The European Food Risk Assessment Fellowship Programme

Series 2

2019-2020

Trusted science for safe food

EU-FORA

efsa

European Food Safety Authority

Luxembourg: Publications Office of the European Union, 2019

Print	ISBN 978-92-9499-101-0	ISSN 2599-7335	doi:10.2805/839438	TM-BC-19-001-EN-C
PDF	ISBN 978-92-9499-100-3	ISSN 2599-7343	doi:10.2805/165612	TM-BC-19-001-EN-N

Table of contents

Please note that each report has a separate numbering system beginning on p.1 in line with the policy of the EFSA Journal

APPROVED: 31 July 2019

doi: 10.2903/j.efsa.2019.e170902

Introduction

We are very happy to present the second special issue of *EFSA journal* devoted to EFSA's fellowship programme (EU-FORA). It brings together the activities of the second cycle of Fellows carried out at the hosting sites, i.e. those scientific institutions across the Member States that participate in the EU-FORA network and devote time and energy to hosting the Fellows.

The technical reports in this issue are a testimony to the high-level, hands-on training that the Fellows received at their host institutions in the scientific fields associated with food safety risk assessment. Through the diversity of the projects covered in this second cycle, we have managed to match the scientific profiles of the Fellows to the work programme of the hosting sites, ensuring the efficient integration and contribution of the Fellows to the programmes.

In most cases, the work programmes presented here served not only as 'training' for the Fellows but also generated new knowledge in the area of risk assessment, as evidenced by the publication of articles in scientific Journals.

Particularly pleasing for this cycle was the participation of new hosting sites which extended further the network of organisations participating in EU-FORA and contributed to the valuable networking opportunities between these organisations and EFSA.

In the last 2 years, the EU-FORA programme has involved more than 200 individual scientists, 100 Member State Competent Organisations and EU Institutions in different roles, making it one of the most extended and successful networking exercises performed by EFSA.

We would like to thank everyone who was involved and contributed to this cycle of EU-FORA and especially the Fellows and Supervisors for the dedication, enthusiasm, professionalism and engagement that they demonstrated throughout. In addition, we would like to extend our gratitude to the members of the EU-FORA Programme Committee for its effective steering of the programme, the EFSA Management Team for supporting it and everyone involved in the training consortium for offering excellent training modules to the Fellows.

On Behalf of EU-FORA Programme

Stylianos Koulouris

EU-FORA Programme Manager

Cristina Alonso Andicoberry

EU-FORA Scientific Coordinator

About the authors: Stylianos Koulouris, EU-FORA Programme Manager and Cristina Alonso Andicoberry, EU-FORA Scientific Coordinator

Correspondence: EU-FORA@efsa.europa.eu

Suggested citation: Koulouris S and Andicoberry CA, 2019. Introduction. EFSA Journal 2019;17(S2): e170902, 1 pp. https://doi.org/10.2903/j.efsa.2019.e170902

ISSN: 1831-4732

APPROVED: 31 July 2019

doi: 10.2903/j.efsa.2019.e170901

Foreword

It gives me great pleasure to introduce this collection of reports from the second round of EFSA's EU-FORA fellowship programme.

The breadth of subjects and experiences described in these 15 documents bears testimony to the success of the programme, which was set up in 2016 as part of EFSA's efforts to nurture the next generation of Europe's food risk assessors.

What is particularly impressive is the relevance of the issues that these early to mid-career scientists have investigated with the assistance of their host institutions. To take just a few examples: the development of chemical exposure assessment by use of probabilistic modelling; the risk assessment of allergenicity from insects as food; the development of risk assessment tools based on molecular typing and whole genome sequencing of *Campylobacter jejuni* genome.

These are challenging real-life issues that speak directly to the current work of risk assessors in Europe. EU-FORA is thus giving valuable experience to the risk assessors of the future, while at the same time expanding and deepening our reservoir of scientific knowledge.

This is a tribute not only to the hard work and dedication of the fellows themselves but also to the enthusiasm with which the host organisations have embraced the programme. Partnership and cooperation are at the centre of everything we do at EFSA, and it is gratifying to see these core values put into practice so productively.

So, congratulations to our second wave of fellows, and thank you to the participating host organisations and the members of the EU-FORA Committee who made this work possible.

EU-FORA is already an established part of EFSA's capacity-building efforts and it will continue to develop for the benefit of the European risk assessment community.

Barbara Gallani

About the author: Barbara Gallani, Head, Communication, Engagement & Cooperation, EFSA

Correspondence: EU-FORA@efsa.europa.eu

Suggested citation: Gallani B, 2019. Foreword. EFSA Journal 2019;17(S2):e170901, 1 pp. https://doi.org/10.2903/j.efsa.2019.e170901

ISSN: 1831-4732

The EFSA Journal is a publication of the European Food Safety Authority, an agency of the European Union.

EU-FORA SERIES 2

APPROVED: 31 July 2019

doi: 10.2903/j.efsa.2019.e170903

Development of food safety risk assessment tools based on molecular typing and WGS of *Campylobacter jejuni* genome

Istituto Zooprofilattico Sperimentale dell' Abruzzo e del Molise "G. Caporale", Teramo, Italy, AI Ardelean, P Calistri, A Giovannini, G Garofolo, A Di Pasquale, A Conte and D MorelliD

Abstract

The 'learning-by-doing' EU-FORA fellowship programme in the development of risk assessment tools based on molecular typing and WGS of *Campylobacter jejuni* genome was structured into two main activities: the primary one focused on training on risk assessment methodology and the secondary one in starting and enhancing the cooperation between the hosting and home organisations, or other joint activities. The primary activities had three subsequent work packages (WPs): WP1 data organisation, WP2 cluster and association analyses, and WP3 development of risk assessment models. The secondary activities have branched into one workshop and the initiation of a cooperation programme between the hosting and home organisations. In the last quarter, the fellow had contributed to the characterisation of some pathogens in possible response to a changing climate, part of the CLEFSA project. The fellow attended various forms of training: online and on-site courses, and also participated at several conferences and meetings for improving his knowledge and skills, contributing to performing the *Campylobacter* risk assessment and source attribution.

Keywords: *Campylobacter*, source attribution, risk assessment

Correspondence: eu-fora@efsa.europa.eu

Acknowledgements: This report is funded by EFSA as part of the EU-FORA programme.

Suggested citation: Istituto Zooprofilattico Sperimentale dell' Abruzzo e del Molise "G. Caporale", Teramo, Italy, Ardelean AI, Calistri P, Giovannini A, Garofolo G, Di Pasquale A, Conte A and Morelli D, 2019. Development of food safety risk assessment tools based on molecular typing and WGS of *Campylobacter jejuni* genome. EFSA Journal 2019;17(S2):e170903, 18 pp. https://doi.org/10.2903/j.efsa.2019.e170903

ISSN: 1831-4732

The EFSA Journal is a publication of the European Food Safety Authority, an agency of the European Union.

Table of contents

1. Introduction

1.1. About EU-FORA

For building the European Union risk assessment capacity and knowledge community, the European Food Safety Authority (EFSA) initiated the European Food Risk Assessment Fellowship Programme (EU-FORA), as part of the EFSA Strategic Objective. The programme is focused on training in risk assessment and in intensifying exchange and cooperation between different organisations and EFSA. The training is based on 'learning by doing' risk assessment methodologies and practices by involving the fellow in one project linked with food safety from the hosting site, where risk assessment is a significant part. The Istituto Zooprofilattico Sperimentale dell' Abruzzo e del Molise 'G. Caporale' (IZSAM) initiated many research projects in this field through the Italian National Reference Centre for Veterinary Epidemiology, Programming, Information and Risk Analysis.

1.2. General framework

The *Campylobacter* spp. are worldwide distributed bacteria and represent one important microbiological hazard linked with food-borne zoonosis. The gastroenteritis caused by *C. jejuni* and *C. coli* consists of 6 days watery or bloody diarrhoea, self-limiting disease in the majority of the cases, with or without associated fever, weight loss, cramps and headache (Man, 2011; MSD, 2019). Furthermore, *C. jejuni* is associated with a range of other gastrointestinal and extra-gastrointestinal infectious conditions including bacteremia and sepsis, also it may lead to autoimmune conditions known as Guillain–Barré syndrome (GBS), Miller Fisher syndrome, irritable bowel syndrome and Bell's palsy (unilateral facial paralysis), and colorectal cancer (Kaakoush et al., 2015; MSD, 2019). Because the precise role of *Campylobacter* species in the development of these clinical conditions is unknown and the highest prevalence of the gastrointestinal reported conditions caused by this genus, more accurate methodologies in their surveillance and monitoring are necessary. For these reasons the surveillance of *Campylobacter* spp. is part of the EFSA strategy. The legal framework for its monitoring is Regulation (EC) 2160/2003 (European Union, 2003a) on the control of Salmonella and other specified food-borne zoonotic agents and Directive 2003/99/EC on the monitoring of zoonosis and zoonotic agents, however, reporting *Campylobacter* infection is not mandatory in all countries (European Union, 2003b).

Considering the concern in public health of the *Campylobacter* spp. contaminations along the production chain of some food products, the IZSAM is currently involved in several epidemiology studies. The following IZSAM's units are involved: the Italian National Reference Centre for Veterinary Epidemiology, Programming, Information and Risk Analysis (COVEPI), the Italian National Reference Centre for Whole Genome Sequencing of microbial pathogens: database and bioinformatics analysis (GENPAT), and the Italian National Reference Laboratory for *Campylobacter* (NRL).

2. Description of work programme

2.1. Aims

The first objective of the work programme was 'learning-by-doing' of the fellow in the food safety risk assessment methodology, including collecting, normalisation, and analysis of the data and involving in developing and validation of a set of risk assessment epidemiological tools based on molecular typing and WGS of *Campylobacter jejuni* genomes.

The work programme had three subsequent work packages (WP), focussed on *C. jejuni*:

- WP1. Data organisation: focuses on reviewing the literature, collecting the raw data available, analysing, normalising and organising the data for subsequent epidemiological analyses.
- WP2. Cluster and association analyses: with the purpose to understanding and gaining practical skills in bioinformatics, becoming familiar with tools used in bioinformatics, being trained by doing various statistical methods and approaches for data analysis.
- WP3. Development of risk assessment models: through involvement in the development and validation of a set of risk assessment model considering the main *C. jejuni* genotypes and estimating their contribution in the whole exposure of consumers from Italy.

The second objective was to enhance relationships among home and hosting organisation. The IZSAM is FAO Reference Centre for Veterinary Epidemiology and OIE Collaborating Centre for

Veterinary Training, Epidemiology, Food Safety and Animal Welfare, and finding a solution for agreement and cooperation between organisations was considered.

The fellow has been part of the COVEPI's team involved in the activities of this working programme. In particular, two senior epidemiologists and a statistician have been part of the team, which worked closely with the bioinformaticians of the GENPAT, and the personnel of the NRL for *Campylobacter*. The two senior epidemiologists of the COVEPI's team have acted as mentors for the fellow, assisting him on a daily basis during all the activities carried out. A coordination meeting among the fellow, mentor and supervisor has been held regularly, on a weekly basis, to verify the work done and discuss the implications of the results obtained and plan subsequent activities. The fellow specifically worked on the analysis of the data generated by the molecular typing of *C. jejuni*, and in developing, testing and validation of different tools for the identification of spatiotemporal clusters of epidemiological relevance. The fellow had followed the whole data production and processing process, from the sequencing activities, carried out at laboratory to the bioinformatics analyses and statistical analyses performed on sequence data. In addition, the fellow had benefited greatly by the participation of IZSAM to the activities of the COHESIVE project, with his involvement in selected project meetings.

2.2. Activities/methods

2.2.1. WP1. Data organisation

In the beginning, the advanced literature search has been conducted regarding the EFSA and international guidance in risk assessment and source attribution based on whole genome sequencing and antimicrobial resistance (AMR), to learn and understand better what is the actual scientific level in the field and what are the best ways to approach the study (and not limited to that, including also the scientific literature in the field) (EFSA BIOHAZ Panel, 2013).

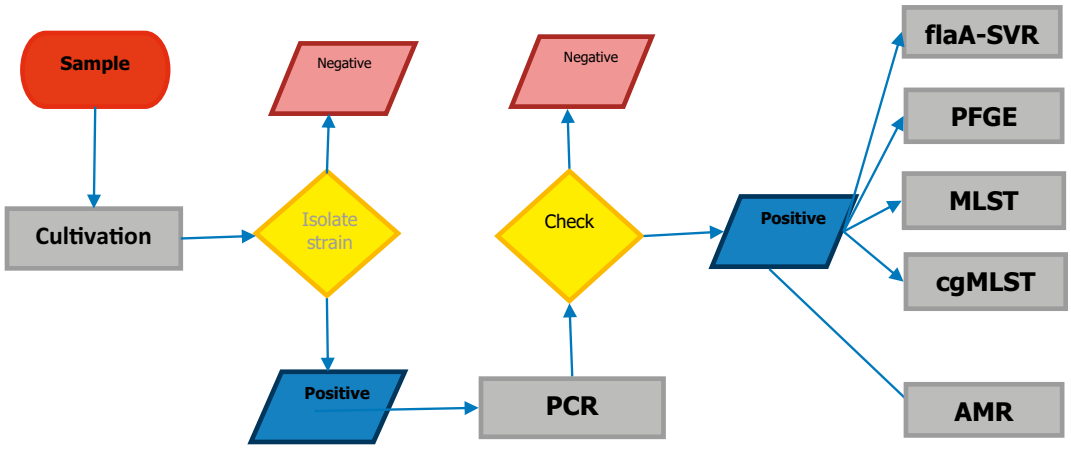


Figure 1: Laboratory flow chart for *Campylobacter* spp. analysis

The purpose of WP1 was to obtain the *Campylobacter* database containing the molecular typing results and related epidemiologically relevant metadata, ready to be analysed. The large part of this WP it was made in the first step at the Italian National Reference Laboratory (NRL) for *Campylobacter*. The protocol to *Campylobacter* spp. surveillance is routinely performed by Italian NRL's and is made by cultivation and identification according to EN ISO 10272 part 1 and 2 method (ISO, 2006: Parts 1 and 2). The genotyping characterisation of the isolates are made through species typing by molecularly confirmation by multiplex polymerase chain reaction (PCR) (Wang et al., 2002), flaA-SVR sequencing (Nachamkin et al., 1993), pulsed-field gel electrophoresis (PFGE) (Institue of Environmental Science and Research, 2013) and BioNumerics software version 7.6 (Applied Maths, 2019), in silico multilocus sequence typing (MLST) (Dingle et al., 2008; Jolley et al., 2018; Seemann, 2019), and since 2017, *C. jejuni* isolates are progressively submitted to Core Genome MLST (cgMLST) for a better discriminatory analysis (O'Mahony et al., 2011; Llarena et al., 2017). Part of these sequencing activities is carried out under various national and international projects. The AMR phenotype characterisation is made by testing of susceptibility to seven antimicrobials with a micro-broth dilution method using the 'Sensititre' automated system (TREK Diagnostic Systems, Biomedical Service, Italy) (Kittl et al., 2013; EFSA, 2019).

To date about 3,000 isolates of *Campylobacter* spp., respectively, collected in entire Italy, are available in the IZSAM's strains collection. In this step, the fellow was actively involved in some specific analytical technique linked with cultivation, genotypic and phenotypic characterisation of *Campylobacter*, having the opportunity to develop and improve his laboratory skills and get acquainted with the National Veterinary Information System (https://www.vetinfo.sanita.it/), managed by IZSAM. During this WP, the fellow had learned and understood the entire laboratory workflow (Figure 1) until obtaining the raw data and their recording into the Italian information system. At the same time, the fellow became familiar with the international *Campylobacter* MLST database (https://pubmlst.org/) (Jolley et al., 2018), and used it for comparison and normalisation of the data. IZSAM collects and registers a well-defined set of standardised data for each sample tested in its laboratories. In addition, several samples are collected in the framework of national control plans and all related data are registered into the National Veterinary Information System. All these factors allow IZSAM to retrieve relevant epidemiological data for all tested samples. These epidemiological metadata are fundamental for a correct interpretation of the microbiological results, including the outcomes of molecular typing and phenotyping (ex: antimicrobial resistance). A second step has been done in COVEPI, where a detailed data analysis plan has been developed, including the description of the dataset to be retrieved, the type of data quality checks to be performed and the format of the resulting validated databases. After extracting the raw data, the fellow retrieved, verified, normalised and organised the data for subsequent epidemiological analyses. These data were used in source attribution for human illness through microbial subtyping. (EFSA, 2008, 2019)

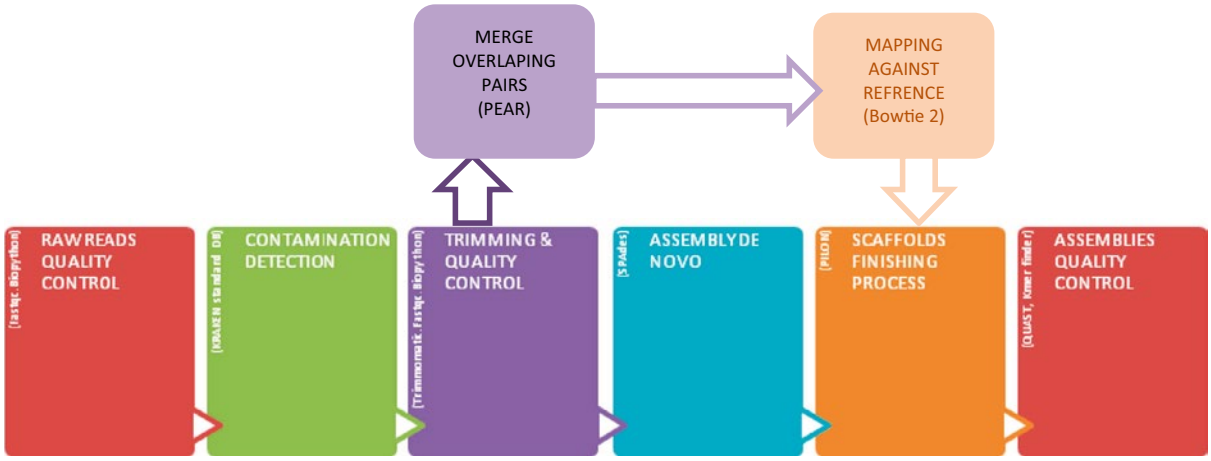


Figure 2: Bioinformatics flow chart for *Campylobacter* spp. analysis

2.2.2. WP2. Cluster and association analyses

WP2 was focused on obtaining the results from the analyses of molecular typing data and identification of main 'epi-clusters' of *C. jejuni* by analysing the genotypes and phenotypes data as well as epidemiological metadata. In the first step, the fellow had the opportunity to work in the Italian National Reference Centre for Whole Genome Sequencing of microbial pathogens (GENPAT). The bioinformaticians of the GENPAT supported the manipulation and analysis of sequencing data and introduced the fellow in bioinformatics and familiarised him to the bioinformatics software (Figure 3). For comparative analysis based on cgMLST data, the fellow learned and used the minimum spanning tree with GrapeTree software (Figure 2) (Zhou et al., 2018). The second step had been in COVEPI's Statistics and GIS Unit where the fellow was trained in using QGIS geographic information system software. During the third step, in the COVEPI, the fellow analysed the data for the purpose of verifying statistically significant genetic clusters of *Campylobacter* spp. (*C. jejuni)* associated with: the particular species, farm types, food production products; specific phenotypic characteristics, like AMR patterns; or specific spatiotemporal patterns and persistence in specific groups of farms. Working with a big amount of data was challenging, prompting to consider to use some business intelligence software like MicroStrategy.

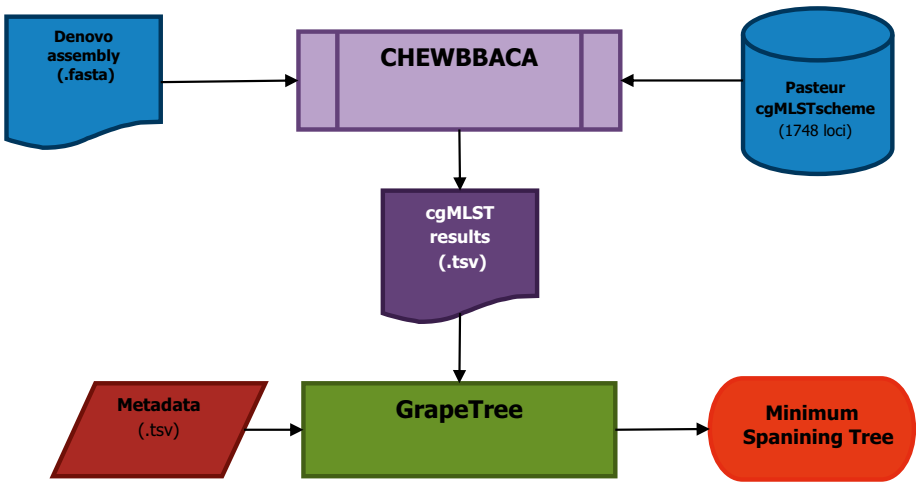


Figure 3: The cgMLST data flow chart for *Campylobacter* spp.

2.2.3. WP3. Development of risk assessment models

During WP3, in COVEPI unit, different source attribution methods had been used for finding and characterisation of the main *C. jejuni* genotypes and estimating their contribution the whole exposure of Italian consumers. The development and validation of a risk assessment model considering the genotypes and phenotypes were considered. In this step, the fellow analysed the microbial genotypic and phenotypic characteristics of the isolates, to find the common fingerprint and to define the 'epi-cluster'. Within the team, different biostatistical methodologies were tested.

Another approach has been attempted in order to define a particular methodology designated to assess the sources of uncertainties in source attribution models used in *Campylobacter* microbial risk assessment (MRA). The considered methodologies were: failure mode effect analysis (FMEA), fault tree analysis (FTA) and key process indicators (KPI)/quality indicators (QIs). Before starting the assessment, the actors and the stages needed to be defined (pre-preanalytical, preanalytical, analytical, postanalytical and post-postanalytical).

2.2.4. Secondary activities

Secondary activities are referred about agreements and cooperation between hosting sites and organisations of origin, cooperation between different organisations and also to other activities not mentioned before, in which the fellow played an active role.

2.2.4.1. Agreements and cooperation

In view of recent epidemics in Europe, taking advantage of this fellowship programme during this period, the foundations have been laid for establishing and strengthening the collaboration between the national reference centres in Italy and Romania, from the IZSAM, Teramo, and the Institute for Diagnosis and Animal Health (IDAH), Bucharest. The first step was to have 2 days 'Animal health risk assessment and vector-borne diseases' workshop during the 2–3 April 2019 for the specialists from IDAH in Bucharest, under scientific coordination of the fellow and participation of the tutors from IZSAM, Paolo Calistri, and Federica Monaco, and from Agricultural Research Council – Onderstepoort Veterinary Research (ARC-OVR), Gert Venter. The second step was the assessment of the needs for professional training in epidemiology for Romanian specialists, for the purpose to find the optimum solution to improve and increase the animal health risk assessment capacity. Depending on the size of the training needs, consideration has been given to the temporary training of a limited number of specialists at the IZSAM, or the initiation of a twinning project, if the needs are more complex. This process is in progress.

In the same context, the European BioSafety Association (EBSA), during their 22nd annual conference at Bucharest, from the total of 42, offered sponsored participation for 24 specialists from Romania including from IDAH and ANSVSA, for participation at the preconference courses (8) in the field of biosafety and biosecurity, and also for participation at the conference (6). Considering the importance of the topics in this region, EBSA increased more than fourth times the numbers of sponsored participation for the persons from Balkan region (Romania, Bulgaria, Republic of Moldova,

Albania, Croatia, Ukraine, Georgia, and Greece) for this year. After this successful experience, different types of collaboration between organisations and their members have started to be considered.

In the last quarter of the EU-FORA programme, the fellow had contributed to the characterisation of some pathogens (like *Campylobacter jejuni*) in possible response to a changing climate, in terms of possible increase in exposure or pathogenicity under a specific climate change scenario, part of the CLEFSA project, coordinated by Angelo Maggiore (EFSA).

2.2.4.2. Additional activities

During 13–15 November 2018, the fellow participated at the Romanian National Sanitary Veterinary and Food Safety Authority (ANSVSA) meeting in Baia-Mare, Romania, for the presentation of the EU-FORA fellowship programme and introduction in risk management, and basic concepts in risk assessment, to the specialists from the national laboratory network.

The fellow participated in the workshop 'Accounting for uncertainty in data-poor scenarios: Case studies on risk analysis in food safety' and at the International Conference on Uncertainty in Risk Analysis, 20–22 February 2019, Berlin at the German Federal Institute for Risk Assessment (BfR), with 'Uncertainty assessment in *Campylobacter* spp. source attribution models: some qualitative approaches' poster presentation at 'Methods for uncertainty analysis' thematic area.

The fellow participated at the 22nd annual international conference 'Burning topics in Biosafety' of the EBSA at Bucharest, Romania, in 2–5 April 2019, where together with the experts Paolo Calistri (IZSAM) and Uwe Mueller-Doblies (MSD), moderated the break-out 'Biosafety and biosecurity in the field in case of an emergency'. Also, the fellow presented two posters: 'The exposure to *Campylobacter* spp. of the food industry workers: a short overview' and 'EFSA EU-FORA – The European Food Risk Assessment Fellowship Programme'.

Like invited guest and collaborator, the fellow participated at the 'Transylvanian Experimental Neuroscience Summer School – TENSS 2019' during 15–16 June 2019, Pike Lake, Romania, organised by the Transylvanian Institute of Neuroscience (TINS).

3. Conclusions

3.1. Risk assessment in *C. jejuni*

The EFSA EU-FORA 'learning by doing' programme is among the few from Europe which is also addressed to mid-career scientists and open to those who do not necessarily come from the academic field, being a real fortune for people from East European countries. This was a great opportunity for the fellow to consolidate his specialised knowledge and skills in food safety and veterinary epidemiology and public health, by working in a prestigious international and national reference centre. He gained experience by participating in the dedicated program and other activities within the host organisation, better understanding the complex workflow in the specific risk assessment and, at the same time, learned how to investigate an outbreak epidemic, its strengths and weaknesses.

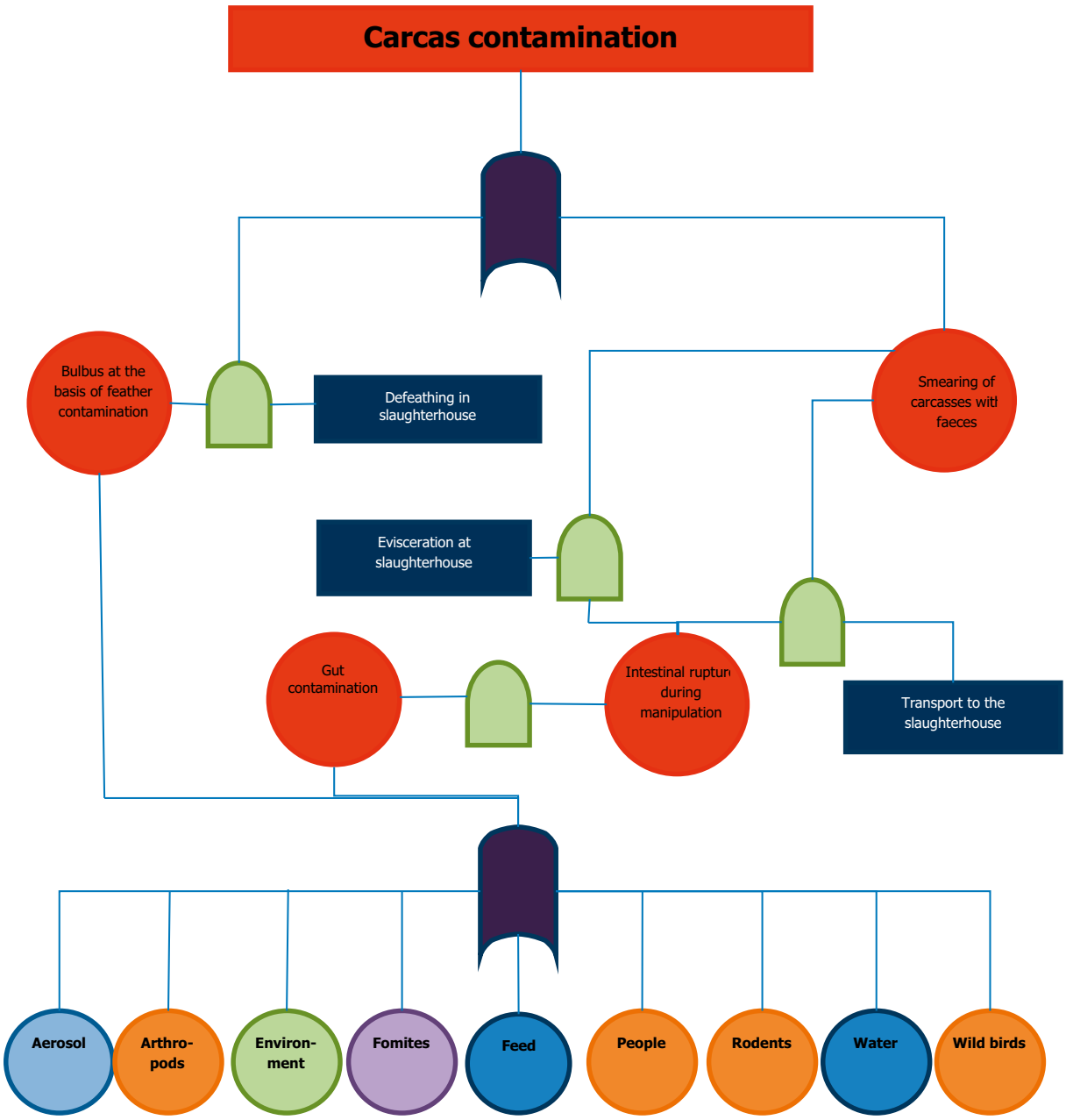


Figure 4: Fault tree analysis diagram in poultry carcass contamination, considering the source of *Campylobacter* spp. from poultry

This training offered the opportunity to work both independently and in a team, to integrate the multidisciplinary and evidence-based veterinary medicine approach into assessing the risk of *Campylobacter* and assigning the source in particular.

Applying microbial subtyping methodology in *Campylobacter* source attribution, some characteristics of its population in Italy has been identified. The complexity and the high volume of data provided by cgMLST, at this time, in the absence of a common database, rendered somehow difficult the source attribution based just on this information, anyway, the evidence indicates the existence within *C. jejuni* population of one 'relatively stable' cluster and one 'very dynamic'. Analysing the AMR fingerprint data of the 'relative stabile' *C. jejuni* population, considering the characteristics of the antimicrobial tested, even in the absence of the direct evidence, has supposed that the population had been selected from strains located from another site than the intestinal tract of the animals. The data gathered were not obtained on the basis of a well-defined sampling program, however, the complexity of the information obtained and analysed allows the initiation of a description of the *C. jejuni* population from Italy, that

will represent the foundation for building the common database and harmonised methodology for sub-typing, analysis and storage the data and, in the future, the development of linkage mechanisms (EFSA BIOHAZ Panel, 2013, 2014).

After applying in microbial risk assessment in poultry of some basic cause-effect and effect-cause models (like FMEA respectively FTA) and key process indicators methodologies, a potential scoring system and KPI have been designed and proposed (Table 1) (Wikipedia, the free encyclopedia, 2019a, b) The results were used to draw the flow chart in source attribution in poultry (Figure 4).

During this programme, the fellow had gained skills in using different software like GrapeTree, QGIS, MicroStrategy and had been initiated in using R.

3.2. Building cooperation

The future agreement and collaboration between the IZSAM, Teramo, and the IDAH, Bucharest, represents a priority especially for the Romanian part. In this context, several fields for collaboration have been designed, like laboratory activity and epidemiology, public health, and risk analysis.

Many fellows from the 2nd series of the EU- FORA programme had worked together at the poster for the EBSA conference, named 'The exposure to Campylobacter spp. of the food industry workers: a short overview'. The greatest gain of this project is represented by the established human relationships and networking. Starting with relationships with the EFSA coordinators experts, continuing with program coordinators, tutors, experts, and personnel from the hosting sites, and last but not least with the fellow colleagues, this series adds a brick at the building of the future of the EU.

Table 1: The list of some key performance indicators (KPI)/quality indicators (QIs) tailored for risk assessment

Stage	Weighting field	KPI/QIs
The KPI with priority 1		
Pre-preanalytical	Weighting the average of the event	a) Number of events, incident or accident/1,000 units of goods b) Number of events, incident or accident/1,000 units of time c) Number of positive *Campylobacter* spp. samples/1,000 units of goods d) Number of positive *Campylobacter* spp. samples/units of time e) Number of positive *Campylobacter* spp. CFU/g per 1,000 units of goods number of positive *Campylobacter* spp. CFU/g per units of time
Pre-preanalytical	Weighting the average of the manufacturing process	a) Number of goods/units of time b) Number of servings/unit of goods c) Number of personnel/organisation d) Number of personnel necessary/1,000 units of goods e) Number of personnel necessary/units of time
Pre-preanalytical	Weighting the average of the transport process	a) Number of goods/units of transport b) Number of units of transport/unit of time c) Number of goods transported/units of time

CFU: colony forming unit.
The KPI was defined with priority 1, compulsory; 2, important; 3, proposed; 4, valuable.

References

Applied Maths, 2019. BioNumerics version 7.6 released. Available online: http://www.applied-maths.com/bionumerics

Dingle KE, McCarthy ND and Cody AJ, 2008. Extended sequence typing of *Campylobacter* spp., United Kingdom. Emerging Infectious Diseases, 14, 1620–1622. https://doi.org/10.3201/eid1410.071109

European Union, 2003a. Directive 2003/99/EC of the European Parliament and of the Council on the monitoring of zoonoses and zoonotic agents, amending Council Decision 90/424/EEC and repealing Council Directive 92/117/EEC. Available online: https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32003L0099&qid=1561878805470&from=EN

European Union, 2003b. REGULATION (EC) No 2160/2003 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL on the control of *Salmonella* and other specified food-borne zoonotic agents. Available online: https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32003R2160&from=EN

EFSA (European Food Safety Authority), 2008. Scientific Opinion of the Panel on Biological Hazards on a request from EFSA on Overview of methods for source attribution for human illness from food borne microbiological hazards. EFSA Journal 2008;17(6):764, 143 pp. https://doi.org/10.2903/j.efsa.2008.764

EFSA (European Food Safety Authority), 2019. Technical specifications on harmonised monitoring of antimicrobial resistance in zoonotic and indicator bacteria from food-producing animals and food. EFSA Journal 2019;17 (6):5709, 122 pp. https://doi.org/10.2903/j.efsa.2019.5709

EFSA BIOHAZ Panel (EFSA Panel on Biological Hazards), 2013. Scientific Opinion on the evaluation of molecular typing methods for major food-borne microbiological hazards and their use for attribution modelling, outbreak investigation and scanning surveillance: Part 1 evaluation of methods and application. EFSA Journal 2013;11 (12):3502, 84 pp. https://doi.org/10.2903/j.efsa.2013.3502

EFSA BIOHAZ Panel (EFSA Panel on Biological Hazards), 2014. Scientific Opinion on the evaluation of molecular typing methods for major food-borne microbiological hazards and their use for attribution modelling, outbreak investigation and scanning surveillance: Part 2 (surveillance and data manage. EFSA Journal 2014;12(7):3784, 46 pp. https://doi.org/10.2903/j.efsa.2014.3784

Institue of Environmental Science and Research, 2013. Standard Operating Procedure for PulseNet PFGE of *Campylobacter jejuni*. (T. I. (ESR), Editor). Available online: http://www.pulsenetinternational.org/assets/PulseNet/uploads/pfge/PNL03_CampyPFGEprotocol.pdf

ISO 10272-1:2006 (en) Microbiology of the food chain — Horizontal method for detection and enumeration of *Campylobacter* spp. — Part 1: Detection method.

ISO 10272-2:2006 (en) Microbiology of the food chain — Horizontal method for detection and enumeration of *Campylobacter* spp. — Part 2: Colony-count technique.

Jolley KA, Bray JE and Maiden MC, 2018. Open-access bacterial population genomics: BIGSdb software, the PubMLST.org website and their applications [version 1; peer review: 2 approved]. Wellcome Open Reserach, 3, 124. https://doi.org/10.12688/wellcomeopenres.14826.1

Kaakoush N, Castaño-Rodríguez N, Mitchell HA and Man SM, 2015. Global epidemiology of *Campylobacter* infection. Clinical Microbiology Reviews, 28, 687–720. https://doi.org/10.1128/cmr.00006-1

Kittl S, Heckel G, Korczak BM and Kuhnert P, 2013. Source attribution of human *Campylobacter* isolates by MLST and Fla-typing and association of genotypes with Quinolone resistance. PLoS ONE, 8, e81796. https://doi.org/10.1371/journal.pone.0081796

Llarena A, Taboada E and Rossi M, 2017. Whole-genome sequencing in epidemiology of *Campylobacter jejuni* infections. Journal of Clinical Microbiology, 55, 1269–1275. https://doi.org/10.1128/JCM.00017-17

Man S, 2011. The clinical importance of emerging *Campylobacter* species. Nature Reviews Gastroenterology and Hepatology, 25, 669–685. https://doi.org/10.1038/nrgastro.2011.191

MSD, 2019. MSD Manual Professional Version. Available online: https://www.msdmanuals.com/professional/infectious-diseases/gram-negative-bacilli/campylobacter-and-related-infections?query=campylobacter%20jejuni

Nachamkin I, Bohachick K and Patton CM, 1993. Flagellin gene typing of *Campylobacter jejuni* by restriction fragment length polymorphism analysis. Journal of Clinical Microbiology, 31, 1531–1536. https://doi.org/0095-1137/93/061531-06$02.00/0

O'Mahony E, Buckley JF, Bolton D, Whyte P and Fanning S, 2011. Molecular epidemiology of *Campylobacter* isolates from poultry production units in Southern Ireland. PLoS ONE, 6, e28490. https://doi.org/10.1371/journal.pone.0028490

Seemann T, 2019. mlst Github. Available online: https://github.com/tseemann/mlst

Wang G, Clark CG, Taylor TM, Pucknell C, Barton C, Price L, Woodward DL and Rodgers FG, 2002. Colony multiplex PCR assay for identification and differentiation of *Campylobacter jejuni*, *C. coli*, *C. lari*, *C. upsaliensis*, and *C. fetus* subsp. *fetus*. Journal of Clinical Microbiology, 40, 4744–4747. https://doi.org/10.1128/jcm.40.12.4744-4747.2002

Wikipedia, the free encyclopedia, 2019a. Failure mode and effects analysis. Available online: https://en.wikipedia.org/wiki/Failure_mode_and_effects_analysis

Wikipedia, the free encyclopedia, 2019b. Fault tree analysis. Available online: https://en.wikipedia.org/wiki/Fault_tree_analysis

Zhou Z, Alikhan NF, Sergeant MJ, Luhmann N and Vaz C, 2018. GrapeTree: visualization of core genomic relationships among 100,000 bacterial pathogens. Genome Research, 28, https://doi.org/10.1101/gr.232397.117

Abbreviations

AMR	antimicrobial resistance
ANSVSA	Romanian National Sanitary Veterinary and Food Safety Authority
ARC-OVR	Agricultural Research Council – Onderstepoort Veterinary Research, South Africa
BfR	German Federal Institute for Risk Assessment
CFU	colony forming unit

cgMLST	Core Genome MultiLocus Sequence Typing
CLEFSA	Climate change as a driver of emerging risks for food and feed safety, plant, animal health and nutritional quality (CLEFSA)' project conducted by the EFSA Scientific Committee - Emerging Risks Unit (SCER)
COHESIVE	Cohesive –One Health Structure In Europe- is a 3-year project, which aims to develop sustainable One Health approaches with respect to signalling, assessing and controlling zoonoses at the national level within EU countries and across borders
COVEPI	Italian National Reference Centre for Veterinary Epidemiology, Programming, Information and Risk Analysis (IZSAM)
EBSA	European BioSafety Association
EU-FORA	The European Food Risk Assessment Fellowship Programme
FAO	The Food and Agriculture Organization of the United Nations
flaA-SVR	The DNA sequence of the flagellin A short variable region
FMEA	failure mode effect analysis
FTA	fault tree analysis
GENPAT	Italian National Reference Centre for Whole Genome Sequencing of microbial pathogens: database and bioinformatics analysis (IZSAM)
IDAH	Institute for Diagnosis and Animal Health, Bucharest, Romania
IZSAM	Istituto Zooprofilattico Sperimentale dell' Abruzzo e del Molise "G. Caporale", Teramo, Italy
KPI	key process indicators
MLST	multilocus sequence typing
MRA	microbial risk assessment
MSD	Merck Sharp & Dohme Corp., a subsidiary of Merck & Co., Inc. Kenilworth, NJ, USA
NRL	National Reference Laboratory
OIE	The World Organisation for Animal Health
PCR	polymerase chain reaction
PFGE	pulsed-field gel electrophoresis
QIs	quality indicators
TEENS-2019	Transylvanian Experimental Neuroscience Summer School –2019
TINS	The Transylvanian Institute of Neuroscience, Cluj-Napoca, Romania
VBD	vector-borne diseases
WGS	whole genome sequencing
WP	work package

Appendix A – The on-site courses and training where fellow attended

Subject	Organisation	Location	Period	Time	Tutor's
Internal training on risk assessment methods	IZSAM	Teramo, Italy	29.10.2018	6 h	Paolo Calistri
Introduction in bioinformatics	IZSAM	Teramo, Italy	8.11.2018	2 h	Adriano Di Pasquale Antonio Rinaldi
The use of GIS and QGIS software	IZSAM	Teramo, Italy	13.3–13.4.2019	16 h	Susanna Tora
Microstrategy Dashboarding Data with Dossiers	Microstrategy	Milano, Italy	11.3.2017	8 h	Stefano Sartorio
Microstrategy Dashboarding Data with Dossiers	Microstrategy	Roma, Italy	6.5.2017	8 h	Stefano Sartorio
Microstrategy Advanced Reporting	Microstrategy	Roma, Italy	7.5.2017	8 h	Stefano Sartorio
Microstrategy Enterprise Mobility	Microstrategy	Roma, Italy	8.5.2017	8 h	Stefano Sartorio
Microstrategy Enterprise Applications	Microstrategy	Roma, Italy	9.5.2017	8 h	Stefano Sartorio
Transylvanian Experimental Neuroscience Summer School – TENSS 2019 (advanced modelling of biological systems, basic and advanced concepts in signal processing, statistical methods for the evaluation of dynamical systems, principal component analysis, machine learning)	TINS	Pike Lake, Romania	15–16 June 2019	6 h	Raul C. Muresan Vasile V.Moca Christian Machens Fede Carnevale

Appendix B – The online courses and training where fellow attended

Subject	Organisation	Period	Time	Tutor's
The FoodEx2 classification system and guidance on its harmonised use	EFSA	26 September 2018	1 h	Sofia Ioannidou Laura Kirwan Alban Shahaj
The FoodEx2 classification system and guidance on its harmonised use	EFSA	3 October 2018	1 h	Sofia Ioannidou Laura Kirwan Alban Shahaj
How to report surveillance data on Transmissible Spongiform Encephalopathies using the EFSA tool	EFSA	21 January 2019	1 h	
Learn more about the risk assessment of phthalates used in plastic food contact materials	EFSA	15 March 2019	1 h	
EFSA's new dedicated support to SMEs	EFSA	24 May 2019	1/2 h	Remigio Marano Patricia Romero
EPI-interactive webinar: Introduction to R Shiny	EPI-Interactive	30 May 2019	1 h	Uli Muellner
IHU BioSecurity Free Webinar	International Hellenic University	15 May 2019	2 h	Gijsbert van Willigen Patrick Rüdelsheim
Medical School Pathology Courses	Dr. Minarcik's Online Medical School Pathology Course	1.9.2018–31.5.2019	24 h	John R. Minarcik
Illness Outbreaks linked to Enteric Zoonoses and the Interconnectedness of Human and Animal Health	Pet Poison Helpline	24 April 2019	1 h	Megin Nichols

Appendix C – The summary agenda of 'Animal health risk assessment and vector-borne diseases' workshop at Bucharest

Subject	Tutors
The *Culicoides* diseases in an endemic area	G. Venter
The vector collection methods	G. Venter
Workgroup schedule	G. Venter/A.I. Ardelean
Epidemiological surveillance and risk factors	P. Calistri
Introduction to risk assessment in animal health	P. Calistri
Lumpy skin disease: epidemiology and control aspects	F. Monaco/P. Calistri
Surveillance of West Nile disease in Italy: example of an integrated One Health approach	F. Monaco/P. Calistri
Geographical information systems: tools for VBD surveillance and control	P. Calistri

VBD: vector-borne disease.

Annex A – Poster Uncertainty assessment in *Campylobacter* spp. source attribution models: some qualitative approaches

"Challenges and Advances in Assessing, Managing and Communicating Uncertainty"
EFSA/BfR International Conference on Uncertainty in Risk Analysis
21–22 February 2019, Berlin, Germany

EU-FORA

Uncertainty assessment in *Campylobacter* spp. source attribution models: some qualitative approaches

Adrian I. Ardelean [1, 2, 3], Paolo Calistri[2], Armando Giovannini[2], Daniela Morelli [2], Guido Di Donato [2], Francesca Dall'Acqua [2], Antonio Maitino[2], Francesca Cito[2], Maria Luisa Danzetta[2], Daria Di Sabatino [2], Simona Iannetti[2], Valentina Zenobio[2]

[1] European Food Safety Authority EU-FORA Fellow
[2] Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise "G. Caporale", Italy
[3] Sanitary Veterinary and Food Safety Directorate Cluj, Romania

Introduction:

In the majority of the EU countries, with the exception of Nordic countries, about 30-50% of broiler flocks are contaminated when tested at the end of the rearing period, before slaughtering. In the contaminated flocks about 80-100% of animals have *Campylobacter* spp. in the caeca and intestines, without any clinical sign. Not all isolates have virulence characteristics and let to be consider the fact the *Campylobacter* spp. can be part from normal gut microbiota. The prevalence of contaminated carcasses varies among countries. In Italy, and similarly to other EU countries, the proportion of contaminated broiler carcasses is around 40%, but with the great majority of these with low levels of contamination. Many hypotheses have been done to explain this high level of contamination in flocks. A different approach considering the organization activities was taken.
Various approaches have been used to assess the contribution of broiler meat to the burder of campylobacteriosis in humans. The continuous improvement of microbiological risk assessment (MRA) and the development of sophisticated source attribution models, incorporating genomic sub-typing data, are more often used as investigating and food safety control prioritization approaches with a certain success. However, these methods are introducing new sources of uncertainties, often difficult to properly evaluate. It has been already postulated by Donald H. Rumsfeld in 2002 "The silent diagnosis" which underlined that there are two types of unknowns: known unknowns, and unknown unknowns. This study has the purpose to define some methodologies designated to assess the sources of uncertainties in source attribution models used in *Campylobacter* MRA. The present assessment is linked to EFSA EU FORA fellowship program cohort 2018-2019

Fig 1. The food cycle

Fig. 2. Cause-effect diagram

Method:

For the purpose of identifying the uncertainty sources were considered some methodologies as: FMEA, FTA, and KPI/ QIs. Before starting the assessment, the entire workflow has been designed, and the key performance indicators/ quality indicators have been defined. The following stages were defined: pre-preanalytical, preanalytical, analytical, postanalytical and post-postanalytical. Also, the actors were defined for each stage.

Table 1. The health effect factor (f_s)

Nr. crt	The category by effect on health	The value of f_s
1	without harm on individual and collective health	1
2	with minimal harm (without morbidity)	0.75
3	with minor harm (minor morbidity)	0.50
4	with moderate harm (moderate morbidity)	0.25
5	with major harm (major morbidity)	0

Results:

During the fault tree analysis (FTA) by using cause-effect diagram, some failure mode events were identified. The standard FMEA does not contain any direct index linked with MRA, therefore, for this purpose the health effect factor (f_s) was considered (Table1). Another useful scoring to use can be CDC Pandemic Severity Index. After the assessment, was tailored the using of the key process indicators (KPI), some of them are listed in Table 2. The KPI linked with the preanalytical, analytical and postanalytical were also considered. The major risk categories were adapted and proposed to be linked with: air, fomites, effluents, people, biologicals and security.

Email: ardelean.adrian-cj@ansvsa.ro

Table 2. The list of some key performance indicators (KPI)/ quality indicators (QIs) tailored for RA

The KPI with priority 1				
1	Pre-preanalytical	1	Weighting the average of the event	a) number of events, incident or accident/ 1000 units of goods b) number of events, incident or accident/ 1000 units of time c) number of positive Campylobacter spp. samples/ 1000 units of goods d) number of positive Campylobacter spp. samples/ units of time e) number of positive Campylobacter spp. UFC/ g / 1000 units of goods f) number of positive Campylobacter spp. UFC/ g / units of time
2	Pre-preanalytical	1	Weighting the average of the manufacturing process	a) number of goods/ units of time b) number of servings/ unit of goods c) number of personnel/ organization d) number of personnel necessary/ 1000 units of goods e) number of personnel necessary/ units of time
3	Pre-preanalytical	1	Weighting the average of the transport process	a) number of goods/ units of transport b) number of units of transport / unit of time c) number of goods transported/ units of time
The KPI were defined with priority 1, compulsory; 2, important; 3, proposed; 4, valuable				

Discussion:

For a powerful MRA a harmonized mutual agreed methodology (referring at FMEA, FTA and KPI) is more than necessary to use, given that it can offer the opportunity to improve it continuously. Considering *Campylobacter* spp. MRA, in particularly for poultry, where there is evidence that contamination occurs in the farm, and it is not very clear how it has happened, the weighting of the entire processes can lead in identifying the source of contamination, also the breaks in the biosecurity measures. New theories presume that the source is linked to surviving in the environment outside or inside the buildings, on the transport vehicles, or/and introducing by personnel during thinning process (the repeated entering of persons into the flock for the selection and removal of animals), so the NGS studies are ongoing trying to explore the accuracy of this hypothesis

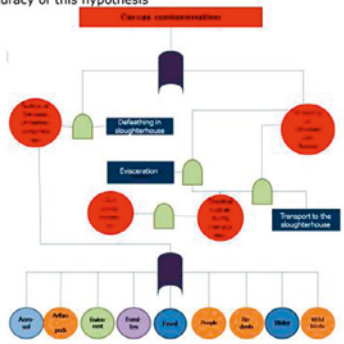


Fig. 3. Fault tree analysis diagram in poultry carcass contamination, considering the source of *Campylobacter* spp. from poultry

This kind of approach has the advantages of defining and monitoring the entire process from an organization, in a uniform way, offering the opportunity to find the gap that can be linked with foodborne diseases. Combining different qualitative methodologies in the MRA, such FMEA, and FTA, and using KPI/ QIs applied to all processes, can lead to enhancing the consistency of the evaluation and can be a useful approach for uncertainty mitigation, proving that these can be useful tools. However, there are some limitations because a deep and exhaustive approach is time and resource consuming. Alternatively, by involving more participants, a standardized approach might enhance in time the MRA through using FMEA and FTA. The KPI/QIs are very useful for total process monitoring and a mutual standardization of them will lead to obtaining benefits.
Through standard definitions and weighting of all processes, a real-time methodology can be developed for risk assessment and for applying more advanced methodologies in the future, such as the one based on the artificial neural network system.

Annex B – Poster EFSA EU-FORA – The European Food Risk Assessment Fellowship Programme

EBSA22- Annual Meeting of the European Biosafety Association

4-5 April 2019

Caro Hotel, Bucharest, Romania

EU-FORA

EFSA EU-FORA – The European Food Risk Assessment Fellowship Programme

Stylianos KOULOURIS[1], Cristina Alonso ANDICOBERRY [1], Christoph UNGER [2], Adrian I. ARDELEAN [3]

EU-FORA Fellowship Programme

[1] European Food Safety Authority
[2] Austrian Agency for Health and Food Safety
[3] European Food Safety Authority EU-FORA Fellow at Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise "G. Caporale", Italy

EU-FORA – The European Food Risk Assessment Fellowship Programme

Is part of the EFSA Strategic Objective to build the European Union risk assessment capacity and knowledge community by attracting early and mid-career scientists to become risk assessors through. Learning by doing, harmonizing risk assessment methodologies and practices across Europe, and intensifying exchange and cooperation among national food agencies and EFSA are some of the activities planned to achieve that goal.

EU-FORA's target audience is focused on the area of biological and chemical food safety, in order to attract scientists with the following backgrounds:

- Molecular Biology,
- Biology,
- Microbiology,
- Biochemistry,
- Chemistry,
- Veterinary/Human Medicine
- Agronomy/Agricultural
- Environmental Science,
- Food Science and Technology,
- Toxicology.

The EU-FORA concept

Is based on learning by doing and practicing through a 1 year fellowship placement in a competent authority of a Member State, with high capacity in performing Food Risk Assessment, accompanied by a specific and uniform risk assessment training programme for 15 fellows (and 15 additional participants in Parma) during the programme:

- three-week induction training in Parma at EFSA premises in September,
- one-week training module in Vienna at Austrian Agency for Health and Food Safety (AGES),
- one-week training module in Berlin at the German Federal Institute for Risk Assessment (BfR), and
- one-week training module in Athens at Hellenic Food Authority (EFET).

Email: ardelean.adrian-cj@ansvsa.ro

The EU-FORA Cohort 2018-2019

Is composed of scientists with professional backgrounds in: veterinary medicine, animal science, agriculture, chemistry, biology, ecotrophology, pharmacy, biotechnology, food science and safety, nutrition, marine biology, and toxicology/ecotoxicology.

The participants come from Cyprus, Germany, Greece, Italy, Portugal, Romania, Spain, and UK.

The EU-FORA Cohort 2019/2020 and 2020/2021

Cohort 3 and 4: EFSA has started a new open call for applications from potential fellows and hosting sites for the cycles 2019/2020 and 2020/2021 of its EU-FORA Fellowship Programme.

Acknowledgment: To the staff of EFSA, Austrian Agency for Health and Food Safety, German Federal Institute for Risk Assessment, The Hellenic Food Authority together with all the tutors and technical personnel involved in the trainings. To the EU-FORA fellows cohort 2018-2019: Elena Anastasi, Ricardo Manuel Abreu de Assunção, Maria Cabral, Giorgiana Mihaela. Cătunescu, Eleni Chatzidimitriou, Laura Escrivá Llorens, Carolin Fechner, Juliana Rodrigues Gadelha, Cristiano Garino, Chrystalleni Hadjicharalambous, Márcia de Jesus Monteiro; Dimitrios Pavlidis, Irina Smeu, Christina Vlachou.

Annex C – Poster The exposure to *Campylobacter* spp. of the food industry workers: a short overview

EBSA22- Annual Meeting of the European Biosafety Association
4-5 April 2019
Caro Hotel, Bucharest, Romania

EU-FORA

The exposure to *Campylobacter* spp. of the food industry workers: a short overview

EU-FORA Fellowship Programme

Adrian I. Ardelean[1], Paolo Calistri[2], Carolin Fechner[1], Dimitrios Pavlidis[1], Chrystalleni Hadjicharalambous[1], Márcia Monteiro[1], Christina Vlachou[1], Laura Escrivá[1], Ricardo Assunção[1], Giorgiana Catunescu[1]

[1] European Food Safety Authority, EU-FORA Fellow
[2] Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise "G. Caporale", Italy

INTRODUCTION
Infection by Campylobacter spp. is the main foodborne zoonotic disease in Europe, despite a still high number of cases remaining non-identified. Therefore the risk of exposure for meat food industry workers (0.84 million employees in the EU) is currently underestimated. This infection presents high relevance due to issues related with:
- human safety and security at work
- risk to remain residual source of contamination

MATERIAL AND METHODS
A literature-based research and a qualitative risk assessment of worker's exposure to this agent were performed based on the established risk assessment steps (Fig. 1).

Fig 1. The risk assessment steps

Target population category
Poultry slaughterhouses employees
Qualitative methods
- supporting method (brainstorming)
- scenario analysis
- function analysis

Control measures evaluated to mitigate the risk
- engineering control
- administrative control
- personal control

RESULTS AND DISCUSSION
Hazard identification/characterization
Campylobacter spp. is a fastidious bacteria, sensitive to desiccation at high/low temperatures with specific growth requirements. Transmission occurs mainly through oral route by ingestion of undercooked poultry meat and poultry products, or aerosol inhalation (infectious dose: 500-800 CFU).

Workflow evaluation (Fig. 2)
→ **Main critical points identified:**
a) place for handling the live bird
b) evisceration
c) digestive tract manipulation
d) waste material manipulation

→ **Possible transmission source:** defeathering process with contaminated aerosols. Further investigations are required.

→ **Control measures for risk mitigation**
a) interruption of the contamination chain
b) engineering control of waste material
c) development of good sewage system
d) good hand hygiene (determinant role in personal exposure)

→ **Early detection measures**
Introduction of an optimized control program for asymptomatic/ symptomatic employees detection according with the level of activity.

Fig. 2. The workflow diagramme
(highlighted the main critical points identified)

CONCLUSION:
The food industry workers, in our case the personnel from the poultry slaughterhouses, can be exposed to different health hazards. From the category of biological agents, *Campylobacter* spp. is one of high relevance.
According to the performed qualitative risk assessment, some main critical points during the poultry slaughter were identified and could present risk for the workers involved in the process, due to *Campylobacter* spp. exposure. Further research, through a quantitative approach, should be implemented to characterize the risk and contribute to the establishment of control measures.

Acknowledgment: EU-FORA fellowship programme (EFSA), cohort 2018-2019.

Email: ardelean.adrian-cj@ansvsa.ro

EU-FORA SERIES 2

APPROVED: 31 July 2019

doi: 10.2903/j.efsa.2019.e170904

Influence of the geographical origin on substance concentrations in herring as basis for dietary exposure assessments

C Fechner[1], S Frantzen[2], O Lindtner[3], GH Mathisen[1] and ITL Lillegaard[1]

[1]Norwegian Institute of Public Health – Norwegian Scientific Committee for Food and Environment, Norway; [2]Institute of Marine Research, Norway; [3]German Federal Institute for Risk Assessment, Germany

Abstract

Previous investigations on agricultural products showed that geographical origin influences concentrations of selected undesirable substances and ultimately dietary exposure assessment. This could also be relevant for fish from different catching areas, as substance concentrations have been found to vary between catching areas. Herring was chosen as an example. Norwegian and German data on consumption and substance concentrations were considered. To investigate if concentrations of substances are different in Norway and Germany, monitoring data between 2012 and 2017 were used. Norway provided data of commercial catching areas from the Norwegian Spring Spawning (NSS) herring stock, while Germany had market data available. Concentrations of cadmium, mercury and selenium tended to be higher in herring from Norway, while lead concentrations were higher in Germany. Polychlorinated dibenzo-*p*-dioxins/polychlorinated dibenzofurans (PCDD/Fs), dioxin-like polychlorinated biphenyls (DL-PCBs) and non-dioxin-like PCBs (NDL-PCBs) tended to have higher concentrations in Germany, while perfluorinated alkylated substances (PFAS) were mostly below quantifiable levels in the two countries. These differences could be attributed to different herring stocks available on the market in Germany and Norway. Country-specific data on consumption and substance concentrations give a basis for a refined exposure assessment covering both the Norwegian and the German situation. This is of special importance if European risk assessments are carried out combining concentration data recorded in several countries without taking origin into account.

