August 2022

Food Safety Science Briefs

Risk Assessment

Assessment of the Applicability of the Threshold of Toxicological Concern for Per- and Polyfluoroalkyl Substances

Isabel A Lea, Ly Ly Pham, Todor Antonijevic, Chad Thompson, Susan J Borghoff. *Regul Toxicol Pharmacol.* 2022 Aug;133:105190. doi: 10.1016/j.yrtph.2022.105190. **Article link**

Significance: A 'threshold of toxicological concern' approach was tested for 27 PFAS compounds and found that broader use of the concept is warranted.

While toxicity information is available for selected PFAS, little or no information is available for most, thereby necessitating a resource-effective approach to screen and prioritize those needing further safety assessment. The threshold of toxicological concern (TTC) approach proposes a de minimis exposure value based on chemical structure and toxicology of similar substances. The applicability of the TTC approach to PFAS was tested by incorporating a data set of no-observed-adverse-effect level (NOAEL) values for 27 PFAS into the Munro TTC data set. All substances were assigned into Cramer Class III and the cumulative distribution of the NOAELs evaluated. The TTC value for the PFAS-enriched data set was not statistically different compared to the Munro data set. Derived human exposure level for the PFAS-enriched data set was 1.3 µg/kg/day. Structural chemical profiles showed the PFAS-enriched data set had distinct chemotypes with lack of similarity to substances in the Munro data set using Maximum Common Structures. The incorporation of these 27 PFAS did not significantly change TTC Cramer Class III distribution and expanded the chemical space, supporting the potential use of the TTC approach for PFAS chemicals.

Foodborne Pathogens

Efficacy of Acidified Oils against *Salmonella* in Low-Moisture Environments

Mrinalini Ghoshal, Shihyu Chuang, Ying Zhang, Lynne McLandsborough. *Appl Environ Microbiol.* 2022 Aug 23;88(16):e0093522. doi: 10.1128/aem.00935-22. **Article link**

Significance: Acidified oils can be cost-effective replacements for dry-sanitation methods and improve the safety of low moisture foods.

When processing low-moisture, high-fat foods such as peanut butter and nuts, water-based sanitization is unsuitable due to the immiscible nature of water and fats. Dry sanitization mainly uses flammable compounds such as isopropanol, requiring equipment cooling before application. The use of oils to deliver antimicrobials against foodborne pathogens enables the use of elevated temperatures, thus eliminating processing downtimes associated with dry sanitization. This study delivered organic acids and medium-chain fatty acids (100, 250, and 500 mM) in peanut oil against *Salmonella enterica* serovar Enteritidis desiccated at 75% relative humidity (RH). Acetic acid in peanut oil (AO) at 45°C was the most effective food-grade acid, causing a 4.4-log reduction in *S.* Enteritidis at 500 mM. AO caused cellular injury and was effective against a variety of *S.* Enteritidis strains. Confocal microscopy demonstrated that cells treated with 50 mM and 250 mM AO had significant membrane damage and reduced cellular respiration compared to untreated controls. Treatment efficacy increased with the increase in acid concentration, treatment duration, and treatment temperature from 20 to 45°C. Transmission electron microscopy after treatment with 100 and 250 mM AO revealed membrane ruffling and leakage in cell membranes, especially at 45°C. Reduction of the RH to 33% during desiccation of *S.* Enteritidis caused a decrease in

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AO efficacy compared to that at 75% RH, while at a higher RH of 90%, there was an increase in the efficacy of AO. Acidified oils can serve as robust, cost-effective replacements for dry-sanitation methods and improve safety of low moisture foods.

Foodborne Illness

Sources and Trends of Human Salmonellosis in Europe, 2015-2019: An Analysis of Outbreak Data

Linda Chanamé Pinedo, Lapo Mughini-Gras, Eelco Franz, Tine Hald, Sara M Pires. *Int J Food Microbiol*. 2022 Oct 16;379:109850. doi: 10.1016/j.ijfoodmicro.2022.109850. **Article link**

Significance: There was a significant increase in the number of foodborne illness outbreaks in Europe reported between 2015 and 2019, by 5 % on average per year.

