Risk Assessment

‘All Chemical Substances Are Harmful.’ Public Appraisal of Uncertain Risks of Food Additives and Contaminants

**Significance:** A mismatch between the level of uncertainty that people expect about food safety and the actual level of scientific uncertainty affects how people evaluate the presence of chemical substances in food.

In toxicological health risk assessment, epistemic uncertainties (e.g. about a chemical’s intrinsic properties or toxicity) often remain, preventing definitive statements about whether a chemical constitutes a risk. In this study, we analyzed public appraisals of uncertain risks of food additives and contaminants. We identified three major characteristics of public appraisal. First, hazard appraisals differed consistently from risk appraisals: respondents were less disturbed by a possible health risk than by the mere presence of the chemical substance in food. Second, while a majority understood that exposure determines whether a chemical constitutes a risk, many respondents thought that all chemicals are equally harmful. This suggests a mismatch between beliefs about exposure and beliefs about toxicity. Finally, the higher people valued certainty about food safety and the less they considered uncertainty about a risk acceptable, the more severe they appraised the presence of the chemical substance in food. This suggests that a mismatch between the level of uncertainty that people expect about food safety and the actual level of scientific uncertainty, affects how people evaluate the presence of chemical substances in food. Following the findings, implications for risk communication are discussed.

Threshold of Toxicological Concern (TTC) for Botanicals - Concentration Data Analysis of Potentially Genotoxic Constituents to Substantiate and Extend the TTC Approach to Botanicals

**Significance:** The use of the Threshold of Toxicological Concern approach was evaluated for safety assessment of botanical preparations.

This paper evaluates use of the Threshold of Toxicological Concern (TTC) approach to assess safety of botanical preparations that may contain potentially genotoxic constituents, based on estimation of the fraction that may be genotoxic. A database of 107 chemical constituents of botanicals was compiled and their potential for genotoxicity evaluated from published data. Forty-three constituents met the criteria for potential genotoxicity. Concentration data on their occurrence in plants provided 2878 data points; the majority were in the low ppm level (range 0.00001-139,965 ppm, by dry weight). Weibull models of the quantitative distribution data were used to calculate 95th percentile values for chemical concentrations, analysing the dataset according to their presence in botanicals (i) as a single chemical, (ii) as two or more chemicals from the same chemical group, or (iii) as two or more chemicals from different chemical groups. The highest 95th percentile concentration value from these analyses was 1.8%. Using the TTC value of 0.15 μg/person per day for potentially genotoxic substances proposed in 2004, this value of 1.8% was used to derive an adjusted TTC value of 10 μg of plant material on a dry weight basis/person per day for assessment of potentially genotoxic substances in botanicals.
An Evaluation Framework for New Approach Methodologies (NAMs) for Human Health Safety Assessment

Significance: A framework that presents a consistent set of criteria, universal across initiatives, to evaluate a new approach methodologies’ fit-for-purpose was developed by a multi-stakeholder group of industry, academic, and regulatory experts.

The need to develop new tools and increase capacity to test pharmaceuticals and other chemicals for potential adverse impacts on human health and the environment is an active area of development. Much of this activity was sparked by two reports from the US National Research Council (NRC) of the National Academies of Sciences, Toxicity Testing in the Twenty-first Century: A Vision and a Strategy (2007) and Science and Decisions: Advancing Risk Assessment (2009), both of which advocated for “science-informed decision-making” in the field of human health risk assessment. The response to these challenges for a “paradigm shift” toward using new approach methodologies (NAMs) for safety assessment has resulted in an explosion of initiatives by numerous organizations, but, for the most part, these have been carried out independently and are not coordinated in any meaningful way. To help remedy this situation, a framework that presents a consistent set of criteria, universal across initiatives, to evaluate a NAM’s fit-for-purpose was developed by a multi-stakeholder group of industry, academic, and regulatory experts. The goal of this framework is to support greater consistency across existing and future initiatives by providing a structure to collect relevant information to build confidence that will accelerate, facilitate and encourage development of new NAMs that can ultimately be used within the appropriate regulatory contexts. In addition, this framework provides a systematic approach to evaluate the currently-available NAMs and determine their suitability for potential regulatory application. This 3-step evaluation framework along with the demonstrated application with case studies, will help build confidence in the scientific understanding of these methods and their value for chemical assessment and regulatory decision-making.

