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Foreword

I am very pleased to introduce this collection of reports from the third round of EFSA's EU-FORA fellowship programme.

EU-FORA started in 2016 as part of EFSA's efforts to support and develop the next generation of Europe's food risk assessors. The variety of subjects and experiences described in these documents bears testimony to the success of the programme, showing how fellows have gained invaluable hands-on experience and knowledge working alongside risk assessors in their host organisation outside their home country.

This year has been particularly challenging for EU-FORA. The COVID-19 pandemic has impacted the fellows' work at their hosting sites, especially where mobility restrictions forced homeworking and reduced social interaction. The great commitment of supervisors and fellows to the programme has been more remarkable than ever, and the quality and relevance of the documents presented are evidence for that.

This special issue is a tribute to the hard work and dedication of the fellows, the enthusiasm with which the host organisations embrace the programme and the support provided by EFSA's colleagues across many different departments. Partnership and cooperation are core values for EFSA, and it is gratifying to see their importance shared by our partners.

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. Our fourth intake of fellows will start on their journey in January, and I am sure that their experiences will be as mutually enriching as those of their predecessors, despite the continued challenging circumstances.

Barbara Gallani

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Introduction

We are very happy to present this third special issue of *EFSA journal* devoted to EFSA's fellowship programme (EU-FORA). It brings together the activities the third wave of fellows has carried out at those institutions across Europe that participate in the EU-FORA network and devote time and energy to host and train the fellows.

The pandemic that is affecting the globe is not alien to the EU-FORA Programme and it has meant an extra challenge for fellows and supervisors. We can only congratulate them and thank them for the professionalism, engagement, passion and resilience they have exhibited during the 12 months of fellowship.

Along this year, fellows have not only significantly increased their knowledge of food risk assessment but the high-quality work programmes presented here have also generated new knowledge in the area, as will be evidenced by the publication of articles in scientific journals in the upcoming months.

Furthermore, this year we have had the pleasure to welcome new organisations in the Programme, thus also making of EU-FORA a successful cooperation tool between them and EFSA. We hope that this special issue of the *EFSA Journal* serves as inspiration for more Article 36 organisations to apply as hosting sites.

Finally, we would like to thank everyone who were involved and contributed to this cycle of EU-FORA; extending our gratitude to the members of the former EU-FORA Programme Committee for their contribution to the Programme since its conception, to the EFSA Management Team for supporting it, and everyone involved in the training consortium for excellent training to the fellows.

On behalf of the EU-FORA Programme

Victoria Villamar

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Review of Potentially Toxic Rare Earth Elements, Thallium and Tellurium in Plant-based Foods

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Abstract

In the last decades, there is an increasing inclusion of various trace metals and metalloids such as thallium, tellurium and rare earth elements (REEs; lanthanides, scandium, and yttrium) in the composition and production of alloys, in agricultural and medicinal applications, as well as in the manufacturing of hi-tech products. All these activities have led to an accumulation of the aforementioned elements both in soil and water bodies and consequently in the food chain, through discharges from mining and mineral processing, liquid industrial waste or disposal of urban and industrial products. It has been demonstrated that chronic exposure to some of these elements, even at low doses, might lead to a wide range of adverse health effects, even from the early stages of life, such as neurotoxicity, neurodevelopmental toxicity and hepatic alterations. Particularly in children, there have been studies suggesting that some of these elements might negatively affect the children's spatial learning and memory ability indirectly. Such effects are triggered by processes like the production of reactive oxygen species (ROS), lipid peroxidation and modulation of antioxidant activities. Nevertheless, the limited data from toxicological studies and their so-far naturally low occurrence levels in the environment acted as a deterrent in measuring their concentrations during routine analyses of metals in foodstuff. Thus, it is important to collect information on their occurrence data both in adults and in children's daily diet. This review sumrises the current knowledge on the concentration of these elements, in plant-based food products to identify whether a potential health risk occurs. As side projects, this Fellowship provided hands-on training on the evaluation of new biocides application and participation in the given advice to the Danish Food and Veterinary Administration, Danish Environmental Protection Agency, the Danish Medical Agency and the European Chemicals Agency.

Keywords: Rare Earth Element, thallium, tellurium, plant-based food, toxicity, trace element

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1. Introduction

The history of human kind has been closely connected with metals and metalloids. Most attention has so far been paid to heavy metals and their toxicity to humans, through the food consumption. Nevertheless, in the last decades, the use of various trace metals and metalloids has increased in medicinal, industrial and agricultural applications (Du and Graedel, 2011). As a result, there has been an elevation in their accumulation in the ecosystem (atmosphere, water, soil), resulting in potential human contamination via food, through discharges from mining and mineral processing, liquid industrial waste or disposal of urban and industrial products (Cheng et al., 2015).

In the current review, we focus on the rare earth elements, thallium, and tellurium. It has been documented that they can pass through the gastrointestinal tract and accumulate in the human body causing short- or long-term structural or functional alterations that can eventually lead to a toxic effect.

1.1. Rare Earth Elements

The REEs are 17 f-block inner transition elements of the periodic table, consisting of the 15 lanthanides: lanthanum (La), cerium (Ce), praseodymium (Pr), neodymium (Nd), promethium (Pm), samarium (Sm), europium (Eu), gadolinium (Gd), terbium (Tb), dysprosium (Dy), holmium (Ho), erbium (Er), thulium (Tm), ytterbium (Yb), and lutetium (Lu), and scandium (Sc) and yttrium (Y) (Connelly and Damhus, 2005). They have similar physicochemical properties and are classified into light (Ce, La, Pr and Nd), medium (Sm, Eu and Gd) and heavy (Tb, Dy, Ho, Er, Tm, Yb, Lu and Y) REEs, according to their ionic radii, which for the trivalent REEs is similar to the one of calcium (Ca). Despite their name, most of the REEs are abundant in Earth's crust, with Ce being more plentiful than copper (Cu). Due to their geochemical properties though, they are dispersed and not found highly concentrated in minerals (Haxel et al., 2020). The REEs and alloys containing them are used in many technological devices, like cell phones, computers and rechargeable batteries for phones, cameras, electric and hybrid vehicles (Zhou et al., 2017).

REEs are distributed to and accumulated at elevated concentrations in the liver, eyes, bone, spleen, lungs, kidneys, testis, brain, heart and adipose tissue (Fei et al., 2011; Kawagoe et al., 2005). The distribution ratio in the organs is higher for the heavier REEs (Nakamura et al., 1997). More specifically, it has been proved that the lighter lanthanides, as well as Eu, accumulate in the microsomal fraction of the liver, in the spleen and other organs rich in reticuloendothelial cells (Ohnishi et al., 2011; Magnusson, 1963; Haley, 1965; Durbin et al., 1956). Yb has been shown to accumulate in the brain, liver and femur, while Tb has been found in liver, lung and spleen, with a very slow elimination rate (Feng et al., 2007; Shinohara et al., 1997).

Seven out of the seventeen REEs (Y, La, Ce, Nd, Gd, Tb and Yb) have been reported to have oxidative stress-related negative impacts. The most analysed endpoints were the lipid peroxidation, reactive oxygen species (ROS) production and formation of antioxidant activities, such as superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) (Marubashi et al., 1998; Tseng et al., 2012; Huang et al., 2011; Wu et al., 2013; Zhao et al., 2011b; Xia et al., 2011; Kumari et al., 2014b).

Furthermore, exposure to elements like Ce and La can lead to a decrease in body weight, accumulate in the liver and brain, and lead to alterations in the histopathology and organ function (Aalapati et al., 2014; Hong et al., 2014; Kumari et al., 2014a; Peng et al., 2014; Sang et al., 2013; Zhao et al., 2011b). La has been shown to affect the spatial learning ability and memory of rats, potentially because of the inhibition of the signalling pathway in the hippocampus (Liu et al., 2014). Another study on La administration, during and after the pregnancy in mice resulted in smaller brain size and indication that this element is a potentially behavioural teratogen (Briner et al., 2000).

The REEs have already been found in edible plant-based foodstuff, like fresh edible fungi, vegetables (leafy, fruiting, legume, root, brassica, and bulb), and cereals (corn, rice and wheat flour) (Jiang et al., 2012; Zhuang et al., 2017; Howe et al., 2005).

1.2. Thallium

Thallium (Tl) is an element belonging to the same family as aluminium, gallium and indium. It has been used as a pesticide for rodents and insects. Nowadays, it is used in the manufacturing of optic lenses and glass, in pharmaceuticals, medicine, alloys and electronics (International Programme on Chemical Safety, 1996; NLM, 1998; EPA, 1991b; Agency for toxic substances and disease registry,

1992). While, naturally, it is found in small concentrations, thallium accumulation in soil has been increased because of human activity, like copper mining, during the smelting of ores (lead, copper, zinc) and petroleum-refining processes (Nriagu, 1998; Queirolo et al., 2009; Kazantzis, 2000). It is considered a highly toxic element and once it enters the body, it is absorbed via gastrointestinal and respiratory tracts and widely distributed. It accumulates in the bone, liver, heart, muscle, lung, central nervous system and renal medulla (Léonard and Gerber, 1997; John Peter and Viraraghavan, 2005; Galvan et al., 2000).

Several mechanisms and modes of action have been proposed to explain the toxicity of Tl. Its chemical properties are similar to those of potassium (K). Thus, Tl can replace K and modify enzymatic activation of the Na+/ K+ ATPases, pyruvate kinase and other proteins, to move via the membrane system and accumulate in the cell (Britten and Blank, 1968; Kayne, 1971). Another mechanism proposes that Tl inactivates sulfhydryl groups, responsible for increasing the permeability of mitochondria, leading to water influx and swelling (Spencer et al., 1973; Maya-López et al., 2018). Exposure to Tl during pregnancy has been linked to decreased mitochondrial DNA in neonates (Wu et al., 2019).

Tl concentrations have been detected in grain and cereal, leafy vegetable (cabbage, lettuce, and kale), oilseed rape, bean and potato samples (Asami et al., 1996; Małuszyński, 2009; Liu et al., 2019; Xiao et al., 2014).

1.3. Tellurium

Tellurium (Te) is a metalloid included in the same group as oxygen, sulfur, selenium and polonium. It has been used as catalyst and pigment for ceramics, in metallurgy as an additive to other metals, in glass optical fibres for telecommunications, as well as in magnetic disk and solar panel manufacturing. Its alloys with other metals are used for nanomaterials, such as quantum dots (Fairhill, 1969; Kominkova et al., 2017; Nishii et al., 1992). Te is distributed to the kidneys, liver, bone, brain and testes (MEDITEXT, 1997). It has been reported that plants that accumulate selenium, can accumulate also Te. Therefore, there is a risk of Te exposure, after consuming a contaminated edible plant (onions, garlic) (Cowgill, 1988). Furthermore, Te's chloride accumulates in brain cells, called astrocytes, and causes cytotoxicity (Roy and Hardej, 2011).

In addition to onions and garlic, Te has been found in baby food samples, citrus fruits, cereals, vegetables, legumes and potatoes (Filippini et al., 2019; Ruiz-de-Cenzano et al., 2017).

2. Description of work programme

2.1. Aims

The main aim of the present work programme was to provide an overview of an area, still not completely explored. More specifically, this report reviews the current literature of the REEs, thallium and tellurium, regarding their potential toxicity after short- or long-term exposure, along with their concentrations in plant-based food including baby food. Moreover, the current work programme allowed the fellow to become familiar with how to conduct assessments in response to a request (public authorities, stakeholders) in compliance with ethical standards to prevent conflicts of interest.

2.2. Activities/methods

The project is comprised of a literature review of relevant publications (scientific papers and reports) published since 1956 on numerous trace metals and metalloids. The search was based on two factors:

1) Their potential toxicity on humans, mainly after oral administration.
2) Their occurrence in the human diet, and more specifically in edible plants.

The initial plan was to perform chemical analysis and retrieve occurrence data of the aforementioned elements in plant-based food. Due to the COVID-19 crisis, there was a lockdown at the university and the performance of chemical analysis in the risk assessment of a specific scenario was not feasible. Therefore, a more theoretical approach, for the final part of the project, had to be adapted.

2.2.1. Secondary activities

Apart from the main project, the fellow participated in weekly group and monthly division meetings and consultations with other colleagues over the entire period of the EU-FORA fellowship programme.

Besides, the hosting institution encouraged the fellow to participate in the postgraduate course 'Risk Analysis in Food Safety', divided in microbiological and chemical risk assessment. The chemical risk assessment module included a group case study, with a final report and poster presentation, elaborating on the risk assessment on a chemical hazard.

The fellow was part of the evaluation of applications and requests, related to biocides' products that meant to be used mainly as disinfectants. Furthermore, the fellow was taking part, monthly, in recommending and consulting the Danish Food and Veterinary Administration, the Danish Environmental Protection Agency, and the Danish Medical Agency regarding residues in food.

During the fellowship programme the fellow participated in numerous secondary activities, provided by EFSA and DTU (Appendix B).

3. Conclusions

The widespread application of the aforementioned elements in numerous industries and agriculture is increasing, by possibly leading to an increase of their concentrations into the environment and consequently our food. Therefore, the fact that these potentially toxic elements have been already detected in several plant-based foodstuff is of concern (Appendix A).

For some of these elements, we have acquired knowledge regarding their adverse effects in human health. However, most of the animal studies up to now are limited to few REE (mostly Ce and La), and short-/medium-term tests (Pagano et al., 2015a; Pagano et al., 2015b).

The little research regarding their potential on humans toxicity combined with their high request in the technological applications has led to an apparent need to extend the research on this field, including hazard evaluation and risk assessment. The same argument applies for Tl and Te, as studies of long-term exposures and life-long observations are yet lacking, while their occurrence in human's diet is evident.

The present study constitutes just the first step of all the steps needed to establish a chemical risk assessment regarding the potential risks posed by REEs, Tl and Te present in plant-based foodstuff, including baby-food. Nevertheless, this is a key step to estimate the size of this risk, according to the current knowledge. The next step would be to evaluate their content in foods that are consumed by the general population and to estimate their actual dietary intake.

From a broader perspective, the EU-FORA programme provided the means to a fast and extensive first-hand knowledge and experience of food risk assessment. During the modules and hands-on training, the EU-FORA fellowship programme offered a unique opportunity of networking and enhancing the cooperation among the food safety agencies. In a multicultural atmosphere, the colleagues of the National Food Institute, DTU provided the expertise and mentoring, and creating the ideal environment for knowledge exchange on food safety. Therefore, National Food Institute makes a suitable host site for future EU-FORA fellows.

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Abbreviations

AGES	Austrian Agency for Health and Food Safety
BfR	German Federal Institute for Risk Assessment
CAT	catalase
DTU	Technical University of Denmark
DW	dry weight
ECHA	European Chemicals Agency
EFET	Hellenic Food Authority
EPA	Environmental Protection Agency
EU-FORA	European Union Food Risk Assessment
fw	fresh weight
GPx	glutathione peroxidase
REEs	rare earth elements
ROS	reactive oxygen species
SOD	superoxide dismutase

Appendix A – Occurrence data for REEs, Tl and Te in plant-based foodstuff

Foodstuff (study)		Element/concentration	
Cereals (wheat, maize, legume) (Zhuang et al., 2017)		REEs: Ce, La, Nd > 90% of total REE for mining area + Gd, Y Mining area 74.22 μg/kg Control area 47.83 μg/kg	
Survey in the major foods in China (Jiang et al., 2012)	Cereals Fresh vegetables	Total REEs 0.039 mg/kg 0.052 mg/kg	
Survey of food crop categories: Fruits, Legumes, Vegetables (leafy & roots) (Howe et al., 2005)		Ce: 0.24 mg/kg (Callaloo) Eu: 2.8 μg/kg (red kidney beans) La: 0.35 mg/kg (Callaloo) Sc: 21 μg/kg (Turnip) Sm: 27 μg/kg (Callaloo)	
Baby food (Ruiz-de-Cenzano et al., 2017)	Puree of fruits (100 g/100 g), (peach, banana and grape juice from concentrate), corn starch and vitamin C	Te: 2 μg/kg (fw)	
	Green beans (40%), skimmed milk (32%), potatoes (27%), onion, milk cream (3%)	Te: 2.94 μg/kg (fw)	
	Skimmed milk (32%), water, beans (14%), peas (10%), onion (5%), pasta (4%), rice	Te: 2.45 μg/kg (fw)	
	Potatoes, skimmed milk, monkfish (10%), tomato, onion, butter, celery	Te: 2.58 μg/kg (fw)	
Survey in Italian population (Filippini et al., 2019)	Cereals and cereal products All vegetables Legumes Potatoes Fresh fruits Dry fruits, nuts, seeds	Te (mg/kg): 0.168 0.246 0.382 0.189 0.185 1.072	Tl (mg/kg): 0.055 0.256 0.001 0.046 0.001 0.648
Pyrite mining area: upstream, midstream and downstream zones (Liu et al., 2019)			
Green cabbage		Tl: 0.85 ± 0.04 mg/kg	
Sweet potato		Tl: 2.78 ± 0.12–0.31 ± 0.01 mg/kg	
Tl-rich sulfide mineralisation area (Xiao et al., 2014)			
Green cabbage		Tl: 338 mg/kg	
Carrot		Tl: 22.1 mg/kg	
Shelled rice		Tl: 2.4 mg/kg	
Waste water-irrigated vegetables (Wang et al., 2013)			
Sweet potato		Tl: 176.7 mg/kg DW	
Green cabbage		Tl: 110 mg/kg DW	
Soya beans		Tl: 51.2 mg/kg DW	
Eggplant		Tl: 56.3 mg/kg DW	
Lettuce		Tl: 22.2 mg/kg DW	

Appendix B – Secondary Activities/Training

	Title	Date
Training sessions provided by EFSA	3-Week induction training at EFSA premises in Parma, Italy	1–20.9.2019
	1-Week training module at the Austrian Agency for Health and Food Safety (AGES) in Vienna, Austria	25–29.11.2019
	1-Week online module organised by the German Federal Institute for Risk Assessment (BfR)	10–14.8.2020
	1-Week online module organised by the Hellenic Food Authority (EFET)	24–31.8.2020
Training sessions	Workshop: Searching for life science and chemistry (Reaxys database – an alternative to Scifinder)	15.1.2020
	Webinar – Metals & their Toxicity	11.2.2020
	Webinar – Rapid Assessment of Contaminant Exposure (RACE) tool	27.4.2020
Other activities	Course: Risk Analysis in Food Safety (Report, Poster presentation)	Winter semester
	Co-advisor of a Master student's report on chemical risk assessment	Spring semester
	Hands-on training on new veterinary medicine and biocides applications	N/A
	Participation in the advice given to the Danish Food and Veterinary administration, the Danish EPA and ECHA	Once per month
	Visit the animal facilities of DTU	19.2.2020

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Risk assessment and toxicological research on micro- and nanoplastics after oral exposure via food products

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Abstract

Plastics are used ubiquitously and have become part of our everyday life. The global production of plastics is rising, which in consequence is leading to increasing amounts of plastics being released into the environment. Recently, the issue of human exposure to micro- and nanoplastic particles and potentially resulting toxicological consequences has been broached, triggered by the discovery of microplastics in foodstuff. In addition to dietary exposure via contaminated food and beverages, other exposure paths such as via air and cosmetics, have to be considered. Currently there is no legislation for microplastics and nanoplastics as contaminants in food. Substantial data gaps with respect to exposure as well as toxicity of such particles impede the risk assessment. Within this EU-FORA fellowship project, a comprehensive data mining approach was followed, focusing on up-to-date knowledge on the occurrence and possible toxic effects associated with micro- and nanoplastics after oral exposure, especially via food products and beverages, in order to provide a basis for risk assessment and to identify important research gaps. The fellowship project was further complemented by practical work aimed at the determination of *in vitro* toxicity of micro-sized polylactic acid particles.

Keywords: microplastics, nanoplastics, food, occurrence, toxicity, risk assessment

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1. Introduction

The present work dedicated to the risk assessment of micro- and nanoplastics after oral exposure via food products was performed in the context of the EFSA EU-FORA fellowship programme. This programme is aimed at early- to mid-career scientists from EU authorities or other Article 36 organisations, to increase their knowledge and experience in food safety risk assessment by practical training (Bronzwaer et al., 2016). The fellow was hosted by the German Federal Institute for Risk Assessment (BfR), Department of Food Safety, jointly by the unit 'Effect-based Analytics and Toxicogenomics' and the Junior Research Group Nanotoxicology.

Plastic material is ubiquitously used in human daily life and is therefore also released into the biosphere. Due to its material properties, in particular its chemical stability, low reactivity and poor degradability, it can accumulate in the environment and reach the animal and human food chain (Bouwmeester et al., 2015). During presence in the environment, various factors can contribute to the decomposition of larger plastic fragments into smaller pieces, the so-called micro- and nanoplastics. The definition of microplastics varies depending on the source. For this reason, in this report microplastics will be referred to plastic particles of different materials and shape, namely fragments, fibres, spheroids, granules, pellets, flakes or beads, in the range of 0.1–5,000 μm, in accordance to the EFSA opinion on the presence of microplastics and nanoplastics in food, with particular focus on seafood, microplastics (EFSA, 2016). A distinction can be made between primary (intentionally produced) and secondary microplastics. According to the European Food Safety Authority (EFSA) and the European Commission's recommendation on nanomaterials definition, nanoplastics can be defined as a material with any external dimension in the nanoscale or having internal structure or surface structure in the nanoscale (0.001–0.1 μm) (EFSA, 2016, European Commission, 2011). In general, there is very little information available on the environmental occurrence and toxicity of nanoplastics.

Recent publications suggest that different types of food and beverages are contaminated with microplastic particles, underlining the relevance of human exposure. Especially smaller particles, which are in a size range of several micro- or millimetre, gained public attention. Up to now, little is known about the risk for human health caused by exposure to micro- and nanoplastics via the diet. With the focus on risk assessment, there are still many data gaps which need to be addressed. First, the detection and quantification of microplastics is still very challenging and available analytical methods are not suitable for all size ranges and food matrices. Detection methods need to be established and validated. Second, microplastics contain a broad mixture of materials, surface properties and material characteristics. Therefore, general statements about 'microplastics' need to be specified in detail to different particle types and cases. Third, little is known about possible effects which can be caused by the oral consumption of microplastic particles. No systematic human exposure studies are available, and data from *in vitro* studies or *in vivo* animal experiments are scarce.

2. Description of work programme

2.1. Aims

The aims of the programme were as follows: first, the fellow should gain experience in performing risk assessment, with a primary focus on micro- and nanoplastics found in food products or beverages. For this purpose, comprehensive literature mining was performed to collect published information and identify data gaps (see Section 2.2.1). Second, the fellow should gain knowledge about practical methods used for the physicochemical characterisation of micro- and nanoplastic particles, as well as for the investigation of their toxicity. In addition, the programme aimed at introducing the fellow to a broader spectrum of risk assessment activities by a combination of in-house and external education activities. The expected scientific outcome of the project was to make a substantial contribution to the risk assessment of micro- and nanoplastics at BfR.

2.2. Activities/methods

2.2.1. Comprehensive revision of available data

Efforts towards a risk assessment of micro- and nanoplastics, i.e. their occurrence as well as their possible health effects when taken up orally via food products, were based on publicly available data, up to and including 1 May 2020. A comprehensive literature search was performed for peer-reviewed articles referring to the presence of micro- and nanoplastics in food and beverages, as well as their

toxicity *in vivo* and *in vitro*. Scientific databases such as 'PubMed', 'Scopus', 'Google Scholar' and 'Web of Science' were searched using various keyword combinations (e.g., "polymer", "microplastics", "nanoplastics", "food", "toxicity", "*in vivo*", "*in vitro*"). Information was extracted from the literature and compiled, and the studies were classified with respect to their quality and suitability for risk assessment. Using data retrieved from the above databases, the fellow had the opportunity to work on a scientific review article jointly authored by the fellow and BfR employees, which has been submitted to a peer-reviewed toxicological journal. In addition, the fellow presented a comprehensive review of available data on the occurrence of micro- and nanoplastics in food and toxicity effects as a poster at the 5th German Pharm Tox Summit held in Leipzig, 2–5 March, 2020.

2.2.2. Practical work, research project on polylactic acid particles

To attain the goal of introduction to micro- and nanoplastics research tools, the fellow has been involved in ongoing research activities of the Department of Food Safety at BfR. The main training objectives included analysis and evaluation of data for preparing working documents, related to the assessment of micro- and nanoplastics via oral exposure, in particular the occurrence and toxicological data. The fellow had the opportunity to learn about *in vitro* models and their applicability to research on micro- and nanoplastics toxicity. To this end, practical work was performed in the laboratory. This included the cultivation of the adherent stable human intestinal cell line Caco-2 in a submerged two-dimensional model as well as the *in vitro* Transwell barrier model. The fellow performed viability testing using colorimetric assays, transepithelial resistance measurements and particle leaching experiments. From the analytical field, the fellow was introduced to dynamic light scattering, element analysis via atomic absorption spectroscopy, and flow cytometry. The fellow was introduced to working with a ball mill and a knife mill for particle grinding. In addition, as part of a visit at the German Federal Institute for Materials Research and Testing, the fellow got insight into artificial *in vitro* digestion, small-angle X-ray scattering and multi-angle light scattering. Polylactic acid (PLA) particles were chosen for experimental investigation.

2.2.3. Training in risk assessment

During the initial phase of the fellowship, the fellow obtained general information on risk assessment activities. This included three weeks of induction training in chemical risk assessment and microbiological risk assessment at the EFSA premises in Parma (2–20 September 2019). An additional 1-week training module on food and feed safety-related risk assessment organised by Austrian Agency for Health and Food Safety (AGES) took place in Vienna (25–29 November 2019). Due to the COVID-19 pandemic, further courses were held as online modules, namely a training module focusing on risk communication and crisis response in Berlin, organised by the BfR (10–14 August 2020), and an online training module focusing on emerging risks in Greece organised by the Hellenic Food Authority (EFET; 24–31 August 2020).

Additionally, at the hosting site BfR, the fellow was acquainted with the risk assessment procedures carried out at the department of food safety. Using current practical examples, the fellow was specifically introduced to the risk assessment of food and feed derived from genetically modified organisms, as well as to the risk assessment of novel foods. The fellow participated in the regular weekly meetings on the current scientific work carried out at the department of food safety at BfR. Moreover, the fellow presented and discussed the results of her project at the department seminar on 7 July, 2020.

Along with the scheduled activities, additional training opportunities were provided by the hosting institution BfR. This helped further improving the fellow's general knowledge on risk assessment. Table 1 presents the supportive training activities organised for the fellow by BfR during the EU-FORA Fellowship.

Table 1: Supportive training activities during the EU-FORA fellowship

	Title	Date
Training sessions	Workshop "Risk Assessment and Risk Management of Genetically Modified Organisms", BfR, Berlin, Germany	9.6.2020
	Workshop "Risk Assessment - Food contamination by plasticisers", BfR, Berlin, Germany	5.5.2020
	10th Berlin Workshop on Developmental Toxicology, BfR, Conference center Berlin Biotechpark	19–20.2.2020
	Creating characters for the BfR as a new line of communication, Berlin, Germany	10.1.2020
	Seminar and workshop "Harmonized exchange of food safety models using web-based services from RAKIP and the AGINFRA + project"	9.12.2019
	An Introduction to the Library of BfR and its Services	15.11.2019
	Testing the study appraisal methodology for the re-evaluation of BPA safety. EFSA webinar	14.11.2019
	Seminar and workshop "Big data and high-throughput driven modeling of health effects of environmental agents"	6.11.2019
Other activities	Submitted review article at Nanoscale Advances-Royal Society of Chemistry "Micro- and Nanoplastics – Current State of Knowledge with the Focus on Oral Uptake and Toxicity"	29.6.2020
	Scientific Conference: One Health EJP Annual Scientific Meeting 2020 (OHEJP ASM 2020), online event	27–29.5.2020
	Scientific Symposium (Poster): 'Risk Assessment review of micro-plastics found in food' presented at the 5th German Pharm-Tox Summit Leipzig, Germany	2–5.3.2020
	Visit at the German Federal Institute for Materials Research and Testing (BAM)	20.11.2019

3. Results and discussion

3.1. Results of the comprehensive literature review

Analysis of the available literature revealed that up to now, there is no sufficient amount of reliable information on the occurrence, composition, particle size and quantity of micro- and nanoplastic particles in food. Ultimately, microplastics can enter the environment in several ways and enter the food chain via the air, seawater, fresh or underground water. It is considered secured that there is an oral exposure to microplastic particles. Microplastics have been detected in a number food products, such as for example drinking water, beverages, honey, mussels, and table salt (see Table 2). Data on nanoplastics is barely available. According to the published data no firm conclusions can be drawn about the quantity and composition of the detected microparticles. Nevertheless, some initial studies worked on the subject of oral exposure. It is obvious that only a small fraction of the broad spectrum of food products has been investigated so far. This includes mainly aqueous and/or simple matrices such as water, beer or honey. The investigation of complex matrices like meat or dough is analytically more challenging. Only for seafood, some results are available, probably due to the high relevance of plastic contaminations in aquatic ecosystems. A comprehensive exposure assessment was not possible since there is a lack of representative data on the occurrence of microplastics in different food groups. Only some selected examples are available (Cox et al., 2019). Another obstacle was that there is no appropriate tool to quantify the dietary exposure of micro- and nanoplastics.

Table 2: Selected results on the occurrence of micro- and nanoplastics in food

Food type	Level of microplastics contamination	Samples	Location	References
Honey	40–660 coloured fibres/kg of honey, with a mean value of 166 ± 147/kg of honey; 0–38 fragments/kg of honey; mean 9 ± 9/kg of honey; fibres and fragments supposed to be synthetic	19	From Germany, France, Italy, Spain and Mexico, from supermarkets (8) or producers (11)	Liebezeit and Liebezeit (2013)
	10–336 fibres/kg and 2–82 fragments/kg supposed to be synthetic	47	From German local supermarkets or beekeepers	Liebezeit and Liebezeit (2015)
	32–108 coloured fibres/kg (mostly cellulose but a minor part being PET fibres); 8–28 blue particles/kg (unknown origin)	5	From 5 locations in Switzerland	Mühlschlegel et al. (2017)
Salt	16–84 item/kg (sea salt), 8–102 item/kg (lake salt) and 9–16 item/kg (rock salt)	16	From Turkish market	Gündoğdu (2018)
	46.7 ± 0.58–806 ± 15.3 particles/kg (average: 212 particles/kg, all positive)	12	From US grocery stores (from North, Celtic, Sicilian, Mediterranean, Utah, Hawaiian, Atlantic, Pacific, Baja Seas and Himalaya)	Kosuth et al. (2018)
	1–10 items/kg (16 positive)	17	From a Malaysian market originating from 8 countries (Australia, France, Iran, Japan, Malaysia, New Zealand, Portugal, South Africa)	Karami et al. (2017)
	50± 7–280± 3 items/kg salt	21	From sea: Atlantic Ocean (Huelva, Cádiz, Lanzarote, La Palma, Galicia), Mediterranean sea (Barcelona, Gerona, Valencia, Alicante, Murcia, Menorca) From well: Alicante, Cuenca, Añana	Iñiguez et al. (2017)
	7–680 items/kg (550-681 particles/kg in sea salts, 43–364 particles/kg in lake salts, and 7-204 particles/kg in rock/well salts)	15	From supermarkets throughout China	Yang et al. (2015)
Sugar	217 ± 123 transparent and coloured fibres/kg of sugar; 32 ± 7 fragments/kg of sugar	5 commercial sugars	–	Liebezeit and Liebezeit (2013)
Fish	1–3 items/contaminated brand (4 contaminated brands – cans containing 2–30 fish [canned sardines and sprats] each)	20 brands (cans containing 2–30 fish each)	From Australian and Malaysian markets (originating from Canada, Germany, Iran, Japan, Latvia, Malaysia, Morocco, Poland, Portugal, Russia, Scotland, Thailand, Vietnam)	Karami et al. (2018)

Food type	Level of microplastics contamination	Samples	Location	References
Beer	2–79 fibres/L (RSDs = 130%), 12–109 fragments/L (RSDs = 205%), 2–66 granules/L (RSDs = 103%)	24	From German local supermarkets	Liebezeit and Liebezeit (2014)
	16 ± 15 fibres/L (Blank sample: 15 ± 9 fibres/L); 21 ± 16 fragments/L (Blank sample: 20 ± 13 fragments/L); 27 ± 10 granules/L (Blank sample: 15 ± 12 granules/L)		–	Lachenmeier et al. (2015)
	0–14.3 particles/L (average: 4.05 particles/L, all positive)	12	From breweries using water from the five Laurentian Great Lakes (US)	Kosuth et al. (2018)
Bottled water	10.4 particles (> 100 μm)/L; 325 particles (6.5–100 μm)/L; 242 bottles contaminated, all single-use plastic bottles except 1 glass bottle	259	From 19 locations in 9 countries (China, USA, Brazil, India, Indonesia, Mexico, Lebanon, Thailand, Germany) – 11 brands	Mason et al. (2018)
	118 ± 88 particles/L (returnable bottles), 14 ± 14 particles/L (single-use plastic bottles), 11 ± 8 particles/L (beverage cartons), 50 ± 52 particles/L (glass bottles)	22 bottles, 3 cartons, and 9 glass bottles	From grocery stores in Germany	Schymanski et al. (2018)
Tap water	0–61 particles/L (average: 5.45 particles/L)	159	From Cuba (1), Ecuador (24), England (3), France (1), Germany (2), India (17), Indonesia (21), Ireland (1), Italy (1), Lebanon (16), Slovakia (8), Switzerland (2), Uganda (26),US (36)	Kosuth et al. (2018)
	Particles > 100 μm: 15.6 particles/50 L (Blank sample: 13.2 particles/50 L) Limit of detection = mean blank + (1.645 × SD for blank) = 29 particles/50 L	17 + 3	Danish tap water	Strand et al. (2018)

Little is known about toxic effects of orally ingested microplastics (Bouwmeester et al., 2015). Due to its inert characteristics, direct effects of the plastic material are only likely at extraordinary high doses, which are often referred to as overload situations. Here, it is not possible to determine a precise molecular mode of action or clear dose–response relationships, since many of these effects are likely to be caused by rather unspecific stress reactions. Another problem is that the vast majority of available studies only show effects of polystyrene particles, since these particles provide the best commercial availability for researchers. Other more abundant materials such as polyethylene, polypropylene or polyvinylchloride are only available as polydisperse powders which are not so well-suitable for experimental investigations unless there are fractioning steps included. Taken together, toxicological investigations of microplastics give only an exemplary insight into possible health impact and do not yet allow for a systematic hazard assessment.