Keywords: dietary exposure, fish, herring, geographical origin, catching area

Correspondence: eu-fora@efsa.europa.eu

Acknowledgements: This report is funded by EFSA as part of the EU-FORA programme. We thank Gisle Solstad from the Norwegian Scientific Committee for Food and Environment (VKM) and Stephanie Taube and Katrin Blume from the German Federal Institute for Risk Assessment (BfR) for their administrative and scientific support during the project.

Suggested citation: Fechner C, Frantzen S, Lindtner O, Mathisen GH and Lillegaard ITL, 2019. Influence of the geographical origin on substance concentrations in herring as basis for dietary exposure assessments. EFSA Journal 2019;17(S2):e170904, 15 pp. https://doi.org/10.2903/j.efsa.2019.e170904

ISSN: 1831-4732

The EFSA Journal is a publication of the European Food Safety Authority, an agency of the European Union.

Table of contents

1. Introduction

Previous investigations on the agricultural products tomatoes, pineapples and kiwis showed effects of the geographical origin, such as country of origin, on concentrations of selected undesirable substances (Fechner et al., 2019). The performed dietary exposure assessment showed a possible refinement by integrating this information in the approach using assumptions on the origin-related consumption behaviour. Another study on dietary cadmium exposure from several food items showed influences on the exposure estimate depending on the use of pooled European concentration data in comparison to country-specific concentration data (Sand et al., 2013). The researchers concluded that both approaches lead to conservative estimates and a low aggregation level of food items is important for the estimation. Depending on the geographical origin of food items and investigated substances, the use of country-specific occurrence data could possibly refine exposure assessments, if there is a geographical variation in substance concentrations related to a country-specific food supply.

An effect of geographical origin on concentrations of selected substances could also apply to fillet of fish from different catching areas, as geographical variations in substance concentrations have been observed. Sunderland et al. (2018) studied geographical origins of seafood on the market in the United States and the concentration of methyl mercury, and showed that seafood was available from different catching areas and contributed to the methyl mercury exposure with different amounts. Several fish species caught in subareas of the Northeast Atlantic were investigated for methyl mercury and concentrations increased from north to south and by fish length (Azad et al., 2019). Another study for the same area showed decreasing concentrations of the brominated flame retardant (BFR) BDE-47 with increasing latitude for eight of 15 fish species investigated (Nøstbakken et al., 2018). For cod caught in the Barents Sea, the highest mercury concentrations were found in the southwest area for all length classes, whereas cod from the eastern area had higher arsenic concentrations (Julshamn et al., 2013). Several deep sea fish species, mainly tusk, ling and haddock, caught in Norwegian waters were analysed for mercury and other metals, polychlorinated dibenzo-*p*-dioxins/polychlorinated dibenzofurans (PCDD/Fs), dioxin-like polychlorinated biphenyls (DL-PCBs), non-dioxin-like PCBs (NDL-PCBs), BFRs and perfluorinated alkylated substances (PFAS) (Frantzen and Måge, 2016). Mercury concentrations in fillet varied geographically with an increasing trend from north to south and in some species from open sea to coast (Frantzen and Måge, 2016; Azad et al., 2019). For the organic contaminants, the concentrations were low in fillets but high concentrations were found in fish liver increasing from north to south and from open sea to fjords in the North Sea area (Frantzen and Måge, 2016). PFAS concentrations were mostly below the limit of quantification (LOQ) in fillet and liver, for perfluorooctanoic acid the LOQ was 2.4 ng/g and for perfluoroctanesulfonic acid 1.8 ng/g, which was below the cut-off values 10 ng/g and 14 ng/g used by EFSA for these two substances (Frantzen and Måge, 2016; EFSA CONTAM Panel, 2018a,b). A similar study was done for Atlantic halibut and concentrations of mercury and organic contaminants in lean and fatty fillet parts increased from north to south and with fish length and weight (Nilsen et al., 2016). Concentrations of PCDD/Fs and DL-PCBs in herring vary by catching area and fishing season (Karl et al., 2002; Frantzen et al., 2011; Karl and Lahrssen-Wiederholt, 2013) and concentrations in farmed salmon decrease if the feed composition is changed (Karl and Lahrssen-Wiederholt, 2013). Fish and fishery products like herring and salmon from the Baltic region contain higher amounts of PCDD/Fs and DL-PCBs than from other areas and therefore special recommendations for monitoring are given (European Commission 2016; EFSA CONTAM Panel, 2018a,b).

Risk benefit assessments of fish are carried out because next to negative effects from various contaminants contained in fish, positive effects are observed because of beneficial substances like omega-3 polyunsaturated fatty acids (VKM 2014). Depending on the catching area of fish and seafood, different environmental factors could influence the composition of substances. Therefore, it is important to investigate to what extent the geographical origin will impact the assessment approach. In this project, substance concentrations in herring from different catching areas and Norwegian and German consumption data were used to evaluate potential effects of herring origin on human exposure to certain substances. Country-specific data enable a comparison of herring as well as for different consumption behaviour as for substance concentrations and provide the basis for a refined dietary exposure assessment accounting for geographical food origin.

2. Description of work programme

2.1. Aims

The Norwegian Scientific Committee for Food and Environment (VKM), a scientifically independent and administrative unit of the Norwegian Institute of Public Health (NIPH), initiated the project 'Faster, better and stronger exposure assessment'. Fish was chosen as the targeted food group for this study. In cooperation with other Norwegian and German scientific institutes, the aim was to compare fish consumption, fish contamination and resulting dietary exposure estimates integrating the influence of fish origin and catching area to develop refined approaches and reduce uncertainties.

As a part of the project, data on selected substances should be prepared to expand the Norwegian food composition database, which is provided by the University of Oslo (UiO). This database also contains consumption data from different Norwegian surveys, data which were used in the project to perform exposure assessments. Parameters contributing to uncertainty in exposure calculations were identified and described.

2.2. Activities/methods

Herring was selected as a case study because it is a wild fish species, because Norwegians and Germans consume it, and because various substances are analysed in monitoring programmes in Germany and Norway. In this project the software IBM SPSS Statistics 25 was used for data analyses.

Data on the consumption of fish and seafood in total and herring in particular were extracted from two national consumption surveys. Both surveys recorded two 24-h recalls and participants who reported only one 24-h recall were excluded. The survey Norkost 3 was conducted during 2010–2011 in Norway (Totland et al., 2012), included 1,787 adults (925 women and 862 men) aged 18–70 years, who completed two 24-h recalls, and the data were provided by the UiO. The German National Nutrition Survey II (NVS II) was conducted during 2005–2006 by Max Rubner-Institut (MRI) (Brombach et al., 2006; Krems et al., 2006) and 13,926 participants (6,897 male and 7,029 female) aged between 14 and 80 years, who completed two 24-h recalls, were considered here. To derive consumption amounts of fish and seafood in total and herring in particular, a version with disaggregated household recipes was used for Germany. That means the amount of pure herring from household recipes consumed was available, while industrial products consumed remained aggregated and the derived consumption amount contained other ingredients next to herring. For Norway both, an aggregated and a disaggregated version for industrial and household recipes was used for herring to derive consumption amounts. Fish and seafood consumption was derived from aggregated data. The consumption of herring was used for dietary exposure assessment on an individual level, per kg body weight (BW) and as mean of the two 24-h recalls.

Concentration data for substances in herring, from national monitoring programmes between 2012 and 2017, were requested in Standard Sample Description format. Norwegian data on substances in herring from the Norwegian Spring Spawning (NSS) herring stock were provided by the Institute of Marine Research (IMR). The herring was sampled from commercial catches in fishery areas by fishermen on contract with the IMR. German data were taken from the authorities in the federal states on the German market. Data of all federal states were submitted to and organised by the Federal Office of Consumer Protection and Food Safety (BVL) and can be used by the German Federal Institute for Risk Assessment (BfR) for exposure assessments. Different codes for the identification of substances were used in Norway and Germany and had to be unified to identify substances investigated in both countries. The substances investigated in both countries were combined in one data file, and each substance was checked for equality of units and if concentrations were equally related to whole weight or fat weight in both countries. Units were equal for Norway and Germany and the concentrations were mostly related to whole weight. NDL-PCBs investigated in Germany in 2012 were reported in fat weight, to have all results in whole weight, the reported fat content in percent of each sample was used to relate results to whole weight. Nickel was investigated in both countries but excluded from further investigations as only two samples from Germany were available and the 50 samples from Norway were neither quantified, nor was a LOQ stated. For all other substances investigated in Norway and Germany lower bound concentrations were calculated replacing results below the limit of detection (LOD) or LOQ by zero. For upper bound concentrations, results below the LOD were replaced by the LOD and results below the LOQ were replaced by the LOQ. In case of missing LOQs and LODs, they were replaced by zero before. Every sample investigated for PCDD/Fs

and DL-PCBs was analysed for all congeners and in this way no sample exclusion was needed for the calculation of sums (EFSA CONTAM Panel, 2018a,b). To weigh the concentrations of the individual congeners according to their different toxicity and to convert the given unit to Toxic Equivalents (TEQ), PCDD/F and DL-PCB lower and upper bound concentrations were multiplied with Toxic Equivalency Factors (TEF) (European Commission 2011) established by the World Health Organization (WHO) in 2005. Afterwards, sums for PCDD/Fs, DL-PCBs and PCDD/Fs and DL-PCBs were calculated. Tables with statistical parameters for country-specific concentrations of all substances investigated in both countries were prepared using the CTABLES command. On this basis, country-specific substance concentrations could be compared and we could also pay attention to the different catching areas. Norwegian parameters on substance concentrations were used to expand the Norwegian food composition database.

The project progress was presented at two conferences as part of the poster sessions; the 'International Conference on Uncertainty in Risk Analysis' 20–22.2.2019 hosted by BfR in Berlin, Germany, and 'The Science of Food Safety – What's our Future? 21–22.8.2019 hosted by the Food Safety Authority of Ireland in Dublin, Ireland. Further investigations and the dietary exposure assessment were based on data of selected metals as well as PCDD/Fs and DL-PCBs using country-specific scenarios to account for uncertainties in exposure assessments related to the use of pooled concentration data.

3. Data for dietary exposure assessment

3.1. Consumption of herring

Country-specific consumption data of Norway and Germany enabled the comparison of the consumption of fish and seafood in total and herring in particular between the countries and provided the basis for the dietary exposure assessment. Table 1 shows the German and Norwegian fish and seafood consumption and the herring consumption calculated from NVS II and Norkost 3. In Germany, there were 13,926 participants, 3,455 (i.e. 24.8%) of them consumed fish and seafood and 596 (i.e. 4.3%) of them consumed herring. In Norway, there were 1,787 participants, while 1158 (i.e. 64.8 %) of them consumed fish and seafood and 99 (i.e. 5.5%) of them were consumers of herring. For Norway, the consumption of herring calculated from aggregated data included other ingredients from industrial products and household recipes, whereas the consumption calculated from disaggregated data was pure herring. Norwegian consumers of herring had a mean herring consumption of 0.31 g/day per kg BW from disaggregated data and 0.41 g/day per kg BW from aggregated data. This difference shows one of the uncertainties in the model. On one hand, factors used to calculate the disaggregated herring consumption may vary between different industrial and household recipes; on the other hand, the aggregated herring consumption contains more ingredients than herring. Which data version to use in dietary exposure assessment depended on model assumptions and in the current case also on the available German consumption data. For Germany, the disaggregated herring consumption included the herring amount from household recipes and industrial products with herring, which were aggregated containing other ingredients as well. German consumers of herring had a mean herring consumption of 0.88 g/day per kg BW, which was higher than in Norway (aggregated and disaggregated). In contrast, the mean fish and seafood consumption of all participants and of consumers was higher in Norway (0.89 g/day per kg BW and 1.37 g/day per kg BW) than in Germany (0.23 g/day per kg BW and 0.92 g/day per kg BW).

3.2. Substance concentrations in herring

For some substances, concentrations vary with geographical food origin. In these cases, country-specific concentration data could help to refine dietary exposure estimates and to see differences related to the origin of consumed food. This is already highlighted by the European Food Safety Authority (EFSA), because Baltic herring is supposed to have higher PCDD/F and DL-PCB concentrations than herring from other catching areas and for the consumption there could be a country-specific focus on certain catching areas (EFSA CONTAM Panel, 2018a,b). Different substance concentrations in herring for Norway and Germany could be due to different geographical origins, named 'catching areas' for fish.

For the substance group PCDD/Fs and DL-PCBs, the German herring samples were from the Baltic Sea and sampled on the German market during 2016 whereas Norwegian herring samples were from the Norwegian Sea and sampled January and February 2014 and 2017. Table 2 shows higher concentrations based on German data compared to Norwegian data for the sum of DL-PCBs, the sum

of PCDD/Fs and the total sum of PCDD/Fs and DL-PCBs. The concentrations for individual PCDD/F and DL-PCB congeners used to calculate the sums shown in Table 2 are described in Appendix A, Table A.1. German mean LOQs were below Norwegian mean LOQs for all congeners. For most of the congeners, German lower and upper bound mean and percentile 95 (P95) concentrations were higher than corresponding Norwegian data except for PCB-123 showing higher lower and upper bound mean and P95 concentrations for Norway. For congeners with only one or two samples quantified in Norway, upper bound mean and P95 concentrations were mostly higher than for Germany, while lower bound concentrations were higher for Germany. This shows the influence of higher LOQs in Norway used in upper bound calculations in comparison with lower quantified concentrations from Germany and represents uncertainty in estimations.

EFSA evaluated PCDD/F and DL-PCB concentrations using pooled European data mostly from Germany, France, Norway and Denmark between 2010 and 2016, and catching areas were not reported and might be mixed (EFSA CONTAM Panel, 2018a,b). For most of the congeners, lower and upper bound mean and P95 concentrations from EFSA (2018) were higher than calculations using German Baltic Sea data. Only for four PCDD/Fs, 1,2,3,4,6,7,8-HpCDD, 1,2,3,4,7,8-HxCDD, 1,2,3,7,8,9-HxCDD and 1,2,3,4,6,7,8-HpCDF, most of the concentration parameters used by EFSA were lower than the German concentrations.

Further substances investigated in herring in Germany and Norway are displayed in Table 3. For Germany, metals were investigated in 2012 and 2017 and Baltic Sea, North Sea, Norwegian Sea and Atlantic were the catching areas reported, while NDL-PCBs were investigated in herring in 2012 in samples from Baltic Sea, North Sea and Atlantic and in 2016 from Baltic Sea. German data for BFR and PFAS were derived from the Atlantic in 2012. For Norway, all substances displayed in Table 3 were investigated in 2014 and 2017 and sampled in the Norwegian Sea. Norwegian mean and percentile 50 (P50) LOQs were below German mean and P50 LOQs for all substances. While the concentrations of the metals arsenic, copper and zinc were similar, the concentrations of cadmium, mercury and selenium tended to be higher in Norway, and lead concentrations were higher in Germany. For BDE-47, a BFR investigated in both countries, only 11 samples including two quantifications were available from Germany. Lower bound concentrations from Norway were higher than Germany, and German upper bound concentrations were higher than the Norwegian and influenced by the higher German LOQs, which represents an uncertainty. For the substance group NDL-PCBs, the German concentrations were higher or concentrations from Norway and Germany were similar. For PFAS, only one sample from Germany was quantified for perfluorooctane sulfonate, whereas the other samples had no quantifiable concentrations for PFAS.

3.3. Requirements for dietary exposure assessment and uncertainties

To investigate a potential relationship between catching areas and substance concentrations in herring, the concentrations were grouped by sampling country and reported catching areas were taken into account instead of pooling all concentration data. In combination with country-specific consumption data, a refined country-specific dietary exposure assessment is possible.

All data for dietary exposure assessments introduce uncertainty to the result of the exposure estimate, e.g. the consumption surveys used in the project were conducted years ago and therefore the current consumption might not be appropriately depicted (e.g. frequencies and amounts might have changed, new food items might be on the market). Additionally, underreporting or misreporting could occur, errors due to measured or self-reported body weights are possible, and the determination of food portion sizes has limitations. The consumption surveys used covered only 2 days, which do not give the full picture of the long-term food consumption. Furthermore, the aggregation level of food consumption might be different in different surveys, influencing the accuracy of derived consumption amounts. In German and Norwegian data, aggregated food items containing other ingredients than herring were included, causing an overestimation of herring consumption. Factors to derive the herring content out of composite foods may be different for various recipes but are unified for a recipe (e.g. amount of fish in different canned fish products). Substance concentration data used were derived from national monitoring programmes, where the LOQs were oriented towards maximum levels and not to the most sensitive analyses. Therefore, concentrations of some substances were below the LOQ and the real concentrations were not known. This effect was visible in the data used, as there were some PCDD/F and DL-PCB congeners with only a few quantified samples for Norway, which resulted in higher upper bound concentrations because of the calculation using the LOQ (Appendix A, Table A.1). Samples from single previous years were taken and might not represent current substance concentrations and

measuring errors appear. Furthermore, the sampling strategy was not representative for all available catching areas and finally consumed processed food items on the German and Norwegian markets, because special catching areas were sampled in Germany and Norway or the catching area was not relevant in the sampling plan in other cases in Germany, which might not depict the current situation properly. Fish length and weight also affect substance concentrations in herring (Frantzen et al., 2011, 2015), but this was not provided in the German data as ready-to-eat fillets might be sampled from the marked. In exposure scenarios assumptions are used for calculations referring to average (P50) and high (P95) consumption as well as concentrations. This might not depict the real situation and causes uncertainties in the model, which could be different using distributions of consumption and concentration. Furthermore, factors of food processing were not part of our model and different food aggregation levels in consumption and concentration data were combined.

4. Conclusions

For some substances, there were distinct differences in the Norwegian and German concentrations. This supports the use of country-specific concentrations paying attention to catching areas in dietary exposure assessment.

To evaluate if there are origin-related differences in substance concentrations, information on catching areas is needed. Depending on the fish supply per country, fish from different catching areas could be available on the country-specific markets. Concentration data used for the current project provided much information on substances in herring and catching areas but the sampling was not related to catching areas available on the country markets. To investigate which substance concentrations vary geographically, a representative sampling for catching areas would be necessary.

However, differences in concentrations may be due to other reasons than catching areas, e.g. season-related. Therefore, seasonal sampling and sampling over several years is needed to evaluate the influence on the substance concentrations. Additionally, information like fish weight and length are important to investigate further influences.

Nevertheless, using the available country-specific concentration data in combination with country-specific consumption data, a first insight in origin-related exposure estimates was possible. This shows the need of country-specific data, if substance concentrations are origin-related, in case of fish, related to different catching areas available.

Because of the globalised markets in Europe, geographical food origin might not be relevant for all foods distributed within the European Member States. Even if there are different levels in fish from different catching areas, this will only affect exposure assessment, if there is a realistic chance for individuals to consume long-term fish from the same catching area.

Depending on the request of the consumer and the substance, it might be more appropriate to do the exposure assessment using country-specific concentrations or in other cases to use information on geographical food origin independent from the place of sampling. However, for EFSA it will be important to ask in calls for data also for information on geographical food origin to be able to consider this in exposure assessment.

Table 1: Consumption of fish and seafood in total and herring in particular in Germany and Norway according to 24-h recalls in the surveys NVS II and Norkost 3

Participants[(a)] (N)	Food item	Consumption (g/day per kg BW)					
		Mean		P50		P95	
		GER	NOR	GER	NOR	GER	NOR
All participants GER: 13926 NOR: 1787	Fish and seafood	0.23	0.89	0.00	0.42	1.39	3.15
	Herring aggregated	–	0.02	–	0.00	–	0.15
	Herring disaggregated	0.04	0.02	0.00	0.00	0.00	0.09
Consumers of fish and sea food/herring GER: 3455/596 NOR: 1158/99	Fish and seafood	0.92	1.37	0.77	1.10	2.21	3.53
	Herring aggregated	–	0.41	–	0.26	–	1.37
	Herring disaggregated	0.88	0.31	0.72	0.19	2.15	1.17

N: sample number; BW: body weight; P50: percentile 50; P95: percentile 95; GER: Germany; NOR Norway; –: no data.

(a): Two 24-h recalls were recorded and an individual mean consumption was calculated. Participants, who reported only one day, were excluded.

Table 2: Sums of PCDD/Fs (dioxins) and DL-PCBs in herring investigated in monitoring programmes in Germany and Norway

Substance sum	Sampling country	Valid N	Substance concentration lower bound (pg WHO_{2005}-TEQ/g)[a]				Substance concentration upper bound (pg WHO_{2005}-TEQ/g)[a]			
			Mean	SD	P50	P95	Mean	SD	P50	P95
DL-PCBs	GER	47	0.92	0.33	0.92	1.37	0.92	0.33	0.92	1.37
	NOR	100	0.43	0.17	0.40	0.74	0.43	0.17	0.40	0.74
PCDD/Fs	GER	47	0.84	0.34	0.87	1.30	0.85	0.33	0.87	1.30
	NOR	100	0.36	0.17	0.34	0.65	0.47	0.15	0.46	0.71
PCDD/Fs and DL-PCBs	GER	47	1.76	0.61	1.68	2.65	1.77	0.61	1.68	2.65
	NOR	100	0.79	0.32	0.75	1.34	0.90	0.31	0.85	1.43

N: sample number; SD: standard deviation; P50: percentile 50; P95: percentile 95; PCDDs: polychlorinated dibenzo-*p*-dioxins; PCDFs: polychlorinated dibenzofurans; DL-PCBs: dioxin-like polychlorinated biphenyls; WHO World Health Organization; TEQ: Toxic Equivalents; GER: Germany; NOR: Norway.
(a): All concentrations are given in whole weight of herring.

Table 3: Substances in herring included in monitoring programmes in both Germany and Norway

Substance group	Substance	Sampling country	Valid N	Quantified N	Mean LOQ[a]	P50 LOQ[a]	Substance concentration lower bound[b]				Substance concentration upper bound[b]			
							Mean	SD	P50	P95	Mean	SD	P50	P95
Metals (mg/kg)	Total Arsenic (As)	GER	140	139	0.02	0.02	1.53	0.43	1.52	2.20	1.53	0.43	1.52	2.20
		NOR	100	100	0.00	0.00	1.50	0.37	1.45	2.10	1.50	0.37	1.45	2.10
	Cadmium (Cd)	GER	140	67	0.01	0.00	0.00	0.01	0.00	0.02	0.01	0.01	0.01	0.02
		NOR	100	100	0.00	0.00	0.02	0.01	0.02	0.04	0.02	0.01	0.02	0.04
	Copper (Cu)	GER	135	112	0.41	0.20	0.67	0.35	0.78	1.06	0.84	0.19	0.87	1.06
		NOR	100	100	0.03	0.03	0.82	0.11	0.80	1.00	0.82	0.11	0.80	1.00
	Total Mercury (Hg)	GER	140	139	0.01	0.01	0.05	0.02	0.05	0.09	0.05	0.02	0.05	0.09
		NOR	100	97	0.00	0.00	0.07	0.03	0.06	0.11	0.07	0.03	0.06	0.11
	Lead (Pb)	GER	140	49	0.01	0.02	0.01	0.01	0.00	0.03	0.01	0.01	0.01	0.04
		NOR	100	20	0.01	0.01	0.00	0.00	0.00	0.00	0.01	0.00	0.01	0.01
	Selenium (Se)	GER	135	129	0.03	0.03	0.34	0.15	0.33	0.59	0.34	0.15	0.33	0.59
		NOR	100	100	0.02	0.02	0.42	0.09	0.41	0.60	0.42	0.09	0.41	0.60
	Zinc (Zn)	GER	135	134	1.10	1.00	7.25	2.61	7.04	11.90	7.26	2.60	7.04	11.90
		NOR	100	100	0.15	0.15	7.23	1.74	7.00	10.50	7.23	1.74	7.00	10.50
BFR (mg/kg)	BDE-47	GER	11	2	0.96	0.97	0.16	0.38	0.00	1.20	3.89	1.91	5.00	5.00
		NOR	112	112	0.01	0.00	0.38	0.18	0.36	0.70	0.38	0.18	0.36	0.70
NDL-PCBs (ng/g)	PCB-101	GER	58	44	0.42	0.40	1.52	1.29	1.45	3.20	1.65	1.15	1.45	3.20
		NOR	100	100	0.02	0.02	1.35	0.60	1.30	2.40	1.35	0.60	1.30	2.40
	PCB-138	GER	58	58	0.42	0.40	3.60	1.68	3.70	6.60	3.60	1.68	3.70	6.60
		NOR	100	100	0.02	0.02	1.39	0.69	1.30	2.50	1.39	0.69	1.30	2.50
	PCB-153	GER	58	56	0.42	0.40	4.18	1.88	4.10	7.70	4.20	1.84	4.10	7.70
		NOR	100	100	0.02	0.02	2.10	1.00	2.00	3.70	2.10	1.00	2.00	3.70
NDL-PCBs (ng/g)	PCB-180	GER	58	45	0.42	0.40	1.14	1.25	0.69	2.90	1.25	1.17	0.70	2.90
		NOR	100	100	0.02	0.02	0.40	0.22	0.36	0.70	0.40	0.22	0.36	0.70
	PCB-28	GER	57	28	0.43	0.50	0.37	0.93	0.00	1.30	0.60	0.87	0.50	1.30
		NOR	100	100	0.02	0.02	0.41	0.24	0.40	0.80	0.41	0.24	0.40	0.80
	PCB-52	GER	58	35	0.42	0.40	0.59	0.94	0.47	1.69	0.81	0.84	0.55	1.69
		NOR	100	100	0.02	0.02	0.79	0.34	0.75	1.30	0.79	0.34	0.75	1.30

Substance group	Substance	Sampling country	Valid N	Quantified N	Mean LOQ[a]	P50 LOQ[a]	Substance concentration lower bound[b]				Substance concentration upper bound[b]			
							Mean	SD	P50	P95	Mean	SD	P50	P95
PFAS (ng/g)	Perfluorobutane sulfonate	GER	21	0	1.00	1.00	0.00	0.00	0.00	0.00	1.00	0.00	1.00	1.00
		NOR	99	0	0.56	0.38	0.00	0.00	0.00	0.00	0.56	0.24	0.38	0.80
	Perfluorohexane sulfonate	GER	21	0	1.00	1.00	0.00	0.00	0.00	0.00	1.00	0.00	1.00	1.00
		NOR	99	0	0.56	0.38	0.00	0.00	0.00	0.00	0.56	0.24	0.38	0.80
	Perfluorooctane sulfonate	GER	40	1	1.28	1.00	0.10	0.60	0.00	0.00	0.97	0.55	1.00	1.00
		NOR	99	0	0.51	0.25	0.00	0.00	0.00	0.00	0.51	0.29	0.25	0.80
	Perfluorohexanoic acid	GER	21	0	1.00	1.00	0.00	0.00	0.00	0.00	1.00	0.00	1.00	1.00
		NOR	99	0	0.56	0.25	0.00	0.00	0.00	0.00	0.56	0.34	0.25	0.90
	Perfluorooctanoic acid	GER	40	0	1.28	1.00	0.00	0.00	0.00	0.00	0.90	0.30	1.00	1.00
		NOR	99	0	0.76	0.25	0.00	0.00	0.00	0.00	0.76	0.54	0.25	1.30
	Perfluorononanoic acid	GER	21	0	1.00	1.00	0.00	0.00	0.00	0.00	1.00	0.00	1.00	1.00
		NOR	99	0	0.56	0.25	0.00	0.00	0.00	0.00	0.56	0.34	0.25	0.90
	Perfluorodecanoic acid	GER	21	0	1.00	1.00	0.00	0.00	0.00	0.00	1.00	0.00	1.00	1.00
		NOR	99	0	0.36	0.25	0.00	0.00	0.00	0.00	0.36	0.14	0.25	0.50
	Perfluoropentanoic acid	GER	21	0	1.00	1.00	0.00	0.00	0.00	0.00	1.00	0.00	1.00	1.00
		NOR	50	0	0.22	0.22	0.00	0.00	0.00	0.00	0.22	0.01	0.22	0.25

N: sample number; LOQ: limit of quantification; SD: standard deviation; P50: percentile 50; P95: percentile 95; BFR: brominated flame retardants; NDL-PCBs: non-dioxin-like polychlorinated biphenyls; PFAS: perfluorinated alkylated substances; GER: Germany; NOR: Norway.
(a): Missing values were replaced by 0.
(b): All concentrations are given in whole weight of herring.

References

Azad AM, Frantzen S, Bank MS, Nilsen BM, Duinker A, Madsen L and Maage A, 2019. Effects of geography and species variation on selenium and mercury molar ratios in Northeast Atlantic marine fish communities. Science of the Total Environment, 652, 1482–1496.

Brombach C, Wagner U, Eisinger-Watzl M and Heyer A, 2006. Die Nationale Verzehrsstudie II. Ernährungs-Umschau, 53, 4–9.

EFSA CONTAM Panel (EFSA Panel on Contaminants in the Food Chain), Knutsen HK, Alexander J, Barregård L, Bignami M, Brüschweiler B, Ceccatelli S, Cottrill B, Dinovi M, Edler L, Grasl-Kraupp B, Hogstrand C, Hoogenboom LR, Nebbia CS, Oswald IP, Petersen A, Rose M, Roudot A-C, Vleminckx C, Vollmer G, Wallace H, Bodin L, Cravedi J-P, Halldorsson TI, Haug LS, Johansson N, van Loveren H, Gergelova P, Mackay K, Levorato S, van Manen M and Schwerdtle T, 2018a. Risk to human health related to the presence of perfluorooctane sulfonic acid and perfluorooctanoic acid in food. EFSA Journal 2018;16(12):e05194. https://doi.org/10.2903/j.efsa.2017.5194

EFSA CONTAM Panel (EFSA Panel on Contaminants in the Food Chain), Knutsen HK, Alexander J, Barregård L, Bignami M, Brüschweiler B, Ceccatelli S, Cottrill B, Dinovi M, Edler L, Grasl-Kraupp B, Hogstrand C, Nebbia CS, Oswald IP, Petersen A, Rose M, Roudot A-C, Schwerdtle T, Vleminckx C, Vollmer G, Wallace H, Fürst P, Håkansson H, Halldorsson T, Lundebye A-K, Pohjanvirta R, Rylander L, Smith A, van Loveren H, Waalkens-Berendsen I, Zeilmaker M, Binaglia M, Gómez Ruiz JA, Horváth Z, Christoph E, Ciccolallo L, Ramos Bordajandi L, Steinkellner H and Hoogenboom LR, 2018b. "Risk for animal and human health related to the presence of dioxins and dioxin-like PCBs in feed and food." EFSA Journal 2018;16(11):5333. https://doi.org/10.2903/j.efsa.2018.5333

European Commission, 2011. Commission Regulation (EU) No 1259/2011 of 2 December 2011 amending Regulation (EC) No 1881/2006 as regards maximum levels for dioxins, dioxin-like PCBs and non dioxin-like PCBs in foodstuffs. Official Journal of the European Union 320, 18–23.

European Commission, 2016. Commission Recommendation (EU) 2016/688 of 2 May 2016 on the monitoring and management of the presence of dioxins and PCBs in fish and fishery products from the Baltic region. Official Journal of the European Union 118, 16–23.

Fechner C, Greiner M, Heseker H and Lindtner O, 2019. Refinement of dietary exposure assessment using origin-related scenarios. Journal of Exposure Science & Environmental Epidemiology, ???, ???–???.

Frantzen S and Måge A, 2016. Fremmedstoffer i villfisk med vekt på kyst - nære farvann. Brosme, lange og bifangstvann. Gjelder tall for prøver samlet inn i 2013–2015. Nasjonalt institutt for ernærings- og sjømatforskning (NIFES), Bergen, NO. pp. 1–116.

Frantzen S, Måge A, Iversen SA and Julshamn K, 2011. Seasonal variation in the levels of organohalogen compounds in herring (Clupea harengus) from the Norwegian Sea. Chemosphere, 85, 179–187.

Frantzen S, Maage A, Duinker A, Julshamn K and Iversen SA, 2015. A baseline study of metals in herring (Clupea harengus) from the Norwegian Sea, with focus on mercury, cadmium, arsenic and lead. Chemosphere, 127, 164–170.

Julshamn K, Duinker A, Nilsen BM, Frantzen S, Maage A, Valdersnes S and Nedreaas K, 2013. A baseline study of levels of mercury, arsenic, cadmium and lead in Northeast Arctic cod (Gadus morhua) from different parts of the Barents Sea. Marine Pollution Bulletin, 67, 187–195.

Karl H and Lahrssen-Wiederholt M, 2013. Factors influencing the intake of dioxins and dioxin-like PCBs via fish consumption in Germany. Journal of Consumer Protection and Food Safety, 8, 27–35.

Karl H, Ruoff U and Blüthgen A, 2002. Levels of dioxins in fish and fishery products on the German market. Chemosphere, 49, 765–773.

Krems C, Bauch A, Götz A, Heuer T, Hild A, Möseneder J and Brombach C, 2006. Methoden der Nationalen Verzehrsstudie II. Ernährungs Umschau, 53, 44–50.

Nilsen BM, Nedreaas K and Måge A, 2016. Kartlegging av fremmedstoffer i Atlantisk kveite (Hippoglossus hippoglossus). Sluttrapport for programmet «Miljøgifter i fisk og fiskevarer» 2013–2015. Nasjonalt institutt for ernærings- og sjømatforskning (NIFES), Bergen, NO. pp. 1–82.

Nøstbakken OJ, Duinker A, Rasinger JD, Nilsen BM, Sanden M, Frantzen S, Hove HT, Lundebye A-K, Berntssen MH and Hannisdal R, 2018. Factors influencing risk assessments of brominated flame-retardants; evidence based on seafood from the North East Atlantic Ocean. Environment International, 119, 544–557.

Sand S, Héraud F and Arcella D, 2013. The use of chemical occurrence data at European vs. national level in dietary exposure assessments: a methodological study. Food and Chemical Toxicology, 62, 7–15.

Sunderland EM, Li M and Bullard K, 2018. Decadal changes in the edible supply of seafood and methylmercury exposure in the United States. Environmental Health Perspectives, 126, 017006–017001-017006-017006.

Totland TH, Melnæs BK, Lundberg-Hallèn N, Helland-Kigen KM, Lund-Blix NA, Myhre JB, Johansen AMW, Løken EB and Andersen LF, 2012. Norkost 3. landsomfattende kostholdsundersøkelse blant menn og kvinner i Norge i alderen 18–70 år, 2010–11. Norwegian Directorate of Health, Oslo, Norway.

VKM, Norwegian Scientific Committee for Food Safety, 2014. "Benefit-risk assessment of fish and fish products in the Norwegian diet – an update. Scientific Opinion of the Scientific Steering Committee." Report 15: 293.

Abbreviations

BfR	German Federal Institute for Risk Assessment (Bundesinstitut für Risikobewertung)
BFR	brominated flame retardant
BVL	Federal Office of Consumer Protection and Food Safety (Bundesamt für Verbraucherschutz und Lebensmittelsicherheit)
DL-PCBs	dioxin-like polychlorinated biphenyls
GER	Germany
IMR	Institute of Marine Research (Havforskningsinstituttet)
LOD	limit of detection
LOQ	limit of quantification
MRI	Max Rubner-Institut
N	sample number
NDL-PCBs	non-dioxin-like polychlorinated biphenyls
NIPH	Norwegian Institute of Public Health (Folkehelseinstituttet)
NOR	Norway
NSS	Norwegian Spring Spawning (herring stock)
P50	percentile 50
P95	percentile 95
PCDDs	polychlorinated dibenzo-*p*-dioxins
PCDFs	polychlorinated dibenzofurans
PFAS	perfluorinated alkylated substances
SD	standard deviation
TEF	Toxic Equivalency Factor
TEQ	Toxic Equivalent
UiO	University of Oslo
VKM	Norwegian Scientific Committee for Food and Environment (Vitenskapskomiteen for mat og miljø)
WHO	World Health Organization

Appendix A – Additional information on PCDD/Fs (dioxins) and DL-PCBs in herring investigated in monitoring programmes in Germany and Norway

Table A.1: Individual congeners of PCDD/Fs (dioxins) and DL-PCBs in herring investigated in monitoring programmes in Germany and Norway

Substance group	Substance	Sampling country	Valid N	Quantified N	Mean LOQ[a]	Substance concentration[b]			
						Mean lower bound	Mean upper bound	P95 lower bound	P95 upper bound
					pg WHO_{2005}-TEQ/g				
PCDDs	1,2,3,4,6,7, 8-HpCDD	GER	47	38	0.000	0.008	0.008	0.004	0.004
		NOR	100	18	0.000	0.000	0.000	0.001	0.001
	1,2,3,4,7, 8-HxCDD	GER	47	40	0.001	0.004	0.005	0.013	0.013
		NOR	100	1	0.005	0.000	0.005	0.000	0.009
	1,2,3,6,7, 8-HxCDD	GER	47	40	0.001	0.014	0.015	0.027	0.027
		NOR	100	61	0.006	0.005	0.008	0.017	0.017
	1,2,3,7,8, 9-HxCDD	GER	47	37	0.001	0.003	0.004	0.010	0.010
		NOR	100	2	0.005	0.000	0.006	0.000	0.010
	1,2,3,7, 8-PeCDD	GER	47	45	0.012	0.223	0.224	0.340	0.340
		NOR	100	60	0.074	0.062	0.098	0.180	0.195
	2,3,7, 8-TCDD	GER	47	43	0.005	0.084	0.084	0.140	0.140
		NOR	100	6	0.048	0.002	0.048	0.029	0.078
	OCDD	GER	47	27	0.000	0.000	0.000	0.000	0.000
		NOR	100	7	0.000	0.000	0.000	0.000	0.000
PCDFs	1,2,3,4,6,7, 8-HpCDF	GER	47	40	0.000	0.001	0.001	0.004	0.004
		NOR	100	5	0.000	0.000	0.000	0.000	0.001
	1,2,3,4,7,8, 9-HpCDF	GER	47	10	0.000	0.000	0.000	0.000	0.000
		NOR	100	2	0.000	0.000	0.000	0.000	0.001
	1,2,3,4,7, 8-HxCDF	GER	47	43	0.001	0.010	0.010	0.021	0.021
		NOR	100	26	0.004	0.001	0.004	0.006	0.007
	1,2,3,6,7, 8-HxCDF	GER	47	43	0.001	0.010	0.010	0.017	0.017
		NOR	100	70	0.004	0.004	0.005	0.010	0.010
	1,2,3,7,8, 9-HxCDF	GER	47	10	0.001	0.001	0.002	0.009	0.009
		NOR	100	1	0.005	0.000	0.005	0.000	0.011
	1,2,3,7, 8-PeCDF	GER	47	45	0.000	0.008	0.008	0.013	0.013
		NOR	100	89	0.003	0.004	0.005	0.009	0.009
	2,3,4,6,7, 8-HxCDF	GER	47	43	0.001	0.011	0.012	0.023	0.023
		NOR	100	74	0.004	0.005	0.006	0.010	0.010
	2,3,4,7, 8-PeCDF	GER	47	47	0.003	0.308	0.308	0.480	0.480
		NOR	100	100	0.028	0.180	0.180	0.300	0.300
	2,3,7, 8-TCDF	GER	47	47	0.001	0.158	0.158	0.270	0.270
		NOR	100	100	0.011	0.098	0.098	0.200	0.200
PCDFs	OCDF	GER	47	24	0.000	0.000	0.000	0.000	0.000
		NOR	100	1	0.000	0.000	0.000	0.000	0.000

Substance group	Substance	Sampling country	Valid N	Quantified N	Mean LOQ[a]	Substance concentration[b]			
						Mean lower bound	Mean upper bound	P95 lower bound	P95 upper bound
						pg WHO_{2005}-TEQ/g			
Non-ortho DL-PCBs	PCB-77	GER	47	45	0.000	0.002	0.002	0.003	0.003
		NOR	100	100	0.000	0.001	0.001	0.001	0.001
	PCB-81	GER	47	45	0.000	0.000	0.000	0.000	0.000
		NOR	100	2	0.000	0.000	0.000	0.000	0.000
	PCB-126	GER	47	47	0.002	0.781	0.781	1.200	1.200
		NOR	100	100	0.043	0.334	0.334	0.550	0.550
	PCB-169	GER	47	47	0.000	0.081	0.081	0.117	0.117
		NOR	100	100	0.008	0.054	0.054	0.104	0.104
Mono-ortho DL-PCBs	PCB-105	GER	47	47	0.000	0.010	0.010	0.016	0.016
		NOR	100	100	0.000	0.009	0.009	0.015	0.015
	PCB-114	GER	47	45	0.000	0.001	0.001	0.001	0.001
		NOR	100	79	0.000	0.001	0.001	0.001	0.001
	PCB-118	GER	47	47	0.000	0.037	0.037	0.055	0.055
		NOR	100	100	0.000	0.029	0.029	0.050	0.050
	PCB-123	GER	47	45	0.000	0.000	0.000	0.001	0.001
		NOR	100	89	0.000	0.001	0.001	0.002	0.002
	PCB-156	GER	47	46	0.000	0.004	0.004	0.006	0.006
		NOR	100	99	0.000	0.002	0.002	0.003	0.003
	PCB-157	GER	47	45	0.000	0.001	0.001	0.002	0.002
		NOR	100	81	0.000	0.001	0.001	0.001	0.001
	PCB-167	GER	47	45	0.000	0.003	0.003	0.004	0.004
		NOR	100	97	0.000	0.001	0.001	0.002	0.002
	PCB-189	GER	47	45	0.000	0.000	0.000	0.001	0.001
		NOR	100	9	0.000	0.000	0.000	0.000	0.001

N: sample number; LOQ: limit of quantification; P95: percentile 95; PCDDs: polychlorinated dibenzo-*p*-dioxins; PCDFs: polychlorinated dibenzofurans; DL-PCBs: dioxin-like polychlorinated biphenyls; WHO World Health Organization: TEQ: Toxic Equivalents; GER: Germany; NOR: Norway.
(a): Missing values were replaced by 0.
(b): All concentrations are given in whole weight of herring.

EU-FORA SERIES 2

APPROVED: 31 July 2019

doi: 10.2903/j.efsa.2019.e170905

Joint venture on the further development of chemical exposure assessment by use of probabilistic modelling

Austrian Agency for Health and Food Safety (AGES),
Christina Vlachou and Daniela Hofstädter

Abstract

Exposure assessment is a fundamental component of the risk assessment process and has a significant contribution to the overall uncertainty of the risk estimates. The aim of the present project, implemented within the framework of the EU-FORA Fellowship, was to develop a structured approach for probabilistic modelling of the dietary exposure to chemical contaminants, which shall be used as a refined alternative to the more conservative deterministic approach or as part of a Tier 2 assessment. The fellow received training and worked in close cooperation with the project team on three case studies of contaminants in food (cadmium, acrylamide and deoxynivalenol). The modelling of the dietary intake was based on relevant EFSA Guidance and employed the Monte Carlo simulation methodology with the use of a standard software tool (Monte Carlo Risk Assessment (MCRA) platform) and/or a tailor-made risk model in the programming language *R*. The strengths and the limitations of every approach were explored and discussed. The conclusion from the critical comparison of the outputs was that the former can be a tool for the generation of fast preliminary estimates of the usual dietary exposure, whereas the latter may be used by the risk assessors as a more sophisticated, 'state-of-the-art' strategy, which will lead to more realistic estimates of the exposure. The outcomes of the project are being currently incorporated in a Guidance Document on probabilistic exposure assessment, which will highly contribute to more informed risk management decisions and to more effective risk communication.

Keywords: risk assessment, probabilistic modelling, dietary exposure

Correspondence: eu-fora@efsa.europa.eu

Acknowledgements: This report is funded by EFSA as part of the EU-FORA programme.

Suggested citation: Austrian Agency for Health and Food Safety (AGES), Vlachou C and Hofstädter D, 2019. Joint venture on the further development of chemical exposure assessment by use of probabilistic modelling. EFSA Journal 2019;17(S2):e170905, 12 pp. https://doi.org/10.2903/j.efsa.2019.e170905

ISSN: 1831-4732

The EFSA Journal is a publication of the European Food Safety Authority, an agency of the European Union.

Table of contents

1. Introduction

This Technical Report presents the workflow and the outcomes of the project 'Joint venture on the further development of chemical exposure assessment by use of probabilistic modelling', which has been implemented within the framework of the EFSA's 2018–2019 EU-Food Risk Assessment Fellowship Programme (EU-FORA). The project was a joint-cooperation among the Austrian Agency for Health and Food Safety (AGES), the German Federal Institute for Risk Assessment (BfR), the Croatian Food Agency (HAH) and the Croatian University of Osijek (UniOS).

The fellow, whose home institution is the General Chemical State Laboratory of Greece, was hosted by AGES and was placed in the Data, Statistics and Risk Assessment Department, where she had the opportunity to exchange views and knowledge with the Austrian experts and gain new skills, more expertise and hands-on experience in chemical risk assessment. During a short hosting period in BfR, she had also the opportunity for a collaboration with the German experts.

2. Description of work programme

2.1. Aims

The aim of this joint initiative was the further development of chemical risk assessment methodologies in food safety, with focus on the probabilistic modelling of dietary exposure. The project included case studies, in which the exposure assessment of a chemical hazard in food would be performed through probabilistic modelling with different software tools, using defined data sets from the participating countries. Critical comparison of the methodologies and of the results and compilation of a Guideline Document on probabilistic exposure assessment including gap analysis were the expected short-term outcomes of the project. As a long-term goal, these results shall be used for capacity building in the participating institutions and in programming tailor-made solutions for the risk assessment of chemical substances in food. Further objectives of the fellowship were to offer relevant training sessions to the fellow, to support her participation in the activities of the hosting organisation and to encourage scientific contributions related to the project.

2.2. Activities/Methods

All chemical risk assessment case studies were conducted with respect to the general principles of Regulation (EC) No 178/2002 and the WHO Human Health Risk Assessment Toolkit (WHO, 2010a) and included the following steps: (i) Problem formulation, (ii) Hazard identification, (iii) Hazard characterisation, (iv) Exposure assessment and (v) Risk characterisation.

Exposure assessment is a fundamental and crucial component of the risk assessment process, as the risk characterisation outcome and any consequent risk management decisions and measures depend largely on the calculated exposure estimates, which should be as close as possible to the 'true' exposure of the population. Deterministic calculation of the dietary intake through generation of point estimates with use of single input data sets is until now the most frequently used methodology for exposure assessment purposes, as it is considered simple to use and understand. However, deterministic methodologies have several limitations, the most important being that they result in substantially more conservative estimates. Probabilistic modelling is a valuable alternative concept, as it utilises distributions for both the occurrence and the consumption data, results in more realistic and precise estimates of the distribution of intake and allows the determination of the primary sources of variability and uncertainty. Despite the unquestionable advantages, probabilistic methodologies have their limitations, such as complexity and data, time and software requirements (Kroes et al., 2002).

The development of probabilistic analysis skills constitutes a strategic decision for the future activities of an organisation, which perform risk assessments with public health relevance. Therefore, the common interest of the participating agencies in this project was to explore as many options as possible, in order to develop and maintain the capacity for scientific modelling, preferably using open source software. A systematic review on the available free of charge software that can be used as a tool for probabilistic exposure assessment and risk modelling was conducted by a postgraduate student from the Croatian University of Osijek. The main functionalities, the possibility of control over the procedures and the availability of technical and support documentation were the main criteria used for the evaluation. Three main categories of non-licenced software for probabilistic modelling of exposure are available: (i) Standard tools, such as the Monte Carlo Risk Assessment (MCRA) software, provided by the Wageningen University (WUR/FERA/RIVM, 2016), (ii) programming languages, such as

R, and (iii) *Excel*-based tools. The first type operates as a 'black box' system, not allowing knowledge or control on the data processing. Non-standard data sets or completely new assessment tasks cannot be handled by those tools. The lack of technical transparency may also pose problems concerning full model documentation, comparison and validation of results. The second type requires expertise on programming, but allows case-specific tailoring and control on the procedures performed in means of a code. *R* packages such as '*fittdistrplus*' and '*mc2d*' provide additional functionalities for probabilistic risk assessment and Monte Carlo simulation. Another *R*-based free software, *rrisk*, falls also in this category. *rrisk* is under development by the BfR as a prototype for quantitative risk assessment and provides all necessary functionalities from model development to documentation of the risk assessment output. The third category includes some *Excel* add-in tools, which suffer from disadvantages regarding lack of info on the usage of certain functionalities or algorithms.

The case studies included in this project comprise exposure assessment based on a standard software tool (MCRA) and/or comparison to a risk model in *R*, according to the EFSA Guidance on the Use of Probabilistic Methodology for Modelling Dietary Exposure. This Guidance recommends the generation of a pessimistic and an optimistic scenario (EFSA, 2012b), constructed according to the upper bound (UB) and lower bound (LB) substitution approach, respectively (EFSA, 2010).

Uncertainty and sensitivity analysis were based on the EFSA Guidance for case-specific assessments (EFSA Scientific Committee, 2018), which requires a systematic identification of all sources of uncertainty, including both the inputs (data, estimates, other evidence) and the methods (statistical methodologies, calculations or models, reasoning) used for the assessment.

2.2.1. Case study 1. Risk assessment on cadmium in food

Cadmium is a toxic heavy metal, which occurs naturally in the earth's crust, accumulates in soils (Tóth et al., 2016) and plants (Shahid et al., 2017), bioconcentrates and bioaccumulates in aquatic organisms (Rubio-Franchini et al., 2016) and occurs ubiquitously as a contaminant in numerous food categories (EFSA, 2009a). Diet and tobacco smoking are the primary sources of human exposure to cadmium (WHO, 2010b), which then accumulates in the body, as it has a half-time of over of 26 years (ATSDR, 2012). The deleterious effects include carcinogenicity (IARC, 2012), end-stage renal failure (Kobayashi et al., 2008), bone demineralisation, (Kjellström, 1992), reproductive and developmental toxicity (Gupta, 2011) and disturbance of metabolism (Edward and Ackerman, 2016) and macro- and micronutrients homeostasis (Kim et al., 2007).

Problem formulation

The objectives of this case study were to estimate whether the chronic dietary exposure of the Austrian adult population to cadmium exceeds the relevant health-based guidance value (tolerable weekly intake (TWI)) and to identify the food categories that mostly contribute to the intake.

Hazard identification and characterisation

A literature review was conducted by the fellow. Due to resources and time limitations, data from the last EFSA Opinion (EFSA, 2009a) on cadmium as well as from the toxicological profiles released by other institutions (ATSDR, 2012, IARC, 2012) were enriched with recent references, in order to account for any new scientific evidence regarding the toxicity of cadmium.

Exposure assessment

The exposure assessment was based on the occurrence levels of cadmium analysed within the framework of the Austrian Official Food Control 2010–2017 and the consumption data from the most recent Austrian dietary survey of 2016. The determination and quantification of cadmium was performed either with inductively coupled plasma mass spectrometry (ICP-MS) or with atomic absorption-graphite furnace technique (GF-AAS) in the ISO 17025 accredited AGES laboratories. The food categories were standardised under EFSA's FoodEx2 hierarchical classification system. The fellow had active participation in the evaluation of the occurrence data for relevance and quality, performed a preliminary summary and delivered descriptive statistics on the analytical results with the programming language *R*. The obtained results provided further insights into the identification of the most contaminated food categories (Vlachou et al., 2019b). The consumption data were available from the national 2-day dietary survey of 2016, conducted by the University of Vienna according to the General principles for the collection of national food consumption data provided by EFSA (2009b). The food categories were coded under FoodEx2. The same data sets of the occurrence and the consumption data were used for the modelling of the intake both for the MCRA and for the *R* application. The results for the optimistic and pessimistic

scenarios with the use of the MCRA software are summarised on Table 1. The model-based usual exposure distribution for the pessimistic scenario is presented in Figure 1.

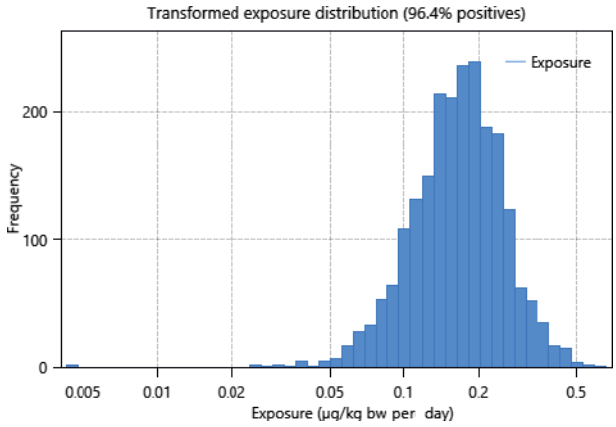


Figure 1: The model-based usual exposure distribution to cadmium for the pessimistic scenario with the use of the MCRA software

In order to account for the other major source of exposure of the general population to cadmium, which is the inhalational exposure to tobacco smoking (WHO, 2010b), the fellow conducted a literature review, summarised the results of the Austrian official control on cadmium in tobacco products, and applied a methodology used by EFSA (2009a) to estimate the magnitude of exposure. The minimum estimated weekly deposition of cadmium in the lungs of a heavy smoker (≥ 20 cigarettes/day) with a body weight of 60 kg b.w. was 0.3 µg cadmium/kg b.w. (Vlachou et al., 2019a). The findings of this study are very important for risk assessment and risk management purposes, as 26% of men and 22% of women > 15 years in Austria are daily smokers, while the percentages for heavy smokers are 12.1% and 6.7%, respectively (EUROSTAT, 2014).

Risk characterisation

The TWI of 2.5 µg/kg b.w. adopted by EFSA was the selected health-based guidance value for the risk characterisation (EFSA, 2009a, 2012a). The results are presented on Table 1. Taking into account, the lack of occurrence data for cadmium in many important food categories and the contribution from the inhalational exposure through tobacco smoking, along with the contribution from other sources, which were not considered for this assessment, we could suggest that the exposure estimates are likely higher, and might be close or even exceeding the TWI.

Table 1: Estimates and 95% CIs for the mean and upper tail (P95) exposure of the Austrian adult population to cadmium (µg/kg b.w. per week) and % contribution to the TWI for the optimistic and the pessimistic scenario with the use of the MCRA software

Scenarios	Mean exposure (µg/kg b.w. per week)	Contribution to the TWI (%)	P95 exposure (µg/kg b.w. per week)	Contribution to the TWI (%)
Optimistic	1.05 (0.9639–1.1389)	42.0 (38.6–45.6)	2.01 (1.7969–2.2267)	80.4 (71.9–89.1)
Pessimistic	1.19 (1.1522–1.3118)	47.6 (46.1–52.5)	2.20 (2.0293–2.3828)	88.1 (81.2–95.3)

CI: confidence interval; TWI: tolerable weekly intake; MCRA: Monte Carlo Risk Assessment; b.w.: body weight.

2.2.2. Case study 2. Risk assessment on acrylamide in food

Acrylamide occurs as a processing contaminant in food, resulting from the Maillard reaction between amino acids and reducing sugars (Mottram et al., 2002). Fried or baked carbohydrate-rich foods are the most contaminated food categories. High levels of acrylamide have been reported in fried potato products, breads, biscuits, breakfast cereals, coffee, cocoa and baby foods (EFSA CONTAM Panel, 2015). Once ingested, acrylamide is readily absorbed and largely distributed in the body. Detoxification after glutathione conjugation and epoxidation to glycidamide (Doerge et al., 2005) are the main metabolic pathways, the latter suggested to be associated with the genotoxicity (COM, 2009) and carcinogenicity (Hogervorst et al., 2010) of acrylamide observed in animal studies. Other deleterious effects include neurotoxicity and reproductive and developmental toxicity (EFSA CONTAM Panel, 2015).

Problem formulation

The objectives of this case study were to estimate the chronic dietary exposure of the Croatian adult population to acrylamide and to identify the food categories that mostly contribute to the intake.

Hazard identification and characterisation

This stage of the risk assessment process has been implemented by the Croatian Food Agency.

Exposure assessment

The exposure assessment was based on data from the Official Croatian Food Control Plan 2014–2016 on acrylamide occurrence in black and espresso coffee, French fries, chips, breakfast cereals, bread & rolls, cookies and snacks, and the Croatian National Food Consumption Survey on adults (NIPNOP 2011–2012, three 24-hours recall, conducted according to EFSA's guidelines (EFSA, 2009b)). The intake estimates were generated with a tailor-made model based on *R* and are summarised on Table 2.

Table 2: Estimates and 95% CIs for the mean and upper tail (P95) exposure of the Croatian adult population to acrylamide (μg/kg b.w. per day) and for the respective MOEs calculated for neurotoxic and neoplastic effects (*R* model, optimistic scenario)

Evaluated risk	Mean exposure (μg/kg b.w. per day)	MOE	P95 exposure (μg/kg b.w. per day)	MOE
Neurotoxic effects	0.0947 (0.0841–0.1060)	4,541 (5,113–4,057)	0.360 (0.316–0.413)	1,194 (1,361–1,041)
Neoplastic effects		1,795 (2,021–1,604)		472 (538–412)

CI: confidence intervals; b.w.: body weight; MOE: margin of exposure.

Risk characterisation

Since acrylamide and its main metabolite, glycidamide, are genotoxic, no safe level and thus no health-based guidance value has been established. The margin of exposure (MOE) approach was used for risk characterisation. The benchmark dose for a 10% response (BMDL10) values of 0.43 and 0.17 mg/kg b.w. per day were used for non-neoplastic effects (neurotoxicity) and for neoplastic effects, respectively (EFSA CONTAM Panel, 2015). All MOEs calculated for neurotoxic effects within the optimistic scenario (Table 2) and the pessimistic scenario are above the adjusted MOE of 125, indicating no health concern for neurotoxicity. However, all MOE values calculated for neoplastic effects are lower than 10 000, indicating a health concern.

