Risk Assessment

An Improved Overall Risk Probability-Based Method for Assessing the Combined Health Risks of Chemical Mixtures: An Example about Mixture of Aflatoxin B₁ and Microcystin LR by Dietary Intake


Significance: An improved overall risk probability-based method was developed that makes it possible to estimate the combined effects of chemical mixtures on human health by dietary pathway.

Previous studies on the risk assessment of chemicals with respect to human health have focused mainly on the safety of individual substances. Recently, public health policy emphasizes the combined effects of mixtures. An overall risk probability (ORP)-based method along with the combined toxicity factor (cuv) can be used to evaluate the combined toxicity of chemical mixtures from the environment and foods on human health. However, the procedure for calculating the cuv accurately and quantitatively in the ORP method is yet unclear. In this study, an improved ORP-based method (IORP) was developed by introducing a variable time t, and the cuv was analyzed quantitatively using simultaneous equations and based on the principle of least squares regression. This phenomenon can be explained based on the example of the mixture of aflatoxin B₁ (AFB₁) and microcystin LR (MC-LR) by dietary intake in order to understand the application of this method. The IORP approach makes it possible for estimating the combined effects of mixtures for human health by dietary pathway.

Foodborne Pathogens

Stress Resistance of Emerging Poultry-Associated Salmonella Serovars


Significance: The findings of this study regarding intraspecific variability and the relative resistance of the different Salmonella strains may help to better understand the physiology and ecology of Salmonella and to design better egg preservation strategies.

In recent years, the on-farm prevalence of some poultry-related Salmonella serovars such as S. Kentucky, S. Heidelberg, S. Livingstone and S. Mbandaka has increased significantly, even replacing S. Enteritidis and S. Typhimurium as the most frequently isolated serovars in some production settings and countries. For this reason, the aim of this work was to determine the resistance to several stressing agents and food preservation technologies, in laboratory media and in egg products, of 4 strains of these emerging Salmonella serovars associated to poultry and poultry products and to make comparisons with 4 S. Enteritidis strains. First, the resistance to acid pH, hydrogen peroxide, NaCl, heat, HHP, PEF and UV of the 8 Salmonella strains studied was determined and compared in laboratory media. From this part of the study, it was concluded that variability in resistance to stress among the 8 studied strains varied depending on the investigated agent/technology. However, differences in resistance (2D-values) were always lower than 3.3-fold. Results obtained also indicated that the strains of the emerging serovars studied would display lower acid and NaCl resistance, higher heat resistance and similar oxidative, HHP, PEF and UV resistance than S. Enteritidis. Then, the resistance of these 8 strains was evaluated and compared in egg, egg products and poultry manure. For some agents — including osmotic stresses, UV and PEF — there was a very good correspondence between the results obtained in laboratory media and in real food matrices and poultry manure (r > 0.85; p < 0.01). A significant relationship was also found for acid and...
HHP resistance ($p < 0.05$) and a trend for heat resistance ($p < 0.10$). Therefore, in general terms, conclusions drawn from the study carried out in laboratory media — regarding intraspecific variability and the relative resistance of the different strains — might be extrapolated, although with caution, to real food scenarios. Results obtained in this investigation would help to better understand the physiology and ecology of Salmonella and to design better egg preservation strategies.

**Survival of Listeria monocytogenes during Storage on Dried Apples, Strawberries, and Raisins at 4 °C and 23 °C**


**Significance:** L. monocytogenes is quickly inactivated during storage on raisins and dried strawberries at 23°C, but it is capable of long-term survival at 4°C.

*This research was supported by the ILSI North America Food Microbiology Committee.*

