Risk Assessment

Cumulative Dietary Risk Assessment Overarching Different Regulatory Silos Using a Margin of Exposure Approach: A Case Study with Three Chemical Silos


Significance: This case study demonstrates that risk assessment of combined chemical exposures can be performed within regulatory silos.

Risk assessment of chemicals occurring in our diet is commonly performed for single chemicals without considering exposure to other chemicals. We performed a case study on risk assessment of combined dietary exposure to chemicals from different regulatory silos, i.e. pesticides (PPRs), persistent organic pollutants (POPs) and food additives (FAs). Chemicals were grouped into the cumulative assessment group (CAG) liver steatosis using a component-based approach. Based on literature, the CAG included 144 PPRs, 49 POPS and 7 FAs for which concentration data were available. For each silo, chronic combined dietary exposure was assessed for adults and children of nine European countries following the most commonly used exposure methodologies in Europe and by using a relative potency factor approach. For risk characterization, a Margin of Exposure (MOE) was calculated. To overarch the risk across silos, a normalised combined margin of exposure (nMOET) approach was proposed. This case study demonstrated that risk assessment of combined exposure to chemicals can be performed within regulatory silos. It also highlighted important differences in the conservatism of exposure scenarios, the derivation of point of departures and the subsequent acceptable MOEs between the silos. To overarch the risk despite these differences, a nMOET approach can be used.

A Generic PBTK Model Implemented in the MCRA Platform: Predictive Performance and Uses in Risk Assessment of Chemicals


Significance: The implementation and use of a physiologically-based toxicokinetic model in the MCRA risk assessment platform is discussed.

Physiologically-based toxicokinetic (PBTK) models are important tools for in vitro to in vivo or inter-species extrapolations in health risk assessment of foodborne and non-foodborne chemicals. Here we present a generic PBTK model implemented in the EuroMix toolbox, MCRA 9 and predict internal kinetics of nine chemicals (three endocrine disrupters, three liver steatosis inducers, and three developmental toxicants), in data-rich and data-poor conditions, when increasingly complex levels of parametrization are applied. At the first stage, only QSAR models were used to determine substance-specific parameters, then some parameter values were refined by estimates from substance-specific or high-throughput in vitro experiments. At the last stage, elimination or absorption parameters were calibrated based on available in vivo kinetic data. The results illustrate that parametrization plays a capital role in the output of the PBTK model, as it can change how chemicals are prioritized based on internal concentration factors. In data-poor situations, estimates can be far from observed values. In many cases of chronic exposure, the PBTK model can be summarized by an external to internal dose factor, and interspecies concentration factors can be used to perform interspecies extrapolation. We finally discuss the implementation and use of the model in the MCRA risk assessment platform.
Foodborne Pathogens

A Toxic Environment: A Growing Understanding of How Microbial Communities Affect Shiga Toxin Expression by *E. coli* O157:H7


**Significance:** Characterizing Shiga toxin-amplifying microbial interactions will improve understanding of *E. coli* O157:H7 infections and elucidate the intricate regulation of pathogenicity.

Enterohemorrhagic *E. coli* (EHEC), including *E. coli* O157:H7, cause severe illness in humans due to production of Shiga toxin (Stx) and other virulence factors. Because Stx is coregulated with lambdoid prophage induction, its expression is especially susceptible to environmental cues. Infections with Stx-producing *E. coli* can be difficult to model due to the wide range of disease outcomes—some infections are relatively mild, while others have serious complications. Probiotic organisms, members of the gut microbiome, and organic acids can depress Stx production, in many cases by inhibiting the growth of EHEC strains. On the other hand, the factors currently known to amplify Stx act via their effect on the stx-converting phage. Here, we characterize two interactive mechanisms that increase Stx production by O157:H7 strains: first, direct interactions with phage-susceptible *E. coli*; and second, indirect amplification by secreted factors. Infection of susceptible strains by the stx-converting phage can expand the Stx-producing population in a human or animal host, and phage infection has been shown to modulate virulence *in vitro* and *in vivo*. Acellular factors, particularly colicins and microcins, can kill O157:H7 cells but may also trigger Stx expression in the process. Colicins, microcins, and other bacteriocins have diverse cellular targets, and many such molecules remain characterized. Identification of additional Stx-amplifying microbial interactions will improve our understanding of *E. coli* O157:H7 infections and help elucidate the intricate regulation of pathogenicity in EHEC strains.