Salmonella remains a major cause of foodborne outbreaks in Europe despite the implementation of harmonized control programmes. Outbreak data are observed at the public health endpoint and provide a picture of the most important sources of human salmonellosis at the level of exposure. To prioritize interventions, it is important to keep abreast of the sources and trends of salmonellosis outbreaks. The objective of this study was to determine the main food sources and recent trends of *Salmonella* outbreaks in Europe. *Salmonella* outbreak data from 34 European countries in 2015-2019 were obtained from the European Food Safety Authority (EFSA). For the source attribution analysis, implicated foods were categorized according to EFSA's zoonosis catalogue classification scheme. An established probabilistic source attribution model was applied using the information on the implicated foods, overall and by region and serotype. To assess significant trends in outbreak occurrence, overall and by region and serotype, mixed-effects Poisson models were used. Overall, the most important food source of salmonellosis outbreaks was eggs (33 %, 95 % Uncertainty Interval [UI]: 31-36 %), followed by pork (7 %, 95 % UI: 6-8 %), and (general) meat products (6 %, 95 % UI: 5-8 %). While eggs were the most important food source in all regions, pork was the second most common food source in Northern and Western Europe, and (general) meat products in Eastern and Southern Europe. Outbreaks caused by *S.* Enteritidis (SE) and other known serotypes (other than SE and *S.* Typhimurium and its monophasic variant [STM]) were mostly attributed to eggs (37 %, 95 % UI: 34-41 % and 17 %, 95 % UI: 11-25 %, respectively), whereas outbreaks caused by STM were mainly attributed to pork (34 %, 95 % UI: 27-42 %). Overall, there was a significant increase in the number of outbreaks reported between 2015 and 2019, by 5 % on average per year (Incidence Rate Ratio [IRR]: 1.05, 95 % Confidence Interval [CI]: 1.01-1.09). This was driven by a significantly increased number of outbreaks in Eastern Europe, particularly those caused by SE (IRR: 1.15, 95 % CI: 1.09-1.22), whereas in Northern and Southern Europe, outbreaks caused by SE decreased significantly from 2015 to 2019 (IRR: 0.72, 95 % CI: 0.61-0.85; IRR: 0.70, 95 % CI: 0.62-0.79, respectively). Regional, temporal and serotype-associated differences in the relative contributions of the different sources were also observed.

Mycotoxins

Simultaneous Determination of Antibiotics, Mycotoxins, and Hormones in Milk by an 8-17 DNAzyme-Based Enzyme-Linked Immunosorbent Assay

Panting Sang, Gang Lu, Dongwei Yu, Xiaodong Song, Yahui Guo 1, Yunfei Xie 1, Weirong Yao, et. al. *Agric Food Chem*. 2022 Aug 29. doi: 10.1021/acs.jafc.2c03833. **Article link**

Significance: Mycotoxins and other contaminants in milk were found using an assay that amplifies their signal while having lower detection limits than conventional methods.

The simultaneous detection of three kinds of small-molecule contaminants (antibiotics, mycotoxins, and hormones) in milk was realized by using an 8-17 DNAzyme-based fluorescent enzyme-linked immunosorbent assay (ELISA), in which 8-17 DNAzyme was utilized as the catalytic enzyme for amplifying the signal. Compared with the conventional ELISA in which horseradish peroxidase is used as the catalyzing factor, this 8-17 DNAzyme-based ELISA could achieve multicolor signal output with lower detection limits. The linearities for chloramphenicol, 17β-estradiol, and aflatoxin M1 were in the range of 0.3 ng/mL-3 μg/mL, 3 ng/mL-3 μg/mL, and 3 pg/mL-3 ng/mL with quantitation limits of 0.3, 3, and 0.003 ng/mL, respectively. This proposed scheme demonstrated that the 8-17 DNAzyme might be an effective substitute for horseradish peroxidase in ELISA for the development of ultrasensitive and multicolor fluorescence immunoassay, which would stimulate the development of ELISA in a new orientation.

