Bundesinstitut für Risikobewertung

Feed and food safety in times of global production and trade

Impressum

BfR Wissenschaft

BfR-Autorinnen und Autoren:
Elisabeth Achten, Sascha Al Dahouk, Bernd Appel, Arnold Bahlmann, Dorina Bodi, Rita Boje-Haderer, Idesbald Boone, Maria Borowiak, Sascha Bulik, Tasja Buschhardt, Anja Buschulte, Miguel de Alba Aparicio, Susanne Esslinger, Alexander Falenski, Carsten Fauhl-Hassek, Carolin Fechner, Matthias Filter, Hendrik Frentzel, Miriam Friedemann, Marcel Fuhrmann, Marion Gottschald, Matthias Greiner, Mirjam Grobbel, Taras Günther, Jens A. Hammerl, Petra Hiller, Bettina Horn, Husam Ibrahem Aroud, Alexandra Irrgang, Annette Johne, Katharina Juraschek, Annemarie Käsbohrer, Sabine Kemmlein, Janine Kowalczyk, Renate Krätke, Monika Lahrssen-Wiederholt, Birgit Lewicki, Oliver Lindtner, Anneluise Mader, Inga Mädge, Burkhard Malorny, Ronald Maul, Anne Mayer-Scholl, Nicole Mazurek, Octavio Mesa Varona, Karsten Nöckler, Jorge Numata, Carolina Plaza Rodríguez, Martin Richter, Janet Riedl, Marco Rügen, Sara Schaarschmidt, Helmut A. Schafft, Silvia Schmoger, Thomas Selhorst, Dina Shamoun, Tanja Skladnikiewicz-Ziemer, Markus Spolders, Eckhard Strauch, Ahmad Swaid, Bernd-Alois Tenhagen, Christian Thöns, Dominic Tölle, Lars Valentin, Armin A. Weiser, Aline Wisniewski, Milena Zupaniec

Weitere Autorinnen und Autoren:
Virginie Desvignes (ANSES), Stylianos Georgiadis (DTU Food), Laurent Guillier (ANSES), Helmut Heseker (Paderborn University), Olaf Mosbach-Schulz (EFSA), Maarten Nauta (DTU Food), Leticia Ungaretti Haberbeck (DTU Food)

Feed and food safety in times of global production and trade

Herausgeber:
Bundesinstitut für Risikobewertung (BfR)
Anstalt des öffentlichen Rechts
Vertreten durch den Präsidenten Professor Dr. Dr. Andreas Hensel
Max-Dohrn-Straße 8–10
10589 Berlin

Telefon: 030 18412 – 0
Telefax: 030 18412 - 99099

E-Mail: pressestelle@bfr.bund.de

Aufsichtsbehörde: Bundesministerium für Ernährung und Landwirtschaft
Ust.-IdNr. des BfR: DE 165893448

V.i.S.d.P: Suzan Fiack

Koordination und Redaktion
Dr. Sigrid Gödert (Bundesinstitut für Risikobewertung, Berlin)

Berlin 2019 (BfR-Wissenschaft 04/2019)
284 Seiten, 64 Abbildungen, 32 Tabellen
€ 15,-

Druck: Inhalt und buchbinderische Verarbeitung
BfR-Hausdruckerei Marienfelde

ISBN 978-3-948484-03-3
ISSN 1614-3795 (Print) 1614-3841 (Online)

DOI 10.17590/20190930-102623

Download als kostenfreies PDF unter www.bfr.bund.de

Content

1 Introduction 5

2 Agricultural Resources, Trade, Transport 9

2.1 Global feed supply chains − Plant resources for animal feed 10

2.2 Importance of food trade for Germany 27

2.3 Container transport - the backbone for smooth running international trade in goods 40

2.4 Bioterrorism and the Food 51

3 Authenticity of Feed and Food 59

3.1 Food Fraud: definitions and requirements 61

3.2 Verification of food and feed authenticity 71

4 Chemical Risks and Toxins in Feed and Food 83

4.1 Transfer of per- and polyfluoroalkyl substances (PFAS) along the food chain 85

4.2 Transfer of natural toxins in aquatic systems 93

4.3 Natural toxins in feed and food processing chains of plant- and blue-green algae-based products 100

4.4 Toxin detection and quantification – regulatory and analytical aspects 118

5 Foodborne pathogens and antimicrobial resistance 139

5.1 Foodborne pathogens in food confiscated from air passenger luggage 141

5.2 Prevalence and Diversity of Pathogenic Bacteria in Seafood – Control of Enteropathogenic Vibrio spp. in Retail Food 149

5.3 Microbiological safety in the spice and dried culinary herb chains 159

5.4 Emergence, distribution and genetic diversity of mcr-1 harboring Escherichia coli from livestock and food in Germany 171

5.5 New trends for the detection and characterization of foodborne pathogens 183

6 Exposure to Humans 199

6.1 Food Origin Information in Times of Global Food Supply – Basis for the Refinement of Dietary Exposure Assessment 201

6.2 Ciguatera food poisoning in Germany caused by imported tropical fish from tropical areas 214

6.3 Foodborne zoonotic infections in different populations in Berlin 227

7 Data and Modelling 235

7.1 Network analysis for food supply networks 237

7.2 Applications of supply chain network modeling for feed and food safety 245
7.3 FoodChain-Lab: an innovative tool to increase food safety through supply chain analyses 259
7.4 Epi-Lab - going the next step. Data collection, analysis and visualization within one platform 268
7.5 Knowledge Plattform RAKIP 272

8 Figures 279

9 Tables 283

1 Introduction

Appel, Bernd

For centuries feed and food were mostly produced and consumed regionally. Since the last two to three decades these are increasingly produced and traded globally, like some brand labels on beverages, chocolate products or fruits. Still a lot of globally produced products are traded and consumed in regional markets. The worldwide demand for high-quality food, available almost anytime and at the same time affordable to most of the consumers of the northern hemisphere, has led to the fact that today both feed and food product chains process raw materials and products from all continents. This development covers all agriculturally used regions worldwide. We therefore are talking about global commodity flows or global feed and food chains.

Why is it important for risk assessment in feed and food to obtain data and knowledge from the increasingly important global production regions, trade routes and structures, the resulting markets and new methods and processes in feed and food production? The answer can be easily deduced, since it is in the interest of all consumers to receive high-quality and safe food from all production regions at all times and this should meet the high standards of feed and food safety in the EU.

That this is not always self-evident has been shown by serious incidents of adulteration or contaminated food over the last 20 years. Melamine as an impermissible substitute for protein in dairy products, toxic colourants as unauthorised additives in spices or food contaminated with pathogens, which have led to life-threatening outbreaks of gastrointestinal diseases and often to deaths, are incidents that demand improved quality controls on the producer side and intervention of responsible risk assessors and management. For example, the serious consequences of the EHEC outbreak in Germany and France in 2011 are still very painfully remembered with almost 4,000 patients and 53 deaths.

However, global food production and trade are not a modern invention. Already in times of the Silk Road between East Asia and regions of today's Europe, the trade routes at that time were used to transport rare and sought-after spices. And in the times of the Roman Empire and later the crusaders of the Teutonic Order, a lively transport and sale of grain from the granaries of today's Eastern Europe to the south and west began.

In recent decades, an improved economic base of consumers in almost all regions of the northern hemisphere, but also in i.e. East Asia, Southeast Asia and South America has led to greater demand for feed and food. Acquaintance with foreign regional food products and consumers customs, caused by increased worldwide travel, is also leading to an increasing demand in food consumption worldwide. In the wealthy countries of the so-called western world, interest in new products or the desire for year-round availability of seasonal food has also become a matter of course. In certain population groups, changes in lifestyle based on increasing economical participation, like i.e. the growing middle-class in China, lead to changes in diet and thus to changes in eating habits.

Increased global demand for safe food requires that the desired products are available on the markets at all times and in sufficient quantities. This is not always the case and there will be increased production and delivery bottlenecks in the future, which will have a clear influence on prices and product quality. Particularly, high-priced products with limited scope of supply, e.g. spices such as saffron, increase the worldwide risk of food deception at the expense of the consumer. In addition, economically strong regions with a high population share, such as some populous states in Asia, will become a noticeable impact on international competition for food markets, i.e. availability and prices. As a consequence, there will be a tremendous increase in competition for raw materials and products available worldwide,

with direct effects for consumers and thus also risk assessment and quality assurance in the food industry.

Structural changes in the international feed and food industry have been observed for years. For example, the majority of salmon production no longer comes from wild catches but from large fish farms in the coastal areas of Norway, Scotland and Canada. Meat, meat products, milk and dairy products as well as other food products are no longer only marketed regionally, but increasingly come from global production and trade activities. As long as a uniform risk assessment and approval system is backing it, as in the EU economic area, consumers can assume that all products produced and marketed in the EU have the same high level of safety.

The experiences of import controls for products originating from areas outside the EU repeatedly indicate that in distant production regions both the quality assurance and monitoring systems are increasingly being made obligatory and that our proven food standards must also be applied in such production areas through global agreements, which is in the responsibility of the producers and traders.

Another important aspect is the global increase in meat consumption, especially in the emerging markets of Asia. Increased consumption of poultry, pork and beef will inevitably lead to an increase in the amount of land used for grazing worldwide or to a sharp increase in the amount of plant feed required. These areas, as well as agricultural land for the cultivation for biofuels, are already and will increasingly compete with growing regions for cereals, vegetables and fruit for direct human consumption. It will be a global social challenge for organisations like FAO, WHO, WTO and the Nations to guide and manage this competition in a politically, ethically, ecologically, and economically sensible and fair manner.

The increase in livestock farming (meat and milk production) in large parts of Africa is already being offset by the steady spread of steppes and desert zones through overgrazing at the expense of arable land. This is in stark contrast to the need to expand agricultural production areas for cereals and vegetables and (water) resources for fish farming on the African continent, especially as the population growth in Africa might increase to 3-4 billion people by the end of the 21st century. As a "neighbouring continent", the EU has a special role to play here in establishing and implementing responsible risk assessment and management in feed and food production and marketing within and with Africa, but also as a mentor for the competitive integration of African countries into global trade. Currently, influences and market shares between African countries and outside countries are still majorized by “old” colonial ties to the UK and France, to the US and, in the recent years, to huge Chinese investments into African land development and agricultural production.

If the economically privileged states, especially Europe, fail to measurably help to improve the nutritional and health conditions in Africa and southern Asia, we are facing an unprecedented migration of peoples from south to north, with culturally and economically significant consequences for Europe, whose population will be rather smaller towards the end of the 21st century than today, based on current population growth indices.

The international competition in the feed and food industry and trading houses for the best raw material batches and products is essentially driven by economical factors and by the competitive securing of market shares. The aim is to use and process the best possible quality raw materials from the world's agricultural regions at economically justifiable prices. However, it is beyond economic planning or logistics when biological (fungal infestation, pests), meteorological (storms, droughts), geographical (earthquakes) or political influences (wars, insurgencies, embargoes) affect regions that are of vital interest for the food production or economy of a region. Such incidents immediately have a strong influence on the availability (and price) of raw materials in feed and food production and availability for the

consumer and thus directly on the safety of the products, as lower quality batches may then have to replace the gaps in the markets.

After all, the digitalisation of all relevant information and data in production and trade has already led to a considerable acceleration of trade processes and a gigantic increase in international trade links and logistics, which hardly anyone outside an affected trade association can look through, especially as these interactions are constantly changing. If one also takes note of the possibilities and procedures of "flaws" in goods (the trader sometimes sees his goods only on paper) and the formation of new batches within the framework of free trade zones in large ports, e.g. Singapore, Rotterdam or Hamburg or elsewhere, then risk assessment and risk management are becoming increasingly important in order to guarantee food safety for the consumers.

In the last decade extensive efforts have been invested in chemical analysis and microbiological and molecular genetic diagnostics, including the overall genome analysis of complex matrices. Moreover, the EU has recognized the challenge of assessing and monitoring new products and raw materials from all over the world and has addressed this issues in the Novel Food directive. Microbiologists and toxicologists are particularly concerned of potentially imported pathogen variants (including resistance properties against antibiotics) or novel pathogens as well as toxic ingredients or contaminants and residues.
Finally, the quality assurance of good agricultural practise and good manufacturing practises at the food producing and trading sides as well as the laboratories of quality control and competent authorities must step up their activities in order to maintain the safety of products in a world of changing and constantly developing markets.. Taking control samples should always be accompanied by statistical and epidemiological expertise and the collection of all data in comparable formats in order to get the best answers out of the necessary numbers of samples, instead of collecting vast amounts of data that might be not helpful to understand and stop for example a foodborne outbreak.

In summary, all these exemplary aspects and facts have a strong and increasingly important influence, directly or indirectly, on the daily work of the supervisory authorities and risk assessment as well as on those responsible for quality assurance in feed and food companies. In future, understanding and knowledge of global feed and food chains must flow even more strongly into the training and application areas to all those involved, ensuring that all responsible institutions will be equal to the challenges of globalisation of animal feed and food.

2 Agricultural Resources, Trade, Transport

Nöckler, Karsten

Introduction

The globalisation of trade and the increasing complexity of supply chains are leading to new challenges for food and feed safety worldwide. In section 2.1, the trade structures of feed materials as an important part of the food supply chain are examined and the trade structures for the feed market in Germany, in the European Union and also in the global context are presented for cereals and in detail for maize. Germany is also one of the world's most important trading partners for the import and export of foodstuffs. Therefore, section 2.2 deals with the development of trade over the last ten years with the most important trading partner countries of Germany. In this context, examples are given to illustrate which factors can have a positive or negative impact on trade relations and what this can imply for food safety. In view of the advancing globalisation and further increase in the volume of trade in feed and food, optimal transport conditions are an indispensable component, with about two thirds of international freight traffic currently being handled by container ships. In addition to the development of requirements for safe transport, section 2.3 also deals with the question of how feed and food can be effectively protected against pests and moulds during transport by fumigation, and which precautionary measures must be considered. Considering the complex chain of feed and food and past experience, the malicious introduction of chemical or biological agents into the food chain cannot be completely ruled out. Such scenarios are very rare so far, but can have a serious impact on public health, international trade and consumer confidence in food safety. Section 2.4 thus deals with appropriate measures for early detection and prevention of possible bioterrorist attacks on the food chain.

Future needs

Trade in feed and food will continue to grow in the coming years associated with the global networking of supply chains. In order to be able to react quickly and adequately to current incidents and changes, the trade data should be available online and up-to-date, at least on a weekly basis. In this context, harmonised product data and clear definitions for corresponding periods (e.g. market year, harvest year) are necessary. Measures in freight transport to protect feed and food, e.g. through the use of chemicals, require preventive measures for occupational safety during loading and unloading as well as valid data on possible exposure of animals and humans to such treated goods. Future research activities should deal, among other things, with the batch-accurate real-time traceability of feed and food along the entire chain on the basis of computer-readable documentation systems. Experimental studies on inactivation of pathogenic organisms in different feed and food matrices should be further developed. On the basis of suitable modelling tools, data obtained from experimental and empiric studies could be used for better prediction of possible events and crises, also taking into account scenarios of targeted damage situations. In the future, the aim will also be to create efficient online platforms for the exchange of relevant data between feed and food producers, competent authorities and the various investigation institutions.

2.1 Global feed supply chains − Plant resources for animal feed

Achten, Elisabeth; Zupaniec, Milena; Mader, Anneluise; Mädge, Inga; Bodi, Dorina; Spolders, Markus; Kowalczyk, Janine; Horn, Bettina; Schafft, Helmut A.

Abstract

The increasing complexity of global supply chains leads to multiple unknown risks for feed and food safety. A preventive approach in consumer protection must therefore include Risk Assessment that considers all steps of global food supply chains. Trade structures as a part of global supply chains that include logistics activities such as transportation and storage are marked by fundamental knowledge gaps. In order to close some of these gaps trade structures of feed material as an important part of food supply chains have been investigated, exemplarily cereals and maize in detail. Further, main agricultural resources for feed material are identified and shifts in cereal-based commodity slows are discussed. Trade relationships of the European Union are shown and the structure of the feed market in Germany is summarized. Trade structures are exemplarily shown for maize, a major feed crop produced and used worldwide. In addition, the transportation and import control system in the EU are explained.

Introduction

The production of feed and subsequent feed safety is continuously gaining importance against the background of global population growth, urbanization and changing consumption patterns towards an increase in meat, milk, eggs, fish and other products of animal origin (FAO and IFIF, 2010; WHO, 2008).
In Europe the focus on feed safety in the overall framework of risk assessment derives from the “farm to fork” approach, embedded in Regulation (EC) No 178/2002 (Eur. Parliament and The Council, 2002d). It implies the management and assessment of safety risks along all steps of food supply chains to pursue a high level of protection for human and animal health. Hence, primary production and production of feed material constitutes a significant element considering the whole food production system. Experience has shown that it is necessary to consider the production, manufacture, transport and distribution of feed given to food-producing animals, including the production of animals which may be used as feed on fish farms. The inadvertent or deliberate contamination of feed, as well as adulteration or fraudulent practices in relation to it, may give rise to a direct or indirect impact on food safety. Feed may contain undesirable substances that are a potential danger to human or animal health. These substances can occur naturally in the environment or contaminate the feed during production, natural as well as accidental or deliberate. Animal products such as dairy products, meat and eggs can be a significant source of food safety risks for the consumer due to the transfer from soil and feed to and the accumulation in the animal body. Examples which gained public attention in Germany are aflatoxin M1 in milk deriving from mycotoxin contaminated maize in 2013 as well as the dioxins incidence in fat for feeding purposes and subsequently in pork and eggs deriving from dioxin contaminated feed in 2011 (BfR, 2011, 2013). Consumers are therefore increasingly aware of the importance of feed safety in producing safe food.

In times of globalisation, the feed sector equal to the food industry gets more and more diverse and connected. Nowadays most countries rely on imports of (agricultural) resources to meet the national feed demand. It can generally be assumed that globalisation increases the complexity of feed and food supply chains which augments the probability of feed and food related safety risks.

On a global scale, the European Union (EU) represents the main feed compound producer after the USA and China and therefore is a key player in the field of feed safety (FEFAC, 2017a). This chapter gives an overview of relevant elements regarding global feed supply chains. It includes the main (agricultural) resources for feed production in Germany and the EU, the most important trading countries for imports into the EU and the main global commodity flows of the most important feed materials worldwide. Further, the feed market structure, the transportation mode and import controls will be discussed. Focus will be set on cereals and especially on maize as one of the most traded raw material for feed purposes worldwide.

The overall aim is to gain knowledge and deepen the understanding of the complexity of global feed supply chains in order to identify relevant areas for research and knowledge gaps to reduce the uncertainty and variability of risk assessment in times of globalization.

Main (agricultural) resources for feed material consumption in Germany and the EU

Feed materials are diverse in nature concerning condition and origin. In general, the main feed materials can be divided in the following categories as provided by the European legislation (EC, 2009a) and European Feed Manufacturers Federation (FEFAC) (FEFAC, 2017a):

- **Cereals** including in particular common wheat, maize, barley, rye, triticale and oat.
- **Cakes and meals** derived from soybeans, rapeseed, palm, maize germ, linseed.
- **By-products** from the food and bioethanol industry, e.g. milling by-products (bran), corn gluten, by-products of the sugar industry (sugar beet pulp, molasses), fruit residues (citrus pulp) and dried distillers grains.
- **Other vegetable raw materials** like grain legumes (peas, field beans, lupines), tapioca, grass meal.
- **Animal protein and by-products thereof** including fish meal, dairy products, blood and plasma products.
- **Oils, fats** like vegetable oils (soybean, rapeseed, palm), fatty acids, mixed fats, animal fats (cat. 3).

Feed additives and minerals as substances that are deliberately added to feed or water to fulfil specific functions (e.g. nature of feed material, livestock performance); feed additives: vitamins, amino acids, enzymes, microorganisms, other technical additives, preservatives, acids, minerals: feed phosphates, Ca-, Na-, Mg-sources, trace elements.

In addition to that, feed may be traded in premixtures or as medicated feedingstuff. Another common categorization is marketable feed and non-marketable feed. Since this report focusses on global supply chains, non-marketable feed material, such as forages and roughages will not be discussed but needs to be in mind from a producer point of view as is it relevant for feed demand (Bundesinformationszentrum Landwirtschaft, 2018a).

Figure 1 shows the proportion of the mentioned feed material categories consumed in Germany and in the EU. Comparison of the data for Germany and the EU reveals similar distributions: Cereals provide the basis to compound feed together with soybeans (“primary products”), followed by cakes and meals and by-products from the food and bioethanol industry (“secondary products”, due to the necessary processing). The feed additives and minerals category accounts for a relatively small portion of the total feed input. From EU’s perspective, the section “Others” comprises amongst others pulses, oils and fats, dried forage and dairy products. As there is no data available on additives and minerals concerning the consumption in Germany, it can be assumed that this feed category is included in the section “Others”.

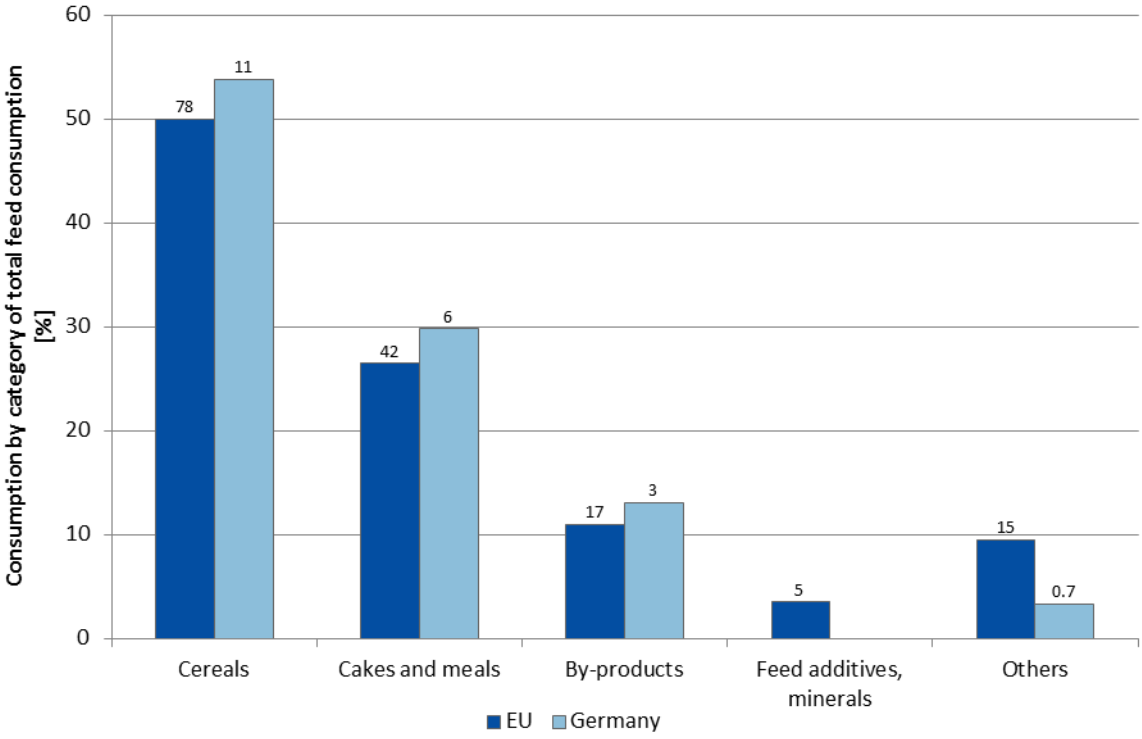


Figure 1: Feed material consumption in the EU and Germany in 2015/16 by category in percent of total feed consumption
Quantities for each feed material category are shown above the bars in mio. tonnes (t). Sources: (Bundesinformationszentrum Landwirtschaft, 2018b; FEFAC, 2017b)

Trade of feed materials in the EU

To meet the feed demand the EU feed sector relies on imports of feed materials. In 2016 the total import volume of feed materials into the EU amounted to 43 mio. t (FEFAC, 2017b). Figure 2 displays the percentage of the previously mentioned categories with regard to the total EU feed import. Additional information on the imported volume of each category and the export countries of each feed input are summarised in Table 1. This Table gives the total volume of grain species (feed grain and grain for human consumption) as there are no data available of the import volume of individual grain species for feed purposes. For the listed by-products main producer countries are shown due to lack of data about the importing countries. Summarised from Figure 2 and Table 1, in 2016 cereals represented almost one third of the total EU feed import (27 %). Here (temporal) supply shortfalls can be balanced by imports from surplus production countries such as Ukraine and Brazil (EU DG AGRI, 2018c).
For cakes and meals there is a bigger supply gap which accounts for more than 50 % of the total EU feed import. Following EU data these comprise 16 mio. t of soybean meals as well as a smaller amount of sunflower and rapeseed meals. Compound feed manufacturer´s count 24 mio. t of cakes and meal imports (FEFAC, 2017b), which opens a gap of around 4 mio. t that cannot be filled by additional data. Beside shortages, economic reasons can drive the feed sector towards global procurement of especially soybeans or soymeal. Soybeans can be produced more efficiently in non-EU countries because of differing growing conditions, especially climatic reasons. This is reflected in the price and in the quality of soybeans, such as the nutritious value or the level of protein. Therefore, soybeans are mainly imported into the EU from South American countries such as Argentina and Brazil and are the most important import feed commodity for the EU (EU DG AGRI, 2018c). To overcome the dependence on imports of protein feed material, strategies have been developed to increase domestic cultivation in Germany (BMEL, 2016).

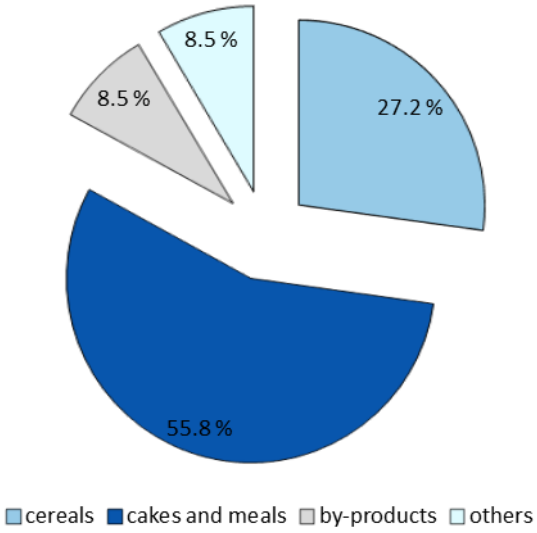


Figure 2: **Percentage of different feed categories of the total EU feed import** (FEFAC, 2017a)

Table 1: **Feed (a) and total (b) import volume of different materials into the EU and the respective main countries of origin and EU trading partners**

Feed material	Import volume [1 000 t]	Countries of origin[b] / EU trading partners[c]
Common Wheat	3 267[b]	Ukraine, Moldova, Canada, USA, Russia
Maize	11 631[b]	Ukraine, Russia, Canada, USA
Barley	421[b]	Ukraine, Moldova, Serbia
Rye	17[b]	Belarus, Russia, Ukraine
Feed Cereals	**11 900[a]**	
Soybeans	12 856[b]	Brazil, USA, Paraguay, Canada, Ukraine
Soymeal	16 706[b]	Argentina, Brazil, Paraguay, India
Sunflower meal	3 659[b]	Ukraine, Russia, Argentina
Rapeseed meal	219[b]	Australia, Ukraine, Canada
Oilcakes and meals	**24 400[a]**	
Dried distillers grains (DDGS)	800[a]	USA, China, Canada
Molasses	1 500[a]	Brazil, India, China, Thailand, USA
Citrus pulp	200[a]	Brazil, USA
Corn gluten feed	600[a]	USA
Dried beet pulp	600[a]	Russia, USA, Egypt
By-products	**3 700[a]**	

[a]: **EU Import volume as feed (FEFAC, 2017b)**.
[b]: **Total import volume, food and feed not explicitly separated (EU DG AGRI, 2018c)**.
[c]: **Only for By-products: main producer countries worldwide** (USDA, 2018c).
Feed categories are highlighted as bold type letters.

From Table 2 it can be seen that on a global scale with the focus on global supply chains, the most important commodities in transportation regarding feed material are cereals and soybeans. Although the production volume of oilcakes and meals (protein meals) is similar to that of soybeans the traded volume is much lower (USDA, 2018a, 2018b).

Table 2: World production and trade of cereals, soy beans and protein meals in 2017/2018 (USDA, 2018a, 2018b)

	Production volume [mio. t]	Export volume [mio. t]
Common wheat	758	183
Coarse grain	1 315	190
Maize	1 033	151
Barley	144	29
Sorghum	58	7
Soybeans	337	152
Protein meals	333	88

Structure of the feed market and supply chains in Germany

The production and supply chains of compound feed are complex. Nevertheless, numerous data are available for the compound feed market of Germany. As it is one of the best recorded markets, it will serve as an example in the following.

In Germany, a country with a high proportion of self-supply, about 24 mio. t of compound feed were produced by 309 compound feed producers in 2016/2017 (BLE, 2017). Data available on the national structures of the raw material origin used in compound feed production include average proportions and numbers of players/producers. These data describe the feed market structure quite impressively and are given in Table 3. One can assume that depending where a contamination takes place, for example at a small cereal producer out of 227 000 or at one of the biggest oil mills, the consequences for the market would be quite different. This also accounts for the size of the company, the batch size and the unit size with which the compound feed is transported (see Table 4).

Table 3: Origin of feed materials in the compound feed industry (adapted from (Schubert, 2012))

Group of raw material	Quantity of compound feed [%]	Number of actors/producers
Cereals	47	227 000 producers, approx. 1 000 trader
Oil meals	28	10 oil mills, 70 „decentralized“ oil presses, imports
By-products of food	Cereal BP 6, pomace 0.5, by-products of sugar 3	580 mills, 14 starch factories, 32 maltings, 1 300 breweries, 378 fruit processors, 20 sugar factories, imports
Other vegetable raw materials	Legumes 0.3, grass meal 1	5 000 producers, 40 drying plants, imports
Animal protein/by-products	Fish- and blood meal 0.1, dairy products 0.5	95 000 milk producers, 230 milk processing plants
Oils, fats	1	10 oil mills, 250 slaughterhouses, (number unknown) fat melts, 10 feed fat producers
Minerals	Minerals 1.5	40 companies (manufacturers), many distributors, imports
Feed additives	Additives/premixes 0.5	40 companies (manufacturers), many distributors, imports

Even though the high volume of feed material that is produced and has to be transported daily in the EU, knowledge on the size of production units and transport capacities can be limited depending of one person’s field of expertise. However, it might be of importance in

case of a natural, accidental or deliberate contamination of feed materials. Therefore, a comprehensive, but not complete, overview on possible units and capacities available is provided in Table 4.

Transportation of feed material

Primary products like cereals and soybeans are agricultural raw materials that can be classified as dry bulk commodities in the world agricultural trade. Dry bulk commodities are transported in ocean-going and inland waterway ships, freight wagons and trucks. Usual capacities can be seen in Table 4. In global transport chains, sea transports present the main part of the route. It can be assumed that 80 % of global trade and 90 % of EU's overall external trade is based on sea transports (Schieck, 2008). Dry bulk commodities are transported in bulk carriers, which hold up to 300 000 dwt (dead weight tons, described the ship´s weight carrying capacity without the ship´s weight). Depending on the load, bulk carriers can be distinguished in dry freight carrier (for cereals, fertilizers, dry chemicals) as well an in “combination freighter“, which besides dry material may also comprise container and wet bulk goods. For the dominating traded agricultural raw materials, regular main traffic routes have developed from the export to the import countries. Permanent exporters for all types of cereals and oilseeds such as soybeans are the USA, Canada, Argentina and Australia. They are characterised by highly export-oriented, large scale agriculture as a result of knowledge base, technical sophistication, access to financial capital as well as favourable production conditions. In contrast, importing countries are deficient in one or more of these requirements. For cereals this applies to parts of Africa, Mediterranean countries, South and South-east Asia, as well as transition countries in Eastern and South-eastern Europe (Woitschützke, 2015). In the case of soybeans Europe is also an import country as the climate is the limiting factor to effective production.

Table 4: Overview on production units and transport capacities in the feed chain of Germany

Origin	Annual production/company [t]	Usual size of a unit [t]
Extraction industry		
for soybean meal	200 000 - 2 000 000	1 000 - 5 000
for rapeseed meal	200 000 - 800 000	40 - 500
Transport		
Trucks		25 - 40[a]
Sea vessels		10 000 - 100 000
Barges		360 - 9 600
Storage		
Silo		2.1 - 6 000[b]
Harbour		10 000 - 500 000
Compound feed producer		
Mixing mills	500 - 300 000	25
Premix industry	5 000 - 50 000	0.025 - 25

[a] 25 t payload = German national standard, 40 t payload = European Modular System

[b] World’s tallest silo holds 8 500 t, Schapfenmühle, Ulm, Germany. The world’s largest grain bin can hold up to 2 mio. bushels, which is equivalent to more than 27 000 t sunflower seeds 54 000 t wheat

Global trade of plant products and EU import control

As a major importer of food and feed the EU has established strict import rules with respect to food and feed hygiene, consumer safety and animal health. Regulation EU 178/2002 (article 11) is laying down that food and feed imported to the EU has to fulfil the same high standards as products from the EU itself (Eur. Parliament and The Council, 2002d). Import controls verifying compliance of food and feed products with relevant requirements for animals and animal products are mandatory since the establishment of the European Community by Maastricht Treaty in 1993 and in particular since the inception of Council Directive 97/78/EC laying down the principles governing the organisation of veterinary checks on products entering the Community from third countries (The Council, 1997). The Commission has to authorize third countries individually for exports of live animals and animal products into the EU. Once authorization is given, countries are listed at the homepage of DG Santé including the specification of products with export permission. By contrast, third countries do not have to be listed for the export of non-animal products. Exceptions are countries importing sprouts to the EU, as a follow-up measure of the EHEC-incidence in 2013 (Regulation (EU) 210/2013). Establishments of food and feed business operators have to be registered no matter if they produce or process food and feed of animal or non-animal origin.

Prior to the import of goods the food and feed business operators have to apply for the health documents required according to Commission Decision 2007/240/EC (veterinary certificates) or Regulation (EU) 669/2009 (common entry document for non-animal food/feed) at the country of origin's competent authority. By signing these documents the authority approves the products' compliance with EU requirements. It is the responsibility of the authority to decide, whether the product has to be checked for conformity e.g. with regulations on pesticide residues or other undesirable substances. In most cases, information on analytical results are not obligatory to be transmitted to the importing country.

For the entry into the EU live animals and products of animal origin have to pass a designated border inspection post (BIP), while non-animal products are imported through designated points of entry (DPE). These BIPs and DPEs are approved and listed in Commission Decision 2009/821/EC and Commission homepage (EC, 2018) according to Regulation (EU) No 669/2009 including the products they are responsible for. Thereby, mandatory channelling through EU approved BIPs and DPEs is accomplished.

With Regulation (EC) No 882/2004 the provision of systematic import control of non-animal derived food and feed was established. An increased risk of contamination of imported food and feed should lead to special attention regarding border inspection of these goods prior to entry into the EU (Eur. Parliament and The Council, 2004). Inspections are supposed to reduce the risk those commodities might pose in relation to animal or plant health respectively.
EU law distinguishes two stages of risk (high and low), when it comes to import control of plant based products. In general, non-animal products with an identified high risk listed under Regulation (EC) No 669/2009 are subject to mandatory border controls. In case of food and feed of plant origin targeted sampling and analysis is carried out on GMOs, radioactive contamination, pesticide residues, and contaminants like nitrate or aflatoxins. This applies, amongst others, for groundnuts and products thereof intended for food and feed purposes imported to the EU from certain countries. A frequency of physical checks including analysis for aflatoxins and identity checks of 10 % to 50 % is prescribed. The consignments have to be accompanied by documents on the results of aflatoxin sampling and analysis performed according to Regulation (EC) No 152/2009 or Regulation (EC) No 401/2006 respectively.

Food and feed of non-animal origin with a low health risk only undergoes random inspections focussing on potential risks according to Regulation (EU) No 882/2004. In order to conduct these random controls and also risk-based controls in the most efficient way, a special sys-

tem was developed at the German DPE at Frankfurt/Main Airport, which is the first point of entry to the EU for many products from non-EU countries.

Here the so called 'bottleneck control' was established in 2007 which implies the systematic testing of non-animal feed and food imported from third countries to the EU. The idea of this approach is to increase efficiency of each control sample and analysis by focussing on large amounts of a product at the point of entry into the EU rather than on monitoring smaller amounts after products have entered the retail (see Figure 3).

