by the reduction of NBT and TBARS assays, respectively. The number of macrophages was evaluated through N-acetyl- $\beta$ -D-glucosaminidase (NAG), and inflammatory cytokines (IL-1 $\beta$  and TNF- $\alpha$ ) were evaluated using ELISA



kits. All essays were performed in spectrophotometry.

**Results:** In the DON-diet, a significant increase in the number of abnormal seminiferous tubules was observed at both ages. The main testicular changes observed were vacuolation of germ cells, flattening of seminiferous epithelium, and the presence of immature cells in the tubular lumen. DON decreased the antioxidant potential (FRAP and ABTS) of adults and prepubertal testicles, increasing the superoxide levels (NBT) only in prepubertal age. In the prepubertal testicles a reduced number of macrophages and increased IL-1 $\beta$  levels were observed. On the other hand, in adults an increased number of macrophages was accompanied by an increase in TNF- $\alpha$  levels.

**Conclusion:** In conclusion, our results indicate that animals of both ages were equally susceptible to DON toxicity. Though, different immune mechanisms are involved in the toxicity of prepubertal and adult testicles. Assessments considering the persistence of the toxic effects in adults after prepubertal exposure still necessary.



## P43 – Aflatoxin B<sub>1</sub> accumulation in *Tenebrio molitor*: a preliminary assessment

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**Keywords:** *T. molitor*, AFB<sub>1</sub>, accumulation, insects

**Introduction:** With the population growth rate, there are some concerns that food production will not be able to keep up with this growth. Edible insects seem to present a sustainable solution. Farming these insects presents an opportunity to drain the production of the by-products by reusing them as bio-feedstocks and reintroducing these components into the food value chain.

However, these products can present several contaminations, including mycotoxins, which can be accumulated in insects after exposure to the contaminant, and be detected at the end of the food chain. The ENTOSAFE project aims to address these concerns and evaluate the potential risk for the consumer.

**Methodology:** The principal aim of this study was to evaluate the mycotoxin aflatoxin B<sub>1</sub> (AFB<sub>1</sub>) accumulation in *Tenebrio molitor* (yellow Mealworm, YMW) exposed to a spiked AFB<sub>1</sub> feed substrate at maximum levels in cereals and products derived from cereals (2 µg/kg) and ten times higher (20 µg/kg). AFB<sub>1</sub> contents were quantified in both feed substrate and *T. molitor* samples, before and after a 14-days of exposure and mycotoxins (aflatoxins and ochratoxin A, OTA), detected by HPLC-FD detection.

**Results:** Results concerning non-contaminated feed substrate revealed absence of AFB<sub>1</sub> and presence of OTA, the latter (0,8 µg/kg) presenting



values below the legislated value of 3  $\mu\text{g}/\text{kg}$ , for cereals and products derived from cereals (European Commission, 2023). AFB<sub>1</sub> spiked feed substrates revealed values slightly higher (4 and 23  $\mu\text{g}/\text{kg}$ ) than the theoretical contamination levels of 2 and 20  $\mu\text{g}/\text{kg}$ . OTA values remain close to the previously reported. No changes occurred in contamination levels at beginning and 14-days AFB<sub>1</sub> exposure assays. Results concerning *T. molitor* larvae, revealed absence of OTA along exposure assays and different AFB<sub>1</sub> contamination levels. AFB<sub>1</sub> contents in low (0.011  $\mu\text{g}/\text{kg}$ ) and high (0.022  $\mu\text{g}/\text{kg}$ ) AFB<sub>1</sub> contamination levels were close, and below the 2  $\mu\text{g}/\text{kg}$  legislated level for cereals and products derived from cereals (European Commission, 2023), after the 14-days exposure.

**Conclusion:** The reported results are preliminary, so several aspects need to be improved as mycotoxin analytical method validation, mycotoxin contamination procedure and a higher number of samples to get representative results on AFB<sub>1</sub> accumulation in insect larvae.

**Acknowledgment:** This study was supported by the project ENTOSAFE (PTDC/CTA-AMB/0730/2021), financially supported by national funds (OE), through FCT/MCTES, as well as for financial support to CESAM by FCT/MCTES (UIDP/50017/2020 + UIDB/50017/2020 + LA/P/0094/2020), through national funds. D. Cardoso was hired under the Scientific Employment Stimulus – Individual Call (CEECIND/01190/2018).



## P44 – Are marine macroalgae affected by the environmental levels of the octocrylene? The case of the food supplement *Ulva rigida*

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**Keywords:** Macroalgae, food supplements, organic sunscreens, environmental concentrations

**Introduction:** Marine macroalgae such as *Ulva rigida* are considered a suitable natural food supplement for humans and aquaculture due to their high content of protein, carbohydrates, vitamins, and minerals and low lipid content. This seaweed is ubiquitous in coastal communities, and is present in marine and estuarine environments. Such ecosystems are considered the ultimate destiny for contaminants, as organic UV filters, which can be found in a wide range of personal care products. These emerging contaminants, including octocrylene are considered toxic for humans and wildlife, but as far as our knowledge goes, no information is available for marine macroalgae. This study aimed to evaluate the potentially toxic effects of octocrylene on *Ulva rigida*, a widely recognized alternative food resource, while assessing its bioaccumulation.

**Methodology:** To accomplish that, we used benzophenone as a proxy for the presence of octocrylene in marine waters by evaluating the photosynthetic efficiency and oxidative stress responses of *U. rigida* after 24 h of exposure to two environmentally relevant concentrations of benzophenone (1 µg/L and 100 µg/L). For the evaluation of the photosynthetic efficiency, we performed chlorophyll fluorescence measurements through pulse amplitude modulated (PAM) fluorometry,



and for oxidative stress, the antioxidant profile (catalase, ascorbate peroxidase, and superoxide dismutase) and membrane damage (lipid peroxidation) were assessed. For assessing the bioaccumulation potential, algae tissues were analyzed by UHPLC-ToF-MS to quantify benzophenone at the end of the experimental trial.