2.2.3. Case study 3. Risk assessment on DON in food

Deoxynivalenol (DON) is a trichothecene-mycotoxin, which is produced by *Fusarium* fungi in cereal grains. DON is relatively heat stable and some industrial processing of the grains can result in increases in concentrations (Abbas et al., 1985; EFSA CONTAM Panel, 2017). Contamination of food and feed with DON is a global issue. Acute intoxication cases have been reported in many countries, mostly in Asia. DON inhibits protein synthesis through binding to ribosomes, and is associated with acute effects on the gastrointestinal system as well as with chronic effects such as intestinal function disruption, immunotoxicity (Antonissen et al., 2014), developmental and reproductive toxicity (SCF, 2002), skeletal abnormalities and postnatal mortality (EFSA CONTAM Panel, 2017). Anorexia and reduced body weight were observed in animal studies following ingestion of feed contaminated with DON. Pigs are the most sensitive species (Rotter et al., 1996; EFSA CONTAM Panel, 2017).

Problem formulation

The objectives of this case study were to estimate the chronic dietary exposure of the Croatian adult population to DON and to identify the food categories that mostly contribute to the intake.

Hazard identification and characterisation

This stage of the risk assessment process has been implemented by the Croatian Food Agency.

Exposure assessment

The exposure assessment was based on Croatian occurrence data on DON and consumption data from the Croatian National Food Consumption Survey. The intake estimates were generated with a tailor-made model based on *R*.

Risk characterisation

The risk characterisation was based on the **group tolerable daily intake (TDI)** of 1 μg/kg b.w. per day established by EFSA CONTAM Panel (2017) for the sum of DON, 3-Ac-DON, 15-Ac-DON and DON-3-glucoside. The selected critical effect was the reduced body weight gain observed in animal studies.

2.2.4. Uncertainty and sensitivity analysis

The uncertainty and sensitivity analysis was based on the recent EFSA Guidance (EFSA Scientific Committee, 2018) and comprises a systematic identification of all potential sources of uncertainty regarding the data and the methods used and of the key factors influencing the output. The main sources of uncertainty associated with the inputs were the absolute lack of occurrence data for some important food categories, the left censoring of the analytical results and the small sample size for some other commodities, the targeted sampling plans, the lack of accepted processing factors, the precision of the description and standardisation in FoodEx2 and the underreporting or misreporting of the consumption in the dietary survey. The main sources of uncertainty associated with the methods were the lack of control on the selection and validation of the model in the MCRA software and potential pitfalls in the structure of the *R* model because no package was available with ready-made functions that would cover all the needs for a risk assessment model. The selection of the statistical methodology of the Monte Carlo simulation was one of the strengths of the project, as this approach allows for the quantification of the uncertainty components and delivers the required estimates accompanied by the respective confidence intervals at a predetermined significance level.

The conclusions of the uncertainty and sensitivity analysis will provide valuable information to the risk managers and will enable prioritisation of uncertainties and decision about the needs for future data collection or research.

2.3. MCRA & R – Comparison of experiences and results

The MCRA software is a web-based platform for probabilistic dietary and/or non-dietary, acute or chronic exposure assessment, which implements the recommendations of EFSA on probabilistic modelling about generation of optimistic and pessimistic scenarios (EFSA, 2012b), or offers the possibility for a custom-made scenario and supports many additional functionalities such as sensitivity analysis, cumulative and aggregated exposure assessment and use of processing factors. It offers a user-friendly interface and is compatible with food categorisation in FoodEx2. The output is very detailed and is presenting the exposure estimates, the uncertainty and the food categories most contributing to the intake. There are some practical limitations: the data shall be prepared in a specific format, which requires some additional effort, and the upload of the files can be challenging. The main detriment is that the user cannot have any knowledge or control over the ongoing procedures.

R is an open source and thus a cost-effective programming language, which is rapidly evolving within a huge community of developers. Learnability and availability of learning resources, extensibility and availability of specified packages (*R* libraries), appropriateness for the handling of large data sets, control over the procedures described through the algorithms are the main advantages of the use of *R* for risk assessment purposes. On the other hand, modelling with *R* is substantially time-demanding and requires the employment of specific skills. The realistic objective of this project was the construction of a tailor-made *R* code under the guidance of the experienced statisticians of AGES, which could be modified and used on demand for probabilistic risk assessment tasks within the current project and in the future. The fellow had introductory and advanced training sessions on R and was able to understand and use the basic functions that are needed in data analysis, algorithms generation and risk simulation. Still, using *R* requires good programming skills. Since no package is available that could cover all the requirements for performing the simulation model, many functions have to be programmed by the user himself.

Comparison of the results on cadmium generated either with the MCRA platform or with *R* revealed that the estimates of the exposure were similar and provided an opportunity for a cross-validation of the two models.

2.4. EU-FORA Fellowship supporting programme

Apart from her participation in the training modules in Parma, Vienna, Berlin and Athens, which were included in the curriculum of the EU-FORA Fellowship programme, the fellow was provided by the hosting organisation AGES with additional training sessions, was enabled to participate in other

activities and benefit from her interaction with colleagues and experts. These supporting activities are presented in Appendix A.

3. Conclusions

3.1. Conclusions from the probabilistic assessments

The EU-FORA Fellowship programme has to a large extent reached the goals predetermined by the participating institutions. The fellow has participated in the establishment of a structured probabilistic risk assessment approach, which is based on a selected standard software tool (MCRA) and a risk model in *R*. The former may be used by the risk assessors as a tool to achieve fast estimates of the usual dietary exposure, whereas the latter shall be used as a refined, state-of-the-art strategy following the commonly used deterministic methodology. The critical comparison of the outputs among the three approaches will contribute to decision-making and risk management. The results of the project are being currently incorporated in a Guidance Document on probabilistic exposure assessment including the gap analysis.

3.2. Conclusions from the participation in the fellowship programme

The fellowship provided the fellow with a unique opportunity to apply and extend her knowledge and elaborate her skills in chemical risk assessment according to European and international guidelines and standards and to widen her hands-on experience in probabilistic assessment of the dietary exposure to contaminants in food through various frameworks. The fellow was fully integrated, had active participation in the activities of the hosting institution, received targeted training, gained valuable insights in methodologies on systematic extraction, evaluation, standardisation, combination and modelling of scientific data and was encouraged to communicate the programme results in poster presentations in conferences, in workshops and in peer-reviewed scientific journals. Furthermore, the fellow had benefit from the dedicated induction training at EFSA and the three further modules offered in Vienna, Berlin and Athens. The outcomes of the programme will contribute to the harmonisation of food risk assessment methodologies and to the capacity building in both the sending and the hosting organisations and could be the basis for future networking and collaborations between them and with EFSA.

3.3. Future goals

Further development and progress of the probabilistic risk assessment methodology, as well as iterative review and refinement of the Guidance Document are the future goals of both the hosting site and the fellow. Meetings and workshops will be scheduled for communication of the results and knowledge transfer to the project team and to the staff of the participating institutions. Publication of the outputs in administrative reports and scientific journals will further contribute to the dissemination of knowledge and experience.

4. Disclaimer

The risk assessment process for the case studies is still ongoing during the last months of the fellowship programme. Therefore, only some limited interim results are included in this report, in order to avoid copyright claims, in case of publication of the final results in other scientific journals.

References

Abbas HK, Mirocha CJ, Pawlosky RJ and Pusch DJ, 1985. Effect of cleaning, milling and baking on deoxynivalenol in wheat. Applied and Environment Microbiology, 50, 482–486.

Antonissen G, Martel A, Pasmans F, Ducatelle R, Verbrugghe E, Vandenbroucke V, Li S, Haesebrouck F, Van Immerseel F and Croubels S, 2014. The impact of fusarium mycotoxins on human and animal host susceptibility to infectious diseases. Toxins, 6, 430–452.

ATSDR, 2012. Toxicological profile for Cadmium. Department of Health and Human Services, Public Health Service, U.S https://www.atsdr.cdc.gov/toxprofiles/tp5.pdf

COM (UK Committee on Mutagenicity of Chemicals in Food, Consumers Products and the Environment), 2009. Statement on the Genotoxicity of Acrylamide. COM/09/S1. Available online: http://www.iacom.org.uk/statements/documents/COM09S1Acrylamide.pdf

Doerge DR, Young JF, McDaniel LP, Twaddle NC and Churchwell MI, 2005. Toxicokinetics of acrylamide and glycidamide in Fischer 344 rats. Toxicology and Applied Pharmacology, 208, 199–209.
Edward J and Ackerman C, 2016. A Review of Diabetes Mellitus and Exposure to the Environmental Toxicant Cadmium with an Emphasis on Likely Mechanisms of Action. Current Diabetes Reviews, 12, 252–258. https://doi.org/10.2174/1573399811666150812142922
EFSA (European Food Safety Authority), 2009a. Scientific Opinion of the Panel on Contaminants in the Food Chain on a request from the European Commission on cadmium in food. EFSA Journal 2009;7(3):980, 139 pp. https://doi.org/10.2903/j.efsa.2009.980
EFSA (European Food Safety Authority), 2009b. General principles for the collection of national food consumption data in the view of a pan-European dietary survey. EFSA Journal 2009;7(12):1435, 51 pp. https://doi.org/10.2903/j.efsa.2009.1435
EFSA (European Food Safety Authority), 2010. Management of left-censored data in dietary exposure assessment of chemical substances. EFSA Journal 2010;8(3):1557, 96 pp. https://doi.org/10.2903/j.efsa.2010.1557
EFSA (European Food Safety Authority), 2012a. EFSA Panel on Contaminants in the Food Chain (CONTAM); Scientific Opinion on tolerable weekly intake for cadmium. EFSA Journal 2011;9(2):1975, 19 pp. https://doi.org/10.2903/j.efsa.2011.1975
EFSA (European Food Safety Authority), 2012b. EFSA Panel on Plant Protection Products and their Residues (PPR); Guidance on the Use of Probabilistic Methodology for Modelling Dietary Exposure to Pesticide Residues. EFSA Journal 2012;10(10):2839, 95 pp. https://doi.org/10.2903/j.efsa.2012.2839
EFSA CONTAM Panel, 2015. Scientific Opinion on acrylamide in food. EFSA Journal 2015;13(6):4104, 321 pp. https://doi.org/10.2903/j.efsa.2015.4104
EFSA CONTAM Panel, 2017. Scientific Opinion on the risks to human and animal health related to the presence of deoxynivalenol and its acetylated and modified forms in food and feed. EFSA Journal 2017;15(9):4718, 345 pp. https://doi.org/10.2903/j.efsa.2017.4718
EFSA Scientific Committee, 2018. Guidance on Uncertainty Analysis in Scientific Assessments. EFSA Journal 2018;16(1):5123, 39 pp. https://doi.org/10.2903/j.efsa.2018.5123
EUROSTAT, 2014. Tobacco Consumption Statistics from the European Health Interview Survey 2014. Available online: https://ec.europa.eu/eurostat/statistics-explained/index.php/Tobacco_consumption_statistics
Gupta CR, 2011. Reproductive and Developmental Toxicology, 1st edition. Acad. Press. pp. 424–426.
Hogervorst JG, Baars BJ, Schouten LJ, Konings EJ, Goldbohm RA and van den Brandt PA, 2010. The carcinogenicity of dietary acrylamide intake: a comparative discussion of epidemiological and experimental animal research. Critical Reviews in Toxicology, 40, 485–512.
IARC, 2012. Monograph: Cadmium and Cadmium Compounds. Available online: https://monographs.iarc.fr/wp-content/uploads/2018/06/mono100C-8.pdf
Kim D-W, Kim K-Y, Choi B-S, Youn P, Ryu D-Y, Klaassen CD and Park J-D, 2007. Regulation of metal transporters by dietary iron, and the relationship between body iron levels and cadmium uptake. Archives of Toxicology, 81, 327–334.
Kjellström T, 1992. Mechanism and epidemiology of bone effects of cadmium. IARC Scientific Publications, 118, 301–310.
Kobayashi E, Suwazono Y, Dochi M, Honda R, Nishijo M, Kido T and Nakagawa H, 2008. Estimation of benchmark doses as threshold levels of urinary cadmium, based on excretion of beta2-microglobulin in cadmium-polluted and non-polluted regions in Japan. Toxicology Letters, 179, 108–112.
Kroes R, Müller D, Lambe J, Löwik MRH, van Klaveren J, Kleiner J, Massey R, Mayer S, Urieta I, Verger P and Visconti A, 2002. Assessment of intake from the diet. Food and Chemical Toxicology, 40, 327–385.
Mottram DS, Wedzicha BL and Dodson AT, 2002. Food chemistry: Acrylamide is formed in the Maillard reaction. Nature, 419, 448–449.
Rotter BA, Prelusky DB and Pestka JJ, 1996. Invited review: Toxicology of deoxynivalenol (vomitoxin). Journal of Toxicology and Environment Health, 48, 1–34. https://doi.org/10.1080/009841096161447
Rubio-Franchini I, López-Hernández M, Ramos-Espinosa MG and Rico-Martínez R, 2016. Bioaccumulation of Metals Arsenic, Cadmium, and Lead in Zooplankton and Fishes from the Tula River Watershed, Mexico, Water. Air & Soil Pollution, 227, 5.
SCF, 2002. Opinion of the Scientific Committee on Food on Fusarium toxins. Part 6: Group evaluation of T-2 toxin, HT-2 toxin, nivalenol and deoxynivalenol. SCF/CS/CNTM/MYC/27. Available online: https://ec.europa.eu/food/sites/food/files/safety/docs/cs_contaminants_catalogue_fusarium_out123_en.pdf
Shahid M, Dumat C, Khalid S, Niazi N and Antunes PMC, 2017. Cadmium Bioavailability, Uptake, Toxicity and Detoxification in Soil-Plant System. Reviews of Environmental Contamination and Toxicology, 241, 73–137.
Tóth G, Hermann T, Da Silva MR and Montanarella L, 2016. Heavy metals in agricultural soils of the European Union with implications for food safety. Environment International, 88, 299–309.
Vlachou C, Wolf J and Hofstädter D, 2019a. Non-Dietary Exposure to Cadmium: Tobacco Smoking. Scientific Symposium of the Austrian Society of Toxicology (ASTOX), Vienna, Austria, 25-26/04/2019.
Vlachou C, Wolf J, Mihats D and Hofstädter D, 2019b. Cadmium levels in foods from the Austrian market: Results of the Official Food Control 2010-2017. 74. ALVA-Jahrestagung, Vienna, 27-28/05/2019.

WHO, 2010a. WHO human health risk assessment toolkit: chemical hazards (IPCS harmonization project document; no. 8).
WHO, 2010b. Exposure to Cadmium: A Major Public Health Concern. Available online: https://www.who.int/ipcs/features/cadmium.pdf?ua=1
WUR/FERA/RIVM, 2016. MCRA 8.2 a web-based program for Monte Carlo Risk Assessment Reference Manual.

Abbreviations

AGES	Austrian Agency for Health and Food Safety
ATSDR	Agency for Toxic Substances and Disease Registry
BfR	German Federal Institute for Risk Assessment
BMDL10	benchmark dose for a 10% response
b.w.	body weight
CI	confidence interval
DON	deoxynivalenol
EU-FORA	European Union Food Risk Assessment
EUROSTAT	The Statistical Office of the European Union
FERA	Food and Environmental Research Agency, The Netherlands
GF-AAS	atomic absorption-graphite furnace technique
HAH	Croatian Food Agency
IARC	International Agency for Research on Cancer
ICP-MS	inductively coupled plasma-mass spectrometry
LB	lower bound
MCRA	Monte Carlo Risk Assessment
MOE	margin of exposure
RIVM	National Institute for Public Health and the Environment, The Netherlands
SCF	Scientific Committee on Food
TDI	tolerable daily intake
TWI	tolerable weekly intake
UB	upper bound
UniOS	University of Osijek
WHO	World Health Organization
WUR	Wageningen University and Research centre

Appendix A – Supporting activities during the EU-FORA Fellowship

	Title	Date
Training sessions	Introduction to Statistics & Software *R*	9–11.10.2018
	Advanced Statistics: Probabilistic Modelling with R	19–23.11.2018
	Strategy-Mission-Tasks of AGES	2.10.2018
	Risk Assessment in AGES	2.10.2018
	FoodEx2 Webinar – Part I	26.9.2018
	FoodEx2 Webinar – Part II	3.10.2018
	Food Control in Austria	11.10.2018
	Literature Search based on 'EndNote'	25.10.2018
	Medical Biometry and Epidemiology	17–18.12.2018
	Introduction to Novel Food	23.1.2019
Other activities	Scientific Symposium (Poster): Vlachou C, Wolf J and Hofstädter D. *Non-Dietary Exposure to Cadmium: Tobacco Smoking*. Scientific Symposium of the Austrian Society of Toxicology (ASTOX), Vienna	25–26.4.2019
	Scientific Conference (Poster and contribution to the proceedings): Vlachou C, Wolf J, Mihats D and Hofstädter D. *Cadmium levels in foods from the Austrian market: Results of the Official Food Control 2010-2017*. 74. ALVA-Jahrestagung, Vienna	27–28.5.2019
	Visit of the AGES Departments in Graz: Data, Statistics and Risk Assessment & Food Safety, Experts Coordination and Fraud Protection Meeting with the experts, exchange of knowledge and views on aspects of food safety and discussions on the course of the fellowship programme with the statisticians.	9–11.10.2018 19–23.11.2018 19.12.2018 27.6.2019
	Visit of the AGES Laboratories in Linz Meeting with the experts, exchange of knowledge and views on aspects of chemical analysis of contaminants in food	25.6.2019

EU-FORA SERIES 2

APPROVED: 31 July 2019

doi: 10.2903/j.efsa.2019.e170906

Quantitative risk assessment of *Listeria monocytogenes* in a traditional RTE product

Department of Veterinary Medicine, University of Perugia, Perugia, Italy,
Chrystalleni Hadjicharalambous, Luca Grispoldi and Beniamino Cenci Goga

Abstract

Ready to Eat (RTE) cooked meat products are among the most consumed RTE food subcategories in the EU. They are also associated with the highest number of listeriosis cases per year. Contamination with *Listeria monocytogenes* may arise from post-processing and its growth is often supported by the pH and water activity of the product. *L. monocytogenes* may grow during refrigeration and reach unacceptable levels at the time of consumption, posing a public health risk. The aim of this study was to conduct a Quantitative Microbiological Risk Assessment (QMRA) of *L. monocytogenes* in a traditional Italian RTE cooked meat product. Data for the risk assessment included prevalence and concentration of the microorganism, temperature-time conditions during transport and storage, information on the growth of the microorganism and its potential for disease (dose–response). These data were obtained from laboratory analysis of product samples (n = 50), a consumer survey (n = 160), recordings of temperatures of domestic refrigerators (n = 60) and were complemented with information from the literature. The data were described with appropriate probability distributions and introduced into a previously described growth model of *L. monocytogenes*. Based on the above components, a probabilistic model was created to evaluate the growth of *L. monocytogenes* at each stage of the product pathway (retail storage, transportation and domestic storage) using Monte Carlo simulations. The model design for this pathogen/food product combination, alongside with the findings of the study are included in a separate publication (manuscript under preparation). The results may help risk managers to apply appropriate control measures to minimise the public health risk. The project contributed to further education of the fellow, especially in the use of QMRA risk analysis tools and laid the foundations for future collaborations between the fellow's home institution, the University of Crete, Greece and the University of Perugia, Italy.

Keywords: *Listeria monocytogenes*, risk assessment, RTE meat, head cheese, QMRA

Correspondence: eu-fora@efsa.europa.eu

Acknowledgements: This report is funded by EFSA as part of the EU-FORA programme.

Suggested citation: Department of Veterinary Medicine, University of Perugia, Perugia, Italy, Hadjicharalambous C, Grispoldi L and Goga BC, 2019. Quantitative risk assessment of *Listeria monocytogenes* in a traditional RTE product. EFSA Journal 2019;17(S2):e170906, 9 pp. https://doi.org/10.2903/j.efsa.2019.e170906

ISSN: 1831-4732

The EFSA Journal is a publication of the European Food Safety Authority, an agency of the European Union.

Table of contents

1. Introduction

Listeria monocytogenes remains a significant public health concern, despite not being among the most commonly reported causes of food-borne illness (Buchanan et al., 2017). Listeriosis may present a mild infection in healthy people, but it can be very serious among susceptible populations, characterised by high mortality and hospitalisation rates. In addition, the number of detected outbreaks is usually low, since most invasive listeriosis cases appear as sporadic infections (EFSA, 2018). Consequently, there is great uncertainty about the true burden of listeriosis and under-reporting has been estimated at around a factor of two in the UK and North America (EFSA BIOHAZ Panel, 2018).

L. monocytogenes is a bacterium that is common in the environment and can be found in agricultural and food production environments, where once established, it tends to persist through the creation of biofilms (Gray et al., 2018). Possible entry routes of the bacterium in the final product are both raw material contamination and cross-contamination during food processing. Ready to Eat (RTE) foods have been shown to be one of the most important vehicles responsible for human infections by several studies (Cenci-Goga et al., 2018; Kurpas et al., 2018). RTE foods typically associated with human listeriosis, include 'meat and meat products' (Cenci-Goga et al., 2008), 'fish and fish products' and 'milk and milk products', as well as foods of plant origin and frozen foods. In the 2018 EFSA scientific opinion on listeriosis (EFSA BIOHAZ Panel, 2018), which considers foods of animal origin, it was reported that cooked meat and heat-treated sausages were the RTE food subcategories with most consumed servings per person and per year in the European Union (EU)/European Economic Area (EEA). Simultaneously, cooked meat products were associated with the largest number of listeriosis cases per year (more than 850). Depending on the formulation and storage conditions, almost all RTE foods may support growth of *L. monocytogenes* and therefore have the potential to cause disease, especially when consumed by the susceptible population (Cenci-Goga et al., 2012, 2015)

L. monocytogenes prevalence and contamination data of various RTE food categories (meat, milk, fish and their products) marketed in the EU is important for estimating the public health risk for listeriosis. Nevertheless, in the 2018 EFSA opinion, it was reported that 41% of listeriosis outbreaks are linked to foods not considered in the Opinion, thus highlighting the need for more QMRA studies to generate data on both meat and plant derived RTE foods (EFSA BIOHAZ Panel, 2018).

Up to date, neither contamination levels nor a risk assessment study for the Italian Head Cheese are available in literature. The traditionally cooked deli meat product, named "Coppa di Testa", is produced seasonally by several small and large processing establishments in Italy, using local pork (or hog) meat. It has been identified as a product that can support the growth of *L. monocytogenes* once contaminated with the bacterium, therefore posing a potential risk for public health (Bardasi et al., 2010). Different variations of the head cheese are also manufactured in several parts of the world, including the EU and the USA. The product has been previously linked to outbreaks of invasive listeriosis in the USA (2011) and more recently in an outbreak that occurred in Italy between May 2015 and March 2016 (CDC, 2011; Duranti et al., 2018). In addition, detection of *L. monocytogenes* during routine testing has resulted in recent Italian head cheese recalls.[1,2]

2. Description of work programme

2.1. Aims

The overall objective of the work programme was to apply 'risk assessment' methodology in order to estimate the public health risk from *L. monocytogenes* following consumption of 'Coppa Di Testa' head cheese. As a scientific process, risk assessment determines the relationship between exposure to a given hazard under a defined set of conditions and the likelihood of an adverse health effect or disease (McLauchlin et al., 2004; Koutsoumanis and Aspridou, 2016). In this work, we applied a Quantitative Microbial Risk Assessment (QMRA) model, which was run on experimental and literature derived data. More specifically, the aim was to:

1) Collect and analyse information on the prevalence of *L. monocytogenes* in product samples, time/temperature storage conditions, consumption data; identify where critical data and/or knowledge is lacking;
2) Characterise the nature and size of the microbial food safety risk due to *L. monocytogenes* in RTE Coppa Di Testa products.

[1] https://www.investireoggi.it/fisco/salume-listeria-coppa-testa-ritirato-dal-mercato-marca-lotto/
[2] http://www.salute.gov.it/portale/news/p3_2_1_3_5_1.jsp?lingua=italiano&menu=notizie&p=avvisi&tipo=richiami&id=505

In addition, through this assessment we aimed to identify factors that contribute most significantly to the risk and suggest potential management strategies to reduce the food safety risk due to *L. monocytogenes*. The main output of the work programme will be the preparation of a manuscript describing the application of a QMRA model for *L. monocytogenes* in RTE Coppa Di Testa, in order to disseminate the results of the project to the wider scientific community. The methodology and the steps applied for the QMRA as well as other activities in which the fellow has been involved are briefly described below.

2.2. Activities/Methods

2.2.1. Production process, sample analysis and consumer survey

In order to better understand the possible entry points for *L. monocytogenes*, the production of 'Coppa di Testa' was carefully recorded during visits of the fellow and other lab members to various production facilities in the area of Umbria, Italy. Coppa di testa is a traditional cooked pork salami seasonally produced by several small and large processing establishments using local pork (or hog) meat. Briefly, it is made by deboned head meat with the addition of tongue and rind. Salt and spices are added and the product is placed in moulds, portioned and vacuum-packaged before distributed to retail stores.

Also, as mentioned above, available literature data on Coppa di Testa are very limited. Therefore, to acquire data on *L. monocytogenes* prevalence required for the stochastic model described below, we sought to collect and directly analyse a number of samples in the laboratory, complementing this analysis with data from local authorities if possible. About 50 random products were purchased from different retail shops either vacuum packed or sliced. Analysis was carried out at the hosting institution and involved measurements on intrinsic factors of the product such as pH and water activity as well as microbiological analysis according to the methods previously described (Cenci-Goga et al., 2012). The ISO 11290 method (ISO, 1996) was used to isolate *Listeria monocytogenes*.

In addition, information on Coppa di Testa domestic storage and consumption habits of consumers was derived after conducting a consumer survey. A relevant questionnaire was prepared, in which consumers were asked to complete information on (i) their personal characteristics (age, gender, susceptible group or not); (ii) the size (portions) and frequency (per week) of the product consumption; as well as (iii) the time required for product transport to home and (iv) the time of domestic storage before consumption for vacuum-packed and sliced products. The anonymous consumer survey was distributed not only via web-based social network platforms such as Facebook, but also in printed form via short consumer interviews during visits to local facilities, i.e. a house for the elderly, supermarkets, medical practices. In total, our survey resulted in more than 160 responses among the Italian population. Responses coming from consumers of other countries were not included. Nevertheless, thanks to the assistance of Dr. Giorgiana Catunescu, also participating in the EU-FORA programme, the questionnaire was made available to a number of Romanian consumers, yielding about 100 responses. Since a product that is similar to Coppa di Testa is produced and consumed in Romania, the survey data may be useful in a future study. Among the 162 Italian participants (39% men and 61% women), 20% of men and 10% of women were > 65 years of age. Briefly, the results of the survey show that the majority (82%) of the consumers do not consume Coppa di Testa on a regular basis. However, about 15% of the participants, consume the product once per week and 3% consume it 2–3 times per week. The majority prefers to consume it mostly during dinner (50%) and lunch (42%) and less frequently during breakfast (8%) with the average slice number/person/week estimated at 3.7 slices.

2.2.2. Application of stochastic mathematical modelling for QMRA – model development

The fellow was involved in the development and application of a stochastic model, as an important tool for quantitative microbial risk analysis. The overall risk assessment was based on four separate stages (in accordance with Codex Alimentarius) namely (i) hazard identification, (ii) hazard characterisation, (iii) exposure assessment and (iv) risk characterisation. More specifically:

- Collection of literature data on listeriosis and the behaviour of *L. monocytogenes* in meat derived RTE foods, especially in Coppa di Testa head cheese (hazard identification);
- Presentation of the potential for *L. monocytogenes* to cause illness in human populations based on literature (hazard characterisation/dose–response);

- Determination of the exposure to *L. monocytogenes* from consumption of head cheese: collection and analysis of data on prevalence and on consumption habits of a sample group among the population (exposure assessment);
- Application of an appropriate mathematical model, integrating exposure assessment and hazard characterisation, in order to estimate the public health risk from consumption of head cheese contaminated with *L. monocytogenes* (risk characterisation).

The product pathway and the risk assessment process is diagrammed in Figure 1.

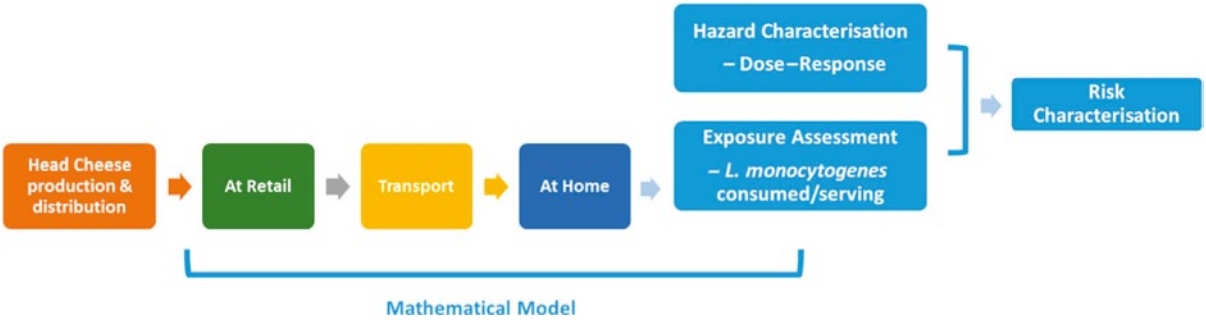


Figure 1: The product pathway and risk assessment process

Initially, the literature was scanned and publications relevant to '*L. monocytogenes* and RTE meat products' were retrieved through searches using the PubMed[3] database, Google Scholar,[4] Scopus[5] and Web of Science[6]. Information was also retrieved from websites belonging to relevant organisations and authorities (e.g. WHO, EFSA, FDA). For hazard characterisation, a dose–response curve was necessary and describes the possibility of illness following consumption of a certain number of pathogenic bacterial cells. Based on several previous QMRA studies, (Giovannini et al., 2007; Ross et al., 2009; Mataragas et al., 2010; Bassett et al., 2012), the general form of the exponential dose–response model by FAO/WHO was selected for this work (FAO/WHO, 2004).

For the exposure assessment, we needed to describe how often and at what levels, consumers in the population consume the hazard in the food of interest (Lammerding and Fazil, 2000). The important output from the exposure assessment was the 'number of *L. monocytogenes* per serving of contaminated Coppa di Testa' was and this was estimated using information about the frequency of contamination (prevalence) and the final contamination levels at the point of consumption. The prevalence was estimated by microbial analysis of samples, whereas predictive microbiology was applied to calculate the final contamination levels from initial contamination levels (at the point of retail or production) as well as growth of *L. monocytogenes* based on product formulation, times and temperatures of distribution and storage prior to consumption. For this purpose, the fellow used data derived from expert opinion (producers and retailers), product labels, as well as temperature data obtained after conducting a study on 60 domestic refrigerators. In addition, information on consumption (size and the number of servings) as well as product storage habits was obtained by the anonymous consumer survey described above. Consumption size and frequency data from the survey were fitted into distributions using @Risk and applied to the model calculations. A schematic overview of the influence diagram for the exposure assessment is presented in Figure 2.

Finally, in order to perform a QMRA, the fellow worked on developing a stochastic model and integrated the steps of hazard characterisation (dose–response relationship) and exposure assessment, leading to the risk characterisation output. The model implemented Monte Carlo simulations and was designed using the @Risk simulation software (@Risk 7.6 for Excel, Palisade, Ithaca, USA), as an add-in to Microsoft Excel. The mathematical model describes the possibility of post-production contamination of Coppa di Testa with *L. monocytogenes* and the effect of temperature and time, during transport and storage, on the growth of the bacterium. The model structure incorporates data from our laboratory sample analysis and from the literature, information derived from questionnaires, previous risk assessments and on consultation with experts. Integrated with the dose–response relationship for

[3] http://www.ncbi.nlm.nih.gov/pubmed
[4] https://scholar.google.com
[5] https://www.scopus.com
[6] https://apps.webofknowledge.com

L. monocytogenes, the model calculates the likelihood of public health adverse effects following consumption of the product. A manuscript currently under preparation includes a comprehensive description of the QMRA study, details on the model structure and mathematical calculations and the results of the study. It is expected to be submitted for publication in a peer-reviewed journal in the coming period.

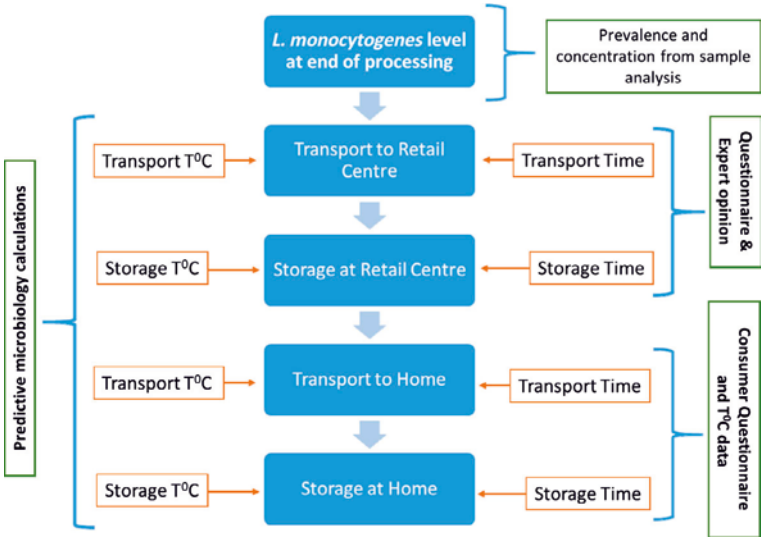


Figure 2: Influence Diagram of the overall structure of the exposure assessment part of the model. The various stages between production and consumption are discretely modelled and the output of each stage is influenced by model inputs as depicted by the arrow points

2.2.3. Other activities during the EU-FORA fellowship

During the fellowship, the fellow had the opportunity to participate to various scientific activities. These included:

- Presentation of the Risk assessment methodology for microbial risks in foods (lecture to undergraduate students at the University of Perugia)
- Participation to the annual European College of Veterinary Public Health (ECVPH) conference, organised in Perugia (October 2018)
- A one-day trip accompanying university students to inspection visits to a large meat-canning factory.
- Visits to the local slaughterhouse accompanied by a tutorial on animal welfare measures, microbial contamination risks and inspection during the process.
- Visits to processed meat production plants and observation of the production procedures for Coppa di Testa and other processed meats. Conducted interviews of producer managers regarding the process and safety measures against contamination.
- Dissemination of the survey questionnaires to the public through visits to supermarkets, a house for the elderly etc.
- Preparation of an abstract/poster for the National conference AIVI (National Congress of the Italian Association of Veterinary Food Hygienists), Bari, Italy September 11–13 (upcoming event).
- Preparation of two research manuscripts: a review manuscript on *L. monocytogenes* (title not available yet) and a second manuscript with results from the main subject of this work programme (Title: Quantitative risk assessment of *Listeria monocytogenes* in ready-to-eat head cheese in Italy). These manuscripts are currently under preparation.
- Preparation of a joint application for funding of a 3-year research project between the institution of the fellow (University of Crete, Greece) and the hosting institution (University of Perugia, Italy), aiming to create future collaboration opportunities between the two organisations. The proposal has been submitted (March 2019) to the Hellenic Foundation for Research and Innovation (HFRI) and at the time of writing, is under review.

Finally, the fellow attended seminars at the Department of Veterinary Medicine and participated in outdoor activities organised by the hosting laboratory.

3. Conclusions

The main focus of the work programme was the development and application of a quantitative microbial risk assessment model in order to estimate the public health risk for listeriosis following consumption of Italian head cheese. The programme has enabled the fellow to gain expertise in risk assessments related to microbiological hazards. Through this process, the fellow learned how to design a risk assessment pathway and to build a QMRA model by combining information, experimental data and mathematical equations while taking into account all input parameters (i.e. temperature, time, microbial population) that affect the output. One important aspect of this was the familiarisation of the fellow with relevant online risk assessment platforms and especially the @Risk software package, often used as a powerful risk analysis tool. Both the fellow and the supervisor agree that the EU-FORA programme was a valuable opportunity to discuss opinions, methodologies and science, and an important step to building a professional network that will serve as a basis for future collaboration in risk assessment studies.

References

Bardasi L, Bonilauri P, Rugna G, Galletti G, Fedrizzi G, Santandrea G, Gandolfi P, Vecchi G and Merialdi G, 2010. Growth of naturally occurring *Listeria innocua* in Coppa di Testa. Italian Journal of Food Safety, 1, 35–39.

Bassett J, Nauta M, Lindqvist R and Zwietering M, 2012. Tools for Microbiological risk assessment. ILSI Europe. ILSI Europe Report Series, In.

Buchanan Robert L, Gorris Leon GM, Hayman Melinda M, Jackson Timothy C and Whiting Richard C, 2017. A review of *Listeria monocytogenes* : An update on outbreaks, virulence, dose-response, ecology, and risk assessments. Food Control, 75, 1–13.

CDC (Centers for Disease Control and Prevention), 2011. Outbreak of invasive listeriosis associated with the consumption of hog head cheese–Louisiana, 2010. MMWR. Morbidity and Mortality Weekly Report, 60, 401–405.

Cenci-Goga BT, Ranucci D, Miraglia D and Cioffi A, 2008. Use of starter cultures of dairy origin in the production of Salame nostrano, an Italian dry-cured sausage. Meat Science, 78, 381–390.

Cenci-Goga BT, Rossitto PV, Sechi P, Parmegiani S, Cambiotti V and Cullor JS, 2012. Effect of selected dairy starter cultures on microbiological, chemical and sensory characteristics of swine and venison (*Dama dama*) nitrite-free dry-cured sausages. Meat Science, 90, 599–606.

Cenci-Goga BT, Karama M, Sechi P, Iulietto MF, Novelli S, Selvaggini R and Mattei S, 2015. Growth Inhibition of Selected Microorganisms by an Association of Dairy Starter Cultures and Probiotics. Italian Journal of Animal Science, 14, 3745.

Cenci-Goga BT, Karama M, Sechi P, Iulietto MF, Grispoldi L, Selvaggini R, Ceccarelli M and Barbera S, 2018. Fate of selected pathogens in spiked <<SALAME NOSTRANO>> produced without added nitrates following the application of NONIT technology. Meat Science, 139, 247–254.

Duranti A, Sabbatucci M, Blasi G, Acciari VA, Ancora M, Bella A, Busani L, Centorame P, Camma C, Conti F, De Medici D, Di Domenico M, Di Marzio V, Filippini G, Fiore A, Fisichella S, Gattuso A, Gianfranceschi M, Graziani C, Guidi F, Marcacci M, Marfoglia C, Neri D, Orsini M, Ottaviani D, Petruzzelli A, Pezzotti P, Rizzo C, Ruolo A, Scavia G, Scuota S, Tagliavento G, Tibaldi A, Tonucci F, Torresi M, Migliorati G and Pomilio F, 2018. A severe outbreak of listeriosis in central Italy with a rare pulsotype associated with processed pork products. Journal of Medical Microbiology, 67, 1351–1360.

EFSA (European Food Safety Authority), 2018. Outcome of the public consultation on the draft Scientific Opinion on Listeria monocytogenes contamination of ready-to-eat foods and the risk for human health in the EU. EFSA Supporting Publications 2018:en-1352, 67 pp. https://www.efsa.europa.eu/en/supporting/pub/en-1352

EFSA BIOHAZ Panel (EFSA Panel on Biological Hazards), Ricci A, Allende A, Bolton D, Chemaly M, Davies R, Escámez Fernández PS, Girones R, Herman L, Koutsoumanis K, Nørrung B, Robertson L, Ru G, Sanaa M, Simmons M, Skandamis P, Snary E, Speybroeck N, Ter Kuile B, Threlfall J, Wahlström H, Takkinen J, Wagner M, Arcella D, Da Silva Felicio MT, Georgiadis M, Messens W and Lindqvist R, 2018. Scientific Opinion on the Listeria monocytogenes contamination of ready-to-eat foods and the risk for human health in the EU. EFSA Journal 2018;16(1):5134, 173 pp. https://doi.org/10.2903/j.efsa.2018.5134

FAO/WHO (Food and Agriculture Organization of the United Nations and World Health Organization), 2004. Risk assessment of Listeria monocytogenes in ready-to-eat foods. TECHNICAL REPORT. Microbiological risk assessment series 5. Food and Agriculture Organization of the United Nations, Viale delle Terme di Caracalla, 00100 Rome, Italy. Available online: http://www.fao.org/es/esn/food/risk_mra_listeria_report_en

Giovannini A, Migliorati G, Prencipe V, Calderone D, Zuccolo C and Cozzolino P, 2007. Risk assessment for listeriosis in consumers of Parma and San Daniele hams. Food Control, 18, 789–799.

Gray JA, Chandry PS, Kaur M, Kocharunchitt C, Bowman JP and Fox EM, 2018. Novel Biocontrol Methods for Listeria monocytogenes Biofilms in Food Production Facilities. Frontiers in Microbiology, 9, 605.

ISO, 1996. ISO 11290–1:1996: Microbiology of food and animal feeding stuffs – horizontal method for the detection and enumeration of Listeria monocytogenes - Part 1: detection method.

Koutsoumanis Konstantinos P and Aspridou Z, 2016. Moving towards a risk-based food safety management. Current Opinion in Food Science, 12, 36–41.

Kurpas M, Wieczorek K and Osek J, 2018. Ready-to-eat meat products as a source of *Listeria monocytogenes*. Journal of Veterinary Research, 62, 49–55.

Lammerding AM and Fazil A, 2000. Hazard identification and exposure assessment for microbial food safety risk assessment. International Journal of Food Microbiology, 58, 147–157.

Mataragas M, Zwietering MH, Skandamis PN and Drosinos EH, 2010. Quantitative microbiological risk assessment as a tool to obtain useful information for risk managers–specific application to Listeria monocytogenes and ready-to-eat meat products. International Journal of Food Microbiology, 141(Suppl 1), S170–S179.

McLauchlin J, Mitchell RT, Smerdon WJ and Jewell K, 2004. *Listeria monocytogenes* and listeriosis: a review of hazard characterisation for use in microbiological risk assessment of foods. International Journal of Food Microbiology, 92, 15–33.

Ross T, Rasmussen S, Fazil A, Paoli G and Sumner J, 2009. Quantitative risk assessment of *Listeria monocytogenes* in ready-to-eat meats in Australia. International Journal of Food Microbiology, 131, 128–137.

Abbreviations

EEA	European Economic Area
EU-FORA	European Food Risk Assessment Fellowship Programme
FAO	Food and Agriculture Organization of the United Nations
FDA	Food and Drug Administration
WHO	World Health Organization
RTE	Ready to eat

EU-FORA SERIES 2

APPROVED: 31 July 2019

doi: 10.2903/j.efsa.2019.e170907

Insects in food and feed – allergenicity risk assessment and analytical detection

German Federal Institute for Risk Assessment (BfR), National Reference Laboratory for Animal protein in Feed, NRL-AP,
Cristiano Garino, Jutta Zagon and Albert Braeuning

Abstract

Insects and insect-based food products have entered in the European market, carrying along issues of safety and the need of establishing a new legal framework. The consumption of massively reared insects can pose chemical and microbiological risks, and insect proteins are likely to represent a hazard for a subpopulation of allergic individuals. All insect-based products are considered 'Novel Food' and fall under EU regulation 2015/2283, according to which a specific application to the European Commission, followed by EFSA scientific evaluation, is needed before the product is put on the market. The recent EU Regulation 2017/893, entered into force on 1 July 2017, allowed a shortlist of seven insect species to be included in the formulation of feeds for aquaculture. Previously, the addition of any insect to any feed for farmed animals was not allowed, due to the risk of prion-derived diseases. The introduction of this new Regulation raises the issue to switch from a classical detection method based on microscopy to a more sophisticated and species-specific method. The overall aims of this EU-FORA project were (i) to set up a new next generation sequencing (NGS)-based molecular method for the identification of insect DNA in feeds for aquaculture; and (ii) to carry out a conceptual work on a probabilistic quantitative risk assessment focused on the allergenicity of yellow mealworm (*Tenebrio molitor*) employed in foods.

Keywords: Insect, Feed, NGS, allergenicity

Correspondence: eu-fora@efsa.europa.eu

Acknowledgements: This report is funded by EFSA as part of the EU-FORA programme. The authors thank Dr. Burkhard Malorny, Dr. Hans Mielke and Dr. Thomas Selhorst for their kind participation and support.

Suggested citation: German Federal Institute for Risk Assessment (BfR), National Reference Laboratory for Animal protein in Feed, NRL-AP, Garino C, Zagon J and Braeuning A, 2019. Insects in food and feed – allergenicity risk assessment and analytical detection. EFSA Journal 2019;17(S2): e170907, 12 pp. https://doi.org/10.2903/j.efsa.2019.e170907

ISSN: 1831-4732

The EFSA Journal is a publication of the European Food Safety Authority, an agency of the European Union.

Table of contents

1. Introduction

The use of insects as food or feed is nowadays widespread around the globe. Entomophagy, the eating of insects, is practiced in more than hundred countries, with over 2,000 documented edible species (Jongema, 2017). United Nations recommended the practice as a potential solution to the shortage of world food supplies (van Huis et al., 2013). In countries like Thailand or Kenya, where insects are largely consumed, people shifted from collecting them in the wild to developing mass-rearing facilities (Dobermann et al., 2017). In Western societies, where protein still derives largely from domesticated land animals, insects are virtually synonymous to nuisance. The European Society generally refuses to accept edible insects as food, and deliberate human entomophagy is rare in westernised societies. Insects are associated with dirt, fear of contamination and disease, along with a psychological and biased thinking regarding taste, odour and colour (Raheem et al., 2018). The feeling of disgust to entomophagy in the West contributes to the common misconception that entomophagy in the developing world is prompted by starvation and is merely a survival mechanism (van Huis et al., 2013). However, insects provide more or less comparable amounts of nutrients (proteins, fat, minerals and vitamins) as other meat sources. Proteins are the main components of the dry matter, and due to their content of essential amino acids insects have a high nutritional value. Fat is rich in polyunsaturated fatty acids (PUFAs), with a profile similar to that of fish. For reared insects, the nutritional profile is strictly dependent on the type of feeding. Finally, insects are to be preferred over large land animals due to their ecological footprint: rearing of insects consumes less land and water, and their greenhouse emissions are far lower. On the other hand, the safety of consuming massively reared whole insects or processed material needs to be assessed. Biological hazards can be represented by pathogenic bacteria, mycotoxin-producing fungi, parasites, viruses, antimicrobial resistance genes, while chemical hazards are heavy metals and generally all toxic chemical compounds. In October 2015, EFSA published a scientific opinion on a risk profile related to the production and consumption of insects as food and feed, concluding that'for both biological and chemical hazards, the specific production methods, the substrate used, the stage of harvest, the insect species, as well as the methods used for further processing will all have an impact on the possible presence of biological and chemical contaminants in insect food and feed products' (EFSA Scientific Committee, 2015). Therefore, a case-by-case risk assessment (RA) is required before a new product is placed on the market. Another potential threat to consumers is the presence of proteins able to trigger allergic reactions in sensitised individuals. The studies so far carried out suggested that the risk is plausible, and that it is especially higher for crustacean-allergic people, even though also other categories of allergic patients might be involved (Verhoeckx et al., 2014).

Insects can also be added to common animal feeds, and recent experiments showed that feeds formulated with insects are viable alternatives for broilers (Rumpold and Schlüter, 2013; Schiavone et al., 2017). Aquaculture (the farming of aquatic animals) is a fast-growing industry that has to rely on feeds today primarily formulated with fishmeal and fish oil. Even though insects are part of the natural diet of fish, their addition in feeds for aquaculture has been banned in Europe until recently, due to the risk of prion-derived diseases. Regarding the risks related to the presence of prions, in its opinion EFSA concluded that, compared to the occurrence of hazards in currently authorised protein sources of animal origin, the occurrence of hazards in non-processed insects is expected to be equal or lower, as long as the insects are fed on substrates that do not harbour material of ruminant or human (manure) origin (EFSA Scientific Committee, 2015). EU Regulation 2017/893, amending previous regulations, allowed a shortlist of seven insect species (*Hermetia illucens, Musca domestica, Tenebrio molitor, Alphitobius diaperinus, Acheta domesticus, Gryllodes sigillatus* and *Gryllus assimilis*) to be included in the formulation of feeds for aquaculture. Accordingly, a specific method to detect the presence of insects in these feeds and to discriminate between allowed and not allowed species needs to be developed.

2. Description of work programme

2.1. Aims

The fellow was enrolled for this EU-FORA fellowship under the working programme 'Use of novel DNA- and mass spectrometry-based detection methods for the identification of potential allergenic species and food authentication'. The German Federal Institute for Risk Assessment (BfR), the fellow's hosting Institution, is home of the National Reference Laboratory for Animal protein in Feed (NRL-AP), which major task is the development and validation of qualitative and quantitative methods for the detection of animal and plant species in food and feed. The fellow research focused on the setting up

of a new next generation sequencing (NGS)-based molecular method for the identification of insect DNA in feeds for aquaculture.

Another important training objective of the fellowship was to learn how to perform an allergenicity RA. Such types of assessment are usually performed for example on genetically modified organisms (GMOs), but an evaluation of the potential ability to trigger allergic reactions is also required when an application for a novel food is submitted to the European Commission. The European Regulation (EU) 2015/2283, applicable as from 1 January 2018 and replacing Regulation (EC) 258/97, stipulates that all insect-based products are considered as 'Novel Food', due to the lack of a significant history of consumption in the EU before 15 May 1997. A new concept of probabilistic quantitative RA for food allergens, aimed at tackling the issue of allergen thresholds in food, has been proposed (Spanjersberg et al., 2007). Moreover, the fellow carried out a conceptual work on a full quantitative RA focused on the allergenicity of yellow mealworm (*T. molitor*) employed in foods.

2.2. Molecular identification of insects in feeds

The setting up of an NGS-based method to detect and identify the presence of insect DNA in a complex matrix such as fish feed required several steps.

2.2.1. Selection of the target sequence

After a brief review of the existing literature on insect metabarcoding, a specific region on the mitochondrial gene coding for the cytochrome oxidase subunit 1 (COI) was selected. This region has been shown to be suitable for the identification of a wide range of animal taxa, from small insects to fish and birds (Nelson et al., 2007). It is one of the most utilised and effective sequences for metabarcoding and it is also the most sequenced one among the various insect species. Information on the COI sequence of all the seven species of insects allowed by EU Reg. 2017/893 could be retrieved from the Barcode of Life Data System (BOLD), a cloud-based data storage and analysis platform developed at the Centre for Biodiversity Genomics in Canada (www.boldsystems.org). The seven allowed species belong to different orders (Coleoptera, Diptera, Orthoptera), which means they are taxonomically and genetically distant from each other, although some of them are more strictly related (for example *Alphitobius* and *Tenebrio* belong to the same subfamily Tenebrioninae, likewise *Acheta*, *Gryllodes* and *Gryllus* all belong to the Gryllinae subfamily). Taxonomical proximity equals reduced genetic variability, and that is why a hypervariable known region like COI was selected.

The number of COI sequences available for each of the seven species under investigation is also important, because it provides information on the individual genetic variability internal to the single species. Such information is crucial when analysing NGS data, because it can enable to distinguish between an informative and a non-informative single nucleotide polymorphism (SNP). Four out of seven species had an extensive number of COI sequences stored in the BOLD database, while in the other three cases (*Alphitobius diaperinus, Gryllodes sigillatus* and *Gryllus assimilis*) only two informative sequences for each species were available. Based on this information, eight primers, able to amplify differently sized products on the COI sequence, were retrieved from the literature and partially re-designed for the purpose of our investigation. All primers were designed to anneal to rather conserved sites of the COI sequence, in order to amplify all insect species but no other arthropods.

2.2.2. Endpoint PCR to evaluate the performances of the primers

The NRL-AP possesses a large collection of samples of insect DNA that was screened for our investigation. Firstly, all DNAs were checked for their quality and amplificability, testing them in endpoint polymerase chain reaction (PCR) with a universal primer pair written on COI and able to amplify a large product of around 700 base pairs. Degraded or low quality DNA samples were substituted with freshly extracted material. The combination of the eight primers purchased gave rise to five different PCR products, three of which resulted successful in practice. These three products were obtained on the seven species allowed and on other not allowed species from the DNA collection. Moreover, one pair performed well also on DNA isolated from complex food matrices containing insects, and on heavily thermally processed samples.

Two of the three successful primer pairs were selected to be further employed in the setting up of the final NGS protocol.

2.2.3. Cloning and sequencing of insect barcoding regions

Insects, source of the DNA samples forming the NRL-AP collection, were mostly purchased online or from local pet feed shops for reptiles in Berlin. Some of them did not have a certified origin, and the species indication was provided only by the seller. Sequencing of the COI region of these insects was performed for two main reasons: (1) to make sure of their real genus and species, and (2) to collect more information on the individual genetic variability within this gene, especially in the case of those species where little information was available from the public databases (*Alphitobius diaperinus*, *Gryllodes sigillatus* and *Gryllus assimilis*). Ten different DNA samples belonging to four alleged species were amplified using the same primer pair employed in the amplificability tests, amplicons were purified from the agarose gel and ligated into a commercial plasmid vector. Plasmids were used to transform chemically competent cells of *Escherichia coli* that were grown on selective agar plates. Positive clones were cultivated in liquid medium and used to prepare plasmid DNA samples, which were then submitted for sequencing. The results showed that only 4 out of 10 samples were actually matching the expected species, while in all other cases problems of mislabelling or of DNA contamination occurred. Moreover, 16 potentially new SNPs of *Acheta domesticus*, *Gryllodes sigillatus* and *Gryllus assimilis* were identified.

2.2.4. Setting up of the NGS protocol

NGS experiments were carried out in collaboration with BfR Unit 4SZ, the Study Centre for Genome Sequencing and Analysis. Selected primer pairs were adapted for the protocol of the preparation of the 16S Metagenomic Sequencing Library for the Illumina MiSeq System. Anchored primers were purchased and preliminarily tested in endpoint PCR to verify their ability of amplifying DNA from different insect species. Twelve different admixtures of DNA from 3 allowed insect species (*H. illucens, Acheta domesticus, T. molitor*) and from other sources (*Drosophila melanogaster*, fish, crustaceans, soybean and fishmeal) were prepared and used as a template for the creation of two libraries, one for each primer pair. These libraries were subsequently sequenced on the Illumina MiSeq instrument using paired 300 base pair reads. In this protocol, the ends of each read are overlapped to generate high-quality, full-length reads in a single 65-hour run. The MiSeq run output is approximately > 20 million reads and can generate > 100,000 reads per sample.

2.3. *Tenebrio molitor* allergenicity RA

The ingestion of whole insects or of insect-derived products may trigger the development of clinical symptoms typical of allergenic reactions in sensitised individuals. Mealworms (*T. molitor, Alphitobius diaperinus* and *Zophobas morio*) are frequently used as animal feed, they are easily reared, have a high nutritional value and a low content of chitin, due to the fact that the insect is collected and processed during the larval stage, before developing the exoskeleton. Several rearing facilities can be found nowadays also in Europe, particularly in the Netherlands.

The first step of RA is the hazard identification (Figure 1). Larvae of the yellow mealworm *T. molitor* displayed the ability of provoking allergic reactions both via inhalation or skin contact (Bernstein, 1983) and via ingestion (Freye, 1996). A recent study showed how individuals can become primarily sensitised to mealworm via contact skin or inhalation, produce specific antibodies (IgEs) against mealworm allergens, and report allergic symptoms after ingestion in a clinical controlled oral food challenge (Broekman et al., 2015).

It is also the only insect so far for which a double-blind, placebo-controlled food challenge (DBPCFC) trial was carried out in clinically allergic patients (Broekman et al., 2016). DBPCFC data are the only ones that can be used to define threshold doses for foods eliciting allergic reactions in susceptible patients. The threshold dose is defined to be one that elicits allergic reactions in a given proportion of susceptible patients (Bindslev-Jensen et al., 2002). The definition of these eliciting doses (EDs) characterises the hazard posed by the food allergen, and is the second step of the probabilistic RA protocol proposed by Spanjersberg et al. (2007). In this type of RA, EDs are compared to several input variables, like the chance that an allergic person consumes a certain product, the amount of the consumed product, or the chance that the food contains allergens (and in which concentration), forming together the 'allergen intake' variable. In this way, the probability of occurrence of an allergic reaction can be calculated (Figure 2). Threshold doses of mealworm proteins able to elicit an allergic reaction in 5, 10 and 50% of the population (ED_5, ED_{10} and ED_{50}) were calculated on a very small group (15 individuals) of shrimp-allergic patients, due to the lack of clinically verified mealworm-allergic

patients (Broekman et al., 2016). Such levels are therefore to be referred to a subpopulation of crustacean allergic individuals consuming foods containing processed mealworms. In order to perform a quantitative probabilistic RA of the allergenicity of yellow mealworm, these EDs need to be treated as distributions and compared with exposure data.

For the third step of the RA (intake assessment), data regarding the number of (potential) allergens, the total amount of proteins and the ability of the allergens to survive different kinds of food processing (allergen stability) were collected from the scientific literature. Moreover, based on the currently available information, the main subgroups of sensitised patients at risk of developing a reaction after the ingestion of mealworm-containing foods were highlighted, and estimates of their prevalence within the general healthy population were retrieved. These are all individuals previously sensitised to allergenic sources (cockroaches, crustaceans, house dust mites (HDM), molluscs, nematodes) that hold the power to cross-react with mealworms proteins, due to their shared three-dimensional structure. Cross-reactivity is the only reason for an allergic reaction that can be used in the quantitative RA of a novel food, where estimates of allergy prevalence are known. However, allergy may also occur through 'de novo' sensitisation of previously healthy individuals, but in this case it is impossible to predict how many unsensitised individuals will become primarily sensitised, because the mechanisms and the reasons of allergy are still largely unknown. Finally, in order to complete the exposure assessment, information about the quantity of the allergenic food within a complex food matrix, as well as estimates of the serving size and prevalence of consumption should be included. All these information are unfortunately unavailable at the moment, since insect-based products placed on the market before 2018 are temporarily allowed only in some of the Member States. These products still represent a small niche of the market, but based on the current applications under evaluation by EFSA (general abstracts can be consulted freely on the EFSA webpage, https://ec.europa.eu/food/safety/novel_food/authorisations/summary-applications-and-notifications_en), it is likely that their diffusion will grow among the European consumers in the next years, even though it is hard to predict at which pace.

For the evaluation of allergenicity, risk characterisation, the last step of RA, can have two major purposes: (1) the characterisation of a risk associated with a defined (range of) level(s) of allergen(s) in a food product and (2) the establishment of (safe) limit levels for allergens in food. Risk characterisation needs to be attuned to the ultimate purpose of the assessment, which needs to be specified at the problem formulation stage (Crevel et al., 2014). The allergenicity RA of yellow mealworm is an activity still ongoing, and it will probably take into consideration potential scenarios to cope with the lack of exposure data. The model will be developed in collaboration with BfR Unit 33, Epidemiology, statistics and exposure modelling.

