Risk Assessment

Recommendations for International Harmonisation, Implementation and Further Development of Suitable Scientific Approaches Regarding the Assessment of Mixture Effects


Significance: This report describes five steps for addressing further development needs and implementation of existing tools for achieving international harmonization of risk assessment of chemical mixtures.

Legal frameworks lay down requirements for risk assessment of combined exposure to multiple chemicals and their implementation where scientific methods are accepted by liable authorities. In order to protect human health, an assessment of potential risks that might result from co-exposure to multiple chemical substances is requested by European legislation. Several approaches for risk assessment of mixtures of chemicals have been proposed, but none has been widely implemented in regulatory risk assessments, so far. EuroMix, an EU Horizon 2020 funded project, contributed to the improvement of internationally harmonised approaches for risk assessment of chemical mixtures. Based on in vitro and in silico tests, an integrated test strategy involving hazard and exposure assessment was developed and a web tool to conduct such assessments was provided. One further task within EuroMix was to make recommendations for international harmonisation, implementation and further development of suitable scientific approaches regarding the assessment of mixture effects. This paper briefly describes objectives and outcome of the EuroMix project as well as recent findings from OECD, WHO and EFSA addressing combined exposure to multiple chemicals. Building on this, five steps addressing further development needs and implementation of existing tools especially for risk managers and policy makers are proposed.

Use of the Kinetically-Derived Maximum Dose Concept in Selection of Top Doses for Toxicity Studies Hampers Proper Hazard Assessment and Risk Management


Significance: This report provides several arguments to demonstrate the Kinetically-Derived Maximum Dose (KMD) concept is invalid for use in toxicity testing.

The KMD (kinetically-derived maximum dose) is an increasingly advocated concept that uses toxicokinetic data in the top dose selection for toxicity testing. Application of this concept may have serious regulatory implications though, especially in the European Union. The basic assumption is that the relationship between internal and external dose (IED) shows an inflection point where linearity transits into non-linearity due to saturation of underlying processes; top doses in toxicity tests should not be above the inflection point, provided human exposures are well below this point. A critical analysis of the KMD concept and its underlying assumptions shows, however, that the IED relationship is non-linear over the whole dose range, without any point of inflection. The KMD concept thus aims to estimate a non-existing point, rendering it invalid for use in toxicity testing. Moreover, the concept ignores the key question in toxicology: What kind of toxic effects occur at which doses? These and several other reservations against the KMD concept are discussed and illustrated with three existing applications of the KMD approach. Hence, we recommend to abolish the KMD concept for selecting top doses in toxicity testing. This requires the updating of regulations, guidance documents and OECD test guidelines.
**Foodborne Pathogens**

**Occurrence of *Listeria monocytogenes* in Ready-to-Eat Meat and Poultry Product Verification Testing Samples From United States Department of Agriculture-Regulated Producing Establishments, 2005-2017**


**Significance:** An analysis of over 13 years of ready-to-eat meat and poultry product sampling in FSIS-regulated establishments through 2017 found that less than 0.4% of samples tested positive for *L. monocytogenes* (*Lm*) in both risk-based and random sampling projects, suggesting the combination of FSIS policies and industry practices may be effective in controlling *Lm*.

Ready-to-eat (RTE) meat and poultry product samples collected between 2005 and 2017 from RTE producing establishments for the Food Safety and Inspection Service’s (FSIS’s) ALLRTE/RTEPROD_RAND (random) and RTE001/RTEPROD_RISK (risk-based) sampling projects were tested for *Listeria monocytogenes* (*Lm*). Data for 45,897 ALLRTE/RTEPROD_RAND samples collected from 3607 unique establishments and 112,347 RTE001/RTEPROD_RISK samples collected from 3283 unique establishments were analyzed for the presence of *Lm*. These data were also analyzed based upon the percentages of establishments with positive samples, as well as annual production volume, sanitation control alternative, geographic location and season/month of sample collection. Results showed low occurrences of *Lm*-positive samples from the random and risk-based sampling projects, with 152 positive samples (0.33%) for ALLRTE/RTEPROD_RAND and 403 positive samples (0.36%) for RTE001/RTEPROD_RISK, respectively. The percentages of positive samples significantly decreased over time, from about 0.7% in 2005-2006 to about 0.2% in 2017 (*P*<0.05). During the 2005-2017 time period, 3.9% of establishments sampled under the ALLRTE/RTEPROD_RAND sampling project had at least one *Lm*-positive sample. Similarly, 10.0% of establishments sampled under the RTE001/RTEPROD_RISK sampling project had at least one positive sample. Positive *Lm* samples were found in all geographic regions in all months. Thus, in 13 years of RTE product sampling in FSIS-regulated establishments (2005-2017), less than 0.4% of samples were positive for *Lm* in both risk-based and random sampling projects. The low percentage of *Lm* in these products suggests that the combination of FSIS policies and industry practices may be effective in controlling *Lm* contamination. Information obtained from these sampling projects is relevant to the ongoing prevention of foodborne *Lm* illnesses from RTE meat and poultry products.

