Food Safety

IAFNS Science Innovation Showcase - 2021 Virtual Event, December 14–15, 2021
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Risk Assessment

Using Existing Knowledge for the Risk Evaluation of Crop Protection Products in order to Guide Exposure Driven Data Generation Strategies and Minimise Unnecessary Animal Testing.

Paul Parsons, Elaine Freeman, Ryan Weidling, Gary L Williams, Philip Gill, Neil Byron. *Crit Rev Toxicol.* 2021 Nov 10;1-22. doi: 10.1080/10408444.2021.1987384. **Article link**

Significance: Leveraging existing knowledge for chemical exposure prediction by combining it with existing hazard data allows assessors to identify key studies needed for human health risk assessment.

Traditionally, human health risk assessment focuses on defining the hazard through mammalian toxicity studies followed by exposure estimation. We have explored ways of predicting exposure based primarily on the use scenario and comparing the exposure to reference dose values derived by various regulatory agencies (US EPA, JMPR, and EU Commission) in order to identify mammalian toxicity studies that are relevant to human health risk assessment. Human dietary exposure was based on existing residue data for substances with comparable use on the same or similar crops. Human occupational exposures were based on the use scenarios and application methods. To provide a point of comparison for the exposure predictions, data were collated for acute, chronic and occupational reference dose values derived by various regulatory agencies (US EPA, JMPR, and EU Commission). The exposure predictions and range of hazard endpoints were compared using risk matrix plots in order to visualise and contextualise the level of potential concern for the exposure prediction. In addition, an approach is proposed to categorise the likelihood of acceptability of risk based on where the exposure sits relative to the distribution of reference dose values. The approaches proposed in this study allow for exposure prediction based on the Good Agricultural Practice (GAP) in conjunction with the use of existing hazard data for crop protection products in order to make an initial determination on acceptability of risk and to identify key studies that are required for human health risk assessment and also opportunities for study waivers.

Foodborne Pathogens

Surveillance of *Listeria monocytogenes:* Early Detection, Population Dynamics, and Quasimetagenomic Sequencing during Selective Enrichment

Eva Wagner, Annette Fagerlund, Solveig Langsrud, Trond Møretrø, Merete Rusås Jensen, Birgitte Moen. *Appl Environ Microbiol.* 2021 Nov 24;87(24):e0177421. doi: 10.1128/AEM.01774-21. **Article link**

Significance: A combination of a short primary enrichment combined with MDA and Nanopore sequencing can accelerate the traditional process of cultivation and identification of *L. monocytogenes*.

In this study, we addressed different aspects regarding the implementation of quasimetagenomic sequencing as a hybrid surveillance method in combination with enrichment for early detection of *Listeria monocytogenes* in the food industry. Different experimental enrichment cultures were used, comprising seven *L. monocytogenes* strains of different sequence types (STs), with and without a background microbiota community. To assess whether the proportions of the different STs changed over time during enrichment, the growth and population dynamics were assessed using dapE colony sequencing and dapE and 16S rRNA amplicon sequencing. There was a tendency of some STs to have a higher relative abundance during the late stage of enrichment when *L. monocytogenes* was enriched

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without background microbiota. When coenriched with background microbiota, the population dynamics of the different STs was more consistent over time. To evaluate the earliest possible time point during enrichment that allows the detection of *L. monocytogenes* and at the same time the generation of genetic information that enables an estimation regarding the strain diversity in a sample, quasimetagenomic sequencing was performed early during enrichment in the presence of the background microbiota using Oxford Nanopore Technologies Flongle and Illumina MiSeq sequencing. The application of multiple displacement amplification (MDA) enabled detection of *L. monocytogenes* (and the background microbiota) after only 4 h of enrichment using both applied sequencing approaches. The MiSeq sequencing data additionally enabled the prediction of cooccurring *L. monocytogenes* strains in the samples. Importance: We showed that a combination of a short primary enrichment combined with MDA and Nanopore sequencing can accelerate the traditional process of cultivation and identification of *L. monocytogenes*. The use of Illumina MiSeq sequencing additionally allowed us to predict the presence of cooccurring *L. monocytogenes* strains. Our results suggest quasimetagenomic sequencing is a valuable and promising hybrid surveillance tool for the food industry that enables faster identification of *L. monocytogenes* during early enrichment. Routine application of this approach could lead to more efficient and proactive actions in the food industry that prevent contamination and subsequent product recalls and food destruction, economic and reputational losses, and human listeriosis cases.