**Results:** After 24h of exposure, neither benzophenone concentrations induced alterations in chlorophyll fluorescence or oxidative stress, demonstrating that short-term exposures to low environmental doses of this compound do not compromise *Ulva rigida's* photosynthetic efficiency or cause oxidative stress. Since *Ulva* species are known for their ability to accumulate contaminants, we predict increased burdens of benzophenone, at least for 100 µg/L concentration.

**Conclusion:** Thus, ingestion of benzophenone-contaminated *Ulva* may lead to the bioaccumulation of this compound and its consequent adverse effects on human health, recommending further evaluation of the impacts and bioaccumulation of this compound on *U. rigida*.



## P45 – Factors associated with lead exposure and health effects in children living in a fishing community in Baía de Todos os Santos, Salvador, Bahia, Brazil

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**Keywords:** exposure assessment; human biomonitoring; health impact assessment; lead; children

**Introduction:** The levels of lead (Pb) exposure of the population, particularly children, are unknown, although studies show the contamination in different environmental compartments of the Baía de Todos os Santos (BTS), in Salvador, Bahia, Brazil. This was the first study conducted in BTS to identify factors associated with lead exposure and as well as its effects on children's health.

**Methodology:** Blood and hair samples from children aged 0 to 6 living in an area of environmental contamination in the northeast of BTS, a fishermen village called Maré Island (MI), were collected. Findings were analyzed and distributed in two subareas: north-east (NE) and south-west (SW) of MI. The children's mothers were asked to answer a questionnaire concerning socioeconomic, demographic and environmental factors, as well food and seafood consumption. Lead concentrations in blood (BPb) and hair (HPb) were determined by means of electrothermal atomic absorption spectrometry (ETAAS) and flame (FAAS), respectively. The iron status measurements included hemoglobin (Hgb) and ferritin (FET).

**Results:** Results indicated high average of Pb concentrations ( $p < 0,001$ ), significantly higher in the children living in the SW ( $19,25 \pm 8,78\mu\text{g/dL}$ ),



as compared to those levels in children from the NE ( $7,58 \pm 5,93\mu\text{g}/\text{dL}$ ). HPb concentrations, just like BPb, were higher in children in the SW area ( $21,24 \pm 14,01\mu\text{g}/\text{g}$ ) than in children in the NE ( $12,93 \pm 10,36\mu\text{g}/\text{g}$ ;  $p < 0,01$ ). This variable showed a positive correlation statistically significant to BPb ( $p < 0,01$ ). Lead exposure negatively affected ferritin; FET and BPb ( $p < 0,05$ ). The consumption of bivalve mollusks in the SW area in MI is considered a high risk factor, because the frequency of weekly consumption in children's diets resulted in  $1,5\mu\text{g}/\text{dL}$  increase in the concentrations of BPb ( $p = 0,008$ ).

**Conclusion:** It is possible that the lifestyle of parents, risk behaviors of children and consumption of fish, coupled with socioeconomic and demographic factors, together, are the main causes of Pb exposure in children in Maré Island. Finally, this study demonstrated that behavioral as well as dietary factors, together, can play an important role in populations exposed to lead, however further investigation is required for this population group.



## P46 – The concepts of food defense and food fraud applied in a meat seasoning factory – a case study.

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**Keywords:** Food defense, food fraud; adulteration; spice export.

**Introduction:** Food Defense is a comprehensive technique developed to safeguard food safety across the entire food production chain. Originating in response to the events of September 11, 2001, in the United States, it encompasses a range of control and quality management strategies within the food industry. Its primary objective is to prevent both intentional and accidental contamination of food. In addition to Food Defense, it is imperative to implement measures to mitigate fraud, commonly referred to as Food Fraud.

This research focuses on the techniques of Food Defense and Food Fraud, aiming to elucidate potential forms of contamination and adulteration of ingredients used in the production of meat seasonings within a small-scale industry setting. The overarching goal is to comply with the new regulations for exports to the United States, establish a standardized framework for the company, and serve as a model for other enterprises engaged in similar relationships with the U.S.

**Methodology:** The study was conducted at a small-scale company that distributes seasonings nationwide, with its headquarters in the United States and operations initiated in Brazil in 2009. Given its position in the supply chain, directly supplying exporting companies, this particular enterprise must adhere to the regulatory requirements of destination countries while simultaneously complying with Brazilian legislation. To identify the vulnerabilities within the company, a comprehensive questionnaire based on the Food Defense Plan Builder



and the Food Fraud Database software was employed. Furthermore, field observations were conducted to assess the various processes employed within the establishment.

**Results:** The obtained results were subsequently analyzed using the aforementioned software, allowing for the identification of potential vulnerabilities and the proposal of mitigation measures. The utilization of the Food Defense Plan Builder software for food manufacturing facilities, although intricate, proved to be both feasible and valuable. Comprehensive engagement with all departments within the industry facilitated the identification of vulnerable points.

**Conclusion:** Moreover, these findings facilitated the development of a comprehensive food defense plan and a fraud prevention plan, outlining potential intentional and unintentional forms of contamination and adulteration associated with the ingredients utilized by the company.



## P47 – Assessment of beef supply in institutional restaurants menus from the perspective of the exposure to pesticide: impacts on human health and the environment

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**Keywords:** pesticides; food system; menus; food service.

**Introduction:** Based on the premise that nutrition is directly related to food systems, the principle of precaution is highlighted, given the lack of epidemiological studies assessing chronic exposure to pesticides and their health impacts. Meat products contain pesticide residues that accumulate in animal tissues through various environmental exposures. To assess the supply of beef in Brazilian institutional menus from the perspective of pesticide exposure.