A selection of available studies which investigated possible toxicological effects are shown in Table 3. Many of these studies have been performed with invertebrates, mussels or fish, which means that the results are difficult to compare with the situation in humans. Only a few studies were performed with mice, and no human data is available. The variety of possible effects ranges from inflammatory responses, disturbance of the reactive oxygen species levels and impairment of the immune system to unspecific cellular damage, like for example cell growth disturbance, disturbance of cellular transport processes of cell division, whereas other studies showed that toxicity did not occur even at high concentrations or doses of microplastic particles. Noteworthy, microplastics might also function as a carrier of environmental contaminants, which is referred to as the so-called 'Trojan horse mechanism'. Since there are still many data gaps concerning dose–response relationships, it is not possible to estimate the risk for human consumers, which are exposed to microplastics via the diet.

Table 3: Selected toxicological effects of micro- and nanoplastics

Toxic effects	Microplastics	Model	Main findings	References
Gastrointestinal toxicity	PE	Blue mussel *Mytilus edulis* L.	Notable histological change and a strong inflammatory response	von Moos et al. (2012)
	PS	Adult male zebrafish	PS microplastics increased the expression of IL-1α, IL-1β and interferon in the gut; indicated microbiota dysbiosis and inflammation	Jin et al. (2018)
	PA, PE, PP, PVC and PS	Zebrafish and nematode	Villi cracking and splitting of enterocytes	Lei et al. (2018)
	PS	Male mice	Accumulation of PS microplastics in mice guts, consequently caused the reduction of intestinal mucus secretion damage of gut barrier function; metabolic disorders in mice	Jin et al. (2019)
	PS	AGS cells	Inflammatory gene expressions such as IL-6 and IL-8	Forte et al. (2016)
Liver toxicity	PS	Zebrafish	Inflammation and lipid accumulation both in 5 μm and 70 nm; oxidative stress and alterations in their metabolic profiles; disturbance of lipid and energy metabolism	Lu et al. (2016)
	PS	*Eriocheir sinensis*	Decreased activities of AChE, CAT, and ALT in *Eriocheir sinensis* liver; antioxidants CAT, SOD, GPx and GST level decreased in the liver; expressions of the genes encoding p38 in the MAPK signalling pathway was upregulated while significantly declined in ERK, AKT and MEK	Yu et al. (2018)
Liver toxicity	PS	Mouse	TG and TCH levels decreased; decreases on key gene expressions related to lipogenesis and TG synthesis in liver indicating mouse hepatic lipid disorder	Lu et al. (2018)
	PS	Zebra mussel *Dreissena polymorpha*	Dopamine concentration increased	Magni et al. (2018)
Neurotoxicity	PS	T98G cells	Increases of ROS, oxidative stress	Schirinzi et al. (2017)
Reproductive toxicity	PS	Oysters	Oocyte number, diameter and sperm velocity decreased in oysters	Sussarellu et al. (2016)
	PS	acs-22 mutant *Caenorhabditis elegans*	Accumulation of nanopolystyrene particles in gonad, dysregulation of some oxidative stress genes	Man et al. (2018)

PA: polyamide; PE: polyethylene; PP: polypropylene; PVC: polyvinylchloride; PS: polystyrene.

3.2. Results of the Analysis of polylactic acid particles

PLA particles were obtained from a commercial provider as PLA granules in the millimetre range and were ground using a knife mill. The resulting particle size was determined using a light microscope and dynamic light scattering for the smaller fraction. The particles showed a very broad size distribution, mostly in the micro- and millimetre range.

Two consecutive cell viability assays were used for the determination of PLA toxicity. Cell viability was first determined by the CellTiter Blue (CTB) assay followed by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay according to a previously developed protocol (Sieg et al., 2018). For this purpose, differentiated Caco-2 cells were incubated with 200 μL of different concentrations of PLA microparticles for 24 h and 48 h in 96-well plates. After incubation, 100 μL of the media was removed and 40 μL CTB reagent was added to the cells. After 30 min, fluorescence was determined with a Tecan plate reader (Ex. 560, Em. 590 nm). Next, 10 μL of the MTT reagent in

PBS was added for another 1 h. After that, whole media was removed and 130 µL pre-warmed desorption solution (0.7% w/v sodium dodecylsulfate in isopropanol) was added. Plates were shaken for 30 min and absorption was measured by a plate reader (570 nm) with subtraction of the background absorption (630 nm). Results were normalised to untreated controls after subtraction of treated cell without reference wells. Triton X-100 solution (0.01%) was used as a positive control. The experimental scheme is shown in Figure 1.

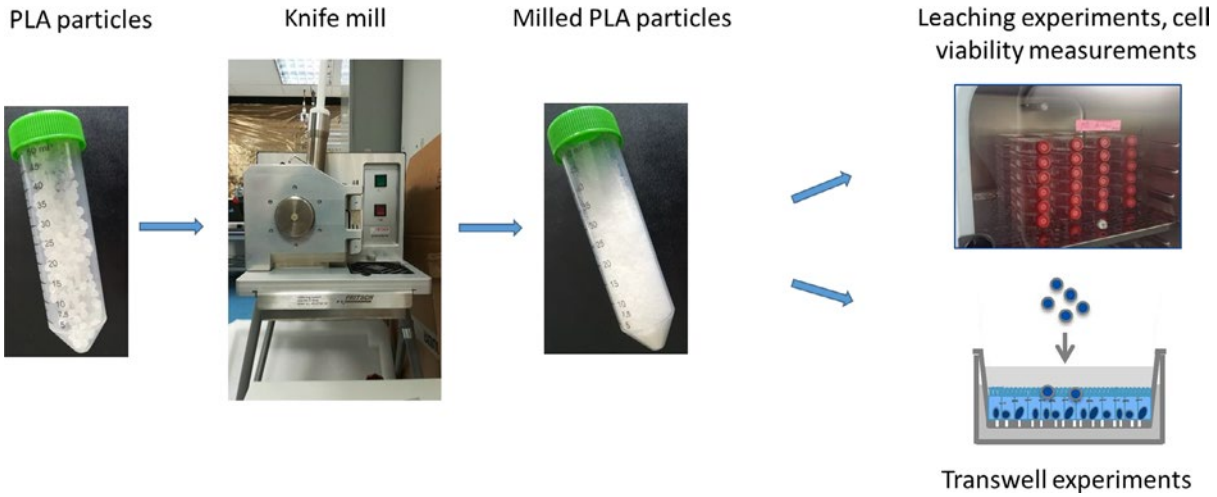

Figure 1: Experimental scheme of PLA particle generation and investigation

None of the PLA microparticles showed substantial cytotoxicity on Caco-2 cells after 24 h or 48 h of treatment. A weak toxic effect was observed with a high concentration of 500 µg/mL only. In addition, leaching experiments in cell culture medium over a period of up to one week showed no altered cell viability on Caco-2 cells when incubated with centrifuged particle supernatant.

4. Conclusions

Overall, the work programme allowed the fellow to gain knowledge on the assessment of micro- and nanoplastics in food. On the one hand, a core area of training was on retrieving, analysing and evaluating available data, focused on the occurrence and toxicological profiles of micro- and nanoscaled plastic particles. On the other hand, the fellow gained knowledge in practical research work on micro- and nanoplastics, with respect to both, particle characterisation and *in vitro* toxicity assessment. The work on plastic particles was embedded into the overall context of food risk assessment and opportunities for scientific networking and collaboration. Results of the fellow's project were presented as a conference poster and will become part of a scientific paper on the oral uptake and toxicity of micro- and nanoplastics to be published in a peer-reviewed scientific journal.

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Abbreviations

AGES	Austrian Agency for Health and Food Safety
BAM	German Federal Institute for Materials Research and Testing
BfR	German Federal Institute for Risk Assessment (Bundesinstitut für Risikobewertung)
CTB	CellTiter Blue assay
EFET	Hellenic Food Authority
EU-FORA	European Union Food Risk Assessment fellowship programme
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay
PA	polyamide
PE	polyethylene
PLA	polylactic acid
PP	polypropylene
PS	polystyrene
PVC	polyvinylchloride

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Risk Assessment of Food Contact Materials

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Abstract

Food contact materials (FCM) can contain chemicals that could migrate from the material itself to the foodstuff posing health concerns if ingested in non-safe quantities by the consumer. FCM include containers, packaging, machinery or kitchenware and can be made from different materials like plastics, paper and board, metal or glass. Printing inks are also an important part of FCM. FCM have an important role in preventing damage or spoilage of the foodstuff and are essential along the food chain. Therefore, their safety needs to be carefully assessed in order to reduce the exposure to potentially hazardous substances and protect the health of the consumer. At the EU level, the legislation on FCM establishes general safety requirements for FCM. In addition, for certain materials, specific measures concerning usage and release of substances have been set. For materials or articles not specifically regulated in this harmonised framework, safety must be proven on a case-by-case basis. National legislations and lists of substances evaluated by competent authorities are important data sources in this context. One of the most important databases are the 'BfR Recommendations on Food Contact Materials' and the soon to come German national regulation on printing inks. BfR Unit 74, besides dealing with chemical risk assessment of FCM, is responsible for the evaluation of application dossiers for including substances into the BfR recommendations on FCM or the substance list of the printing inks regulation. Through the proposed work programme the fellow has been involved in risk assessment of substances that migrate from FCM into foodstuff gaining experience in the methodologies used to perform the scientific data evaluation as well as to support the BfR Unit 74s work.

Keywords: chemical risk assessment, food contact materials, *in silico* toxicology

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1. Introduction

Food contact materials (FCM) are materials and articles intended to come into contact with food at any level of the food chain including processing, preparation, storage, serving, etc. Chemical substances used in the production of FCM are several thousand considering both intentionally added as well as known and unknown non-intentionally added substances (NIAS) (Geueke et al., 2014). Migration of these substances or their breakdown or reaction products into foods can occur. In order to protect the consumer health an appropriate chemical risk assessment needs to be undertaken.

The framework regulation for FCM is the regulation (EC) No 1935/2004 (European Commission, 2004) which lays down the general safety principles for all FCM – first of all, that FCM must not endanger human health. Another EU regulation, which FCM have to comply with, is regulation (EC) No 2023/2006 on 'Good manufacturing practice' (GMP) (European Commission, 2006a) that applies to all stages in their manufacturing chain. Besides the general legislation, specific European Union (EU) measures exist for some FCM such as plastic materials (also recycled), ceramics, regenerated cellulose films, active and intelligent materials as well as for some substances including bisphenol A, epoxy derivatives and nitrosamines. However, there are many materials and substances not specifically regulated in a harmonised way and in these cases risk assessment has to be conducted on a case by case basis.

BfR Recommendations on Food Contact Materials (https://bfr.ble.de/kse/faces/DBEmpfehlung_en.jsp) represent a standard for the production of materials not subjected to any specific legislation and are well accepted by other European Commission member states according to the mutual recognition principle. BfR Recommendations are not legal norms but 'reflect the current state of science and technology for the conditions under which consumer products made of high polymer materials meet the requirements of § 31.1 of the German Foods, Consumer Articles and Feed Act (LFGB) and Article 3.1 of Regulation (EC) 1935/2004 on Materials and Articles Intended to Come into Contact with Food as to their safety for human health'.

The BfR unit 74 'Safety of Food Contact Materials', where the fellow has been placed in, deals with risk assessment of chemical substances that migrate from FCM into food or food simulant. The unit is part of the Department 7 'Chemicals and Product Safety' of the BfR that assesses substances in context of the REACH regulation and is involved in the assessment of the health risks relative to chemicals, cosmetics, FCM, toys and other consumer products.

One important task of the BfR Unit 74 is to evaluate dossiers for including substances into the BfR Recommendations. The dossier evaluation process includes checking compliance with the requirements set by the EFSA 'Note for Guidance For the Preparation of an Application for the Safety Assessment of a Substance to be used in Plastic Food Contact Materials' (https://www.efsa.europa.eu/en/efsajournal/pub/rn-21) as well as assessing the scientific information provided by the applicant. The evaluated dossiers are then further discussed with external experts in the Panels 'Toxicology' and 'Petitions' of the BfR Committee for Consumer Products (BeKo). The unit elaborates also scientific opinions about health risk of substances who need a review or an up-to-date.

2. Description of the work programme

2.1. Aims

The overall aim of the programme was to learn how the risk assessment workflow of substances from FCM works. To achieve this purpose, the fellow has been involved in the ongoing activities and projects of the BfR unit 74. Specifically, the training objectives to be addressed included the dossier evaluation process, insight into the analytical and toxicological data, the use of *in silico* tools such as QSAR Toolbox, Toxtree, Derek and Sarah Nexus and practical experience in the German National Reference Laboratory for Materials in contact with food (NRL-FCM). Moreover, the training purpose of the work programme has been a suitable opportunity for the fellow to take part actively to the projects and to contribute to current assessment issues of the host unit.

2.2. Activities/methods

In order to accomplish the training objectives the following activities, concerning relevant risk assessment issue of the unit, were carried out:

i) scientific evaluation of two dossiers (confidential) from current issues. The evaluation consisted in reviewing analytical and toxicological data provided by the applicant in

accordance to the EFSA note for guidance. The dossiers are submitted for substances to be included in the BfR recommendations on food contact materials.

ii) review and update of the risk assessment for certain substances, used for printing inks in FCM (presented at the 5th German Pharma-Tox Summit in Leipzig, 2–5 March 2020, see Annex A)

iii) review and update of the risk assessment of styrene oligomers with special focus on *in silico* genotoxicity prediction

iv) determination of migration of aluminium and boron from paper and board FCM by means of cold-water extract in combination with inductively coupled plasma mass spectrometry (ICP-MS).

Because of data confidentiality concerning the dossiers, only the projects (ii)–(iv) will be further elaborated.

2.2.1. Risk Assessment review for printing inks in FCM (see Annex **A**)

Printing inks are complex chemical mixtures that can include pigments, solvents, monomers, photoinitiators and others. They are mainly applied to the non-food contact surface of the food packaging but, although the printed surface is not in direct contact with the foodstuff, migration can still occur through diffusion, partition or set-off phenomena (Aznar et al., 2015; Lago et al., 2015).

At the European level, a specific regulation for printing inks in FCM is missing. As a final component of a packaging material, they have to comply with the EU Regulation (EC) No 1935/2004 (European Commission, 2004) on FCM and the EU regulation (EC) No 2023/2006 (European Commission, 2006a) on GMP. In absence of European specific regulation for printing inks in FCM, evaluations of national authorities and national legislations are used to prove the safety of the inks according to regulation (EC) 1935/2004.

The most complete national legislation in Europe is the Swiss Printing Inks Ordinance (https://www.blv.admin.ch/blv/en/home/gebrauchsgegenstaende/materialien-in-kontakt-mit-lebensmitteln.html). Specifically, Annex 10 of the Swiss Ordinance deals with printing inks included in a positive list which is further divided into List A (evaluated substances for which a specific migration limit is given) and List B (non-evaluated substances).

The BfR started its own evaluation on printing inks in 2010 with a positive list based on the Swiss list A. Since 2012 BfR and Swiss Federal Food Safety and Veterinary Office (FSVO) started a joint evaluation for printing inks in FCM in order to harmonise the two national positive lists. The German and the Swiss authorities independently carry out evaluations for the new substances and twice a year the submitted dossiers are discussed and approved in a joint meeting.

For some of the already evaluated substances, the basis for the positive listing is old or unknown. In order to fill the possible gaps and to better harmonise the two lists, a risk assessment review for these substances has been performed.

As a starting point, substances that have been already evaluated under the OECD high production volume (HPV) chemicals Programme with an existing assessment (OECD-SIDS), have been selected. Then, a tiered approach for toxicological data was applied based on the EFSA note for guidance. According to that, in case of low migration of the substance into food (< 0.05 mg/kg food) only absence of genotoxicity has to be proven. For migration values between 0.05 and 5 mg/kg food, an *in vivo* subchronic study and data on accumulation in men have to be provided in addition to the genotoxicity tests, while for high migration values (> 5 mg/kg per food) a full toxicological data set (including information on absorption, distribution, metabolism and excretion (ADME), studies on reproductive toxicity, teratogenicity, chronic toxicity/carcinogenicity studies) is needed.

For each substance, a careful revision of the toxicological data provided in the OECD-SIDS was performed by the fellow. In addition, a literature search for new data was conducted with a focus on *in vitro* and *in vivo* genotoxicity tests, 90-day repeated oral toxicity and carcinogenicity studies. The reliability of each test was a key point of the review. Overall, 16 substances were re-evaluated (Table 1).

Two pigments have been assessed considering the category approach and three solvents using the analogue approach meaning that data on chemicals belonging to the same category or to metabolites or analogues were considered for the evaluation. Particular attention has been given to the genotoxicity endpoint, which needs to be ruled out. As already mentioned, reliability has been a crucial point; it reflects data quality and can deeply influence the overall outcome. The reviewed specific migration limit (SML) values have been reported and compared with the ones already published in the Annex 10 of the Swiss Ordinance. These preliminary results will be discussed with the FSVO in order to further refine and harmonise the two lists.

Table 1: Results of the toxicological assessment review considering studies required for the evaluation

Name CAS	SML		SIDS	*In vitro*			*In vivo*	90 days	Carc.
	FSVO	BfR		Ames	Mamm	ca			
Hexylene glycol 147-14-8	5	0.05*	Yes	✓R	×	✓R	×	✓R	×
Pigment Blue 15 147-14-8		ND*	Yes	✓NR	×	✓NR	×	✓NR	✓NR
Natural Blue 1 482-89-3		ND*	Yes	✓NR	×	✓NR	✓NRm	×	✓NR
Pigment Yellow 13 5102-83-0		ND*	Yes	✓RR	×	×	×	×	×
Pigment Yellow 83 5567-15-7		ND*	Yes	✓R	×	×	×	×	✓NR
Pigment Yellow 12 6358-85-6, same category	NL	NL	Yes	✓RR	×	✓RR	×	×	✓RR
Pigment Yellow 14 5468-75-7		ND	NO	×	×	×	×	×	×
Cyclohexane 110-82-7	1	1*	**SIAP** EU-RAR	✓NR	✓NR	×	✓NRca	✓NR	✓NR
Tributyl phosphate 126-73-8	0.05	0.05**	Yes	✓R	✓NR	✓NR	✓Rca	✓R	✓R
Dibutyl adipate 105-99-7	0.05	0.05**	Yes	✓NR	×	✓NR	×	×	×
N-vinyl-2-pirrolidone 88-12-0		ND*	**SIAP** EU-RAR	✓NR	✓NR	✓NR	✓NRm	✓RR	✓NR
n-Propyl acetate 109-60-4		*	Yes	✓R	×	×	×	×	×
n-Propyl alcohol 71-23-8, analogue		(10/2011)	NO	×	×	✓RR[1]	×	×	×
n-Butyl acetate 123-86-4, analogue		(10/2011)	Yes	×	×	✓RR	×	✓R	×
Ethyl acetate 141-78-6, analogue		(10/2011)	Yes	×	×	✓RR	✓RRm	✓R	×
Isobutyl acetate 110-19-0	1	1*	Yes	✓RR	×	×	×	×	×
Isobutanol 78-83-1, analogue	1	1	Yes	×	×	×	✓Rm	✓RR	×
2-Butanol 78-92-2	1	1*	Yes	✓R	×	✓R	Unknown NRm	×	×
2-Butanone 78-93-3, analogue	5	5	Yes	✓NR	✓NR	✓NR	✓Rm	✓R	×
Pigment Yellow 53 8007-18-9		ND*	Yes	✓R	✓RR	✓R	×	✓RR	×
Pigment Green 7 1328-53-6		ND**	Yes	✓R	×	✓R	×	✓RR	×
Bis(2ethylexyl)azelate 103-24-2	0.05	0.05**	Yes	✓R	×	✓R	×	×	×

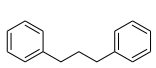
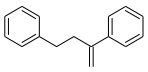
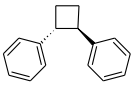
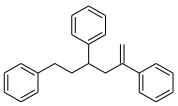
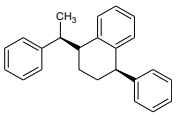
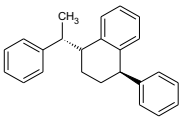
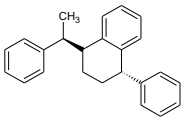
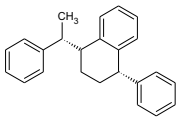
✓R = negative and reliable; ✓NR = negative and NOT reliable; ✓RR = negative and reliable with restrictions; ✓R = positive and reliable; ✓NR = positive and NOT reliable; × = not performed; ND = not detectable < 10 ppb; 1 = reported in the SIDS of n-propyl acetate; SIAP = SIDS Initial Assessment Profile; EU-RAR = EU Risk Assessment Report; ___ category approach; ___ analogue approach; grey = analogues not included in the list but used for the risk assessment * no accumulation; ** possible bioaccumulation; NL = not listed; (10/2011) = covered by reference to reg. (EU) No 10/2011; ca = chromosomal aberration test; m = micronucleus test; Ames = bacterial reverse mutation test; Mamm = gene mutations in mammalian cells.

2.2.2. *In silico* genotoxicity prediction of styrene oligomers

Styrene oligomers are NIAS produced during the manufacturing process of polystyrene. The latter can be used for FCM, e.g. packaging or take-away tableware. The Commission Regulation (EU) No 10/2011 on food contact plastics requires the risk assessment of NIAS by the business operator (Article 19 (European Commission, 2011)).

Recently, an official German laboratory for food surveillance (CVUA-MEL) performed migration tests on 7 different styrene (Table 2) from 12 commercially available polystyrene FCM. A summed migration of up to 51 μg/kg food simulant (Ethanol 50%, 2 hours at 70°C) was measured. Considering that, in 2016 BfR performed the risk assessment of these investigated styrene oligomers (BfR, 2016). Based on the level of migration and on the available toxicological data, there was no evidence for health risk.

Table 2: Chemical structure of styrene dimers and trimers

Dimers					
Trimers					

In 2019, a new risk assessment on styrene oligomers from polystyrene containers was published (Gelbke et al., 2019). A detailed literature search was conducted updating the information on migration and toxicological studies. Margin of safety (MoS) were calculated considering different approaches (no observed adverse effect level (NOAEL) or threshold of toxicological concern, TTC) and different tiers of exposure were applied (migration from food simulants, concentration in foodstuff, FACET methodology). Overall, the performed risk assessment showed a low risk for consumers.

Only two genotoxicity studies on styrene dimers and trimers are available so far. In 1990, Grifoll and co-workers performed Ames tests using the *Salmonella* Typhimurium strain TA98 with metabolic activation on chromatographic fractions isolated from river and marine sediments (Grifoll et al., 1990). In Nakai et al. (2014), investigated styrene oligomers extracted from polystyrene intended for FCM use in an Ames test and in an *in vitro* chromosomal aberration test according to the relative OECD guidelines. In both studies, no evidence for genotoxicity was observed but considering each specific assay, many limitations apply.

When data on genetic toxicity of NIAS are scarce, computational (*in silico*) methods can be used in combination with existing data in order to improve the confidence of the outcome. In recent years, these *in silico* methodologies have been promoted by regulatory authorities (European Commission, 2006b; EFSA, 2017; EMA, 2018) and have been used in many regulatory fields (impurities in pharmaceuticals, NIAS).

The main goal of this project was to review of the literature on styrene oligomers focusing on migration, (geno)toxicity and endocrine disruptor activity data as well as to use *in silico* tools for genotoxicity prediction in order to strengthen the risk assessment. For genotoxicity prediction four independent software programs were used, the knowledge-based OECD (Q)SAR (quantitative/ qualitative structure activity relationships) Toolbox v. 4.4 and DEREK Nexus v. 6.0.1 (Lhasa Limited 2.2.2) and the statistically based Sarah Nexus v. 3.0.0 (Lhasa Limited 2.2.2) and DTU (Q)SAR database (http://qsar.food.dtu.dk). The results are intended to be presented in a publication later on.

2.2.3. Content determination of aluminium and boron from cold-water extracts in paper and board

Paper and board are commonly used as FCM, and they can contain certain elements or their salts as residues derived from the production process. If they migrate into food, some of them could rise toxicological concerns because of their potential for accumulation in the cells and organs and for causing many diseases and disorders even at low concentration.

In the BfR recommendation XXXVI on Paper and Paperboard, guidance values for the maximum content or migration are given for many substances, including aluminium- and boron-compounds (BfR-Recommendations). In this context, the aim of this study was to determine the aluminium and boron

release from paper samples. The work has been performed at the NRL-FCM, which develops and validates analytical methods for the determination of the release of chemicals from FCM, conducts comparative laboratory tests or proficiency tests and establishes a national network within Germany for laboratories responsible for FCM.

Cold-water extraction was performed according to CEN standard EN 645 (Gelbke et al., 2019).

ICP-MS (iCAP™ TQ Thermo Scientific™) was used for the determination of aluminium and boron in the extracts according to a standard operating procedure. For each paper sample 12 replicate extractions were carried out, and measurements were performed in triplicate.

3. Conclusions

Overall, the work programme allowed the fellow to get a deep insight into chemical risk assessment applied to food contact materials. Specifically, it has provided the fellow to experience the dossier evaluation procedure and the decision-making process for the inclusion of substances into the 'BfR recommendations on Food Contact Materials'. Then, other projects were performed: the risk assessment review for printing inks, that implied to retrieve relevant data from existing assessments with a specific attention given to their reliability, and the *in silico* genotoxicity prediction for styrene oligomers, that allowed the fellow to learn using *in silico* tools for the toxicological assessment of NIAS. Furthermore, some practical work in the NRL-FCM has been performed to complete the training objectives.

Beside the scientific training and achievements, the programme has been a unique opportunity for the fellow to build a strong network with the experts gaining a worthwhile experience and a growth mind-set that will be valuable beyond the fellowship itself.

4. Disclaimer

The results of the *in silico* genotoxicity prediction of styrene oligomers are intended to be published in a peer-review journal. In order to avoid copyright claims they were not included in the technical report.

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Abbreviations

ADME	absorption, distribution, metabolism and excretion
BeKo	BfR Committee for Consumer Products
BfR	German Federal Institute for Risk Assessment
CEN	European Committee for Standardization
CVUA-MEL	Chemical and Veterinary Investigation Office Münsterland-Emscher-Lippe
DTU	Technical University of Denmark
FACET	Flavours, Additives, and food Contact materials Exposure Task
FCM	food contact materials
FSVO	Federal Food Safety and Veterinary Office
GMP	Good Manufacturing Practice
HPV	high production volume
ICP-MS	inductively coupled plasma-mass spectrometry
MoS	margin of safety
NIAS	non-intentionally added substances
NOAEL	no observed adverse effect level
NRL-FCM	German National Reference Laboratory for Materials in contact with food
OECD	Organisation for Economic Co-operation and Development
QSAR	quantitative/qualitative structure activity relationships
SIDS	Screening Information Datasheet
SML	specific migration limit
TTC	threshold of toxicological concern

Appendix A – Training activities

What	Title	Contribution	Where	Date
Seminar	Big data and high-throughput-driven modeling of health effects of environmental agents Prof. Roland Grafström, Karolinska Institut Stockholm	Attendance	BfR	6.11.2019
Meeting	Toxicological Subcommittee meeting BfR unit 74	Oral presentation	BfR	12.11.2019
Webinar	Testing the study appraisal methodology for the re-evaluation of BPA safety EFSA	Attendance	On-line	14.11.2019
Seminar	An introduction to the BfR library and its services Benedikt Hummel, Head of BfR Library	Attendance	BfR	15.11.2019
Workshop	Harmonized exchange of food safety models using web-based services from RAKIP and the AGINFRA+ project BfR	Attendance	BfR	9.12.2019
Seminar/ Workshop	Creating characters for the BfR as a new line of communication" plus Workshop Claudio Canales Rios, BfR	Attendance	BfR	10.1.2020
Seminar	Trust – how we understand, measure and build it Michelle Patel (Food Standards Agency, UK)	Attendance	BfR	29.1.2020
Workshop	10th Berlin Workshop on Developmental Toxicology BfR	Attendance	BfR	19–20.2.2020
Conference	5th German Pharm-Tox Summit 2020 – Leipzig	Poster presentation	University of Leipzig	2–5.2.2020
Webinar	Introductory GastroPlus® Simulation and Modeling Maxime Le Merdy	Attendance	Online	13.4.2020
Workshop	Risk Assessment- Food contamination by plasticisers Dr. Zellmer, Dr. Pirow	Attendance	BfR	5.5.2020
Conference	One Health EJP Annual Scientific Meeting 2020	Attendance	Online	27–29.5.2020
Workshop	Risk Assessment and Risk Management of Genetically Modified Organisms (GMO) Hermann Broll	Attendance	BfR	9.6.2020

Annex A – Poster presented at the 5th German Pharma-Tox Summit in Leipzig, 2-5 March 2020

GERMAN FEDERAL INSTITUTE FOR RISK ASSESSMENT

Risk assessment review for printing inks

BfR Bundesinstitut für Risikobewertung

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EU-FORA European Food Risk Assessment Fellowship Programme

Introduction

Printings inks are complex chemical mixtures that include photoinitiators, solvents, monomers, polymerization stabilizers, colorants. They are usually applied to the non-food contact surface of food packaging, however, migration of ink ingredients could occur due to diffusion, partition and set-off phenomena (Aznar, Vera et al. 2011, Aznar, Domeño et al. 2015).
There is no specific regulation for printing inks on food contact materials (FCM) at European level. In general, printing inks for FCM have to comply with the EU Regulation (EC) No 1935/2004 (EC 2004) on FCM and the EU regulation (EC) No 2023/2006 (EC 2006) on Good Manufacturing Practice (GMP). In Absence of European harmonization for inks in FCM national legislations are used as legal standard in Europe.
The most complete national legislation in Europe is the Swiss Printing Inks Ordinance with positive lists of substances that may be used in printing inks applied to the non-food-contact side. The lists were compiled from lists of the Council of Europe (Res AP(2005)2) and refined together with industry (EuPIA). All substances which could be evaluated by the Swiss Federal Food safety and Veterinary office (FSVO) are included in a list A. After publication of the Swiss Ordinance the German Federal Institute for Risk Assessment (BfR) evaluated in 2010 printing ink substances on its own and compiled likewise a list of evaluated substances. Thereafter, harmonization of the two national lists is under progress. Since 2012 new substances are jointly evaluated.

Swiss Ordinance on FCM
Annex 10: printing inks
List A (specific migration limits are given)
List B (not-evaluated substances)

CH

Draft German Ordinance
Based on List A of the Swiss positive list

DE

JOINT EVALUATION

FSVO FSVO BfR BfR

The German Federal Institute for Risk assessment (BfR) and the Swiss Federal Food safety and Veterinary office (FSVO) cooperate in evaluating substances used for printing inks in order to harmonize the authorized substances (List A only) and the SML values between German and Swiss authorities.

The evaluations are separately carried out by BfR and FSVO and discussed twice a year in the subcommittee for toxicology of the BfR committee for consumer products. Data requirements for the safety evaluation are based on the EFSA Note for guidance.

Methodology

The evaluation of some substances is based on positive listing in various European or national legislation, the basis of the listing being either unknown or rather old. Therefore, this work aims to compile publicly available toxicological data to support these evaluations. As a starting point, substances that have been evaluated under the OECD High Production Volume Chemicals (HPV) Programme have been selected. This was done by comparing the printing ink lists with the 2007 OECD list (OECD 2009) and selecting all matching substances for which a Screening Information Data Set (SIDS) was available. The work was then refined by examining the OECD HPV database and compiling the available toxicological information.

Chemicals were reviewed using the **existing assessments,** such as the OECD-SIDS evaluations, as well as according to the **publicly available** toxicological data.

OECD-SIDS

A **tired approach** was applied according to the EFSA note for Guidance for Food Contact Materials. Required data:
- SML ≤ 0.05 mg/kg food: genotoxicity tests.
- 0.05 < SML ≤ 5 mg/kg food: a 90-day oral repeated dose study and accumulation data in men need to be added to the genotoxicity tests.
- SML > 5 mg/kg food: a full data set is needed, genotoxicity tests, 90-day toxicity, ADME, reproduction / developmental and long-term / carcinogenicity studies.

TOXICOLOGY DATA

Reconsidered SML values will be discussed with the Swiss authorities in order to further refine and harmonize the two lists.

REVIEWED SML

Results

Table 1. Results of the toxicological assessment review considering studies required for the evaluation.

