Risk Assessment

Incorporating New Approach Methodologies in Toxicity Testing and Exposure Assessment for Tiered Risk Assessment Using the RISK21 Approach: Case Studies on Food Contact Chemicals


Significance: This study uses case studies of two indirect food additive chemicals to highlight the potential utility of the RISK21 approach for interpretation of the ToxCast high-throughput screening data.

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Programs including the ToxCast project have generated large amounts of in vitro high-throughput screening (HTS) data, and best approaches for the interpretation and use of HTS data, including for chemical safety assessment, remain to be evaluated. To fill this gap, we conducted case studies of two indirect food additive chemicals where ToxCast data were compared with in vivo toxicity data using the RISK21 approach. Two food contact substances, sodium (2-pyridylthio)-N-oxide and dibutyltin dichloride, were selected, and available exposure data, toxicity data, and model predictions were compiled and assessed. Oral equivalent doses for the ToxCast bioactivity data were determined by in-vitro in-vivo extrapolation (IVIVE). For sodium (2-pyridylthio)-N-oxide, bioactive concentrations in ToxCast assays corresponded to low- and no-observed adverse effect levels in animal studies. For dibutyltin dichloride, the ToxCast bioactive concentrations were below the dose range that demonstrated toxicity in animals; however, this was confounded by the lack of toxicokinetic data, necessitating the use of conservative toxicokinetic parameter estimates for IVIVE calculations. This study highlights the potential utility of the RISK21 approach for interpretation of the ToxCast HTS data, as well as the challenges involved in integrating in vitro HTS data into safety assessments.

Modified Reference Point Index (mRPI) and a Decision Tree for Deriving Uncertainty Factors: A Practical Approach to Cumulative Risk Assessment of Food Contaminant Mixtures


Significance: A proposed modified Reference Point Index combines the advantages of the Hazard Index and the Reference Point Index and is presented with a decision tree for the determination of specific uncertainty factors.

Risk assessment of chemical mixtures remains a challenging task in all areas of food and consumer safety. So far, no general method has been developed that is best suited to several subject areas (e.g. food contaminants, additives and supplements, plant protection products). Especially for mixtures of food contaminants sophisticated methods are typically not applicable due to a general lack of complete toxicological data sets. We developed a new approach, the modified Reference Point Index (mRPI), that combines the advantages of the Hazard Index and the Reference Point Index. Furthermore, we developed a decision tree for the determination of specific uncertainty factors that makes the mRPI an easy to use method for cumulative risk assessment even in a data poor field such as food contaminants. To further characterise the estimated cumulative risks, the Maximum Cumulative Ratio (MCR) was adapted to be applied on the mRPI, and the modified Maximum Cumulative Ratio (mMCR) was established to identify whether the risks are dominated by a single substance. We present two case studies assessing the nephrotoxic and neurotoxic risks for the Austrian population originating from food contaminant mixtures. Calculations could not rule out potential cumulative risks, yet, they seemed to be dominated by single substances.
**Foodborne Pathogens**

**A Hybrid Sub-Lineage of Listeria monocytogenes Comprising Hypervirulent Isolates**


**Significance:** This work characterizes a hypervirulent sub-lineage of *Listeria monocytogenes*.

The foodborne pathogen *Listeria monocytogenes* (Lm) is a highly heterogeneous species and currently comprises of 4 evolutionarily distinct lineages. Here, we characterize isolates from severe ovine listeriosis outbreaks that represent a hybrid sub-lineage of the major lineage II (HSL-II) and serotype 4h. HSL-II isolates are highly virulent and exhibit higher organ colonization capacities than well-characterized hypervirulent strains of Lm in an orogastric mouse infection model. The isolates harbour both the Lm Pathogenicity Island (LIPI)-1 and a truncated LIPI-2 locus, encoding sphingomyelinase (SmcL), a virulence factor required for invasion and bacterial translocation from the gut, and other non-contiguous chromosomal segments from another pathogenic species, *L. ivanovii*. HSL-II isolates exhibit a unique wall teichoic acid (WTA) structure essential for resistance to antimicrobial peptides, bacterial invasion and virulence. The discovery of isolates harbouring pan-species virulence genes of the genus *Listeria* warrants global efforts to identify further hypervirulent lineages of Lm.