**Methodology:** Per capita meat consumption data were obtained from the preparation technical sheets of the weekly institutional menus planning. The analysis of permitted pesticides was conducted using the Codex database using values from Maximum Residue Limits (MRL) and Acceptable Daily Intake (ADI). The estimation of the Maximum Theoretical Daily Intake was determined through the relationship between MRL and the quantity offered. Risk exposure was determined by analyzing the ingestion and corresponding ADI of the active ingredient. The octanol-water partition coefficient (Kow), an indicator of bioaccumulation in animal organisms, was assessed using data from PubChem platform.

**Results:** A total of 120 institutional menus were analyzed. The average meat supply per capita was 103g. The analysis of the intake estimate



revealed potential contamination with residues of bifenthrin (46.2% of ADI); indoxacarb (30.8% of ADI); cypermethrins (15% of ADI); carbaryl (9.6% of ADI); 2,4-D (3.1% of ADI); terbufos (2.6% of ADI); acetamiprid (1.1% of ADI), and acephate (0.3% of ADI). Active ingredients that exceeded the acceptable intake include aldrin and dieldrin, chlordane, DDT, haloxyfop, and heptachlor, despite being banned due to their toxicity to health and the environment, they persist in various aquatic and terrestrial ecosystems. The bioaccumulation potential of these residues was classified as high ( $\log Kow > 3.0$ ), facilitating your accumulation in the human and animal body for long periods.

**Conclusion:** A legislation is required to decrease the quantity of pesticides in food, particularly those used in pastures. Furthermore, structural changes at systemic and intersectoral levels should be prioritized to bring about transformations in the food system. Managers of food and nutrition units are called upon to join the fight against the use of pesticides in food, prioritizing the purchase of local, seasonal, and especially agroecological-based foods.



## P48 – Exposure assessment to inorganic arsenic from rice consumption in Uruguay

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**Keywords:** risk assessment, food safety, inorganic arsenic, rice, Uruguay, exposure assessment.

**Introduction:** Arsenic is a metalloid naturally present in air, soil, water, and food. Its inorganic form (iAs) is carcinogenic to humans and can be found in water and different foods, including rice products. Within the framework of a food safety risk assessment, this work summarizes the results of the exposure to iAs of the Uruguayan population from white rice consumption, according to gender and age group, and the evaluation of the potential health risk, based on the Margin of Exposure (MOE). The MOE is the ratio between the toxicological point of departure (POD) – defined as the point on the dose-effects curve for an adverse effect (here 0.0003-0.008 mg/kg/day, EFSA) – and the calculated exposure. The lower the MOE, the greater the probability of adverse health effects.

**Methodology:** A deterministic evaluation was carried out, with values of consumption and body weight obtained from national data and extrapolations from international studies, when national data was unavailable. iAs concentration (0.066 mg/kg, below the European limit of 0.1 mg/kg for infants) is the mean of 150 values from Uruguayan raw white rice of two harvests, with 80% bioavailability. Domestic rice practices (e.g., soaking, rinsing) were not considered.

**Results:** iAs exposure from rice consumption was below the POD for the entire population considering mean of both consumption and concentration, with MOE  $\geq$  2.4. Likewise, in the chronic evaluation



throughout life, the exposure never exceeded the lower limit of such toxicological range (0.0003 mg/kg/day). This study was the first to estimate the exposure to iAs from rice consumption in all Uruguayan population by age group and gender, demonstrating the usefulness of this tool to assess the risk in food safety and the relevance of research on these issues.

**Conclusion:** It is worth to highlight the importance of having national data on rice consumption and body weight by age group and gender, as well as household preparations and cooking practices, in order to minimize uncertainties of the evaluation and improve results of iAs health consequences in the different and possible scenarios.



## P49 – Interaction effects of 3-MCPD and glycidol co-exposure on the cytotoxicity in hepatoma-derived HuH-7 cell line

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**Introcuction:** Food processing induces the formation of toxic chemical compounds depending on the food composition and the type of processing performed. Some processing contaminants, such as esters of 3-MCPD (3-MCPDE) and glycidol (GE), are induced by the same conditions from acylglycerols and their intermediates when foods high in lipids are heated up to 200 °C or more, especially refined vegetable oils. Thus, the co-occurrence of 3-MCPDE and GE is highly probable in these oils and food containing them. 3-MCPDE is known to have the potential to induce nephrotoxicity, reproductive toxicity and carcinogenicity, while GE has genotoxic and hepatotoxic properties. However, the interaction effects of 3-MCPDE and GE co-exposure still need to be investigated.

**Methodology:** To verify whether simultaneous acute exposure to these contaminants affect the viability of liver cells, we used the Huh-7 cell line and the Chou-Talalay method. To evaluate cell viability, we used the calcein AM assay, which also defined the unaffected fraction (fu) from the Chou-Talalay method. The requirement graph from the linearization of the equation of Mean Effect for isolated and combined exposure demonstrated three parallel lines, which satisfied the method condition, confirming its application.

**Results:** The results showed synergism between 3-MCPD and glycidol when the affected fractions were greater than 52%, 64%, and 72% in a 1:1 ratio at 3, 4, and 5 mM concentrations, respectively. On the



other hand, the method predicted that the affected fractions had antagonistic effects up to 40% of toxic compounds ( $CI > 1$ ). The Chou-Talalay method also suggested that the additive effect occurred when the affected proportion was approximately 50% at an equal ratio of 3-MCPD and glycidol with 3 mM.

**Conclusion:** Therefore, we can conclude that there are interaction effects between 3-MCPD and glycidol in the 1:1 ratio, resulting in antagonism and synergism depending on the affected fraction.