Induction of the Viable but Non-Culturable State in *Salmonella* Contaminating Dried Fruit. Victor Jayeola, J M Farber, S Kathariou. *Appl Environ Microbiol*. 2021 Nov 3; AEM0173321. doi: 10.1128/AEM.01733-21. **Article link**

Signi icance: Unique stressors on dried fruit can induce the viable but non-culturable state in *Salmonella*. In addition to culture-based methods, microscopic and molecular methods are needed for the accurate detection of *Salmonella* contaminating dried fruit.

This work was supported by the IAFNS **Food Microbiology Committee**

Salmonella can become viable but non-culturable (VBNC) in response to environmental stressors but the induction of the VBNC state in *Salmonella* contaminating ready-to-eat dried fruit is poorly characterized. Dried apples, strawberries and raisins were mixed with a five-strain cocktail of *Salmonella* at 4% volume per weight of dried fruit at 109 CFU/g. The inoculated dried fruit were then dried in desiccators at 25°C until the water activity (aw) approximated that of the uninoculated dried fruit. However, *Salmonella* could not be recovered after drying, not even after enrichment, suggesting a population reduction of approx. 8 log CFU/g. To assess the potential impact of storage temperature on survival, dried apples were spot-inoculated with the *Salmonella* cocktail, dried under ambient atmosphere at 25°C and stored at 4 and 25°C. Spot-inoculation permitted recovery of *Salmonella* on dried apple after drying, with the population of *Salmonella* decreasing progressively on dried apples stored at 25°C until it was undetectable after about 46 days, even following enrichment. The population decline was noticeably slower at 4°C, with *Salmonella* being detected until 82 days. However, fluorescence microscopy and laser scanning confocal microscopy with the LIVE-DEAD BacLight Bacterial Viability system at timepoints at which no *Salmonella* could be recovered on growth media even following enrichment, showed that a large proportion (56-85%) of the *Salmonella* cells on the dried fruit were viable. The data suggest that the unique combination of stressors in dried fruit can induce large numbers of VBNC cells of *Salmonella*. Importance: *Salmonella* is a leading foodborne pathogen globally causing numerous outbreaks of foodborne illnesses and remains the leading contributor to deaths attributed to foodborne disease in the United States and other industrialized nations. Therefore, efficient detection methods for *Salmonella* contaminating food are critical for public health and food safety. Culture-based microbiological methods are considered the gold standard for the detection and enumeration of *Salmonella* in food. Findings from this study suggest that unique stressors on dried fruit can induce the VBNC state in *Salmonella*, thus rendering it undetectable with culture-based methods even though the bacteria remain viable. Therefore, strong consideration should be given to using, in addition to culture-based methods, microscopic and molecular methods for the accurate detection of all viable and/or culturable cells of *Salmonella* contaminating dried fruit, as all these cells have the potential to cause human illness.

Foodborne Illness

Consumption of High-Risk Foods in the Canadian Population, Foodbook Study, 2014 to 2015. Megan Tooby, Vanessa Morton, Andrea Nesbitt, Nadia Ciampa, M Kate Thomas. *J Food Prot*. 2021 Nov 1;84(11):1925-1936. doi: 10.4315/JFP-21-101. **Article link**

Significance: Additional consumer food safety measures may be warranted alongside current messaging to address elevated high-risk food consumption behaviors (fresh seafood, deli meats, etc.). Using multifaceted

communications (e.g., social media and information pamphlets) and highlighting the large incidence and severity of foodborne illnesses in Canada may be important.

