

3rd INTERNATIONAL CONFERENCE ON
FOOD CONTAMINANTS: CHALLENGES
ON RISK ASSESSMENT

ICFC 2019
International Conference on Food Contaminants
Challenges on Risk Assessment



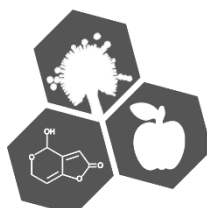
BOOK OF ABSTRACTS



➤ 26-27 SEPTEMBER
AVEIRO, PORTUGAL

ICFC 2019

International Conference on Food Contaminants
Challenges on Risk Assessment



26 – 27 SEPTEMBER 2019
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CONFERENCE THEME

Challenges on Risk Assessment

ICFC2019 Book of Abstracts

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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) and Braga (ICFC2017) under the seal of the National Institute of Health Doutor Ricardo Jorge (INSA). Therefore, the Organization expresses its recognition to the role played by INSA, namely the Chairman and Vice-Chairman of the Executive Board, Dr Fernando Almeida and Dra Cristina Abreu Santos, respectively.

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Dear Participants,

On behalf of the National Institute of Health Dr Ricardo Jorge (INSA), we are honored and delighted to welcome you to the 3rd International Conference on Food Contaminants (ICFC2019) and to receive you in the beautiful city of Aveiro.

ICFC2019 is organized in a close collaboration with CESAM, the Centre for Environmental and Marine Studies of the University of Aveiro and will focus on the topic of Challenges on Risk Assessment.

Food is a complex mixture of a large variety of substances. It contains potentially hazardous chemicals but also beneficial substances (e.g. macronutrients and micronutrients). For the chemical risk assessment, toxicity assessment (hazard identification and characterization) and exposure assessment are performed. Their outcomes are compared to characterize the risk, and the uncertainties of the assessment are described and, if possible, quantified. However, since consumers are daily exposed to a large mixture of chemicals and beneficial compounds a risk-benefit assessment integrating food risks and benefits in the areas of toxicological, microbiological and nutritional components, is vital.

In the frameworks for human health RA of chemicals, the default approach is either to consider only external exposure or to infer internal exposure from the external exposure measurements from different sources and via different routes. Inclusion of human biomonitoring (HBM) data can improve RA hence providing a more accurate estimate of the body burden.

We hope that ICFC2019 represents an opportunity to network, discuss new ideas and develop future research actions, in an attempt to contribute to improve RA strategies promoting the evidence based crucial for policy maker decisions in order to protect human health of European citizens.

We wish you an excellent Conference!

Fernando de Almeida

Chairman of the Executive Board of the National Institute of Health Doutor Ricardo Jorge, I.P.



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CESAM – Centre for Environmental and Marine Studies; UA – University of Aveiro; INSA – National Institute of Health Doutor Ricardo Jorge; INRA - Institut National de la Recherche Agronomique; EFSA – European Food Safety Authority; UM – University of Minho; UBA – German Environment Agency; UG – University of Ghent; DTU – Technical University of Denmark



The Organizing Committee would like to welcome all of you and express a sincere appreciation for the choice of Aveiro city, Portugal, as the place for the 3rd International Food Contaminants Conference.

This meeting will be an international forum to gather researchers from around the world, to share and discuss their findings regarding the broad and interdisciplinary field of food contaminants under the theme “Challenges on Risk Assessment”.

Following the rationality on the impact of food contaminants on human health of ICFC conferences (ICFC2015 <http://hdl.handle.net/10400.18/3214> and ICFC2017 <http://hdl.handle.net/10400.18/4882>), ICFC2019 will address the challenges related to i) new perspectives on risk assessment (RA) of foods; ii) human exposure to chemical contaminants; iii) other agents (microbiological and nutritional) affecting risks and iv) risk-benefit assessment in foods in Europe.

Participants at this conference are cordially invited to contribute with a full manuscript to the Special Issue “Food Safety & Risk Assessment” on Food Research International.

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

We look forward to welcoming and hosting you in Aveiro, one of the most attractive cities in Portugal, and hope this meeting becomes an unforgettable moment.

Paula Alvito and Artur Alves

Chairs of ICFC2019



26.09.2019 – Day 1

08.30 - 09.15	Registration
09.15 - 09.30	Welcome and Opening Session

Session 1 - Challenges on risk assessment in foods: new perspectives

Chairs: Paula Alvito & Filipa Vasconcelos

09.30 - 10.00	L1	Challenges on risk-benefit and risk assessment for food safety (Hans Verhagen, EFSA, Italy)
10.00 - 10.30	L2	Challenges of risk and risk-benefit assessment of foods in Portugal (Isabel Castanheira, INSA, Portugal)
10.30 - 11.00	L3	Development of new indicators of exposure for risk assessment (Céline Brochot, INERIS, France)
11.00 - 11.30		COFFEE BREAK & POSTER SESSION
11.30 - 12.00	L4	Emerging technologies, emerging risks: Challenges for future risk communications (Rui Gaspar, UCP, Portugal)
12.00 - 12.15	OC1	Chemical risks associated to ready-to-eat vegetables: quantitative analysis to estimate disinfection-by-products during washing (Juliana Gadelha, CIMAR, Portugal)
12.15 - 12.30	OC2	Ensuring food safety in community canteens – An example of a public health intervention program in ACES Baixo Vouga (Rui Leitão, USP - ACES Baixo Vouga, Portugal)
12.30 - 14.00		LUNCH & POSTER SESSION



Session 2 – Human exposure to chemical food contaminants

Chairs: Susana Loureiro & Marthe De Boevre

14.00 - 14.30	L5	Science and policy for a healthy future: the European joint human biomonitoring initiative HBM4EU (Marika Kolossa, UBA, Germany)
14.30 - 15.00	L6	Mycotoxin exposure in a developing world (Marthe De Boevre, Ghent University, Belgium)
15.00 - 15.30	L7	Challenges in mixtures of food contaminants in Europe (Jacob van Klaveren, RIVM, The Netherlands)
15.30 - 16.00		COFFEE BREAK & POSTER SESSION
16.00 - 16.30	L8	Analytical advances in mycotoxin biomarker analysis (Franz Berthiller, BOKU, Vienna, Austria)
16.30 - 17.00	L9	We are what we eat: opportunities and challenges towards unraveling the food exposome to empower precision prevention (Denis Sarigiannis, AUTH, Greece)
17.00 - 17.15	OC3	Mycotoxins in adult population from Portugal: Overall results and determinants of exposure (Carla Martins, INSA, Portugal)
17.15 - 17.30	OC4	Bidirectional relationship between gut microbiota and fish contamination with brominated flame retardants (Rebeca Cruz, REQUIMTE, Portugal)
17.30 - 17.45	OC5	Citrinin: New occurrence, toxicokinetic and toxicity data to refine its risk assessment (Celine Meerpoel, Ghent University, Belgium)
17.45 - 18.00	OC6	Assessment of exposure to pesticides: residues in 24h duplicate diets versus their biomarkers in 24h urine (Rosalie Nijssen, Wageningen University & Research, The Netherlands)
19.00		SOCIAL EVENT



27.09.2019 – Day 2

Session 3 – Other agents (microbiological and nutritional) affecting risks

Chairs: Artur Alves & Géraldine Boué

		Estimating the burden of foodborne disease at the national level: an integrated approach (Sara Pires, DTU, Denmark)
09.00 - 09.30	L10	
		Sperm quality is dangerously low worldwide: is there and environmental/food factor? (Marco Alves, ICBAS, Portugal)
09.30 - 10.00	L11	
		Integration of omics into microbiological risk assessment (Sandrine Guillou, INRA, France)
10.00 - 10.30	L12	
10.30 - 11.00		COFFEE BREAK & POSTER SESSION
		Marine bivalves produced in aquaculture: a nutritious food source or a potential public health risk? (Rui Rocha, UA, Portugal)
11.00 - 11.30	L13	
		Relationship between food and prevention of neurodegenerative diseases (Cláudia Nunes dos Santos, CEDOC, NMS-UNL, Portugal)
11.30 - 12.00	L14	
		Monitoring of Shiga toxin-producing <i>Escherichia coli</i> in food in Southern Italy in 2015-2018 (Gaia Nobili, IZSPB, Italy)
12.00 - 12.15	OC7	
		Influence of altitude and shade in the physical and chemical characteristics of Gorongosa coffee (Ana Ribeiro-Barros, ISA, Portugal)
12.15 - 12.30	OC8	
12.30 - 14.30		LUNCH & POSTER SESSION



Session 4 – Risk-benefit assessment in foods in Europe

Chairs: Hans Verhagen & Sara Pires

14.30 - 15.00	L15	Risk-Benefit assessment computation methods (Jeljer Hoekstra, RIVM, The Netherlands)
15.00 - 15.30	L16	Building capacity in risk-benefit assessment of foods: lessons learned from the RB4EU Project (Géraldine Boué, INRA, France)
15.30 - 16.00	L17	Risk-benefit assessment of cereal-based foods consumed by children - a case study under RB4EU project (Ricardo Assunção, INSA, Portugal)
16.00 - 16.30		COFFEE BREAK & POSTER SESSION
16.30 - 17.00	L18	Food substitution in Risk Benefit assessment – why and how (Lea Jakobsen, DTU, Denmark)
17.00 - 17.15	OC9	Risk-benefit assessment of fish consumption in Italy: connecting the dots to reduce uncertainty in assessment (Olimpia Vicentini, ISS, Italy)
17.15 - 17.30	OC10	Novel Foods as Red Meat Replacers: an insight using Risk- Benefit Assessment Methods. The NovRBA Project. (Ermolaos Ververis, NKUA, Greece)
17.30 - 18.00		Conclusions and Best Poster Award
18.00		Closing Session



INVITED LECTURES AND ORAL COMMUNICATIONS



L1 - Challenges on risk-benefit and risk assessment for food safety

Hans Verhagen

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EFSA's mission is to contribute to the safety of the EU food chain by providing scientific advice to risk managers, by communicating on risks to the public, and by cooperating with Member States and other parties to deliver a coherent, trusted food safety system in the EU. This mission has been a constant since EFSA started in 2002. As the environment within which EFSA operates is changing rapidly and, in some respects, dramatically, this presents new challenges for EFSA in terms of the risks that need to be addressed and the way in which EFSA operates.

In 2018, EFSA hosted its third Scientific Conference on Science, Food and Society in Parma, Italy (<https://efsa.onlinelibrary.wiley.com/toc/18314732/2019/17/S1>;

<https://efsa.onlinelibrary.wiley.com/doi/10.2903/j.efsa.2019.e170723>). This event resulted in many recommendations on future actions and research priorities. The conference started with a plenary session on 'where science meets society: putting risk assessment in context' and closed with the session on 'staying relevant in a changing world'. Alongside, there were seven breakout sessions five of which aimed at advancing risk assessment science in the areas of human health, the environment, biological hazards, nutrition and managing evidence, and two of which were dedicated to the societal aspects of risk assessment: engaging with society and envisioning the expertise of the future. The conference addressed the complex interplay between science, risk assessment, policy and society, and explored how to advance food safety risk assessment to address the challenges of a changing world and ensure preparedness. Acknowledging that good science alone is no longer sufficient to ensure fit-for-purpose food safety risk assessments, EFSA must further build on its current five Strategic Objectives. To ensure that its risk assessments remain scientifically robust and sound, EFSA should strive for robust and fit-for-purpose risk assessments; consider food in the context of safety, nutrition and sustainability; and explore further how EFSA can work with other organizations to achieve the One Health/One Environment goals. In addition, EFSA should base scientific risk assessments on reliable science while capitalizing on scientific advances; address scientific uncertainties; and fully publish the evidence and data used. In line with societal expectations, EFSA, in coordination with risk managers, should frame risk assessments through clear policy goals and problem formulation; be explicit about value judgements; communicate clearly and consistently and in coordination with risk assessors and risk managers; involve society; avoid conflicts of interest; and follow trustworthy processes.

Keywords: Food Safety, Risk Assessment, Future, Challenges



L2 - Challenges of risk and risk-benefit assessment of foods in Portugal

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Food safety is threatened by numerous pathogens and toxins which are associated to a variety of acute and chronic foodborne diseases. The concept of risk assessment in the context of food safety, despite clearly described, poses challenges to the national authorities involved in the process of warranty the safety of foods. On the other hand, the concept of risk-benefit assessment as an integrated approach was recently introduced by EFSA, WHO and FAO and gained an enormous impact at European and global level. Risk-benefit assessment adds to the traditional risk assessment the beneficial perspective that inherently every food product could present.

The purpose of this presentation is to give an overview of the current strategies and policies adopted in Portugal in the field of risk and risk-benefit evaluation. The National Institute of Health (INSA) addresses critical questions for food safety managers covering foods, food groups or diets.

At INSA, different studies are being developed in the field of harmonization to integrate data sources from microbiology, nutrition, toxicology, and epidemiology. The work carried out under research infrastructures for metrology METRO FOOD-RI is presenting as a facilitator to align risk and risk-benefits with methodological approaches of analytical data. Quality Management procedures and requirements has been an essential tool as a communication gate between nutrient information available in food composition data and the occurrence of contaminants obtainable in a total diet study. Data on NewGenomeSequencing and whole genome sequencing carried out in inter-departmental projects are crucial to identify animal or plant food species with environmental and health risks and benefits.

Considering the current needs to establish clear recommendations, as restrict diets or adherence to plant-based foods and new habits, international cooperation under the framework of EFSA projects and initiatives is an important contribution under these fields. Attempts to strength the linkage between health effects with economy, sustainability, and consumer is addressed at global level.

Building on both the success and the lessons learned, the experience obtained through the different projects developed at INSA will be discussed, focusing the main challenges and the associated opportunities for the risk and risk-benefit assessment of foods in Portugal.

Keywords: food data; food safety; measurements; international cooperation



L3 - Development of new indicators of exposure for risk assessment

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Introduction: Exposure assessment is a key step in the risk assessment process. Human exposure to chemicals can be assessed via large-scale biomonitoring studies that usually rely on biomarkers measurements from single time points. Reverse dosimetry approaches aim at reconstructing the external exposure from such biomarkers using physiologically based pharmacokinetic (PBPK) models accounting for the processes that the chemical undergoes in the human body and the individual characteristics of the studied population. Such approaches are also valuable to simulate exposure between biomarker measurement time points, especially during critical windows of exposure. In the HELIX project on building the early-life exposome, we aimed at estimating the early-life exposure of children to perfluorooctanesulfonic acid (PFOS) and perfluorooctanoic acid (PFOA).

Methodology: Our study involved 1239 mother-child pairs living in 6 European countries. PFOS and PFOA were measured in maternal plasma at the time of pregnancy and in child plasma at the age of 6-10 years old. Realistic exposure scenarios were defined for each participant. A lifetime PBPK model including childhood, pregnancy, and lactation periods was parameterized for PFOS and PFOA, and for each woman and child based on their individual characteristics (e.g., age, weight, birth weight of the child).

Results: We calculated new indicators of exposure during the in utero life and childhood. We showed that the pattern of prenatal PFOA and PFOS exposures varies greatly among the children and that the actual measurements do not correlate well with the predicted indicators during the first years of life. Our results also showed the importance of accounting for the individual history of mothers and children as different exposures can result in similar measured concentrations at one time point. An adequate use of individual information (e.g. breastfeeding) with a mechanistic TK model can rebuild realistic exposure scenarios and reduce uncertainty.

Conclusion: Neglecting inter-individual differences and actual exposures can lead to large exposure misclassification problems, reducing badly the power of subsequent dose-response or association analyses. Our simulated fetal and child exposures bring additional information to the measured spot concentrations and can help to better characterize the exposure in target organs during windows of susceptibility.

Keywords: exposure assessment; PBPK model; PFAS; reverse dosimetry; in utero; breastfeeding



L4 - Emerging technologies, emerging risks: Challenges for future risk communications

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Introduction: Emerging risks to human health can be defined as: “A risk resulting from a newly identified hazard to which a significant exposure may occur, or from an unexpected new or increased significant exposure and/or susceptibility to a known hazard.” (EFSA, 2007). Due to their novelty and uncertainty regarding their consequences, various challenges emerge on how to communicate these risks to the population, to enable their prevention. A current source of emerging risks in society is new technologies that, if used in unconventional settings in unconventional ways, may induce the emergence of new hazards and associated risks.

Methodology: As an example, we performed a non-systematic review of the literature focused on emerging technologies use by the “biohacking” movement, comprised of “loose collectives of scientists, engineers, artists, designers, and activists experimenting with biotechnology outside of conventional institutions and laboratories” (Ireland, 2017). Often, Do It Yourself (DIY) biology experimentations occur in unconventional settings, such as households, by using new technologies such as the Crispr technique for gene editing. Such use may present new hazards to other people (genes manipulation of bacteria that may contaminate food or other materials/substances) and hazards to the experimenter (e.g. non-conventional uses such as biological enhancement of the human body through self-editing of genes).

Results: Existent literature shows that risk acceptance varies according to the technological application type (e.g. gene therapy vs. synthetic blood), with both risks and benefits being recognized. However, the literature is still scarce regarding perceived risks of DIY biology and related citizen movements. Differently, experts frame DIY and other unconventional science initiatives as beneficial for science, although ethical discussions persist.

Conclusions & relevance: To implement communication strategies for unconventional uses of emerging technologies, further comprehension should exist regarding public and experts' views of these initiatives. This understanding can allow reducing the inherent uncertainty regarding personal and societal consequences of emerging technologies, used for example in DIY biology activities.

Keywords: Emerging risks; Risk uncertainty; Risk communication; DIY biology; Biohacking



OC1 - Chemical risks associated to ready-to-eat vegetables: quantitative analysis to estimate disinfection-by-products during washing

Gadelha JR¹, Allende A², López-Gálvez F², Fernández P², Gil M.I.², Egea JA²

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Fresh products can become contaminated with disease-causing microorganisms and chemical contaminants at every step of the production and processing chain and in a variety of ways, including through contact with contaminated process water. Water quality is critical to prevent microbial and chemical risks in any of the postharvest and processing operations related to fresh and fresh-cut fruits and vegetables. The wash process requires a high volume of water. To maintain the microbiological quality of the process water, intervention strategies are needed. Chemical disinfection is the most common method to maintain the microbial quality of process water. However, the use of chemicals leads to the formation/accumulation of disinfection-by-products (DBPs) in the process wash water such as trihalomethanes (THMs) and chlorates, which can be absorbed by the washed vegetables. The presence of high concentrations of DBPs in vegetables has led to an intensive debate on current disinfection practices and how DBPs may enter the food supply chain, becoming a potential health risk for consumers.

To assess the risk associated to the formation/accumulation of DBPs in process wash water, a quantitative analysis was done, to perform a mathematical model to predict the wash water by-products formation during disinfection process. Available data has been used to develop a mathematical model to predict the formation/accumulation of DBPs (chlorates and THMs) in process water due to the use of chlorine derived compounds. The obtained results have identified an underestimation of the accumulation of chlorates in the process water. In the case of THMs, already published kinetic models that described the formation of THMs in process water have been further developed. The present study tries to simulate the agro-industry wash water process and highlighted the human consumption and associated risks.

Keywords: DBPs; Chlorination; Chlorates; THMs; Model



OC2 - Ensuring food safety in community canteens – An example of a public health intervention program in ACES Baixo Vouga

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Introduction: There is a wide range of diseases caused by unsafe food. Several agents (bacteria, viruses, parasites) may contaminate food and cause disease in humans, putting at risk vulnerable groups like children, elderly and the sick. Food safety for all is one of the main goals of Public Health, as stated in the “10 essential public health operations” by the World Health Organization. We aim to describe an intervention program implemented in the Community Health Centre Group of Baixo Vouga (ACES BV), designed by our Public Health Unit to assess food risk in community canteens.

Methodology: The main goal of this program is to ensure and improve food safety conditions in all community canteens in the 11 counties of ACES BV. This approach is based on three major strategies: Food handlers' training and assistance, microbiological surveillance and quality assessment of structures, equipment and procedures.

Results: Relevant information regarding community canteens was collected and registered. A risk assessment analysis was performed based on the results of a routine sampling schedule (food, utensils and surfaces) and the application of an assessment tool in every canteen (general conditions, kitchen, food storage, sanitary facilities, document analysis, food handlers and food distribution). Food safety training was offered to all professionals working in community canteens. We identified 420 canteens serving children and/or elderly people, handling over 28.000 meals a day. In 2018, 211 canteens were evaluated using the assessment tool. From these, 108 needed to implement corrective measures. A total of 371 samples (main dishes, salads and surfaces/utensils) were collected and analyzed. Globally, 67% of the canteens obtained a “Very good” classification, 24% obtained “Good” and 9% obtained “Acceptable”. Since 2014, over 250 canteens had at least one professional attending our food safety workshops.

Conclusions: This community intervention program helps community canteens comply with food safety standards. Despite the reasonably overall good classification of our canteens, there are still areas where improvements can be achieved.

Relevance: Foodborne diseases are an important cause of morbidity and mortality worldwide. This program is a Public Health response to this issue, ensuring food safety in a vulnerable population setting.

Keywords: Public Health; Food Safety; Community Intervention Program; Food Risk Assessment; Community Canteens; ACES Baixo Vouga.



L5 - Science and policy for a healthy future: the European joint human biomonitoring initiative HBM4EU

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People in Europe are still substantially exposed to various chemicals from different sources and via different exposure pathways. Some of these chemicals have already been restricted or cannot be reasonably further regulated, others raised concern just recently or are not even known as pollutants relevant for human health. Targeted and non-targeted human biomonitoring (HBM) analyses provide information on the aggregate exposure from all sources and via all pathways. HBM is therefore an excellent basis to identify the real exposure of European citizens, to improve risk assessment procedures for chemicals, and thus to protect people's health.

More than 110 partners from 28 countries cooperate in the European Joint Programme HBM4EU to generate the knowledge decision makers need to improve Europe's environment and health actions and programs. As an innovative approach, HBM4EU was designed after consultation with European and national policy makers to identify the policy needs and priority chemicals. Inclusion of stakeholders aims at facilitating the use of scientific results by different target groups, including the general public.

HBM data are currently fragmented in Europe. Therefore, HBM4EU develops a network of analytical laboratories that are qualified to deliver high quality and comparable exposure biomarker results for 18 priority substances. Up to now, first important sources for reduced comparability have been identified, which will be tackled by tailored training activities developed by HBM4EU. Study centers in 21 countries collect and analyze new samples in a harmonized way. Data for three age groups will jointly be analyzed and deliver comparable HBM data. An occupational study on exposure to chromium is conducted in 8 countries. 5 countries investigate internal exposure of mothers and their children to pesticides. Interpretation of HBM data is supported by derivation of European HBM Health Based Guidance Values (HBGV), exposure modelling and physiologically based pharmacokinetic modelling, mechanistic studies, identification of appropriate effect markers, and improving novel analytical techniques to screen for emerging chemicals and their metabolites in human samples.

Through 2021 and well beyond, HBM4EU contributes directly to the improvement of health and well-being of European citizens.

The project has received funding from the European Unions' Horizon 2020 research and innovation Programme under grant agreement No 733032 HBM4EU (www.hbm4eu.eu).



L6 - Mycotoxin exposure in a developing world

Marthe De Boevre, De Saeger S

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Mycotoxins are naturally occurring toxic secondary metabolites produced by different fungal species including *Aspergillus*, *Fusarium*, *Penicillium* and *Claviceps spp.* These fungi and related mycotoxins contaminate crops in the field all over the world, but are also present post-harvest due to bad storage and transportation conditions. This results in contaminated cereal crops, spices, nuts, and fruits not suitable for human consumption and thus, enormous economic losses worldwide. These mycotoxins are causing a variety of toxic effects in humans and animals ranging from acute to chronic (e.g. carcinogenic, immunotoxic, hepatotoxic, stunting in children), and can even cause death. Therefore, the co-occurrence of different types of mycotoxins results in serious food safety issues all over the world, with Sub-Saharan Africa being one of the regions at high risk.

Moreover, in Africa and other developing countries, the possibilities for regular mycotoxin analysis are scarce to non-existent: analytical tests are expensive, there is a lack of expertise and training, there is insufficient technical support from companies selling analytical instruments and the focus is mainly put on aflatoxins, while other mycotoxins are being neglected.

To obtain a substantial mycotoxin reduction, a holistic approach is needed in which all different research fields of mycotoxicology, together with (international) stakeholders such as food industry and governments work together. MYTOX-SOUTH (<https://mytoxsouth.org>) is an intercontinental, multi-disciplinary partnership striving to improve food security and food safety through mitigation of mycotoxins at global level with the following long-term goals: 1) building human and infrastructural capacity through training of South partners, 2) bridging the gap between research and the development and 3) stimulate the environment for a fruitful public-private partnership to create a sustainable network.

This presentation will highlight the results of some multi-mycotoxin biomonitoring studies that have been performed at Ghent University in different regions of the world, and will give examples of projects developed together with the South to study risk management strategies. Mainly, it will highlight the need for capacity development and how this can be practically achieved through training and education.

Keywords: mycotoxin; developing world; Africa; MYTOX-SOUTH; biomonitoring



L7 - Challenges in mixtures of food contaminants in Europe

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We still don't know much about the consequences of exposure to chemical mixtures. Research under the EuroMix project will help stakeholders closing the gap.

EuroMix aimed to establish novel testing and assessment strategies for chemical mixtures of contaminants and pesticide residues in food, to develop appropriate mixture risk assessment methodology and to implement this in a well-accessible inter-operational data and model platform.

Human biomonitoring studies show that people have a considerable number of man-made chemicals in their bodies, and European regulations stipulate the need to consider the potential mixture effect. However, animal testing should be reduced, and new testing methods are required. For the EuroMix team, the question was simple: How do we translate these good intentions to effective actions?

The EuroMix project delivered a test strategy to generate missing toxicity data needed for future risk assessments. They tested many chemicals and ran several case studies with a large number of chemicals affecting three adverse outcomes; liver steatosis, skeletal malformation and endocrine disruption. They also developed a novel risk modelling approach aligned with needs in Europe and elsewhere.

The approach starts with *in silico* modelling grouping chemicals into cumulative assessment groups. Then *in vitro* assays are used to investigate the appropriateness of the dose addition assumption and to derive the relative toxicity of chemicals. Results from *in vitro* testing are then compared with data from animal studies.