Food Pathogens
Tracking Bacteriome Variation Over Time in Listeria monocytogenes-positive Foci in Food Industry

Significance: Study of microbial composition in biofilms could be used to improve existing sanitation protocols or for the design of novel strategies.

The variation in microbial composition over time was assessed in biofilms formed in situ on selected non-food and food contact surfaces of meat and fish industries, previously identified as Listeria monocytogenes-positive foci. First, all samples were analysed for the detection and quantification of L. monocytogenes using ISO 11290-1 and ISO 11290-2 norms, respectively. Although the pathogen was initially detected in all samples, direct quantification was not possible. Psychrotrophic bacteria counts were among resident microbiota in meat industry samples (Mean max = 5.85 log CFU/cm²). Visual analysis of the biofilms using epifluorescence microscopy revealed a trend to form microcolonies in which damaged/dead cells would act as anchoring structures. 16S rRNA gene metagenetic analysis demonstrated that, although Proteobacteria (71.37%) initially dominated the bacterial communities at one meat industry location, there was a dramatic shift in composition as the biofilms matured, where Actinobacteria (79.72%) became the major phylum present in later samples. This change was largely due to an increase of Nocardiaceae, Micrococcaceae and Microbacteriaceae. Nevertheless, for the other sampling location, the relative abundance of the dominating phylum (Firmicutes) remained consistent over the entire sampling period (Mean = 63.02%). In fish industry samples, Proteobacteria also initially dominated early on (90.69%) but subsequent sampling showed a higher diversity in which Bacteroidetes and Proteobacteria were the most abundant phyla accounting for the 48.04 and 37.98%, respectively by the last sampling period. Regardless of the location, the community profiles of the endpoint samples were similar to those reported previously. This demonstrated that in a given industrial setting there is a trend to establish a determinate biofilm structure due to the environmental factors and the constant incoming microbiota. This information could be used to improve the existing sanitisation protocols or for the design of novel strategies.

Whole Genome Sequence Analysis of Antimicrobial Resistance Genes, Multilocus Sequence Types and Plasmid Sequences in ESBL/AmpC Escherichia coli Isolated From Broiler Caecum and Meat

Significance: 520 caecal swabs and 85 vacuum-packed broiler meat samples were investigated at the slaughterhouse level. WGS of the bacterial isolates revealed acquired antimicrobial resistance genes, multilocus sequence types, and plasmid sequences.
Plasmid-encoded extended-spectrum β-lactamase and AmpC gene-carrying Escherichia coli (ESBL/AmpC E. coli) is an increasing cause of human infections worldwide. Increasing carbapenem and colistin resistance further complicate treatment of these infections. The aim of this study was to assess the occurrence of ESBL/AmpC E. coli in different broiler flocks and farms, as well as in broiler meat, in a country with no antimicrobial usage in broiler production. An additional goal was to assess the genetic characteristics of ESBL/AmpC E. coli isolates by using whole genome sequencing (WGS). Altogether 520 caecal swabs and 85 vacuum-packed broiler meat samples were investigated at the slaughterhouse level. WGS of the bacterial isolates revealed acquired antimicrobial resistance (AMR) genes, multilocus sequence types (MLST) and plasmid sequences. ESBL/AmpC E. coli was identified in 92 (18%) of the caecum and 27 (32%) of the meat samples. ESBL/AmpC E. coli-carrying birds derived from six (33%) out of 18 farms. Of the two bla(ESBL/AmpC) genes detected by PCR, bla_{CMY-2} (96%) was predominant over bla_{CTX-M-1} (4%). Furthermore, WGS revealed an additional AMR gene sul2. Carbapenemase, colistin, and other AMR genes were not detected from the isolates of either the caecal or meat samples. Altogether seven MLSTs (ST101, ST117, ST212, ST351, ST373, ST1594 and an unknown ST) and a variety of different plasmid sequences (IncB/O/K/Z, IncI1, IncFII, IncII, IncFIB, IncFIC, IncX1 and an additional set of Col-plasmids) were detected. This is the first study on genomic epidemiology of ESBL/AmpC E. coli on broiler farms and flocks with no antimicrobial usage, by using WGS analysis. Results show that ESBL/AmpC E. coli occurrence is common both in the caecum and in the packaged meat. However, compared to other European countries, the occurrence is low and the presence of AMR genes other than bla_{CMY-2} and bla_{CTX-M-1} is rare. More studies are needed to understand the ESBL/AmpC E. coli occurrence in broiler production to prevent the meat from contamination during slaughter and processing, thereby also preventing zoonotic transmission of ESBL/AmpC E. coli. Additionally, more studies are needed to understand the ecology and fitness cost of Enterobacteriaceae plasmids in animal production in order to prevent their acquisition of plasmid-encoded antimicrobial resistance genes such as carbapenem and colistin resistance genes, as this would pose a great hazard to food safety.