Many foods have the potential to cause foodborne illness; however, some pose a higher risk. Data were collected through the Foodbook study, a population-based telephone survey conducted between 2014 and 2015 that assessed 10,942 Canadians' food exposures using a 7-day recall period. The 19 foods included in the survey were identified as high risk for common foodborne pathogens in Canada. Results were analyzed by age group, gender, region of residence, income, and education. Consumption proportions of high-risk foods ranged from 0.4% (raw oysters) to 49.3% (deli meats). Roughly 94% of the population reported consuming one or more high-risk food in the past week. Certain high-risk food behaviors were associated with demographic characteristics. High-risk adults such as those 65 years or older still report consuming high-risk foods of concern, including deli meats (41.8%), soft cheeses (13.7%), and smoked fish (6.3%). Consumption of certain foods differed between genders, with males consuming significantly more deli meats, hot dogs, and raw or undercooked eggs and females consuming significantly more prebagged mixed salad greens. The overall number of high-risk foods consumed was similar, with both genders most frequently consuming three to five high-risk foods. High-risk food consumption was seen to increase with increasing household income, with 14.2% of the highest income level consuming six-plus high-risk foods in the past week, compared with 7.1% of the lowest income level. If a respondent had heard of a risk of foodborne illness associated with a food, it did not affect whether it was consumed. Additional consumer food safety efforts put in place alongside current messaging may improve high-risk food consumption behaviors. Enhancing current messaging by using multifaceted communications (e.g., social media and information pamphlets) and highlighting the large incidence and severity of foodborne illnesses in Canada are important strategies to improve behavior change.

Mycotoxins

Detection, Contamination, Toxicity, and Prevention Methods of Ochratoxins: An Update Review.
Melvin S Samuel, Kanimozhi Jeyaram, Saptashwa Datta, Narendhar Chandrasekar, Ramachandran Balaji, Ethiraj Selvarajan. *J Agric Food Chem.* 2021 Nov 24;69(46):13974-13989. doi: 10.1021/acs.jafc.1c05994. **Article link**

Significance: Mold development and mycotoxin contamination occur in improperly stored crops or cereals subject to, for example, high levels of humidity or temperature. This paper describes the potential health threats of Ochratoxin A.

Ochratoxins (OTs) with nephrotoxic, immunosuppressive, teratogenic, and carcinogenic properties are thermostable fungal subordinate metabolites. OTs contamination can occur before or after harvesting, during the processing, packing, distribution, and storage of food. Mold development and mycotoxin contamination can occur in any crop or cereal that has not been stored properly for long periods of time and is subjected to high levels of humidity and temperature. Ochratoxin A (OTA) presents a significant health threat to creatures and individuals. There is also a concern of how human interaction with OTA will also express the remains of OTA from feedstuffs into animal-derived items. Numerous approaches have been studied for the reduction of the OTA content in agronomic products. These methods can be classified into two major classes: inhibition of OTA adulteration and decontamination or detoxification of food. A description of the various mycotoxins, the organism responsible for the development of mycotoxins, and their adverse effects are given. In the current paper, the incidence of OTA in various fodder and food materials is discussed, which is accompanied by a brief overview of the OTA mode of synthesis, physicochemical properties, toxic effects of various types of ochratoxins, and OTA decontamination adaptation methods. To our knowledge, we are the first to report on the structure of many naturally accessible OTAs and OTA metabolism. Finally, this paper seeks to be insightful and draw attention to dangerous OTA, which is too frequently neglected and overlooked in farm duplication from the list of discrepancy studies.

Food Packaging

Sterilization of Food Packaging by UV-C Irradiation: Is *Aspergillus brasiliensis* ATCC 16404 the Best Target Microorganism for Industrial Bio-Validations?

Irene Racchi, Nicoletta Scaramuzza, Alyssa Hidalgo, Massimo Cigarini, Elettra Berni. *Int J Food Microbiol.* 2021 Nov 2;357:109383. doi: 10.1016/j.ijfoodmicro.2021.109383. **Article link**

Significance: Multi-layer inoculums proved most resistant to UV-C irradiation compared to those with single layers, calling into question the best target microorganisms for industrial validation.

In food industries UV-C irradiation is used to achieve decontamination of some packaging devices, such as plastic caps