Although *in-vitro* assays already allow generation of new hazard data on yet-untested chemicals, their results need to be extrapolated from internal exposure concentrations to external doses before being used in mixture risk assessment. For *in vitro* to *in vivo* extrapolation (IVIVE) substance-specific kinetic models have been developed.

EuroMix has delivered an open web-based data and model platform for exposure, hazard and risk assessment. The modular system includes *in silico* modelling, dose-response modelling of *in vitro* test and *in vivo* results, the derivation of relative potency factors, an IVIVE modelling approach and the combination of hazard characterizations with dietary and non-dietary exposure assessments into margins of exposure. Eleven European Member States performed dietary exposure assessments using EuroMix data and models. Case studies addressing multiple exposure routes of bisphenols and pesticides, a comparison with human biomonitoring data and a feasibility study on simultaneous exposure to pesticides, additives and contaminants regulated under different regulatory sectors will be further explored. Stakeholders have been successfully trained on the EuroMix models.



L8 - Analytical advances in mycotoxin biomarker analysis

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Introduction: Biomarkers are objective, quantifiable characteristics of biological processes, medical states or exposure, which can be measured accurately and reproducibly. While biomarkers of effect (quantifiable changes in biological processes in an individual) and biomarkers of exposure (parent toxins, their metabolites or reaction products) or known, only the latter are commonly used in mycotoxin research. The objectives of this presentation is to show the latest developments in the usage of biomarkers to study to exposure to the important *Fusarium* mycotoxins deoxynivalenol (DON) and zearalenone (ZEN), as well as to their masked forms.

Methodology: We studied the metabolism of DON and ZEN using liver microsomes with subsequent LC-MS/MS determination. Various animal trials (chicken, turkeys, rats, mice, cows and pigs) were performed for DON and trials ZEN and masked mycotoxins in pigs. Urine and faeces samples were collected using metabolic cages and the samples were analysed with LC-MS/MS thereafter.

Results: Whereas deepoxydation and glucuronidation of DON have been known for over a decade, sulfation (chicken) and sulfonation (rats) have only lately been discovered as metabolization pathways of DON. In addition, we could identify novel DON-, DOM-, iso-DON and iso-DOM glucuronides in urine of rats, mice, cows and pigs. Besides minor formation of α - and/or β -zearalenol, glucuronidation is the main metabolic pathway of ZEN in mammals. All investigated masked mycotoxins were completely hydrolyzed in the gastrointestinal tract of swine, thus contributing to the overall toxicity of DON or ZEN, respectively.

Conclusions and relevance: Iso-DON-3-glucuronide proved to be a major DON metabolite in rats and iso-DON-8- glucuronide in mice. DOM-3- glucuronide was the dominant DON metabolite in cows. The discovery of several novel DON-derived glucuronides in animal urine requires adaptation of the currently used methods for DON-biomarker analysis of livestock. The plant and fungal metabolites DON-3-glucoside, ZEN-14-glucoside, ZEN-16-glucoside and ZEN-14-sulfate were readily hydrolyzed to ZEN in the GI tract of pigs. Despite slightly reduced toxicity compared to the parent toxins, the investigated modified forms contribute to the total mycotoxin burden. Hence, we suggest considering the sum of DON or ZEN and its naturally-occurring metabolites as a group guidance (or maximum) value.

Keywords: liquid chromatography – mass spectrometry; metabolism; excretion; toxicokinetics



L9 - We are what we eat: opportunities and challenges towards unraveling the food exposome to empower precision prevention

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The exposome represents the totality of exposures from conception onwards. Unraveling it will help us understand the intricate web of relationships between environmental and dietary exposures, lifestyle, genetics and disease. Such a process implies that environmental and dietary exposures and genetic variation are reliably measured and linked through mechanistic analysis of toxicity pathways. To understand the interaction between environmental and dietary exposure and disease, we need to capture the biological perturbations initiated by exposure to environmental and food-related chemical stressors; and identify which of these perturbations overcome the homeostasis barrier, resulting in observed alterations of the cell/tissue environment and eventually to pathologic phenotypes. Integrated exposure biology provides the methodological elements for the surveillance of changes at different levels of biological organization through the use of the full array of –omics and post-omics technologies including epigenomics. Starting from untargeted transcriptomics and metabolomics we proceed with joint analysis of biological processes induced by exposure to xenobiotics at the molecular level and of metabolic processes induced in parallel. Dynamic flux balance analysis is a key for joint interpretation of gene expression data and metabolite profiles. Thus, putative pathways of toxicity can be identified; they need to be verified by targeted multi-omics and functional assays. Identification of the functional links among the data derived from different high throughput testing platforms and their proper interpretation are supported by advanced bioinformatics such as support vector machines and clustering algorithms and systems biology models.

Our objective is to support the development of precision prevention strategies that protect public health while ensuring cost-effectiveness and addressing health inequities. The exposome and the genome are the two main determinants of human health. Effectively coupling genetic information with environmental and food exposure-related data, as well as capturing epigenetic perturbations and linking them with mechanisms of toxicity and adverse outcome pathways maybe the solution towards the development of efficient and precise prevention policies.

This is facilitated by the advent of high throughput analytical and multi-sensing methods, which are integrated in the quest to create the premise for delivering precision medicine. Even though this is a true revolution for the promotion of good health and cost-effective healthcare on the individual level, public health



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promotion warrants paying equal attention to prevention and avoidance/reduction of human exposure to the stressors that contribute to ill health.

Keywords: exposome; high throughput; cross-omics; toxicity pathways; precision prevention



OC3 - Mycotoxins in adult population from Portugal: Overall results and determinants of exposure

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Human biomonitoring (HBM) is recognized as an important tool to assess the Human exposure to chemicals, contributing to describe trends and patterns of exposure over time and to identify population groups that could be under risk. Mycotoxins produce toxic effects in humans and are found in foods usually consumed worldwide in a daily basis. In Portugal, scarce data are available regarding exposure to mycotoxins and the determinants of this exposure.

In the scope of the National Food, Nutrition, and Physical Activity Survey of the Portuguese General Population (2015-2016), 24h-urine samples from 94 participants were analyzed by liquid chromatography–mass spectrometry (LC-MS/MS) for the simultaneous determination of 37 urinary mycotoxins' biomarkers and data obtained used to estimate the probable daily intake as well as the risk characterization applying the Hazard Quotient approach. Participants performed two face-to-face interviews and dietary intake was obtained by two non-consecutive 24-hours recalls with a time interval between 8 and 15 days, complemented with a Food Propensity Questionnaire. Results revealed the exposure of Portuguese population to zearalenone, deoxynivalenol, ochratoxin A, alternariol, citrinin and fumonisin B₁ through the quantification in 24h urine samples. Risk characterization data revealed a potential concern to some reported mycotoxins since the reference intake values were exceeded by some of the considered participants. The present study generated, for the first time and within a HBM study, reliable data on internal exposure to mycotoxins at individual level for the Portuguese population. These data were crucial to characterize the determinants of exposure and to support risk managers to establish preventive policy measures that contribute to ensure the public health protection.

Acknowledgments: This research was performed with the financial support to CESAM (UID/AMB/50017/2019), to FCT/MEC through national funds, and the co-funding by the FEDER, within the PT2020 Partnership Agreement and Compete 2020. This research was also supported by the project MYTOX-SOUTH, Ghent University Global Minds programme. IAN-AF national survey was funded by the EEA Grants Program, Public Health Initiatives (PT06 - 000088SI3).

Keywords: Human Biomonitoring; Mycotoxins; Risk Assessment; Portugal



OC4 - Bidirectional relationship between gut microbiota and fish contamination with brominated flame retardants

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Environmental health is increasingly compromised by persistent toxic substances, which may have serious implications in food safety and, thus, in human health. Polybrominated diphenyl ethers (PBDEs) are anthropogenic contaminants with endocrine disruption abilities and are commonly found in seafood, the main route of human exposure. Growing evidence points out that the human gut microbiota interacts with xenobiotics, which may lead to impairment of host homeostasis if functions of microbiota become compromised.

The aim of this study was to ascertain if gut microbiota could metabolize PBDEs from a digested fish meal as if these chemical hazards may affect the physiological balance of gastrointestinal microorganisms.

Experiments were conducted using the duodenal non-bioaccessible fraction obtained after static *in vitro* digestion of fish artificially contaminated with tri- to hexa-BDEs. Fermentation was performed in a batch closed-system using an inoculum made from fresh human stool. Contaminant analyses were performed by gas chromatography (tandem) mass spectrometry using QuEChERS-like extraction or dispersive liquid-liquid microextraction for solid or liquid matrices, respectively. The volatilomic profile was analysed by solid-phase microextraction coupled to gas chromatography-mass spectrometry. Samples were subjected to quantification of mesophilic bacteria in Plate Count Agar and quantification of Gram-negative bacteria and coliforms in MacConkey agar.

Gut microbiota reduced the amounts of all tested flame retardants (up to 82 %) from the contaminated fish meal, though no metabolism by-products were found. In addition, the exposure to PBDEs resulted in an imbalance in sulphur, acids and terpenic compounds, changing the microbial volatilome in a dose- and time-dependent manner. Moreover, total counting in culture media showed a decrease of bacterial growth in the presence of PBDEs.

This study highlighted the role of gut microbiota as a degradation and transformation pathway of environmental contaminants. Furthermore, our work underlined the significance of volatile profile to detect signs of contaminants exposure in microorganisms, thus indicating its potential to be included in ecotoxicology and risk assessment studies.



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Keywords: PBDEs; Fish; In vitro digestion; Human gut microbiota; Volatolomics



OC5 - Citrinin: New occurrence, toxicokinetic and toxicity data to refine its risk assessment

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Citrinin (CIT) is a nephrotoxic mycotoxin with a frequent occurrence in human urine samples. However, the risk assessment for this mycotoxin is incomplete, since occurrence and toxicity data are lacking (EFSA, 2012). Therefore, this research aims to collect data on the occurrence of CIT in Belgian food and feed and to determine its toxicokinetic profile in pigs and chickens, in order to enable refinement of the risk assessment.

LC-MS/MS methods were developed and validated for analysis of CIT in food and feed matrices. Next, 300 Belgian food and 100 feed samples were collected and analyzed. Following food products were included, based on literature data on CIT occurrence: cereal products, herbs, spices, nuts, seeds, vegetarian products, meat products, baby food, alcoholic beverages, food supplements, fruit and vegetable juices. CIT was detected in a high number of food products (60%), the highest concentration was found in a red yeast rice food supplement (<LOQ - 1787 µg/kg). Other contaminated food sources were cereal products (<LOQ - 1 µg/kg), vegetarian products (<LOQ - 1.5 µg/kg), spices (<LOQ - 4.5 µg/kg) and meat (<LOQ - 1 µg/kg). Toxicokinetic studies were performed on two relevant animal species, namely pigs and broiler chickens. The absolute oral bioavailability of CIT was determined to be 52% in pigs and 100% in chickens. A longer mean elimination half-life was observed in pigs compared to chickens (12h versus 4h respectively). These results showed a remarkable inter-species variability.

Concerning food safety, it is important to screen for residues of CIT in edible tissues of animal origins. Therefore, steady-state studies were performed on 16 pigs, 16 broiler chickens and 16 laying hens. CIT contaminated feed was administered to the animals for 3 weeks. After this period, the animals were euthanized and all edible tissues were collected, including eggs from laying hens. Carry-over to tissues for human consumption was demonstrated for all species. In addition, toxicity was evaluated by histopathology. This study can contribute to a refinement of the risk assessment of CIT, since new crucial data concerning occurrence and toxicity was gathered.

Acknowledgements: This research was funded by the Belgian Federal Public Service of Health, Food Chain Safety and Environment through the contract RT 16/6308 (CITRIRISK).

Keywords: Citrinin; Occurrence; Toxicity; Carry-over; Risk Assessment



OC6 - Assessment of exposure to pesticides: residues in 24h duplicate diets versus their biomarkers in 24h urine

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Exposure assessment of the general population to pesticides is typically done based on residue data from food monitoring and food consumption databases. To a lesser extent, total diet studies (market basket surveys) are used to estimate exposure. Human biomonitoring (HBM) is an alternative and can bring added value for chemical risk assessment because it can reduce the assumptions regarding consumption rates, residue occurrence and processing effects, and it integrates exposures from different sources (diet, household use, environmental).

To investigate this, a pilot study was conducted in which 24 hour urine was collected from 35 subjects. In parallel, duplicate diet samples were collected from the same persons on the same day. First, non-targeted measurement of both types of samples was done using LC and GC combined with full scan high resolution mass spectrometry. The diet was screened for pesticide residues using existing methods^{1,2} and target libraries. Urine was screened for pesticide biomarkers. For this a target database was compiled, containing over 1500 urinary biomarkers known from draft assessment reports and literature.

Multiple residues were identified in most duplicate diet samples. Multiple biomarkers were detected in urine samples but only partially fully identified due to lack of analytical standards. Nevertheless, additional analyses of the urine samples with deconjugation, targeted MS/HRMS measurement, and comparison with data from the corresponding diets, facilitated provisional identification. Examples of pesticide residues and their biomarkers detected in >50% of duplicate diets and urine samples are boscalid (hydroxy-boscalid), carbendazim (5-HBC), chlorpropham (4-HSA), chlorpyrifos-methyl (TCPy) and pirimiphos-methyl (desmethyl).

The study provided new insights in possibilities, added value, but also into the challenges of HBM for (dietary) exposure assessment.

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Keywords: dietary exposure; biomonitoring; pesticide; biomarkers



L10 - Estimating the burden of foodborne disease at the national level: an integrated approach

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Foodborne diseases cause substantial health and economic burden worldwide. Recent estimates by the World Health Organization show that 1 in 10 people get ill from food contaminated with pathogens or chemicals annually, resulting in 600 million cases and 420,000 deaths worldwide. While these estimates are crucial to raise awareness and guide policies, they are the product of an enormous research initiative that faced substantial data gaps. Precise national disease burden estimates are essential to inform policy-makers and allocate food safety resources.

Deriving these estimates is challenging in many ways. First, only a fraction of those who become sick from food seek medical care. Secondly, some hazards cause chronic disease and establishing a link between exposure to a hazard and the occurrence of symptoms after a long latency period is difficult. Thirdly, comparing diseases requires harmonized metrics that integrate incidence, mortality, and disability. Lastly, most diseases can be caused by several foods, and estimating the contribution of each is determinant to inform interventions.

With its long-established integrated surveillance and rich health and monitoring registries, Denmark is in a unique position to develop methods and produce such evidence. We present the Danish framework to estimate foodborne disease burden, designed to 1) estimate the burden of microbial and chemical -related diseases; 2) attribute this burden to relevant sources; and 3) rank diseases and foods accordingly. Risk ranking results, methodologies and challenges are discussed.



L11 - Sperm quality is dangerously low worldwide: is there an environmental/food factor?

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According with WHO statistics, sperm quality is decreasing worldwide even among noninfertile men. There is not a single factor contributing for these alarming statistics nor there is only one sperm parameter affected. Sperm concentration is one of the most widely studied parameters. Although fertility is poorly correlated with sperm concentration as normal spermatozoa are able to fertilize independently of concentration; it is relevant to discuss what happens before and beyond fertilization, including during sperm maturation and the establishing of the hormonal milieu necessary for the success of reproduction. Spermatogenesis is a complex, highly regulated process sensitive to internal and external cues, as well as to a variety of genetic and epigenetic factors. Among those, obesity, smoking and a wide range of environmental pollutants (including products from the chemicals industry, preservatives, insecticides, herbicides and therapeutic agents) have been highlighted as responsible for the decreasing in sperm quality. In addition, the role of daily nutrient exposure, dietary quality and diet has a vehicle for environmental contaminants/chemicals and exogenous antiandrogenic compounds have been a matter of intense debate. Indeed, studies provide evidence that nutrition and lifestyle have a crucial role on the normal functioning of the male reproductive system. For instance, sperm count and other semen parameters have been strongly associated with multiple environmental and nutritional factors, including endocrine disrupting chemicals, diet, stress, obesity or even air pollution. Several of those factors alter the epigenetic/genetic information carried by sperm and thus, uncontrolled deleterious effects can affect the offspring. Finally, the health and wellbeing of the offspring together with perpetuation of the infertile phenotype into future generations and the quality of the reproductive event in itself have been overlooked and deserve special attention from researchers, politicians and stakeholders. In this talk, we will discuss the latest works focused on analyzing trends in sperm quality in modern societies and how environmental and nutritional factors may have direct effects in those statistics. In addition to clinical studies, we will present some mechanistic-based studies performed in animal models by our team. We will further discuss some of the implications and future perspectives on sperm quality in modern societies.

Keywords: sperm; spermatogenesis; nutrition; toxicology;



L12 - Integration of omics into microbiological risk assessment

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Introduction: Microbiological Risk Assessment (MRA) is a structured process for determining the public health risk associated with foodborne pathogens. In recent years, there has been a strong tendency in providing food safety decisions based upon quantitative assessment. Especially, variability and uncertainty inherent to biological processes have been integrated in food safety management through the use of powerful statistical and probabilistic techniques.

In parallel, with the availability of modern technologies that are able to deeply investigate microorganisms at molecular level, we are experiencing an exciting momentum in which there is the opportunity to exploit such detailed information to better understand the ecology, the behaviour and the physiology of foodborne pathogens. Even more intriguing is the possibility to integrate molecular data (genomics, transcriptomics, proteomics and metabolomics, generally defined as Omics) into risk assessment schemes.

Methodology: In this talk, the possibility of integrating Omics into MRA will be illustrated through various examples. One of them will be based on the high potential of biomarkers. Indeed, biomarker identification and characterization will enable soon to build strain-level exposure assessment models and then to include better pathogen behaviour variability into MRA. The discussion will be based on conclusions drawn by a group of more than 20 scientists brought together by IAFP, ILSI-Europe and ICFMH (see special issue of Int. J. Food Microbiol. Vol 287, December 2018).

Conclusions and relevance: Such Omics approach may be able to tackle existing problems in microbiological risk assessment such as source attribution and root cause analysis, strain variability of foodborne pathogens, behavioural changes in terms of virulence as influenced by the surrounding environment...etc.

Keywords: Predictive model; strain variability; biomarker; exposure assessment



L13 - Marine bivalves produced in aquaculture: a nutritious food source or a potential public health risk?

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Marine bivalves have been promoted worldwide as a healthy and sustainable food. Consequently, bivalve consumption has substantially increased in recent years, which led to an intensification of its production. However, as filter feeders, bivalves can accumulate chemical and microbiological contaminants, which might represent a risk to human health. According to European legislation, bivalve production areas are classified as A, B or C, based on presence or absence of *Salmonella* sp. and on *Escherichia coli* load, which must not exceed 230, 4600 and 46000 *E. coli*/100 g of flesh and intravalvular liquid, in class A, B and C areas, respectively. Bivalves produced in B areas must be depurated before being commercialized for human consumption. Still, bivalves produced in C areas must be transposed to relying areas for more than two months prior to depuration. This study, supported by the Biodepura and Molbipack projects, aimed to assess whether it is possible to depurate marine bivalves reared in class C areas, reducing *E. coli* to legally accepted levels in 24 h, and also looking for the suitable water temperature for each species.

Bivalves (*Ruditapes decussatus*, *Solen marginatus* and *Cerastoderma edule*) were harvested in class C zones and depurated during 24 h in 250 L modular depuration systems, in different temperatures: 10, 15, 20, and 25 °C. Before and after the depuration, bivalves were sampled to determine the most probable number of *E. coli* (ISO 16649-3:2015).

After 24 h it was possible to depurate bivalves from class C areas, regarding *E. coli* values, but the optimal temperature for depuration differ between species. *R. decussatus* depurated better at 20 °C, *S. marginatus* presented lower values of *E. coli* at 10 and 15 °C and *C. edule* depuration was more efficient at 20 and 25 °C.

In sum, temperature can have a preponderant effect in depuration process, which might be related to physiological requirements of each species. Obtained results also strength the feasibility to depurate bivalves harvested in class C areas in 24 h, using adequate methodologies, guarantying the quality and safety of the product for human consumption.

Keywords: aquaculture; bivalve; depuration; E. coli; food safety;



L14 - Relationship between food and prevention of neurodegenerative diseases

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Currently a big concern of our aging society is to efficiently delay the onset of neurodegenerative diseases, which are progressively rising in incidence. The paradigm that a diet rich in the phenolics, prevalent e.g. in fruits, is beneficial to brain health has reached the public. However their mechanistic actions in brain functions remain to be seen, particularly since the nature of those acting in the brain remains overlooked.

The difficulty in demonstrating (poly)phenols true effects can also be justified by the uncertain metabolic fate that dietary (poly)phenols can have. In fact, it is necessary to identify the bioavailable metabolites resulting from (poly)phenol ingestion through the diet, as well as their ability to overcome and/or interact with cellular barriers and reach target tissues, in this case reach the brain. Having this in mind, it will be reviewed the current knowledge on the molecular mechanisms underlying (poly)phenol metabolites effects and their role on neuroinflammation one central hallmark, common in all neurodegenerative diseases.

On the other hand the intense research dedicated to this topic revealed the fact that food components are linked to some problematic aspects, such as toxicological side effects when associated with classical synthetic drugs. The food–drug interactions are submitted to complex issues regarding pharmacokinetic interactions leading to changes in absorption, distribution, metabolism and excretion processes with direct impact on a protective effect and toxicological potential.

Keywords: polyphenol metabolites; neuroinflammation; brain



OC7 - Monitoring of Shiga toxin-producing *Escherichia coli* in food in Southern Italy in 2015-2018

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Introduction: Shiga toxin-producing *Escherichia coli* (STEC) are the fourth most common zoonoses reported in Europe in 2017. In Apulia (Southern Italy), in recent years there was an increase of STEC infections and in 2013 the largest outbreak of European epidemic caused by *E. coli* O26 exploded. In 2016 and 2018, 3 children of about 2-year-old were died. The source of infections has never been identified. Therefore, this study aims to provide information on the prevalence and virulence characteristics of STEC in foods produced and/or marketed in Apulia and Basilicata.

Methodology: From 2015 to 2018, 1761 food samples, including 699 meat samples, 798 dairy products, 137 vegetable samples, 127 environmental samples (irrigation water, surface swabs) were collected from local retail markets and farmers' markets of Apulia and Basilicata. The samples were analyzed according to ISO/TS 13136:2012 for *stx*, *eae* and TOP FIVE genes. The identification of O104, O45, O55, O91, O104, O113, O121, O128 and O146 serogroups was performed following the European Union Reference Laboratory procedure. In addition, *stx* gene subtypes were determined according to the protocol described by Scheutz et al.

Results: 25 out of 1761 (1,48%) samples were positive to STEC, not linked to HUS cases. Results showed that STEC were isolated from 3 raw milk samples, 8 dairy samples and 14 raw meat samples.

13 out of 25 STEC isolated resulted positive to *stx2* gene, 6 to *stx1* and *stx2*, 5 to *stx1* and one to *stx1* and *eae*. Subtyping of *stx* genes showed the presence of different variants: 5 strains positive to *stx1a*, 5 to *stx1c*, 3 to *stx2a*, 8 to *stx2b*, 6 to *stx2c*, 5 to *stx2d* and 3 to *stx2g*. Only 6 STEC strains were positive for the serogroup-associated genes tested (1-O26, 2-O55, 2-O91, 1-O113).

Conclusions and relevance: The presence of positive samples in this study showed that STEC represent a severe hazard to public health in Apulia and Basilicata. Monitoring of food-producing animals and strictly hygiene standards should be carefully considered throughout the entire food chain, in order to prevent and reduce the transmission of STEC to consumers in the "One Health" perspective.

Keywords: STEC; VTEC; Food security; Real-Time PCR



OC8 - Influence of altitude and shade in the physical and chemical characteristics of Gorongosa coffee

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Introduction: Deforestation rates in tropical regions are among the highest in the world, with strong negative impacts on the environment. The implementation of the coffee crop under agroforestry system in the Gorongosa Mountains, Mozambique, aims at reconciling biodiversity conservation with human development through the consociation of native and coffee trees and to develop a sustainable model to produce fair trade, organic, certified, highest quality coffee. In this context, we have analysed the influence of shade and altitude in bean quality, and developed and validated an analytical method to determine ochratoxin A (OTA), an important contaminant of coffee bean.

Methods: Mature fruits of *Coffea arabica* cv. Costa Rica, from plants cultivated at altitude of ca. 650, 825 and 935 m, under dense shade, moderate shade, and full sun exposure, were harvested, and several physical (weight of 100 grains and grain size, apparent density, color) and chemical (caffeine, trigonelline, chlorogenic acids, caffeic, p-coumaric and ferulic acid, pH, and ochratoxin A) parameters were evaluated.

Results: Globally, the weight of 100 grains, density, caffeine content and total phenols increased with altitude. The bean color suggested an improvement in grain quality with increasing altitude. Trigonelline, caffeic, p-coumaric and ferulic acids and soluble solids contents were influenced by both shade and altitude, with significant increases with shade at 935 m. Chlorogenic acids were not affected by either altitude or shade, whereas mineral contents decreased with altitude. The validation of the analytical method for OTA was performed in a subset of samples by HPLC-FD, and the levels of this contaminant were below the maximal allowed contents (5 -10 µg kg⁻¹; Reg CE 1881/2006).

Conclusions: In general the results suggest that bean quality increases with altitude, with some contribution of shade. Gorongosa coffee meets the quality standards for trading. These findings will be further confirmed in the following production years.

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Tecnologia (FCT, Portugal) through the research units UID/AGR/04129/2013 (LEAF), UID/GEO/04035/2013 (GeoBioTec) and UID/AMB/50017/2019 (CESAM), as well as FCT/MEC through national funds and the co-funding by the FEDER, within the PT2020 Partnership Agreement and Compete 2020.

Keywords: Agroforestry; altitude; Coffea arabica; coffee bean quality; deforestation; shading; sustainability



L15 - Risk-Benefit assessment computation methods

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The standard set-up of a risk-benefit assessment is that an intervention or policy is considered, where the intervention is expected to introduce risks and benefits. The intervention could be e.g. fortification of a food or giving a diet recommendation. The assessors are subsequently asked to assess the risk and benefits of the intervention compared to the reference, mostly the status-quo.