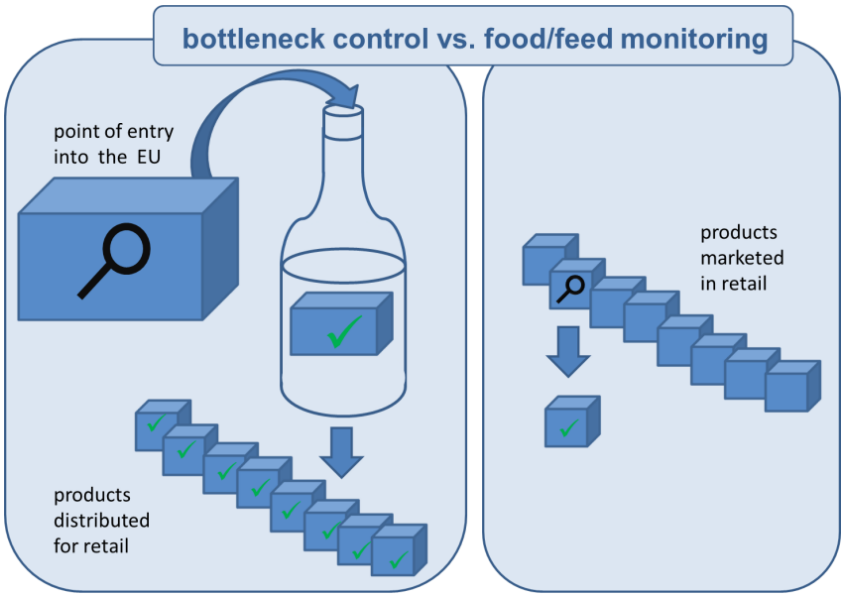


Figure 3: Advantageous efficiency of samples analysed for bottleneck control on the left compared to monitoring on the right

The bottleneck control implemented by means of a collaboration of the Hesse State Laboratory and the Hesse Ministry for the Environment, Energy, Agriculture and Consumer Protection is representing a unique situation in Europe. In contrast to mandatory controls, consignments randomly chosen for sampling and analysis are not stopped at the DPE, provided that documents comply with the respective requirements. Thereby, unnecessary disruption of trade is avoided. Analysis of the samples, e.g. for aflatoxins or pesticide residues, has to be performed within four weeks. In case of a result non-compliant the following three consignments of the same product from the same producer are stopped at the DPE due to suspected exceedance of maximum levels of undesired substances. The producer has to provide certificates of analysis issued by a laboratory accredited according to ISO17025 proving the consignments' compliance with the maximum levels in order to release them from the storage at the DPE. The imported products are sent back to the country of origin or destroyed if no such certificate is presented (Heinzler et al., 2011).

More than 10 years after implementation of the bottleneck system, it can be resumed that the number of samples claimed non-compliant has decreased significantly (Heinzler et al., 2011), which is demonstrated in Figure 4 for pesticide residues. Both, the Hesse State Laboratory's results from bottleneck control of pesticides (LHL, 2017) as well as the Federal Office of Consumer Protection and Food Safety's (BVL) national reports on pesticide residues in food (BVL, 2018a) show decreasing numbers of non-compliant samples. Plant products which exceeded the maximum levels of pesticide residues most frequently in the frame of the bottleneck control were also included in the annex I of Regulation (EU) No 669/2009. Those are categorized as feed and food of non-animal origin with a known risk and therefore subject to

an increased level of official controls at the point of entry into the EU. Another proof of the system's success is the decreasing number of notifications via the Rapid alert system for food and feed (RASFF) due to determined pesticide residues in samples from the bottleneck control at Frankfurt/Main Airport from 10 notifications in 2010 to 1 notification in 2016 (LHL, 2017).

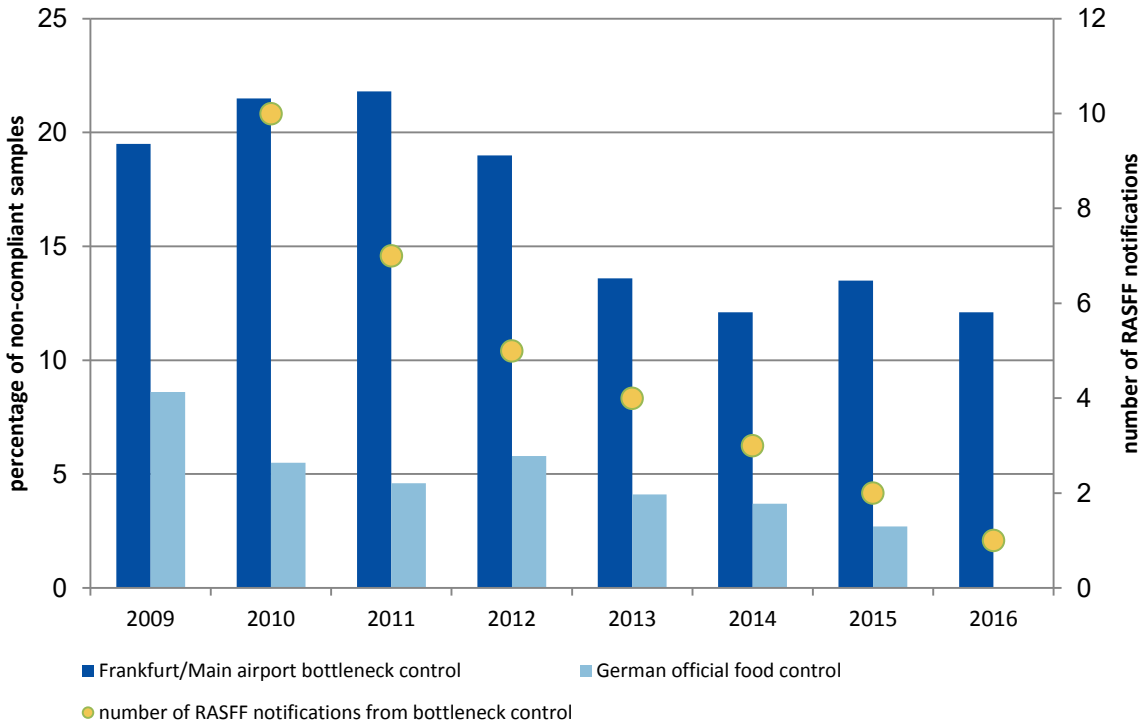


Figure 4: Percentage of food and feed samples of non-animal origin imported from third countries (random sampling of low health risk products for bottleneck control) reported non-compliant due to exceeding maximum levels of pesticide residues and resulting number of RASFF notifications.
Bottleneck control reported by (LHL, 2017) and official food control reported by (BVL, 2018a).

The decreased number of samples claimed non-compliant as a result of the bottleneck control at Frankfurt/Main Airport DPE is presumably due to different factors. Preferably pesticide control in the countries of origin, cause analysis within the chain of production or cancellation of supply contracts with producers delivering high residue products have caused this positive development. However, it could be assumed that producers and traders might change the point of entry into the EU for products containing high pesticide levels. This would result in a shift of commodity flows.

Shifts of global commodity flows

Despite the relatively stable main commodity flows, trading partners of agricultural raw materials, as adopted of the EU, may vary. Shifts in global commodity flows are attributable to the quality, price and market conditions of the specific commodity which to a large extent depend on production conditions.

Production conditions firstly relate to on-site conditions or ecological conditions, such as climate/weather, soil, geography and topography. Especially climate has a great impact on all components of *crop production* such as area, intensity and yield. The ongoing climate change including extreme weather conditions such as drought and heavy rain increasingly

provoke unfavourable conditions for agriculture (Alexandratos and Bruinsma, 2012). Extreme weather may cause poor harvest and subsequent fluctuations or rather disruptions of for example the cereal supply which was the case in the maize damage caused by drought in the USA in 2012/2013 (Raja, 2013). Weather-related crop failures have often been the reason why crop production in major producer countries (as indicated above) gradually declines. In contrast, new producer countries arise in Latin America and in the black sea region (Schmid and Goldhofer, 2017; USDA, 2018c).

Furthermore, weather related factors such as the distribution and amount of rain, temperature, relative humidity and daylight do not only affect crop yield, but also plant health. Adverse conditions may weaken the plant and make it less resistant to diseases and infestation. Beside ecological conditions, agricultural cultivation practices play an important role in the quantity and safety of products: The application of agricultural inputs such as pesticides and fertilizers, the use of technology and machinery and the timing of sowing, irrigation and harvest all codetermine crop yield and the contamination of crops (e.g. pesticide residues, mycotoxins).

Cereals − a global commodity

Cereal production and trade have experienced a continuous worldwide growth over the last decades. On the demand side this is a result of increasing world population and rising prosperity in developing countries that is associated with higher meat demand (indirect cereal consumption). Whereas on the production side the steady increase of produced cereals is mainly due to the growth in crop yields and crop intensification and less due to expansion in the arable land (FAO Statistical Yearbook, 2013).

The greatest part of cereals produced within the EU and imported from third countries reaches the feed industry (see Figure 5). In detail, in 2016 approximately 61 % of imported and produced cereals (173 mio. t) was used for the feed industry 23 % flew in food industry and the rest (16 %) in industrial use and seeds (EU DG AGRI, 2018b; Schmid and Goldhofer, 2017). Hereby, the food industry comprises in particular the mill industry, starch industry and malt houses. In comparison to this, Germany's cereal consumption in feed industry was about 52 % of the total cereal amount (25 mio. t) in 2016/2017 (Bundesinformationszentrum Landwirtschaft, 2018c).

On a global scale the picture is different: In 2016/2017, 43 % of produced cereals were used by food industry, whereas a relatively smaller amount of 35 % was utilised by feed industry and the rest (22 %) was assigned to the industrial sector, seeds and losses (see Figure 5b) (Schmid and Goldhofer, 2017). This is due to the fact that especially in developing countries more than 75 % of cereals are usually used for human consumption (Schmid and Goldhofer, 2017) although its proportion in the feed sector is increasing with the ongoing growing animal feed demand (OECD-FAO, 2016). That is for instance the case for maize which traditionally constitutes a basic foodstuff in many parts of the world (such as in Latin America and Africa). In contrast, in the EU maize represents one of the major feed material and the most imported grain with an import volume up to 12 mio. t (see Table 1). In the compound feed production cereals are mutual convertible when lacking essential nutrients are supplemented. Therefore the consumption and the imports are greatly influenced by the respective availability of each cereal.

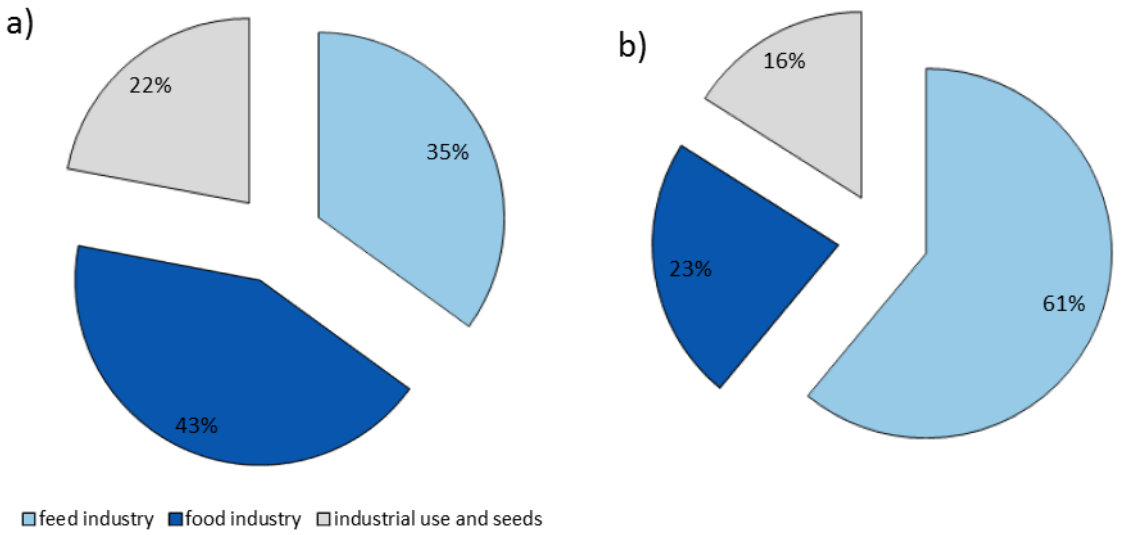


Figure 5: Use of cereals a) on a global scale and b) in the EU (Schmid and Goldhofer, 2017)

Maize − a major feed material

As it was previously shown, on a global scale maize is one of the most important feed crops. Maize can be fed both, as silage as well as grain maize. Because maize silage is mainly produced on-site and therefore not subject to international trade it will not be examined further here. Grain maize is used as whole kernels, in some cases also as corn-cob mix, ground and mixed with other feed components as compound feed. The chemical and nutritional composition of grain maize shows the relevance as feed (see Table 5), it can be used as energy crop but also supplies small amounts of fat and proteins for the animals. The composition of the nutrients varies by harvest years, climate and soil conditions, thus needs to be known for accurate ration formulation (Watson, 2003).

Table 5: **Composition of yellow dent corn kernels from seven American midwest hybrids** (Earle et al., 1946; Watson, 2003)

Compound	Dry weight [%]
Starch	73.4
Fat	4.4
Protein	9.1
Ash	1.4
Sugar	1.9
Other	9.8

To compare maize with similar cereal grains used as feed, their crude protein content and metabolisable energy content are summarised in Table 6. Maize has a lower crude protein content compared to the other cereals but mostly slightly higher energy values. This can be explained by the higher contents of fibre in the other grains that reduce the digestibility (Kellems and Church, 2010).

Table 6: **Crude protein and metabolisable energy of cereal grains as relative values compared to maize** (Kellems and Church, 2010; National Research Coucil, 1982)

Grain	Crude protein	Metabolisable energy		
		Ruminants	Pig	Poultry
Maize	100	100.0	100.0	100.0
Barley	124	96.2	88.6	74.5
Millet, proso	118	96.2	89.0	86.5
Milo	114	98.8	96.3	96.7
Oats	122	87.1	80.9	75.0
Tricitale	161	96.2	91.2	92.2
Rye	127	96.2	89.3	78.6
Wheat	132	101.5	97.4	94.8

Considering the overall topic of global supply chains, maize can be used as demonstrator for feed materials as it is widely grown, traded and used. The crop is grown on six continents with the most producing states and economic regions being the USA, China, Brazil, the EU and Argentina, which have also the highest use of maize as feed (see Table 7, (Agricultural Market Information System, 2018)).

Data for the EU are provided by EUROSTAT about the production area and amount of grain maize for each member state. These are summarised in Table 8 and illustrated in Figure 6 (EU DG AGRI, 2018a). This source includes grain maize as exclusive type of maize, however, the later use is not clearly stated and may cause confusion with other statistical sources when the data summarises grain maize used as feed and food such as in case of maize flour.

Table 7: **Maize production in selected states worldwide in 2016/2017** (Agricultural Market Information System, 2018)

Country/region	Production [mio. t]	Feed use [mio. t]
Argentina	40	15
Brazil	63	42
Canada	14	8
China	220	143
Egypt	8	12
European Union	61	54
India	26	10
Indonesia	21	9
Kazakhstan	1	1
Mexico	28	19
Nigeria	10	2
Philippines	8	5
Republic of Korea	0	8
Russian Federation	15	8
Saudi Arabia	0	3
South Africa	8	5
Thailand	5	4
Turkey	6	6
Ukraine	28	6
United States of America	385	139
Viet Nam	5	10

Table 8: Production volume of grain maize in the European Union in 2017 (EU DG AGRI, 2018a) and import and export volume in 2017/18 (EU DG AGRI, 2018c)

Country	Production volume [t]	Import volume [t]	Export volume [t]	Difference export - import volume [t]
Austria	2 019 900	13 494.8	5 636.8	−7 858.0
Belgium	598 170	416 651.0	10 125.4	−406 525.6
Bulgaria	2 562 750	15 536.2	123 801.3	108 265.1
Croatia	1 556 000	1 722,5	23 565.2	21 842.7
Cyprus	0	74 980.6	0.1	−74 980.5
Czech Republic	588 110	637.9	1.1	−636.8
Denmark	38 900	33 229.4	145.0	−33 084.4
Estonia	0	8 531.9	0.0	−8 531.9
Finland	0	370.1	0.0	−370.1
France	14 538 360	6 855.4	139 097.3	132 241.9
Germany	4 547 600	306 816.9	18 686.4	−288 130.5
Greece	1 423 810	119 557.3	2 021.2	−117 536.1
Hungary	6 811 340	32 500.1	51 070.2	18 570.1
Ireland	0	0.0	0.0	0.0
Italy	6 048 500	1 705 794.9	32 600.7	−1 673 194.2
Latvia	0	98 650.7	1.7	−98 649.0
Lithuania	56 970	50 008.2	59.2	−49 949.0
Luxembourg	750	0.0	0.0	0.0
Malta	0	5 954.8	0.0	−5 954.8
Netherlands	164 667	296 6471.1	3 222.9	−2 963 248.2
Poland	3 983 800	125 474.8	22 343.4	−103 131.4
Portugal	778 200	1 377 982.2	3 988.5	−1 373 993.7
Romania	14 841 420	21 868.2	742 664.6	720 796.4
Slovakia	1 079 070	107.0	417.7	310.7
Slovenia	272 180	120 483.9	461.7	−120 022.2
Spain	3 700 000	4 654 168.8	50 365.4	−4 603 803.4
Sweden	8 800	10473.4	213.2	−10 260.2
United Kingdom	24 000	744 929.8	16 226.3	−728 703.5

Country/Region	Production in [mio. t]
Argentina	40
Brazil	63
China	220
European Union	61
USA	385

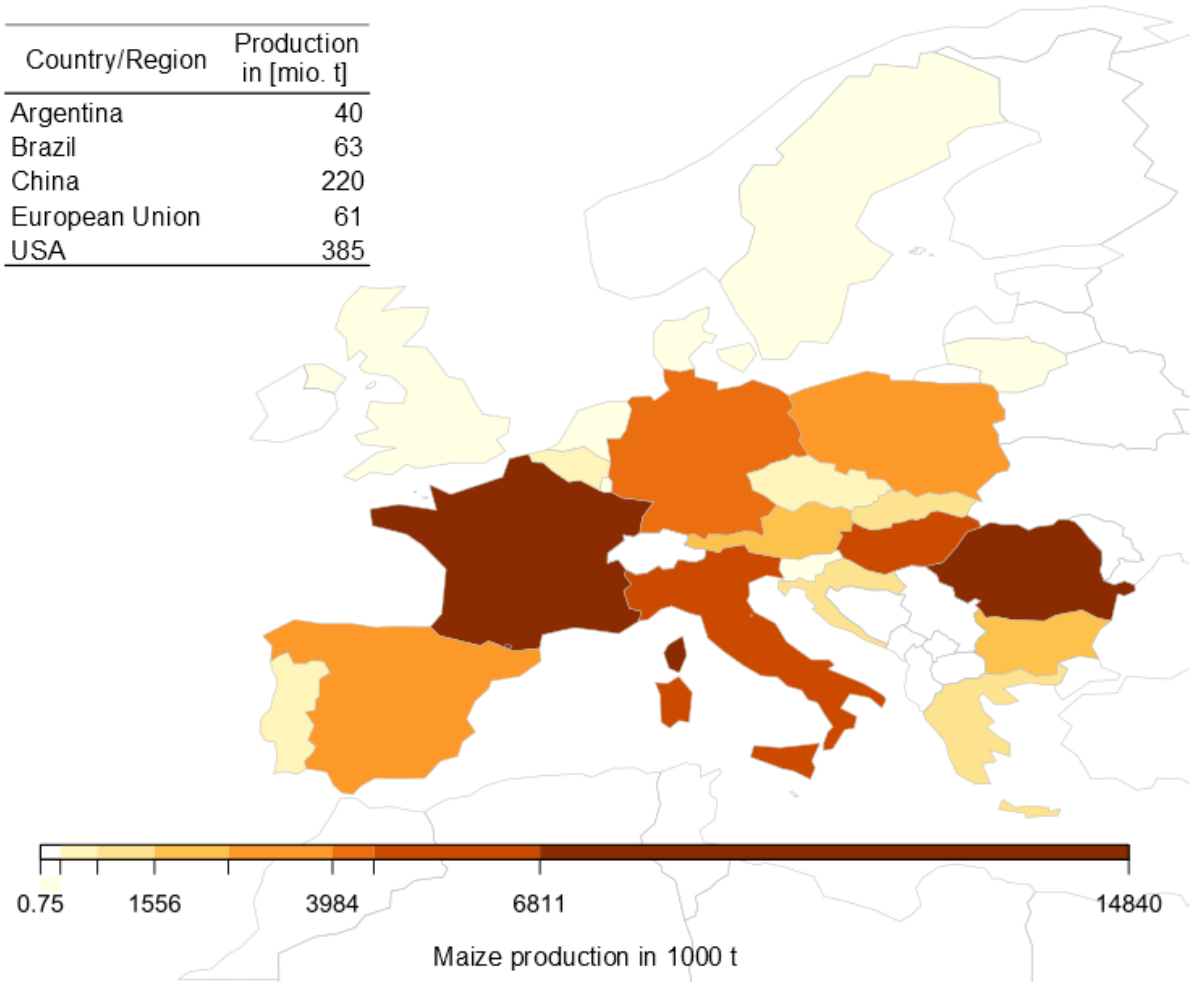


Figure 6: Production volume of grain maize in the European Union in 2017
Countries are coloured according to their production quantity. Full information about production worldwide and in the EU can be found in Table 7 and Table 8 in the Supplementary Material, respectively (Agricultural Market Information System, 2018; EU DG AGRI, 2018a).

Comparison of the global data (see Table 7) with data from the EU market (see Table 8) obviously shows a similar range for the production in the EU. Global data collection lists 61 mio. t of maize being produced in the EU (Agricultural Market Information System, 2018), whereas the EUROSTAT database provides more detailed production values for each member state. These add up to 65 mio. t in 2017 (EU DG AGRI, 2018a). The discrepancy may be related to differences in the time range for data collection that corresponds to the growing season on both hemispheres for global data and the northern hemisphere for EU data.
Maize is grown all over Europe with the exception of the Britannic islands as well as Nordic and Baltic countries that grow maize only in the most southern latitudes in a small amount (below 50 000 t). Most important producers in the EU are France and Romania yielding more than 14 mio. t maize each per harvest year. In addition, Ukraine, Russia and Turkey are also high producing countries on the European continent producing 28 mio. t, 15 mio. t and 6 mio. t of maize in 2016/17, respectively. Although these are no members of the EU but trading partners, their data are also included in the EUROSTAT database.

About the EU domestic market no data are collected but statistics list the import and export of member states with non-EU countries (see Table 8) (Agricultural Market Information System, 2018). Comparing import and export values for the EU each member state show an export surplus for Romania, France and Bulgaria with Romania exporting grain maize mostly to the non-EU countries Turkey, Lebanon and New Zealand. An excess of imports from non-

EU countries can be seen for Spain, Netherlands and Portugal, among others, which import a multiple of their own production. Using Spain as example it can be seen that in addition to the production volume of 3.7 mio. t another 4.6 mio. t of grain maize are imported. These imports originate mostly from Brazil, Ukraine and the USA. The available data allow the tracking of imports and exports with non-EU countries but the actual use as feed cannot be derived. On the one hand there is no information about the EU domestic market available and on the other hand the maize may be used in food products or as raw material in the renewable energy sector as well. In cases of interchangeable uses and dependence of the market on the demand and supply, controlling bodies need to be aware of that as different threshold values for contaminants need to be applied (i.e. maize kernels as food: 5 µg/kg aflatoxin B1; grain maize as feed: 20 µg/kg (EC, 2002b, 2009a)).

In Germany the total production of 4.5 mio. t grain maize in 2017 is distributed over most federal states with the highest production of more than 1.2 mio. t maize in Bavaria (Statistisches Bundesamt, 2017). In addition to this production, a net import of around 300 000 t adds to the total usable amount of grain maize in Germany. 2.3 mio. t of the total amount of grain maize is used in compound feed plants in 2016/17 (Bundesinformationszentrum Landwirtschaft, 2018b). The fate of the other 2.5 mio. t of maize is not covered by available statistics.

As it can be seen on maize as demonstrator for feed, there is currently no consistent database to track goods and follow the supply chain. The definitions which product group is listed and what particular versions of that product are included, are not clearly and especially feed use is often not stated at all. This adds uncertainty to the traceability of the supply chain. Without a fraud-resistant document-based traceability other strategies are needed to supervise the supply chains and ensure safety for animals and consumers. This might be done with authenticity testing based on instrumental analysis. For further information about this topic see section 3.2.

Conclusion

Trade of feed material is an important part in global food supply chains. On a European scale oilcakes and meals are the main import goods whereas on a global scale cereals are the most traded feed commodity with maize and common wheat as the dominant products. Global trade is influenced by many factors, such as production conditions that may result in shifts in the global flow of goods especially due to the ongoing climate change.

Current and future risk assessment needs to be aware of these changes as they can result in changing or emerging challenges to the safety of products and subsequent potential harm to human or animal health. Inconsistent and missing data concerning the global trade at the same time might increase uncertainty in reliable risk assessment. To ensure health and welfare for both animal and human consumer, feed safety should be included and harmonized in the relevant legislation.

Trading volumes and increasing numbers of trading partners from non-EU countries have shown that the international logistic sector plays a significant role regarding global feed supply chains. As there are many knowledge gaps concerning the transportation, storage and handling, international commodity flows are identified as a relevant research area in terms of feed safety.

Due to its significance in animal nutrition, the worldwide cultivation, use and global trade maize is a prime demonstrator for feed materials to research feed safety risks, i.e. mycotoxin contamination throughout global commodity flows as well as analytical methods for geographical authentication of feed in the context of global supply chains.

Abbreviations

BIP	Border inspection point
BVL	Federal Office of Consumer Protection and Food Safety
DPE	Designated point of entry
dwt	Dead weight tons
EU	European Union
EUROSTAT	Statistical office of the European Union
FEFA	European Feed Manufacturers´ Federation
GMO	Genetically modified organism
USA	United States of America

References

Agricultural Market Information System. (2018). http://statistics.amis-outlook.org/data/index.html

Alexandratos, N., and Bruinsma, J. (2012). *World agriculture towards 2030/2050: the 2012 revision*.

BfR, Bundesinstitut für Risikobewertung. (2011). Current dioxin issue: consumers do not have to worry. https://www.bfr.bund.de/en/press_information/2011/04/current_dioxin_issue__consumers_do_not_have_to_worry-59506.html

BfR, Bundesinstitut für Risikobewertung. (2013). Increased aflatoxin concentrations detected in raw milk. Information No. 08/2013 of 01 Mar. 2013.

BLE, Bundesanstalt für Landwirtschaft und Ernährung. (2017). Struktur der Mischfutterhersteller 2017. In (pp. 82). Berlin: Bundesministerium für Ernährung und Landwirtschaft (BMEL).

BMEL, Federal Ministry of Food and Agriculture. (2016). *Beans, Peas and Co. - The Federal Ministry of Food and Agriculture´s Protein Crop Strategy for promoting the cultivation of pulses in Germany*.

Bundesinformationszentrum Landwirtschaft. (2018a). *Bericht zur Markt- und Versorgungslage Futtermittel 2018*.

Bundesinformationszentrum Landwirtschaft. (2018b). Struktur der Mischfutterhersteller - Wirtschaftsjahr 2015/2016-2016/2017.

Bundesinformationszentrum Landwirtschaft. (2018c). Versorgungsbilanz für Getreide Wirtschaftsjahr 2016/2017.

BVL, Bundesamt für Verbraucherschutz und Lebensmittelsicherheit. (2018a). Archive of reports on pesticide residues. https://www.bvl.bund.de/DE/01_Lebensmittel/01_Aufgaben/02_AmtlicheLebensmittelueberwachung/07_PSMRueckstaende/02_nb_psm_archiv/nbpsm_archiv_node.html

Earle, F. R., Curtis, J. J., and Hubbard, J. E. (1946). Composition of the component parts of the coin kernel. *Cereal chemistry, 23*, 504-511.

EC, European Commission. (2002b). DIRECTIVE 2002/32/EC OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 7 May 2002 on undesirable substances in animal feed. *Official Journal of the European Communities, L 140*, 10-22.

EC, European Commission. (2009a). REGULATION (EC) No 767/2009 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 13 July 2009 on the placing on the market and use of feed, amending European Parliament and Council Regulation (EC) No 1831/2003 and repealing Council Directive 79/373/EEC, Commission Directive 80/511/EEC, Council Directives 82/471/EEC, 83/228/EEC, 93/74/EEC, 93/113/EC and 96/25/EC and Commission Decision 2004/217/EC. *Official Journal of the European Union, L 229*, 1-28.

EC, European Commission. (2018). Commission Homepage on Food and feed of non-animal origin - Designated Points of Entry. https://ec.europa.eu/food/safety/official_controls/legislation/imports/non-animal_en

EU DG AGRI. (2018a). EU Cereals production, area and yield. https://ec.europa.eu/agriculture/market-observatory/crops/cereals/statistics_en

EU DG AGRI. (2018b). EU Crops Market Observatory - Cereals - Balance sheets 2007/08 to 2018/19. https://ec.europa.eu/agriculture/market-observatory/crops/cereals/balance-sheets_en

EU DG AGRI. (2018c). EU Crops Market Observatory - Cereals - Cereals exports and imports (Eurostat - COMTEXT). https://ec.europa.eu/agriculture/market-observatory/crops/cereals/statistics_en

Eur. Parliament, and The Council. (2002d). Regulation (EC) No 178/2002 of the European Parliament and the council of the European Union of 28 January 2002 laying down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety. *Official Journal of the European Communities, L 31*, 1-24.

Eur. Parliament, and The Council. (2004). REGULATION (EC) No 882/2004 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 29 April 2004 on official controls and other official activities performed to ensure the application of food and feed law, animal health and animal welfare rules. *Official Journal of the European Union, L165*, 1-141.

FAO and IFIF. (2010). Good practices for the feed industry–Implementing the Codex Alimentarius Code of Practice on Good Animal Feeding. *FAO Animal Production and Health Manual*(9 s 79).

FAO Statistical Yearbook. (2013). World food and agriculture. Food And Agriculture Organization Of The United Nations, Rome, Part 3, Feeding the world. In.

FEFAC, European Feed Manufacturers Federation. (2017a). Annual Report 2016-2017. In.

FEFAC, European Feed Manufacturers Federation. (2017b). Feed & Food Statistical Yearbook 2016. In.

Heinzler, M., Gerlach, D., and Brunn, H. (2011). New border control system for food originating from third world countries. *Environmental Sciences Europe, 23*(1), 14. doi:10.1186/2190-4715-23-14

Kellems, R. O., and Church, D. C. (2010). High-Energy Feedstuffs. In R. O. Kellems and D. C. Church (Eds.), *Livestock feeds and feeding* (6 ed., pp. 55-83). Upper Saddle River, NJ: Prentice Hall.

LHL, Landesbetrieb Hessisches Landeslabor (2017). Pflanzenschutzmittelberichte. *PSM in Importproben.* https://lhl.hessen.de/tgsh/perishable-center/pflanzenschutzmittel-importproben-2015-2017

National Research Coucil. (1982). *United States-Canadian Tables of Feed Composition: Nutritional Data for United States and Canadian Feeds, Third Revision*. Washington, DC: The National Academies Press.

OECD-FAO, Food Agriculture Organization of the United Nations,. (2016). *OECD-FAO Agricultural Outlook 2016-2025*.

Raja, U. (2013, 18.02.2013). Which drought-hit US states suffered the worst crop damage? *The Guardian*.

Schieck, A. (2008). *Internationale Logistik: Objekte, Prozesse und Infrastrukturen grenzüberschreitender Güterströme*: Oldenbourg Verlag.

Schmid, W., and Goldhofer, H. (2017). *2 Getreide*.

Schubert, K. (2012). *Mischfutteroptimierung unter geänderten Vorzeichen*. Paper presented at the Sächsischer Schweinetag, Groitzsch. https://www.landwirtschaft.sachsen.de/landwirtschaft/download/DrSchubertTiernahrung.pdf

Statistisches Bundesamt. (2017). Land- und Forstwirtschaft, Fischerei - Wachstum und Ernte - Feldfrüchte. In.

The Council. (1997). *COUNCIL DIRECTIVE 97/78/EC of 18 December 1997 laying down the principles governing the organisation of veterinary checks on products entering the Community from third countries*. Official Journal of the European Communities. https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:31997L0078&from=DE

USDA, United States Department of Agriculture – Office of Global Analysis. (2018a). Grain: World Markets and Trade. https://apps.fas.usda.gov/psdonline/circulars/grain.pdf

USDA, United States Department of Agriculture – Office of Global Analysis. (2018b). Oilseeds: World Markets and Trade. https://apps.fas.usda.gov/psdonline/circulars/grain.pdf

USDA, United States Department of Agriculture – Office of Global Analysis. (2018c). Commodities and Products. https://www.fas.usda.gov/commodities

Watson, S. A. (2003). Description, development, structure and composition of the corn kernel. In P. J. White & L. A. Johnson (Eds.), *Corn: chemistry and technology* (2nd ed., pp. 69-106). St. Paul, Minnesota: American Association of Cereal Chemists.

WHO, World Health Organization. (2008). *Animal feed impact on food safety. Report of the FAO/WHO Expert Meeting, FAO Headquarters, Rome, 8-12 October 2007.* Paper presented at the Animal feed impact on food safety. Report of the FAO/WHO Expert Meeting, FAO Headquarters, Rome, 8-12 October 2007.

2.2 Importance of food trade for Germany

Wisniewski, Aline; Buschulte, Anja

Abstract

Germany is one of the world's most important trading partners in the import and export of foodstuffs. Therefore, the food trade is of great importance in terms of food safety. Since only a small percentage of imported foodstuffs can be checked at customs offices, it is important to know the country of origin of the food and whether there are comparable food safety requirements. The following text describes which countries are Germany's most important trading partners and how the import and export of its major trading products has developed over the last ten years. Building on this, the question of which factors can influence trade will be examined and examples are given to show the extent to which these factors can affect the fragile construct of trade.

Trade statistics of agri food imports and exports to Germany for the years 2008 to 2017

The German food industry plays a crucial role in the global food trade: For years, Germany has been named as world's number three in import and export of goods of the agriculture and food industry (hereinafter also referred to as agri food) (BMEL, 2017). In the database of Destatis, the German Federal Statistical Office, agri food is summarized as „Warengruppen und -untergruppen der Ernährungs- und Gewerblichen Wirtschaft“ (EGW). According to this database, agri food comprises the following product categories: live animals, food of animal origin, food of vegetable origin as well as luxury food. The classification of the individual product classes is carried out according to the specifications of the 2-digit list of Destatis. The development of the trade value and the trade mass of Germany over the last ten years is shown in Figure 7. In 2017, Germany exported agri food and beverages worth around 73.02 billion EUR.

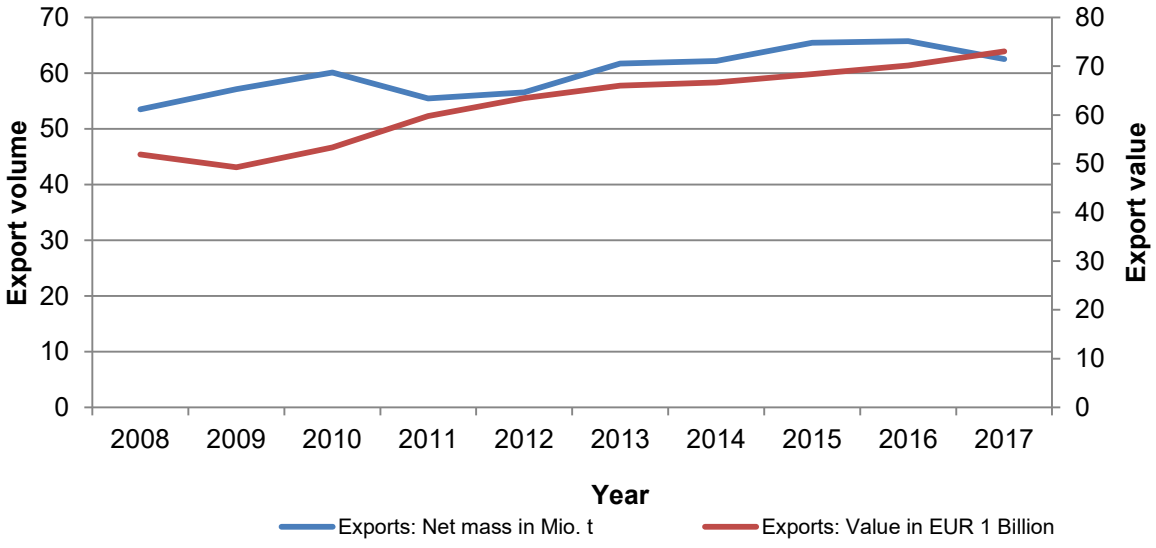


Figure 7: Export volume of agricultural goods over the last 10 years
(Expressed in volume (in million tons) and value of goods (in billion EUR))

At the same time Germany imported agri food and beverages worth around 84.83 billion EUR (see Figure 8). Both the import and export of agricultural goods have shown a steady increase over the last ten years, except in 2009. The decrease of trade in this year was strongly influenced by the global economic and financial crisis (BMEL, 2017) and is demonstrated by the declining trade value in EUR currency (see Figure 7 and 8).