### Chemical Contaminants

**A Review on Acrylamide in Food: Occurrence, Toxicity, and Mitigation Strategies**


**Significance:** This review summarizes the occurrence of acrylamide and potential mitigation strategies of its formation in foods.

Acrylamide (AA) is a food contaminant present in a wide range of frequently consumed foods, which makes human exposure to this toxicant unfortunately unavoidable. However, efforts to reduce the formation of AA in food have resulted in some success. This review aims to summarize the occurrence of AA and the potential mitigation strategies of its formation in foods. Formation of AA in foods is mainly linked to Maillard reaction, which is the first feasible route that can be manipulated to reduce AA formation. Furthermore, manipulating processing conditions such as time and temperature of the heating process, and including certain preheating treatments such as soaking and blanching, can further reduce AA formation. Due to the high exposure to AA, recognition of its toxic effect is necessary, especially in developing countries where awareness about AA health risks is still very low. Therefore, this review also focuses on the different toxic effects of AA exposure, including neurotoxicity, genotoxicity, carcinogenicity, reproductive toxicity, hepatotoxicity, and immunotoxicity.

**Serum Levels of Perfluoroalkyl Substances (PFAS) in Adolescents and Young Adults Exposed to Contaminated Drinking Water in the Veneto Region, Italy: A Cross-Sectional Study Based on a Health Surveillance Program**


**Significance:** The major predictors of PFAS serum levels were gender, municipality, duration of residence in the affected area, and number of deliveries.

In spring 2013, groundwater of a vast area of the Veneto Region (northeastern Italy) was found to be contaminated by perfluoroalkyl substances (PFAS) from a PFAS manufacturing plant active since the late 1960s. Residents were exposed to high concentrations of PFAS, particularly perfluorooctanoic acid (PFOA), through drinking water until autumn 2013. A publicly funded health surveillance program is under way to aid in the prevention, early diagnosis, and treatment of chronic disorders possibly associated with PFAS exposure. The objectives of this paper are: a) to describe the organization of the health surveillance program, b) to report serum PFAS concentrations in adolescents and young adults, and c) to identify predictors of serum PFAS concentrations in the studied population. The health surveillance program offered to residents of municipalities supplied by...
contaminated waterworks includes a structured interview, routine blood and urine tests, and measurement of 12 PFAS in serum by high-performance liquid chromatography-tandem mass spectrometry. We studied 18,345 participants born between 1978 and 2002, 14-39 years of age at recruitment. Multivariable linear regression was used to identify sociodemographic, lifestyle, dietary, and reproductive predictors of serum PFAS concentrations. The PFAS with the highest serum concentrations were PFOA [median 44.4 ng/mL, interquartile range (IQR) 19.3-84.9], perfluorohexanesulfonic acid (PFHxS) (median 3.9 ng/mL, IQR 1.9-7.4), and perfluorooctanesulfonic acid (PFOS) (median 3.9 ng/mL, IQR 2.6-5.8). The major predictors of serum levels were gender, municipality, duration of residence in the affected area, and number of deliveries. Overall, the regression models explained 37%, 23%, and 43% of the variance of PFOA, PFOS, and PFHxS, respectively. Serum PFOA concentrations were high relative to concentrations in populations with background residential exposures only. Interindividual variation of serum PFAS levels was partially explained by the considered predictors.