Usually, a risk-benefit assessment follows a tiered approach such as proposed by the BRAFO project or by EFSA. When necessary, in the last tier, a common metric is computed in which risk and benefits can be quantified. A common metric makes the weighing between the risks and benefits of a reference and at least one alternative policy scenario possible. By comparing the outcomes for the different scenarios, an advice can be given on which is the preferred policy. There are several potential metrics but in many risk-benefit assessments, the DALY (Disability Adjusted Life Year) is used.

The DALY is a measure that incorporates the severity, the duration of a disease and premature death due to the disease and of course, how many people suffer from it. Although the DALY metric makes it straightforward to compare the risks and benefits in the different scenarios, it can be complicated to do.

Risk and benefits have to be expressed as diseases that can be turned into DALYs. Somehow, data from animal experiments and epidemiological studies that form the basis for the risk and benefits, are converted in disease prevalence in both reference and alternative scenario. Furthermore, the disease characteristics such as severity, probability of death, age of onset need to be available so that DALYs can be calculated. Several case studies have been performed which show how this can be done.

Keywords: risk-benefit quantification; DALY; health metric



L16 - Building capacity in risk-benefit assessment of foods: lessons learned from the RB4EU Project

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Risk-Benefit Assessment (RBA) of food, food ingredients and diets, has recently emerged to address multidisciplinary public health issues. RBA aims to assess in a global perspective potential risks and benefits related to microbiology, toxicology and nutrition; in order to evaluate different options and to support decision-making process. At this time, significant methodological progress have been made but only few research groups have experience in RBA. To promote knowledge transfer, the RiskBenefit4EU project was funded by the European Food Safety Authority (EFSA).

The objective of the presentation will be to introduce the strategy developed to capacitate a new team in RBA and to give an overview on lessons learned from this experience. The new team was comprised of experts in risk assessment in toxicology, microbiology or nutrition, epidemiology, dietary assessment or data analysis to answer to a specific multidisciplinary public health issue on cereal-based foods consumed by young children considering breakfast and infant cereals. Activities of the capacity building experience were designed by experienced researchers in RBA to transfer a harmonized methodology.

Two weeks of face-to-face training were organized, one on theoretical concepts used in RBA and one on their application to the cereal case study. This experience has highlighted the necessity to build a common language within the team by harmonizing important concepts including hazard, health effect, adverse health effect, beneficial health effect, risk, benefit, health and health impact. It was also important that all participant learn basis of all area of expertise used in RBA. A harmonized RBA stepwise approach was introduced with key steps illustrated with examples of previous RBA performed. Finally, the RBA case study was initiated to design a shared protocol.

The learning by doing process experienced under the RB4EU project enabled to create the first training in RBA. The strategy developed, with materials and method used, can now be re-used to capacitate other new teams in RBA and can be considered as a robust basis to build on.



Acknowledgements: “RiskBenefit4EU – Partnering to strengthen the risk-benefit assessment within EU using a holistic approach” funded by EFSA (GA/EFSA/AFSCO/2017/01 – GA02). The authors declare that this study reflects only the authors’ view and EFSA is not responsible for any use that may be made of the information it contains.

Keywords: risk-benefit; food safety; nutrition; methodology; knowledge transmission; experience



L17 - Risk-benefit assessment of cereal-based foods consumed by children - a case study under RB4EU project

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Foodstuffs, as cereal-based products, may present both potential risks and benefits to consumers. Cereal-based foods including breakfast and infant cereals, represent important components of human diets and are also among the first solid foods that young children usually eat.

From the risk components, cereals are one of the most frequent foodstuffs contaminated by mycotoxins. Aflatoxins, a frequent mycotoxin found in cereals, are undoubtedly the most toxic and are associated to genotoxic, carcinogenic and immunosuppressive toxic effects. Besides chemical hazards, cereal-based foods also potentially contain microbiological hazards, as e.g. *Bacillus cereus*. Despite the fact that organisms cannot easily grow in low moisture foods as e.g. breakfast and infant cereals, *B. cereus* can persist for long periods of time and can cause illness due to possible subsequent temperature abuse that allows the organism to grow.

Cereal-based products are also a source of several nutrients, including sodium, fibre and free sugars. High sodium intake is linked to increased blood pressure and also represents a risk factor of cardiovascular diseases. Regarding free sugars, WHO strongly recommends a reduced intake, up to 10% of total energy. Dietary fibre intake contributes to significant immediate and future health benefits, as e.g. promotion of normal gastrointestinal function, prevention of childhood obesity and risk reduction for future chronic diseases.

The present case study, developed under RiskBenefit4EU project and funded by EFSA, aimed to assess the risks and the benefits associated to the consumption of cereal-based products by the Portuguese young children. The risks posed by breakfast and infant cereals, due to aflatoxins, *B. cereus*, sodium and free sugars, were evaluated against the benefits of the intake of fibre.

Obtained results showed that moving from the current consumption to the considered alternative scenarios could result in a gain of healthy-life years.



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Keywords: risk-benefit; cereal-based foods; children; mycotoxins; Bacillus cereus; sodium; fibre; free sugars



L18 - Food substitution in Risk Benefit assessment – why and how

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Risk-benefit assessment (RBA) of foods may be applied to estimate the health impact associated with a change in intake of a particular food. This change in intake is usually the change from current to an alternative intake. Up until now, most RBAs have ignored substitution, that is the fact that a change in intake of one food usually results in a concomitant change in intake of other foods, which in turn may impact health risks and benefits. We present approaches to account for substitution and the results of two case studies: 1) the substitution of red and processed meat by fish, and 2) the substitution of white rice by brown rice; both estimated for the adult Danish population.

We investigate how nutritional risks and benefits, and risk of chemical contaminants change when substituting one food for another and estimate the overall health impact of the substitutions in DALY. In the first case study, we consider the beneficial effects of decreasing meat intake on colorectal (CRC) and stomach cancer and of the concomitant increase in fish intake on cardiovascular disease (CVD) and fetal neurodevelopment, and its negative effect of increased exposure to methyl mercury and dioxin. In the second case study, we consider the beneficial effect of decreasing white rice intake on type 2 diabetes (T2D) and of the concomitant increase in brown rice on CRC, CVD and T2D, and its negative effect of increased exposure to inorganic arsenic.

Substituting meat by fish prevents approximately the loss of 7000 healthy life years, whereas the substitution of white rice by brown has less public health impact, also in high consumption scenarios.

We discuss the impact of substitution approaches in RBA, the implication that accounting for substitution has on the formulation of food-based dietary guidelines, as well as identified future challenges and opportunities.

Keywords: risk-benefit; food substitutions; nutrition; toxicology; DALY



OC9 - Risk-benefit assessment of fish consumption in Italy: connecting the dots to reduce uncertainty in assessment

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The recent National Total Diet Study revealed that dietary exposure to methylmercury in the Italian population merits attention. The average methylmercury exposure in children is above the EFSA TWI, and up to 20% of adults might also exceed this, with females (including women of childbearing age) having 20% higher intakes than males. One uncertainty in this study was that total mercury was measured and assumed to be 100% and 80% methylmercury in fish and other seafood, respectively.

A subsequent literature study on nutrient and contaminant profiles of fish and seafood items of current importance in the Italian market revealed the growing importance of 'new' species (from the Indo-Pacific, South America and Africa). Only for a few species was it possible to weigh the risk of exceeding the TWI for methylmercury against the benefit of meeting DRVs for eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA); the overall assessment was accompanied by a significant uncertainty.

On this basis, ISS designed a two-year nationwide study to assess the benefits of EPA and DHA intake compared to the risks of methylmercury exposure associated with fish consumption. The study focuses on 10 key fish products (corresponding to >20 individual species) and its design considered variables such as season and product origin (geographical, fishing/farming) to reduce the uncertainty related to the representativeness of sampling. Another key issue is methylmercury, which is being measured by speciation analysis and compared to total mercury to generate robust estimates of the proportion of mercury present as methylmercury. Currently, such data do not exist but are of critical importance, along with analytically determined concentrations of EPA and DHA, to improve the accuracy of the risk-benefit assessment.

Validating and optimizing eco-innovative, sustainable solutions for nutritious and safe marine and aquaculture-derived food products is the goal of SEAFOODTOMORROW. Within this ongoing EU project, ISS is working on (i) developing a consistent analytical platform and quality assurance strategy, (ii) validating with analytical data, the new eco-innovative solutions by comparison with the corresponding 'business-as-usual' products, and (iii) contributing to risk-benefit analysis.

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Keywords: Fish; Risk-benefit; Methylmercury; n-3 long chain fatty acids



OC10 - Novel Foods as Red Meat Replacers: an insight using Risk-Benefit Assessment Methods. The NovRBA Project*

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The NovRBA project was launched in the beginning of 2019. Academic and research institutes of four European member states (Denmark, France, Germany, Greece) form the project's multidisciplinary consortium. Under the coordination of Greece, the group focuses on applying risk-benefit assessment (RBA) methods in the context of dietary replacement options.

More specifically, the project aims to estimate the overall health impact of replacing red meat with other protein sources, with a particular focus on novel foods. In an effort to reflect both current and emerging societal and environmental needs and concerns, the replacement of red meat by insects will be used as a case study. The project takes a multifaceted approach, reflecting the health impact of the dietary replacement, considering nutrition, toxicology and microbiology in a combined assessment.

In addition to the scientific aspects of the project, NovRBA will develop strategies to communicate the results. Communication will focus on scientific excellence and transparency, leaving space for further cooperation among all interested parties. The communication strategy will contribute to the development of better tailored and contextualised tools and to disseminate RBA findings to managers and the general public. This joint initiative is expected to create the environment for the fit-for purpose exchange and transfer of knowledge by introducing multichannel interactions among RBA experts, epidemiologists and risk communicators, contributing thus in increasing transparency on assumptions, data used and uncertainties



in RBA outputs. Furthermore, it will provide insights into new perspectives towards the establishment of RBA as a consolidated scientific tool, capable of facilitating risk management decisions and risk communication in the field of food safety and nutrition.

Keywords: Risk-Benefit Assessment (RBA); novel foods; substitution; replacement; red meat consumption; edible insects; risk communication; human health



POSTERS



P1 - Availability and information needs in the chemical contaminants domain – PortFIR assessment

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Introduction: Chemical contaminants (Chc) can be naturally present and/or be introduced in food and feed as result of the various stages of its production, processing and transport and/or due to environmental contamination. The analysis of Chc laboratory data allows to generate information for continuous improvement of food safety and protection of public health. In order to define priorities for development of the Contaminants Database of the Portuguese Food Information Resource (PortFIR), it was decided to identify and assess the availability and information needs on food and feed Chc of the main Portuguese stakeholders in the area.

Material and Methods: PortFIR Information Management Working Group (GTGI) developed a questionnaire to evaluate the availability and information needs of Portuguese stakeholders, from different sectors. The survey was implemented online in August-September 2017.

Results: 93.4% of 457 respondents are "Economic operators" and 56.9% have "Production and processing of foodstuffs (including supplements)" as main activity. Only 0.9% indicated to be in the "Production and processing of feed" sector. In addition, 55.2% indicated to produce/have available analytical data on Chc due to their professional activities. Regarding the information needs, 42.5% indicated the need of more information on the data they produce/have and simultaneously 57.6% pointed that same need on Chc in general. The "Maximum Permissible Values/Maximum Residue Limits" and the "Main contaminants by food/product groups" were indicated in 17.5% and 39.8% of the valid responses, respectively. Finally, 96.9% signalled the importance of having a national platform that can provide useful data and information in this domain and 65.7% indicated to be available to share data/information with this platform.

Conclusions: A significant number of stakeholders (55.2%) produce and/or have available information on Chc but the majority (57.6%) need more information on it. It was stated (96.9% respondents) the importance of having a national platform that can provide useful data, information and knowledge in Chc domain.

Relevance: A national contaminants database within PortFIR, developed and updated accordingly to stakeholders needs, can constitute an important resource for assessment of risk/risk-benefit associated with food and feed, for defining recommendations and enabling economic operators to a continuous improvement of food safety and for allowing consumers to make choices that are more informed.

Keywords: Chemical contaminants; food and feed; availability and information needs; PortFIR Contaminants Database; Information Management Working Group; Survey



P2 - Ranking of chemical substances in foods, drinks and dietary supplements for use in monitoring based on health risks, vulnerable groups and knowledge gaps

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Introduction: Food shall not contain levels of undesirable chemical substances that may be of health concern. In order to prioritize which substances to monitor, the Norwegian Food Safety Authority (NFSA) needed a risk-based ranking of substances that may pose a potential health risk for the consumers. Therefore, the Norwegian Scientific Committee for Food and Environment (VKM) was requested to provide a ranked list of undesirable chemical substances in foods, drinks and dietary supplements, using expert judgement.

Methodology: The ranking of single substances or groups of substances was performed by experts in the scientific panels of VKM with expertise within the various substance groups. The ranking was based on potential health risk, using quantitative or qualitative information of toxicity and exposure, as well as knowledge of vulnerable groups in the population and lack of data on toxicity and/or exposure. When available, quantitative data for toxicity and exposure were used to calculate the margin of exposure (MOE) or acceptable/tolerable daily or weekly intake (ADI, TDI or TWI) values were used. A scoring system was established with varying points for each of five questions with three levels of severity, and the total score (9.0-2.0 points) was used to make the final ranking from high to low priority for monitoring.

Results: The ranked list included food additives, flavourings, substances in food contact materials, environmental contaminants, process-induced substances, natural toxins and 'other substances'. The types of foods most relevant for monitoring of the various substances, adequate sample numbers and other factors important for sampling, such as which part of the food is most relevant to sample and seasonal variations, were also provided to ensure representativeness for exposure in Norway.

Conclusions: A list of ranked substances (>70) with scoring of 8.5 to 3.0 points was established. This list will be used by NFSA for planning their food monitoring in 2020, and will be regularly updated and developed based on the obtained experience.

Relevance: The large number of undesirable chemical substances in foods with potential health risk is a challenge for the risk management authorities in prioritizing of monitoring. Therefore, a ranking of their risk is needed.

Keywords: chemicals, expert judgement, food, monitoring, risk ranking



P3 - earlyMYCO project - Early-life exposure to mycotoxins: a neglected issue?

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Recent studies under MYCOMIX project reported that Portuguese children until 3 years old are exposed to multiple mycotoxins through food consumption, constituting a potential health threat. Aflatoxins (carcinogenic toxins) represented the main risk contributors and deoxynivalenol (a non-carcinogenic toxin associated with immunological and gastrointestinal toxic effects) showed the highest daily intake of the studied mycotoxins. These results opened new research perspectives and emphasized the need to accurately assess the prenatal and lactational exposure to mycotoxins in a critical and vulnerable period of life.

Early-life exposure of children occurs during gestation through transfer of toxic substances present in the maternal diet to the fetus and later on, during lactation, through the breast milk. Considering this, the national project earlyMYCO – Early-life exposure to MYCOtoxins and its impact on health aims at assessing the risk of early-life exposure to mycotoxins. earlyMYCO proposes to answer several key questions including what extent are pregnant women and infants until six months exposed to mycotoxins in Portugal? Is this exposure a health threat? With this purpose, earlyMYCO gathered a multidisciplinary team with expertise on medical sciences, public health and toxicology to perform i) an epidemiological study, including the recruitment of pregnant women and infants, food survey and biological sample collection and ii) mycotoxin exposure assessment in pregnant women and infants using biomarkers of exposure. The epidemiological study was approved by INSA's Ethical Committee and will be conducted in the Primary Health Care of Central Lisboa. The biomonitoring study will use advanced analytical methodologies and will provide data to perform the exposure assessment.

Due to the increasing prevalence in food commodities, mycotoxins appear to be important, but often neglected contaminants in terms of health impact on human population especially in vulnerable groups as



children. It is expected that results obtained within earlyMYCO will contribute to understand the impact of mycotoxin early-life exposure.

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Keywords: Early-life exposure; mycotoxins; biomarkers, exposure assessment



P4 - Dietary exposure of the Portuguese Population to Aflatoxins, Ochratoxin A and Patulin based on a Total Diet Study

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Introduction: Mycotoxins are secondary metabolites of fungi that contaminate food, are usually thermostable, tend to persist during the transformation and processing of contaminated plants and are usually not eliminated during cooking and sterilization. Exposure to mycotoxins may be associated with carcinogenic, immunosuppressant and estrogenic effects.

The objective of this study is to evaluate, by the Total Diet Study approach, the dietary exposure of the adult Portuguese population to six mycotoxins (AFB₁, AFB₂, AFG₁, AFG₂, AFM₁, OTA and PAT) and the risk concerning the international health-based guidance values (HBGV).

Methodology: To estimate the dietary exposure of the Portuguese population to mycotoxins, based on the Total Diet Study approach, 164 composite samples were prepared “as consumed” and analysed by HPLC. The analysed foods were representative of the consumption habits of the Portuguese population and were based on the national food consumption survey “Portuguese Population’s Food Habits and Lifestyles”. Exposure was assessed by combining consumption data with analytical results, using upperbound (UB) assumptions for left-censorship management. Average and high percentile intakes were calculated using the MCRA software and foods that most contribute to exposure were identified. Exposure data were compared with available HBGV.

Results: The evaluation of average and high percentiles (99 and 99.9) for exposure to the mycotoxins studied were all lower than the respective HBGV. For OTA only 0.12% of the population studied reaches half of the HGBV value. For mycotoxins, AFB₁, AFB₂, AFG₁, AFG₂, AFM₁ and OTA, the foods that contributed to ingestion were milk, coffee, bread and herbal tea. Juices were the foods that contributed to Patulin exposure.

Conclusions: Considering that the values estimated in this work for the exposure do not exceed the health-based guidance values, the risk linked with the exposure to AFs, OTA, PAT was considered to be out of concern for the adult Portuguese population.

Relevance: The exposure assessment conducted in the present work by the TDS approach provided the first dietary exposure estimate of the adult Portuguese population to seven mycotoxins.

Acknowledgements: Carla Martins, Maria João Borges and Paula Alvito from the Food and Nutrition Department, National Institute of Health Doutor Ricardo Jorge, IP Portugal.

Keywords: Exposure; TDS; MCRA; Mycotoxins



P5 - Spanish newborns exposure to parabens through lactation

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Introduction: Parabens are a group of preservatives frequently used in personal care products, food and pharmaceuticals. Exposure to parabens has been associated with estrogenic effects in studies with animals. In humans, the main routes of exposure are dermal absorption and ingestion, and are rapidly eliminated through urine, however, levels of parabens in other biological fluids as breast milk have been found. The main objectives of the present study were to determine the levels of parabens in human milk of mothers from Valencia (Spain) and estimate the exposure of breastfed infants to parabens.

Methodology: Breast milk samples were provided by 120 lactating mothers two weeks after birth in the framework of the BETTERMILK project (2015). All the participants signed an informed consent and the study was approved by the ethical and scientific committees. Levels of methyl (MP), ethyl (EP), propyl (PP) and butyl (BP) were determined using liquid chromatography coupled to mass spectrometry (LC-MS/MS). Statistical analysis were performed using the R software (version 3.3.1) and IBM SPSS (version 17.0). The newborns estimated daily intake (EDI) was calculated considering the detected levels of parabens in breast milk and a 95th percentile milk intake rate of breast-feeding of 190 mL/kg bw-day.

Results: The detection frequencies of parabens in breast milk ranged from 61% (BP) to 89% (MP) and the geometric mean of paraben concentrations ranged from 0.10 (BP) to 0.36 (MP) ng/mL. The infants paraben EDIs at the 95th percentile ranged from 0.06 (BP) and 3.0 (MP) µg/kg bw-day.

Conclusions: The EDI of lactating infants to parabens is far below the 10,000 µg/kg bw-day acceptable daily intake established by EFSA for the sum of MP and EP. Nevertheless, reference values for PP and BP are needed

Relevance: As far as we know, this is the largest biomonitoring study of parabens in human milk which estimates the newborns exposure to parabens through lactation.

Keywords: parabens; breast milk; breastfed infants; biomonitoring; mothers; risk assessment



P6 - Furan content in selected commercial food

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Introduction: Furan is a naturally occurring compound found in many heat-processed foods and can significantly contribute to food flavour. Studies conducted to date suggest that the major pathway for the furan formation is the Maillard reaction of reducing sugars, thermal degradation of some amino acids and thermal oxidation of ascorbic acid and polyunsaturated fatty acids. The presence of furan in food has received considerable attention because it was classified “as possible carcinogenic to humans (type 2B)” by the International Agency for Research on Cancer. The potential harmful effect on human health has justified the realization of studies in order to obtain information about the levels of this contaminant in food.

Methodology: Food samples (14 jarred baby food samples and 26 canned/jarred beans samples) were analysed by gas chromatography-mass spectrometry and headspace sampling, under the optimized extraction conditions. Quantification was performed by internal standard calibration using d4-furan as internal standard. The method was selective for the analyte. Good precision was obtained both in terms of repeatability ($RSD \leq 6.2\%$) and intermediate precision $RSD \leq 11\%$). Limit of detection and limit of quantification were 0.2-0.4 $\mu\text{g/kg}$ and 0.5-2.0 $\mu\text{g/kg}$, respectively. The mean of recoveries ranged from 83% to 110%.

Results: The level of furan was in the range of 6.8 $\mu\text{g/kg}$ to 98.6 $\mu\text{g/kg}$ in baby food samples and in the range of 3.5 $\mu\text{g/kg}$ to 20.1 $\mu\text{g/kg}$ in canned beans samples.

Conclusions: The present study reports the occurrence of furan in two different groups of foods. The results show a considerable variation between individual foodstuffs within the same food product group. For baby food samples, more furan was found in the samples containing meat/vegetables compared to fruit based foods.

Relevance: Knowledge of contaminants content is crucial to make a reliable estimate of furan exposure through food and risk associated with these contaminants in food.



P7 - Use of Human Biomonitoring in food risk assessment: assessing exposure to bisphenols in Portugal

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Bisphenols are among the chemicals with the highest production volume worldwide and are used to make polycarbonate plastic containers for food use, such as beverage containers and plastic dinnerware, and in the internal coatings of food and beverage cans among other products.

According to the scientific literature, small amounts of these compounds migrate from packaging into food resulting in human exposure. Although numerous studies have been carried out in order to assess its effects on human health, there are still uncertainties concerning the possible toxic effects of these compounds. Nevertheless, the most commonly used bisphenol, bisphenol A (BPA), is considered an endocrine disrupting compound. As a consequence, current European legislation prohibits the use of BPA in baby bottles, infant sipping cups and in the coating of food containers for children up to 3 years old and significantly tightens the restrictions on the use of BPA in other food contact materials. This has led to the replacement of BPA by other bisphenols, such as BPS and BPF, whose health effects are still largely unknown.

Considering the above and that there is no data on the exposure of the Portuguese population to these compounds, a new project named INSEF-ExpoQuim is currently being developed by the National Health Institute Doutor Ricardo Jorge, in cooperation with the five Regional Health Administrations and the Regional Health Secretariats of the Autonomous Regions of the Azores and Madeira, as part of the European Human Biomonitoring Initiative HBM4EU with the aim to assess exposure to bisphenols in the Portuguese population and contribute to the food risk assessment of bisphenols in Portugal.

It is expected that project results will contribute to the reduction of the impact on the health of the Portuguese population that could result from the exposure to these chemicals, by producing high quality data on the actual exposure of the population, in order to support the development and implementation of policy measures aimed at minimizing exposure.



Acknowledgments: HBM4EU has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 733032.

Keywords: Human biomonitoring; food risk assessment; bisphenols; food containers; Portugal



P8 - Biomonitoring of polycyclic aromatic hydrocarbons in lactating mothers: urinary levels and risk assessment

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Introduction: Polycyclic aromatic hydrocarbons (PAHs) are persistent organic pollutants generated during the incomplete combustion of organic materials. The human organism may be exposed to PAHs through inhalation of polluted air or cigarette smoke, dietary intake and dermal contact. Since several PAHs are considered by International Agency of Research on Cancer as carcinogenic or potentially carcinogenic in humans, a risk assessment of exposure to PAHs in vulnerable population is necessary.

Methodology: First-morning urine samples were collected from 110 lactating mothers participating in the BETTERMILK project in Spain (2015). The urine sample (2.5 ml) was spiked with labeled internal standards and subjected to an enzymatic treatment. After that, a liquid-liquid extraction was carried out and the total organic layer was evaporated under N₂ stream. The dry residue was redissolved with the mobile phase. In total, 11 urinary metabolites of PAHs (1-, 2-hydroxynaphthalene, 2-, 3-hydroxyfluorene, 1-, 2-, 3-, 4-, 9-hydroxyphenanthrene, 1-hydroxypyrene and 3-hydroxybenzo[a]pyrene) were analyzed in the final extract using a Finnigan Surveyor High-Performance Liquid Chromatography system and Finnigan TSQ Quantum Ultra detector (HPLC-MS/MS) (Thermo Fisher Scientific) equipped with a KINETEX F5 column (2.1 x 150 mm, 2.6 µm) (Phenomenex) and ESI (Electrospray ionization) source. An Estimated Daily Intake (EDI) was calculated for interpretation of urinary levels of PAHs metabolites in a risk assessment context.

Results: Detection frequencies ranged from 2% (3-hydroxybenzo[a]pyrene) to 100% (1-hydroxypyrene), and geometric means were between 0.04 ng/ml (4-hydroxyphenanthrene) and 7.15 ng/ml (2-hydroxynaphthalene). The highest derived EDI was obtained for naphthalene (152 ng/kg-day).

Conclusions: Similar urinary levels of the 11 PAHs metabolites were found in other populations such as USA (0.02 – 4.29 ng/ml) and Czech Republic (0.10 – 5.9 ng/ml). Spanish population seemed to be more exposed to naphthalene. Calculated EDIs were far below their oral exposure Reference Doses established by EPA, so PAHs does not raise a health concern for lactating women living in Spain.



Relevance: Biomonitoring of PAHs metabolites in urine should continue in order to evaluate tendencies and to derive the associated risk, especially for vulnerable population such as children and pregnant/lactating women, related with the attentions and actions on food safety control and/or air quality.

