

Food Safety Briefs

Risk Assessment

Relevance of *In Vitro* Transcriptomics for *In Vivo* Mode of Action Assessment

Luijten M, Wackers PFK, Rorije E, Pennings JLA, Heusinkveld HJ. *Chem Res Toxicol.* 2021 Feb 15;34(2):452-459. doi: 10.1021/acs.chemrestox.0c00313. [Article link](#)

Significance: Categorizing chemicals by mode of action can be done with toxicogenomic methods and allows for comparison of automated assays with those in test animals to support chemical risk assessments.

Recently, we reported an *in vitro* toxicogenomics comparison approach to categorize chemical substances according to similarities in their proposed toxicological modes of action. Use of such an approach for regulatory purposes requires, among others, insight into the extent of biological concordance between *in vitro* and *in vivo* findings. To that end, we applied the comparison approach to transcriptomics data from the Open TG-GATEs database for 137 substances with diverging modes of action and evaluated the outcomes obtained for rat primary hepatocytes and for rat liver. The results showed that a relatively small number of matches observed *in vitro* were also observed *in vivo*, whereas quite a large number of matches between substances were found to be relevant solely *in vivo* or *in vitro*. The latter could not be explained by physicochemical properties, leading to insufficient bioavailability or poor water solubility. Nevertheless, pathway analyses indicated that for relevant matches the mechanisms perturbed *in vitro* are consistent with those perturbed *in vivo*. These findings support the utility of the comparison approach as tool in mechanism-based risk assessment.

Foodborne Pathogens

Nature versus Nurture: Assessing the Impact of Strain Diversity and Pregrowth Conditions on *Salmonella enterica*, *Escherichia coli*, and *Listeria* Species Growth and Survival on Selected Produce Items

Harrand AS, Guariglia-Oropeza V, Skeens J, Kent D, Wiedmann M. *Appl Environ Microbiol.* 2021 Feb 26;87(6):e01925-20. doi: 10.1128/AEM.01925-20. [Article link](#)

Significance: Pathogen growth and survival data generated using multiple pre-growth conditions will strengthen microbial risk assessments and more accurately account for uncertainty.



Inoculation studies are important when assessing microbial survival and growth in food products. These studies typically involve the pre-growth of multiple strains of a target pathogen under a single condition; this emphasizes strain diversity. To gain a better understanding of the impacts of strain diversity (“nature”) and pre-growth conditions (“nurture”) on subsequent bacterial growth in foods, we assessed the growth and survival of *Salmonella enterica* ($n = 5$), *Escherichia coli* ($n = 6$), and *Listeria* ($n = 5$) inoculated onto tomatoes, pre-cut lettuce, and cantaloupe rind, respectively. Pre-growth conditions included (i) 37°C to stationary phase (baseline), (ii) low pH, (iii) high salt, (iv) reduced water activity, (v) log phase, (vi) minimal medium, and (vii) 21°C. Inoculated tomatoes were incubated at 21°C; lettuce and cantaloupe were incubated at 7°C. Bacterial counts were assessed over three phases, including initial reduction (phase 1), change in bacterial numbers over the first 24 h of incubation (phase 2), and change over the 7-day incubation (phase 3). *E. coli* showed overall decline in counts (<1 log)

over the 7-day period, except for a <1-log increase after pre-growth in high salt and to mid-log phase. In contrast, *S. enterica* and *Listeria* showed regrowth after an initial reduction. Pre-growth conditions had a substantial and significant effect on all three phases of *S. enterica* and *E. coli* population dynamics on inoculated produce, whereas strain did not show a significant effect. For *Listeria*, both pre-growth conditions and strain affected changes in phase 2 but not phases 1 and 3.

Importance: Our findings suggest that inclusion of multiple pregrowth conditions in inoculation studies can best capture the range of growth and survival patterns expected for *Salmonella enterica* and *Escherichia coli* present on produce. This is particularly important for fresh and fresh-cut produce, where stress conditions encountered by pathogens prior to contamination can vary widely, making selection of a typical pregrowth condition virtually impossible. Pathogen growth and survival data generated using multiple pregrowth conditions will allow for more robust microbial risk assessments that account more accurately for uncertainty.