or laminated foils, and of those smooth surfaces that can be directly irradiated. Since its effectiveness can be checked by microbial validation tests, some ascospore-forming molds (*Aspergillus hiratsukae, Talaromyces bacillisporus, Aspergillus montevidensis,* and *Chaetomium globosum*) were compared with one of the target microorganisms actually used in indus-trial bio-validations (*Aspergillus brasiliensis* ATCC 16404) to find the species most resistant to UV-C. Tests were carried out with an UV-C lamp (irradiance = 127 µW/cm2; emission peak = 253.7 nm) by inoculating HDPE caps with one or more layers of spores. Inactivation kinetics of each strain were studied and both the corresponding 1D-values and the number of Logarithmic Count Reductions (LCR) achieved were calculated. Our results showed the important role played by the type of inoculum (one or more layers) and by the differences in cell structure (thickness, presence of protective solutes, pigmentation, etc.) of the strains tested. With a single-layer inoculum, *Chaetomium globosum* showed the highest resis-tance to UV-C irradiation (1D-value = 100 s). With a multi-layer inoculum, *Aspergillus brasiliensis* ATCC 16404 was the most resistant fungus (1D-value = 188 s), even if it reached a number of logarithmic reductions that was higher than those of some ascospore-forming mycetes (*Aspergillus montevidensis, Talaromyces bacillisporus*) tested.

Heavy Metals

Climate Change and Emerging Food Safety Issues: A Review

Ramona A Duchenne-Moutien, Hudaa Neetoo. J *Food Prot*. 2021 Nov 1;84(11):1884-1897. doi: 10.4315/JFP-21-141. **Article link**

Significance: This review discusses the impacts of climate change on existing and emerging food safety risks and considers potential mitigation and adaptation strategies.

Throughout the past decades, climate change has been one of the most complex global issues. Characterized by world-wide alterations in weather patterns, along with a concomitant increase in the temperature of the Earth, climate change will undoubtedly have significant effects on food security and food safety. Climate change engenders climate variability: significant variations in weather variables and their frequency. Both climate variability and climate change are thought to threaten the safety of the food supply chain through different pathways. One such pathway is the ability to exacerbate foodborne diseases by influencing the occurrence, persistence, virulence and, in some cases, toxicity of certain groups of disease-causing microorganisms. Food safety can also be compromised by various chemical hazards, such as pesticides, mycotoxins, and heavy metals. With changes in weather patterns, such as lower rainfall, higher air temperature, and higher frequency of extreme weather events among others, this translates to emerging food safety concerns. These include the shortage of safe water for irrigation of agricultural produce, greater use of pesticides due to pest resistance, increased diffi-culty in achieving a well-controlled cold chain resulting in temperature abuse, or the occurrence of flash floods, which cause runoff of chemical contaminants in natural water courses. Together, these can result in foodborne infection, intoxication, antimicrobial resistance, and long-term bioaccumulation of chemicals and heavy metals in the human body. Furthermore, severe climate variability can result in extreme weather events and natural calamities, which directly or indirectly impair food safety. This review discusses the causes and impacts of climate change and variability on existing and emerging food safety risks and also considers mitigation and adaptation strategies to address the global warming and climate change problem.

Chemical Contaminants

Sample Preparation Techniques for Suspect and Non-Target Screening of Emerging Contaminants

Parvaneh Hajeb, Linyan Zhu, Rossana Bossi, Katrin Vorkamp. *Chemosphere*. 2021.;287(Pt 3):132306. doi: 10.1016/j.chemosphere.2021.132306. **Article link**

Significance: Sequential solvent extraction and a combination of different SPE sorbents can cover a broad range of chemicals in sampling for emerging contaminants, which more powerful spectrometers are better at detecting.

The progress in sensitivity and resolution in mass spectrometers in recent years provides the possibility to detect a broad range of organic compounds in a single procedure. For this reason, suspect and non-target screening techniques are gaining attention since they enable the detection of hundreds of known and unknown emerging contaminants in various matrices of environmental, food and human sources. Sample preparation is a critical step before analysis as it can significantly affect selectivity, sensitivity and reproducibility. The lack of generic sample preparation protocols is obvious in this fast-growing analytical field, and most studies use those of traditional targeted analysis methods. Among them, solvent extraction and solid phase extraction (SPE) are widely used to extract emerging contaminants from solid and liquid sample types, respectively. Sequential solvent extraction and a combination of different SPE sorbents can cover a broad range of chemicals in the samples. Gel permeation chromatography (GPC) and adsorption chromatography, including acidification, are typically used to remove matrix components such as lipids from complex

matrices, but usually at the expense of compound losses. Ideally, the purification of samples intended for non-target analysis should be selective of matrix interferences. Recent studies have suggested quality assurance/quality control measures for suspect and non-target screening, based on expansion and extrapolation of target compound lists, but method validations remain challenging in the absence of analytical standards and harmonized sample preparation approaches.