The survival of Listeria monocytogenes was assessed during long-term storage on three dried fruits: dried apples, raisins and dried strawberries. Using sand as a carrier, the dried fruits were dry-inoculated with a four-strain cocktail of L. monocytogenes to achieve numbers of 4.0 to 4.6 log CFU/g. The inoculated foods were stored at 4 °C, 25–81% relative humidity (RH) and 23 °C, 30–35% RH for 336 days. Colonies of L. monocytogenes could not be recovered from the dried apples after inoculation, i.e., day 0. Concentrations of L. monocytogenes decreased rapidly on the raisins and dried strawberries during storage at 23 °C, with enhanced survival observed at 4 °C. Linear rates of decline for populations of L. monocytogenes during storage at 4 °C on the raisins and dried strawberries were 0.1 and 0.2 log CFU/g/month, respectively. The relative distribution of the four L. monocytogenes strains making up the cocktail was determined by multiplex PCR at the beginning of storage and after 336 days on the dried fruits. At day 0, L. monocytogenes populations were predominantly composed of the serotype 1/2a and 3a strains on both the raisins and dried strawberries. After long-term storage at 4 °C, a relative decrease in serotype 1/2a was observed on both fruits, coupled with relative increases in the serotype 3a strain during storage on both fruits, in addition to the serotype 1/2b strain on the raisins. These results demonstrate that L. monocytogenes is rapidly inactivated during storage on raisins and dried strawberries at 23 °C, but it is capable of long-term survival at 4 °C. Improved knowledge on the survival of L. monocytogenes on these commodities is important for predictive modeling and can be used to better inform microbial health risk assessments.

**Foodborne Illness**

**Modeling Invasion of Campylobacter jejuni into Human Small Intestinal Epithelial-Like Cells by Bayesian Inference**


**Significance:** An infection process of C. jejuni, the invasion behavior of the bacteria in intestinal epithelial cells, was revealed, and a predictive mathematical model of the cell-invading pathogen counts was developed for the purpose of providing part of a dose-response model for C. jejuni.

Current approaches used for dose-response modeling of low-dose exposures of pathogens rely on assumptions and extrapolations. These models are important for quantitative microbial risk assessment of food. A mechanistic framework has been advocated as an alternative approach for evaluating dose-response relationships. The objectives of this study were to investigate the invasion behavior of Campylobacter jejuni, which could arise as a foodborne illness even if there are low counts of pathogens, into Caco-2 cells as a model of intestinal cells and to develop a mathematical model for invading cell counts to reveal a part of the infection dose-response mechanism. Monolayer-cultured Caco-2 cells and various concentrations of C. jejuni in culture were cocultured for up to 12 h. The numbers of C. jejuni bacteria invading Caco-2 cells were determined after coculture for different time periods. There appeared to be a maximum limit to the invading bacterial counts, which showed an asymptotic exponential increase. The invading bacterial counts were higher with higher exposure concentrations (maximum, 5.0 log CFU/cm²) than with lower exposure concentrations (minimum, 0.6 log CFU/cm²). In contrast, the ratio of invading bacteria (number of invading bacteria divided by the total number of bacteria exposed) showed a similar trend regardless of the exposure concentration. Invasion of C. jejuni into intestinal cells was successfully demonstrated and described by the developed differential equation model with Bayesian inference. The model accuracy showed that the 99% prediction band covered more than 97% of the observed values. These findings provide important information on mechanistic pathogen dose-response relationships and an alternative approach for dose-response modeling. One of the infection processes of C. jejuni, the invasion behavior of the bacteria in intestinal epithelial cells, was revealed, and a mathematical model for prediction of the cell-invading pathogen counts was developed for the purpose of providing part of a dose-response model for C. jejuni based on the infection mechanism. The developed predictive model showed a high accuracy of more than 97% and successfully described the C. jejuni invading counts. The bacterial invasion predictive model of this study will be essential for the development of a dose-response model for C. jejuni based on the infection mechanism.
**Mycotoxins**

*Mycotoxins in Food: A Review on Liquid Chromatographic Methods Coupled to Mass Spectrometry and Their Experimental Designs*


**Significance:** A first review on experimental design for the development of multi-mycotoxin approaches, this approach could be useful in the development and optimization of LC-MS/MS methods.

The development of a multi-mycotoxins method using LC-MS/MS is necessary and it is clear that the development of such method involves many compromises in the choice of the different parameters. This review summarizes applications using conventional experimental designs and some recent studies using response surface methodology (RSM) as a mathematical modeling tool for the optimization of extraction procedures. The authors also discuss pros and cons of the different procedures. To our knowledge, it is the first review on experimental design for the development of multi-mycotoxin methods. This review could be useful in the development and optimization of LC-MS/MS methods with the aim of describing experimental design and variables (factors) that are likely to affect sensitivity and specificity.

**Food Packaging**

*Nanomaterials for Food Packaging Applications: A Systematic Review*


**Significance:** Although nanotechnology-based food packaging has many advantages for public health, the associated toxicity due to migration—especially in acidic conditions—is considerable.

