Risk Assessment

Testing the Thresholds of Toxicological Concern Values Using a New Database for Food-Related Substances

Significance: This report confirms the applicability of Threshold of Toxicological Concern values to substances relevant to food safety.

The Threshold of Toxicological Concern (TTC) concept integrates data on exposure, chemical structure, toxicity and metabolism to identify a safe exposure threshold value for chemicals with insufficient toxicity data for risk assessment. The TTC values were originally derived from a non-cancer dataset of 613 compounds with a potentially small domain of applicability. There is interest to test whether the TTC values are applicable to a broader range of substances, particularly relevant to food safety using EFSA’s new OpenFoodTox database. After exclusion of genotoxic compounds, organophosphates or carbamates or those belonging to the TTC exclusion categories, the remaining 329 substances in the EFSA OpenFoodTox database were categorized under the Cramer decision tree, into low (Class I), moderate (II), or high (III) toxicity profile. For Cramer Classes I and III the threshold values were 1000 μg/person per day (90% confidence interval: 187-2190) and 87 μg/person per day (90% confidence interval: 60-153), respectively, compared to the corresponding original threshold values of 1800 and 90 μg/person per day. This confirms the applicability of the TTC values to substances relevant to food safety. Cramer Class II was excluded from our analysis because of containing too few compounds. Comparison with the Globally Harmonized System of classification confirmed that the Cramer classification scheme in the TTC approach is conservative for substances relevant to food safety.

Foodborne Pathogens

Worldwide Epidemiology of Salmonella Serovars in Animal-Based Foods: A Meta-Analysis

Significance: This study identifies the prevalence of Salmonella serovars in four animal-based food matrices and highlights opportunities for improving global interventions to control Salmonella along the food chain.

Salmonella spp. are among the most important foodborne pathogens and the third leading cause of human death among diarrheal diseases worldwide. Animals are the primary source of this pathogen, and animal-based foods are the main transmission route to humans. Thus, understanding the global epidemiology of Salmonella serovars is key to controlling and monitoring this bacterium. In this context, this study aimed to evaluate the prevalence and diversity of Salmonella enterica serovars in animal-based foods (beef, pork, poultry, and seafood) throughout the five continents (Africa, the Americas [North and Latin America], Asia, Europe, and Oceania). The meta-analysis consisted of a chemometric assessment (hierarchical cluster analysis and principal component analysis) to identify the main epidemiological findings, including the prevalence and diversity of the Salmonella serovars in each matrix. Regarding the serovar distribution, S. Typhimurium presented a cosmopolitan distribution, reported in all four assessed matrices and continents; poultry continues to play a central role in the dissemination of the Enteritidis serovar to humans, and Anatum and Weltevreden were the most frequently found in beef and seafood, respectively. Additionally, we recommended careful monitoring of certain serovars, such as Derby, Agona, Infantis, and Kentucky. Finally, given the scientific data regarding the most frequently reported serovars and which matrices constitute the main vehicles for the transmission of this pathogen, control programs may be improved, and specific interventions may be implemented in an attempt to reduce the risk of this pathogen reaching humans. Importance: Salmonellosis is caused by Salmonella spp. and is the third leading cause of death...
among food-transmitted diseases. This pathogen is commonly disseminated in domestic and wild animals, and the infection’s symptoms are characterized by acute fever, nausea, abdominal pain, and diarrhea. The animals are the primary source of salmonellae, and animal-based foods are the main transmission route to humans. Therefore, data collected from these sources could contribute to future global interventions for effective control and surveillance of Salmonella along the food chain. In light of this, the importance of our research is in identifying the prevalence of Salmonella serovars in four animal-based food matrices (pork, poultry, beef, and seafood) and to evaluate the importance that each matrix has as the primary source of this pathogen to humans.

**Pathogen Detection**

Multicolor and Ultrasensitive ELISA Based on Fluorescence Hybrid Chain Reaction for Simultaneous Detection of Pathogen


**Significance:** An assay was developed for ultrasensitive, specific, and simultaneous detection of multiple target pathogens.

Various pathogens may coexist in one sample, however, detection methods that rely on traditional selective culture media or immune agents designed specifically for a certain target are unsuitable for multiple targets. It is important to develop simultaneous and sensitive detection method of multiple pathogens. Here, a multicolor and ultrasensitive ELISA platform based on fluorescence hybridization chain reaction (HCR) was developed. In the assay, multicolor fluorescence concatemers formed as signal amplifiers and signal reporters in the presence of target pathogens. When HCR occurred, *Escherichia coli O157:H7*, *Salmonella choleraesuis*, and *Listeria monocytogenes* were detected simultaneously with three different fluorescences. Additionally, the limits of detection (LODs) for *Escherichia coli O157:H7*, *Salmonella choleraesuis*, and *Listeria monocytogenes* were 3.4×10¹, 6.4×10⁰, and 7.0×10¹ CFU/mL, respectively. The assay achieved ultrasensitive, specific, and simultaneous detection of three pathogens and can be applied to the detection of pathogens in milk samples. Therefore, this multicolor and ultrasensitive ELISA platform has great potential in the application of simultaneous detection of pathogens.