Can Abundance of Sensory Proteins Distinguish between Pathogenic and Non-Pathogenic Bacteria?


**Significance:** A new metric termed the ‘Sensory Protein Index’ was found to be correlated to *E. coli* virulence.

Signal Transduction Systems are essential for microorganisms to respond to their ever changing environment. It can be distinguished into one-component systems, two-component system, and extracytoplasmic-function or factors. Abundances of a few signal-transducing proteins, termed herein as Sensory Proteins (SPs), have previously been reported to be correlated to the genome size and the ecological niche of the certain gram-positive bacteria. No such reports are available for gram-negative bacteria. The current study tries to investigate the relationship of the abundances of SPs to genome size in *Escherichia coli*, and the bacterial patho- or phylo-types. While the relationship between SP abundance and genome size could not be established, the ‘Sensory Protein Index’ (SPI), a new metric defined herein, was found to be correlated to *E. coli* virulence. In addition, significant association among the distribution of SPs and *E. coli* pathotypes were observed. Results indicated that such associations might be due to genomic re-arrangements to best utilize the resources available in a given ecological niche. Overall, the study provides an in-depth analysis of the occurrence of different SPs among pathogenic and non-pathogenic *E. coli*. Possibilities of using the SPI as a marker of identifying pathogenic strains from amongst an organism complex have also been discussed.

Changes in Salmonella Contamination in Meat and Poultry Since the Introduction of the Pathogen Reduction; Hazard Analysis and Critical Control Point Rule


**Significance:** An overall reduction of *Salmonella* on meat and poultry products has been observed since the implementation of the HACCP rule, but the magnitude and direction of change have not been consistent over time or across commodities.

In 1996, the Food Safety and Inspection Service published its Pathogen Reduction; Hazard Analysis and Critical Control Point (PR;HACCP) rule. The intention of this program was to reduce microbial contamination on meat, poultry, and egg products. The program was implemented in stages between January 1998 and January 2000, with sampling for *Escherichia coli* (*E. coli*) O157:H7 and/or *Salmonella* in large production establishments beginning in 1998. As the PR;HACCP program begins its third decade, it is reasonable to question whether there have been reductions in the frequency of pathogen-contaminated meat and poultry products reaching consumers. This study summarizes the results for over 650,000 samples collected by the Food Safety and Inspection Service between 2000 and 2018 in slaughter and processing establishments across the United States and compares these results to the roughly 100,000 retail samples collected by the Food and Drug Administration between 2002 and 2017. The data demonstrate that there has been an overall reduction in the occurrence of *Salmonella* on meat and poultry products, but the direction and magnitude of change has not been consistent over time or across commodities. While the available data do not support the identification of causal factors for the observed changes, a historical review of the timing of various factors and policy decisions generates potential hypotheses for the observed changes.
Detection and Prevalence of *Listeria* in US Produce Packinghouses and Fresh-Cut Facilities


**Significance:** An environmental “routine sampling” plan was implemented to collect information on the detection, prevalence and distribution of *L. monocytogenes* in packinghouses and fresh-cut facilities.