Keywords: PAHs, Urine, Human Biomonitoring, Lactating mothers, Risk Assessment



P9 - Biomonitoring of PCDD/Fs and dioxin-like PCBs in human milk

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Introduction: Polychlorinated dibenzo-p-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs) and polychlorinated biphenyls (PCBs) are Persistent Organic Pollutants of great concern because of their toxicity and tendency to bioaccumulate through the food chain. Therefore, these compounds enter the human body and accumulate in fatty tissues. They may also concentrate in human milk, which is the main food source for infants.

Methodology: A total of 120 breastfeeding women were recruited in Valencia (2015) as part of the BETTERMILK project. Samples were collected in polypropylene containers and stored at -80°C until analysis. The breast milk (25 g) was lyophilized and spiked with ¹³C¹²-labeled internal standards, blended with diatomaceous earth and extracted with an accelerated solvent extractor (ASE). After solvent evaporation, fat content was determined gravimetrically. Power-Prep/6 (Fluid Management Systems) automated lipid removal and enrichment procedure using multilayered silica gel column (acidic, basic, and neutral silica) and alumina column coupled to an AX-21 carbon column was used for the clean-up. Resultant fractions containing PCDD/F or dl-PCBs were evaporated under nitrogen stream and redissolved with the corresponding recovery standards. The analysis was performed on a DFS Magnetic Sector GC-HRMS (Thermo Scientific), using selected ion monitoring (SIM) at 10000 resolving power (10% valley).

Results: Each set of samples was analysed under QA protocols, including procedural blanks and standard reference materials (SRM1953 and SRM1954, corresponding to organic contaminants in non-fortified and fortified human milk, respectively).

The upper-bound concentrations for the sum of PCDD/F and dl-PCBs ranged from 1.56 pg TEQ₂₀₀₅ g⁻¹ lipid to 13.48 pg TEQ₂₀₀₅ g⁻¹ lipid with a mean value of 4.82 pg TEQ₂₀₀₅ g⁻¹ lipid.

Conclusions: The PCDD/F and dl-PCBs levels found in the human milk of mothers from Valencia are similar to those reported in Italy, Canada, France and Sweden (6-30 pg TEQ₁₉₉₈ g⁻¹ lipid, 2.2-27, 2.55-51.84, 3.7-56 pg TEQ₂₀₀₅ g⁻¹ lipid, respectively).

Relevance: Although recent biomonitoring data of PCDD/F and dl-PCBs in human milk seem to indicate a global decline, efforts should be maintained in order to reduce dioxin emission and human exposure to protect the environment and human health in this region. Future studies can help to evaluate the effectiveness of measures adopted.



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Keywords: PCDD/F, dl-PCBs, human milk, human biomonitoring, infants



P10 - Aflatoxins in *Salicornia* - A food safety concern

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Salicornia L. is a genus of annual halophytes that develop naturally along the salt marshes, from the Arctic to the Mediterranean. *Salicornia* spp. are characterised by a simple morphology with succulent and only apparently leafless shoots, which resemble green asparagus. They have been occasionally harvested in coastal areas, to use as food, forage, and medicine. However, in recent years *Salicornia*'s young fleshy tips are in high demand, not only in gourmet cuisine for its salty taste, but also, and increasingly, in the food and nutraceutical industries because of its nutritional value and richness in bioactive compounds. Furthermore, the current crises of soil salinisation and freshwater scarcity have also triggered a new interest in *Salicornia* use and cultivation, owing to its inherent extreme salt tolerance and high economical yield. However, alongside this consumption growth, food safety concerns also arise.

The aim of this study was to examine the mould profile and to assess the occurrence of aflatoxins (B₁, B₂, G₁, and G₂) in *Salicornia* in the Portuguese market. The fungal contamination was evaluated by direct plating in selective media. The mycotoxin levels were determined by UHPLC-ToF-MS.

The results revealed that, should any failures occur in its supply chain, *Salicornia* is susceptible to fungal spoilage, mostly by fungi of the *Aspergillus* genera. The growth of fungi induces changes in *Salicornia* sensory characteristics, leading to the loss of its market value, but also, and especially, raises concerns regarding the production of mycotoxins, owing to the carcinogenic, toxic, immunosuppressive, and mutagenic effects of these fungal metabolites. Indeed, high levels of aflatoxins were detected in several *Salicornia* samples (>5 µg/Kg of Aflatoxin B₁ and >10 µg/Kg for the sum B₁, B₂, G₁ and G₂), which may be reduced by adopting simple measures, as e.g. an appropriate harvest time and refrigerated transport.

In conclusion, our findings reveal that *Salicornia* is a possible source of exposure to mycotoxins, namely aflatoxins, which has so far been neglected.

This study brings to light an urgent need to establish both legal limits and control strategies regarding the presence of mycotoxins in *Salicornia* in order to protect the consumer's health.

Keywords: Salicornia; fungi; Aspergillus; mycotoxins; aflatoxins; Portugal



P11 - Urinary Trace metals and heavy metals levels in obese and non-obese Portuguese Children

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Introduction: Some metals have been associated with obesity and associated comorbidities. However, typically those relations have typically focused on individual metals. Thereat, there is a growing interest in evaluating the health effects of cumulative exposure to metals in trace levels.

Methodology: The aim of the study was to determine the exposure to suspected or confirmed (endocrine disruptors and/or obesogens. Children were recruited from the pediatric appointment at Hospital de S. João, and several local schools, in 2014 and 2015. Children lived in Oporto and Aveiro, two Portuguese districts, in the North and Central region of the country.

The children were divided in two groups: the group “regular diet” included healthy children which were normal-/underweight not changing their regular diet; the group “healthy diet” included children diagnosed for obesity/overweight without other known associated diseases, counselled for healthy and balanced nutrition and thus set on a prescribed diet, for at least 3 months. First morning urine samples were collected from each participating child. The study was approved by the ethics committee of the Centro Hospitalar S. João/FMUP and all the parents provided written consent. Urine analysis was performed by inductively coupled plasma-mass spectrometry (ICP-MS).

Results: Urinary concentrations of several elements (Cadmium, Cobalt, Molybdenum, Lead, Manganese, Nickel, Cooper, Arsenic, Tin, Antimony and Thallium) were investigated in 110 urine samples, from 4 to 18 years old Portuguese children. The study group was composed of 55 % girls and 55 % boys, with a median age of 10 years old. The majority of the children was overweight/obese and underwent a diet with nutritional guidance (healthy diet group) (61 %; n=67). Statistical differences (in µg/L) were observed ($p < 0.05$), between the two studied groups, for Manganese, Cooper, Tin and Antimony. Obese/overweight children on a healthy diet presented higher median values for these compounds compared to the normal-weight children following their regular diet.



Conclusions and relevance: Risk assessment showed that the maximum values determined for the Portuguese children were some orders of magnitude above reference values thus, alerting for further need of research on this topic/field (future monitoring interventions in the country).

Keywords: Urine; metals; diet; obesity; biomonitoring



P12 - Can p,p'-DDE induce endoplasmic reticulum stress in rat liver, muscle and adipose tissue?

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The interest in the potential effect of chemicals on health, such as persistent organic pollutants (POPs), arose after the industrial revolution, where they were introduced, whether intentional or unintentional to the environment (UNEP, 2005). POPs include organochlorine pesticides, such as dichlorodiphenyltrichloroethane (DDT) and its metabolite p,p'-dichlorodiphenyldichloroethylene (p,p'-DDE). p,p'-DDE, a metabolic disruptor, is involved in several metabolic syndrome morbidities. Disruption of endoplasmic reticulum homeostasis leads to endoplasmic reticulum stress (ERS), which is often related to these diseases.

This study aimed to evaluate if chronic exposure to sustained low levels of p,p'-DDE could contribute to ERS in target organs (liver, adipose tissue and muscle).

To this purpose, thirty male Wistar rats were randomly divided into four groups, subjected to p,p'-DDE (100 µg/kg/day), either in presence or absence of high-fat diet for a total of 12 weeks.

RNA from liver, adipose tissue and muscle was extracted and the expression of key genes involved in ERS were analysed by real-time PCR: binding immunoglobulin protein (BiP), X-box-binding protein-1 (XBP1) and enhancer-binding protein homologous protein (CHOP).

Detection of significant changes of BiP, XBP-1 and CHOP in the group of animals treated with p,p'-DDE and HF diet, suggested a contribution of this exposure to the appearance and exacerbation of ERS in muscle, adipose tissue and liver. AT and liver had a better adaptive response to handle toxicity than muscle.

Our data revealed a new possible mechanism underlying obesity and insulin resistance supported by the contribution of p,p'-DDE in high-fat diet context to the ERS development or aggravation.

Keywords: p,p'-DDE; Metabolic disruptor; Endoplasmic reticulum stress; Adipose Tissue; Liver



P13 - Exposure and risk assessment of bisphenols in urine of breastfeeding mothers

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Introduction: Bisphenols are man-made chemicals used in various consumer products. BPA is used in manufacturing of polycarbonate plastic and epoxy resins and is found in different products like cans, dental sealants, thermal receipts, food packaging and personal care products. Due to BPA toxicity, bisphenols analogues like BPS and BPF are used as a substitution for BPA. To date, most human biomonitoring studies for bisphenols in urine are focused on population different from breastfeeding women.

Methodology: A total of 180 breastfeeding women aged between 20 and 45 were recruited from June to November 2015 at the University and Polytechnic Hospital "La Fe" (Valencia, Spain) as a part of the Bettemilk project. Urine sample collection was obtained between 2 and 8 weeks after birth. Statistical analysis was performed using R software. Biomonitoring Equivalents (BE) are one of the guidance values used for risk assessment studies. BE values have been described for BPA. Hazard Quotient (HQ) have been calculated as the ratio between biomarker concentration (95th percentile) to specific BE value.

Results: Bisphenol A (BPA), Bisphenol F (BPF) and Bisphenol S (BPS) were detected in 76 % (BPA) and 20% (BPF,S) of the mothers. Average bisphenols concentrations ranged from 0.042 ng mL⁻¹ (BPF) to 0.381 ng mL⁻¹ (BPA). For BPA, risk assessment was estimated, obtaining Hazard quotients (HQ) < 1.

Conclusions: BPA was the highly detected bisphenol in urine of the studied lactating mothers. Low frequencies of detection have been observed for BPF and BPS. The calculated hazard quotient demonstrated that no risk was derived from the estimated exposure in the studied breastfeeding population.

Relevance: There are very few papers studying urinary levels of bisphenols in breastfeeding mothers. This presentation provides more data in this specific and relevant group of population. In addition, human biomonitoring data has been put in a context of risk assessment.

Keywords: Biomonitoring; Breastfeeding mothers; Bisphenols; Urine



P14 - Mycotoxin exposure and human cancer risk: a systematic review of epidemiological studies

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Introduction: In recent years, there has been an increasing interest to investigate mycotoxins' carcinogenicity in humans. This systematic review aims to provide the scientific community with observations in potential associations between multiple mycotoxin exposure and human cancer development.

Methodology: Publications (upon 2018) were identified in PubMed and EMBASE, on-topic case-control studies were screened and quality was assessed according to the Newcastle-Ottawa scale. Exclusion criteria comprised animal, cross-sectional and molecular studies. A compilation of 15 articles was drafted with 13 case-control, 1 longitudinal cohort study and 1 meta-analysis.

Results: Main focus was set towards associations of mycotoxin exposure with primary liver, breast and cervical cancer. A positive association between the consumption of aflatoxin-contaminated foods and primary liver cancer risk was verified. Two case-control studies in Africa investigated the associations between zearalenone and its derivatives with breast cancer risk, however conflicting observations were found. Two case-control studies investigated the association between hepatocellular carcinoma and fumonisin B1 exposure, but no significant associations were observed.

Conclusions: This poster provide clear observations in associations between aflatoxins and liver cancer risk, given sufficient evidence for the IARC Monograph evaluation. Unfortunately, only few human epidemiological studies investigated associations between mycotoxin exposures and cancer risk. To cover this research gap, more in depth research is needed to unravel too scarce to non-existent evidence for other mycotoxins such as deoxynivalenol and ochratoxin A.

Relevance: The link between mycotoxin exposures and cancer risk has mainly been established in experimental studies, and need to be confirmed in human epidemiological studies to allow evidence-based public health strategies.

Keywords: mycotoxins; exposure; fungal metabolites; cancer; food; aflatoxin; fumonisins



P15 - Chronic (multi-)mycotoxin exposure and colorectal and liver cancer risk in a multi-national European cohort

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Introduction: Mycotoxins have been suggested to contribute to a diversity of adverse health effects in humans, even at low concentrations.⁽¹⁾⁽²⁾⁽³⁾ The recognition of multiple mycotoxins being carcinogenic, over singularly present mycotoxins, was echoed in recent research and reviews.⁽²⁾⁽⁴⁾⁽⁵⁾ The aim of this study was to assess potential effects of single and multiple mycotoxin exposures in the European Prospective Investigation into Cancer and Nutrition (EPIC) cohort.⁽⁶⁾

Methodology: Questionnaire data from the EPIC study were matched to mycotoxin food occurrence data compiled by EFSA from EU Member States to assess long-term dietary mycotoxins exposures, and relate them to the risk of colorectal (CRC, n=6,291 cases) and liver (LC, n=834) cancers and their different sub-sites. Analyses were conducted using Cox proportional hazards regression models to compute hazard ratios (HR) and 95% confidence intervals (95% CI) and with mycotoxin exposures expressed as µg/kg body weight/day.

Results: The most important food groups contributing to mycotoxin exposures were cereals and cereal-based products, vegetables, non-alcoholic beverages (including fruit juices) and fruits. A significantly increased risk for hepatocellular carcinoma (HCC) was found with multi-mycotoxin exposures, and a borderline increased risk for colon cancer. For individual mycotoxin groups, deoxynivalenol (DON) showed the strongest association with CRC risk, and in particular with proximal colon cancer risk. DON exposure was also positively associated with liver cancer risk and in particular with HCC risk. Patulin (PAT) was strongly positively associated with rectum cancer risk and with HCC risk.

Conclusions: These results showed an increased cancer risk associated with long-term dietary mycotoxin exposures, and will possibly help to raise awareness of these hazardous contaminants in the general public, as well as among food business operators and regulatory bodies. However, further research investigating potential mechanisms underlying these putative associations is warranted. DON presents a potential threat to human health, mainly when co-occurring with other mycotoxins in the consumers' diet.

Relevance: The acquired results are representing revolutionary and innovative findings regarding potential associations between several ubiquitously present mycotoxins and CRC risk, through basically confirming the hypothesised harmful effects of co-occurrence of different mycotoxins in the human diet.



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Keywords: mycotoxins, epidemiology, prevention colorectal cancer



P16 - Ergot alkaloids in wheat and rye derived products in Italy

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Introduction: Genus *Claviceps* is a plant pathogen able to produce a group of toxins, ergot alkaloids (EAs), whose effects have been known since the Middle Ages (ergotism). *Claviceps purpurea* is the most important representative specie, known to infect more than 400 monocotyledonous plants including economically important cereal grains (e.g., rye, wheat, triticale). EAs are not regulated as such, but maximum limits are in the pipeline of the EU Commission. This study aimed to investigate the presence of the six principal EAs (ergometrine, ergosine, ergocornine, α -ergocryptine, ergotamine and ergocristine) and their relative epimers (-inine forms) in rye- and wheat-based products of the Italian market.

Methodology: A total of 71 samples of flour and bread, all 100% wheat or rye, were collected. Following the FoodEx level 2 codes, "grain milling products" and "bread and rolls" were taken. In particular 20 flour samples (16 wheat and 4 rye milling products) and 51 bread samples (39 wheat and 12 rye bread and rolls) were available.

The collected samples were analyzed by LC-MS/MS following a previously published method which was in-house verified for the sampled matrices.

Results and conclusions: From all the samples, 85% resulted positive for at least one of the EAs. Wheat bread was the product with the highest number of positivity (56%), followed by wheat flour (26%). The results of our study show generally low risk, especially for rye-based products (range for EAs sum 2.6–188.6 $\mu\text{g/kg}$) and wheat flour (range for the EAs sum 2.5–28.6 $\mu\text{g/kg}$). Wheat bread was the most critical food category (range for the EAs sum 2.5–1142.6 $\mu\text{g/kg}$) both for the presence of a very high value (1142.6 $\mu\text{g/kg}$) and for the number of products ($n = 4$) above a possible maximum limit of 150 $\mu\text{g/kg}$ for cereal based products intended for direct human consumption.

Relevance: This study presents a first survey on EAs in rye- and wheat-based products in Italy. The monitoring of EAs in food products is recommended for supporting exposure calculation in the framework of risk assessment also in view of EU regulatory limits.

Keywords: ergot alkaloids, mycotoxins, wheat, rye, UHPLC-MS/MS



P17 - Exposure of Portuguese population to aflatoxins: the contribution of human biomonitoring to estimation of burden of disease

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Human biomonitoring (HBM) is recognized as an important tool to assess the Human exposure to chemicals, contributing to describe trends and patterns of exposure over time and to identify population groups that could be under risk. Natural chemicals as mycotoxins, fungi metabolites that produce toxic effects in humans, are important compounds that could be found in foods usually consumed worldwide in a daily basis. Mycotoxins as aflatoxins (AFTs) are genotoxic, carcinogenic and immunosuppressive compounds. Hepatocellular carcinoma (HCC) is one of their main health toxic effects and is the third leading cause of cancer deaths worldwide. In Portugal, scarce data are available regarding exposure to AFTs and none previous study used HBM data to characterize comprehensively the burden associated to this exposure.

In the scope of the National Food, Nutrition, and Physical Activity Survey of the Portuguese General Population (2015-2016), 24h-urine samples from 94 participants were analyzed by liquid chromatography–mass spectrometry (LC-MS/MS) for the simultaneous determination of AFTs (B1, B2, G1, G2, M1). A model was developed to estimate the health impact of the exposure of Portuguese population to aflatoxins, estimating the number of cases of HCC and DALYs attributed to AFTs exposure.

AFTs were detected in 12.8% (AFB1), 16.0% (AFB2) and 19.1% (AFM1) of the 24h-urine samples. The estimated number of extra cases of HCC attributed to this exposure ranged from 17 to 65 cases/year; the associated DALYs for the Portuguese population ranged from 284 to 1802 years.

The present study generated, for the first time and within a HBM study, reliable data on internal exposure to AFTs at individual level for the Portuguese population. These data were crucial to characterize the burden derived from this exposure and to support risk managers to establish preventive policy measures that contribute to ensure the public health protection.



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Keywords: Human Biomonitoring; Health Impact Assessment; Aflatoxins; Portugal



P18 - Emerging marine biotoxins in seafood from Portugal and challenges for their regulation in the European Union

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Marine biotoxins can cause human acute intoxications after consumption of contaminated seafood. Bivalve mollusc are traditionally considered the main vectors of biotoxins, and for this reason most coastal countries have a tight monitoring program in place to survey the presence and variability of the EU regulated toxins, namely the amnesic shellfish poisoning toxins, the paralytic toxins and the lipophilic-group toxins (okadaic acid, yessotoxins and azaspiracids). However, new toxins are emerging in the European waters and legislation is scarce to control their presence in seafood. In this sense European Commission asked European Food Safety Authority (EFSA) for a scientific opinion on the risks to public health related to the presence of the new emerging toxins which may occur in seafood in Europe. This is the case of the highly potent neurotoxins tetrodotoxins (TTX) and ciguatoxins (CTX). TTX are commonly known in pufferfish in Asian countries and CTX are endemic of tropical and subtropical regions such as Polynesia and south Pacific, Indian Ocean and the Caribbean Sea. In this study, occurrence data of TTX in bivalves, gastropods and pufferfish from the Portuguese coast will be reported for the first time. The presence and identification of CTX levels in fish from Madeira archipelago will be discussed at the light of risk to human consumption and challenges to scientific community and governmental authorities with responsibilities at regulation and official control level.



P19 - Exposure of Portuguese children to Glyphosate: evidence from a cross-sectional biomonitoring survey

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Glyphosate (Group 2A 'probably carcinogenic to humans', IARC, 2015), introduced in 1974 has since become the most commonly and intensively used herbicide worldwide, for vegetation control in agriculture and in urban areas (e.g. public parks and footpaths) and for grain desiccation to increase harvest yield. The development of different glyphosate-resistant genetically-modified crop varieties, and latter the emergence of glyphosate-resistant weeds, resulted in the need to increase frequency and volume of applications of glyphosate. Glyphosate has thus become ubiquitous in the environment and food supply, and subject of public debate, societal concern and scientific controversy (Arazona et al., 2017; Abraxis, 2019).

The recent review work of Gillezeau et al. (2019) highlighted the paucity of data on glyphosate levels among individuals exposed occupationally, para-occupationally, or environmentally to the herbicide. The authors further underlined that it is challenging to fully understand the extent of exposure overall and in vulnerable populations such as children.

As part of an ongoing Portuguese biomonitoring surveillance of children, regarding different contaminants, the occurrence of glyphosate in 41 samples of children's urine, aged 2-13 years old, lived in central Portuguese was investigated. The first morning urine was collected between July 2018 and January 2019. The participation of children depended on the parents' signed consent form and a filled questionnaire (A.anthropometric data and individual characteristics; B.socio-demographic, residency and household use of chemicals data; C.data relating to food consumption). For this initial screening phase, a direct competitive ELISA kit was applied following the manufacturer's instructions (Abraxis, PA, EUA).

Results showed that 97.6% (n=40) were contaminated (LOD=0.6µg/L) at an average level of 1.72±0.83 (range=0.75-4.58µg/L). A similar study (Knudsen, 2017) conducted in 14 Danish children reported comparable urinary levels (mean=1.96µg/L; range=0.85–3.31µg/L). Non-occupational exposure in adult populations ranged from 0.16 to 7.6µg/L, as recently reviewed (Gillezeau et al., 2019).

Further results of this ongoing infants biomonitoring surveillance work will continued to be analysed and published, given the added value that human biomonitoring can bring to support Public health policy, for



chemical risk assessment in different food safety areas (e.g. exposure assessment), and in the application of a needed systematic post market monitoring approach.

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Keywords: Children; Exposure assessment; Glyphosate; Human biomonitoring; Portugal; Public health; Urine



P20 - Probabilistic approach for exposure assessment to deoxynivalenol (DON)

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Introduction: Deoxynivalenol (DON) is a *Fusarium* mycotoxin that can contaminate cereals and cereal-based foodstuff. Cereals products are widely represented in the Italian diet strongly based on pasta, bread, pizza, polenta, cakes, biscuits, etc. The scores of cereal consumption rates in all population groups combined with the constant presence of DON in wheat, barley, oats and corn makes it compulsory the exposure assessment estimates. The dietary exposure is typically carried out following the deterministic approach, using single values or point estimates in the exposure equation. Probabilistic assessment represents a more complex approach where exposure estimates are performed using data distributions for input variables.

Methodology: Individual consumption data of seven food categories (namely breakfast cereals, bread, alternative to bread, pasta, biscuits, cereals, beers and beverages) were taken from the Comprehensive National Consumption Data (2005-2009). Occurrence data of DON contamination in cereal-based products derived from the National Official Mycotoxins Control Plan (2016-2018), which collects data from monitoring and surveillance plans on food contaminants (including DON). The probabilistic statistical model was used to multiply impute non-detectable values (<Limit of Quantification) of the truncated contamination distributions. Combining the obtained complete imputed contamination data with the normalized consumption data observed (individual consumption and body weight), probabilistic exposures were estimated.

Results: Probabilistic estimates of single food category contributions and global dietary exposure estimates (GE, sum of exposure estimates for each food) were reported as mean values and 95ile (including confidence intervals).

Conclusions: Largest contributions to the GE exposure were given by pasta and pasta-substitutes and bread and alternative to bread, while breakfast cereals, biscuits, cakes and bakery products gave consistently lower values. The mean GE estimate of children was higher than those obtained for the other groups. This is due to the fact that while consumption rates among population groups are comparable, children body weights are sensibly lower negatively impacting on the exposure values.

Relevance: Estimates of GE for adolescents, adults and elderly were lower than the DON Tolerable Daily Intake of 1000 ng/kg bw. Children's GE were higher than the TDI evidencing a possible risk for this population group.

Keywords: dietary exposure assessment, probabilistic assessment; mycotoxin; deoxynivalenol; cereal based products



P21 - Contribution of commercial fish species to human mercury exposure: A long-term evaluation near the Mid-Atlantic Ridge

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Fish consumption is linked to the prevention of some human diseases, especially regarding cardiac and circulatory disorders, due to the content of high-quality protein, vitamins and ω -3 fatty acids. At the same time, fish consumption is considered a major pathway of mercury (Hg) exposure in human. More than 90% of the Hg present in fish tissue is found essentially in its organic form (methylmercury – MeHg), which is the most toxic form of Hg. Due to the potential adverse human health effects, international agencies have established Reference doses as recommendations to Hg intake.

Azores archipelago is located in the North Atlantic Ocean close to the Mid-Atlantic Ridge. Last fishery statistics shows that each Azorean consumes about 80 kg of fish per year being the region with the highest consumption of fishery products in Portugal.

This study aims to evaluate the contribution of 28 commercial fish species (discharges from 1994 to 2018) to human Hg exposure in Azores.

The [Hg] in the muscle of fish species reported in this study results from a review of all published articles indexed in Web of Science using the keywords “Mercury AND Azores”, and new data measured in fish obtained by recreational fishing.

An average of 10300 tonnes per year of these 28 fish species are discharged at the Azorean ports. Despite the low Hg levels found in fish muscle, the population of this area is exposed to more than 1700g of Hg per year via fish consumption. The fish species *Mora moro* and *Zeus faber* exhibit higher values than the permitted for fish consumption, however, species with the highest [Hg] are not always those that contribute to a higher human exposure. Furthermore, data show that carnivores fish species generally exhibit higher [Hg] than omnivores fish species and demersal fish species demonstrated higher [Hg] than pelagic fish species.

Finally, the target hazard quotient is < 1 for all fish species studied, meaning that the level of exposure is lower than the reference dose, indicating that the daily exposure is not likely to cause any negative health effects during a lifetime in the human population.