Heavy Metals

A Systematic Review of Adverse Health Effects Associated with Oral Cadmium Exposure

Heather R Schaefer, Brenna M Flannery, Lynn Crosby, Olivia E Jones-Dominic, Cecile Punzalan, Karlyn Middleton. *Regul Toxicol Pharmacol*. 2022 Aug 15;134:. doi: 10.1016/j.yrtph.2022.105243. **Article link**

Significance: A review of human and test animal studies found seven studies were of sufficient quality to derive a toxicological reference value for cadmium.

Scientific data characterizing the adverse health effects associated with dietary cadmium (Cd) exposure were identified in order to make informed decisions about the most appropriate toxicological reference value (TRV) for use in assessing dietary Cd exposure. Several TRVs are available for Cd and regulatory organizations have used epidemiologic studies to derive these reference values; however, risk of bias (RoB) evaluations were not included in the assessments. We performed a systematic review by conducting a thorough literature search (through January 4, 2020). There were 1714 references identified by the search strings and 328 studies identified in regulatory assessments. After applying the specific inclusion and exclusion criteria, 208 studies (Human: 105, Animal: 103) were considered eligible for further review and data extraction. For the epidemiologic and animal studies, the critical effects identified for oral Cd exposure from the eligible studies were a decrease in bone mineral density (BMD) and renal tubular degeneration. A RoB analysis was completed for 49 studies (30 epidemiological and 19 animal) investigating these endpoints. The studies identified through the SR that were considered high quality and low RoB (2 human and 5 animal) can be used to characterize dose-response relationships and inform the derivation of a Cd TRV.

Food Packaging

Investigation of Eight Cellulose Nanomaterials' Impact on Differentiated Caco-2 Monolayer Integrity and Cytotoxicity

Ninell P Mortensen, Maria Moreno Caffaro, Klinton Davis, Shyam Aravamudhan, Susan J Sumner, Timothy R Fennell. *Food Chem Toxicol*. 2022 Aug;166:113204. doi: 10.1016/j.fct.2022.113204. **Article link**

Significance: Cellulose nanomaterials in food packaging present a possible source of human exposure. The researchers found that three types of nanomaterials induce cytotoxicity in certain cells, warranting further study.

The potential applications of cellulose nanomaterials (CNMs) as food additives or in food packaging, present a possible source of human ingestion. While micron- and macro-scale cellulose products are classified as Generally Regarded As Safe, the safety of ingested nano-scale cellulose is largely unknown. Using fully differentiated Caco-2 cells, the perturbation of intestinal barrier function and cytotoxicity was investigated for four nanocellulose crystals (CNCs) and four nanocellulose fibrils (CNFs) following 24 h of exposure at 50 µg/mL. Scanning electron microscope showed some aggregation of both CNCs and CNFs. X-ray photoelectron spectroscopy analyses showed that carbon and oxygen were the main elements. The zeta-potential for CNMs formulated in cell culture medium showed a negative surface charge. Two CNMs increased cell membrane permeability and three CNMs decreased the cell metabolic activity. While three CNMs lead to cytotoxic responses, no changes in apparent permeability coefficient (Papp) for dextran or tight junction integrity were found. Our results show that three CNMs induce cytotoxicity in differentiated Caco-2 cells, demonstrating the need to understand the role of size and shape. The interaction between CNMs and the intestinal epithelium needs to be evaluated to understand potential intestinal barrier dysfunction and resulting health implications following CNM ingestion.

Caffeine

The Magical Smell and Taste: Can Coffee be Good to Patients with Cardiometabolic Disease?

Marcia Ribeiro, Livia Alvarenga, Ludmila F M F Cardozo, Julie A Kemp, Ligia S Lima, Jonatas S de Almeida, Viviane de O Leal, et. al. *Crit Rev Food Sci Nutr*. 2022 Aug 5;1-22. doi: 10.1080/10408398.2022.2106938. **Article link**

Significance: Coffee is rich in caffeine, antioxidants, and phenolic compounds, which can affect the composition of the gut microbiota and reduce both inflammation and oxidative stress.