**SeqSero2: Rapid and Improved Salmonella Serotype Determination Using Whole Genome Sequencing Data**


**Significance:** Several updates to SeqSero software are presented for rapid Salmonella serotype determination from whole genome sequencing data.

SeqSero, launched in 2015, is a software tool for *Salmonella* serotype determination from whole genome sequencing (WGS) data. Despite its routine use in public health and food safety laboratories in the United States and other countries, the original SeqSero pipeline is relatively slow (minutes per genome using sequencing reads), is not optimized for draft genome assemblies, and may assign multiple serotypes for a strain. Here we present SeqSero2 (github.com/denglab/SeqSero2; denglab.info/SeqSero2), an algorithmic transformation and functional update of the original SeqSero. Major improvements include: 1) additional sequence markers for identification of *Salmonella* species and subspecies and certain serotypes; 2) a k-mer based algorithm for rapid serotype prediction from raw reads (seconds per genome) and improved serotype prediction from assemblies; and 3) a targeted assembly approach for specific retrieval of serotype determinants from WGS for serotype prediction, new allele discovery, and prediction troubleshooting. Evaluated using 5,794 genomes representing 364 common US serotypes, including 2,280 human isolates of 117 serotypes from the National Antimicrobial Resistance Monitoring System, SeqSero2 is up to 50 times faster than the original SeqSero while maintaining equivalent accuracy for raw reads and substantially improving accuracy for assemblies. SeqSero2 further suggested that 3% of the tested genomes contained reads from multiple serotypes, indicating a use for contamination detection. In addition to short reads, SeqSero2 demonstrated potential for accurate and rapid serotype prediction directly from long nanopore reads despite base call errors. Testing of 40 nanopore-sequenced genomes of 17 serotypes yielded a single H antigen misidentification. **Importance:** Serotyping is the basis of public health surveillance of *Salmonella*. It remains a first-line subtyping method even as surveillance continues to be transformed by whole genome sequencing. SeqSero allows the integration of *Salmonella* serotyping into a whole genome sequencing-based laboratory workflow while maintaining continuity with the classic serotyping scheme. SeqSero2, informed by extensive testing and application of SeqSero in the United States and other countries, incorporates important improvements and updates that further strengthen its application in routine and large scale surveillance of *Salmonella* by whole genome sequencing.

**Mycotoxins**

**Worldwide Contamination of Food-Crops With Mycotoxins: Validity of the Widely Cited ‘FAO Estimate’ of 25**


**Significance:** This study confirms the Food and Agriculture Organization’s (FAO) estimate of global food crop contamination with mycotoxins above European Union and Codex limits, but reports that FAO’s figure greatly underestimates the occurrence above the detectable levels.
Prior to 1985 the Food and Agriculture Organization (FAO) estimated global food crop contamination with mycotoxins to be 25%. The origin of this statement is largely unknown. To assess the rationale for it, the relevant literature was reviewed and data of around 500,000 analyses from the European Food Safety Authority and large global survey for aflatoxins, fumonisins, deoxynivalenol, T-2 and HT-2 toxins, zearalenone and ochratoxin A in cereals and nuts were examined. Using different thresholds, i.e. limit of detection, the lower and upper regulatory limits of European Union (EU) legislation and Codex Alimentarius standards, the mycotoxin occurrence was estimated. Impact of different aspects on uncertainty of the occurrence estimates presented in literature and related to our results are critically discussed. Current mycotoxin occurrence above the EU and Codex limits appears to confirm the FAO 25% estimate, while this figure greatly underestimates the occurrence above the detectable levels (up to 60–80%). The high occurrence is likely explained by a combination of the improved sensitivity of analytical methods and impact of climate change. It is of immense importance that the detectable levels are not overlooked as through diets, humans are exposed to mycotoxin mixtures which can induce combined adverse health effects.

**Food Packaging**

**Recent Advances and Challenges on Applications of Nanotechnology in Food Packaging. A Literature Review**


**Significance:** This review highlights recent advancements in the application of engineered nanoparticles (ENPs) in food and beverage packaging, including current demands for risk assessment strategies associated with the use of ENPs in food contact materials.