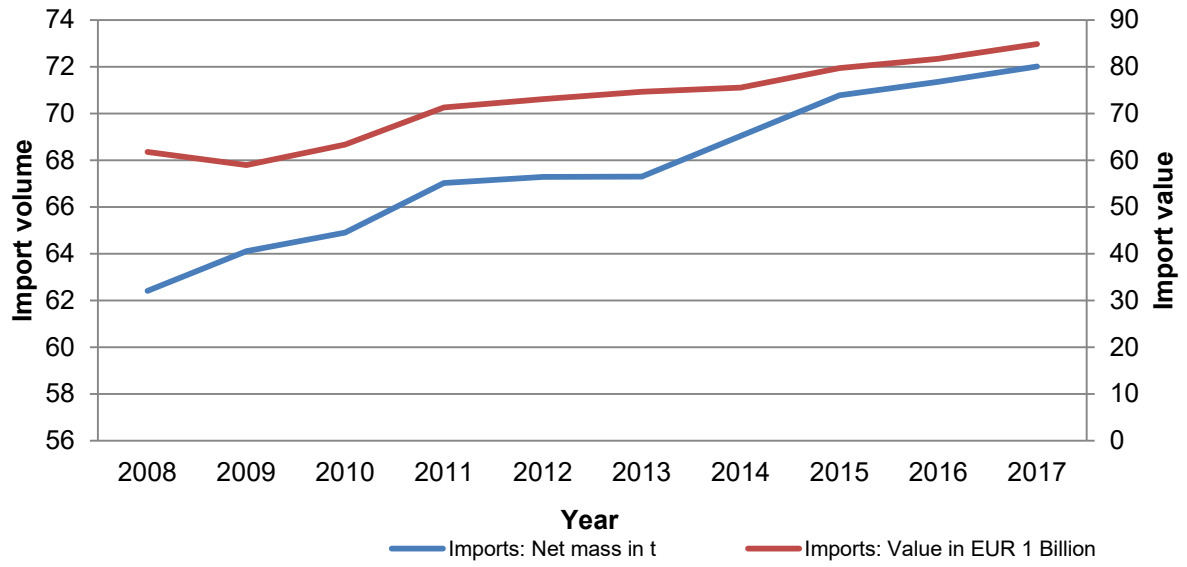


Figure 8: Import volume of agri goods over the last 10 years
(Expressed in volume (in million tons) and value of goods (in billion EUR))

The top trading partners of Germany based on trade value in the sector of agri food products for export

Germany is one of the world`s largest export countries particularly in the food sector. Table 9 shows the most important trading partners over the last 10 years, based on the value of traded agri food goods. The first five places are consistently occupied by the following trading partners: European Union (EU), Russian Federation (RU), Switzerland (CH), United States of America (USA), People's Republic of China (CN) and Saudi Arabia (SA). While CN has risen to the top 5 in the last 4 years, the importance of RU has steadily decreased since 2013. This might be caused by tightened regulation on veterinary measures. As an example, on January 2013, the RU issued a ban on imports of fresh, chilled meat from Germany. At the same time, a large number of approved suppliers were banned in the context of alleged insufficient official veterinary surveillance to ensure compliance with specific Russian requirements in exporting establishments (Beckhove, 2013). In addition, the Crimean conflict intensified in 2013 and also affected the trade relations between the RU and Germany. The impact with relation to that will be further explained in chapter 3.

The top trading partners of Germany based on trade value in the sector of agri food products for import

Especially with regard to food safety, it is particularly important for the importing country to know the origin of food as this may have a direct impact on food safety and/or food fraud.
Table 10 lists the top 10 trading partners of Germany for import over the last 10 years. The Table expresses that the EU is so far Germany's largest trade partner for the import of agri food. Almost 75 % of all agri foods are imported from the EU. Brazil (BR), US, CN, CH and

Turkey (TR) are the most important trading partners after the EU. They follow the EU in changing order from 2nd to 6th place in the rankings over the last 10 years. All these countries have been exporting more than a billion EUR worth of agri food products to Germany every year for at least eight years.

Table 9: Germany's most important trading partners in agri-food exports by year and trade value in billion EUR

Order	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017
1	EU 42076422	EU 39786086	EU 42130446	EU 46573824	EU 48662213	EU 51020651	EU 51561507	EU 52188582	EU 53733820	EU 56431508
2	Russian Federation 1649596	Russian Federation 1335006	Russian Federation 1777259	Russian Federation 1913567	Russian Federation 1864851	Switzerland 1785932	Switzerland 1791232	Switzerland 1828331	Switzerland 1907644	Switzerland 2031956
3	Switzerland 1269701	Switzerland 1321055	Switzerland 1443146	Switzerland 1630430	Switzerland 1655182	United States 1586885	United States 1634253	United States 1731211	United States 1817085	United States 1919887
4	United States 1089596	United States 1048520	United States 1267781	United States 1501970	United States 1616748	Russian Federation 1586821	Russian Federation 1150836	Saudi Arabia 1373195	China, People's Republic of 1756687	China 1588667
5	Japan 532524	Japan 439913	Saudi Arabia 461206	Saudi Arabia 677989	Saudi Arabia 755966	Saudi Arabia 1104385	China 933741	China 1362063	Saudi Arabia 1289370	Russian Federation 979974
6	Norway 405812	Norway 410824	Norway 438624	Norway 540264	China 668337	China 942853	Saudi Arabia 875257	Russian Federation 862800	Russian Federation 881808	Saudi Arabia 947838
7	Saudi Arabia 368416	Saudi Arabia 345747	Japan 425407	Turkey 449479	Norway 607779	Norway 633110	Islamic Republic of Iran 645550	Norway 573687	Norway 661980	Korea, Republic of 649266
8	Ukraine 366311	Ukraine 292403	Turkey 365483	Japan 443216	Turkey 501005	Ukraine 429423	Norway 618646	Turkey 460122	Korea, Republic of 551961	Norway 507950
9	United Arab Emirates 238630	Turkey 291653	Ukraine 340450	Ukraine 394413	Ukraine 458212	Turkey 406954	Korea, Republic of 422385	Korea, Republic of 439038	Turkey 488663	Hong Kong 477542
10	Turkey 229819	Islamic Republic of Iran 245994	South Africa 268540	Hong Kong 389275	Japan 420609	Japan 366342	Turkey 417530	Algeria 420048	Japan 405385	Japan 474370

Table 10: Germany's most important trading partners in agri-food imports by year and trade value in billion EUR

Order	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017
1	EU 42841263	EU 41188301	EU 43638507	EU 48021086	EU 49753707	EU 52241600	EU 52373381	EU 54407081	EU 56573356	EU 59480904
2	Brazil 3034759	Brazil 3080966	Brazil 3022524	Brazil 3795853	Brazil 3544201	Brazil 2885338	Brazil 3534873	Brazil 3495553	Brazil 2973908	Brazil 2735316
3	United States 1640899	United States 1298834	United States 1507772	United States 1719727	United States 2034447	United States 2341081	United States 2300327	United States 2758855	United States 2538609	United States 2635911
4	China 1269276	China 1191876	China 1370352	China 1520285	China 1548947	China 1460762	China 1477588	Turkey 1750499	China 1646629	China 1645698
5	Argentina 1056131	Switzerland 981522	Turkey 1048988	Switzerland 1195312	Switzerland 1312579	Switzerland 1398520	Switzerland 1439909	China 1639895	Turkey 1529884	Switzerland 1559777
6	Turkey 997037	Turkey 917529	Switzerland 1035201	Turkey 1170395	Turkey 1222995	Turkey 1266580	Turkey 1349927	Switzerland 1440253	Switzerland 1502142	Turkey 1462189
7	Switzerland 941595	Argentina 892189	Argentina 893420	Indonesia 1008201	Argentina 915737	Argentina 844401	Indonesia 767728	Viet-Nam 866742	Viet-Nam 978841	Viet-Nam 1045632
8	Indonesia 917449	Indonesia 737227	Indonesia 824151	Argentina 896366	Indonesia 892438	Indonesia 763974	Viet-Nam 760330	Indonesia 828338	Indonesia 837130	India 841193
9	Colombia 557902	Viet-Nam 428529	Norway 534221	Cote d'Ivoire 637445	Viet-Nam 786202	Viet-Nam 674721	Argentina 746587	Argentina 748017	Argentina 776493	Indonesia 801331
10	New Zealand 495638	Peru 426580	Peru 517787	Viet-Nam 624452	India 620586	India 595535	India 638086	Cote d'Ivoire 693924	Cote d'Ivoire 702478	Argentina 702250

The most popular food products for export from Germany

Among the most important export goods in the field agri food goods counts:

- meat
- dairy produce, eggs and honey
- cocoa and cocoa preparations
- preparations of cereals, pastrycooks' products
- miscellaneous edible preparations and
- beverages, spirits and vinegar (see Figure 9).

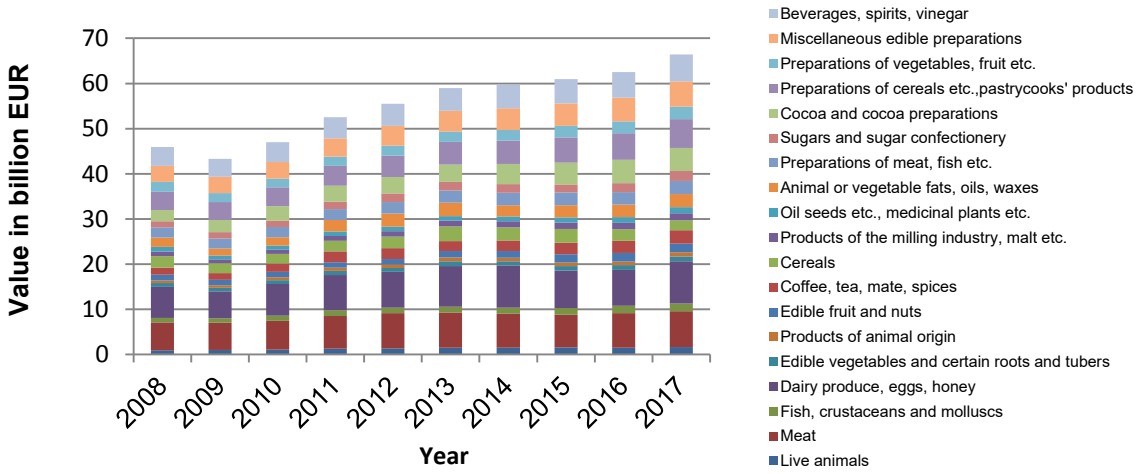


Figure 9: Value (in billion EUR) of food goods exported from Germany in the last 10 years

Each of these product classes was exported from Germany in 2017 with an export value over 5 billion EUR. For these product categories, it was also listed which products were exported particularly often. For this purpose, the years 2008 to 2017 and the corresponding commodity class codes (6-digit code) were selected for the survey at the Destatis database. The results are presented in Table 11.

Table 11: Germany`s top seller (6 digit code) in the top category`s for export for the year 2017

Category	Main trading partners in 2017	Export value in billion EUR	Top seller commodity class (6-digit code)	Export value in billion EUR
Meat	EU	6.33	meat from pigs (fresh or chilled)	2.09
	CN	0.50	frozen meat of swine	1.18
	KR[1]	0.32	meat from cattle boneless, freshly chilled	0.54
Dairy produce, eggs and honey	EU	7.76	cheese and cream cheese	2.96
	CN	0.26	milk and cream	1.04
	CH	0.11	milk powder	0.58
Cocoa and cocoa preparations	EU	4.07	chocolate (weight ≤ 2kg)	1.82
	US	0.16	chocolate, i.e. plates, unfilled (≤ 2kg)	1.02
	RU	0.15	chocolate, i.e. plates, filled (≤ 2kg)	0.91
Preparations of cereal and pastry cooks' products	EU	5.03	bakery products (whether or not containing coco)	2.25
	CN	0.27	cookies and similar biscuits (sweetened)	0.74
	CH	0.22	food preparations of flour	0.69
Miscellaneous edible preparations	EU	4.07	food preparations	2.98
	CH	0.20	extracts, essences and concentrates of coffee	0.77
	RU	0.19	seasonings	0.65
Beverages, spirits and vinegar	EU	4.21	beer	1.14
	US	0.33	fresh grape wine (≤ 2l)	0.80
	CH	0.2	non-alcoholic beverages and water (incl. mineral water)	0.79

[1] Republic of [1] [1]Korea

The most popular food products for import to Germany

The following food categories have regularly been among the most relevant imported agri food goods to Germany in the last 10 years:

- edible fruit and nuts
- edible vegetables and certain roots and tubers
- dairy produce, eggs and honey
- meat and
- beverages, spirits and vinegar (see Figure 10)

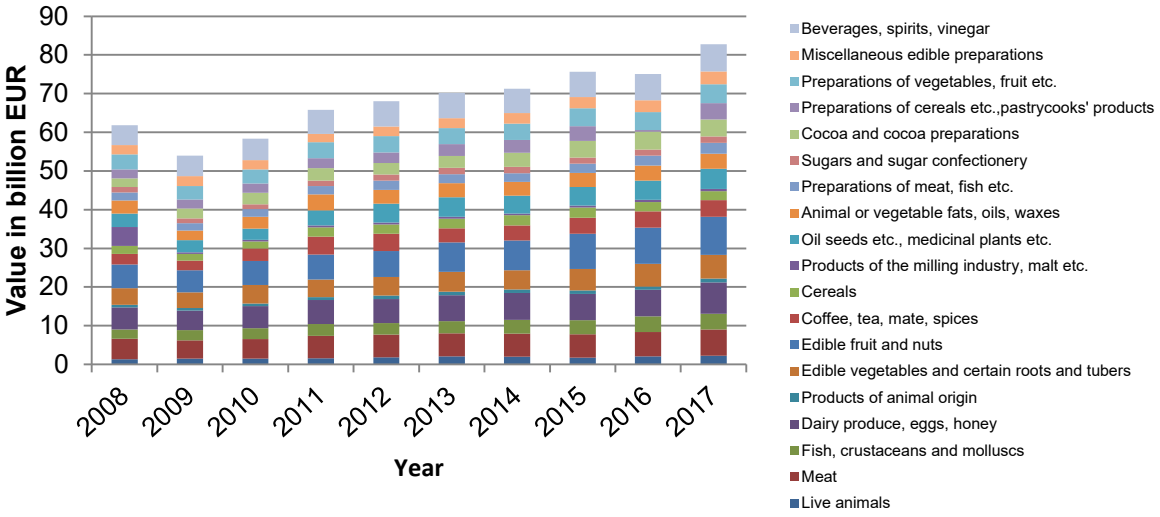


Figure 10: Value (in billion EUR) of food goods imported to Germany in the last 10 years

Each of these product classes was imported in 2017 with an import value over 6 billion EUR. For these product categories, it was also listed which products were imported particularly often. For this purpose, the years 2008 to 2017 and the corresponding commodity class codes (6-digit code) were selected for the survey at the Destatis database. The results are presented in Table 12.

Table 12: Germany`s top seller (6 digit code) in the top category`s for import for the year 2017

Category	Main trading partners in 2017	Import value in billion EUR	Top seller commodity class (6-digit code)	Import value in billion EUR
Edible fruit and nuts	EU	4.77	bananas	0.90
	US	0.83	grapes	0.64
	TR	0.55	fresh apples	0.60
Edible vegetables,	EU	5.35	tomatoes, fresh or chilled	1.31
certain roots and tubers	CN	0.17	fruits of the genera capsicum, pimenta, fresh	0.74
	MA[1]	0.11	cucumbers and gherkins, fresh or chilled	0.50
Dairy produce, eggs	EU	7.62	cheese (not elsewhere specified)	2.96
and honey	CH	0.27	milk and cream	1.04
	MX[2]	0.04	butter (excluding dehydrated butter and ghee)	0.58
Meat	EU	5.67	boneless beef, fresh or chilled	1.20
	NZ[3]	0.26	carcases of pigs, fresh or chilled	0.72
	AR[4]	0.26	chicken parts, fresh or chilled	0.55
Beverages, spirits and vinegar	EU	6.05	wine of fresh grapes (< 2 l)	1.6
	US	0.38	ethyl alcohol (alcohol ≤ 80% Vol.)	0.60
	ZA[5]	0.10	water (incl. mineral waters)	0.56

[1]Morocco,[2]Mexico, [3]NewZealand, [4]Argentina, [5]SouthAfrica

Influences on trade

Trade relations form a very complex network, which is subjected to many different influences. There are many factors that can have an impact on trade. The most obvious factor is the demand, which, for example, is subject to the influence of consumer trends. External factors such as political conflicts (wars, riots), climatic fluctuations (droughts, frosts), animal or plant diseases, water availability, access to exploring technologies and resources to produce or harvest products are also to consider. Two examples from the area of import and export illustrate how far-reaching such factors may influence trade.

- **Political conflicts**

In spring of 2014, the RU annexed the Ukrainian peninsula of Crimea after an internationally unrecognized referendum in Crimea in March 2014. This action and Russian support of separatists in parts of eastern Ukraine prompted a number of governments and international organizations, to impose sanctions against Russia (Deutscher Bundestag, 2017). In this context, the EU imposed sanctions on Russian individuals, such as account and access barriers, but also on Russian companies. Economic and financial sanctions were imposed as well. As a countermeasure to these sanctions introduced by the EU, in August 2014 the Russian government banned imports of various food and agricultural products (fruits, vegetables, meat, fish and dairy products) from the EU (Oja, 2015). These sanctions also affected other countries, such as the US, Norway, Canada and Australia and were originally intended to be effective for one year. The import ban was initially extended for a further year in June 2015, as political conditions had hardly changed (Europäisches Parlament, 2018). In May 2016, the Russian government eased food sanctions and allowed the import of certain goods for further processing as for example baby food. Later in 2016, the Russian President extended the sanctions against the EU for another year until 31 December 2017.

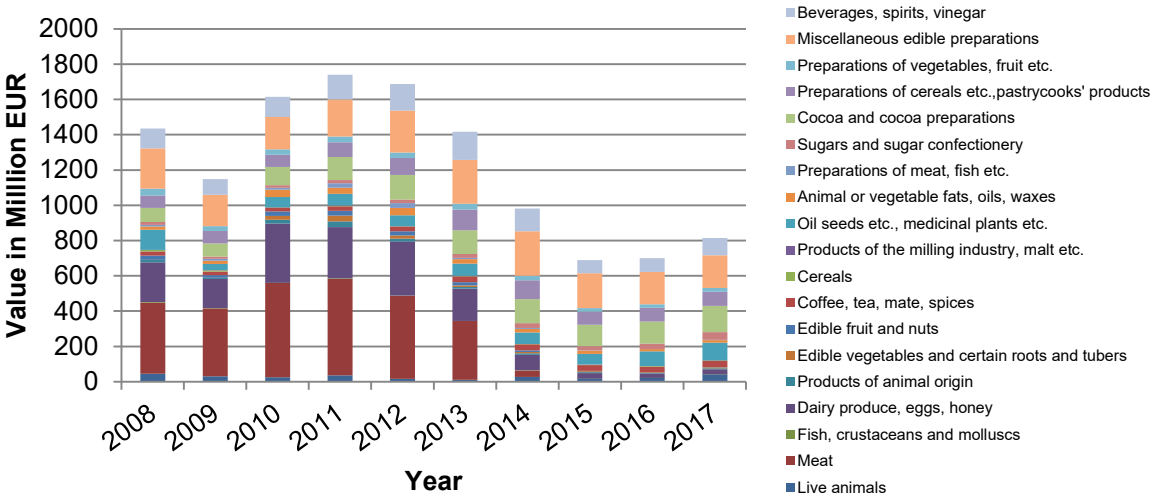


Figure 11: Value (in million EUR) of food goods exported to Russia in the last 10 years

The import bans of Russia become apparent very well in the trade data from the last 10 years. There was already a slight decline in trading value in 2009, caused by the global economic and financial crisis. In February 2013, Russia enacted far-reaching veterinary sanctions regulating imports of fresh meat. In the course of Russia's economic sanctions against the EU, imports of selected agricultural products were rigorously suspended in August 2014. This development can be seen in Figure 11 and shows how strongly political conditions can

affect economic relations. Until 2012 Russia was still the strongest consumer of meat from Germany after the EU (approximately (approx.) 0.5 Billion EUR).
Figure 11 clearly shows that the trade of meat has almost completely collapsed in the wake of political developments since 2014.

- **Climatic fluctuations**

TR is one of the top 6 import trading partners of Germany in the field of agri food. The most important import food goods from TR are preparations of vegetables, fruit, edible fruits and nuts (shown in Figure 12).

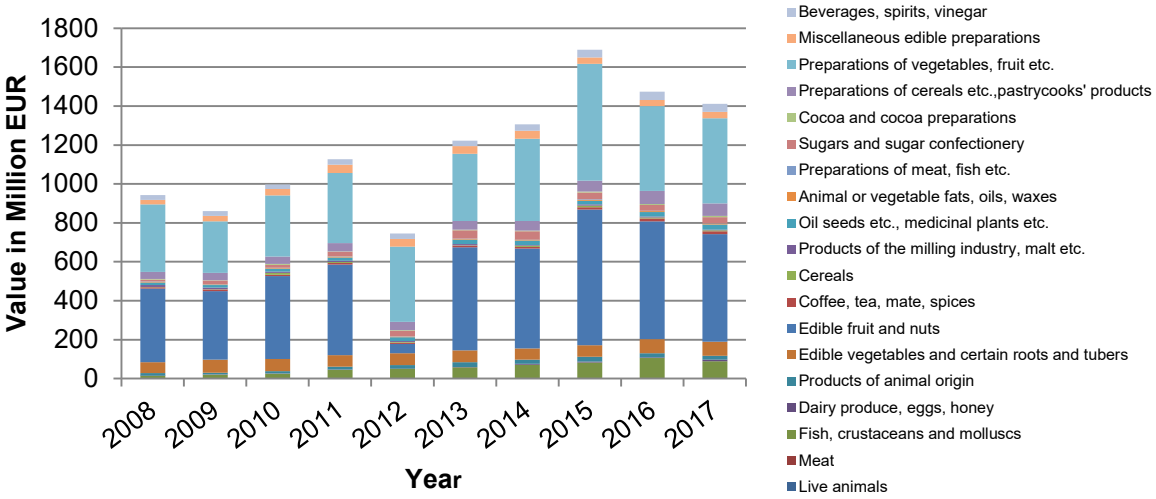


Figure 12: Value (in million EUR) of food goods imported from Turkey to Germany in the last 10 years

For the latter product category the focus lies on the import of hazelnuts, figs, apricots, cherries, spices, apples, strawberries, sultanas, melons, pistachios, walnuts, cucumbers, chick peas and lentils (BMEL, 2016). Based on the data of the last 5 years approx. 72 % of the hazelnuts worldwide were produced in TR. Germany is one of the world`s largest purchaser of hazelnuts (INC, 2018). According to Destatis, Germany imported approx. 38.000 tons of hazelnuts from TR, 17.000 tons from Italy (IT), 4.000 tons from Azerbaijan (AZ) and 4000 tons from several other countries in 2017 (Destatis, 2018b).

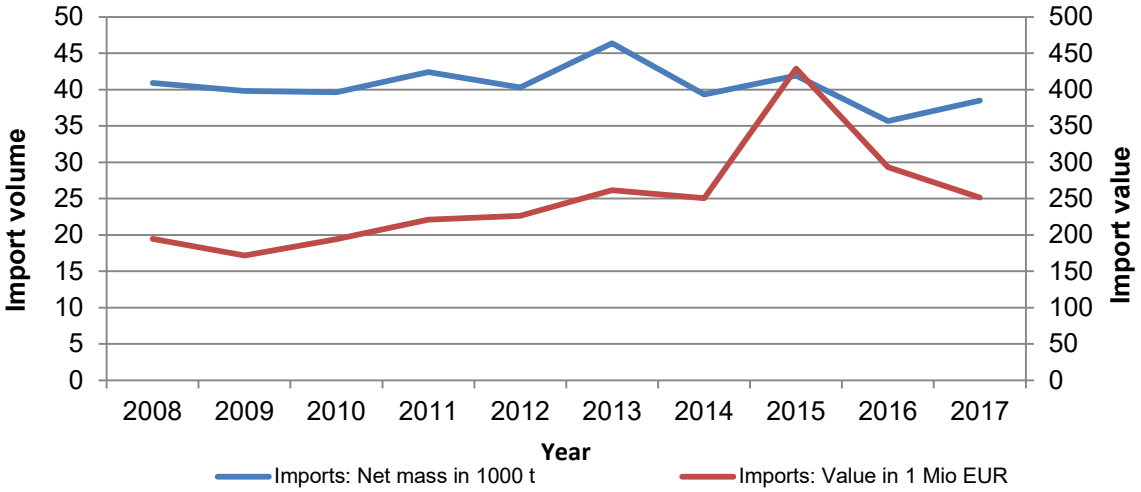


Figure 13: Imported volume of hazelnuts without shell, fresh or dried from TR to Germany during the last 10 years, expressed in 1000 tons and value in EUR 1 million

Since 2010, the prize of hazelnuts on the world market has risen steadily with a dramatic increase in 2014, when a sudden frost strongly decimated the hazelnut harvest in TR. At the same time, the demand in Germany continued to rise. While in 2006 approx. 35.000 tons of hazelnuts without shell were imported from TR, this quantity increased by more than 12 % to approx. 38.000 tons in 2017 (Destatis, 2018b). Figure 13 shows the increasing prise over time. As suppliers are bound by the fulfilment of their contracts, such events can lead to an increased probability of the occurrence of food fraud. On the one hand, the harvest loss makes it difficult to provide the agreed delivery quantities of hazelnuts, which could result in contractual penalties. On the other hand, the sharp rise in price may provide a further incentive for food fraud (see chapter 3.1).

Conclusion

On the basis of the data obtained, it became clear that the EU is by far Germany's most important trading partner in terms of food imports and exports. Since the same safety regulations and standards apply in the EU as in Germany, it can be assumed that these goods have the same level of food safety. However, in addition to the EU, third countries such as the USA, the People's Republic of China and Switzerland are also very important trading partners for Germany. Particularly for these third country partners, it must be ensured that the legal requirements for food safety are comparable to those in Germany and that they are implemented. Germany's most important export goods are animal products such as meat and dairy products, which are also imported in large quantities. Germany's most important imported goods are fruit and vegetables. Two examples were used to illustrate the enormous impact that external factors such as political conflicts or climate fluctuations can have on imports or exports. These examples already illustrate the complexity of international commodity chains. The world is becoming increasingly interconnected and the issue of food safety can no longer be dealt with on a local or regional basis but must be addressed globally.

References

Beckhove, A. (2013). Deutsche Fleischexporte nach Russland gehen weiter zurück.

BMEL, Bundesministerium für Ernährung und Landwirtschaft. (2016). *Länderbericht Türkei*. https://agrarexportfoerderung.de//fileadmin/SITE_MASTER/content/files/Laenderberichte2016/L%C3%A4nderbericht_T%C3%BCrkei_Mai_2016.pdf

BMEL, Bundesministerium für Ernährung und Landwirtschaft. (2017). *Agrarexporte 2017*. https://www.bmel.de/SharedDocs/Downloads/Broschueren/Agrarexporte_2017.pdf?__blob=publicationFile

Destatis. (2018b). *Imported volume of hazelnuts without shell, fresh or dried from TR to Germany during the last 10 years*. https://www.destatis.de

Deutscher Bundestag. (2017). *Sanktionen im Zusammenhang mit den Ereignissen in der Ukraine*. https://www.bundestag.de/resource/blob/515820/75b18131eead30b280426665c489b2c2/wd-2-052-17-pdf-data.pdf

Europäisches Parlament. (2018). Ukraine: Chronologie eines Konflikts.

INC, International Nut and Dried Fruit Council Foundation, (2018). *Statistical Yearbook 2016/2017*. https://www.nutfruit.org/files/multimedia/1510229514_1497859419_Statistical_Yearbook_2016-2017.pdf

Oja, K. (2015). No milk for the bear: the impact on the Baltic states of Russia's counter-sanctions. *Baltic Journal of Economics, 15*(1), 38-49. doi:10.1080/1406099X.2015.1072385

2.3 Container transport - the backbone for smooth running international trade in goods

Mazurek, Nicole; Boje-Haderer, Rita; Krätke, Renate

Abstract

Global markets and any increase in trade growth is depending on optimal transport conditions over sea and on inland waters linked to transport by rail and road. Nowadays this is achieved by multimodal transport chains based on standardized containers which can be efficiently loaded and unloaded, stacked, transported and reloaded between different modes of transport. Modern transport has its origins in the container transport of the 19th century which was further developed in the early 20th century for standardized container transport and distributed worldwide after the Second World War. This so-called container revolution or containerization led to a significant reduction in transport costs followed by an accelerated globalization of general cargo. This development changed the character of ports worldwide. In the meantime, two-thirds of international cargo transport is handled by container ships. Since 1996, the number of container ships has doubled. The container market grew three times faster than the global economy. Based on forecasts with regard to future transport volumes, it can be expected that container transport will experience further growth rates. Main routes for global see transport are the Far East - Europe, the Trans-Pacific and the Trans-Atlantic route. On these routes goods of interest for the public include food, feed and consumer products like textiles. In order to protect goods during transport from pests and molds, some ship containers are fumigated with pesticides. However, the fumigation may cause harm to workers, if the necessary safety measures are disregarded when opening containers or fumigated containers are not labelled appropriately. BfR is involved in the development of requirements for safe transport and in issues of classification of toxic and corrosive dangerous goods.

Introduction

Containerization originated several centuries ago but was not well developed or widely applied until after World War II, when it dramatically reduced the costs of transport, supported the post-war boom in international trade, and was a major element in globalization. Already in the 18th century, wooden outer packaging boxes were used in England. These precursors of today's containers allowed a faster transfer of goods from railroad to horse transport. In the 19th century, in various countries early container forms were used in freight transport by rail.

The first ship specially built for container transport was put into service in Denmark in 1951, while the first container ship for intermodal containers was built in 1955 in Montreal. On its first voyage, 600 containers were transported and loaded to container freight cars for transport to the Yukon. This is reported to be the first intermodal transport using trucks, ships and railways (Wikipedia, 2018).

Modern multimodal containers were invented in 1956 by the American Malcom McLean, a freight forwarder and owner of a small shipping company. The first 56 containers were shipped with the Ideal X, a converted tanker, from Newark (New Jersey) to Houston (Texas). The inventions of McLean were the breakthrough for modern container ships and the standardisation of containers (planet wissen, 2017).

The American containership Fairland was the first containership that reached Europe in 1966. It stopped in Rotterdam and Bremen were it unloaded 99 containers

(WirtschaftsWoche, 2016). The first German container ship was the Bell Vanguard which was built in the same year in Hamburg (Wikipedia, 2018).

In the meantime different container types have been developed with varying dimensions. The most frequently used containers are 20 (6.1 m) or 40 (12.2 m) feet long and can carry up to 22 or 27 tons, respectively. The standard measure for container cargo is the Twenty-foot Equivalent Unit (TEU). This value refers to an equivalent unit of cargo which is 20 feet long and 8 feet wide.

Maritime transport and container handling

Over the last three decades, world seaborne trade developed in line with developments in the world economy. With over 80% of global trade by volume and more than 70% of its value being carried on board ships and handled by seaports worldwide, the maritime transport plays an important role for trade and development. The United Nations Conference on Trade and Development publishes data on maritime transport regularly since 1968 with the aim of fostering the transparency of maritime markets, analyzing relevant developments and provides an overview on the changes in global cargo transport.

According to the latest report, in 2016 world seaborne trade reached more than 10,000 million tons with developing economies being mayor players covering 59% of cargoes for export and 64% for import. Over the last decades, growth rates in international seaborne trade were about 3% per year in average with some declines in years suffering from economic crisis (UNCTAD, 2017).

Table 13 shows the development of international seaborne trade between 1980 and 2016. When comparing the different rates of growth, the importance of container transport becomes obvious as containerized trade increased nearly 17-fold while total seaborne trade increased three-fold only.

Table 13: International seaborne trade, selected years
Data in millions of tons

Vessel Type / Year	1980	1990	2000	2010	2016
Container	102	234	598	1280	1720
Other dry cargo	1123	1031	1928	2022	2339
Five mayor bulks	608	988	1295	2335	3172
Oil and gas	1871	1755	2163	2772	3055

Source: (UNCTAD, 2017)

The growth in international trade for the different types of cargoes is also reflected by the world fleet growth and by changes in the corresponding vessel types. An analysis of the commercial value of the world fleet provides another perspective to the traditional market share in terms of cargo-carrying capacity, the dead-weight ton (dwt; weight a ship can carry). Main growth is reported for container ships as percentage share of dwt increased from 1.6% in 1980 to 13.2% in 2017 (see Table 14). In general, dwt is considered the relevant indicator for shipping, because it represents the relevance of maritime transport for international trade volumes. In terms of dwt, the world fleet is dominated by dry bulk carriers, oil tankers and container ships (see Figure 14) transporting iron ore or coal.

Table 14: World fleet by principle vessel type, 1980-2017
Percentage share of dwt

Vessel Type / Year	1980	1990	2000	2010	2017
Container Ships	1.6	3.9	8.0	13.3	13.2
Oil tankers	49.7	37.4	35.4	35.3	28.7
Dry bulk carriers	27.2	35.6	34.6	35.8	42.8
General cargo ships	17.0	15.6	12.7	8.5	4.0
Other	4.5	7.5	9.4	7.2	11.3

Source: (UNCTAD, 2017)

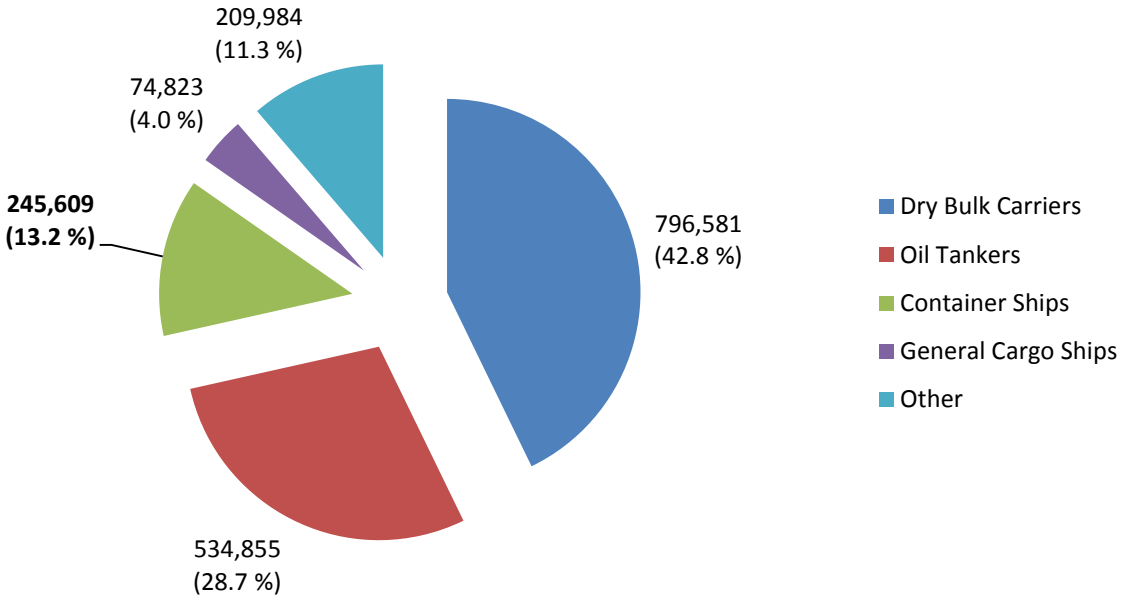


Figure 14: World fleet by principle vessel type in 2017
Thousands of dwt and percentage share. Source: (UNCTAD, 2017)

Regarding world fleet ownership and operation, Greece continued to be the largest shipowning country in terms of cargo-carrying capacity (309 million dwt in 2017), followed by Japan (224 million dwt), China (165 million dwt), Germany (112 million dwt) and Singapore (104 million dwt). In terms of vessel numbers, China is the leading ship owning country with 5,206 ships of 1,000 gross tons and above (UNCTAD, 2017). With respect to container ships, the drivers for global chain values and trade of manufactured goods, Germany continues to be the largest owner with a market share of 21.46%, followed by China and Greece. A completely different picture appears, when looking for the leading fleet countries. More than 76.2% of the world fleet tonnage is registered in developing countries like Panama, Liberia and the Marshall Islands.

Great changes during the last decades are related to the container ship sizes. The largest container ship in 1968 - a converted tanker - had a transport capacity of 700 TEU at a maximum speed of 20 knots. In 1981, the Hapag Lloyd AG's Frankfurt Express was the biggest container ship in the world with a storage capacity of 3430 TEU. Emma Mærsk, 398 m long and 56.4 m wide was the largest container ship by 2012 and had a storage capacity of more than 13,000 TEU. The biggest container ship ever in the history of the port of Hamburg is the 400 meters long and 59 meters wide "CMA CGM Antoine de Saint Exupéry" and the current-

ly greatest containership of the world with a 21,413 TEU-capacity reached Wilhelmshaven in 2017.

Container ships can generally be divided in two groups: (1) Neo-Panamax ships which are able to transit the expanded locks of the Panama Canal (with up to a maximum 49 m beam and 366 m in length overall) and (2) Panamax ships above 3,000 20-foot equivalent units with a beam below 33.2 m (the largest size of vessel able to transit the former locks of the Panama Canal). Meanwhile, there is the even larger Triple E-Class, which has a length of 400 m and a width of 59 m. This can transport up to 18,000 TEU. It is predicted that these ships will reach the Malaccamax size, which is limited only by the draft of the Strait of Malacca. These ships will reach a length of 470 m and a width of 60 m.

Since 1996, the number of container ships has doubled. The container market grew three times faster than the global economy. In 2005, around 20 million containers traveled around the world on 200 million journeys, of which around three quarters were on container ships.

Global ports handle over 80% of global merchandise trade in volume and more than two thirds of its value. Therefore, the importance of well-functioning seaports for merchandise trade, globalized production processes and economic growth cannot be overlooked. As key nodes in global transport chains, which are providing access to markets, support supply chains and link consumers and producers, ports are under constant pressure to adapt to changes in the economic, institutional, regulatory and operating landscape. The Altenwerder container terminal in the Port of Hamburg, for example, increased its turnover from 4.7 to 7.0 million TEU following the completion of the modernization between 2002 and 2004.

The world port container traffic is finally spread over six regions (see Fig. 15). In 2016, Asia accounted for 64% of world container port throughput followed by Europe and North America.