Evaluation of *Enterococcus faecium* NRRL B-2354 as a Surrogate for *Salmonella* in Ground Black Pepper at Different Water Activities

Wei X, Vasquez S, Thippareddi H, Subbiah J. *Int J Food Microbiol.* 2021 Feb 23;344:109114. doi: 10.1016/j.ijfoodmicro.2021.109114. [Article link](#)

Significance: This study found that *E. faecium* is a suitable surrogate for *Salmonella* in thermal processing validation in ground black pepper.

Thermal inactivation kinetics of *Salmonella* in low moisture foods are necessary for developing proper thermal processing parameters for pasteurization. The effect of water activity on thermal inactivation kinetics of *Salmonella* and *Enterococcus faecium* NRRL B-2354 in ground black pepper has not been studied previously. Identification of a suitable surrogate assists in conducting in-plant process validations. Ground black pepper was inoculated with a 5-serotype *Salmonella* cocktail or *E. faecium* NRRL B-2354, equilibrated to water activities of 0.25, 0.45 or 0.65 in a humidity-controlled chamber, and isothermally treated at different temperatures. The survivor data were used for fitting the log-linear models to obtain the D and z-values of *Salmonella* and *E. faecium* in ground black pepper. Modified Bigelow models were developed to evaluate the effects of temperature and water activity on the thermal inactivation kinetics of *Salmonella* and *E. faecium*. Water activity and temperature showed significant negative effects on the thermal resistance of *Salmonella* and *E. faecium* in ground black pepper. For example, significantly higher D values of *Salmonella* were observed at water activity of 0.45 ($D_{70^{\circ}\text{C}} = 20.5$ min and $D_{75^{\circ}\text{C}} = 7.8$ min) compared to water activity of 0.65 ($D_{70^{\circ}\text{C}} = 3.9$ min and $D_{75^{\circ}\text{C}} = 2.0$ min). D-values of *E. faecium* were significantly higher than those of *Salmonella* at all three water activities, indicating that *E. faecium* is a suitable surrogate for *Salmonella* in thermal processing validation.

Mycotoxins

A PCR Method to Identify Ochratoxin A-Producing *Aspergillus westerdijkiae* Strains on Dried and Aged Foods

Susca A, Anelli P, Haidukowski M, Probyn CE, Epifani F, Logrieco AF, et al. *Int J Food Microbiol.* 2021 Feb 23;344:109113. doi: 10.1016/j.ijfoodmicro.2021.109113. [Article link](#)

Significance: A PCR method is presented that can identify *A. westerdijkiae* and distinguish between ochratoxin A-producing and ochratoxin A-nonproducing chemotypes.

Ochratoxins are a group of mycotoxins that frequently occur as contaminants in agricultural commodities and foods, including dry-cured meats and cheeses. The fungus *Aspergillus westerdijkiae* is frequently isolated from aged foods and can produce ochratoxin A (OTA). However, individual strains of the fungus can have one of two OTA production phenotypes (chemotypes): OTA production and OTA nonproduction. Monitoring and early detection of OTA-producing fungi in food are the most effective strategies to manage OTA contamination. Therefore, we examined genome sequence data from five *A. westerdijkiae* strains isolated from the surface of cheese from southern Italy to identify genetic markers indicative of the two OTA chemotypes. This analysis revealed a naturally occurring deletion of the OTA regulatory gene, *otaR*, in an OTA-nonproducing isolate. We used this information to design a polymerase chain reaction (PCR) method that could identify *A. westerdijkiae* and distinguish between the two OTA chemotypes. In this method, the PCR primers were complementary to conserved sequences flanking *otaR* and yielded different-sized amplicons from strains with the different chemotypes. The primers did not yield *ota*-region-specific amplicons from other OTA-producing species. Because the method is specific to *A. westerdijkiae* and can distinguish between the two OTA chemotypes, it has potential to significantly improve OTA monitoring programs.

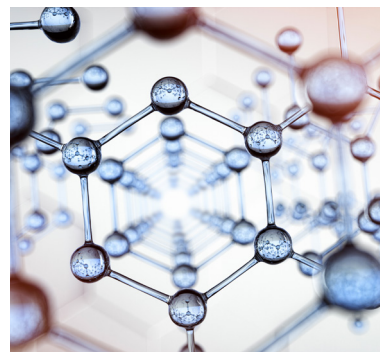
Food Packaging

Regulatory Landscape of Nanotechnology and Nanoplastics from a Global Perspective

Allan J, Belz S, Hoeveler A, Hugas M, Okuda H, Patri A, et al. *Regul Toxicol Pharmacol.* 2021 Feb 19;122:104885. doi: 10.1016/j.yrtph.2021.104885. [Article link](#)

Significance: The harmonization of methodologies for quantification and risk assessment of nanomaterials and micro/nanoplastics—along with the documentation of regulatory science studies and sharing databases—are important steps needed to advance global safety and regulatory efforts.