CAS	name	SML mg/kg/day FSVO	SML mg/kg/day BfR	SIDS	*in vitro* gene mutations In bacteria	*in vitro* gene mutations in mammalian cells	*in vitro* chromosomal aberrations	*in vivo*	90-days oral study	carcinogenicity
107-41-5	Hexylene glycol	5	0.05 *	yes	✓R	✗	✓R	✗	✓R	✗
147-14-8	Pigment Blue 15		ND *	yes	✓NR	✗	✓NR	✗	✓NR	✓NR
482-89-3	Natural Blue 1		ND *	yes	✓NR	✗	✓NR	✓NR Micronucleus	✗	✓NR
5102-83-0n	Pigment Yellow 13		ND *	yes	✓RR	✗	✗	✗	✗	✗
5567-15-7	Pigment Yellow 83		ND *	yes	✓R	✗	✗	✗	✗	✓NR
same category	*Pigment Yellow 12*	NL	NL	yes	✓RR	✗	✓RR	✗	✗	✓RR
5468-75-7	Pigment Yellow 14		ND	NO	✗	✗	✗	✗	✗	✗
110-82-7	Cyclohexane	1	1 *	SIAP EU-RAR	✓NR	✓NR	✗	✓NR chromosome abb.	✓NR	✓NR
126-73-8	Tributyl phosphate	0.05	0.05 **	yes	✓R	✓NR	✓NR	✓R chromosome abb.	✓R	✓R
105-99-7	Dibutyl adipate	0.05	0.05 **	yes	✓NR	✗	✓NR	✗	✗	✗
88-12-0	N-vinyl-2-pirrolidone		ND *	SIAP EU-RAR	✓NR	✓NR	✓NR	✓NR Micronucleus	✓RR	✓NR
109-60-4	n-propyl acetate		*	yes	✓R	✗	✗	✗	✗	✗
	n-propyl alcohol		(10/2011)	NO	✗	✗	✓RR¹	✗	✗	✗
analogues	*n-butyl acetate*		(10/2011)	yes	✗	✗	✓RR	✗	✓R	✗
	ethyl acetate		(10/2011)	yes	✗	✗	✓RR	✓RR Micronucleus	✓R	✗
110-19-0	Isobutyl acetate	1	1 *	yes	✓RR	✗	✗	✗	✗	✗
analogue	*Isobutanol*	1	1	yes	✗	✗	✗	✓R Micronucleus	✓RR	✗
78-92-2	2-butanol	1	1 *	yes	✓R	✗	✓R	unknown NR Micronucleus	✗	✗
analogue	*2-Butanone*	5	5	yes	✓NR	✓NR	✓NR	✓R Micronucleus	✓R	✗
8007-18-9	Pigment Yellow 53		ND *	yes	✓R	✓RR	✓R	✗	✓RR	✗
1328-53-6	Pigment Green 7		ND **	yes	✓R	✗	✓R	✗	✓RR	✗
103-24-2	Bis(2ethylexyl)azelate	0.05	0.05 **	yes	✓R	✗	✓R	✗	✗	✗

✓ R = negative and reliable; ✓ NR = negative and NOT reliable; ✓ RR = negative and reliable with restrictions; ✓ R = positive and reliable; ✓ NR = positive and NOT reliable; ✗ = not performed; ND = not detectable < 10 ppb; 1 = reported in the SIDS of n-propyl acetate; SIAP = SIDS Initial Assessment Profile; EU-RAR = EU Risk Assessment Report; ____ category approach; ____ analogue approach; * no accumulation; ** possible bioaccumulation; NL = not listed; (10/2011) = covered by reference to reg. (EU) No 10/2011.

Conclusion

A toxicological risk assessment review for chemical compounds used in printing inks for Food Contact Materials using publicly available toxicological data was performed. The evaluation has been mainly based on data presented in the available OECD-SIDS documents. The results are presented in Table 1. These results will be discussed with the Swiss authorities in order to further refine and harmonize the two lists.

Acknowledgments: We would like to thank the European Food Safety Authority (EFSA) for financing the EU-FORA fellowship.

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Risk assessment regarding the use of *Annona muricata* in food supplements

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Abstract

The current risk assessment was performed in the context of the European Food Risk Assessment Fellowship Programme (EU-FORA) supported by EFSA and was intended to evaluate possible health risks associated with the consumption of *Annona muricata* L. (Annonaceae) and derived food supplements. *A. muricata* grows as a tree and is native to the Caribbean and Central America. Preparations made from different plant parts of *A. muricata* (i.e. fruit, leaves, bark, roots) have been used as herbal medicine and are also marketed worldwide as over-the-counter food supplements that have been purported to support general health or to treat a wide range of health conditions, particularly cancer and parasitic infections. However, open questions remain regarding the safety of *A. muricata*-based food supplements, since Annonaceae have been reported to contain potentially neurotoxic compounds, i.e. acetogenins. The assessment conducted within the present fellowship programme shows that substantial uncertainties exist regarding the safe use of *A. muricata*-based supplements. The available data provide indications of neurotoxic potential of certain *A. muricata* preparations. The paucity of adequate studies, particularly related to long-term use of *A. muricata* supplements, currently does not allow the establishment of a safe intake level. Within this technical report a workflow of the project is presented.

Keywords: *Annona muricata* L., annonacin, botanical preparation, food, food supplement, neurotoxicity, risk assessment

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1. Introduction

1.1. European Food Risk Assessment Fellowship Programme (EU-FORA)

The EU-FORA programme supported by the European Food Safety Authority (EFSA) offers an opportunity to professionals within the European Union (EU) to increase their knowledge and experience in food safety risk assessment (Bronzwaer et al., 2016). The aim of the programme is to contribute to the expansion of the EU's community of scientists working in the field of risk assessment and thus to increase both the pool of experts and to support cooperation with respect to food safety risk assessment activities at both the national and EU levels (Bronzwaer et al., 2016).

The EU-FORA fellow was hosted by the German Federal Institute for Risk Assessment (BfR), Department of Food Safety, Unit of Nutritional Risks, Allergies and Novel Foods. Within the agreed work programme that was entitled 'Risk assessment of botanical preparations used in food supplements and fortified foods', the main task of the fellow was the preparation of a monograph regarding the risk assessment of *Annona muricata* and preparations derived thereof with respect to use in food supplements.

1.2. General background regarding the risk assessment

The use of herbal preparations has gained popularity in industrialised countries as complementary or alternative approach to pharmacotherapy involving synthetic, monosubstance pharmaceuticals (WHO, 2013). With a high demand driven by consumer's health concerns, cultural factors, the belief that herbal preparations are natural and thus safe, and since herbal products are often viewed as being balanced and moderate home remedies, thousands of herbal products, including herbal food supplements, are advertised, marketed and distributed via various channels, including pharmacies, natural herbal shops, online retail stores and social media platforms (Raclariu et al., 2018). Herbal preparations often contain a complex mixture of natural chemicals, the composition of which depends, among others, on plant growth conditions, the part of the plant used for processing and the conditions of processing, i.e. conditions of extraction from the plant. Despite their popularity, the assessment of their safety requires a thorough multidisciplinary scientific investigation and validation of their chemical and biological activities, including potential pharmaceutical, pharmacological and toxicological activities. However, in cases where herbal products are sold as food supplements, surveillance of such herbal products with respect to potential adverse effects (nutrivigilance) remains difficult because these products do not require a medical prescription and are sold as over-the-counter products. Legislative frameworks that take into account monitoring, consistent documentation and evaluation of adverse effects associated with food supplements are not in place in many EU countries.

Herbal products' regulations vary greatly between countries and continents. In the EU/EEA, herbal products fall into two main categories, depending on their primary intent of use: i) 'herbal medicines' that are regulated under medicinal products' legislation and ii) 'herbal food supplements' that are covered by the provisions of food legislation. The European Directive 2002/46/EC defines food supplements as concentrated sources of nutrients or other substances with nutritional or physiological effect whose purpose is to supplement the normal diet. Regarding more specific provisions for nutrients or other substances, the Directive 2002/46/EC so far only regulates which vitamins and minerals may be added to food supplements and which vitamin/mineral substances or compounds may be used. Daily maximum amounts for vitamins and minerals in individual food supplement products have not yet been established at the EU level. With respect to 'other substances with a nutritional or physiological effect', current specific provisions (with only a few exceptions) are lacking as to which 'other substances' may be used in food supplements or regarding daily maximum amounts for individual substances in food supplement products.

Though the quality and safety of herbal food supplements need to fulfil the requirements of food legislation, these are, however, considerably less stringent compared to the medicinal products regulations. Thus, unlike drugs, which must be approved by the competent authorities before they can be marketed, food supplement products do not require premarket review or approval within the EU and their safety and conformity with the food law requirements is under the responsibility of manufactures and suppliers.

Annona muricata L. (Annonacea), known as graviola (Portuguese), guanabana (Spanish), Stachelannone (German) or soursop (English), grows as a tree and is endemic to the warmest areas of the tropics of South and Central America and the Caribbean. In addition, it has been distributed very early to eastern and western Africa, Asia and to south-east China (Wahab et al., 2018). Various

preparations from fruits and other plant parts of *A. muricata* are marketed worldwide as over-the-counter food supplements that are purported to support general health or to treat a wide range of health conditions, particularly cancer and parasitic infections (Badrie and Schauss, 2009; Coria-Téllez et al., 2018). However, open questions remain regarding their safety since Annonaceae have been reported to contain potentially neurotoxic compounds, i.e. acetogenins. *A. muricata* is also listed in the *EFSA Compendium of botanicals reported to contain naturally occurring substances of possible concern for human health when used in food and food supplements* (EFSA, 2012).

The current risk assessment is intended to evaluate possible health risks associated with the consumption of *A. muricata*-based food supplements, based on available published data.

2. Description of the work programme

The hosting Unit in which the work programme was carried out (BfR Unit Nutritional Risks, Allergies and Novel Foods) has long-standing experience in risk assessment of food supplements and fortified foods. One of the major areas of its research relates to the risk assessment of 'other substances' with specific nutritional or physiological effects, including the safety assessment of secondary plant ingredients and plant preparations.

2.1. Aims

The aims of the work programme were for the fellow to i) gain experience in performing risk assessment of 'other substances' used in food supplements or fortified foods, with a focus on substances of plant origin ('botanicals', i.e. plant preparations and secondary plant compounds); ii) to specifically assess the possible health risks associated with the consumption of *Annona muricata*-based food supplements; and iii) to set up further networking and build professional collaboration with the host institution, as well as with other experts in various fields of food risk assessment.

The activities described below were in line with the aims of the work programme proposed by the BfR.

2.2. Activities/methods

2.2.1. Preparation of a monograph for the risk assessment of *Annona muricata*

Possible health risks associated with the consumption of *A. muricata*-containing food supplements were evaluated based on available published data. Risk assessment was performed following the 'BfR Guidance Document for Health Assessments' (BfR, 2010) as well as the EFSA 'Guidance on Safety assessment of botanicals and botanical preparations intended for use as ingredients in food supplements' (EFSA, 2009). A detailed monograph on *A. muricata* was prepared, forming the basis for a publication to be submitted to a peer-reviewed scientific journal.

In the course of the training, the fellow gained experience in i) searches in scientific databases and search strategies to identify relevant scientific publications, ii) systematic data extraction from scientific publications, iii) structuring of scientific data, iv) evaluation of scientific data from individual studies as well as from the overall data situation (i.e. assessment of observed effects, identification of toxicological key parameters, characterisation of dose–response relationships), v) methods for derivation of health-based guidance values, vi) identification of potentially sensitive groups and groups at risk, vii) structure and content of risk assessment monographs, and viii) scientific writing.

The corresponding detailed risk assessment report on *A. muricata* is to be submitted for publication in a peer-reviewed scientific journal shortly. Therefore, in the context of the present technical report, the workflow, methodology and results of the risk assessment are summarised in brief in the following:

Scientific databases such as `PubMed/Medline', 'Scopus', 'Google Scholar' and 'Web of Science' were searched in order to retrieve relevant publications, with the last update being in mid-March 2020. Numerous search strategies were used, including different names of the plant species of interest (i.e. '*Annona muricata*', 'soursop', 'graviola') in connection with terms related to the endpoints of interest, as for instance 'adverse effects', 'toxicity' or 'safety'. This represented the basis for identifying scientific evidence provided in peer-reviewed scientific publications in relation to compounds of potential concern contained in *A. muricata*, alongside with toxicological data and studies reporting adverse health outcomes in humans. Additionally, scientific abstracts, reports as well as pertinent evaluations performed by scientific bodies or national and international authorities dealing with food and drug safety were checked as well. Moreover, the key grey literature was considered, including articles published in non-scientific journals, project reports, and other forms of documentation outside of scientific literature.

Data from the literature studies included in the present risk assessment were retrieved, summarised and arranged, based on the following criteria: 1) chemical composition of constituents of *A. muricata* and of the derived herbal preparations, 2) human studies, 3) acute, subchronic and chronic toxicological animal studies, 4) *in vitro* studies, and 5) potential pharmakokinetic and pharmacodynamic interaction.

With respect to constituents of *A. muricata*, about 200 bioactive secondary plant compounds have been isolated and described from *A. muricata*, with the most abundant being annonaceous acetogenins (ACGs), followed by alkaloids, flavonoids and phenols (Leboeuf et al., 2007; Bonneau et al., 2017; Coria-Téllez et al., 2018). ACGs have been proposed to have cytotoxic, antitumoral, antimalarian, antiparasitic, antiviral, antimicrobial or immunosuppressant activities, or as pesticidal agents, and some are well known to be potent inhibitors of the mitochondrial complex I (NADH-quinone-oxidoreductase) in the respiratory chain (Bermejo et al., 2005; McLaughlin, 2008). Among ACGs, annonacin has been identified as the most abundant in *A. muricata* (Yamada et al., 2014; Coria-Téllez et al., 2018).

Several human observational studies were identified (Caparros-Lefebvre and Elbaz, 1999; Chaudhuri et al., 2000; Caparros-Lefebvre et al., 2001; Caparros-Lefebvre and Lees, 2005). These studies suggested an association between the long-term consumption of fruits and infusions made from other plant parts *of A. muricata* (i.e. leaves) and an increased incidence of movement disorders that resembled Parkinson's disease. In one of these studies. the post-mortem neuropathological and biochemical examination of some affected patients showed an accumulation of tau proteins in the midbrain (Caparros-Lefebvre et al., 2001). However, causality in relation to *A. muricata* is difficult to prove and information provided in the observational studies is insufficient in this respect.

Regarding the use of *A. muricata* in food supplements, scientific information from human intervention studies, which might be used for safety evaluation of *A. muricata,* is currently lacking. This represents a major data gap that impedes risk assessment, especially in the case when long-term use of high supplemental *A. muricata* doses is intended. Along these lines, the importance to include a detailed documentation regarding the incidence of adverse effects and measurements of clinical safety parameters in any future intervention studies is underlined.

Among retrieved animal studies, some 'classical' toxicological *in vivo* studies showed that the exposure to annonacin (whether in the form of *A. muricata* extracts or as purified phytochemical), induced serious neuropathologies in rodents (Champy et al., 2003; Lannuzel et al., 2006; Yamada et al., 2014). For instance, after i.v. application of annonacin over 28 days to rats, annonacin accumulated in the brain parenchyma, decreased brain ATP levels, induced neuropathological abnormalities in the basal ganglia and loss of nigral and striatal neurons in exposed animals (Champy et al., 2003). Moreover, following 1-year oral exposure to *A. muricata* fruit juice, increased numbers of neurons with phosphorylated tau proteins in several brain regions were observed in wild-type and human tau protein transgenic mice (Rottscholl et al., 2016). No further toxicological long-term animal studies with *A. muricata* preparations were identified.

In vitro, annonacin extracted from the root of *A. muricata* promoted death of dopaminergic neurons in embryonic rat mesencephalic cultured cells and caused tau protein pathology in cultured rat striatal neurons (Lannuzel et al., 2003, 2006). A high degree of toxicity on Lund human mesencephalic cells was observed after exposure to preparations from marketed dietary supplements containing leaves and stems of *A. muricata* (Höllerhage et al., 2015).

Regarding *A. muricata* preparations that might be used in food supplements, the composition of such preparations may differ considerably, depending, among others, on conditions of plant growth, conditions of harvest, part of plant used, method of extraction and further processing of the preparation. Reliable toxicological data, human studies or toxicokinetic *in vivo* data, however, are currently lacking for specific food supplement products based on *A. muricata* preparations. There is also a lack of reliable *in vitro* and *in vivo* studies with respect to potential interactions between *A. muricata* preparations and conventional drugs.

In cases where relevant information from safety testing is lacking for certain botanicals and thus the data situation does not provide a sufficient basis for a comprehensive risk assessment, it has been suggested by EFSA to follow a presumption of safety approach. This approach implies that for botanicals traditionally consumed as food, it is assumed that intake, i.e. via supplements or fortified foods, that corresponds to the intake via traditional or normal diet does not pose a risk (EFSA, 2014). However, due to the lack of information on traditional or background exposure to *A. muricata* or constituents thereof via food, the application of the presumption of safety approach for the use of *A. muricata* and derived preparations, including preparations marketed as herbal food supplements, is currently not feasible.

In conclusion, risk assessment regarding the consumption of certain *A. muricata*-based food supplement products faces a number of challenges, including the lack of standardisation of composition of *A. muricata* preparations used in food supplements and the lack of information from reliable studies with preparations actually used in food supplements.

2.2.2. EU-FORA Fellowship supporting programme

During the introductory phase of the fellowship, the fellow obtained general information on risk assessment activities performed within the BfR Department of Food Safety, as well as in the hosting Unit Nutritional Risks, Allergies and Novel Foods. Furthermore, the fellow was supported by appropriate supervision to obtain experience in risk assessment of 'other substances' used in food supplements or fortified foods, with a focus on substances of plant origin ('botanicals', i.e. plant preparations and secondary plant compounds) and regular consultations in this regard with the supervisor as well as with other colleagues. At the BfR, the fellow participated in regular short seminars organised by the Department of Food Safety, with presentations on food safety-related (*in vitro* or *in vivo* experimental) ongoing research activities carried out at different units of the department. During the regular meetings of the host Unit of Nutritional Risks, Allergies and Novel Foods, the fellow presented the EU-FORA fellowship programme, as well as the intermediary results of her project on risk assessment of *A. muricata*. Moreover, the fellow was also given the opportunity to present and discuss the final results of her project within the EU-FORA programme at a departmental seminar on 7 July 2020.

Complementary to the 'learning by doing' placement at the BfR, a 3-week general induction training was arranged by EFSA in Parma (Italy) at the start of the Programme (September 2019), as well as other three specific training modules spread over the rest of the 12-month period.

In addition to the scheduled activities regarding the scientific project, the hosting institution, BfR, provided an additional training curriculum, and also enabled the fellow to participate in some other activities that played an important role in developing the general knowledge on risk assessment. For example, the fellow attended the 5th German Pharm-Tox Summit 2020 in March 2020 and presented results of her project as a conference poster on that occasion (Raclariu-Manolica et al., 2020). This also provided the opportunity for her to interact with a broad scientific community from academia as well as from regulatory institutions.

The following Table 1 provides an overview on the supporting activities organised or facilitated for the fellow by the BfR during the EU-FORA Fellowship.

Table 1: Supporting activities during the EU-FORA Fellowship

	Event title	**Date, place**	**Note**
Scientific meetings	One Health EJP Annual Scientific Meeting 2020	27–29 May 2020, Online	URL: www.ohejp2020.com
	5th German Pharm-Tox Summit 2020	2–5 March 2020, Leipzig University	URL: https://www.gpts-kongress.de/ Poster presentation: Risk assessment regarding the use of *Annona muricata* in food supplements (Abstract: Raclariu-Manolica et al., 2020)
Workshops/ Colloquium	Workshop 'Risk Assessment and Risk Management of Genetically Modified Organisms (GMO)'	9 June 2020, BfR, Berlin	Tutor: Hermann Broll
	Workshop 'Risk Assessment - Food contamination by plasticisers'	5 May 2020, BfR, Berlin	Tutors: Dr. Ralph Pirow, Dr. Sebastian Zellmer
	10th Berlin Workshop on Developmental Toxicology	19–20 February 2020, BfR, Conference center Berlin Biotechpark	URL: https://www.devtox.org/workshops_en.php
	Trust – how we understand, measure and build it	29 January 2020, BfR, Berlin	Speaker: Michelle Patel (Food Standards Agency, UK)

	Event title	Date, place	Note
	Creating characters for the BfR as a new line of communication	10 January 2020, BfR, Berlin	Speaker: Claudio Canales Rios
	Harmonized exchange of food safety models using web-based services from RAKIP and the AGINFRA + project	9 December 2019, BfR, Berlin	Tutors: Estibaliz Lopez de Abechuco Garrido, Esther Sundermann, Lars Valentin, Miguel de Alba Aparicio, Thomas Schüler, Tasja Buschhardt, Matthias Filter
	Big data and high-throughput-driven modeling of health effects of environmental agents	6 November 2019, BfR, Berlin	Speaker: Prof. Roland Grafström, Karolinska Institut, Stockholm, Sweden
Other activities	Nanopore Sequencing in Plants: From Greenhouse to Genome	18 February 2020, Webinar from Technology Networks	Speaker: Maximilian Schmidt
	An introduction to the BfR library and its services	15 November 2019, BfR, Berlin	Tutor: Mr. Benedikt Hummel, BfR library

3. Conclusions

3.1. Conclusions from *A. muricata* risk assessment

The results of the assessment show that substantial uncertainties exist regarding the safe use of *A. muricata*-based food supplements. The present data provide strong indications of neurotoxic potential of certain *A. muricata* preparations. However, the paucity of adequate studies, particularly related to long-term use of *A. muricata* supplements, currently does not allow the establishment of a safe intake level.

3.2. Conclusions from the participation in the EU-FORA programme

Participation in the EFSA EU-FORA work programme was a valuable opportunity for the fellow to obtain experience in risk assessment of herbal food supplements and other plant preparations. This was also an excellent opportunity for the fellow to consolidate her specialised knowledge and skills in food safety, particularly in herbal food supplements, by working according to European and international guidelines and standards. The general risk assessment methodology applied for this specific project is expected to be further extended and applied by the fellow to other substances with nutritional or physiological effected added to foods and food supplements.

Moreover, the EU-FORA programme provided a great environment to build a strong professional and personal network that will be used for future collaborations.

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Abbreviations

ACG	acetogenin
BfR	Bundesinstitut für Risikobewertung (German Federal Institute for Risk Assessment)
EEA	European Economic Area

EU-FORA	European Union Food Risk Assessment
i.v.	intravenous
RA	risk assessment
WHO	World Health Organization

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Integration of tools and social science into food safety risk assessments

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Abstract

The European Food Risk Assessment (EU-FORA) Fellowship work programme 'Integration of tools and social science into food safety risk assessments' was proposed and delivered by the Food Standards Agency (FSA), UK. The Food Standards Agency is a non-ministerial government department of the UK, responsible for protecting public health in relation to food in England, Wales and Northern Ireland. The programme was tailored to several different activities to provide an overview of the different tools that can be employed in food safety risk assessment also accounting for the interaction between risk assessment and social science. In order to structure the proposed work, the programme was split into four modules to run over the 12-month period of 'learning-by-doing'. In the first module, the fellow was introduced to Microbiological Risk Assessment (MRA), in the second to Chemical Risk Assessment (CRA), in the third to Social Science, and finally, in the fourth to the Risk Prioritization Tools and Networks in UK - National Dietary Data (NDNS), collection methodology, coding and analysis. The fellow was assigned to the Risk Assessment Unit within the Science, Evidence and Research Department which brings together specialist expertise from Microbiological, Chemical Risk Assessment, and Analytics Units, under one department together with additional staff from the food allergy and radiological risk assessment fields. The aim was to be fully integrated in the organisation's work gaining first-hand experience, increase knowledge of scientific aspects relevant to food safety risk assessment, and more importantly, to enhance network connection activities in the EU food risk assessment environment.

Keywords: risk assessment, exposure assessment, norovirus in lettuce, risk perception

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1. Introduction

The European Food Risk Assessment (EU-FORA) Fellowship work programme 'Integration of tools and social science into food safety risk assessments' was proposed and delivered by the Food Standards Agency (FSA), UK. The FSA is an independent Government department working across the countries of England, Wales and Northern Ireland to protect public health and consumers' wider interests in food. It is led by a board appointed to act in the public interest. The Agency is advised by a number of independent expert committees, including: the Advisory Committee on the Microbiological Safety of Food (ACMSF), the Committee on Toxicity (COT), the Advisory Committee on Novel Foods and Processes (ACNFP) and the Advisory Committee on Social Sciences (ACSS). The work of the independent committees and working groups that advise the FSA helps to ensure that advice to consumers is always based on the best and most recent scientific evidence. The FSA-EU-FORA working programme was tailored to several different activities to provide an overview of the different tools that can be employed in food safety risk assessment and the interaction between risk assessment and social science. It was split into four modules to run over the 12-month period covering a wide range of aspects related to risk assessment (rapid assessments undertaken for food incidents and outbreaks, longer term strategic food risk assessment projects and papers prepared to be submitted to scientific advisory committees). A lead supervisor, Dr Amie Adkin, was responsible for the general monitoring of the programme, while specialist supervisors tutored the fellow within each module.

2. Description of work programme

The EU-FORA work programme 'Integration of tools and social science into food safety risk assessments', as already mentioned was comprised in four modules. The fellow worked within discrete teams (microbiological risk assessment (MRA) team, chemical risk assessment (CRA) team and social science team), attending Teams and Unit meetings, as well as all associated social events. In addition, as an integral member of the FSA, the fellow followed a wide range of continuous internal risk assessment training programmes, workshops, forums, food production site visits, presentations and webinars. Moreover, the fellow was given the opportunity to present several topics.

2.1. Aims

Each of the four modules represented an independent project and had specific deliverables and outcomes. Consequently, this diverse programme had several aims providing the opportunity to cover a wide range of aspects of food safety risk assessment.

Modules aims

The aim of Module 1, 'Systematic review tools and development of microbial food incident risk assessments', was to provide an in-depth knowledge of how to conduct a systematic review following the Prisma method regarding a microbiological risk and how the data collected through the review (evidence) can be used to support risk assessments.

The aim of Module 2, 'Tools for development of Exposure Assessments and ad-hoc national chemical food safety risk assessment', was to train the fellow on standard methodologies internationally adopted, and available tools used to estimate the quantitative exposure assessment within the exposure assessment team. Moreover, the aim of module 2 was to understand the chemical food incident work conducted within the CRA team by shadowing the rapid response to several incidents during the three months of the training in the CRA team.

The aim of Module 3, 'Understanding the role of social science in risk assessment', was to learn about risk perception models and how these models influence risk perception and consequently risk assessment and risk management.

The aim of Module 4, 'Prioritisation of Risk Tools and Networks in the UK', was amended. This learning objective was replaced by the fellow learning more about the wider area of how national dietary data is collected, coded and analysed to feed into exposure assessments.

2.2. Activities/Methods

Module 1: Systematic review tools and development of food incident risk assessments

During the first module, the fellow was placed within the MRA team, supervised by Dr Paul Cook, with the support of Dr Anthony Wilson as a (deputy). The first deliverable of the module was the

writing of a systematic review regarding the effectiveness of washing practices on the removal/inactivation of norovirus on green leafy vegetables. The overall aim of the review was to further the work initiated by the recent FSA NoVAS research project which looked at unwashed, whole lettuce and to understand the impact of different washing treatments on norovirus decontamination of raw leafy green vegetables and how this might impact the risk to consumers. The review was written following the principles of a systematic review methodology (Moher et al., 2009).

In addition, the fellow was trained to perform rapid reactive incident type qualitative risk assessments.

Moreover, part of the first module deliverables was to learn about the MRA team workflow management tools to assist in the management of staff resources in both risk assessment incident response and long-term research work.

Overall, during this module, the fellow was provided various training opportunities in order to strengthen her RA knowledge.

More specifically, the fellow attended learning opportunities and other activities (Appendix A):

- Various internal meetings: twice per month the MRA workload planning meetings; joint FSA & Food Standards Scotland (FSS) meeting; food outbreak meetings; team paper discussion on microbiological incidents at the FSA; discussion meetings regarding the development of a handy guide, aimed to provide common information needed for MRAs covering pathogens including (norovirus, *C. botulinum*, aerobic colony counts (ACC) indicators); and research prioritisation discussion meetings.
- Presentations on various RA topics (regulated products overview, incident review, Norovirus FSA project, series of FSA Strategic Surveillance presentations on prioritisation of immediate food risks and signal prioritisation phase 2).
- Workshops and training sessions: Introduction to incidents; RAU incident workshop; Quality workshop-introduction to Government Statistical Service (GSS) guidance; and the UK government guidance references Aqua, Green and Magenta books.
- A webinar on food-borne viruses' detection, risk assessment and control options in food processing.
- The 'Risky Bites' informal lunch club on various topics (Aqua book, Norovirus reflections on lessons learnt).
- The 'Food for Thought' internal FSA seminars: Seeing is (not always) believing…multispectral imaging (MSI) for food screening; UK soft drinks.
- Other activities: UK Civil service-learning online courses; Field visit to Northampton-Greencore chilled foods.
- Attendance of internal and external meetings: ACMSF, COT, Analytics Unit (AU), Science Evidence and Research Division (SERD), Risk Assessment Unit (RAU).
- The fellow presented the EFSA Foodex 2 food classification and description system to the exposure assessment team.

Module 2: Tools for development of Exposure Assessments and ad hoc national chemical food safety risk assessment

Module 2 was co-supervised by Cath Mulholland team leader of the CRA with Chara Tsouli (Senior Toxicological Risk Assessor) and Chloe Thomas (member of the exposure assessment team) also assisting in the training process. During this module, the fellow was assigned to the CRA team in order to be trained by the exposure assessment group using the quantitative data sets and exposure assessment tools such as CRÈME. Furthermore, the fellow has undertaken several chemical related incidents alongside FSA staff, covering pesticides, supplements, contaminants and veterinary meds, and getting familiarised with the pesticide residue intake models (EFSA PRIMo and the NESTI). The fellow has also worked on developing the exposure assessment element of the risk assessment paper submitted to the Committee on Toxicity regarding the consumption of plant-based drinks (soya drink) in children aged 6 months to 5 years of age.

The main challenge in the assessment of the safety of these drinks is the lack of information regarding dietary intakes (soya drink) for children following dairy-free or plant-based diets. At the suggestion of the Scientific Advisory Committee on Nutrition (SACN) Secretariat, the exposure calculations have been revisited, using information from several sources including the First Steps Nutrition Trust Eating Well: vegan infants and under 5s (2020), Vegan Society Food tips for vegan children (2017) and Public Health England's (PHE) published example menus for Early Years Settings in

England (EYS) (2017). The above sources provided guidance for frequency of consumption as well as portion sizes estimation for children under 5 considered that are high consumers of plant-based drinks. These recommendations aim to achieve a well-balanced, nutritious diet and they were used to provide an indication of more realistic exposures to isoflavones. In addition to the exposure to isoflavones from soya milk itself, the contribution of soya-based products such as soya alternatives to other dairy products or meat to the diet has also been considered.

Moreover, for understanding the role of national committees for assurance of risk assessments and openness of government decision making, the fellow has also attended several COT meetings as an observer.

Finally, during this module, the fellow was provided by further training opportunities in order to strengthen her RA knowledge *(Appendix A)*:

- Various departmental, unit and team meetings.
- Presentation on the Economic Impact of COVID-19.
- Workshops and training sessions (Potency estimation and PBPK workshop followed via teams, Introduction to Chemical risk Assessment, Introduction to food survey data collection and use by the FSA, Introduction to exposure assessment, Quantitative Risk Assessment workshop, Crème software training, Almond drinks exposure assessment, Dairy alternative exposure assessment; Probabilistic Quantitative Risk Assessment course – Exercise 1 and Exercise 2).
- Webinar on Food Safety Colloquium Challenges in Food and Ingredient Safety (FDA).
- The 'Risky Bites' informal lunch club (Feed governance and Animal feed incidence session).
- The Food for Thought' internal FSA seminars (Information-based regulation-Food standards hygiene ratings, Eating out with Food Allergy, Sociology of Nutrition and Food Choices).
- Lunch time seminar: Economic impact of COVID-19; Eliciting consumers' valuation of food safety regulation.
- Presenting to members of the exposure assessment team the FOODEX2 categorisation and description system.

Module 3: Understanding the role of social science in risk assessment

Module 3 was co-supervised by Michael Patel and Rebeca Gillespie with the main objective being to understand the importance of social science to risk assessment. Typically, food safety risk assessments are carried out within a four-step, technical framework, as detailed by Codex. However, the technical framework presumes a level of 'objective risk', while neglecting to take into account the psychological factors that often explain the biases and fallacies associated with decisions and judgments made from different perceptions of risk (Slovic, 1999). This is where the social sciences play a significant role, by revealing the psychological and social factors that impact the representation, perception and interpretation of risk across all agents in the risk analysis process.

For Module 3, the two fellows assigned to FSA conducted a joint literature review in order to understand lay's people risk perception (part A) and what might affect those performing risk assessments – expert bias (part B). The reviews' objective was to provide guidance on the possible solutions to i) improve the effectiveness of risk communication by risk managers taking into account the mental models affecting the risk perception of the public and ii) mitigate the biases affecting risk assessors during the process of risk assessment.

In addition, during this module the fellow also had training opportunities, committee meetings and other activities *(Appendix A)*.

More specifically, the fellow followed the following activities:

- Various Food for Thought' internal FSA seminars (The Role of Trust in People's Response to COVID-19 Communication, Moments of Change' and Food-Related Behaviours, COVID-19: food safety & fraud risk, Can digital technologies improve healthy diet?).
- The 'Risky Bites' informal lunch club on (Risk: The Game, Understanding the policy profession).
- Lunch time session on Food and Mood.
- Webinar on how do we estimate the cost of food crime to the UK.
- Qualitative risk assessment training course which was an introduction to risk and risk analysis.
- Parma Summer School.
- Unit and department meetings.
- Remote presentation to members of the unit regarding the entire EU-FORA/FSA work programme.

Module 4: Prioritisation of Risk in the UK - National Dietary Data (NDNS), collection methodology, coding and analysis

Only a small part of this module was dedicated to risk prioritisation tools as the fellow followed throughout the year several sessions on FSA Strategic Surveillance presentations on the prioritisation of immediate food risks and the signal development prioritisation using big data. The fellow has also had the opportunity to try the signal prioritisation dashboard through a demo & support session aiming to identify how to track, monitor and analyse incident alerts by combining large datasets. Furthermore, this Module has been adapted to accommodate the fellow's interest regarding methodology of collecting dietary data in the UK. The fellow followed a four-session seminar on NDNS and Intake24 provided by the MRC Cambridge team working on NDNS. The session covered various topics providing an NDNS overview – NDNS Year 12 fieldwork model; An Introduction to Intake24 development and functionality, The rationalisation of food lists; Modelling usual dietary intakes, Statistical efficiency: stratification, clustering, weighting of the NDNS data; The development of the NDNS nutrient databank; Recipes database Nutrient databank, UK food composition database, and Recipes calculation. Moreover, the fellow was also invited to provide external seminar to CEDAR/MRC Epidemiology Unit (via Zoom) introducing to the MRC to the Greek National Survey on Health and Nutrition-Methodology and Development (the HYDRIA Project).

In addition, the fellow followed the work that has been started with the literature review conducted during Module 1 and worked to incorporate dietary data into a quantitative risk assessment (QRA) (norovirus in lettuce) to furthering her skills in spreadsheet stochastic QRAs using @Risk. More specifically worked on identifying NDNS food codes that fall within the definition of uncooked leafy greens according to the EFSA definitions of leafy greens. Used the data retrieved from Crème to be entered and manipulated using various functions and pivot tables to get the eating occasions and average portion size for leafy greens with recipes, lettuce with recipes, which was then plugged into the norovirus QRA model.