Nanotechnology applied to food and beverage packaging has created enormous interest in recent years, but in the same time there are many controversial issues surrounding nanotechnology and food. The benefits of engineered nanoparticles (ENPs) in food-contact applications are accompanied by safety concerns due to gaps in understanding of their possible toxicology. In case of incorporation in food contact polymers, the first step to consumer exposure is the transfer of ENPs from the polymer to the food. Hence, to improve understanding of risk and benefit, the key questions are whether nanoparticles can be released from food contact polymers and under which conditions. This review has two main goals. Firstly, it will present the current advancements in the application of ENPs in food and beverage packaging sector to grant active and intelligent properties. A particular focus will be placed on current demands in terms of risk assessment strategies associated with the use of ENPs in food contact materials (FCMs), i.e. up-to-date migration/cytotoxicity studies of ENPs which are partly contradictory. Food matrix effects are often ignored, and may have a pronounced impact on the behaviour of ENPs in the gastrointestinal tract (GIT). A standardized food model (SFM) for evaluating the toxicity and fate of ingested ENPs was recently proposed and herein discussed with the aims to offer an overview to the reader. It is therefore clear that further systematic research is needed, which must account for interactions and transformations of ENMs in foods (food matrix effect) and in the gastrointestinal tract (GIT) that are likely to determine nano-biointeractions. Secondly, the review provides an extensive analysis of present market dynamics on ENPs in food/beverage packaging moving beyond concept to current industrial applications.

**Caffeine**

**Bioassay for Determining the Concentrations of Caffeine and Individual Methylxanthines in Complex Samples**


**Significance:** A bioassay is presented that links *Escherichia coli* growth to methylxanthine demethylation to determine the amounts of individual methylxanthines in complex mixtures or beverages.

Caffeine and other methylxanthines are stimulant molecules found in formulated beverages, including sodas and energy drinks, and in brewed beverages, such as coffee and teas. Previously, we developed a bioassay for caffeine that involves monitoring the growth of a ΔguaB mutant of *E. coli* defective in *de novo* guanine biosynthesis. When supplemented with a plasmid expressing the genes for an N-demethylation pathway from *Pseudomonas putida* CBB5, these bacteria demethylate caffeine (1,3,7-trimethylxanthine) and other methylxanthines into xanthine, which is then converted into guanine to support cell growth. A major limitation of this bioassay was that it could only measure the total concentration of all methylxanthines in a mixture. Therefore, it could not be used to measure the caffeine content of beverages like teas, which contain substantial quantities of multiple methylxanthines. To overcome this limitation, we created seven new plasmids containing all subsets of the three demethylase genes (ndmA, ndmB, and ndmC). We show that strains of ΔguaB *E. coli* containing each plasmid are able to demethylate specific subsets of methylxanthines and that they can be used to determine the concentrations of individual methylxanthines in complex mixtures containing multiple methylxanthines, including coffee doped with additional methylxanthine. While validating this assay, we discovered an unexpected demethylation event at the 1-methyl position when NdmB and NdmC were expressed in the absence of NdmA. The improved cell-based bioassay is cheap, easy to use, and gives results comparable to standard
HPLC methods for measuring methylxanthine concentrations. **Importance:** Caffeine (1,3,7-trimethylxanthine) is the dominant neurostimulant found in coffee, teas, sodas, and energy drinks. Measuring the amount of caffeine and other methylxanthines in these beverages is important for quality assurance and safety in food science. Methylxanthines are also used in medicine and as performance-enhancing drugs, two contexts in which accurately determining their concentrations in bodily fluids is important. Liquid chromatography is the standard method for measuring methylxanthine concentrations in a sample, but it requires specialized equipment and expertise. We improved a previous bioassay that links *E. coli* growth to methylxanthine demethylation so that it can now be used to determine the amounts of individual methylxanthines in complex mixtures or beverages, such as coffee.

**Food Allergens**

**Sustained Outcomes in Oral Immunotherapy for Peanut Allergy (POISED Study): A Large, Randomised, Double-Blind, Placebo-Controlled, Phase 2 Study**


**Significance:** Continuation or reduction of oral immunotherapy may increase the likelihood of regaining clinical reactivity to peanut.

