

# Shaping the Future of Food Safety, Together

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#### 40. Communication of scientific uncertainty: international case studies on the development of folate and vitamin D Dietary Reference Values

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##### ABSTRACT

**Objectives:** Transparent evidence-based decision making has been promoted worldwide to engender trust in science and policy making. Yet, little attention has been given to transparency implementation. The degree of transparency (focused on how uncertain evidence was handled) during the development of folate and vitamin D Dietary Reference Values was explored in three a priori defined areas: (i) value request; (ii) evidence evaluation; and (iii) final values. **Materials and methods:** Design: Qualitative case studies (semi-structured interviews and desk research). A common protocol was used for data collection, interview thematic analysis and reporting. Results were coordinated via cross-case synthesis. Setting: Australia and New Zealand, Netherlands, Nordic countries, Poland, Spain and UK. Subjects: Twenty-one interviews were conducted in six case studies. **Results:** Transparency of process was not universally observed across countries or areas of the recommendation setting process. Transparency practices were most commonly seen surrounding the request to develop reference values (e.g. access to risk manager/assessor problem formulation discussions) and evidence evaluation (e.g. disclosure of risk assessor data sourcing/evaluation protocols). Fewer transparency practices were observed to assist with handling uncertainty in the evidence base during the development of quantitative reference values. **Conclusions:** Implementation of transparency policies may be limited by a lack of dedicated resources and best practice procedures, particularly to assist with the latter stages of reference value development. Challenges remain regarding the best practice for transparently communicating the influence of uncertain evidence on the final reference values. Resolving this issue may assist the evolution of nutrition risk assessment and better inform the recommendation setting process.

#### 41. Consumer willingness-to-pay for farm animal welfare: a systematic review and meta-analysis

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##### ABSTRACT

**Objectives:** Farm animal health and welfare (FAW) is an important area of public policy, and increasingly so from a demand perspective. A meta-analysis and systematic review was conducted to establish the public's willingness-to-pay (WTP) for FAW, piloting a tool to assess the uncertainty and strength of evidence in consumer decision-making. Data synthesis and integration methodologies are becoming increasingly important in ensuring that information from the ever expanding body of literature is presented in a more useable format, enabling policy makers and researchers to assess both the strength of evidence and uncertainty. The application of robust methodologies ensures only reliable data are included. **Materials and Methods:** Multiple databases were searched to identify relevant studies. Following a two stage screening process using a set of pre-determined inclusion criteria, 70 studies with 59 unique populations were included in the final analysis, with the strength of evidence and uncertainty for each study being assessed. Meta-regression based on random effects meta-analysis explored heterogeneity in relation to animal species, welfare measures, socio-demographic and socio-economic characteristics. Akaike's Information Criterion (AIC) was used to minimise over fitting. Sensitivity analyses were conducted to assess the risk of bias where appropriate. A cumulative meta-analysis was conducted to establish changes in WTP over time. **Results:** Variation in WTP estimates were reported in relation to animal species and participant characteristics. Reporting standards of studies were mixed. Only 65% of studies reported statistics to enable the weighting of evidence. 25% failed to report socio-demographic characteristics that would enable in-depth exploration of the data. **Conclusions:** A positive WTP for FAW was

demonstrated, varying in relation to a number of factors. An evidence gap was highlighted in relation to WTP for specific animal production diseases associated with the intensification of production, along with the need for more consistent reporting standards.