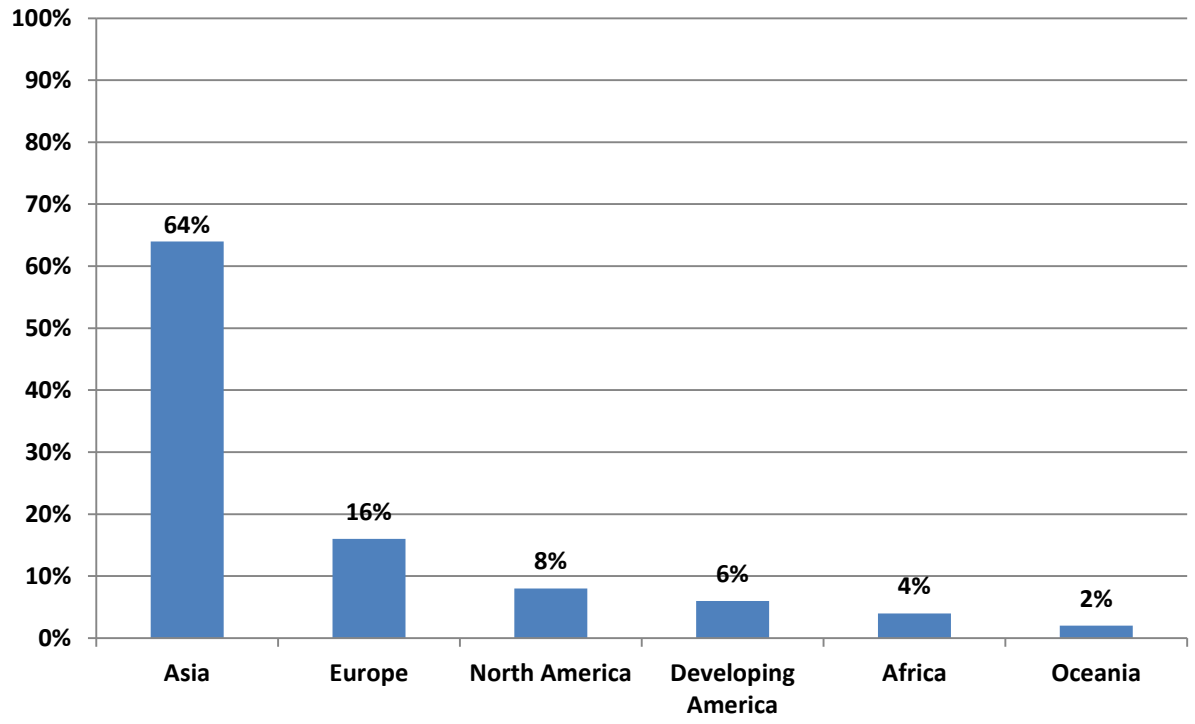


Figure 15: Container port volumes in 2016, percentage shares
Source: (UNCTAD, 2017)

In 2016, the top 40 container ports worldwide handled a total of 415.9 million TEU which equals 60% of the world total container throughput. With ports in Shanghai, Shenzhen, Ningbo, Hong Kong, Guangzhou, Qingdao and Tianjin, seven of the top ten container ports are located in China. German ports of Hamburg and Bremerhaven are top 17 and top 27, respectively, handling a total of about 14.4 million TEU (see Figure 16), representing 3.46% of the total container port volumes worldwide in 2016.

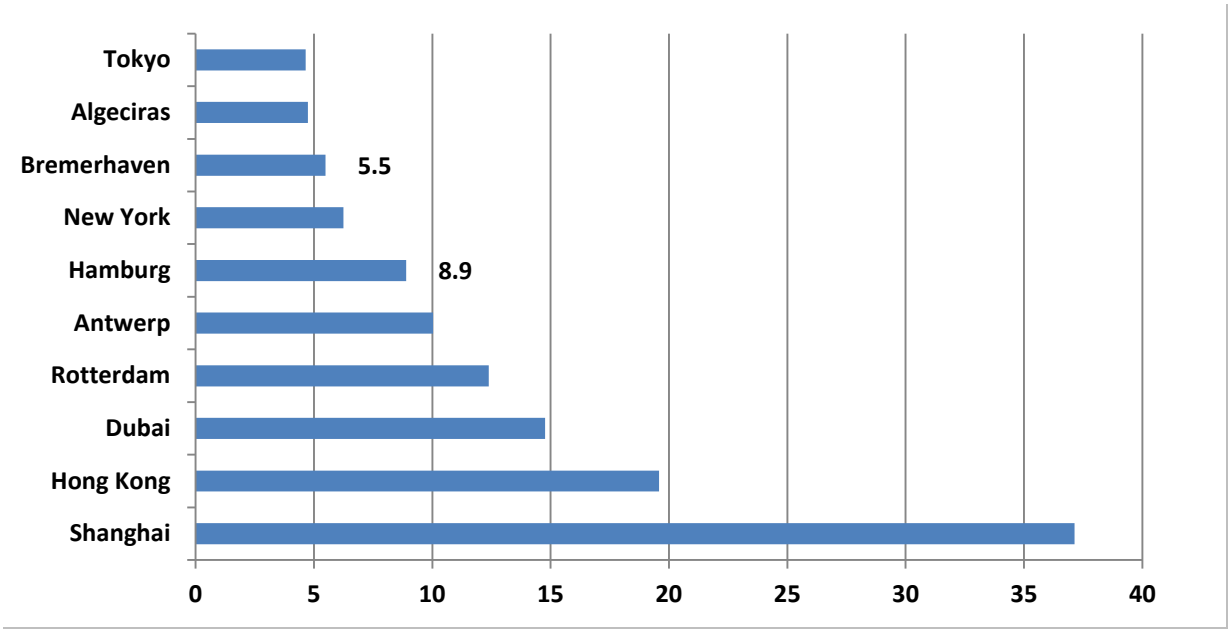


Figure 16: Container port volumes in million TEU at selected container terminals in 2016
Source: (UNCTAD, 2017)

Changes in container throughput in the port of Hamburg for selected years are shown in Table 15. Highest values are reported for 2007. During the financial crisis beginning in 2008 the port volumes decreased worldwide, but were declining over the last years.

Table 15: Container throughput in the port of Hamburg in 1,000 TEU

Year	1990	2000	2007	2010	2017
Import	1034	2192	5118	4075	4579
Export	935	2056	4772	3821	4237
Total	1969	4248	9890	7896	8816

Source: (Port of Hamburg, 2018)

Three major east-west container trade routes determine container traffic and have a significant influence on the supply and demand situation in maritime shipping. These are Trans-Pacific Route (Far East - North America; 26.1 million tons in 2017), the Asia - Europe Route (23.1 million tons in 2017) and the Transatlantic - Route (Europe - North America; 7.4 million tons in 2017) (UNCTAD, 2017).

In the meantime, there are hardly any restrictions on transport in the container, unless the goods are so big, heavy or long that they do not fit in the metal box, such as steel pipes or complete systems. Coffee sacks, bananas and cotton & Co have long been shipped in containers worldwide. Just 20 years after the first container handling, around 50% of the total landing volume was already containerized. By transporting goods in such huge quantities, costs are minimized. The costs of transporting a bottle of wine from Australia to Europe today

is 12 cents, a pound of coffee from Central America 3 cents (Wikipedia, 2018). For electronic devices transport costs are usually less than 1% of the market price (World Ocean Review, 2010).

Container transport reduced and accelerated international transport costs, especially for consumer goods and bulk cargoes. The world shipping fleet provides not only transport connectivity to global trade but also livelihoods to the people working in maritime business in developed and developing countries. Before highly automated container handling was invented, crews of 20 to 22 workers were needed for loading and unloading the ship. After the introduction of container traffic, they were no longer needed and the port facilities as well as job descriptions were completely transformed. Cut in wages for loaders and unloaders in the ports is still ongoing.

Containerization also dramatically changed the character of port cities worldwide. In the harbours long piers for loading and unloading were no longer needed, but space for the containers became necessary. In 2010 the container terminal Bremerhaven was registered as the world's largest contiguous container terminal in the Guinness Book of Records.

Seagoing vessels are comparatively energy-efficient means of transport. Nevertheless, the marine environment in general is considerably burdened by the growing maritime shipping. Dangerous chemicals in the ship's paint, the introduction of alien species with the ballast water, the introduction of wastewater and waste into the sea and the pollutants from exhaust gases or oil contaminants affect the marine environment. Ship traffic on the world's oceans is reported to be responsible for over 2% of global CO_2 emissions (Umweltbundesamt, 2016).

Transport safety and tasks of BfR

The transport of dangerous goods is part of global trade and requires specific safety measures for humans and for the environment. For smooth transitions between different countries and modes of transport, common standards are an indispensable prerequisite. The development of worldwide standards for the transport of dangerous goods occurs under the umbrella of the United Nations Economic and Social Council where an expert committee is establishing and updating the recommendations for the transport of dangerous goods, the so-called model regulations, which were published in 2017 in the 20th revision. The model regulations contain the physico-chemical and toxicological criteria and classification rules for the nine dangerous goods classes, the dangerous goods list currently with more than 3,500 entries (UN-numbers), as well as requirements for packaging and shipping, including loading, unloading and handling of dangerous goods. The model regulations aim at presenting a basic scheme of provisions that will allow uniform national and international regulations governing the various modes of transport.

For transport by road, rail and inland waterways European agreements were established, i.e. the European Agreement concerning the International Carriage of Dangerous Goods by Road (ADR), the European Agreement concerning the International Carriage of Dangerous Goods by Inland Waterways (ADN) and the Règlement concernant le transport international ferroviaire de marchandises Dangereuses (RID).

In Germany, the transport of dangerous goods is regulated by the law on the transport of dangerous goods (Règlement concernant le transport international ferroviaire de marchandises Dangereuses - GGBefG) which is the basis for specific regulations on the transport of dangerous goods like the Regulation on the National and International Carriage of Dangerous Goods by Road, Railways and Inland Waterways (Gefahrgutverordnung Straße, Eisenbahn und Binnenschifffahrt - GGVSEB) and the Ordinance on the Transport of Dangerous Goods by Seagoing Ships (Gefahrgutverordnung See – GGVSee).

For transport by see specific codes have been developed under the umbrella of the International Maritime Organization (IMO), a specialized agency of the United Nations. Based on the model regulations the International Maritime Dangerous Goods Code (IMDG Code) regulates the transport of packaged dangerous goods by see, also covering the transport of cargoes in containers. The transport of solid bulk cargoes, however, is regulated by the International Maritime Solid Bulk Cargoes Code (IMSBC Code), which in turn falls within the area of the IMDG Code while the transport of hazardous chemicals and liquid bulk cargoes is regulated in the International Code for the Construction and Equipment of Ships carrying Dangerous Chemicals in Bulk (IBC Code).

The BfR is involved in the development of criteria and classification rules for health hazards of dangerous goods. Experts are members in national and international bodies dealing with classification, labelling and packaging of dangerous goods. Furthermore, the institute is involved in the provisions for medical first aid after accidents with dangerous goods on board of ships. The BfR also gives advice with regard to precautions, stowage and segregation of dangerous goods during sea transport as well as to the protection of food and feed from dangerous goods on board or in containers. One topic of high concern is the risk associated with the fumigation of containers.

Fumigation of transport containers

To ensure the preservation and quality of goods and to prevent unwanted harmful organisms from spreading worldwide, containers are treated with toxic gases before being shipped. To effectively fulfill their purpose of combating undesirable organisms, fumigants per se must have high biocidal efficacy and therefore may provide toxic properties which may also harm humans. The special risks of fumigated sea containers became obvious from poisoning reports to the BfR in 2007 (BfR, 2007). Cargoes that have to be fumigated may include foodstuffs, leather goods, handicrafts, textiles, timber or cane furniture, luxury vehicles and cargo in timber cases or on timber pallets. When opening fumigated containers for unloading or for inspections port and warehouse workers, employees of control authorities or bystanders may come into contact with fumigant residues and suffer health damage.

An overview of relevant legislation regarding fumigation in different countries is provided in the report of the European Agency for Safety and Health at Work (EU-OSHA). This report also provides preventive actions and strategies and identifies health and safety risks from fumigation (EU-OSHA, 2018).

Chemicals used for fumigation are methyl bromide, sulfuryl fluoride, phosphine and 1,2-dichloroethane and less frequently also formaldehyde or hydrogen cyanide (hydrocyanic acid). In Germany, occupational exposure limits for methyl bromide and phosphine are 3.9 and 0.14 mg/m³, respectively (AGS, 2018). For 1,2-dichloroethane values for occupational exposure limits have been withdrawn due to the carcinogenic properties of the chemical.

EU legislation does not allow treatment with methyl bromide, to be carried out within the EU, as from 18. March 2010. In Germany, the fumigation with methyl bromide is already prohibited since 2006.

In Germany, the Technical Rules for Hazardous Substances on Fumigation (TRGS 512) specify the fumigation regulations in Annex I No. 4 of the Hazardous Substances Ordinance (GefStoffV). These regulations address both, the freight containers fumigated for export in Germany and containers which are fumigated in the exporting country for import via German ports.

TRGS 512 contains among others also information on the determination of the hazard potential of freight containers that are fumigated with high probability and those in which fumigation cannot be ruled out. Measures to be taken into account to protect the health and safety of workers and other persons are described for both situations and include:

- Examination of the interior of the closed transport unit by a qualified person by means of a sufficiently selective external measuring system, e.g. by introducing a measuring probe at a suitable location.
- Definition of a safety area of at least 10 m around the cargo door to be opened.
- Determination of the ventilation time by a specialist.
- Opening and ventilation of the unit under suitable respiratory protection.
- Determination of fumigant residual concentration after the ventilation phase and release of the cargo unit.

The prerequisite for the release of fumigated transport containers and transport goods is that the desorption of the fumigant used has advanced so far that in the ambient air the concentration has been reduced to a safe level which is 0.14 mg/m^3 (0.1 ppm) for hydrogen phosphide, 3.9 mg/m^3 (1 ppm) for bromomethane, 2.1 mg/m^3 (1.9 ppm) for hydrogen cyanide and 10 mg/m^3 for sulfuryl difluoride.

For the international transport, the UN Model Regulations list with UN number 3359 a specific entry for fumigated cargo transport units. The associated special provision 302 defines the term "fumigated cargo transport unit" and Section 5.5.2 contains the special provisions for their transport. Most important requirements are:

- Persons engaged in the handling of fumigated cargo transport units shall be trained commensurate with their responsibilities.
- A fumigated cargo transport unit shall be marked with a specific mark, affixed at each access point in a location where it will be easily seen by persons opening or entering the cargo transport unit. This mark shall remain on the cargo transport unit until the fumigated cargo transport unit has been ventilated **and** fumigated goods or materials have been unloaded.
- Ventilated cargo units shall be marked with the date of ventilation on the fumigation warning mark.
- When the fumigated goods or materials have been unloaded, the fumigation warning mark shall be removed.
- Date and time of fumigation, the type and amount of fumigant used and instructions for disposal of any residue fumigant must be documented for fumigated cargo transport units that have not been completely ventilated before transport.

Special provisions for maritime transport are laid down in the IMDG Code and comprise in addition to the general aspects of the TDG the following:

- Cargo transport units shall be fumigated and handled according to the IMO Revised Recommendations on the safe use of pesticides in ships.
- When fumigated cargo transport units are stowed under deck, equipment for the detection of fumigant gases shall be carried on the ship.
- Fumigants shall not be applied to the contents of a cargo transport unit once it has been loaded aboard the ship.
- A fumigated cargo transport unit shall not be allowed on board until a sufficient period has elapsed to attain a reasonable uniform gas concentration throughout the cargo in it. Twenty-four hours is normally sufficient for this purpose.
- Stowage clear of living quarters.

- No stowage under deck on ships with more than 25 passengers (or more than 1 passenger per 3 m of overall length).

Despite the introduction of a requirement to label containers fumigated in international dangerous goods law, this risk remains unpredictable, as experience has shown that the provisions are often not being complied with. Unfortunately, in practice the marking is often illegible, damaged or completely missing. Even if a container has already been ventilated, outgassings, for example from goods or packaging materials, can again accumulate within the container after several hours. It is estimated that between 10% and 20% of all containers arriving European harbours contain volatile toxic substances above the exposure limit values (Baur et al., 2015). In 2011, measurements of 123,349 import containers in different European countries resulted in 13% of containers with hazardous substances over the respective occupational exposure limits (OEL). Most frequently detected were Carbon Monoxide (4.2%), 1,2-Dichloroethane (3.8%) and Formaldehyde (3.7%). Phosphine and methyl bromide were measured in concentrations over the OEL in 1,5% with a maximum of 329 ppm and 0,4% with a maximum of 82 ppm, respectively (Otto Mück, 2012).

It is obvious that this hazardous situation in freight transport requires preventive steps. In order to improve awareness and relevant knowledge there is a need for more comprehensive information on chemical hazards and a broader implementation of the already existing regulations and guidelines, such as those from ILO, IMO, and national authorities. It is also necessary to have regular controls by the authorities on a worldwide scale, which should be followed by sanctions in case of disregarding regulations.

BfR is involved in the further development of international requirements regarding fumigation of transport cargo units. The institute for example initiated changes in the IMDG Code to ensure that the fumigation warning mark is waterproof in the future. In the latest version of the Code, mandatory since 2018, the following amendment was internationally agreed:

“The method of marking shall be such that this information will still be identifiable on cargo transport units surviving at least three months’ immersion in the sea. In considering suitable marking methods, account shall be taken of the ease with which the surface of the cargo transport unit can be marked.”

Conclusions

Containerization has changed the global transport considerably over the last decades and led to a replacement of other types of transport. It also contributed to the growth of the total transport volume as well as to the structural change in trade and the production of goods through considerable cost savings. According to widespread opinion, this development has not yet reached its end point. But the weak trade economy since the 2008 recession and the overcapacity of the shipping industry has continued to limit growth in shipping. The throughput within EU (28), for example, decreased from 3,966 Mio tons in 2007 to 3,790 Mio tons in 2014 (Eurostat, 2017). Nevertheless, the supply of ship-carrying capacity increased faster than demand, leading to a continued situation of global overcapacity and downward pressure on freight rates and earnings. The current low demand–high overcapacity environment has constrained freight rates and dampened profitability in most shipping market segments. The collective operating loss reported by the container-shipping market in 2016 amounted to $3.5 billion (UNCTAD, 2017).

Despite some encouraging signs in early 2017 for most segments, the market situation is still challenging. Rates and demand levels remain low. In the container ship segment, new mergers and acquisitions and mega alliances established in 2016 and 2017 may lead to better

handling of supply and fleet utilization, which in turn could lead to improved markets and profitability for the container shipping sector and services for shippers (UNCTAD, 2017).

UNCTAD forecasts an estimated annual growth rate for maritime transport of 3.2% between 2017 and 2022. Cargo flows are set to expand across all segments, with containerized and major dry bulk commodities trades recording the fastest growth (UNCTAD, 2017). The average annual growth rate between 2017 and 2022 of the worldwide container traffic is estimated depending on the reference area between 4 and 5 percent. The strongest annual growth rate of 8% is predicted for the route between Europe and Asia (Statista, 2018).

It is worth to note that economic and political influences can also affect trade. Trade restrictions, including import or export bans on selected goods by individual countries, can affect maritime transport and thus global food and feed flows. Important goods like food or animal feed and living animals were transported via rail, air, road or ship but other transport forms than container are usually used. Therefore, this aspect of transport is not further addressed in this chapter.

Within the framework of the Maritime Traffic Forecast 2030, initiated by the German Federal Ministry of Transport and Digital Infrastructure, the container throughput of the German seaports is supposed to increase from 13.0 million TEU in 2010 to 30.1 million TEU in 2030, representing an annual growth of 4.3% (BMEL, 2014). However, the trend towards globalization of value chains seems to play a minor role in the future. This is depending on changes in growth of the major emerging economies, demographic changes in industrial nations, and the development towards domestic products in emerging markets (Quitzau, 2018). It is discussed that the age of the container may be replaced by the age of new technologies (Quitzau, 2018; UNCTAD, 2017). The 3D printing technology is likely to shift the importance between container, bulker and tanker transport capacities. On top of that, digital goods are expected to play a bigger role in the future. Unlike physical goods, they do not have to be shipped. For these reasons, projected growth in world seaborne trade remains subject to uncertainty and several downside risks. Nevertheless, seaborne trade is of strategic economic importance and will play an important role in world economy also in the future.

Abbreviations

3D	Three-dimensional space
ADN	European Agreement concerning the International Carriage of Dangerous Goods by Inland Waterways
ADR	European Agreement concerning the International Carriage of Dangerous Goods by Road
BfR	The German Federal Institute for Risk Assessment (Bundesinstitut für Risikobewertung)
BMVI	Federal Ministry of Transport and Digital Infrastructure (Bundesministerium für Verkehr und digitale Infrastruktur)
dwt	dead-weight ton
EU	European Union
EU-OSHA	European Agency for Safety and Health at Work
GefStoffV	Hazardous Substances Ordinance
GGBefG	Règlement concernant le transport international ferroviaire de marchandises Dangereuses
GGVSEB	Regulation on the National and International Carriage of Dangerous Goods by Road, Railways and Inland Waterways
GGVSee	Ordinance on the Transport of Dangerous Goods by Seagoing Ships
IBC Code	International Code for the Construction and Equipment of Ships carrying Dangerous Chemicals in Bulk

ILO	International Labour Organization
IMDG Code	International Maritime Dangerous Goods Code
IMO	International Maritime Organization
IMSBC Code	International Maritime Solid Bulk Cargoes Code
m	meter
m^3	cubic metre
mg	milligram
OEL	Occupational Exposure Limit
ppm	parts per million
RID	Règlement concernant le transport international ferroviaire de marchandises Dangereuses
TDG	Transport of Dangerous Goods
TEU	Twenty-foot Equivalent Unit
TRGS	Technical Rules for Hazardous Substances
UBA	German Federal Environment Agency (Umweltbundesamt)
UN	United Nations
UNCTAD	United Nations Conference on Trade and Development
UN number	United Nations number

References

AGS, Ausschuss für Gefahrstoffe,. (2018). Technische Regeln für Gefahrstoffe (TRGS) 900. *Gemeinsames Ministerialblatt (GMBl)*.

Baur, X., Budnik, L. T., Zhao, Z., Bratveit, M., Djurhuus, R., Verschoor, L., . . .and Jepsen, J. R. (2015). Health risks in international container and bulk cargo transport due to volatile toxic compounds. *Journal of Occupational Medicine and Toxicology (London, England), 10*. doi:10.1186/s12995-015-0059-4

BfR, Bundesinstitut für Risikobewertung. (2007). Ärztliche Mitteilungen bei Vergiftungen 2007.

BMEL, Bundesministerium für Ernährung und Landwirtschaft. (2014). Seeverkehrsprognose 2030.

EU-OSHA, European Agency for Safety and Health at Work. (2018). Health risks and prevention practices during handling of fumigated containers in ports. doi:10.2802/16959

Eurostat. (2017). Gross weight of goods handled in all ports by direction - annual data.

Otto Mück, A. S. (2012). Occupational safety in import containers containing fumigants, other gases and volatile substances: practical experiences. *Navarro S, Banks HJ, Jayas DS, Bell CH, Noyes RT, Ferizli AG, Emekci M, Isikber AA, Alagusundaram K, [Eds.] Proc 9th. Int. Conf. on Controlled Atmosphere and Fumigation in Stored Products, Antalya, Turkey. 15 – 19 October 2012, ARBER Professional Congress Services*.

planet wissen. (2017). Containerschifffahrt.

Port of Hamburg. (2018). Container handling 1990 to 2017.

Quitzau, J. T., Niklas; Vöpel, Henning; Jahn, Malte; Otto, Alkis Henri; Wolf, André. (2018). Schifffahrt in Zeiten des digitalen Wandels. *Strategie 2030 - Vermögen und Leben in der nächsten Generation, No. 25*.

Statista. (2018). Prognose zum durchschnittlichen jährlichen Transportvolumen der weltweiten Containerschifffahrt im Zeitraum der Jahre 2017 bis 2022 nach Routen.

Umweltbundesamt. (2016). Seeschifffahrt.

UNCTAD, United Nations Conference on Trade and Development,. (2017). Review of Maritime Transport.

Wikipedia. (2018). Containerization. https://en.wikipedia.org/wiki/Containerization

WirtschaftsWoche. (2016). Vor 50 Jahren begann das Containerzeitalter. https://www.wiwo.de/schifffahrt-vor-50-jahren-begann-das-containerzeitalter/13563904.html

World Ocean Review. (2010). Was Schiffe laden – Öl, Container und Trockenfracht.

2.4 Bioterrorism and the Food

Buschulte, Anja; Mader, Anneluise; Appel, Bernd

Abstract

Since the September 11 attacks, people all over the world became aware of the terrorist threat. The food chain has been recognized as a possible target for bioterrorism (WHO, 2008). The malicious contamination of the food chain with biological agents is an unlikely but nonetheless, a possible event. In case of occurrence it could have a tremendous impact on public health, the international food trade and the consumers trust in food. Negative political and economic implications could follow. Due to this, it is in the public interest to use every available option to further secure the food chain and to develop appropriate measures and tools. Against this background, the Federal Institute for Risk Assessment (Bundesinstitut für Risikobewertung, BfR) has been dealing with bioterrorism and food for many years. One focus of the work is the development of suitable measures for the early detection and prevention of possible bioterrorist attacks on the food chain. In this context, the existing food safety systems were evaluated with regard to their suitability for the prevention of bioterrorist attacks. Furthermore suitable measures for the prevention of such attacks were developed and various national and international research projects were initiated or carried out.

Introduction

In recent years, international organisations such as the World Health Organisation (WHO), the Food and Agriculture Organisation (FAO) and the World Organisation for Animal Health (OIE) have raised repeated concerns that deliberate contamination of the food chain could harm the civilian population and cause enormous economic losses. They have therefore worked with various international and national institutions and organisations to assess the risks associated and to develop strategies to combat the risks of deliberate food contamination. Awareness that the food chain could be the target of bioterrorist activities and is vulnerable to deliberate contamination has led to several guidelines on how to respond to these threats. These include the “Report of the CBRN (Chemical, Biological, Radiological and Nuclear) Task Force” (EC, 2009b), “Terrorist Threat to Food” (WHO, 2008), “Green Paper on Bio-preparedness” (EC, 2007), “Communication on Strengthening Coordination on Generic Preparedness Planning for Public Health Emergencies at EU Level (EC, 2005b), “Communication on Cooperation in the European Union on Preparedness and Response to Biological and Chemical Agent Attacks” (EC, 2003) and the “White Paper on Food Safety” (EC, 2000). The research needs addressed therein are related to prevention, monitoring and surveillance, detection, biological traceability and clinical treatment. The four main challenges with respect to biopreparedness are: threats to humans; threats to animals, and food and feed for the animals; threats to crops, food and feed; as well as biological detection (EC, 2009b). Nevertheless the European Community concluded: “There is no need to establish new systems, but rather to adjust the current mechanisms in order to improve their functioning, taking into account the threat of bioterrorism” (EC, 2003). Securing food chains from primary production to ready-to-eat food against deliberate contamination is directly linked to food safety, especially as the complexity of food supply chains is constantly increasing due to the globalisation of the food trade. The available (legal) requirements, measures and resources to ensure food safety must therefore be assessed, optimised and, if necessary, redeveloped in relation to the particular risks posed by bioterrorism.

Legal Requirements

According to European food law, food companies that produce, process or market food are responsible for the safety of their products. They are responsible for ensuring food safety at all stages of production against biological, chemical or physical hazards. In order to identify and control these hazards, procedures are set up within the framework of self-monitoring based on the principles of the Hazard Analysis Critical Control Point (HACCP) system (see "Basic Regulation" (EC) No. 178/2002)(Eur. Parliament and The Council, 2002d). In addition, food business operators are obliged to comply with certain microbiological food safety and hygiene criteria, which are specified in more detail in Regulation (EC) No 2073/2005 (EC, 2005a). In Germany, the Food and Feed Code (BMJV, 2013) contains corresponding legal provisions at national level. Accordingly, it is prohibited to produce or treat food for others in such a way that its consumption is harmful to health (§ 5 LFGB). In addition, in-house controls are required, including the performance of microbiological tests (§ 36 LFGB). However, these tests are not suitable for detecting every microbial contamination, in particular with pathogens that do not occur naturally in foodstuffs. The deliberate contamination of foodstuffs with highly pathogenic agents would therefore hardly be detected within the framework of in-house controls or official monitoring. The strategy of self-monitoring, which is designed to minimise risk, cannot rule out a residual risk for this type of contamination. The term "deliberate contamination" is not explicitly mentioned in the aforementioned legal provisions and has not yet been queried in the context of official controls in Germany.

Requirements of international food standards

The international food standards refer to the prevention of deliberate contamination of food with the term "food defence". The Global Food Safety Initiative (GFSI) is an initiative for the continuous improvement of food safety management systems. In the area of private sector standards, GFSI stipulates in its Guidance Document (GFSI Guidance Document, Version 6.41, Chapter FSM 21 Food Defense), valid since 2011 and replaced in February 2018 by the GFSI Benchmarking Requirements Guidance Document Version 7.1, that food defence should be part of the food standards. The Guidance Document defines food defence as “the process to ensure the security of food and drink and their supply chains from all forms of intentional malicious attack including ideologically motivated attack leading to contamination or supply failure”. The relevant Clause Number constitutes that “the standard shall require that the organisation has a documented risk assessment procedure in place to address food defence risks and establish, implement and maintain a system to reduce or eliminate the identified risks.” GFSI is an international initiative of the trade which aims to achieve comparability and mutual recognition of food safety standards. It is thus a kind of superordinate body for the individual standards of trade. One of the GFSI objectives is delivering equivalence and convergence between effective food safety management systems by benchmarking them. During the benchmarking process related schemes are compared to the GFSI Guidance Document to determine equivalence. Therefore the International Food Standards recognised by GFSI like the British Retail Consortium’s (BRC) Technical Standards, Food Safety System Certification 22000 (FSSC) and the International Food Safety Standard (IFS) included food defence requirements in their audits. The requirements relating to food defence shall include, amongst others:

- an evaluation of the security of companies,
- adequate protection of defined safety-critical areas,
- the establishment of procedures for the protection against falsification and sabotage or for the identification thereof,
- the establishment of procedures for staff and visitor security,
- the creation of a food defence plan,

- the establishment of procedures for dealing with external controls and
- conducting a documented hazard or vulnerability analysis.

On the basis of the latter analysis, security-critical areas for deliberate contamination are to be identified, evaluated and measures to control, reduce or eliminate the identified hazards should be implemented. How the actual analysis is carried out, which contents are queried in detail and, above all, which system or procedure is used for this, remains the decision of the company. Although the available guidelines and questionnaires of the standards provide information on implementation, they do not contain comprehensive instructions, recommendations for action or procedures.

Vulnerability assessment in relation to food defence

There are several methods available to conduct a vulnerability assessment for example the Vulnerability Analysis Critical Control Point (VACCP) – System, the Threat Assessment Critical Control Point (TACCP) – System (BSI, 2017), the Failure Mode and Effect Analysis (FMEA, w.d.) or the Vulnerability Assessment Software tool of the Food and Drug Administration (FDA, 2014). Each of them has advantages and disadvantages but all of them are more or less complex and difficult to implement especially for small and medium sized enterprises (SME). Our own experiences have shown that the software tool of FDA enables on one hand the food industry companies to identify their most susceptible points for bioterrorist attacks. But on the other hand it needs substantial adaptions to be useful for German requirements and it has a low usability especially for small companies (Buschulte et al., 2012). An easy to use approach hasn`t been available in Germany and so implementing the necessary measures in order to fulfil the standard commitments regarding food defence (German: Produktschutz) presents a major challenge especially for small sized companies with limited financial and personnel resources.

Based on practical experience gained by the BfR in carrying out vulnerability analyses in various types of food companies, BfR developed an easy-to-use method for the identification of vulnerabilities in SME. In addition, the requirements of the international food standards and related standards should be covered. Overall, the method should meet the following requirements:

- enable food companies to perform a self-check regarding food defence,
- the identification of measures for risk mitigation,
- easy to use in terms of comprehensibility,
- user friendly user interface,
- easy technical installation on almost all types of computer,
- no need of additional software and
- no need of user experience.

Initially available systems, methodologies and procedures which are dealing with the topic of deliberate contamination or vulnerability assessment in the food chain have been analysed by BfR with regard to content, structure and work flow. In addition a review of the literature relevant to the topic food defence and relevant criteria and questions for a malicious contamination was made. Based on the literature survey the criteria most important for the realization of a deliberate contamination of the food chain have been identified. The analysis showed that some criteria could be attached to higher-ranked categories. The identified categories and their criteria built the basis for a questionnaire. Expert rounds and workshops with experts in the fields of quality management, food hygiene, food technology and operational management were used to discuss these categories and criteria and also to include

their respective points of view, experiences and requirements. The structure of the questionnaire was developed according to Multiple-criteria decision-making (MCDM). The questionnaire and all other information were then compared with other publically available tools such as FDAs Vulnerability Assessment Software tool, the Food Defense Plan Builder of FDA (FDA, 2014) and the requirements of related international food standards to include additional topics and identify further needs. Measures, criteria, categories, and further content assessed as relevant were then implemented in a Microsoft Office Excel file. After evaluating all existing methods dealing with identifying vulnerable points in the food chain it was concluded that a checklist is the most practical tool to perform a vulnerability analysis within a food business establishment. The food companies are used to work with checklists and use them mainly in the field of quality management. Due to the limited resources of SME as well as competent authorities which will be the end user, the Excel program was used for the implementation of the checklist.

The developed checklist contains a questionnaire of sixty-four questions which are attached to nine criteria (1. Management; 2. Staff; 3. Public e.g. supplier, customers, visitors; 4. Location, building construction; 5. Production; 6. Incoming goods and vendors; 7. Storage; 8. Finished goods, goods outward and 9. Access to computer systems) and three categories (Management; People on site; Factory and operation). Based on the answers to the questionnaire, the results for each criterion are displayed in a coloured bar chart so that the user can easily identify the areas where action is needed. After downloading the checklist from the BfR website and saving it on the user`s computer's local hard drive or other location as selected by the user it resides only on these storage media. Therefore it is the user's responsibility to ensure that the completed questionnaire with probably sensitive information of the company is saved in a secure location. The BfR does not track or monitor the use of the checklist and does not have access to any content developed by using it. The developed checklist enables especially SME with limited budget to perform an assessment concerning the threat of deliberate contamination. Answering the questionnaire of the checklist gives an overview about points or areas in which appropriate measures are already implemented or on the other hand should be implemented. The checklist furthermore offers the opportunity to identify and specify needed actions, to assign responsibilities and to determine deadlines for implementation. It therefore can be used to perform a documented vulnerability assessment which is a requirement of the international food standards. The availability of explanations for each question which clarify the background and the intention of the specific topic support the user. Finally the graphical illustration of the questionnaire and the status of implementation enhance the usability. Areas for which further information/clarification or action is needed are easily identified. The German version of the checklist is available free of charge from the website of the BfR. An English version is under development and will be released soon.

BfR-projects dealing with food defence and related issues

Several national and international research projects, dealing with different aspects of food safety and defence, have been coordinated by the BfR. Some of these projects are shortly described in the following. In addition, BfR has been involved as a partner in several other national and international projects dealing with the topic as some examples in the timeline below show (Figure 17).

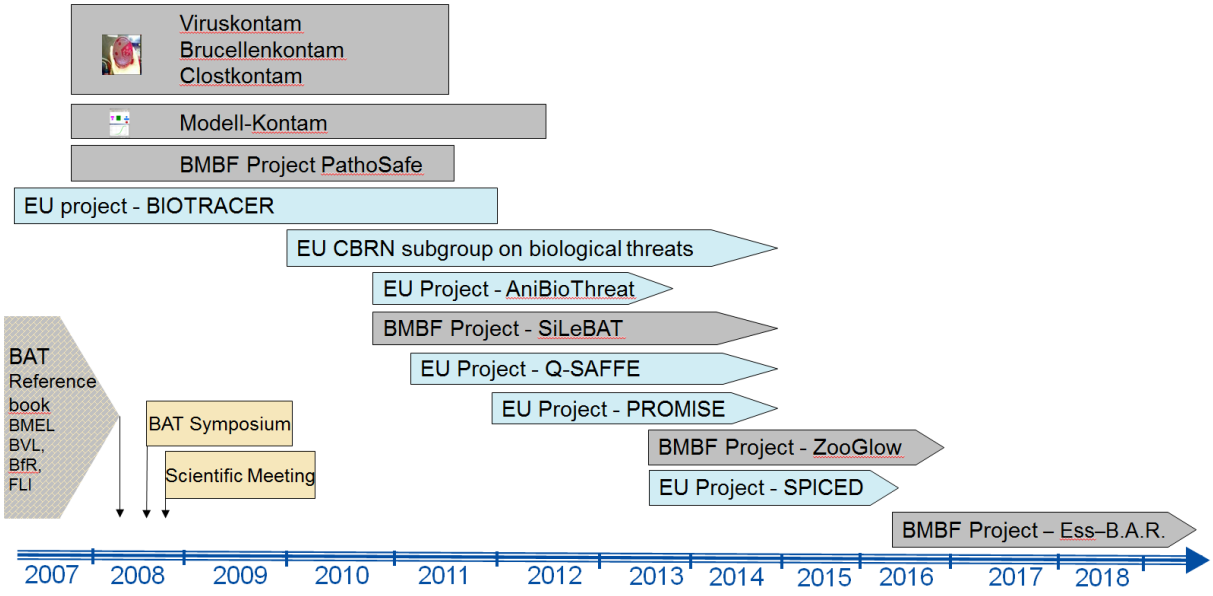


Figure 17: Bio- and agroterroristic research initiatives coordinated or conducted by BfR since 2007

SiLeBAT – Ensuring the safety of the food and feed supply chain in case of damage resulting from bio or agro terrorism attacks

Commonly, bioterrorism is a form of terrorism, in which microorganisms or their toxins are used for attacks against people or for deliberate contamination. Agroterrorism is defined as the deliberate spread of disease in plants and animals. Feed and its supply facilities can therefore be a target of deliberate contamination through agroterrorism whereas food and supply facilities might be the target of deliberate contamination through bioterroristic agents.