*Listeria monocytogenes* (LM) contamination of produce can often be traced back to the environment of packinghouses and fresh-cut facilities. As there is limited information on the detection, prevalence, and distribution of this pathogen in produce operations, environmental “routine sampling” plans for LM and other *Listeria* spp. were developed and implemented in three packinghouses and five fresh-cut facilities in the US. For “routine sampling,” a total of 2,014 sponge samples were collected over 6-8 separate samplings per operation, performed over one year; vector and pre-production samples (n=156) were also collected as needed to follow up on positive findings. In addition, a single “validation sampling” visit by an outside expert was used to evaluate the routine sampling. Among the 2,014 routine sponge samples collected, 35 and 30 were positive for LM and *Listeria* species other than LM (“LS”), respectively. LM prevalence varied from 0.8% to 5.8% for packinghouses and <0.4% to 1.6% for fresh-cut facilities. Among the 394 validation sponge samples, 23 and 13 were positive for LM and LS, respectively. Validation sampling found statistically significantly higher LM prevalence compared to routine sampling for 3/8 operations. For all samples collected, up to eight isolates per sample were characterized by sequencing of sigB, which allowed for classification into sigB allelic types. Among the 97 samples with more than one *Listeria* isolate characterized, 28 had more than one sigB allelic type present, including 18 sponges that were positive for LM and another *Listeria* spp and 13 sponges that were positive for more than one LM subtype. This indicates that collection of multiple isolates is necessary to capture *Listeria* diversity present in produce operations. Additionally, 17/77 sponges that were positive for LM were positive at only one enrichment time (i.e., 24 or 48h), indicating that LM testing after two different enrichment times provides enhanced sensitivity.

Inactivation of Shiga Toxin-Producing *Escherichia coli* and *Listeria monocytogenes* within Plant versus Beef Burgers in Response to High Pressure Processing


**Significance:** These data suggest that time and pressure level validated for control of *L. monocytogenes* and STEC in ground beef are likely to be equally effective towards these same pathogens in plant-based burgers.

We evaluated high pressure processing to lower levels of Shiga toxin–producing *Escherichia coli* (STEC) and *Listeria monocytogenes* inoculated into samples of plant or beef burgers. Multistrain cocktails of STEC and *L. monocytogenes* were separately inoculated (~7.0 log CFU/g) into plant burgers or ground beef. Refrigerated (i.e., 4°C) or frozen (i.e., −20°C) samples (25 g each) were subsequently exposed to 350 MPa for up to 9 or 18 min or 600 MPa for up to 4.5 or 12 min. When refrigerated plant or beef burger samples were treated at 350 MPa for up to 9 min, levels of STEC were reduced by ca. 0.7 to 1.3 log CFU/g. However, when refrigerated plant or beef burger samples were treated at 350 MPa for up to 9 min, levels of *L. monocytogenes* remained relatively unchanged (ca. ≤0.3-log CFU/g decrease) in plant burger samples but were reduced by ca. 0.3 to 2.0 log CFU/g in ground beef. When refrigerated plant or beef burger samples were treated at 600 MPa for up to 4.5 min, levels of STEC and *L. monocytogenes* were reduced by ca. 0.7 to 4.1 and ca. 0.3 to 5.6 log CFU/g, respectively. Similarly, when frozen plant and beef burger samples were treated at 350 MPa up to 18 min, reductions of ca. 1.7 to 3.6 and ca. 0.6 to 3.6 log CFU/g in STEC and *L. monocytogenes* numbers, respectively, were observed. Exposure of frozen plant or beef burger samples to 600 MPa for up to 12 min resulted in reductions of ca. 2.4 to 4.4 and ca. 1.8 to 3.4 log CFU/g in levels of STEC and *L. monocytogenes*, respectively. Via empirical observation, pressurization did not adversely affect the color of plant burger samples, whereas appreciable changes in color were observed in pressurized ground beef. These data confirm that time and pressure levels already validated for control of STEC and *L. monocytogenes* in ground beef will likely be equally effective towards these same pathogens in plant burgers without causing untoward effects on product color.