3. Conclusions

Before EU Regulation 2017/893 entered into force (1 July 2017), feeds could be inspected for the presence of insect contamination by using simple microscopy, looking for small insect body parts like wings or legs. Since the allowance of seven species for their productions, detection methods can no longer inform just about the presence of unidentified insects, but they must as well be able to identify these species. Given that thousands of insect species exist but only seven are allowed for inclusion in feeds, the method needs the power to distinguish between genetically distant but also very close species (for example between two species of *Gryllus*, one allowed and one not allowed). Most importantly, the method needs to be open, because in the future more insects might be added to the current list. This shortlist was in fact drawn up by the European Commission based on the existing scientific evidences on the insects currently reared in EU, and it is easy to predict that more species will be introduced in the near future. Molecular investigations using DNA guarantee enough specificity for species identification, but classical endpoint or real-time PCR have the limitation to be closed protocols, which need to be re-established and re-validated every time a new species is added to the existing list. NGS technology has been established already since several years, and applications in food and feed authenticity testing are increasing. The main advantage of this technique is that it can provide genetic information on whatever organism is present within the analysed sample, without the need of a predefined target (untargeted analysis), making it theoretically able to detect any unexpected contamination. However, PCR-free procedures like whole genome sequencing (WGS) require a large amount of high quality starting material, which is difficult to obtain from processed foods or feeds in most cases. Conversely, DNA metabarcoding performed exploiting the high-throughput capacity of NGS platforms is a powerful tool for characterising the biodiversity, and it relies on the preparation of libraries of amplicons targeting specific conserved regions, in which DNA or RNA

fragments are coupled to adapters to allow PCR amplification and sequencing. The identification of the obtained sequence relies on the existence of a large updated database, through which sequences can be compared. Global efforts at storing and sharing DNA sequence data have been underway for several decades, and today GenBank, managed by the United States National Center for Biotechnology Information (NCBI), together with other curated, application-specific DNA sequence databases hosted by other organisations (e.g. Greengenes, BOLD or the JRC GMO-Amplicons database), hold several terabases of nucleic acid sequences completely free for consultation (Haynes et al., 2019). The availability of a sound number of reference sequences is of utmost importance for this kind of analysis, and its lack could represent a major drawback.

The protocol proposed by the NRL-AP is based on the NGS of a small region included in the COI gene, a mitochondrial gene well known to be able to discriminate animals at the species level, which has been already tested successfully on insects (Nelson et al., 2007). The protocol still needs to be tested on model feeds prepared at known concentrations of insects, and subsequently validated on commercial feed samples. At the moment, the EU Reg. 2017/893 does not indicate a maximum tolerated amount of prohibited insect material, which could be translated into a zero tolerance policy. However, the presence of several insects in the facilities where feeds are prepared is common, and it is likely to expect that they can fall into the final product and contaminate it. NGS is a powerful and sensitive technology, and the chance that DNA coming from these unintentionally added insects is indeed amplified and sequenced is not negligible. This might represent an issue for the application of the method in official investigations.

Allergenicity RA is an activity so far considered marginal and included within the broadest safety assessment of GMOs. Its primary goal is to prevent the transfer of an existing allergen or celiac-inducing protein into a new food source and to protect those who are allergic or have celiac disease (Goodman and Tetteh, 2011). This RA has been developed within the past two decades and is currently based on a weight of evidence (WoE) approach, an integrated, stepwise, case-by-case approach that relies upon various criteria used in combination, since no single criterion is sufficiently predictive on either allergenicity or non-allergenicity (Codex Alimentarius Commission, 2003). It includes several steps, but the main idea is that the applicant needs to prove that its product is highly unlikely to contain a new potentially allergenic protein based on its primary amino acid sequence. Currently the same strategy is applied for the evaluation of potential allergenicity of novel foods, but several authors have questioned it, claiming that it is not adequate, that there are limitations to be acknowledged and gaps to be filled (Mazzucchelli et al., 2018). From one side, the protocol is too conservative, because linear sequence similarity does not always lead to *in vitro* cross-reactivity, and even that does not automatically indicate clinical cross-reactivity. From another side, however, the protocol is based on a comparison with known allergens, and it has a limited applicability for novel proteins lacking homology to already identified proteins, disregarding the so-called'de novo' sensitisation. Recently, the group led by Verhoeckx proposed a conceptual strategy for novel foods that combines the WoE for food derived from GM plants and other strategies previously published in the literature. The main point raised was the need to introduce *in vitro* functional IgE testing, such as basophil activation test (BAT) and skin prick tests (SPT), and *in vivo* food challenges (DBPCFC) to verify clinical reactivity in sensitised patients. Also, an accurate preliminary collection of information on the history of the food is advised, in order to assess its potential ability to 'de novo' sensitise new individuals (Verhoeckx et al., 2016).

Whether the WoE approach currently applied by EFSA is used, or a more comprehensive strategy is chosen to assess potential allergenicity, the final outcome of this RA will anyway be deterministic and qualitative. Without available data on a threshold dose for a specific food, it is neither possible to conduct RA nor to focus quality control on efforts which bring the greatest benefit to the allergic consumer. In September 1999, 12 clinical allergists and other interested parties were invited to participate in a roundtable conference to share existing data on threshold doses and to discuss clinical approaches that would have allowed the acquisition of that information. The participants concluded that'thresholds for common allergenic foods are finite, measurable and above zero. However, attempting to reach consensus [...] on the basis of the existing data would probably be premature' (Taylor et al., 2002). A similar conclusion was reached by EFSA few years later (EFSA, 2004). Fifteen years later the situation has not changed much, and scientists still struggle in the identification of precise amounts of allergenic protein, the intake of which will have no effects on 100% of the allergic population. In the same years, some groups tried to propose alternative methods to estimate a threshold dose for foods eliciting allergic reactions in susceptible patients. Bindslev-Jensen and colleagues proposed to use clinical food challenge data coming from *in vivo* human studies to model a

statistical distribution of minimum EDs in the allergic population, introducing for the first time an element of hazard characterisation (Bindslev-Jensen et al., 2002). This bottom-up protocol is borrowed by classical chemical and microbiological risk assessment, and has elements of both approaches. Hazard characterisation for allergens relies on human data from DBPCFC studies, so no animal to man extrapolation of the results is needed. The concept of predicted population EDs is used, where EDp refers to the dose of allergen that is predicted to produce a response in p% of the allergic population. Uncertainty still exists regarding the lowest amount to which sufferers will react, the proportion of sufferers reacting to a defined dose, as well as the relationship between dose and severity for any given individual. However, by applying the probabilistic modelling, uncertainty and variability associated with each input variable are included in the model, therefore there's no need to apply, often arbitrary, uncertainty factors to the risk assessment output (Crevel et al., 2014).

Few years later, starting from this concept of hazard characterisation, a group from TNO (the Netherlands organisation for applied scientific research) developed a probabilistic quantitative risk assessment to predict the likelihood of an allergic reaction, resulting in a quantitative assessment of the risk associated with unintended exposure to food allergens. The intent was to overcome the conservative approach based on worst-case values, generally overestimating the actual risk, by proposing a quantitative RA that put together data collected from controlled food challenges and expressing a'safe' dose distribution, and epidemiological data collected from sampling and measuring the quantity of the allergen source within a certain food or group of foods. A case study on hazelnut proteins in chocolate spread was presented as a proof of concept (Spanjersberg et al., 2007). Since this publication, the same approach has been replicated to assess other food matrices, such as peanut in chocolate tablets (Rimbaud et al., 2010), gluten traces in gluten-free wheat substitutes (Gibert et al., 2013) and recently peanut proteins in highly refined vegetable oil (Blom et al., 2017).

The conceptual work on a full quantitative RA of the allergenicity of yellow mealworm (*T. molitor*) tries to apply the concepts of the stochastic RA to a novel food matrix with a postulated allergenicity power. The main limitation is represented by the scarcity of data, both for the hazard characterisation and for the exposure assessment. To overcome these limitations, the model tries to use all retrievable information, either numerical or non-numerical, and needs to run scenarios that take into account the uncertainty.

The EU-FORA Project consists in a practical programme addressed at early- to mid-career scientists working in food safety organisations across Europe, which aims to increase the expertise and capacity available to risk assessment bodies at both the European and national levels (Bronzwaer et al., 2016). Selected candidates are fully integrated into the work of the hosting site, and thanks also to the training modules spread during the year, they develop their knowledge and experience in food risk assessment. BfR actively contributed to the programme since its early development stage, and it provided a suitable training environment. The presence of highly qualified personnel with different scientific backgrounds has encouraged the fellow to widen the spectrum of the investigation and propose collaborations with two other units, specifically the Study Centre for Genome Sequencing and Analysis (Unit 4SZ) and the Epidemiology, statistics and exposure modelling unit. Overall, the fellow acquired the tools and the ability to carry out an independent qualitative risk assessment, and received the necessary training to understand and collaborate to a full quantitative stochastic risk assessment, regardless on the topic. Particularly, the aspect of allergenicity food assessment has been deepened over the all year, following as well the natural inclination that derived from the personal scientific background of the fellow.

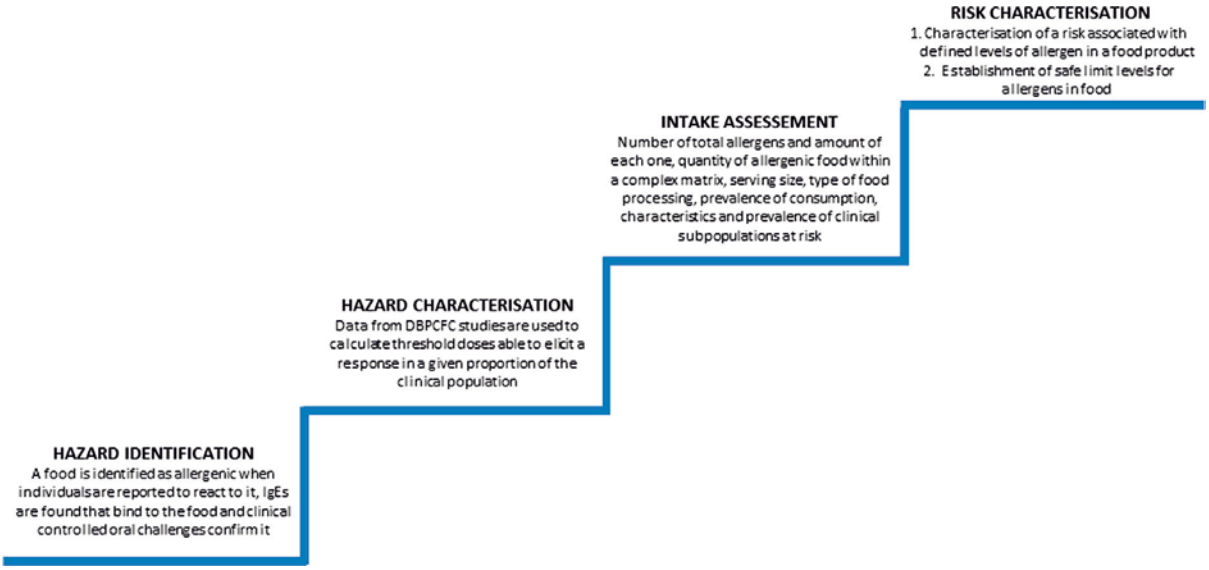


Figure 1: Steps of allergenicity risk assessment

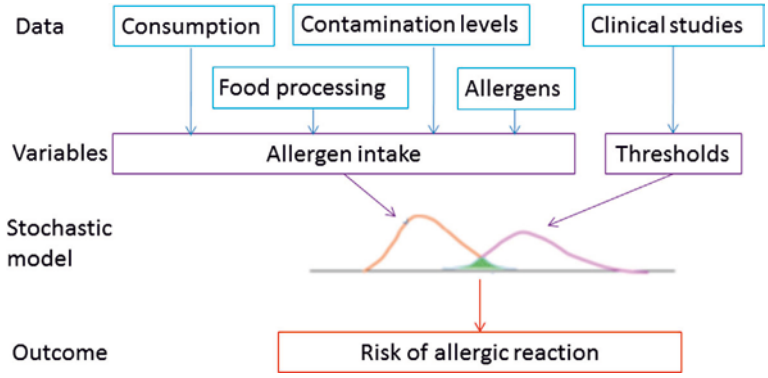


Figure 2: Probabilistic risk assessment for food allergens

References

Bernstein IL, 1983. Mealworm asthma: clinical and immunologic studies. Journal of Allergy and Clinical Immunology, 72, 475–480.

Bindslev-Jensen C, Briggs D and Osterballe M, 2002. Can we determine a threshold level for allergenic foods by statistical analysis of published data in the literature? Allergy, 57, 741–746.

Blom WM, Kruizinga AG, Rubingh CM, Remington BC, Crevel RWR and Houben GF, 2017. Assessing food allergy risks from residual peanut protein in highly refined vegetable oil. Food and Chemical Toxicology, 106, 306–313.

Broekman HC, Knulst A, Jager SD, Gaspari M, Kruizinga A, Bruijnzeel-Koomen C, Houben G and Verhoeckx K, 2015. Insect exposure causes primary food allergy. Allergy, 70, 283–283.

Broekman HC, Verhoeckx KC, den Hartog Jager CF, Kruizinga AG, Pronk-Kleinjan M, Remington BC, Bruijnzeel-Koomen CA, Houben GF and Knulst AC, 2016. Majority of shrimp-allergic patients are allergic to mealworm. Journal of Allergy and Clinical Immunology, 137, 1261–1263.

Bronzwaer S, Le Gourierec N and Koulouris S, 2016. Editorial: The European Food Risk Assessment Fellowship Programme (EU-FORA). EFSA Journal, 14(11), e14111.

Codex Alimentarius Commission, 2003. Guideline for the conduct of food safety assessment of foods derived from recombinant-DNA plants (CAC/GL 45). Food and Agriculture Organisation, Joint FAO/WHO Food Standards Program, Rome.

Crevel RWR, Baumert JL, Baka A, Houben GF, Knulst AC, Kruizinga AG, Luccioli S, Taylor SL and Madsen CB, 2014. Development and evolution of risk assessment for food allergens. Food and Chemical Toxicology, 67, 262–276.

Dobermann D, Swift JA and Field LM, 2017. Opportunities and hurdles of edible insects for food and feed. Nutrition Bulletin, 42, 293–308.

EFSA (European Food Safety Authority), 2004. Opinion of the Scientific Panel on Dietetic Products, Nutrition and Allergies on a request from the Commission relating to the evaluation of allergenic foods for labelling purposes. EFSA Journal 2004;2(3):32, 197 pp. https://doi.org/10.2903/j.efsa.2004.32

EFSA Scientific Committee, 2015. Scientific Opinion on a risk profile related to production and consumption of insects as food and feed. EFSA Journal 2015;13(10):4257, 60 pp. https://doi.org/10.2903/j.efsa.2015.4257

Freye HB, 1996. Anaphylaxis to the ingestion and inhalation of *Tenebrio molitor* (mealworm) and *Zophobas morio* (superworm). Allergy and Asthma Proceedings, 17, 215–219.

Gibert A, Kruizinga AG, Neuhold S, Houben GF, Canela MA, Fasano A and Catassi C, 2013. Might gluten traces in wheat substitutes pose a risk in patients with celiac disease? A population-based probabilistic approach to risk estimation. American Journal of Clinical Nutrition, 97, 109–116.

Goodman RE and Tetteh AO, 2011. Suggested Improvements for the Allergenicity Assessment of Genetically Modified Plants Used in Foods. Current Allergy and Asthma Reports, 11, 317–324.

Haynes E, Jimenez E, Pardo MA and Helyar SJ, 2019. The future of NGS (Next Generation Sequencing) analysis in testing food authenticity. Food Control, 101, 134–143.

van Huis A, Itterbeek JV, Klunder H, Mertens E, Halloran A, Muir G and Vantomme P, 2013. Edible insects: Future prospects for food and feed security. FAO Forestry paper, 171. Food & Agriculture Organization, Rome, Italy.

Jongema Y, 2017. List of edible insects of the world – WUR. Available online: www.wur.nl/en/Expertise-Services/Chairgroups/Plant-Sciences/Laboratory-of-Entomology/Edible-insects/Worldwide-species-list.htm

Mazzucchelli G, Holzhauser T, Cirkovic Velickovic T, Diaz-Perales A, Molina E, Roncada P, Rodrigues P, Verhoeckx K and Hoffmann-Sommergruber K, 2018. Current (Food) Allergenic Risk Assessment: Is It Fit for Novel Foods? Status Quo and Identification of Gaps. Molecular Nutrition & Food Research, 62, 1700278.

Nelson LA, Wallman JF and Dowton M, 2007. Using COI barcodes to identify forensically and medically important blowflies. Medical and Veterinary Entomology, 21, 44–52.

Raheem D, Carrascosa C, Bolanle Oluwole O, Nieuwland M, Saraiva A, Millán R and Raposo A, 2018. Traditional consumption of and rearing edible insects in Africa, Asia and Europe. Critical Reviews in Food Science and Nutrition, 15, 1–20.

Rimbaud L, Heraud F, La Vieille S, Leblanc J-C and Crepet A, 2010. Quantitative Risk Assessment Relating to Adventitious Presence of Allergens in Food: A Probabilistic Model Applied to Peanut in Chocolate. Risk Analysis, 30, 7–19.

Rumpold BA and Schlüter OK, 2013. Potential and challenges of insects as an innovative source for food and feed production. Innovative Food Science and Emerging Technologies, 17, 1–11.

Schiavone A, Cullere M and De Marco M, 2017. Partial or total replacement of soybean oil by black soldier fly larvae (*Hermetia illucens* L.) fat in broiler diets: effect on growth performances, feed-choice, blood traits. Italian Journal of Animal Science, 16, 93–100.

Spanjersberg MQI, Kruizinga AG, Rennen MAJ and Houben GF, 2007. Risk assessment and food allergy: the probabilistic model applied to allergens. Food and Chemical Toxicology, 45, 49–54.

Taylor SL, Hefle SL, Bindslev-Jensen C, Bock SA, Burks AW, Christie L, Hill DJ, Host A, Hourihane JO'B, Lack G, Metcalfe DD, Moneret-Vautrin DA, Vadas PA, Rance F, Skrypec DJ, Trautman TA, Malmheden Yman I and Zeiger RS, 2002. Factors affecting the determination of threshold doses for allergenic foods: How much is too much? Journal of Allergy and Clinical Immunology, 109, 24–30.

Verhoeckx KCM, van Broekhoven S, den Hartog-Jager CF, Gaspari M, de Jong GAH, Wichers HJ, van Hoffen E, Houben GF and Knulst AC, 2014. House dust mite (Der p 10) and crustacean allergic patients may react to food containing yellow mealworm proteins. Food and Chemical Toxicology, 65, 364–373.

Verhoeckx K, Broekman H, Knulst A and Houben G, 2016. Allergenicity assessment strategy for novel food proteins and protein sources. Regulatory Toxicology and Pharmacology, 79, 118.

Abbreviations

BAT	basophil activation test
BfR	Bundesinstitut für Risikobewertung
BOLD	Barcode of Life Data System
COI	cytochrome oxidase subunit 1
DBPCFC	double-blind placebo-controlled food challenge
ED	eliciting dose
GMO	genetically modified organism
HDM	house dust mites
IgE	immunoglobulin E
NCBI	National Center for Biotechnology Information
NGS	next generation sequencing
NRL-AP	National reference laboratory for animal protein
PCR	polymerase chain reaction
PUFAs	polyunsaturated fatty acids

RA	risk assessment
SNP	single nucleotide polymorphism
SPT	skin prick test
TNO	the Netherlands organisation for applied scientific research
WGS	whole genome sequencing
WoE	weight of evidence

EU-FORA SERIES 2

APPROVED: 31 July 2019

doi: 10.2903/j.efsa.2019.e170908

Application of data science in risk assessment and early warning

Bundesinstitut für Risikobewertung (BfR) (German Federal Institute for Risk Assessment), Dimitrios E Pavlidis, Matthias Filter and Anja Buschulte

Abstract

The food supply chain has been recognised by the EU as a critical infrastructure, and its complexity is the main cause of vulnerability. Depending on the food matrix, natural and/or deliberate contamination, food-borne diseases or even food fraud incidents may occur worldwide. Consequently, robust predictive models and/or software tools are needed to support decision-making and mitigating risks in an efficient and timely manner. In this frame, the fellow participated in data collection and analysis tasks, so as to provide additional predictive models. The working programme, covered a wide range of aspects related to risk assessment including identification of emerging risks (quantitative), microbiological risk assessment, authenticity assessment, spatio-temporal epidemiological modelling and database formation for hosting predictive microbial models. The training and close integration, in the open-source, in-house (German Federal Institute for Risk Assessment (BfR)) developed software tools under the framework of FoodRisk-Labs (https://foodrisklabs.bfr.bund.de.) for data analysis, predictive microbiology, quantitative microbiological risk assessment and automatic data retrieval purposes allowed for the independent use. Moreover, the fellow actively contributed to the update of the upcoming *Yersinia enterocolitica* risk assessment, and also in authenticity assessment of edible oils. Over the course of the year, the fellow was closely involved in international and national research projects with experts in the above-mentioned disciplines. Lastly, he consolidated his acquired knowledge by presenting his scientific work to conferences, and BfR-internal meetings.

Keywords: risk assessment, *Yersinia enterocolitica*, fermented products, traditional food, PMM-Lab, KNIME, authenticity

Correspondence: eu-fora@efsa.europa.eu

Acknowledgements: This report is funded by EFSA as part of the EU-FORA programme.

Suggested citation: Bundesinstitut für Risikobewertung (BfR) (German Federal Institute for Risk Assessment), Pavlidis DE, Filter M and Buschulte A, 2019. Application of data science in risk assessment and early warning. EFSA Journal 2019;17(S2):e170908, 10 pp. https://doi.org/10.2903/j.efsa.2019.e170908

ISSN: 1831-4732

The EFSA Journal is a publication of the European Food Safety Authority, an agency of the European Union.

Table of contents

1. Introduction

1.1. General Description of the followed programme

The project took place in the department Biological Safety (Dep. 4) of the German Federal Institute for Risk Assessment (BfR) and was supervised by the Unit Food Hygiene and Technology, Supply Chains, Food Defense (Unit 41) of this department. The department Biological Safety deals with health risks for humans, which may arise more particularly from microorganisms, the toxins formed by them and other microbial metabolites. This includes bacteria but also viruses, parasites and TSE pathogens. The department is involved in establishing the cause of outbreaks of food-borne diseases and zoonoses. It has a number of national reference laboratories for the diagnosis and fine typing of pathogens, antibiotic resistance and the microbiological contamination of foods (a task anchored in food legislation). In this scope, Unit 41 deals with the identification and evaluation of hazards that may be present in food. Performing vulnerability assessments concerning these hazards and developing risk mitigation strategies are related unit's tasks. Unit 41 is furthermore involved in microbiological risk assessments (MRAs) and provides the national expert for the European Food Safety Authority (EFSA) Scientific Network on MRA. Other key foci are national and international research projects that aim at the development of new data and knowledge-driven models supporting the efficient generation of risk assessments. In this context, several open-source software tools have been developed under the umbrella of FoodRisk-Labs (https://foodrisklabs.bfr.bund.de) which aim to facilitate the generation of quantitative microbial risk assessments (QMRAs). Examples are Predictive Microbial Modelling Lab (PMM-Lab; aims to ease and standardise the statistical analysis of experimental microbial data and the development of predictive microbial models), FoodProcess-Lab (for the application of predictive microbial models on food process chains), Food Safety Knowledge Lab (FSK-Lab: an open-source software supporting exchange of risk assessment models) and the open Food Safety Model Repository (openFSMR; a community-driven search engine for predictive microbial models). Parallel activities are the creation of data analysis pipelines supporting food safety research questions and national and international research projects, like: FoodAuthent, RAKIP, AGINFRA+, DEMETER. Under the internal BfR structure, the Unit 41 is highly connected with other units of BfR, such as Unit 83, performing among others assessments of food integrity by means of untargeted analytical methods.

The fellow being part of the Unit's risk assessment team was supervised by two senior research scientists of Unit 41: Dr. Anja Buschulte, Veterinarian, Senior Research Scientist and Matthias Filter, Biochemist, Senior Research Scientist. Further support was given by other research scientists of the Unit with many years of professional background in the field of either performing MRA and/or data analysis or software development. Cross-unit activities gave the opportunity to get a closer insight into other risk assessment-related issues.

1.2. *Y. enterocolitica* in various food matrices

Codex Alimentarius recommends following and applying risk-based approaches and metrics that can more directly and transparently establish the stringency of control measures (Codex Alimentarius, 1999). This supports the statement by Roberts and Jarvis (1983), 'a mathematical model that quantitatively describes the combined effect of the environmental parameters can be used to predict growth, survival or inactivation of a microorganism and thereby contribute important information about product safety and shelf-life'. In the end, such models could be applied in the whole food-supply chain. The employment of models may be useful for decision-making purposes to prevent risks for human and animal health (Prandini et al., 2008). Indeed, numerous publications, models and data sets describing the behaviour of microbial hazards in food already exist. However, this information is widely spread in the scientific literature and usually not available in a harmonised format. Therefore, structured and annotated databases that bring this information together could efficiently close this gap.

According to annual reports of the European Food Safety Authority (EFSA and ECDC, 2018), *Campylobacter* spp., *Salmonella* spp. and *Yersinia enterocolitica* are the three most common enteropathogenic bacteria responsible for food-borne disease. *Y. enterocolitica* belongs to the family Yersiniacea and the genus Yersinia. Despite the numerous species of this genus, only virulent strains of the species *Y. pestis*, *Y. pseudotuberculosis* and *Y. enterocolitica* have been found to be human pathogens (Cornelis et al., 1998; von Altrock et al., 2015). *Y. enterocolitica* and, to a much lesser extent, *Y. pseudotuberculosis* are the causative agents of the so-called Yersiniosis, a gastrointestinal disease of humans. Despite this fact, the identification of virulent strains is still a challenge (Bancerz-Kisiel et al., 2018).

Epidemiological studies have shown that *Y. enterocolitica* occurs in various niches, such as humans, animals, food and the environment (Rahman et al., 2011). The majority of isolates recovered from asymptomatic carriers, infected animals, contaminated food, untreated water and contaminated environmental samples are non-pathogenic having no clinical importance (Fredriksson-Ahoma and Korkeala, 2003). Other non-food-related sources of *Y. enterocolitica* could be domestic animals, such as dogs and cats (von Altrock et al., 2015; Duan et al., 2017). The main reservoir of *Y. enterocolitica* is the pig. Even though Yersiniosis can be caused by different foods and water, there is evidence of the link between pigs, pork carcasses and wild boars and associated products (Fredriksson-Ahomaa et al., 2007; Virtanen et al., 2012; EFSA BIOHAZ Panel, 2014; von Altrock et al., 2015). Prevalence data in pork and minced meat and a study by the Robert Koch Institute (RKI; Rosner et al., 2012) indicate that the consumption of raw minced pork, probably as so-called 'Mett' or 'Hackepeter', can be considered the most important risk factor for Yersiniosis in Germany (DE). These traditional German pork products are 'ready to eat' and considered as eaten raw, as they consist of raw pork meat, which has only be prepared, e.g. with the addition of spices. Besides, *Yersinia* spp. is also found in raw milk, which can serve as an excellent medium for reproduction due to its pH value and chemical composition. Furthermore, outbreaks linked to fresh produce (ready to eat salads, spinach) have also been documented (Sakai et al., 2005; MacDonald et al., 2012; Espenhain et al., 2019). For instance, vegetables can be contaminated with this pathogen through direct contact with wildlife faeces or through contaminated water, e.g. during irrigation, harvesting or transport (EFSA, 2007).

Y. enterocolitica is able to grow in a wide temperature range (0–42°C) and the ability of this species to multiply at low temperatures is of considerable concern. In this context, it is particularly important that *Yersinia* spp. can even grow at −2°C in fresh pork (EFSA BIOHAZ Panel, 2014) and can survive and remain infectious for several weeks in frozen food (BfR, 2013). Common heating methods, such as cooking and pasteurisation, eliminate the pathogen. Heating to a core temperature of at least +70°C for 2 min is deemed to be sufficient to eliminate *Yersinia* (BfR, 2013). However, insufficient cooking and pasteurisation (BfR, 2013; Longenberger et al., 2014), and/or post-process cross-contamination still remain a threat. The species *Y. enterocolitica* consists of six biotypes (BT) and more than 50 different serotypes which are very heterogeneous in terms of their pathogenic potential. At European level, the strains of BT 4 (serotype O:3) and BT 2 (serotype O:9) are often associated with clinical cases in humans (EFSA, 2007). In DE and in the European Union (EU), *Y. enterocolitica* strains of bioserovar 4/O:3 are the most common cause of Yersiniosis (RKI, 2019). In terms of pathogenesis, *Y. enterocolitica* is responsible for a wide variety of clinical manifestations, ranging from mild gastroenteritis (abdominal pain and diarrhoea) to invasive syndromes like terminal ileitis and mesenteric lymphadenitis to death (CDC, 2016). At present, only limited data on real prevalence are available. This may be explained by the low recovery rates of isolates from food samples, which in turn may be due to the limited sensitivity of cultivation methods Fredriksson-Ahoma and Korkeala (2003). The ail gene, which is associated with the pathogenicity of *Y. enterocolitica*, has in general a low prevalence among the *Y. enterocolitica* isolates (Bancerz-Kisiel et al., 2018). To our knowledge, little information related to the minimum infective dose is published. Robins-Browne (2013) reported that a median infective dose for Yersiniosis in humans is not known but is likely to exceed 4 log colony-forming units (cfu). The Public Health Agency of Canada states an infective dose of 10^6 cells (Public Health Agency of Canada). Lastly, Bhunia (2008) reported that generally a high dose of seven to nine log cells of *Y. enterocolitica* is necessary to cause the disease. Furthermore, it can be assumed that the infective dose depends on the food matrix and the immune status of exposed consumer groups (BfR, 2013).

In summary, the consumption of raw minced pork has been shown to be the main risk factor for Yersiniosis infections in DE (Rosner et al., 2012; BfR, 2013). The EFSA estimates that the largest proportion of food-borne outbreaks occurring in the EU originate in the household, with meat and meat products being the most frequently implicated food (EFSA, 2016). Typical raw pork products such as Mett, fresh raw sausages and fermented products, which are very popular in German-speaking countries, therefore pose food safety challenges (Lücke and Zangerl, 2014). Against this background, the question arises to what extent these typical products must be considered as potential sources of infection and what influence household practices have in this context.

1.3. Food integrity in the food supply chain/Vulnerability assessment in various food matrices

Globalisation and the growing complexity of the food chain, as well as the recent food scandals (e.g. melamine in milk, horsemeat in lasagne, fipronil in eggs and adulterated edible oils) have become

a challenge for food safety authorities. Food fraud/adulteration is according to Elliott (2014) considered as food crime, thus, violation of the general food law (European Commission, 2002). In parallel, EFSAs emerging risks infrastructure has categorised food authenticity and fraud as 'medium-term issues' (medium levels of uncertainty) based on the knowledgeability (EFSA, 2018). One of the most reported Rapid Alert System of Food and Feed (RASSF) cases, especially at border inspections, is related to mislabelling and/or food fraud topics. Among the several outcomes of the emerging risks framework include issues related to edible oils (EMA, 2017). Taking into account all the above, one of the proposed actions is a predictive analysis that allows to derive trends or patterns from the data which help to determine what the driving activities and behaviour are in order to predict which future problems/threats are likely to emerge (EFSA, 2018). In this concept, BfR is involved in the development of a so-called Emerging Risk Knowledge Exchange Platform (ERKEP) within the DEMETER project and explores rapid, simple and in-field applicable fingerprinting methodologies to identify food fraud, e.g. by performing preliminary screening of edible oils.

2. Description of work programme

2.1. Aims

The overall aims of the fellowship project were:

- Learning best practice on data analysis principles (including transparency, harmonisation, validation, documentation and good data analysis practices).
- Getting insights about tasks of the department concerning MRA with emphasis on the use of scientific data for risk assessment.
- Getting familiar with open-source software tools for data analysis and data mining – KNIME, R and emerging risk identification frameworks as e.g. SiLeBAT-NewsRadar.
- Getting expertise in tools for predictive microbial modelling (PMM-Lab, Combase and FSK-Lab).
- Getting expertise in relevant tools for QMRA (FSK-Lab, FDA-iRISK) and spatio-temporal epidemiological modelling (STEM).
- Going deeper in the databases concepts and participate in the update of the openFSMR.
- Participation in experimental research together with data analysis tasks in a microbiological and a chemometrical lab.

The Unit 41 aimed to integrate the fellow in upcoming MRA tasks of BfR, such as an update of the BfR '*Y. enterocolitica* risk assessment'. In this way, the fellow extends his knowledge in the field of MRA, acquires additional theoretical background and additional lab experience. Finally, it was agreed to offer to the fellow the opportunity to participate in the exploration of the potential of a new handheld NIR sensor for the rapid and reliable authentication and identification of possible adulterations of edible oils.

2.2. Activities/methods

The fellow got close insight into the area of (quantitative) MRA. He participated in BfR internal activities in this area and explored the currently ongoing software tools for the related community. Fellow-specific trainings were organised from the Unit 41, such as on KNIME, FSK-Lab, PMM-Lab, STEM and KNIME-based FoodAuthent workflows. In addition, the fellow was closely involved in related international and national research projects (AGINFRA+, DEMETER, RAKIP and ORION) carried out in Unit 41 and participated actively in workshops and trainings organised in these projects. Apart from that the fellow joined actively the weekly team meetings that monitor the status of the group members` work. Other related training opportunities provided by Unit 41, Department 4 and BfR were various seminars, symposia and meetings, such as Junior Research Group Meetings, PreDoc Symposium, Colloquium presentations, Modellers Meeting and *Yersinia* risk assessment meetings. Additionally, the fellow participated in externally activities proposed by BfR researchers. For instance, he attended the winter semester 2018/2019 course on 'Infectious disease epidemiology' and Colloquium for Statistic Methods at the RKI, Berlin, DE. Last but not least, the fellow participated in two International Conferences:

i) the conference co-organised by EFSA and BfR on 'Uncertainty in Risk Analysis', with a workshop on 'Accounting for uncertainty in decision making' and the title: 'Accounting for uncertainty in data-poor scenarios: cases studies on risk analysis in food safety' in Berlin, DE
ii) the 'European Symposium on Food Safety' of the International Association of Food Protection (IAFP) in Nantes, France.

In detail, the fellow pursued the following research during the 1-year internship:

A) Update of the openFSMR. openFSMR is a user-friendly tabular web portal, containing detailed meta-information on each predictive microbial model that is available in a third party software tool or as a publicly accessible files in the Predictive Modelling in Food Markup Language (PMF-ML) format. PMF-ML is a software-independent information exchange format for models specifically designed for predictive microbial models. Following the proposal of Plaza-Rodriguez et al. (2015) to establish food safety model repositories, a screening of the literature for integrated microbial models in software took place. Extracted information from Tenenhaus-Aziza and Ellouze (2015), and other known and unknown sources lead to 17 publicly available or commercial software tools. Each was screened individually for each separate model. The different input and outputs model parameters were recorded and grouped. A data matrix was prepared in the openFSMR Google sheet, with rows representing the integrated tertiary model describing the metadata following MIRIAM guidelines (Le Novere et al., 2005) and the input parameters of each model. The results of this study as well as the final database (https://sites.google.com/site/openfsmr/) were presented as a poster at the European Symposium of Food Safety of the International Association for Food Protection (IAFP) 2019 in Nantes, France. The aim of this database is to provide risk assessment authorities and food business operators a mapping of ready to use available predictive microbial models.

B) Research on data sources to support modelling and potential risk assessments on Hepatitis E and A virus (HAV and HEV) in foods. As only limited data could be identified to estimate the exposure to HEV from food, this research was used to get deeper understanding of the FDA-iRisk risk assessment tool. In parallel, published data related to inactivation of the viruses were collected and analysed with PMM-Lab.

C) Re-implementation of published microbial models.
Published validated microbial models were re-implemented using the in-house software PMM-Lab, FoodProcess-Lab and R – specifically, the models on growth and survival of *Listeria monocytogenes* and *Y. enterocolitica* under dynamic growth/death-inducing conditions, in Italian style fresh sausage (Iannetti et al., 2017), and *Hepatitis A* inactivation in spinach (Bozkurt et al., 2015).

D) Collection and data analysis of publicly available data sets related to growth/inactivation/survival of *Y. enterocolitica*, in order to develop additional ready to use predictive microbial models. It is well known that *Y. enterocolitica* is a ubiquitous agent (EFSA and ECDC, 2018) and can survive and/or multiply in various food matrices. Thus, models that can be used to predict its behaviour are of interest. Data on microbial growth/survival of *Y. enterocolitica* were collected from the scientific literature and COMBASE (https://www.combase.cc/index.php/en) and processed with the Predictive Microbial Modelling Lab software v1.06 (https://foodrisklabs.bfr.bund.de/pmm-lab_de/). PMM-LAB is an extension of the open-source platform KNIME (http://www.knime.org), providing tailored functionality of microbial data analysis in a transparent, modular way. The collected datasets cover specific food matrices: raw and cooked meat, seafood, milk and 'uncategorized food' for a wide range of environmental conditions. Following a two-step modelling approach, the data points were initially fitted through different primary equations, and then, the dependency of relevant environmental parameters (mainly temperature) was modelled through a secondary equation. Alternatively, a one-step modelling approach was followed. Part of the results will be presented orally and as poster at the Junior Researcher's Zoonoses Meeting 2019 in Berlin, DE.

E) Participation in the process of updating a QMRA of *Y. enterocolitica* in typical German ready to eat products. Experimental data from various challenge tests related to the behaviour of the hazard in fermented sausages and salami were utilised for predictive modelling purposes. In parallel, the fellow participated in a lab experiment related to 'Mett', by spiking different levels of *Y. enterocolitica* in spiced raw minced pork. The final step of this project foresees an in-depth study of the current available scientific articles on the bacterial survival in similar products. The ultimate aim is to develop a precise mathematical model which can explain and describe the behaviour of the pathogen in certain matrices or assess the observed values with already existing models from the openFSMR repository.

F) Authenticity assessment of edible oils. The need to establish on-site procedures to mitigate the risk of fraudulent practices have triggered the application for in-field methodologies. The fellow participated in a feasibility study to explore the potential of a handheld NIR sensor

measuring from 740 to 1070 nm for the robust and reliable authentication of edible oils, which are widely consumed in DE. The preliminary results were presented orally at the Junior Research Group Meeting 'Authenticity along the supply chain' and will also be part of a presentation during the National Food Chemistry Conference 2019 in Dresden, DE.

3. Conclusions

Globalisation has led to a wide distribution of raw materials and goods. The higher the complexity of the food supply chain, the higher its vulnerability and the more difficult risk assessments become. Consequently, robust predictive models and/or software tools are needed to support decision-making in an efficient and time-effective manner.

3.1. *Y. enterocolitica* response in various food matrices

The occurence of *Y. enterocolitica* in traditional German products still remains a threat for the public. The experiments performed at BfR, revealed that these typical products can pose a risk to the public health. Preliminary results are in line with those of other authors for similar products (Lindqvist and Lindblad, 2009; Ivanovic et al., 2015; Mitrovic, 2016) reporting that the use of starter cultures to produce sausages significantly affects the survival of microbial pathogens such as *Y. enterocolitica*. In other products, such as Mett, the presence of respective amounts of salt could not lead to a significant reduction and/or elimination of *Y. enterocolitica*. Taking into consideration that the reduction of pathogen levels may be insufficient if fermented sausages undergo inadequate maturation prior to refrigerated storage (Lindqvist and Lindblad, 2009), production procedures should be re-evaluated to ensure safe food. Therefore, such products should preferably not be consumed by particularly risk groups (such as pregnant women, small children, elderly and immunocompromised persons). Address-oriented risk communication is particularly important in this context, as these products are consumed especially in rural areas also by small children and senior citizens who are often unaware of the risk of a food-related illness. Last but not least, own experimental work confirmed that the detection methods for *Y. enterocolitica* need to be improved. With regard to other aspects, it was shown that the growth of *Y. enterocolitica* differs according to the food matrix. New consumer trends, such as the consumption of raw milk (a matrix which can support the growth of this and other pathogens), should be critically evaluated by the authorities, as they could pose a risk and thus lead to an increase of food-borne diseases.

3.2. Assuring authenticity in the food supply chain

In the past laborious, time-consuming and sophisticated lab methodologies were a bottleneck to ensure authenticity of food products. Nowadays convenient, portable, easy to use untargeted analysis approaches become available that might provide solutions in that area and can increase the number of controls with limited budget in a time-efficient period at all stages of the supply chain. In experiments performed by the fellow, the inter sensor analytical variability and repeatability was investigated by measuring the same samples with a new sensor, yielding similar results. The experimental research carried out revealed that there is a high potential for reliable screening of edible oils by applying miniaturised NIR sensors.

Summarising, this was a very fruitful year. Overall the project was very inspiring, allowing a deep insight in the activities of a Federal authority dealing with food safety. Thanks to the EU-FORA programme and the expertise of the hosting site the fellow gain a lot of experience in various areas. For instance, the activities carried out allowed him to expand his scientific knowledge both in practice and on a theoretical level. During the fellowship, the fellow was introduced to and gained experience in the use of modern open-source software tools for MRA. He became more familiar with cloud computing in a data-driven community. He adopted concepts such as transparency, harmonisation and documentation and exposed himself in a highly academic environment, having interaction with scientists from different disciplines. By joining and actively participating in various meetings and seminars organised internally or externally throughout the year, new ideas and questions were raised. The fellow is convinced that he will be able to use the knowledge in the near future to support food safety at national and European level. For sure the fellow will disseminate his acquired knowledge in the different software tools. Both the fellow and the supervisors agree that the EU-FORA programme provides an excellent opportunity to exchange opinions, experiences and methodologies on relevant public health issues and to build a professional and personal network that can serve as a basis for future cooperation.

References

von Altrock A, Seinige D and Kehrenberg C, 2015. *Yersinia enterocolitica* Isolates from Wild Boars Hunted in Lower Saxony. Germany, Applied and Environmental Microbiology, 81, 4835–4840.

Bancerz-Kisiel A, Szczerba-Turek A, Platt-Samoraj A, Michalczyk M and Szweda W, 2018. Characterisation of ail-positive *Yersinia enterocolitica* of different biotypes using HRMA. International Journal of Food Microbiology, 269, 46–51.

BfR, 2013. *Yersinia* in food: recommendations for protection against infections BfR Opinion No. 002/2013, 18 January 2013.

Bhunia AK, 2008. Foodborne microbial pathogens. Mechanisms and pathogenesis. 1st Edition. Springer Science Business media LLC., New York.

Bozkurt H, Ye X, Harte F, D'Souza HD and Davidson M, 2015. Thermal inactivation kinetics of *hepatitis A* virus in spinach. International Journal of Food Microbiology, 193, 147–151.

CDC, 2016. *Yersinia enterocolitica*. Available online: https://www.cdc.gov/yersinia

Codex Alimentarius, 1999. Principles and guidelines for the conduct of microbiological risk assessment (CAC/GL-30). 6 pp.

Cornelis GR, Boland A, Boyd AP, Geuijin C, Iriarte M, Neyt C, Sory M and Stanier I, 1998. The virulence plasmid of *Yersinia*, an antihost genome. Microbiology and Molecular Biology Reviews, 62, 1315–1352.

Duan R, Liang J, Zhang J, Chen Y, Wang J, Tong J, Guo B, Hu W, Wang M, Zhao J, Liu C, Hao H, Wang X and Jing H, 2017. Prevalence of *Yersinia enterocolitica* Bioserotype 3/O:3 among Children with Diarrhea, China, 2010–2015. Emerging Infectious Diseases, 23, 1502–1509.

EFSA (European Food Safety Authority), 2007. Monitoring and identification of human enteropathogenic *Yersinia* spp. EFSA Journal 2007;5(12):595, 30 pp. https://doi.org/10.2903/j.efsa.2007.595

EFSA (European Food Safety Authority), 2016. The European Union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2015. EFSA Journal 2016;14(12):4634, 231 pp. https://doi.org/10.2903/j.efsa.2016.4634

EFSA BIOHAZ Panel (EFSA Panel on Biological Hazards), 2014. Scientific Opinion on the public health risks related to the maintenance of the cold chain during storage and transport of meat. Part 2 (minced meat from all species). EFSA Journal 2014;12(7):3783, 30 pp. https://doi.org/10.2903/j.efsa.2014.3783

EFSA and ECDC (European Food Safety Authority and European Centre for Disease Prevention and Control), 2018. The European Union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2017. EFSA Journal 2018;16(12):5500, 262 pp. https://doi.org/10.2903/j.efsa.2018.5500

EFSA (European Food Safety Authority), Donohoe T, Garnett K, Lansink AO, Afonso A and Noteborn H, 2018. Scientific report on the emerging risks identification on food and feed – EFSA. EFSA Journal 2018;16(7):5359, 37 pp. https://doi.org/10.2903/j.efsa.2018.5359

Elliott C, 2014. Review into the integrity and assurance of food supply networks- final report. A National Food Crime Prevention Framework. The National Archives, Kew, London. Available online: https://www.gov.uk/government/publications/elliott-review-into-the-integrity-and-assurance-of-food-supply-networks-final-report

EMA, 2017. The economically motivated adulteration (EMA) database. Available online: http://ema.foodshield.org/

Espenhain L, Maximilian R, Luise M, Soledad C, Steen E, Eva L, Cecilia J, Sharon K-B, Mats L, Kühn H N, Mia T and Jansson M M, 2019. Cross-border outbreak of *Yersinia enterocolitica* O3 associated with imported fresh spinach, Sweden and Denmark, March 2019. Euro Surveillance 24, pii=1900368. https://doi.org/10.2807/1560-7917.es.2019.24.24.1900368

European Commission, 2002. Regulation (EC) No 178/2002 of the European Parliament and of the Council of 28 January 2002 laying down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety. OJ L 31, 1.2.2002, p. 1–24.

Fredriksson-Ahoma M and Korkeala H, 2003. Low occurrence of pathogenic *Yersinia enterocolitica* in clinical, food, and environmental samples: a methodological problem. Clinical Microbiology Reviews, 16, 220–229. https://doi.org/10.1128/cmr.16.2.220-229.2003

Fredriksson-Ahomaa M, Stolle A and Stephan R, 2007. Prevalence of pathogenic *Yersinia enterocolitica* in pigs slaughtered at a Swiss abattoir. International Journal of Food Microbiology, 119, 207–212.

Iannetti L, Salini R, Sperandii AF, Santarelli GA, Neri D, Di Marzio V, Romantini R, Migliorati G and Baranyi J, 2017. Predicting the kinetics of *Listeria monocytogenes* and *Yersinia enterocolitica* under dynamic growth/death-inducing conditions, in Italian style fresh sausage. International Journal of Food Microbiology, 240, 108–114.

Ivanovic J, Janjic J, Djordjevic V, Dokmanovic M, Boskovic M, Markovic R and Baltic M, 2015. The effect of different packaging conditions, pH and *Lactobacillus* spp. on the growth of *Yersinia enterocolitica* in pork meat. Journal of Food Processing and Preservation, 39, 2773–2779.

Le Novere N, Finney A, Hucka M, Bhalla SU, Campagne F, Collado-Vides J, Crampin JE, Halstead M, Klipp E, Mendes P, Nielsen P, Sauro H, Shapiro B, Snoep LJ, Spence DH and Wanner LB, 2005. Minimum information requested in the annotation of biochemical models (MIRIAM). Nature Biotechnology, 23, 1509–1515.

Lindqvist R and Lindblad M, 2009. Inactivation of *Escherichia coli*, *Listeria monocytogenes* and *Yersinia enterocolitica* in fermented sausages during maturation/storage. International Journal of Food Microbiology, 129, 59–67.

Longenberger A H, Gronostaj M P, YEE GY, Johnson LM, Lando JF, Voorhees RE, Walker K, Weltman AC, Moll M, Lyss SB, Cadwell BL, Gladney LM and Ostroff S M, 2014. *Yersinia enterocolitica* infections associated with improperly pasteurized milk products: southwest Pennsylvania, March–August, 2011. Epidemiology and Infection, 142, 1640–1650. https://doi.org/10.1017/S09502688130026

Lücke F-K and Zangerl P, 2014. Food safety challenges associated with traditional food in German-speaking regions, Review. Food Control, 43, 217–230.

MacDonald E, Heier BT, Nygård K, Stalheim T, Cudjoe SK, Skjerdal T, Wester AL, Lindstedt B-A, Stavnes T-L and Vold Line, 2012. *Yersinia enterocolitica* outbreak associated with ready-to-eat salad mix, Norway, 2011. Emerging Infectious Diseases, 18, 1496–1499. https://doi.org/10.3201/eid1809.120087

Mitrovic R, 2016. Investigation of the possibilities inactivation of Yersinia enterocolitica in fermented sausages. PhD thesis. Faculty of Veterinary Medicine. University of Belgrade. Serbia.

Plaza-Rodriguez C, Thoens C, Falenski A, Weiser AA, Appel B, Kaesbohrer A and Filter M, 2015. A strategy to establish Food Safety Model Repositories. International Journal of Food Microbiology, 204, 81–90.

Prandini A, Sigolo A, Filippi L, Battilani P and Piva G, 2008. Review of predictive models for *Fusarium* head blight and related mycotoxin contamination in wheat. Food and Chemical Toxicology, 47, 927–931.

Public Health Agency of Canada. Available online: Available online: http://www.phac-aspc.gc.ca/msds-ftss/msds168e-eng.php)

Rahman A, Bonny ST, Stonsaovapak S and Ananchaipattana C, 2011. Yersinia enterocolitica: Epidemiological Studies and Outbreaks, Review Article. Journal of Pathogens, SAGE-Hindawi Access to Research, 2011, 11. Article ID 239391. https://doi.org/10.4061/2011/239391

RKI. 2019. Yersiniose. RKI-Ratgeber. Available online: https://www.rki.de/DE/Content/Infekt/EpidBull/Merkblaetter/Ratgeber_Yersiniose.html;jsessionid=060D97271FA33578F55FB4F69671DB55.1_cid381#doc11934216bodyText2

Roberts T and Jarvis B, 1983. Predictive Modelling of Food Safety with particular reference to Clostridium botulinum in model cured meat systems. In: Roberts TA and Skinner FA (eds.). Food Microbiology: Advances and Prospects. Academic Press, Orlando, FL. pp. 85–95.

Robins-Browne RM, 2013. *Yersinia enterocolitica*. In: Doyle MP and Buchanan RL (eds.). Food microbiology: Fundamentals and frontiers. 4th Edition. ASM Press, Washington, DC.

Rosner BM, Stark K, Höhle M and Werber D, 2012. Risk factors for sporadic *Yersinia enterocolitica* infections, Germany 2009-2010. Epidemiology and Infection, 140, 1738–1747.

Sakai T, Nakayama A, Hashida M, Yamamoto Y, Takebe H and Imai S, 2005. Outbreak of food poisoning by *Yersinia enterocolitica* serotype O8 in Nara Prefecture: the first case report in Japan. Japanese Journal of Infectious Diseases, 58, 257–258.

Tenenhaus-Aziza F and Ellouze M, 2015. Software for predictive microbiology and risk assessment: a description and comparison of tools presented at the ICPMF8 Software Fair. Food Microbiology, 45, 290–299.

Virtanen S, Salonen L, Laukkanen-Ninios R, Fredriksson-Ahoma M and Korkeala H, 2012. Piglets are a source of pathogenic *Yersinia enterocolitica* on fattening-pig farms. Applied and Environmental Microbiology, 78, 3000–3003.

Abbreviations

AGINFRA+	Accelerating user-driven e-infrastructure innovation in Food and Agriculture
BfR	German Federal Institute for Risk Assessment, Berlin, Germany
CDC	Centres for Disease Control and prevention
cfu	colony-forming units
DE	Deutschland
DEMETER	DEtermination and METrics of Emerging Risks
EMA	Economically Motivated Adulteration
FSK	Food Safety Knowledge
FSMR	Food Safety Model Repository
HAV	Hepatitis A virus
HEV	Hepatitis E virus
IAFP	International Association of Food Protection
MRA	Microbiological Risk Assessment
PMM	Predictive Microbial Modelling
QMRA	Quantitative Microbiological Risk Assessment
RAKIP	Risk Assessment Knowledge Integration Platform
RASSF	Rapid Alert System of Food and Feed
RKI	Robert-Koch Institute
STEM	spatio-temporal epidemiological modelling

EU-FORA SERIES 2

APPROVED: 31 July 2019

doi: 10.2903/j.efsa.2019.e170909

Nanomaterials in Food – Prioritisation & Assessment

ANSES- French Agency for Food, Environmental and Occupational Health & Safety, France,
Eleni Anastasi, Gilles Riviere and Bruno Teste

Abstract

Nanomaterials (NMs) are of significant economic interest and have a huge impact on many industries including the food industry. The main application in food industry includes food additives and food packaging. However, the effects of NMs on human health are highly discussed, as well as the need of harmonised analytical methods and risk assessment methodologies. In line with these discussions, the French Agency for Food, Environmental and Occupational Health & Safety (ANSES) has started in 2017 a 2-year project focusing on NMs in food, to which the fellow was involved under the framework of the European Food Risk Assessment Fellowship Programme (EU-FORA). This technical report contains a description of the working program, the aims and the activities to which the fellow was involved during this placement. The main aims of the programme were to be involved in different steps of risk assessment process, to improve knowledge regarding food process, analytical and toxicological methods and to learn how to conduct expert assessments. All aims were linked with different kind of activities. Gaining hands-on experience on food risk assessment was achieved mainly by collecting occurrence data and performing exposure assessment calculations for the 'of concern' NMs, while scheduled visits to laboratories specialising in analytical methods of nanoparticles and toxicological studies helped to improve knowledge in these fields. Regular participation in the Working Group (GT) related to NMs in food and interaction with experts within ANSES facilitated the learning process of how to conduct collective expertise as well as to be further trained in risk assessment processes. Furthermore, apart from knowledge gained in risk assessment and NMs, the fellow was able to obtain transferable skills and knowledge that can be used to increase the scientific capacity of the fellow's home institute as well as to expand her scientific network, which could lead to collaboration opportunities in the future well beyond this fellowship.

Keywords: exposure assessment, food additives, nanomaterials, occurrence data, risk assessment

Correspondence: eu-fora@efsa.europa.eu

Acknowledgements: This report is funded by EFSA as part of the EU-FORA programme.

Suggested citation: ANSES- French Agency for Food, Environmental and Occupational Health & Safety, France, Anastasi E, Riviere G and Teste B, 2019. Nanomaterials in Food – Prioritisation & Assessment. EFSA Journal 2019;7(S2):e170909, 10 pp. https://doi.org/10.2903/j.efsa.2019.e170909

ISSN: 1831-4732

The EFSA Journal is a publication of the European Food Safety Authority, an agency of the European Union.

Table of contents

1. Introduction

Nanotechnology is a rapidly developing field and nanomaterials (NMs), are of significant technological and economic interest and have a huge impact on many industries including the food industry. The current use of NMs in the food sector is associated with three main areas: food additives and food packaging (Calzolai et al., 2012; Mattarozzi et al., 2017). While the nanotechnology has many applications and benefits for the food sector, there are also some concerns about their safety. The main concerns arise from the lack of knowledge regarding the interactions of NMs at the molecular or physiological levels and the fact that new NMs and applications are constantly being produced (Mattarozzi et al., 2017). Additionally, while the risk of particle inhalation has received much attention, there are still gaps of knowledge regarding possible adverse health effects due to oral exposure to nanoparticles (Winkler et al., 2018). Moreover, it is foreseen that due to the expanding commercialisation of NMs as part of the modern diet, their oral intake will increase worldwide (Winkler et al., 2018). In addition, since nanotechnology is a new field, there are still a lot of discussions on what is the definition of a NM especially from the regulatory point of view.

The International Organization for Standardization (ISO) has defined NM as a material with any external dimension on the nanoscale ('nano-object') or having an internal or surface structure in the nanoscale ('nanostructured material') (ISO, 2015). In particular, a nano-object is defined as a discrete piece of material with one, two or three external dimensions on the nanoscale. 'Nanoparticles' are nano-objects with all external dimensions on the nanoscale, where the lengths of the longest and shortest axes do not differ significantly. If the dimensions differ significantly, typically by more than a factor of 3, other terms, such as 'nanofibre' (two external dimensions in the nanoscale) or 'nanoplate' (one external dimension on the nanoscale) may be preferred to the termanoparticle. In turn, a 'nanostructured material' is defined as a material having internal or surface nanostructure, i.e. a composition of interrelated constituent parts in which one or more of those parts is a nanoscale region. 'Nanoscale' is defined as ranging from approximately 1 to 100 nm (ISO, 2015).

The European Commission issued a Recommendation (currently under review) for a definition of a NM in 2011 to provide a common basis for regulatory purposes across most areas of European Union (EU) policy where 'nanomaterial' means a natural, incidental or manufactured material containing particles in an unbound state or as an aggregate or as an agglomerate and where, for 50% or more of the particles in the number–size distribution, one or more external dimensions is in the size range 1–100 nm. In specific cases and where warranted by concerns for the environment, health, safety or competitiveness, the number–size distribution threshold of 50% may be replaced by a threshold between 1 and 50%. Moreover, engineered NMs are a subset of the 'nanomaterial' that is defined in the European Commission's Recommendation of 2011 (European Commission, 2011).

In the Novel Food Regulation (EU) No 2015/22837 and referring to Regulation (EU) No 1169/20118 on the Provision of Food Information to Consumers, Engineered NM means 'any intentionally produced material that has one or more dimensions of the order of 100 nm or less or that is composed of discrete functional parts, either internally or at the surface, many of which have one or more dimensions of the order of 100 nm or less, including structures, agglomerates or aggregates, which may have a size above the order of 100 nm but retain properties that are characteristic of the nanoscale (European Union, 2011, 2015).

To ensure sustainable development and use of nanotechnology, especially in the food sector, this requires control and monitoring of NMs, as well as risk assessments on their application, which in turn requires information about their toxicity and an exposure assessment (Bouwmeester et al., 2009; Mattarozzi et al., 2017). Even though a number of analytical methods for the detection and characterisation of NMs exist (Calzolai et al., 2012; Mattarozzi et al., 2017), it is clear that it is necessary to improve and harmonise the analytical methods as well as to find methodologies to perform nanospecific risk assessments for the implementation of future regulations (Mattarozzi et al., 2017). In line with this, European Food Safety Authority (EFSA) has recently produced a guidance for the risk assessment of nanoscience and nanotechnology applications in the food and feed chain (EFSA, 2018a).