Nanotechnology and more particularly nanotechnology-based products and materials have provided a huge potential for novel solutions to many of the current challenges society is facing. However, nanotechnology is also an area of product innovation that is sometimes developing faster than regulatory frameworks. This is due to the high complexity of some nanomaterials, the lack of a globally harmonised regulatory definition and the different scopes of regulation at a global level. Research organisations and regulatory bodies have spent many efforts in the last two decades to cope with these challenges. Although there has been a significant advancement related to analytical approaches for labelling purposes as well as to the development of suitable test guidelines for nanomaterials and their safety assessment, there is still a need for greater global collaboration and consensus in the regulatory field. Furthermore, with growing societal concerns on plastic litter and tiny debris produced by degradation of littered plastic objects, the impact of micro- and nanoplastics on humans and the environment is an emerging issue. Despite increasing research and initial regulatory discussions on micro- and nanoplastics, there are still knowledge gaps and thus an urgent need for action. As nanoplastics can be classified as a specific type of incidental nanomaterials, current and future scientific investigations should take into account the existing profound knowledge on nanotechnology/nanomaterials when discussing issues around nanoplastics. This review was conceived at the 2019 Global Summit on Regulatory Sciences that took place in Stresa, Italy, on 24-26 September 2019 (GSRS 2019) and which was co-organised by the Global Coalition for Regulatory Science Research (GCRSR) and the European Commission's (EC) Joint Research Centre (JRC). The GCRSR consists of regulatory bodies from various countries around the globe including EU bodies. The 2019 Global Summit provided an excellent platform to exchange the latest information on activities carried out by regulatory bodies with a focus on the application of nanotechnology in the agriculture/food sector, on nanoplastics and on nanomedicines, including taking stock and promoting further collaboration. Recently, the topic of micro- and nanoplastics has become a new focus of the GCRSR. Besides discussing the challenges and needs, some future directions on how new tools and methodologies can improve the regulatory science were elaborated by summarising a significant portion of discussions during the summit. It has been revealed that there are still some uncertainties and knowledge gaps with regard to physicochemical properties, environmental behaviour and toxicological effects, especially as testing described in the dossiers is often done early in the product development process, and the material in the final product may behave differently. The harmonisation of methodologies for quantification and risk assessment of nanomaterials and micro/nanoplastics, the documentation of regulatory science studies and the need for sharing databases were highlighted as important aspects to look at.



Chemical Contaminants

Exploring the Potential of ToxCast Data in Supporting Read-Across for Evaluation of Food Chemical Safety

Firman JW, Punt A, Cronin MTD, Boobis AR, Wilks MF, Hepburn PA, et al. *Chem Res Toxicol*. 2021 Feb 15;34(2):300-312. doi: 10.1021/acs.chemrestox.0c00240. [Article link](#)

Significance: This study evaluated the utility of high-throughput screening (HTS) data for application in toxicological read-across in food-relevant chemicals, including the extent to which HTS data could provide information regarding the definition of the boundaries of chemical space across which bioactivity could reliably be extrapolated.

The intention of this study was to determine the utility of high-throughput screening (HTS) data, as exemplified by ToxCast and Tox21, for application in toxicological read-across in food-relevant chemicals. Key questions were addressed on the extent to which the HTS data could provide information enabling (1) the elucidation of underlying bioactivities associated with apical toxicological outcomes, (2) the closing of existing toxicological data gaps, and (3) the definition of the boundaries of chemical space across which bioactivity could reliably be extrapolated. Results revealed that many biological targets apparently activated within the chemical groupings lack, at this time, validated toxicity pathway associations. Therefore, as means of providing proof-of-principle, a comparatively well-characterized end point—estrogenicity—was selected for evaluation. This was facilitated through the preparation of two exploratory case studies, focusing upon groupings of paraben-gallates and pyranone-type compounds (notably flavonoids). Within both, the HTS data were seen to reflect estrogenic potencies in a manner which broadly corresponded to established structure-activity group relationships, with parabens and flavonoids displaying greater estrogen receptor affinity than benzoate esters and alternative pyranone-containing

molecules, respectively. As such, utility in the identification of out-of-domain compounds was demonstrated, indicating potential for application in addressing point (3) as detailed above.