Finally, during Module 4, the fellow had the opportunity to visit the Animal and Plant Health Agency (APHA) at Weybridge for a day, following presentations from members of the APHA to become familiar with the UK government specialists in quantitative animal health risk assessment. Special emphasis was given to introducing to the fellow on how qualitative risk assessment is performed at the APHA.

During this module, the fellow was also provided with several training opportunities in order to strengthen her RA knowledge *(Appendix A).* More specifically the fellow attended the one day 'Food programme' at Leatherhead Food Research, which was an introduction to food science. The training day' covered various aspects such as: Sensory evaluation, Nutrition for non-nutritionist, Ingredients and food improvement agents, and Food safety.

The fellow also followed several training sessions and presentations such as: Introduction to norovirus QRA model; Norovirus QRA workshop; One Health: Strengthening Animal & Plant Health Surveillance workshop – APHA (London); The 'Risky Bites' informal lunch club-EFSA research needs 2030.

3. Conclusions

The working programme 'Integration of tools and social science into food safety risk assessments' followed a modular 'learning by doing' approach. The four modules included activities that successfully trained the EU-FORA fellow in: conducting systematic literature reviews by using the prisma methodology implementing automatic paper screening, extracting and collating the retrieved data; efficiently extracting the required data from NDNS using exposure assessment tools; reformulating exposure calculations for implementing realistic scenarios when specific food exposures are not available by the national dietary database; promptly responding to both microbiological and chemical occurring incidents; conducting both qualitative and quantitative risk assessments; understanding the role of model development in risk assessment; preparing risk assessment papers for independent National Committees.

Attending the meetings of several scientific national advisory committees ensured the fellows' in depth understanding on the interaction between different networks of risk assessors (not just from the FSA but across UK government and academia). It should be noted that the activities carried throughout the year allowed her to expand her scientific knowledge both on a theoretical level and in practice. The fellow was successfully integrated in the day-by-day workflow of FSA's Risk Assessment Unit, gaining first-hand experience in a multicultural and interdisciplinary context. This enabled a productive exchange of good practices and contributed to the building of a European risk assessment network. This fellowship represented a unique opportunity for the fellow to consolidate and broaden

her knowledge on various aspects of risk assessment. Moreover, the involvement to the workflow management of staff resources in the area of risk assessment provided her with valuable skills, required to work as a risk assessor at a national authority. Overall, the plethora of training opportunities provided the fellow a proper insight into risk assessment conferring important transferable skills that she will be able to use in the near future to support food safety at national and European level.

Finally, the EU-FORA programme was an excellent opportunity for the fellow to acquire and exchange opinions, experiences and methodologies on the risk assessment field and to build a professional and personal network that can serve as a basis for future cooperation.

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Abbreviations

ACMSF	Advisory Committee on the Microbiological Safety of Food
APHA	Animal and Plant Health Agency
AU	Analytics Unit
CEDAR/MRC	Centre for Diet and Activity Research/Medical Research Council
COT	Committee on Toxicity
CRA	chemical risk assessment
EU-FORA	European Union Food Risk Assessment
FDA	Food and Drug Administration US
FSA	Food Standards Agency
FSS	Food Standards Scotland
MRA	microbiological risk assessment
NDNS	National Diet and Nutrition Survey
NoVAS	Norovirus Attribution Study
PRIMo	Pesticide Residue Intake Model
PBPK	physiologically based pharmacokinetic modelling
RAM	Risk Assessment Meeting
RAU	Risk Assessment Unit
QRA	quantitative risk assessment
SACN	Scientific Advisory Committee on Nutrition
SERD	Science Evidence and Research Division

Appendix A

	Title	Date
Training sessions (workshops, webinars, presentations, meetings)	Introduction to incidents	20.11.2019
	Webinar-Foodborne Viruses: Detection, Risk Assessment, and Control Options in Food Processing	12.11.2019
	Introduction to GSS guidance and the Aqua, Green and Magenta books workshop (London)	16.1.2020
	Probabilistic Quantitative Risk Assessment course – Exercise 1	2.2.2020
	Probabilistic Quantitative Risk Assessment course – Exercise 2	9.4.2020
	Introduction to Chemical risk Assessment	3.3.2020
	Introduction to food survey data collection and use by the FSA	31.3.2020
	Introduction to Exposure Assessment	6.4.2020
	Introduction to norovirus QRA model	17.2.2020
	Norovirus QRA workshop	25.2.2020
	Food Science training – Leatherhead Food Research (Epsom)	6.2.2020
	Workshop-One Health: Strengthening Animal & Plant Health Surveillance workshop – APHA (London)	26.2.2020
	Quantitative Risk Assessment workshop (London)	4.3.2020
	APHA visit – Presentation on qualitative risk assessment	5.3.2020
	Potency estimation and PBPK workshop (followed via teams)	11.3.2020
	Dairy alternative exposure assessment	9.4.2020
	Almond drinks paper exposure assessment	15.4.2020
	Crème software training	24.4.2020
	Workshop- SOT FDA Food Safety Colloquium: Artificial Intelligence Applications in Food and Cosmetic Safety workshop	29.4.2020
	Seminar on NDNS and Intake24 – MRC Cambridge/FSA	14.5.2020
	Parma summer school	9–10.06.2020
	Seminar "The cost of food crime in UK"	10.7.2020
	Regulated products overview – internal presentation	1.11.2019
	Lines to Take: Norovirus	21.10.2019
	Lines to Take: ACC indicators	12.12.2019
	Lines to Take: *C. botulinum*	29.1.2010
	Lunch & Learn: Economic impact of COVID-19	1.5.2020
	Lunch & Learn: Eliciting consumers' valuation of food safety regulation	20.5.2020
	Lunch & Learn: Food and Mood	22.7.2020
	Food for thought seminars: Seeing is (not always) believing… multispectral imaging (MSI) for food screening	21.11.2019
	Food for thought seminars: Information-based regulation	19.3.2020
	Food for thought seminars: The Sociology of Nutrition and Food Choices	13.5.2020
	Food for thought seminars: The Role of Trust in People's Response to COVID-19 Communication	1.6.2020
	Food for thought seminars: Moments of Change and Food-Related Behaviours	10.6.2020
	Food for thought seminars: COVID-19: A food safety and fraud risk?	15.6.2020
	Food for thought seminars: Can digital technologies improve healthy diets?	2.7.2020
	Risky Bites sessions: Norovirus: Reflections on lessons learnt	9.12.2019
	Risky Bites sessions: Aqua Book	15.10.2019
	Risky Bites sessions: EFSA Research Needs 2030	12.2.2020

	Title	Date
	Risky Bites sessions: Feed governance and Animal feed incidence	21.5.2020
	Risky Bites sessions: Understanding policy profession	21.7.2020
	Risky Bites sessions: Risk: The Game	26.6.2020
Fellow's Presentations	EFSA Foodex 2 food classification	29.10.2019
	CEDAR/MRC Epidemiology Unit External Seminar Series (via Zoom) 'The Greek National Survey on Health and Nutrition (the HYDRIA Project)'	9.6.2020
	EU-FORA/FSA Work Programme Journey	16.7.2020
Other activities	Field visit to Northampton-Greencore Chilled Foods	15.1.2020
Meetings	COM October meeting	10.10.2019
	ACMSF October meeting	17.10.2019
	AU November meeting	18.11.2019
	COT December meeting	3.12.2019
	SERD December meeting	10.12.2019
	Science Council meeting	17.12.2019
	Risk Assessment Meeting	14.1.2020
	AU January meeting	27.1.2020
	RAU March meeting	3.3.2020
	COT March meeting	10.3.2020
	CRA March meeting	27.3.2020
	SERD April meeting	27.4.2020
	COT May meeting	5.5.2020
	CRA May meeting	18.5.2020
	RAM May meeting	21.5.2020
	AU June meeting	8.6.2020
	COT July meeting	7.7.2020
	SERD meeting	9.7.2020

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Integration of computational tools, data analysis and social science into food safety risk assessment

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Abstract

The EU-FORA Fellowship Programme 'Integration of tools and social science into food safety risk assessments' was proposed by the Food Standards Agency (FSA), the government department responsible for food safety in the UK. The working programme was organised into four modules, covering different areas of risk assessment, including microbiological risk assessment, chemical risk assessment, exposure assessment, risk prioritisation and the integration of risk assessment with social science. During this period, the fellow had the unique opportunity to gain experience in different fields of risk assessment, namely how to conduct a systematic review, to assess the risk of microbiological and chemical hazards, to make use of modelling tools for exposure assessment and risk prioritisation, to write scientific reports for committees and networks at the national level and to understand the role of social science in risk assessment. In addition, the fellow was able to attend several meetings, seminars, courses and workshops that helped him to gain further insight in the field of food science. The complete programme enabled a fast learning curve that allowed the fellow to have an overview of the different tools that can be employed in the wide field of food safety risk assessment, in order to acquire skills and competences that can be used in his future career.

Keywords: modelling, risk assessment, systematic review, mycotoxins, social science

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Table of contents

1. Introduction

The fellow was enrolled in the EU-FORA fellowship programme working on the project 'Integration of tools and social science into food safety risk assessments' at the Food Standards Agency (FSA) in London. The FSA is an independent UK Government department that works across England, Wales and Northern Ireland to protect public health and consumers' wider interests in relation to food. Risk assessment at FSA is organised in teams, including Microbiological Risk Assessment (MRA), Chemical Risk Assessment (CRA), Exposure Assessment Team (EAT) and Regulated Products, all of which belong to the Risk Assessment Unit (RAU). The RAU is embedded within the Science, Evidence and Research Directorate (SERD), which also comprises the Analytics Unit (AU), which includes statisticians, economists and social scientists. The different units actively interact in an interdisciplinary environment forming virtual teams for specific work areas. The work carried out at FSA is supported by Scientific Advisory Committees (SACs), which are independent committees and working groups comprised of scientists, practitioners, medics and academics. The function of a SAC is to help FSA access, interpret and understand the full range of relevant scientific information and to make judgements about its relevance, potential and application. There are multiple SACs in the UK associated with food safety, including the Committee on Toxicity (COT), Committee on Carcinogenicity (COC), Committee on Mutagenicity (COM), Scientific Advisory Committee on Nutrition (SACN) and the Advisory Committee on the Microbiological Safety of Food (ACMSF). In addition, there are networks where the different UK departments discuss and share information about risk and risk prioritisation, such as the Human-Animal Infections and Risk Surveillance Group (HAIRS), Veterinary Risk Group (VRG) and Epidemiology of Foodborne Infections Group (EFIG).

The programme proposed for the EU-FORA fellow was tailored to provide an overview on the activities carried out within SERD, focusing on the different areas of food safety risk assessment and the interaction between risk assessment and social science. In addition, during his period at the FSA, the fellow was invited to attend a series of trainings, workshops, seminars and meetings, including meetings of the SACs, Strategic Surveillance and UK Risk Network, providing a complete spectrum of knowledge within the field of food safety risk assessment and the work carried out at FSA. The fellow was also involved in the 'Food for Thought' seminars, targeting innovative food science research projects, and 'Risky Bites', an informal lunch club which encourages the transfer of risk assessment methods. At the end of the programme, the fellow was also invited to give a presentation at a Risky Bites on his EU-FORA experience. To allow the fellow to work on the different areas of food safety at FSA, the EU-FORA Programme was split into four modules. Each module was coordinated by a co-supervisor and deputies, whose role was to follow progress and to arrange meetings and training for the fellow.

For the first module, the fellow was placed within the MRA team. His first task was to review the tools available to complete systematic reviews in the field of food risk assessment, including tools that apply machine learning and artificial intelligence methods, and to provide guidance to the unit. In addition, the fellow performed a systematic review on the prevalence of methicillin-resistant *Staphylococcus aureus* (MRSA) in recent years in Italy and reported the results in the form of a manuscript. The fellow was also trained on rapid risk assessment for food incidents and outbreaks, which is the everyday work of the unit. The final task of the module consisted of an introduction to the various tools used in the unit for the organisation of the workflow and the coordination of research projects.

The aim of the second module was for the fellow to gain a better understanding of the work of the CRA and the EAT at FSA. For this reason, the fellow was placed within the CRA team for 3 months. During this period, he was trained on using the tools available to undertake a quantitative exposure assessment and the use of data from national dietary surveys for carrying out bespoke exposure assessments. He was also trained on carrying out rapid risk assessments for chemical hazards and provided with realistic examples of toxicological incidents to assess and guide the drafting of risk assessments. The fellow was given a topic on which he had to draft a longer risk assessment to present to the COT, with guidance from scientific officers from the CRA and EAT. This provided the fellow with a good knowledge of the processes involved in the presentation and discussion of scientific reports submitted to the national committees.

In the third module, the fellow had the opportunity to work closely with the social science team at FSA. The main objective of this module was for the fellow to comprehend the interconnection between food risk assessment and social science, with a particular focus on public risk perception. This is crucial for understanding the psychological and social factors influencing the public when providing an expert

judgement, therefore affecting the interpretation of the overall risk analysis process. For this reason, the fellow reviewed the literature on the mental models affecting the risk perception of lay people and the biases associated with expert judgement and drafted a report on the topic.

The fourth and final module allowed the fellow to become more familiar with the on-farm stage of the farm-to-consumption risk assessment process, as well as attending meetings on risk prioritisation for pathogens of animal health importance. For this task the fellow was placed at the Animal and Plant Health Agency (APHA), where he applied sensitivity analyses to the results of a quantitative microbial risk assessment (QMRA) model to assess the transmission of ESBL-producing *Escherichia coli* on commercial pig farms. The fellow also had the chance to attend the meetings of SACs and different networks by which the UK government departments discuss the prioritisation of risk and share information related to risk, such as HAIRS and ACMSF.

2. Description of the work programme

2.1. Aims

The modules were organised in tasks and deliverables, targeting the main topics and activities of the specific area.

- Module 1: Investigation of the tools to complete a systematic review and development of food incident rapid risk assessment on microbiological hazards
- Module 2: Review of the tools used for exposure assessment and development of a risk assessment on a chemical hazard within the Toxicology team
- Module 3: Understanding the role of social science in risk assessment, with a focus on the mental models of the public and the expert judgement
- Module 4: Computational tools for the risk prioritisation and risk networks in the UK

2.2. Activities

2.2.1. Module 1

For the first module of the EU-FORA fellowship, the fellow was placed in the MRA team who deliver the national food safety risk assessment function for microbiological, prion and physical hazards. The MRA team also acts as the Secretariat to the ACMSF. The main task and deliverable for the EU-FORA programme consisted of a review of the tools and software available for carrying out a systematic review on a specific topic concerning a microbiological hazard, with reference to making use of machine learning techniques. Systematic reviews are a type of literature review used to identify, evaluate and synthesize the findings using systematic and reproducible methods (Grant and Booth, 2009, Jaspers et al., 2018). Machine learning techniques can provide excellent help for carrying out a broad systematic review, especially for the phases of title and abstract screening. The EFSA machine learning tool for systematic review (EFSA 2015, Jaspers et al., 2018) was tested by the fellow. The fellow then produced guidelines for using the tool and organised a training session for the MRA team.

The programme also included practical training on the process of conducting systematic reviews (Grant and Booth, 2009). For this reason, the fellow had to produce a review on a relevant microbiological hazard, which was chosen in the field of antimicrobial resistance (AMR); an emerging and global concern for both animals and humans (ECDC 2018, EFSA and ECDC 2019). Specifically, the fellow drafted a manuscript on the prevalence of MRSA in recent years in Italy, where MRSA infection in humans is one of the highest in Europe (ECDC 2018). The fellow carried out the literature search and screened the papers retrieved according to three review questions; i) targeting the epidemiology and characteristics of MRSA, ii) the prevalence of MRSA in livestock and humans in Italy and iii) advances in effective antimicrobials to use against MRSA. The papers selected were then analysed by the fellow in order to extract the relevant information and data, which were reported in the form of a manuscript that was presented and discussed within the MRA team.

Furthermore, training was provided in the field of microbiological food incident rapid risk assessment. MRA, and in particular quantitative MRA, is a multidisciplinary approach used for assessing the risks to human health from food-borne pathogens and can be used in the refinement of standards and regulations for food in international trade. The training consisted of a presentation on the process of qualitative and quantitative risk assessments, attending meetings on incidents and outbreaks occurring in the UK in that period and a series of meetings on a quantitative model developed within

FSA for transmission of norovirus in food products. After the initial training, the fellow was given an incident on a microbiological hazard (mould in fruit juice) on which to perform a rapid risk assessment and drafted a report for the risk management team to take action.

The fellow was also introduced to the various tools used by the MRA team for organising their day to day workload and research projects. These tools are necessary to plan and distribute the work within the team, especially when considering projects that are expected to last for some years in the future and require a lot of planning. Also, the weekly workload of each scientific officer of the team is planned and organised, so that the team manager is able to optimise the work of each researcher in the best way possible.

Additional training on probabilistic quantitative risk assessment modelling was provided, in which the fellow was trained in using the software @Risk and ComBase Predictive Models. This training allowed the fellow to gain a better knowledge on the quantitative modelling tools used to assess the risk of microbiological hazards in food.

2.2.2. Module 2

For the second module of the EU-FORA programme, the fellow was embedded within the CRA who deliver the national food safety risk assessment function for chemicals, allergens and radiological hazards. CRA also acts as the Secretariat to the COT. In this regard, CRA prepares and presents scientific papers and reports on toxicological relevant topics for the committee to discuss and review.

The second module started with training on the CRA activities, beginning with an introduction to the risk assessment process for chemicals (FAO/WHO 2009). Risk assessments usually comprise of four steps, namely hazard identification, hazard characterization, exposure assessment and risk characterization (FAO/WHO 2009). Hazard identification aims at collecting and summarising information on the hazard of interest, also in relation to the endpoints of concern for the specific risk assessment. In the hazard characterization section, the endpoints of concern are further evaluated in relation to the hazard; estimating the nature, severity and duration of adverse effects, also considering the subpopulations more at risk. The exposure assessment is focused on quantifying the exposure to the hazard of concern via a specific diet. The important information required is the concentration of the hazard and which food commodities are involved, in order to provide mean values of exposure to the hazard by different population groups, both for acute (short-term) and chronic (long-term) exposure. Finally, the risk characterisation assesses if there is a risk from the consumption of the food and, if there is, the magnitude and the population groups more at risk, together with an analysis of uncertainties. This process has been implemented by international health and food organisations, including EFSA, FAO and WHO. Risk assessments are an integrated part of the work in the CRA. For this reason, after having received the training, the fellow was presented with realistic examples of toxicological incidents for which he had to prepare rapid risk assessments with realistic deadlines and compare the results with the official ones produced for the risk management team by the CRA scientific officers. These risk assessments included topics such as supplements, pesticides, additives, contaminants and veterinary medicine residues.

Furthermore, the fellow received training by the EAT, focusing on the steps to follow for carrying out a quantitative exposure assessment, together with the tools commonly used at FSA, and the FSA dietary data on which the assessments are based on. The first training was on the use of Crème, a scientific software tool used within the FSA to obtain food safety exposure and intake assessment, using the national consumption survey. The Crème database includes additives, flavourings, contaminants, pesticides, novel food and ingredients. The data are used to model and predict the exposure and consumption of different populations, and the statistical analysis can be tailored by the user based on their needs. The fellow received both a theoretical and a practical training on Crème, from which he comprehended the different steps of an exposure assessment and how to analyse and present the results. The other training organised by EAT regarded the FSA dietary data, with a special focus on the National Diet and Nutrition Survey (NDNS). The NDNS is a continuous programme funded by Public Health England (PHE) and the FSA. It is carried out jointly by the MRC Epidemiology Unit through the Cambridge NIHR BRC Innovation Programme for the measurement of diet, physical activity and nutrition, and NatCen Social Research. NDNS is designed to assess the diet, nutrient intake and nutritional status of the general population aged 1.5 years and over living in private households in the UK. The fellow was also introduced to a new tool that can fully automate the collection of food consumption data and the coding of foods and portion sizes, called Intake 24. This tool uses a 24-h multiple pass recall method to obtain dietary data from participants, replacing the 4-day paper diary.

The training also provided a practical session, in which the fellow could see how the survey is carried out, together with the extraction and analysis of the data.

The EU-FORA programme included the development of a risk assessment on a chemical hazard to present at the COT, for which the fellow collaborated with scientific officers within the CRA and the EAT. The topic of the scientific report drafted was 'Potential risks from aggregated dietary exposure to mycotoxins' and focused on the UK population. Mycotoxins are toxic secondary metabolites produced by fungi and can cause adverse health effects in both humans and animals (Lee and Ryu, 2017, Palumbo et al., 2020, Smith et al., 2016, Shi et al., 2019, Battilani et al., 2020). Cereals are often the most severely affected crops. Acute and chronic exposure to mycotoxins can lead to several adverse effects in humans, including carcinogenic, teratogenic, hepatotoxic, nephrotoxic and cytotoxic effects. Natural co-occurrence of mycotoxins in food and feed is quite common and occurs for three main reasons: i) some fungi can produce more than one mycotoxin, ii) food commodities can be contaminated by several fungi and iii) animal and human diets usually consist of multiple commodities (Lee and Ryu, 2017, Alassane-Kpembi et al., 2017, Battilani et al., 2020). The fellow, together with another scientific officer within the CRA and with the help of the EAT, prepared a Discussion Paper for the COT on this subject. The paper contained details on the chemicals of interest, their relevance to human health, details on regulatory parameters previously assessed by COT or other international bodies, toxicological information, exposure assessment, risk characterisation and conclusions. The general format is for the contents of the paper to be discussed by the COT members during a meeting. Depending on the outcome of the discussions, further data and information may be requested. Ultimately, a statement will be prepared setting out the final views of the Committee. The paper on co-occurrence of mycotoxins prepared by the FSA scientific officers and the fellow was presented at the July COT meeting.

2.2.2. Module 3

In the third module, the fellow had the chance to work within the Social Science team at the FSA. In addition, the fellow attended the meetings of the AU, in order to gain insights on the different fields of research carried out by the experts in economics, statistics and social science working within the SERD.

The main task of this module was to perform a literature review on the mental models affecting the risk perception of lay people and the biases associated with expert judgement. The aim was to produce a manuscript gathering all the information on the subject, in order to provide recommendations to risk assessors for conducting unbiased risk assessments and to effectively communicate the results to the public.

This module allowed the fellow to gain insights into the work carried out within the AU, with a special focus on the importance of social science for conducting effective risk assessments. The fellow studied both the biases affecting the experts during their risk evaluations and the perception of the risk by lay people. A good knowledge of these two aspects by risk assessors would allow them to identify the biases affecting their judgements during the risk assessment process, in order to apply methods enabling the mitigation of those biases and thus to evaluate the risk of events in a more objective way. In addition, knowing the mental models affecting lay people's perception of the risk, risk assessors would be able to effectively communicate the results of their assessments, so that the public would grasp the true risk of an event.

2.2.3. Module 4

For the fourth module, the fellow had the opportunity to carry out a small project at the APHA, where the fellow was placed for 2 weeks. This project was part of the RaDAR (Risk and Disease burden of Antimicrobial Resistance) One Health European Joint Project (OHEJP) on AMR, which includes several European organisations. The deliverable for APHA is to develop a computational model that looks at the transmission of ESBL-producing *E. coli* on pig farms. The assignment for the fellow was to perform sensitivity analyses on the preliminary results of the model using the software R.

Sensitivity analysis (SA) can be used to evaluate how robust risk assessment and management strategies are, by assessing how variation of a model output can be attributed to variations in the different input factors (Tsao et al., 2019, Feyissa et al., 2012, Pianosi et al., 2016, Carlucci et al., 1999). This can be used for the ranking of the input factors, screening the input factors in order to identify which have a negligible influence on the output and for mapping the region of the input variability space that produces significant output values. There are several different methods of SA,

including regression analysis, difference in log-odds ratio (ΔLOR), scatter plots and analysis of variance (ANOVA) (Frey and Patil, 2002, Patil and Frey, 2004, Wu et al., 2013).

For this project, the fellow got the opportunity to read through and understand a large, complex, stochastic mathematical model showing farm-level transmission of pathogens of One Health importance. Then, he produced R scripts for the different methods of SA applied, namely scatter plots, regression analysis, ANOVA and heat maps. These scripts were then used for the evaluation and optimisation of the parameters of the model. This project allowed the fellow to receive an overview on the numerous methodologies for SA, to acquire a deeper knowledge on the analysis of models for the transmission of food-borne diseases and to use R.

Throughout the whole EU-FORA programme, the fellow had the opportunity to participate in various SACs meetings, including COM, COT, Science Council and ACMSF, and also a meeting of the UK Risk Network HAIRS. The fellow also attended the periodic seminars organised by FSA, 'Food for Thought' and 'Risky Bites', and the workshops and courses organised by the different teams of FSA. At the end of the programme, the fellow had the opportunity to share his personal experience; presenting the work carried out for the fellowship at a 'Risky Bites' seminar. Another important part of the module was his participation in Strategic Surveillance meetings; a series of sessions on a risk prioritisation surveillance tool developed for the FSA and other stakeholders to monitor data sources and learn about food risk signals (country – commodity – hazard) based on what has already occurred in places elsewhere.

3. Conclusions

The working programme 'Integration of tools and social science into food safety risk assessments' was an opportunity for the fellow to work in the different units of SERD at FSA and to gain experience on their day-to-day work in carrying out rapid risk assessments and longer term assessments. In particular, the first module of the programme was tailored for the fellow to learn how to carry out a systematic review and a rapid risk assessment for incidents and outbreaks. Furthermore, in the second module, he was introduced to chemical risk assessments; producing a series of risk assessments on hazards of toxicological interest and a scientific paper on the co-occurrence of mycotoxins that was presented at a COT meeting. The fellow also received training on several aspects of exposure assessment, both theoretical and practical, on the tools used for performing a quantitative exposure assessment (Crème) and on the national surveys in the UK (NDNS, TDS, Intake24), from which data are used for evaluating the exposure to the hazards by population type. The third module aimed at offering the fellow an understanding of the role of social science in the framework of food safety risk assessment. During this module, the fellow developed a literature review on the mental models affecting the risk perception of the public and the biases associated with expert judgement. The results were then used to provide recommendations to risk assessors, in order for them to examine the possible biases affecting their judgement during the process of a risk assessment and try to mitigate them, and also consider the risk perception of lay people in order to deliver an optimised communication of the risk. The fellow also attended the AU meetings to have an understanding of the activities carried out by the experts in economics, statistics and social science. For the fourth and final module, the fellow had the opportunity to take part in a small project at the APHA. The main task was to perform a sensitivity analysis for a model (developed by APHA researchers) assessing the spread of ESBL-producing *E. coli* within pig farms in UK. The fellow was trained on the theoretical methods for sensitivity analysis and then designed R scripts to evaluate the model parameters that have the largest and the least effect on the output. Moreover, the fellow had the opportunity to attend several meetings, including SACs, RAU and UK Risk Network, workshops and additional trainings that allowed him to improve his skills and expertise on the different steps of the risk assessment process. Overall, the programme was a useful overview for the fellow to gain insight on the different lines of work in the field of national food safety. At the end of the fellowship, he was able to perform a systematic review, to carry out risk assessments within realistic deadlines, to interact with people with different expertise to obtain the information needed and to understand the importance of social science in the field of risk assessment.

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Abbreviations

ΔLOR	difference in log-odds ratio
ACMSF	Advisory Committee on the Microbiological Safety of Food
ANOVA	analysis of variance
APHA	Animal and Plant Health Agency
AU	Analytics Unit
BRC	Biomedical Research Centre
COC	Committee on Carcinogenicity
COM	Committee on Mutagenicity
COT	Committee on Toxicity
CRA	Chemical Risk Assessment team
EAT	Exposure Assessment Team
EFIG	Epidemiology of Foodborne Infections Group
EU-FORA	European Union Food Risk Assessment

ESBL	Extended Spectrum Beta-Lactamases
FAO	Food and Agriculture Organization
FSA	Food Standards Agency
HAIRS	Human animal infections and risk surveillance group
MRA	Microbiological Risk Assessment
MRC	Medical Research Council
MRSA	methicillin-resistant *Staphylococcus aureus*
NatCen	National Centre for Social Research
NDNS	National Diet and Nutrition Survey
NIHR	National Institute of Health Research
OHEJP	One Health European Joint Project
PHE	Public Health England
QMRA	quantitative microbial risk assessment
RaDAR	Risk and Disease burden of Antimicrobial Resistance
RAU	Risk Assessment Unit
SA	sensitivity analysis
SAC	Scientific Advisory Committee
SACN	Scientific Advisory Committee on Nutrition
SERD	Science, Evidence and Research Directorate
VRG	Veterinary Risk Group
WHO	World Health Organization

Appendix A – Trainings and activities

	Title	Date
Training sessions	Introduction to incidents	20.11.2019
	Probabilistic Quantitative Risk Assessment course – Part 1	19.11.2019
	Probabilistic Allergy Risk Assessment course	5.12.2019
	ComBase training	21.01.2020
	Food Science training - Leatherhead Food Research (Epsom)	6.2.2020
	Introduction to Chemical risk Assessment	3.3.2020
	Introduction to food survey data collection and use by the FSA	31.3.2020
	Introduction to Exposure Assessment	6.4.2020
	Crème software training	24.4.2020
	Parma summer school	9-10.6.2020
	Qualitative risk assessment training course – session 1 - "An introduction to risk and risk analysis"	28.7.2020
Seminars and workshops	*Risky Bites* "More than one piece of career"	25.9.2019
	Risky Bites "Aqua Book"	15.10.2019
	Food for thought "Seeing is (not always) believing… multispectral imaging (MSI) for food screening"	21.11.2019
	Risky Bites "Norovirus: Reflections on lessons learnt"	9.12.2019
	Introduction to GSS guidance and the Aqua, Green and Magenta books workshop (London)	16.1.2020
	Risky Bites "EFSA Research Needs 2030"	12.2.2020
	One Health: Strengthening Animal & Plant Health Surveillance workshop – APHA (London)	26.2.2020
	Quantitative Risk Assessment workshop (London)	4.3.2020
	AMR Programme workshop	9.3.2020
	Potency estimation and PBPK workshop (Manchester)	11.3.2020
	Food for thought "Information-based regulation"	19.3.2020
	SOT FDA Food Safety Colloquium: Artificial Intelligence Applications in Food and Cosmetic Safety workshop	29.4.2020
	Food for thought "The Sociology of Nutrition and Food Choices"	13.5.2020
	Seminar on NDNS and Intake24 – MRC Cambridge/FSA	14.5.2020
	Risky Bites "Feed governance and Animal feed incidence"	21.5.2020
	Food for thought "The Role of Trust in People's Response to COVID-19 Communication"	1.6.2020
	Food for thought "Moments of Change and Food-Related Behaviours"	10.6.2020
	Food for thought "COVID-19: A food safety and fraud risk?"	15.6.2020
	Risky Bites "Risk: The Game"	26.6.2020
	Food for thought "Can digital technologies improve healthy diets?"	2.7.2020
	Seminar "The cost of food crime in UK"	10.7.2020
	Risky Bites "Understanding policy profession"	21.7.2020
	Food for thought "The impact of the FSA's Food Allergy and Intolerance Research Programme over the past 10 years"	4.8.2020

	Title	Date
Meetings	COM October meeting	10.10.2019
	ACMSF October meeting	17.10.2019
	AU November meeting	18.11.2019
	COT December meeting	3.12.2019
	SERD December meeting	10.12.2019
	Science Council meeting	17.12.2019
	Risk Assessment Meeting	14.1.2020
	AU January meeting	27.1.2020
	RAU March meeting	3.3.2020
	COT March meeting	10.3.2020
	CRA March meeting	27.3.2020
	SERD April meeting	27.4.2020
	COT May meeting	5.5.2020
	CRA May meeting	18.5.2020
	RAM May meeting	21.5.2020
	Levels and trends of Antimicrobial Resistance in Campylobacter *spp.* from chicken reared in the UK	27.2.2020
	AU June meeting	8.6.2020
	COT July meeting	7.7.2020
	SERD meeting	9.7.2020
Other activities	Chilled Food Production Site Visit	15.1.2020

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Training in tools to develop Quantitative Risk Assessment using Spanish ready-to-eat food examples

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Abstract

Unsafe food poses global health threats, potentially endangering consumers. The great majority of people will experience a food-borne disease at some point in their lives. Ready-to-eat (RTE) food is the one intended by the producer or the manufacturer for direct human consumption without the need for cooking or other processing effective to eliminate or reduce the concentration of pathogenic microorganisms. Prepared foods are often complex and may contain multiple components that make them vulnerable for growth of pathogenic microorganisms. Among all the pathogenic microorganisms that may be present in RTE foods, *Listeria monocytogenes* is of special interest because it is the causative agent of listeriosis and it has the ability to survive and replicate at refrigeration and low pH conditions. We performed a quantitative microbial risk assessment (QMRA) in RTE dry-fermented sausage to measure the risk of listeriosis associated to the consumption of this product. The starting point of our investigation was the storage at the factory, after the end-product was produced and before distribution to retail. The stochastic model was implemented in MicroHibro, an online tool for QMRA. Because *L. monocytogenes* concentration and prevalence can vary greatly between different studies and different types of fermented sausages, we tested different scenarios to show the importance of low prevalence and concentration of the pathogen at the final product. Our results show that the risk estimates are very sensitive to the modelling hypotheses used to describe this process. Therefore, the development of accurate probabilistic models describing the initial concentration of *L. monocytogenes* shall largely reduce the uncertainty associated to the QMRA of listeriosis in this type of product.

Keywords: *Listeria monocytogenes*, risk assessment, RTE meat, QMRA, food safety

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1. Introduction

Unsafe food poses global health threats, potentially endangering consumers. As a result, the great majority of people will experience a food-borne disease at some point in their lives. This highlights the importance of making sure the food we eat is safe from potentially harmful bacteria, parasites, viruses, toxins and/or chemicals (World Food Summit, 1996). Food safety remains as one of the main concerns in every Member State of the European Union, including Spain. In 2018, EU Member States reported 5,146 food-borne outbreaks affecting 48,365 people (EFSA & ECDC, 2019). A food-borne disease outbreak is defined as an incident during which at least two people suffer the same illness after ingesting the same contaminated food or drink. Food-borne disease has long represented a considerable burden to public health and continues to challenge health systems worldwide. Although anyone may contract a food-borne disease, vulnerable populations such as small children, elderly people, pregnant women, immunocompromised people and those living in poverty or who are food insecure are particular vulnerable (WHO, 2017). The global scale of the supply chain in modern food production has reduced the cost and increased the variety of food available, but this integration of the food supply enable food-borne pathogens and toxins to infect and poison large numbers of consumers (Ercsey-Ravasz et al., 2012; Garre et al., 2019a).