**Food Packaging**

**The Role of Smart Packaging System in Food Supply Chain**

**Significance:** This review highlights how innovative smart packaging solutions can improve the quality and safety of the food supply by enhancing product traceability and reducing the amount of food loss and waste.

Food supply chain is a rapidly growing integrated sector and covers all the aspects from farm to fork, including manufacturing, packaging, distribution, storing, as well as further processing or cooking for consumption. Along this chain, smart packaging could impact the quality, safety, and sustainability of food. Packaging systems have evolved to be smarter with integration of emerging electronics and wireless communication and cloud data solutions. Although there are many factors causing the loss and waste issues for foods throughout the whole supply chain of food and there have been several articles showing the recent advances and breakthroughs in developing smart packaging systems, this review integrates these conceptual frameworks and technological applications and focuses on how innovative smart packaging solutions are beneficial to the overall quality and safety of food supply by enhancing product traceability and reducing the amount of food loss and waste. We start by introducing the concept of the management for the integrated food supply chain, which is critical in tactical and operational components that can enhance product traceability within the entire chain. Then we highlight the impact of smart packaging in reducing food loss and waste. We summarize the basic information of the common printing techniques for smart packaging system (sensor and indicator). Then, we discuss the potential challenges in the manufacturing and deployment of smart packaging systems, as well as their cost-related drawbacks and further steps in food supply chain.

**Heavy Metals**

**A New Method for Evaluating the Bioaccessibility of Different Foodborne Forms of Cadmium**

**Significance:** The toxicity of different cadmium forms commonly found in food was evaluated.

The bioabsorption and biotoxicity of cadmium are closely related to its binding form. Currently, total concentration is used as the indicator for evaluating cadmium toxicity in food, but it might not accurately reflect cadmium’s toxic effects. This study attempted to evaluate the toxicity of the different forms of cadmium including cadmium-malate, cadmium-glutathione, and cadmium-metallothionein that are commonly found in food. The in vitro physiologically based extraction test (PBET) combined with Visual MINTEQ modeling was used to predict the toxicity of different forms of cadmium, and acute toxicity testing was performed in mice for validating their results. The in vivo experimental results showed that different forms of cadmium had diverse biotoxicities of which PBET was a good predictor. In particular, the simulation of cadmium ions in PBET using the MINTEQ software revealed that the free cadmium ion content in the simulated intestinal fluid had a superior linear relationship than the total cadmium concentration with the toxicology indexes. Verification using the other two forms of cadmium confirmed the accuracy of the prediction of their biotoxicity. These findings hopefully provide an important reference for a more accurate and rapid safety assessment of cadmium in food.