2. Description of work programme

In line of these discussions, French Agency for Food, Environmental and Occupational Health & Safety (ANSES) started at late 2017 a 2-year project focusing on NMs in food. The main objectives of this project are:

a) To identify the NMs present in food
b) To prioritise them in terms of potential health concern
c) To estimate exposures and if possible to perform a health risk assessment for the 'of concern' NMs in food.

A dedicated ad hoc working group (Nanomaterials in food) was set to deal with this issue and the project was split into two different phases:

First phase: The focus was on the use of NMs in the food industry in order to identify the type of NMs involved in the food process. For this, database analyses were performed (French and international, i.e. Global New Products Database, GNPD) as well as industrial and Non-Governmental Organisation (NGO) hearings.

Second phase: The focus was on the social impact of NMs as well as on the scientific and regulatory issues that arise from this. During this phase, based on literature searches, a prioritisation of the 'of concern' NMs concerning food safety was performed. Another aim of this working group is to coordinate specific research activities to complement information extracted from bibliography, in order to improve NMs dietary exposure data and if data allow it, to perform a health risk assessment for the selected NMs.

The fellow was involved in the second phase of the project, placed in the UERALIM unit (Food Risk Assessment Unit) at DER (Risk Assessment Department) for 1 year. During this period, the fellow was supervised by Dr Gilles Riviere and Dr Bruno Teste and the tasks of her work were under the framework of the working group (WG) (Nanomaterials in food) activities and in close collaboration with the experts. The work programme was set in collaboration with the Laboratory of Genetic Toxicology at the Institut Pasteur de Lille de Lille, led by Dr Fabrice Nesslany, who is also the chairman of the working group.

Prioritisation of nanomaterials in food

After the prioritisation of the substances containing nanoparticles, it has been decided that the work of the fellow would focus on the following food additives:

a) Titanium Dioxide, TiO_2 (E171)
b) Silicon Dioxide, SiO_2 (E551)

Titanium dioxide-TiO2-E171

TiO_2 is a white powder, which is mainly used in products to give a white background colour. Titanium dioxide in bulk form is approved as a food additive with number E171 and it is used on a large scale as a whitener and as a colorant to impart brightness to food products (Mattarozzi et al., 2017). More specifically according to the EC Regulation 1333/2008 (European Union, 2008), TiO_2 is authorised as a food additive (E171) in EU in quantum satis, in 51 food categories. Therefore, since there is no max permitted limit (MPL), the concentration in food can vary a lot. Examples of food products containing TiO_2 could be chewing gum, ice cream and confectionary products like candies, chocolate products, cakes pastries and biscuits (Lomer et al., 2000; Weir et al., 2012; Fiordaliso et al., 2018). Other food products could include sauces, dressings, spreads, cheese or even fish products like surimi (Yin et al., 2017).

The food additive E171 consists of TiO_2 particles and can be present in two major crystalline forms, anatase and rutile. Literature data show a great variability in the proportion of crystalline forms as well as in the particle size distribution in the different commercially available batches of E171 since the size of the particles can vary from a few dozen to several hundred nanometres (Weir et al., 2012; Yang et al., 2014; Fiordaliso et al., 2018). Therefore, since part of the food-grade TiO_2 material has been shown to be nanosized, this has created many discussions whether the food additive is considered as a NM or not and if it is safe for human consumption. (European Commission, 2017).

Silicon Dioxide-SIO2-E551-SAS

The food additive, silicon dioxide (E551 or SAS-synthetic amorphous silica), is used in the food industry as an anticaking agent, thickener or carrier of flavours, therefore is often added to foods that are in powder form e.g. salt, vegetable powder, egg powder, creamer, coffee powder etc. (Dekkers et al., 2011; Mattarozzi et al., 2017).

More specifically according to the EC Regulation 1333/2008 (Annex II), SiO_2 (E 551) is authorised as a food additive in 22 food categories. Several food categories are authorised at MPLs ranging from 2,000 to 30,000 mg/kg and others at quantum satis (QS). Silicon dioxide (E 551) can be authorised together with silicates (E 552, E 553a and E 553b) in different forms like fumed (pyrogenic) silica and hydrated silica (precipitated silica, silica gel and hydrous silica) depending on the process (thermal or

wet) used for their manufacture. In addition, E551 is authorised for other application in Parts 1–5 of Annex III e.g. as a carrier in emulsifiers and colours or as a food additive in nutrients.

According to EFSA Opinion, E551 is a material comprised of aggregated nanosized primary particles. These aggregates can further agglomerate to form larger structures. The sizes of the aggregates and agglomerates are normally greater than 100 nm. However, depending on the starting material and/or on the manufacturing process, it cannot be totally excluded that some aggregates of primary particles could be smaller than 100 nm in size (EFSA, 2018b).

2.1. Aims

The main aims of the working program 'Nanomaterials in Food – Prioritisation & Assessment' were the following:

a) The fellow to be involved in different steps of risk assessment process (e.g. collection of occurrence data, hazard identification and characterisation, estimating exposures based on food consumption data and risk characterisation) according to specific guidelines (e.g. 'Guidance on risk assessment of the application of nanoscience and nanotechnologies in the food and feed chain') (EFSA, 2018a). More specifically, the idea was to perform literature searches (scientific and grey literature) to gather occurrence data, for the selected NMs, which can be used to calculate the exposure of the general French population and/or the exposure of specific populations (e.g. children).
b) To be further trained in current risk assessment methodologies in place at Anses (for nanotechnologies but also for non-nano chemicals) and to improve knowledge regarding food process, analytical and toxicological methods.
c) Learn how to conduct expert assessments in response to a request (public authorities, internal, stakeholders) based on compliance with ethical standards to prevent conflicts of interest and to ensure independence, collective multidisciplinary expertise, independency, quality etc.

2.2. Activities/Methods

Learning objectives were achieved by the activities described below, in line with the aims of the program.

2.2.1. Involvement in different steps of risk assessment process

The fellow was mainly involved in the collection of occurrence data and exposure assessment calculations for the selected NMs (TiO_2 and SiO_2) as described below. The remaining risk assessment steps: hazard identification, hazard characterisation and risk characterisation of the selected NMs are under process since the WG dealing with NMs in food is currently trying to set up a methodology for them. If time allows, the fellow will be further involved in these tasks for the remaining time of the EU-FORA fellowship.

2.2.1.1. Collection of occurrence data

As previously described, one of the aims of the ad hoc working group (Nanomaterials in food) was to improve NMs dietary exposure data and to perform a health risk assessment for the selected NMs, if data allow it. Along with this aim, the fellow undertook this task for collecting occurrence data for the food additives Titanium Dioxide (TiO_2) and Silicon Dioxide (SiO_2), in food products by performing a literature search.

The aim of this activity was to collect occurrence data for each food category for which TIO_2/SiO_2 is authorised according to the EC Regulation 1333/2008. For the collection of the occurrence data, two approaches were used: a) reported industry use levels of E171 and b) food analysis data corresponding to individual food products. For the literature search, the databases PubMed and Scopus were used and searches were restricted to the English language while no restriction was applied for the time of publication. Additional information was retrieved from: a) relevant web pages of National/ European Agencies/Bodies (e.g. EFSA, RIVM), b) grey literature from Google Scholar (e.g. Published NGO's data), c) data provided by DGCCRF (Directorate-General for Competition, Consumer Affairs and Fraud Control) inspections.

Information about each food product was individually collected (whenever it was possible) and registered in an excel file. Information included details about the product (description of product, labelling, photos, brand, point of sale, origin etc.), the concentration of the additive (in mg/Kg or mg/L)

as well as information about the method of analysis (validation data, LOQ/LOD) and sample preparation information. After registration, all food products were categorised according to Food Categorisation System (FCS) and sorted according to the number of the food category. The food categorisation was based on the 'Guidance document describing the food categories in Part E of the Annex II to Regulation (EC) No. 133/2008 on Food Additives' (European Commission, 2017). Finally, a min, mean and max value was calculated for the concentration of additive for each food category. For the mean value, the weighted approach was used to combine values between different sources when this was applicable.

Developing a methodology for the collection of occurrence data was the biggest challenge of this task, since many obstacles had to be overcome (combining data from different sources, quality/validity of data, food categorisation etc.) during the procedure.

Concerning TiO_2 the methodology used for the collection of occurrence data along with the results, the uncertainties and limitations of the study were published in an ANSES Opinion (ANSES, 2019) where detailed information can be found. According to these results, the highest concentrations of E171 are found in the following food categories: Confectionery products, dairy analogues, flavoured drinks, sauces, decorations coating and fillings as well as in food supplements in which the maximum concentration measured was 26,950 mg/kg.

The second aim of this activity was to collect information concerning the concentration of the nanoparticles of TiO_2/SiO_2 (either in food products or in the food additives itself) by performing a literature search. Concerning the concentration of Nps of TiO_2 expressed in % by number, the results were published in an ANSES Opinion (ANSES, 2019). These results indicate that on average 25% of the particles in E171 are nanoparticles (with a dimension less than 100 nm) while the min and max values reported in the literature were 6% and 55%, respectively.

Moreover, the fellow was involved in setting up a methodology to calculate a percentage of Nps by mass in E171 from available data and she was also involved in the final calculations.

The results of these tasks are not included in this report and are not discussed further since its part of the current work of the WG (NMs in food), the results of which will be published in an ANSES Opinion by the end of 2019.

2.2.1.2. Exposure Assessment Calculations

The fellow used the data collected for TiO_2/SiO_2 to perform an exposure assessment for different French population groups. The calculations for TiO_2 were performed by using the FAIM template (Food Additives Intake Model) for which an authorisation of use was requested form EFSA. Different scenarios were examined and the results shall be used for a future risk assessment of nano-TiO_2 in food. The calculations for SiO_2 are currently in progress.

The results obtained from the exposure assessment calculations are not included in the report and are not discussed further since its part of the current work of the working group (Nanomaterials in food), and the results of which will be published in an ANSES Opinion by the end of 2019.

2.2.2. Improve knowledge regarding food process, analytical and toxicological methods and further training in current risk assessment methodologies in place at ANSES

2.2.2.1. Improve knowledge on analytical methods for nanomaterials

The fellow visited the Laboratoire National de Metrologie et d' Essais-LNE (Paris, 27 of March, 2019) in order to gain further knowledge on the methods that can be used for the characterisation and quantification of nanoparticles in food products. This was very important for the fellow since the characterisation of NMs is an important step in nanospecific risk assessment and specifically for the steps of hazard identification.

During the visit, the principles of the main methods used by LNE (SEM-EDX, AFM, Zeta meter, DLS, BET instrument, XRD) to characterise the key parameters of NMs (size, shape, charge, concentration, specific surface, porosity, surface condition) were explained and a short demonstration of sample preparation procedure was performed (extraction of TiO_2 from a candy).

2.2.2.2. Improve knowledge on toxicological methods

The fellow was invited to visit the laboratory of Genetic Toxicology, Institut Pasteur de Lille (Lille, 18-20 of June, 2019), where she had the opportunity to gain knowledge on genotoxicity testing.

The principles of the main genotoxicity tests where explained and the guidelines associated with these tests were indicated (e.g. OECD). Moreover, it was explained how the interpretation of results is

done and what is required (e.g. criteria) in order to conclude that substance is genotoxic/mutagenic or not. During the visit, the fellow followed the laboratory procedure for the in vivo comet assay (Primary DNA damage) and part of the in vitro Ames test (Gene mutation test) and in vivo (bone marrow) and in vitro (mammalian cells) micronucleus assay (Chromosomal aberration test). Moreover, the principles of the in vivo Transgenic Mice and Pig-A tests (Gene mutation tests) were explained.

Overall, the visit to the Laboratory of Genetic Toxicology helped the fellow not only to increase knowledge on toxicological methods but also to better understand how these tests can be used for the hazard identification and characterisation. Moreover, the knowledge acquired from this training will help the fellow to be more familiar with toxicological results while reading literature in the future.

2.2.2.3. Further training in risk assessment methodologies in place at ANSES

During the 1-year's placement, the follow was encouraged to arrange independent meetings with ANSES colleagues, who have knowledge and expertise on different fields relevant with RA methodology. This interaction helped not only to increase knowledge of the fellow but enabled her also to extend her network within ANSES. Overall, the fellow was able to increase knowledge and to discuss on the following subjects:

a) Collection of food consumption data at ANSES – INCA studies: The aims of the last survey (INCA3) were explained as well as the methodology used to collect the data and how these can be utilised. In addition, the fellow received information on how food products can be classified according to FoodEx2 categories and was able after to use the EFSA browsing catalogue for food categorisation.
b) Total diet studies performed at ANSES (TDS): Several aspects concerning TDS were discussed. The fellow received information concerning the study design and the methodology used for this kind of studies (e.g. prioritisation of food and chemicals, sampling, food analysis etc.) as well as on the utilisation of these kinds of data for RA purposes. Moreover, the advantages of using TDS studies versus using data from monitoring and control programs were analysed.
c) Database for occurrence data – CONTAMINE database: The data from national surveillance and control plans are collected and standardised by ANSES in contamine database, along with the data from TDS studies. The fellow discussed several aspects concerning the Contamine database like, its uses and applications, the quality of data as well as how the data are registered and categorised.
d) Hazard identification and hazard characterisation: Members of the UERALIM unit devoted time to explain to the fellow the major toxicological studies and how their use for hazard identification and characterisation.
e) PBPK models: The fellow received information on the most common uses and applications of PBPK models for chemical RA.
f) Social aspects of NMs: The role of the Social Sciences, Expertise & Society Unit of ANSES was explained and information was provided for the activities of the unit concerning NMs.

Additionally, the fellow was involved in another project on the risk assessment of Cadmium (Cd) due to the consumption of seaweed food products. This arrangement was in agreement with the fellow, in order to get a broader knowledge on risk assessment methodologies in place at Anses and to have a better understanding on expert assessments processes. For that, the fellow had followed the procedures for the collection of occurrence and consumption data, which will be used for an exposure assessment calculation. During this process, the fellow gained knowledge on how you exclude or include occurrence data depending on the quality of results and on the criteria that is used for this decision.

2.2.3. Conduct expert assessments

ANSES provides collective expertise in the Agency's fields of competence in response to requests from the public authorities and stakeholders entitled to submit requests, as well as in the context of internal requests. The scientific expert assessments are conducted collectively in conjunction with Expert Committees (published as opinions on the ANSES website) and lead to recommendations designed to assist the competent authorities for risk management decisions.

During this 1 year of placement, the fellow attended several meetings of the WG (NMs in food) and was regularly presenting the results of her work to the experts. During these meetings, the fellow was involved in brainstorming processes and discussions with experts to determine the ways to conduct expertise. The fellow was therefore in this way taking part in the collective multidisciplinary expert

assessment process and has got hands-on experience and understanding of ANSES's expert assessment process which was a unique a highly valuable experience.

Moreover, she has followed the work of the ad hoc 'TiO_2' emergency collective expert appraisal group (GECU) which was created in response to a formal request in order to provide scientific and technical support on the risks associated with ingestion of the food additive E171. GECU's work was published in an ANSES Opinion (ANSES, 2019) which included the fellow's work results concerning the occurrence of TiO_2 in food as previously described. After the issue of this Opinion, the French Authorities decided to suspend the marketing of foods containing (titanium dioxide – TiO_2, E 171) as of January 2020 for 1 year, a decision that highlights the connection between risk assessment and risk management and the importance of risk assessment on policy decision.

2.3. Other activities

Learning objectives were further achieved by the following activities:

a) Participation in a symposium on 'Food Contaminants - Emerging Approaches to Know and Prevent Risk' (Paris, 19 December 2018)
b) A seminar on literature search called 'Agency Information Resources' given by a staff member at ANSES (ANSES, 31 October 2019)
c) Participation in Parma Summer School on Risk Benefit in food safety and nutrition (Parma, 11–13 June 2019)

3. Conclusions

The working program of 'Nanomaterials in Food – Prioritisation & Assessment' has provided a unique opportunity for the fellow to gain insights into the whole risk assessment process and linked principles and practices as well as to obtain knowledge in the field of NMs. NMs as a subject was the most challenging part of the project, since this is a new field with a lot of scientific interest. In addition, the obtained transferable skills and new knowledge could be further used to increase the scientific capacity of the fellow's home institute

The activities of the fellow proceeded in accordance with the work program. All aims were achieved and part of the fellow's work has been published in an ANSES Opinion and has been linked with a risk management decision as previously described.

Moreover, the EU-FORA program and this 1-year placement in ANSES provided an opportunity for the fellow to interact with experts having a long experience not only in NMs but also in risk assessment in general, enabling the fellow to expand her scientific network. Active participation in a WG was a unique and a highly valuable experience.

Finally, this experience gained at ANSES could also lead to future collaboration opportunities well beyond this fellowship.

References

ANSES, 2019. Opinion on the risks associated with ingestion of the food additive E171 (Request 2019-SA-0036).

Bouwmeester H, Dekkers S, Noordam MY, Hagens WI, Bulder AS, de Heer C, Ten Voorde SE, Wijnhoven SW, Marvin HJ and Sips AJ, 2009. Review of health safety aspects of nanotechnologies in food production. Regulatory Toxicology and Pharmacology, 53, 52–62. https://doi.org/10.1016/j.yrtph.2008.10.008

Calzolai L, Gilliland D and Rossi F, 2012. Measuring nanoparticles size distribution in food and consumer products: a review. Food Additives & Contaminants. Part A, Chemistry, Analysis, Control, Exposure & Risk Assessment, 29, 1183–1193. https://doi.org/10.1080/19440049.2012.689777

Dekkers S, Krystek P, Peters RJ, Lankveld DP, Bokkers BG, van Hoeven-Arentzen PH, Bouwmeester H and Oomen AG, 2011. Presence and risks of nanosilica in food products. Nanotoxicology, 5, 393–405. https://doi.org/10.3109/17435390.2010.519836

EFSA (European Food Safety Authority), 2018a. Guidance on risk assessment of the application of nanoscience and nanotechnologies in the food and feed chain: Part 1, human and animal health (1831-4732). EFSA Journal 2018;16(7):5327, 95 pp. https://doi.org/10.2903/j.efsa.2018.5327

EFSA (European Food Safety Authority), 2018b. Re-evaluation of silicon dioxide (E 551) as a food additive. EFSA Journal 2018;16(1):e05088, 70 pp. https://doi.org/10.2903/j.efsa.2018.5088

European Commission, 2011. Commission Recommendation 2011/696/EU of 18 October 2011 on the definition of nanomaterial. OJ L 275, 20.10.2011, p.38–40, under revision.

European Commission, 2017. Guidance document describing the food categories in Part E of Annex II to Regulation (EC) No 1333/2008 on Food Additives. Available online: https://ec.europa.eu/food/sites/food/files/safety/docs/fs_food-improvement-agents_guidance_1333-2008_annex2.pdf

European Union, 2008. Regulation (EC) No 1333/2008 of the European Parliament and of the Council of 16 December 2008 on food additives, 354 C.F.R. 2008.

European Union, 2011. Regulation (EU) No 1169/2011 of the European Parliament and of the Council of 25 October 2011 on the provision of food information to consumers, amending Regulations (EC) No 1924/2006 and (EC) No 1925/2006 of the European Parliament and of the Council, and repealing Commission Directive 87/250/EEC, Council Directive 90/496/EEC, Commission Directive 1999/10/EC, Directive 2000/13/EC of the European Parliament and of the Council, Commission Directives 2002/67/EC and 2008/5/EC and Commission Regulation (EC) No 608/2004 OJ L 304, 2011. 22.11.2011, p. 18–63.

European Union, 2015. Regulation (EU) 2015/2283 of the European Parliament and of the Council of 25 November 2015 on novel foods, amending Regulation (EU) No 1169/2011 of the European Parliament and of the Council and repealing Regulation (EC) No 258/97 of the European Parliament and of the Council and Commission Regulation (EC) No 1852/2001 (Text with EEA relevance). OJ L 327, 2015. 11.12.2015, p. 1–22.

Fiordaliso F, Foray C, Salio M, Salmona M and Diomede L, 2018. Realistic Evaluation of Titanium Dioxide Nanoparticle Exposure in Chewing Gum. Journal of Agriculture and Food Chemistry, 66, 6860–6868. https://doi.org/10.1021/acs.jafc.8b00747

ISO (International Organization for Standardization), 2015. ISO/TS 80004-2:2015 Nanotechnologies Vocabulary – Part 2: Nano-objects. 10 pp. In.

Lomer MC, Thompson RP, Commisso J, Keen CL and Powell JJ, 2000. Determination of titanium dioxide in foods using inductively coupled plasma optical emission spectrometry. Analyst, 125, 2339–2343.

Mattarozzi M, Suman M, Cascio C, Calestani D, Weigel S, Undas A and Peters R, 2017. Analytical approaches for the characterization and quantification of nanoparticles in food and beverages. Analytical and Bioanalytical Chemistry, 409, 63–80. https://doi.org/10.1007/s00216-016-9946-5

Weir A, Westerhoff P, Fabricius L, Hristovski K and von Goetz N, 2012. Titanium dioxide nanoparticles in food and personal care products. Environmental Science and Technology, 46, 2242–2250. https://doi.org/10.1021/es204168d

Winkler HC, Notter T, Meyer U and Naegeli H, 2018. Critical review of the safety assessment of titanium dioxide additives in food. J Nanobiotechnology, 16, 51. https://doi.org/10.1186/s12951-018-0376-8

Yang Y, Doudrick K, Bi X, Hristovski K, Herckes P, Westerhoff P and Kaegi R, 2014. Characterization of food-grade titanium dioxide: the presence of nanosized particles. Environmental Science and Technology, 48, 6391–6400. https://doi.org/10.1021/es500436x

Yin C, Zhao W, Liu R, Liu R, Wang Z, Zhu L and Liu S, 2017. TiO2 particles in seafood and surimi products: Attention should be paid to their exposure and uptake through foods. Chemosphere, 188, 541–547. https://doi.org/10.1016/j.chemosphere.2017.08.168

Abbreviations

AFM	Atomic Force Microscope
ANSES	French Agency for Food, Environmental and Occupational Health & Safety
DER	Risk Assessment Department
DGCCRF	Directorate-General for Competition, Consumer Affairs and Fraud Control
DLS	Dynamic Light Emitting
EU-FORA	The European Food Risk Assessment Fellowship Program
FAIM	Food Additives Intake Model
FCS	Food Categorisation System
FoodEx2	Version 2 of the EFSA Food Classification and description system for exposure assessment
MPL	max permitted limit
NGO	Non-Governmental Organisations
NMs	Nanomaterials
OECD	Organisation for Economic Co-operation and Development
QS	quantum satis
RA	Risk Assessment
RIVM	Dutch Institute of Public Health and the Environment
SEM-EDX	EDX Scanning Electron Microscope Coupled with Energy-dispersive X-ray Spectroscopy
SiO_2	Silicon Dioxide
TDS	Total Diet Studies
TiO_2	Titanium Dioxide
UERALIM Unit	Food Risk Assessment Unit
XRD	X-Ray Diffraction

EU-FORA SERIES 2

APPROVED: 31 July 2019

doi: 10.2903/j.efsa.2019.e170910

Assessment of combined risk to pesticide residues through dietary exposure

French Agency for Food, Environmental and Occupational Health & Safety (ANSES), France, Regulated Products Assessment Department, Residues and Food Safety Unit,
E Chatzidimitriou, A Mienne, S Pierlot, L Noel and X Sarda

Abstract

Plant protection products (PPPs) are preparations intended to protect plants and their products including one or more active substances. The use of PPPs may cause direct or indirect risks. Residues that can remain in or on food might pose a danger to human health through consumption and acute or/and chronic exposure. Authorisation of active substances and PPPs are decided at European and national level, respectively. Risk assessment of dietary exposure to residues of PPPs is regulated by a very extensive legal framework, ensuring consumer safety. The review and evaluation of the residue section of active substance monographs and the dossiers for PPP authorisations within the French Agency for Food, Environmental and Occupational Health & Safety (ANSES) helped gain hands-on experience on food risk assessment, as previewed in the framework of the European Food Risk Assessment Fellowship Programme (EU-FORA). The programme also focused on the cumulative effects of acute exposure to pesticides in food on the human nervous system using probabilistic methodology and it was in continuation of the work carried out by ANSES and the regulated products department residue unit. Using the European Database for processing factors for pesticides in food was one of the main challenges in order to approach a more realistic scenario of exposure. The probabilistic methodology followed was used in accordance with the European Food Safety Authority harmonised guidance.

Keywords: plant protection products, pesticide residues, dietary risk assessment, combined dietary risk assessment, processing factors

Correspondence: eu-fora@efsa.europa.eu

Acknowledgements: This report is funded by EFSA as part of the EU-FORA programme.

Suggested citation: French Agency for Food, Environmental and Occupational Health & Safety (ANSES), France, Regulated Products Assessment Department, Residues and Food Safety Unit, Chatzidimitriou E, Mienne A, Pierlot S, Noel L and Sarda X, 2019. Assessment of combined risk to pesticide residues through dietary exposure. EFSA Journal 2019;17(S2):e170910, 8 pp. https://doi.org/10.2903/j.efsa.2019.e170910

ISSN: 1831-4732

The EFSA Journal is a publication of the European Food Safety Authority, an agency of the European Union.

Table of contents

1. Introduction

The overall objective of the European Food Risk Assessment Fellowship Programme (EU-FORA) offered by the European Food Safety Authority (EFSA) is to give an opportunity to young and mid-career researchers to gain hands-on experience and skills on risk assessment in a food safety framework. This specific programme focused firstly on risk assessment of dietary exposure to residues of plant protection products (PPPs) on a regulatory basis and secondly, on combined dietary risk assessment of multiple pesticide residues.

The work was performed at the French Agency for Food, Environmental and Occupational Health & Safety (ANSES) in the Residues and Food Safety Unit (URSA) within the Department of Regulated Products. URSA is responsible for the activities related to assessment of risks to human health via dietary exposure in the area of plant protection active substances and products, non-indigenous macro-organisms considered beneficial to plants and introduced into the environment, biocidal active substances and products, fertilisers, growing media and similar products. It is also responsible for assessing marketing authorisation application dossiers in the area of PPPs in the framework of the regulations in force.

This report will start with general background information on regulations concerning PPPs. The framework for PPPs authorisation in relation to their respective maximum residue levels (MRLs) will be briefly described focusing on the dietary exposure and risk assessment of residues. Finally, the issue of dietary exposure to multiple pesticides will be presented, summarising the available methodologies in the framework of regulatory science on European level. The need of refining the risk to be assessed towards more realistic approaches will also be discussed alongside with the surrounding uncertainties.

1.1. Regulation of plant protection products

Plant protection products are preparations intended to protect plants and their products. Herbicides, insecticides, fungicides, plant growth regulators and biocontrol are all grouped under pesticides, a group which also includes biocides.

Preparations consist of one or more active substances responsible for the action and use of the PPP. The risk assessment process is undertaken at two stages. Firstly, according to Regulation (EC) No 1107/2009[1] concerning the placing of PPPs on the market, prior to the authorisation of PPPs, active substances should be approved by the European Commission. In parallel, food and/or feed cannot be placed on the market without setting MRLs for the requested specific substance. Therefore, according to Regulation (EC) No 396/2005 on MRLs of pesticides in or on food and feed of plant and animal origin, MRLs for each combination of active substance and food should be set a priori using a harmonised approach in the European Union. The MRLs set assure that the remaining residues after the most critical agricultural practice pose no unacceptable risk for the consumers. Finally, as long as the active substance of the preparation is approved, the risk assessment associated with the specific and requested use of the PPPs is performed within different European geographical zones. At the end, the relevant Member states decide their authorisation at national level. France although typically belongs to the Southern zone, data of both northern and southern geographical zones are required to evaluate consumer exposure.

The Member States, the EFSA and the European Commission all have an active role in the procedure for approving active substances, setting MRLs and placing PPPs in the market.

1.2. Dietary risk assessment of pesticide residues

The use of PPPs may cause direct or indirect risks. Residues that can remain in or on food might pose a risk to human health through consumption and acute or/and chronic exposure.

For the authorisation of an active substance, the applicant needs to submit the dossier to a Rapporteur Member State (RMS), which will peer review the application and upon completion will discuss it with other member States and EFSA. At the end, EFSA will publish a conclusion to support risk managers and the final decision at European level. The PPPs risk assessment is similar, but the respective authorisation is decided on a national level upon geographical zonal requirements and recommendations.

[1] Regulation (EC) No 1107/2009 of the European Parliament and of the Council of 21 October 2009 concerning the placing of plant protection products on the market and repealing Council Directives 79/117/EEC and 91/414/EEC. OJ L 309, 24.11.2009, p. 1–50.

A very extensive legal framework regulates the risk assessment of active substances, PPPs and their respective residues in food and the environment. The data requirement to evaluate the risk because of PPP residues include evaluation of studies on the metabolism of the active substance in crops, animals and rotational crops to identify the relevant metabolites, as well as studies on processed and stored commodities. Residue trials in crops, rotational corps and animal feeding studies are amongst others required to estimate the final residues levels of the active substance in food according to the intended agricultural practice. These residue levels are then to be used in mathematical models to calculate the human chronic and acute exposure, in relation to relevant human health toxicological threshold values, usually expressed as a contribution percentage of the acceptable daily intake (ADI) and Acute Reference Dose (ARfD), respectively.

The critical evaluation of the data provided and the data treatment are both crucial for a transparent and harmonised approach within PPPs residues risk assessment.

1.3. Combined dietary risk assessment of multiple pesticide residues

In the framework of PPPs authorisation, Article 4 of Regulation (EC) No 1107/2009 specifies that combined or as previously used, cumulative effects of the residues of the PPPs should not pose a risk to human health as long as a appropriate scientific methodology is available. Similarly, article 14 of Regulation (EC) No 396/2005 specifies that cumulative effects in the framework of MRL setting must be taken into account.

EFSA has published a guidance document on the probabilistic approaches that should be applied when evaluating the combined exposure to pesticides (EFSA PPR Panel, 2012). Similarly, the European Commission (2018) has agreed that the approach for combined risk should be a tiered level approach (Tier I and Tier II) starting from the most conservative scenario so as to screen the overall global exposure, progressively reaching more realistic scenarios, still including some degree of uncertainty.

Under European funding (7th Framework Programme for Research), the statistical software Monte Carlo Risk Assessment (MCRA) was released by the Dutch National Institute for Public Health and Environment (RIVM). This is a statistical software that allows probabilistic analyses with a tiered approach according to EFSA's recommendations and the Committee's requirements on the risk assessment of dietary exposure to multiple pesticides. Exposure estimates are calculated for specific cumulative assessment groups (CAG) (e.g. nervous system) as described by EFSA PPR Panel (2013) on grouping active substances on the basis of their toxicological profile. Exposure estimates are calculated for specific percentiles of exposure distribution and as suggested by the risk managers, conclusions should be drawn using the 99.9th percentile. The expression of exposure can be based on margin of exposure or other type of threshold values and measurements. Uncertainties can be found in all steps of the risk assessment, both in hazard characterisation (CAG formation and residue definition) and the exposure calculation. The high non-detect and missing values in the monitoring programs, the actual use of the authorised pesticides on each crop, the extrapolation that could potentially be used and the effect of processing on the resides of the consumed commodity are some of the uncertainties that are systematically being evaluated. In this respect processing factors are applied to each food commodity and active substance combination. A comprehensive European database of reliable processing factors for multiple active substances was recently published (Scholz et al., 2018) and should be applied in the framework of a combined risk assessment, even at Tier I level.

Being still in the pilot phase of the systematic implementation of probabilistic approach, RIVM will soon publish cumulative exposure assessments to pesticide residues in food regarding acute and chronic effects on nervous and thyroid systems using MCRA software and based on CAGs published by EFSA PPR Panel (2013). Similarly, EFSA will publish a similar report after public consultation duplicating the methodology of RIVM but using RAS software and taking into consideration an updated CAG and new approaches agreed at European level. RIVM will soon release the final version of MCRA 8.3 taking into consideration more parameters and the aforementioned approaches agreed at European level. Using probabilistic approaches to evaluate the risk caused by dietary exposure to multiple pesticides and set MRLs for PPP on a regulatory basis poses a challenge. Significant steps have been made to systematically approach a realistic characterisation of the risk to be evaluated towards a harmonised way of methodology as proposed by EFSA at a national and European level (EFSA Scientific Committee, 2019).

2. Description of work programme

2.1. Aims

The objective of the work programme was to work on the dietary risk assessment of (a) residues of PPPs (b) multiple residues of PPPs using a probabilistic approach.

During the first part of the project, the objective was to understand and familiarise with the risk assessment procedures and European regulations of active substances and PPPs at European and national/zonal level. During the second part of the project, the aim was to assess the dietary exposure of the French population to multiple pesticides using the MCRA software while looking into the effect of processing factors by incorporating the newly published European database of processing factors for pesticides in food (Scholz et al., 2018)

Integration in the projects and activities within the regulated products residue and consumer safety unit at ANSES was the core objective of the programme.

2.2. Activities/Methods

2.2.1. Dietary risk assessment of pesticide residues

In the framework of active substance renewal and PPP evaluation, there was a training period on EU regulations and technical guides concerning active substances and placing PPPs on the market. Using the Organisation for Economic Co-operation and Development (OECD) guidelines on crops, rotational crops and livestock metabolism, residue definition in plant and animal commodities, stability of residues, processing studies, residue and feeding trials was in the core of the methodology used for the evaluations.

Part of this work consisted of reviewing the residue section of two active substance monographs that were under the renewal period; one fungicide and one insecticide. All sections concerning the residue section were considered and the comments were made in a reporting table format, which was delivered to EFSA and the respective RMS. After the respective periods of consultation and addressing of comments, EFSA decided if the points made were adequately discussed and in some cases, that expert consultation is further needed. Numerous evaluations of PPPs containing one or more active substances were also undertaken in the framework of zonal authorisation and mutual recognition procedures under Regulation (EC) 1107/2009. Zonal and national evaluations were made providing opinions at both southern zonal European level and at national level concerning France (including northern and southern zones). Conformity to in-force EU MRLs was checked using the OECD MRL calculator as proposed by EFSA for a harmonised approach. Chronic and acute exposure assessments were undertaken using the calculation model developed by EFSA (PRIMo – Pesticide Residue Intake Model).

Participation at the preparative discussions within URSA to support the representative experts that participated at one pesticide peer-review experts meeting (PRAS) teleconference was a valuable experience and opportunity to understand the mechanisms and procedures of such meetings organised by EFSA at European level.

In both cases, during the evaluation of all aspects compromising the residue risk assessment section, consultation of fellow risk assessors was encouraged. The structure and expertise of the residue unit in ANSES offered a valuable opportunity for discussion and expertise exchange between risk assessors on a regular basis. Final evaluations were always discussed and validated by more experienced risk assessors within URSA.

2.2.2. Combined dietary risk assessment of multiple pesticide residues

The project on combined risk assessment started with a thorough understanding of EFSA's publications on harmonised methodologies for risk assessment to multiple chemicals. In parallel, there was training on the use and applications of MCRA software for probabilistic risk evaluations.

The project was focused on cumulative effects of acute exposure to pesticides in food on the human nervous system and it was in continuation of the work carried out within URSA in the framework of regulatory risk assessment. In the framework of this pilot project, the exposure studied was limited to the French population (consumption data 2006–2007; concentration data in food 2013–2015) and the CAG of human nervous system established by EFSA (EFSA PPR Panel, 2013). A tiered approach – starting with the most conservative to the most realistic – was followed, in accordance with

the approach agreed by the European Member States (European Commission, 2018). In view of refining and making more realistic the risk under evaluation, the effects of processing on the total residues have to be taken into account. The main objective of the current project was to incorporate in the analysis the processing factors published in the European food database (Scholz et al., 2018). The challenges arsing were (a) translating the processing factor data (provided in a FOODEX2 system) to a form compatible with the available concentration and consumption data currently used by France (FOODEX1) and (b) interpreting the results taking into consideration that actually very limited information on the specific combination of process and active substance within each food commodity is actually available. Initially, communication with experts from RIVM, responsible for MCRA support was initiated in so as to correctly insert the data into the software. In view of the aforementioned challenges, a meeting with an expert of coding of food consumption and concentration data was organised within ANSES, in order to elucidate the possibilities of translating FOODEX 2 to FOODEX1 coding. On another occasion, a telephone meeting was organised with experts from the combined risk assessment team in EFSA to discuss possibilities and uncertainties of such translation but also overall limitations of the evaluation because of existing uncertainties.

Overall, the participation in the unit's working group of combined risk gave an insight of the work carried out so far by ANSES, RIVM and EFSA and allowed for discussions on the potential of a probabilistic approach of combined risk in the framework of regulatory pesticide risk evaluation.

3. Conclusions

This fellowship being part of the EU-FORA programme and taking place at URSA within the Regulated Products Department at ANSES gave the opportunity to gain not only knowledge on the regulatory procedures but also professional experience on the risk assessment of PPPs.

The initial training of the programme on chemical risk assessment was crucial for the better incorporation in this working programme. A comprehensive view of the procedures of chemical risk assessment fundamentally helped to apply the new knowledge and methodology acquired during the training.

The critical evaluation of active substance monographs and the PPP authorisation dossiers using harmonised European procedures, allowed for a profound understanding of the principles covering the risk assessment of PPP products. Furthermore, the participation in URSA's working group of combined risk assessment in parallel with the advancement of the probabilistic analysis approach with the refinement of the risk has given the opportunity to discuss on the potentials of setting a standard approach for the combined risk in the framework of regulatory pesticide risk evaluation.

The working environment and fellow colleagues were key to a pleasant and rewarding environment on a daily basis. The successful integration in the team has undoubtedly led to productive discussions, knowledge exchange and the foundations of a network and future collaborations between experts in food risk assessment.

References

EFSA PPR Panel (EFSA Panel on Plant Protection Products and their Residues), 2012. European Food Safety Authority Panel on Plant Protection Products and their Residues (PPR). Guidance on the Use of Probabilistic Methodology for Modelling Dietary Exposure to Pesticide Residues. EFSA Journal 2012;10(10):2839, 95 pp. https://doi.org/10.2903/j.efsa.2012.2839. Available online: www.efsa.europa.eu/efsajournal

EFSA PPR Panel (EFSA Panel on Plant Protection Products and their Residues), 2013. *European Food Safety Authority Panel on Plant Protection Products and their Residues. Scientific Opinion on the identification of pesticides to be included in cumulative assessment groups on the basis of their toxicological profile* (1831-4732). EFSA Journal 2013;11(7):3293, 131 pp. https://doi.org/10.2903/j.efsa.2013.3293. Available online: https://efsa.onlinelibrary.wiley.com/doi/abs/10.2903/j.efsa.2013.3293

EFSA Scientific Committee, More SJ, Bampidis V, Benford D, Bennekou SH, Bragard C, Halldorsson TI, Hernández-Jerez AF, Koutsoumanis K, Naegeli H, Schlatter JR, Silano V, Nielsen SS, Schrenk D, Turck D, Younes M, Benfenati E, Castle L, Cedergreen N, Hardy A, Laskowski R, Leblanc JC, Kortenkamp A, Ragas A, Posthuma L, Svendsen C, Solecki R, Testai E, Dujardin B, Kass GEN, Manini P, Jeddi MZ, Dorne J-LCM and Hogstrand C, 2019. Guidance on harmonised methodologies for human health, animal health and ecological risk assessment of combined exposure to multiple chemicals. EFSA Journal 2019;17(3):e05634, 77 pp. https://doi.org/10.2903/j.efsa.2019.5634

European Commission, 2018. Commission working document on risk management aspects related to the assessment of cumulative exposure. SANTE-2015-10216 rev. 7.

Scholz R, van Donkersgoed G, Herrmann M, Kittelmann A, von Schledorn M, Graven C and Michalski B, 2018. European database of processing factors for pesticides in food. *EFSA Supporting Publications*, 15(11), 1510E. https://doi.org/10.2903/sp.efsa.2018.EN-1510

Abbreviations

ADI	acceptable daily intake
ANSES	French Agency for Food, Environmental and Occupational Health & Safety
ARfD	Acute Reference Dose
CAG	cumulative assessment groups
EU-FORA	The European Food Risk Assessment Fellowship Programme
MCRA	Monte Carlo Risk Assessment
MRLs	maximum residue levels
OECD	Organisation for Economic Co-operation and Development
PPPs	plant protection products
PRAS	pesticides peer-review experts meeting
PRIMo	Pesticide Residue Intake Model
RIVM	Dutch National Institute for Public Health and Environment
RMS	Rapporteur member State
URSA	Residues and Food Safety Unit (ANSES, France)

EU-FORA SERIES 2

APPROVED: 31 July 2019

doi: 10.2903/j.efsa.2019.e170911

Risk assessment methodologies in the field of contaminants, food contact materials, technological ingredients and nutritional risks

Department of Nutrition and Bromatology, Toxicology and Legal Medicine Faculty of Pharmacy, Universidad de Sevilla, Spain,
Giorgiana M Cătunescu, Ana M[a] Troncoso and Angeles Jos

Abstract

The programme aimed at training the fellow in the risk assessment guidelines proposed by the EFSA in the field of contaminants, food contact materials, technological ingredients and nutritional risks. It had a modular 'learning by doing' approach and a balanced learning/case studies and theory. Module 1 offered an insight into chemical risk assessment and conferred transferable skills for a proper application of the framework. The hands-on activities consisted of three case studies that went from a simple exercise on an official opinion, to working in a team with experts to produce a new opinion, to an individual work to obtain a publishable review manuscript. Module 2 was a training in experimental toxicology designed to create a toxicological basis and to enable the fellow to perform toxicological studies for risk assessment purposes. She joined the team working on cyanotoxins, gained experience with both EFSA and Organization of Economic Cooperation and Development (OECD) guidelines on genotoxicity and an insight into the developing of analytical methods suitable for risk assessment purposes. During module 3, the fellow was trained in nutritional risk assessment and involved in experimental work in chemical characterisation, biomarkers and mechanisms of action of bioactive compounds. This developed the critical perspective when assessing nutritional and health claims related the design of experiments, methods used, interpretation of results and human relevance. Module 4 provided a 'hand-on experience' in scientific risk communication as the fellow was encouraged and supported in the participation at local, national and international workshops and congresses presenting the outcomes of the three modules. Thus, the fellow was successfully integrated in the day-by-day workflow of the department, gaining first-hand practical experience in risk assessment in a multicultural and interdisciplinary context. This enabled a productive exchange of good practices and contributed to building a European risk assessment community.

Keywords: cyanotoxins, anthocyanins, EU-FORA, nanomaterials, nutrition, risk assessment, toxicology

Correspondence: eu-fora@efsa.europa.eu

Acknowledgements: This report is funded by EFSA as part of the EU-FORA programme.

Suggested citation: Department of Nutrition and Bromatology, Toxicology and Legal Medicine Faculty of Pharmacy, Universidad de Sevilla, Spain, Cătunescu GM, Troncoso AM[a] and Jos A, 2019. Risk assessment methodologies in the field of contaminants, food contact materials, technological ingredients and nutritional risks. EFSA Journal 2019;17(S2):e170911, 11 pp. https://doi.org/10.2903/j.efsa.2019.e170911

ISSN: 1831-4732

The EFSA Journal is a publication of the European Food Safety Authority, an agency of the European Union.

Table of contents

1. Introduction

The European Food Risk Assessment Fellowship (EU-FORA) Programme was created by EFSA to build a European pool of expert risk assessors and a knowledge community. It is intended for scientists with less than 15 years of experience in various fields: biology, chemistry, veterinary or human medicine, food technology, toxicology, agricultural or environmental science and offers dedicated training and hands-on experience in chemical and microbiological risk assessment (Bronzwaer et al., 2016).

The training programme 'Risk assessment methodologies in the field of contaminants, food contact materials, technological ingredients and nutritional risks' was developed and implemented at the Department of Nutrition and Bromatology, Toxicology and Legal Medicine (DNBTLM) Faculty of Pharmacy, Universidad de Sevilla (US), as hosting site, under the supervision of Profs. Angeles Jos and Ana Mª Troncoso. The team at the DNBTLM has extensive expertise in the field of experimental toxicology; nanomaterials; nutrition; risk assessment of food contaminants; and assessment of nutritional and health claims. Thus, they were able to offer a complementary insight and transferable practical skills to the EU-FORA fellow who had a very different background – food technology – and was coming from a life science university as sending organisation (University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, Romania).

The programme was designed starting from methodologies and guidelines proposed by EFSA and adapted to the expertise of the team at DNBTLM. The objectives of the training were considered in relation to the two groups at DNBTLM: Area of Toxicology and Area of Nutrition and Food Science, while the activities were grouped in modules based on common intended outcomes. A 'learning by doing' approach was consistent for all the modules.

2. Description of work programme

2.1. Aims

The aim of the working programme proposed by the DNBTLM was to train the EU-FORA fellow in the risk assessment methodologies and guidelines proposed by EFSA in the field of contaminants, food contact materials, technological ingredients and nutritional risks. To achieve this purpose, six learning objectives were formulated: (1) to efficiently use the main information sources and databases useful for risk assessment purposes; (2) to be able to identify the hazards of main concern for chemical and nutritional risk assessment; (3) to acquire substantial knowledge on the different phases of the food safety risk assessment of contaminants, food contact materials, technological ingredients and nutritional risks; (4) to acquire practical skills in experimental toxicology (*in vitro* assays, genotoxicity assays, analytical determinations etc.) for hazard characterisation; (5) to be able to interpret and discuss experimental results in a risk assessment perspective; (6) to gain oral and written communication skills to present scientific and risk assessment results. The working programme had a modular 'learning by doing' approach to meet the six objectives. For each of the four modules, a balance between interactive learning/case studies and theory was guaranteed.

2.2. Module 1 – Insight into chemical food safety risk assessment

Module 1 was focused on chemical risk assessment and included both activities designed to deepen the theoretical knowledge of the fellow and 'learning by doing' case studies. These activities were designed to provide the fellow a proper insight into the four parts of chemical risk assessment and confer transferable skills that will enable the proper application of the framework to any other chemicals.

The fellow benefited from an individual introductory session with the supervisor prof. Jos at the beginning of the fellowship which complemented the initial training at EFSA. The goal was to discuss and clarify aspects of the chemical risk assessment; EFSA's framework; and reliable scientific sources and databases (EFSA guidelines, OCDE protocols, European Commission, IARC, Codex Alimentarius) for risk assessment purposes. During the whole year, the fellow was engaged in meetings and discussions with the team of experts from the DNBTLM for a deeper insight on particular topics of the risk assessment of contaminants. In addition, she attended two undergraduate courses from the Degree in Pharmacy curricula: Food Safety; Toxicology and one course in Technological ingredients for food processing from the line of study on Food and Health included in Professional Master's in Pharmacy. The courses provided a complementary overview to the fellow's background in food technology.

The hands-on experience on chemical food safety risk assessment consisted of three case studies that went from a simple exercise on an already published official opinion, to working in a team with experts with the aim of producing a new opinion to an individual work under the guidance of the supervisor prof. Jos with the aim of obtaining a publishable review manuscript.

The first activity was an in-depth study of EFSA's opinion on acrylamide in food (EFSA, 2015) because it presented all the steps in the risk assessment framework of contaminants, with detailed methodological explanation that could be easily followed and replicated. In addition, the management approach is detailed in the Commission Regulation (EU) 2017/2158 establishing mitigation measures and benchmark levels for the reduction of acrylamide in food (Commission Regulation, 2017).

The case study was valorised by two lectures given by the fellow to the participants of two workshops organised by the Area of Toxicology from the DNBTLM, US (Table 1). The attendance to the workshops provided complementary information for the chemical risk assessment and a proper background for debating and networking activities. Following her participation at the IIIrd Workshop on Toxicology, the fellow co-authored a poster on creative education in Pharmacy (Table 1).

The second case study involved the fellow in the workings of the Scientific Committee of the Spanish Agency for Food Safety and Nutrition (AESAN) on emerging risks. The chemical hazards of interest in food safety in Spain were already identified by the Committee, and the fellow was assigned to work on the emerging *Fusarium* mycotoxins enniatins (ENNs) and nivalenol (NIV); and pyrrolizidine alkaloids (PAs). ENNs and NIV were of interest because either they are not yet regulated, and/or they can co-occur with other mycotoxins (CONTAM Panel, 2014, 2017a), while PAs are a large group of plant secondary metabolites highly toxic to humans and animals (CONTAM Panel, 2017b). A literature search was conducted to identify relevant scientific data on ENNs, NIV and PAs relevant for food safety in Spain. The results and the conclusion obtained under the supervision of prof. Jos, the coordinator of the working group, were approved by the Committee and published in a report on emerging chemical hazards of concern in Spain (Jos et al., 2018) (Table 1).

The third case study required the fellow to apply EFSA's guidance on risk assessment of the application of nanoscience and nanotechnologies in the food and feed chain covering mainly the human exposure via the oral route (EFSA Scientific Committee, 2018) on a nanomaterial used in food contact materials. The aim of the activity was to develop a scientific opinion in the form of a publishable manuscript in a peer-reviewed scientific journal. The topic was chosen because of the safety concerns associated with the characteristics of nanomaterials related to their nanoscale, both physicochemical (size, chemical composition, aspect ratio, surface properties, crystallinity, solubility, clustering properties) and biological (particle–cell interactions, mobility, persistence in the body, bioavailability). The guidelines are recent and there is no concern of producing a research paper over imposing with other current publication. In addition, the supervisor prof. Jos has ample expertise in the toxicology and safety of food contact materials and can proficiently guide the fellow. A systematic search of the scientific literature was performed using Web of Science, Science Direct, PubMed and PubChem electronic databases. The scientific data were extracted and structured according to EFSA's three-step framework proposed for hazard identification and characterisation of nanoparticles with a preliminary step (Step 0) that firstly determines the existence of nanospecific properties (EFSA Scientific Committee, 2018). In Step 0, data were collected and evaluated to determine the *in vitro* rate of degradation of the nanomaterial to the non-nanomaterial under representative gastrointestinal tract conditions. In Step 1, a literature review was performed on the carcinogenicity, mutagenicity, reprotoxicology of the nanomaterial. The aim was to either provide sufficient information to perform the risk assessment or to identify key toxicological aspects needed to be explored in the *in vivo* oral study (Step 2). Step 2 comprises of the review of data on *in vivo* studies: pilot 14-day studies aimed at dose finding and assessment of absorption, tissue distribution and accumulation, elimination phase (Step 2a) and modified 90-day toxicity tests in rodents with emphasis on liver, brain, testis and spleen (Step 2b). The goal was to identify a reference point: lower boundary of the benchmark dose confidence interval (BMDL) or a no observed adverse effect level (NOAEL). Step 3 consisted of the reviewing of in-depth targeted studies (long-term exposure toxicokinetic studies, neurotoxicity, immunotoxicity or endocrine-mediated effects) designed to decreasing the uncertainty of the risk assessment. In addition, data were collected on the effects on the gut microbiome as the nanomaterial showed antimicrobial activity. Partial results of the study will be presented in a poster at the XXIIIrd Spanish Congress of Toxicology at the end of June 2019 (Table 1).

A valuable insight into the field of nanotechnology was offered by the participation at the Workshop on Nanotechnology in Food Industry organised by the National Network of Food Nanotechnology and the Platform Food4Life Spain at the Ministry of Science in Madrid in the 14 March 2019.

2.3. Module 2 – Experimental toxicology in risk assessment

Module 2 was focused on a 'hands-on' training in experimental toxicology offered by the Area of Toxicology from the DNBTLM, to complement the fellow's background. The aim of the module was to create a sound toxicological basis and to enable her to appropriately and critically evaluate toxicological studies for risk assessment.

Similar to module 1, the fellow was systematically engaged into meetings and discussions with the supervisor prof. Jos and the team of experts from the DNBTLM for a deeper insight into experimental toxicology: protocols; methods and assays; doses and administration routes; analysing of samples and data interpretation in the framework of risk assessment. The attendance to the undergraduate course on Toxicology completed her theoretical training.

The fellow joined the team working on the toxicity of cyanotoxins, mainly cylindrospermopsin (CYN) and mixtures of microcystins (MCs) and CYN. This topic was chosen because these toxins pose rising health-related concerns because of the increasing spread and frequency of cyanobacteria blooms caused by to eutrophication and climate change (Buratti et al., 2017). Humans may be exposed to cyanotoxins via many routes, but the oral exposure by contaminated water and foods (mainly fish, sea food and vegetables) is prevalent (Testai et al., 2016).

CYN is considered by an External scientific EFSA Report (Testai et al., 2016) as a potential emerging risk because of its cytotoxicity, neurotoxicity, pro-genotoxicity and potential carcinogenicity (Puerto et al., 2018). However, the mode of action is not yet fully understood, mainly in respect to its genotoxicity, which is a key point in the risk assessment. Some *in vitro* studies suggested genotoxicity due to DNA fragmentation, mediated by previous metabolism (Puerto et al., 2018). In addition, EFSA recommended the performing of new *in vivo* genotoxicity studies of CYN because previous results were either inconclusive and contradictory (Testai et al., 2016). Thus, the fellow was involved in the study of CYN genotoxicity *in vivo* following the EFSA guidelines on genotoxicity testing (EFSA Scientific Committee, 2011). Rats were used as experimental model and the induced potential genotoxic effects were evaluated after oral administration of pure CYN standard, by application of a combined micronucleus (MN)-comet assay following OECD 474 (OECD, 2016a) and OECD 489 (OECD, 2016b) guidelines. The fellow gained experience in working with both EFSA and OECD guidelines on genotoxicity testing and was trained in the using of OLYMPUS BX61 microscope for MN assay; the microscope together with the CometAssay IV software for comet assay; and Graph-Pad InStat software for the statistical analysis of the results. In addition, there was an active exchange of best practices in scientific reference and data management between the fellow and the team of experts at the DNBTLM. Some of the results of this experiment were already presented in two posters (Table 1) and included in a manuscript co-authored by the fellow: '*In vivo* genotoxicity evaluation of Cylindrospermopsin in rats using a combined micronucleus and comet assay' currently under review for a peer-reviewed journal. In addition, results will be presented as an additional poster at the EUROTOX2019 Congress in September (Table 1).

CYN and MCs frequently co-occur (Testai et al., 2016) and mixtures are a more probable exposure scenario. In addition, the toxic effects may be different than those observed for single cyanotoxins. An External scientific EFSA Report has concluded that more data are needed on the toxicity of cyanotoxin mixtures (Testai et al., 2016). The fellow is involved in a second ongoing *in vivo* genotoxicity testing on mixtures of CYN and MCs, following the previously protocol described. Partial results will be presented as a poster at the XXIIIrd Spanish Congress of Toxicology at the end of June 2019.

The same External scientific EFSA Report (Testai et al., 2016) showed the need for new analytical methods for sample preparation, routine detection and quantification of CYN and mixtures of MCs – CYN in complex matrices for both control procedures and risk assessment. Thus, the fellow participated in the activities related to the development and optimisation of a method to determine the content of CYN and MCs – CYN in mussel matrix based on a solid-phase extraction method and quantification by ultra-performance liquid chromatography–tandem mass spectrometry previously optimised and validated by the team at DNBTLM (Diez-Quijada et al., 2018; Prieto et al., 2018). The fellow gained a close insight into the developing and validating of analytical methods with suitable limit of quantification (LOQ) and limit of detection (LOD) for risk assessment purposes. The fellow was proactively involved in the design of the experiment for the optimisation study by carrying out the response surface methodology.

The fellow had the opportunity to be involved in a biotransformation and bioaccessibility study under simulated digestion. The experiment was an *in vitro* digestion model, including salivary; gastric

and duodenal phases; and colonic fermentation under lactic acid bacteria (Maisanaba et al., 2018). Thus, the fellow had an insight into the protocol, design of experiments and data interpretation that conferred skills to critically evaluate simulated digestion studies for risk assessment purposes.

In addition, both the fellow and the team from Area of Toxicology from the DNBTLM have already undergone steps in the continuing of their collaboration: the fellow applied for funding for a future mobility to the US after the ending of the fellowship, while the members of Area of Toxicology included the fellow as an external collaborator in a funding proposal.

2.4. Module 3 – Insight into nutritional risk assessment

In module 3, the Area of Nutrition and Food Science of the DNBTLM offered training in exposure assessment, nutritional risk assessment and assessment of nutritional and health claims. Similar to the previous two modules engaged the fellow in both theoretical and practical aspects of: food composition and food consumption databases; novel foods; dietary reference values; nutrients bioavailability; biomarkers; mechanisms of action of bioactive compounds; nutritional and health claims.

The fellow participated in discussions on specific characteristics of the nutritional risk assessment with the supervisor prof. Troncoso and the team of experts from the DNBTLM. In addition, she attended the undergraduate course from the Degree in Pharmacy curricula on Nutrition, Dietetics and Dietotherapy and two Master courses of the line of study of Food and Health included in Professional Master's in Pharmacy: Bioactive compounds and functional foods; Nutritional risk assessment. During the courses the fellow received a complementary theoretic background, and practical skills during the seminars and practical courses on: evaluating and designing diets; working with food consumption database; assessing nutritional labels and health claims of foodstuffs; identifying and quantifying biomarkers in human samples. The fellow had the opportunity to engage with both Spanish and international Erasmus students and scholars during the group activities, contributing to her working experience in multicultural environments and sharing of best practices.

In addition, the fellow collaborated with the experts from the Area of Nutrition and Food Science of the DNBTLM in their ongoing experimental work in the field of chemical characterisation, biomarkers and mechanisms of bioactive compounds. In particular, she was involved in two experiments.

The first study dealt with the assessment of health protective properties of dietary polyphenols consumed as part of human diets in cardiovascular diseases. The topic was chosen because the scientific evidence is the most robust compared to the multitude of other biological activities demonstrated *in vitro*. In most of the *in vitro* studies, the bioactivity was observed in supra-physiological concentrations and/or for non-physiological compounds and the bioactivity remained theoretical with no demonstrated *in vivo* mechanisms (Cerezo et al., 2015). The study aimed to identify the molecular mechanism of polyphenols (melatonin and hydroxytyrosol) related to the inhibition of angiogenesis, a process involved in the development and destabilisation of atherosclerotic plaques. The vascular endothelial growth factor (VEGF) – the most important pro-angiogenic growth factor in humans – exerts its angiogenic effects by stimulating VEGF receptor 2 (VEGFR-2) (Cerezo et al., 2015). The inhibition of VEGF-induced VEGFR-2 activity was tested on human umbilical vein endothelial cells (HUVECs). The fellow participated in the preparing and treatment of HUVECs; protein content determination; phosphorylated VEGFR-2 in lysates measurement by ELISA; and Western blot analysis for VEGR-2 endothelial nitric oxide synthase (eNOS) following the procedures described by (Cerezo et al., 2015, 2017; Moyle et al., 2015).