Ready-to-eat (RTE) food is food intended by the producer or the manufacturer for direct human consumption without the need for cooking or other processing effective to eliminate or reduce to an acceptable level microorganisms of concern (2019/229 of 7 February 2019 amending regulation (EC) No 2073/2005). The lack of a cooking step prior to consumption makes this type of product especially susceptible for being a source of food-borne disease. *Listeria monocytogenes* is a ubiquitous organism that is widely distributed in the environment. It can contaminate foods and cause gastroenteritis (a mild, non-invasive illness) or listeriosis (a severe, invasive illness) (World Health Organization, & Food and Agriculture Organization of the United Nations, 2004). The most important characteristic of listeriosis is the relatively high mortality rate compared to illnesses caused by most other food-borne pathogens (15.6% compared to < 1% for *Salmonella* or *E. coli* O157) (EFSA & ECDC, 2019). Furthermore, mortality may be underestimated among elderly in nursing homes and care facilities (EFSA BIOHAZ Panel, 2018), while having an underling disease is a risk factor for increased mortality (Goulet et al., 2012). In 2018, 28 Member States reported, 2,549 confirmed invasive human cases of listeriosis with an EU notification rate of 0.47 cases per 100,000 population (EFSA & ECDC, 2019). The capacity of *L. monocytogenes* to grow under extreme conditions such as low temperature, low pH and high salt is among the most important factors affecting the risk of human listeriosis associated with consumption of RTE foods. Growth may occur both in foods and in the environment, while other elements affecting *L. monocytogenes* growth include water activity, concentration of antimicrobials, storage temperature and time (EFSA, 2018). On 16 August 2019, Regional Health Authorities in Andalusia, Spain, reported an outbreak of listeriosis, caused by the bacteria *L. monocytogenes*, associated with the consumption of a chilled roasted pork meat product manufactured in Spain and sold under the brand name 'La Mechá'. A total of 222 confirmed cases linked to this outbreak were reported, including three deaths among elderly persons who were ill with listeriosis at the time of death (Ministerio de Sanidad, Consumo y Bienestar Social, 2019).

Quantitative microbial risk assessment (QMRA) is a mathematical modelling approach used to estimate the risk of infection when a population is exposed to microorganisms. The process of QMRA involves identifying and characterising the hazards, assessing exposure, and characterising the risk (European Commission Scientific Committee for Food, 1997). QMRA is based on a quantitative description of the microbial response to the different conditions encountered during each step of the field-to-fork chain of the product based on mathematical models. However, this system is inherently stochastic. For that reason, it is essential to include (and ideally separate) uncertainty and variability in the analysis (Benford et al., 2018). In this context, variability refers to sources of variation that are inherent to the system (e.g. biological differences between microbial cells), whereas uncertainty is related to lack or imprecise knowledge (Nauta, 2000; Koutsoumanis and Aspridou, 2017). This can be accomplished by a stochastic modelling approach where the relevant kinetic parameters are described using probability distributions (van Boekel, 2020; Garre et al., 2020). Numerous studies have pointed out that the prediction of the outbreak size may depend on the way that uncertainty and variability are separated, and that a major outbreak may be overlooked if the distinction between uncertainty and variability is neglected (Nauta, 2000; Benford et al., 2018; Garre et al., 2020).

2. Description of work programme

2.1. Aims

As part of the EU-FORA fellowship, the focus of this study was for the fellow to be involved in all the activities required for the risk assessment of RTE foods, from data analysis to modelling alternatives, establishing different scenarios and performing a QMRA. The final outcome will be the estimation of the risk based on different scenarios for susceptible groups: persons over the range of 60–65, infants, pregnant women and immunocompromised patients. This objective will focus on establishing the health risks on the basis of conditions included in the study using existing web-based tools (MicroHibro) and the data and models already available in the scientific literature. This will allow the establishment of risk interpretation of the impact of variability and uncertainty on a QMRA. The fellow will gain skills related to the interpretation and communication of the outcome of a QMRA using software tools broadly used by the community.

2.2. Activities/methods

As part of the fellowship, the priority of the hosting site was to provide the fellow with the basic theoretical background required to perform a QMRA. The fellow joined a working team with proved expertise in the use of risk assessment tools and received training on specific topics such as:

- Handling of available databases (EFSA, FAO, the group's database for microbial inactivation, etc.);
- Optimal Experimental Design (including the bioOED software, developed in the group) applied to inactivation experiments;
- Growth and inactivation modelling (such as Combase or Bioinactivation, the latter developed in the group);
- Statistical analysis using Monte Carlo and Bayesian methods and risk ranking methodologies;
- Software tools specific for risk assessment (e.g. MicroHibro and FDA-iRISK);
- Separation between variability and uncertainty, the quantification of these terms and the incorporation in predictions from the point of view of experimental design and statistical analysis.

2.2.1. Laboratory experience gained

In order to better understand the empirical methods required to gather the data needed to build predictive models for QMRA, part of the training included the characterisation of the response of *L. monocytogenes* to thermal inactivation treatments at both constant and varying temperatures. Experiments were performed using *L. monocytogenes* CECT 4032, supplied by the Spanish Type Culture Collection.

Training at bacterial strains and culture conditions

A freeze-dried sample was transferred to 10 mL of tryptic soy broth (TSB) (Scharlab Chemie S.A., Barcelona Spain) for rehydration during 30 min. Then, 5 mL of culture were inoculated in 500 mL of TSB and incubated for 21 h at 37°C with constant stirring at 200 rpm. At that time, cells reached stationary growth phase. Subsamples of the culture were frozen with glycerol (1:1) at −20°C and stored until use. To perform experiments, a frozen sample was inoculated in a tryptic soy agar (TSA) plate and incubated at 37°C for 24 h. Then, a colony was selected, inoculated in TSB and grown with constant stirring overnight until cells reached the stationary growth phase.

Thermal treatments and enumeration of survivors

Thermal treatments were carried out using a Mastia thermoresistometer (Conesa et al., 2009). This device allows to perform thermal treatments in liquid media with a temperature profile that can be programmed within the maximum heating and cooling rates of the equipment (40°C/min). The vessel of the thermoresistometer is constantly stirred during the treatment, ensuring a homogeneous temperature distribution. Before starting the treatment, the vessel was filled with 400 mL of the heating medium. Sterile TSB was used as heating media.

Isothermal experiments were performed at 55, 57.5, 60, 62.5 and 65°C. For each treatment, the thermoresistometer was programmed at constant temperature. Once the temperature in the vessel

was stable, a 0.2 mL volume of the microorganism suspension was inoculated. For non-isothermal conditions, eight different temperature profiles were tested, that can be grouped in two different categories: monophasic profiles with constant heating rate, and biphasic profiles with a holding phase after the initial heating rate. Viable counts were determined following the same procedure for both isothermal and dynamic profiles. Samples with a volume of 3 mL were taken at preset times and collected in sterile test tubes, which were immediately cooled in ice. They were based on duplicate counts from appropriate dilutions, of 1 mL aliquots, that were plated and mass homogenised in TSA. Plates were incubated at 37°C for 24 h and then counted (Garre et al., 2019b).

2.2.2. Mathematical modelling

The fellow was involved in the development and application of stochastic mathematical modelling for QMRA. We used MicroHibro (González et al., 2019), a web tool for QMRA based on stochastic models. This type of model uses a bottom-up (or forward) approach considering the steps of initial microbial concentration (including prevalence), growth, transfer, reduction and dose–response.

L. monocytogenes has been studied extensively during the past decade, regarding its potential to produce food-borne illnesses and it is being closely monitored by health authorities worldwide (Buchanan et al., 2017; Churchill et al., 2019; Garre et al., 2019b). For this reason, we performed a literature review, in order to find and use the most appropriate data for the QMRA.

The prevalence of *L. monocytogenes* in cured meats has been studied in different Spanish regions with substantial variation between the study findings. In Navarra, for example, they found a prevalence of 6.7% in cured meats ('salami', 'chorizo', 'salchichon'), while prevalence dropped to 2.7% in vacuum-packed deli meat products and increased to 8.5% in opened deli meat products (Vitas and Garcia-Jalon, 2004). A study from Catalonia showed that 1/9 (11.1%) of pork luncheon meat, 11/65 (16.9%) of cured dried pork sausage and 3/24 (12.5%) of cooked ham were *L. monocytogenes* positive (Cabedo et al., 2008). In Zaragoza, 6 out of 35 RTE cooked meat products (17.14%), 21 out of 57 RTE raw-cured meat products (36.84%), and 9 out of 37 RTE dry-cured, salted meat products (24.32%) were *L. monocytogenes* positive (Gomez et al., 2015).

The starting point of our exercise was the storage at the factory, after the final product was ready and before distribution to retail (Figure 1). We decided to use the data available from a recent study coming from Córdoba, Spain (Possas et al., 2019) which built a probabilistic model to predict the fate of *L. monocytogenes* in Spanish chorizo sausage from mixing of raw materials up to consumption (Table 1).

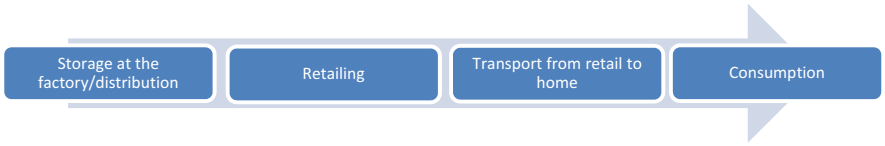

Figure 1: Flow chart of the product pathway and distribution chain

The data used to build the QMRA model consists of the following main parameters: 1) the prevalence of the pathogen at retail level; 2) the concentration of the pathogen at retail level; 3) the growth of the pathogen during storage; 4) the serving size; 5) the total number of annual servings; 6) the population of interest; and 7) the dose–response based on an exponential model. Additional data information were also extracted from EFSA's Scientific Opinion on the development of a risk ranking toolbox (EFSA BIOHAZ Panel, 2015).

Table 1: Overview of the probabilistic model input variables

Model phase	Input variable	Description	Distribution/ model/value	Unit	References
Initial concentration	N_0	Initial concentration	Normal (2.42, 1.016)	log CFU/g	EFSA BIOHAZ Panel (2015)
	P	Prevalence	7.5	%	Possas et al. (2019)
	L_g	Lag time	0	–	Possas et al. (2019)
	W	Sausage weight	100	Grams	–
	G	Growth at storage	0.425	log10 CFU/g	EFSA BIOHAZ Panel (2015)
Storage at factory	t_{st}	Storage at the factory duration	Uniform (0, 36)	h	Nauta et al. (2003)
	T_{st}	Storage temperature at the factory	5	°C	Marcos et al. (2013)
Retail	t_R	Storage time at retailing	Uniform (2, 6)	h	Possas et al. (2019)
	T_R	Temperature at retailing	Normal distribution (3.7, 1.78)	°C	Possas et al. (2019)
Transport from retail to home	t_{TR}	Transport to home time	Uniform (0.25, 2)	h	Possas et al. (2019)
	T_{TR}	Transport to home temperature	Triangular distribution (10; 4; 25)	°C	Nauta et al. (2003)
Consumption	t_H	Household storage time	Normal distribution (4.3, 2.6)	h	Nauta et al. (2003)
	T_H	Household temperature	Normal (6.62, 2.56)	°C	Carrasco et al. (2007)
	S_z	Serving size	Normal distribution (50, 5)	Grams	–
	D_R	Dose–response	**Equation:** 1–exp(–r × pow (10, dose) × serving)	–	World Health Organization & Food and Agriculture Organization of the United Nations (2004)
Population	Population of interest	Spanish population with an estimate that 10% is considered highly susceptible	47.000.000	Persons Persons	–
	Simulations	Number of model simulations	100	–	–

CFU: colony forming unit.

Effect of prevalence and initial concentration of *L. monocytogenes*

As evidenced from the preliminary review of the scientific literature, *L. monocytogenes* concentration and prevalence can vary greatly between different studies and different types of fermented sausages. Therefore, in order to assess how different modelling hypotheses regarding the initial concentration levels affect the risk estimates, a scenario analysis where different distributions were compared was applied. Originally, we defined a normal distribution (2.42, 1.016) as in the study by (EFSA BIOHAZ Panel, 2015) As an alternative scenario, we used a uniform distribution of the initial concentration (Uniform (0,3)) as in the study by (Possas et al., 2019).

3. Conclusions

3.1. Conclusions from the probabilistic assessments

The contamination of RTE meat products with *L. monocytogenes* is a major concern for the food industry. Mathematical modelling simplifies processes occurring in the physical world through a series of hypotheses. The aim of this project for the fellow was to develop technical skills relevant in risk assessment stages: i) hazard identification, ii) hazard characterisation, iii) exposure assessment and iv) risk characterisation.

Our model predicted a risk of 0.002363 illness per serving (min = 3.96E-07, max = 0.085165, SD = 0.01) and a total number of 416 cases, in the Spanish population (using input parameters of Table 1). This number is higher than the 372 cases of listeriosis that was recorded in Spain for the year 2018 (EFSA & ECDC, 2019). Overestimation of the predicted cases may be due to the different resources, databases and distributions of the input variables that were used for the estimation of listeria QMRA in this report.

An example of how much the predicted risk can be influenced by the type of input variables and distributions, we analysed the sensitivity of the number of cases to the hypotheses used to describe the initial concentration in the product. When we applied a Uniform distribution with a range from 0 to 3 log CFU/g, the predicted risk of cases per serving decreased to 0.000111 (min = 7.26307E-07, max = 0.000772, SD = 0.00018) with only 20 predicted cases. As expected, the prevalence of the final product at the retail level plays a significant role in the expected risk of infection. Apart from the original prevalence (Table 1), seven more prevalence concentrations of *L. monocytogenes* were tested based on the findings of different studies. The first group of prevalence tested less than 7.5%, namely 1.5%, 3% and 5% showed the same risk per serving (0.00236 illness/serving) with 83, 167 and 278 predicted cases, respectively. On the other hand, prevalence concentration of 10%, 17%, 24.3% and 36.84% increased the predicted number of cases at 555, 944, 1,349 and 2,045, respectively.

Therefore, the hypotheses used to describe the initial concentration in the product can largely influence the predicted risk. This is especially relevant considering the large variability in the prevalence levels estimated in different studies published in the scientific literature, which can range from 2.7% to 38.84%. The uncertainty about the outbreak size increases with increasing uncertainty in the input distributions (Nauta, 2000). Hence, the development of more accurate probabilistic models to describe the initial concentration of *L. monocytogenes* in this type of product shall greatly reduce the uncertainty associated to the risk estimates.

3.2. Scientific activities during fellowship

During the fellowship, the fellow had the opportunity to participate to various scientific activities. These included attending various conferences/seminars/webinars:

- IV Jornada Cátedra AgroBank. *Technological challenges in the cultivation and post-harvest of fruit and vegetables*. Universidad Politécnica de Cartagena, Spain, 12 November 2019.
- Escuela Técnica Superior de Ingeniería Agronómica. *The use of mathematical models and statistical tools to navigate an ocean of data.* Universidad Politécnica de Cartagena, Spain, 18 November 2019.
- Escuela Técnica Superior de Ingeniería Agronómica. *Concepts regarding food shelf life (definitions, legislation, date marking, quality and safety issues, sensory vs consumer panels and finally shelf life estimation methods).* Universidad Politécnica de Cartagena, Spain, 4 December 2019.
- Escuela Técnica Superior de Ingeniería Agronómica. *Modelling approaches to estimate shelf life for a specific food product based on a set of experimental data.* Universidad Politécnica de Cartagena, Spain, 5 December 2019.
- Misión Posible: *De Horizonte 2020 a Horizonte Europa*. Universidad Politécnica de Cartagena Spain, 23 January 2020.
- Presentación del Programa PRIMA: *The Partnership for Research and Innovation in the Mediterranean Area,* Universidad Politécnica de Cartagena, Spain, February 13 2020.
- VectorNet with ECDC & EFSA collaboration*: Webinar 1 - VectorNet Maps: What are they and how to use them.* Online course 18 February 2020.

- GRUPOGAM. *Prevention and Control of Contamination by Listeria in Food,* Universidad de Murcia, Spain, 20 February 2020.
- Escuela Técnica Superior de Ingeniería Agronómica. *Modeling and control of irrigation and fertilizers in greenhouses.* University of Almería, February 28, 2020.
- Escuela Técnica Superior de Ingeniería Agronómica. *International Seminar: Technological advances in the post-harvest fruit and vegetable.* Universidad Politécnica de Cartagena Spain, 11 March 2020.
- EFSA. Webinar: *Rapid Assessment of Contaminant Exposure (RACE) tool,* 12 March 2020.
- One Health EJP: *Annual Scientific Meeting 2020,* Online Congress, Prague, 27–29 May 2020.
- EFSA Online Course: *Parma Summer School 2020 'One Health'. 9–10 June 2020*.

3.3. Conclusions from the participation in the fellowship programme

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 Spanish ready to eat fermented sausage. The work plan provided training and knowledge on all the steps and tools required to perform a QMRA, taking as an example food/ pathogen combinations relevant for Spanish ready-to-eat products. The fellow was involved in all the activities of the risk assessment process, from data analysis to modelling alternatives, establishing different scenarios and performing a QMRA. He had a very significant contribution during all the steps of the programme.

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Abbreviations

EU-FORA	European Union Food Risk Assessment
FAO	Food and Agriculture Organization
QMRA	quantitative microbiological risk assessment
RTE food	ready-to-eat food
TSA	tryptic soy agar
TSB	tryptic soy broth

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Risk assessment of phthalates based on aggregated exposure from foods and personal care products and comparison with biomonitoring data

Norwegian Institute of Public Health,
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Abstract

Phthalates are a group of diesters of phthalic acid and have been widely used by the industry as plasticisers giving flexibility and durability to polyvinyl chloride (PVC) plastics. Commonly their uses vary from plasticisers in food contact materials and toys to emulsifying agents in personal care products. Phthalates are not covalently bound to PVC, thus they can migrate into the air, skin, water, food and the environment. The omnipresence of phthalates results in human exposure via multiple pathways such as dermal, oral and inhalation for prolonged periods. There is evidence that phthalates can induce disruption in oestrogenic activity, reproductive, developmental and liver toxicity both in experimental animals and potentially in humans. The aim of this technical report is to summarise the activities of the fellow performed at the Norwegian Institute of Public Health (NIPH). The goals of the work programme were collecting concentration levels on five specific phthalates from the scientific literature and combining them with consumption/use data reported in a biomonitoring study part of a Horizon 2020 project (EuroMix), and finally, estimate the aggregate phthalate exposure from food and personal care products and compare them with the measured phthalate levels in urine samples collected in the biomonitoring study.

Keywords: phthalates, exposure, food, cosmetics, Monte Carlo, risk assessment

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1. Introduction

Phthalates are diesters of phthalic acid and have been widely used in the industry as plasticisers giving flexibility and durability to polyvinyl chloride (PVC) plastics. Commonly their uses range from plasticisers in plastics, including food contact materials and toys, as emulsifying agents and solvents in personal care products (PCPs), to excipients used in the pharmaceutical industry. This widespread use leads to a ubiquitous, constant and virtually unavoidable exposure in humans. Phthalates can migrate into the air, water and foodstuff, and humans can be exposed via multiple pathways such as dermal, oral and inhalation.

The phthalate plasticiser global market currently stands at approximate 5.5 million metric tonnes per year (OECD, 2018). The biggest market being the People's Republic of China accounting for 45% of all use, followed by Europe and the USA with a combined 25% use. There is evidence in the literature that phthalates can induce disruption in oestrogenic activity, reproductive, developmental and liver toxicity both in experimental animals and in humans (Gray et al., 2000; Heudorf et al., 2007; Lyche et al., 2009; Chen et al., 2014). Di-2-ethylhexyl phthalate (DEHP), one of the most widely used phthalates, has been found to cause liver carcinogenicity in rodents and has also been classified by IARC as possibly carcinogenic to humans (Category 2B). Although, it is disputed if the mechanism involved (peroxisome proliferation) is relevant for humans (IARC, 2013). Even if phthalates were authorised for use as food contact materials in the European Union (EU) in 2011 (EC 10/2011), for the aforementioned reasons, DBP, DEHP and DIBP should not be placed on the market in the EU after July 2020 individually or in any combination, in concentrations equal or greater than 0.1% by weight of plasticised material (EU 2018/2005). Thus, various phthalate substitutes have emerged such as di (isononyl)cyclohexane-1,2-dicarboxylate (DINCH), tributyl *O*-acetylcitrate, triethyl 2-acetylcitrate, trihexyl *O*-acetylcitrate (Schutze et al., 2012; Kim et al., 2019).

In order to evaluate qualitatively or quantitatively the likely human exposure of biological, chemical and physical agents via food and PCPs, exposure assessment was performed. In exposure assessments the magnitude, frequency and duration of human exposure to a chemical agent is modelled and the different exposure pathways, as inhalation, ingestion of water or food and dermal contact for PCPs are taken into account. Exposure is a crucial aspect in risk assessment since if there is no exposure; even a serious health hazard can be classified as a non-risk. In order to estimate chemical exposure, occurrence, product concentration data and use/consumption data are needed. A very popular tool to estimate exposure is probabilistic exposure assessment. Probabilistic analyses use more complicated modelling approaches than the deterministic (point estimates) and rely on distributions of data as input in place of single values. The outcome is a distribution of possible exposure estimates and assists in characterising variability and uncertainty providing an insight into the overall picture in the population. In this way, the outcome is influenced at a lesser degree from possible outliers leading to overestimation or underestimation of the actual exposure. The use of statistical methods, i.e. Monte Carlo simulations, also provides greater credibility in comparison with deterministic approaches and/or expert judgement, which may be led by subjectivity. Even though probabilistic methods can provide a more reliable exposure estimate, it should be mentioned that availability of consumption and exposure data is paramount and limited concentration data can lead to a higher uncertainty in the final exposure estimate.

A biomonitoring study (BM) was performed in Norway between September 2016 and November 2017 as part of the EuroMix project financed by H2020 programme. The study included males and females, who recorded their food consumption (including weights), and cosmetic use and collected all 24 h urine for two non-consecutive days (Husøy et al., 2019). The consumption data from this study along with the concentration data from the literature were used for the probabilistic exposure estimates for five phthalates for males and females on both days. The selected phthalates were di(2-ethylhexyl) phthalate (DEHP), di-iso-nonyl phthalate (DINP), diethyl phthalate (DEP), di-*n*-butyl phthalate (DBP) and butyl-benzyl-phthalate (BBP).

2. Description of work programme

2.1. Aims

The main aims of this work were to estimate the exposure of a sample of the Norwegian population to the most important phthalates recorded in the EuroMix biomonitoring study (DEHP, DINP, DBP, BBP and DEP) and the phthalate substitute DINCH. Finally, the risk characterisation had to be determined for each phthalate. In order to achieve the main goal, the following tasks were performed;

1) Identifying the most important phthalate sources and exposure pathways.
2) Collecting concentrations of phthalates and DINCH in food and PCPs.
3) Performing aggregate probabilistic exposure modelling for the most important phthalates.
4) Comparing the modelling outcome with the two 24-h urine measurements of phthalate and DINCH metabolites.
5) Discussing the potential impact on the risk characterisation after comparing the aggregate exposure with the reference values provided by EFSA in their 2019 opinion on phthalates (under public consultation).

2.2. Activities/methods

2.2.1. Biomonitoring study

A biomonitoring study was performed in Norway between September 2016 and November 2017 as part of the EuroMix project financed by H2020 programme. The study included 144 participants (44 males and 100 females) participating the first study day and of these 140 participants (43 males and 97 females) completed the second study day. There was a 2- to 3-week interval between the sampling and during the two study sessions. The participants gave detailed weighted records on the food consumed and personal care products usage. All urine was collected for both study days and blood samples were taken at the end of each 24-h period. A detailed description of the EuroMix BM study can be found in the paper published by Husøy et al. (2019). The phthalates measured in the urine with the highest concentrations were the metabolites sums of DEHP, DBP, DEP, DINP and BBP.

2.2.2. Systematic literature search

A systematic literature search was performed in October 2019 in order to collect concentration data on phthalates in foods and PCPs. The search included DBP, BBP, DEHP, DEP, DINP and DINCH for the period 2010–2019. The databases used were Web of Science and PubMed. An additional search was completed at the end of November 2019 for the abovementioned compounds starting from 2008; including databases such as Embase, Cochrane, Medline and Web of Science. The retrieved papers were organised in an EndNote 9 file to ensure traceability, and duplicates were removed. Finally, the phthalate concentrations in food and PCP item/category were extracted to an excel table, where information on the country of origin, type of analytical method, number of samples and the type of descriptive data (median, mean, minimum, maximum) were also collected.

2.2.3. Data analysis

The collected concentrations were weighted by adjusting the phthalate concentrations with the number of samples tested in each respective study, and finally, lower bound (LB), middle bound (MB) and upper bound (UB) phthalate concentrations were calculated using R (3.6.4 version). Summary data were calculated, such as P50, P5 and P95 quantiles, mean, standard deviation, minimum, maximum and when possible the geometric mean and geometric standard deviation, for LB, MB and UB for each phthalate using R. The P5, P50 and P95 were used for the probabilistic exposure estimates. For the exposure estimates of the five phthalates the consumption data from the EuroMix study were combined with the concentration data from the literature using the following equation (1).

$$\text{Diet exposure} = \sum \frac{x \times C}{BW} \left[\frac{\mu g}{kg\ bw\ day}\right], \quad (1)$$

where C is the concentration of phthalates in foods (μg/g); x is the gram food eaten (g/day), and BW is the body weight (kg).

Whereas for the exposure estimates from PCPs, the following equation (2) was used.

$$\text{Dermal exposure} = \sum \frac{C \times PCP_{fr} \times PCP_{a} \times ABS \times R_{f}}{BW} \left[\frac{\mu g}{kg\ bw\ day}\right] \quad (2)$$

where C is the concentration of DEHP in PCPs (μg/g); PCP_{fr} is the frequency of application (application/day); PCP_{α} is the amount per application (g/application); ABS is the dermal absorption factor (non-dimensional); R_f is the retention factor for rinse-off products (non-dimensional) which were taken from SCCS (2016), and BW is the body weight (kg).

The individual exposure estimates for each phthalate were modelled using 1,000 Monte Carlo interactions, and the triangular type of distribution was based on the P5, P50 and P95 as parameters values. Triangular distributions were used due to the limited availability of concentration data in foods. Triangular distribution is a continuous probability shaped as a triangle and with the Pert distribution, can be used when minimum, maximum and the mode are available (Borek et al., 2014) and is being used in the phthalate exposure estimation (Martinez et al., 2017).

In order to compare the exposure estimates with the phthalate levels found in the urine, the reported phthalate metabolite concentration in the urine were back calculated (μg/kg bw) to external exposure of their respective parent compounds by taking into account toxicokinetic parameters such as absorption and the % excretion represented by the measured phthalate metabolites in the urine.

Further statistical analysis was performed by calculating the linear regression between middle bound and urine for males and females on both days. Two-way ANOVA tests were used to calculate any correlation between the sexes and the 2 days with the levels of phthalates found in the biomonitoring study. In addition, one-way ANOVA tested any significant within day correlation variations in the levels of phthalates for males and females on both days. All calculations were made using R version 3.6.4.

3. Conclusions

The fellow has completed the objectives specified in the project proposal. By accomplishing this, the fellow gained experience in writing a project protocol, performing systematic literature review and acquired practical experience with probabilistic exposure modelling both for single and aggregate exposure. Moreover, the fellow gained theoretical and practical experience in R, along with statistical methods for data treatment. As the part of the results (dietary exposure) was presented at a conference in January 2020 organised by the Norwegian society for Pharmacology and Toxicology at Beitostølen. Phthalate exposure was estimated for food, PCPs and their aggregate and was compared with the measured phthalate metabolites found in the urine. Additionally, their risk was characterised not only for the individual compounds but also for the mixture. The outcome of these activities is going to be published in a peer review journal and the manuscript currently is in preparation. Additionally, part of the results (dietary exposure) were used as a chapter in the fellow's PhD thesis titled 'New developments in harmonised risk assessment of emerging chemical hazards: Chemical mixtures' for the University of Parma, which was the sending institute.

An objective that was amended during the fellowship was the use of the Monte Carlo Risk Assessment tool (MCRA). Due to data protection issues, the fellow was able to use the tool only for training purposes. This did not affect the work outcome since the objectives were met by using R. Additionally the COVID-19 pandemic did not significantly hinder the work progress, since there were frequent online interdepartmental meetings. Overall, the fellow did not have any adjustment issues; he integrated at the NIPH and collaborated well with colleagues from the Section of Toxicology and Risk Assessment.

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Abbreviations

BBP	butyl-benzyl-phthalate
BW	body weight
DBP	di-*n*-butyl phthalate
DEHP	di(2-ethylhexyl) phthalate
DEP	diethyl phthalate
DINCH	di(isononyl)cyclohexane-1,2-clicarboxylate
DINP	di-iso-nonyl phthalate
EU-FORA	European Food Risk Assessment Fellowship Programme
IARC	International Agency for Research on Cancer
LB	lower bound
MB	middle bound
MCRA	Monte Carlo Risk Assessment
NIPH	Norwegian Institute of Public Health
OECD	Organisation for Economic Co-operation and Development
PCPs	personal care products
PVC	polyvinyl chloride
SCCS	Scientific Committee on Consumer Safety
UB	upper bound

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Modelling and magnitude estimation of cross-contamination in the kitchen for quantitative microbiological risk assessment (QMRA)

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Abstract

In the kitchen of the consumer, two main transmission routes are relevant for quantitative microbiological risk assessment (QMRA): the *cross-contamination* route, where a pathogen on a food product may evade heating by transmission via hands, kitchen utensils and other surfaces, e.g. to non-contaminated products to be consumed raw; and the *heating* route, where pathogens remain on the food product and are for the most part inactivated through heating. This project was undertaken to model and estimate the magnitude of cross-contamination in the domestic environment. Scientific information from the relevant literature was collected and analyzed, to define the cross-contamination routes, to describe the variability sources and to extract and harmonise the transfer fractions to be included as model parameters. The model was used to estimate the relative impact of the cross-contamination routes for different scenarios. In addition, the effectiveness of several interventions in reducing the risk of food-borne diseases due to cross-contamination was investigated. The outputs of the model showed that the cutting board route presents a higher impact compared to other routes and replacement of the kitchen utensils is more effective than other interventions investigated; the transfer to other surfaces and objects, which can house bacteria in the environment, is also described. Laboratory cross-contamination trials have been performed to estimate bacterial transfer via cutting, from the external surface of the meat to the cutting surfaces and to the knife. The results, obtained from the laboratory trials, show magnitudes of and differences in the bacterial transfer fraction to the knife and the cutting surface in relation to which side of the meat is contaminated. Despite the complexity of factors which influence bacterial transfer, the combination of laboratory work with mathematical modelling enhanced scientific understanding and appreciation of the uncertainty of the estimates. QMRA methodology results in magnitude estimation of cross-contamination in the kitchen and evaluation of intervention strategies.

Keywords: QMRA, cross-contamination, food-borne pathogens, risk assessment

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1. Introduction

Quantitative microbiological risk assessment (QMRA) is a methodology used to organise and analyse scientific information to both estimate the probability and severity of an adverse event as well as prioritise efforts to reduce the risk of food-borne pathogens (Habib et al., 2020). Modern food safety considerations are based on 'farm to fork' (or 'stable to table') QMRA and encompass all the steps of the agrifood chain until the food is consumed. However, when the food reaches the domestic environment, how to estimate the risk carried by the 'fork' directly into the mouth of the consumer? The European Union One Health 2018 Zoonoses Report publishes information on food-borne and waterborne outbreaks as provided by EU Member States according to Directive 2003/99/EC, and states that 40.5% of strong-evidence outbreaks occurred at home and 15.6% of the outbreak cases were due to contaminated food within the domestic environment. 'Cross-contamination' is identified as one of the contributory factors of the strong-evidence outbreaks.

The term 'cross-contamination' stands for the transfer of bacteria or viruses from a contaminated food, raw material, kitchen utensil or person, to other foods, whether it occurs directly or indirectly (Manios et al., 2015). Bloomfield and Scott (1997) state that, when assessing the risk of food-borne diseases associated with cross-contamination, the microbial contamination level on the surfaces and the probability of the transfer to the food should be taken into account. In addition to this, the estimation of the bacterial fraction transmitted to the different cross-contamination routes (i.e. hand to salad, meat to cutting board, etc.) appears of paramount importance for the correct estimation of the overall impact of cross contamination.

The available literature which describes bacterial transfer between contaminated source (e.g. meat) and recipient (e.g. cutting board) shows that the cross-contamination process is complex and affected by many factors associated with the characteristics of the pathogen, the surfaces and the contact process (e.g. pressure applied, duration of the contact) where findings might be also contradictory (e.g. the effect of the initial bacterial concentration on the transfer fraction). Quantitative data to describe the transfer of microorganisms from a contaminated source to a recipient are fundamental to carry out a risk assessment.

Mathematical modelling is a powerful tool for further investigations by including different scenarios and comparison between interventions. Data to feed the model are often not readily available and in some cases also contradictory, thus making explicit the difficulties in describing the phenomenon (Pérez-Rodríguez et al., 2008, Hoelzer et al., 2012). In addition, the variability in experimental set up used by researchers does not allow for a straightforward ascertainment of the effect of different variability sources using these cross-contamination experiments. Monte Carlo simulation can be performed to describe the uncertainty and variability associated with risk (Vose, 2008), in this case the risk of cross-contamination.

2. Description of work programme

During food preparation in the kitchen of the consumer, two main transmission routes leading to human ingestion are possible: the *cross-contamination* route, where a pathogen may evade heating by transmission via hands, taps, raw vegetables, etc., and the *heating* route, where pathogens remain on the food product (e.g. meat) and are partly inactivated through heating.