## P50 – Bioaccessibility of trace elements of plant-based yogurts

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**Keywords:** aluminum, barium, molybdenum, nickel, in vitro digestion, ICP-MS

**Introduction:** The search for alternatives to products of animal origin has made the consumer prefer foods that are both tasty and supply their nutritional needs. However, non-essential inorganic elements can have toxic effects when ingested and absorbed by the body. The challenge related to alternative, plant-based (PB) foods is that there is still no regulation that defines their quality parameters. Among the PB products, plant milk is the most widely consumed food, but other products, such as plant-based yogurt, are already being marketed.

**Methodology:** Thus, the objective of the study was to evaluate the trace element contents of aluminum (Al), barium (Ba), molybdenum (Mo), and nickel (Ni) in commercial PB yogurt samples, as well as to estimate the bioaccessible fraction using the INFOGEST 2.0 protocol. For this, the samples were mineralized, using a simple and rapid method, by ultrasound-assisted acid digestion (80°C for 35 min) and trace element determination by ICP-MS. The method was validated according to INMETRO and the figures of merit obtained were: LOQ of 200 µg/kg for Al and 4 µg/kg for the other elements; recovery of 80 to 110%; precision (n=7) of 6-15%, attending INMETRO specifications. The PB yogurts showed total contents (µg/kg) ranging from Al (<200-6716), Ba (136-1085), Mo (7.3-329) and Ni (41-491). Regarding the bioaccessible fraction (µg/kg), the contents ranged from Al (<200-747), Ba (104-360), Mo (6.4-256) and Ni (19-252).



**Results:** The results of the total contents show variation between yogurt samples analyzed. In relation to bioaccessibility, the highest percentages observed were: 11% for Al, 76% for Ba, 88% for Mo, and 90% for Ni. The high bioaccessibility found for the studied trace elements is worrying, because these elements can present toxic effects, causing harmful impacts on human health.

**Conclusion:** Thus, it is of utmost importance to know and establish the composition of PB foods in order to ensure the safety and health of its consumers.

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## P51 – Antibacterial potential of essential oils for lactic acid bacteria contaminants of sugarcane juice

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**Keywords:** Fermentation; *Origanum vulgare*; *Thymus vulgaris*

**Introduction:** The production of bioethanol in Brazil from the fermentation of sugarcane led the country to be responsible for 30% of the world's production of biofuels, in addition to its high production of premium cachaça. However, this must is rich in contaminating microorganisms, mostly lactic acid bacteria (LAB), during fermentation they compete with commercial yeast and promote a stressful environment, which can compromise production yield and generate undesirable sensory compounds in beverages. The production of organic ethanol grows, as does the export of organic cachaça, leading to the search for alternatives such as natural antimicrobials, including essential oils (OE). The EOs showed a great antibacterial effect, especially those consisting of carvacrol and thymol, such as the oregano (OEO) and thyme (OET) EOs, but few studies have applied them in BAL.

**Methodology:** We investigated the use of oregano and thyme EO in three BAL strains isolated from fermentative must from sugar and alcohol plants, coded as CLUAR1, CLUBR2 and CIUBR3. We evaluated the antibacterial activity of the EOs using the disk diffusion method in Mueller-Hinton agar culture medium with 5µl of the EOs individually, as well as performing the sensitivity test using the macrodilution method in GLT medium at concentrations of 0.1 , 0.15, 0.2, 0.25, 0.3 and 0.4µl/mL of each EO. The sensitivity of the microorganisms for minimum inhibitory concentration (MIC) determination was performed by macrodilution measured by the turbidity of the inoculum (OD 540 nm spectrometry), at time 0 and after 6 hours of incubation at 35°C.



**Results:** Inhibition zones for OEO ranged from 30 to 32mm, while for OET ranged from 24 to 33mm, confirming its antibacterial activity. For MIC values, 0.25, 0.2 and 0.4 $\mu$ l/mL were found for CLUAR1, CLUBR2 and CIUBR3 respectively, while for OET the values were 0.25, 0.25 and 0.4 $\mu$ l/mL respectively.

**Conclusion:** The data indicate an antibacterial effect of EO's against LAB with lower MIC values found in the literature for other microorganisms, indicating a high effectiveness of essential oils for these contaminants.



## P52 – Raw milk cheeses from Beira Baixa, Portugal – A contributive study for the microbiological quality assessment

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**Keywords:** cured raw milk cheeses; whole-genome sequencing; surveillance; food safety; tracing sources

**Introduction:** In Portugal, traditional cheeses are part of the gastronomic culture, with the accessibility to products of high microbiological quality being valued to guarantee the safety of the consumer's population. These artisanal cheeses are normally made from raw milk, having a complex microbiota that will influence the characteristics, quality and shelf life of the final manufactured products.

**Methodology:** In this study we evaluated the presence of *Escherichia coli*, Coagulase Positive Staphylococci (CPS), *Listeria monocytogenes* and *Salmonella* spp. in 98 cured raw milk cheeses produced in the Beira Baixa region. Conventional methods (based on ISO standards) and alternative methods (TEMPO®, VIDAS® and VITEK®2) for the quantification, detection or identification of each bacterium were used. Subsequent phenotypic characterization (serotyping and antimicrobial resistance – AMR), as well as genotyping by Whole-Genome Sequencing (WGS) were also performed.

**Results:** The results obtained indicate the presence of *E. coli*, CPS, *L. monocytogenes* and *Salmonella* spp. in 64.3%, 51.0%, 4.1% and 1.0% of the samples, respectively. AMR was present in 41.5% of the *E. coli*



isolates, from which 5.1% were multidrug resistant. In the 2 samples with  $CPS > 4.9 \times 10^4$  cfu/g, staphylococcal enterotoxins (A, B, C, D, E) were not detected in 25 g. Salmonella (*S. enterica enterica* ser. Duisburg) was detected in one sample. Furthermore, WGS and bioinformatics analysis of *L. monocytogenes* isolates unveiled clusters of high closely related isolates and correlated links to cheese processing facilities.