**Cadmium: Mitigation Strategies to Reduce Dietary Exposure**

**Significance:** This review highlights current and new mitigation efforts that have the potential to reduce exposures of cadmium throughout the food supply chain.
Cadmium has long been recognized as an environmental contaminant that poses risks to human health. Cadmium is of concern since nearly everyone in the general population is exposed to the metal through the food supply and the ability of the element to accumulate in the body over a lifetime. In support of the United States Food and Drug Administration’s (FDA) Toxic Element Working Group’s efforts to reduce the risks associated with elements in food, this review sought to identify current or new mitigation efforts that have the potential to reduce exposures of cadmium throughout the food supply chain. Cadmium contamination of foods can occur at various stages, including agronomic production, processing, and consumer preparation for consumption. The presence of cadmium in food is variable and dependent on the geographical location, the bioavailability of cadmium from the soil, crop genetics, agronomic practices used, and postharvest operations. Although there are multiple points in the food supply system for foods to be contaminated and mitigations to be applied, a key step to reducing cadmium in the diet is to reduce or prevent initial uptake by plants consumed as food or feed crops. Due to complex interactions of soil chemistry, plant genetics, and agronomic practices, additional research is needed. Support for field-based experimentation and testing is needed to inform risk modeling and to develop practical farm-specific management strategies. This study can also assist the FDA in determining where to focus resources so that research and regulatory efforts can have the greatest impact on reducing cadmium exposures from the food supply. The presence of cadmium in food is highly variable and highly dependent on the geographical location, the bioavailability of cadmium from the soil, crop genetics, and agronomic practices used. This study can assist the FDA in determining where to focus resources so that research and regulatory efforts can have the greatest impact on reducing cadmium exposures from the food supply.

**Food Allergens**

**Mass Cytometry Reveals Cellular Fingerprint Associated With IgE+ Peanut Tolerance and Allergy in Early Life**


**Significance:** High-dimensional mass cytometry analysis is useful in interrogating the cellular interactions that are associated with allergic sensitization and clinical food allergy in the first year of life.

IgE-mediated peanut allergic is common, often serious, and usually lifelong. Not all individuals who produce peanut-specific IgE will react upon consumption of peanut and can eat the food without adverse reactions, known as sensitized tolerance. Here, we employ high-dimensional mass cytometry to define the circulating immune cell signatures associated with sensitized tolerance and clinical allergy to peanut in the first year of life. Key features of clinical peanut allergic are increased frequency of activated B cells (CD19^hi^HLADR^hi^), overproduction of TNFα and increased frequency of peanut-specific memory CD4 T cells. Infants with sensitized tolerance display reduced frequency but hyper-responsive naive CD4 T cells and an increased frequency of plasmacytoid dendritic cells. This work demonstrates the utility and power of high-dimensional mass cytometry analysis to interrogate the cellular interactions that are associated with allergic sensitization and clinical food allergy in the first year of life.

**Critical Reviews and Recent Advances of Novel Non-Thermal Processing Techniques on the Modification of Food Allergens**


**Significance:** Rather than boiling or steaming, several studies have shown that novel processing techniques generally have better performance in retaining original characteristics of food and improving the efficiency of eliminating allergens.

Nowadays, the increasing prevalence of food allergy has become a public concern related to human health worldwide. Thus, it is imperative and necessary to provide some efficient methods for the management of food allergy. Some conventional processing methods (e.g., boiling and steaming) have been applied in the reduction of food immunoreactivity, while these treatments significantly destroy nutritional components present in food sources. Several studies have shown that novel processing techniques generally have better performance in retaining original characteristics of food and improving the efficiency of eliminating allergens. This review has focused on the recent advances of novel non-thermal processing techniques including high-pressure processing, ultrasound, pulsed light, cold plasma, fermentation, pulsed electric field, enzymatic hydrolysis, and the combination processing of them. Meanwhile, general information on global food allergy prevalence and food allergy pathology are also described. Hopefully, these findings regarding the modifications on the food allergens through various novel food processing techniques can provide an in-depth understanding in the mechanism of food allergy, which in turn possibly provides a strategy to adapt in the reduction of food immunoreactivity for the food industries.
Mycotoxins

Effects of Zinc Chelating Nutrients on Aflatoxin Production in Aspergillus flavus

Significance: Natural zinc chelators can be used as harmless aflatoxin-production inhibitors.