Keywords: Mercury; Mid-Atlantic Ridge; Fish consumption; Target Hazard Quotient; Risk Assessment



P22 - Exposure to mercury induces developmental and behavioural alterations on zebrafish embryos

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Introduction: Mercury (Hg) is a toxic non-essential metal ubiquitously distributed in the environment. Humans are exposed to Hg through air, water and food, mainly seafood. Chronic exposure can lead to its bioaccumulation and subsequently adverse effects at a neurological and reproductive level. Zebrafish (ZF) has been considered an ideal model organism for studying potentially hazardous compounds and their effects on both human health and the environment. Thus, the aim of this study was to identify the effects of Hg exposure on ZF embryo development, neurobehavior and enzymatic markers levels, under a human health perspective.

Methodology: ZF embryos were exposed to different Hg²⁺ concentrations (up to 401 µg/L, added as HgCl₂) following the OECD 236 protocol. Toxicity and developmental endpoints were assessed every 24h over a 96h exposure period. The effects on locomotor activity are related to neurotoxicity and were assessed using a video tracking system (Zebrafish) at concentrations where no toxicity was observed (up to 12 µg/L) after 120h of exposure. To assess Hg-induced alterations in Acetylcholinesterase, also related to the nervous system, Catalase, Glutathione-S-transferase, Glutathione-Reductase, Phosphoprotein Phosphatase-1-gamma (PPP1) and Glutathione Peroxidase-4 (GPx4), ZF were exposed to 0, 10 and 100 µg/L, collected every 24h for up to 96h of exposure and were snap-frozen prior to analysis.

Results: The control group presented no mortality or abnormal embryonic development. ZF exposed to Hg, were affected mainly at the highest concentrations (100-401 µg/L). At 24 hpf, embryos' exposure to 201 µg/L and 401 µg/L induced 13% and 100% mortality, respectively. Also, our results showed that Hg caused developmental, locomotor and biomarkers levels alterations. For the first time, we identified that Hg induced alterations in the PPP1CC2 and GPx4 levels at the highest concentration.

Conclusions: Our study provides evidence that exposure to Hg induced developmental, locomotor and biomarkers levels alterations. Such results may indicate alterations in a reproduction level, once PPP1CC2 is a protein involved in spermatogenesis.

Relevance: The use of ZF in biomedical research is common. ZF gene functions show a high degree of similarity with human genes and there is a close similarity of reproductive regulation on zebrafish and human.

Keywords: Mercury; Neurotoxicity; Embryotoxicity; Fish embryo test



P23 - Aflatoxins in Portuguese small ruminant¹ herds: is carry-over into milk a risk?

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The Mediterranean region is a major world producer of ewe and goat milk, with acknowledged healthy benefits and contribution to sustainable livelihoods. Although dairy ruminant species are less susceptible to mycotoxins, these can still carry-over to milk. After consumption, the mycotoxin aflatoxin B₁ (AFB₁) is biotransformed into the derivative aflatoxin M₁ (AFM₁) excreted in milk. Both are IARC group 1 carcinogens.

The main objective of the present work was to survey AFM₁ occurrence in ewe and goat milk. Bulk-tank milk samples (n=62) from sheep (n=46) and goat (n=16) herds were collected at the middle of lactation cycle in four Portuguese mainland regions between 2017 and 2018. Epidemiologic questionnaire was added to characterize samples. Quantification of AFM₁ in milk samples were performed by a competitive ELISA kit (I'screen®, TECNA, LOD=5 ng/L), performed according to enclosed instructions.

Twenty-six samples were found positive (41.9%) with a mean value of 7.1±2.0 ng/L. None surpassed the EU Maximum level (50 ng/L; 1881/2006/EC), with the maximum detected value being 4-times lower (12.6 ng/L). Sheep milk featured higher mean (7.2±2.1 vs. 6.6±0.5 ng/L) and incidence (45.7 vs. 31.3%) of AFM₁, when comparing with goat milk. Data obtained in 2018 was similar regarding the previous year. Positive samples were associated to concentrates and forage. Total incidence values obtained were higher than those observed in Italy (4.6%; Viridis et al., 2014) and Iran (31%; Rahimi et al., 2012). The calculation of extrapolated values of AFB₁ concentration in feeds from back calculation of the values of AFM₁ obtained from analysis of milk samples was based on 0.33% transference (Battacone et al., 2005). Results showed lower values than EU maximum limit (5 µg/kg for dairy animals; 2002/32/EC). Human exposure was estimated (0.5 ng/kg bw/day) considering the annual sheep and goat cheese consumption in Portugal (1,782 Kg per capita). Nevertheless, AFM₁ concentration in cheese is estimated to be 2 times higher than in milk (AFM₁ associates with milk proteins), and sheep feature a higher concentration of proteins in milk than cows (Battacone et al., 2005). Considering that almost all the small ruminants' milk is destined to cheese manufacture, further studies should follow.

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Keywords: Aflatoxin; cheese; exposure; goat; milk; sheep.



P24 -Paralytic Shellfish Poisoning due to ingestion of contaminated mussels: a case report in Caparica (Portugal)

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In Portugal, the potent paralytic shellfish toxins (PSTs) have appeared irregularly since the onset of a national monitoring program for marine biotoxins in 1986. In years where high contamination levels were attained in bivalves, sporadic cases of human poisonings have been recorded, as in 1994 and 2007. The reappearance of high contamination levels led to the appearance of new cases during the autumn of 2018. This study reports the case of two patients that ingested mussels from the Portuguese southwest coast and required hospitalization due to the severity of symptoms. Details of toxin ingestion, symptomatology and toxin metabolism in the fluids are described. The diagnosis was confirmed by ELISA in plasma and urine samples. In mussel samples, the toxin profile obtained by HPLC-FLD displayed a wide diversity of toxins, typical of *Gymnodinium catenatum* ingestion. However, in the urine samples toxin profile was reduced to B1 and dcSTX. Abundant compounds in mussels having an O-sulfate at C11, such as C1+2 and dcGTx2+3, were absent in urine. In plasma, PSTs were not detected by HPLC-FLD. Calculated toxin ingestion, resulting from consumption of an estimated 200-gram portion, was in the range of 104-120 µg STX eq./kg b.w. This study alerts physicians to be aware of this human syndrome with only sporadic occurrence in Portugal.

Keywords: Paralytic shellfish poisoning; saxitoxin; seafood poisoning; human samples; HPLC; ELISA; Portugal



P25 - Occurrence of aflatoxins and ochratoxin A in selected spices consumed in Morocco

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Introduction: Spices are dried herbs used for flavor, color and preservation of foods for centuries. Their quality may be affected by the presence of mycotoxins produced by toxigenic fungi especially aflatoxins (AF) and ochratoxin A (OTA). This study aims to investigate for first time the presence of AF and OTA in four selected spices (paprika, ginger, pepper and curcuma) consumed in Morocco.

Methodology: In total, 407 spices samples were purchased in four areas in Morocco (El Jadida, Kénitra, Meknès and Marrakech) and investigated for the presence of mycotoxins. OTA and AF were extracted by an-in house method, and then quantified by LC coupled to a fluorescence detector. Positive samples were confirmed by LC-MS/MS technique.

Results: Results showed that AF and OTA contaminated spices samples originated from all investigated areas. In total, 20.6% of paprika samples, 6.8% of ginger samples, and 10.3% of pepper samples exceeded the MRL of AFB₁ (5 µg/kg) recently set by Moroccan regulations (BO 2016). Regarding OTA presence in spices, the toxin was not detected in paprika, ginger and curcuma samples. However, it was detected in pepper samples from El Jadida and Kénitra areas. Out of a total pepper samples, only two samples exceeded the MRL of OTA (15 µg/kg) set by Moroccan regulations (BO 2016).

Conclusions and relevance: This study provided the first data on the presence of AF and OTA in selected spices marketed in different areas in Morocco. It is difficult to assess the risk of these mycotoxins through spices intake because of the absence of official consumption data. However, according to our knowledge, and since the daily average consumption of each spice is very low (some grams/day) in comparison to some widely consumed foods (like cereals), the risk attributed to these mycotoxins through spices consumption would be very low.

Keywords: Aflatoxins; Ochratoxin A; Contamination; Spices; Morocco



P26 - Food Contaminants Levels in Portugal

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Chemicals contaminants can enter the food chain from multiple sources. Contamination can occur during primary production, from various environmental sources including pollution, waste from factories, fires, contaminated land from natural occurrence, contaminated water, plant diseases, contamination from weeds, plant toxins, climatic conditions.

Chemical contaminants can also enter the food chain at the secondary production stage e.g. during cooking and processing, storage, transportation and handling.

Chemical contaminants may be harmful to health at certain levels. It was therefore necessary to define maximum levels of contaminants achievable through the pursuit of good agricultural, fisheries and manufacturing practices, taking into account the risk associated with food consumption. Furthermore, in the case of contaminants considered as genotoxic carcinogens or where the exposure of the population or vulnerable groups is close to or exceeds the tolerable intake, those maximum levels should be set at a level which is as low as reasonably achievable (ALARA) .

The European Commission defined Maximum Limits (ML) for chemical contaminants in foodstuffs in the Commission Regulation (EC) No 1881/2006 of 19 December. To ensure effective protection of public health and to reduce exposure to consumers, foods containing contaminants that exceed those maximum levels shall not be placed on the market as such or mixed with other foodstuffs or used as a food ingredient.

This work presents the level of contaminants present in foodstuffs available to the Portuguese consumer. The data was determined through the official controls promoted by the Directorate-General for Food and Veterinary (DGAV), which is responsible for coordinating the National Multi-Annual Control Plan that includes all the checks carried out by the Competent Authorities in the Food area.

Keywords: Food contaminants; food safety; official control; occurrence; exposure



P27 - Exposure risk assessment to ochratoxin A through the consumption of three cereal products available on the Moroccan market

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Introduction: Mycotoxins are currently considered as the most contaminants of cereals and derivatives available worldwide. Recently, increased efforts have been made by Moroccan scientists to investigate the presence of selected mycotoxin in food and feed. The presence of ochratoxin A (OTA) in Moroccan foods has been confirmed by previous studies. However, few reports evaluated the exposure of Moroccan local populations to this mycotoxin and characterized the risk associated with this exposure. In this context, the present study was carried out in 2017 to provide answers regarding the exposure of Moroccans to OTA through the consumption of three cereal derivatives widely consumed in the country.

Methodology: The OTA contamination levels of 470 samples of three cereal derivatives (bread, pasta and semolina) randomly obtained in different regions of Morocco, were obtained after OTA analysis by liquid chromatography coupled to fluorescence detection. National consumption data obtained from the Office of the High Commissioner for Planning were used. Exposure to OTA was estimated through a deterministic model based on the crossover of consumption and contamination data under different scenarios.

Results: Results showed that the global weekly intake of OTA through cereal derivatives is 13.88 ng/kg b.w. Bread is considered the main vector of OTA exposure (80%) despite its low contamination level (0.277 ng/g) comparing to pasta (0.645 ng/g) and semolina (0.334 ng/g). At this contamination levels, the risk of OTA nephrotoxic effects through the consumption of the three cereal derivatives in the Moroccan context seems to be very low.

Conclusions and relevance: Despite some uncertainties related to the unavailability of some data, this risk assessment of deterministic type made it possible to assess in a global manner the level of risk associated with the contamination of three cereal derivatives widely consumed in Morocco. This should be complemented by future studies of other food products that could act as vectors of exposure to OTA to further refine this exposure risk assessment.

Keywords: Ochratoxin A; Exposure; Risk assessment; Cereal Derivatives; Morocco



P28 - Optimization of QuEChERS method for determination of pesticides in breast milk

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Introduction: The use of pesticides in forestry, public health, the domestic sphere, and in agriculture results in the appearance of residues of these xenobiotics in foods and human samples. Thus, several methods have been developed for monitoring them; however, most are tedious and expensive. By contrast, the QuEChERS (Quick, Easy, Cheap, Effective, Rugged, and Safe) methodology involves a microextraction that yields small samples and has been applied for the analysis of various xenobiotics. QuEChERS has shown advantages over other techniques including fast sample preparation, reduced needs for reagents and labware, and versatility. The subject of this research project is to develop an analytical method for the quantification of organochlorine (OCP) and organophosphate (OPP) pesticides in breast milk in order to assess the exposure of mother and child.

Methodology: Because of the complexity of the milk samples, the optimization of an easy and fast method for the purification and detection of these pesticides is required. The analytical method developed and optimized in this work was performed in two steps. Isolation and purification of the pesticides from their matrix were obtained by a QuEChERS based methodology (1st step) evaluating three different QuEChERS compositions (original, AOAC and EN) as well as two clean-up compositions (2nd step). Afterward, determination and quantification were done by gas chromatography (GC) using two detectors (electron capture detector (ECD) and Flame photometric detector (FPD)).

Results: The method validation for breast milk showed good linearity with $R^2 > 0.99$, limit of detection in the range 2.4-10.3 µg/L and limit of quantification between 7.8-34.4 µg/L.

Conclusions: This study concludes that the European EN QuEChERS composition combined with clean-up 2 (PSA and Q-Sep) gave the best recoveries for all pesticides.

Relevance: A new validated method is now available for OCP and OPP determination in breast milk. These results are undoubtedly a significant contribution to better understand the contaminants present in food, to assess the potential risk and to improved guidelines.

Keywords: breast milk; organochlorine pesticides; organophosphate pesticides; QuEChERS; Q-Sep; GC



P29 - Food mercury contamination through contaminated soil

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Mercury derived from both natural and anthropological sources is widely distributed around the world. The protection of the entire food from contamination by mercury is an important task. In Slovakia there are several ecologically contaminated areas, which include Central Spiš region (district Gelnica and district Spišská Nová Ves). Mercury content in the soils of Slovakia is higher in comparison with the soils of other world regions taking into account geochemical abnormalities and industry influence – the concentration in soils was checked as up to 130 mg/kg. The average contents of mercury in different types of soils from all over the world range between 0.58 and 1.8 mg/kg.

The aim of the study was to recognize the amount of mercury in plant food collected from Slovakia region. The flameless atomic absorption spectrometry (AAS) method at a wavelength of 254nm (UV region) was used.

The concentration of Hg was measured in different samples of plants. The Hg concentrations in the leaves of plants ranged from 0.14-10.61 mg/kg (dandelion leaves were the highest). The lowest values of mercury were obtained in the roots of carrot, parsley, one year onion and kohlrabi (in the range from 0.02 mg/kg to 0.46 mg/kg). However, in total of 896 samples of fruits and vegetables the range of Hg checked in samples was 0.001-1.779 mg/kg. Potatoes, which are commonly used in daily diet, have Hg (11 samples) from 0.0015-0.0241mg/kg.

We conclude that increasing concentration of mercury in the soil leads to increased absorption of mercury by plants.

Keywords: mercury; soil; plant food



P30 - Assessment of lead and cadmium contamination levels in breakfast cereals available in Rabat, Morocco

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Introduction: The role of breakfast cereals in a balanced diet has been recognized for many years. Such food should have healthy properties and not contain toxic substances, especially trace elements. Among these elements, lead (Pb) and cadmium (Cd) are two important inorganic food contaminants. In this study, the contamination levels of breakfast cereals by Pb and Cd was assessed for the first time in commercialized samples available in Rabat, Morocco.

Methodology: A total of sixty two (n=62) samples of breakfast cereals purchased in different markets in Rabat in Morocco were surveyed for their lead and cadmium content. The amount of two trace elements was determined by Atomic Absorption Spectrophotometer (GF-AAS) after total mineralization of samples.

Results: Results showed that out of total samples, 77.4% were contaminated with Pb concentrations in the range 0.016-1.057 µg/g. The rest of samples (22.6%) were under the limit of quantification (LOQ) of Pb. In the case of Cd, more than 66 % of samples were contaminated with levels that ranged between 0.011 to 0.123 µg/g. In the present study, results showed that 6.45% of positive samples of Pb are above the maximum limit (0.2 µg/g) set by the European Commission Regulation No 1881/2006 for this element in cereals. However for the concentration of Cd, only one sample exceeded this limit (0.1 µg/g).

Conclusions and relevance: This is the first study on the co-occurrence of two trace elements in breakfast cereal samples commercialized in Morocco. The levels compare well with those reported for similar foodstuffs from some other parts of the world. Finally, ready breakfast cereals may constitute an exposure source of Pb and Cd in human diet. It is important that the long-term exposure of these elements be kept to minimal.

Keywords: Lead; Cadmium; breakfast cereals; Contamination; Morocco



P31 - Determination of the cadmium content by atomic absorption spectrometry by flame in edible seafoods coming from China, Korea and Japan, available at Brazilian market

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Cadmium is a heavy metal, considered an environmental pollutant, generated from anthropogenic action. It can be found in several foods, this being the main route of exposure for the non-smoker population. From a toxicological point of view, it is considered carcinogenic and the main affected organs are lung, kidney and liver.

In this work, the methodology of flame atomic absorption spectrometry was used to determine cadmium in edible marine algae. Twelve specimen units of dehydrated marine algae from China, Korea and Japan were available in Brazilian market. The species analyzed were hijiki (*Hizikia fusiformis*), kombu (*Lamina-ria japonica*), nori (*Porphyra tenera*, *Porphyra yezoensis*) and wakame (*Undaria pinnatifida*).

Algae nori and wakame presented high contamination rates, up to 1.93 mg.Kg⁻¹, and the hijiki and kombu algae presented levels of cadmium within the Brazilian legislation, less than 1.0 mg.Kg⁻¹. Of the total samples analyzed, 58.3% had cadmium concentration levels above those acceptable for human consumption and 41.7% were determined with an in-standard level, considering Decree 55871 of March 26, 1965 (BRAZIL, 1965).

The results point to the importance of carrying out actions to monitor the levels of cadmium in food and suggest the need to carry out a risk assessment of the exposure of the Brazilian population to this contaminant through food.

Keywords: Algae; Heavy metal; Cadmium; Atomic flame absorption spectrometry



P32 - Official control of contaminants in food from the Portuguese market between the years 2016-2018

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Contaminants may be present in foodstuffs at toxicologically relevant concentrations and, for this reason, are one of the focus of legislation and regulations in the European Union, where Maximum Levels (MLs) are set to control these levels in food.

The Portuguese Economic and Food Safety Authority (ASAE) collects samples under the National Sampling Plan (PNCA), in order to ensure that foodstuffs placed on the market do not pose health risks, as well as to ensure the interests of consumers at the level of correct and adequate food information. The PNCA takes into account the risk associated with food, the non-compliance rate of previous years, the analytical capacity to detect the hazard and, nowadays, the national food consumption data.

As regards to contaminants, the laboratory results, in addition to providing information and experience to future monitoring and control activities, in particular at the level of the priorities to be established, are a national source of information on the occurrence of contaminants in different food groups. These occurrence data together with the consumption data currently available in Portugal through the National Food, Nutrition, and Physical Activity Survey (IAN-AF), allow the assessment of dietary exposure of consumers to contaminants.

The aim of this work is to present occurrence data of contaminants in different food matrices in the Portuguese market between 2016-2018. All samples were obtained from local/national markets and the analysis was carried out in ASAE's in-house Food Safety Laboratory (LSA), applying accredited testing methods for the determinations of: mycotoxins (aflatoxins, ochratoxin A, zearalenone, deoxynivalenol, fumonisins and patulin); metals (lead, mercury and cadmium); dioxins and PCBs; nitrate; polycyclic aromatic hydrocarbons (PAH) and erucic acid. Between 2016-2018, ASAE planned and collected a total of 2103 samples for the control of contaminants in food, and a total of 14615 determinations were performed. The results over this period show that the non-compliant samples found were in the range of 1-3%, and the main noncompliance detected was aflatoxins in the group of dried fruits and nuts.

Keywords: Contaminants; Dietary exposure; LSA; Occurrence; PNCA; Risk assessment; Sampling plan



P33 - Romanian maize cropping system: A three years survey on total aflatoxins

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Introduction: There is an increasing awareness of the hazards to both human and animal welfare represented by mycotoxins present in food and feed. Mycotoxin contamination represents a clear public health concern. Aflatoxins, mainly produced by *Aspergillus flavus* and *A. parasiticus*, contaminate a wide range of agricultural products. In this context, the purpose of this study was to investigate the incidence of these mycotoxins within the Romanian 2016-2018 maize crop.

Methodology: Maize samples from all around Romania, along with information regarding the specific location of fields and the applied agronomic practices were collected and investigated three consecutive years. A total of 394 maize samples was assessed for the occurrence of total aflatoxins using ELISA method (RIDASCREEN® Aflatoxin Total kit, R-Biopharm AG, Germany).

Results: For the 2016 harvest, 14 samples from the southern, southeastern and southwestern regions registered total aflatoxin levels higher than the maximum accepted limit of 10.00 µg/kg (Commission Regulation (EC) No 1881/2006). The highest concentration was 397.56 µg/kg, noted by a sample from the southern region (Argeş county). In 2017, only two samples exceeded the maximum level: one sample from the southern region (Prahova county: 18 µg/kg) and one sample from the northwestern region (Bihor county: 30 µg/kg). In 2018, 21 samples noted a total aflatoxin contamination, with concentrations between 1.80 - 5.48 µg/kg. Macroregion 3 (the southern regions) noted the highest number of contaminated samples (18.18% of the total number of samples from this region). Macroregion 4 (the south-western and western areas) noted the smallest number of contaminated samples.

Conclusions: When referring to the analyzed samples, the total aflatoxin contamination was independent of the type of hybrid, but strongly influenced by the differences of pedo-climatic conditions. The southern counties proved to represent critical risk areas for aflatoxin contamination on maize crops.

Relevance: This study highlights the importance of an effective and sustainable mycotoxin management along the food and feed chain, as well as the need of mapping the mycotoxin risk areas. Within a sustainable development approach, alternative practices that maintain the quality of crops with respect to the environment, humans and animal welfare have to be developed.

Keywords: aflatoxins; maize; mycotoxins



P34 - Evaluation of a dispersive-solid phase extraction cleanup procedures in polybrominated diphenyl ethers and novel brominated flame retardants analysis with QuEChERS in *Capsicum* cultivars

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Introduction: Brominated flame retardants (BFRs) are mixtures of chemicals that are added to a wide variety of products to make them less flammable. BFRs have raised great concerns for their persistence, bioaccumulation, and harm to human health. In the European Union the use of certain BFRs is banned or restricted. The attention in monitoring BFRs is growing and the European Commission has adopted a recommendation (118/2014/EU). The contamination of BFRs in food results not only from the environment but also during food processing or storage.

Methodology: Different QuEChERS (original, AOAC and EN), and different sorbent compositions for the cleanup step were evaluated in terms of recovery and matrix effects. The best results were obtained with citrate-European version QuEChERS and a cleanup step, with 150 mg MgSO₄, 50 mg PSA, 50 mg C₁₈, and 10 mg of carbon. Gas chromatography (GC) coupled with an electron capture detector was used for the detection and the quantification of the BFRs.

Results: The limit of detection was between 1.39-9.10 µg/kg and R²>0.99. Recoveries and matrix effects were between 67-95% and 0.58-2.18, respectively. The relative standard deviations from repeatability and reproducibility studies were lower than 20 %. GC coupled with a mass spectrometer was used to confirm the presence of the BFRs in the samples. The method was applied to 24 samples of red capsicum cultivars [12 Chili peppers, 6 red sweet peppers (*Capsicum annum* L), 3 *Capsicum frutescens* L., 3 *Capsicum Chinese* L.]. A novel BFR, was observed in a chromatogram of two samples of *Capsicum annum* L and *Chili peppers*. According to the matrix calibration curve, the concentrations were 3.62 and 3.85 µg/kg.

Conclusions: The use of 10 mg of carbon instead of 50 mg, permitted to meet the objective in terms of recoveries, ME and precision verified by the RSDs, recommended by the analytical guidelines.

Relevance: For the first time, a simple, fast and cost-effective sample preparation method is proposed as an extraction technique to determine 12 BFRs (7 congeners polybrominated diphenyl ether (PBDE) and 5 novel BFRs) in *Capsicum* cultivars.

Keywords: brominated flame retardants; Novel brominated flame retardants; chili peppers; Capsicum annum L.; QuEChERS; GC



P35 - Optimization of extraction procedure for Organochlorine pesticides evaluation on Shrimp samples

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Introduction: Organochlorine pesticides (OCPs) were widespread used around the world and very persistent in the environment. OCPs are, lipophilic, and hydrophobic compounds that can accumulate in biota, become biomagnified through the food chain. Despite several OCPs have been banned, they can still be detected in sediments, plants and animals. Shrimp is one of the most consumed shellfish. It is quite nutritious, rich in proteins, omega-3 fatty acids and a good source of phosphorus, choline, copper, selenium, zinc, iodine, astaxanthin and vitamins. However, they can also accumulate pollutants such as OCPs. The shrimp specie *Palaemon serratus* is widely distributed in Portugal coastal areas and estuaries, is the subject of small-scale fisheries.

Methodology: The OCP residues were extracted by using Quick, Easy, Cheap, Effective, Rugged and Safe (QuEChERS) approach. Three different QuEChERS (AOAC, EN and Original) were tested. The best results were obtained for AOAC QuEChERS. A volume of 1.5mL of prepared aliquot was sampled from the upper layer into a 2-mL centrifuge vial containing a clean-up sorbent with graphitized carbon black. The amount of carbon was optimized in order to use the smaller quantity that are capable of remove the pigments from the samples. An aliquot of the supernatant was transferred to a vial, and the extract was concentrated just to dryness. The sample residue was reconstituted in n-hexane and placed into an auto sampler vial for analysis. The extent of the environmental contamination was reached through the quantification of 14 OCPs by using gas chromatography coupled with electron-capture detector.

Results: The limits of detection and quantification obtained were between Endosulfan I (4.7 µg/L and 15.5 µg/L) and β-Hexachlorocyclohexane (10.7 µg/L and 35.5 µg/L) respectively. Recovery testes were made for 20, 30, 40 and 50 µg/L levels. Recoveries ranged from 56% for Hexachlorobenzene and 112% for α-Hexachlorocyclohexane.

Conclusion: A simple and faster analytical methodology to analyze pesticides in shrimp samples was developed.

Relevance: Shrimp is much appreciated and widely consumed, and for that reason is important to guarantee the safety of these foods.