**Mycotoxins**

**Aflatoxin, Fumonisin, Ochratoxin, Zearalenone and Deoxynivalenol Biomarkers in Human Biological Fluids: A Systematic Literature Review, 2001-2018**


**Significance:** A comprehensive overview of global mycotoxin biomonitoring studies is presented.

Human exposure to mycotoxins occurs mostly through dietary intake, although exposure through dermal and inhalation routes has also been shown. Depending on the type of mycotoxins, the applied dose and duration of exposure, a particular toxin can cause either chronic or acute illnesses such as kidney failure and cancer. Thus, understanding the biotransformation of mycotoxins and identification of reliable biomarkers in the human body is important for accurate risk assessment of mycotoxin exposure. This review provides a comprehensive overview of worldwide aflatoxins, fumonisins, ochratoxin, zearalenone and deoxynivalenol mycotoxin biomonitoring studies reported in the last 18 years. The studies performed in Africa, Europe, Asia and America are based on the measurement of a limited number of mycotoxin biomarkers and do not provide a comprehensive risk assessment of the mycotoxin exposure. Although the findings represent a small segment of a much larger health risk of mycotoxins exposure, it is acknowledged that a multianalyte approach covering bioconjugated and other metabolites of most often occurring mycotoxins would better reflect the extent of the global exposure problems with these highly toxic compounds.

**The Mycotoxin Patulin: An Updated Short Review on Occurrence, Toxicity and Analytical Challenges**


**Significance:** This review highlights the patulin occurrence profile and analytical challenges related to patulin detection.

Patulin (PAT) is a common mycotoxin in fruit products, especially in apples and apple-based products. The European Commission has set maximum levels for PAT in food. Nevertheless, worrying PAT levels were recently recorded in diverse foods across the world. Therefore, a worldwide follow-up of PAT levels in foods should be considered. Because of PAT’s high probability in food products, the toxicological implications for humans need to be addressed as well. Recent studies proved adverse health effects of PAT, such as hepatotoxicity, gastrointestinal alterations and immunotoxicity. In comparison to the toxicity of other mycotoxins
such as ochratoxin A, PAT’s immunotoxicity can be even more outspoken destructive. In addition, PAT is a low-molecular-weight and highly polar molecule, resulting in many analytical challenges for its detection. As the analytical techniques are continuously improving, PAT determination in multi-mycotoxin analysis has advanced using liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) during the last year. Finally, the presence and toxicity of PAT requires a biomarker method to assess its exposure among the population. To date, however, there is no information regarding PAT biomarkers in biological samples. This short review highlights the PAT-occurrence profile, toxicological discoveries and analytical challenges of 2014 until to date.

**Food Packaging**

**Styrene Monomer Migration From Polystyrene Based Food Packaging Nanocomposite: Effect of Clay and ZnO Nanoparticles**


**Significance:** Organoclay and zinc oxide nanoparticles were found effective in reducing styrene monomer migration into food simulants.

Inhibition from migration of plastic ingredients such as styrene monomer (SM) is very important in food packaging industry. Styrene monomer is one of the substances which can potentially migrate from polystyrene based packaging. In the present study, organoclay and zinc oxide nanoparticles (ZnO-NPs) were used for decreasing of the SM migration into food simulants (10 and 50% ethanol (v/v)). A used GC-FID method for measuring of the migrated SM showed good precision and accuracy. Maximum reduction of SM migration into 10% and 50% ethanol (24 h storage at 40 °C) were observed in the polystyrene/nanoclay and polystyrene/ZnO samples, respectively. The SM migration data in 50% ethanol at 5 °C followed from Fickian diffusion law and the lowest diffusion coefficient (2.89 × 10^{-14} \text{cm}^2/\text{s}) was observed in the polystyrene/ZnO/nanoclay samples.

**Heavy Metals**

**Market Basket Survey of Arsenic Species in the Top Ten Most Consumed Seafoods in the United States**


**Significance:** This study found that inorganic arsenic concentrations in commonly consumed seafood products are much lower than the tolerable intake set by the Joint FAO/WHO Expert Committee.

The study focused on the determination of arsenic species in the top ten most consumed seafoods in the United States. Fifty-four samples were collected from local supermarkets, and their species identities were confirmed by DNA barcoding. The total arsenic in the samples varied greatly in the range of 8–22200 ng/g (wet mass). Speciation analysis based on extraction of water-soluble and nonpolar arsenic showed that inorganic arsenic (iAs) was found only in clams and crabs, while arsenobetaine (AsB) predominates in most samples. Among the other arsenicals, trimethylarsionipropionate (TMAP) was found in most matrices with higher concentrations in crabs, and arsenosugars existed in most clams and crabs. Nonpolar arsenic accounted for 1–46% of the total arsenic in the samples. The accuracy of the analytical results was evaluated using standard reference materials and spike recovery tests. The survey showed that the iAs concentrations in America’s most consumed seafood products are much lower than the tolerable intake set by the Joint FAO/WHO Expert Committee, even at the highest levels found in this study.