**Conclusion:** The findings of this study may indicate, among other factors, a lack of adherence to good hygiene and manufacturing practices along one or more phases of the food chain, e.g. in the milk collection/storage, in the cheesemaking process and in the distribution, or during shelf-life whilst placed in the market. The results also highlight for the importance of the information provided by the WGS data integration, helping in public health surveillance while revealing possible infection contamination routes and epidemiological links, which contributes to the investigation of potential foodborne outbreaks.



## P53 – Enhancing the food safety of bivalves: Successful use of a phage cocktail to inactivate human pathogenic bacteria during depuration

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**Keywords:** Bacteriophages, Food Safety, Bivalves, Depuration, Phage Cocktail

**Introduction:** Bivalve molluscs are filter feeding animals which are commonly consumed raw or lightly cooked and are frequently farmed in contaminated waters due to the growing scarcity of pristine farming areas. Consequently, these organisms are often implicated in food-borne disease transmission to humans. The recurrent emergence of infection outbreaks associated with the consumption of shellfish is of extreme importance for public health. Although the elimination of bacteria mostly relies on the depuration of bivalves, this process is not sufficiently efficient to eliminate some microorganisms present in their tissues. Moreover, several bacterial species are known to be resistant to this process forming strong biofilms in depuration systems. Bacteriophage (or phage) treatment represents a promising, tailor-made approach to control human pathogens in bivalves, but its success depends on a deep understanding of the kinetics resulting from the interaction between bacteria, phages and bivalves.

**Methodology:** The present study investigated the potential application of a cocktail of phages to inactivate human pathogenic bacteria commonly detected in bivalves. For that, 4 phages, belonging to 4 different hosts (*Escherichia coli*, *Salmonella* Typhimurium, *Aeromonas hydrophila* and *Vibrio parahaemolyticus*) were characterized and tested. The bacteria-phage cocktail dynamics was assessed *in vitro* for pure and mixed cultures of bacteria in liquid media and in artificial marine water.



**Results:** When the pure cultures of bacteria were challenged with this cocktail, a maximum inactivation of 7.3, 6.1, 6.7 and 4.8 Log CFU/mL was observed for *A. hydrophila*, *S. Typhimurium*, *E. coli* and *V. parahaemolyticus* respectively. Comparatively to the use of each phage individually, the cocktail, in general, improved the inactivation potential for each individual bacterium. When cocktail was used to inactivate the mixed cultures of bacteria in artificial marine water a maximum inactivation of 1.5 Log CFU/mL was observed. However, there was no visible growth of bacteria during the 12 h assay.

**Conclusion:** The results of this study suggest that phage therapy can be an effective alternative to control human pathogenic bacteria during depuration. By controlling bacterial growth, phages can also improve the success of standard depuration techniques. This new approach may provide consumers with safer shellfish products that are valued by being traded live.



## P54 – Phage therapy for the inactivation of *Vibrio alginolyticus* in live feeds: preliminary studies

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**Keywords:** Bacteriophages, Food Safety, Phage Therapy, Live feed, *Vibrio Alginolyticus*

**Introduction:** *Vibrio* species are responsible for the most common diseases in marine fish and invertebrate hatcheries, leading to substantial economic losses. Furthermore, these bacteria can be transmitted to humans through the ingestion of undercooked or raw seafood, posing a potential health risk. *Artemia nauplii*, used as live food in aquaculture, has been considered a possible source of *Vibrio spp.* contamination, including *V. alginolyticus*. It serves as a vehicle for these bacteria to enter culture tanks, causing disease outbreaks and, possibly, reach consumers. To overcome this issue, antibiotics are still commonly used in several countries, either for treatment of disease outbreaks or prophylactically, increasing the antimicrobial resistance of pathogens. Therefore, alternative environmentally-friendly biological strategies to control bacterial infections need to be implemented. Phage therapy is a proven eco-friendly alternative approach to prevent and control pathogenic bacteria in aquaculture. The application of phages in live feeds can reduce bacterial load and, consequently, improve microbiological safety of aquaculture food products.

**Methodology:** In this study two new phages (TDD and SRI) were isolated, characterized and their efficacy was evaluated separately and in cocktail (TDD/SRI) to control *V. alginolyticus*.

**Results:** Both phages were effective against *V. alginolyticus*, but phage TDD (reduction of 5.7 log CFU/mL after 10 h treatment) was more



effective than phage SRI (reduction of 4.6 log CFU/mL after 10 h treatment). The use of the cocktail was not significantly more effective than the use of single phages.

**Conclusion:** The results of this study suggest that the phages TDD and SRI can be an effective alternative to control *V. alginolyticus*. However, further trials are necessary to assess the effectiveness of these phages in controlling *V. alginolyticus* infections in *Artemia franciscana*, validating their potential for application on live feed.



## P55 – *In vitro* activity of natamycin against emerging *Candida* species

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**Keywords:** Natamycin, *Candida* spp, Susceptibility profile

**Introduction:** Natamycin is an antifungal with very distinct applications, namely in medicine against fungal infections and in food industry to prevent mold contamination of cheese and other nonsterile foods. It belongs to the class of polyene antifungals like amphotericinB but its mechanism of action is yet unknown. The study aim is to assess the efficacy of natamycin against emerging *Candida* species.

**Methodology:** Thirty-two *Candida* strains with different susceptibility profiles to commonly used clinical antifungals were used, including 8 *C. auris* (7 from the NCPF, 1 from DSMZ collection), 4 *C. glabrata* (3 clinical isolates and 1 ATCC2001), 5 *C. parapsilosis* (4 clinical isolates and 1 ATCC22019), 7 *C. albicans* (5 clinical isolates and 2 ATCC10231, ATCC90028), 1 *C. krusei* ATCC6258, 5 *C. dubliniensis* and 2 *C. guilliermondii* (clinical isolates). Minimal Inhibitory Concentration (MIC) and Minimal Lethal Concentration (MLC) of natamycin were determined according to protocol M27-A3 and M27-S4 of the Clinical and Laboratory Standards Institute (CLSI). Visual readings were performed after 24h and 48h of incubation. MIC was defined as the lowest concentrations that produced total inhibition of cellular growth (MIC100) and a reduction above 50% (MIC50), compared to cells not exposed to the antifungal (positive control). Assays were performed in duplicate in 3 different days.