Coffee is a beverage consumed globally. Although few studies have indicated adverse effects, it is typically a beneficial health-promoting agent in a range of diseases, including depression, diabetes, cardiovascular disease, and obesity. Coffee is rich in caffeine, antioxidants, and phenolic compounds, which can modulate the composition of the gut microbiota and mitigate both inflammation and oxidative stress, common features of the burden of lifestyle diseases. This review will discuss the possible benefits of coffee on complications present in patients with diabetes, cardiovascular disease and chronic kidney disease, with the social and emotional benefits attributed to caffeine consumption.

Chemical Contaminants

Glycidamide and cis-2-butene-1,4-dial (BDA) as Potential Carcinogens and Promoters of Liver Cancer - An *in vitro* Study

Sofia Gouveia-Fernandes, Armanda Rodrigues, Carolina Nunes, Catarina Charneira, João Nunes, Jacinta Serpa, Alexandra M Antunes. Food Chem Toxicol. 2022 Aug;166:113251. doi: 10.1016/j.fct.2022.113251. **Article link**

Significance: This study provides insights on the carcinogenic capacity of glycidamide and BDA and also point out their effect in favoring hepatocellular carcinoma progression.

Acrylamide and furan are environmental and food contaminants that are metabolized by cytochrome P450 2E1 (CYP2E1), giving rise to glycidamide and cis-2-butene-1,4-dial (BDA) metabolites, respectively. Both glycidamide and BDA are electrophilic species that react with nucleophilic groups, being able to introduce mutations in DNA and perform epigenetic remodeling. However, whereas these carcinogens are primarily metabolized in the liver, the carcinogenic potential of acrylamide and furan in this organ is still controversial, based on findings from experimental animal studies. With the ultimate goal of providing further insights into this issue, we explored *in vitro*, using a hepatocyte cell line and a hepatocellular carcinoma cell line, the putative effect of these metabolites as carcinogens and cancer promoters. Molecular alterations were investigated in cells that survive glycidamide and BDA toxicity. We observed that those cells express CD133 stemness marker, present a high proliferative capacity and display an adjusted expression profile of genes encoding enzymes involved in oxidative stress control, such as GCL-C, GSTP1, GSTA3 and CAT. These molecular changes seem to be underlined, at least in part, by epigenetic remodeling involving histone deacetylases (HDACs). Although more studies are needed, here we present more insights towards the carcinogenic capacity of glycidamide and BDA and also point out their effect in favoring hepatocellular carcinoma progression.

Food Allergens

IL-4 Receptor Alpha Signaling Alters Oral Food Challenge and Immunotherapy Outcomes in Mice

Varsha Ganesan, Ankit Sharma, Sunil Tomar, Charles F Schuler 4th, Simon P Hogan. *Allergy Clin Immunol.* 2022 Aug 4;S0091-6749(22)01021-1. doi: 10.1016/j.jaci.2022.07.011. **Article link**

Significance: Developing a mouse test animal that mimics the disease course of food allergies and treatment in humans could help identify biomarkers that predict reactivity.

Background: Food allergy diagnosis and management causes a number of social an emotional challenge for individuals with food allergies and caregivers. This has led to increasing interest in the development and usage of approaches to accurately predict food allergy diagnosis, severity of food allergic reactions and treatment outcomes. However, the utility of these approaches is somewhat conflicting. **Objective:** Develop and utilize a murine model that mimics the disease course of food allergy diagnosis and treatment in humans and to identify biomarkers that predict reactivity during food challenge and responsiveness during oral immunotherapy and how these outcomes are modified by genetics. **Methods:** Skin sensitized Intestinal IL-9Tg (IL9Tg) and IL9Tg mice backcrossed onto the IL4RαY709F background received single intragastric exposure of egg antigen (OVA), underwent oral food challenge (OFC) and oral immunotherapy (OIT) and food allergy severity, mast cell activation and OVA-specific IgE levels were examined to determine the predictability of these outcomes in determining reactivity and treatment outcomes. **Results:** We show that s.c. sensitization and a single intragastric allergen-challenge of egg antigen to BALB/c IL-9Tg mice and IL4RαY709F IL9Tg induced a food allergic reaction. We show that enhanced IL-4Rα-signaling altered the symptoms induced by the first oral exposure, decreased the cumulative antigen dose and increased severity of reaction during OFC and altered side effect frequency and OIT outcomes. Analysis of the biomarkers following the first oral exposure revealed that only the severity of the initial reaction significantly correlated with the cumulative dose of the OFC. **Conclusion:** Collectively, these data indicate that SNPs in IL-4Rα can alter clinical symptoms of food allergic reactions, severity and reactive dose during food challenge and OIT and that severity of first reaction can predict the likelihood of reaction during a food challenge in mice with IL-4Rα gain of function.