#### 42. The importance of an integrated approach for genotoxic testing

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##### ABSTRACT

The main goal of hazard identification is the realistic assessment of the potential risks a compound might have. Testing for genotoxicity is an essential part of hazard identification and is defined as the process in which the structure and/or information of the DNA gets altered. A range of in vitro and in vivo assays have been developed to identify substances which could trigger genotoxicity. The present study demonstrates the importance of a more integrated approach in the investigation of genotoxic potential of compounds, here okadaic acid (OA) and azaspiracid (AZAs). In contrast to other studies two additional non-genotoxic assays were included to reduce false positive/negative results by eliminating possible alternative explanation for the observed DNA damage, such as overt cytotoxicity and apoptotic processes. To investigate genotoxicity in the present study, DNA fragmentation was detected using the COMET assay. Additionally, the Trypan Blue Exclusion assay was used to provide information on possible cytotoxicity and cell number. Flow cytometer analysis was included to detect the possible involvement of apoptotic processes. In house data for all endpoints were established using positive controls. Three different cell lines, Jurkat T cells, CaCo-2 cells and HepG-2 cells, representing the main target organs, were exposed to OA and AZA1-3 at different concentrations and exposure times. Data obtained from the COMET assay showed an increase in DNA fragmentation for all phycotoxins, indicating a modest genotoxic effect. However, the data obtained from the Trypan Blue Exclusion assay showed a clear reduction in cell viability and cell number at the concentrations where DNA fragmentation was observed, indicating the involvement of cytotoxic and/or apoptotic processes. This was further supported by data obtained by flow cytometer analysis. All phycotoxins showed signs of early/late apoptosis. Therefore, the combined observations made in the present study indicate that OA and AZA1-3 are not genotoxic per se. Apoptotic processes appear to make a major contribution to the observed DNA fragmentation. Genotoxic testing is a key component of risk assessment and results of this study stress the importance of using a more integrated approach, including cytotoxicity and apoptosis studies to avoid false positive results due to other factors than direct DNA strand breaks.

#### 43. Assessing multiple sources of cadmium exposure in an Italian population

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##### ABSTRACT

**Objectives:** Cadmium (Cd) is a heavy metal representing a serious environmental hazard to the human. Even though food and cigarette smoking are usually by far the main sources of exposure, outdoor air pollution could be an additional important source to be taken into account. Main anthropogenic sources of outdoor air cadmium are non-ferrous metal industrial production and fossil fuel combustion, followed by ferrous metal and cement production, and waste incineration. The aim of our study was

to assess the influence of outdoor air pollution on a biomarker of cadmium exposure. **Material and Methods:** Outdoor exposure to particulate matter  $\leq 10 \mu\text{m}$  (PM10) from motorized traffic was assessed for fifty subjects randomly selected from Modena municipality residents, aged 35-70. We geocoded the residence of these subjects and modeled the corresponding ambient air PM10 concentration using the CALifornia LINE Source Dispersion Model version 4 (CALINE-4) as a proxy of environmental air Cd level. We compared these estimate with the serum Cd, measured with inductively coupled plasma – sectorfield – mass spectrometry. Information on smoking habits and cadmium dietary intake were collected with a semi-quantitative food frequency questionnaire in order to assess possible confounding factors. We used both crude and multivariate linear regression models to determine the influence of outdoor PM10 levels, smoking and dietary Cd intake on serum Cd. **Results:** Median values (25th–75th) for serum and dietary Cd were 40.60 ng/l (30.05 - 53.5) and 13.36  $\mu\text{g}/\text{die}$  (10.45 - 16.77). Crude  $\beta$ -coefficients were 0.617 (95% CI -0.194–1.428,  $P=0.133$ ), 0.026 (-0.827–0.829,  $P=0.952$ ) and 6.962 (-0.022–13.945,  $P=0.051$ ) for PM10, diet and smoking, respectively. Adjusted values were 0.463 (-0.365–1.292,  $P=0.266$ ), -0.036 (-0.866–0.793,  $P=0.930$ ) and 6.057 (-1.175–13.289,  $P=0.099$ ), respectively. **Conclusion:** In our population the most important factor influencing Cd serum content appears to be cigarette smoking, followed by outdoor air pollution (measured by PM10 levels) and lastly diet, possibly for the limitations of dietary assessment methodology. In addition, other unmeasured factors could have influenced serum Cd content, such as a slow release from liver and kidney due to long term exposure.

#### 44. Effects of chemical mixtures in hymenoptera, reptiles and amphibians and variability associated

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##### ABSTRACT

Animal and ecological populations are subject to a multi-chemical exposure which may have adverse effects. The risk assessment of chemical mixtures raises several questions and it would be necessary to develop methodological tools that could improve the evaluation. The systematic review methodology was used to gather data on the effects of chemical mixtures in hymenoptera, reptiles and amphibians, and to make their assessment. Sixty-one publications mostly studying mortality parameters and reproductive and developmental toxicity in response to pesticides' mixtures were included (29, 12 and 20 studies for hymenoptera, reptiles and amphibians, respectively). If the most often measured effect in the context of chemical mixtures was the dose or response addition, synergistic effects were also found in significant number, mainly for pesticides' mixtures. Antagonistic effects were also reported, especially for mixtures of pharmaceuticals. Variability and magnitude of interaction were estimated according to different criteria (e.g., kinds of chemicals in association, toxicological targets...). These results were then compared with those of several groups of species like birds, molluscs, fishes, worms... , representing different ecological compartments and various relevant trophic levels to assess inter-species variability. Specific variability factors associated to the estimation of magnitudes of effects following combined exposure to multiple chemicals could be applied to specific groups of chemicals and/or species showing the most important variability, in order to improve hazard assessment.