**Results:** Minimal Inhibitory Concentration values to natamycin were very consistent among the 7 different species of *Candida* studied,



including *C. auris*, at 24h and 48h of incubation: both MIC100 and MIC50 ranged between 4-8  $\mu\text{g}/\text{mL}$  (Figure 1). Concerning MLC, values ranged between 4-32  $\mu\text{g}/\text{mL}$  at 24h and 4-128  $\mu\text{g}/\text{mL}$  at 48h.

**Conclusions:** The comparison of natamycin MIC and MLC results, following 24h, indicates a fungicidal effect, for all *Candida* species tested. However, the development of antifungal resistance to the different classes of antifungals poses a real threat in different scenarios, mainly in industry, and in veterinary and human medicine. Thus, the uncontrolled/unrestricted use of natamycin in food industry must be avoided at all cost.



## P56 – Assessment of multiple mycotoxins in maize flour samples from Portugal under SafeGrains project

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**Keywords:** *Tribolium castaneum*, *Aspergillus flavus*, aflatoxins, stored maize flour

**Introduction:** One of the most relevant problems in stored grains is the contamination by insects and mycotoxin-producing fungi. *Tribolium castaneum* is one of the most common insect pests of stored products. Its presence makes cereals more susceptible to the spread of the fungi *Aspergillus flavus*, which may produce mycotoxins. The aim of this work was to evaluate the mycotoxin profile of maize flour infested by *T. castaneum* adults, alone or in combination with mycotoxigenic *A. flavus* strain (MUM-UMinho).

**Methodology:** Maize collected directly from fields was stored at 4°C and then ground and sieved to obtain maize flour. Multiple mycotoxin profile of maize flour was determined in three different assays: i) controls (n=8), ii) inoculated with *T. castaneum* adults (insect assay, n = 24), iii) inoculated with *A. flavus* conidia (fungus assay, n = 8) and iv) inoculated with both organisms (mixed assay, n = 24). Samples were analyzed by liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) according to Franco *et al.* (2019) for determination of aflatoxins (AFs) B<sub>1</sub>, B<sub>2</sub>, G<sub>1</sub> and G<sub>2</sub>, deoxynivalenol (DON), zearalenone



(ZEN) and fumonisins (FBs) B<sub>1</sub> and B<sub>2</sub>. The limits of detection (LOD) and quantification (LOQ) of the analytical method were 6.1 and 18.0 µg/kg for DON, respectively. For AFs, ZEN and FBs, LOD and LOQ varied between 0.2 – 0.9 and 0.6 – 2.5 µg/kg, respectively.

**Results:** DON, ZEA, FB<sub>1</sub> and FB<sub>2</sub> were detected in control assays at mean levels ranging from 33-50, 2-6, 112-144 and 49-75 µg/kg, respectively. AFs were not detected in controls. Insect, fungi and mixed assays presented the same mycotoxin profile (with contents in the same order of magnitude), except for AFB<sub>1</sub>, which was detected only in mixed assays. Although the levels of mycotoxins are below the legislated ones (Commission Regulation (EU) 2023/915), they point out to the presence of multiple mycotoxins in raw maize flour and to a potential impact between insects and mycotoxigenic strains in which concerns AFB<sub>1</sub>. Data presented here agree with previous assays from this team describing significantly higher concentration in the maize flour inoculated with both organisms (Duarte et al, 2021).

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## P57 – Contamination of Food Supplements: Recreational Athletes' Safety Perceptions

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**Keywords:** Food Supplements, Safety Perception, Contamination; Adulteration; Recreational Athletes.

**Introduction:** In the last decades, the popularity of food supplements (FS) among recreational athletes has increased, however, the usefulness of different FS is not clear in the scientific community. FS could lead to health concerns, through some intrinsic toxicity, but also by the risk of contamination or adulteration. Thus, this work aims to study the perception of recreational athletes about the main concepts of FS, as well as the risk of its contamination or adulteration and its impact on human health.

**Methodology:** The present study consisted of a cross-sectional analysis involving 303 gym-goers from Lisbon, Portugal, being 133 women and 170 men (30.82 ± 12.84 years old). Data were collected by trained researchers through face-to-face interviews.

**Results:** FS were consumed by 71.90 % of the population, being men as the main consumers. Among all the participants, 95.05% were not aware of the full and correct definition of FS. Individuals looking to improve their wellness did not use FS ( $p = 0.006$ ), meanwhile, those looking to build muscle did ( $p < 0.001$ ). Significant differences were found in beliefs among those who do not consume FS *versus* those who consume. Individuals who do not consume FS are aware of potential interactions between FS and drugs, consider that FS can be used as a substitute for a healthy diet, and express concerns about the possibility of death resulting from FS overdose. A high proportion of participants are unaware of market regulation (46.50 %) and control (40.90 %).



Individuals that do not consume FS worry about the possibility of their contamination with industrial pollutants, while consumers believe that FS could include forbidden ingredients that change nutritional value.

**Conclusion:** A substantial proportion of participants were unaware of the full and correct definition of FS and present a lack of knowledge regarding market regulation and control. The study participants believe that industrial pollutants and prohibited substances are the most prevalent contaminants in FS.

**Topic relevance:** Promoting awareness about the risks of contamination of FS and their impact on human health, ensuring a safe and beneficial fitness journey.