The second study focused on the nutritional value of anthocyanins extracted from blueberries because of the growing scientific and economic interest in their potential beneficial effects on human health. Anthocyanins are water-soluble plant pigments found in red, blue or purple fruits and vegetables. They are present predominantly in the skin of the fruit, but in berries, they are present in both the skin and flesh (Cassidy, 2018). They are glycosides of anthocyanidins and only six of them seem to be relevant to the human diet (cyanidin, delphinidin, malvidin, pelargonidin, peonidin, petunidin) (Cassidy, 2018). However, the anthocyanin content and profile vary widely with growing and storage condition; thus, it is of interest to evaluate local varieties. Thus, the aim of the study was to determine the content of anthocyanins from four Spanish blueberry varieties; to identify the anthocyanin profile; and to investigate the *in vitro* bioactivity in relation to EFSA's Guidance for the scientific requirements for health claims related to antioxidants, oxidative damage and cardiovascular health (EFSA Panel on Dietetic Products Nutrition, 2018). The anthocyanin fraction was prepared and the antioxidant activity was performed (Cerezo et al., 2010). The experiment is ongoing, and the total anthocyanin content and determination and quantification of the individual anthocyanin compounds will follow shortly. Other

assays related to their antibacterial activity will be performed in the fellow's home institution, as this experiment builds on her previous experience in the study of anthocyanins and their health benefits. The results of the case study are to be included in a scientific manuscript co-authored by researchers from both sending and hosting institution, contributing to the continuous co-operation.

In addition, the fellow was invited to attend the Workshop 'Towards the search for new bioactive properties in fermented foods' organised by the Area of Nutrition and Food Science of the DNBTLM on 24 May 2019 with the aim of completing her theoretical and practical experience and contributing to the extension of her scientific network.

The fellow gained practical experience with the testing of bioactive compounds for their bioactivity, bioavailability and mechanisms of action. This contributed to the development of a critical perspective on studies needed to assess nutritional and health claims in relation to the design of experiment, methods used, interpretation of results and extrapolation for human relevance. In addition, a productive exchange of good practices took place between the fellow and the experts of the DNBTLM in relation to laboratory practices and data processing. Moreover, the fellow shared her personal practices in references management in a seminar on EndNote reference manager software (Table 1).

2.5. Module 4 – Scientific risk communication

Module 4 was designed to provide a 'hand-on experience' in scientific risk communication. The fellow was encouraged and supported in the participation at local, national and international workshops and congresses (Table 1). Thus, all the case studies used for the training of the fellow were materialised into different forms of scientific communication: lectures, posters oral, presentations, scientific opinions and research manuscripts.

In addition, the fellow was able to gain experience by being involved in the organisation of a scientific congress as the DNBTLM was the host of the XXIII Spanish and VII Iberoamerican Congress of Toxicology, organised by the Spanish Association of Toxicology (AETOX) and the Area of Toxicology of the Faculty of Pharmacy of the US in 26-28.06.2019.

Table 1: Scientific communications delivered or co-authored by the EU-FORA fellow

Date of communication	Type of communication	Scientific context	Title of communication
19.11.2018	lecture	VIIIth Workshop on Food Safety: Risk assessment, [VIII Jornadas de Seguridad Alimentaria: Análisis de Riesgos], DNBTLM, US, Spain	Acrylamide formation in foods during thermal processing
28.11.2018	scientific report	Revista del Comité Científico de la AESAN	Report of the Scientific Committee of the Spanish Agency for Food Safety and Nutrition (AESAN) on the prospection of chemical hazards of interest in food safety in Spain
2-5.04.2019	poster	European Biophysical Societies' Association EBSA22 Conference Bucharest, Romania	*In vivo* genotoxicity of cylindrospermopsin by the comet assay
26.04.2019	lecture	IIIrd Workshop on Toxicology and Society: Abuse drugs and molecular toxicology (Forum and toxicology colloquium), [III Jornadas de Toxicología y Sociedad: Drogas de Abuso y Toxicología Molecular (Foro y Olimpiadas de Toxicología)], DNBTLM, US, Spain	Acrylamide formation in foods during thermal processing
5-10.05.2019	poster	The 11th International Conference on Toxic cyanobacteria, in Krakow, Poland	DNA damage induced by cylindrospermopsin in rats

Date of communication	Type of communication	Scientific context	Title of communication
15-17.05.2019	poster	The 25th European Association of Faculties of Pharmacy (EAFP) Annual Conference 2019: Creative education: Towards competences in patient-oriented pharmacy education, Krakow, Poland	Celebration of the III Meeting on Toxicology and Society: Drugs of abuse and molecular toxicology (Toxicology Forum and olympiad)
11.06.2019	seminar	DNBTLM, US, Spain	Overview of using reference management software: EndNote Case Study
26-28.06.2019	oral presentation	XXIIIrd Spanish and VIIth Iberoamerican Congress of Toxicology; section: toxicology education, [XXIII Congreso Español de Toxicología y VII Iberoamericano; sección de Educación en Toxicología], US, Spain	Experience of an European food risk assessment (EU-FORA) fellow at the University of Seville
26-28.06.2019	poster	XXIIIrd Spanish and VIIth Iberoamerican Congress of Toxicology; section: food safety, [XXIII Congreso Español de Toxicología y VII Iberoamericano; sección de Educación en Toxicología], US, Spain, sección de Seguridad Alimentaria, US, Spain	EFSA Scientific Committee's (2018) stepwise framework for nano-related hazard identification and characterisation in food and feed
	poster		Analysis of DNA damage in rats by simultaneous exposure to cylindrospermopsin and microcystin-LR [Análisis del daño en el ADN en ratas tras la exposición simultánea a cilindrospermopsina y microcistina-LR]
8-11.09.2019	poster	55th Congress of the European Societies of Toxicology, EUROTOX2019: Toxicology – Science Providing Solutions, Helsinki, Finland	Cylindrospermopsin induces genotoxic damage in rats by the comet and micronucleus tests

3. Conclusions

The working programme 'Risk assessment methodologies in the field of contaminants, food contact materials, technological ingredients and nutritional risks' managed by the DNBTLM, US, employed a modular 'learning by doing' approach. The four modules (chemical risk assessment; experimental toxicology; nutritional risk assessment and risk communication) included activities that successfully trained the EU-FORA fellow in: the efficient use of scientific databases for risk assessment; identifying hazards; using the food safety risk assessment framework for contaminants, food contact materials, technological ingredients and nutritional risks; acquiring practical skills in experimental toxicology; experimental data interpretation for risk assessment; and communication of risk assessment results.

This is supported by the outcomes of the working programme: two lectures on risk assessment in thematic workshops; co-authoring as an external collaborator of a published Report of the Scientific Committee of the Spanish Agency for Food Safety and Nutrition (AESAN) on the prospection of chemical hazards of interest in food safety in Spain; six posters at international conferences on risk assessment topics; one oral presentation at an international conference on risk assessment training; a submitted manuscript to a peer-reviewed journal and other papers on preparation; and participation at four thematic workshops.

The activities were designed to provide the fellow a proper insight into risk assessment and confer transferable skills. In addition, she was successfully integrated in the day-by-day workflow of the department, gaining first-hand experience. The fellow attended both undergraduate and Master courses that were complementary to the fellow's background in food science and technology. This created a good environment for professional and social interaction between the team at the DNBTLM, the fellow and the students. It helped with the integration of the fellow in the daily routine of the DNBTLM, but also provided a multicultural and interdisciplinary context. This enabled a fruitful

exchange of good practices in teaching, laboratory and communicating in a higher education environment in general, and risk assessment in particular.

Thus, the professional and pleasant working environment of the DNBTLM has guaranteed the success of the EU-FORA training programme. It has set the stage for future collaboration between the fellow and her home institution, on one side, and the team at DNBTLM and the hosting institution, on the other side, such as common project proposals that currently under evaluation; opinion and research manuscripts under preparation and future European funding opportunities accessed in consortium. This contributes significantly to building a European risk assessment community by engaging in common goals and using harmonised practices.

References

Bronzwaer S, Le Gourierec N and Koulouris S, 2016. Editorial: The European Food Risk Assessment Fellowship Programme (EU-FORA). EFSA Journal 2016;14(11):e14111, 2 pp. https://doi.org/10.2903/j.efsa.2016.e14111

Buratti FM, Manganelli M, Vichi S, Stefanelli M, Scardala S, Testai E and Funari E, 2017. Cyanotoxins: producing organisms, occurrence, toxicity, mechanism of action and human health toxicological risk evaluation. Archives of Toxicology, 91, 1049–1130. https://doi.org/10.1007/s00204-016-1913-6

Cassidy A, 2018. Berry anthocyanin intake and cardiovascular health. Molecular Aspects of Medicine, 61, 76–82. https://doi.org/10.1016/j.mam.2017.05.002

Cerezo AB, Cuevas E, Winterhalter P, Garcia-Parrilla MC and Troncoso AM, 2010. Isolation, Identification, and Antioxidant Activity of Anthocyanin Compounds in Camarosa Strawberry. Food Chemistry, 123, 574–582. https://doi.org/10.1016/j.foodchem.2010.04.073

Cerezo AB, Winterbone MS, Moyle CW, Needs PW and Kroon PA, 2015. Molecular structure-function relationship of dietary polyphenols for inhibiting VEGF-induced VEGFR-2 activity. Molecular Nutrition & Food Research, 59, 2119–2131. https://doi.org/10.1002/mnfr.201500407

Cerezo A, Hornedo-Ortega R, Álvarez-Fernández M, Troncoso A and García-Parrilla M, 2017. Inhibition of VEGF-induced VEGFR-2 activation and HUVEC migration by melatonin and other bioactive indolic compounds. Nutrients, 9, https://doi.org/10.3390/nu9030249

Commission Regulation E, 2017. 2017/2158 of 20 November 2017 establishing mitigation measures and benchmark levels for the reduction of the presence of acrylamide in food.

Diez-Quijada L, Guzman-Guillen R, Prieto Ortega AI, Llana-Ruiz-Cabello M, Campos A, Vasconcelos V, Jos A and Camean AM, 2018. New method for simultaneous determination of microcystins and cylindrospermopsin in vegetable matrices by SPE-UPLC-MS/MS. Toxins (Basel), 10, https://doi.org/10.3390/toxins10100406

EFSA (European Food Safety Authority), 2015. Scientific Opinion on acrylamide in food. EFSA Journal, 13, https://doi.org/10.2903/j.efsa.2015.4104

EFSA CONTAM Panel (EFSA Panel on Contaminants in the Food Chain), 2014. Scientific opinion on the risks to human and animal health related to the presence of beauvericin and enniatins in food and feed. EFSA Journal 2014;12(8):3802, 174 pp. https://doi.org/10.2903/j.efsa.2014.3802

EFSA CONTAM Panel (EFSA Panel on Contaminants in the Food Chain), 2017a. Appropriateness to set a group health based guidance value for nivalenol and its modified forms. EFSA Journal 2017;15(4):4751, 25 pp. https://doi.org/10.2903/j.efsa.2017.4751

EFSA CONTAM Panel (EFSA Panel on Contaminants in the Food Chain), 2017b. Risks for human health related to the presence of pyrrolizidine alkaloids in honey, tea, herbal infusions and food supplements. EFSA Journal 2017;15(7):4908, 34 pp. https://doi.org/10.2903/j.efsa.2017.4908

EFSA Panel on Dietetic Products Nutrition, Turck D, Bresson JL, Burlingame B, Dean T, Heinonen M, Hirsch-Ernst KI, Mangelsdorf I, McArdle HJ, Naska A, Neuhäuser-Berthold M, Nowicka G, Pentieva K, Sanz Y, Sjödin A, Stern M, Tomé D, Strain JJ, Vinceti M, Willatts P, Martin A, Heng L, Valtueña Martínez S, Siani A, 2018. Guidance for the scientific requirements for health claims related to antioxidants, oxidative damage and cardiovascular health. EFSA Journal 2018;16(1):5136, 21 pp. https://doi.org/10.2903/j.efsa.2018.5136

EFSA Scientific Committee, 2011. Scientific opinion on genotoxicity testing strategies applicable to food and feed safety assessment. EFSA Journal 2011;9(9):2379, 68 pp. https://doi.org/10.2903/j.efsa.2011.2379

EFSA Scientific Committee, 2018. Guidance on risk assessment of the application of nanoscience and nanotechnologies in the food and feed chain: Part 1, human and animal health. EFSA Journal 2018;16(7):5327, 95 pp. https://doi.org/10.2903/j.efsa.2018.5327

Jos Á, Conchello P, Martín O, Ruiz MJ and Catunescu GM, 2018. Report of the Scientific Committee of the Spanish Agency for Food Safety and Nutrition (AESAN) on the prospection of chemical hazards of interest in food safety in Spain. Revista del Comité Científico de la AESAN, 28, 69–127.

Maisanaba S, Guzman-Guillen R, Valderrama R, Meca G, Font G, Jos A and Camean AM, 2018. Bioaccessibility and decomposition of cylindrospermopsin in vegetables matrices after the application of an in vitro digestion model. Food and Chemical Toxicology, 120, 164–171. https://doi.org/10.1016/j.fct.2018.07.013

Moyle CW, Cerezo AB, Winterbone MS, Hollands WJ, Alexeev Y, Needs PW and Kroon PA, 2015. Potent inhibition of VEGFR-2 activation by tight binding of green tea epigallocatechin gallate and apple procyanidins to VEGF: relevance to angiogenesis. Molecular Nutrition & Food Research, 59, 401–412. https://doi.org/10.1002/mnfr.201400478

OECD, 2016a. Test No. 474: Mammalian Erythrocyte Micronucleus Test. https://doi.org/10.1787/9789264264762-en

OECD, 2016b. Test No. 489: In Vivo Mammalian Alkaline Comet Assay. https://doi.org/10.1787/9789264264885-en

Prieto AI, Guzman-Guillen R, Diez-Quijada L, Campos A, Vasconcelos V, Jos A and Camean AM, 2018. Validation of a method for cylindrospermopsin determination in vegetables: application to real samples such as lettuce (Lactuca sativa L.). Toxins (Basel), 10, https://doi.org/10.3390/toxins10020063

Puerto M, Prieto AI, Maisanaba S, Gutierrez-Praena D, Mellado-Garcia P, Jos A and Camean AM, 2018. Mutagenic and genotoxic potential of pure Cylindrospermopsin by a battery of *in vitro* tests. Food and Chemical Toxicology, 121, 413–422. https://doi.org/10.1016/j.fct.2018.09.013

Testai E, Buratti FM, Funari E, Manganelli M, Vichi S, Arnich N, Biré R, Fessard V and Sialehaamoa A, 2016. Review and analysis of occurrence, exposure and toxicity of cyanobacteria toxins in food. EFSA Supporting Publications, 13, 998E. https://doi.org/10.2903/sp.efsa.2016.EN-998

Abbreviations

AESAN	Spanish Agency for Food Safety and Nutrition
BMDL	Benchmark dose confidence interval
CYN	Cylindrospermopsin
DNBTLM	Department of Nutrition and Bromatology, Toxicology and Legal Medicine
ENNs	Enniatins
eNOS	Endothelial nitric oxide synthase
HUVEC	Human umbilical vein endothelial cells
LOD	Limit of detection
LOQ	Limit of quantification
MCs	Microcystin
MN	Micronucleus
NIV	Nivalenol
NOAEL	No observed adverse effect level
OECD	Organization of Economic Cooperation and Development
PAs	Pyrrolizidine alkaloids
US	Universidad de Sevilla
VEGF	Vascular endothelial growth factor
VEGFR-2	Vascular endothelial growth factor receptor 2

EU-FORA SERIES 2

Animal & Plant Health Agency

EU-FORA

EFSA Journal

APPROVED: 31 July 2019

doi: 10.2903/j.efsa.2019.e170912

Livestock, food chain and public health risk assessment

Animal and Plant Health Agency (APHA), Biomathematics and Risk Research workgroup, United Kingdom,
Irina Smeu and Rachel A Taylor

Abstract

The EUropean FOod Risk Assessment (EU-FORA) Fellowship work programme 'Livestock, food chain and public health risk assessment', founded by EFSA was proposed by the Animal and Plant Health Agency (APHA), United Kingdom (UK). A scientist working in the field of food safety was selected to work within the Department of Epidemiological Sciences, under the guidance of an experienced risk assessor. The programme was structured in four different modules that covered a wide range of aspects related to risk assessment (RA). Taken together, all modules ensured a broad overview of the various methodologies, tools and applications of RA. Thus, the learning-by-doing working programme in RA allowed the fellow to develop her knowledge in RA, to diversify her competencies and to extend her scientific network for future collaborations in the field of RA.

Keywords: risk assessment, risk ranking, livestock health, food chain

Correspondence: eu-fora@efsa.europa.eu

Acknowledgements: This report is funded by EFSA as part of the EU-FORA programme.

Suggested citation: Animal and Plant Health Agency (APHA), Biomathematics and Risk Research workgroup, United Kingdom, Smeu I and Taylor RA, 2019. Livestock, food chain and public health risk assessment. EFSA Journal 2019;17(S2):e170912, 11 pp. https://doi.org/10.2903/j.efsa.2019.e170912

ISSN: 1831-4732

The EFSA Journal is a publication of the European Food Safety Authority, an agency of the European Union.

Table of contents

1. Introduction

This Technical Report represents a description of the EUropean FOod Risk Assessment (EU-FORA) Fellowship work programme 'Livestock, food chain and public health risk assessment', founded by the European Food safety Authority (EFSA). It was proposed by the Animal and Plant Health Agency (APHA), UK, one of the four executive agencies working for the Department for Environment, Food and Rural Affairs (Defra) and also on behalf of the Scottish Government and Welsh Government. The agency is responsible for safeguarding animal and plant health in the UK, providing support for the delivery of their animal health and welfare and bee health policies. Within the EU-FORA fellowship, the fellow, Dr. Irina Smeu from the National R&D Institute for Food Bioresources – IBA Bucharest in Romania, was placed at the APHA, within the Biomathematics and Risk Research (BRR) workgroup, part of the Department for Epidemiological Sciences (DES). It is a nationally and internationally recognised group of risk analysts, modellers and statisticians providing high quality scientific evidence for policy formulation and outbreak response, as well as specialist support to research and operations in the area of animal health. The work programme was supervised by Dr. Rachel Taylor, Senior Risk Analyst within the BRR workgroup. The programme consisted of four different modules based on on-going risk assessment (RA) project work and previous research interests at APHA, including the development of several RAs supported and funded by EFSA to underpin significant RA research work and European Commission policy support.

2. Description of work programme

The EU-FORA work programme 'Livestock, food chain and public health risk assessment' developed by APHA was structured in four different modules that covered a wide range of aspects related to RA. Taken together, all modules ensured a broad overview on the various methodologies, tools and applications of RA. Each module was organised into various related activities that were addressed step by step. Over the course of the year, Dr. Taylor monitored the progress of the programme and managed the evolution of the project's activities. Weekly meetings analysed in greater detail the progress of each module's deliverables and outcomes according to the programme timeline. Furthermore, specialists were chosen to co-supervise each module based on their experience and relevance.

The work programme with the included modules is presented in Figure 1.

Figure 1: Schematic presentation of the work programme

2.1. Aims

Each module of the 'Livestock, food chain and public health risk assessment' work programme represented an independent RA project and had specific deliverables and outcomes, as follows:

Module 1 aimed to consolidate the RA knowledge of the fellow gained through the EFSA training, by guidance and practice in understanding the basic principles of qualitative and quantitative RA, the RA methodology and the different tools that are often used to perform RA. During this module, the fellow attended various courses and training opportunities in order to strengthen their RA knowledge.

Module 2 focused on the understanding of available risk ranking tools for exotic animal diseases cross-bordering Europe and how they can be incorporated into the decision making process. The fellow attended various meetings of different governmental groups, and had a close collaboration with Defra, which facilitated progress on this module. A review paper on risk ranking tools for animal diseases has been submitted for publication.

Module 3 addressed different RA models and techniques that are available to relate *Salmonella* isolates identified in food products with those that are prevalent in causing human salmonellosis. For this purpose, a collaboration with Public Health England (PHE) was formed as part of the work programme, as it allowed the use of whole genome sequencing (WGS) data on isolates of *Salmonella* from food commodities and human cases. Different levels of genetic relatedness were identified, allowing the ranking of food isolates by order of public health risk.

Module 4 focused on the RA process from start to finish through the scenario of onward transmission of *Leishmania* parasites if entry were to occur into the UK. The module involved working alongside risk assessors for developing the risk pathways for the pathogen of interest, which offered a rapid learning of RA methodologies applied within RA situations.

2.2. Activities/methods

Module 1: Principles and Terminology of Quantitative and Qualitative Risk Assessment

The first module of the working programme was co-supervised by Dr. Robin Simons, Lead Risk Analyst within the BRR Workgroup, APHA. The module was dedicated to structured lectures and practical sessions on RA methodologies for both qualitative and quantitative RA. The lectures were part of the Royal Veterinary College (RVC)'s Master of Science (MSc) course in Veterinary Epidemiology, held at the London campus of the RVC. The training consisted of both theoretical and practical sessions. The practical exercises provided experience in how to produce a qualitative RA report and how to replace descriptive analysis of the risk pathways and qualitative risk estimates with their mathematical description and numerical risk estimates.

During the one year fellowship, the fellow also attended meetings of various governmental groups such as the National Emergency Epidemiology Group (NEEG), the Human-Animal Infections and Risk Surveillance Group (HAIRS) and the Veterinary Risk Group (VRG) from Defra. The NEEG coordinates and reports on the epidemiology of exotic notifiable disease outbreaks to describe and anticipate disease frequency and distribution, providing epidemiological advice and assessment on the determinants, level and distribution of disease to the National Expert Group (NEG) (Scottish Government, 2017). The HAIRS is a multiagency and cross-disciplinary horizon-scanning group, comprising numerous governmental agencies such as PHE, Defra, APHA, Food Standards Agency (FSA) and the Department of Health and Social Care. The group identifies and discusses infections with potential for interspecies transfer (Welsh and Morgan, 2005). The VRG is a UK group managed and delivered by APHA and directly supported by a network of risk management teams. Its role is to identify, assess, escalate and prioritise new and re-emerging animal-related threats in the UK, in order to decrease their impact to society and economy (Kosmider et al., 2017). Thus, the attendance at these meetings represented an important training in RA, especially in outbreak or high-impact situations, which acted as a support for carrying out the following activities. Various RA situations which were presented in these meetings offered the chance to identify key elements in the control and prevention of animal diseases and to learn how various RA methodologies are applied to real situations. It also served to indicate how RA is used to support policy and the roles risk assessors and risk managers play in these situations.

In addition to the scheduled activities, the fellow participated in various meetings and consultations with other colleagues over the entire period of the EU-FORA fellowship programme which had numerous advantages for the fellow. The hosting institution provided additional training which played an important role in improving the fellow's knowledge of RA (Table 1).

Table 1: Supporting activities organised or facilitated by the hosting site, Animal and Plant Health Agency, during the EU-FORA fellowship

Type of event	Title	Date
Workshops	Workshop on the use of pig movement data for modelling and demographics	3.10.2018
	2019 APHA Modelling Symposium: 'Global Thinking: Modelling pathogen risk and spread across borders'	7.2.2019
Seminars	Epidemiology training day	6.11.2018
	Videoconference on generic risk models, in collaboration with EFSA and Wageningen Bioveterinary Research	15.1.2019
	Department of Epidemiological Sciences seminar day: 'Epidemiology as a Collaborative Science'	30.4.2019
	Science Engagement Group (SEG) seminars	Monthly
	Department for Epidemiological Sciences Taster Club presentations	Fortnightly
Other activities	Civil Service Learning online courses	3.10.2018–8.10.2018
	Science Open Day, APHA Weybridge	7.11.2018
	Career Q&A session with Dr. Francesca Gauntlett	13.11.2018
	FutureLearn online course: 'Animal viruses'	3.12.2018–11.1.2019
	DataCamp online courses	3.12.2018–30.4.2019
	Training session: 'Leadership skills'	19.2.2019

The EU-FORA training programme was supported by 4 training sessions provided by EFSA: a 3-week induction training at EFSA premises in Parma, Italy and 1-week training modules at the Austrian Agency for Health and Food Safety (AGES) in Vienna, Austria, the German Federal Institute for Risk Assessment in Berlin, Germany and the Hellenic Food Authority in Athens, Greece.

Furthermore, the training programme was completed with participation in the Parma Summer School 2019 'Risk-benefit in food safety and nutrition', organised at the EFSA's premises on the 11–13 of June 2019. The event was coordinated by EFSA, the University of Parma and the School of Advanced Studies on Food and Nutrition, with the collaboration of the Catholic University Sacro Cuore of Piacenza, the Technical University of Denmark, the National Food Agency, Sweden, the University of Barcelona and the Instituto Superiore di Sanità. The course broadened the fellow's knowledge and understanding of the risk-benefit approach in food safety and nutrition and highlighted the importance of a close collaboration between risk assessors and benefit assessors in order to ensure that the generated data can be used in a broader risk-benefit assessment context.

Module 2: Hazard Identification and Risk Prioritisation Methods

Module 2 was co-supervised by Dr. Helen Roberts, Equine, Pets and New and Emerging Diseases, Science and Risk Adviser, within the Exotic Disease Control team of Defra. Defra is a UK ministerial department supported by 33 agencies and public bodies and is responsible for safeguarding the natural environment, promoting the food and farming industry and sustaining the rural economy (Defra, 2019).

This module was focused on an extensive study of risk ranking methods and tools for prioritising animal diseases. There were explored various tools which are maintained and designed specifically for the UK to prioritise pathogens of highest risk, on a regular basis, and which feed into the specific contingency plans within the Outbreak National Response. The International Disease Monitoring (IDM) 'Risk of Incursion' tool was closely studied by the fellow. The tool deals with rapid RAs for animal health, being currently used in the assessment of risk incursions for the UK (Roberts et al., 2011). Thus, a case study was assessed by the fellow, taking the IDM 'Risk of Incursion' tool as a template to analyse the risk of incursion of various animal diseases into Romania through an associated livestock or product of animal origin. For this purpose, 2017 international trade data for Romania were obtained from COMEXT, a freely available online reference database for detailed statistics on international trade in goods run by Eurostat, and the Trade Control and Expert System (TRACES) European Union (EU) database (Eurostat, 2017; European Commission, 2019).

Furthermore, various EU risk ranking tools for animal diseases were studied and the ones available online were tested in order to identify their advantages and limitations. Several generalised risk ranking

frameworks proposed by various international agencies were also assessed, in order to identify their general recommendations for a prioritisation approach of animal diseases in the selected risk ranking tools. These activities facilitated the completion of a comprehensive overview of various tools developed within the EU over time to rank animal diseases. The study entitled 'Best practices in risk ranking animal diseases: An analysis of ten risk ranking tools' has been submitted for publication.

Horizon scanning methods were also explored within module 2 for assessing future risks for animal and public health. These activities were co-supervised by Dr. Paul Gale, Senior Risk Analyst within the BRR workgroup. The fellow was introduced to the International Forward Look (IFL), a programme developed at the request of the Government Chief Scientific Adviser, in partnership with Government Office for Science. IFL represents a cross-government approach which joins up horizon-scanning scientists and risk assessors from various governmental departments for identifying, flagging and anticipating emerging global natural threats which could require a response from government departments working overseas. Since July 2015, a group of scientists from various government agencies such as the Met Office, British Geological Survey, PHE and APHA perform weekly a natural hazard identification document for government departments. To support this, a Microsoft Excel®-based summary was created by the fellow, containing information on animal health supplied by the World Organisation for Animal Health (OIE) international surveillance system and alerts through the OIE World Animal Health Information System (WAHIS), an internet-based computer system which processes data on animal health in real time and informs the international community. Thus, data on animal diseases and their serotypes, countries of origin, status of the report, date of the event, affected animal species, number of affected animals, outbreak status and other observations were centralised, along with the maps of outbreak locations, for feeding into the weekly natural hazard risk identification document. This activity allowed the fellow to learn a routine approach that highlights the current situation of emerging global natural threats in order to improve the situational awareness of decision-makers.

Module 3: Public Health and Food Chain Risk Assessment

Module 3 was co-supervised by Dr. Robin. Simons, Lead Risk Analyst within the BRR Workgroup on behalf of APHA and Dr. Lesley Larkin, Surveillance Lead, Gastrointestinal Infections, Tuberculosis, Acute Respiratory, Gastrointestinal, Emerging/Zoonotic Infections and Travel Health Division (TARGET), on behalf of PHE. The work was carried out between the two governmental agencies, APHA and PHE, and involved a 2-week secondment at PHE in Colindale, London. PHE is an executive agency of the Department of Health and Social Care and a distinct organisation with operational autonomy. PHE is the key provider for public health surveillance data and information to parametrise aspects of public health.

Non-typhoidal Salmonellae are major zoonotic pathogens which cause a significant global public health concern. The European Centre for Disease Prevention and Control (ECDC) noted that salmonellosis is the second most commonly reported gastrointestinal infection in the EU/European Economic Area (EEA). The Annual Epidemiological Report for 2016 noted an EU/EEA notification rate of 20.4 cases per 100,000 population, where the highest notification rate of salmonellosis was noticed among young children 0–4 years, with 89.9 cases per 100,000 population, seven times higher than in adults 25–64 years (ECDC, 2019).

In April 2014, WGS was implemented by PHE for the characterisation of isolates of *Salmonella* (McLauchlin et al., 2019). Since 2015, WGS is the primary typing tool used for public health surveillance. *Salmonella* serovar determination is predicted based on the *Salmonella* eBURST group (eBG) or sequence type (ST) (Achtman et al., 2012; Ashton et al., 2016) as a replacement for traditional serotyping. PHE currently holds databases on *Salmonella* isolates, including more than 1,000 isolates from food and approximately 50,000 isolates from cases of human salmonellosis. The activities of this module aimed to use WGS data on isolates of *Salmonella* from food commodities and enumerate the number of cases of human salmonellosis reported, analysed at different levels of genetic relatedness.

The method employed by PHE is single nucleotide polymorphisms (SNP) typing. A bioinformatics application, SnapperDB, has been developed to quantify SNP relatedness and derive an isolate level nomenclature termed the 'SNP Address' (Dallman et al., 2018). This applies multi-threshold single linkage clustering to describe an isolate's position in the population structure of a given *Salmonella* eBG. Single-linkage clustering is performed at seven descending thresholds of SNP distance: 250, 100, 50, 25, 10, 5 and 0. This clustering results in a discrete seven-digit code where each number represents the cluster membership at each descending SNP distance threshold. Thus, clusters of cases that are microbiologically linked can be detected using the 'SNP address' to indicate how closely related genetically an isolate is to other isolates in the database (Figure 2).

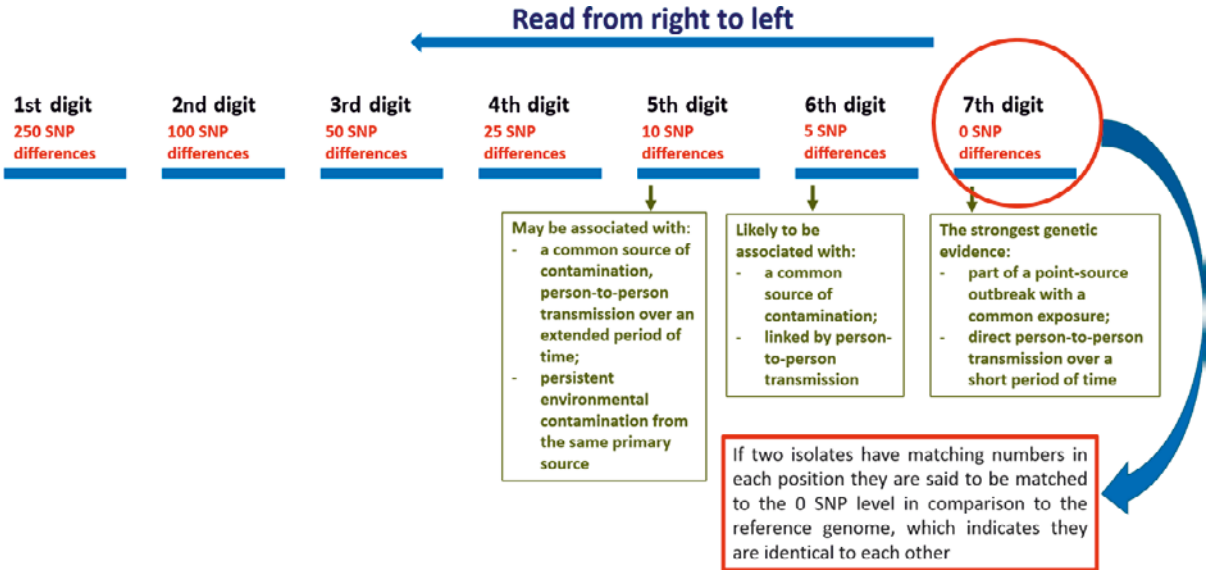


Figure 2: Schematic description of a Single Nucleotide Polymorphism (SNP) address

Data provided by the Gastrointestinal Bacteria Reference Unit's web-based result reporting system, Gastro Data Warehouse (GDW), was used to facilitate the detection and assessment of clusters identified by whole genome sequencing on 2016–2018 human and food isolates of *Salmonella*. The food isolates were ranked by considering the *Salmonella* serotype, the food category, the reason for sampling and the country of origin of the food isolate. Based on SNP addresses, clinical isolates identical or with less than 10 SNP differences were linked to some of the food commodities, demonstrating how higher resolution SNP typing could efficiently be used for surveillance and global tracking of important food-borne pathogens, by having an increased discriminatory power over traditional typing techniques (Waldram et al., 2018).

Module 3 also considered the role of quantitative microbiological risk assessments (QMRAs) in the area of food-borne pathogens, such as *Salmonella*. APHA has extensive expertise in the development of farm-to-consumption QMRAs, including leading the development of a QMRA for EFSA for *Salmonella* in pigs, (Hill et al., 2010). The fellow studied this model and then conducted a review to obtain the input data necessary to parameterise the model for Romania. Thus, data including information regarding Romanian pig farms, pig population, meat processing and meat consumption were obtained from the official websites of the Romanian authorities (Ministry of Agriculture and Rural Development, the National Institute of Statistics, the National Sanitary Veterinary and Food Safety Authority). As all these documents were in Romanian, this specific task would have been particularly difficult for APHA staff. Furthermore, the fellow was introduced to R, version 3.4.4 (R Core Team, 2018) and used it to analyse and plot various maps of these input data (Figure 3). These maps contribute to the RA by visualising relevant data to aid understanding by users without an in-depth knowledge of the subject matter, generating a level of transparency and facilitating the investigation of interventions at different points of the food chain.

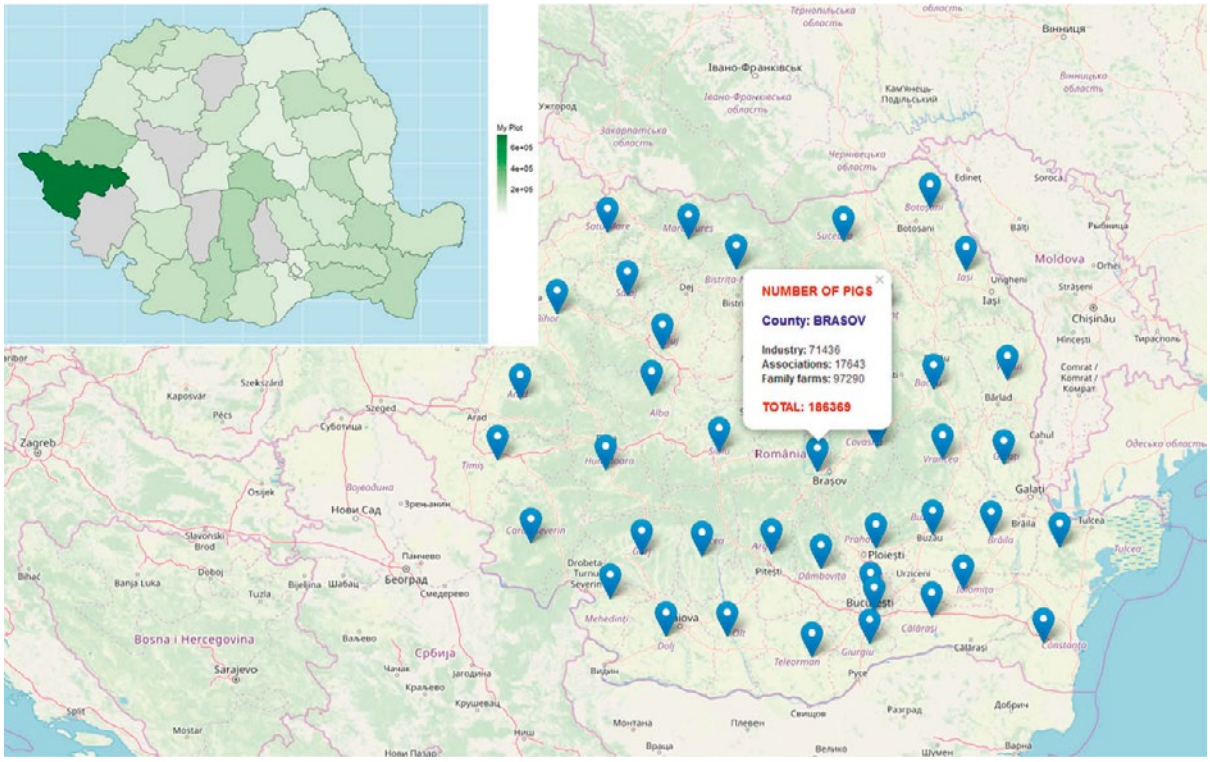


Figure 3: Mapping the 2017 Romanian pig livestock population using R (ggplot2 package, 2019)

Module 4: Import Risk Assessment

The fourth module of the working programme was co-supervised by Verity Horigan, a Risk Analyst in the BRR workgroup within APHA and specialist in microbial and food safety RAs.

This module was focused on the production of a qualitative RA which addressed the likelihood of introduction and onward transmission of *Leishmania* parasites within the UK. This assessment represented a case study for the EU 'COHESIVE – One Health Structure In Europe' project, within The One Health European Joint Programme (JIP2 COHESIVE, 2018; OHEJP, 2018).

Leishmaniasis is caused by the protozoan *Leishmania* parasites (over 20 *Leishmania* species) which are transmitted by the bite of infected female phlebotomine sandflies. The epidemiology of leishmaniasis depends on the characteristics of the parasite and sandfly species, the local ecological characteristics of the transmission sites, current and past exposure of the human population to the parasite, and human behaviour. Over 90 sandfly species are known to transmit *Leishmania* parasites and some 70 animal species, including humans, have been found as natural reservoir hosts of *Leishmania* parasites (WHO, 2019). Thus, there is the potential that this disease could enter new regions due to multiple pathways, namely entry via infected humans, pets or vectors.

Within this study, the fellow analysed the risk of onward transmission of leishmaniasis in the UK, taking into account biogeographic and epidemiological consequences in relation to the risk of leishmaniasis introduction into the UK. The potential transmission cycles and various risk pathways were identified and described, based on the available literature, and a qualitative RA was performed. Thus, this module provided the chance to work alongside staff of a national risk research group while performing a RA from start to finish, and thus represented the best opportunity in understanding and practising the RA principles outlined during the previous modules.

3. Conclusions

The EU-FORA 'learning-by-doing' programme enabled a fast and extensive knowledge and experience of RA. An overview of the major RA methodologies and various risk assessment and risk ranking tools was facilitated by the 'Livestock, food chain and public health risk assessment' working programme alongside the BRR workgroup within APHA, UK.

The interdisciplinary approach of the working programme as well as its diversity of the research fields will provide a complementary perspective to future cross-disciplinary research projects and future collaborations.

References

Achtman M, Wain J, Weill FX, Nair S, Zhou Z, Sangal V, Krauland MG, Hale JL, Harbottle H, Usebeck A, Dougan G, Harrison LH, Brisse S, the S. enterica MLST study group. 2012. Multilocus sequence typing as a replacement for serotyping in *Salmonella* enterica. PLoS Pathogens, 8, e1002776. https://doi.org/10.1371/journal.ppat.1002776

Ashton PM, Nair S, Peters TM, Bale JA, Powell DG, Painset A, Tewolde R, Schaefer U, Jenkins C, Dallman TJ, de Pinna EM and Grant KA, Salmonella Whole Genome Sequencing Implementation Group, 2016. Identification of *Salmonella* for public health surveillance using whole genome sequencing. Peer Journal, 4, e1752. https://doi.org/10.7717/peerj.1752

Dallman T, Ashton P, Schafer U, Jironkin A, Painset A, Shaaban S, Hartman H, Myers R, Underwood A, Jenkins C and Grant K, 2018. SnapperDB: a database solution for routine sequencing analysis of bacterial isolates. Bioinformatics, 34, 3028–3029. https://doi.org/10.1093/bioinformatics/bty212:bty212-bty212

Defra (Department for Environment, Food and Rural Affairs), 2019. About us. Available online: https://www.gov.uk/government/organisations/department-for-environment-food-rural-affairs/about (Accessed: May 2019)

ECDC (European Centre for Disease Prevention and Control), 2019. Salmonellosis. In: ECDC. Annual epidemiological report for 2016. Stockholm. Available online: https://ecdc.europa.eu/sites/portal/files/documents/AER_for_2016-salmonellosis.pdf (Accessed: May 2019)

European Commission, 2019. TRACES: TRAde Control and Expert System. Available online: https://ec.europa.eu/food/animals/traces_en (Accessed May 2019)

Eurostat, 2017. COMEXT trade data. Available online: https://ec.europa.eu/eurostat/data/bulkdownload (Accessed November 2018)

Hill A, Simons R, Ramnial V, Tennant J, Denman S, Cheney T, Snary E, Swart A, Evers E, Nauta M, Swanenburg M, Leusden F, Vigre H, Domingues AR, Barfod K, Bo Petersen U, Wingstrand A and Hald T. (EFSA Supporting Publications), 2010. Quantitative microbiological risk assessment on *Salmonella* in slaughter and breeder pigs: final report. EFSA Supporting Publication 2010;7(4):EN-46, 7, 463 pp. Available online: https://www.efsa.europa.eu/en/supporting/pub/en-46 (Accessed: May 2019)

JIP2 COHESIVE (Joint Integrative Project COHESIVE), 2018. COHESIVE: One Health Structure In Europe. Available online: https://onehealthejp.eu/projects/jip02-cohesive (Accessed: May 2019)

Kosmider R, Gibbens J and Avigad R, 2017. Identification, assessment and management of new and re-emerging animal-related risks: UK perspective. Veterinary Record, 181, 67. https://doi.org/10.1136/vr.104258

McLauchlin J, Aird H, Andrews N, Chattaway M, de Pinna E, Elviss N, Jørgensen F, Larkin L and Willis C, 2019. Public health risks associated with *Salmonella* contamination of imported edible betel leaves: analysis of results from England, 2011–2017. International Journal of Food Microbiology, 298, 1–10.

OHEJP (One Health EJP), 2018. One Health European Joint Programme. Available online: https://onehealthejp.eu (Accessed: May 2019)

R Core Team, 2018. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. Available online: https://www.r-project.org/ (Accessed: January 2019)

R package, 2019. ggplot2 version 3.1.1. Available online: https://CRAN.R-project.org/package=ggplot2 (Accessed: January 2019)

Roberts H, Carbon M, Hartley M and Sabirovic M, 2011. Assessing the risk of disease introduction in imports. Veterinary Record, 168, 447–448. https://doi.org/10.1136/vr.d1784

Scottish Government, 2017. Exotic Animal Disease Contingency Framework Plan v5. Available online: https://www.gov.scot/publications/scottish-governments-exotic-diseases-animals-contingency-framework-plan/pages/2 (Accessed: May 2019)

Waldram A, Dolan G, Ashton PM, Jenkins C and Dallman TJ, 2018. Epidemiological analysis of *Salmonella* clusters identified by whole genome sequencing, England and Wales 2014. Food Microbiology, 71, 39–45. https://doi.org/10.1016/j.fm.2017.02.012

Welsh AL and Morgan D, 2005. Identifying hazards, assessing the risks. The Veterinary Record, 157, 684–687. https://doi.org/10.1136/vr.157.22.684

WHO (World Health Organization), 2019. Leishmaniasis. Available online: https://www.who.int/news-room/fact-sheets/detail/leishmaniasis (Accessed: May 2019)

Abbreviations

AGES	Austrian Agency for Health and Food Safety
APHA	Animal and Plant Health Agency
BRR	Biomathematics and Risk Research workgroup

Defra	Department of Environment, Food and Rural Affairs
DES	Department for Epidemiological Sciences
ECDC	European Centre for Disease Prevention and Control
EEA	European Economic Area
EU-FORA	European Food Risk Assessment Fellowship Programme
FSA	Food Standards Agency
GDW	Gastro Data Warehouse
HAIRS	Human-Animal Infections and Risk Surveillance Group
IFL	International Forward Look
MSc	Master in Science
NEG	National Expert Group
NEEG	National Emergency Epidemiology Group
OIE	World Organisation for Animal Health
OHEJP	One Health European Joint Programme
PHE	Public Health England
QMRA	Quantitative Microbiological Risk Assessments
RVC	Royal Veterinary College
SNP	single nucleotide polymorphism
TARGET	Tuberculosis, Acute Respiratory, Gastrointestinal, Emerging/Zoonotic Infections and Travel and Migrant Health Division
TRACES	Trade Control and Expert System
VRG	Veterinary Risk Group
WGS	Whole Genome Sequencing
WAHIS	World Animal Health Information System
WHO	World Health Organization

EU FORA SERIES 2

APPROVED: 31 July 2019

doi: 10.2903/j.efsa.2019.e170913

Chemical risks associated with ready-to-eat vegetables: quantitative analysis to estimate formation and/or accumulation of disinfection byproducts during washing

Research Group on Quality, Safety and Bioactivity of Plant Foods, Department of Food Science and Technology, CEBAS-CSIC (Spanish National Research Council), Campus Universitario de Espinardo 25, 30100 Murcia, Spain.

Juliana Rodrigues Gadelha, Spain,
JR Gadelha, A Allende, F López-Gálvez, P Fernández, MI Gil and JA Egea

Abstract

Fresh produce can become contaminated with disease-causing microorganisms and chemical contaminants at every step of the production and processing chain and in a variety of ways, including through contact with contaminated process water. Water quality is critical to prevent microbial and chemical risks in any of the postharvest and processing operations related to fresh and fresh-cut fruits and vegetables. The wash process requires high volumes of water, which are usually reduced by water reuse. To maintain the microbiological quality of the process water, intervention strategies are needed. Chemical disinfection is the most common method to maintain the microbial quality of process water. However, the use of chemicals leads to the formation/accumulation of disinfection byproducts (DBPs), which can be absorbed by the washed vegetables. This is the case of trihalomethanes (THMs) and chlorates. The presence of high concentrations of DBPs in vegetables has led to an intensive debate on current disinfection practices and how DBPs may enter the food supply chain, becoming a potential health risk for consumers. To assess the risk associated with the formation/accumulation of DBPs in process water, a quantitative analysis was done. Available data have been used to develop mathematical models to predict the formation/accumulation of DBPs (chlorates and THMs) in process water due to the use of chlorine-derived compounds. Preliminary models have been developed, but adjustments are still needed to refine them. The present study contributes more information related to the development of a mathematical model for the accumulation of chlorates and THMs in process water.

Keywords: Modellisation, Quantitative Risk Assessment, Chemical Hazard, byproducts, Disinfection

Correspondence: eu-fora@efsa.europa.eu

Acknowledgements: This report is funded by EFSA as part of the EU-FORA Program and the project: Inside the project MINECO (Project AGL2016-75878-R) the Center for Produce Safety Grant Agreement (Project 2017-01) and CSIC (Intramural 201670E056).

Suggested citation: Gadelha JR, Allende A, López-Gálvez F, Fernández P, Gil MI and Egea JA, 2019. Chemical risks associated with ready-to-eat vegetables: quantitative analysis to estimate formation and/or accumulation of disinfection byproducts during washing. EFSA Journal 2019;17(S2):e170913, 12 pp. https://doi.org/10.2903/j.efsa.2019.e170913

ISSN: 1831-4732

The EFSA Journal is a publication of the European Food Safety Authority, an agency of the European Union.

Table of contents

1. Introduction

1.1. Chemical sanitisers to maintain the microbiological quality of process wash water

Chemical disinfection, especially using chlorine-derived compounds, is a usual practice to maintain the microbiological quality of process water in the fresh-cut vegetable industry. Chlorine-derived compounds are inexpensive and very efficient reducing microorganisms present in water, when used under the recommended operational standards (Garrido et al., 2019). In general, fresh produce industry uses large volumes of water in the different steps of the postharvest and processing activities, such as washing. The maintenance of an optimal minimum chlorine concentration in the washing tank guarantee the microbiological quality of the process wash water while avoiding the cross-contamination of the different product batches that are washed in the same washing tank (Gil et al., 2016, 2019). However, the use of chlorine-derived compounds has been linked to an increase of chemical risk due to the formation/accumulation of Disinfection byproducts (DBPs). Although other disinfection technologies have been suggested to maintain the microbiological quality of the process water, chlorine-derived compounds are still highly demanded by fresh produce processors and the potential chemical risks should be evaluated (López-Gálvez et al., 2018, 2019).

1.2. Disinfection byproducts

Disinfection byproducts are formed by the reaction of chemical disinfectants with byproduct precursors. The use of chlorine-derived compounds leads to the formation/accumulation of different types of DBPs, which can be generated by two pathways: (1) formation of chlorates in chlorinated water as a result of chlorine degradation and (2) formation of halogenated DBPs such as trihalomethanes (THMs) and haloacetic acids (HAAs) due to the reaction of chlorine with the organic matter. In the case of chlorates formation, the main mechanism is the disproportionation of the free chlorine (HCLO/CLO), whose balance is determined by the pH. Therefore, to quantitatively predict the accumulation of chlorates in process water, it is important to determine the needed addition of free chlorine to maintain the quality of the water. Regarding THMs and HAAs, the natural organic matter (usually measured as total organic carbon (toc)) and inorganic matter (bromide) are the most significant disinfection byproduct precursors. They react with naturally present fulvic and humic acids, amino acids, and other natural organic matter, as well as iodide and bromide ions, to produce THMs, HAAs, bromate and chlorite. Most of the commonly used chemical disinfectants (e.g. sodium hypochlorite, calcium hypochlorite and chlorine gas) react with organic matter and/or bromide to varying degrees to form different DBPs (Morris, 1966; Adam and Gordon, 1999; Black and Veatch Corporation, 2010) (Table 1).

Chlorine is the most common disinfectant used worldwide, and chlorates, THMs and HAAs are the DBP classes formed at the highest concentrations after chlorination. The DBPs could be originated by organic and inorganic compounds: (1) Halogenated compounds: THMs, HAAs, halonitromethanes, haloaldehydes and haloacetones, haloacetamides, haloacetonitriles and haloalcohols; (2) Non-halogenated compounds: aldehydes and ketones of low molecular weight, other carboxylic acids, keto acids, nitriles and nitrosamines and (3) inorganic byproducts: decyanogen chloride, chlorites, chlorates and bromates.

Additionally, the so-called 'emerging' DBPs such as halonitromethanes, haloacetonitriles, haloamides, halofuranones and iodo-acids such as iodoacetic acid, iodo-THMs (iodotrihalomethanes), nitrosamines and others could also be formed.

1.2.1. Chlorates

Chlorates are substances with high power of oxidation and were widely used as a pesticide in the past, but in European Union, they are banned since 2008. This why, currently, the use of chlorine derived as water disinfectants is by far, the principal source of chlorates in fruits and vegetables. Despite this, the use of chlorine-derived compounds is still widely use in Europe to maintain the quality of process water (Gil et al., 2016).

In the European Union, there is a current debate regarding the maximum residue level (MRL) for chlorate in different fruits and vegetables, because the previous MRL of 0.01 mg kg^{-1} is not valid since 2014. In the United States, the regulatory limit for chlorate and chlorite only applies for drinking water and is establish at 700 μg/L for each. Chlorates levels, included in the US Environmental Protection

Agency's monitoring of unregulated contaminants and on the contaminant candidate list, could potentially receive a regulatory determination in the near future. The present report, using available literature along with past and current monitoring data, assesses the presence of chlorate in drinking water and the potential impact of its regulation. Data are still missing regarding the maximum levels that could be recommended in process water in contact with fresh fruits and vegetables (Tables 2 and 3).

There is scarce information regarding the real risk that accumulation of chlorates in fresh fruits and vegetables represents for consumers. Based on available studies conducted to estimate the carcinogenicity and genotoxicity of these compounds in rodents, BPDs seem to be a concern for the human health (SCHER/SCCP/SCENIHR, 2008).

1.2.2. Trihalomethanes

Trihalomethanes are a group of four chemicals that are formed along with other DBPs when chlorine-derived compounds are used to maintain the microbiological quality of process water containing high concentrations of organic and inorganic matter. These compounds have been defined as carcinogenic compounds, becoming a relevant concern for the public health. Epidemiological evidence has shown a consistent association between long-term exposure to THMs and the risk of bladder cancer, although the causal nature of the association is not conclusive. Evidence concerning other cancer sites is insufficient or mixed (Villanueva et al., 2015). Numerous studies have evaluated reproductive implications, including sperm quality, time to pregnancy, menstrual cycle and pregnancy outcomes such as fetal loss, fetal growth, preterm delivery and congenital malformation. The body of evidence suggests only minor effects from high exposure during pregnancy on fetal growth indices such as small for gestational age (SGA) at birth. THM formation can be minimised by avoiding the use of pre-chlorination.

Regulations in developed countries governing DBPs have established varying thresholds for the THM presence in drinking water. The maximum contaminant levels (MCL) of total THMs (the sum of chloroform, bromodichloromethane, dibromochloromethane and bromoform) have been set at 80 μg/L in the United States and 100 μg/L in the European Union (EU) (EPA, 2011). While no parametric values have been set for DPBs other than THMs and bromate, there is a requirement under Regulation 13 of the Drinking Water Regulations that 'any contamination from DPBs is kept as low as possible without compromising the disinfection, in accordance with any such directions as the supervisory authority may give' (EPA, 2011).

1.3. Prediction Model

Mathematical models are abstract representations of physical or chemical systems able to predict the system's response to some conditions without need of performing new experiments. During the model-building process, one of the key steps is the calibration of the proposed model through experimental data. We can distinguish two main groups of mathematical models: (i) Deterministic models, which are based in mass and energy balances as well as in physical laws and kinetics, and (ii) Empirical models, which usually fit experimental data to certain mathematical functions. In the first case, the mechanism of the process is known, and such models are able to make predictions in different conditions from those used to calibrate the model. In the case of empirical models, they are useful when the process mechanisms are not known. Its application to different conditions from the calibration one must be cautious.

Mathematical modelling is a mathematical and statistical method of studying events and predicting outcomes in different scenarios without the need of retrieving new experimental data.

Time series data of free chlorine (FC), Chemical Oxygen Demand (COD), pH and ultraviolet (UV) absorbance of process water as well as the chlorates and THMs concentrations have been used to construct mathematical models able to predict the DBP's concentration along experiments of wash water disinfection of different processing lines of leafy greens. The results of the study would help to improve the confidence on a disinfection system predicting the DBPs formation and reducing the risks to human health.

In the present study, available data have been used to develop mathematical models able to predict the formation/accumulation of DBPs (chlorates and THMs) in process water due to the use of chlorine-derived compounds. A mechanistic model was developed in the case of chlorates whereas an empirical model based on multiple linear regression was built for THMs quantitative prediction.

2. Description of work programme

2.1. Aims

The goals of the current project were to collect the data generated in the last 2 years in the CEBAS-CSIC research group (the host institution) and use them to develop mathematical models that can explain the formation/accumulation of chlorates and THMs in process wash water obtained from different processing lines of fresh produce (Gil et al., 2016, 2019; López-Gálvez et al., 2018, 2019). Four different process, wash water have been evaluated including: iceberg lettuce (*Lactuca sativa L*. var. *capitata*), a mix of three types of baby leaves (rocket; *Eruca sativa Mill*., red oak leaf; *Lactuca sativa L.* cv. oak leaf and red swiss chard; *Beta vulgaris L.* var. *cycla*), red cabbage (*Brassica oleracea* var. *capitata f. rubra*) and diced onion (*Allium cepa L.*). Based on the available data, the aim was to determine the potential risks associated with the accumulation of chlorates and THMs in different types of process water. Different scenarios were evaluated taking into account the different types of products, physico-chemical parameters of process water, format of the fresh produce, the washing time as well as the concentration of DBPs generated in the process wash water. Therefore, the aim of the project was to develop mathematical models able to predict the formation/accumulation of DBPs (chlorates and THMs) in process water due to the use of chlorine-derived compounds.

2.2. Activities/methods

The experimental data used for the development of the model were obtained in lab scale experiments simulating the industrial fresh produce wash systems (Tudela et al., 2019). In these experiments, the disinfectant concentrations needed to avoid cross-contamination in the washing tank between different product lots was established as well as the potential formation and/or accumulation of DBPs formed during the washing. The selected DBPs were chlorates (inorganic DPBs) and THMs (halogenated DPBs). Both are chlorine DBPs and relevant for human health. With the use of probabilistic distributions, a model was developed to describe how different factors may or may not contribute to the risk. Model predictions provided an estimation of the disinfectant concentrations necessary to eliminate the microorganisms but assuring the chemical safety to human being.