The SiLeBAT project developed concrete solution approaches to ensure a continued food supply to the population in case of bio- or agroterroristic (BAT) attacks. All partner and associated partners involved in the project agreed that in case of damage through bio- or agroterrorism, effective and coordinated action is only possible if comprehensive and valid expert information is available quickly. For this reason, one key area of activities was the systematic collection and development of relevant expert information in the areas of epidemiology, the ability to survive of harmful microorganisms in the food chain and the durability of biotoxins in food matrices as well as information on the availability and effectiveness of detection methods, sample preparation procedures and decontamination processes. The idea – which could be realised - was that this expert information should then be used to make suitable recommendations for countermeasures and to make these recommendations available online. In addition, computer-based processes for assessing the risks and available response options were developed. In areas where the availability of expert information was patchy or non-existent, experimental work was conducted to supplement the missing information. A further goal was to develop an IT solution, which - in case of need – would enable the participating project partners to access all resources developed and released as part of the project via a specially designed, secure and extendable information platform. For the purpose of practical application, an example of a practice and training programme was devised and implemented. Additional information on the IT solutions for improving feed and food safety whose development began as part of the SiLeBAT project, can be found in chapters 7.2, 7.3, 7.5 and at https://foodrisklabs.bfr.bund.de/frl/.

Commonly, the preventive measures investigated within SiLeBAT are based on scenarios involving on the one hand direct contamination of food and on the other hand indirect contamination of food via domestic cattle. Besides that, a focus was set on feed by investigating the possibilities to use feed for agroterroristic actions. The main question regarding this complex farm to fork chain was, if it is possible to contaminate feed with a direct impact on the health of consumers.

In general, the feed chain has basic similarities with the food chain, but it also differs in essential points, which should be taken into account in the risk assessment against bioterroristic agents. The most important difference is that the agent spread deliberately has to contaminate animal derived products without having an impact on the animal before slaughter as well as on the meat quality thereafter. Further common challenges are the strong globalisation of trade of feed and feed materials, the consequent use of a high proportion of imported components and the use of bulk commodities in addition to extremely heterogeneous minor components, each of which requires a specific characterization of inherent risks. From the scientific point of view, fundamental knowledge on various aspects of feed and the related production, processing and supply chains is available. However the knowledge on infection doses, tenacity and behaviour of agents in specific matrices as well as during the feed production and processing is still limited. Sampling procedures could be improved and last but not least there is a lack of detection methods for specific agent-matrix-combinations and of useful decontamination methods. In addition, the feed industry could improve its biosafety by improving prevention measures, crises management, emergency plans as well as measures for sampling and supporting forward and backward tracing within their production facilities and between various actors of the supply chain. Further, knowledge on batch size and unit size can be of specific importance, as discussed in chapter 2.1, especially Table 4.

The consortium of the four years Federal Ministry of Education and Research (BMBF)-project SiLeBAT was coordinated by the BfR and included partners from industry, academia and food authorities. The nine project partners were supported by associated partners with significant knowledge on production, processing and supply chains of feed and food.

SPICED – Securing the spices and herbs commodity chains in Europe against deliberate, accidental or natural biological and chemical contamination

At the beginning of food market globalisation, spices and dried culinary herbs might have played one of the most important roles. Therefore they are an interesting example, when dealing with global food supply chains.

Due to their low water activity, which inhibits biological growth, spices and herbs are natural products that can be contaminated with several microorganisms, among them pathogenic species (see also chapter 5.3). Further, also chemical contaminations may occur, mainly due to natural or unintentional inclusion, but also because of economic benefits. Besides various common hazards also the highly toxic naturally occurring lectin ricin has to be taken into account as a possible food contaminant. Because of the close resemblance between the castor beans of *Ricinus communis* and nutmeg seeds, a spice commonly used in Europe, it might occur as natural spice contaminant. Ricin is listed as a schedule 1 controlled substance in the Biological and Toxin Weapons Convention (BTWC, 1972) and Chemical Weapons Convention (CWC, 1997). According to the European Food Safety Authority the fatal oral dose for humans is about 1 mg ricin/kg body weight (5-10 castor beans) (EFSA, 2008a).

In general, spices and dried culinary herbs contaminations can take place at numerous vulnerable points within the production, processing and/or supply chain and can pose a tremendous risk for farmers, producers and consumers, leading to e.g. severe foodborne infections and intoxications. As they are contained in almost every processed food, including ready-to-

eat products, consumers can be directly exposed to contaminated spices and herbs. Especially, due to the fact that they can be added at the end of the production line and therefore no further decontamination step might reduce or eliminate a contamination.

However, the identification of condiments - as a cause of a natural, accidental or deliberate outbreak - is difficult, as consumers and experts that are investigating the outbreak often focus on major food ingredients instead of minor components, as seen during the enterohemorrhagic *Escherichia coli* crisis in Germany in 2011 (Weiser et al., 2013). By the way spices and herbs have been the source of various foodborne outbreaks (see chapter 5.3). Identifying - especially minor components - as a cause of a disease outbreak can be difficult, as the detection methods available for various pathogen/food matrix-combinations are limited, as for example the detection of ricin in the complex matrix of spices and herbs. Thus, qualitative and quantitative detection methods for various biological and chemical hazards, including ricin, were tested for herb/spice matrices including the development of sample preparation methods within the EU-project.

The consortium of the three years EU-project SPICED was coordinated by the BfR and included partners from industry, academia and food authorities from several European countries. The eleven project partners were supported by associated partners with significant knowledge of the world wide spices and herbs markets including all levels of production and sale. For further information please see http://spiced.linux17.webhome.at/, the BfR-Abstracts of the SPICED Symposium in Berlin 2016 and the Food Control Special Issue: Environmental and Food Safety of Spices and Herbs along Global Food Chains published 2018.

Conclusion

The deliberate contamination of food with biological agents to achieve policy objectives is an unlikely but possible scenario. Although the legal requirements and the associated procedures and measures in Germany and the EU guarantee a high level of food safety, they are only partially suitable for the threat of bioterrorism. The national, European and international activities to minimise the risk of bioterrorism via the food chain have raised awareness of this form of contamination. They can be used as a basis for the implementation of suitable protection strategies. The measures and tools developed by the BfR in various projects can additionally serve in particular to identify and prevent possible bioterrorist attacks on the food supply chain. The implementation of suitable measures to protect against deliberate contamination is becoming increasingly important, as the ever increasing trade in foodstuffs does not permit isolated solutions and requires international, coordinated strategies and solutions.

Acknowledgements

The SiLeBAT-project was sponsored by the Federal Ministry of Education and Research (BMBF) (No. 13N11202).

The SPICED-project has received funding from the European Union‘s 7th Framework Programme for research, technological development and demonstration under grant agreement No. 312631.

References

BMJV, Bundesministerium der Justiz und für Verbraucherschutz. (2013). Lebensmittel- und Futtermittelgesetzbuch in der Fassung der Bekanntmachung vom 3. Juni 2013 (BGBl. I S.

1426), das zuletzt durch Artikel 1 des Gesetzes vom 30. Juni 2017 (BGBl. I S. 2147) geändert worden ist.

BSI, British Standards Institution. (2017). PAS 96:2017 Guide to protecting and defending food and drink from deliberate attack. 58.

BTWC. (1972). Biological and Toxin Weapons Convention.

Buschulte, A., Muller-Wahl, B., Kasbohrer, A., and Appel, B. (2012). Vulnerability Assessment in the Food Chain - A Way to Do It. *Future Security, 318*, 448-453. doi:10.1007/978-3-642-33161-9_63

CWC, Chemical Weapons Convention,. (1997). 3. Convention on the Prohibition of the Development, Production, Stockpiling and Use of Chemical Weapons and on their Destruction. *Chapter XXVI*.

EC, European Commission. (2000). *White paper on food safety of 12 January 2000 [COM/99/0719 final - Not published in the Official Journal]*. European Commission. https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=LEGISSUM:l32041

EC, European Commission. (2003). *Communication from the Commission to the Council and the European Parliament on cooperation in the European Union on preparedness and response to Biological and Chemical agent attacks (Health security) /* COM/2003/0320 final*. EUR-Lex. https://eur-lex.europa.eu/legal-content/EN/TXT/?qid=1561549213019&uri=CELEX:52003DC0320

EC, European Commission. (2005a). Commission Regulation (EC) No 2073/2005 of 15 November 2005 on microbiological criteria for foodstuffs. *Official Journal of the European Communities, L 338*, 1-26.

EC, European Commission. (2005b). *Communication from the Commission to the Council, the European Parliament, the European Economic and Social Committee and the Committee of the Regions on strengthening coordination on generic preparedness planning for public health emergencies at EU level /* COM/2005/0605 final */*. EUR-Lex. https://eur-lex.europa.eu/legal-content/EN/TXT/?qid=1561549429565&uri=CELEX:52005DC0605

EC, European Commission. (2007). *Green Paper on Bio-Prepardness*. EUR-Lex.

EC, European Commission. (2009b). Report of the CBRN Task Force. 1-98.

EFSA, European Food Safety Authority. (2008a). Scientific Opinion of the Panel on Contaminants in the Food Chain on a request from the Europea Commission on ricin (from Ricinus communis) as undesirable substances in animal feed. *The EFSA Journal, 726*, 1-38.

Eur. Parliament, and The Council. (2002d). Regulation (EC) No 178/2002 of the European Parliament and the council of the European Union of 28 January 2002 laying down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety. *Official Journal of the European Communities, L 31*, 1-24.

FDA, U.S. Food and Drug Administration. (2014). Vulnerability Assessment Software. https://www.fda.gov/Food/FoodDefense/FoodDefensePrograms/ucm376791.htm

FMEA. (w.d.). Failure Mode and Effect Analysis (FMEA). https://en.wikipedia.org/wiki/Failure_mode_and_effects_analysis

Weiser, A. A., Gross, S., Schielke, A., Wigger, J.-F., Ernert, A., Adolphs, J., . . . and Greiner, M. (2013). Trace-back and trace-forward tools developed ad hoc and used during the STEC O104:H4 outbreak 2011 in Germany and generic concepts for future outbreak situations. *Foodborne Pathogens and Disease, 10*(3), 263-269. doi:10.1089/fpd.2012.1296

WHO, World Health Organization. (2008). *Terrorist threats to food: guidance for establishing and strengthening prevention and response systems. Revision May 2008*. http://www.who.int/foodsafety/publications/general/en/terrorist.pdf

3 Authenticity of Feed and Food

Lahrssen-Wiederholt, Monika; Mader, Anneluise

Introduction

One can assume that food authenticity will be on the top political and legislative topics in the upcoming years. That's because on the one hand the number of food fraud incidents is increasing - with regard to the increasing complexity of supply chains and the number of partners involved in the global market - and on the other hand the technical progress of the past years enables important improvements in detection. Therefore, feed and food authentication has its relevance at all stages of the production process.

The possibilities and limits within this field of research will further change and the BfR is willing to and prepared to take over an important role in defining standardizing terms, describing concepts and strategies, establish techniques and methodologies, as well as support developing coherent legislation.

An aspect which is important when dealing with authenticity of feed and food is food fraud as it can have a major impact on feed and food safety. That is why, in section 3.1 definitions and requirements (e.g. laws, regulations, standards) related to food fraud are provided, including examples of incidents which adversely and non-adversely affected the consumer health and compromised the consumer confidence not only in the respective product but also in feed and food safety. Food fraud can have a direct impact on the consumer behaviour and therefore, can influence the global feed and food trade. Despite these potential consequences, neither a precise definition of the term food fraud exists in Europe, nor a clear description of facts attributed to this term.

Additionally the verification of feed and food authenticity is of growing interest as a consequence of recent incidences. Section 3.2 describes working definitions and descriptions of terms, an overview on analytical methods including targeted and non-targeted approaches and the issue of reference databases in the field of food and feed authenticity.
Moreover, it is discussed that approaches can rely on considerations of so called authenticity ranges – and not on the determination of exogenous substances – of single ingredients or metabolic patterns.

Future needs

A first prerequisite for combating food fraud is the adoption of a legal definition of the term and the facts associated with it. Establishing an instrument for example an early warning system or a kind of checklist would be helpful to identify a possible risk of fraud. Part of this could also be the development of a tool that would allow information exchange between industry and authorities and allow companies to respond quickly to confirmed incidents by inspecting their own goods.

Commonly accepted terms and definitions need to be ensured as well as the recognition of the scientific field "authentication" as an own part of feed and food analysis and its relevance for the public. Further, developing, validating and assessing flexible and reliable analytical methods for food and feed authentication which are suitable for raw material as well as for processed food/feed and for detecting unknown and unforeseen adulterants have to be strengthened. In addition, reliable and available databases with comparative data and compatible data formats for referencing the authenticity range of genuine food and feed products are needed.

Data exchange between supply chain analysis / vulnerability analysis and analytical method development could be improved to profit from the information generated with each methodology. Last but not least the BfR supports an overall integration of analytical authentication in the supply chain analysis.

3.1 Food Fraud: definitions and requirements

Wisniewski, Aline; Buschulte, Anja

Abstract

Food fraud (FF) has existed since hundreds of years. It can have a major impact on food safety, as the melamine scandal of 2008 has shown. But even if consumer health is not adversely affected, consumer confidence in food safety can be compromised. This can have a direct impact on the purchasing behaviour of consumers and in some cases may even influence the global food trade, resulting in strong negative economic effects. Despite these potential consequences, neither in Germany nor in Europe a precise definition of the term FF exists, nor a clear description of the facts attributed to this term. Against this background, this text discusses which facts should be assigned to the term and which laws, regulations and standards can be used to combat food fraud.

Introduction

FF has been around ever since people began trading in food. FF often does not pose a health hazard (counterfeit weights of scales, adding of water to milk or fish, false declaration of conventional products as organic goods) but may also lead to a severe health risk in some cases, as countless examples from the past decades confirm. In 1981 about 800 people died in Spain, and around 25.000 were poisoned, as cheap industrial rapeseed oil was sold as pure olive oil (Dalziel, 2009). In Italy, 22 people died and over 100 needed medical treatment in hospitals when red wine was mixed with methanol (methyl alcohol) in 1986 (Wright, 1994). In 2008, at least 6 babies in China died of kidney failure and around 300.000 children fell ill due to added melamine in infant formula (Ingelfinger, 2008). These few examples alone already illustrate the major issues that FF entails.

First of all, the health effects can be dramatic and in some cases even lead to death. But even if no direct harm to humans occurs, FF that has become known can lead to a significant loss of consumer confidence. Then, there are almost endless ways of adulterating food, which makes it difficult to detect these adulterations and to estimate how much FF actually reaches the consumer. It is assumed that the number of documented cases is likely to be only a small fraction of the actual amount (Johnson, 2014). Furthermore, it can also have a huge economic impact on the food business, the production line or even the whole corresponding industry, as the consumer may stop buying certain or even all products from the company or the production line or restrictions on trade are established. Current estimates suggest that the problem of FF costs the companies worldwide between $ 30 billion and $ 40 billion each year (Food Safety Magazine, 2017).

In addition the different types of FF make it difficult to create a legal definition which forms the basis for a coordinated and consistent strategy against FF.

The definition of Food fraud

A major problem in tackling FF is that a universally and legally accepted definition of FF is still missing in Germany (DE) and in the European Union (EU) as well. One reason for that is that the regulatory framework is essentially aimed at ensuring food safety and therefore focused to a great extent on risks to human health. However, in order to tackle FF, the European Commission (EC) issued the following working definition in 2013: “Food Fraud is an intentional violation of the rules referred to in Art 1 of Regulation 882/2004, for the purpose of

financial or economic gain" (Eur. Parliament, 2013). As the four operational criteria for FF were identified:

1. Violation of EU Food Law
2. Intention
3. Economic Gain
4. Deception of Consumers.

Another reason for the lack of a uniform definition is that it has not been determined yet which facts or types of adulteration are to be summarised under this term. As a result, there is still no uniform classification or categorisation of the issues belonging to FF available. Another complicating factor is that even when FF cases are categorised, they can certainly be sorted into more than one category. Table 16 gives an overview of possible classifications of FF cases and presents examples. This Table demonstrates how difficult classification can be, as the categories sometimes overlap, making a clear assignment to a single category often not possible. At present, therefore, the concept of FF is a collective term that is defined in many different ways and to varying degrees of detail depending on the observer (Spink and Moyer, 2013).

Table 16: Different types of Food Fraud, their explanation and examples
(modified according to (Wisniewski and Buschulte, 2019))

FF Type	Explanation	Example
Substitution of substances	Replacement of a high-priced component or part of the product by a cheaper component (exogen or endogen)	- Replacing saffron with turmeric - Replacing honey with syrup, fructose or glucose - Replacing beef with cheaper meat from alternative animal species
Omitting substances	Omitting a valuable part of the product or an ingredient	- Extra virgin olive oil mixed with oil of lower quality
Concealment of inferior quality	Hiding the low quality of a product or a part of it by adding illegal substances	- Use of harmful or non-harmful coloring substances (i.e. Sudan red dyes for coloring paprika, chili powders, curries or fuchsine for meat coloring) - After diluting milk with water addition of melamine to artificially increase the protein content
Mislabeling/Label Manipulation	Use of false information (i.e. about origin or method of production) on the packaging using otherwise regular products and packaging	- Declaration of red tuna as yellowfin tuna - Declaration of Turkish oil as Italian oil - Declaration of conventional, non-organic products as organic products (i.e. eggs) - Declaration of a synthetically derived flavor chemical as being “naturally” derived
Counterfeiting/ Imitation of food	Violating Intellectual Property Rights (IPR)	- Copies of popular food, such as copying the brand name, the packaging concept, the recipe, processing methods or others
Selling stolen goods	Selling of products stolen from their original producers	- Selling of meat that has been stolen
Redirection of food	Sale or distribution of legitimate products outside their intended markets	- Merchandise intended for a particular distribution channel is shifted to be sold somewhere else without the knowledge or permission of the primary vendor
Illegally imported food	Selling illegally imported goods circumventing the official control bodies	- Import of products without official border controls

Effects of food fraud

The primary intention of FF is to gain a financial benefit, whereby this "source of income" should preferably not be detected in order to be able to operate FF for as long as possible. Therefore food fraudsters are generally concerned not to harm the health of the consumer. Nevertheless, contamination, adulteration or pure ignorance of chemical substances can pose health risks for the consumer (Everstine et al., 2013; Moyer et al., 2017; Spink et al., 2015). The effects can vary from relatively harmless gastrointestinal complaints (e.g. from microbial contamination) to organ damage (e.g. from melamine) or even death (e.g. from anaphylactic shock caused by allergens or renal failure due to melamine).

Apart from the health effects, FF may cause a loss of the consumer trust in official food controls and the relevant authorities, the entire food supply chain, but also the industry and trading partners. All this can influence purchasing and trading behavior and lead to market and trading disruptions.

Laws and regulations

Food produced and traded in DE is subject to the legal regulations of the EU and national regulations (see below). In the EU, the so-called General Food Law (GFL) Regulation (EC) No 178/2002 forms, among others legal texts, the basis of all food safety requirements. This GFL stipulates that labelling, advertising, presentation and packaging must not "mislead consumers" and that consumers must be protected from practices of fraud and deception, the adulteration of food and all other practices which may mislead consumers (Eur. Parliament and The Council, 2002d). The obligation for true, accurate and not misleading labelling is referred to in more detail in Regulation (EU) No 1169/2011. Article (Art) 7 lays down, that food information shall not be misleading. In detail this concerns the characteristics of the food and, in particular, its nature, identity, properties, composition, quantity, durability, country of origin or place of provenance, method of manufacture or production (Eur. Parliament, 2011).

However, the practical implementation of this legislation varies widely between Member States (MS), making it difficult to prosecute infringements. In addition, the number of controls in this area is very limited, as they involve a high level of personnel and financial expenditure (Eur. Parliament, 2013). As a result, cases of FF are rarely detected, especially when there is no impact on food safety, which makes it difficult to assess the current level of FF in the EU (ibid.) One example of the impact of such inconsistent interpretations of existing legislation was particularly evident in the horsemeat scandal in 2013, when several European countries found undeclared horse meat in food declared as beef products. In the course of the investigations, undeclared proportions of other types of meat and medicines such as phenylbutazone were also found in some cases. This particularly affected frozen foods and sauces containing minced meat. As a result a very divergent interpretation of Art 19 GFL "Responsibilities for food: food business operators" has emerged between the MS (van der Meulen et al., 2015). While Greece (GR), the Netherlands (NL) and Portugal (PT) were of the opinion that the contaminated products did not comply with food safety and therefore had to be withdrawn as a consequence of Art 19 GFL, Ireland (IE), Italy (IT), DE and France (FR) disagreed with this interpretation as they were of the opinion that these products did not pose a health risk to the consumer.

DE and FR, however, argued that they could recall the products concerned on the basis of national legislation. But IT and IE did not have national legislation to recall the products from the market (van der Meulen et al., 2015). This incident shows that the different interpretation of the laws by MS poses a major problem as it leads to different legislative and institutional arrangements. Due to the lack of definition, the approach to control FF varies greatly. As consequence of the horsemeat scandal, the authorities responded with the adoption of a

wide-ranging action plan consisting of targeted policy, legislative and enforcement measures. To better coordinate these measures, the European FF Network, composed of representatives of the EC, the MS and Europol officials, was set up. The network aims at facilitating the investigation of cross-border fraud cases across Europe (EC, 2013). Therefore, the network was provided with an IT tool - the Administrative Assistance and Cooperation (AAC) System – for handling FF cases. Since its introduction, the number of reported cases has increased every year. Figure 18 also shows that a large proportion of the reported cases are due to mislabelling.

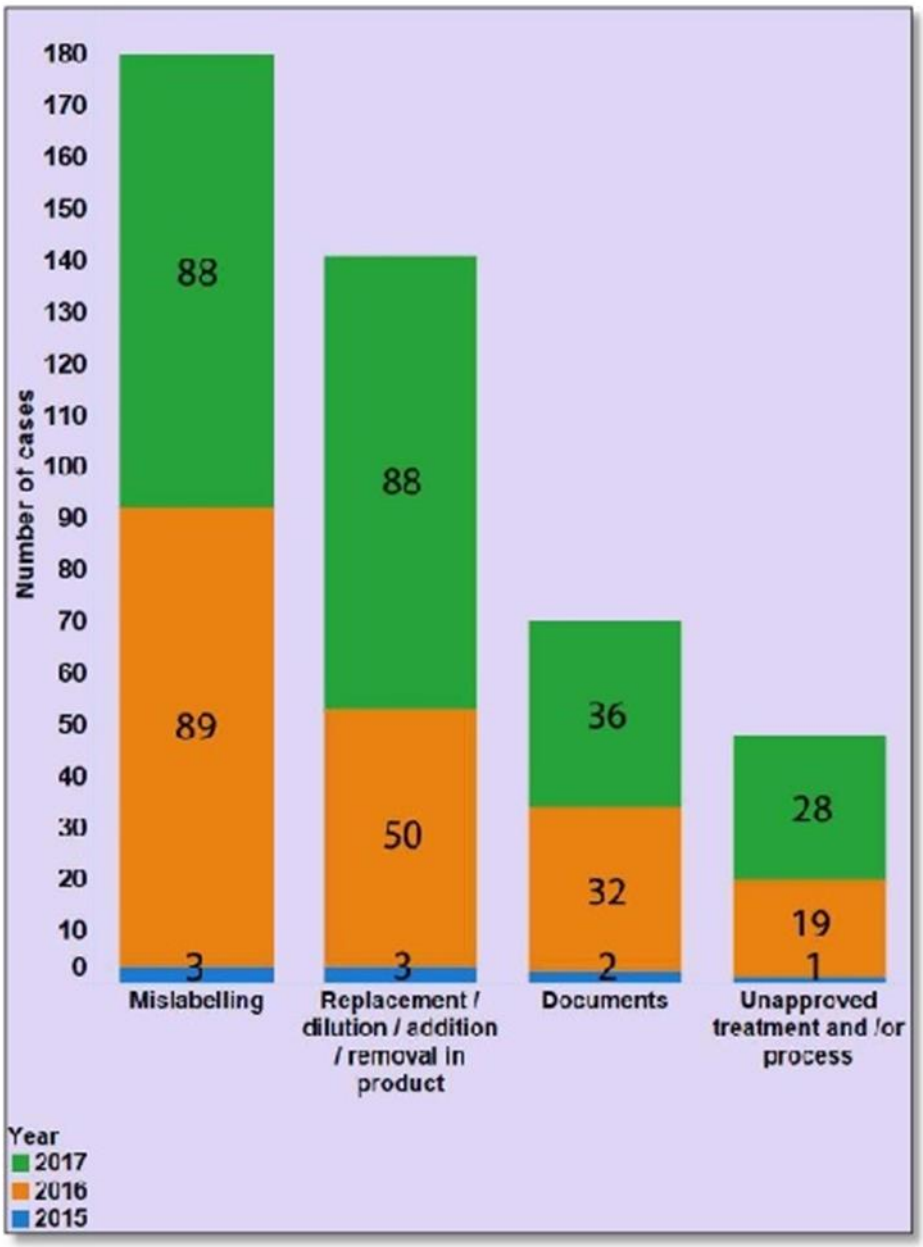


Figure 18: Food Fraud cases in the Administrative Assistance and Cooperation (AAC) System in 2015, 2016 and 2017 (EC, 2018)

Competent authorities are obliged to carry out appropriate and risk-based controls. These controls are based on EU Control Regulation (EC) No 882/2004, which was revised in 2016.

On March 2017, the new EU Control Regulation (EU) No 2017/625 was published and will enter into force on 14 December 2019 (Eur. Parliament and The Council, 2017). This new regulation takes into account the FF scandals of recent years and explicitly points out that possible infringements due to fraudulent or misleading practices must be investigated. Art 9 (2) of this regulation requires the competent authorities to carry out regular checks, which must be risk-based and explicitly include control measures against possible breaches of European food chain rules due to fraudulent or misleading practices. Unfortunately, however, this regulation also lacks a detailed definition of FF and what is meant by fraudulent or misleading practices.

At national level, the German Food and Feed Code (Lebensmittel-, Bedarfsgegenstände- und Futtermittelgesetzbuch (LFGB)) protects consumers from FF. Art 11 of the LFGB, summarises the provisions on fraud protection. It states that it is forbidden to place unfit foods, products with misleading or false information on the German market or to advertise them in such a way as to mislead consumers. Furthermore, counterfeit products as well as products that differ in their composition from commercially available products and whose value, in particular their nutritional value, gustatory qualities or usability, is therefore significantly reduced, and food that suggest a better quality than their actual, are prohibited in Germany without sufficient labelling (BMJV, 2013). Failure may result in a custodial sentence of up to one year or a fine. In accordance with Art 14 (2a) GFL the LFGB generally prohibits in Art 5 to manufacture or treat consumer goods in such a way that they are harmful to health or to place products on the market which are not fit for human consumption.

In Art 263 of the German Criminal Code (Strafgesetzbuch (StGB)), the term fraud is explicitly dealt with. It states "Whosoever with the intent of obtaining for himself or a third person an unlawful material benefit damages the property of another by causing or maintaining an error by pretending false facts or by distorting or suppressing true facts shall be liable to imprisonment not exceeding five years or a fine” (STGB, 1998). However, the term fraud presupposes financial loss, which in the case of FF is difficult to prove in most cases. Therefore, the majority of FF cases fall within the scope of the LFGB and not within the scope of the Criminal Code (Sulzer, 2017).

Requirements of international standards

In addition to the legal requirements at national, European or international level, various private international food standards address FF. The basis are no legal regulations, but an economic representation of interests in which all companies must comply with the same standard requirements, regardless of their national legislation.

In the field of private sector standards, the Global Food Safety Initiative (GFSI), an international initiative of commerce, acts as a superordinate body that strives for comparability (benchmarking) and mutual recognition of food safety standards. This Initiative was launched in 2000 by a group of industry chief Executive Officers from the Consumers Goods Forum to help optimise and harmonise food safety standards (Spink et al., 2016). The aim of this organisation is to increase food safety, ensure consumer protection and thereby strengthen consumer confidence. In order to implement this, the GFSI developed a guideline defining the most important requirements for food safety (Borggrewe, 2014). FF is a major and serious issue for GFSI. In June 2012, the Food Fraud Think Tank (FFTT) was established to provide guidance and recommendations to inform companies how to protect consumers from potential harm caused by FF (GFSI, 2014). GFSI first published a position paper called “GFSI Position Paper on Mitigation of public health risk of FF” in 2014. In this paper the GFSI Board recognized “the importance of food fraud mitigation and the urgency to start performing food fraud vulnerability assessments and implementing associated control plans” (GFSI, 2018). The position paper deals with the subject of FF which covers a wide range of inci-

dents and includes adulteration (specifically dilution, substitution, concealment, unapproved enhancements), mislabelling, grey market (including diversion, parallel trade, etc.), smuggling, theft, and counterfeiting (GFSI, 2014). Two key elements in the Fight against FF were identified. The first element is to carry out a FF vulnerability assessment. For this the introduction of a documented procedure is necessary to assess susceptibility to FF and to implement measures to prevent it. The second element relates to the drawing up of a specific plan to prevent FF. On the basis of the vulnerability assessment, this plan must establish measures to reduce the risks to public health arising from the identified vulnerable points in the area of FF (GFSI, 2014). This plan must cover the relevant GFSI scope and be supported by the companies' food safety management systems.

All standards which seek recognition by GFSI, like the International Featured Standard (IFS, e.g. IFS Food Standard Version 6.1, IFS PACsecure Version 1.1 & IFS Logistics Version 2.2), the British Retail Consortium Global Standard (e.g. BRC Global Standard for Food Safety Issue 8, BRC/IOP Global Standard for Packaging and Packing Materials Issue 5), the Food Safety System Certification 22000, CanadaGAP (CanadaGAP Scheme Version 7.1 Options B, C and D and Program Management Manual Version 7.1), SQF Institute (SQF Code 8TH Edition), Global Red Meat Standard (GRMS 4th Edition Version 4.2), GlobalG.A.P. (GlobalG.A.P Integrated Farm Assurance Standard V 5.1, Produce Safety Standard version 4 and Harmonized Produce Safety Standard), Global Aquaculture Alliance Seafood (Global Aquaculture Alliance Seafood BAP Seafood Processing Standard) and PrimusGFS Standard (PrimusGFS Standard) have to include these two elements in the requirements catalogue for their certification in the future (Sulzer, 2017). In addition, the guidance document includes a definition of GFSI on FF and defines it as “A collective term encompassing the deliberate and intentional substitution, addition, tampering or misrepresentation of food, food ingredients or food packaging, labelling, product information or false or misleading statements made about a product for economic gain that could impact consumer health” (GFSI, 2016).

One of the most important standards in the German food industry is the IFS, which was developed by German retailers for the verification and auditing of private label manufacturers. IFS released version 6.1 IFS Food in November 2017. The latest requirements of the GFSI were taken into account and chapter 4.21 FF was added. In addition to the requirements of a vulnerability assessment and a documented FF mitigation plan, IFS requires that in the event of an increased risk of FF, the vulnerability assessment must be reviewed and adapted. In general, IFS demands an annual review of this assessment (https://www.ifs-certification.com). In DE the BRC and the International Organisation for Standardization (ISO) are important in addition to IFS. Both also implemented the requirements of the GFSI.

In conclusion, the most important standards in DE demand a documented assessment of the companies' vulnerable points based on the requirements of GFSI. On the basis of this assessment, measures must be defined and implemented to reduce or eliminate the risks to public health arising from the identified weaknesses in the area of FF. The challenge here is that there are no precise specifications how the vulnerability assessment should be carried out, which content must be queried in detail and which system or procedure should be used for this. Rather, the implementation of these procedures is the sole responsibility of the companies.

In addition to GFSI other organisations working on food safety and food authenticity as well. The first is SSAFE. It is a global, non-profit organisation founded in 2006. SSAFE is not officially affiliated with GFSI, but some GFSI members are also SSAFE members and GFSI is an important partner of SSAFE. In 2014, GFSI publishes the position paper "GFSI Position on Mitigation of Public Health Risk of FF", which makes clear that the GFSI board supports the initiative of SSAFE. SSAFE, PricewaterhouseCoopers (PwC) and Wageningen University developed a semi-quantitative FF Vulnerability Assessment Tool (FFVA) based on GFSI pa-

rameters to provide companies with practical guidelines to assess and control FF vulnerabilities and supply chains (Wageningen University, 2016). It does not offer specific mitigation techniques to detect FF or to predict future incidents, but it offers indications how and where to find solutions (Withworth, 2016). The FFVA requires the organisation to have a documented FF vulnerability assessment procedure to identify potential vulnerabilities and prioritise FF mitigation measures. It contains three main elements for combating fraud: the identification of opportunities for FF, motivations for FF and the elimination of the lack of anti-fraud measures. The FFVA online tool is free for any company and covers most but not all types of FF. A note in the information document states that "grey market production/theft/diversion" does not fall within the scope of this evaluation instrument. It includes dilution, substitution, concealment, unauthorized improvement and infringement of intellectual property rights by food counterfeiting.

Another important organisation is the U.S. Pharmacopeial Convention (USP). USP is a non-profit scientific organisation founded in 1820. Its mission is to set standards for the identity, strength, quality and purity of medicines, food ingredients and food supplements produced, distributed and consumed worldwide (USP, w.d.). To this end, USP defines specifications, test methods and reference samples for product quality, including food ingredients (i.e. unfinished products), food supplements and pharmaceuticals. To fulfil its mission, the USP has established several expert committees and expert panels composed of volunteers from industry and government agencies (USP, w.d.). One of these is the Intentional Adulteration Expert Panel, which has prepared a FF Mitigation Guidance to provide manufacturers and retailers with a comprehensive, practical four-step approach to conduct a vulnerability analysis and develop a tailored FF mitigation plan (USP, 2015). Additionally this Panel has created a FF database that deals with published test methods for the detection of counterfeits and specific substances (USP, 2016). For this purpose, the period 1980-2010 was first examined for FF cases. The database was set up in 2012 and has been continuously updated since then. Necessary documents are compiled from publicly available sources such as scientific literature, media publications, regulatory reports, court records and business associations from around the world. The information that companies can obtain from this database should help to identify problematic ingredients and take appropriate countermeasures. The data contain the corresponding case numbers, the report categories, classifications of the report subjects, the ingredient categories and ingredients, the counterfeits, the types of fraud and the years of notification (Sulzer, 2017). Since the introduction of version 2.0 in summer 2016, the use of the database is subject to a fee. As of 2018, the database is owned by Decernis and operated by this private company (Decernis, 2019). Furthermore, the USP has established a collection of approximately 1200 internationally recognized monographs on the identity and purity of food additives, such as flavours, colorants, flavour enhancers, nutrients and preservatives, the Food Chemical Codex (USP FCC, w.d.). The FCC and its associated reference materials enable suppliers and manufacturers to verify the identity, quality and purity of the food ingredients they buy and sell. The attached FCC annexes describe suitable tests together with appropriate reference material to ensure the reliability of the specific analytical methods. This helps to safeguard the overall security and integrity of the food ingredient supply chain. A working group of the FCC also dealt with a vulnerability analysis for FF, similar to that of the BRC, which evaluates the vulnerability of the product against adulteration using specific evaluation criteria. These criteria are divided to “can be” influenced and “cannot be” influenced (Sulzer, 2017).

Conclusion and perspective

FF has existed since people started trading food. It can be harmless or have serious consequences for consumer health, as demonstrated by the melamine scandal in China in 2008. Even if the fraudsters do not intentionally endanger the health of consumers, such a risk cannot be ruled out due to unawareness of the fraudsters. For example, the addition of pri-

marily non-toxic substances can pose a considerable risk for allergy sufferers. Due to the diverse possibilities in the field of FF, it is simply not possible to permanently monitor all food for any kind of fraud, especially as counterfeiters are often well informed and adapt the counterfeiting methods to the advanced detection methods. In recent years, numerous national and international efforts have been made in the field of FF in order to gain new knowledge on prevention, early detection and the development of risk reduction measures. For example, the EU Food Fraud Network and the AAC System have been established at European level. In Germany the Expert Advisory Board for Food Fraud was founded in 2015 and the National Reference Centre for Authentic Food (NRZ) at the Max Rubner Institute (MRI) was established in 2018 among other national initiatives.

As part of a doctoral thesis at Freie Universität Berlin, an online survey on FF was conducted in cooperation with the BfR in autumn 2017. The aim was to find out which types of FF are assigned to the term FF in the official food control institutions in order to be able to suggest a uniform definition. For this purpose, questionnaires were sent to the state laboratories and the competent authorities. The survey also asked participants whether they would favour a tool to improve early detection of fraudulent intentions so that FF can be averted as early as its emergence. Such a tool would be welcomed by a large majority of participants. In order to implement such a tool, it is important to identify the factors that can lead to FF. Various German institutions, such as the Federal Institute for Risk Assessment (BfR) or the Bavarian State Office for Health and Food Safety (LGL) have been working on this field of research for years to protect consumer safety. It is planned to develop suitable measures (e.g. checklists, software tools) in the coming years to support official controls and self-checks of food companies and to enable them to carry out a targeted analysis of vulnerabilities in the area of FF. The results of this survey will be published in near future.

Despite all these national and international initiatives a clear and legally binding definition of FF is still missing and therefor a consistent and coordinated combat remains challenging.

References

BMJV, Bundesministerium der Justiz und für Verbraucherschutz. (2013). Lebensmittel- und Futtermittelgesetzbuch in der Fassung der Bekanntmachung vom 3. Juni 2013 (BGBl. I S. 1426), das zuletzt durch Artikel 1 des Gesetzes vom 30. Juni 2017 (BGBl. I S. 2147) geändert worden ist.

Borggrewe, B. (2014). Zertifizierungsstandards in der Lebensmittelindustrie. *Der Lebensmittelbrief*.