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# ROUND TABLES

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*RT1 – “Brazil meets Europe – Exposure assessment and Health impact”*

## RT1 – OC1 – Risk management actions to reduce aflatoxin exposure

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**Keywords:** mycotoxin; dietary exposure; management actions

Aflatoxins, metabolites produced by *Aspergillus* fungi, are the most potent mutagenic and carcinogenic substances known. Besides the chronic effects, acute aflatoxicosis outbreaks have been reported, especially in Africa. In these latter cases, high concentrations levels of aflatoxins were necessary, while chronic effects are associate with low levels. On its 83rd meeting, the Joint FAO/WHO Expert Committee on Food Additives (JECFA) evaluated that only five food commodities (maize, peanuts, rice, sorghum and wheat) were each responsible for more than 10% of international dietary exposure estimates for more than one GEMS/Food cluster diet, for either total aflatoxins (AFT) or aflatoxin B1 (AFB1). Although lower concentrations are found in cereals compared to nuts, the high consumption of cereals may account for up to 80% of dietary exposure for some countries. Based on JECFA recommendations, the Codex Committee on Food Contaminants (CCCF) undertook several management actions to reduce aflatoxin exposure. One of the actions was to revise the Code of practice for the prevention and reduction of mycotoxin contamination in cereals, including in 2016 the Annex 5, which included recommended practices based on good agricultural practices (GAP) and good manufacturing practices (GMP) in order to prevent and reduce contamination by aflatoxins in cereal grains. More recently, in 2022, maximum levels (MLs) for aflatoxins were set by CCCF in maize grain, maize products



(flour meal, semolina and flakes), rice (husked and polished), sorghum grain and cereal-based food for infants and young children. The establishment of MLs balanced exposure reduction and food availability, also considering year-to-year and regional variations. In this process, aflatoxins occurrence levels in cereals were analysed taking into account possible rejection rates if different MLs were set. In 2023, CCCF agreed upon sampling plans for total aflatoxins in certain cereals and cereal-based products, which needed to consider the heterogenous pattern of aflatoxin contamination. All of these actions together aim to reduce aflatoxin exposure and, consequently, the risk of developing hepatocellular carcinoma and other adverse effects related to aflatoxin and its metabolites.



## RT1 – OC2 – Occurrence, dietary exposure, and risk characterization of polycyclic aromatic hydrocarbons in grilled and fried meats in São Paulo, Brazil

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**Keywords:** polycyclic aromatic hydrocarbons, exposure, incremental lifetime cancer risk

Polycyclic aromatic hydrocarbons (PAHs) constitute a class of organic compounds characterized by a structure of carbon and hydrogen atoms forming two or more fused rings without the presence of a heteroatom or substituent. The International Agency for Research on Cancer (IARC) classifies Benzo(a)Pyrene (BaP) as a carcinogen in humans (Group 1), Benzo(a)Anthracene (BaA), Chrysene (Chr) and Benzo(b)Fluoranthene (BbF), are classified as possible human carcinogens (Group 2B).

This study aimed to evaluate the incidence of PAH4 (BaA, Chr, BbF and BaP) in ready-to-consumption beef and chicken meat, to estimate the levels of exposure of the adult population of Ribeirão Preto to these compounds by ingestion, and to characterize the risk associated with this exposure.

One hundred samples ( $n = 100$ ) of meat products were collected in the local market and HPA4 concentrations were determined using high performance liquid chromatography with fluorescence detection. Meat consumption was estimated by applying a Quantitative Food Consumption Frequency Questionnaire to 386 volunteers over 18 years of age.

The results of the BaP concentration varied between  $<LOD$  and  $3.1 \mu\text{g}\cdot\text{kg}^{-1}$ , while the sum of PAH4 varied between  $<LOD$  and  $45.9 \mu\text{g}\cdot\text{kg}^{-1}$ . The estimated daily intake for the evaluated population varied between 0 and  $36 \text{ ng}\cdot\text{day}^{-1}$  for BaP and between 39 and  $2611 \text{ ng}\cdot\text{day}^{-1}$



for PAH4. The margin of exposure (MoE) and the combined margin of exposure (MoET) were used to characterize the risk of exposure to BaP and HPA4, respectively. The estimated MoET for high meat consumers showed values below the reference value (10,000), which is considered a priority for risk management actions. The incremental lifetime cancer risk (ILCR) was also calculated and the values obtained indicated that exposure to PAHs by the consumption of meat foods does not represent a priority risk. However, the results observed for high meat consumers may pose a potential risk.

Additional studies, including additional classes of foods, can be carried out to expand the population's exposure estimates to these contaminants. The deterministic calculations used in this work may underestimate or overestimate the exposure data.



## RT1 – OC3 – Control of prenatal exposure to mercury: Portuguese results from the HBM4EU-mom project

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**Introduction:** Methylmercury is a substance of high concern globally. Nowadays, the main source of human exposure is through the consumption of seafood. Seafood consumption during pregnancy



can expose the foetus to methylmercury and result in neurological damage. However, with suitable dietary advice, its benefits outweigh the risks, as seafood also provides essential nutrients for optimal neurodevelopment. Being the highest consumer of seafood in the EU, Portugal participated in the HBM4EU-mom project – a pilot harmonized intervention study that combined seafood consumption advice to pregnant women with human biomonitoring aiming to assess and control prenatal exposure to methylmercury.

**Methodology:** In Portugal, 135 women participated in phase 1 (first trimester) and 113 in phase 2 ( $\geq 12$  weeks after). Participation in each phase involved providing a hair sample for measuring total mercury and answering a questionnaire on nutrition, lifestyle and health. Participants were randomly assigned to an intervention (received seafood consumption recommendations) or control group (standard care).