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Keywords: Shrimp; Organochlorine pesticides; QuEChERS



P36 - Assessing the exposure and vulnerability of children with celiac disease to inorganic arsenic from gluten-free cereal foods: a novel approach combining biomarkers of exposure and of methylation capacity

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In the general population the main source of inorganic arsenic (iAs) is food, with rice playing an important role depending on consumption figures. Chronic oral exposure to iAs, depending on duration and magnitude, is associated to several adverse outcomes and a range of BMDL01 values has been established by EFSA for lung and other cancers, and skin lesions. Rice is used as a substitute for wheat in the diet of children affected by celiac disease (CD), a condition caused by an abnormal immune response to gluten. This pediatric population may have a higher dietary exposure to iAs, due to the higher consumption of rice-based foods, compared to non-celiac children of the same age. Along with exposure, vulnerability to iAs toxicity, which is largely dependent upon the individual methylation capacity (i.e. the iAs detoxification efficiency), is a major determinant of risk.

A multicentric nationwide study involving 170 CD and 173 healthy children aged 3-10 years has been undertaken. A Food Frequency Questionnaire was administered to identify the types and amount of rice and gluten-free products eaten by CD children (food groups belonging to the main category 'cereals and cereal products'). Seventy-three major food items were selected based on consumption frequency and, after cooking when appropriate, were characterized for iAs content by arsenic speciation analysis. Concentrations of iAs in biscuits, cakes and sweet snacks, flours, bread, pizza, and savoury fine bakery were substantially higher than those measured in the recent National Total Diet Study and rice was the key ingredient for such higher levels. To quantify the magnitude of iAs exposure, nail arsenic was used since it is not affected by the DMA present in rice, which hampers the use of speciated urinary arsenic (i.e. the sum of iAs and its methylated metabolites, MMA and DMA) as a biomarker of exposure. Nail arsenic was significantly higher in CD children (34% greater average content compared to other children). Urines of children were collected after a 5-d diet with exclusion of food containing (or being metabolized to) DMA and analyzed for iAs and methylated metabolites. The relative proportions of the species were used as measure of the individual methylation capacity and showed that CD children do have a better detoxification of ingested iAs.

Keywords: Celiac disease, children, arsenic, speciation analysis, biomarkers, exposure assessment



P37 - Determination of Bisphenol A in Canned Fish Food

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Introduction: Food contact materials should be monitored to ensure product quality and safety, with the ultimate objective of ensuring that no damage will occur to consumers' health. Bisphenol A (2,2-bis(4-hydroxyphenyl)propane), known as BPA, is authorized by the European Commission according to Commission Regulation (EU) no. 2018/213 to be used in the manufacture of plastic food contact materials (FCM) with a specific migration limit (SML) of 0.05 mg/kg. This chemical can be transferred to food from some types of containers such as polycarbonate containers and metal cans with epoxy resin coatings. The health concern related to BPA is due to the fact that its chemical structure allows it to fit in the estrogen receptor behaving as an endocrine disruptor.

Methodology: In this work, canned fish (tuna and sardines) was analyzed to evaluate the migration of BPA from the metallic cans. An analytical method based on ultra-high performance liquid chromatography with fluorescence detection (UHPLC-FLD) was optimized for BPA identification and quantification. A UPLC® BEH Shield RP18 pre-column (2.1 x 5 mm, 1.7 µm) and an Acquity UPLC® BEH Shield RP18 column (2.1 x 100 mm, 1.7 µm) were used. The mobile phase was a gradient of ultrapure water (solvent A) and acetonitrile (solvent B). The BPA was quantified at $\lambda_{\text{emission}}$ 273.1 nm and at $\lambda_{\text{absorption}}$ 317 nm. The method was validated regarding the following parameters: linear range, linearity, limit of detection (LoD), limit of quantification (LoQ), precision and accuracy.

Results: The LoD of the proposed method was 0.02 mg/kg, indicating it is suitable to monitor the safety of the product, since both are below the SML established in accordance with EU legislation for FCM. All analyzed fish samples presented BPA values below the LoD, complying with the established SML. Fortification tests were performed at four different levels: 0.4, 0.6, 0.8 and 1.2 mg/kg. The recoveries varied between 106%-110%.

Conclusions: The results of this study show that we are dealing with a suitable method for the determination of bisphenol A in canned fish. Furthermore, they demonstrate the analyzed canned tuna and sardine are safe for consumption regarding BPA migration.

Keywords: Bisphenol A; Canned tuna; Migration; UHPLC



P38 - The natural occurrence of ochratoxin A in rice commercialized in six cities of Morocco

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Introduction: In Morocco, a North African country surrounded by the Atlantic Ocean and Mediterranean Sea, has a climate characterized by high humidity and high temperature which favor growth of fungi that produce Ochratoxin A (OTA), mainly *Aspergillus ochraceus* and *Penicillium verrucosum*. OTA is a mycotoxin detected in a variety of food like cereals, olives and dried fruits. The aim of this study is to evaluate the occurrence of OTA in 70 samples of rice commercialized in Morocco.

Methodology: Seventy samples of rice were collected from different local markets in six Moroccan cities: Rabat (n=14), Errachidia (n=12), Kénitra (n=11), Mohammadia (n=11), Tangier (n=10), and Casablanca (n=12). All collected samples were analyzed for the presence of OTA. Samples were extracted with mixture of acetonitrile/acetic acid/water (50/49/1 v/v/v). Then the OTA was identified and quantified with liquid chromatography (LC) coupled to fluorescence detection.

Results: Analytical results from the natural occurrence of OTA in rice commercialized in Morocco showed that this mycotoxin were present in 42.8% of total analyzed samples, the rest of samples (57.2%) were under the limit of quantification (LOQ=0.05 ng/g). The frequencies of contamination of samples with OTA varied between 91% and 8.3%. OTA levels varied between 0.23 and 2.47 ng/g. The previous results illustrated that the highest frequency of positive samples (91%) and the highest OTA value (2.47 ng/g) was found in samples of Mohammadia.

Conclusion: This survey summarized that rice samples commercialized in Morocco could be contaminated with OTA. From the results above, we concluded that the Moroccan population is exposed to the toxic effects of OTA, for this reason studies on the effects of these toxins are needed to assess the impact of the contamination on human health.

Keywords: Ochratoxin A; Occurrence; Rice; Morocco



P39 - Transfer of cannabinoids from industrial hemp into cow's milk

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Introduction: Industrial hemp fibers are coming back into focus for a variety of uses, and seeds are increasingly used as food. Permission of using industrial hemp relies on a maximum content of 0.2 % of tetrahydrocannabinol (THC). However, there is concern about the possible transfer of cannabinoids into food of animal origin when hemp or hemp by-products are used as feed. We conducted an experiment studying the transfer of different cannabinoids in cow's milk after feeding industrial hemp.

Methodology: A feeding trial was conducted with ten lactating Holstein Frisian dairy cows. All animals received a hemp-free diet for seven days before a period of feeding whole plant hemp silage with low cannabinoid content (Δ 9-THC: 58 mg/kg DM, CBD: 805 mg/kg DM) for seven days (adaption period). Subsequently, animals were separated into two groups receiving hemp silage produced from leaves, flowers and seeds with higher cannabinoid content (Δ 9-THC: 1255 mg/kg DM, CBD: 8304 mg/kg DM) at either 0.84 (group 1) or 1.68 kg DM/animal/day (group 2) respectively for six days (experimental period). Thereafter, both groups were fed hemp-free diets for another eight days. Milk samples were taken regularly and animal health was recorded. The concentration of six different cannabinoids and two metabolites of Δ 9-THC was determined.

Results: During the first two weeks, animals showed no clinical signs and only low cannabinoid levels were detected in the milk. During the period with high cannabinoid exposure, the average intake of Δ 9-THC was approximately 1000 mg/animal/day for group 1 and 1700 mg/animal/day for group 2, respectively. Concomitantly, average CBD intake was approximately 6900 (group 1) and 11 000 mg/animal/day (group 2), respectively. As a consequence, the cannabinoid concentration in cow's milk reached over 2 mg/L for CBD and nearly 0.5 mg/L for Δ 9-THC. Other cannabinoids were detected as well. In addition, a substantial response of the animals (feed intake, heart rate) was observed.

Conclusions and relevance: Feeding industrial hemp silage to dairy cows can result in considerable Δ 9-THC and CBD contents in the milk. Moreover, animal health could be affected. The relevance of the results for consumer safety and animal welfare requires further investigation.

Keywords: THC; cannabinoids; industrial hemp; cow's milk



P40 - Veterinary drugs stability upon cooking and in vitro digestion of chicken meat

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Veterinary drugs (VDs) as β -lactams, sulfonamides (SA), tetracyclines (TC), macrolides, fluoroquinolones (FQ) and coccidiostats are widely used in poultry livestock production to prevent and treat diseases [1]. Their administration via feed often leads to their presence as residues in animal tissues and consequent carry-over to humans causing allergic reactions in hypersensitive individuals, destruction of the dynamic balance of gastrointestinal flora, and increasing of microbial resistance to antibiotics [1]. As chicken meat is cooked before consumption, it is important to study the effect of the different cooking practices VD residues stability, a subject scarcely reported in bibliography. Moreover, the addition of herbs/beer has shown to reduce humans' exposure to contaminants (e.g. mycotoxins, polycyclic aromatic hydrocarbons) in cooked meat [2,3], however no information was found concerning VDs.

This study evaluated the impact of oven cooking (200°C, 15 min) and microwaving (600 W, 1.15s) chicken burgers with/without herbs/beer on the stability and bioaccessibility of 14 VDs. Two VD concentration were tested: 100 μ g/kg and 1 mg/kg. The in vitro digestion was performed according to INFOGEST protocol [4]. The VDs were quantified by liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) and high-resolution mass spectrometry analyses were performed to identify the degradation products.

VDs were quite stable to cooking except amoxicillin, chlortetracycline and tylosin with reductions higher than 50%. The rearrangement of molecules and dechlorination reactions are probable transformation products derived from cooking. Adding herbs before cooking had a negative impact on almost all VDs, avoiding their reduction.

Concerning the in vitro digestion, the interaction between VDs and matrix components as cations, proteins and bile salts contributed to low bioaccessibilities (<60%). Therefore, concerning these interactions, the prediction of VDs bioaccessibility cannot be based on its free form as VD interaction with bile salts is known to increase the absorption of lipophilic compounds.

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Keywords: microwaving; oven cooking; oregano; beer; antibiotics; bioaccessibility; transformation products; LC-MS/MS; HRMS



P41 - Assessment of heavy metal contamination in groundwater samples around Oum azza controlled landfill (NW, Morocco) and related human risk

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Introduction: Developing countries such as Morocco deal with hazard effects of socioeconomic development on environment. Groundwater contamination in the vicinity of the controlled landfill poses a Substantial risk to local resource users and to the natural environment; including the risk related to heavy metal pollution especially that water wells are used for irrigation and animal watering. The aim of this study was to assess the level of heavy metal in groundwater samples and their hazardous effects on surrounding environment in Oum azza area, NW of Morocco. The purpose of this study was to investigate the potential environmental and human health risks caused by heavy metal pollution.

Methodology: Samples were collected from twelve wells in dry and wet seasons from Oum azza area near controlled landfill. We assessed levels of trace metals by measuring Pb, Cd, Cr and Zn. The concentrations of these metals were determined using SpectrAA-20 (Varian) atomic absorption spectrometer.

Results: From the heavy metal concentration of the water samples, it was observed that all assessed metal were present but with different levels, Except Pb concentration being more than the standards water drinking (10 ug/L) especially in the first two campaigns, posing health hazards to the communities who rely on the water. The following order of the heavy metal concentration is not the same for all seasons; the Pb was the most important element; however, the cadmium presented the lowest concentration.

Conclusion: Except lead, the concentration of these elements does not exceed recommended standards. Nevertheless, there is health-hazard risk of local population; by bioaccumulation effect, especially as water wells are used for irrigation and animal watering.

Keywords: Heavy metal; human health; contamination; environmental risks



P42 - Occurrence and profile of PSP toxins in ascidians *Microcosmus spp.* harvested in western waters of istrian peninsula

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Many edible solitary ascidians such as *Microcosmus spp.* represent an easily available, highly nutritious food source, rich in proteins and different micronutrients. Considering that they are filter-feeding marine animals, ascidians can accumulate contaminants such as marine biotoxins and become their vectors to consumers. The aim of this study was to determine the occurrence and profile of hydrophilic phycotoxins causing Paralytic shellfish poisoning syndrome (PSP) in 47 samples of *Microcosmus spp.* harvested in the waters of the western Istrian peninsula in the period from April to September 2018. According to our knowledge, this was the most comprehensive study considering the number of samples and the length of study. The analysis has been carried out using high performance liquid chromatography with fluorescence detection (HPLC-FLD) and pre-column oxidation method. PSP toxins were detected in 32 samples in concentrations far below the regulatory limit of 800 µg STX eq. kg⁻¹ set by the European legislation. Detected analogs were decarbamoylsaxitoxin (dcSTX), gonyautoxin 2,3 (GTX2,3) and gonyautoxin 5 (GTX5). The most dominant analog among them was less potent dcSTX detected in 26 samples, the most potent GTX2,3 was detected in 10 samples and the least potent GTX5 in 16 samples. Given the ability of ascidians to accumulate phycotoxins, the fact they are often harvested in the wild and consumed by coastal populations, there is a need for monitoring PSPs in these species, to ensure food safety and protection of consumer's health.

Keywords: PSP; phycotoxins; contaminants; ascidians; HPLC-FLD; food safety



P43 - Acrylamide: will the golden cookie be a cancerous cookie?

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Acrylamide is one of the main food contaminants in products made at high temperatures, such as cookies. The most important route for acrylamide formation is Maillard reactions. These are complex and in many cases desirable because of the organoleptic characteristics that they confer to the food. However, due to its high presence in several foods, acrylamide is classified as a possible carcinogen, which reveals the importance of its detection, quantification and mitigation.

Aim: Check the favorable conditions for the formation of acrylamide in wafers, and the associated toxicity levels.

Methods: The research was made through the databases "Google Scholar", "PubMed" and "Science Direct", scientific and review articles were searched using the keywords "Acrylamide", "Cookies", "Toxicity", "Maillard reactions" and "Asparagine". Articles published before 2014 were excluded as well as patents and citations. The selected articles were chosen by title, abstract and then extensive reading.

Through the literature review it is possible to verify that acrylamide formation is favorable at more basic pH (pH = 8), and that at lower pH, formation is not as significant in certain recipes. It is also apparent that the amount of acrylamide may increase to a certain temperature, such as 170° C, but after a prolonged exposure, about 20 minutes, it tends to decompose, thereby decreasing its concentration.

Although there are so many techniques to mitigate acrylamide, it should be noted that many of these strategies are also linked to possible formation of undesirable compounds. As a carcinogenic and genotoxic compound, it is not possible to establish a tolerable or safe daily intake. There are no current recommendations as the European Food Safety Authority acknowledges that human testing is unconscious and limited because of the results obtained from animal studies. Legislation governing the limits of acrylamide is necessary because it is a concern for the public health.

Keywords: acrylamide; crackers; toxicity; Maillard reactions; asparagine



P44 - Contamination of food supplements by androgens

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Introduction: Nutritional supplement are any oral substance ingested that contains elements capable of supplementing a diet. Androgenic Anabolic Steroids (AAS) are hormones that share a common mechanism of action with testosterone, advertised as having muscle growth properties can be found in nutritional supplements. According to Autoridade Antidopagem de Portugal (ADOP) rules, these AAS are part of a class of prohibited anabolizers.

Aim: To understand the effects contamination of food supplements by AAS can have on human health with a focus on athletes. **Methology:** Research in database "Google Scholar" and "Pubmed" with the keywords "androgens" AND "nutritional supplements" AND "athletes" until 21, February 2019. Selection was made from title, abstract and full-text.

Results: Non-hormonal supplements may contain undeclared prohormones, which can be metabolized in the prohibited AAS. A study found that 14.8% supplements contained 0.01-190 ug/g leading to a positive result in doping results for several hours.(1) There is no mandatory regulation for their control, it is performed by third parties who evaluate them as to purity, quality and composition, but participation is voluntary. Prolonged use of adulterated supplements with AAS affects both genders, having sexual, metabolic, cardiovascular, neurological, psychiatric and dermatological consequences.

Conclusion: Before supplementing, it is essential that the athlete is evaluated as to their need. If a dietary supplement is needed or desired, its use should be done with caution and only after careful evaluation as it may cause adverse effects.

Keywords: androgens; nutritional supplements; athletes; contamination; hazard



P45 - Biological/Organic products: safer than conventional counterparts?

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During the last years, social concerns with sustainability, safety and quality of conventionally-grown fruits and vegetables increased notably. This has led to a growing recognition of the need to develop alternative agricultural practices such as biological/organic production. The organic food market is projected to increase as consumers associate organic food with “natural”, care for the environment and animal welfare and absence of pesticides and fertilizers. If on one hand, some studies correlate organic production systems with lower environmental impact, on the other hand, these can be considered as a risk to the population due to the use of animal manure as fertilizers that may contain enteric bacteria or other pathogens responsible for foodborne diseases. This is a major concern for fresh fruits or vegetables eaten raw such as lettuce.

In order to evaluate if there were any microbiological differences between samples of organic and conventional products, 10 samples of each production mode were purchased in Porto (19 in supermarkets and one in a biological market) and analyzed for a wide range of parameters, including pathogens and indicator organisms. Microbiological analysis for enumeration of mesophilic bacteria, lactic acid bacteria, *Pseudomonas spp.* yeasts and molds, Enterobacteriaceae and *Escherichia coli*, and detection of *Salmonella spp.* and detection/enumeration of *Listeria monocytogenes* were performed. In addition, determination of nitrate concentration in lettuce samples was also performed.

Significant differences ($p < 0.05$) between conventional and organic samples were only detected for total microorganisms at 30 °C. Pathogenic microorganisms like *Salmonella spp.* and *L. monocytogenes* were absent in all samples. No significant differences ($p > 0.05$) were detected in nitrate concentration between organic and conventional products.

Based on the samples analyzed and for the parameters investigated, with the exception of total mesophilic counts, organic and conventional lettuce do not differ significantly.

Keywords: Organic lettuce, conventional-grown lettuce, microbiological safety



P46 - *Y. enterocolitica* and a traditional German raw pork product – a risk?

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Introduction: According to EFSA, *Yersinia* (*Y.*) *enterocolitica* is the 3rd most common bacterial enteropathogen in the European Union (EU), causing the so-called yersiniosis. In some parts of Germany, as well as in other Member States of the EU (e.g. Belgium), it is common to eat raw minced pork (so-called "Mett"). In parallel, such products have been identified as the main risk factor for yersiniosis in DE. Against this background, the question arises to what influence household practices have on Mett as a possible source of infection.

Methodology: Fresh Mett batches were prepared according to German standard recipes from raw pork minced meat, spices and onions. Subsequently, they were inoculated with monocultures of two *Y. enterocolitica* strains (human strains, Biotype 4, Serotype O:3 and O:9) with 104 and 106 cfu g⁻¹, respectively. The household practices were simulated by storing the Mett at room temperature (~ 22°C) up to six hours. Sampling was based on the modified ISO-method for cultural detection of pathogenic *Y. enterocolitica*. Suspicious colonies were confirmed by means of Maldi-ToF. In addition, the values of pH and water activity (aw) were determined.

Results: Preliminary results indicate growth of *Y. enterocolitica* during storage, which seems to be uniform in both strains and for both inoculum sizes. At the same time, a slight decrease of the aw-value and the pH was observed.

Conclusions: The initial results indicate that Mett stored at household conditions does not appear to inhibit the growth of *Y. enterocolitica*. Since pigs are the main reservoir for *Yersinia*, raw pork may be contaminated with the pathogen and thus Mett may pose a risk to public health. Therefore, such products should preferably not be consumed by risk groups (pregnant women, small children, elderly and immunocompromised persons). In this context, address-oriented risk communication is particularly important, as these traditional products are consumed especially in rural areas by small children and the elderly, who are often unaware of these food-related risks.

Acknowledgement: This research has been funded by the EU-FORA fellowship programme and the German Federal Institute for Risk Assessment, Germany.

Keywords: Yersinia enterocolitica; Mett; risk assessment; household practices



P47 - Assessment of histamine and spermidine levels in fishery products from Portuguese markets by Enzyme Linked Immunosorbent Assay (ELISA)

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Introduction: There is a global concern regarding food spoilage, which makes food consumption inadequate, and may pose a serious risk to public health [1]. As fishery products deteriorate, they undergo a complex series of events leading to the production of biogenic amines (BAs) [1]. Therefore, there is a strong need to screen BA in seafood using reliable methodologies to assess fish quality. In the present study, we determined the levels of histamine and spermidine in several seafood products (fresh fish and mollusks), purchased in traditional Portuguese markets.

Methodology: Fresh fishery products (*Sardina pilchardus*, *Trachurus trachurus*, *Sparus aurata*, *Ruditapes decussata*) were purchased in Portuguese markets and transported to the laboratory in refrigerated containers. Fifteen specimens were sampled (from each species) and processed for analysis by homogenizing in phosphate-buffered saline solution and analyzed for the presence and content of histamine and spermidine using an indirect enzyme-linked immunosorbent assay (ELISA) [3]. Statistical analysis was performed using the Statistica 8.0 software (Statsoft, USA).

Results: As expected, the presence of the selected BAs (histamine and spermidine) were variable between selected species but also between samples from the same species. The presence of at least one of the selected BAs was detected in most of the samples analyzed. The highest levels of histamine were detected in *T. trachurus*.

Discussion and Conclusions: For several samples, the detected levels of the biogenic amines were below the limits established by the FDA [4,5] and the EU Commission Regulation (EC) No 1441/2007. Although there are no safety limits established for spermidine all species analyzed showed high levels of this biogenic amine.

Relevance: The relevance of this study is based on the importance of assuring the quality of fishery products, avoiding risks to the consumers.

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Keywords: fishery products; histamine; spermidine; ELISA; quality



P48 - Dietary supplements for weight loss with synephrine available in the Portuguese market: risks and toxicity

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Introduction: Synephrine is the main active compound present in *Citrus aurantium*, and is included in many dietary supplements that advocate the promotion of weight loss. Some authors report a thermogenic effect, associated with an increase of basal metabolism and lipolysis, and consequent weight loss. However, studies that prove the thermogenic effect and safety of synephrine are lacking. Importantly, several international food safety authorities issued recommendations on the maximum daily levels.

Objective: Study the composition of dietary supplements containing synephrine available in the Portuguese market and analyze the adverse effects associated with their synephrine daily doses.

Materials and Methods: A research at physical stores and internet websites, between March 2018 and April 2018, was conducted to find dietary supplements for weight loss containing synephrine. For the bibliographic support, the scientific articles were searched in the PubMed, B-on, Science direct and Google Scholar data bases.

Results: 37 supplements containing synephrine were found, but it was only possible to infer about the amount of synephrine in 16 supplements. It was assessed that the *Citrus aurantium* daily dose recommended by the producer and/or distributor corresponded to a median value of 200 mg, with a minimum value of 6 mg and a maximum value of 840 mg. The median amount of daily synephrine recommended is 12 mg, with a maximum value of 60 mg and a minimum value of 0.6 mg. Anhydrous caffeine and synephrine were combined in 9 supplements. Several supplements exceed the daily dose limits of synephrine recommended by food safety authorities, of note that 13 of the 16 supplements exceed the limits recommended by the German BfR authority.

Conclusion: Synephrine daily levels present in various supplements can be dangerous to health consumers. The cardiovascular risk of synephrine is increased in combination with caffeine. Consumers of these supplements should be informed of adverse effects, especially at the cardiovascular and cerebrovascular levels.

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Keywords: synephrine; Citrus aurantium; dietary supplement; body weight; cardiovascular toxicity



P49 - Quality of cockles can be affected by harvesting and transport techniques

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In Portugal, harvesting of cockles (*Cerastoderma edule*) is still carried out using artisanal techniques. These techniques can induce stress in bivalves as they are exposed to air and temperature fluctuations, from the harvesting time to arrival at the depuration and expedition centres.

Thus, to evaluate the stress caused by these procedures, two strategies were tested: usual transport at ambient temperature vs transport in a cooled environment. Animals from the two groups were sampled after arriving the expedition centre in Ria Formosa (3h after harvesting). Posteriorly, the two groups of bivalves were transported in cooled environment ($6\pm1^{\circ}\text{C}$) to the experimental depuration centre Aveiro (7h) and sampled at arrival. After, bivalves were depurated during 24h in recirculated 250L tanks at 15°C and posteriorly sampled. Finally, bivalves of the two groups were stored for 6 days at $5\pm1^{\circ}\text{C}$ and sampled at the end of this shelf life period. In each sampling, edible tissue samples were immediately frozen in liquid nitrogen and kept at -80°C until biochemical determinations. Comet assay was performed to evaluate the DNA damage. Lipid peroxidation and cellular energy allocation (ratio between energy reserves and energy consumption) were measured. Cockles transported in a cooler environment presented lower levels of DNA damage after both transport and shelf life period.

Results of sampling after arriving experimental depuration centre revealed that energy consumption in bivalves transported in a cooler environment after harvesting was lower when compared to the values obtained in bivalves transported at ambient temperature. Higher values of energy reserves were measured in the cooled group comparing to ambient temperature group after the transport and also after 24h depuration. Regarding the energy budget it is possible to observe a significant increase during the ice cooled transport comparing to the ambient temperature. Additionally, the transport in cooler conditions seemed to increase the quality of cockles until 6 days of shelf life, as lipid peroxidation (LPO) was lower in these group of *C. edule*. Thus, the transport of harvested *C. edule* in a cooled environment seems to be an effective measure to minimize all the stress associated with handling procedures.