Caffeine

Coffee Consumption and the Risk of All-Cause and Cause-specific Mortality in the Korean Population

Seong-Ah Kim, Li-Juan Tan, Sangah Shin. *J Acad Nutr Diet*. 2021 Nov;121(11):2221-2232.e4. doi: 10.1016/j.jand.2021.03.014. **Article link**

Significance: This study of Korean subjects provides evidence that greater coffee consumption is associated with a decreased risk of all-cause mortality. Moderate consumption around 3 cups per day was associated with a decreased risk of CVD mortality in the study.

Background: There is a dearth of information regarding the association between coffee consumption and its health effects with respect to mortality among Korean people. **Objective**: The aim of this study was to examine the association between coffee consumption and all-cause mortality and cause-specific mortality risks in the Korean population. **Design:** This prospective cohort study had a median follow-up period of 9.1 years. Participants/setting: In total, 173,209 participants aged 40 years and older from the Health Examinees study were enrolled between 2004 and 2013. The analytic sample included 110,920 participants without diabetes, cardiovascular disease (CVD), or cancer at baseline who could be linked with their death information. **Main outcome measures**: Deaths of participants until December 31, 2018 were ascertained using the death certificate database of the National Statistical Office. Cause of death was classified according to the International Classification of Diseases, 10th Revision. **Statistical analyses performed**: Participants were categorized according to the amount and type of coffee consumed. Cox proportional hazards regression analysis was performed to estimate the hazard ratio (HR) and 95%CI of all-cause mortality and cause-specific mortality, such as CVD and cancer mortality. **Results**: Compared with nonconsumers of coffee, participants who consumed > 3 cups/day had a reduced risk of all-cause mortality (HR 0.79, 95% CI 0.66 to 0.95). Participants who consumed ≤1 cup/day and 1 to 3 cups/day had a reduced risk of CVD mortality (≤1 cup/day: HR 0.58, 95% CI 0.69 to 0.94; 1 to 3 cups/day: HR 0.62, 95% CI 0.41 to 0.96). **Conclusions**: This study provides evidence that greater coffee consumption is associated with a decreased risk of all-cause mortality and moderate coffee consumption (approximately 3 cups/day) is associated with a decreased risk of CVD mortality, regardless of the type of coffee, in a Korean population.

Food Allergens

Metabolomic Profiling Revealed Altered Lipid Metabolite Levels in Childhood Food Allergy

Haerin Jang, Eun Gyul Kim, Mina Kim, Soo Yeon Kim, Yoon Hee Kim, Myung Hyun Sohn, Kyung Won Kim. J *Allergy Clin Immunol*. 2021 Nov 26; S0091-6749(21)01817-0. doi: 10.1016/j.jaci.2021.10.034. **Article link**

Significance: Lipid metabolite profiles are closely related to childhood food allergy and this study suggests potential predictive biomarkers and mechanisms to further elucidate allergic reactions and resolutions.

Background: The pathophysiology of childhood food allergy (FA) and its natural history are poorly understood. Clarification of the underlying mechanism may aid in identifying novel biomarkers and strategies for clinical intervention in children with FA. **Objective:** This study aimed to identify metabolites associated with the development and resolution of FA. Methods: The metabolomic profiles of 20 children with FA and 20 healthy controls were assessed using liquid chromatography-mass spectrometry. Comparative analysis was performed to identify metabolites associated with FA and FA resolution. For subjects with FA, serum samples were collected at the time of diagnosis and after resolution to identify the changes in metabolite levels. The selected metabolites were then quantified in a quantification cohort to validate the results. Finally, genome-wide association analysis of the metabolite levels was performed**. Results**: The study demonstrated a significantly higher level of sphingolipid metabolites and a lower level of acylcarnitine metabolites in children with FA than those in healthy controls. At diagnosis, subjects with resolving FA had a significantly high level of omega-3 metabolites and a low level of platelet-activating factors (PAF) compared to persistent FA. However, the level of omega-3 metabolites decreased in children with resolving FA but increased in children with persistent FA during the same time. The quantification data of omega-3 derived resolvins, PAF, and PAF acetylhydrolase activity further supported these results. **Conclusion**: The lipid metabolite profile is closely related to childhood FA and FA resolution. This study suggests potential predictive biomarkers and provides insight into mechanisms underlying childhood FA.