The importance of the *heating* route has been described by the research conducted at RIVM within the EU-FORA fellowship programme 2017–2018, while the investigation of the *cross-contamination* route for theoretical and practical implementation of this aspect in food safety QMRA was the purpose of the EU-FORA fellowship programme 2019–2020.

The work programme was carried out at the Centre for Zoonoses and Environmental Microbiology (Z&O) at RIVM, The Netherlands, which has an extensive experience in performing risk assessments in food, water and environment.

The work programme was structured as follows:

i) Define which cross-contamination routes (hand to tap, knife to vegetables, etc.) are potentially relevant;
ii) Define a theoretical model of cross-contamination for each transmission route, taking into account that results are presented in a rather variable way in cross-contamination literature;
iii) Define the variability that is to be included in the estimation of the intensity of cross-contamination. This concerns, e.g. differences between pathogens, whether pathogens are located only on the surface of a product or also inside, and differences in the preparation

and heating process in the kitchen of the consumer. The variability to be included is limited by the availability of variability information for the description of the realistic kitchen process;

iv) Perform a literature study on cross-contamination, during which the starting points above can be updated. The output of this literature study will be the set of quantitative data and estimates that are available at present in literature to describe cross-contamination.

v) Combine and integrate the information and estimates obtained into a framework model that describes the network of cross-contamination routes that occur in the kitchen of the consumer and estimates the final intensity of cross-contamination. This framework model will be customisable, in terms of both transmission routes and parameter values, as a function of the relevant variability aspects defined before.

vi) Estimation of the cross-contamination magnitude by simulating different scenarios.

vii) Estimation of the effect of (hygiene) interventions.

The work programme was extended with *laboratory experiments* conducted at Z&O at RIVM, The Netherlands, describing the bacterial transfer from meat spiked on the external surface to the cutting surface during the process of slicing with a knife. The laboratory trials included the setting up of the experiments, the estimation of the recovery performances of the methods used and the analysis of the original data using modelling and statistics, for future scenario analysis and integration in food safety QMRA.

2.1. Aims

The activities of the work programme were aimed at estimating the magnitude of cross-contamination in the kitchen, during the preparation of a meal, for QMRA.

For the *modelling* part of the working programme, the aim was:

i) To assess the fraction of bacteria that was in the raw food and overpassed all the steps till arriving from the same product/other products/utensils/hands to the mouth of the consumer;

   a) By calculating what is the number of colony forming units (CFU) that reach the mouth of the consumer due to cross-contamination in the kitchen, in relation to the number on the raw meat.

ii) To compare scenarios and assess the effect of interventions on the fraction of bacteria that are cross-contaminated.

For the *laboratory* part of the working programme, the aim was to investigate a specific cross-contamination step, estimating:

i) The fraction of bacteria on meat surface transferred to cutting surface during meat slicing, as these bacteria will experience a different heating regime during meat preparation compared to the bacteria that remain on the original meat surface;

ii) Transfer from meat to knife and subsequent transfer from knife to meat. This last aspect is important as bacteria remaining on the knife will be transferred also to uncontaminated pieces of meat at following cuts made by this knife.

2.2. Activities/methods

2.2.1. Modelling cross-contamination in the kitchen

In order to model and to estimate the magnitude of cross-contamination in the kitchen for QMRA, the approach was structured as described in the work programme.

i) *List of cross-contamination routes*: The starting point of the model is a contaminated food (raw meat) which can be in contact with hands ('meat to hands'), with the cutting board ('meat to cutting board') and with the knife ('meat to knife') during the preparation of a meal. After the contact with the contaminated source, hands, cutting board and knife can transfer bacteria to other products (i.e. salad) which are not exposed to any further heat treatment before reaching the mouth of the consumer; routes which are defined as 'knife to salad', 'cutting board to salad', 'hand to salad'.

In addition, the hands can transfer bacteria to any surface or other inanimate objects which will become contaminated and hold bacteria partly in the environment (such as handlers of the drawers,

kitchen utensils or kitchen counter), so called 'hand to fomite' route. The possibility of a direct contact between hand and mouth of the consumer has also been included and described by the route 'hand to lip'.

Taking into account that after the first contact the transfer of bacteria will occur also in the opposite direction (e.g. 'hand to meat'), bidirectionality of the transfer has been included.

ii) The *model* for transmission from source to recipient is the fraction (1) and the uncertainty is described by Beta-distribution.

$$\text{Transfer fraction} = \frac{\text{no. of CFU on the recipient}}{\text{no. of CFU on the source}} \quad (1)$$

iii) *Variability sources*. Bacterial transfer from one surface to another is a complex process: given the differences between the experimental set up available in investigations published in literature, the variability sources for each cross contamination steps have been investigated within the same experimental trial. This analysis allows the definition of the variability sources to be included in the mathematical model, within dedicated scenarios.

iv) Available data on bacterial transfer have been extracted from the scientific literature and collected in a *literature database*, structured to include information on the food-borne pathogen, the cross-contamination route (source, recipient, description of the step), values as reported by the authors, values expressed as mean, standard deviation, alpha and beta parameters of the beta distribution after harmonisation of the data, and relevant details on the paper. Each row of the file describes in detail one step of cross-contamination. The literature database allows to retrieve information by selecting the field of interest (for example, authors or cross-contamination step). Data from papers which describe transfer for cumulative or combined steps (i.e. from meat to salad or from meat to cutting board and knife) are presented in the literature database but not included in the mathematical model. Even though many papers describe cross-contamination events, in order to be able to include the data in the model, harmonisation of the data from the literature was necessary using consistent criteria.

v) *Mathematical model.* The model was developed in Excel and @Risk, as deterministic and probabilistic model and it is structured using 8 steps which mimic the preparation of a meal, which starts with raw meat and ends with a salad. The final output of the model is the fraction of CFU originating from the raw meat that reaches the mouth of the person preparing and eating the meal. It is also possible to describe the distribution of CFU among the different recipients included in the model, expressed as number of CFU per recipient and as a percentage of the number on the raw meat.

vi) *Scenarios.* The 'chicken-salad' scenario was defined as baseline scenario to describe a condition of surface contamination while the 'ground beef-salad' scenario was included for contamination of the surface and the interior of the raw meat. The model allows to include also the investigation of the estimation of cross-contamination in case of a 'next meal scenario', which stands for the preparation of a meal, using the same utensils after a certain timeframe. An example of the output of the Monte Carlo simulation done in @Risk is represented in Figure 1.

vii) *Interventions* included in the model are washing hands, cutting board or knife – by describing the fraction of bacteria which remains after washing – replacement of the kitchen utensils and the order of actions.

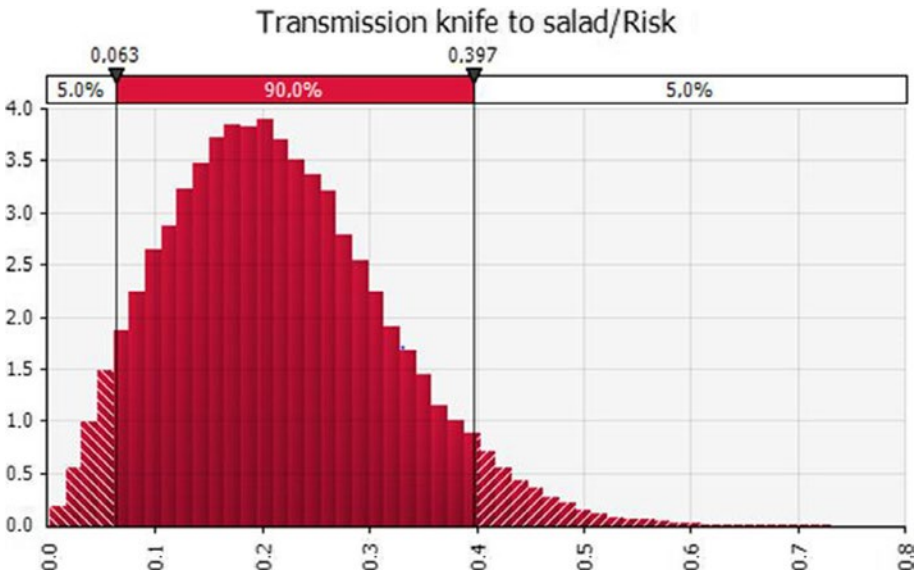

Figure 1: Probability density function describing variation of fraction of bacteria transmitted from knife to salad

2.2.2. Laboratory experiments

The presence of food-borne pathogens on food and food contact surfaces represent a concern. Many factors can influence the adhesion of microorganisms and the process of attachment can start in less than 5 seconds and can vary according to different substrates (Miranda and Schaffner, 2016). Laboratory trials available in literature describe cross-contamination and bacterial transfer with different approaches and laboratory techniques), and some authors provides insight on the bacterial transfer 'from meat (or vegetable) to knife' via the cutting process (Zhao et al., 1998; Luber et al., 2006; Ravishankar et al., 2010; Zilelidou et al., 2015; Sarjit and Dykes, 2017).

From October 2019 to July 2020, experimental trials have been conducted at RIVM laboratories focusing on the *cutting process* and estimating not only the bacterial transfer 'from meat to knife' but also 'from knife to the cutting surfaces' (Figure 2). Importantly, as a preliminary step, the performance of different recovery methods has been investigated to choose the preferable one to implement.

2.2.2.1. Recovery performance trials

Recovery of CFU from spiked meat and knife have been performed as follow: after spreading a solution with known concentration of CFU/ml of *Escherichia coli* O111:H2 on the meat, quantification of the bacterial strain was conducted by a direct contact method (i.e. agar stamp) (Figure 3) and by swabbing (using a cotton swab to recover bacteria and to release them into a physiological solution during vortexing, followed by plating). The recovery from a spiked knife was conducted by rinsing, by combining rinsing and swabbing, and by swabbing. Viable plate count (the number of CFU on the agar plates) was compared with the number of CFU on the inoculum to quantify the recovery performance of the method.

2.2.2.2. Meat slicing experiment

As mentioned in the previous section, the aim of the *meat slicing experiment* was to estimate the transfer fraction from an externally spiked meat to the cutting surfaces, taking into account also the intermediate transfer step to the knife.

After the inoculum of a known concentration of CFU/ml of *E. coli* O111:H2 was spread on the surface of beef meat and overnight storage at refrigeration temperature, the meat was cut to obtain two symmetrical slices, exposing two cutting surfaces (A and B); the four sides of the meat (top side, front side, back side and bottom side) were investigated separately as contamination source. Immediately after cutting, the two cutting surfaces were put in contact on Tryptone Bile X-Glucuronide (TBX) Medium agar (agar stamp method), followed by incubation at 37°C for 18 h and viable plate counting on the next day. The process of spiking and cutting was performed always by the same operator, to eliminate this variability source.

In order to investigate the transfer to the knife, the blade of the knife was rinsed on both sides with physiological salt solution and plated on TBX agar, being the preferred method. The trials have been repeated at least four times for each sides.

The analysis of the data was aimed to obtain the values of two parameters: t_1, which describes the transfer from the spiked area of the meat to the knife, and t_2, which estimates the transfer fraction from knife to the cutting surface of the meat. For the enumeration of the fraction of bacteria transferred from the contaminated source to the recipient aspects such as the relative contact surface and the recovery performance of the methods have been taken into account.

Figure 2: Process of cutting (*from left to right*) resulting in exposure of the cutting surfaces

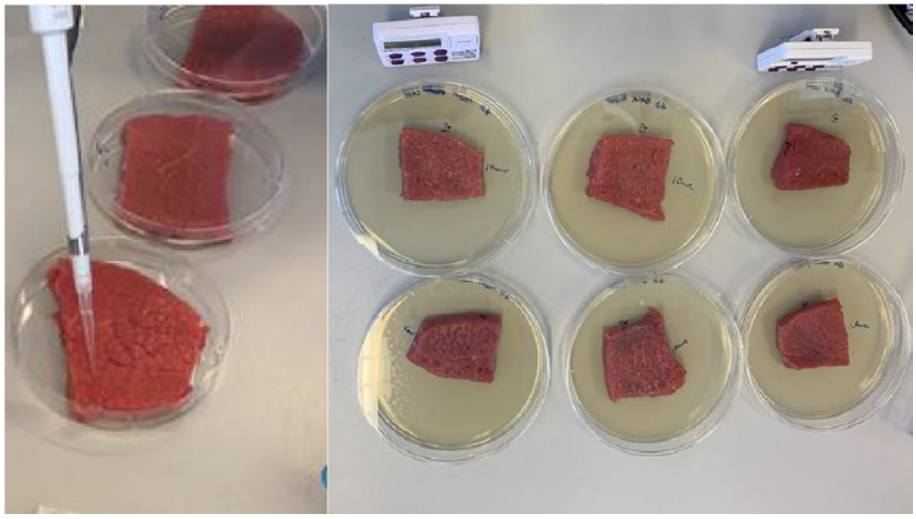

Figure 3: Recovery performance experiment: spiking of the meat *(left)* and meat agar stamp method application *(right)*

3. Conclusions

Transfer of bacteria from a source to a recipient is considered as a cause of food-borne disease (Pérez-Rodríguez et al., 2008). Cross-contamination refers to the direct or indirect transfer of bacteria/ viruses from a contaminated food product to a non-contaminated product (Pérez-Rodríguez et al., 2008, Evers, 2015). This phenomenon is usually associated with contaminated equipment and poor hygiene practices and its occurrence in the consumers' kitchen can be related to disease cases.

The project executed within the EU-FORA fellowship programme provides new valuable modelling and data on cross contamination for QMRA. The cross-contamination model is able to estimate the fraction of bacteria that reaches the consumer for the different scenarios and allows to estimate the importance of the different cross-contamination routes in the transfer of bacteria from contaminated meat to the final dish. The cutting board route presents a higher impact compared to other routes;

moreover, the transfer route 'from hand to fomites' should not be neglected, given the fact that bacteria from the kitchen environment can be reversely transferred to food. Furthermore, the model allows also to estimate the effect of the interventions applied and can help risk managers in defining the best advices to reduce the impact of cross-contamination.

The laboratory recovery trials conducted gave more insight in the microbiological detection methods which could be applied to bacterial transfer investigation. Concerning the results of the trials, it is possible to conclude that the agar stamp method could be an alternative to sampling by a destructive method, giving the possibility to define exactly the area of investigation of the product, represented by the relative contact surface and taking into account only the bacteria available for the transfer. Concerning the knife sampling, the rinsing methods showed higher recovery values compared to swabbing and the combination of rinsing and swabbing. The laboratory experiments on meat cutting provide insight in the complexity of the action of cutting. The transfer from the relative contact surface of the meat (spiked area in contact with the knife) to the knife, named t_1, is high and similar among the sides with the exception of the bottom part which appears lower. The transfer from knife to cutting surfaces, named t_2, is very high, probably due to the characteristic of the blade which enables the detachment of bacteria during the slicing.

The EU-FORA programme allowed the fellow to familiarise with QMRA with a 'learn by doing' approach: from the collection, analysis and harmonisation of data from the scientific literature to the setup of a mathematical model on cross-contamination. Furthermore, combining laboratory work with mathematical modelling can boost scientific understanding and appreciation of the underlying processes and uncertainty of the estimates.

The fellow was exposed also to the best-suited statistical methods to describe the uncertainty associated with microbiological data. In addition, the fellow had the opportunity to become familiar to @Risk, software for risk assessment, which allows for Monte Carlo simulations. The fellow was actively involved in the activities carried out at Z&O (more details in the Appendix section), and attended meetings and seminars organised during the year. The EU-FORA fellowship programme provided an opportunity for a fruitful exchange and collaboration between fellow and supervisor.

3.1. Future goals

The EU-FORA fellowship programme set the basis for future collaboration between the fellow and the hosting site. Further steps foreseen are the publication of the cross-contamination model and the results of the laboratory experiments in scientific journals.

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Abbreviations

CFU	colony forming unit
ECDC	European Centre for Disease Control
EU-FORA	European Food Risk Assessment
QMRA	Quantitative microbiological risk assessment
RIVM	Rijksinstituut voor Volksgezondheid en Milieu – National Institute for Public Health and the Environment, The Netherlands
Z&O	Centre for Zoonoses and Environmental Microbiology

Appendix A

	Description	Date
Training sessions	Workshop Next Generation Sequencing: One tool fits all!	23.1.2020
	Git basic course (RIVM)	7.4.2020
Meetings with scientific presentations	Weekly meeting with Z&O Modelers, with scientific presentations, active interactions and exchange of advices; including discussions and updates on the course of the fellowship project	On Mondays
	Weekly research meeting with the members of the Voedselgroup (Food group), with scientific presentations, exchange of knowledge, including updates on the course of the fellowship programme	On Thursdays until March and on Mondays from April
	Monthly meeting of the centrum Z&O. Meeting with two scientific presentations from the members of the centrum on the activities conducted at RIVM	1.10.2019 5.11.2019 10.12.2019 7.1.2020 4.2.2020 9.4.2020 7.5.2020 11.6.2020 9.7.2020
	'Interactions among infectious agents: Why they're important and how to detect them' (University of Georgia)	10.10.2019
	'Microbiome-mediated defence against enteric infections' (LUMC)	15.10.2019
	'Metagenome analysis for parasites' (Z&O, RIVM)	19.11.2019
	'Climate & Health' (Santé Publique France & RIVM)	16.1.2020
	ZOMAR meeting with presentations of the results from research conducted on antimicrobial resistance	15.10.2019 11.2.2020 17.12.2019
	SIM (Statistiek, Informatica en Modellering) colloquium	14.2.2020
	EPI (Epidemiologie en Surveillance van Infectieziekten) referee presentations	14.11.2019 14.5.2020 25.6.2020
Conferences	One Health EJP – Annual Scientific Meeting	27–29.5.2020
Scientific presentations by the fellow at RIVM	Presentation on previous scientific achievements at the research meeting of the 'Food group' of Z&O centrum	10.10.2019
	Presentation to the 'Modelers group' on the EU-FORA project with focus on the mathematical model	11.5.2020
	Presentation at the research meeting of the 'Food group' of Z&O centrum on the EU-FORA project with focus on the laboratory trials results	8.6.2020
	Presentation 'Cross contamination in the kitchen: modelling and measuring' at the Z&O centrum meeting	9.7.2020
Invited presentation	'EFSA: esperienze a confronto' – Invited contribution on the experiences related to EFSA, online event organized by the University of Perugia (IT)	22.5.2020

	Description	Date
Webinars	Introduction to Risk Analysis using @RISK	22.10.2019
	Webinar global webinar: COVID-19 and companion animals – what we know today (WSAVA)	17.4.2020
	Emerging respiratory viruses, including COVID-19: methods for detection, prevention, response and control (WHO)	17.4.2020
	EFSA Webinar Rapid Assessment of Contaminant Exposure (RACE)	27.4.2020
	Practical use of NGS (One Health EJP)	30.4.2020
	Webinar on Coronavirus detection methods (Istituto Superiore di Sanità, IT)	18.5.2020
	Webinar on Novel Foods and new plant breeding techniques (Foodhub)	3.6.2020
	EFSA webinar – High-risk plants – how does the EU carry out risk assessment of plant commodities?	26.6.2020
	Pandemic! A one health view of emerging infectious diseases	30.6.2020

EU-FORA SERIES 3

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Identification of risk factors and hotspots of antibiotic resistance along the food chain using next-generation sequencing

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Abstract

Bacterial antimicrobial resistance (AMR) is considered to be very alarming following an upward trend and thus posing a primary threat to public health. AMR has tremendous adverse effects on humans, farm animals, healthcare, the environment, agriculture and, thus, on national economies. Several tools have been proposed and adopted by numerous countries after comprehending the need for antimicrobial stewardship and for a rational use of antibiotics. These tools include diagnostics for infections or AMR detection, for measuring and monitoring antibiotic consumption (e.g. surveillance tools) and for guiding medical doctors and veterinarians in selecting suitable antibiotics. In addition, it has been known that the food chain represents a leading vector for the transmission of pathogens to humans via various routes (direct or indirect). Considerable efforts have been made and are still in progress both at international and national levels in order to control and mitigate the spread of pathogens and thus ensure food safety. During the last decades, a new concern has risen regarding the food chain playing a potential major role in the transmission of resistant bacteria as well as resistance genes from the animal kingdom to humans. Several recent studies highlight the role of food processing environments as potential AMR hotspots contributing to this spread phenomenon. Next-generation sequencing (NGS) technologies are becoming broadly used in the AMR field, since they allow the surveillance of resistant microorganisms, AMR determinants and mobile genetic elements. Moreover, NGS is capable of providing information on the mechanisms driving and spreading AMR throughout the food chain. In the current work programme, the aim was to acquire knowledge and skills to track AMR genes and mobile genetic elements in the food chain through NGS methodologies in order to implement a quantitative risk assessment and identify hotspots and routes of transmission of AMR along the food chain.

Keywords: antimicrobial resistance, next-generation sequencing, food chain, risk assessment

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1. Introduction

According to the World Health Organization (WHO), antimicrobial agents are indispensable drugs for the protection of human and animal health. Yet, the persisting emergence and spread of pathogenic microorganisms that are resistant to antimicrobial agents constitute a growing global concern (WHO, xxxx). Antimicrobial resistance (AMR) is one of the major health issues of the 21st century. A major factor leading to the spread of AMR is the use of antimicrobial drugs in veterinary medicine and human medicine, where misuse and overuse of antibiotics have been detected. However, another hotspot for AMR can be the food production environment.

Nevertheless, the relative drivers and the potential sources of AMR bacteria in the food chain are not well established yet. Overprescription of antimicrobial agents by clinicians is considered one the main sources of selection for AMR, and some antimicrobials are used in larger quantities in food production. These resistant bacteria can afterwards reach humans via the environment, food or other means (Hudson et al., 2017). According to the Center for Disease Control and Prevention (CDC), antibiotic-resistant bacteria can spread through the food chain in various ways. For instance, in the process of animal slaughtering and meat processing, contamination of meat (or other animal products) as well as processing equipment can occur by resistant bacteria. In addition, animal waste can be a source of resistant bacteria which can contaminate the surrounding environment. Furthermore, vegetables and fruits can get contaminated while being in contact with water, soil or fertilisers containing animal waste contaminated with resistant bacteria (Centers for Disease Control and Prevention, online). In other words, food and food processing environments (FPEs) could be reservoirs and vehicles of transmission of AMR to humans causing a major public health impact.

In order to bring antibiotic-resistant bacteria under control several actions could be implemented, such as in the fields of i) education and training, ii) surveillance, monitoring and record-keeping, iii) legislation and regulations, or iv) optimisation and reduction of irrational antibiotic use (Caniça et al., 2019).

Among the surveillance and monitoring tools, omics technologies (e.g. genomics, metagenomics and transcriptomics), serve the purpose of monitoring and controlling AMR in various One Health settings, mainly in respect to the selection and distribution of AMR in food-related settings. In addition, omics could facilitate the unravelling of the associated AMR mechanisms (Caniça et al., 2019).

Major improvements have been made in sequencing technologies during the last decade. These technologies are referred to as next-generation (or high-throughput) sequencing (NGS). NGS has considerably revolutionised the analysis not only of bacterial genomes but also of complex bacterial communities (i.e. intestinal or environmental microbiota). Regarding the AMR field, NGS facilitates both the identification of already known resistance genes and it also enables the prediction of novel ones. NGS has advanced the capacity of tracking bacterial clones (e.g. multidrug-resistant clones), and the identification of new antibiotic resistance genes (ARGs) as well as their genetic carriers (i.e. plasmids). Therefore, NGS paves the way for new perspectives in risk assessment and AMR surveillance while simultaneously it enables the comprehension of the AMR dynamics between pathogens and commensals stemming from various sources (e.g. environment, animals and humans) (Ruppé et al., 2019). The main advantages offered by NGS are: i) it facilitates clinical decision-making by providing various levels of information to guide the treatment with the appropriate antimicrobial; ii) it improves outbreak investigation and guides the interventions to control them; iii) it allows a retroactive analysis once new information appears (storing NGS data for future analysis); iv) it has the potential to link various fields such as clinics, food, environment and animals; v) it provides mechanistic information on the resistance. Regarding these latter advantages, unlike phenotypic tests – providing information only related to resistance/susceptibility to antimicrobials – NGS can shed light on the molecular basis for the resistance. Moreover, NGS has the potential to characterise novel resistance mechanisms once they arise (via the sequencing of isolates proved to be phenotypically resistant). This is an outstanding added value compared to conventional nucleic-acid based techniques (e.g. polymerase chain reaction, PCR) (Berendonk et al., 2017).

2. Description of work programme

2.1. Aims

The aim of the work programme was to acquaint the fellows in the execution of assessments to identify risk factors present in the food chain that could allow occurrence and spread of antibiotic-resistant microorganisms, AMR genes and mobile genetic elements in FPEs. The main focus was to

investigate the potential of NGS methodologies as a tool for the identification and characterisation of resistant bacteria and their AMR gene repertoire. The outcomes of the work programme may assist the design of knowledge-based interventions facilitating the reduction of the dispersal of multidrug-resistant microorganisms in the food industry.

2.2. Activities/methods

The objectives of the work programme are listed below:

Objective 1: Training of the fellows on the risk assessment methodologies routinely used by the mentor and other collaborators at the host institution.

The research fellows gained in-depth training in specific tools commonly employed by laboratory members of the supervisor and collaborators. The fellows were familiarised with the use of several software tools such as: Monte Carlo simulation distribution software (@Risk, Crystal Ball), software programs for quantitative microbiology and risk assessment models (Combase, FSSP) and last but not least risk ranking tools (RiskRanger).

Moreover, they received training in the use of the SPSS software program for processing the acquired data from the performed experiments in order to identify any correlations among the various parameters being analysed. In addition, several simulations were ran regarding various case studies employing the @Risk software allowing the fellows to obtain a more clear view and acquire hands-on experience on Monte Carlo simulations and the importance of distribution functions in the risk assessment process.

Objective 2: Execution of a qualitative risk assessment approach on AMR in the food chain.

A qualitative assessment of the risk posed by extended-spectrum β-lactamase (ESBL)-producing Enterobacteriaceae was undertaken, focused on hazard identification and exposure assessment. Exposure assessment modelling was carried out regarding the presence of ESBL-producing *E. coli* in pork meat products within the European Union (EU). A systematic literature review was conducted where the *Scopus* database was used for the article search. The extracted data were latter used for building an exposure assessment model. Software (@Risk and Crystal Ball) were used for the simulation part. Apart from the literature review conducted related to the prevalence of ESBL-producing *E. coli* on pork meat, the data from EFSA Comprehensive European Food Consumption Database (EFSA, 2020) were also taken into account in order to perform the exposure assessment.

Objective 3: Phenotypic and genotypic characterisation of a collection of isolates from FPEs.

The fellows characterised a wide collection of isolates from FPEs with classical microbiology tools and NGS (Figure 1). These included culture-based methods -pure culture isolation, determination of resistance to antibiotics (growth in media supplemented with antibiotics (chromogenic media)), and molecular-based methods (PCR). In more details, the two fellows isolated pure cultures of Enterobacteriaceae*, Enterococcus* spp. and *Staphylococcus* spp. from samples obtained from FPEs stemming from 30 industries from different food sectors. Over 500 pure isolates were screened on chromogenic media. Pure isolates were verified by matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry (MALDI-TOF). Pure isolates were obtained and preserved. DNA isolation was performed for all the isolates which exhibited resistance in the chromogenic media. PCR was performed for Enterobacteriaceae isolates to determine presence of $bla_{CTX\text{-}M}$ and bla_{SHV} genes. Regarding the *Enterococcus faecalis* and *Enterococcus faecium*, the presence of the *vanA* and *vanB* genes was examined. 15 *E. coli* and 45 *E. faecalis* and *E. faecium* isolates were screened against a wide array of antimicrobials following the broth microdilution method. The most interesting isolates were analysed through whole genome sequencing (WGS), specifically using the Illumina HiSeq system. The acquired whole genome sequences were analysed by bioinformatic tools. In Figure 1, the flow chart followed for the experimental part is depicted.

Objective 4: Integration of metadata provided in Objective 3 into risk assessment models.

Main hazards were identified along the food production chain in different food industries. Analyses of a questionnaire on hygiene and sanitation practices filled by food producers were performed. Correlations between different variables were assessed, binary logistic regression, as well as some statistical analyses were performed. Regression analyses were conducted in order to investigate any possible correlations between the industry status in relation to the occurrence of Enterobacteriaceae and *Enterococcus* spp., respectively and the metadata on the food safety practices employed by each industry. The FSSP software was used to perform the analyses.

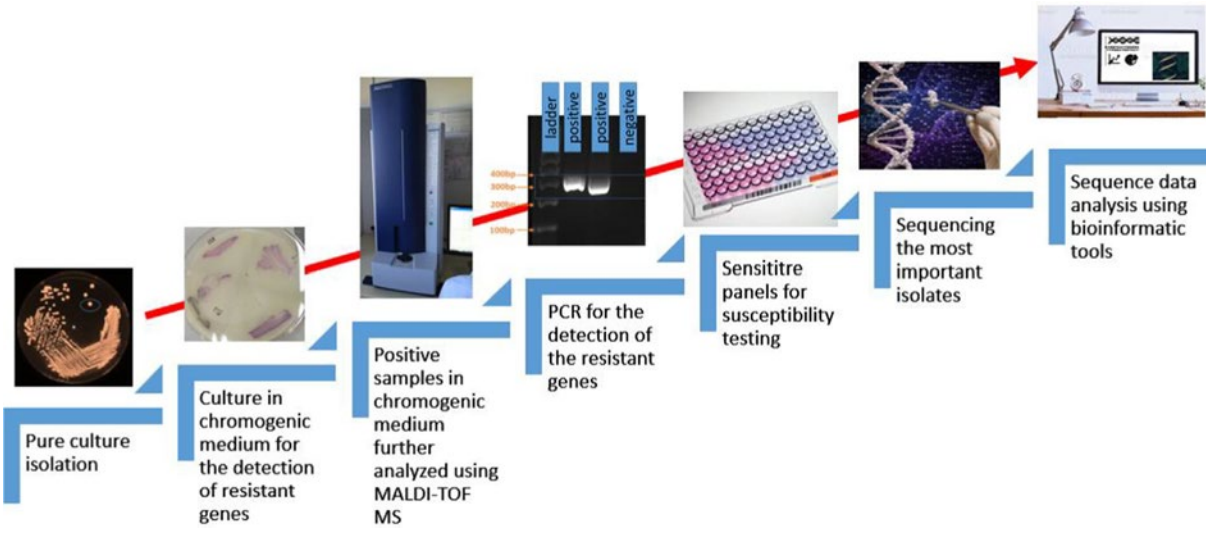

Figure 1: Flow chart of the experimental work undertaken under objective 3 of the work programme

Objective 5: Communication, dissemination and outreach activities.

The fellows participated in dissemination and outreach activities. Two review articles with the following titles 'A review on Extended Spectrum β-Lactamase (ESBL) producing *Escherichia coli* in pigs and pork meat in the European Union' and 'Rapid methods for antimicrobial resistance diagnostics' will be sent for publication during September in peer reviewed journals. The former review covers the analysis of the prevalence of ESBL-producing *E. coli* in pork meat and pigs in the EU, and dissemination pathways of these bacteria along the pork production chain. The latter review article epitomises the newly introduced rapid methods such as NGS, Fourier-transform infrared (FTIR) spectroscopy, MALDI-TOF and lab-on-a-chip (LoC) technologies addressing the detection of AMR in a fast and reliable manner. In Table 1, the courses, conferences and seminars attended by the fellows are summarised.

Fellows were also actively participating in outdoor activities hosted by members of the laboratory and by the work programme supervisors.

Table 1: Summary of courses, conferences and seminars attended by the fellows

Course/seminar	Type of presentation/ courses	Topic	Location
Seminar	Oral presentation, both fellows	Research interests and background	ICTAL, Leon, Spain. November 2019
International Scientific Symposium, Science To Strengthen Sustainable And Safe Food Systems	Poster presentation with previous EU-FORA fellow (Janis Ruško)	EU-FORA fellows of Latvia	BIOR, Riga, Latvia, 30–31 January 2020
	Oral presentation, by Georgia Kaprou	Integrated, fast, cost effective, semi-automated Lab on a Chip for foodborne pathogen detection	
MALDI-TOF instrument as a tool to identify bacterial species	On site courses organised by the Head of the Microorganism culture collection group Laura Alksne	MALDI-TOF use to identify bacteria species and AMR protein fingerprint detection	BIOR, Riga, Latvia, 4 February 2020

Course/seminar	Type of presentation/ courses	Topic	Location
Antimicrobial resistance theory and methods	Online courses	Antimicrobial action and resistance, antimicrobial susceptibility testing and interpretation, quality assurance	DTU, February–March 2020
WGS of bacterial genomes, tools and applications	Online courses	Typing of bacteria, use of WGS for surveillance of bacterial pathogens and AMR, MLST typing, phylogenetic tree building	DTU, February-March 2020
SPSS training on data interpretation	Online courses organised by E.A. Alexa, fellow from the 1st EU-FORA cohort	Chi-Square Test, One-way Anova and Binary logistic regression analyses	University of León, León, Spain. 18 May 2020
WGS analyses	Online courses organised by Jose Francisco Cobo-Diaz, bioinformatician at the hosting site	Assembling raw reads of sequences, single nucleotide polymorphism analyses, AMR gene detection, serotype identification	University of León, León, Spain. 16–17 June 2020
Monte Carlo simulation (distribution functions) using @Risk & Crystalball software	On site and online courses organised by Miguel Prieto Maradona	Data integration in existing models and building a model on ESBL-producing *E. coli* in pork meat	University of León, León, Spain. February–August 2020
Annual Scientific Meeting (ASM) of the One Health European Joint Programme (OHEJP)	Online event	Food-borne zoonoses, antimicrobial resistance and emerging threats	27–29 May 2020

MALDI-TOF: matrix-assisted laser desorption/ionisation time-of-flight; AMR: antimicrobial resistance; WGS: whole genome sequencing; MLST: multilocus sequence typing; ESBL: extended spectrum β-lactamase.

3. Conclusions

Both fellows have successfully fulfilled the objectives and tasks of the proposed work programme. Activities performed allowed the fellows to acquire skills related to the execution of risk assessments and the communication and dissemination of results through various actions (seminars, seminars, publications). More specifically, one review article is going to be submitted in the peer-reviewed *Journal of Antibiotics* (impact factor 3.893) and the second one in the *Journal of Microbiological Methods* (Impact Factor: 1.707). Moreover, Fellows were familiarised with risk assessment and Monte Carlo simulation software for data processing. Furthermore, short trainings were performed regarding NGS analyses as well as MALDI-TOF apparatus use.