Emerging Science Areas

Emerging Area: Food Safety Abroad

A Realist Review of Voluntary Actions by the Food and Beverage Industry and Implications for Public Health and Policy in Low- and Middle-Income Countries

Erzse, A., Karim, S.A., Foley, L. et al. *Nat Food* 3, 650–663 (2022). August 5, 2022. doi.org/10.1038/s43016-022-00552-5. **Article link**

Significance: A global review of 20 food and beverage industry voluntary actions (VA) to improve public health using a conceptual framework with three stage literature and VA search following the RAMESES guidelines found

lack of evidence of VAs effectiveness in safeguarding public health. It may have in fact weakened responses and policy substitution. Vague language and a lack of enforcement mechanisms suggests VAs have the potential for negative influence. The study concludes that mandatory measures should be put in place instead of industry voluntary approaches.

The increasing availability of unhealthy processed food products is linked to rising rates of non-communicable diseases and obesity in low- and middle-income countries. Voluntary actions (VAs) are often adopted in lieu of regulating the composition, production, marketing and sale of unhealthy commodities, but their effectiveness is unclear. This realist review examines VAs adopted by the food and beverage industry in low- and middle-income countries. We developed a conceptual framework and followed a three-stage search to identify literature and VAs and, adhering to the Realist and Meta-narrative Evidence Syntheses: Evolving Standards (RAMESES) guidelines, we produced a synthesized analysis of VAs. VAs, often initiated in response to governments' efforts to introduce regulations, were difficult to evaluate due to vague language and a lack of enforcement mechanisms. The review found no evidence indicating that VAs are effective in safeguarding public health. Yet their implementation has resulted in weaker responses and policy substitution, and so we suggest that VAs have the potential to negatively influence public health and policy. The United Nations should rescind their endorsement of industry involvement and mandatory measures should be favored over VAs.

Engage with IAFNS

- **FNCE**
  October 8-11, 2022
  Orlando Florida
    - Two IAFNS science projects will be presented at FNCE 2022! Each fall, the Academy of Nutrition and Dietetics sponsors the world's largest meeting of food, nutrition and dietetics experts to address key issues affecting the health of individuals and communities throughout the country and around the world. The two IAFNS sessions are:

      Are We Ready? Dietary Recommendations Based on Direct-to-Consumer Gut Microbiome Tests

      Flavanols and Cardiometabolic Health: Examining the First Ever Dietary Bioactive Guideline

      Find out more information about FNCE 2022 **here.**

- **Sample Collection, Preservation, and Data Analysis in Gut Microbiome Research: Current Methods and Potential Impact on Results**
  October 13, 2022. 12:00-2:30 pm ET.
  Virtual, Event
    - Part of the American Society for Nutrition's NUTRITION 2022 Annual Satellite Series.
    - In this session, the current state of knowledge related to sample collection, utility of standards, sequencing, and bioinformatic and biostatistical approaches will be reviewed by experts working in the field. Speakers will expand on how selection among the various methodological options can impact study results. Find out more information about the event **here.**

- **IAFNS Science Innovation Showcase – 2022**
  December 13-15, 2022.
  Virtual, Event
    - This science-first and science-focused event will bring together scientists from multiple sectors, at all stages of their careers from graduate students to professors, technical experts to CEOs. Attendees will have the opportunity to engage in dialogue and discussion on the data, the technology and science being applied across the food and beverage ecosystem including plant-based proteins and other alternatives. Join us to learn about next generation possibilities! Find out more information about the Science Innovation Showcase **here.**