The application of nanotechnology in food packaging is widely considered during the last two decades. In this regard, numerous studies have been conducted regarding applying nanomaterials such as zinc oxide, clay, silver, carbon nanotube, titanium dioxide, and copper, and copper oxide in food packaging which were summarized in the current study. The employing of nano food packaging increases the physicochemical quality of food (color, flavor, moisture content, weight, bioavailability, and texture) and reduces the microbial load by cell-membrane function, Trojan-horse, and reactive oxygen mechanisms while they improve the barrier/mechanical properties of food packaging. Although nano food packaging has many advantages for public health, the associated toxicity due to migration, especially in acidic conditions, is considerable. Further studies regarding the advantages and disadvantages of this technique are recommended.

**Chemical Contaminants**

*Heavy Metal and Pesticide Levels in Dairy Products: Evaluation of Human Health Risk*


**Significance:** A new methodology was applied to calculate source hazard quotients and an adversity specific hazard index for contaminants in cow’s milk.

Cattle milk’s health benefits can be compromised by the presence of contaminants. The levels of cadmium, copper, lead and zinc, and residues of dichlorodiphenyldichloroethylene (DDE), dichlorodiphenyldichloroethane (DDD), dichlorodiphenyltrichloroethane (DDT) were determined in soil, milk and cheese samples collected from cow farms from 3 Romanian areas with industrial and agriculture tradition. A new methodology was applied for the determination of the corrected estimated daily intake (cEDI) corresponding to the aggregate dietary exposure. For the risk assessment, we calculated the source hazard quotient (HQs) for each contaminant and the adversity specific hazard index (HIA). Cadmium, copper, lead and zinc, and the sum of DDT levels in soil samples were below maximum residue levels (MRLs). The MRLs of lead and DDD were exceeded in milk and cheese samples from all the 3 areas. The MRLs of copper and zinc were exceeded in cheese samples from area 2 and 3. HQs >10 for lead indicates increased risk, while HQ > 1 for copper and sum of DDT indicates moderate risk for both milk and cheese. By calculating the HIA, we identified a moderate and increase risk for nephrotoxicity, hepatotoxicity, hematotoxicity, cardiotoxicity and reproduction toxicity after consumption of the dairy products from the 3 areas.
Heavy Metals

**Associations of Prenatal Methylmercury Exposure and Maternal Polyunsaturated Fatty Acid Status with Neurodevelopmental Outcomes at 7 Years of Age: Results from the Seychelles Child Development Study Nutrition Cohort 2**


**Significance:** Methylmercury in fish poses risks to the developing brain but in this study no significant association between MeHg exposure and polyunsaturated fatty acids status was present.

**Background:** Fish is a primary source of protein and n-3 PUFA but also contains methylmercury (MeHg), a naturally occurring neurotoxicant to which, at sufficient exposure levels, the developing fetal brain is particularly sensitive. **Objectives:** To examine the association between prenatal MeHg and maternal status of n-3 and n-6 PUFA with neurodevelopment, and to determine whether PUFA might modify prenatal MeHg associations with neurodevelopment. **Methods:** We examined the Seychelles Child Development Study Nutrition Cohort 2 (NC2) at age 7 y. We used a sophisticated and extensive neurodevelopmental test battery that addressed 17 specific outcomes in multiple neurodevelopmental domains: cognition, executive and psychomotor function, language development, behavior, scholastic achievement, and social communication. Analyses were undertaken on 1237 mother-child pairs with complete covariate data (after exclusions) and a measure of at least 1 outcome. We examined the main and interactive associations of prenatal MeHg exposure (measured as maternal hair mercury) and prenatal PUFA status (measured in maternal serum at 28 weeks’ gestation) on child neurodevelopmental outcomes using linear regression models. We applied the Bonferroni correction to account for multiple comparisons and considered P values <0.0029 to be statistically significant. **Results:** Prenatal MeHg exposure and maternal DHA and arachidonic acid (20:4n-6) (AA) status were not significantly associated with any neurodevelopmental outcomes. Findings for 4 outcomes encompassing executive function, cognition, and linguistic skills suggested better performance with an increasing maternal n-6:n-3 PUFA ratio (P values ranging from 0.004 to 0.05), but none of these associations were significant after adjusting for multiple comparisons. No significant interaction between MeHg exposure and PUFA status was present. **Conclusions:** Our findings do not support an association between prenatal MeHg exposure or maternal DHA and AA status with neurodevelopmental outcomes at age 7 y. The roles of n-6 and n-3 PUFA in child neurodevelopment need further research.