**Foodborne Illness**

Assessment of the Risk of Salmonellosis Linked to the Consumption of Liquid Egg Products Made from Internally Contaminated Shell Eggs Initially Stored at 65°F (18°C) Compared with Eggs Stored at 45°F (7°C)


**Significance:** The diversion of eggs from broiler production to human consumption under the current storage practices of 65°F (versus 45°F) would present a substantial overall increase in the risk of salmonellosis.
According to the U.S. Food and Drug Administration’s (FDA’s) rule on “Prevention of Salmonella Enteritidis in Shell Eggs during Production, Storage, and Transportation,” shell eggs intended for human consumption are required to be held or transported at or below 45°F (7.2°C) ambient temperature beginning 36 h after time of lay. Meanwhile, eggs in hatcheries are typically stored at a temperature of 65°F (18.3°C). Although most of those eggs are directed to incubators for hatching, excess eggs have the potential to be diverted for human consumption as egg products through the “breaker” market if these eggs are refrigerated in accordance with FDA’s requirement. Combining risk assessment models developed by the U.S. Department of Agriculture’s Food Safety and Inspection Service for shell eggs and for egg products, we quantified and compared Salmonella Enteritidis levels in eggs held at 65°F versus 45°F, Salmonella Enteritidis levels in the resulting egg products, and the risk of human salmonellosis from consumption of those egg products. For eggs stored 5 days at 65°F (following 36 h at 75°F [23.9°C] in the layer house), the mean level of Salmonella Enteritidis contamination is 30-fold higher than for eggs stored at 45°F. These increased levels of contamination lead to a 47-fold increase in the risk of salmonellosis from consumption of egg products made from these eggs, with some variation in the public health risk on the basis of the egg product type (e.g., whole egg versus whole egg with added sugar). Assuming that 7% of the liquid egg product supply originates from eggs stored at 65°F versus 45°F, this study estimates an additional burden of 3,562 cases of salmonellosis per year in the United States. A nominal range uncertainty analysis suggests that the relative increase in the risk linked to the storage of eggs at higher temperature estimated in this study is robust to the uncertainty surrounding the model parameters. The diversion of eggs from broiler production to human consumption under the current storage practices of 65°F (versus 45°F) would present a substantive overall increase in the risk of salmonellosis.

**Mycotoxins**

**Changes in Wheat Nutritional Content at Elevated [CO2] Alter Fusarium graminearum Growth and Mycotoxin Production on Grain**


**Significance:** Rising atmospheric [CO2] may result in increased mycotoxin production and reduce the nutritional content of wheat.

Rising atmospheric [CO2] has been shown to impact plant primary metabolism and the severity of Fusarium head blight (FHB) in wheat. In this study, we evaluated how changes in grain nutritional content due to growth at elevated [CO2] affected Fusarium graminearum growth and mycotoxin production. Susceptible (Norm) and moderately resistant (Alsen) hard spring wheat grains that had been grown at ambient (400 ppm) or elevated [CO2] (800 ppm) were independently inoculated with two F. graminearum fungal strains, which produce the trichothecene mycotoxin, deoxynivalenol. Under higher [CO2], FHB-susceptible and moderately resistant wheat had disproportionate losses in protein and mineral contents, with Alsen being more severely impacted. Furthermore, the F. graminearum strain 9F1 had increased mycotoxin biosynthesis in response to the loss of wheat nutritional content in Alsen. Our results demonstrate that future [CO2] conditions may provide a strain-specific pathogenic advantage on hosts, with greater losses in nutritional content.

**Food Packaging**

**Migration of Volatile Compounds from Natural Biomaterials and Their Safety Evaluation as Food Contact Materials**


**Significance:** Migration of compounds from wheat pulp and wood were found to be lower than existing specific migration limits, and their use may thereby be suitable as food contact materials.