Certain objectives were slightly amended due to the COVID-19 pandemic; however, the fellows successfully fulfilled the main objectives and tasks of the work programme. Apart from the two review papers which are going to be submitted during September, the fellows are planning to continue collaborating in the following months in order to publish the results stemming from the activities regarding the objectives 2 and 3; more specifically, the results of the analysis on the occurrence of AMR from FPEs and the WGS analyses of the most interesting isolates, as well as those from the exposure assessment modelling for ESBLs in pork products. The results of this ongoing effort will very likely lead to 2–3 more publications.

3.1. Future goals

The close cooperation between the fellows and the hosting site personnel will be maintained. Hopefully, research grant proposals between sending and hosting institutions will be drafted. Moreover, close collaboration between the hosting site and the home institution of the fellows is envisioned in order to further proceed with the outcomes of the work programme and finalise the remaining publications.

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Abbreviations

AMR	antimicrobial resistance
ARGs	antibiotic resistance genes
ASM	Annual Scientific Meeting
BIOR	Institute of Food Safety, Animal Health and Environment
CDC	Center for Disease Control and Prevention
DTU	Technical University of Denmark
ESBL	extended-spectrum β-lactamase
EU-for a	European Union Food Risk Assessment
FPE	food processing environment
FTIR	Fourier-transform infrared spectroscopy
ICTAL	Institute of Food Science and Technology
LoC	lab-on-a-chip
MALDI-TOF	matrix-assisted laser desorption/ionisation time-of-flight
MLST	multilocus sequence typing
NGS	next-generation sequencing
OHEJP	One Health European Joint Programme
PCR	polymerase chain reaction
WGS	whole genome sequencing
WHO	World Health Organization

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Risk assessment related to food additives and food processing-derived chemical contaminants exposure for the Portuguese population

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Abstract

The European Food Risk Assessment Fellowship Programme (EU-FORA) is an initiative by EFSA, aimed at building scientific capacity in food safety risk assessment in the EU. Current paper reports on the activities of this fellow, undertaken in participation of the third, 2019–2020 cycle of the EU-FORA programme while placed at the University of Porto, Faculty of Nutrition and Food Sciences, in Portugal. The work programme offered by the hosting site was related to risk assessment on food additives and contaminants. The fellow's hands-on work consisted of two practical exercises, which aimed to assess the exposure to the 10 intense sweeteners authorised in the EU and a process contaminant, acrylamide, for the Portuguese general population.

Keywords: food additives, intense sweeteners, process contaminants, acrylamide, dietary exposure

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1. Introduction

The European Food Risk Assessment (EU-FORA) Fellowship Programme is an initiative by the European Food Safety Authority (EFSA), aimed at building scientific capacity in food safety risk assessment in the European Union. During a 12-month period, the fellows enrolled in the programme are placed in a food safety risk assessment organisation outside their own country, to be integrated in the hosting site's activities for a hands-on work experience. Additionally, the fellows benefit from four dedicated training modules that cover a wide range of topics related to risk assessment.

Current paper reports on the activities of this fellow, undertaken in participation of the third, 2019–2020 cycle of the EU-FORA programme. The fellow, whose home institution is the National Institute for Health Development in Estonia, was placed at the University of Porto, Faculty of Nutrition and Food Sciences, in Portugal. The work programme offered by the hosting site was related to risk assessment on food additives and contaminants.

Food additives are substances that are intentionally added to food for a specific technological purpose, e.g. to prevent spoilage, preserve the food's structure or improve its organoleptic properties. In the European Union (EU), rules for the use of food additives are laid down by the European Parliament and Council Regulation (EC) No 1333/2008 (European Union, 2008), and only substances that are listed in Annex II to that regulation may be placed on the market. Food additives must be safe when used and therefore should be kept under continuous observation (European Union, 2010), considering not only new scientific information but also potential changes in their intake by the population.

The functional class 'sweeteners' includes food additives used to impart a sweet taste to foods or in table-top sweeteners (European Union, 2008). The class may be further divided into two groups: bulk and intense sweeteners. The former group consists of sugar alcohols (or polyols) that are usually slightly less sweet than sucrose and that are therefore used in similar volume to sugars for achieving the desired sweetness and texture of the food (Grembecka, 2015). Intense sweeteners, also referred to as non-nutritive sweeteners, are in contrast hundreds to thousands of times sweeter than sucrose and include substances with diverse chemical structures and of both synthetic and natural origin (Mortensen, 2006; Carocho et al., 2017).

In the recent decades, the prevalence of obesity has increased both among adults and children in most countries around the world, and excessive body weight contributes to the escalating public health burden of non-communicable diseases such as diabetes, cardiovascular disease and many cancers (GBD 2015 Obesity Collaborators, 2017). One of the main drivers of this obesity pandemic is an easy access to energy-dense foods in the modern food environment (Swinburn et al., 2011). This realisation had led to an array of intervention strategies (Sisnowski et al., 2017), including the taxation of sugary foods. This, however, may considerably change the exposure to sugar substitutes in the population.

In contrast to additives that are intentionally added to food, contaminants are unwanted chemical substances that may be introduced into food during various stages of production, processing, transport or storage (European Union, 1993). Process contaminants are formed when food components undergo chemical changes due to the processing of food, either at home or by the industry – for example, when foods are smoked, cured, fermented or heated. Some examples of process contaminants include heterocyclic aromatic amines, acrylamide and polycyclic aromatic hydrocarbons that are formed when starchy and protein-rich foods are subjected to high heat, e.g. fried, roasted or grilled (Koszuka and Nowak, 2019). This leads to an appealing flavour and texture and consequently to the popularity of such foods; however, many process contaminants have been identified as possible or probable human carcinogens (Jägerstad and Skog, 2005). Therefore, efforts are needed to monitor and minimise the intake of such contaminants by the population.

Acrylamide is formed when starchy foods are subjected to temperatures above 120°C at low moisture, mainly due to the Maillard reaction involving asparagine (Zhang and Zhang, 2007). Acrylamide is a probable genotoxic carcinogen in humans and a neurotoxicant (EFSA CONTAM Panel, 2015) that was highlighted as a food-related health risk in the early 2000s (Tareke et al., 2002). Subsequently, several risk assessments on acrylamide have been carried out in different countries (e.g., Mojska et al., 2010; Hirvonen et al., 2011; Claeys et al., 2016), including an extensive scientific opinion by EFSA (EFSA CONTAM Panel, 2015) that identified exposure to acrylamide as a concern for neoplastic effects in the European population.

2. Description of work programme

2.1. Aims

Although the general topic for the fellow's hands-on exercises remained unchanged – namely, risk assessment on food additives and contaminants, the particulars of the work programme underwent several developments during the roll-out. First, changes were proposed due to delays in field work for the population-based birth-cohort Generation XXI, the source of food intake data according to the initial work programme which was titled 'Risk assessment related to food additives and contaminants exposure during infancy and adolescence'. Later, the focus for food contaminants shifted from heterocyclic amines and polycyclic aromatic hydrocarbons to acrylamide. This was prompted by the University of Porto being asked to assist in the analysis of epidemiological data from the COVID-19 outbreak in support of the national health authorities, which required a prioritisation of the university's resources. Consequently, the work programme that was realised included the following two parts: a risk assessment on the intense sweeteners currently authorised in the EU and a risk assessment on a process contaminant, acrylamide, both of which were concerned with the Portuguese general population.

In support of the practical exercises, the fellow also followed additional learning objectives. For instance, as the practical work consisted mainly of computational tasks, the fellow worked through several handbooks to improve her data wrangling skills and ability to write clean script in the R programming language, attended an online course on R Markdown for creating dynamic documents with R, and familiarised herself with some of the available software for the assessment of habitual dietary intake. Furthermore, by a combination of onsite and online classes and seminars, the fellow gained knowledge on a wide range of other relevant topics, such as the concepts of epidemiology, conducting systematic reviews and meta-analysis, food security, One Health, etc. In addition, the fellow benefited from the four EU-FORA dedicated training modules: the induction training on 2–20 September 2019 in Parma, module 1 on 25–29 November 2019 in Vienna, and modules 2 and 3 on 10–14 and 24–28, 31 August 2020 as online events due to restrictions related to the COVID-19 outbreak.

2.2. Activities/methods

2.2.1. Practical exercise: risk assessment on intense sweeteners

This practical exercise aimed to assess the exposure to intense sweeteners authorised in the EU and to characterise the risk for the Portuguese general population. In Portugal, a tax on sugar-sweetened beverages was introduced in February 2017 (Diário da República, 2016); consequently, changes in consumer preferences and the reformulation of products by the food industry may have led to an increased intake of intense sweeteners in the recent years.

There are currently 10 intense sweeteners authorised for the use in food in the EU (European Union, 2008). Approved food additives, including intense sweeteners, have been subject to a safety assessment by EFSA or its predecessor, the Scientific Committee on Food (SCF). Therefore, as the first step of the risk assessment, the fellow located and read the latest safety assessment for each of these sweeteners, to gain an overview of their toxicology and to extract their acceptable daily intake (ADI). The results from this literature survey are listed in Table 1. No ADI is specified for thaumatin as no adverse health effects are known. The safety of aspartame-acesulfame salt has not been separately assessed as its constituent moieties are covered by the safety assessments for aspartame and acesulfame K; therefore, aspartame-acesulfame salt was also considered contributing to the exposure to aspartame and acesulfame K in the current risk assessment.

Subsequently, the fellow carried out an exposure assessment that utilised individual-level food consumption data from the Portuguese National Food, Nutrition and Physical Activity Survey (Lopes et al., 2017). The survey collected high-resolution food intake data, including the specific brand of the consumed food products, from a nationally representative sample of the Portuguese population aged three months to 84 years. The exposure assessment was based on 24-h food intake data collected on two non-consecutive days from a total of 5,811 respondents.

As occurrence data, maximum usage levels for each permitted food category were extracted from the Regulation (EC) No 1333/2008 on food additives (European Union, 2008). The fellow was also provided with some auxiliary databases, including one that mapped the food items of the Portuguese

food composition database, that were consumed by the respondents of the dietary survey, to the legislative food categories.

Since the hosting organisation had compiled a database of label information for brand products reported in the dietary survey, the exposure assessment on intense sweeteners was further refined – namely, the presence of a sweetener in each food item consumed by each respondent was determined by the ingredients list of the reported brand product. Missing product information had been filled in by multiple imputations, and the fellow was given the food consumption database that included five imputations for each sweetener. The label information database was also the source of the usage levels for the sweetening agents in table-top sweeteners, since the use of intense sweeteners in table-top products is permitted *quantum satis* (i.e. at a level that is needed to achieve the intended purpose), meaning that no numerical maximum limit is specified by the legislation.

Table 1: Toxicological characteristics of the 10 intense sweeteners currently authorised for the use in food in the European Union

Name	E-number	Critical adverse health effect	ADI, mg/kg bw	Reference
Acesulfame K	E 950	Not specified	9	SCF (2000a)
Aspartame	E 951	Reproductive and developmental toxicity	40	EFSA ANS Panel (2013)
Cyclamic acid and its calcium and sodium salts	E 952	Reproductive toxicity	7[(a)]	SCF (2000b)
Saccharin and its sodium, calcium, and potassium salts	E 954	Carcinogenicity (non-genotoxic)	3.8[(b)]	SCF (1997)
Sucralose	E 955	Reduced body weight gain	15	SCF (2000c)
Thaumatin	E 957	None that are known	Not specified	EFSA ANS Panel (2015)
Neohesperidin DH	E 959	Not specified	5	SCF (1989)
Steviol glycosides	E 960	Changes in organ weights	4[(c)]	EFSA ANS Panel (2010)
Neotame	E 961	Potential hepatotoxicity	2	EFSA (2007)
Aspartame-acesulfame salt	E 962	Not specified	Not specified	Not available

ADI: acceptable daily intake; bw: body weight.
(a): As cyclamic acid.
(b): As free acid.
(c): As steviol equivalents.

Before proceeding to exposure estimation, the fellow combined the various input databases to obtain each respondent's intake of each sweetener per kg bw per each of the two survey days. To improve the transparency and reproducibility of the assessment, this preparatory data wrangling as well as subsequent calculations were organised as computational notebooks that provided a comprehensive and thoroughly annotated description of the workflow, detailing also any assumptions and data corrections made during this process. The computations were carried out using the R statistical programming language (R Core Team, 2018) and formatted as R notebooks. An R notebook is a document in the R Markdown language that contains plain text with independently and interactively executable code chunks and that can be rendered to a shareable format such as html, pdf or a Word document (Xie et al., 2018).

The mean and the 75th and 95th percentiles of sweeteners' habitual intake were estimated using the two-part model for episodically consumed food components implemented in the SPADE (Statistical Program to Assess Dietary Exposure) software, for the general population and by specific age groups. SPADE is an R-based program developed at the National Institute for Public Health and the Environment of the Netherlands (Dekkers et al., 2014), which aims to estimate the habitual (i.e. long-term) population intake based on short-term measured intake data. To estimate the within-person variability of intake, which is necessary for this kind of modelling, a sufficient number of respondents

with non-zero intakes on both of the two survey days is required. Therefore, the observed individual means (OIM; EFSA PPR Panel, 2012) model was used instead habitual intake estimation for some of the more rarely consumed sweeteners. Furthermore, mean intakes by food category were calculated using the OIM model to characterise the main sources of exposure. For each sweetener, exposure was estimated based on each of the five imputations of the food consumption database, applying survey weights to better approximate the results to the Portuguese population. Later the estimates were combined using Rubin's rules (Marshall et al., 2009).

Besides the computational notebooks, the fellow compiled a short summary that presented the main results of the exposure assessment, characterised the risk for the Portuguese population and discussed the uncertainties. According to the brand database of product label information, two sweeteners, thaumatin and neotame, were not consumed by the respondents of the dietary survey. The mean intake of the rest of the sweeteners (acesulfame K, aspartame, cyclamic acid, saccharin, sucralose, neohesperidin DC and steviol glycosides) by the general population aged 0–84 years and for each of the five imputed datasets is presented in Figure 1. Dietary exposure remained far below the ADI in the general population as well as in the 0- to 9-, 10- to 17-, 18- to 64- and 65- to 84-year age groups. The 75th and 95th percentiles did not exceed the ADI, either, further suggesting that the population is not at risk. The main source of intake for most of the intense sweeteners was the food category 'flavoured drinks' that mainly referred to soft drinks in the Portuguese food consumption database. Further results are not presented in this report in order not to compromise any future scientific publications by the hosting site.

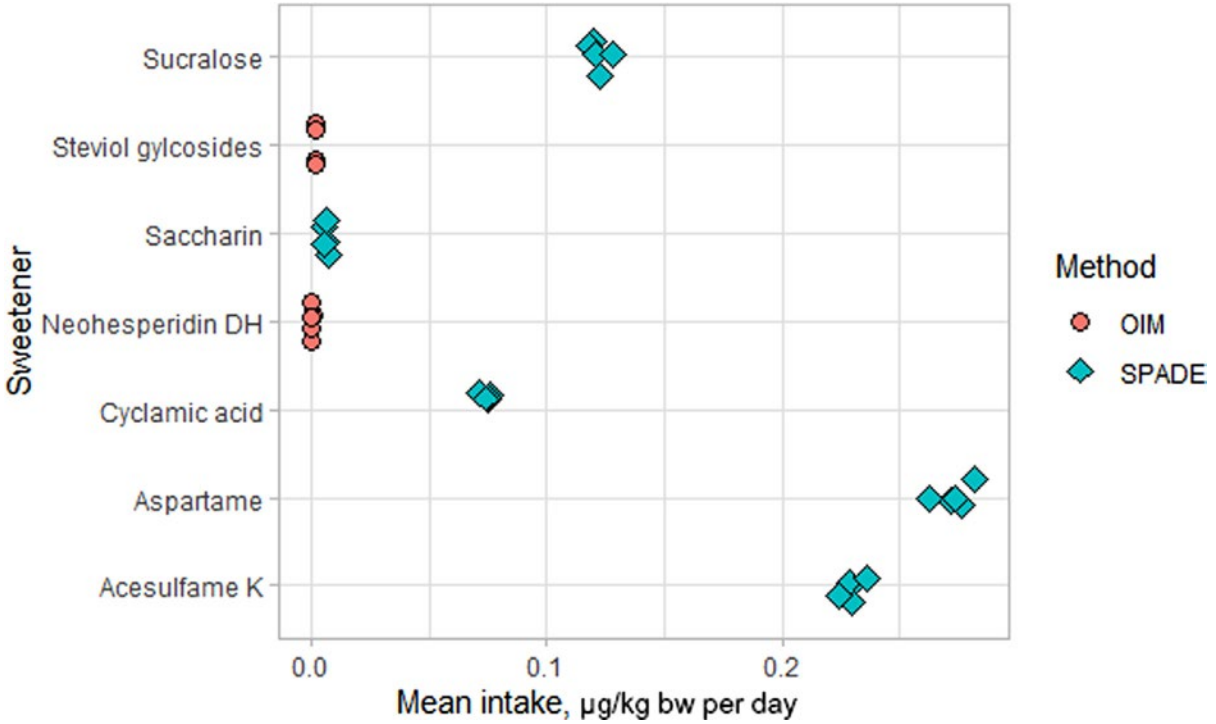

Figure 1: Mean intake of intense sweeteners by the 0- to 84-year age group, results from five imputed data sets per sweetener

2.2.2.. Practical exercise: risk assessment on acrylamide

The other practical exercise aimed to assess the dietary exposure to acrylamide and characterise the risk for the Portuguese general population. The exposure assessment again utilised the individual-level food consumption data from the Portuguese National Food, Nutrition and Physical Activity Survey, and occurrence data corresponding to the lower, medium and upper bound scenarios for various food groups were extracted from the EFSA scientific opinion on acrylamide (EFSA CONTAM Panel, 2015). The $BMDL_{10}$ (benchmark dose lower confidence limit 10%) for the neurotoxic effects (430 µg/kg bw) and neoplastic effects (170 µg/kg bw) of acrylamide were similarly obtained from the scientific opinion.

As for the previous practical exercise, the fellow first combined the input databases to calculate each respondent's intake of acrylamide per kg bw per each of the two survey days, and the workflow

was detailed in a computational notebook. Subsequently, the mean and the 75th and 95th percentiles of habitual intake were estimated using the one-part model for daily consumed food components in the SPADE software. Intakes were estimated for the lower, medium and upper bound scenario and for the general population aged 0–84 years as well as by age group and sex. Mean intakes by food category were calculated using the OIM model to characterise the main sources of exposure.

Again, the fellow compiled a summary of the assessment that described the results of the exposure assessment, calculated the margins of exposure (MOE) to characterise the risk, and briefly discussed the uncertainties. For genotoxic effects, such as the neoplastic effects of acrylamide, an MOE of 10,000 could be considered of low concern from a public health perspective, and an MOE of 100 is considered safe for non-genotoxic effects (EFSA, 2005). Regardless of the population group and scenario, MOEs for both neurotoxic and neoplastic were far below these safe margins, and the main sources of dietary exposure to acrylamide proved to be various cereal-based products such as soft bread, biscuits and crackers, etc. This indicates the need for a more refined exposure assessment on acrylamide for the Portuguese population; however, such assessment fell out of the scope of this fellowship.

3. Conclusions

The EU-FORA programme offered this fellow an opportunity to familiarise herself with risk assessment on two classes of substances found in food: additives and contaminants. This included both reviewing literature, to gain an understanding of the type of substances, their health effects, and the regulatory framework, as well as practical, hands-on exercises on risk assessment. The latter also provided the fellow an opportunity to develop her data science related skills, which will benefit her professional development as a data analyst. In addition, the fellow gained an overview of various topics related to food safety risk assessment by attending the EU-FORA dedicated training modules.

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Abbreviations

ADI	acceptable daily intake
$BMDL_{10}$	benchmark dose lower confidence limit 10%
bw	body weight
EU-FORA	The European Food Risk Assessment
OIM	observed individual means
MOE	margin of exposure
SCF	Scientific Committee on Food
SPADE	Statistical Program to Assess Dietary Exposure

Appendix A – Additional learning activities

Type of activity	Title	Organised by	Date/Duration
On-site courses	Fundamentals of Epidemiology	University of Porto, part of Master's in Public Health	31.10.2019–8.1.2020
	Systematic Review and Meta-Analysis	University of Porto, part of Doctoral program in Public Health	9.1.2020–30.1.2020
On-site seminars	Institute of Public Health open seminars	University of Porto, Institute of Public Health	October 2019–February 2020, monthly
	Institute of Public Health PhD Students' seminars	University of Porto, Institute of Public Health	October 2019–February 2020, monthly
	Nutrition and Obesity Epidemiology research group's seminars	University of Porto, Institute of Public Health	October–December 2019, monthly
Online courses	Reproducible Templates for Analysis and Dissemination	Emory University, at Coursera	~ 20 h
	Estimation of Measurement Uncertainty in Chemical Analysis	University of Tartu, MOOC	~ 26 h
	Sustainable Food Security: Crop Production	Wageningen University and Research, at edX	~ 42 h
	Sustainable Food Security: Food Access	Wageningen University and Research, at edX	~ 42 h
	Sustainable Food Security: The value of systems thinking	Wageningen University and Research, at edX	~ 54 h
	Julia Scientific Programming	University of Cape Town, at Coursera	~ 21 h
Online conferences and seminars	One Health EJP Annual Scientific Meeting	The One Health European Joint Programme	27–29.5.2020
	Getting to know the Global Dietary Database	The Global Nutrition and Policy Consortium	11.6.2020

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Livestock Health and Food Chain Risk Assessment

Animal and Plant Health Agency (APHA), Biomathematics and Risk Research workgroup, United Kingdom,
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Abstract

The EUropean FOod Risk Assessment (EU-FORA) Fellowship work programme 'Livestock Health and Food Chain Risk Assessment', funded by EFSA was proposed by the Animal and Plant Health Agency (APHA), UK. A scientist with a PhD in Food Science was selected to work within the Biomathematics and Risk Research group, under the guidance of a senior risk assessor. The programme consisted of four different modules that covered a wide range of aspects related to risk assessment (RA). The aims, activities and conclusions obtained during the year are described in this article. The learning-by-doing approach in RA allowed the fellow to discover a broad pool of methodologies, tools and applications while developing his own knowledge in RA, as well as gaining scientific network for future collaborations in the field.

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

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1. Introduction

This Technical Report consists of a description of the activities conducted under the EUropean FOod Risk Assessment (EU-FORA) Fellowship work programme entitled: 'Livestock Health and Food Chain Risk Assessment', funded by the European Food safety Authority (EFSA). The work programme was proposed by the Animal and Plant Health Agency (APHA), UK. APHA is 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. It is devoted to safeguarding animal and plant health in the UK, for the benefit of people, the environment and the economy, providing support for the delivery of their animal and plant health, welfare and bee health policies.

Within the EU-FORA fellowship, the fellow, Dr. Juan Manuel Martínez from the Food Technology Department of University of Zaragoza in Spain, was placed at the APHA, within the Biomathematics and Risk Research (BRR) workgroup, part of the Department for Epidemiological Sciences (DES). The workgroup 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, a Senior Risk Analyst within the BRR workgroup.

The Food Standards Agency (FSA) was a partner in the proposal. Its commitment is in protecting public health by making sure food is safe and is what it says it is. The Risk Assessment Unit (RAU) at FSA delivers risk assessment and technical advice relating to chemical and microbiological hazards of food and feed, food intolerance and allergy and radiological risk assessment.

The programme consisted of four different modules based on ongoing 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 Health and Food Chain Risk Assessment' designed by APHA was organised in four different modules that covered a wide range of aspects related to RA. Altogether, the modules aim to provide a broad overview of the various methodologies, tools and applications of RA. Each module built on the skills learnt in the previous modules and other training activities. Over the course of the year, Dr. Taylor monitored the progress of the programme and supervised the evolution of the project's activities. Furthermore, specialists were chosen to co-supervise each module based on their experience and relevance. Weekly meetings, both with Dr. Taylor and the co-supervisors, assessed in greater detail the progress of each module's deliverables and outcomes according to the programme schedule.

2.1. Aims

Each one of the four modules of the work programme consisted of a specific set of deliverables and outcomes, as follows:

Module 1: Principles and Terminologies of Quantitative and Qualitative Risk Assessment. The objective of this task was to complete and consolidate the RA knowledge of the fellow gained through the EFSA training, by getting practice in understanding the principles of qualitative and quantitative RA, the RA methodology and the different tools that can be used to perform RA. As part of this module, the fellow attended various courses and had access to learning materials and opportunities in meetings to improve his familiarity with RA.

Module 2: Hazard identification and Risk Prioritisation Methods. This module aimed to understand risk ranking methodologies and how risk ranking tools are implemented by adapting an existing risk ranking tool to the country of origin of the fellow. Specifically, the International Disease Monitoring (IDM) 'Risk of Incursion' tool (Roberts et al., 2011), was adapted to Spain. In addition, the fellow performed an updated overview of the risk ranking methods available regarding import of animal diseases in Europe and how they can be incorporated into the decision making process. The fellow reviewed ranking methods in literature, had the opportunity to use most of them, and explored horizon scanning methods and qualitative and semi-quantitative techniques for assessing food importation risks and other threats to animal and public health. The fellow also attended various meetings of different governmental groups and had a close collaboration with Defra, which facilitated progress on this module.

Module 3: Public Health and Food Chain Risk Assessment. This unit addressed the development of quantitative microbiological risk assessments (QMRAs) from farm-to-consumption. The fellow reviewed a farm-to-consumption *Salmonella* QMRA (Snary et al., 2016) and adapted it to data and conditions of Spanish pig farms. It is an individual-based stochastic model developed in Matlab programming language. In addition, the fellow had the opportunity to learn advanced visualisation techniques for spatial quantitative risk assessment.

Module 4: Import Risk Assessment. This module focused on the assessment of risk via importation of pathogens through different pathways. Specifically, the fellow considered the case study of importation of SARS-CoV-2 into the EU via human travel. This work was part of a European-funded Horizon 2020 project, the Versatile Emerging infectious disease Observatory (VEO), and built upon work from a previous project (Taylor et al., 2020). The fellow took into account uncertainty due to the lack of knowledge of new pathogens. The module involved learning how to extract and manipulate data from different sources, such as air passengers travel data from different countries to the EU and records of prevalence at different dates.

2.2. Activities/Methods

2.2.1. Module 1. Principles and Terminology of Quantitative and Qualitative Risk Assessment

The first module of the working programme was co-supervised by Dr. Louise Kelly, Senior Risk Analyst, part-time employee of APHA, Lecturer at University of Strathclyde and has previously provided consultancy to the World Health Organization, the Food and Agricultural Organization of the United Nations, EFSA and the OIE.

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. The qualitative RA lectures were undertaken in November 2019 at the RVC campus. The quantitative RA lectures were given online during May 2020, due to social distance guidance related to the COVID-19 pandemic. 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. As well as the lecture sessions of the Qualitative RA course, the fellow performed a rapid qualitative RA exercise at BRR for Crimean Congo Haemorrhagic Fever (CCHF) and discussed this with senior risk analysts in the workgroup. The RA assessed the annual risk of CCHF being introduced into the UK from Spain and infecting a human via ticks carried by wild birds. The Quantitative RA part of the RVC course also comprised lectures, and practical exercises, including the use of the @Risk software.

The fellow attended departmental training sessions, including the DES Taster Club and other seminars, where staff members or visitors present current research and it was possible to discuss and ask questions in a very interactive and rich debate. Likewise, the fellow also attended face-to-face and online meetings of various governmental groups. In November and December 2019, the fellow attended meetings of the National Emergency Epidemiology Group (NEEG), Human-Animal Infections and Risk Surveillance Group (HAIRS) and the Veterinary Risk Group (VRG). 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 group 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 the economy (Kosmider et al., 2017). During these meetings, various RA situations that were of importance at that moment were presented. The fellow was offered the opportunity to participate in the identification of the key elements of control and prevention of animal and human diseases and participated in the RA methodologies that were applied in these cases in real conditions. Likewise, the fellow understood how RA serves to support policy and the responsibilities of risk assessors and risk managers in these situations. Thus, the attendance at these meetings gave the

fellow the opportunity to understand the function of each group in identifying, assessing and prioritising new threats to the UK. The different members of the groups talked to the fellow about their roles, especially in outbreaks or emergencies of high-impact for health. In addition to these scheduled activities, the fellow participated in other meetings and consultations with colleagues over the course of the EU-FORA fellowship programme. 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

	Title	Date
Training sessions	Introduction to UK Civil service	October 2019
	Various Datacamp courses on R	October–December 2019
	Royal Veterinary College (RVC), Qualitative Risk Assessment (RA) Course	14 November 2019
	Qualitative RA exercise at BRR	11 December 2019
	Working from home and engaging colleagues course	5 May 2020
	RVC, Quantitative RA Course (virtualised)	May 2020
	Data visualization in R (Part I-III)	June 2020
Regular meetings	BRR workgroup meeting	Every month
	DES Team meeting	Every month
	DES Taster Club (talks of different topics)	Every two weeks
Other meetings	Meeting in Nobel House (DEFRA), IDM tool discussion with Helen Roberts (Advisor Exotic Disease Control)	21 October
	Veterinary Risk Group meeting in Nobel House	5 November
	HAIRS and EpiRisk meeting in Nobel House	4 December
Conferences	One Health European Joint Programme Annual Scientific Meeting 2020	27–29 May
	Society for Veterinary Epidemiology and Preventive Medicine conference	12 May–16th June (every Tuesday)
One Health Webinars	Managing a raging pandemic: a steep learning curve Risks of future pandemic threats and how to prepare	13 July 16 July

In addition, the EU-FORA training programme was supported by four training sessions provided by EFSA. Due to the unprecedented situation derived from the COVID-19 pandemic, two of these were performed as scheduled but the other two had to take place online. The fellow attended a 3-week induction training at EFSA headquarters in Parma, Italy and a 1-week training module at the Austrian Agency for Health and Food Safety (AGES) in Vienna, Austria. The training sessions due to be held at the German Federal Institute for Risk Assessment in Berlin, Germany and the Hellenic Food Authority in Athens, Greece were adapted to take place online during the month of August.

2.2.2. 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 focused on studying the IDM 'Risk of Incursion' tool (Roberts et al., 2011) and adapting this for Spain. This Microsoft Excel®-based tool was originally developed in 2009 to provide a rapid, semi-quantitative measure of the risk of disease introduction to the UK. The tool was based on the hypothesis that the primary routes by which an exotic animal disease (EAD) could be introduced into the UK would be legal or illegal trade, wild bird migration or vectors (e.g. mosquitoes, midges, ticks). The tool has been regularly updated, with further adaptions implemented, and the current version is still being used by the Veterinary Advice & Surveillance Group to assess the risk of incursions of EADs to the UK.

After having the opportunity to work with the tool, the fellow analysed and described the function, identified the strengths and drawbacks and studied the criteria and the inputs and outputs. Second, the fellow assessed the feasibility of adapting the IDM tool to analyse the risk of incursion of various animal diseases into Spain through an associated livestock or product of animal origin.

The list of diseases assessed by the tool was revised to be relevant to the Spanish situation. Diseases already present in Spain, such as Brucellosis (*Brucella abortus* and *B. melitensis*), West Nile Virus, Aujeszky's Disease, Contagious Agalactia, HP-PRRS, *Trichinella* and *Leishmania* were removed, while others of more relevance to Spain than the UK, such as *Salmonella abortusovis*, theileriosis and trypanosomosis, were added. The 2018 international trade data for Spain 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, 2018; European Commission, 2019). The model included not only all the legal trade partners of Spain, but also countries where illegal trade may come from. The countries are separated into regions based on the volume of their trading with Spain. Each region is assigned a score based on its disease status, namely whether the disease is only found in wildlife, is sporadic in livestock or is endemic in the livestock population. This indicates the overall geographical distribution for each considered disease. Various risks of trade routes are taken into account, such as imports of livestock, products of animal origin (POAOs) (meat, milk and eggs), genetic material (semen, ova and embryos), biological material (serum and plasma), transport vehicles, food waste and zoo animals. Furthermore, the potential for movement of wild animals, vectors, or migration of birds is scored from each region. A negative score is included to indicate forms of mitigation actions against the targeted disease which are in place in the region of origin of the imported commodity. All the resultant scores are input into an algorithm to calculate the overall risk of incursion level for Spain of each disease, in relation to its geographical distribution. Of great relevance was the fact that the fellow included new factors in the pathway in order to consider the effect of the interaction of vectors and migratory birds. One of the main differences between the results of original UK IDM tool and the new version adapted by the fellow lies in the vector-borne diseases. This is likely due to the location of Spain in the southern part of Europe and its warm weather.

In addition, the fellow also considered other tools available and contributed to an extensive study of risk ranking methods and tools for prioritising animal diseases. The routine horizon scanning methods undertaken to ensure emerging issues are captured were investigated. This approach includes risk ranking, horizon scanning methods and qualitative and semi-quantitative techniques for assessing food importation risks and other threats to animal and public health. It increased the fellow's knowledge of risk prioritisation methods, data sources and incorporation of uncertainty into outputs.

Various tools were explored and used, some of which are maintained and designed specifically for the UK and others developed by EU countries and international agencies. These tools prioritise pathogens of highest risk, on a regular basis, and feed into the specific contingency plans within the Outbreak National Response. The tools available online were tested in order to identify their advantages and limitations. These risk ranking frameworks were assessed in order to identify their general recommendations for a prioritisation approach of animal diseases in the selected risk ranking tools. These accomplishments facilitated the completion of a comprehensive overview of a wide pool of tools developed within the EU over time to rank animal diseases. The fellow commented and provided his opinion to the study entitled 'Best practices in risk ranking animal diseases: An analysis of ten risk ranking tools' that is intended to be submitted for publication and whose main author was the former EU-FORA fellow.

2.2.3. Module 3. Public Health and Food Chain Risk Assessment

Module 3 was supervised by Dr. Catherine M^{c}Carthy, Risk Analyst within the BRR Workgroup of APHA.