Allergens

**Allergen Risk Assessment: Food Intake Levels of the General Population Represent Those of Food Allergic Patients**


**Significance:** Food consumption data from the general population can be useful in food allergen risk assessment and will not lead to a relevant under- or overestimation of the risk for the food allergic population.

Unintentional intake of allergens through food products poses a daily risk for allergic patients. Models estimating the risk of reactions mostly use intake data from general population surveys. Our study evaluates the comparability of food intake levels in the general population to those in the food allergic population. Data were collected by a 24-h recall method on 2 non-consecutive days in 38 cow’s milk and/or hen’s egg and 35 peanut and/or tree nut allergic adult patients. All products were assigned to food groups previously developed for allergen risk assessment. Food intake distributions from the allergic populations and a matched sample from the Dutch National Food Consumption Survey were compared, and risk assessments were performed. Food intake data was obtained for 92% of the food groups. Comparison of the intake showed no statistically significant differences between either of the two allergic populations and the general population. Consequently, only small variations in estimated risks were found, that would not result in different risk management decisions. In conclusion, food intake data from the general population can be used for food allergy risk assessment and will not lead to a relevant under- or overestimation of the risk for the food allergic population.

**Evaluating Potential Risks of Food Allergy of Novel Food Sources Based on Comparison of Proteins Predicted from Genomes and Compared to www.AllergenOnline.org**


**Significance:** The findings of this study highlight the need for changes in the allergen databases or methods of identifying matches for risk evaluation of new food sources to improve risk assessment of food allergies.
Potential proteins from three novel food sources (Chlorella variabilis, Galdieria sulphuraria, and Fusarium strain flavolapis) were predicted from genomic sequences and were evaluated for potential risks of allergic cross-reactivity by comparing the predicted amino acid sequences against the allergens in the www.AllergenOnline.org (AOL) database. The preliminary analysis used CODEX Alimentarius limits of >35% identity over 80 amino acids to evaluate the predicted proteins which include many evolutionarily conserved proteins. Regulators might expect clinical serum IgE tests based on identity matches above the criteria if the proteins were introduced in genetically engineered crops. Some regulators have the same expectations for proteins in novel foods. To address the inequality of extensively conserved sequences, we compared the predicted proteins from curated genomes of 23 highly diverse allergenic species from animals, plants and arthropods as well as humans to AOL sequences and compiled identities. Identity matches greater than CODEX limits (>35% ID over 80 AA) are common for many proteins that are conserved through extensive evolution but are not predictive of published allergy risks based on observed taxonomic cross-reactivity. Therefore, we recommend changes in the allergen databases or methods of identifying matches for risk evaluation of new food sources. Our results provide critical data for redefining allergens in AOL or for providing guidance on more predictive sequence identity matches for risk assessment of possible risks of food allergy.

Full Range of Population Eliciting Dose Values for 14 Priority Allergic Foods and Recommendations for Use in Risk Characterization


Significance: Displaying the full range of eliciting dose values for 14 allergenic foods and providing recommendations for their use, this research aids in characterizing risks of concentrations of (unintended) allergenic proteins in food products.

Previously, we published selected Eliciting Dose (ED) values (i.e. ED01 and ED05 values) for 14 allergic foods, predicted to elicit objective allergic symptoms in 1% and 5%, respectively, of the allergic population (Remington et al., 2020). These ED01 and ED05 values were specifically presented and discussed in the context of establishing Reference Doses for allergen management and the calculation of Action Levels for Precautionary Allergen Labeling (PAL). In the current paper, we publish the full range of ED values for these allergenic foods and provide recommendations for their use, specifically in the context of characterizing risks of concentrations of (unintended) allergenic proteins in food products. The data provided in this publication give risk assessors access to full population ED distribution information for 14 priority allergenic foods, based on the largest threshold database worldwide. The ED distributions were established using broad international consensus regarding suitable datapoints and methods for establishing individual patient’s NOAELs and LOAELs and state of the art statistical modelling. Access to these ED data enables risk assessors to use this information for state-of-the-art food allergen risk assessment. This paper contributes to a harmonization of food allergen risk assessment and risk management and PAL practices.