Factors which influenced DBP formation include:

Type of disinfectant used*;

- Concentration of disinfectant used*;
- Concentrations of organic matter and other DBP precursors in water to be disinfected;
- Water temperature (seasonality)*;
- pH*;
- Contact time;
- Length of the distribution network;
- Vegetable type
- Bromide ion concentration (THMs case)

*controlled parameter

Briefly, the experimental set-up aimed to simulate a real agro-industry disinfection system (Tudela et al., 2019). First, an aqueous solution with a high concentration of free chlorine was elaborated. This concentrated chlorinated solution was used to maintain a constant free chlorine concentration in the washing tank. Four different concentrations of free chlorine were selected (0, 10, 20 and 30 ppm). A washing tank of approximately 15 L capacity was used to simulate a washing tank of the fresh produce industry. The washing tank was filled with about 6 L of pipe water (with undetectable concentrations of chlorates and THMs). Following a dynamic system, a concentrated aqueous solution of organic matter (about 2,000 mg/L, COD) obtained after washing approximately 10 kg of produce in a small volume of water was constantly added to the washing tank to simulate the continuous entrance of produce in the washing tank. At the same time, the selected concentration of free chlorine (0, 10, 20 and 30 ppm) was constantly maintained by adding chlorine from the concentrated chlorinated solution. The system was running for 80 min for each chlorine concentration. In order to measure and assure the controlled conditions, physico-chemical parameters, such as free chlorine, COD and pH, were measured each 5 minutes. Water samples were collected every 20 minutes to measure the DPB concentrations. Phosphoric acid was also added to the solution, in order to keep the pH constant at ~~6.5~~ 5.6 in order to guarantee a high concentration of hypochlorous acid in the washing

solution, which is guarantee of the efficacy of chlorine. Microbiological parameters were also evaluated to determine the antimicrobial capacity of the chlorine solution (Tudela et al., 2019).

The working plan of the fellow included: (1) to learn microbiological techniques to get familiar with the microbiological parameters involved in a disinfection process, (2) to understand the microbial data and to determine the best methods to analyse microbiological data, (3) to develop mathematical models able to predict the formation/accumulation of DPBs in process water used to wash fresh produce, (4) to get familiar with different tools used to elaborate statistical analyses of data including Bioinactivation (Garre et al., 2018) and R programming language (R Core Team, 2018). To this, the fellow has performed a Statistical course related to use the R software on March 11–15 (hosting by CIIMAR, Porto-Portugal) to improve her knowledge in the topic and finally to apply the concepts 'learning-by-doing' using the previous data produced to design a mathematical model.

This research project aims to give a tool to the industry that allow the prediction of the formation/ accumulation of DPBs in process water. This can lead to the establishment of good handling practices in order to avoid the presence of these compounds in the final product, reducing the exposure of the consumers to these DBPs.

3. Conclusions

This work is still in draft form and the main conclusions are not yet formulated since the analysis is ongoing. The information presented here is therefore to be considered provisional. Indeed, our system has a dosing scheme to replenish the possible losses of free chlorine or to maintain its level within a desired range.

We identify the sources of production or depletion of chlorates in process water obtained during the washing of different leafy greens. Each type of vegetable generates a different type of process water, which might affect the formation of DBPs in the water and consequently to have a different impact on the chemical risk for consumer.

The present study constitutes only one step of all the steps needed to establish a Chemical Risk Assessment regarding the risks posed by DPBs present in process water which can be absorbed by fresh fruits and vegetables in contact with the water. However, this is a relevant step in order to estimate the formation and accumulation of DPBs in process water. The final objective will be to determine risks linked to the consumption of fresh fruits and vegetables.

Table 1: Disinfectant and the respective byproducts generated by the disinfection process (Adapted from USEPA Drinking Water Guidance on Disinfection byproducts)

Disinfectant	Disinfectant byproducts	Disinfectant byproducts
Chlorine (e.g. gas, sodium hypochlorite, tablets, OSEC)	Trihalomethanes, Haloacetic Acids, Chloramines1, Chlorinated Acetic Acids, Halogentated Acetonitriles, Chloral Hydrate, Chlorophenols, MX2, bromate3, chloropicrin, halofurans, bromohydrins.	Trihalomethanes, Haloacetic Acids, Chloramines1, Chlorinated Acetic Acids, Halogentated Acetonitriles, Chloral Hydrate, Chlorophenols, MX2, bromate3, chloropicrin, halofurans, bromohydrins.
Chlorine Dioxide	Chlorite, Chlorate and Chloride	Chlorite, Chlorate and Chloride
Ozone	Bromate, Formaldehyde, Aldehydes, Hydrogen Peroxides, Bromomethanes.	Bromate, Formaldehyde, Aldehydes, Hydrogen Peroxides, Bromomethanes.
Chloramines	Dichloramines, Trichloramines, Cyanogen Chloride, Chloral Hydrate.	Dichloramines, Trichloramines, Cyanogen Chloride, Chloral Hydrate.

Table 2: Components of DBPs in drinking water, their effects and regulatory limits (Adapted from Chowdhury et al., 2009)

Main Group	Compounds	Acronym	Main disinfectant*	Effects	Toxicity to Human			Regulations (µg/L)				
				Animal	Human	RfD	SF	HC (2007)	USEPA (2006)	WHO (2004)	Aus-NZ (2004)	UK (2000)
Trihalomethanes (THMs)	Chloroform	TCM	Chlorine	Liver tumors	B-2	0.01	0.01			300		
	Bromodichloromethane	BDCM	Chlorine	Kidney tumors	B-2	0.02	0.062	16		60		
	Bromoform	DBCM	Chlorine	Colon tumor	B-2	0,02	0.0079			100		
	Dibromochloromethane	TBM	Ozone, Chlorine	Liver tumors	C	0.02	0.0084			100		
TTHMs								100	80		250	100
Haloacetic acids (HAAs)	Bromochloroacetic acid	BCAA	Chlorine	Liver tumors								
	Bromodichloroacetic acid	BDCAA	Chlorine	Liver tumors								
	Chlorodibromoacetic acid	CDBAA	Chlorine	Liver tumors								
	Dibromoacetic acid	DBAA	Chlorine	Liver tumors								
	Dichloroacetic acid	DCAA	Chlorine		B-2	0.004	0.05			50	100	
	Monobromoacetic acid	MBAA	Chlorine									
	Monochloroacetic acid	MCAA	Chlorine									
	Tribromoacetic acid	TBAA	Chlorine								150	
	Trichloroacetic acid	TCAA	Chlorine	Liver tumors	C					100	100	
HAA5									60			
Haloacetonitriles (HANs)	Bromochloroacetonitrile	BCAN	Chlorine	Embryo-death								
	Dibromoacetonitrile	DBAN	Chlorine	Skin tumor						70		
	Dichloroacetonitrile	DCAN	Chlorine	Embryo-death						20		
	Trichloroacetonitrile	TCAN	Chlorine	Embryo-death	C							
Haloketones (HKs)	1,1-dichloropropanone	DCP	Chlorine									
	1,1,1-trichloropropanone	TCP	Chlorine									
Aldehydes	Formaldehyde		Ozone, Chlorine							900	500	
	Acetaldehyde		Ozone, Chlorine									
	Glyoxal		Ozone, Chlorine									
	Methyl glyoxal		Ozone, Chlorine									
Carboxylic acids	Formate		Ozone									
	Acetate		Ozone									
	Oxalate		Ozone									
Nitrosaminas	Nitrosodimethylamine		Chloramine		B-2		51					
			Chloramine									
Cyanogen halides	Cyanogen chloride		Chloramine							70	80	
	Cyanogen bromide		Chloramine									
Chloral hydrate		CH	Chlorine							10	20	
Bromate			Ozone		B-2	0.004	0.7	10	10	10	20	10
Chlorate			Chlorine Dioxide							700		
Chlorite			Chlorine Dioxide		D	0.03	-		1000	700	300	

Table 3: Basic information and attributes of disinfectants (Adapted from Chowdhury et al., 2009)

Issue		Chlorine	Chloramine	Chlorine dioxide	Ozone	Ultraviolet radiation	Reference
Application		Most commom	Commom	Occasional	Commom	Emerging use	USEPA (2006)
Cost		Lowest	Moderate (>chlorine)	High	High	Extremely High	Clark et al. (1994)
Disinfection efficiency	Bacteria (V. chloerae, Coliform, E. coli, etc)	Excellent	Good	Excellent	Excellent	Good	MWH (2005), Sadiq and Rodriguez (2004)
	Viruses (Polio virus, Rota virus, MS2 coliphase, etc)	Excellent	Fair	Excellent	Excellent	Fair	
	Protozoa (G. lamblia, C. parvum, E. intestinalis, etc)	Fair to poor	Poor	Good	Good	Excellent	
	Endospore	Good to poor	Poor	Fair	Excellent	Fair	
Organisms regrowth		Unlikely	Unlikely	Likely	More likely	More likely	MWH (2005)
Limits on free residuals		4 mg/L	4 mg/L	0.8 mg/L	-	-	USEPA (2006)
Byproducts	Regulated	4 THMs, HAAS	Traces of THMs and HAAs	Chlorite	Bromate	None	USEPA (2006)
	Unregulated	Many	Many: cyanogen halides, NDMA	Many: chlorate	Biodegradables organics	None know	Richardson (2005)
Oxidation		Strong	Weak	Selective	Strongest	None	Chlorine Chemistry Council (2003)
Odor and taste removal		Excellent	Good	Excellent	Good to poor	None	
Stability		Stable	Stable	Unstable	Unstable	Unstable	

References

Adam LC and Gordon G, 1999. Hypochlorite ion decomposition: effects of temperature, ionic strength, and chloride ion. Inorganic Chemistry, 38, 1299–1304.

Black and Veatch Corporation, 2010. Chemistry of Aqueous Chlorine. In White's Handbook of Chlorination and Alternative Disinfectants, 68–173.

Chowdhury S, Champagne P and McLellan PJ, 2009. Models for predicting disinfection by-product (DBP) formation in drinking waters: a chronological review. Science of Total Environmental, 1, 4189–4206.

EPA, 2011. Drinking Water Guidance on Disinfection By-Products. Advice Note No.4. Version 2. EPA Advice Note on Disinfection By-Products in Drinking Water.
Garre R, Clemente-Carazo M, Fernández PS, Lindqvist R and Egea JA, 2018. Bioinactivation FE: a free web application for modelling isothermal and dynamic microbial inactivation. Food Research International, 112, 353–360.
Garrido Y, Marín A, Tudela JA, Allende A and Gil MI, 2019. Chlorate uptake during washing is influenced by product type and cut piece size, as well as washing time and wash water content. Postharvest Biology and Technology, 151, 45–52.
Gil MI, Marín A, Andujar S and Allende A, 2016. Should chlorate residues be of concern in fresh-cut salads? Food Control, 60, 416–421.
Gil MI, López-Gálvez F, Andújar S, Moreno M and Allende A, 2019. Disinfection by-products generated by sodium hypochlorite and electrochemical disinfection in different process wash water and fresh-cut products and their reduction by activated carbon. Food Control, 100, 46–52.
López-Gálvez F, Andújar S, Marín A, Tudela JA, Allende A and Gil MI, 2018. Disinfection by-products in baby lettuce irrigated with electrolysed water. Journal of Science and Food Agriculture, 98, 2981–2988.
López-Gálvez F, Tudela JA, Allende A and Gil MI, 2019. Microbial and chemical characterization of commercial washing lines of fresh produce highlights the need for process water control. Innovative Food Science and Emerging Technology, 51, 211–219.
Morris JC, 1966. The Acid Ionization Constant of HOCl from 5 to 35°. The Journal of Physical Chemistry, 70, 3798–3805.
R Core Team, 2018. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna. Available online: https://www.R-project.org (Accessed: 11 March 2019).
SCHER/SCCP/SCENIHR, 2008. Scientific opinion on the risk assessment methodologies and approaches for mutagenic and carcinogenic substances. European Commission, 47 p.
Tudela JA, López-Galvez F, Allende A, Hernández N, Andújar S, Marín A, Garrido Y and Gil MI, 2019. Operational Limits of sodium hypochlorite for different fresh produce wash water based on microbial inactivation and disinfection by-products (DBPS). Food Control, 104, 300–307.
Villanueva CM, Cordier S, Font-Ribera L, Salas LA and Levallois P, 2015. Overview of disinfection by-products and Associated Health Effects. Current Environmental Health Reports, 2, 107–115.

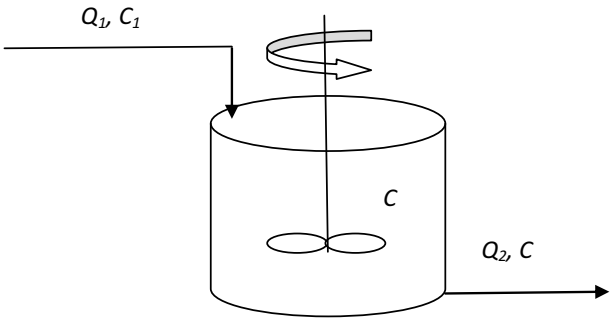
Abbreviations

DBPs	Disinfection byproducts
CEBAS	Centro de Edafología y Biología Aplicada del Segura
CIIMAR	Interdisciplinary Centre of marine and Environmental Research
COD	Chemical Oxygen Demand
CRA	Chemical Risk Assessment
CSIC	Consejo Superior de Investigaciones Científicas
EU	European Union
FC	Free Chlorine
HAAs	Haloacetic Acids
MCL	Maximum Contaminant Level
MRL	Maximum Residue Level
SGA	Small for Gestational Age
THMs	Trihalomethanes
toc	total organic carbon
USEPA	United States Environmental Protection Agency
UV	Ultraviolet radiation

Annex A – Mechanistic model for chlorates quantitative prediction

Evolution of chlorates

The following scheme represents the system used in this study: A Stirred continuous tank.



Where Q_1 and Q_2 represent, respectively, the inlet and outlet flow rate. We assume that the liquid volume (V) remains constant inside the thank, thus $Q_1 = Q_2$ (we call them Q). C_1 is the chlorates concentration of the inlet stream (which we assume it is zero or very close) whereas C is the chlorates concentration $[ClO_3^-]$ of the outlet stream, which varies with time and equals the concentration inside the tank if it is perfectly stirred.

We have to identify the sources of production or depletion of chlorates in the solution.

1) Dilution of chlorates by continuous inlet stream free of chlorates.
2) Increase of chlorates for reaction of hypochlorite to produce chlorates. If we consider a first order kinetics:

$$\frac{d[ClO_3^-]}{dt} = K_{obs}[FC] \quad (1)$$

Where *[FC]* is the free chlorine concentration which is controlled in our system by addition of a concentrated NaOCl solution. In this study, the value of *[FC]* has been considered as a constant value calculated as the mean value of the measured free chlorine concentration along the experiment. The constant (K_{obs}) is estimated using the experimental data to minimise the differences between the measured and the predicted values of chlorates concentration.

3) Increase of chlorates by addition of mother solution of NaClO which contains a certain concentration of chlorates, C_M.

Indeed, our system has a dosing scheme to replenish the possible losses of free chlorine or to maintain its level within a desired range. The 'mother' solution of Na_2ClO contains a certain concentration of chlorates, C_M, due to chlorine disproportionation. We applied a dosing strategy with a fixed period $\tau = 5min$ that consist of adding an amount of mother solution with a given rate every 5 min during a very short time, $\tau_0 \approx 1s$. Then, the increase of chlorates concentration in the tank due to this fact can be expressed as:

$$\frac{d[ClO_3^-]}{dt} = \frac{1}{V}\sum_{k=1}^{N}(Q_{M,k} \cdot C_M)\chi[k\tau, k\tau + \tau_0] \quad (2)$$

Where V is the volume of the tank, $Q_{M,K}$ is the mother solution flow rate added each time k, C_M is the chlorates concentration in the mother solution and $\chi[k\tau, k\tau + \tau_0]$ is an indicator function, taking the value 1 on time interval $[k\tau, k\tau + \tau_0]$ for some small time increment τ_0 and zero elsewhere, N is the number of doses added.

The times when we measured the THMs were on 0, 20, 40, 60 and 80 minutes, where the chlorine addition was made each 5 minutes.

Summarising all the considered terms, the change of chlorates concentration with time in our system can be represented as:

$$\frac{d[ClO_3^-]}{dt} = -\frac{Q}{V}[ClO_3^-] + Kobs[FC] + \frac{1}{V}\sum_{k=1}^{N}(Q_{M,k} \cdot C_M)\chi[k\tau, k\tau + \tau_0], \quad (3)$$

which corresponds with the points 1, 2 and 3 considered above.

The following plots show the experimental data (dots) and the model prediction (solid line) for the lettuce case in the three controlled scenarios (10, 20 and 30 ppm of free chlorine in the wash water).

Annex B – Power model to predict THMs concentration

According to the experimental data retrieved in the laboratory, we propose a power model to predict THMs concentration as a function of the identified relevant variables: COD, UV absorbance, theoretical FC concentration and time (Abbreviation list). The proposed power model has the following form.

$$THM = \beta_0 \cdot DQO^{\beta_1} \cdot UV^{\beta_2} \cdot FC^{\beta_3} \cdot (times + 10)^{\beta_4}. \quad (1)$$

Similar to some of the models presented in the review by Chowdhury et al. (2009).

Note: We add a constant term equal to 10 to avoid numerical problems in later logarithmic transformations.

Taking logarithms from Equation 1 we obtain:

$$\log(THM) = \log(\beta_0) + \beta_1 \cdot \log(DQO) + \beta_2 \cdot \log(UV) + \beta_3 \cdot \log(FC) + \beta_4 \cdot \log(time + 10) \quad (2)$$

This formulation allows a multiple linear regression analysis. Preliminary results with lettuce indicate that there are highly correlated explanatory variables. In particular, DQO, UV and time are highly correlated. After performing a model selection procedure, the identified most accurate model contains de variables UV and time. For lettuce, the fitted model is:

$$\log(THM) = 3.45 + 0.125 \cdot \log(UV) + 0.303 \cdot \log(FC). \quad (3)$$

The coefficient of determination is $R^2 = 0.79$. All the coefficients are statistically significant ($p < 0.001$) and the residual analysis indicates that the normality and heteroscedasticity assumptions are met.

Similar models will be developed for the other substrates (Lettuce, baby leaves, onion and col) and a model comparison procedure will be carried out to check whether these different substrates have an influence in the THMs concentration.

EU-FORA SERIES 2

APPROVED: 31 July 2019

doi: 10.2903/j.efsa.2019.e170914

Assessment of the endocrine disrupting properties of Bisphenol AF according to the EU criteria and ECHA/EFSA guidance

Karolinska Institutet, Institute of Environmental Medicine, Sweden,
Laura Escrivá, Annika Hanberg, Johanna Zilliacus and Anna Beronius

Abstract

Endocrine disruptors (EDs) are exogenous compounds that interfere with the hormone system, affecting human health and environment. Specific legislative obligations have been introduced in the European Union (EU) to gradually eliminate EDs in water, industrial chemicals and pesticides. However, identification of EDs is the first and essential step towards regulation and appropriate risk management. Scientific criteria and guidance for ED assessment have recently been established for pesticides in the EU. In this project, the ED properties of the non-pesticide chemical Bisphenol AF (BPAF), analogue and potential substitute of Bisphenol A were evaluated by the application of the EU criteria and guidance in the frame of human health risk assessment. A data dossier was built by a systematic literature review (WOS, Scopus, Pubmed, Embase), title/abstract screening (RAYYAN) and full-text examination. All relevant information was extracted and systematically reported, and reliability and relevance of data were assessed (SciRAP). Data were synthesised into lines of evidence for (i) endocrine activity, (ii) adversity and (iii) general toxicity, and weight of evidence evaluation was applied. The initial analysis of the evidence showed potential endocrine adverse effects and endocrine activity, meeting the ED criteria and leading the assessment to the mode of action (MoA) analysis. The biological plausibility of the link between the adverse effects and the endocrine activity was investigated based on current scientific knowledge. Empirical support for dose–response and temporal concordance was evaluated, and the key events were assessed in terms of essentiality, consistency, analogy and specificity. Finally, an overall conclusion of the ED properties of BPAF was drawn. The EU criteria and guidance for EDs assessment were successfully applied to BPAF demonstrating its endocrine activity and adversity based on weight of evidence methodology and MoA analysis. The Fellow greatly increased her knowledge and hands-on experience on ED assessment in the EU regulatory context contributing to implement transparency and structure in health risk assessment.

Keywords: bisphenol AF, endocrine disruptor, risk assessment

Correspondence: eu-fora@efsa.europa.eu

Acknowledgements: This report is funded by EFSA as part of the EU-FORA programme.

Suggested citation: Karolinska Institutet, Institute of Environmental Medicine, Sweden, Escrivá L, Hanberg A, Zilliacus J and Beronius A, 2019. Assessment of the endocrine disrupting properties of Bisphenol AF according to the EU criteria and ECHA/EFSA guidance. EFSA Journal 2019;17(S2): e170914, 10 pp. https://doi.org/10.2903/j.efsa.2019.e170914

ISSN: 1831-4732

The EFSA Journal is a publication of the European Food Safety Authority, an agency of the European Union.

Table of contents

1. Introduction

The hormone system has an essential role in the regulation of many physiological functions such as body development, growth, reproduction, metabolism, immunity, inflammation and behaviour (Chrousos, 2007). Endocrine disruptors (EDs) are exogenous compounds that interfere with any aspect of endogenous hormone system, including hormones production, release, transport, metabolism, binding, action or elimination, negatively affecting human health (Lee, 2018; Pouzaud et al., 2018). They represent a special and challenging form of toxicity as their effects depend on both the level and timing of exposure, being especially critical in developmental stages (WHO/UNEP, 2012).

EDs are highly heterogeneous chemicals – including pesticides, fungicides, plastics, plasticisers and heavy metals – with diverse applications at industrial, agricultural, pharmaceutical and cosmetic level, which result in contaminant residues in food and other consumer products leading to human exposure to ED mixtures (Schug et al., 2016).

Scientific understanding of the health impacts of ED substances has been growing in recent years and progressively raised awareness of ED-related concerns (Gore et al., 2015). The European Commission initiated in 1999 a strategy to develop a legislative framework on EDs pursuing the harmonisation of hazard-based criteria for EDs identification. The European Commission's Endocrine Disrupters Expert Advisory group described the three elements required to identify an ED, in line with the World Health Organization definition (WHO, 2002). Accordingly, an ED substance has to show (i) endocrine adverse health effects in individuals and/or their offspring, (ii) endocrine activity through an endocrine mode of action (MoA) and (iii) a plausible and clear-established link between the adverse effects and the endocrine MoA (Munn and Goumenou, 2013). However, to demonstrate that a given substance is an ED represents a huge challenge due to the complex and critical roles of the endocrine system in maintaining the homeostasis of all biological processes, as well as the multiple pathways and mechanisms involved (Beausoleil et al., 2018). Scientific criteria to identify substances with ED properties have been recently implemented in plant protection products (PPP) regulation (European Union, 2009), and biocidal products (BP) regulation (European Union, 2012) applying from June 2018 and November 2018, respectively. The European Commission entrusted the European Food Safety Authority (EFSA) and the European Chemicals Agency (ECHA) to develop a guidance document for the implementation of ED criteria pursuant to the PPP and BP regulations. The recent published ECHA/EFSA guidance intends to reduce subjectivity and conflicting procedures for determining ED properties by guiding applicants and assessors of the competent regulatory authorities, contributing to the harmonisation between industry, authorities and academia with regard to ED toxicity assessment (ECHA/EFSA, 2018).

2. Description of work programme

The work programme was based on the application of the EU criteria and ECHA/EFSA guidance for ED identification to a non-pesticide compound in the frame of health risk assessment. Although the ED criteria cover all ED effects, the guidance mainly addresses EATS (estrogen, androgen, thyroid, steroidogenesis) modalities due to their relatively good mechanistic understanding and the availability of standardised in vivo and in vitro test guidelines with broad scientific agreement.

2.1. Aims

The aim of the project was **(**i) to identify strengths and specific challenges in the process described in the ECHA/EFSA guidance for ED assessment, and (ii) to explore its application on a non-pesticide model substance, Bisphenol AF (BPAF), by evaluating the ED properties for human health according to the EU scientific criteria.

2.2. Activities and methods

2.2.1. Selection of the model substance

Selecting the model substance was the very first and extremely important step of the project. The ideal model substance should have been extensively investigated with enough scientific data to allow the whole ED evaluation; however, due to personnel and time resources, data amount should also be handling and not extremely large to be completely analysed by the fellow during the EU-FORA programme.

Under the European regulation of chemicals REACH (Registration, Evaluation, Authorisation and Restriction of Chemicals), substances having ED properties have to be identified as Substances of Very

High Concern (SVHC) as first step towards regulation and appropriate risk management. The use of SVHC is controlled by temporary authorisations conditioning its uses and strongly encouraging its substitution (European Union, 2006; Beausoleil et al., 2018). Although REACH regulation does not provide any specific ED criteria, the European Commission last communication on EDs claimed the development of a horizontal approach for ED identification across EU legislation built on the pesticides criteria (EC Communication, 2018).

With the objective of applying the ED scientific criteria on a REACH chemical, information sources were consulted including the SVHC list (https://echa.europa.eu/candidate-list-table), SIN (Substitute It Now) list (https://chemsec.org/sin-list) and REACH chemicals regulation (European Union, 2006) collecting data on several compounds about their registration status (candidate list, authorisation list, restriction list, CoRAP list, full registration, intermediate registration, notification of new substance), production amounts (tonnes/year), hazard category classification and reason for inclusion in the SVHC or SIN list when it applies. At this point, compound search was framed on phthalates and bisphenols after analysing systematic reviews (Rochester and Bolden, 2016; Skledar and Masic, 2016; NTP 2017) that highlighted the wide use of these substances despite the lack of comprehensive knowledge on their ED properties. In order to increase the value of the work and provide useful information to both the scientific community and competent authorities, we informed the Swedish Chemicals Agency (KemI) about the ongoing project asking their interest and opinion with regard to the regulatory relevance of our final substance candidates: Bisphenol AF, Bisphenol B, Bisphenol F and Bisphenol S. Finally, according to our preliminary data search and the KemI suggestion, BPAF (Figure 1) was selected as the model substance for the ED assessment. The cooperation with KemI is an example of successful communication between academia and national authorities increasing work efficiency and ensuring regulatory relevance by sharing knowledge and expertise in risk assessment. The project workflow for the further steps is shown in Figure 2.

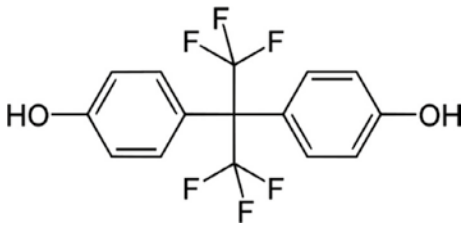


Figure 1: Bisphenol AF (BPAF) chemical structure. *IUPAC name*: 4-[1,1,1,3,3,3-hexafluoro-2-(4-hydroxyphenyl)propan-2-yl]phenol; *CAS number*: 1478-61-1

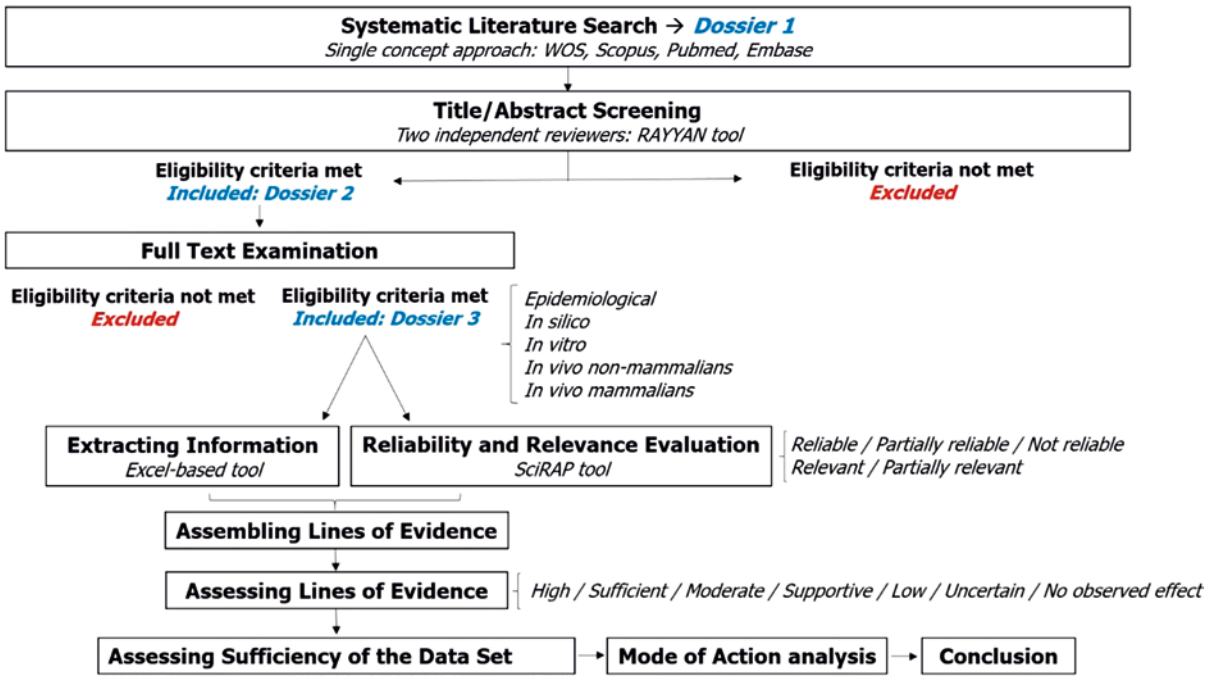


Figure 2: Workflow for Bisphenol AF assessment based on the ECHA/EFSA guidance

2.2.2. Systematic literature search

Data for assessing potential ED properties of BPAF was gathered by a structured literature review based on the principles of systematic review methodology in the electronic databases WOS, Pubmed, Scopus and Embase. Information experts from Karolinska Institutet library service were consulted at this point to guarantee that the methodology that fits best the search purpose was applied. A single concept approach by using several search terms (CAS number, IUPAC name and chemical name synonyms) was applied as a first step, according to the ECHA/EFSA guidance. Since the number of hits retrieved by the single concept search was not excessively large, additional search refinement was not required for further screening steps. The individual studies from all databases were transferred into the electronic reference management software EndNote (http://kib.ki.se) where reference duplicates were removed to obtain the preliminary dossier (dossier 1).

2.2.3. Screening and selection of the studies

Two steps were applied for the studies selection from the preliminary dossier: (i) screening by title and abstract, and (ii) full-text examination (Figure 2). Title and abstract screening was independently performed by two reviewers at different institutions and countries (RIVM – National Institute for Public Health and the Environment, The Netherlands; KI – Karolinska Institutitet, Sweden) by RAYYAN tool (https://rayyan.qcri.org/). The included and excluded studies were critically identified after defining the problem formulation (scope, scientific needs/objectives and feasibility), the PECO (population, exposure, comparator and outcome) statements and the eligibility (inclusion/exclusion) criteria, according the EFSA systematic review methodology (EFSA 2010).

Studies meeting the eligibility criteria were kept for next screening step. Studies clearly not relevant to the problem formulation or meeting the exclusion criteria were excluded. When exclusion could not be made based on the title/abstract, studies were kept for subsequent full-text examination performed by the fellow. Studies in conflict between the two reviewers were resolved by discussion and the final included studies formed the title and abstract dossier (dossier 2). It should be noted that cooperation between institutions (RIVM and KI) was essential at this step, providing the fellow experience in networking and external scientific collaboration.

A deep examination at full-text level was then performed by the fellow for the screened studies, where those considered that met the eligibility criteria were included into the full-text dossier (dossier 3) and preliminary classified into epidemiological, in silico, in vitro, in vivo mammalians and in vivo non-mammalians.

2.2.4. Extracting and reporting the information

All the information from dossier 3, including relevant endocrine-related parameters, as well as general toxicity endpoints was extracted and systematically reported for both positive and negative results. The supplementary Excel-based tool from the ECHA/EFSA guidance document (Appendix E – Excel template for reporting the available information relevant for ED assessment) was used to collect all data describing each parameter in one single row as recommended in the guidance. Once all the parameters were entered, a data matrix was applied into the Excel tool to clearer reorganise the information and data clustering.

2.2.5. Evaluating reliability and relevance

The relevance (appropriateness of the data for the intended purpose of the assessment) and reliability (inherent quality of the test method and level of reporting) of each individual study included in dossier 3 was assessed by the online web tool Science in Risk Assessment and Policy – SciRAP (http://www.scirap.org). SciRAP provides predefined criteria and a colour coding tool aimed to promote structure and transparency in the evaluation of ecotoxicity and toxicity (in vitro and in vivo) studies for hazard and risk assessment of chemicals. When a study contained both in vitro and in vivo information, two parallel SciRAP evaluations were performed.

Relevance evaluation allows the classification into three categories (relevant, partially relevant, not relevant) based on five criteria: substance identity, concentrations, test system/animal model, administration route and studied endpoint. However, according to the systematic review methodology, studies were efficiently assessed for relevance against inclusion criteria in two steps: (i) screening of titles and abstracts for relevance to the study question and (ii) full-text examination for the eligibility of studies (EFSA 2010). Therefore, assessment of relevance at this stage was considered as a

confirmation and only two categories (relevant and partially relevant) were included since the not relevant studies were excluded at the initial steps of the protocol.

Reliability consisted of two blocks evaluating 23 or 30 criteria for reporting quality, and 15 or 18 criteria for methodological quality, for in vitro and in vivo studies, respectively. The output for each assessed study was provided as an Excel file containing a colour profile (qualitative evaluation) and a ranking score (quantitative evaluation) used to rate the studies as (i) reliable, (ii) partially reliable and (iii) not reliable. Five pure in silico and four epidemiological studies, not applicable for SciRAP tool, were manually evaluated by similar SciRAP-based criteria.

2.2.6. Assembling lines of evidence

The extracted parameters along with the study quality assessment scores were assembled into lines of evidence for (a) endocrine activity, (b) adversity and (c) general toxicity. Each group was subdivided into categories based on the nature of the data as shown in Table 1.

Table 1: Lines of evidence classification in groups and subgroups

Lines of evidence classification			
Groups	**Endocrine activity**	**Adversity**	**General toxicity**
Subgroups	*In silico*	*In vivo:* EATS-mediated	Cellular toxicity
	In vitro mechanistic	*In vivo:* EATS-sensitive but not diagnostic	Target organ toxicity
	In vivo mechanistic	Epidemiological	Systemic toxicity

2.2.7. Assessing lines of evidence

Each individual line of evidence was assessed considering the quantity and quality of both the studies and the included parameters, as well as their coherence and/or conflicting information. In this way, a ranking composed of seven categories was defined based on the examples from the guidance and a weight of evidence document from the European Commission Scientific Committee on Health, Environmental and Emerging Risks (SCHEER 2018). Ranking categories were designed to categorised each assessed line of evidence after summarising the information included in each, and weight of evidence was described as follows: (1) *high:* several studies in different species/strains indicating clear and coherent evidence in the absence of conflicts; (2) *sufficient:* more than two studies in different species/strains indicating clear and coherent evidence; (3) *moderate:* two or more studies not necessarily in different species/strains showing coherent evidence; (4) *supportive:* one or more studies showing clear trend or indication of evidence but not enough available data; (5) *low:* one or more studies showing slight trend or indication of evidence not clearly demonstrated with not enough available data; (6) *uncertain:* one or more studies showing conflicting and not coherent results hindering evidence assessment; (7) *no observed effect:* one or more studies showing no effects observed.

After assessing each individual line of evidence, a similar approach was applied to the subgroup evaluation and the assessment of the integrated lines of evidence for each group: endocrine activity, adversity and general toxicity (Table 1).

2.2.8. Assessment of sufficiency of the data set

According to the ECHA/EFSA guidance, the sufficiency of the collected data set – lines of evidence – should be assessed with regard to EATS-mediated adversity and EATS-related endocrine activity, in order to identify the specific next-step scenario that should be followed to proceed with the assessment. Six different scenarios are described in the guidance, represented as a decision tree, which evaluates: (i) if endocrine activity and adversity have been sufficiently investigated, (ii) if endocrine activity and adversity have been observed.

With this aim, the amount and type of available information was evaluated for adversity and endocrine activity. The final dossier generated in the present work included studies covering all the levels established in the OECD conceptual framework (CF) for the testing and assessment of ED chemicals (OECD 2012). Level 1 (existing data and non-test information) was filled by in silico studies while epidemiological studies were considered as supportive data. In vitro studies performed in several cell lines from mice, rats, monkeys and mainly humans were classified as level 2 – in vitro assays providing mechanistic data. Additional mechanistic data were obtained from ToxCast studies and yeast bioassays. OECD CF level 3 (in vivo assays providing data about selected endocrine mechanism and pathways), level

4 (in vivo assays providing data on adverse effects on endocrine relevant endpoints) and level 5 (in vitro assays providing more comprehensive data on adverse effects on endocrine relevant endpoints over more extensive parts of the life cycle of the organism) were represented by in vivo studies in fish (medaka and mainly zebrafish), amphibian and in vivo mammalian studies in mice and rats.

Overall, it was concluded that the available information showed potential endocrine-related adverse effects and endocrine activity; therefore, ED criteria were met and a MoA analysis was required according to the ECHA/EFSA guidance.

2.2.9. Mode of action analysis

As the guidance describes, a MoA consists of a sequence of measurable events at molecular, cellular and individual levels that link the molecular initiating event (MIE), to the adverse outcome (AO) through intermediate key events (KEs). MoA analysis consists of two steps: (1) postulate a MoA and (2) evaluate the MoA by the establishment of a biologically plausible link between endocrine activity and adverse effect.

To postulate the MoA, the adverse effects that showed the highest weight of evidence were initially selected as AOs. The information in the lines of evidence that was considered biologically connected to the AOs was organised into biological levels and a preliminary hypothesis was drawn defining the events chain from the molecular/cellular level to the individual/population AO (Figure 3).

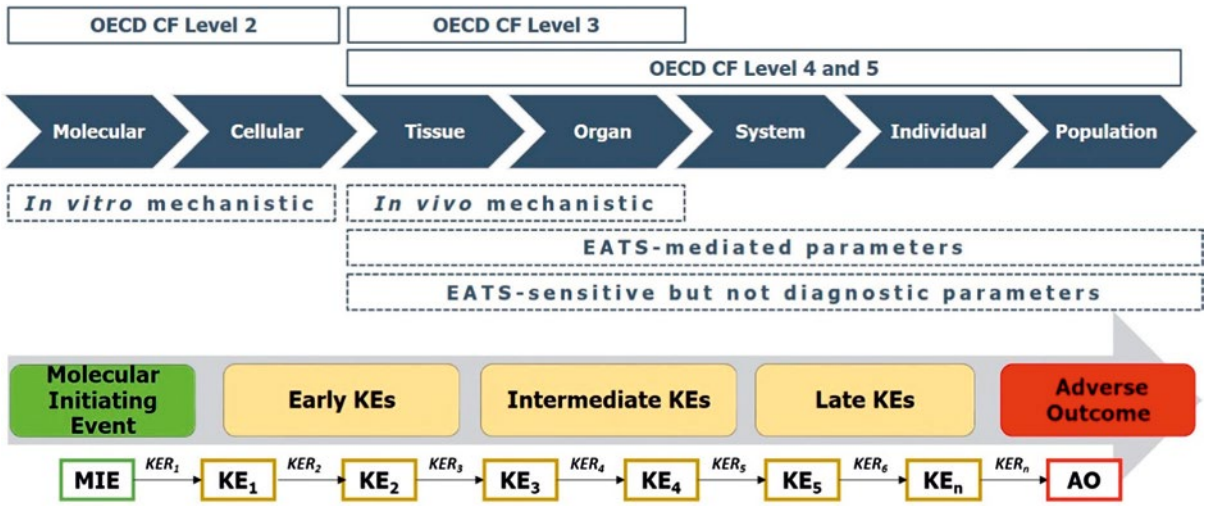


Figure 3: Scheme of the MoA postulation based on the ECHA/EFSA guidance for EDs assessment

To evaluate the MoA, the biological plausibility of the link between each adverse effect and the endocrine activity was investigated based on current scientific knowledge by applying weight of evidence approach. Each KE in the postulated MoA was identified, briefly described and evaluated based on the supporting evidence from the available literature. Biological plausibility of the KE relationships (KERs) was assessed based on the broader knowledge of biology, this means the current understanding of physiology, endocrinology and toxicology. As recommended in the guidance, biological plausibility of KERs was weighted as: (i) *strong:* if is there is extensive understanding of the KER based on extensive previous documentation and broad acceptance, (ii) *moderate:* if the KER is plausible based on analogy with accepted biological relationships, but scientific understanding is not completely established and (iii) *weak:* if the structural or functional relationship between the KEs is not understood. At this step, empirical support for dose–response/incidence concordance (earlier KEs are observed at below or similar doses than later KEs and AO) and temporal concordance (earlier KEs are observed in studies of similar or shorter duration than later KEs and AO) was evaluated as strong, moderate or weak. The individual KEs in the MoA were assessed with regard to (i) *essentiality:* the sequence of events in the postulated MoA is reversible if dosing is stopped or a KE prevented; (ii) *consistency:* repeatability of the KE in different studies/species/strains/systems; (iii) *analogy:* the postulated KEs also occur for other substances for which the same MoA has already been established; and (iv) *specificity:* the MoA for the adverse effect is endocrine related and not an indirect result of other non-endocrine-mediated toxicity. Finally, the overall conclusion of the ED properties of BPAF was reported based on the weight of evidence that supported the postulated MoA.

2.2.10. EU-FORA Fellowship additional activities

In addition to the work at the Unit of Biochemical Toxicology at the Institute of Environmental Medicine (IMM), Karolinska Institutet, the Fellow attended the four EU-FORA modules organised by EFSA (Italy), AGES (Austria), BfR (Germany) and EFET (Greece) where a wide training in risk assessment provided her extremely useful knowledge and practice. Moreover, additional activities positively contributed the work development and results dissemination, as well as her training and learning:

- Course on *Health Risk Assessment of Reproductive Toxicity and Endocrine Disruptors*, Karolinska Institutet (IMM), Sweden.
- Lecture at the *Doctoral Programme Seminar – Environmental Factors and Health* (EFH), Karolinska Institutet (IMM), Sweden.
- Lecture at the *Master of Food Quality and Safety*, University of Valencia (UV), Spain.
- Study visit to the *Swedish Chemicals Agency* (KemI), Sweden.
- Open lecture attendance; *Toxicology: should we really take the risk to communicate?* – Lucia de Luca. Karolinska Institutet (IMM), Sweden.
- *Webinar on BioSecurity* – International Hellenic University, Greece.
- Poster presentation at the *XXIII Spanish Congress of Toxicology* (AETOX – Spanish Association of Toxicology), Spain.
- Poster presentation at the *55th Congress of the European Society of Toxicology* (EUROTOX – Federation of European Toxicologists & European Societies of Toxicology), Finland.

3. Conclusions

The ECHA/EFSA guidance for ED assessment was successfully applied to evaluate the ED properties for human health of BPAF, a potential substitute for BPA, as an illustration of its application on a non-pesticide model substance. According to the EU criteria and guidance, BPAF showed endocrine activity and adversity based on systematic review approach, weight of evidence methodology and MoA analysis. The Fellow gained extensive knowledge and hands-on experience on EDs assessment in the EU regulatory context as part of chemical risk assessment, and as an essential step towards regulation and appropriate risk management. This included the application of methodologies to increase transparency and structure in health risk assessment, such as systematic review, weight of evidence, and AO Pathways (AOPs). Moreover, a remarkable collaboration network was developed within the project demonstrating successful scientific communication and cooperation between different research groups and institutions (RIVM), as well as between academia and national authorities (KemI).

References

Beausoleil C, Emond C, Cravedi JP, Antignac JP, Applanat M, Appenzeller BR, Beaudouin R, Belzunces LP, Canivenc-Lavier MC, Chevalier N, Chevrier C, Elefant E, Eustache F, Habert R, Kolf-Clauw M, Le Magueresse-Battistoni B, Mhaouty-Kodja S, Minier C, Multigner L, Schroeder H, Thonneau P, Viguié C, Pouzaud F, Ormsby JN, Rousselle C, Verines-Jouin L, Pasquier E and Michel C, 2018. Regulatory identification of BPA as an endocrine disruptor: context and methodology. Molecular and Cellular Endocrinology, 475, 4–9.

Chrousos GP, 2007. Organization and integration of the endocrine system: the arousal and sleep perspective. Sleep Medicine Clinics, 2, 125–145.

EC Communication, 2018. 734 final. Communication from the Commission to the European Parliament, the Council, the European Economic and Social Committee and the Committee of The Regions. Towards a Comprehensive European Union Framework on Endocrine Disruptors. Available online: http://ec.europa.eu/transparency/regdoc/rep/1/2018/EN/COM-2018-734-F1-EN-MAIN-PART-1.PDF

ECHA/EFSA (European Chemicals Agency/European Food Safety Authority), 2018. Guidance for the identification of endocrine disruptors in the context of Regulations (EU) No 528/2012 and (EC) No 1107/2009. EFSA Journal 2018;16(6):5311, 135 pp. https://doi.org/10.2903/j.efsa.2018.5311

EFSA (European Food Safety Authority), 2010. Application of systematic review methodology to food and feed safety assessments to support decision making. EFSA Journal 2010;8(6):1637, 90 pp. https://doi.org/10.2903/j.efsa.2010.1637

European Union, 2006. Regulation (EC) No 1907/2006 of the European Parliament and of the Council of 18 December 2006 concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH), establishing a European Chemicals Agency, amending Directive 1999/45/EC and repealing Council Regulation (EEC) No 793/93 and Commission Regulation (EC) No 1488/94 as well as Council Directive 76/769/EEC and Commission Directives 91/155/EEC, 93/67/EEC, 93/105/EC and 2000/21/EC.

European Union, 2009. Regulation (EC) No 1107/2009 of the European Parliament and of the Council of 21 October 2009 concerning the placing of plant protection products on the market and repealing Council Directives 79/117/EEC and 91/414/EEC.
European Union, 2012. Regulation (EU) No 528/2012 of the European Parliament and of the Council of 22 May 2012 concerning the making available on the market and use of biocidal products.
Gore AC, Chappell VA, Fenton SE, Flaws JA, Nadal A, Prins GS, Toppari J and Zoeller RT, 2015. EDC-2: The Endocrine Society's Second Scientific Statement on Endocrine-Disrupting Chemicals. Endocrine Reviews, 36, E1–E150.
Lee DH, 2018. Evidence of the possible harm of endocrine-disrupting chemicals in humans: ongoing debates and key issues. Endocrinology and Metabolism, 33, 44–52.
Munn S and Goumenou M, 2013. Key scientific issues relevant to the identification and characterisation of endocrine disrupting substances- Report of the Endocrine Disrupters Expert Advisory Group. Toxicology Letters, 221, S170.
NTP (National Toxicology Program), 2017. NTP Research Report on Biological Activity of Bisphenol A (BPA) Structural Analogues and Functional Alternatives.
OECD Series on Testing and Assessment, 2012. OECD Conceptual Framework for Testing and Assessment of Endocrine Disrupters (as revised in 2012).
Pouzaud F, Thierry-Mieg M, Burga K, Vérines-Jouin L, Fiore K, Beausoleil C, Michel C, Rousselle C and Pasquier E, 2018. Concerns related to ED-mediated effects of Bisphenol A and their regulatory consideration. Molecular and Cellular Endocrinology, 475, 92–106.
Rochester JR, Bolden AL, 2016. Bisphenol S and F: a systematic review and comparison of the hormonal activity of bisphenol A substitutes. Environmental Health Perspectives, 123, 643–650.
SCHEER, 2018. Scientific Committee on Health, Environmental and Emerging Risks SCHEER. Memorandum on weight of evidence and uncertainties. Revision 2018.
Schug TT, Johnson AF, Birnbaum LS, Colborn T, Guillette Jr LJ, Crews DP, Collins T, Soto AM, Vom Saal FS, McLachlan JA, Sonnenschein C and Heindel JJ, 2016. Minireview: endocrine disruptors: past lessons and future directions. Molecular Endocrinology, 30, 833–847.
Skledar D and Masic P, 2016. Bisphenol A and its analogs: do their metabolites have endocrine activity? Environmental Toxicology and Pharmacology, 47, 182–199.
WHO, 2002. International Programme on Chemical Safety. In: Damstra T, Barlow S, Bergman A, Kavlock R, Van Der Kraak G. Global Assessment of the State of the Science of Endocrine Disruptors. Available online: http://www.who.int/ipcs/publications/new_issues/endocrine_disruptors/en/
WHO/UNEP, 2012. State of the science of endocrine disrupting chemicals – 2012. An assessment of the state of the science of endocrine disruptors prepared by a group of experts for the United Nations Environment Programme and World Health Organization.

Abbreviations

AO	Adverse Outcome
AOP	Adverse Outcome Pathway
BP	biocidal products
BPA	Bisphenol A
BPAF	Bisphenol AF
EATS	Estrogen, Androgen, Thyroid, Steroidogenesis
ECHA	European Chemicals Agency
ED	Endocrine Disruptor
KemI	Swedish Chemicals Agency
KEs	Key Events
KERs	Key Events Relationship
MIE	Molecular Initiating Event
MoA	Mode of Action
OECD CF	Organisation for Economic Co-operation and Development Conceptual Framework
PPP	Plant protection products
REACH	Registration, Evaluation, Authorisation and Restriction of Chemicals
RIVM	National Institute for Public Health and the Environment, The Netherlands
SciRAP	Science in Risk Assessment and Policy
SIN	Substitute It Now
SVHC	Substances of Very High Concern

EU-FORA SERIES 2

APPROVED: 31 July 2019

doi: 10.2903/j.efsa.2019.e170915

Analysis and Risk Assessment of Seaweed

National Food Institute, Technical University of Denmark, Denmark,
M Sá Monteiro, J Sloth, S Holdt and M Hansen

Abstract

During the last decade, the interest on the use of seaweed as food or feed, which was before limited to certain European regional subpopulations, has experienced a significant increase in other regions of the EU. In fact, the growing awareness and interest on sustainable and alternative food sources, healthier lifestyles and changes on dietary patterns brought seaweed to the spotlight for the general worldwide cuisine. Due to their high biosorption and accumulation capacity, seaweed can be an important source of increased exposure to persistent and potential harmful elements, such as cadmium (Cd), lead (Pb), mercury (Hg) and inorganic arsenic (iAs), or even some micronutrients, particularly iodine (I), to which an antioxidant role as been described in seaweed. This concentration potential has raised the interest of several Food Authorities regarding the risk of increased exposure to these elements. Moreover, the European Commission requested the collection of monitoring data on their levels aiming to aid the performance of better risk assessments and potentially set maximum levels on the European Legislation. This work aimed to obtain levels of these elements in species of seaweed (*Fucus vesiculosus, Fucus serratus, Fucus spiralis, Fucus evanescens, Saccharina latissima, ulva lactuca* and *Ccladophora* sp.) cultivated and harvested in Denmark, following European Commission's request. Additionally, a collaboration between Denmark, Ireland, France and the Netherlands was initiated to review and collect all the data available on scientific papers regarding the levels of these contaminants in seaweed worldwide. The final result of this work would be the publication of a review article. This Fellowship also provided on-the-job training on the evaluation of applications of new biocides and participation in the science based advises given to the Danish Food and Veterinary Administration, Danish EPA, the Danish Medical Agency and ECHA.

Keywords: seaweed, persistent contaminants, iodine, risk assessment

Correspondence: eu-fora@efsa.europa.eu

Acknowledgements: This report is funded by EFSA as part of the EU-FORA programme 2018/2019.

Suggested citation: National Food Institute, Technical University of Denmark, Denmark, Sá Monteiro M, Sloth J, Holdt S and Hansen M, 2019. Analysis and Risk Assessment of Seaweed. EFSA Journal 2019;17(S2):e170915, 11 pp. https://doi.org/10.2903/j.efsa.2019.e170915

ISSN: 1831-4732

The EFSA Journal is a publication of the European Food Safety Authority, an agency of the European Union.

Table of contents

1. Introduction

Up to now, mostly used by specific subpopulations in Europe (namely in Iceland, Scotland, Ireland, Wales and France) (Mahadevan, 2015; Tiwari and Troy, 2015), seaweeds or macroalgae have recently experienced and increased interest regarding their use as food and feed. In fact, after being used for centuries as a staple food particularly in Asian countries, seaweeds are expected to become a relevant food and food ingredient in the European market. Seaweeds were brought to the spotlight in the Western world due to their marketing and perception as 'superfood', increased interest in healthier diets and lifestyles as well as on more sustainable food sources and production (Mahadevan, 2015; Mendis and Kim, 2011; FAO, 2018). As a result, a wide variety of seaweed-based or containing products is now more easily available to European consumers, from the traditional sushi to salads, breads pasta, chips and drinks (Bouga and Combet, 2015).

With high nutritional value due to the presence of important macro- and micronutrients including vitamin B12, omega-3 and -6 fatty acids, selenium, iodine and dietary fibre (Aguilera-Morales et al., 2005; Peña-Rodríguez et al., 2011; Gil et al., 2015), seaweeds are also studied as a source of several bioactive compounds with potential health benefits/applications (Holdt and Kraan, 2011; Brown et al., 2014). Seaweeds can also be a source of increased dietary exposure to potential harmful and persistent contaminants (such as inorganic arsenic, lead, cadmium and mercury) as well as some nutrients, such as iodine. In fact, due to the specific characteristics of their cell wall and structure, seaweeds present a high concentration potential for minerals and trace elements present in the surrounding waters. As a result, the levels of these elements are on average several orders of magnitude higher in seaweed than in the water (Jadeja and Batty, 2013; Malea et al., 2015; Bonanno and Orlando-Bonaca, 2018). This concentration potential is behind the extended use of macroalgae in biomonitoring and bioremediation protocols, from where most of the knowledge on the uptake of contaminants by seaweeds has been gathered (Hamdy, 2000; Sheng et al., 2004; Chakraborty et al., 2014; Holan et al., 1993). So far, studies report high intra- and interspecies differences, as well as geographic and seasonal variability in the concentration of different elements in macroalgae (Brito et al., 2012; Ryan et al., 2012; Chakraborty et al., 2014; Malea et al., 2015; Chen et al., 2018).

Iodine is an essential micronutrient for the synthesis of thyroid hormones, which in turn are important for growth, development and metabolism, particularly vital during earlier stages of life (WHO, 2007). Iodine can cause the dysfunction of thyroid gland at high levels of exposure. This is the reason why in 2002, EFSA's Scientific Committee on Food (SCF) suggested a tolerable upper intake level (UL) for adults of 600 μg iodine/day and adjusted this for the remaining age groups based on differences on body surface area (body weight$^{0.75}$) (European Commission, 2002). Mercury, lead, cadmium, and inorganic arsenic are completely deprived of biological activity in humans and harmful even at trace levels (Bilal et al., 2018), being elements of greater interest for food safety authorities. Inorganic arsenic (IARC, 2012) is classified as carcinogenic for humans while methylmercury (MeHg) (IARC, 1993) and inorganic lead (IARC, 2006) have been classified as possibly carcinogenic for humans, besides being characterised by several other toxic effects in humans, e.g. neurotoxicity and nephrotoxicity.

The toxicological profile and the relative high exposure from other sources of these elements has raised the interest of several Food Authorities concerned with the exposure to excessive levels of these contaminants due to seaweed consumption (FSAI, 2015; Duinker et al., 2016; ANSES, 2018). However, maximum levels for heavy metals and metalloids have been set by the Commission Regulation No 1881/2006 (European Commission, 2006) as amended by Regulation No 629/2008 (European Commission, 2008), in a range of foodstuffs including seafood, seaweeds are not included on the list. Despite being more frequently performed, speciation of arsenic and mercury is still frequently not included despite its importance for the evaluation of the risk associated with consumption ad increase consumers' protection. In conclusion, nowadays in Europe, there are no regulation on the maximum levels of these elements in seaweeds as food, besides a maximum limit level of 3.0 mg/kg wet weight for cadmium in 'food supplements consisting exclusively or mainly of dried seaweed or of products derived from seaweed' (European Commission, 2008). Recognising the emergent interest in seaweed and the lack of data on the levels of these contaminants in seaweeds available and or produced in the European market, monitoring data for the most common edible species of seaweeds have been requested by the European Commission to all member states during the period of 2018 to 2020 (European Commission, 2018). The final result of this monitoring action could be the setting of maximum levels for arsenic, lead, cadmium, mercury and iodine for seaweeds as well as providing more data to improve the risk assessments regarding the consumption of this food.

Still, owing to their nutrient density, the invaluable potential of edible seaweeds as a food source should not be neglected. Hence gathering knowledge about contamination patterns and distribution would be of great value to enhance their safe use and health benefits. The main goal of the EU-FORA Fellowship Programme was to monitor the levels of inorganic arsenic, cadmium, lead, mercury and iodine in samples of edible seaweeds cultivated and harvested in Denmark.

2. Description of work programme

2.1. Aims

The main aim of the work programme was the analysis of lead, mercury, inorganic arsenic, cadmium and iodine in samples of seaweed cultivated and harvested in Denmark, following the request on monitoring data by the European Commission (2018). Defining a hypothetical consumption scenario, a risk assessment was performed for the different species of seaweed included in the present study.

2.2. Activities/methods

The work programme included two parts:

1) Analysis of iodine mercury, cadmium, lead, and total arsenic using inductively coupled plasma mass spectrometry (ICP-MS). The analysis of inorganic arsenic (in preparation) would be performed using anion-exchange high-performance liquid chromatography (HPLC) coupled to ICP-MS.

    a) Samples of *Fucus vesiculosus, Fucus serratus, Fucus spiralis, Fucus evanescens, Saccharina latissima, Ulva lactuca* and *Cladophora* sp. were harvested, freeze dried, pulverised and quantified. The quality of the analytical methods was assured by simultaneous analysis of certified reference materials and adherence to European standard methods (EN15763, EN15111 and EN16802[1]). The results are present as μg/g freeze dried weight (fdw);

2) Risk assessment regarding the dietary exposure to iodine, cadmium, lead, and mercury due to seaweed consumption was performed considering a single serving size of 5 g of fdw. Species-specific exposure was estimated using the average content for each species as well as the 95th percentile. A similar approach was followed, considering all different species together, as a very rough indicative scenario of what might happen when consumers buy seaweed in store, as frequently the species are not identified and can be picked randomly. The adult population was considered and, when relevant specific high-risk subgroups where discussed.

3. Conclusions

3.1. Occurrence of iodine, cadmium, lead, mercury and total arsenic in Danish seaweed samples

Table 1 summarises the content of iodine, total arsenic, cadmium, mercury and lead analysed for the different species of seaweed. In general, interspecies variability is greater than variability within the same species. Considering all the samples, the levels for the different elements decreased in the order: iodine ranged (17.2–4782 fdw) > total arsenic (3.2–116.7 μg/g fdw) > lead (0.072–9.6 μg/g fdw) > cadmium (0.017–1.97 μg/g fdw) > mercury (0.003–0.042 μg/g fdw).

Despite not useful to perform a risk assessment, the levels of total arsenic were included. This data is important to assess the relative amount of arsenic as inorganic arsenic and better characterise potential inter- and intraspecies variability. However, the content of inorganic arsenic, the species with toxicological relevance to perform a risk assessment, were not available at the time this report was written. Therefore, a risk assessment regarding exposure to inorganic arsenic due to seaweed consumption was not performed on Section 3.2.