Dalziel, G. R. (2009). *Food Defence Incidents, 1950-2008: A Chronology and Analysis of Incidents Involving the Malicious Contamination of the Food Supply Chain*: S. Rajaratnam School of International Studies, Nanyang Technology University.

Decernis. (2019). Food Fraud Database. https://decernis.com/solutions/food-fraud-database/

EC, European Commission. (2013). EU Food Fraud Network. https://ec.europa.eu/food/safety/food-fraud/ffn_en

EC, European Commission. (2018). *Food Fraud Network Activity Report 2017*. https://ec.europa.eu/food/safety/food-fraud/reports_en

Eur. Parliament. (2011). Regulation (EU) No 1169/2011 of the European Parlament and the Councel of 25 October 2011 (LMIV) on the provision of food information to consumers, amending regulations (EC) No 1924/2006 and (EC) No 1925/2006 of the European Parliament and of the Council, and repealing Commission Directive 87/250/EEC, Council Directive 90/496/EEC, Commission Directive 1999/10/EC, Directive 2000/13/EC of the European Parliament and of the Council, Commission Directives 2002/67/EC and 2008/5/EC and Commission Regulation (EC) No 608/2004. *Official Journal of the European Union, L304*, 18-63.

Eur. Parliament. (2013). *Report on the food crisis, fraud in the food chain and the control thereof*. http://www.europarl.europa.eu/sides/getDoc.do?type=REPORT&reference=A7-2013-0434&format=PDF&language=EN

Eur. Parliament, and The Council. (2002d). Regulation (EC) No 178/2002 of the European Parliament and the council of the European Union of 28 January 2002 laying down the general principles

and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety. *Official Journal of the European Communities, L 31*, 1-24.

Eur. Parliament, and The Council. (2017). Regulation (EU) 2017/625 of the European Parlament and the Council of 15 March 2017 on official controls and other official activities performed to ensure the application of food and feed law, rules on animal health and welfare, plant health and plant protection products. *Official Journal of the European Union, L95*, 1-142.

Everstine, K., Spink, J., and Kennedy, S. (2013). Economically motivated adulteration (EMA) of food: common characteristics of EMA incidents. *Journal of Food Protection, 76*(4), 723-735. doi:10.4315/0362-028x.jfp-12-399

Food Safety Magazine. (2017, 2017-06-13). Tackling Food Fraud on a Global Level. *Food Safety Magazine(6)*. https://www.foodsafetymagazine.com/magazine-archive1/junejuly-2017/tackling-food-fraud-on-a-global-level/

GFSI. (2014). *GFSI Position Paper on Mitigating the Public Health Risk of Food Fraud*. http://www.mygfsi.com/images/mygfsi/gfsifiles/July_2014_-_GFSI_Position_Paper_-_Mitigating_the_Impact_of_Food_Fraud.pdf

GFSI. (2016). *Benchmarking Requirements - GFSI Guidance Document Version 7*. http://www.mygfsi.com/schemes-certification/benchmarking/gfsi-guidance-document.html

GFSI. (2018). *Tackling food fraud through food safety management systems*. https://www.mygfsi.com/news-resources/news/news-blog/1396-tackling-food-fraud-through-food-safety-management-systems.html

Ingelfinger, J. R. (2008). Melamine and the global implications of food contamination. *N Engl J Med, 359*(26), 2745-2748. doi:10.1056/NEJMp0808410

Johnson, R. (2014). *Food Fraud and Economically Motivated Adulteration of Food and Food Ingredients*. https://www.fas.org/sgp/crs/misc/R43358.pdf

Moyer, D. C., DeVries, J. W., and Spink, J. (2017). The economics of a food fraud incident – Case studies and examples including Melamine in Wheat Gluten. *Food Control, 71*, 358-364. doi:10.1016/j.foodcont.2016.07.015

Spink, J., and Moyer, D. C. (2013). Understanding and combating food fraud. *Food Technology, 67*(1), 30-35.

Spink, J., Moyer, D. C., Park, H., Wu, Y., Fersht, V., Shao, B., . . . and Edelev, D. (2015). Introducing Food Fraud including translation and interpretation to Russian, Korean, and Chinese languages. *Food Chemistry, 189*, 102-107. doi:10.1016/j.foodchem.2014.09.106

Spink, J., Moyer, D. C., and Speier-Pero, C. (2016). Introducing the Food Fraud Initial Screening model (FFIS). *Food Control, 69*, 306-314. doi:10.1016/j.foodcont.2016.03.016

STGB. (1998). Strafgesetzbuch in der Fassung der Bekanntmachung vom 13. November 1998 (BGBl. I S. 3322), das zuletzt durch Artikel 14 des Gesetzes vom 18. Dezember 2018 (BGBl. I S. 2639) geändert worden ist.

Sulzer, G. (2017). Food Fraud in Normen. In U. Nöhle (Ed.), *Food Fraud - Lebensmittelbetrug in Zeiten der Globalisierung* (pp. 115 et sqq.). Hamburg: Behr`s Verlag.

USP. (2015). Food Chemicals Codex 9: Appendix XVII: Food Fraud Mitigation Guidance. In *Appendix XVII: Food Fraud Mitigation Guidance* (pp. 36).

USP. (2016). New USP Food Fraud Database Helps Industry and Regulators Mitigate Risk of Food Adulteration [Press release]. http://www.usp.org/news/new-usp-food-fraud-database-helps-industry-and-regulators-mitigate-risk-food-adulteration

USP. (w.d.). About USP. http://www.usp.org/about-usp

USP. (w.d.). USP volunteers.

van der Meulen, S., Boin, G., Bousoula, I., Conte-Salinas, N., Paganizza, V., Montanari, F., . . . and van der Meulen, B. (2015). Fighting Food Fraud. *European Food & Feed Law Review, 10*(1), 2.

Wageningen University. (2016). Tool for estimating vulnerability to food fraud. https://www.wur.nl/en/newsarticle/Tool-for-estimating-vulnerability-to-food-fraud.htm

Wisniewski, A., and Buschulte, A. (2019). Dealing with Food Fraud - Part 2. *European Food and Feed Law Review, 14*(2), 103-117.

Withworth, J. J. (2016). Food fraud vulnerability assessment created by SSAFE and PwC. http://www.foodqualitynews.com/Industry-news/SSAFE-and-PwC-tool-helps-assess-vulnerability-to-food-fraud

Wright, S. (1994). *Handbook of Organic Food Processing and Production* (First Edition ed.). London: Springer Science & Business Media.

3.2 Verification of food and feed authenticity

Fauhl-Hassek, Carsten; Esslinger, Susanne; Riedl, Janet; Horn, Bettina

Abstract

The verification of food and feed authenticity is of growing interest as consequence of recent food fraud incidences. With regard to the increasing complexity of supply chains in the global market the risk of fraudulent practices is enhanced, not at least due to the more and more involved operators and respective opportunities for the application of fraud. Therefore, food and feed authentication has its relevance at all stages of the production process.

Initially, this chapter introduces working definitions and descriptions of terms in the field of food and feed authenticity because there still is a lack of official ones. Then, a short overview about analytical methods for food and feed authentication is provided. Hereby, a classification of these methodologies into targeted and non-targeted approaches is promoted, which allows a systematic assignment. Moreover, the issue of reference data bases is discussed as many of the analytical methods for authenticity verification, and in particular the new non-targeted approaches, rely on considerations of so called authenticity ranges – and not on the determination of exogenous substances – of single ingredients or metabolic patterns. Particularly, the challenges and requirements related to non-targeted methods, such as validation and jointly usable databases are still regarded as relevant research gaps with respect to the application of these techniques in routine analysis. Several research activities of the BfR address these gaps and are comprehensively presented in the subsequent chapter.

Introduction

In terms of (preventive) consumer (health) protection, strategies are required in food and feed control which enable the verification of product declarations at all stages of the supply chain. So-called authentication of food and feed products is typically performed by complementary approaches. The analytical verification is based on the chemical analysis of product characteristics of each samples matrix, whereas the record-based procedure relies on documentation made in the supply chain. The investigation of the product composition and the verification of the product origin, including biological and geographical origin as well as production processes, are essential aspects of food and feed authentication along the supply chain.

Due to the globalisation and increasing complexity of supply chains, special attention is paid on the detection of adulterations as well as the proof of the geographical origin of products. The more actors and points of trade are involved in the supply chain the higher the risk for criminal activity becomes. Although the motivation for fraudulent practices is economic gain, the result is a real public health vulnerability (Spink and Moyer, 2011). Especially adulteration of products creates the potential for harm and can pose a known or possible health risk to consumers (Manning and Soon, 2014). Only recently, a systematic classification of 1294 adulterants which were reported beforehand in a comprehensive food fraud database over a period of five years showed that 45% of the substances or fraudulent materials were potentially hazardous. Of those 45%, 27% had a documented history of causing consumer illness or even death, or were involved in safety-related regulatory management actions, or were classified as allergens (Everstine et al., 2018). This scientific investigation based on reported adulteration shows systematically that in almost half of the cases at least a potential health risk was indicated and thereby clearly underpins that potential health implications in relation to food fraud are not at all negligible. The geographical origin is a product characteristic closely linked to its value which might influence acceptance by the consumer, and therefore the decision to buy (Bitzios et al., 2017). Moreover, the geographical origin of falsified prod-

ucts of previously reported food fraud incidences might be a hint for vulnerabilities in a supply chain analysis or for an in-depth hazard analysis on fraudulent material (Everstine et al., 2018).

A particular challenge in food and feed authentication is the identification of unexpected deviations of a product in addition to the examination of issues that are already known. Therefore, there is a general need for developing, validating and assessing flexible and reliable analytical methods for food/feed authentication along the supply chain which are also applicable in routine analysis. The following chapter focusses on established as well as upcoming analytical approaches for food and feed authentication.

Context, terms and definitions

Although the authentication of food and feed is an integral part of official control as well as ongoing research and development efforts, there is still a fundamental need for a clear definition of the terminology. Accordingly, several of the most frequently used terms in this field are described below.

Food/Feed authenticity is the agreement of the products attributes and the claimed product descriptions.

Food/Feed authentication is the process of verifying the authenticity of the food or feed product. It comprises the confirmation of requirements regarding the legal product description or the detection of fraudulent claims or statements (Esslinger et al., 2014), particularly in view of adulteration and false claims of origin.

Food/Feed adulteration is a subcategory of food fraud (for detailed information see Section 3.1) in which the composition of the product is changed during the period of growth, storage, processing, transport or distribution of the food or feed products by i) addition of substances, ii) substitution of ingredients and/or iii) dilution of ingredients. Consequences of adulteration may include economic, health, and religious concerns.

i) **Addition** is the process of adding (illegal) substances to fake a higher quality or to cover up blending with lower value materials. For example, dairy products are often subjected to adulteration for financial gain. In the recent past melamine was found to be added to dairy products to increase the apparent protein content and cover up blending with lower value material (e.g. dilution of milk with water, see below). This addition has the potential to cause serious health-related problems as was shown in course of the China's melamine-tainted milk scandal in 2008: It was reported that more than 50.000 children had become sick and were hospitalised, and 6 infants had died due to kidney damages after being fed with melamine-tainted infant formula (Gossner et al., 2009).

ii) **Substitution** is the process of replacing an (often high value) ingredient of the food/feed product with an (often lower value) component. For instance, in the beginning of 2013 Europe went through a scandal regarding the authenticity of processed beef products, which contained undeclared portions of horse meat. Species substitution in meat products using sources of low-priced meats in high-value meat products is a common problem reported worldwide (Dalsecco et al., 2018).

iii) **Dilution** is the process of increasing the quantity of an (often low value) ingredient to increase weight or volume and thereby to reach a greater profit. As mentioned above, water was found to be the most common adulterant in milk. Dilution of milk with water leads to a substantially poorer quality with a lower nutritional value (Handford et al., 2016).

Food/Feed origin comprises attributes of food and feed products with respect to i) geographical origin, in particular food/feed from specific regions, ii) animal or botanical origin (species, varieties) and iii) respective production processes. Another subcategory of food fraud is the deliberate change of origin claims. Consequences of such misdescriptions may include economic, health, ethnical and religious concerns.

i) **Geographical origin**: The latest food fraud incidents that go hand in hand with the globalisation of supply chains led to an increased interest as well as sensitisation of consumers with regard to the geographical origin of food such as country, region and local origin (Fernqvist and Ekelund, 2014). The reasons for that range from patriotism up to specific culinary or organoleptic properties connected with regional products as well as a lack of trust in the quality and safety of products which were made outside of a particular local region or country. Besides the increasing consumer interest in the geographical origin of products, it has been suggested for industry to consider the country of origin in systematic hazard evaluations on fraudulent adulteration (Everstine et al., 2018).

ii) **Animal or botanical origin (species)**: The previously mentioned horse meat scandal from 2013 demonstrates that it is necessary to verify the species of food products of animal origin. In addition to this, there are similar issues in the field of plant-derived food which are also of great economic and scientific interest, such as the verification of the declared grape variety of wine, the identification of alkaloid-containing plants in tea mixtures or the determination of the plant varieties in honey. Other examples are the declaration of mozzarella made of cow's milk as genuine mozzarella *di bufala*, of Asian truffles (*Tuber indicum*) as expensive Perigord truffles (*Tuber melanosporum*) or of common wheat (*Triticum aestivum*) as hard wheat (*Triticum durum*) in pasta products.

iii) **Production process**: Verification of the origin of a food or feed product with regard to its production process poses a particular challenge for analytical chemistry. To do so, strategies and methods are required which enable the control of various manufacturing approaches, such as the distinction between organically and conventionally produced food, wild or farmed fish production or the identification of genetically modified organisms. Another example is the manufacture of seed oils by means of cold pressing or solvent extraction.

Traceability means "the ability to trace and follow a food, feed, food-producing animal or substance intended to be, or expected to be incorporated into a food or feed, through all stages of production, processing and distribution" (Eur. Parliament and The Council, 2002d). Traceability approaches are usually record-based approaches that rely on the labelling of products and documentation of the process steps in the food and feed company's quality management system, on other suitable documentation systems which allow the identification of every lot or batch, or on the maintenance of in-house databases. The appropriate implementation of these systems and thereby compliance with legal regulations on trade of foodstuffs are verified by official food control. This "control of control" is achieved by the inspection of the food and feed business operators and by taking of samples which are then examined using various analytical and diagnostic methods (Figure 19).

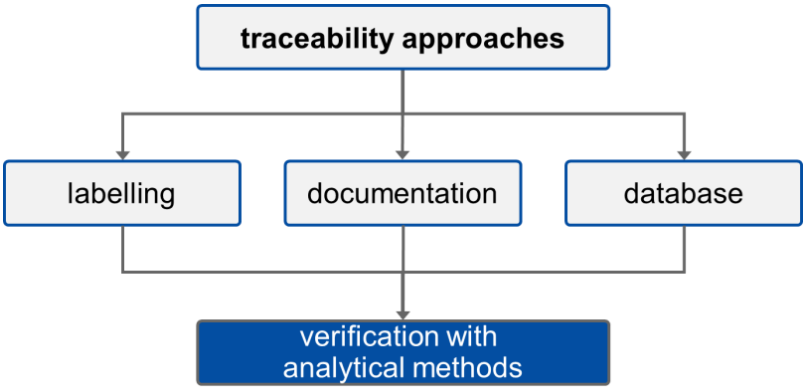


Figure 19: Current traceability approaches in connection with analytical methods for food and feed authentication
(Fauhl-Hassek et al., 2015).

Analytical approaches

There are a number of analytical methods that are currently used in routine analysis to verify the authenticity of food and feed products. Examples are listed below:

- stable isotope analysis by means of site-specific natural isotopic fractionation (SNIF) nuclear magnetic resonance (NMR) spectroscopy and stable isotope ratio mass spectrometry (IRMS) for confirmation of geographical as well as botanical origin and detection of adulterations,
- elemental analysis by means of mass spectrometry with inductively coupled plasma (ICP-MS), atomic absorption spectroscopy (AAS), optical emission spectrometry with inductively coupled plasma (ICP-OES) for confirmation of geographical origin,
- fatty acid composition determination by the means of gas chromatographic (GC) analysis for confirmation of botanical origin,
- real time quantitative polymerase chain reaction (qPCR) and other PCR-based methods for species differentiation,
- DNA-based methods based on the detection of highly conserved sequences composed of mitochondrial DNA with subsequent restriction fragment length polymorphism for species differentiation, and
- enzyme immunological methods (including enzyme-linked immunosorbent assay, Western Blot) for species differentiation.

In general, the analytical approaches in food and feed authentication can be distinguished in targeted and non-targeted analysis and furthermore in the following three approaches according to the type of analyte(s) (Figure 20):

- targeted analysis of specific exogenous marker compounds,
- targeted determination of authenticity ranges of natural ingredients, and
- non-targeted analysis of metabolic pattern (e.g. fingerprints)

which are discussed below in detail.

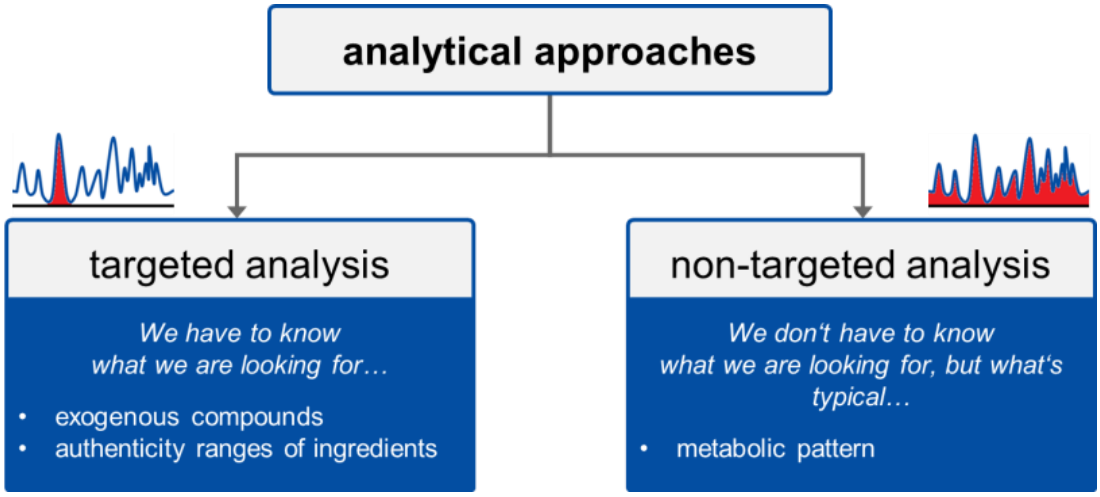


Figure 20: Systematic of analytical approaches for food and feed authentication

The different methodologies used for targeted and non-targeted analysis for food and feed authentication are applied to many different products over all stages in the supply chain. Of course the product type and its processing stage also determine and limit the relevant approaches. It is obvious that piece goods (e.g. apples) as raw product are less vulnerable to adulteration (addition, substitution, dilution) compared to more processed products such as juices. Species verification or the determination of the geographical origin is a product characteristic that is often related to the origin of the raw material, but its verification is as important for processed and complex foods as well. Therefore, analytical approaches need to address raw material verification as well as processed food verification, whereby the latter is far more challenging and needs more attention in future.

Targeted analysis of specific exogenous marker compounds

Food and feed authentication by determination and/or quantification of exogenous compounds is based on the targeted analysis of specific substances which are no natural product ingredients. This approach forms the basis of routine analysis as well as official food control and comprises a selective sample preparation, chemical analysis using a validated method followed by simple data evaluation (e.g. univariate regression in case of quantification). The result – the absence or the presence of the target marker compound – is scientifically accepted. This approach is in particular relevant for the detection of adulterations. While following this approach already known issues can be investigated effectively and efficiently. However, the big disadvantage is that only known compounds or known adulterants can be detected and the threat for public health from unknown and unforeseen adulterants remains.

For example, synthetic dyes such as Sudan are known adulterants in paprika powder and analytical methods to detect and quantify these carcinogenic substances in spices are readily available (Rebane et al., 2010) and routinely applied. Furthermore, lead oxide has been used to fraudulently manipulate paprika powder (Kákosy et al., 1996) and many more substances are conceivable to dilute or cover up blending of paprika powder with lower value product material (Horn et al., 2018). However, if not suspected, these adulterants will not be observed with routine targeted analysis.

Targeted determination of authenticity ranges of natural ingredients

This targeted approach, in which the analyte is a natural ingredient of the product, is currently the classical way of assessing the authenticity of food or feed products: It comprises the comparison of an actual measured value with a previously determined control limit (authenticity range) for a certain parameter. The natural range of a specific parameter of authentic reference samples is examined by targeted analysis using validated analytical methods. Through comparison of a new sample with the established reference values authenticity assessment is possible. This approach is often used to determine the botanical origin or processing methods as well as to detect specific adulterations.

Applications of this approach are long known in the field of wine authentication. For example, the detection of illegal addition of water to wine is performed routinely (in official control) by targeted analysis of stable isotopes (standard method OIV-MA-AS2-12). The addition of water can be easily uncovered by determining the oxygen isotope ratio $^{18}O/^{16}O$ (expressed as $\delta^{18}O‰$) of wine water using IRMS and comparison with the reference data defined by the official wine databank (EC, 2008). Tap water highly depletes the original $\delta^{18}O$ value of wine so that diluted wine samples fall below the limit defined by the EU wine databank (Perini and Camin, 2013).

Non-targeted analysis of metabolic pattern

The trickiness in the detection of food fraud using targeted analysis is that only substances that are being investigated are usually found. That is, because using classical targeted approaches food samples are tested for the presence or absence of specific compounds, but especially unknown or unforeseen adulterants can be overlooked. Therefore, a lot of effort has been put into the development of non-targeted (fingerprinting) approaches. In recent years fingerprinting techniques have been demonstrated to be powerful screening tools for answering various authenticity questions (Danezis et al., 2016; Esslinger et al., 2014; Sobolev et al., 2017).

Basically, food fingerprinting describes the non-targeted analysis of a food sample using a spectroscopic or spectrometric method followed by multivariate data analysis. This approach is based on the detection of a large quantity of analytical data which comprehensively describe the chemical composition of the examined food or feed matrix. Depending on the statistical data evaluation method used, the fingerprinting approach enables an assignment to a specific origin, as well as the identification of any deviation from the expected product, including both known and unknown adulterants. The most frequently used techniques for non-targeted analysis (fingerprinting) are based on NMR and vibrational (near infrared, mid infrared, Raman) spectroscopy as well as on mass spectrometry coupled with chromatographic separation methods (Danezis et al., 2016; Esslinger et al., 2014; Sobolev et al., 2017).

The fingerprinting approach is not implemented yet in routine analysis. At the current state, these methods might be best applied as a rapid tool to screen food and feed samples for anomalies. Current research is often based on feasibility studies that focus on demonstrating the high potential of fingerprinting approaches for food and feed authentication (see “BfR activities”). Despite the high potential for broad application of non-targeted methods − especially in view of globalisation and increasing complexity of supply chains − their use in routine analysis is currently limited to a few products. The reason for this is the lack of important prerequisites, such as

- comprehensive method validation, effective proficiency tests, and quality assurance measurements,
- reliable databases of representative authentic samples and of genuinely adulterated material, and
- uniform data exchange formats for jointly usable databases (see also “Databases for analytical authentication”).

Nonetheless, recently these gaps were recognized by the scientific community and activities were initiated towards potential solutions, e.g.:

- first validation concepts and guidance documents have been developed for non-targeted analysis and multivariate data evaluation including sampling, chemical analysis and validation (Alewijn et al., 2016),
- recent research projects (e.g. FoodIntegrity and FoodAuthent, for more information see “BfR activities”) investigate and bundle options for harmonisation of non-targeted analytical approaches,
- in the field of metabolomics standardization activities are to be mentioned which might be transferred to the authenticity domain, e.g. the COSMOS project developed policies to ensure that metabolomics data are encoded in open standards, supported by open-source data management tools and disseminated in open-access databases (COSMOS, 2018; Salek et al., 2015).
- accreditation bodies have acknowledged first approaches based on non-targeted testing methods. As a rare example for a commercial solution, the NMR FoodScreenerTM (Bruker, Rheinstetten) applies a non-targeted approach for characterising wine, fruit juice and honey using NMR spectroscopy.
- inter-laboratory comparisons, in particular for NMR spectroscopy (Gallo et al., 2015), have been organised and conducted, according to internationally agreed procedures.

Databases for analytical authentication

In particular non-targeted approaches for determining characteristic fingerprints and targeted methods for the investigation of trace elements by means of ICP-MS as well as stable isotope ratio analysis by SNIF-NMR and IRMS are suitable for solving various authenticity issues. There is a need for available reliable databases of comparative reference data to use these strategies for confirming a food or feed product as authentic. Because there are numerous factors that influence the various analytical parameters (e.g. molecular fingerprints and stable isotope ratios are affected by geographic and climatic factors), the continuous update (use and maintenance) of these databases are required for a reliable and contemporary assessment of current authenticity issues, in particular the geographical origin. In addition, a sufficient number of representative samples covering the major relevant sources of variance expected for the specific case (such as different geographical origin, production processes, storage conditions, seasoning, etc.) or comparative data are crucial for making statistically reliable statements. Therefore, there are also high demands on the validity of the measured results and the compatibility of the data formats used.

With regard to depositing and probably even sharing authenticity data in common repositories, a further challenge is how to realise such (jointly used) databases with regard to administration and access rights (who is authorised to upload analytical data or restricted to read permission). These questions are intensified in the field of non-targeted analysis through the challenge of uniform data exchange formats of each set of spectral data, as well as the additional communication of necessary metadata. Therefore, creation of databases containing fingerprinting data is a topic of current scientific activities (see also ”BfR activities”).

BfR activities

For more than ten years, the BfR has been continuously participating in research projects on the authenticity of food and feed in terms of geographical origin, species differentiation, adulteration, development of reference databases, supply chain analysis and early risk detection. With regard to preventive consumer and public health protection, BfR focuses in particular on the method development for detecting anomalies in food and feed products through verification of geographical origin, detection of unknown (potentially hazardous) adulterants including identifying unauthorized additions in animal feed. Moreover, the harmonization of innovative non-targeted approaches including validation efforts and database development is of major interest. These approaches are relevant to all stages in the supply chain and of high interest for raw products such as feeding materials as well as for highly processed food such as wine. Some of the very recent projects are presented in Figure 21 and described below.

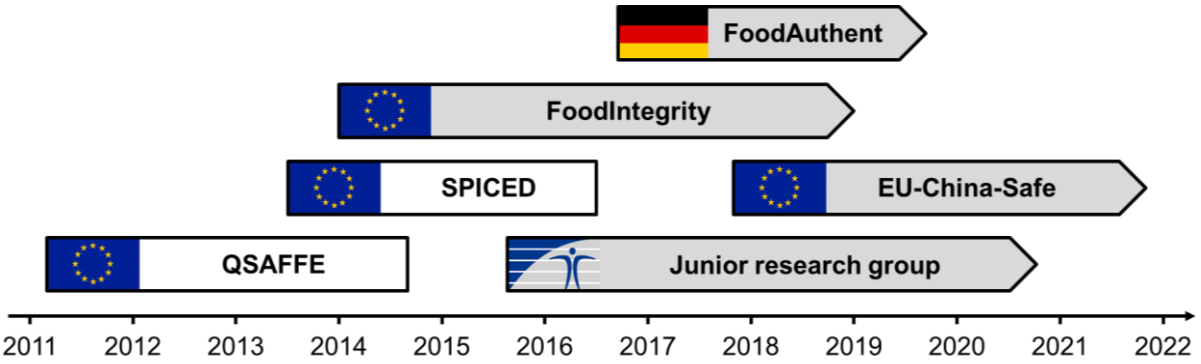


Figure 21: International, national and in-house research projects of BfR on food and feed authentication in the context of global supply chains
Ongoing research projects are highlighted in grey.

Ongoing research projects on food and feed authentication

Junior research group "Authenticity along the Supply Chain":

Since 2015 the BfR junior research group “Authenticity along the Supply Chain” is active in developing innovative strategies and analytical methods for authentication issues including authentication of animal proteins in food and feed, verification of the geographical origin, and detection of (unknown) adulteration using spectrometry and spectroscopy. Further, for taking forward the standardisation of non-targeted methods a focus is the development of suitable concepts for validation and investigations concerning the comparability of fingerprinting techniques (Riedl et al., 2015).

For example, a research project of BfR aims at developing a strategy to verify the geographical origin of grain maize. Maize is a major feedstuff, which is produced and traded worldwide. Furthermore, it serves as a crude material for many food products such as corn oil, flour, cornflakes and popcorn. Classical methods for the verification of the geographical origin, like stable isotope analysis or element analysis, reach their limits at separating regions that are similar in terms of climate or geology. Therefore, spectroscopic methods (mid infrared and NMR) are combined with multivariate techniques for classification of maize and its origin on a global, European and national base. The long-term objective is to use these models for predicting the geographical origin of unknown samples or to verify the origin of con-

spicuous samples or to identify suspicious samples (e.g. no match between claim and analytical verification).
In recent years progress in the development of non-targeted approaches has been made for food and feed authentication. Still, a big challenge is the standardisation of these techniques. While there are clear guidelines for the method validation and comparability of targeted analyses, they are missing for non-targeted methods. Furthermore, the comparability of the results of non-targeted methods, e.g. between instrument types or laboratories, has to be ensured. Because this aspect is scarcely addressed in previous publications, another recently started project of BfR will focus on investigations of the comparability of non-targeted analytical methods.

FoodAuthent:

The 3-year project FoodAuthent is supported by funds of the Federal Ministry of Food and Agriculture (BMEL) based on a decision of the Parliament of the Federal Republic of Germany via the Federal Office for Agriculture and Food (BLE) under the innovation support programme. FoodAuthent is being carried out in cooperation with five German project partners from research and industry and follows major BfR research goals to facilitate the broader up-take of non-targeted methods for authentication in routine application. The project's main objectives include the development of new analytical methods for spirits, edible oils and dairy products, comprehensive strategies for quality assurance and comparability of analytical results, and the establishment of a cloud-based database for broad use. It aims to protect consumers from adulterated or mislabelled food (FoodAuthent, 2018).

EU-China-Safe:

Comprising 15 participants from Europe and 18 partners from China the recently started 4-year project EU-China-Safe (EU's Horizon 2020 programme) will mobilise resources in Europe and China aiming at an implementation of improved detection capabilities for chemical/microbiological hazards and food fraud in a harmonised way. Implementation is to be realised on the basis of an EU-China Joint Laboratory Network, which is to be set up and that will achieve and demonstrate equivalency of results, and will develop a state of the art virtual laboratory, with interchangeable staff from two continents, that will be used as a "showcase" to communicate and demonstrate best practice.
Main objectives are to develop new/improved food authenticity surveillance systems as well as to improve transparency in management of the supply chain through the development of innovative traceability tools. Herewith, the project focuses on the most commonly reported food linked to chemical and microbiological contaminations and fraud such as dairy products and infant formula, processed meat, vegetables, wine, honey and spices. As the national senior expert office for the import control of wine, BfR contributes to EU-China-Safe with product knowledge and experience in respect of wine analysis and authenticity assessment. In addition the BfR carries out research on the traceability tools dedicated for fraud detection in the wine supply chain Europe – China, as well as on the vulnerability analysis along the wine production steps (EU-China-Safe, 2018).

Recently finished research projects on food and feed authentication

FoodIntegrity:

With regard to standards and databases in food authentication, BfR promotes research within the European 5-year project FoodIntegrity (EU's 7th framework programme) together with more than 60 collaboration partners from Austria,

Belgium, China, Czech Republic, Denmark, France, Germany, Great Britain, Hungary, Iceland, Ireland, Italy, the Netherlands, Norway, Poland, Portugal, Spain and others. The project aims to establish an international network of experts and stakeholders in food authenticity, safety and quality as well as to collect and evaluate analytical methods for authentication in research and official control. Within the FoodIntegrity project BfR is involved in

- preparation of scientific opinions on crucial authentication topics for various stakeholders (Camin et al., 2017; McGrath et al., 2018),
- development of a knowledge base for analytical methods, and
- determination of present and future food integrity gaps.

Hereby, the project contributes to improve the security of national and international food chains in terms of the authenticity and quality of food and to protect the food chain from being adulterated (FoodIntegrity, 2018).

QSAFFE:

The major goal of the European project QSAFFE (EU's 7th framework programme) was to deliver better, faster and more economically viable means of ensuring the quality and safety of animal feed in Europe. Therefore, the consortium aimed to develop an integrated approach to the reduction and management of chemical and microbiological contamination in animal feed by developing strategies for early quality and safety assurance in the feed chain. Existing testing methods and emerging technologies such as fingerprinting were used to deliver a comprehensive analytical strategy for monitoring of safety as well as authenticity issues at ports, feed mills and laboratories. This project was the first EU research that included authenticity issues - adulteration and origin - to feed material, what will become more important in future, particularly in view of early risk assessment and management in the food and feed chain in case of health relevant fraudulent practices (QSAFFE, 2018).

SPICED:

BfR was coordinator of the European project SPICED (EU's 7th framework programme). The 3-year project aimed at securing the spices and herbs commodity chains in Europe against deliberate, accidental or natural biological and chemical contamination (SPICED, 2018; Székács et al., 2018).

The SPICED project introduced non-targeted methodologies to the very sensitive spice supply chains and in due of the project and related research studies several fraudulent practises (e.g. the addition of olive leaves in oregano) were discovered. One analytical objective of the project was the development of high-throughput methods for spice authentication and broad anomaly testing. For this, various spectroscopic and spectrometric-based fingerprinting techniques have been tested for the purpose of determining deviations from typical product characteristics, particularly the addition of unknown adulterants. For example, BfR combined Fourier transform infrared (FTIR) spectroscopy and a one-class classification technique (soft independent modelling of class analogy (SIMCA)) for the non-targeted detection of paprika adulteration. For this, a representative set of commercially available paprika powders was investigated to determine the data space of non-adulterated samples and define the critical limits for further classification. The performance of the established model for adulteration detection was tested by predicting artificially spiked samples with various adulterants both organic and inorganic colourants and bulking materials (gum arabic, lead chromate, lead oxide, polyvinyl chloride, silicon dioxide, Sudan I and Sudan IV). Further, the influence of data preprocessing on the model performance was investigated. The results of the study

demonstrate the potential of the fingerprinting approach for a broad anomaly testing (Horn et al., 2018).

Conclusion

Because food fraud is a global issue and prediction of incidents is hardly possible, authenticity testing of food and feed products from the raw material to the processed food/feed product becomes of increasing interest worldwide. Although economically motivated in the first place, a subsequent threat of public health is always possible. In the past, fraudulent practices have been revealed which indeed posed a serious health risk to consumers. Therefore, lots of effort has been directed in the development of analytical approaches for the verification of the authenticity of food and feed. The targeted analysis of exogenous marker compounds as well as the determination of authentic ranges of natural ingredients are already used in official control for food and feed authentication. In the last few years non-targeted analysis using fingerprinting techniques obtained increasing importance. The application of fingerprinting is an evolving trend in food and feed authentication and is considered to be especially advantageous in the detection of unknown and unforeseen adulterants at all stages of the supply chain. However, the use of these methods in routine analysis and food surveillance is still limited because of lacking important prerequisites, such as method validation strategies and reliable databases. BfR will continue to contribute to solutions by performing excellent research in national and international projects.

References

Alewijn, M., van der Voet, H., and van Ruth, S. (2016). Validation of multivariate classification methods using analytical fingerprints – concept and case study on organic feed for laying hens. *Journal of Food Composition and Analysis, 51*, 15-23. doi:10.1016/j.jfca.2016.06.003

Bitzios, M., Jack, L., Krzyzaniak, S.-A., and Xu, M. (2017). Country-of-Origin Labelling, Food Traceability Drivers and Food Fraud: Lessons from Consumers' Preferences and Perceptions. *European Journal of Risk Regulation, 8*(3), 541-558. doi:10.1017/err.2017.27

Camin, F., Boner, M., Bontempo, L., Fauhl-Hassek, C., Kelly, S. D., Riedl, J., and Rossmann, A. (2017). Stable isotope techniques for verifying the declared geographical origin of food in legal cases. *Trends in Food Science and Technology, 61*, 176-187. doi:10.1016/j.tifs.2016.12.007

COSMOS. (2018). Developing an efficient e-infrastructure, standards and data-flow for metabolomics and its interface to biomedical and life science e-infrastructures in Europe and world-wide. *https://cordis.europa.eu/project/rcn/104204_de.html*.

Dalsecco, L. S., Palhares, R. M., Oliveira, P. C., Teixeira, L. V., Drummond, M. G., and de Oliveira, D. A. A. (2018). A Fast and Reliable Real-Time PCR Method for Detection of Ten Animal Species in Meat Products. *Journal of Food Science, 83*(2), 258-265. doi:10.1111/1750-3841.14001

Danezis, G. P., Tsagkaris, A. S., Brusic, V., and Georgiou, C. A. (2016). Food authentication: state of the art and prospects. *Current Opinion in Food Science, 10*, 22-31. doi:10.1016/j.cofs.2016.07.003

EC, European Commission. (2008). Commission Regulation (EC) No 555/2008 of 27 June 2008 laying down detailed rules for implementing Council Regulation (EC) No 479/2008 on the common organisation of the market in wine as regards support programmes, trade with third countries, production potential and on controls in the wine sector. *Official Journal of the European Union, L 170*, 1-80.

Esslinger, S., Riedl, J., and Fauhl-Hassek, C. (2014). Potential and limitations of non-targeted fingerprinting for authentication of food in official control. *Food Research International, 60*, 189-204. doi:10.1016/j.foodres.2013.10.015

EU-China-Safe. (2018). Delivering an Effective, Resilient and Sustainable EU-China Food Safety Partnership. *https://cordis.europa.eu/project/rcn/210500_en.html*.