The concern for environmental conservation is increasing, and a very important factor to consider is the search for alternatives to the use of plastics in the food packaging industry. A good option is the manufacture of containers of biodegradable materials, such as the so-called biomaterials made of vegetable fibre such as wheat, wood, bamboo or palm leaf pulp. The migration of compounds from food packaging can cause alterations in food safety and acceptability. Therefore, their control through studies of specific migration is definitely important in the food industry. Specific migration has been studied in two types of dishes (wheat pulp and wood) in contact with three liquid simulants (ethanol 10%, acetic acid 3% and ethanol 95%). The analysis of migration extracts have been carried out by solid-phase microextraction coupled to gas chromatography (SPME-GC-MS) in the most suitable working conditions. In addition, those identified compounds considered of interest according to existing legislation have been quantified in order to assess whether exceed or not the migration limits established for some of them. The results obtained show that the quantified compounds are well below the specific migration limits (SML) set by the legislation, thereby showing the safety in use of this type of biodegradable dishes.
Chemical Contaminants

Early Life Exposure to Perfluoroalkyl Substances (PFAS) and ADHD: A Meta-Analysis of Nine European Population-Based Studies


Significance: Early life exposure to PFOS and PFOA was not associated with a higher incidence of attention-deficit/hyperactivity disorder (ADHD) in the overall analysis, but increased prevalence of ADHD in association with PFAS exposure was found in specific subgroups.

Introduction: To date, the evidence for an association between perfluoroalkyl substances (PFAS) exposure and attention deficit and hyperactivity disorder (ADHD) is inconclusive. Objective: We investigated the association between early life exposure to perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA), and ADHD in a collaborative study including nine European population-based studies, encompassing 4,826 mother–child pairs. Methods: Concentrations of PFOS and PFOA were measured in maternal serum/plasma during pregnancy, or in breast milk, with different timing of sample collection in each cohort. We used a validated pharmacokinetic model of pregnancy and lactation to estimate concentrations of PFOS and PFOA in children at birth and at 3, 6, 12, and 24 months of age. We classified ADHD using recommended cutoff points for each instrument used to derive symptoms scores. We used multiple imputation for missing covariates, logistic regression to model the association between PFAS exposure and ADHD in each study and combined all adjusted study-specific effect estimates using random-effects meta-analysis. Results: A total of 399 children were classified as having ADHD, with a prevalence ranging from 2.3% to 7.3% in the studies. Early life exposure to PFOS or PFOA was not associated with ADHD during childhood [odds ratios (ORs) ranging from 0.96 (95% CI: 0.87, 1.06) to 1.02 (95% CI: 0.93, 1.11)]. Results from stratified models suggest potential differential effects of PFAS related to child sex and maternal education. Conclusion: We did not identify an increased prevalence of ADHD in association with early life exposure to PFOS and PFOA. However, stratified analyses suggest that there may be an increased prevalence of ADHD in association with PFAS exposure in girls, in children from nulliparous women, and in children from low-educated mothers, all of which warrant further exploration.

Chemical Food Contaminants during Food Processing: Sources and Control


Significance: A comprehensive overview of known chemical food contaminants during food processing is provided.

With the development in international food trade, there has been emerging risks in the food chain. Food contamination can be caused by several factors in a complex food chain. This article provides a comprehensive review of known chemical contaminants from the production of raw materials to the consumption of food products as well as prevention and control measures. Specifically, this review discusses the following topics, raw material contamination caused by environmental pollution, endogenous food contamination caused by processing methods, and cold chain system challenges in food e-commerce.

Heavy Metals

Dietary Exposure to Total and Inorganic Arsenic via Rice and Rice-Based Products Consumption


Significance: Concentrations of total and inorganic arsenic in rice and rice products are presented, along with dietary exposure estimates for infant and adult populations.