[1] The method employed to analyse inorganic arsenic was developed by a group of researchers at the National Food Institute, Technical University of Denmark, in a project under the European Committee for Standardization (CEN). This method has been approved as the European analytical standard [CEN standard (EN16802:2016)] for measuring inorganic arsenic in foodstuffs.

Table 1: Average (±SD) and range levels (μg/g fdw) of iodine, total arsenic, mercury, lead and cadmium

	No. samples	Iodine		Total arsenic*		Mercury		Lead		Cadmium	
		Average (± SD)	Range (min–max)	Average (± SD)	Range (min–max)	Average (± SD)	Range (min–max)	Average (± SD)	Range (min–max)	Average (± SD)	Range (min–max)
Phaeophyta (brown algae)											
Saccharina latissima	16	2,302.5 (1,098.18)	333.0–4,782.2	38.324 (8.713)	22.504–54.117	0.016 (0.005)	0.007–0.023	0.257 (0.222)	0.072–0.708	0.682 (0.216)	0.231–0.966
Fucus vesiculosus	27	274.9 (75.87)	137.8–451.2	28.379 (19.690)	10.358–116.677	0.012 (0.007)	0.003–0.042	0.897 (1.730)	0.189–9.601	0.780 (0.372)	0.299–1.969
Fucus spiralis	1	209.52	–	8.940	–	0.019	–	0.956	–	0.464	–
Fucus evanescens	1	394.16	–	14.084	–	0.008	–	0.506	–	0.520	–
Fucus serratus	14	366.46 (197.92)	105.2–961.4	30.269 (9.579)	21.457–56.277	0.009 (0.003)	0.005–0.015	0.465 (0.177)	0.236–0.865	1.044 (0.339)	0.628–1.561
Chlorophyta (green algae)											
Ulva lactuca	2	18.97 (2.52)	17.2–20.8	3.399 (0.293)	3.192–3.606	0.007 (0.002)	0.005–0.008	0.078 (0.019)	0.064–0.092	0.038 (0.030)	0.0168–0.059
Cladophora sp.	1	140.27	–	7.069	–	0.007	–	1.447	–	0.782	–

SD: standard deviation.
*: Data on inorganic arsenic is in preparation.

Table 2: Estimated average and 95th percentile exposure to iodine (μg/day), mercury, lead and cadmium (μg/kg bw per day) due to the consumption of a single serving size of 5 g fdw of seaweed, considering each species individually and altogether. For species with only one representative sample, the total content was considered for exposure calculation

	Iodine (μg/day)		Mercury (μg/kg bw per day)		Lead (μg/kg bw per day)		Cadmium (μg/kg bw per day)	
	Average	**95th percentile**	**Average**	**95th percentile**	**Average**	**95th percentile**	**Average**	**95th percentile**
Species-specific exposure								
Fucus vesiculosus	1,374.6	274.9	0.00100	0.0015	0.0747	0.14424	0.0650	0.0928
Fucus serratus	1,832.2	2,523.2	0.00099	0.0010	0.0387	0.05998	0.0870	0.1217
Fucus spiralis	1,047.6	–	0.00161	–	0.0796	–	0.0387	–
Fucus evanescens	1,970.8	–	0.00069	–	0.0421	–	0.0433	–
Saccharina latissima	11,512.3	18,677.2	0.00135	0.0018	0.0214	0.05380	0.0568	0.0772
Ulva lactuca	94.9	86.0	0.00055	0.0004	0.0065	0.00536	0.0031	0.0014
Cladophora sp.	701.3	–	0.00062	–	0.1205	–	0.0652	–
Population of samples								
	4,052.7	13,631.1	0.0010	0.0016	0.0521	0.0786	0.0652	0.1194

bw: body weight.

3.2. Health Risk Assessment

An assessment of potential health risks associated with the consumption of unprocessed seaweed was performed (Table 2). An average consumption of seaweed of 5.2 g/adult per day for Chinese, 4 g/adult per day for Japanese and 8.5 g/adult per day for South Korean has been estimated (Roleda et al., 2019). However, seaweed consumption data for Europeans is absent and, in this report, a single serving size of 5 g (fdw), once a week, was assumed to perform this risk assessment. A body weight (bw) of 60 kg was considered as average body weight for an adult.

Regarding exposure to lead, the previously accepted provisional tolerable weekly intake (PTWI) of 25 μg/kg bw per week was considered, in 2010, by the EFSA Panel on Contaminants in the Food Chain (CONTAM Panel) no longer appropriate. The Panel determined a benchmark dose (BMD) lower bound associated with 1% extra risk (BMDL01) for neurodevelopmental effects in children of 12 μg/L in blood, corresponding to 0.50 μg/kg bw per day. A margin of exposure (MOE) of 10 was considered sufficient (exposure ≤ 0.05 μg/kg bw per day) (EFSA CONTAM Panel, 2010). Cereals are the main dietary source of dietary exposure to lead in Europe, with an average dietary exposure in European adults ranging between 0.36 up to 1.24 μg/kg bw per week and 0.80 to 3.10 μg/kg bw in children (EFSA CONTAM Panel, 2010). For high consumers, dietary exposure can reach 2.43 μg/kg bw per week (adults) and 5.51 μg/kg bw per week (children). Therefore, however not being the main dietary source of exposure, increased exposure to lead and its health effects cannot be excluded due to seaweed consumption. This would be of particular relevance for frequent and high consumers and for specific at high risk subgroups, namely children (1–7 years old) and pregnant women due to its critical effects on the neurodevelopment and lower body weight of foetus and children.

Cadmium is primarily toxic to the kidneys and bones and, for non-smokers, diet is also the main source of exposure to this element. Cadmium's TWI was set by EFSA to 2.5 μg/kg bw per week, being reported that, in adults, average exposure to cadmium in Europe is already close or slightly above the TWI. Rice, grains and vegetables are the main food sources of cadmium and, therefore, vegetarians and vegans are at higher risk of exceeding the TWI, as well as children, smokers and people living in highly contaminated areas (EFSA, 2009). Considering the species included in this study, a single serving would contribute with an average intake of 0.003 μg/kg bw to 0.087 μg/kg bw, 1.2–3.5% of the TWI, which is insignificant compared to other sources.

Methylmercury has a defined TWI of 1.3 μg/kg bw per week (neurodevelopmental effects in children), while total mercury has a TWI of 4 μg/kg bw per week (EFSA CONTAM Panel, 2012). In fish and seafood, most of the mercury is considered to be in the form of methylmercury, however, for seaweeds scarce data is available on speciation of this element. However, even when considering the total amount present as methylmercury, in average, the contribution of a single serving of seaweed to total exposure to mercury would be negligible.

On the other hand, intake of iodine due to a single serving of seaweed might easily exceed the UL for iodine (600 μg/day for adults and 200 μg/day for children), as observed for all of the species of brown algae and particularly for *Saccharina latissima* (average > 11,000 μg/day and 95th percentile > 18,000 μg/day). In general, consumption of brown seaweeds rich in iodine, namely *Saccharina latissima*, once a week would not represent a problem for the general healthy population. However, when considering at high risk subgroups: pregnant women (due to the importance of thyroid hormones in fetal development), children and individuals with thyroid dysfunction, a more careful evaluation regarding the species, amount and frequency of seaweed consumed should be done. Species with lower iodine content should be selected and iodine-rich species should be avoided due to lack of knowledge on the long-term exposure effects in fetus and children.

Here, only data on total arsenic was available, which comprises both organic and inorganic arsenic. Inorganic arsenic is the species most well characterised toxicologically and to which health effects have been associated. Therefore, inorganic arsenic is the arsenical form of relevance. Nonetheless, arsenosugars, the most relevant form of organic arsenic in seaweed, undergo extensive metabolism in humans (Leffers et al., 2013 Jul; Van Hulle et al., 2004; Raml et al., 2005, 2009; Wei et al., 2003), and more data on the toxicokinetics and toxicological profile of arsenosugars and their metabolites are warranted.

It is important to caveat that considering the unprocessed seaweed biomass is a conservative approach for exposure assessment, likely leading to overestimation, as the effects of cooking and processing on the final content as well as bioavailability are not taken into account. In fact, it is known that washing and cooking can significantly reduce the levels of several of these elements, namely iodine. Additionally, several other uncertainties exist, particularly regarding the exposure assessment

related with lack of data on iodine content (inter-species and intra-species, seasonal and geographic variability) and consumption data (species, frequency and amount of seaweed consumed) in Europe.

In general, we can conclude that seaweed consumption, by the general population, would be of low health risk for mercury, cadmium, and lead. However, due to their health effects, monitoring the levels of these elements should be performed and potentially, in the future, maximum levels set up for seaweed for food in Europe. For iodine, additionally to setting up maximum levels, the identification of the species on the label of the commercial products could be suggested as a way to decrease the exposure to high levels of iodine. Moreover, this preliminary study highlights the need for more data to be collected so that a more robust risk assessment can be performed. It is considered that the priorities would be the collection of species-specific consumption data and a better characterisation of the content of seaweed on these elements, including speciation data for arsenic.

3.3. Secondary activities

Additional activities taken during this working programme were:

1) A literature review of scientific papers and reports published since 1998 on the levels of iodine, cadmium, lead, mercury and inorganic arsenic in seaweeds of interest for human consumption (according to their commercial value, extension of consumption as well as availability of data). Regarding arsenic only studies reporting speciation and levels of inorganic arsenic were included as, so far, this is the species with toxicological relevance. The aim was to gather data for a better characterisation regarding species, season-variability and geographic origin (in preparation for submission);
2) Participation on the postgraduate course 'Risk Analysis in Food Safety' including a module in microbiological risk assessment and a second module in chemical risk assessment. Each module included two case studies intended to the elaboration of a risk assessment on a specific microbiological/chemical hazard and elaboration of a report;
3) Participation with a poster presentation on the *Open Day* of the National Food Institute (Denmark) as part of the commemorations of its 60th anniversary. The poster was on the work develop by the National Food Institute and DTU on the monitorisation of chemical contaminants in seaweed (particularly iodine), evaluation of efficacy of different processing/ preparation methods, risk assessment and elaboration of advises to consumers regarding seaweed consumption.
4) On-the-job training on the evaluation of applications and requests, related with biocides products, mainly destined to be used as disinfectant/cleaning agents. Taking part in advice giving to the Danish food and veterinary administration, the Danish Environment Protection Agency and the Danish Medical Agency in a range of different settings.

3.4. Disclaimer

The individual results of the analysis are not included in this report to avoid copyright claims as this research is part of an ongoing research project (being the EU-FORA Fellowship Programme) and the results are intended to be subsequently published in other scientific journals.

References

Aguilera-Morales M, Casas-Valdez M, Carrillo-Domínguez S, González-Acosta B and Pérez-Gil F, 2005. Chemical composition and microbiological assays of marine algae *Enteromorpha* spp. as a potential food source. Journal of Food Composition and Analysis 18, 79–88. Available online: http://www.sciencedirect.com/science/article/pii/S0889157504000122

ANSES, 2018. Opinion of the French Agency for Food, Environmental and Occupational Health and Safety: on the risk of excess iodine intake from the consumption of seaweed in foodstuffs.

Bilal M, Rasheed T, Sosa-Hernandez JE, Raza A, Nabeel F and Iqbal HMN, 2018. Biosorption: an interplay between Marine Algae and potentially toxic elements-A review. Mar Drugs, 16, 65.

Bonanno G and Orlando-Bonaca M, 2018. Chemical elements in Mediterranean macroalgae. A review. Ecotoxicology and Environmental Safety, 148, 44–71.

Bouga M and Combet E, 2015. Emergence of Seaweed and Seaweed-Containing Foods in the UK: focus on labeling, iodine content, toxicity and nutrition. Foods (Basel, Switzerland), 4, 240–253.

Brito GB, de Souza TL, Bressy FC, Moura CWN and Korn MGA, 2012. Levels and spatial distribution of trace elements in macroalgae species from the Todos os Santos Bay, Bahia, Brazil. Marine Pollution Bulletin, 64, 2238–2244.

Brown EM, Allsopp PJ, Magee PJ, Gill CIR, Nitecki S, Strain CR and McSorley EM, 2014. Seaweed and human health. Nutrition Reviews, 72, 205–216. https://doi.org/10.1111/nure.12091

Chakraborty S, Bhattacharya T, Singh G and Maity JP, 2014. Benthic macroalgae as biological indicators of heavy metal pollution in the marine environments: a biomonitoring approach for pollution assessment. Ecotoxicology and Environmental Safety, 100, 61–68. Available online: http://www.sciencedirect.com/science/article/pii/S0147651313005277

Chen Q, Pan X-D, Huang B-F and Han J-L, 2018. Distribution of metals and metalloids in dried seaweeds and health risk to population in southeastern China. Scientific Reports, 8, 3578.

Duinker A, Roiha I, Amlund H, Dahl L, Lock E-J, Kögel T, Maage A and Lunestad BT, 2016. Potential risks posed by macroalgae for application as feed and food – a Norwegian perspective. Report from National Institute of Nutrition and Seafood Research. Norway.

EFSA (European Food Safety Authority), 2009. Scientific Opinion of the Panel on Contaminants in the Food Chain on a request from the European Commision on cadmium in food. EFSA Journal 2009;7(3):980, 139 pp. https://doi.org/10.2903/j.efsa.2009.980

EFSA CONTAM Panel (EFSA Panel on Contaminants in the Food Chain), 2010. Scientific Opinion on Lead in Food. EFSA Journal 2010;8(4):1570, 151 pp. https://doi.org/10.2903/j.efsa.2010.1570

EFSA CONTAM Panel (EFSA Panel on Contaminants in the Food Chain), 2012. Scientific Opinion on the risk for public health related to the presence of mercury and methylmercury in food. EFSA Journal 2012;10(12):2985, 241 pp. https://doi.org/10.2903/j.efsa.2012.2985

European Commission, 2002. Opinion of the Scientific Committee on Food on the Tolerable Upper Intake Level of Iodine. Brussels.

European Commission, 2006. Commission Regulation (EC) No 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs. Official Journal of European Union, 364/5.

European Commission, 2008. Commission Regulation (EC) No 629/2008 of 2 July 2008 amending Regulation (EC) No 1881/2006 setting maximum levels for certain contaminants in foodstuffs. Official Journal of European Union, L 215, 4–8.

European Commission, 2018. Commission Recommendation (EU) No 2018/464 of 19 March 2018 on the monitoring of metals and iodine in seaweed, halophytes and products based on seaweed. Official Journal of European Union 78, 16–18.

FAO, 2018. The global status of seaweed production, trade and utilization. Globefish Research Programme Volume 124. Rome, Italy. 120 pp. Licence: CC BY-NC-SA 3.0 IGO.

FSAI, 2015. Consumption of Hiziki Seaweed [Internet] [cited 2018 Sep 21]. Available online: https://www.fsai.ie/faq/hijiki_seaweed.html

Gil MN, Torres AI, Commendatore MG, Marinho C, Arias A, Giarratano E and Casas GN, 2015. Nutritive and xenobiotic compounds in the alien algae *Undaria pinnatifida* from Argentine Patagonia. Archives of Environmental Contamination and Toxicology, 68, 553–565.

Hamdy AA, 2000. Biosorption of heavy metals by marine algae. Current Microbiology, 41, 232–238.

Holan ZR, Volesky B and Prasetyo I, 1993. Biosorption of cadmium by biomass of marine algae. Biotechnology and Bioengineering, 41, 819–825.

Holdt SL and Kraan S. 2011. Bioactive compounds in seaweed: functional food applications and legislation. Journal of Applied Psychology, 23, 543–597. https://doi.org/10.1007/s10811-010-9632-5

IARC, 1993. Mercury and Mercury Compounds. IARC Monographs on the Evaluation of Cracinogenic Risks to Humans. Volume 58.

IARC(Inorganic and Organic Lead Compounds), 2006. In: IARC Monographs on the evaluation of carcicogenic risks to humans. Volume 87.

IARC, 2012. Arsenic and arsenic compounds.

Jadeja RN and Batty L, 2013. Metal content of seaweeds in the vicinity of acid mine drainage in Amlwch, North Walwa, U.K. Indian Journal of Geo-Marine Sciences, 42, 16–20.

Leffers L, Ebert F, Taleshi MS, Francesconi KA and Schwerdtle T, 2013. In vitro toxicological characterization of two arsenosugars and their metabolites. Molecular Nutrition and Food Research, 57, 1270–1282.

Mahadevan K, 2015. Chapter 13 Seaweeds: a sustainable food source. In: Seaweed Sustainability [Internet]. pp. 347–364. Available online: https://app.dimensions.ai/details/publication/pub.1008475047

Malea P, Chatziapostolou A and Kevrekidis T, 2015. Trace element seasonality in marine macroalgae of different functional-form groups. Marine Environment Research, 103, 18–26. Available online: http://www.sciencedirect.com/science/article/pii/S0141113614001883

Mendis E and Kim S-K, 2011. Present and future prospects of seaweeds in developing functional foods. Advances in Food and Nutrition Research, 64, 1–15.

Peña-Rodríguez A, Mawhinney TP, Ricque-Marie D and Cruz-Suárez LE, 2011. Chemical composition of cultivated seaweed *Ulva clathrata* (Roth) C. Agardh. Food Chemistry 129, 491–498. Available online: http://www.sciencedirect.com/science/article/pii/S0308814611006704

Raml R, Goessler W, Traar P, Ochi T and Francesconi KA, 2005. Novel thioarsenic metabolites in human urine after ingestion of an arsenosugar, 2′,3′-dihydroxypropyl 5-deoxy-5-dimethylarsinoyl-beta-D-riboside. Chemical and Research in Toxicology, 18, 1444–1450.

Raml R, Raber G, Rumpler A, Bauernhofer T, Goessler W and Francesconi KA, 2009. Individual variability in the human metabolism of an arsenic-containing carbohydrate, 2′,3′-dihydroxypropyl 5-deoxy-5-dimethylarsinoyl-beta-D-riboside, a naturally occurring arsenical in seafood. Chemical Research in Toxicology, 22, 1534–1540.

Roleda MY, Marfaing H, Desnica N, Jónsdóttir R, Skjermo J, Rebours C and Nitschke U, 2019. Variations in polyphenol and heavy metal contents of wild-harvested and cultivated seaweed bulk biomass: health risk assessment and implication for food applications. Food Control, 95, 121–134. Available online: http://www.sciencedirect.com/science/article/pii/S0956713518303694

Ryan S, McLoughlin P and O'Donovan O, 2012. A comprehensive study of metal distribution in three main classes of seaweed. Environmental Pollution 167, 171–177. Available online: http://www.sciencedirect.com/science/article/pii/S026974911200187X

Sheng PX, Ting Y-P, Chen JP and Hong L, 2004. Sorption of lead, copper, cadmium, zinc, and nickel by marine algal biomass: characterization of biosorptive capacity and investigation of mechanisms. Journal of Colloid and Interface Science, 275, 131–141. Available online: http://www.sciencedirect.com/science/article/pii/S0021979704000943

Tiwari BK and Troy DJ, 2015. Chapter 1 - Seaweed sustainability – food and nonfood applications. In: Tiwari BK and Troy DJ (eds.). Seaweed Sustainability [Internet]. Academic Press, San Diego. pp. 1–6. Available online: http://www.sciencedirect.com/science/article/pii/B9780124186972000015

Van Hulle M, Zhang C, Schotte B, Mees L, Vanhaecke F, Vanholder R, Zhang XR and Cornelis XR. 2004. Identification of some arsenic species in human urine and blood after ingestion of Chinese seaweed Laminaria. Journal of Analytical Atomic Spectrometry, 19, 58–64. https://doi.org/10.1039/b307457a

Wei C, Li W, Zhang C, Van Hulle M, Cornelis R and Zhang X, 2003. Safety evaluation of organoarsenical species in edible Porphyra from the China Sea. Journal of Agricultural Food and Chemistry, 51, 5176–5182.

WHO (World Health Organization), 2007. Iodine deficiency in Europe. A continuing public health problem.

Abbreviations

BMD	benchmark dose
BMDL01	benchmark dose lower bound
bw	body weight
CONTAM	EFSA Panel on Contaminants in the Food Chain
FAO	Food and Agriculture Organization
fdw	freeze dried weight
HPLC	high-performance liquid chromatography
ICP-MS	inductively coupled plasma-mass spectrometry
MOE	margin of exposure
PTWI	provisional tolerable weekly intake
SCF	Scientific Committee on Food
SD	standard deviation
TWI	tolerable weekly intake
UL	tolerable upper intake level
WHO	World Health Organization

EU-FORA SERIES 2

APPROVED: 31 July 2019

doi: 10.2903/j.efsa.2019.e170916

Risk assessment of exotic disease incursion and spread

Hosting Institution: Wageningen Bioveterinary Research, Wageningen University & Research, the Netherlands,

M Cabral, R Taylor* and CJ de Vos

Abstract

This Technical Report describes the activities developed in the scope of the EU-FORA Fellowship, within the work programme of risk assessment (RA) of exotic disease incursion and spread, developed at Wageningen Bioveterinary Research (WBVR). The programme focused on the work carried out in the Generic risk assessment for introduction of animal diseases (G-RAID) project, which brings together a number of different generic RA tools from multiple European partners. The aim of the fellowship was to gain understanding of veterinary import risk assessment by using different RA tools and to learn how different algorithms can be used to calculate disease incursion risks. G-RAID's tools cover a wide range of RA methodologies; from purely qualitative, to semi-quantitative and fully stochastic quantitative methods, which allowed the fellow to understand a variety of algorithms used to produce the final risk estimate. The fellowship programme provided the fellow with the chance to learn in detail about how generic RAs are performed across Europe, understanding how to deal with the uncertainty and variability involved in RAs and the potential problems of data availability and reliability. The fellow made an inventory of publicly available databases on disease occurrence and international trade that could be used for import RA and assessed their quality and usefulness for the different generic RA tools. The programme also provided the fellow the opportunity to perform several import risk assessments using the RA tools of G-RAID. She completed a RA on African swine fever using the MINTRISK model developed by WBVR. Furthermore, she assessed the risk of foot and mouth disease introduction using the Rapid Risk Assessment Tool (RRAT) model developed by WBVR and the COMPARE model developed by the Animal and Plant Health Agency (APHA). To this end, the fellow completed a short-term visit to APHA, enabling her to have additional training in quantitative RA and to expand her professional network in this area.

Keywords: Import risk assessment, generic model, validation, livestock disease, introduction risk

Correspondence: eu-fora@efsa.europa.eu

* Animal and Plant Health Agency, United Kingdom.

Acknowledgements: This report is funded by EFSA as part of the EU-FORA programme.

Suggested citation: Hosting Institution: Wageningen Bioveterinary Research, Wageningen University & Research, the Netherlands, Cabral M, Taylor R and de Vos CJ, 2019. Risk assessment of exotic disease incursion and spread. EFSA Journal 2019;17(S2):e170916, 8 pp. https://doi.org/10.2903/j.efsa.2019.e170916

ISSN: 1831-4732

The EFSA Journal is a publication of the European Food Safety Authority, an agency of the European Union.

Table of contents

1. Introduction

Increasing globalisation and international trade contribute to the rapid expansion of animal diseases. Introduction of exotic animal diseases into naive livestock populations can result in large-scale epidemics with serious economic and socio-ethical impact. Hence, preparedness is warranted to prevent, detect and control outbreaks of exotic animal diseases. If outbreaks are detected promptly, interventions can be made to reduce the size of the outbreak and mitigate its consequences.

Import risk assessments are useful tools to inform risk managers on exotic animal disease threats providing information on e.g. relevant pathways or regions most at risk. Results can be used to assign resources for prevention and surveillance to those pathways, regions or diseases that pose the highest risk or to identify targets for additional research.

In recent years, several generic risk models or frameworks have been developed that can easily be applied to assess the incursion risk for multiple diseases (De Vos et al., 2012; Simons et al., 2019; Taylor et al., 2019). In contrast to bespoke models, these generic risk assessment (RA) tools are not disease specific, but can be used for a range of diseases, allowing for a more rapid response to newly emerging or re-emerging diseases. Rapid risk assessments are needed to help risk managers prepare and respond swiftly to new disease events that pose a potential health threat to their territory.

The development of generic RA tools is faced with three major challenges: (i) the need for extensive and real-time data sets on global disease presence and movements of humans, animals and products, (ii) the use of algorithms to combine all input data into either a qualitative or a quantitative risk estimate and (iii) the validation of results.

The EU FORA fellow participated in the 'Generic approaches for Risk Assessment of Infectious animal Disease introduction' (G-RAID) project (GA/EFSA/AFSCO/2017/01-GA01), which brings together a number of different generic RA tools from multiple European partners, including Wageningen Bioveterinary Research (WBVR) and the Animal Plant Health Agency (APHA). The RA tools in G-RAID cover a wide range of RA methodologies; from purely qualitative, to semi-quantitative and fully stochastic quantitative methods, which allowed the fellow to understand a variety of algorithms used to produce the final risk estimate. The main aim of G-RAID is to compare and contrast the different tools and, where possible, propose areas for standardisation and validation of generic RA tools. The fellow contributed to these objectives by inventorying global databases on disease occurrence worldwide and international trade in animals and animal products. Furthermore, the fellow performed several risk assessments, using three different generic RA tools.

2. Description of work programme

2.1. Aims

The overall objective of the fellowship was to gain understanding of veterinary import RA by using different RA tools and to learn how different algorithms can be used to calculate disease incursion risks. To achieve this objective, the work programme was divided into three modules: (1) qualitative and quantitative RA through the lens of generic models; (2) impact of uncertainty in data inputs for RA; and (3) comparison and validation of different generic models.

By taking advantage of the wide range of RA models that are involved in the G-RAID project, the specific aims of the modules were:

1) To introduce the fellow to qualitative, semi-quantitative and quantitative RA with a focus on data requirements, algorithms and output, and how generic models can be used to prioritize, diseases, locations or pathways for risk management purposes. In particular, to understand the role of generic RAs and the different approaches to generic RA across Europe;
2) To clarify which publicly available databases are used for animal disease RA and to assess their quality and usefulness for the different generic models. To understand the concepts of variability and uncertainty;
3) To gain understanding of several generic models of G-RAID and to discern how different algorithms can be used to calculate the incursion risk. To gain a wide range of skills in relation to creating RAs, such as programming skills, working with large data sets and interpretation of results, by performing a RA from the sourcing of data up to the final production for a specific model.

2.2. Activities/Methods

2.2.1. Qualitative and Quantitative Risk Assessments through the lens of Generic Models

The initial task of the work programme was to further consolidate the European Food Safety Authority (EFSA) induction training, and to obtain an understanding of the basic principles of both qualitative and quantitative RA. This was achieved by using the resources available at WBVR, such as text books, scientific meetings, departmental seminars, course notes and practical sessions in both qualitative and quantitative RA.

The fellow started by studying guidelines and methods for animal health RA (Murray, 2004; Vose, 2008). More detailed understanding was provided through involvement in the G-RAID project. Seven generic RA tools, developed in four different European countries (the Netherlands, UK, Sweden and Finland), were included in the G-RAID project. The seven generic RA tools included two quantitative models (SPARE, COMPARE), four semi-quantitative tools (RRAT, MINTRISK, IDM, NORA) and one qualitative tool (SVARRA). Although all of these tools can be used to address the incursion risk of exotic livestock diseases, they were originally developed for different purposes ranging from immediate response to new disease events to prioritisation of diseases and horizon scanning. Therefore, input, algorithms and endpoints of the tools differed.

The fellow read the G-RAID report describing the generic RA tools, watched the Webinar on 'Rapid risk assessment tools for animal disease outbreaks' organized by EFSA (2017), had a 1-day training on MINTRISK, and had the opportunity to participate in a videoconference in which some of the generic RA tools of G-RAID were presented and discussed (SPARE, COMPARE and RRAT). In addition, the fellow contributed to the preparation of a 1-day symposium (SYMPOSIUM: Generic risk assessment for introduction of animal diseases, 2019) to disseminate results of the G-RAID project to risk managers and risk assessors. The fellow was particularly involved in the workshop on data sources for generic RAs.

Thus, the fellow was able to take advantage of the wide range of RA models available in G-RAID to gain knowledge of performing risk assessments, the differences between qualitative, semi-quantitative and quantitative risk assessments in terms of data requirements, algorithms and output, and how generic models can be used to prioritize diseases, locations or pathways for risk management purposes.

Finally, the fellow also worked on a bespoke model using the @Risk (Palisade Corp) add-in for Excel (@RISK for Risk Analysis). The objective was to perform a quantitative RA for the introduction of African swine fever (ASF) into the Netherlands, United Kingdom and Finland by legal import of live pigs. A scenario tree for the probability of introduction of ASF into the country of destination by the import of live pigs was developed. Data from COMEXT were used as an input for trade, OIE data were used to assess disease prevalence in source areas and a literature review was performed to estimate disease-specific parameters.

2.2.2. Impact of Uncertainty in Data Inputs for Risk Assessments

Data required for animal RA relate to movement and disease occurrence. The first category provides information on how many animals/products etc. are moved from infected areas (area of origin) to the area under study (target area) regardless of whether or not they are infected, for each pathway considered. For some pathways, this will be related to trade flows. The second category provides information on disease occurrence in the areas of origin, which is then used to estimate the probability that animals/products etc. are infected upon arrival in the target area.

Several global databases exist providing information on international trade and disease occurrence and risk assessors have to decide which databases to use in their models. The choice of data set can have significant impact on model results, and therefore, the risk assessor needs to weigh up the pros and cons of each. Considerations for using different data sets include accessibility, quality of data, level of detail, scope of the information, accuracy, confidentiality and time spent to retrieve up-to-date data.

Global databases have different purposes and are under different legal frameworks, all with their own strengths and limitations. However, the lack of harmonization across the different data sources and the often insufficient resolution/detail hinder the ability to be used in analytical epidemiology and risk assessments. In addition, the uncertainty associated with the data poses additional challenges. One of the main issues with generic RAs is that the data sources must have a broad scope (e.g. multiple diseases or countries). This means that many detailed data sources (e.g. national statistics on animal movements) are not appropriate as the scope is too narrow whereas at the broader scale, the

lack of detail can lead to high uncertainty. The different purposes/modes of collecting the data can lead to fairly substantial differences between data sources that aim to provide similar information. As such, the choice of data source can have an impact on the model results. The lack of standardization between data sources also means it can be difficult to combine them in order to utilize them all.

Therefore, the fellow clarified which publicly available databases are used for animal RA and assessed their quality and usefulness for different generic models. The fellow reviewed different data sources available, with a particular focus on trade data, including COMEXT (Eurostat) (European Commission, 2019a), FAOSTAT (FAO) (FAOSTAT, 2019), TRACES (EU) (European Commission, 2019b) and COMTRADE (UN) (United Nations Statistics Division, 2019), and disease outbreak reports, including the WAHIS database (OIE) (OIE, 2019), EMPRES-i (FAO) (FAO, 2019), ADNS (EU) (European Commission, 2019c), HealthMap (Freifeld et al., 2008) and ProMed (Yu and Madoff, 2004). The criteria considered were the availability and accessibility of data, the quality of data, the reasons for its creation, the pros and cons of each data source and the potential issues when used in RA models.

The fellow completed this task by producing a report summarising the different data sources available, comparing the European and global data sources on aspects such as usability or reliability of the data and examining the impact of their uncertainty and reliability on the results of the bespoke model for ASF.

2.2.3. Comparison and Validation of Different Generic Models

The fellow had the opportunity to work with three generic RA tools.

The fellow used the Method for INTegrated RISK assessment of vector-borne diseases (MINTRISK) (de Vos-de Jong et al., 2016), a semi-quantitative RA tool developed at WBVR, and participated in the validation of generic RA tools for animal disease incursion based on a case study for ASF. The fellow collected data on ASF and performed the RA for ASF introduction for the Netherlands and Finland for the 2017 situation and for two hypothetical scenarios in which ASF cases were reported in wild boar and/or domestic pigs in Germany, in MINTRISK. Results were used for cross-validation with the other tools in the G-RAID project. The pathways considered were trade in live pigs and wild boar movements. The results will be published in a peer-reviewed paper drafted by the G-RAID Consortium.

In addition, the fellow had the opportunity to complete a RA for Foot and Mouth disease (FMD) introduction in the Netherlands and the UK, using a generic model developed at WBVR (the RRAT model) and a second developed at APHA (the COMPARE model) (Taylor et al., 2019). The fellow collected data on disease prevalence in source areas; pathway movements from source areas to the target areas; susceptible animals in the target areas; and FMD disease-specific parameters. Then, the fellow proceeded to perform the RA and to draft a paper to disseminate the results of the FMD case study for a joint WBVR/APHA publication in a peer-reviewed journal.

The fellow's work on the COMPARE model was possible through the participation in a short-term mission (STM) awarded by the Med-Vet-Net association (Med-Vet-Net Association for Zoonoses Research, 2009). The fellow visited the Department of Epidemiological Sciences at APHA to work with the Biomathematics and Risk Research workgroup. During this 2-week STM, the fellow was introduced to the COMPARE model by Dr Rachel Taylor. The aim of the fellow's visit was to understand the model's framework, algorithms and data requirements, and to practice running the model.

The STM also enabled the fellow to expand her professional network in the area of risk assessment. The fellow was introduced to both the Biomathematics and Risk Research workgroup and the Epidemiology workgroup in the Department of Epidemiological Sciences, attended department meetings and had the opportunity to participate in a meeting with the UK's Chief Veterinary Officer. Furthermore, the fellow presented her research topics in a meeting and attended presentations of different projects currently ongoing within the Department of Epidemiological Sciences. In addition, the fellow was able to consolidate her knowledge of spatial modelling in R.

3. Conclusions

The fellowship programme provided the fellow with expertise and experience in veterinary import RA through a 'learning-by-doing' approach. It provided the chance to learn in detail about how different generic RA tools for disease incursion are performed across Europe, as well as understanding how to deal with uncertainty and variability within RAs and the potential problems of data availability and reliability.

Most importantly, the programme provided the fellow with the opportunity of experiencing the whole process of performing RAs, with a focus on the introduction of infectious diseases. The

programme complemented the previously gained knowledge in the theory-based training programmes at EFSA, by providing the opportunity to get a thorough insight into a number of different generic RA tools from multiple European partners, particularly the ones developed by WBVR and APHA.

The activities proceeded in accordance with the work programme and the expected time frame in a stimulating scientific working atmosphere at the Department of Bacteriology and Epidemiology of WBVR. Through the daily exchange with experts of different veterinary epidemiology aspects, group meetings and the opportunity to participate in different seminars, the fellow was able to learn new methodologies, gain more expertise and start building a network in the European food safety community.

The fellowship also introduced the fellow to the 'One Health' concept, a collaborative, multisectoral, and transdisciplinary approach with the goal of achieving optimal health outcomes recognising the interconnection between people, animals, plants and their shared environment.

Finally, the EU-FORA fellowship provided a unique opportunity for expanding the fellow's network in the field of food safety RA during the induction training at EFSA and three further training modules hosted by the national food safety authorities in Vienna, Berlin and Athens, the placement at WBVR and the STM at APHA.

References

De Vos C, Hoek M, Fischer E, de Koeijer A and Bremmer J, 2012. Risk assessment framework for emerging vector-borne livestock diseases. Wageningen UR, Central Veterinary Institute.

EFSA (European Food Safety Authority), 2017. Webinar: Rapid risk assessment tools for animal disease outbreaks 2017. Available online: https://www.youtube.com/watch?v=KVmp9nOfZ50

European Commission, 2019a. COMEXT 2019. Available online: https://ec.europa.eu/eurostat/web/international-trade-in-goods/data/focus-on-COMEXT

European Commission, 2019b. Trade Control and Expert System (TRACES) 2019. Available online: https://ec.europa.eu/food/animals/traces_en

European Commission, 2019c. Animal Disease Notification System (ADNS) 2019. Available online: https://ec.europa.eu/food/animals/animal-diseases/not-system_en

FAO, 2019. EMPRES Global Animal Disease Information System (EMPRES-i) 2019. Available online: http://empres-i.fao.org/eipws3g/

FAOSTAT, 2019. FAO Global Statistical Yearbook 2019. Available online: http://www.fao.org/faostat/en/

Freifeld CC, Mandl KD, Reis BY and Brownstein JS, 2008. HealthMap: global infectious disease monitoring through automated classification and visualization of Internet media reports. Journal of the American Medical Informatics Association, 15, 150–157.

Med-Vet-Net Association for Zoonoses Research, 2009. Available online: http://www.medvetnet.org/

Murray N, 2004. Handbook on import risk analysis for animals and animal products: quantitative risk assessment. Office International des Épizooties.

OIE, 2019. World Animal Health Information System 2019. Available online: http://www.oie.int/

Vose D, 2008. Risk analysis: a quantitative guide. John Wiley & Sons.

@RISK for Risk Analysis. Available online: https://www.palisade.com/risk/

Simons RR, Horigan V, Ip S, Taylor RA, Crescio MI, Maurella C, Mastrantonio G, Bertolini S, Ru G, Cook C and Adkin A, 2019. A spatial risk assessment model framework for incursion of exotic animal disease into the European Union Member States. Microbial Risk Analysis.

SYMPOSIUM: Generic risk assessment for introduction of animal diseases, 2019. Available online: https://www.wur.nl/en/show/SYMPOSIUM-Generic-risk-assessment-for-introduction-of-animal-diseases.htm

Taylor RA, Berriman AD, Gale P, Kelly LA and Snary EL, 2019. A generic framework for spatial quantitative risk assessments of infectious diseases: Lumpy skin disease case study. Transboundary and Emerging Diseases, 66, 131–143.

United Nations Statistics Division, 2019. United Nations Commodity Trade Statistics Database 2019. Available online: https://comtrade.un.org/db/help/uReadMeFirst.aspx

de Vos-de Jong C, van Roermund H, de Koeijer A and Fischer E, 2016. Risk assessment of seven emerging vector-borne animal diseases for The Netherlands: A structured approach.

Yu VL and Madoff LC, 2004. ProMED-mail: an early warning system for emerging diseases. Clinical Infectious Diseases, 39, 227–232.

Abbreviations

ADNS	Animal Disease Notification System
APHA	Animal and Plant Health Agency
ASF	African Swine Fever

EMPRES-i	FAO's EMPRES Global Animal Disease Information System
EU-FORA	European Food Risk Assessment Fellowship Programme
FAOSTAT	Food and Agriculture Organization Corporate Statistical Database
FMD	Foot and Mouth disease
G-RAID	Generic risk assessment for introduction of animal diseases
MINTRISK	Method for INTegrated RISK assessment of vector-borne diseases
OIE	Office International des Epizooties
ProMED	The Program for Monitoring Emerging Diseases
RRAT	Rapid Risk Assessment Tool
TRACES	Trade Control and Expert System
UN Comtrade	United Nations Trade Statistics Database
WAHIS	World Animal Health Information System
WBVR	Wageningen Bioveterinary Research

EU-FORA SERIES 2

APPROVED: 31 July 2019

doi: 10.2903/j.efsa.2019.e170917

Risk-Benefit Assessment of Foods

National Food Institute, Technical University of Denmark (DTU Food), Denmark,
R Assunção, SM Pires and M Nauta

Abstract

Food is an elementary requirement for human life, providing nutrients and essential energy needed for optimal health. But at the same time, food can also be a vehicle of hazardous substances or pathogens that could affect human health negatively. Risk-benefit assessment (RBA) of foods, a relatively new methodology for decision support, integrates nutrition, toxicology, microbiology, chemistry and human epidemiology for a comprehensive health impact assessment. By integrating health risks and benefits related to food consumption, RBA facilitates science-based decision-making in food-related areas and the development of policies and consumer advice. The present work programme aimed to allow the fellow to become acquainted with the process of RBA and the associated tools needed to assess quantitatively the risks and the benefits through three main activities (i) to learn the different methodologies used for RBA; (ii) to apply these methodologies to a specific case-study – RBA of raw milk consumption; and (iii) to participate in the main activities of the Risk-Benefit research group at DTU Food regarding risk-benefit issues. For the RBA of raw milk consumption, microbiological pathogens (*Listeria monocytogenes*, *Salmonella* spp., *Campylobacter jejuni* and Shiga toxin-producing *Escherichia coli*), probiotic bacteria and nutritional components (vitamins B2 and A) were considered, as well as the potential impact of raw milk consumption in the reduction of the allergies' prevalence. Two major approaches were applied: the bottom-up (estimating the disease incidence due to the exposure) and the top-down (using epidemiological and incidence data to the estimate the number of cases attributable to a certain exposure). Through all the training and hands-on activities performed, the present work programme enabled the fellow to extend the knowledge on the quantitative RBA, specifically in the context of raw milk consumption. EU-FORA programme also provided an exceptional opportunity of networking and establishment of future research lines of collaboration.

Keywords: Risk-benefit assessment, food, toxicology, microbiology, nutrition, raw milk

Correspondence: eu-fora@efsa.europa.eu

Acknowledgements: This report is funded by EFSA as part of the EU-FORA programme.

Suggested citation: National Food Institute, Technical University of Denmark (DTU Food), Denmark, Assunção R, Pires SM and Nauta M, 2019. Risk-Benefit Assessment of Foods. EFSA Journal 2019;17 (S2):e170917, 8 pp. https://doi.org/10.2903/j.efsa.2019.e170917

ISSN: 1831-4732

The EFSA Journal is a publication of the European Food Safety Authority, an agency of the European Union.

Table of contents

1. Introduction

Food is an elementary requirement for human life, providing the nutrients and the essential energy needed for optimal health. At the same time, food may also be associated with adverse health effects, due to e.g. natural toxins, hazardous chemical substances or pathogenic microorganisms that could be present in foods and consequently affecting human health negatively. Moreover, dietary intake of specific nutrients in foods could be too low or too high, resulting in potential deficiencies or toxicity symptoms (Nauta et al., 2018). Therefore, methodologies and tools as risk-benefit assessment (RBA) constitute important contributions in the integrated research of risks and benefits, supporting the decision under food-related areas and in the development of food policies and consumer advice. The development of new food products and the support to consumers considering dietary changes are also important aspects that could take advantage of a RBA (Hoekstra et al., 2013).

1.1. Risk-benefit assessment of foods

Risk-benefit assessment of foods is a relatively new-decision support tool that intends to estimate the human health benefits and risks following exposure (or lack of exposure) to a particular food or food component and to integrate them in comparable measures (Boué et al., 2015; Pires et al., 2019). The beneficial and adverse health effects may occur concurrently from the intake of a single food item or a single food component, within the same population. This means that any policy action directed at the adverse effects also affects the degree of beneficial effects and vice versa. RBA integrates knowledge on nutrition, toxicology, microbiology, chemistry and human epidemiology for comprehensive health impact assessments (Pires et al., 2019). It constitutes one of the three pillars of the Risk-Benefit Analysis paradigm that combines RBA, risk-benefit management and risk-benefit communication, mirroring the risk analysis paradigm (EFSA Scientific Committee, 2010; Fransen et al., 2010; Nauta et al., 2018; Pires et al., 2019).

Figure 1 illustrates the proposed procedure for a RBA which consists of two separate and independent arms of assessing the risk and the benefit, respectively.

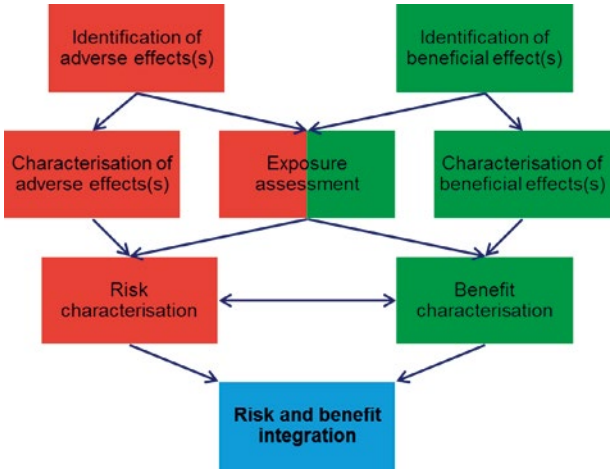


Figure 1: Risk-benefit assessment paradigm. Adapted from (EFSA Scientific Committee, 2010; Thomsen, 2019)

Generally, RBA process starts with the problem formulation corresponding to the clear description of the problem. The problem formulation, a critical step under RBA, should ensure that its outcome is useful and relevant (EFSA Scientific Committee, 2010). Under this step, the risk-benefit question (RBQ) should be defined, describing the purpose, scope and limitations of the assessment. Additionally, RBQ should define the population of interest, the level of aggregation (i.e. if the assessment should concern a food component, a food product or a diet) and the exposure scenarios (including the reference and the alternative scenarios). After the problem formulation, and based on the weight and quality of the scientific evidence, the identification of the health effects associated to the food component/food product/diet considered in the RBQ should be performed (identification of adverse/beneficial effect(s)). The relationship between the exposure to a food component or a food product and the associated health impact (usually known as dose–response assessment) should be established (characterisation of

adverse/beneficial effect(s)). For the considered scenarios, the exposure to the food component or food product should be assessed, using consumption data for the considered foods and concentration of the substances in the referred food products (exposure assessment). Combining the information regarding the dose–response relationship and the exposure assessment, the probability of occurrence of an adverse or beneficial health effect and the consequences of that effect should be estimated (risk characterisation; benefit characterisation). Finally, the risks and the benefits should be integrated, combining them if expressed in a common health metric, and the considered scenarios should be compared.

1.2. Risk-benefit assessment of raw milk consumption

Consumer demand for organic and natural foods, i.e. including minimal food processing, has been growing last years. Despite in the perception of some consumers these products are safer than the conventional, this is not necessarily correct (Claeys et al., 2013; Costard et al., 2017). Some evidences of high rates of food-borne illnesses associated to some 'natural foods' as, e.g. raw milk, reflect that despite the increase popularity, these food products are not exempt of risks.

According to the Regulation (EC) No 853/2004, raw milk is defined as milk produced by the secretion of the mammary gland of farmed animals that has not been heated to more than 40°C or undergone any treatment that has an equivalent effect (European Commission, 2004). Regarding the consumption of raw milk, there is currently a debate on the potential health benefits when compared to pasteurised milk. The preference to the raw milk is mainly associated with several perceived health benefits that are believed to be destroyed upon heating. Claimed health benefits are, e.g. 'higher nutritional value', especially in terms of vitamins' contents, 'beneficial microflora' as probiotic bacteria, and 'allergy prevention'. Oppositely, there are significant concerns by regulatory and public health organisations regarding the potential risk of contracting milk-borne diseases due to raw milk contamination with human pathogens. Although some previous studies compared the health risks and benefits of raw milk, and found that the risks are larger than the benefits, the overall health effect of drinking raw milk instead of pasteurised milk has never been quantified, and the benefits compared to the risks continue to be an issue for debate.

2. Description of work programme

2.1. Aims

The present work programme was prepared to allow the fellow to become acquainted with the process of RBA of foods and the associated tools needed to assess the risks and the benefits in a quantitative way. In order to attain this core objective, three main specific objectives were considered, namely: (i) to learn the different steps of RBA and the different methodologies applicable to estimate the risks and benefits associated to foods; (ii) to apply these process and methodologies to a specific case-study; and (iii) to integrate the main activities of the research group regarding different risk-benefit issues. The entire work programme was carried out in the Research Group for Risk-Benefit of the National Food Institute, Technical University of Denmark (DTU Food).

The case-study selected to be performed under the present work programme was the quantitative RBA of raw milk consumption. In addition to the originality of this work, the added value of such a quantitative assessment is that consumers can be informed on the magnitude of the risk and the expected health impact, and make informed decisions based in scientific evidence.

2.2. Training in risk-benefit assessment in foods

As an initial step of the training process in the RBA of foods, some literature search regarding the methodology and the different aspects of RBA was performed. The main aspects of RBA were discussed in one-to-one meetings between the fellow and his supervisor. In order to deeper learn and harmonise the concepts and methodologies of RBA of foods, namely to identify and quantify beneficial and adverse health effects of foods, food constituents or nutrients, and to measure their risk-benefit balance, the fellow attended the course 'Risk-Benefit Assessment in Foods: methods for quantifying health effects' (November 6–15, 2018, Lyngby, Denmark). This 8-day intensive course was taught by Maarten Nauta (supervisor of the present work programme and the course responsible) and other researchers from the Research Group for Risk-Benefit from DTU Food. The course considered a total study load of 2.5 ECTS (equivalent to 70 h of study). In addition to theoretical lectures, hands-on

exercises, group works and discussions were used to introduce the RBA and its steps, covering chemical, microbiological and nutritional important perspectives for RBA. Additional aspects as burden of disease and disability-adjusted life years (DALY) calculations, quantitative and stochastic assessment and variability and uncertainty were also addressed.

2.3. Risk-benefit assessment of raw milk consumption

The objective of this assessment was to quantify the risk-benefit balance and the health impact of raw milk consumption in terms of DALY. A stepwise approach was used to perform the RBA of raw milk consumption, following the scheme described previously by EFSA (Figure 1). Firstly, the problem was defined, stating the scope of the assessment and the RBQ to be answered. The scenarios to be considered were also described, including a reference (corresponding to the consumption of pasteurised milk) and an alternative scenarios (corresponding to the consumption of raw milk). Through literature review, different components usually present in milk (raw and/or pasteurised) were identified and the associated health effects were selected. In addition to the literature search, specific documents produced by national and international authorities were considered (Ministry for Primary Industries, 2013a,b; EFSA BIOHAZ Panel, 2015). Some criteria of inclusion and exclusion were established for the identification of the health effects associated to the considered food components and particular attention was dedicated to the degree of evidence and quality of data. The microbiological hazards *Listeria monocytogenes*, *Salmonella* spp., *Campylobacter jejuni* and Shiga toxin-producing *Escherichia coli* (STEC) were considered next to potentially beneficial components such as probiotic bacteria (*Lactobacillus* species) and vitamins B2 and A. The potential effect of the consumption of raw milk in the reduction of the prevalence of allergies was also considered as a potential beneficial effect of raw milk consumption. Mathematical modelling, including predictive modelling of bacterial inactivation and growth and modelling of dose–response using epidemiological data, were used to quantify the DALYs associated to the consumption of raw milk directly from vending machines. Two major approaches were applied: (i) the bottom-up approach estimating the incidence of disease due to the exposure via dose-response models (used for the microbiological hazards); and (ii) the top-down approach that starts from the epidemiological and incidence data and estimates the number of attributable cases of a certain disease due to an exposure (used for the nutritional components) (Nauta et al., 2018). Published data were used to perform the exposure assessment. BCoDE, software developed by the European Centre for Disease Prevention and Control (ECDC), was used to estimate the associated DALYs (ECDC, 2019). Dutch food composition database was used to estimate the vitamins A and B2 intake through milk consumption. Modelling resources and the GBD Results Tool were used to establish the associated risk prevention and to estimate the associated DALYs, respectively. Finally, the integration of risks and benefits expressed in DALYs was obtained, comparing the considered different scenarios, through the calculation of the difference between alternative and reference scenarios (expressed in ΔDALY). Due to the assumptions and approximations included in the RBA model, required to accommodate the lack of knowledge or data, the associated uncertainty was identified and characterised.

Some of the obtained results were presented orally in the division seminar at DTU Food, and also as a case study in the Parma Summer School 'Risk-benefit in food safety and nutrition' (June 11–13, 2019, Parma, Italy).

2.4. Other activities related with risk-benefit assessment of foods

Additional activities were accomplished related with the main goal of the present work programme – the establishment of a solid knowledge foundation under RBA. Integrating the usual activities of the research group, the fellow also attended and participated actively in the weekly group meetings, journal club (every month) and scientific division meetings (every 14 days). Regarding the journal club, a presentation and associated discussion of a paper related with the health effects associated with Mediterranean diet was performed.

RiskBenefit4EU (RB4EU), a collaborative project, funded by EFSA under the Partnering Grants, joins together DTU Food and National Institute of Health Dr. Ricardo Jorge (fellow's home institution). Taking advantage of some planned activities of RB4EU, the fellow also participated in training on RBA, collaborated in the organisation and mentoring of a short-term scientific mission from Portugal to DTU and in the development of a case-study in the RBA of cereal-based foods intended to be consumed by young children.

A quick quantitative RBA of nuts in Portugal was also performed and the obtained results were presented orally in an international conference (41st Mycotoxins Workshop, May 6–8, 2019, Lisboa, Portugal).

The fellow is also part of the team responsible to perform a systematic review on the RBAs of fish, developed under the International Network on Risk-Benefit Assessment of Foods.

3. Conclusions

On a broader perspective, research in RBA of foods is promising and future evolution is expected. The present work programme developed at the Research Group for Risk-Benefit of the National Food Institute, Technical University of Denmark, had as main focus the capacitation of the fellow in the RBA of foods. The programme provided the opportunity to get a thorough insight into the work performed in an international research group dealing with RBA. Through all the activities performed, the present work programme enabled the fellow to gain first-hand experience on RBA, extending the knowledge on the quantitative RBA of raw milk consumption. Detailed description of the outputs obtained in the RBA of raw milk consumption will be made available in a peer-reviewed publication.

In addition to the scientific achievements regarding the acquired knowledge through training as well as the hands-on activities, the EU-FORA programme also provided an exceptional opportunity of networking and establishment of future research lines of collaboration. In a pleasant and multicultural atmosphere, DTU Food provided the expertise, mentoring as well as working conditions, promoting the ideal environment to knowledge exchange and research on food safety and nutrition domains. For these reasons, DTU Food is completely aligned with the purposes of the EU-FORA programme and an adequate host site for future fellows.

References

Boué G, Guillou S, Antignac J-P, Bizec B and Membré J-M, 2015. Public Health Risk-benefit Assessment Associated with Food Consumption–A Review. European Journal of Nutrition & Food Safety, 5, 32–58. https://doi.org/10.9734/ejnfs/2015/12285

Claeys WL, Cardoen S, Daube G, De Block J, Dewettinck K, Dierick K and Herman L, 2013. Raw or heated cow milk consumption: review of risks and benefits. Food Control, 31, 251–262. https://doi.org/10.1016/j.foodcont.2012.09.035

Costard S, Espejo L, Groenendaal H and Zagmutt FJ, 2017. Outbreak-Related Disease Burden Associated with Consumption of Unpasteurized Cow's Milk and Cheese, United States, 2009–2014. Emerging Infectious Diseases, 23, 957–964. https://doi.org/10.3201/eid2306.151603

ECDC (European Centre for Disease Prevention and Control), 2019. BCoDE toolkit [software application]. Version 1.7. Solna: European Centre for Disease Prevention and Control. Available online: https://ecdc.europa.eu/en/publications-data/toolkit-application-calculate-dalys

EFSA BIOHAZ Panel (EFSA Panel on Biological Hazards), 2015. Scientific Opinion on the public health risks related to the consumption of raw drinking milk. EFSA Journal 2015;13(1):3940, 95 pp. https://doi.org/10.2903/j.efsa.2015.3940

EFSA Scientific Committee, 2010. Guidance on human health risk-benefit assessment of foods. EFSA Journal 2010;8(7):1673, 40 pp. https://doi.org/10.2093/j.efsa.2010.1673

European Commission, 2004. Regulation laying down specific hygiene rules for the hygiene of foodstuffs, 853/2004/EC.

Fransen H, De Jong N, Hendriksen M, Mengelers M, Castenmiller J, Hoekstra J, van Leeuwen R and Verhagen H, 2010. A tiered approach for risk-benefit assessment of foods. Risk Analysis, 30, 808–816. https://doi.org/10.1111/j.1539-6924.2009.01350.x

Hoekstra J, Hart A, Owen H, Zeilmaker M, Bokkers B, Thorgilsson B and Gunnlaugsdottir H, 2013. Fish, contaminants and human health: quantifying and weighing benefits and risks. Food and Chemical Toxicology, 54, 18–29. https://doi.org/10.1016/j.fct.2012.01.013

Ministry for Primary Industries, 2013a. An Assessment of the Effects of Pasteurisation on Claimed Nutrition and Health Benefits of Raw Milk. Wellington.

Ministry for Primary Industries, 2013b. Assessment of the microbiological risks associated with the consumption of raw milk. New Zealand. Retrieved from Ministry for Primary Industries, Wellington.

Nauta MJ, Andersen R, Pilegaard K, Pires SM, Ravn-Haren G, Tetens I and Poulsen M, 2018. Meeting the challenges in the development of risk-benefit assessment of foods. Trends in Food Science & Technology, 76, 90–100. https://doi.org/10.1016/j.tifs.2018.04.004

Pires SM, Boué G, Boobis A, Eneroth H, Hoekstra J, Membré J-M, Persson IM, Poulsen M, van Klaveren Thomsen ST and Nauta MJ, 2019. Risk Benefit Assessment of foods: key findings from an international workshop. Food Research International, 116, 859–869. https://doi.org/10.1016/j.foodres.2018.09.021

Thomsen ST, 2019. Risk-benefit assessment of food substitutions. Technical University of Denmark. Available online: http://orbit.dtu.dk/en/publications/riskbenet-assessment-of-food-substitutions(b8150e23-9548-4449-ada1-7daa663489a6).html

Abbreviations

DALY	Disability-adjusted life years
DTU Food	National Food Institute, Technical University of Denmark
ECDC	European Centre for Disease Prevention and Control
RB4EU	RiskBenefit4EU
RBA	risk-benefit assessment
RBQ	risk-benefit question
STEC	Shiga toxin-producing *Escherichia coli*

Getting in touch with the EU

In person

All over the European Union there are hundreds of Europe Direct information centres. You can find the address of the centre nearest you at: https://europa.eu/european-union/contact_en

On the phone or by email

Europe Direct is a service that answers your questions about the European Union. You can contact this service:

– by freephone: 00 800 6 7 8 9 10 11 (certain operators may charge for these calls),
– at the following standard number: +32 22999696 or
– by email via: https://europa.eu/european-union/contact_en

Finding information about the EU

Online

Information about the European Union in all the official languages of the EU is available on the Europa website at: https://europa.eu/european-union/index_en

EU publications

You can download or order free and priced EU publications at: https://publications.europa.eu/en/publications. Multiple copies of free publications may be obtained by contacting Europe Direct or your local information centre (see https://europa.eu/european-union/contact_en).

EU law and related documents

For access to legal information from the EU, including all EU law since 1952 in all the official language versions, go to EUR-Lex at: http://eur-lex.europa.eu

Open data from the EU

The EU Open Data Portal (http://data.europa.eu/euodp/en) provides access to datasets from the EU. Data can be downloaded and reused for free, for both commercial and non-commercial purposes.

European Food Safety Authority

Via Carlo Magno 1A
43126 Parma
ITALY

Tel. +39 0521 036 111
Fax +39 0521 036 110
www.efsa.europa.eu

Publications Office
of the European Union