**Foodborne Illness**

**Micro/Nanoencapsulation Strategy to Improve the Efficiency of Natural Antimicrobials Against *Listeria monocytogenes* in Food Products**


**Significance:** A review of new encapsulation techniques for natural antimicrobials to control the growth of *L. monocytogenes* is presented.

*Listeria monocytogenes* (*Lm*), the etiological agent of listeriosis diseases in humans, is a serious pathogenic microorganism threatening the food safety especially in ready-to-eat food products. Adhesion on both biotic and abiotic surfaces is making it a potential source of contamination by *Lm*. Also, this bacterium has become more tolerant in food processing conditions, including in the presence of adverse conditions such as cold and dehydration. One of the attractive and effective methods to inhibit the growth of *Lm* in the food products is using natural antimicrobial agents, which can be a suitable alternative to synthetic preservatives for producing organic food products. The use of pure natural antimicrobials has some limitations including low stability against harsh conditions, low solubility and absorption, and un-controlled release, which can decrease their functions. These limitations have been overcome by using new advanced encapsulation techniques, which have boosted the anti-listerial activity of natural agents. Therefore, the current paper is aiming to review the results of recent studies conducted on using natural antimicrobials added directly or as encapsulated forms into the food formulation to control the growth of *Lm*. The information of current study can be used by the researchers as well as the food companies for the optimization of food formulations through encapsulation strategies to control *Lm* and potentially produce safe foods for the consumers.

**Mycotoxins**

**Fusarium Mycotoxins Enniatins: An Updated Review of Their Occurrence, the Producing *Fusarium* Species, and the Abiotic Determinants of Their Accumulation in Crop Harvests**

Significance: This review highlights recent advances concerning the abiotic determinants and genetic regulation of enniatin biosynthesis by Fusarium species.

Cereal grains and their processed food products are frequently contaminated with mycotoxins produced by the Fusarium genus. Enniatins (ENNs), which belong to the so-called “emerging mycotoxins” family, are among the most frequently found in small grain cereals. Health hazards induced by a chronic exposure to ENNs or an association of ENNs with other major mycotoxins is a risk that cannot be excluded given the current toxicological data. Thus, efforts must be pursued to define efficient control strategies to mitigate their presence in cereal grains. A key condition for achieving this aim is to gain deep and comprehensive knowledge of the factors promoting the appearance of ENNs in crop harvests. After an update of ENN occurrence data, this review surveys the scientific literature on the Fusarium species responsible for ENN contamination and covers the recent advances concerning the abiotic determinants and the genetic regulation of ENN biosynthesis.

Food Packaging

Freshness Monitoring Technology of Fish Products in Intelligent Packaging

Significance: This report summarizes research progress on intelligent packaging approaches for fish including three advanced technologies—indicator, sensor and radio frequency identification (RFID).

Fish products are one of the preferred products in modern healthy diets, because they contain unqualified proteins, polyunsaturated fatty acids and a variety of vitamins and minerals. However, because of their vulnerability to deterioration, methods to maintain their freshness have attracted wide attention. Intelligent packaging can effectively monitor the quality and safety of fish products, provide warning, and has a great market and development potential. Therefore, this paper reviews the research progress of intelligent packaging technology used to monitor the freshness of fish products. The quality attributes of freshness of fish products are summarized. The classification, principle and latest application progress of three advanced technologies, indicator, sensor and radio frequency identification (RFID), are summarized. In addition, the advantages and disadvantages of the intelligent packaging technology for monitoring the freshness of products are discussed, and the current research results are summarized and prospected.

Chemical Contaminants

Maternal Plasma Perfluoroalkyl Substances and Miscarriage: A Nested Case-Control Study in the Danish National Birth Cohort

Significance: Maternal exposures to high concentrations of PFOA, PFHpS and PFAS mixtures were associated with the risk of miscarriage, particularly among women who have already given birth.

Per- and polyfluoroalkyl substances (PFAS) are widespread persistent organic pollutants and endocrine disruptors. High doses of perfluorooctanesulfonic acid (PFOS) and perfluorooctanoic acid (PFOA) exposure can cause pregnancy loss and infant deaths in animals, but the associations between PFAS exposures and risk of miscarriage in humans are not well studied. Using a case-control study nested within the Danish National Birth Cohort (DNBC, 1996-2002), we compared 220 pregnancies ending in miscarriage during weeks 12-22 of gestation, with 218 pregnancies resulting in live births. Levels of seven types of PFAS [PFOS, PFOA, perfluorohexane sulfonate (PFHxS), perfluoroheptane sulfonate (PFHpS), perfluorononanoic acid (PFNA), perfluorodecanoic acid (PFDA), and perfluorooctanesulfonic acid (PFOSA)] were measured in maternal plasma collected in early gestation (mean gestational week 8). We estimated the odds ratios (ORs) and 95% confidence intervals (CIs) for miscarriage and each PFAS as a continuous variable or in quartiles, controlling for maternal age, parity, socio-occupational status, smoking and alcohol intake, gestational week of blood sampling, and maternal history of miscarriage. Stratification by parity and PFAS mixture analyses using weighted quantile sum (WQS) regression were also conducted. We observed a monotonic increase in odds for miscarriage associated with increasing PFOA and PFHpS levels. The ORs comparing the highest PFOA or PFHpS quartile to the lowest were 2.2 (95% CI: 1.2, 3.9) and 1.8 (95% CI: 1.0, 3.2). The ORs were also elevated for the second or third quartile of PFHxS or PFOS, but no consistent exposure-outcome pattern emerged. An interquartile range (IQR) increment in the WQS index of seven PFAS was associated with 64% higher odds for miscarriage (95% CI: 1.15, 2.34). The associations were stronger in parous women, while findings were inconsistent among nulliparous women. Maternal exposures to higher levels of PFOA, PFHpS, and PFAS mixtures were associated with the risk of miscarriage and particularly among parous women. Larger replication studies among nulliparous women are needed to allay concerns about confounding by reproductive history.
### Heavy Metals