Diet is the major route of exposure to arsenic (As), with rice and rice products as food groups with relatively high As levels. This study was aimed at determining the concentrations of total arsenic (total As) and inorganic arsenic (InAs) in rice and rice products. The dietary exposure and health risks for infant and adult population were also estimated. Brown varieties of rice showed higher As levels than white rice (189 vs 132 μg/kg). Toddlers and infants presented the highest dietary exposure to total As (4.08 and 3.99 μg/day, respectively), but unlike the rest of population groups, the main contributor was organic arsenic. Focusing on the contribution of each food item, rice represents the major contributor to InAs exposure by the adult population, while baby cereals and breakfast cereals are the most important contributors for infant exposure. Anyhow, none of the population groups exceeded...
the lower limit of the BMDL\textsubscript{01} range (from 0.3 to 8.0 μg/kg body weight/day) set by EFSA in any of the three exposure scenarios (high, mean, and low) hereby considered. Finally, consumption of white rice varieties or pre-cooked rice, as well as washing rice before cooking, are recommended in order to minimize the exposure to arsenic.

**Caffeine**

**Metabolites, Nutrients, and Lifestyle Factors in Relation to Coffee Consumption: An Environment-Wide Association Study**


**Significance:** Analysis of the Third NHANES found that regular coffee and carbonated beverage consumption correlated with smoking, serum lead levels, and poorer dietary habits.

Coffee consumption has been inversely associated with various diseases; however, the underlying mechanisms are not entirely clear. We used data of 17,752 Third National Health and Nutrition Examination Survey participants to investigate the association of 245 metabolites, nutrients, and lifestyle factors with coffee consumption. We used data from the first phase (n = 8825) to identify factors with a false discovery rate of <5%. We then replicated our results using data from the second phase (n = 8927). Regular coffee consumption was positively associated with active and passive smoking, serum lead and urinary cadmium concentrations, dietary intake of potassium and magnesium, and aspirin intake. In contrast, regular coffee consumption was inversely associated with serum folate and red blood cell folate levels, serum vitamin E and C, and beta-cryptoxanthin concentrations, Healthy Eating Index score, and total serum bilirubin. Most of the aforementioned associations were also observed for caffeinated beverage intake. In our assessment of the association between coffee consumption and selected metabolites, nutrients, and lifestyle factors, we observed that regular coffee and caffeinated beverage consumption was strongly associated with smoking, serum lead levels, and poorer dietary habits.

**Food Allergens**

**Epitopes with Similar Physicochemical Properties Contribute to Cross Reactivity between Peanut and Tree Nuts**


**Significance:** Cross-reactivity to peanuts and tree nuts may result from repeats of shared peptide sequences.

Many individuals with peanut (PN) allergy have severe reactions to tree nuts (TN) such as walnuts or cashews. Although allergenic proteins in TN and PN have overall low identity, they share discrete sequences similar in physicochemical properties (PCP) to known IgE epitopes. Here, PCP-consensus peptides (cp, 13 aa and 31 aa) were identified from an alignment of epitope rich regions of walnut vicilin, Jug r 2, leader sequence (J2LS) and cross-reactive epitopes in the 2S albumins of peanut and synthesized. A peptide similarity search in the Structural Database of Allergenic Proteins (SDAP) revealed a network of peptides similar (low property distance, PD) to the 13 aa cp (13cp) in many different plant allergens. Peptides similar to the 13cp in PN and TN allergens bound IgE from sera of patients allergic to PN and TN in peptide microarray analysis. The 13cp was used to produce a rabbit consensus peptide antibody (cpAB) that detected proteins containing repeats similar to the 13cp in western blots of various nut extracts, in which reactive proteins were identified by mass spectrometry. The cpAB bound more specifically to allergens and nut extracts containing multiple repeats similar to the 13 cp, such as almond (Pru du 6), peanut (Ara h 2) and walnut (Jug r 2). IgE binding to various nut extracts is inhibited by recombinant J2LS sequence and synthetic 31cp. Thus, several repeated sequences similar to the 13cp are bound by IgE. Multiple similar repeats in several allergens could account for reaction severity and clinically relevant cross-reactivity to PN and TN. These findings may help improve detection, diagnostic, and therapeutic tools.