Aflatoxins are carcinogenic metabolites produced by Aspergillus and Penicillium spp. Aflatoxin contamination of food is a serious health hazard. Some metal ions (such as Zn\(^{2+}\)) affect Aspergillus growth and aflatoxin biosynthesis. Presence of zinc in the growth medium incites aflatoxin production. This study investigates the effect of zinc binding amino acids and peptides on aflatoxin synthesis in indigenous toxigenic Aspergillus species isolated from agro-ecological zones in Northern Iran. Zinc (II) chelating nutrients (such as Histidine (His), Cysteine (Cys), Histidine-Cysteine (His-Cys), and triple peptide (Asn-Cys-Ser)) were added to the growth medium of toxigenic Aspergillus isolates and incubated at temperature range of 25-40 °C. Aflatoxin production on different culture media was tested using ELISA. Addition of cysteine to Sabouraud dextrose broth (SDB) medium significantly reduced aflatoxin production, which could be related to its zinc chelating property. Aflatoxin production was drastically restrained at high concentration of His, especially in combination with Cys, at high pH values and incubation temperature (pH = 7.5, temperature = 40 °C). Aflatoxin production was low in presence of triple peptide (Asn-Cys-Ser) at concentration of 500 mg/L. From the application perspective, natural zinc chelators can be used as harmless aflatoxin-production inhibitors.

Caffeine

Caffeine as a Tool to Investigate Sarcoplasmic Reticulum and Intracellular Calcium Dynamics in Human Skeletal Muscles

Significance: This review focuses on the mechanism of action of caffeine on sarcoplasmic reticulum and its use to study intracellular calcium dynamics in human muscle.

Caffeine is worldwide used for its power to increase cognitive and physical performance. The ergogenic effects of caffeine, however, do not depend on a direct action on muscles. Actually, the actions of caffeine on skeletal muscles, take place at millimolar concentrations which are far above the micromolar level reached after a regular consumption of coffee or similar drinks, and close to a lethal concentration. At millimolar concentrations caffeine exerts a powerful effect on sarcoplasmic reticulum (SR) activating the release of calcium via ryanodine receptors and, possibly, inhibiting calcium reuptake. For this reason caffeine has become a valuable tool for studying SR function and for diagnostics of SR related muscle disorders. This review aims to briefly describe the effects and the mechanism of action of caffeine on sarcoplasmic reticulum and to focus on its use to study intracellular calcium dynamics in human muscle fibers in physiological and pathological conditions.

Effects of Caffeine on Neuromuscular Function in a Non-Fatigued State and During Fatiguing Exercise

Significance: Caffeine modulates inhibitory mechanisms of the central nervous system, recovery of M-wave amplitude, and perception of effort.

Purpose: Caffeine enhances exercise performance but its mechanisms of action remain unclear. This study investigated its effects on neuromuscular function in a non-fatigued state and during fatiguing exercise. Methods: Eighteen males participated in this randomised, double-blind, placebo-controlled crossover trial. Baseline measures included plantarflexion force, drop jump, squat jump, voluntary activation of triceps surae muscle, soleus muscle contractile properties, M-wave, alpha-motoneuron excitability (H-reflex), corticospinal excitability, short-interval intracortical inhibition (SICI), intracortical facilitation (ICF), silent period evoked by transcranial magnetic stimulation (SP) and plasma potassium and caffeine concentration. Immediately after baseline testing, participants ingested caffeine (6 mg kg\(^{-1}\)) or placebo. After a 1-h rest, baseline measures were repeated, followed by a fatiguing stretch-shortening cycle exercise (sets of 40 bilateral rebound jumps on a sledge apparatus) until task failure. Neuromuscular testing was carried out throughout and after the fatigue protocol. Results: Caffeine enhanced drop jump height (4.2%) and decreased SP (12.6%) in a non-fatigued state. A caffeine-related decrease in SP and SICI prior to the fatiguing activity was associated with an increased time to task failure. The participants who benefited from an improved performance on the caffeine day, reported a significantly lower sense of effort during exercise and had an accelerated post-exercise recovery of M-wave amplitude. Caffeine modulates inhibitory mechanisms of the central nervous system, recovery of M-wave amplitude and perception of effort. This study lays the groundwork for future examinations of differences of caffeine-induced neuromuscular changes between those who are deemed to benefit from caffeine ingestion and those who are not.