Keywords: biochemical biomarkers; DNA damage; Cerastoderma edule; packaging



P50 - Biochemical status of *Solen marginatus* harvested in class C areas, after a depuration process testing water temperature and organism positioning in tanks

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Bivalve molluscs represent a highly important fishery resource in the national economy and are present in many traditional recipes of Portuguese gastronomy. However, bivalve consumption may represent a risk to human health. European legislation requires the classification of production areas in class A, B or C, according to microbiological load (content of *Escherichia coli* and in the presence or absence of *Salmonella* sp.), to ensure food safety. Bivalves produced in B areas must to be depured before being commercialized for human consumption. Still, bivalves produced in C areas theoretically cannot be simply depured, and must be transpose into class A areas, for a period up to 4 months, before being depured and commercialized. However, due to associated costs and mortality, this process is not economically viable. This study aimed to assess whether it is possible to depurate *Solen marginatus* reared in class C areas. There were tested four temperatures: 10, 15, 20 and 25 °C; and two organisms positioning in the tanks, for temperature 15 °C: sets of *S. marginatus* placed horizontally - as they are usually placed in commercial depurators, or placed vertically - as they are in their habitat. In all the remaining temperatures, bivalves were placed in a vertical position. The depuration was performed during 24 h in 250L recirculated tanks, individually equipped with filtration (UV-C unit and protein skimmer) and temperature control systems. Before and after the depuration process, edible tissue from bivalves were sampled to analysis of microbiological content, genetic damage, and biochemical biomarkers. *S. marginatus* depurated better at 10 and 15 °C, presenting the lowest values of *E. coli*. At the end of depuration, organisms placed at 15 °C vertically had a higher protein content than the ones placed horizontally. Similarly, the lower values of DNA damage were observed for *S. marginatus* at 15 °C vertically. Overall, results were promising showing that is possible depurate organisms from class C areas in 24 h. This study also highlights the relevance of adapting depuration practices to physiological requirements of each species in order to improve the biochemical composition and the survival during commercialization period.

Keywords: Bivalve molluscs; purification; food safety; DNA damage; biochemical biomarkers



P51 - Temperature effects on the depuration and shelf-life of *Ruditapes decussatus* from class C areas, to ensure food quality and safety

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Bivalves are healthy and nutritious food sources with high commercial value in world markets. However, when microbial contamination from harvesting areas are passed to consumers, bivalves can cause foodborne diseases. Therefore, harvesting areas in Europe are grouped into class A, B and C based on *Escherichia coli* load, which must not exceed 230, 4600 and 46000 *E. coli*/100 g of flesh in A, B and C areas, respectively. Harvesting from class C is usually avoided as bivalves will require a transposition of more than 2 months to relaying areas before depuration and commercialization for human consumption, which involves major economic costs. The present study aimed at developing an efficient method for depuration of class C *Ruditapes decussatus* at minimal time and cost. Further, the effects of water temperature on clam depuration and posterior shelf life were assessed through the measurement of oxidative stress biomarkers. Clams were subjected to depuration for 24 h in recirculating tanks equipped with filters and UV-C system at 10, 15, 20 and 25 °C. Samples were collected for: microbiological analyses at 0 and 24 h; biochemical biomarkers and DNA damage at 0, 24 h (depuration period), 2, 4 and 6 days (shelf life). Results showed that 24 h depuration, at all tested temperatures, is enough to reduce microbial load of class C to class A areas with no adverse effects on survival (100 % survival in all treatments) at the end of shelf life period. In general, oxidative defenses decreased during the experimental period and clams purified at 25 °C presented significantly higher values of lipid peroxidation (LPO) and DNA strand breaks. The present study showed that 15°C is a suitable depuration temperature for *R. decussatus* and can help to reduce time and cost in purifying clams from class C, thus making them safe for human consumption. Overall, these findings are promising to invigorate the depuration process and can contribute to improve product quality and reduce food losses, through survival, biochemical profile, and antigenotoxic biomarkers analyses.

Keywords: bivalve; food loss reduction; microbiological load; commercialization



P52 - Prevalence of *Campylobacter* in chickens from different production systems

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Campylobacter is the major cause of gastroenteritis from bacterial origin, known as campylobacteriosis, since 2005 and was responsible for 72.8% of the reported cases in 2017 in Europe. Poultry are the biggest reservoir of *Campylobacter*, which makes the consumption of raw or undercooked chicken and ready-to-eat foods that have been in contact with raw chicken the most common source of infection [1].

This study aimed to investigate the prevalence of *Campylobacter* in chickens from different production systems: commercial broiler, free range and backyard chickens. Samples were purchased in local supermarkets or kindly provided by people who raise chickens at home. Detection and quantification of *Campylobacter* spp. was performed by cultural methods using chicken skin following the ISO 10272-2:2017, using the modified charcoal-cefoperazone-deoxycholate agar with Bolton enrichment broth. *Campylobacter* species confirmation was performed by multiplex PCR.

An overall prevalence of 90% of *Campylobacter* was obtained for the 30 samples tested. All samples from commercial broiler and free-range chickens were positive for *Campylobacter* (100% of prevalence) with levels of contamination of $3.0E^{+03}$ CFU/g and $4.4E^{+02}$ CFU/g, respectively. Regarding backyard chickens, 70% (7/10) of the samples were positive with an average of $4.2E^{+04}$ CFU/g. *Campylobacter jejuni* and *Campylobacter coli* were the only species of *Campylobacter* detected in all samples.

Free-range chickens had the lowest levels of *Campylobacter*. Even though the prevalence of *Campylobacter* was lower for the backyard poultry samples this type of production presented the higher levels of contamination. Commercial broiler chicken and backyard chicken presented, in average, levels of contamination above the limit set by the European legislation for process hygiene criteria ($1.0E^{+03}$ CFU/g), which presents a risk for *Campylobacter* foodborne illness.

[1] EFSA and ECDC 2018. The European Union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2017. EFSA Journal 2018;16(12):5500, 262 pp

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Keywords: Campylobacter; broilers; chicken



P53 - Evaluation of salt content in Portuguese hospital patient menus

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Measures intended to reduce salt consumption are a Public Health priority, in order to address chronic diseases, related to excessive salt utilization. As patients totally rely on meals provided by Hospital caterings, Health institutions should provide nutritional well-balanced menus, in order to help patient's recovery, to increase their quality of life and reduce the incidence of malnutrition and possible health complications, as cardiovascular diseases and hypertension specially related to excessive salt intake.

The aim of this study was to evaluate sodium, and correspondent salt levels, in commonly served Hospital patient's menus, and to evaluate if the amounts are in agreement with established salt recommendations.

Samples of hospital's meals (n=26) were collected between 2017 and 2019, and included soup, fruit, bread, salad and a composite dish, grinded all together and homogenized. These meals were mostly a general diet, intended to patients who do not require specific food modifications.

Analyses were performed in quality management system, according to EN ISO 17025. Sodium levels were determined using an Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES). Salt content, in g/100 g of food, was calculated by the formula: salt = sodium × 2.5.

Sodium contents ranged from 69.0 to 303 mg/100g of food, and salt amounts from 0.2 to 0.8 g/100g of food. Meal portion size ranged from 500 to 900 g and salt levels from 2.0 to 5.0 g/meal.

Taking into account the WHO recommendations of 5 g salt/day, the consumption of 100 g of a composite dish can represent about 30% of the daily salt intake. Also, salt consumption in Portugal almost doubles WHO recommended maximum level of intake, what is in agreement with presented results, where salt levels in one meal, generally covers recommendations for one day.

As the prescription of inadequate diets and lack of flexibility of hospitals feeding service can contribute to patient's malnutrition, early intervention in hospital diets is critical to prevent worsening of patients' nutrition status and can provide an opportunity to adopt healthy eating habits, what should include a salt reduction strategy.

Keywords: salt levels; Portuguese hospital meals; nutrition



P54 - Assessment of the salt content in school meals

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School lunch is an important opportunity for start learning healthy eating habits and shall ensure a nutritionally balanced food supply. Balanced school meals are linked to improved concentration in class and improved academic outcomes. The report from FAO 2019 "Nutrition guidelines and standards for school meals" refers that more emphasis should be given on setting upper limits for saturated fat, sugar and sodium, especially in contexts where overweight and obesity are prevalent among schoolchildren.

According to IAN-AF (2015-2016) the average daily consumption estimated of salt is for children (<10 years) 5.4 g/day and for adolescents (11-17 years) 7.2 g/day. The WHO recommends maximum level intake of 5 g/day of salt in adults should be adjusted downward based on the energy requirements of children relative to those of adults, in order to control blood pressure.

The purpose of this study was to assess the salt content of the main components of school meals, collected in different schools of Lisbon region and to evaluate the contribution of the different components to the salt content of meal. These results were also compared to established guidelines and nutritional recommendations for school meals.

Meals were analyzed from 12 different schools, consisting of bread, soup and main course. The samples were analyzed by optical emission spectrometry with coupled inductive plasma (ICP-OES) after acid digestion, for determination of sodium content, in accordance with the requirements of EN ISO / IEC 17025. Salt content was calculated by formula $\text{salt} = \text{sodium} \times 2.5$.

The amount of salt provided from the components of the school meals ranged between 0.2 and 0.9 g/meal in bread, 0.2 and 1.7 g/meal in soup and 0.6 and 2.9 g/meal in main course. The component that contributed most to the sodium content of the meal was the main course.

These results suggest that the salt content of school meals should be controlled by monitoring, with gains in children's health. Once the values remain far above the WHO recommendation for salt consumption, it is necessary to go even further in salt reduction and extend this intervention to other Portuguese schools.

Keywords: salt; school meals; children; soup; bread; main course



P55 - Antimicrobial activity of industrial disinfectants against microorganisms isolated from slaughterhouse

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A disinfectant can be defined as a product that reduces the number of viable microorganisms on a surface, to a level specified as appropriate for further use. The aim of this study was to verify the efficacy of different disinfectants used in the food industry against isolated microorganisms in a slaughterhouse. In this way, the antimicrobial activity was determined according to European standard EN 1276, 2009: Chemical disinfectants and antiseptics - Quantitative suspension test for the evaluation of bactericidal activity of chemical disinfectants and antiseptics used in food, industrial, domestic and institutional areas - Test method and requirements (phase 2, step 1). Three different disinfectants (P3-manodes, OxyDes Rapid and P3-topax 91 (0.5%, 1% and 2%)) were used against different microorganisms (*Salmonella* spp., *Listeria monocytogenes*, *Staphylococcus* spp. and *Escherichia coli*). Regarding the results obtained, P3-manodes and P3-topax 91 were effective against all the target microorganisms occurring a reduction ≥ 5 log. For P3-topax 91 only the concentration of 0.5% was used since at this concentration it was quite effective. Oxydes Rapid does not have antimicrobial activity against any of the tested microorganisms.

According to the results obtained it is possible to claim that P3-manodes and P3-topax 91 disinfectants have a good antimicrobial activity. However, more studies should be carried out since the application of disinfectants in free cells may not have the same effect when applied in biofilms occurring food processing environments.

Keywords: Industrial disinfectants; antimicrobial activity; slaughterhouse



P56 - Microbiological quality of fishes sold on public market at Northwest of Brazil

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Introduction: Fish is a highly digestible food and a source of high biological value and polyunsaturated fatty acids. On the other hand, spoiling and pathogenic microorganisms attack this food due to chemical composition and higher pH. In addition, other aggravating factors for microbiological growth observed in the street market in northwest of Brazil were the damaged refrigeration conditions and wide insect presence. The present paper evaluated the microbiology of fishes sold on public market at northwest of Brazil by quantifying mesophilic heterotrophic microorganisms and *Staphylococcus aureus*. This study can provide technical support for Brazilian authorities through specific microbiological standards concerning the consumption of raw fish.

Methodology: From January 2018 to February 2019, a number of twenty samples of non-specific fish species were collected from the local street retail market in Sao Lourenco da Mata city, in the northwest of Brazil. From each sampling unit were chosen two portions weighing approximately 100g. In addition, the samples were properly packed in ice, placed inside isothermal boxes. Next, they were taken to the laboratory to be analyzed. The microbiological determinations for detecting *Staphylococcus* and mesophilic microorganisms were carried out according to the plate count method, according to the official methodology planned by official methods of Brazilian authorities. The results were expressed by colony forming units (CFU/g) of the sample.

Results: The population of mesophilic heterotrophic microorganisms counted in the analyzed samples were higher than 103 CFU/g indicating errors in fish handling. The *Staphylococcus aureus* count also present higher colony forming units (CFU/g) according to Brazilian current legislation (103 CFU/g).

Conclusion: Considering the high populations of mesophilic heterotrophic and *Staphylococcus aureus* found in fish samples commercialized in Northwest of Brazil it can to affirm that are errors during fish handling as well as refrigeration condition and others.

Relevance: In this paper became evident the application of good handling practices to reduce the contamination levels by mesophilic and *Staphylococcus aureus* microorganisms to avoid fish deterioration and the risks of food disease.

Keywords: mesophilic heterotrophic microorganisms; Staphylococcus aureus; public market, food safety, food spoilage



P57 - Microbiological Analysis and Health Aspects of Fishes put on Sale in the Street Market of São Lourenço da Mata -PE/BRASIL

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Introduction: The fish is a food with high nutritional content because of its high content of proteins, vitamins, essential fatty acids and mineral salts, therefore, becomes of great importance to the population consumption. Markets and street markets are important centers of fish marketing, however, this product distribution requires special attention, once it is part of the group of highly perishable food group, and must receive highest attention from the Sanitary Surveillance bodies. The present work has got as an objective to evaluate the sanitation aspects of the fish put on to sell on the street market at São Lourenço da Mata – PE/Brasil.

Methodology: There were evaluated twenty samples of fishes of two separate points, collected in a street Market of São Lourenço da Mata, from February of 2018 to January of 2019. The microbiological determinations to detect Total Coliforms, *Escherichia coli* and yeasts, filamentous fungi, obeying to the official methodology planned by the Ministry of Agriculture, Livestock and Food Supply data. For samples quality evaluation, were observed the items, as: equipment, utensils, personal hygiene and good production practices use fixed by the Technical Regulation of Identity and Quality of fresh fish.

Results: The results obtained in the present study for Total Coliforms and thermotolerant regarding to the two analyzed points, 60% of the sample are out of current standards patterns, that establish a limit of 102/g for *Escherichia coli*. The filamentous fungi analysis in the samples of fish presented maximum results of 104 UFC/g, although the ones for yeasts presented high results, coming to uncountable colonies.

Conclusion: The sample presented a high index of contamination for Total Coliforms and thermotolerant and fungi representing food risk for the consumer. The fish exposition in sell points, exposed to public without any kind of packaging or protection, also contributed to the increase of microbial load.

Relevance: The commercialization and manipulation of fishes in the street Market of São Lourenço da Mata - PE is under worrying conditions, existing sanitary problems in the fish exposure, sector organization, hygiene of the observation place and the handler hygiene.

Keywords: Fungi; Coliforms; Fish; Technical Regulation



P58 - Microbiological evaluation of sushis marketed in the southern region of Florianópolis (Brazil)

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The consumption of sushi has grown a lot in recent years, so the number of establishments with this type of cuisine has increased in several places in the world. There are several sushi restaurants options in Florianópolis (SC - Brazil), so the present work aims a microbiological evaluation of the sushis commercialized in the southern region of Florianópolis and to verify the susceptibility of isolated strains against different antimicrobials.

Microbiological analyzes were performed according to Silva et al. (2017). The antibiotic resistance profile was performed with antibiotics disks (DME®) and the inhibition zone were measured according to the manufacturer's instructions. The multiple resistance index was calculated according to Krumperman (1983). The results were compared to Brazilian Microbiological Standards to ready-to-eat foods (RDC 12/2001).

Of the 30 analyzed samples, 12 shown thermotolerant coliforms parameters above those allowed, 5 of them being confirmed the presence of *Escherichia coli*, in 4 samples it was verified presence of *Salmonella sp.* in 25g. *Staphylococci* coagulase positive were found in 6 samples, however all were within the parameters of the legislation. Thus 43% of the samples were unfit for consumption, coming from 80% of the researched establishments. *E. coli* strains were tested to 11 antimicrobial, all of them (100%) shown resistance profile to rifampicin and vancomycin, and 63.6% of the isolates demonstrated sensitivity or intermediate sensitivity profile to 9 antibiotics, and the antibiotic multiple resistance (MAR) index varied from 18.2 to 36.4%. To *Staphylococci* coagulase positive, 100% had a sensitivity profile for chloramphenicol and resistance for vancomycin, the MAR index ranged from 16.7 to 58.3%. Data shown that 83.3% of strains had values above 35%, demonstrating an increasing resistance to antibiotics and the need of its use control, so that a progressive adaptation of the bacteria to these drugs is not obtained.

The results demonstrate the need of observation of GMP to ensure the safety of this kind of food. It is extremely important to take care of the raw material quality, cleaning of the utensils, to avoid cross contamination and by the manipulator, besides the storage of the sushi under temperature control until consumption.

Keywords: Sushi; Microorganisms; Good Manufacturing Practices; Antimicrobials; Bacterial resistance



P59 - Natural extracts as alternative antimicrobials to conventional chemicals against yeasts

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Introduction: Nowadays, consumers are very concerned about their health and they are aware of the risks associated to the consumption of some chemical preservatives used by the food industry. In order to replace the chemical preservatives and satisfy their clients' needs, food companies have been using natural antimicrobials. The aim of this work was to study the effect of some natural antimicrobials against yeasts previously isolated from fruit preparations (strawberry, blueberry, raspberry and blackberry).

Methodology: The effect of some natural extracts (cloves, lemon, mint, oreganos, thyme and grapefruit, a mixture of thyme and lemon and a mixture of thyme and grapefruit) against yeasts (*Pichia anomala*, *Meyerozyma caribbica* and *Candida intermedia*) was screened by the disk diffusion assay. The minimum inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) was determined for the extracts that displayed the highest antimicrobial activity (i.e. inhibition halos > 10 mm).

Results: Results demonstrated that the antimicrobial effect of each extract varied according to the target yeast. In the disk diffusion assay, each tested extract produced inhibition halos >10 mm for the three yeasts. As for the MIC assay, the mixture of thyme and lemon and the mixture of thyme and grapefruit demonstrated a high antimicrobial activity, i.e. lower MICs values against *Pichia anomala*. For the three yeasts, the lowest MICs values were observed when using oreganos extract. Similar MICs were registered for the other extracts.

Most of the extracts demonstrated a bacteriostatic effect against *M. caribbica* and *C. intermedia* and bactericide effect against *P. anomala*.

Conclusions and relevance: The natural extracts tested, mainly oreganos, can be interesting to food processors due to their antimicrobial characteristics, since they prevent or reduce the development of microorganisms. The extract mixtures can be used not only as antimicrobial agents but also used as flavoring alternatives.

Keywords: Fruit preparations; yeasts; natural antimicrobial



P60 - Management of gluten labeling in catering businesses: the importance in considering cross-contamination and good manufacturing practices

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The incidence of food allergies has been increasing in recent years and among food allergens, gluten has been identified as a major public health concern. Because there is no cure for gluten allergy, its avoidance is the best strategy to allergic individuals. Recently, non-packaged foods sold in the catering businesses were required by law to declare allergens. However, since gluten can be unintentionally introduced into food due to the cross-contamination and non-compliance of good manufacturing practices, improper label declaration may occur. The aim of this study was to assess the potential (cross-) contamination of foods with gluten due to the shared activities with utensils/equipment and the non-compliance of good manufacturing practices.

Samples of non-packaged foods (n= 35) and surfaces of utensils and equipment (n=50) were collected in four kitchens belonging to a collective catering company. ELISA kits were employed to perform the gluten analyses.

In general, all food samples have gluten, including garnish, salads and soups in which would not be expected to find this allergen. This suggests that gluten-free products are susceptible to cross-contamination, probably due to the unsystematic activities developed in these establishments and the non-strictly compliance of good manufacturing practices. However, the concentration of gluten in most foods was <20 ppm, which allows classifying them as gluten-free. Likewise, in all surfaces traces of gluten were found. Nevertheless, although cleaning is not absolutely effective in removing gluten, this process can reduce its content to significantly lower levels.

In conclusion, for the proper labeling of gluten in non-packaged food sold in catering business, periodic training programs and routine supervision should be applied to ensure that procedures are being carried out effectively. It is also crucial the regular food testing and the assessment of the effectiveness of sanitation to ensure that cross contamination are not occurring at levels of risk.

Keywords: Catering business; cross contamination; gluten; good manufacturing practices; labelling



P61 - Chemical and Microbiological Contamination in Limpet (*Patella* spp.)

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Marine invertebrate production areas can suffer anthropogenic contamination from urban, agro-industrial and leisure activities. Some contaminants, such as organisms or chemical substances intrinsic to the environment, might also have a telluric origin. Non-filter feeding gastropods, such as limpets, which are very appreciated in Portugal, are excluded from provisions on the classification of production areas, but can also present some contamination. Thus, the aim of this study was to characterize the microbiological and chemical contaminations in limpets from three production areas by estimating the effect of temporal and seasonal variation.

Limpets analyzed in this study were collected in 2019 from three Portuguese coastal production areas, under IPMA responsibility. Cadmium, lead and mercury were performed by atomic absorption spectrometry based on the standard methodologies (NP EN 14084, 2003; EPA method 7473, 2007). *Escherichia coli* was determined by most probable number (MPN) in live species (ISO 16649-3:2015).

Results indicated that microbiological contamination of fecal origin was low (below the detection level). Contents of mercury and lead in limpets from the three areas studied, over several months, were always below the limits of 0.50 mg/kg and 1.5 mg/kg allowed by the EU, respectively. However, limpets from one of the studied areas presented lead levels statistically inferior to those of limpets from the other two areas. Regarding cadmium levels, these were always above the limit of 1.0 mg/kg, reaching about 3.0 mg/kg in some samples. These values probably indicate contamination from telluric origin (soil or rocks) in these production areas. Thus, potential risk associated with their consumption, taking into account the cadmium tolerable monthly intake (TMI), is possible and more data are needed to deepen this study. However, it should be noted that due to these high cadmium values, capture of these species has been forbidden.

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Keywords: limpets, mercury, lead, cadmium, E. coli



P62 - Food packaging materials use in the Portuguese population

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Introduction: In the last decades, the population's exposure to chemicals derived from food contact materials with a potentially negative impact on health has been growing. This study aims to analyze the utilization of different food packaging materials by the Portuguese population.

Methodology: A representative sample of the Portuguese population aged between 3 months and 84 years old (n=5811) was evaluated within the National Food, Nutrition and Physical Activity Survey 2015-2016. Dietary data was collected by 2 nonconsecutive days of food diaries (children) / 24-hour recalls (adolescents+adults), including information regarding the food packaging material. The use of food packaging materials was measured according to the number of reported food items with packaged versus the amount of packaged foods. A decision tree was used to generate a multiple imputation model to handle missing information about packaging material. The food packaging utilization was weighted for the population.

Results: The majority of food items (67%) were report with a package associated. Plastic was the most frequent food contact material, followed by glass and multilayer materials. The food groups most frequently reported with plastic packaged were "Added salt" and "Fats and Oils", for glass was "Wine" and for multilayer materials "Milk". "Fruit" and "Vegetables" were the food items most frequently reported without package. These results were compared with those adjusted to the amount of food.

Conclusion: The Portuguese population presents a large utilization of packaged foods. This trend increases the exposure to chemicals from food contact materials that migrate to foodstuffs, mostly during transport and storage.

Relevance: Given the absence of information, this study becomes relevant to better understand the individual intake of packaged foods in view of the exposure assessment to chemicals derived from food packaging materials in the Portuguese population.

Financing: Programa EEA Grants-Iniciativas de Saúde Pública, FEDER (POCI-01-0145-FEDER-031949)

Keywords: food packaging materials; exposure assessment; dietary survey



P63 - Liver histopathology under combined effect of copper sulphate and temperature in Mozambique tilapia (*Oreochromis mossambicus*)

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Greenhouse gases (GHG) emissions are one of the main causes of atmospheric temperature increase in last decades, which is inducing climate changes, and also alterations in pollutant chemistry, hypoxia in fish, and more toxics bioavailable for aquatic organisms, among others.

One main goal of this study was to test the impact of temperature increase on the toxic effect of copper sulphate on Mozambique's tilapia (*Oreochromis mossambicus*) liver and implement a grading system to compare toxicant effects in fish. For this, 70 animals, with an average weight of 40.51 ± 10.1 g, were exposed to two concentrations of copper sulphate (1.1 and 3.6 mg/L) and two different water temperatures (25°C and 32°C). For the histopathological liver assessment, a semi-quantitative analysis was used, based on a grading system of 6 levels ("grade 0" for normal structure and "grade 5" for high level of changes observed). Muscle was collected to assess copper content.

The body condition and hepatosomatic index show no statistical differences among groups ($P > 0.05$). Several hepatic lesions/alterations were observed, namely macrophage aggregation, necrosis, hepatocyte vacuolization, hyalinization and presence of nonspecific granulomas. The analyses of the grading data showed some modifications in macrophage aggregates, necrosis and nonspecific granulomas, although not statistically significant. The hyalinization was the lesion with higher grade and with marked variations among the different treatments and times of exposure. It was possible to detect higher grades of hyalinization with the increase of temperature in fish exposed to copper sulphate. Vacuolization was observed with higher grade after 14 days of exposure.

Histopathological results did not allow presuming a relationship between the increase of some lesions and the studied variables, except for hyalinization. Liver require some time to show detectable changes in its structure, mainly for low levels of copper related with relevant environmental concentrations. Muscle copper data is not ended.

Nevertheless, more studies have to be done in order to better understand the effect of temperature on pollutants toxicity and accumulation in fish, mainly in muscle and liver, because this problem can be relevant for fish population and human health, considering future scenarios of climate changes.

Keywords: temperature; copper sulphate; tilapia; liver; histopathology



P64 - Applicability of the harmonized *in vitro* digestion method to titanium dioxide nanomaterials

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Titanium dioxide nanomaterials (TiO₂ NMs) have a high potential for ingestion by human populations, due to their increasing use as food additives, inclusion in dietary supplements and food packaging materials. Whether this oral exposure may lead to adverse local or systemic outcomes, has been the subject of research. *In vitro* studies have generated contradictory results, possibly due to differences in the physicochemical properties of the TiO₂ NMs studied, which can be additionally affected by the surrounding matrix and interactions during digestion.

INGESTnano is a national project aimed at investigating the nano-bio interactions of nanomaterials on intestinal cells, at the cellular and molecular levels, after the digestion process, to better understand their potential impacts on human health. Three TiO₂ NMs were selected as case-studies to setup a workflow for addressing nanosafety concerns of ingested NMs, while considering the nano-bio interactions under physiological conditions. As an alternative to *in vivo* testing, this project is focused on the use of the harmonized *in vitro* digestion method for simulating the human digestion of NMs. This digestion comprises three compartments: a) mouth (pH 7.0, alpha-amylase, salts); b) stomach (pH 3, HCl, pepsin, salts) and c) small intestine (pH 7.5, bile salts, pancreatin, salts). The final product of digestion is tested in bioassays using intestinal cells, to ascertain its toxicity.