The fellow had the opportunity to review and adapt a *Salmonella* QMRA for Spain, which was previously produced by APHA for EFSA and implemented for four European Union member states, but not Spain (Snary et al., 2016). The EFSA QMRA included a cost-benefit component to help aid decision makers as to the best control measures to implement (Gavin et al., 2018).

Salmonella is an important zoonotic pathogen that can cause human illness with potential hospitalisation and even may lead to the death of the patients in severe cases. Food-borne-outbreaks (FBO) and pork meat in particular are frequent sources of human infection. The fellow carried out a QMRA that could assist the development of national control plans for *Salmonella* in pigs in Spain. The

original farm transmission model (Hill et al., 2016) was adapted by the fellow using Spanish pig production parameters taking into account the particularities of the pig industry in the country.

The original farm model was an individual-based stochastic susceptible-infected-susceptible model (SIS) taking account of i) multiple changing populations and ii) intermittent shedding of *Salmonella*. The model was implemented using Monte Carlo simulation, where each iteration represents production from one farm over a 500-day period, incorporating farrowing, weaning, grower and finisher production. It considers sows, feed and external environment as sources of infection, as well as allowing for infection from bacteria in the pen environment. A range of different farm management practices were considered. The outputs obtained included within-batch prevalence along rearing and at the slaughter age and shedding rate. The farm management factors were estimated from different Spanish production databases. The weightings for apportioning farm types were taken from data collected from the EFSA baseline survey for breeding pigs (EFSA, 2009) and the Agriculture Spanish ministry survey 2016 (Spanish Agriculture, Fishing and Food Ministry). The breeding herd prevalence of Spain was taken from the EFSA breeding pig survey and compared with data from the literature (Andreoletti et al., 2008; Vico et al., 2011) and Agriculture Spanish ministry, as well as the within herd prevalence and the contamination of feed. The issues regarding sampling and data collection were addressed by taking into account the uncertainty and fitting distributions. Mass of faeces defecated by the pigs and amount consumed per day at different stages have been extracted from Spanish data from IRTA (Institute of Agrifood Research and Technology). Once a pig has been infected then the magnitude of shedding is assigned, by sampling from a distribution derived from observed values and according to the dose with which the pig was infected. It was assumed that infected pigs excrete intermittently during the whole time period of infection, with a highly variable profile between individual pigs.

The model adapted to Spain found that the breeding herd prevalence plays a major role as a source of infection. The results obtained were compared to an example of a high-prevalence Member State (MS) from the original model. The Spanish model estimated a higher level of *Salmonella* infection, highlighting the need to control this pathogen on farm. Sow prevalence was found to be a strong indicator of slaughter pig prevalence and this model is a promising tool to evaluate the effectiveness of interventions.

The fellow wrote a report and made a presentation to Dr. Robin Simons (acting lead risk analyst), Dr. Catherine McCarthy and Dr. Rachel Taylor at the end of the module, providing an opportunity to discuss and receive comments in order to improve the understanding and quality of the model. It is expected that the fellow will continue working on the model and submit an article.

Originally, within Module 3, the fellow intended to take a short-term secondment within the Microbial Risk Assessment team at FSA to work on the analysis of *Salmonella* Typhimurium data from human *Salmonella* outbreak cases. This would have allowed time to explore different 'what if' scenarios in order to feed this knowledge back into the quantitative model at APHA. However, this secondment could not take place due to the COVID-19 pandemic. Instead, the fellow was able to spend additional time learning detailed visualisation methods for spatial quantitative risk assessment. Using the R software package, the fellow produced maps such as one presenting the *Salmonella* prevalence in EU members states (Figure 1).

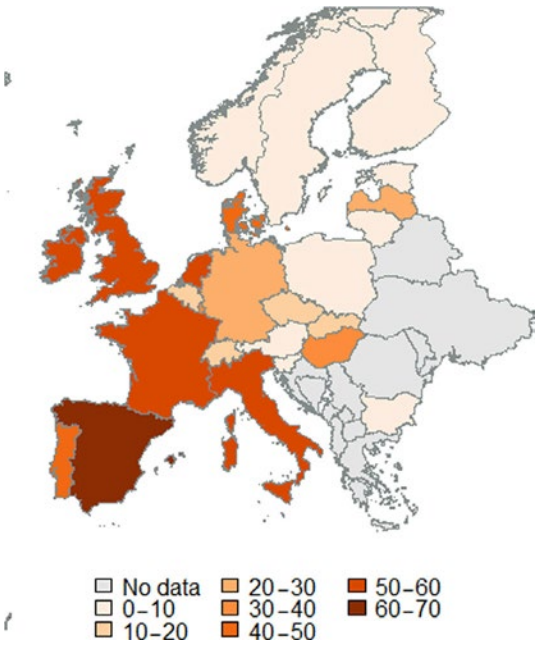

Figure 1: Mapping the 2016 EFSA baseline *Salmonella* prevalence in the EU member states using R

2.2.4. Module 4. Import Risk Assessment

In this module, the fellow has been involved in an on-going RA project at APHA, as part of the VEO Horizon 2020-funded project (https://www.veo-europe.eu/).

The role of the fellow in this project consisted of testing APHA's previously developed generic risk assessment framework (Taylor et al., 2020), in a Disease X scenario. Disease X is defined as a fast spreading exotic infectious disease (EID), with high case fatality by an unknown pathogen. In the light of the COVID-19 pandemic, it was decided to focus on the RA process of introduction of SARS-CoV-2 in the EU/EEA and the UK, specifically to address the question: In the event of an emergence, can we rapidly apply the tools developed to support pandemic response? In particular, the fellow performed a RA answering two questions:

i) What was the risk, as of 31 January 2020, of introduction of SARS-CoV-2 by human travel into the EU/EEA and UK taking into account the information that we knew at that time?
ii) What was the risk, as of 31 January 2020, of introduction of SARS-CoV-2 by human travel into the EU/EEA and UK taking into account the information that we know now (June 2020)?

The two different dates were chosen in order to understand the effect of uncertainty due to lack of disease knowledge in a newly emerging disease outbreak.

Given the short timeframe, this module only focused on the entry of the pathogen. The risk pathway of entry due to human travel was designed in line with the generic framework that has been previously developed at APHA (Taylor et al., 2019). Data on prevalence and human travel was collated. The data on prevalence, number of cases detected and new cases were extracted from the Johns Hopkins records (Johns Hopkins University of Medicine, 2020), health ministry reports (Spain and the UK), ECDC reports, WHO reports, published scientific literature and BBC news, Spanish news or social media. Travel data was extracted from Eurostat for official records of air passengers (Eurostat, 2020).

This module also involved attending online group meetings for the VEO project which took place during this time.

At the time of writing, the fellow is finalising the model and anticipates writing a short communication article on the work.

3. Conclusions

This EU-FORA fellowship has provided an opportunity to learn RA methodologies across a range of RA topics, such as chemical and microbial risk assessment, risk communication, import risk assessment and risk ranking tools. This intensive year of training, utilising the 'learning-by-doing' approach within a

hosting institute allowed for an increased knowledge of all areas of RA, and hands-on experience with different types of risk assessment projects and questions. The fellow learnt both qualitative and quantitative skills, such as data collation, data manipulation in R, plotting maps, understanding inputs and outputs of risk assessment, and effective risk communication. This was all helped by a dedicated hosting institute and the atmosphere of undertaking this learning when surrounded by experienced and knowledgeable risk assessors. Although the scope of work programme was impacted by the COVID-19 pandemic, the experiences and skills gained by the fellow are invaluable and have presented numerous opportunities for future research projects and collaborations.

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Abbreviations

AGES	Austrian Agency for Health and Food Safety
AMR	antimicrobial resistance
APHA	Animal and Plant Health Agency
BfR	German Federal Institute for Risk Assessment
BRR	Biomathematics and Risk Research workgroup
CCHF	Crimean Congo Haemorrhagic Fever
DEFRA	Department of Environment, Food and Rural Affairs
EAD	exotic animal disease
ECDC	European Centre for Disease Prevention and Control
EIDs	emerging infectious diseases
EU-FORA	European Union Food Risk Assessment
EUROSTAT	The Statistical Office of the European Union
FSA	Food Standard Agency
HAIRS	Human-Animal Infections and Risk Surveillance Group
NEG	National Expert Group
NEEG	National Emergency Epidemiology Group
OIE	World Organisation for Animal Health
QMRA	quantitative microbiological risk assessment
RVC	Royal Veterinary College
SIS	susceptible-infected-susceptible
TRACES	Trade Control and Expert System
VRG	Veterinary Risk group
VEO	Versatile Emerging infectious disease Observatory
WHO	World Health Organization

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Study of the different evaluation areas in the pesticide risk assessment process

National Institute for Agricultural and Food Research and Technology (INIA), Roberto Molteni and José-Luis Alonso-Prados

Abstract

Approval of active substances and authorisation of plant protection products in the EU is made based on a strict risk assessment of the agronomic use of the plant protection products. Regulation 1107/2009 regulates the procedure in the EU with complex procedures involving many actors. 'The Farm to Fork strategy' and 'The Biodiversity for 2030 strategy', that are the heart of the 'European Green Deal', aiming to make food systems fair, healthy, environmentally friendly and put Europe's biodiversity on the path to recovery by 2030, for the benefit of people, climate and the planet. Therefore, 'The Farm to Fork strategy' and 'The Biodiversity for 2030 strategy' represents a challenge for the evaluation and authorisation of plant protection products in which the risk management will constitute a key element on the approval of active substances and authorisation of plant protection products. The aim of the work was to get knowledge of the large body of EU legislation and guidelines in the plant production products, identifying the most critical points of the pesticide evaluation in each of its areas, analysing the complexity and the interaction between these different areas. This study allowed to have a global and clearer vision of these procedures, with the focus on highlighting inconsistency and to propose speed up alternatives. Finally, this work will also facilitate not only the risk assessment but also the decision-making on the approval of active substances and the authorisation of plant protection products.

Keywords: authorisation, active substance, approval, plant protection products, maximum residue level, risk assessment, pesticide

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1. Introduction

The focus of the European Food Risk Assessment Fellowship Programme (EU-FORA) is to provide hands-on skills in chemical and microbiological risk analysis for food safety, especially focus on risk assessment, with an overview of risk management and risk communication.

This specific project *Study of the different evaluation areas in the pesticide risk assessment process* was performed at the National Institute for Agricultural and Food Research and Technology (INIA), the unique Independent Evaluation Institution authorised in Spain to carry out the assessment in the areas of identity and physical-chemical properties, methods of analysis, residues, fate and behaviour in the environment, ecotoxicology and efficacy in the field of the active substances, plant protection products (PPP), safeners and synergists (Resolución 2015, complying with Real Decreto 971/2014). While the Ministry of Health, Consumer Affairs and Social Welfare carries out the assessment in the area of human toxicology.

In Spain, the Ministry of Agriculture Fisheries and Food is the National Competent Authority, in charge of the implementation of Regulation (EC) No 1107/2009 concerning the placing of PPPs on the market; while the competent authorities at Autonomous Community level are responsible for implementing all other controls (e.g. on marketing, maximum residue level (MRL)).

Details of the Spanish organisation for authorisation and control of PPPs and residues are provided in the Appendix A.

PPPs are *pesticides* that protect crops or desirable or useful plants. They are primarily used in the agricultural sector but also in forestry, horticulture, amenity areas and in-home gardens. They contain at least one active substance and have one of the following functions: 1) protect plants or plant products against weeds, pests and diseases, before or after harvest; 2) influence the life processes of plants (such as substances influencing their growth, excluding nutrients); 3) preserve plant products; and 4) destroy or prevent growth of undesired plants or parts of plants.

Pesticides contain and at least one active substance, such as any chemical, plant extract, pheromone or microorganism (including viruses), that has action against *pests* or on plants, parts of plants or plant products. They may also contain other components including safeners, synergists and co-formulants.

It is also important define the term *pesticide*, that is often used interchangeably with *plant protection product*. However, pesticide is a broader term which also covers non plant/crop uses, for example biocides. Nevertheless, the most common use of pesticides is in the form of PPPs.

For active substances and PPPs (chemical and microbiological), respectively, a risk assessment must be carried out to ensure that these substances/products do not have harmful effects on human or animal health or unacceptable effects on the environment.

2. Description of work programme

The work follows the uniform principles for evaluation and authorisation of PPPs as in the wide EU legislation and guidelines which regulate the authorisation, use of PPPs and their residues in food. Those areas of evaluation are under responsibility of INIA.

The first step of the work was the participation in the extensive training *corpus iuris* delivered by the coordination team of the *Unidad de Productos Fitosanitarios* (UPF) in INIA allowed to get a global view of the different areas of evaluation.

This global view is a key point for interpreting the results of evaluations, with their uncertainty, in order to ensure that the chances of failing to detect adverse effects or of under-estimating their importance are reduced to a minimum. All this picture shall be taken into consideration in the decision-making process, identifying critical decision points or items of data for which uncertainties could lead to a false classification of risk.

The training was complemented with the analysis of some specific cases for which Spain is the rapporteur Member State (RMS) in order to identify critical aspects of the risk assessment process when applied.

2.1. Aims

The aim of the work was to get knowledge of the large body of European Union (EU) legislation and guidelines in the plant production products, identifying the most critical points of the evaluation in each of the its areas, analysing the interaction between the different areas and defining the most appropriate risk mitigation measures, based on the good agricultural practice. This study will allow to

have a global and clearer vision of the risk assessment results and of the risk mitigation measures that should be established, with the focus on highlighting inconsistency and to propose speed up alternatives. Finally, this work will also facilitate not only the risk assessment but also the decision-making on the approval of active substances and the authorisation of PPPs.

2.2. Regulation of plant protection products

Plant protection products and their residues are regulated at the EU level by Regulation (EC) No 1107/2009 and Regulation (EC) No 396/2005, respectively. They are complemented by the so-called Sustainable Use Directive 2009/128/EC that requires Member States (MSs) to establish National Action Plans for the sustainable use of pesticides by promoting the adoption of Integrated Pest Management (IPM) and alternative approaches or techniques.

Regulation (EC) No 1107/2009 is in force since June 2011 and lays down harmonised rules for the approval of active substances and the placing on the EU market of PPPs. While Regulation (EC) No 396/2005 is in force since April 2005 and lays down the rules and procedures for setting maximum levels of pesticide residues (MRL) in or on food and feed of plant and animal origin, taking into account also international Codex Alimentarius levels.

The main objectives of these regulations are the following: 1) to ensure safety for operators, workers, bystanders, residents, consumers (including vulnerable groups of consumers) non-target species and the environment; 2) to allow an efficient use of resources for risk assessment and risk management in the policy area of pesticides; and 3) to shorten the time for new products to come on the market. Their purpose is also to facilitate the free movement of PPPs and plant products treated with PPPs and their availability in MSs, and to safeguard the competitiveness of EU agriculture.

These regulations clearly define the European Food Safety Agency (EFSA), MSs and Commission' responsibilities for risk assessment, risk management and control for active substance approvals, product authorisations and MRL setting. The regulations set a centralised procedure for active substance approvals and MRL setting, which avoids fragmentation of the internal market for food products and difficulties for importers having to deal with differing national rules on MRLs. While all PPPs undergo a double authorisation procedure before they can be placed on the market.

The first step is in the applicant' hands – the company that has commercial interest in placing the new substance on the market – submits an *application* (dossier) with the required data, described in the Regulation (EU) No 283/2013 for active substances and Regulation (EU) No 284/2013 for PPPs, to a MS of its choice (RMS) and to the European Commission, then a comprehensive assessment of the active substance is carried out by experts of MSs; while for renewals, the Commission, not the applicant, assigns the dossiers on pesticide active substances to the MSs on the basis of a country quota rule. EFSA then performs a final peer review and adopts a conclusion on whether the substance meets the approval criteria.

The following step is taken under the examination procedure: The Commission makes a proposal for approval to the MSs representatives in the Standing Committee on Plants, Animals, Food and Feed (SCoPAFF), who votes on the proposal; a positive vote by a qualified majority results in the approval of the pesticide active substance at the EU level.

Once an active substance has been approved, an authorisation of individual formulations based on the active substance at the level of the individual MSs can be requested and one MS of the zone carries out an assessment on behalf of the other MSs of the same zone (zonal evaluation). Authorisations are granted on a national basis because local environmental conditions and the occurrence of pests (therefore, use of pesticides) may differ. Each MS can do it at its national level, indifferently of how huge country is and how those differences can be also inside itself are, e.g. in Spain, *Ministerio de Agricultura, Pesca y Alimentación* authorises the PPPs that can be used in region with different environmental conditions, where the occurrence of pests may differ such as *Comunidad Autónoma de Galicia* or *Comunidad Autónoma de Andalucía*.

Authorisation of PPPs by MSs follows the provisions of the zonal evaluation of the Regulation (EC) No 1107/2009 (Articles 33–39). The zones for the evaluation of PPPs are defined in the Annex 1 of this regulation: Zone A –North (Denmark, Estonia, Latvia, Lithuania, Finland, Sweden); Zone B – Centre (Belgium, the Czech Republic, Germany, Ireland, Luxembourg, Hungary, the Netherlands, Austria, Poland, Romania, Slovenia, Slovakia, the United Kingdom); Zone C – South (Bulgaria, Greece, Spain, France, Croatia, Italy, Cyprus, Malta, Portugal). EU countries assess applications on behalf of other countries in their zone and sometimes on behalf of all zones. Mutual recognition is an important part of this: an authorisation in one MS can be used for mutual recognition in another MS, either in the

zone or even in another zone if the product is used in greenhouses, as post-harvest treatments, for treatment of empty storage rooms, or for seed treatments. Mutual recognition is built on the assumption that any assessment which was already done by one MS shall not be repeated by another MS when recognising an authorisation. The procedure could be improved with a well structure and easily public available database, to ensure best exchange of information for zonal application and particularly important for interzonal applications. Sharing zonal and interzonal elements of assessment is highly recommended, providing these and others information to EFSA, with a clear reorganisation of the arrangements for risk assessment (SAPEA, 2018).

Another issue which was examined was the emergency authorisation for an active substance that is under evaluation at the EU level or for a substance not approved at the EU level, as long as the provisions in art. 53 of Regulation (EC) No 1107/2009 are guaranteed: e.g. 1,3-dichloropropene in *Región de Murcia* (Spain), granted for the same crops, functions and methods for the periods from 24 January 2018 to 15 April 2018; from 1 January 2019 to 15 April 2019 and from 1 January 2020 to 30 March 2020 (Database of Emergency Authorisations).

Several provisions of the two regulations have not been implemented yet, and several others cannot be fully enforced. Moreover, timelines for procedures is challenging, particularly for MSs and legal timelines are widely exceeded, especially those set for the mutual recognition of authorisations. This hinders innovation and affects the capacity of the sector to replace hazardous substances with either other substances or alternative methods. The lack of innovative solutions may have a negative effect on the objectives of improving agricultural production and safeguarding the competitiveness of the European agriculture.

Therefore, there is a need to reduce the complexity through a better coordination of work. This could reduce duplications, improve effectiveness and foster efficiency. Simplification is necessary to tackle the future work overload in the risk assessment, but the risks of pesticide use vary considerably from one pesticide to another, depending on the intrinsic characteristics of the active substances (toxicity, persistence, etc.) and on the use patterns (applied volumes, application period and method, crop and soil type, etc.). Most of the intrinsic characteristics of active substances are known, although they are not always easily available.

Simplification can also be achieved through the implementation of tools, useful for a quick screening of the hazard, as the hazard approach is foreseen by Regulation (EC) No 1107/2009, and can be useful for focusing on the critical areas of the assessment. One of such tools, it has been proposed at the 13th European Pesticide Residues Workshop (EPRW, 2020).

2.3. Regulation on residues

Regulation (EC) No 396/2005 covers compliance with legal limits for pesticide residues in food and feed, including provisions on official controls of pesticide residues in food (plant or animal origin).

The regulation defines the roles of the MSs, EFSA and the Commission in setting of MRLs, and sets a common EU assessment scheme for all agricultural products.

The procedure foresees that applicant proposes MRL, providing experimental data on the expected residues when the pesticide is applied according to Good Agricultural Practice (GAP) and on toxicological reference values. One MS evaluates this application and produces an evaluation report that it is verified by EFSA. The evaluation performed by the MS verifies that residues are safe for all European consumer groups, including vulnerable groups such as babies, children and vegetarians. When a risk is established for any consumer group, the MRL application is rejected and the pesticide may not be used on that crop and MRL is set at the lowest limit of analytical quantification (LOQ): default lowest limit in EU law is 0.01 mg/kg. That is the MRL also for crops on which the pesticide has not been used or when its use has not left detectable residues.

The data requirements for the analytical methods are set in Regulation (EU) No 283/2013, Regulation (EU) No 284/2013 and in the guidance SANCO/825/00 rev. 8.1.

However, a current trend in monitoring food for chemical residues and contaminants is to combine as many analytes as possible into a single method with an emphasis on developing laboratory methods which simultaneously analyse compounds from multiple categories including pesticides, veterinary drugs, mycotoxins, and other organic chemicals in a variety of food commodities (Turnipseed and Jayasuriya, 2020). A review describes several methods developed for simultaneous analysis of veterinary drugs and pesticide residues (Garrido Frenich et al., 2014.

Improvements in instrumentation and in data processing software, for both liquid chromatography–mass spectrometry (LC–MS) and gas chromatography–mass spectrometry (GC–MS), have facilitated

the ability to quickly query the mass data for hundreds of analytes in an automated manner and to find out unexpected analytes. This will allow regulatory agencies to better ensure the safety of the global food supply. For example, the United States Food Drug Administration (US FDA) recommends developing these analytical methods for imported products (FDA, 2019). In the light of the management adage that 'if you can't measure it, you can't change it'.

Regulation (EC) No 396/2005 establishes harmonised Community provisions relating to maximum levels of pesticide residues in or on food and feed of plant and animal origin. However, the note in Annex I specify that MRLs do not apply to products or part of products that by their characteristics and nature are used exclusively as ingredients of animal feed, until separate MRLs are set in the specific category 1,200,000. It should be also noted that article 1 (Subject matter) of the regulation mentioned only 'maximum levels of pesticide residues in or on food and feed of plant and animal origin', therefore, it excludes feed of mineral origin. These drawbacks could be overcome with a risk analysis (Circulaire, 2019), but nowadays a European harmonised approach is missing, although working for harmonisation is on-going.

Regarding the risk assessment for surface and ground water, some details are provided in the Appendix B.

PPPs can also contain safeners, synergists, adjuvants and co-formulants for which there are not MRL at the EU level. Nowadays, they can be only regulated at national level, although working for harmonisation is ongoing.

2.4. Regulations, guidance and procedures

Risk assessment methodologies are methods of – and criteria for – evaluating data, which form the basis of regulatory decision-making. They are written into hard law (legislation and implementing acts) and in soft law (non-legally binding guidance documents, administrative and peer-reviewed scientific literature), with a significant role left to the latter (Robinson et al., 2020). Deviations from non-legally binding guidelines are allowed provided a full description and scientific justification in the risk assessment.

Regulation (EC) No 1107/2009 art. 12(2) stipulates that EFSA ('the Authority') shall adopt a conclusion in its opinion on the substance 'in the light of current scientific and technical knowledge using guidance documents available at the time of application'. In this regulation, an article dedicated to guidance is the art. 77 (Guidance documents), which concerns micro-organisms, pheromones and biological products. Annex II of the same regulation mentions also 'any further guidance developed in the framework of the SCoPAFF for the purposes of refining, where relevant, the risk assessments'. In addition, the art. 78 states: 'Any further measures necessary for the implementation of this Regulation may be adopted'. Therefore, Commission can initiate the work to produce new guidance documents at any time, although guidance documents for risk assessment are made normally by EFSA, under a mandate from EU Commission or by its own initiative. Pesticide Steering Network Group, integrated by EFSA, all MS and EU Commission has identified the list of guidance documents that are necessary and has prioritised them.

Regulation (EU) No 284/2013 also refers to specific guidelines adopted by international organisation, i.e. European and Mediterranean Plant Protection Organization (EPPO) and Organisation for Economic Co-operation and Development (OECD).

There are national guidance, too, e.g. Guidance for the Comparative Assessment and Substitution of Plant Protection Products, implementing Regulation (EC) No 1107/2009. In Spain, it is nowadays used the *Guía complementaria de evaluación comparativa y sustitución de productos fitosanitarios en España*, which is based on the guidance document SANCO/11507/2013 (rev. 12) and on the EPPO standard – PP1/271 (1) as well as on the guidance elaborated by the UK (Comparative Assessment and substitution: guide for UK applicants for PPPs authorization) and Portugal (Comparative assessment and substitution – Guide for Plant Protection Product authorization).

The International guidelines can help the harmonisation of specific pesticide topics and even improving the procedure with the joint review of pesticide (OECD, 2011).

Science moves fast and the guideline update is fundamental, taking into consideration also the relevant scientific literature (Court of Justice of the European Union, 2018).

A European audit within the legal framework defined by the Regulation (EC) No 1107/2009 and Regulation (EU) No 546/2011 identified weaknesses for prioritisation of official controls, co-ordination and co-operation between and, in some cases, within Competent Authorities due to the complex highlighted system. In particular, with regard to PPP authorisation, the significant delays of MSs in the

evaluation or re-evaluation of PPPs highlight the difficulty to implement authorisation systems based on EU legislation (SANTE, 2017).

3. Conclusions

Pesticide risk assessment is governed by hard and soft laws, with complex procedures involving many actors, and these procedures have been analysed to get the global view of the process.

Specific areas of evaluation, within the remit of the UPF, such as method of analysis, efficacy, residues, ecotoxicology, environmental fate and behaviour, have been analysed. As result of this analysis, several points that should be improved have been highlighted, such as the coordination with other legislative areas, the effective strategies for replacement of substances of concern or how to use monitoring data, to reduce the risk assessment burden.

In conclusion, this report has analysed regulations, guidance and procedures of the pesticide risk assessment in the areas of which the UPF in INIA is in charge. Finally, the report has highlighted some shortcoming of the process and has also proposed some ways to overcoming them, such as tools (EPRW, 2020), simplification procedures (SAPEA, 2018) and suggestion for the next European audits to be comprehensive of the whole process.

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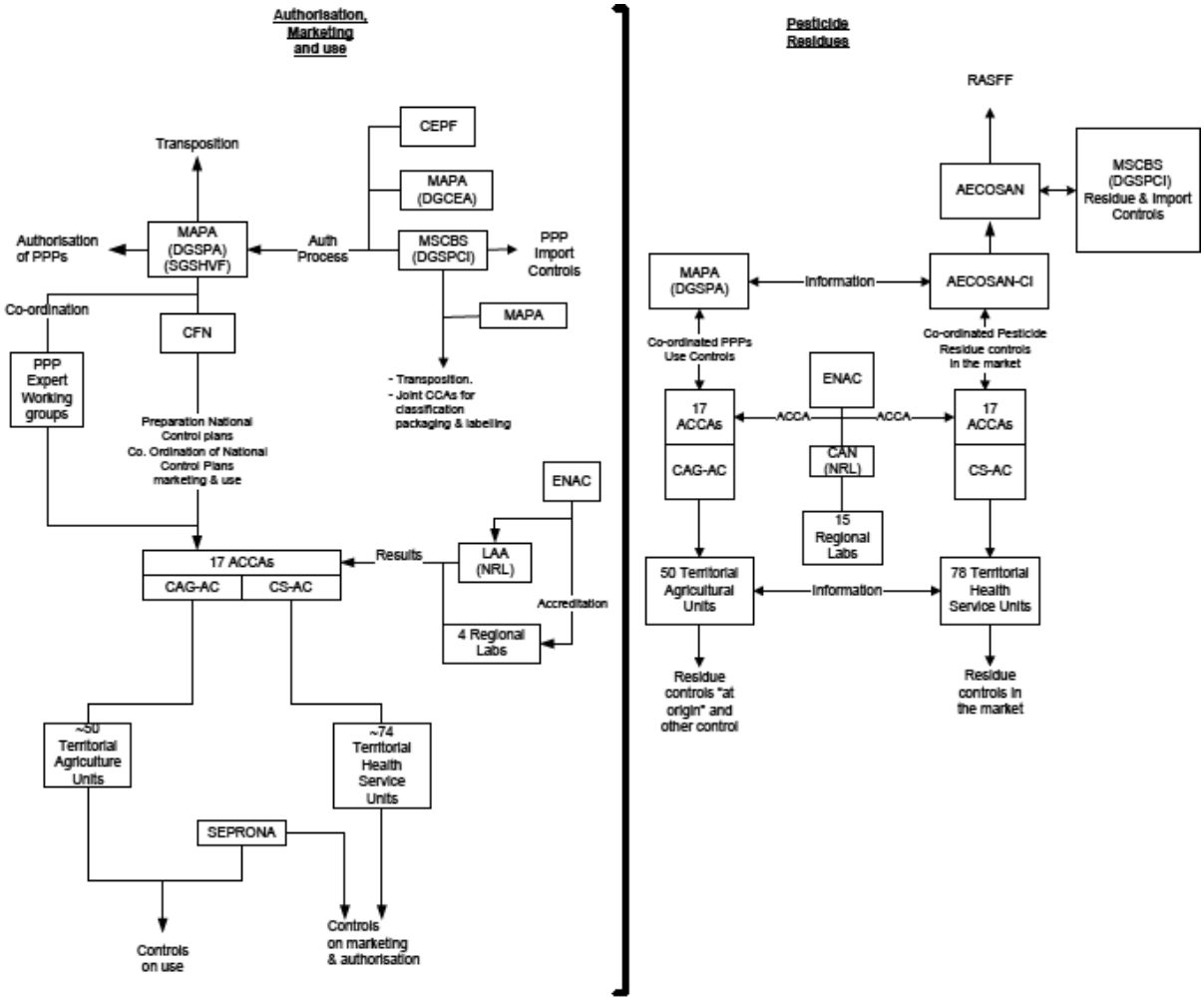
Abbreviations

EPPO	European and Mediterranean Plant Protection Organization
EPRW	European Pesticide Residues Workshop
EQS	environmental quality standard
ERO	ecological recovery option
ETO	ecological threshold option
EU-FORA	European Union Food Risk Assessment
GAP	Good Agricultural Practice
GC–MS	gas chromatography–mass spectrometry
IPM	Integrated Pest Management
LC–MS	liquid chromatography–mass spectrometry
LOQ	limit of quantification
MRL	maximum residue level
OECD	Organisation for Economic Co-operation and Development
OJ	Official Journal of the European Union
RAC	regulatory acceptable concentration
RMS	rapporteur Member State
SCoPAFF	Standing Committee on Plants, Animals, Food and Feed
SPG	specific protection goals
US FDA	United States Food Drug Administration
WFD	EU Water Framework Directive

Appendix A – Control system for PPPs and residues



AC	Autonomous Community
ACCA	Autonomous Community Competent Authority
AECOSAN	Spanish Agency for Consumers, Food Safety and Nutrition
AECOSAN (-CI)	Spanish Agency for Consumers, Food Safety and Nutrition (- Institutional Committee).
CAG-AC	Ministry of Agriculture and Livestock of the AC
CEPF	Commission for Evaluation of Phytosanitary Products
CFN	National Phytosanitary Committee
CS-AC	Ministry of Health of the AC
CNA	National Food Centre
DGCEA	Directorate-General for Quality and Environmental Assessment of MAPA
DGSPA	Directorate-General for Agricultural Production Health
DGSPCI	Directorate General for Public Health, Quality and Innovation
SGSE	Sub-directorate-General for External Health
ENAC	National Body for Accreditation
LAA	Agri-Food Laboratory in Madrid
MAPA	Ministry of Agriculture, Fisheries and Food
MSCBS	Ministry of Health, Consumer Affairs and Social Welfare
NRL	National Reference Laboratory
PPP	Plant protection products
SEPRONA	Civil Guard Environmental Protection Service
SGSHVF	Sub-directorate-General for Plant and Forestry Health and Hygiene
RASFF	Rapid Alert System for Food and Feedingstuffs

(Source: DG(SANTE)/2018-6516 Final Version date: August 2018).

Appendix B – Risk assessment for water

In Europe, different legislations have been developed with different methodologies to assess the aquatic risks of PPPs. In particular, these differences are apparent when comparing the authorisation criteria for the compartment water according to the Regulation (EC) No 1107/2009 and the water quality standards according to the Directive 2000/60/EC or, for short, the EU Water Framework Directive (WFD). These criteria and standards are a reflection not only of differences in the use of data on environmental fate and ecotoxicology of PPPs, but also of different policy decisions about the acceptance of risks in relation to formulated protection goals. Although the generic protection goals of the WFD and PPP Regulation do not differ substantially, the specific protection goals (SPGs) of the Plant Protection Product Regulation do not exclude that under certain conditions short-term effects followed by recovery are acceptable ecological recovery option (ERO), while environmental quality standard (EQS) setting within the context of the WFD in principle is based on the ecological threshold option (ETO) (EFSA, 2013).

The PPP Regulation has its focus on edge-of-field surface waters in agricultural landscapes assessment schemes and the EFSA guidance allow for the derivation of regulatory acceptable concentrations (RACs) (EFSA, 2013).

The EU WFD Water Framework Directive (2000/60/EC) aims to ensure good chemical status of both surface water and groundwater bodies across Europe. For surface waters, this goal is defined by limits on the concentration of certain pollutants relevant across the EU, known as priority substances. Good chemical status means that the concentrations of all of the priority substances and certain other pollutants do not exceed the environmental quality standards (EQSs). Priority substances are set out in the Directive 2008/105/EC and are defined as those substances presenting a significant risk to or via the aquatic environment. The Groundwater Directive 2006/118/EC, as a daughter of the WFD, established specific measures to prevent and control groundwater pollution. The Drinking Water Directive 98/83/EC sets special quality requirements for water for human consumption. These directives set enforcement limits for the drinking water and the groundwater at 0.1 μg/L. The Drinking Water Directive sets also concentration limit for total pesticides at 0.5 μg/L (EEA, 2018).

The limits are default legal limit, although the detection systems based on mass spectrometric techniques such as tandem mass spectrometry and quadruple-time-of-flight mass spectrometry can have lower LOQ with high sensitivity and selectivity (Alcântara et al., 2019).

Other polices and regulations that are not specifically aimed at protecting the environmental *medium water*, but are significant concerning chemicals in water, e.g. Directive on the Sustainable Use of Pesticides 2009/128/EC, are listed in the European Environment Agency Report (EEA, 2018).

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