Eur. Parliament, and The Council. (2002d). Regulation (EC) No 178/2002 of the European Parliament and the council of the European Union of 28 January 2002 laying down the general principles and requirements of food law, establishing the European Food Safety Authority and laying

down procedures in matters of food safety. *Official Journal of the European Communities, L 31*, 1-24.

Everstine, K., Abt, E., McColl, D., Popping, B., Morrison-Rowe, S., Lane, R. W., . . . and Chin, H. B. (2018). Development of a Hazard Classification Scheme for Substances Used in the Fraudulent Adulteration of Foods. *Journal of Food Protection, 81*(1), 31-36. doi:10.4315/0362-028x.Jfp-17-173

Fauhl-Hassek, C., Esslinger, S., Herold, T., Jäger, M., and Hensel, A. (2015). Herkunftsanalyse von Lebens- und Futtermitteln - Stand der Technik und Perspektiven. *Rundschau für Fleischhygiene und Lebensmittelüberwachung, 9*, 331-333.

Fernqvist, F., and Ekelund, L. (2014). Credence and the effect on consumer liking of food – A review. *Food Quality and Preference, 32*, 340-353. doi:10.1016/j.foodqual.2013.10.005

FoodAuthent. (2018). Entwicklung eines Systems zur Sammlung, Analyse und Verwertung von Produktauthentizitätsdaten. *https://www.foodauthent.de*.

FoodIntegrity. (2018). Ensuring the Integrity of the European food chain. *https://cordis.europa.eu/project/rcn/110951_en.html*.

Gallo, V., Intini, N., Mastrorilli, P., Latronico, M., Scapicchio, P., Triggiani, M., . . . and Valerio, M. (2015). Performance Assessment in Fingerprinting and Multi Component Quantitative NMR Analyses. *Analytical Chemistry, 87*(13), 6709-6717. doi:10.1021/acs.analchem.5b00919

Gossner, C. M.-E., Schlundt, J., Ben Embarek, P., Hird, S., Lo-Fo-Wong, D., Beltran, J. J. O., . . . and Tritscher, A. (2009). The Melamine Incident: Implications for International Food and Feed Safety. *Environmental Health Perspectives, 117*(12), 1803-1808. doi:10.1289/ehp.0900949

Handford, C. E., Campbell, K., and Elliott, C. T. (2016). Impacts of Milk Fraud on Food Safety and Nutrition with Special Emphasis on Developing Countries. *Comprehensive Reviews in Food Science and Food Safety, 15*(1), 130-142. doi:10.1111/1541-4337.12181

Horn, B., Esslinger, S., Pfister, M., Fauhl-Hassek, C., and Riedl, J. (2018). Non-targeted detection of paprika adulteration using mid-infrared spectroscopy and one-class classification – Is it data preprocessing that makes the performance? *Food Chemistry, 257*, 112-119. doi:10.1016/j.foodchem.2018.03.007

Kákosy, T., Hudák, A., and Náray, M. (1996). Lead intoxication epidemic caused by ingestion of contaminated ground paprika. *Journal of Toxicology - Clinical Toxicology, 34*(5), 507-511. doi:10.3109/15563659609028008

Manning, L., and Soon, J. M. (2014). Developing systems to control food adulteration. *Food Policy, 49*, 23-32. doi:10.1016/j.foodpol.2014.06.005

McGrath, T. F., Haughey, S. A., Patterson, J., Fauhl-Hassek, C., Donarski, J., Alewijn, M., . . .and Elliott, C. T. (2018). What are the scientific challenges in moving from targeted to non-targeted methods for food fraud testing and how can they be addressed? – Spectroscopy case study. *Trends in Food Science and Technology, 76*, 38-55. doi:10.1016/j.tifs.2018.04.001

Perini, M., and Camin, F. (2013). δ18O of Ethanol in Wine and Spirits for Authentication Purposes. *Journal of Food Science, 78*(6), C839-C844. doi:10.1111/1750-3841.12143

QSAFFE. (2018). Quality and Safety of Feeds and Food for Europe. *https://cordis.europa.eu/project/rcn/97821_en.html*.

Rebane, R., Leito, I., Yurchenko, S., and Herodes, K. (2010). A review of analytical techniques for determination of Sudan I-IV dyes in food matrixes. *Journal of Chromatography A, 1217*(17), 2747-2757. doi:10.1016/j.chroma.2010.02.038

Riedl, J., Esslinger, S., and Fauhl-Hassek, C. (2015). Review of validation and reporting of non-targeted fingerprinting approaches for food authentication. *Analytica Chimica Acta, 885*, 17-32. doi:10.1016/j.aca.2015.06.003

Salek, R. M., Neumann, S., Schober, D., Hummel, J., Billiau, K., Kopka, J., . . . and Steinbeck, C. (2015). COordination of Standards in MetabOlomicS (COSMOS): facilitating integrated metabolomics data access. *Metabolomics, 11*(6), 1587-1597. doi:10.1007/s11306-015-0810-y

Sobolev, A. P., Circi, S., Capitani, D., Ingallina, C., and Mannina, L. (2017). Molecular fingerprinting of food authenticity. *Current Opinion in Food Science, 16*, 59-66. doi:10.1016/j.cofs.2017.08.002

SPICED. (2018). Securing the spices and herbs commodity chains in Europe against deliberate, accidental or natural biological and chemical contamination. *https://cordis.europa.eu/project/rcn/108959_en.html*.

Spink, J., and Moyer, D. C. (2011). Defining the Public Health Threat of Food Fraud. *Journal of Food Science, 76*(9), R157-R163. doi:10.1111/j.1750-3841.2011.02417.x

Székács, A., Wilkinson, M. G., Mader, A., and Appel, B. (2018). Environmental and food safety of spices and herbs along global food chains. *Food Control, 83*, 1-6. doi:10.1016/j.foodcont.2017.06.033

4 Chemical Risks and Toxins in Feed and Food

Lahrssen-Wiederholt, Monika; Mader, Anneluise

Introduction

The challenge of bioaccumulative substances that enrich in animals and humans is to understand its origin (natural/anthropogenic) and the path along the food chain including the so-called biomagnification. Even if research is quite complex when dealing with supply chains, the BfR is convinced that the global supply chain approach will improve the risk assessment. It enables a holistic provision of information as well as point up different action options either for the consumer as well as the risk manager.

In section 4.1 and 4.2 the transfer of chemical agents and natural toxins along the food chain of animal-derived products is discussed using the emerging issue of per- and polyfluoroalkyl substances (PFAS) and natural toxins in aquatic systems as examples. Both, the industrial chemicals PFAS, with its versatile application in consumer-related products and industrial processes, as well as the natural marine biotoxins, in fish from tropical and subtropical fishing regions, which are gaining in popularity on the EU market, requires an understanding of the global substance flows both in the food web and the food chain in order to identify the origin of contamination and contributing factors.

Section 4.3 is dealing with natural toxins in feed and food processing chains of plant-based products. Several natural toxins can occur in feeds and foods, and are either due to contamination with microorganisms (as in the case of mycotoxins) or being produced directly by the organisms the feed/food is derived from (like for cyanotoxins). Mycotoxins are for example typically found in cereals and fruits and therefore, a brief overview on exemplified processing chains of wheat bread and apple juice describe their behavior in the feed and food chain. Cyanotoxins are secondary metabolites generated by some genera of cyanobacteria (blue-green algae). In order to protect consumers from allergic reactions, liver damages, and other consequences, regular analytical controls of blue-green algae-based food supplements (BGAS) are necessary.

In Section 4.4 one focuses on toxin detection and quantification – regulatory and analytical aspects. It covers a selection of items that are directly or indirectly linked to each other in order to meet these measures at a national and international level as well. General aspects and challenges regarding the various analytical approaches with a special emphasis on new “emerging” toxins and the corresponding work and interconnections of standardization bodies are also discussed.

Future needs

To allow better work within the field of PFAS, it is recommended to establish maximum values for PFCAs and PFSAs in food, at least of PFOA and PFOS and to develop appropriate analytical methods for the determination of “total” PFAS concentration in different matrices. Further an establishment of scientific knowledge on the degradation of precursor compounds is needed to assess their impact on the supply chain and their additive effects on the PFCAs and PFSAs.

For marine biotoxins the development and validation of alternative screening methods for the control and of confirmatory methods as well as the availability of analytical standards especially for emerging ones are of interest. Further it is recommended to generate occurrence data of emerging toxins like TTX and CTX to enable comprehensive risk assessment, develop sampling strategies for ciguatoxic fish, e.g. supported by marine biology, meteorology and

oceanography data, and to improve risk communication and educational measures in third countries as fish and shellfish producers.

More reliable data on modified and emerging mycotoxins regarding occurrence/fate during (industrial) processing as well as on toxicology are needed. This is especially true for more data on mycotoxins other than aflatoxins regarding a potential transfer from feed via food-producing animals to humans. In addition, analytical methods used for these controls require adequate reference materials, but availability of the latter is very limited so far.

4.1 Transfer of per- and polyfluoroalkyl substances (PFAS) along the food chain

Kowalczyk, Janine

Abstract

Substances with bioaccumulative properties are able to enrich in animals and humans. If species are in trophic relationships, bioaccumulative substances are passed along the food chain. The so-called biomagnification occurs mainly for lipophilic substances, which are only slowly degraded and excreted by the body. Hence, species within the highest trophic level enriches higher concentrations of bioaccumulative substances than animals within lower trophic levels. For consumer health protection, it has to be kept in mind that humans are at the top of the food chain. The consumption of food of animal origin, either of aquatic or terrestrial origin, can therefore be expected to increase human exposure to lipophilic substances, which is critical for those being highly persistent and/or toxic. For health risk assessment, the challenge in addition to the assessment of the toxicological risks of bioaccumulative substances for humans is to understand its origin (natural / anthropogenic) and path along the food chain.

The industrial chemicals, such as per- and polyfluoralkyl substances (PFAS), with its versatile application in consumer-related products and industrial processes requires an understanding of the global substance flows both in the food web and the food chain in order to identify the origin of contamination. Such approach will improve the risk assessment because it enables a holistic provision of information as well as point up different action options either for the consumer as well as the risk manager.

The emerging issue of PFAS

Per- and polyfluoralkyl substances (PFAS) assign to anthropogenic chemicals that contain a carbon chain of different length on which all the H atoms are substituted by fluorine. The family of persistent organic chemicals has been produced since the mid-20th century. PFAS exhibit unique properties for industrial and consumer applications imparted by the C-F bond, the strongest bond in nature, and the variety of perfluoroalkyl moieties (CnF2n+1-). Worth particular mention here are the extremely low surface tension resulting in water- and oil-repellency and the thermal stability (OECD, 2018). Based on desired functionality and manufacturing process PFAS are applicated e.g. in the fluoropolymer production, electroplating, various fire-Fighting foams, cleaning and impregnating agents or as surface treatment of textile, leather, and papers (Kotthoff et al., 2015; Wang et al., 2014). The wide variety of PFAS for industrial applications and its usage in numerous consumer products led to ubiquitous distribution around the world in matrices like soils and sediments, surface and groundwater, plants, wildlife and humans (Bjerregaard-Olesen et al., 2017; Cai et al., 2012; Rankin et al., 2016; Sedlak and Greig, 2012). PFAS undergo long-range transport via ocean currents or atmosphere and subsequently deposit in the environment even in remote regions such as the Arctic or alpine lakes that are not directly impacted by humans (Benskin et al., 2011; Wong et al., 2018; Yeung et al., 2006). Studies of PFAS biomagnification in marine and terrestrial food webs show increasing PFAS concentrations along the trophic levels with highest levels in top predators (Houde et al., 2006; Müller et al., 2011). Among the entire PFAS family, multiple long-chain PFASs of the subgroups perfluoroalkyl carboxylic acids (PFCAs) with seven or more perfluorinated carbons, and perfluoroalkyl sulfonic acids (PFSAs) with six or more perfluorinated carbons are recognized as contaminants of high concern due to their persistence, accumulation potential, toxicity in mammals and biomagnification along the food chain (Ahrens and Bundschuh, 2014; EFSA, 2008b; Houde et al., 2006; Müller et al., 2011; Pérez et al., 2013). The most frequently detected PFCA and PFSA are perfluorooctanoic acid (PFOA) and perfluorooctane sulfonic acid (PFOS), respectively. Because of concern

regarding their hazardous health and environmental impacts various actions have been taken by the industry and regulatory agencies to restrict the environmental and human exposure to PFOA and PFOS and their precursor compounds. For instance, since 2000-2002, the major global producers of PFCAs and fluoropolymers have voluntarily stopped the production of long-chain PFCAs and PFSAs, including PFOA and PFOS, and implemented techniques to reduce industrial emissions and PFAS-impurities in products. In addition, PFOS and related compounds were included in the Annex B (restricted production and use) of the Stockholm Convention on Persistent Organic Pollution in 2009. Moreover, PFCAs with a chain length of 11 to 14 carbons as well as PFOA and its ammonium salt (AFPO) have been added to the Candidate List of Substances of Very High Concern (SVHCs) for Authorization under REACH (ECHA, 2018).

Global emission and entrance into the food chain

Analyses of environmental samples indicate a widespread distribution of PFAS in different matrices throughout the globe. Numerous efforts have been made to identify the industrial sources and understand the environmental fate of PFAS. For PFOA and PFOS two emission sources have been identified. Firstly, direct emission sources where PFOA and PFOS are emitted during the manufacturing process or the industrial application as processing aids or from consumer products (Paul et al., 2009; Wang et al., 2014). Furthermore, when fluorochemicals were commercially synthesized e.g. by electrochemical fluorination, a complex PFAS mixture is generated which does not only contain the major constituent but also up to 30% homologues; those impurities may include PFOA or PFOS (D'Eon and Mabury, 2007; Paul et al., 2009). In consequence of the legal restriction and voluntary phase-out of industry in Japan, Western Europe and United States the direct emission of PFOA and PFOS decrease in these regions. At the same time, a geographical shift in manufacturing PFCAs, fluoropolymer and other PFAS could be observed from the industrialized countries to the emerging economies in particular Asia, e.g. China and India (Wang et al., 2014). Wang and coworkers have estimated the total global emission of PFCAs from 1951 – 2030 (Figure 22). They demonstrated that the annual PFCA emission increases much faster in the emerging economies than it declines in the industrial countries, thus causing a re-increase of the global emission in total (Wang et al., 2014). Reasons discussed were economic and political factors like emerging market with low costs of raw material as well as of well-educated workers plus low regulation of the chemical industry (Wang et al., 2014).

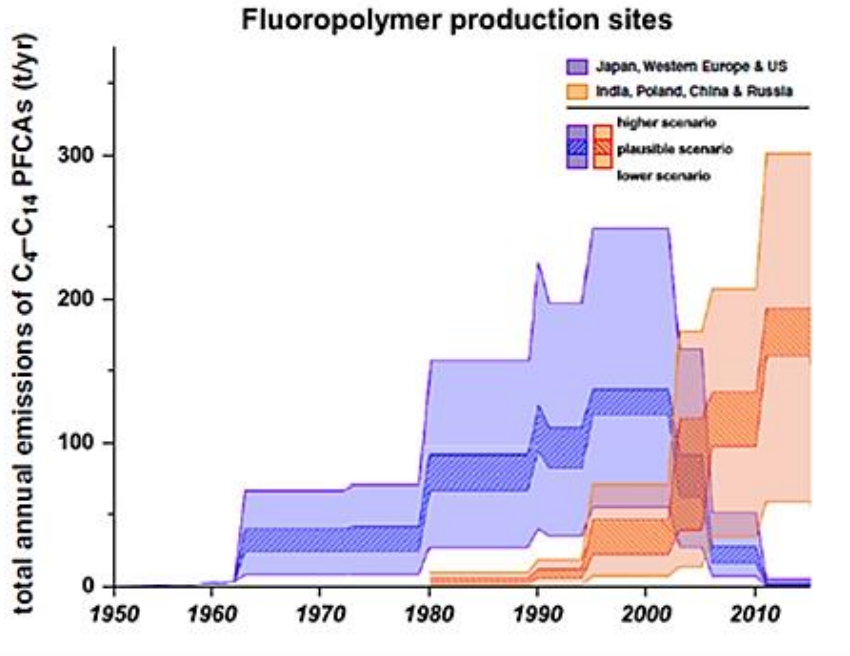


Figure 22 Estimated annual release of PFCAs from fluoropolymer production sites in the United States (US), Western Europe and Japan (purple) as well as in China, Russia, Poland and India (orange)
The colored areas represent the estimated ranges of annual emissions based on the full ranges of the ammonium perfluorooctanoic acid (APFO) / sodium perfluorooctanoic acid (NaPFO) use rate of 0.3 wt% of relevant fluoropolymers produced (plausible scenario). (Adapted from Figure 6 of Wang et al. 2014 (Wang et al., 2014))

Indirect sources that have been discussed are the degradation of precursor compounds to the not further degradable substances PFCAs or PFSAs such as PFOA and PFOS, respectively either through the atmospheric transformation of volatile precursors, the thermolysis of fluoropolymers or the biodegradation of precursors in biota (Butt et al., 2014; Scott et al., 2006; Young and Mabury, 2010). Here, PFOA and PFOS are expected to be released into the environment as residuals or by commercial fluorochemicals through subsequent biotransformation during the product life-cycle (D'Eon and Mabury, 2007; Wang et al., 2014).

Regardless of direct and indirect sources or on which stage of the product's life-cycle the emission occurs, PFAS can enter into the environment via air, water and soil, and thereby the food chain (Figure 23). Depending on the physico-chemical properties (water solubility, vapor pressure, sorption to particles) PFAS undergo long-range transport in different ways, and therefore, can be detected ubiquitously around the world independently of the manufacturing locations and the global trade routes of PFAS containing goods. Apart from former and current manufacturing sites, the usage of PFAS in consumer products and disposal are considered to be important for the total global PFAS emissions (Paul et al., 2009). The usage of PFAS containing consumer products is noticeable by detection of fluorochemicals in wastewater treatment plants (WWTPs), which cannot be removed efficiently during wastewater treatment (Bossi et al., 2008; Guo et al., 2010; Schultz et al., 2006; Yu et al., 2009). Hence, the discharge of sewage from WWTPs is recognized to be a significant source for PFAS in the environment. Furthermore, if sewage sludge is spread on agricultural fields PFAS can directly enter into the food chain. Here, the product life-cycle closes, see Figure 23.

Next to emissions at the stage of manufacturing, that is at least limited to technically unavoidable emissions, and the usage stage the current and past extensive inputs of PFAS into the food chain can be attributed to point-source pollutions. In Germany, point-source pollu-

tions are related to the utilization of PFAS containing aqueous film-forming foams (AFFFs) on e.g. former military training grounds or military/commercial airports, but also to illegal disposal of PFAS containing industrial sludge on agricultural farmland, as happened in the Region Sauerland in 2006 and in North and Middle Baden in 2013 (RP-Karlsruhe, 2018; Wilhelm et al., 2008). From the time on the first serious German PFAS pollution case in 2006, the BfR dedicated itself intensively to the PFAS transfer along the food chain.

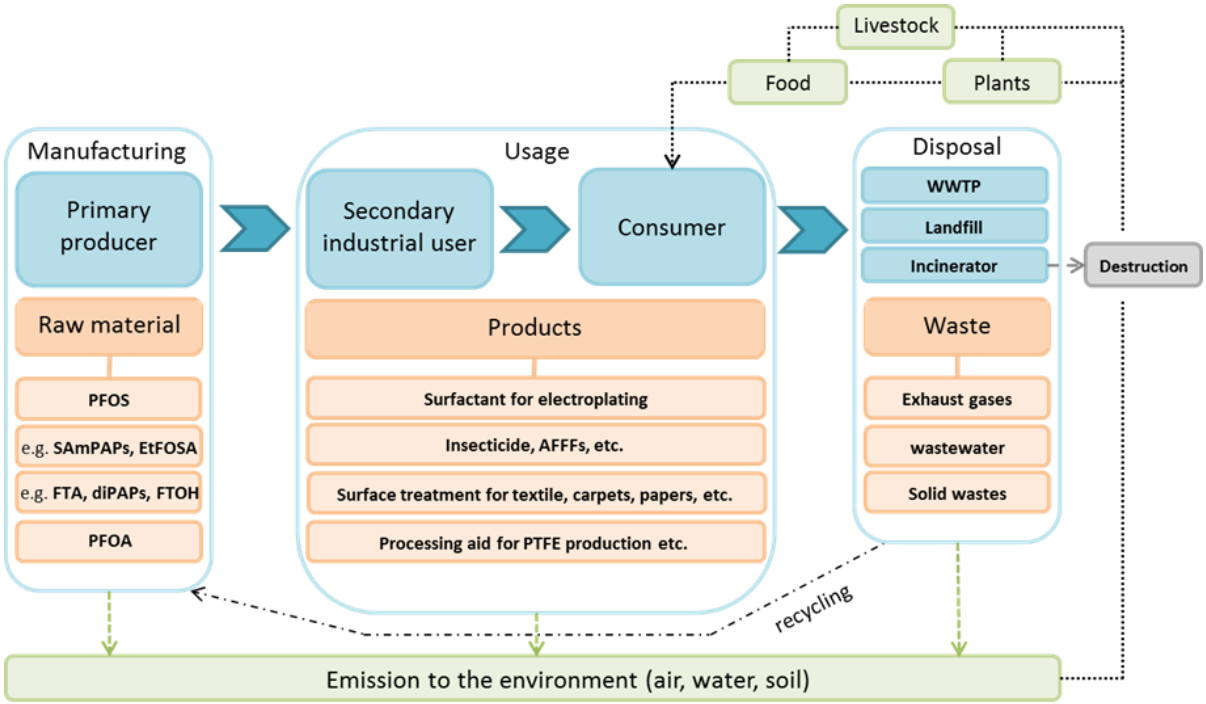


Figure 23 **Scheme of the fate of PFAS through product life-cycle, their emissions and entering into the food chain (Adapted from Figure 1, Figure S2 and Figure S9 of Wang et al. 2014 (Wang et al., 2014) and Figure 1 of Wang et al. 2017 (Wang et al., 2017))**
PFOS = perfluorooctane sulfonic acid; SAmPAPs = Perfluorooctane sulfonamidoethanol-based phosphate esters; EtFOSA = N-ethyl perfluorooctane sulfonamide; FTA = fluorotelomer acrylate; diPAPs = polyfluoroalkyl phosphoric acid diesters; FTOH = fluorotelomer alcohol; PFOA = perfluorooctanoic acid; AFFFs = aqueous film-forming foams; PTFE = polytetrafluoroethylene; WWTP = waste water treatment plant

Transfer of PFAS along the food chain

The soil is an environmental sink for persistent pollutants and concurrently an important starting point of the food chain. From the consumer risk assessment perspective, the concern of soil pollution grows if it is directly related to human health. Therefore, the BfR conducts research to investigate the path of contaminants from farm to fork. A particular emphasis is to track the path of PFAS from contaminated soil to feed and to food of animal origin. The need of knowledge about the path of PFAS along the food chain has arisen when the first big environmental pollution case became evident in the Sauerland region in 2006. At that time, hardly information existed about the fate of PFAS along the soil-plant-animal/food-chain.

Since that time, the scientific knowledge about PFAS has been increased. Transfer studies have shown that there is an uptake of PFAS from soil to plant (Krippner et al., 2014; Stahl et al., 2013). If livestock were fed those plants PFAS will transfer into animal tissue and corresponding food of animal origin. However, studies indicate a different PFAS transfer from feed to animal-derived foods that depend on, for example, the chemical structure of the respective PFAS compounds, its toxicokinetics in the different livestock species, the type of animal food and the duration of feeding the PFAS-containing feed. Within the scope of the EU project

INTEREGG, the BfR examined the transfer of PFCAs and PFSAs from feed to food of animal origin like milk, meat and eggs by conducting feeding studies on dairy cattle, fattening pigs and laying hens.

The results show large differences in the toxicokinetic behavior of the substances depending on the animal species. In the case of cattle, for example, only a small proportion of the ingested PFCAs and PFSAs have been transferred into the milk, while laying hens excreted a significantly larger proportion of the alimentary PFCAs and PFSAs via the eggs (Kowalczyk et al., 2013). In pigs, the half-life of these substances in the body was significantly longer compared to other animal species (years vs. hours) and was of the same magnitude as in humans (Numata et al., 2014).

Humans are primarily exposed to PFAS via drinking water and food (EFSA, 2008b). PFAS are detectable in both foodstuffs of plant and animal origin. The PFAS levels are below the analytical limits of quantification in most food groups (EFSA, 2012a). However, the consumption of foods with low levels of PFAS for an extended period of time may result in an increase in body burden for these compounds due to their long half-lives in the human body.

The persistence, mobility and accumulation of the substances in the environment as well as the long half-lives in the human body are supposed to be a sufficiently critical issue from the consumer health perspective to justify the demand to avoid as far as possible further inputs of PFAS into the environment.

Studies conducted by the BfR with wild boars of the North, South and West of Germany as well as in redfish and cod of the Barents Sea are an indicator of environmental pollution in both civilian and remote regions (Kowalczyk, Flor, et al., 2018; Kowalczyk, Numata, et al., 2018).

Challenges

In 2018, the OECD published a comprehensive global database of PFAS that shows the identification of in total 4730 PFAS-related CAS numbers, which includes PFAS with diverse structure and at least one perfluoroalkyl moiety (OECD, 2018). The analytically well-measurable PFAS are currently limited to PFCA and PFSA of chain length C4 to C14. At present, only a very few laboratories are able to analyze precursors. These currently include the most prominent precursors for PFOA and PFOS. To its own estimates, less than 1% of the PFAS identified by OECD 2018 are analytically detectable. In addition, there are wide gaps in knowledge for the degradability of precursor in the analytically measurable and toxicologically well characterized PFCAs and PFSAs. This fact poses two challenges for the authorities: a) appropriate analytical methods have to be developed for the determination of the total PFAS concentration; and b) scientific knowledge on the degradation of precursors is needed to assess their impact on the supply chain and their additive effects on the PFCAs and PFSAs.

Acknowledgements

The BfR greatly acknowledges the financial support from the INTERREG IV-A project SafeGuard (Förderkennzeichen der EU: INTERREG IVa III-2-03=025).

Abbreviations

AFFFs	aqueous film-forming foams
APFO	ammonium perfluorooctanoic acid
diPAPs	polyfluoroalkyl phosphoric acid diesters
ETFOSA	N-ethyl perfluorooctane sulfonamide
FTA	fluorotelomer acrylate
FTOH	fluorotelomer alcohol
LC-MS/MS	Liquid Chromatography Tandem Mass Spectrometry
NaPFO	sodium perfluorooctanoic acid
PFAS	per- and polyfluoralkyl substances
PFCAs	perfluoroalkyl carboxylic acids
PFOA	perfluorooctanoic acid
PFOS	perfluorooctane sulfonic acid
PFSAs	perfluoroalkyl sulfonic acids
PTFE	polytetrafluoroethylene
SAmPAPs	Perfluorooctane sulfonamidoethanol-based phosphate esters
SVHCs	Substances of Very High Concern
WWTP	wastewater treatment plant

References

Ahrens, L., and Bundschuh, M. (2014). Fate and effects of poly- and perfluoroalkyl substances in the aquatic environment: a review. *Environ Toxicol Chem, 33*(9), 1921-1929. doi:10.1002/etc.2663

Benskin, J. P., Phillips, V., St. Louis, V. L., and Martin, J. W. (2011). Source Elucidation of Perfluorinated Carboxylic Acids in Remote Alpine Lake Sediment Cores. *Environmental Science & Technology, 45*(17), 7188-7194. doi:10.1021/es2011176

Bjerregaard-Olesen, C., Bossi, R., Liew, Z., Long, M., Bech, B. H., Olsen, J., . . . and Bonefeld-Jørgensen, E. C. (2017). Maternal serum concentrations of perfluoroalkyl acids in five international birth cohorts. *International Journal of Hygiene and Environmental Health, 220*(2, Part A), 86-93. doi:10.1016/j.ijheh.2016.12.005

Bossi, R., Strand, J., Sortkjær, O., and Larsen, M. M. (2008). Perfluoroalkyl compounds in Danish wastewater treatment plants and aquatic environments. *Environment International, 34*(4), 443-450. doi:10.1016/j.envint.2007.10.002

Butt, C. M., Muir, D. C. G., and Mabury, S. A. (2014). Biotransformation pathways of fluorotelomer-based polyfluoroalkyl substances: A review. *Environmental Toxicology and Chemistry, 33*(2), 243-267. doi:10.1002/etc.2407

Cai, M., Yang, H., Xie, Z., Zhao, Z., Wang, F., Lu, Z., . . . and Ebinghaus, R. (2012). Per- and polyfluoroalkyl substances in snow, lake, surface runoff water and coastal seawater in Fildes Peninsula, King George Island, Antarctica. *Journal of Hazardous Materials, 209-210*, 335-342. doi:10.1016/j.jhazmat.2012.01.030

D'Eon, J. C., and Mabury, S. A. (2007). Production of Perfluorinated Carboxylic Acids (PFCAs) from the Biotransformation of Polyfluoroalkyl Phosphate Surfactants (PAPS): Exploring Routes of Human Contamination. *Environmental Science & Technology, 41*(13), 4799-4805. doi:10.1021/es070126x

ECHA, European Chemicals Agency. (2018). Candidate List of substances of very high concern for Authorisation.

EFSA, European Food Safety Authority. (2008b). *Perfluorooctane sulfonate (PFOS), perfluorooctanoic acid (PFOA) and their salts. Scientific Opinion of the Panel on Contaminants in the Food chain*.

EFSA, European Food Safety Authority. (2012a). Perfluoroalkylated substances in food: occurrence and dietary exposure. *EFSA Journal, 10*(6: 2743), 55. doi:10.2903/j.efsa.2012.2743

Guo, R., Sim, W.-J., Lee, E.-S., Lee, J.-H., and Oh, J.-E. (2010). Evaluation of the fate of perfluoroalkyl compounds in wastewater treatment plants. *Water Research, 44*(11), 3476-3486. doi:10.1016/j.watres.2010.03.028

Houde, M., Bujas, T. A. D., Small, J., Wells, R. S., Fair, P. A., Bossart, G. D., . . . and Muir, D. C. G. (2006). Biomagnification of Perfluoroalkyl Compounds in the Bottlenose Dolphin (Tursiops

truncatus) Food Web. *Environmental Science & Technology, 40*(13), 4138-4144. doi:10.1021/es060233b

Kotthoff, M., Muller, J., Jürling, H., Schlummer, M., and Fiedler, D. (2015). Perfluoroalkyl and polyfluoroalkyl substances in consumer products. *Environ Sci Pollut Res Int, 22*(19), 14546-14559. doi:10.1007/s11356-015-4202-7

Kowalczyk, J., Flor, M., Karl, H., and Lahrssen-Wiederholt, M. (2018). Perfluoroalkyl substances (PFAS) in beaked redfish (Sebastes mentella) and cod (Gadus morhua) from arctic fishing grounds of Svalbard. *in prep.*

Kowalczyk, J., Numata, J., Zimmermann, B., Klinger, R., Habedank, F., Just, P., . . . and Lahrssen-Wiederholt, M. (2018). Suitability of Wild Boar (Sus scrofa) as a Bioindicator for Environmental Pollution with Perfluorooctanoic Acid (PFOA) and Perfluorooctanesulfonic Acid (PFOS). *Arch Environ Contam Toxicol*. doi:10.1007/s00244-018-0552-8

Kowalczyk, J., Schafft, H., and Lochotzke, H.-M. (2013). Akkumulation von Perfluoralkylsäuren (PFAAs) in essbaren Geweben landwirtschaftlicher Nutztiere. *UMID, 4*, 31-37.

Krippner, J., Brunn, H., Falk, S., Georgii, S., Schubert, S., and Stahl, T. (2014). Effects of chain length and pH on the uptake and distribution of perfluoroalkyl substances in maize (Zea mays). *Chemosphere, 94*, 85-90. doi:10.1016/j.chemosphere.2013.09.018

Müller, C. E., De Silva, A. O., Small, J., Williamson, M., Wang, X., Morris, A., . . . and Muir, D. C. G. (2011). Biomagnification of Perfluorinated Compounds in a Remote Terrestrial Food Chain: Lichen–Caribou–Wolf. *Environmental Science & Technology, 45*(20), 8665-8673. doi:10.1021/es201353v

Numata, J., Kowalczyk, J., Adolphs, J., Ehlers, S., Schafft, H., Fuerst, P., . . . and Greiner, M. (2014). Toxicokinetics of seven perfluoroalkyl sulfonic and carboxylic acids in pigs fed a contaminated diet. *J Agric Food Chem, 62*(28), 6861-6870. doi:10.1021/jf405827u

OECD, Organisation for Economic Co-operation and Development,. (2018). Towards a new comprehensive global database of per- and polyfluoroalkyl substances (PFASs): Summary report on updateing the OECD 2007 list of per- and polyfluoroalkyl substances (PFASs). *ENV/JM/MONO(2018)7*.

Paul, A. G., Jones, K. C., and Sweetman, A. J. (2009). A First Global Production, Emission, And Environmental Inventory For Perfluorooctane Sulfonate. *Environmental Science & Technology, 43*(2), 386-392. doi:10.1021/es802216n

Pérez, F., Nadal, M., Navarro-Ortega, A., Fàbrega, F., Domingo, J. L., Barceló, D., and Farré, M. (2013). Accumulation of perfluoroalkyl substances in human tissues. *Environment International, 59*(0), 354-362. doi:10.1016/j.envint.2013.06.004

Rankin, K., Mabury, S. A., Jenkins, T. M., and Washington, J. W. (2016). A North American and global survey of perfluoroalkyl substances in surface soils: Distribution patterns and mode of occurrence. *Chemosphere, 161*, 333-341. doi:10.1016/j.chemosphere.2016.06.109

RP-Karlsruhe. (2018). Überblick zur PFC-Problematik in Mittel- und Nordbaden. *Stabstelle PFC des Regierungspräsidium Karlsruhe*.

Schultz, M. M., Higgins, C. P., Huset, C. A., Luthy, R. G., Barofsky, D. F., and Field, J. A. (2006). Fluorochemical Mass Flows in a Municipal Wastewater Treatment Facility†. *Environmental Science & Technology, 40*(23), 7350-7357. doi:10.1021/es061025m

Scott, B. F., Spencer, C., Mabury, S. A., and Muir, D. C. G. (2006). Poly and Perfluorinated Carboxylates in North American Precipitation†. *Environmental Science & Technology, 40*(23), 7167-7174. doi:10.1021/es061403n

Sedlak, M. D., and Greig, D. J. (2012). Perfluoroalkyl compounds (PFCs) in wildlife from an urban estuary. *Journal of Environmental Monitoring, 14*(1), 146-154. doi:10.1039/C1EM10609K

Stahl, T., Riebe, R. A., Falk, S., Failing, K., and Brunn, H. (2013). Long-term lysimeter experiment to investigate the leaching of perfluoroalkyl substances (PFASs) and the carry-over from soil to plants: results of a pilot study. *J Agric Food Chem, 61*(8), 1784-1793. doi:10.1021/jf305003h

Wang, Z., Boucher, J. M., Scheringer, M., Cousins, I. T., and Hungerbuhler, K. (2017). Toward a Comprehensive Global Emission Inventory of C4-C10 Perfluoroalkanesulfonic Acids (PFSAs) and Related Precursors: Focus on the Life Cycle of C8-Based Products and Ongoing Industrial Transition. *Environ Sci Technol, 51*(8), 4482-4493. doi:10.1021/acs.est.6b06191

Wang, Z., Cousins, I. T., Scheringer, M., Buck, R. C., and Hungerbuhler, K. (2014). Global emission inventories for C4-C14 perfluoroalkyl carboxylic acid (PFCA) homologues from 1951 to 2030, Part I: production and emissions from quantifiable sources. *Environ Int, 70*, 62-75. doi:10.1016/j.envint.2014.04.013

Wilhelm, M., Kraft, M., Rauchfuss, K., and Hölzer, J. (2008). Assessment and Management of the First German Case of a Contamination with Perfluorinated Compounds (PFC) in the Region

Sauerland, North Rhine-Westphalia. *Journal of Toxicology and Environmental Health, Part A, 71*(11-12), 725-733. doi:10.1080/15287390801985216

Wong, F., Shoeib, M., Katsoyiannis, A., Eckhardt, S., Stohl, A., Bohlin-Nizzetto, P., . . . and Hung, H. (2018). Assessing temporal trends and source regions of per- and polyfluoroalkyl substances (PFASs) in air under the Arctic Monitoring and Assessment Programme (AMAP). *Atmospheric Environment, 172*, 65-73. doi:10.1016/j.atmosenv.2017.10.028

Yeung, L. W. Y., So, M. K., Jiang, G., Taniyasu, S., Yamashita, N., Song, M., . . . and Lam, P. K. S. (2006). Perfluorooctanesulfonate and Related Fluorochemicals in Human Blood Samples from China. *Environmental Science & Technology, 40*(3), 715-720. doi:10.1021/es052067y

Young, C. J., and Mabury, S. A. (2010). Atmospheric perfluorinated acid precursors: chemistry, occurrence, and impacts. *Rev Environ Contam Toxicol, 208*, 1-109. doi:10.1007/978-1-4419-6880-7_1

Yu, J., Hu, J., Tanaka, S., and Fujii, S. (2009). Perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA) in sewage treatment plants. *Water Research, 43*(9), 2399-2408. doi:10.1016/j.watres.2009.03.009

4.2 Transfer of natural toxins in aquatic systems

Bodi, Dorina; Mädge, Inga

Abstract

Aquatic systems are important sources of food and feed supply, either through fishery or aquaculture. Therefore, potential health risks arising from marine and fresh water products have to be taken into account when it comes to safety in the global food and feed chain.