**Perspective on Cadmium and Lead in Cocoa and Chocolate**


**Significance:** Lead and cadmium concentrations from chocolate and cocoa sourced in Latin America can be elevated and more work to identify successful mitigation efforts is warranted.

Cocoa and chocolate contain cadmium (Cd) and lead (Pb) from natural and anthropogenic sources. This perspective provides background on the origin, occurrence, and factors affecting Cd and Pb levels in chocolate products as well as ongoing international efforts to mitigate Cd and Pb in these popular foods, particularly the higher Cd levels observed in some cocoa and chocolate originating from parts of Latin America. Information on factors contributing to higher Cd levels in Latin America, including elevated soil Cd, is increasing, but more work is needed to identify successful mitigation methods.

### Caffeine

**Caffeine Content in Newborn Hair Correlates With Maternal Dietary Intake**


**Significance:** Caffeine content in newborn hair samples reflects maternal third trimester dietary caffeine intake, introducing a new method to assess fetal cumulative caffeine exposure.

High-maternal caffeine intake during pregnancy may be harmful for perinatal outcomes and future child health, but the level of fetal cumulative exposure has been difficult to measure thus far. Here, we present maternal dietary caffeine intake during the last trimester and its correlation to caffeine content in newborn hair after birth. Maternal third trimester diets and dietary caffeine intake were prospectively collected in Kuopio Birth Cohort (KuBiCo) using a 160-item food frequency questionnaire (n = 2840). Newborn hair was collected within 48 h after birth and analyzed by high-resolution mass spectrometry (HRMS) for caffeine (n = 316). Correlation between dietary caffeine intake and neonatal hair caffeine content was evaluated from 203 mother-child pair. Mean dietary caffeine intake was 167 mg/days (95% CI 162-172 mg/days), of which coffee comprised 81%. Caffeine in the maternal diet and caffeine content in newborn hair correlated significantly (r = 0.50; p < 0.001). Older, multiparous, overweight women, and smokers had the highest caffeine levels in the maternal diet, as well as in their newborn babies’ hair. Caffeine exposure, estimated from newborn hair samples, reflects maternal third trimester dietary caffeine intake and introduces a new method to assess fetal cumulative caffeine exposure. Further studies to evaluate the effects of caffeine exposure on both perinatal and postnatal outcomes are warranted, since over 40% of pregnant women consume caffeine more than the current suggested recommendations (European Food Safety Association, EFSA recommendations).

### Food Allergens

**Biomarkers of Severity and Threshold of Allergic Reactions During Oral Peanut Challenges**


**Significance:** The basophil activation test diagnosed peanut allergenicity with high specificity, and was the best biomarker for peanut allergy severity among several methods tested.

Oral food challenge (OFC) is the gold-standard to assess peanut allergy (PA) but involves a risk of allergic reactions of unpredictable severity. To identify biomarkers for risk of severe reactions or low dose threshold during OFC to peanut. We assessed LEAP, LEAP-On and PAS participants with basophil activation test (BAT), skin prick test (SPT), peanut-specific IgE (sIgE) and Ara h 2-sIgE and peanut-specific IgG4 and analyzed the utility of the different biomarkers in relation to PA status, severity and threshold dose of allergic reactions to peanut during OFC. Using a previously defined optimal cut-off, BAT diagnosed PA with 98% specificity and 75% sensitivity. BAT identified severe reactions with 97% specificity and 100% sensitivity. SPT, Ara h 2-sIgE, peanut-sIgE and IgG4/IgE ratios also had 100% sensitivity but slightly lower specificity (92%, 93%, 90% and 88% respectively) to predict severity. Participants with lower threshold of reactivity had higher basophil activation to peanut in vitro. SPT and BAT were the best individual predictors of threshold. Multivariate models were superior to individual biomarkers and were used to generate nomograms to calculate the probability of serious adverse events during OFC for individual patients. BAT diagnosed PA with high specificity and identified severe reactors and low threshold with high specificity and high sensitivity. BAT was the best biomarker for severity, surpassed only by SPT in predicting threshold. Nomograms can help estimate the likelihood of severe reactions and reactions to low dose of allergen in individual peanut allergic patients.