Heavy Metals

Derivation of Biomonitoring Equivalents for Aluminium for the Interpretation of Population-level Biomonitoring Data

Podalgoda D, Hays SM, Kirman C, Chander N, Nong A. *Regul Toxicol Pharmacol*. 2021 Feb 27;122:104913. doi: 10.1016/j.yrtph.2021.104913. [Article link](#)

Significance: Pharmacokinetic and mass balance models were developed to derive blood and urine Biomonitoring Equivalents for established guidance values, which may be useful in interpreting population-level biomonitoring data in a health risk context.

Aluminium is widely used in many consumer products, however the primary source of aluminium exposure to the Canadian general population is through food. Aluminium can cause neurotoxicity and reproductive toxicity at elevated exposure levels. Health-based exposure guidance values have been established for oral exposure to aluminium, including a Minimal Risk Level (MRL) by the Agency for Toxic Substances and Disease Registry (ATSDR), a Provincial Tolerable Weekly Intake (PTWI) by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) and a Tolerable Weekly Intake (TWI) by the European Food Safety Authority. Aluminium concentration in blood and urine can be used as a tool for exposure characterization in a population. A pharmacokinetic (PK) model was developed based on human dosing data to derive blood Biomonitoring Equivalents (BEs), whereas a mass balance approach was used to derive urine BEs for the above guidance values. The BEs for blood for daily intake consistent with the MRL, PTWI and TWI were 18, 16 and 8 µg/L, respectively. BEs for urine for the same guidance values were 137, 123 and 57 µg/L, respectively. The derived BEs may be useful in interpreting population-level biomonitoring data in a health risk context and thereby screening and prioritizing substances for human health risk assessment and risk management.

Caffeine

Caffeinated Coffee Consumption and Health Outcomes in the US Population: A Dose–Response Meta-Analysis and Estimation of Disease Cases and Deaths Avoided

Di Maso M, Boffetta P, Negri E, La Vecchia C, Bravi F. *Adv Nutr*. 2021 Feb 11:nmaa177. doi: 10.1093/advances/nmaa177. [Article link](#)

Significance: Current patterns of caffeine consumption in the United States would account for a fraction of avoided diseases/deaths ranging from 6% to 12%. The study confirms the beneficial health effects of consumption in the US population on several health outcomes considered in the research.



To explore the role of coffee on health outcomes in the United States, where coffee consumption is common, we conducted a meta-analysis of prospective studies investigating the magnitude (any compared with no consumption) and the dose–response shape (cups per day) of the associations between caffeinated coffee consumption and incidence/mortality of cardiovascular disease (CVD), as well as incidence of type 2 diabetes (T2D), hepatocellular carcinoma (HCC), endometrial cancer, melanoma, and nonmelanoma skin cancer. We selected the desirable health outcomes that have been shown to be positively associated with coffee consumption. Studies were identified by searching PubMed/Embase databases up to September 2019. Inclusion criteria included prospective studies that investigated the relation of ≥ 3 categories of caffeinated coffee consumption and the outcomes of interest. Twenty-six studies (42 distinct cohorts), with 93,706 cases/deaths and 3,713,932 participants, met the inclusion criteria. In any coffee consumers, there was a significant inverse association with the risk of CVD (RR = 0.90; 95% CI: 0.84, 0.96), T2D (RR = 0.90; 95% CI: 0.85, 0.96), endometrial cancer (RR = 0.85; 95% CI: 0.78, 0.92), melanoma (RR = 0.89; 95% CI: 0.80, 0.99), and nonmelanoma skin cancer (RR = 0.92; 95% CI: 0.89, 0.95). Coffee consumption was also inversely associated with HCC (RR = 0.93; 95% CI: 0.80, 1.08), without reaching statistical significance. The dose–response relation was nonlinear uniquely for CVD (P -nonlinearity = 0.01). In particular, the largest risk reduction was observed for 3–4 cups/d (~120 mL/cup) and no reduction thereafter. For other outcomes, the risk decreased linearly over the whole coffee consumption range. Current patterns of consumption in the United States would account

for a fraction of avoided cases/deaths ranging from 6% to 12% according to the outcome considered. This study confirms the beneficial health effects of caffeinated coffee consumption in the US population on the health outcomes considered, and quantifies their possible magnitude.