**Results:** In phase 1, geometric means of  $1.61 \mu\text{g/g}$  and  $1.58 \mu\text{g/g}$  were observed for the intervention and control groups, respectively. Mercury levels observed in phase 2 were lower ( $1.39 \mu\text{g/g}$  for the intervention and  $1.35 \mu\text{g/g}$  for the control group). Portugal registered the highest mercury levels among the five European countries participating in the study, with 36% of the samples exceeding EFSA's health-based guidance value ( $1.8 \mu\text{g/g}$ ) in phase 1 and 27% in phase 2, with no significant differences between groups. The overall frequency of seafood consumption decreased slightly between phase 1 and phase 2, similarly in both groups, and was positively associated with mercury levels in both phases.

**Conclusion:** Results showed that methylmercury exposure and seafood consumption were high in pregnant women living in Portugal. This study showed that it is possible to raise awareness about the health risks of methylmercury exposure during pregnancy, thereby contributing to minimize prenatal exposure.

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*RT2 – “Networking and societal concern of food contaminants”*

**RT2 – OC1 – “Networking as a strategy to strengthen Public Policies on Food and National Security in Portuguese speaking African Countries”**

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**Keywords:** Hunger, Food Systems, ESAN-CPLP, Academic network

The reduction of hunger, poverty, inequalities, and the protection of natural systems and all forms of life on the planet have challenged science and generated a growing incentive for scientific and technological development, focusing on joint research essential for the formulation and implementation of public policies. The Mechanism for Facilitating University Participation in the Food and Nutritional Security Council of the Community of Portuguese Language Countries (MU-CONSAN-CPLP) consists of a network of researchers who have the freedom to develop their work, with representation in the CPLP’s Food and Nutritional Security Council (CONSAN). CONSAN is a platform of multiple actors, composed of State Ministers responsible for Food and Nutritional Security (SAN) policies, as well as representatives from Civil Society, Universities, the Private Sector, and Parliamentarians to coordinate actions developed in the area of SAN within the scope of CONSAN-CPLP and to provide assistance to the governments of this geopolitical bloc for the implementation of the CPLP’s Food and Nutritional Security Strategy (ESAN-CPLP). The main activities of MU-CONSAN-CPLP are as follows: a) Development of joint positions and proposals to CONSAN; b) Training, exchange of experiences, and



shared learning; c) Participation in specific working groups of CONSAN; d) Monitoring the implementation of ESAN-CPLP. Researchers from almost all CPLP countries actively participate in MU-CONSAN, except for East Timor and Equatorial Guinea. The focus of the work is on the three pillars of the social function of universities: teaching, research, and extension/interaction with society. MU-CONSAN meets monthly, especially the planning and monitoring of formative processes, and the construction of guidelines for SAN research in CPLP. Researchers from MU-CONSAN do not receive additional compensation for their work; they operate through collective cooperation. So far, there have been financial contributions from the Brazilian government and operational support from the universities involved in offering courses at the extension, undergraduate, and postgraduate levels, as well as exchanges of researchers and students. The networking efforts of MU-CONSAN have proven to be an important ally in strengthening SAN policies in CPLP.



## RT2 – OC2 – “Coffee Agroforestry and Sustainable Development: the case of Gorongosa, Mozambique”

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**Keywords:** Biodiversity; *Coffea* spp.; Conservation; Deforestation; Development

Gorongosa National Park (GNP) is one the greatest successful stories of wildlife restoration in Africa. Among others, a coffee agroforestry system (CAFS) with native trees has been implemented in the fragmented rain forest of Mount Gorongosa aiming at reconciling biodiversity conservation and human development, through the implementation of a sustainable model to produce fair trade and high-grade quality coffee.

Through a transdisciplinary approach, Key Performance Indicators (KPIs) are being monitored to evaluate the sustainability of the CAFS. These include: (i) climate models; (ii) phenological and eco-physiological analyses; (iii) biodiversity (flora, fauna and microbes) and carbon (vegetation and soils) dynamics; (iv) coffee bean quality; (v) capacity building and advanced training; (vi) beneficiary families; and (vii) trading.

Higher altitudes (825 and 935 m) and moderate shading improved the performance of coffee plants. All studied climate scenarios pointed to an absence of adequate areas under full sun management by 2040, whereas the AFS may be sustainable until the end of the century, despite a loss of *ca.* 30% in the cropping area by 2060. Biodiversity indicators (microbes, insects, birds), as well as carbon and biomass



(vegetation and soil) values approached those observed in the native forest. The bean quality meets the standard requisites in terms of physico-chemical quality and absence of contaminants. The CAFS has been successfully implemented and shows a high potential to boost the recovery of the tropical rain forest and biodiversity. Coffee is traded in national and international markets, with a direct impact on the livelihoods of *ca.* 1000 families, and on the economy of the entire region.

The model is being replicate in other conservation areas. The project runs under a triangular cooperation program involving several institutions from Mozambique, Brazil, and Portugal.

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## RT2 – OC3 – “Tackling societal concerns on food contaminants: a perspective of the European Partnership for the Assessment of Risks from Chemicals”

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The use of chemicals in a wide range of products, from fields of application as varied as biomedicine, agriculture, or food technology has brought undeniable benefits to society. However, it is of utmost importance to assess, control and minimize potential costs, in terms of long-term impacts on human health and the environment quality. Concerning both naturally produced (e.g., mycotoxins) and synthetic chemicals (e.g., pesticides, residues from food contact materials) that may contaminate food, there has been a world-wide effort to assess and manage potential risks and put in place regulation to ensure food safety. The European Partnership for the assessment of Risks from Chemicals (PARC) aims to advance chemicals risk assessment and management based on scientific evidence. Considering the relevance of food products as a source of human exposure to a wide range of contaminants, an overview of some approaches planned under PARC to understand the societal concerns on food contaminants through surveys, investigate the real exposure of citizens using human biomonitoring, as well as their adverse effects through toxicological studies using classical and more advanced in silico, in vitro, in vivo models, will be briefly presented. The joint effort of PARC and the networking with other external research initiatives is expected to expand the knowledge on food contaminants and reduce human exposure with the ultimate goal of protecting public health.

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