**Caffeine**

**Caffeine, Type of Coffee and Risk of Ovarian Cancer: A Dose-Response Meta-Analysis of Prospective Studies**


**Significance:** This meta-analysis of prospective studies found no association between caffeine intake and risk of ovarian cancer.

Context: Prospective studies on caffeine and different types of coffee intake in relation to the risk of ovarian cancer have shown conflicting results. Objective: The aim of this study was to perform a dose-response meta-analysis of cohort studies on the association between dietary caffeine intake, different types of coffee consumption and risk of ovarian cancer. Data sources: PubMed/Medline, ISI Web of Science, Scopus and EMBASE were searched to identify relevant articles published until October 2018. Study selection: Prospective cohort studies that considered caffeinated and different types of coffee as the exposure variable
and ovarian cancer as the main outcome variable or as one of the outcome variables were included in this systematic review and meta-analysis. Two authors independently screened 9344 publications. A total of 14 cohort studies were included in the meta-analysis. Data extraction: Two authors independently extracted the data. Any disagreements were resolved in consultation with the principal investigator. Results: In total, 940359 participants, including 4434 subjects with ovarian cancer, aged >20 years were included in this systematic review and meta-analysis. Combining 13 effect sizes, we found no significant association between coffee consumption and risk of ovarian cancer (RR: 1.08; 95% CI: 0.89, 1.33). Also, one additional cup per day of coffee consumption was marginally significantly associated with increased risk of ovarian cancer (RR: 1.02; 95% CI: 0.99, 1.05; P=0.21; I²=0.0%; P heterogeneity=0.68). No significant association was observed between caffeine intake, caffeinated or decaffeinated coffee consumption and risk of ovarian cancer. Conclusions: We found no significant association between caffeine, different types of coffee and risk of ovarian cancer.

**Food Allergens**

**Validation of Bioinformatic Approaches for Predicting Allergen Cross-Reactivity**

**Significance:** A standardized method for evaluating the sensitivity of different bioinformatic algorithms using a comprehensive database of known allergen sequences is proposed.

Part of the allergenicity assessment of newly expressed proteins in genetically engineered food crops involves an assessment of potential cross-reactivity with known allergens. Bioinformatic approaches are used to evaluate the amino acid sequence identity or similarity between newly expressed proteins and the sequences of known allergens. To be useful, such approaches must be sensitive to detecting cross-reactive potential, but also capable of excluding low-risk sequences. One difficulty in comparing the effectiveness of different bioinformatic approaches has been the lack of a standardized validation and evaluation method. Here, we propose a standardized method for evaluating the sensitivity of different bioinformatic algorithms using a comprehensive database of known allergen sequences. We combine this with a previously described method for evaluating selectivity using sequences from a crop not known to commonly cause food allergy (e.g. maize) to compare the standard “>35% identity-criterion over sliding-window of ≥80 amino acids” bioinformatic approach with the previously described “one-to-one (1:1) FASTA” similarity approach using an E-value threshold of 1E-9. Results confirm the superiority of the 1:1 FASTA approach for selectively detecting cross-reactive allergens. The validation methods described here can be applied to other algorithms to select even better fit-for-purpose approaches for evaluating cross-reactive risk.

**High Dietary Fat Intake Induces a Microbiota Signature That Promotes Food Allergy**

**Significance:** In germ-free mice, a high-fat diet-associated gut microbiome was sufficient to confer enhanced susceptibility to food allergy.

**Background:** Diet-induced obesity and food allergies increase in tandem, but a potential cause-and-effect relationship between these diseases of affluence remains to be tested. Objective: We sought to test the role of high dietary fat intake, diet-induced obesity, and associated changes in gut microbial community structure on food allergy pathogenesis. Methods: Mice were fed a high-fat diet (HFD) for 12 weeks before food allergen sensitization on an atopic dermatitis–like skin lesion, followed by intragastric allergen challenge to induce experimental food allergy. Germ-free animals were colonized with a signature HFD or lean microbiota for 8 weeks before induction of food allergy. Food-induced allergic responses were quantified by using a clinical allergy score, serum IgE levels, serum mouse mast cell protease 1 concentrations, and type 2 cytokine responses. Accumulation of intestinal mast cells was examined by using flow cytometry and chloroacetate esterase tissue staining. Changes in the gut microbial community structure were assessed by using high-throughput 16S ribosomal DNA gene sequencing. Results: HFD-induced obesity potentiates food-induced allergic responses associated with dysregulated intestinal effector mast cell responses, increased intestinal permeability, and gut dysbiosis. An HFD-associated microbiome was transmissible to germ-free mice, with the gut microbial community structure of recipients segregating according to the microbiota input source. Independent of an obese state, an HFD-associated gut microbiome was sufficient to confer enhanced susceptibility to food allergy. Conclusion: These findings identify HFD-induced microbial alterations as risk factors for experimental food allergy and uncouple a pathogenic role of an HFD-associated microbiome from obesity. Postdieting microbiome alterations caused by overindulgence of dietary fat might increase susceptibility to food allergy.