#### 45. Evidence synthesis and GMO impact assessment

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##### ABSTRACT

The European regulatory framework for the market authorization of genetically modified organisms (GMO) and products derived thereof demands a comprehensive risk assessment. Even though a large body of GMO safety data has been accumulated, it is difficult for stakeholders, risk assessors and the general public to fully overview the evidence base. The EU-funded research project GRACE (GMO Risk Assessment and Communication

of Evidence) identifies the need to impartially compile existing evidence of potential impacts, including risks and benefits, caused by the deliberate release of GM plants on human/animal health, the environment and socio-economy. The evidence synthesis performed in the frame of GRACE is based on the outcomes of national, EU and international research activities. Systematic evidence synthesis approaches are already established in other research fields to support evidence-based decision-making. They represent powerful tools to collect, evaluate and summarize accessible research results in order to address a specific scientific question in a transparent, reproducible and unbiased manner. Thus, their adaption to and implementation in the impact assessment process for GM plants and products derived thereof aims to increase the transparency and supports the updating of science-based decision-making processes. GRACE aims to identify and to integrate the most appropriate evidence synthesis approaches in a unified framework for the impact assessment of GM plants by drawing on and adapting existing concepts and general guidelines. A set of research questions referring to health, environmental and socio-economic impacts is reviewed by GRACE and stakeholders are actively involved at both planning and interpretation stages. Review teams and stakeholders are supported in preparing and using reviews through the open-access database CADIMA (Central Access Database for the Impact Assessment of Crop Genetic Improvement Technologies) that mirrors the entire evidence synthesis process. The database will be permanently established beyond the lifetime of the GRACE project.

#### 46. Weighting evidence can shape results: how to deal with 0 estimates in proportion meta-analysis

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##### ABSTRACT

**Objectives:** Systematic reviews are effective tools to provide qualified overviews of scientific evidence. In case of specific (closed framed) questions they allow a quantitative synthesis of data through meta-analysis. Proportion (one arm) meta-analysis can provide useful estimates to fill data gaps in risk assessment models but some problem could arise in combining studies with high heterogeneity, different sample size and comprising 0 estimates. Aim of this work is to analyse different transformations of prevalence data for the synthesis of literature. **Materials and Methods:** A dataset of data describing the prevalence of *Toxoplasma* in cattle was used for this exercise. Meta-analysis was performed with R ('metafor' package [1]). As the use of raw prevalence proportions are problematic for several reasons [2] applying three different transformation (logit, arcsine and double arcsine) on both raw data and data corrected according to 0.5 continuity correction [3,4]. For each transformation, forest plot with and without moderators (geographic area and analytical technique), funnel plot, cumulative meta-analysis (ordered by decreasing sample number), qq-plot, radial plot and sensitivity analysis were performed and compared. **Results:** The prevalence estimate is strongly dependent on the applied transformations. 0.5 continuity correction has a marked effect due to the presence in the dataset of studies with small N and 0 estimates. This influence is even higher if moderator or subgroup analysis is performed as results depend on the distribution of 'problematic' studies in different groups. **Conclusions:** As meta-analytical methods rely on the weight of single studies, variance represents a critical parameter, and its calculation has to be addressed carefully. Particular problems arise when datasets comprise studies with 0 estimates that are at risk of outweighing. Our work highlights the importance of transforming data with arcsine or double arcsine functions that give a more reliable weight to studies and do not need correction for 0 estimates. References[1] Viechtbauer W. Conducting Meta-Analyses in R with the metafor Package. J Stat Softw 2010;36. [2] Barendregt JJ, et al. Meta-analysis of prevalence. J Epidemiol Community Health 2013;67:974-8. [3] Cox DR, and Snell JE. The analysis of multivariate binary data. CRC; 1989. [4] Andreano A, et al. Measures of single arm outcome in meta-analyses of rare events in the presence of competing risks. Biom J 2015;00:1–12