A high level of toxicity of the final digestion product challenged the applicability of the digestion product in the biological assays. The results revealed that digestion products without the NM showed cytotoxic effects above the concentration of 10% in cell culture medium. Several modifications to the initial protocol were thus performed to overcome this issue. The results suggested that the addition of bile salts accounted for most of the toxicity observed. The applicability of the harmonized *in vitro* digestion method is discussed in view of its potential use as a tool for addressing the toxicity of ingested NMs or other food contaminants, mimicking the physiological processes, in alternative to animal models.

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Keywords: nanomaterials; gastrointestinal tract; harmonized in vitro digestion method; titanium dioxide



P65 - Setting up of a molecular method to identify insect species in food and feeds

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The use of insects as food or feed is nowadays widespread, and entomophagy is practiced in more than hundred countries. The United Nations recommended the practice as a potential solution to the shortage of world food supplies. So far in Europe, only 5 countries explicitly permit the marketing and consumption of insects. However this condition is only provisional, since EU Regulation 2015/2283 stipulates that all insect-based products are considered as “Novel Food”, and anyone who wants to put them on the EU market needs to submit a specific application to the European Commission, which will decide based on EFSA’s scientific evaluation. The Netherlands is home to some insect farms for human consumption, as well as some start-ups active in the marketing and production of edible insects. Moreover insects, previously reared mainly as pet food, can also be formulated in feeds for farmed animals. However, the use of insects is still banned for farmed land animals, and it has only recently been approved for fish from aquaculture. EU Reg. 2017/893 allowed a shortlist of 7 insect species (*Hermetia illucens*, *Musca domestica*, *Tenebrio molitor*, *Alphitobius diaperinus*, *Acheta domesticus*, *Gryllobates sigillatus* and *Gryllus assimilis*) to be included in the formulation of feeds for aquaculture, but so far a specific and official method to detect the presence of insects in these feeds and to discriminate between allowed and not allowed species is still lacking. Within the EU-FORA EFSA Fellowship Programme, an EU-funded initiative aiming at increasing the expertise to food risk assessment bodies, a project was initiated focusing on the setting up of a new Next Generation Sequencing (NGS)-based molecular method for the identification of insect DNA in feeds for aquaculture. The protocol is based on the amplification of the barcode region COI, well known to be able to discriminate animals at the species level and already successfully tested on insects. The ultimately validated protocol will be applicable to all feeds, and possibly also to several foods for human consumption that are currently waiting for a proper regulation, contributing to their safety and to the safety of the consumers.

Keywords: insect; feed; detection; NGS



P66 - Water temperature affects differently the depuration and oxidative stress status of two bivalve species

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Depuration of harvested bivalves is essential to assure food security and good practices are regulated among the European countries. Yet, these practices do not consider the physiological requirements of the diverse commercialized species (e.g.: optimum temperature range), which can compromise the quality of the final product. Further, bivalves harvested in class C areas are usually avoided by stakeholders due to economical constraints, as they must be transposed to relaying areas for a minimum of two months and depurated, before being commercialized for human consumption. Thus, we wanted to evaluate: i) if bivalves harvested from class C areas were able to purify in 24 h, and ii) if water temperature will affect not only the purification capability but also the oxidative stress status. We selected two bivalve species, with broad distribution and high economic value in Europe, clam - *Ruditapes decussatus* and razor clam - *Solen marginatus*. The efficacy of purification of each species was influenced by water temperature, with *R. decussatus* presenting the lower microbiological load between 15 and 20°C and *S. marginatus* between 10 and 15°C. Clams seemed to have a higher range of temperature tolerance, with DNA damage increasing only at 25°C, whereas for razor clams temperatures above 20°C caused increase in DNA strain breaks. Antioxidant defences of razor clams were also impaired with increasing temperatures. Regarding energy consumption, clams presented a significantly higher activity of electron transport system than razor clams, although no significant changes in energy consumption were observed due to water temperature. In sum, *R. decussatus* were more tolerant to water temperature changes than *S. marginatus*, highlighting the relevance of considering species-specific requirements to improve purification, welfare, and quality of commercialized bivalves.

Keywords: purification; temperature; bivalve; biochemical biomarkers; food safety



P67 - Biocontrol of *Pseudomonas syringae* pv. *actinidiae* infection of kiwifruit plants using phage $\Phi 6$: *in vitro* and *ex vivo* experiments

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In the last decade, the worldwide production of kiwifruit has been highly affected by *Pseudomonas syringae* pv. *actinidiae*, a phytopathogenic bacterium responsible by bacterial canker of kiwifruit. The available treatments for this disease are still scarce, with the most common involving frequently spraying the orchards with copper derivatives and/or antibiotics. These treatments should be avoided due to their high toxicity to the environment and promotion of bacterial resistance. Phage therapy may be an alternative approach to inactivate Psa. The present study investigated the potential application of the already commercially available phage $\phi 6$ to control Psa infections. The inactivation of Psa was assessed *in vitro*, using liquid culture medium, and *ex vivo*, using artificially contaminated kiwifruit leaves with two biovar 3 (a highly aggressive pathogen) strains (CRA-FRU 12.54 and CRA-FRU 14.10). As the plants are exposed to the natural variability of physical and chemical parameters, the influence of pH, temperature, solar radiation and UV-B irradiation on phage $\phi 6$ viability was also evaluated in order to develop an effective phage therapy protocol. In *In vitro* experiments, the phage $\phi 6$ was effective against both strains (maximum reduction of 2.2 and 1.9 CFU/mL for CRA-FRU 12.54 and CRA-FRU 14.10, respectively). In the *ex vivo* tests, the decrease was lower (maximum reduction 1.1 log and 1.8 CFU/mL for CRA-FRU 12.54 and CRA-FRU 14.10, respectively). The viability of phage $\phi 6$ was mostly affected by exposure to UV-B irradiation (decrease of 7.3 log PFU/mL after 8 h), exposure to solar radiation (maximum reduction of 2.1 PFU/mL after 6 h) and high temperatures (decrease of 8.5 PFU/mL after 6 days at 37 °C, but decrease of only 2.0 log PFU/mL after 67 days at 15 °C and 25 °C). The results of this study suggest that the commercially available phage $\phi 6$ can be an effective alternative to control Psa infections in kiwifruit orchards. Although the stability of phage $\phi 6$ was affected by UV-B and solar radiation, this can be overcome by the application of phage suspensions at the end of the day or at night.

Keywords: Phage therapy; phage $\phi 6$; kiwifruit; kiwi canker; Psa



P68 - Antibacterial activity of natural plant extracts from *Arbutus unedo* against *Escherichia coli* and *Salmonella enterica* serovar Typhimurium

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Agri-food and forestry industries are among the most important sectors for the sustainable development of the worldwide economy. Nowadays, there are numerous of such industries that produce high quantities of by-products that are under-exploited. These by-products are considered one of the main causes of environmental problems. Several studies on the literature had already showed that plant extracts have important biological properties, such as antimicrobial, antioxidant, antitumoral as well can act as anti-inflammatory agents. This work presents the antibacterial activity studies of methanolic extracts of leaves and branches of *Arbutus unedo* against *Escherichia coli* and *Salmonella enterica* serovar Typhimurium. The bactericidal profile after 24 h of incubation with branch extract of *A. unedo* had shown that this extract was not capable to inactivate *E. coli*. However, leaves extract of *A. unedo* at 7.5 mg/mL had promoted the inactivation of *E. coli* until the detection limit of the method. In the case of *S. Typhimurium*, the bactericidal profile after 24 h of incubation of these extracts had shown that branch extract of *A. unedo* at 7.5 mg/mL and leaves extract of *A. unedo* at 5 mg/mL were capable to inactivate this bacterium until the detection limit of the methodology. These results led us to consider the potential application of these extracts as components of food packaging avoiding the growth of foodborne pathogens *E. coli* and *S. Typhimurium*.

Keywords: Natural plant extract; E. coli; S. Typhimurium; Antibacterial activity



P69 - Identification of risks and benefits associated to the consumption of raw milk: the first step of a risk-benefit assessment

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Milk is a highly nutritious food. Due to consumers' perception that raw milk (RM) is a better source of nutrients and other active components there is currently considerable debate on the potential health benefits of consumption of RM compared to pasteurized milk (PM). Current trends advocating for "consuming natural" and "purchasing locally" have contributed to the increased popularity of RM in some countries (e.g. Italy, Estonia and the US). Claimed health benefits are e.g. "higher nutritional value", especially in terms of vitamins' contents, "beneficial microflora" as probiotic bacteria, and "allergy prevention". However, several human pathogens can be present in RM and have been identified as the cause of several foodborne outbreaks. Consequently, this new trend encourages a proper assessment of the associated risks and benefits through a quantitative risk-benefit assessment (RBA). Traditionally, RBA considers as a first step the identification of the risks and the benefits posed by the considered food product, gathering scientific evidence for the inclusion or exclusion of each food component.

This study aimed to identify the risks and the benefits associated to the consumption of RM when compared to PM. Through literature search, the nutritional, toxicological and microbiological food components that could be present in RM and PM were identified. For each identified food component, scientific evidence were analysed to support the decision about the inclusion or exclusion of each food component.

Microbiological pathogens (*Listeria monocytogenes*, *Salmonella* spp., *Campylobacter jejuni* and Shiga toxin-producing *Escherichia coli*), probiotic bacteria (*Lactobacillus* spp.) and nutritional components (vitamins B2 and A) were identified, as well as the potential impact of RM in the reduction of the allergies' prevalence. Since it is not expected that heat-treatment could affect the occurrence of chemical toxic compounds in milk, no toxicological components were identified in the assessment.

We identified the main components that should be considered in RBA of RM consumption. Such a quantitative RBA will contribute to inform the consumers about the magnitude of the risk and the expected health impact.

Acknowledgments: this study was performed under EU-FORA – The European Food Risk Assessment Fellowship, funded by EFSA.

Keywords: risks; benefits; health; nutrition; toxicology; microbiology; raw milk



P70 - Nut consumption in Portugal: the balance between the risks and the benefits regarding liver cancer and cardiovascular disease

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Nuts are rich sources of cis-unsaturated fatty acids, fibre, vitamins, minerals, and a number of bioactive substances and the regular consumption of nuts are being associated with a reduction in all-cause mortality, particularly cardiovascular disease (CVD). At the same time, the occurrence of mycotoxins in nuts, including the most potent carcinogenic aflatoxins (AFTs), has been reported by several authors worldwide. According to the National Food, Nutrition and Physical Activity Survey (IAN-AF 2015-16), the usual consumption of nuts by the Portuguese adults is 2.7 g/day. Some recent epidemiological studies (e.g. PREDIMED) suggested that a daily nuts consumption of 30 g could reduce the incidence of CVD.

The present study aimed to quantify the health impact in terms of Disability-adjusted life years (DALY) of increasing nut consumption to 30 g/day by adult Portuguese population, when compared to the current intake. Regarding nut consumption, two scenarios were established: current consumption (CS) – 2.7 g/day; alternative scenario (AS) – 30 g/day. AFTs occurrence in nuts available in Portuguese market and nut consumption data were used to estimate AFTs intake. Epidemiological and National population data were used to estimate the DALYs, considering two different endpoints: the occurrence of hepatocellular carcinoma due to exposure to AFTs (IPSC/WHO) and the prevention of acute myocardial infarction due to nuts' consumption (PREDIMED).

A mean daily intake of AFTs of 0.013 (CS) and 0.142 (AS) ng/kg bw/day was predicted. This intake is estimated to cause 0.013 (CS) and 0.15 (AS) extra cases of Hepatocellular Carcinoma, corresponding to 0.2 (CS) and 2.7 (AS) annual DALYs. In contrast, the estimated number of preventable cases of myocardial infarction due to the increasing of nut consumption to 30 g/day was 1402 and the DALYs gained was 5658. Integrating risks and benefits, 5656 annual DALYs could be potentially saved. These results suggest an



overall beneficial health effect of increased nut consumption in Portugal. Therefore, it seems not advisable to reduce exposure to AFTs by recommending a reduced intake of nuts.

Keywords: risk-benefit; nuts; aflatoxins; liver cancer; cardiovascular diseases



P71 - Benefits and risks of tuna intake regarding mercury, inorganic arsenic and selenium

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As a result of the high level of tuna consumption, as well as its economic value, tuna has become one of the fish species of greater importance. Regulatory agencies have advised to limit tuna consumption particularly in risk groups. The reason is the bioaccumulation of mercury (Hg), that tuna shows as predatory fish. However, tuna is rich in selenium (Se), an essential element that might counteract the harmful effects of Hg. Therefore, regarding both elements Health Benefit Value (HBVSe) and molar ratios have been proposed as criteria to consider fish healthy or not. In a lower degree than Hg, tuna might also accumulate other pollutants like inorganic arsenic (iAs). Fortunately, in mammals the jointly co-excretion between Se and iAs has been observed, this is thanks to the formation of the compound $[(GS)_2AsSe]^-$. This mechanism might be considered to perform risk assessments; however, should be confirmed in humans.

Samples of fresh and processed tuna commercialised in Spain were analysed by ICP-MS to determine the concentrations of these elements (iAs was considered 3% of total As).

The surplus of Se with respect to Hg and iAs was evident in both tuna presentations. The mean molar concentrations (expressed in wet weight) in fresh tuna were $15.7 \mu\text{mol kg}^{-1}$, $3.81 \mu\text{mol kg}^{-1}$ and $1.52 \mu\text{mol kg}^{-1}$ for Se, Hg and iAs respectively. Processed tuna presented $14.8 \mu\text{mol kg}^{-1}$, $1.52 \mu\text{mol kg}^{-1}$ and $0.461 \mu\text{mol kg}^{-1}$ in the same sequence of elements. Therefore, fresh tuna offers a Se surplus of $10.3 \mu\text{mol kg}^{-1}$ and molar ratio between Se and the sum of Hg and iAs of 2.96 (ratios >1 might be considered healthy). Processed tuna displayed a Se surplus of $12.9 \mu\text{mol kg}^{-1}$ and the molar ratio was 7.47. Considering only the surplus of Se, to calculate its estimated daily intake for the average Spanish adult consumer, tuna would contribute with 13.5% of the adequate Se intake proposed by EFSA.

In conclusion, tuna is a good source of Se. Moreover, enough Se seems to be present in tuna to counteract the possible adverse health effects of Hg and iAs on consumers.

Keywords: Tuna; Se surplus; Hg; iAs



P72 - Risk vs Benefit Assessment of Seaweed as Food Taking into account Bioaccessibility: Arsenic vs Iodine

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Introduction: Seaweeds are an important ocean resource. They are currently used for various purposes: as biofertilizer; as a source of colloids; for extraction of bioactives; and as food - especially in sushi. The application as food comprises edible brown seaweed, such as *Halopteris scoparia*, *Petalonia binghamiae*, and *Saccharina latissima*, green seaweed, such as *Ulva* spp., and red seaweed, such as *Osmundea pinnatifida*. Recent studies have shown that some of these seaweeds are rich in antioxidants, minerals, and iodine. However, arsenic content raises some public concerns. These elements have different impacts on health, which require assessment. Thus, it is important to ponder benefits against risks in algal consumption. Hence, this study aimed to assess the main risks (As, excessive I) vs benefits (adequate I) associated to the consumption of a representative array of seaweed species, taking into account bioaccessibility.

Methodology: The methodology used in this assessment was based on the most advanced mathematical-statistical modelling and the available non-digested and digested (bioaccessible) As and I data concerning the selected species of seaweed. This enabled the evaluation of the risk-benefit binomial for different consumption scenarios using thresholds such as the I recommended daily intake (RDI) or the I Tolerable Upper Intake Level (TUIL).

Results: While As bioaccessibility varied in a narrow range and was high, I bioaccessibility was very variable. This study led to advances in the estimation of beneficial consumption levels of the selected seaweed species. In particular, concerning I, species, such as *Halopteris scoparia* and *Petalonia binghamiae* were shown to guarantee the full benefit with less than 20 g dry seaweed/week or 100 g steamed seaweed/week. Regarding As, there must be some caution, taking into account the geographical source of the seaweeds. For *Ulva* spp., excessive I and As risks were low, thus not representing an obstacle for their consumption, especially taking into account that very high consumption levels of dry seaweed are implausible.

Conclusions: For seaweeds from less polluted areas (Portugal), benefits seem to outweigh any possible risk.

Relevance: This study enabled to identify an advisable consumption frequency interval for several seaweed species found in Portuguese waters.

Keywords: Risk-Benefit Assessment; Seaweed; Arsenic; Iodine



P73 - Different Approaches in Assessing Risk and Benefit: The Case Study of Tuna

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Introduction: Fish are rich in omega-3 polyunsaturated fatty acids, particularly eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), which have a beneficial role in the neurocognitive development of children and in the prevention of cardiovascular disease. Fish are also a source of essential elements, such as selenium (Se), which lessens the effects of methylmercury (MeHg). Indeed, fish are a dietary source of MeHg, exposure to which can be deleterious to the neurocognitive development of children. Therefore, it is important to weigh benefits versus risks in fish consumption. Tuna (*Thunnus* sp.) contains substantial levels of Se, EPA, and DHA as well as of MeHg. Thus, tuna is similar to fish in general. This study sought to compare different approaches in assessing risk vs benefit using tuna as a case study.

Methodology: Three alternative methodological approaches were used and compared: 1) the probabilistic approach based on the calculation of a probability of exceeding a threshold or reference dietary value as defined by the Extreme Value Theory (EVT); 2) the estimation of Quality-Adjusted life Years (QALY)/Disability-Adjusted life Years (DALY) through the estimation of dose-response curves and specific factors; and 3) the calculation of particular risk/benefit effects using particular health endpoints with relevance for a given situation. Tuna, raw and subjected to different culinary treatments (boiling, grilling, canning), was used as model.

Results: According to the EVT-based probabilistic approach, in tuna and for a 50 g weekly meal, boiling and grilling led to higher probabilities of surpassing the MeHg Tolerable Weekly Intake than canning, 10-11% vs <0.1%. The probability of surpassing the EPA+DHA recommended daily intake was always higher than 17%. The Se recommended daily allowance was met with a probability >6% in boiled and grilled tuna. Moreover, a 50g weekly meal of tuna canned in water prevented almost 100 deaths per year and million individuals, thereby offsetting negative impacts on IQ. The same positive outcome was reached by the QALY/DALY method.

Conclusions: According to used methodologies, high Se, EPA, and DHA contents after boiling and grilling may outweigh MeHg risk.

Relevance: This study showed the validity of different approaches for assessing risk-benefit.

Keywords: Risk-Benefit Assessment; Tuna; Probabilistic Approach; QALY/DALY; Alternative Metrics



P74 - Risk-benefit in food safety and nutrition; Parma Summer School 2019

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Food contains necessary and beneficial ingredients and may also contain potentially adverse ingredients. Moreover, one food or food ingredient may have both beneficial and adverse effects. Risk-benefit assessment is the comparison of the risk of a situation to its related benefits. The risk-benefit analysis paradigm mirrors the classical risk analysis one: risk-benefit assessment goes hand-in-hand with risk-benefit management and risk-benefit communication.

The European Food Safety Authority (EFSA), the University of Parma, with the collaboration of the Catholic University Sacro Cuore of Piacenza, the Technical University of Denmark, the National Food Agency, Sweden, and the University of Barcelona organised a Summer School with the objective to provide an opportunity to learn from some of the most prominent experts in the field of risk-benefit approach in food safety and nutrition, including theory, case studies, and communication of risk-benefit assessments.

The various health effects associated with food consumption, together with the increasing demand for advice on healthy and safe diets, have led to the development of different research disciplines in food safety and nutrition. In this sense, there is a clear need for a holistic approach, including and comparing all of the relevant health risks and benefits. The risk-benefit assessment of foods is a valuable approach to estimate the overall impact of food on health. It aims to assess the combined negative and positive health effects associated with food intake by integrating chemical and microbiological risk assessment with risk and benefit assessment in food safety and nutrition.

The event proved very successful and participants became well-informed in this modern area of risk assessment. Moreover, whereas tools and approaches are developed now, more and more case studies are performed that can form an inherent validation of the risk-benefit approach. Recent risk-benefit assessments apply the characteristics developed a decade ago: problem formulation (with at least 2 scenarios), tiered approach until a decision can be made, one currency to describe effects (DALYs in most instances). It was concluded that the risk-benefit assessment in food safety and nutrition is gaining more and more momentum.

Keywords: Risk-benefit, Food Safety, Nutrition, Parma, Summer School



P75 - EMERTOY - Emergent Marine Toxins in the North Atlantic and Mediterranean

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Introduction: Episodes of human poisoning caused by Harmful Algal Blooms have been commonly recorded in the last century mostly because of the lack of regular monitoring programs. Today, the cases of human poisonings are sporadic, usually because of violations of national health authorities' regulations imposing the closure of harvesting areas and seafood commercialization. Nevertheless, the occurrence of emergent toxins and the respective producing organism in the North Atlantic and Mediterranean, such as tetrodotoxins, ciguatoxins, palytoxins and a diversity of congeners is being increasingly reported either by scientific studies or by events of human intoxications.

Expected Results: The project EMERTOY, funded by H2020 under the RISE program, will create a robust and sustainable network of experts with excellent complementary competencies on marine algal toxins and the detection of the organisms producing these toxins. The network will collaborate not only with national authorities but also with European ones, such as EFSA, for the assessment and management of risks associated to emerging toxins and the species that produce them. Current risks assessment relating to emerging harmful algae and predicting future scenarios will be fundamental for EFSA, which will recommend whether these emerging toxins should be monitored in Europe, and for the development appropriate strategies to protect human health. EMERTOY aims to map the actual situation in emergent marine toxins and the producing organisms, develop new approaches to assess their occurrence and predict the possible future scenarios in the framework of global warming. The consortium, formed by a multidisciplinary team, will produce a joint research and innovation project that will exploit the complementary expertise of the participants and will create synergies among them.

The main relevance are: i) to assess the current situation on potentially harmful algae and bacteria and the relevant emerging toxins in 9 countries belonging to different but geographically connected areas; ii) to develop innovative approaches to sample, and analyze the producing organisms and their toxins by chemical and biological methods including immunoassays and sensors; iii) to predict different future scenarios based on molecular data (routes of dispersion) and modelling.

Keywords: emergent toxins; global changes; tetrodotoxins; ciguatoxins; palytoxins



P76 - Tetrodotoxins expansion and occurrence in benthic non-traditional vectors in the North Atlantic – Update

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Introduction: Tetrodotoxin (TTX) is a potent alkaloid typically from tropical ecosystems, being more prevalent in the temperate waters of the North Atlantic in the last decade. The European Food Safety Authority (EFSA) stressed its last scientific opinion, the need for data regarding TTX prevalence in European waters.

Methodology: To address EFSA's concerns, benthic organisms such as mollusks, crustaceans, echinoderms and fish with different feeding habits were collected (≈ 30 species) along the Portuguese continental coast, islands (São Miguel, Azores, and Madeira) and the northwestern Moroccan coast. A total of 299 samples were analyzed by LC-MS/MS, UHPLC-HRMS and UHPLC-MS/MS.

Results and Conclusions: Geographical tendencies were detected as follows, by descending order: S. Miguel Island (Azores), Moroccan coast, Portuguese continental coast and Madeira Island. The toxin amounts detected were significant, above the Dutch limit value established in 2017, showing the importance and the need for continuity of these studies to gain more knowledge about the prevalence of these toxins, unraveling new vectors, in order to better assess human health risk. This work represents a general overview of new TTX bearers (9) most of them in gastropods (*Patella depressa*, *Nucella lapillus*, *Onchidella celtica*, *Aplysia depilans*, *Phorcus lineatus* and *Gibbula umbilicalis*), followed by echinoderms (*Echinus esculentus* and *Ophidiaster ophidianus*) and puffer fish *Sphoeroides marmoratus*.

Keywords: Tetrodotoxins; Hyphenated chromatographic Techniques; New Vectors, North Atlantic



P77 - Multi-mycotoxin contamination of green tea and risk assessment in Moroccan population

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Introduction: Tea is an aromatic beverage commonly prepared by pouring hot or boiling water over cured leaves of the *Camellia sinensis*, an evergreen bush native to East Asia. It is an everyday drink for almost everyone. Tea is generally divided into categories based on fermentation degree. The most familiar kinds are black, white, oolong, green, and Pu-erh (post-fermented) tea. China, India, Kenya, Sri Lanka, and Turkey are the world's largest tea producers. Moroccans remain the first consumers of Chinese green tea in the world. The volume of tea imports recorded a considerable increase and reached 60.000 tons of green tea in 2108. In this study, the multi-mycotoxin contamination of green tea samples by LC-MS/MS was investigated as well as the risk assessment for the population.

Methodology: One hundred and eleven samples (n=111) of green tea purchased from different markets in Rabat area (Morocco) were extracted with a based on a dispersive liquid-liquid microextraction procedure, and mycotoxins identification and quantification was carried out by liquid chromatography tandem mass spectrometry (LC-MS/MS).

Results: Analytical results showed that out of 111 total samples, 81 were contaminated by at least one mycotoxin. Among positive samples, 67 samples were contaminated with Alternariol, 48 samples with Zearalenone, 3 samples with aflatoxin G₁, 2 samples with Enniatin B as well as for Aflatoxin B₂, and one sample with Aflatoxin B₁ as well as HT-2 and Tentoxin. The multi-presence of mycotoxins was also observed in some positive samples.

Conclusions and Relevance: This is the first study on the multi-presence of mycotoxins in commercialized green tea samples from Morocco. It was observed that the contamination levels in positive tea samples were below the maximum limits set by EU regulations. Further studies are necessary to assess completely the situation. Risk exposure assessment of regulated mycotoxins showed that the risk is moderate to low because of the low intake of green tea (2 kg/capita/year) by local inhabitants.

Keywords: Multi-mycotoxin; Green tea; Contamination; LC-MS/MS; Risk assessment; Morocco

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