Allergens

Unsupervised Modeling and Genome-Wide Association Identify Novel Features of Allergic March Trajectories

Gabryszewski SJ, Chang X, Dudley JW, Mentch F, March M, Holmes JH, et al. *J Allergy Clin Immunol*. 2021 Feb;147(2):677-685.e10. doi: 10.1016/j.jaci.2020.06.026. [Article link](#)

Significance: Novel associations were identified between race and progression along distinct allergic trajectories. These results uncover important health disparities, refine the concept of the allergic march and represent a step toward developing individualized medical approaches for these conditions.

Background: The allergic march refers to the natural history of allergic conditions during infancy and childhood. However, population-level disease incidence patterns do not necessarily reflect the development of allergic disease in individuals. A better understanding of the factors that predispose to different allergic trajectories is needed. **Objective:** Our aim was to determine the demographic and genetic features that are associated with the major allergic march trajectories. **Methods:** Presence or absence of common allergic conditions (atopic dermatitis [AD], IgE-mediated food allergy [IgE-FA], asthma, and allergic rhinitis [AR]) was ascertained in a pediatric primary care birth cohort of 158,510 subjects. Hierarchic clustering and decision tree modeling were used to associate demographic features with allergic outcomes. Genome-wide association study was used to test for risk loci associated with specific allergic trajectories. **Results:** We found an association between self-identified black race and progression from AD to asthma. Conversely, Asian or Pacific Islander race was associated with progression from AD to IgE-mediated food allergy, and white race was associated with progression from AD to AR. Genome-wide association study of trajectory groups identified risk loci associated with progression from AD to asthma (rs60242841) and from AD to AR (rs9565267, rs151041509, and rs78171803). Consistent with our epidemiologic associations, rs60242841 was more common in individuals of African ancestry than in individuals of European ancestry, whereas rs9565267 and rs151041509 were more common in individuals of European ancestry than in individuals of African ancestry. **Conclusion:** We have identified novel associations between race and progression along distinct allergic trajectories. Ancestral genetic differences may contribute to these associations. These results uncover important health disparities, refine the concept of the allergic march, and represent a step toward developing individualized medical approaches for these conditions.

Prevalence and Characteristics of Peanut Allergy in US Adults

Warren C, Lei D, Sicherer S, Schleimer R, Gupta R. *J Allergy Clin Immunol*. 2021 Feb 3;S0091-6749(20)32412-X. doi: 10.1016/j.jaci.2020.11.046. [Article link](#)

Significance: The prevalence of peanut allergy is estimated at 4.6 million US adults—over 800,000 of whom developed it after age 18. More research on phenotypic differences between childhood-onset and adult-onset PA may improve prevention and management.

Background: Peanut allergy (PA) is the leading pediatric food allergy and a common cause of anaphylaxis. Little is known, however, on the prevalence and characteristics of PA in the adult population and whether phenotypic differences exist between adult-onset and childhood-onset PA. **Objectives:** This study describes the current US population-level burden of adult PA. **Methods:** A cross-sectional food allergy survey was administered via phone and web in 2015 and 2016, resulting in nationally representative complex-survey weighted data for 40,443 adults. Reported food allergies were considered “convincing” if symptoms to specific allergens were consistent with an IgE-mediated reaction. **Results:** The prevalence of current self-reported PA was 2.9% among US adults, with 1.8% having convincing PA. Over 17% of adults with peanut allergy reported onset of their PA in adulthood. In adults with childhood-onset PA, 75.4% reported physician-diagnosed PA, compared with only 58.9% of adult-onset PA. Despite a similar frequency of food allergy-related emergency department visits within the past year (approximately 1 in 5 adults with PA allergy), adults with childhood-onset PA were significantly more likely to have a current epinephrine prescription compared with those with adult-onset PA (56% vs 44% respectively; $P = .02$) and were more likely to use an epinephrine autoinjector (48% vs 35%, $P = .01$). **Conclusions:** Approximately 4.6 million US adults have PA—over 800,000 of whom appear to have developed their PA after age 18 years. Further examination of phenotypic differences between childhood-onset and adult-onset PA may improve understanding and management of adult PA.