**Background:** Dietary avoidance is recommended for peanut allergies. We evaluated the sustained effects of peanut allergy oral immunotherapy (OIT) in a randomised long-term study in adults and children. **Methods:** In this randomised, double-blind, placebo-controlled, phase 2 study, we enrolled participants at the Sean N Parker Center for Allergy and Asthma Research at Stanford University (Stanford, CA, USA) with peanut allergy aged 7–55 years with a positive result from a double-blind, placebo-controlled, food challenge (DBPCFC; ≤500 mg of peanut protein), a positive skin-prick test (SPT) result (≥5 mm wheal diameter above the negative control), and peanut-specific immunoglobulin (IgE) concentration of more than 4 kU/L. Participants were randomly assigned (2:4:1:4:1) in a two-by-two block design via a computerised system to be built up and maintained on 4000 mg peanut protein through to week 104 then discontinued on peanut (peanut-0 group), to be built up and maintained on 4000 mg peanut protein through to week 104 then to ingest 300 mg peanut protein daily (peanut-300 group) for 52 weeks, or to receive oat flour (placebo group). DBPCFCs to 4000 mg peanut protein were done at baseline and weeks 104, 117, 130, 143, and 156. The pharmacist assigned treatment on the basis of a randomised computer list. Peanut or placebo (oat) flour was administered orally and participants and the study team were masked throughout by use of oat flour that was similar in look and feel to the peanut flour and nose clips, as tolerated, to mask taste. The statistician was also masked. The primary endpoint was the proportion of participants who passed DBPCFCs to a cumulative dose of 4000 mg at both 104 and 117 weeks. The primary efficacy analysis was done in the intention-to-treat population. Safety was assessed in the intention-to-treat population. This trial is registered at ClinicalTrials.gov, NCT02103270. **Findings:** Between April 15, 2014, and March 2, 2016, of 152 individuals assessed, we enrolled 120 participants, who were randomly assigned to the peanut-0 (n=60), peanut-300 (n=35), and placebo groups (n=25). 21 (35%) of peanut-0 group participants and one (4%) placebo group participant passed the 4000 mg challenge at both 104 and 117 weeks (odds ratio [OR] 12·7, 95% CI 1·8–554·8; p=0·0024). Over the entire study, the most common adverse events were mild gastrointestinal symptoms, which were seen in 90 of 120 patients (50/60 in the peanut-0 group, 29/35 in the peanut-300 group, and 11/25 in the placebo group) and skin disorders, which were seen in 50/120 patients (26/60 in the peanut-0 group, 15/35 in the peanut-300 group, and 9/25 in the placebo group). Adverse events decreased over time in all groups. Two participants in the peanut groups had serious adverse events during the 3-year study. In the peanut-0 group, in which eight (13%) of 60 participants passed DBPCFCs at week 156, higher baseline peanut-specific IgG4 to IgE ratio and lower Ara h 2 IgE and basophil activation responses were associated with sustained unresponsiveness. No treatment-related deaths occurred. **Interpretation:** Our study suggests that peanut OIT could desensitise individuals with peanut allergy to 4000 mg peanut protein but discontinuation, or even reduction to 300 mg daily, could increase the likelihood of regaining clinical reactivity to peanut. Since baseline blood tests correlated with week 117 treatment outcomes, this study might aid in optimal patient selection for this therapy. **Funding:** National Institute of Allergy and Infectious Diseases.

**Gut Microbiome**

**Effects of Single and Combined Toxic Exposures on the Gut Microbiome: Current Knowledge and Future Directions**

Significance: This report identifies studies that are needed to comprehensively evaluate the effects of chemical pollutants and food additives on the gut microbiome.

Human populations are chronically exposed to mixtures of toxic chemicals. Predicting the health effects of these mixtures require a large amount of information on the mode of action of their components. Xenobiotic metabolism by bacteria inhabiting the gastrointestinal tract has a major influence on human health. Our review aims to explore the literature for studies looking to characterize the different modes of action and outcomes of major chemical pollutants, and some components of cosmetics and food additives, on gut microbial communities in order to facilitate an estimation of their potential mixture effects. We identified good evidence that exposure to heavy metals, pesticides, nanoparticles, polycyclic aromatic hydrocarbons, dioxins, furans, polychlorinated biphenyls, and non-caloric artificial sweeteners affect the gut microbiome and which is associated with the development of metabolic, malignant, inflammatory, or immune diseases. Answering the question ‘Who is there?’ is not sufficient to define the mode of action of a toxicant in predictive modeling of mixture effects. Therefore, we recommend that new studies focus to simulate real-life exposure to diverse chemicals (toxicants, cosmetic/food additives), including as mixtures, and which combine metagenomics, metatranscriptomics and metabolomic analytical methods achieving in that way a comprehensive evaluation of effects on human health.