It is important to consider not only potentially toxic compounds of anthropogenic origin, but also natural toxins, e.g. from marine and fresh water algae, can accumulate along the food chain posing risks to humans and animals.

Some of these compounds can cause intoxications which might not be recognized as such, due to unspecific symptoms resembling those of microbiologically induced food poisoning. Others among the variety of marine and fresh toxins can cause severe up to life-threatening symptoms, which can be highly specific for a certain group of toxins.

Increasing or varying occurrence of certain toxins in marine animals and marine regions is caused by a complex combination of ecological, climatic, meteorological and also natural factors. An increased growth of phytoplankton and makroalgae is often but not necessarily connected to toxin production.

Analytical determination of marine and fresh water toxins is of major importance when it comes to risk assessment and monitoring in food and feed products originating from aquatic systems. The German National Reference Laboratory for Marine Biotoxins is engaged in this wide field to enable control of maximum toxin levels in shellfish, to develop, improve and standardize analytical methods and to identify strategies for consumer protection in case of lacking analytical methods. The approaches applied differ depending on the toxin groups. Furthermore, understanding the formation of aquatic toxins along the food web and the metabolic fate of marine toxins in the human body are key factors for risk assessment.

Sources of marine biotoxins in different marine products

Food poisoning after consumption of seafood is not always caused by microbiological contamination but often related to marine toxins from microalgae. So called harmful algae blooms (HABs) develop under certain conditions regarding climate and other environmental factors. Those conditions lead to an exponential growth of microalgae and the production of toxic metabolites by some species among the so called marine phytoplankton. As the first stage of the marine food chain, microalgae including the toxin producing species are ingested by other organisms like fish and shellfish, hence accumulating the toxins.

Symptoms after ingestion of marine biotoxin containing seafood can be gastrointestinal like diarrhea, nausea, vomiting and abdominal pain, neurologic like tingling sensations, headaches, and dizziness, up to severe muscular or respiratory paralyses, which can end fatal. The toxin groups are named diarrhetic shellfish poisoning and paralytic shellfish poisoning toxins accordingly. There are a number of other neurologic toxins, e.g. tetrodotoxins known as the pufferfish toxin, and Ciguatoxins which can cause the unique symptom of reversal of cold and hot sensation among other numerous symptoms.

Approaches for consumer protection

In order to protect the consumer from intoxications with marine biotoxins at European level there are health standards for live bivalve molluscs laid down in the European legislation including maximum limits for several toxin groups and the need for reference methods to be recommended by the network of reference laboratories (Eur. Parliament and The Council, 2004a).

- **Lipophilic marine biotoxins**

According to the recommendations of EFSA, maximum levels for lipophilic marine biotoxins and the mouse bioassay (MBA) as the reference methods were laid down in Commission Decision 2002/225/EC (EC, 2002c). In Germany's official control it had not been used in routine analysis since the 1990s, only case of dispute, sample analysis by MBA was carried out mainly by contract laboratories. Due to animal welfare reasons it had never been used in the German National Reference Laboratory (NRL) for marine biotoxins. Results of interlaboratory comparison studies demonstrated that the MBA cannot be validated for the analysis of diarrhetic shellfish poisoning toxins (LeDoux and Hall, 2000) and that its sensitivity is not sufficient to monitor e.g. okadaic acid toxin limits at acceptable false negative and false positive result rates (Hess et al., 2009; Jorgensen and Jensen, 2004). Several incidents uncovered susceptibility issues of the assay.(Lawrence et al., 1994; Mcculloch et al., 1989) With the technical progress and development of liquid chromatographic tandem mass spectrometric (LC-MS/MS) methods drawbacks of the MBA became even more obvious. Quantitative analysis has major advantages when it comes to monitoring of maximum limits and also sensitivity of LC-MS/MS is superior to that of the bioassay and thereby improving consumer protection.

In 2005 BfR asked EFSA for the scientific evaluation of the ability and fitness for purpose of the MBA to be used as a reference method for the surveillance of regulated marine biotoxins. (BfR, 2005) In 2009 the Panel on Contaminants in the Food Chain (CONTAM Panel) noted that this bioassay has shortcomings and is not considered an appropriate tool for control purposes because of the high variability in results, the insufficient detection capability and the limited specificity. (EFSA CONTAM, 2009) Furthermore, in its scientific opinion on marine biotoxins in shellfish EFSA considered current maximum limits too high to avoid exceeding the acute reference dose (EFSA CONTAM, 2009).

Finally, by end of 2014, after a three year transition period, MBA was amended as the reference method for the control of lipophilic marine biotoxins and was replaced by an LC-MS/MS method (EC, 2011a). In 2019 MBA as the reference method for saxitoxin group toxins will be replaced by an precolumn oxidation HPLC-FLD method (EC, 2017a). This accomplishment is amongst many other parties merit of BfR acitivities.

Where control during production or anywhere else along the food chain fails to detect the presence of marine toxins, eventually contaminated shellfish is put on the market where it might lead to intoxication of consumers. Follow-up investigations of intoxications are necessary to prove the toxins to be the original cause of effects in the patients. This proof is necessary to ensure consequences for responsible parties in the production and trade chain and to enable for measures of improvement in the control system. Using respective information traders can communicate issues to producers, establish approaches to prevent the repetition of toxic events in the future or change partners on the production side. An unambiguous proof for the cause of intoxication includes the combination of symptoms observed in the patient and detection of respective toxins in remnants of the suspect meal or stomach content of the affected person.

In many cases no food remnants or stomach contents are available to perform analyses of the potentially causative meal. Alternatively, body fluids could be analysed for evidence of the intoxication. However, it has to be taken into account that the toxin itself might not be present e.g. in urine, blood or serum of an intoxicated individuum due to biotransformation of toxic compounds. These toxin metabolites can be analysed instead prerequisite knowledge can give evidence for the intoxication.(F. Kolrep et al., 2016).

Research groups at BfR including the NRL for Marine Biotoxins have been investigating the biotransformation of marine biotoxins in the human and other organisms for several years (Kittler et al., 2010; F. Kolrep et al., 2016). A major task was the development of a mass spectrometric strategy to determine compounds expected according to basic rules of the metabolic pathways. Most of the predicted metabolites are not commercially available, so unambiguous confirmation is impossible. However, by means of the knowledge of the routes of metabolism and chemical structure of the toxins including their mass spectrometric behavior, potential metabolites were predicted for six representatives of lipophilic toxins (okadaic acid, dinophysis toxin 1 and 2, azaspiracid 1, yessotoxin and pectenotoxin 2). In-vitro metabolisation of the toxins using rat S9-liver homogenate to imitate the responsible enzyme composition were carried out. A two-stage mass spectrometric analysis of the S9-incubates followed including several tandem-mass spectrometric experiments on a triple quadrupole mass spectrometer and full scan experiments on a high resolution mass spectrometer. By means of this approach a total of 47 metabolites (phase I and phase II) from six toxins were regarding the sum formula, the added or removed structural feature and its position in the carbon skeleton of the molecule. (Kittler et al., 2010) This initiative work was continued and complemented by identification of metabolites formed by the human enzymatic system (F. Kolrep et al., 2016; Franziska Kolrep et al., 2017) and by investigating the passage of lipophilic marine biotoxins through the human gastrointestinal barrier via active (Ehlers et al., 2014) and passive (Ehlers et al., 2011) transport systems. Results are not only steps to the initial aim of supporting the clarification of intoxication cases but may also improve the knowledge on effects of marine biotoxins due to chronic exposure to low toxin levels (Franziska Kolrep et al., 2017).

- **Ciguatoxins**

Globally, regulation of toxin groups e.g. regarding maximum limits obviously depends on the occurrence of respective toxins in aquatic organisms of the respective region. However, globalization of trade and changes in the marine environment for example due to climate change, might lead to the occurrence of toxins prior unknown to a specific area. This is the case amongst other for ciguatoxin (CTX) containing fish. The demand of consumers for exotic fish in highly industrialized countries caused a series of outbreaks (Friedemann, 2016) of Ciguatera Fish Poisoning (CFP) in Europe.

Monitoring of CTX in fish is a challenging task, especially, as accumulation and biotransformation towards this toxin starting from gambiertoxin containing benthic algae ends in carnivorous fish (Figure 24). Those top predators tend to change locations frequently over their life time which they mostly spent outside schools of fish.

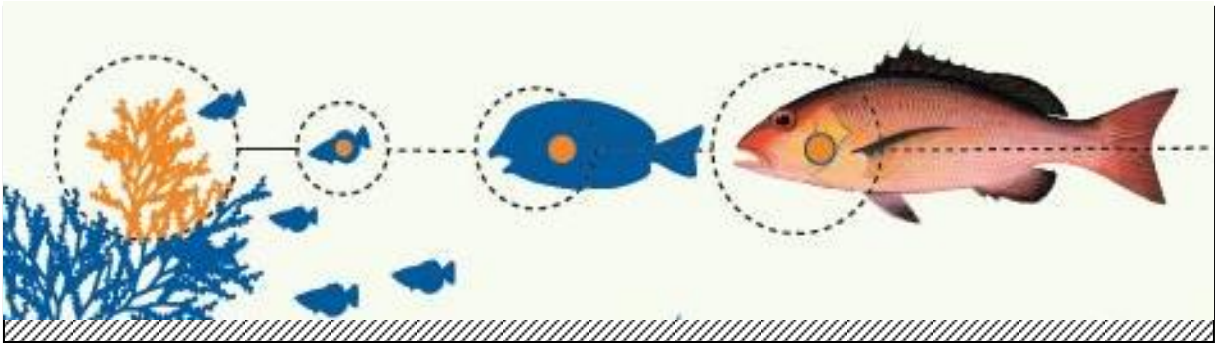


Figure 24: Transformation of gambiertoxins to ciguatoxins through the food web (FAO/WHO/Codex alimentarius)

These circumstances lead to heterogeneous catches regarding regional origin, fish species, size and age making distribution of possibly toxin contaminated individuals unpredictable. Even with powerful analytical methods available the issue of representative sampling is impeding effective consumer protection by toxin analysis. However, in case of emerging toxins analytical methods are not necessarily available. One example, also dealt with at BfR, is the protection of consumers from CFP (Querverweis Kapitel 6.2). Fish species with a high risk to contain CTX are moray eel (*Murarena* spp.), snapper (*Lutjanus* spp.) and barracuda (*Sphyraena* spp.). Various snapper species are very popular for European consumers and, therefore, imported frequently. After several CFP outbreaks in Germany from 2012 (Friedemann, 2016), a first meeting of involved parties was organized by BfR in 2015 in order to support the German official control laboratories regarding their monitoring tasks.

The state of the art regarding CTX analysis, methods for species identification, recent CFP cases, the general course of intoxication, operational controls and import controls of fresh fish, and legal rules for trading and marketing of the latter. It was consensually concluded, that the present rules are not appropriate to fulfill the tasks of official control (also see chapter 2.1) as demanded by the “control regulation” (EC) No 882/2004 (Eur. Parliament and The Council, 2004).

Excursus: EU import control

The import control system is summarized under the term ‘customs control’ which serves among other purposes to check compliance with health requirements, veterinary, phytosanitary and quality regulations as well as the correct description of imported goods and their origin. Procedures for veterinary checks at Community border inspection posts on products imported from third countries are laid down in Commission Regulation (EC) No. 136/2004 referring to Council Directive 97/78/EC on veterinary checks on products entering the Community from third countries. Since 2004 it is mandatory to use the online tool TRACES (Trade control and expert system) to export animals and animal products to the EU and to trade those goods between EU member states. All export certificates and import documents are organized through the online system, movements (e. g. shipment documents) and other information of about goods are documented. Thereby, traceability with respect to the ‘trace back and forth’ approach is ensured and administrative procedures are sped up. In case of non-conformities measures can be taken more quickly. Another advantage of TRACES is the availability of trading data in terms of annual statistics about traded commodity flows including food and feed categories, trading countries and percentage of conformities and non-conformities. Those are valuable information for risk assessment and management. Another tool helping to identify risks from imported food and feed and their origin is the database of the Rapid alert system for food and feed (RASFF) (D'Amico et al., 2018).

A series of meetings followed in 2017 and 2018 involving different authorities, producers and traders imaging the interests involved in successive stages of trade chain. As a result communication between involved parties was improved with regard to awareness of each position and the issues they are facing. The urgent need for the development and validation of potent screening methods for CTX in fish was expressed. Up to now there is no analytical laboratory in Europe, nor outside Europe, which is accredited according to ISO 17025 (CEN, 2017) for CTX analysis using biological (like mouse bioassay or neuroblastoma cell assay) or chemical methods (like liquid chromatography mass spectrometry, LC-MS/MS). Meanwhile, it was consensus to put more emphasis on the control of the species declared on the consignment and to increase the frequency of controls in case of imports of potentially ciguatoxic fish consignments. This was considered the most effective way to improve consumer protection from CFP. However, currently, methods for fish species identification like protein or DNA analysis are time consuming and therefore not able to provide results for immediate or at least quick decision on the import authorization. A promising alternative is being developed by researchers of the Department of Safety and Quality of Milk and Fish Products of the Max-Rubner-Institute in cooperation with the University of Hamburg. By means of this DNA microarray (DNA chip) screening test 10 fish species (including one potentially CTX producing species) and 2 shrimp species can be distinguished. After validation of the test it is suitable for the use as a screening test in control of incoming goods and at border inspection posts. Another outcome of the numerous discussions was the agreement on mutual support of the parties and cooperation in CFP cases. One possible action could be the provision of fish material which species has been unambiguously classified or which is suspected to contain CTX, both for the development of the required analytical methods. BfR as an independent institute has taken the mediating role between involved parties at the various points of the fish production and trade chain.In order to support elucidation of intoxication cases, NRL for Marine Biotoxins in BfR has started its activities concerning the development of CTX detection methods.

The aim of the NRL MB is the establishment of a two-tiered analytical approach as applied e.g. by the US Food and Drug Administration involving the mouse neuroblastoma cell assay as a screening test and an LC-MS/MS method for subsequent confirmation. This procedure proved to be efficient in the very few expert laboratories worldwide. However, validation and harmonization of those methods in close cooperation with the experienced laboratories is necessary.

A more proactive and therefore promising measure of consumer protection is the monitoring of toxic *Gambierdiscus* species and the presence of CTX or CTX precursor toxins in fishing areas.

Conclusions

Reliable and harmonized analytical methods are a key prerequisite to monitor marine and fresh water toxins in food and feed products from aquatic systems to protect consumer health. The approach of toxin control at pre-harvest, pre-market and post-market control of compliance with maximum toxin levels in shellfish is unique compared to other food and feed products taking into account the severe health risks of acute toxins. When it comes to emerging toxins and toxin metabolites even more powerful and innovative analytical methods are necessary. The development of methods for both routine and research is being tackled in the German NRL for marine biotoxins and other research groups of the BfR, thereby supporting consumer protection and creating the essential base for comprehensive risk assessment.

However, precautionary analytical control for marine toxins is not always feasible to ensure consumer protection. For example the distribution of ciguatoxic fish in one specific catch is too inhomogeneous to get a representative sample thus involving a high risk to miss contam-

inated fish for sampling. Other strategies are necessary to be developed to avoid CFP intoxications which is a current task of the BfR coordinating the mutual work of the concerned parties in Germany from various authorities to economic players.

Abbreviations

CFP	ciguatera fish poisoning
CTX	ciguatoxin
LC-MS/MS	Liquid Chromatography Tandem Mass Spectrometry
MB	marine biotoxins
MBA	mouse bioassay
NRL	National Reference Laboratory
PSP	paralytic shellfish poisoning
TTX	tetrodotoxin

References

BfR, Bundesinstitut für Risikobewertung. (2005). Stellungnahme Nr. 032/2005 des BfR vom 26. Mai 2005: Maus-Bioassay als Standard-Referenzmethode für die routinemäßige Analyse von Algengiften in Muscheln nicht geeignet. In (Vol. BfR032/2005).

CEN, European Committee for Standardization. (2017). EN 14526:2017 Determination of saxitoxin and dc-saxitoxin in mussel – HPLC method using pre-column derivatization with peroxide or periodate oxidation. In (Vol. 2017-01).

D'Amico, P., Nucera, D., Guardone, L., Mariotti, M., Nuvoloni, R., and Armani, A. (2018). Seafood products notifications in the EU Rapid Alert System for Food and Feed (RASFF) database: Data analysis during the period 2011-2015. *Food Control, 93*, 241-250. doi:10.1016/j.foodcont.2018.06.018

EC, European Commission. (2002c). COMMISSION DECISION 2002/225/EC laying down detailed rules for the implementation of Council Directive 91/492/EEC as regards the maximum levels and the methods of analysis of certain marine biotoxins in bivalve molluscs, echinoderms, tunicates and marine gastropods. *Official Journal of the European Communities,, L75*, 62-64.

EC, European Commission. (2011a). COMMISSION REGULATION (EU) No 15/2011 amending Regulation (EC) No 2074/2005 as regards recognised testing methods for detecting marine biotoxins in live bivalve molluscs. *Official Journal of the European Union, L 6*, 3-6.

EC, European Commission. (2017a). COMMISSION REGULATION (EU) 2017/1980 amending Annex III to Regulation (EC) No 2074/2005 as regards paralytic shellfish poison (PSP) detection method. *Official Journal of the European Union, L 285*, 8-9.

EFSA CONTAM, European Food Safety Authority's Panel on Contaminants in the Food Chain. (2009). Scientific Opinion of the Panel on Contaminants in the Food Chain on a request from the European Commission on Marine Biotoxins in Shellfish – Summary on regulated marine biotoxins. *The EFSA Journal, 1306*, 23. doi:10.2903/j.efsa.2009.1306

Ehlers, A., Scholz, J., These, A., Hessel, S., Preiss-Weigert, A., and Lampen, A. (2011). Analysis of the passage of the marine biotoxin okadaic acid through an in vitro human gut barrier. *Toxicology, 279*(1-3), 196-202. doi:10.1016/j.tox.2010.11.001

Ehlers, A., These, A., Hessel, S., Preiss-Weigert, A., and Lampen, A. (2014). Active elimination of the marine biotoxin okadaic acid by P-glycoprotein through an in vitro gastrointestinal barrier. *Toxicology Letters, 225*(2), 311-317. doi:10.1016/j.toxlet.2013.12.019

Eur. Parliament, and The Council. (2004). REGULATION (EC) No 882/2004 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 29 April 2004 on official controls and other official activities performed to ensure the application of food and feed law, animal health and animal welfare rules. *Official Journal of the European Union, L165*, 1-141.

Eur. Parliament, and The Council. (2004a). Regulation (EC) No 853/2004 of the European Parliament and of the Council of 29 April 2004 laying down specific hygiene rules for on the hygiene of foodstuffs. *Official Journal of the European Union, L 139*, 55-201.

Friedemann, M. (2016). First ciguatera outbreak in Germany in 2012. *Bundesgesundheitsblatt-Gesundheitsforschung-Gesundheitsschutz, 59*(12), 1556-1565. doi:10.1007/s00103-016-2456-y

Hess, P., Butter, T., Petersen, A., Silke, J., and McMahon, T. (2009). Performance of the EU-harmonised mouse bioassay for lipophilic toxins for the detection of azaspiracids in naturally contaminated mussel (Mytilus edulis) hepatopancreas tissue homogenates characterised by liquid chromatography coupled to tandem mass spectrometry. *Toxicon, 53*(7), 713-722. doi:10.1016/j.toxicon.2009.02.015

Jorgensen, K., and Jensen, L. B. (2004). Distribution of diarrhetic shellfish poisoning toxins in consignments of blue mussel. *Food Additives and Contaminants, 21*(4), 341-347. doi:10.1080/02652030410001665919

Kittler, K., Preiss-Weigert, A., and These, A. (2010). Identification Strategy Using Combined Mass Spectrometric Techniques for Elucidation of Phase I and Phase II in Vitro Metabolites of Lipophilic Marine Biotoxins. *Analytical Chemistry, 82*(22), 9329-9335. doi:10.1021/ac101864u

Kolrep, F., Hessel, S., These, A., Ehlers, A., Rein, K., and Lampen, A. (2016). Differences in metabolism of the marine biotoxin okadaic acid by human and rat cytochrome P450 monooxygenases. *Archives of Toxicology, 90*(8), 2025-2036. doi:10.1007/s00204-015-1591-9

Kolrep, F., Rein, K., Lampen, A., and Hessel-Pras, S. (2017). Metabolism of okadaic acid by NADPH-dependent enzymes present in human or rat liver S9 fractions results in different toxic effects. *Toxicology in Vitro, 42*, 161-170. doi:10.1016/j.tiv.2017.04.009

Lawrence, J. F., Chadha, R. K., Ratnayake, W. M. N., and Truelove, J. F. (1994). An incident of elevated levels of unsaturated free fatty acids in mussels from Nova Scotia and their toxic effect in mice after intraperitoneal injection. *Natural Toxins, 2*(5), 318-321. doi:10.1002/nt.2620020511

LeDoux, M., and Hall, S. (2000). Proficiency Testing of Eight French Laboratories in Using the AOAC Mouse Bioassay for Paralytic Shellfish Poisoning: Interlaboratory Collaborative Study. *Journal of AOAC International, 83*(2), 305-310.

Mcculloch, A. W., Boyd, R. K., Defreitas, A. S. W., Foxall, R. A., Jamieson, W. D., Laycock, M. V., . . . and Richard, D. J. A. (1989). Zinc from Oyster Tissue as Causative Factor in Mouse Deaths in Official Bioassay for Paralytic Shellfish Poison. *Journal of the Association of Official Analytical Chemists, 72*(2), 384-386.

4.3 Natural toxins in feed and food processing chains of plant- and blue-green algae-based products

Schaarschmidt, Sara; Ibrahem Aroud, Husam; Bodi, Dorina

Abstract

Natural toxins are naturally produced by living organisms, which themselves are not affected by the toxic compounds. However, other organisms, including humans, might be harmed by the toxins. Several natural toxins can occur in feeds and foods, and are either due to contamination with microorganisms (as in the case of mycotoxins) or being produced directly by the organisms the feed/food is derived from (like for cyanotoxins).

Mycotoxins are common feed and food contaminants produced by toxigenic fungi of different species. They are typically found in cereals and fruits and can be a threat to the health of animals and humans. Numerous mycotoxins are known, including traditional and emerging mycotoxins and modified forms. Mycotoxins can be produced in the field and/or during transport and storage. During processing, mycotoxins can be degraded, transformed, or released from or bound to the feed/food matrix. The current chapter gives a brief overview on the potential impact of processing in cereal and fruit chains as exemplified by the processing chains of wheat bread and apple juice.

Cyanotoxins are secondary metabolites generated by some genera of cyanobacteria (blue-green algae). One prominent subgroup, the microcystins (MCs) consists of more than 100 representatives with different toxicity. All of them have a monocyclic heptapeptide base structure. Organic food supplements based on micro algae (*Chlorella* sp.) and cyanobacteria (*Spirulina* sp., *Aphanizomenon flos-aquae*) are supposed to have health promoting effects (Raja et al., 2016). Therefore, the demand for these products has increased. However, especially regarding supplements from cyanobacteria, serious concerns were expressed that contaminations of these products with toxins like MCs, nodularin, or anatoxin-a could cause health risks to the consumers (Buratti et al., 2017; Testai et al., 2016). In order to protect consumers from allergic reactions, liver damages, and other consequences, regular analytical controls of blue-green algae-based food supplements (BGAS) are necessary. After development of a multi-MC determination method, a collection of BGAS products was analysed to get an impression on the current situation on the market.

Mycotoxins in cereal chains and the fate of deoxynivalenol in wheat bread production

Cereals like wheat and maize are often contaminated with mycotoxins. These toxins can be produced by phytopathogenic fungi in the field, particularly by *Fusarium* species, and by commensal fungi, such as *Aspergillus* and *Penicillium* spp. that colonise plants without obvious affection of plant health or which infect the crops after harvest as storage fungi. *Aspergillus* and *Penicillium* species can produce for example aflatoxins – including the most carcinogenic mycotoxin aflatoxin B1 – and ochratoxin A. Other “traditional mycotoxins” cover the *Fusarium* toxins deoxynivalenol (DON), nivalenol, T-2 and HT-2 toxins, zearalenone, and fumonisins. Besides, so-called “emerging mycotoxins” (e.g. enniatins, beauvericin, and moniliformin), which are to date less analysed, and modified forms of mycotoxins (e.g. DON-3-glucoside, 3-acetyl-DON, and 15-acetyl-DON) can occur (Berthiller et al., 2013; Jestoi, 2008; Rychlik et al., 2014). Typically, cereals are contaminated with multiple mycotoxins simultaneously. The contamination pattern can depend, besides other factors, on the cereal species and on the growing region and climate. The *Fusarium* toxin DON is probably the most common mycotoxin in wheat and wheat-based products in the European Union (EU). *Aspergillus* spp. and aflatoxin contaminations are of main significance in grains produced in warmer climates, such as maize (for further information on the maize chain, see Subchapter 2.1). Also

fumonisins are common in maize, but are typically not present (or only at very low levels) in wheat. Due to the high impact of climate, yearly variations of the mycotoxin concentrations in cereal raw materials are typical and climate change is usually predicted to exacerbate the mycotoxin problem. However, more data is needed to more precisely estimate the impact of climate change on food safety and food sustainability for different regions (Angel et al., 2017).

Exposure of humans to mycotoxins takes place directly by the consumption of contaminated cereals or other plant-based products, or indirectly by contaminated feed materials and a carry-over to foods of animal origin. The conversion of aflatoxin B1 to aflatoxin M1 and the carry-over into milk is a well-known problem. However, also other mycotoxins appear to a certain extent to be transferred from feed to food in the milk chain, such as free and modified forms of zearalenone and DON (for an overview see Flores-Flores et al., 2015). Moreover, mycotoxins can be transferred to eggs or other foods of animal origin as reviewed elsewhere (Escrivá et al., 2017; Völkel et al., 2011). More data is still needed on potential transformation and carry-over of mycotoxins other than aflatoxins from feed to food chains.

To avoid adverse effects from mycotoxin exposure on the consumers' health, several mycotoxin maximum levels are set for cereals and cereal products. On a global level, maximum levels for DON, fumonisins B1 + B2, and ochratoxin A were established for some raw cereals and cereal-based food products by the Codex Alimentarius Commission (CAC) by its General Standard for Contaminants and Toxins in Food and Feed (CAC, 2017b). In the EU, legal levels for mycotoxins in cereals and cereal-based foods are laid down for aflatoxins, DON, zearalenone, fumonisins B1 + B2, and ochratoxin A; moreover, the permitted number of ergot sclerotia in cereals excluding maize and rice is limited (EC, 2006c, 2017b). Further, to protect the health of (food-producing) animals and to avoid an excessive transfer of mycotoxins to the food chains, maximum levels exist for aflatoxins and ergot sclerotia in feed materials (EC, 2017b). For DON, zearalenone, T-2 and HT-2 toxins, fumonisins B1 + B2, and ochratoxin A, guidance levels are available for feed materials in the EU (EC, 2006a). In many countries, national legal mycotoxin limits or action levels exist that cover at least aflatoxin B1 in case of non-EU member states (see e.g. FDA, 2015a, 2015b; Ministério da Saúde. República Federativa do Brasil, 2011; USDA, 2011) or mycotoxins/products not covered by EU law in case of EU member states (for example, see BMJV, 2010).

Several measures can help to minimise mycotoxin contaminations in cereals (see e.g. CAC, 2017a; EC, 2006b). They cover (i) pre-harvest measures, such as *Fusarium*-tolerant/-resistant cultivars, crop rotation, the application of fungicides or biological control agents, weed and pest control, and appropriate irrigation (if required) to limit crop stress, (ii) proper harvest strategies, as well as (iii) post-harvest measures. The latter include for example drying and cleaning of cereals before storage and appropriate storage conditions. Further, processing of cereals can affect mycotoxin concentrations and the binding, transformation, or degradation of mycotoxins. Because processing can not only lower, but also elevate mycotoxin levels and might facilitate the formation of new, potentially even more toxic forms, the processing chain – as part of the feed/food chain – needs to be carefully considered when looking at mycotoxins in global feed/food chains. An integrated approach in developing, improving, and implementing measures for mycotoxin reduction in cereal feed and food chains is taken by the EU-funded project MyToolBox (www.mytoolbox.eu). Besides actions to prevent mycotoxin contaminations and detoxification strategies for feed materials and biofuel production, new (pre-)milling technologies and improved baking procedures are in focus of MyToolBox (for an overview on the project's objectives, see also Krska et al., 2016). The impact of current processing procedures on mycotoxin contaminations of cereals, with focus on DON in wheat bread production, was recently reviewed (Schaarschmidt and Fauhl-Hassek, 2018) and is summarised as follows.

In general, cleaning and milling operations do not affect total mycotoxin amounts, but can lead to a redistribution of mycotoxins in the different fractions. The infection of grain with toxigenic and phytopathogenic *Fusarium* spp. in the field is usually accompanied by an impaired grain development and discolouring of grains. Thus, removal of small, shrivelled, and low-density grains by mechanical cleaning operations based on sieving, gravity separation, and/or separation by indented cylinders or discs (Kent and Evers, 1994; Posner, 2009) can reduce *Fusarium* toxin concentrations in the cleaned batch. Same is true for the removal of foreign material, such as weed seeds, straw, dust, and dirt adhering to the grain surface (by using also aspirators and scourers), which is often as well highly mycotoxin-contaminated. Optical sorting technologies enable the specific removal of discoloured grains and/or of grains with specific defects etc., which can improve mycotoxin reduction in the cleaned batch. The removed low-quality grains and foreign material, typically characterised by elevated mycotoxin levels, is often used as feed material. Thus, mycotoxin concentrations should be carefully monitored to avoid excessive contamination of feed. The mycotoxin distribution pattern among the different cleaning fractions is strongly dependent on the quality of the raw batch, the initial mycotoxin contamination levels, the applied cleaning technology, and the stringency of cleaning (which is also an economic factor). Compared to the raw grain, mycotoxin concentrations in the cleaned batch can be lowered by only few percent to around 80% or more (for an overview on the impact of wheat cleaning, see Schaarschmidt and Fauhl-Hassek, 2018).

Dry milling typically aims on the production of white flour or semolina. Because outer grain layers are usually higher contaminated with mycotoxins than the inner ones, milling products derived from the endosperm often have lower mycotoxin concentrations compared to whole grain. In case of white wheat flour, DON is in the literature described to be reduced approximately by up to 70% (often by 20–50%) compared to the whole grain. In contrast, the bran that is often used as feed material, but which can be also a source for human nutrition, is typically characterised by elevated mycotoxin levels. In case of DON, an increase by up to 3-fold or more (often by 1.2- to 2.5-fold) is found in the wheat bran fraction. If wheat milling products are intended to be marketed in the EU for direct human consumption, DON must not exceed 750 µg/kg, whereas the permitted DON levels in unprocessed grain (which includes cleaned grain) amounts to 1,250 µg/kg according to EU legislation (EC, 2017b). Thus, monitoring of mycotoxin levels is highly recommended for potentially high contaminated milling products, such as bran, before marketing for direct human consumption. If bran and by-products of grain cleaning are used as feed material, it is recommended to keep the DON level below 8,000 µg/kg (EC, 2006a).

For most secondary processed cereal-based foods, stricter mycotoxin limits are laid down by EU law compared to raw materials and milling products. For wheat bread, the EU maximum level for DON is set at 500 µg/kg (EC, 2017b). Because the maximum level is based on the product as is, higher moisture content and the dilution with other, uncontaminated or less mycotoxin-contaminated ingredients contribute to a lower mycotoxin level in the final product. Typically, bread has a moisture content of around 40% (not considering crisp bread or other low-moisture bakery products), whereas the moisture in flour is around 12.5% (Scudamore et al., 2009). Other ingredients used in bread production usually amount to up than 10% based on dry weight (BMEL, 2005). Besides such dilution effects, the procedures applied during secondary processing can impact the mycotoxin pattern and concentrations.

Fermentation using baker's yeast and/or sourdough can affect mycotoxins like DON by microbial action, which can cause (i) a (partial) degradation of mycotoxins, (ii) transformation from/into modified forms, (iii) binding to the microorganisms, or (iv) release of mycotoxins from grain cell walls or other cell components. Further, the microbial activity can also lead to changes in the pH level that in return might affect mycotoxin stability or transformation. The potential impact of fermentation appears to be mainly dependent on the mycotoxins and the microflora present in the dough, on the conditions of fermentation regarding temperature and

duration, and on the composition including an optional addition of enzyme-containing bakery improvers. The latter can promote the release of mycotoxins from matrix-bound forms. Due to the many influencing factors and the different type of actions, mycotoxin concentrations can be reduced, not affected, or even enhanced during the fermentation step. For DON, the change during fermentation was found to range from a reduction by around 80% up to an increase by around 80%; often the data in literature ranged from 50% loss to 40% increase. Changes in mycotoxin concentrations caused by transformation processes or by the binding/release of toxins during the fermentation step can, however, be transient and reversed during the baking step (L'vova et al., 1998; Vidal et al., 2014a; Vidal et al., 2014b).

Besides affecting transformation or binding processes, due to thermal action, baking can cause a certain degradation of mycotoxins. The extent of degradation depends on the heat-stability of the mycotoxins, their potential protection by the matrix, and its heat-penetration properties. Typically, a higher mycotoxin reduction is found at higher temperature and longer heating duration as well as in the crumb of bread compared to the inner part. However, in case of DON, the degradation effect is usually relatively low due to the high heat stability of this toxin. Often, no effect or a reduction by up to 40% is described for baking. The entire range regarding the change in DON concentration during the baking step in wheat bread production spans, according to the literature, from a reduction by around 50% to an increase by around 100%. Studies that analysed the impact of both processes, fermentation and baking, found a change in DON concentration of approximately 70% loss to 80% increase; mostly DON was between 50% reduced and 20% enhanced. An enhancement of DON during baking was typically preceded by a reduction during fermentation.

By using the information on how DON is affected by the individual steps of processing, the processing factor from cleaned wheat grain to wheat bread can be estimated and compared with legal requirements (Figure 25). In doing so it becomes obvious that – due to the lack of fractionation during dry milling – it is typically more difficult to comply with legal limits in case of wholemeal bread. Compliance can also become challenging in case of low-moisture bakery products with high cereal content. Monitoring of mycotoxins, the selection of raw materials of higher quality, and the application of improved technologies or procedures (as under investigation in the MyToolBox project) can help to overcome compliance issues.

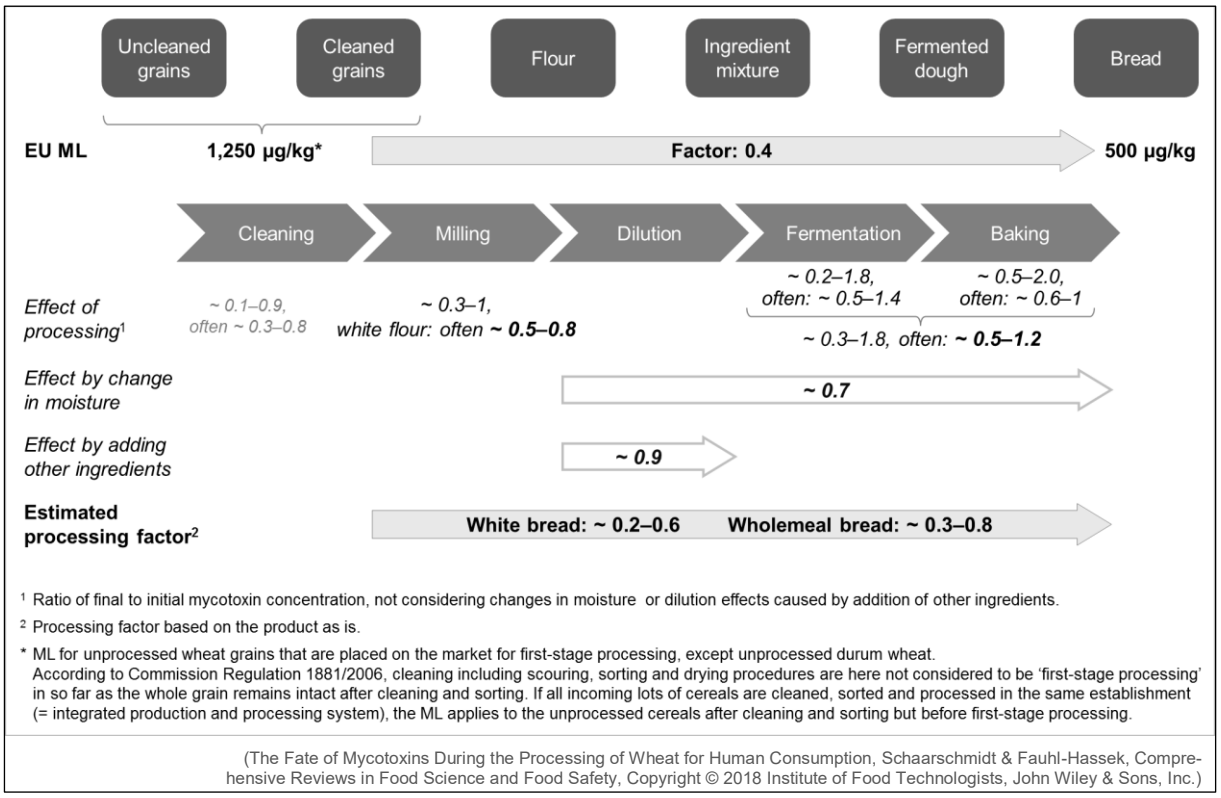


Figure 25: Estimation of the DON processing factor in the production of wheat bread and comparison with legal obligations in the EU
The effect of different processing steps can considerably vary depending on many factors. Thus, the provided ranges are only approximate data taken from the literature (for more information on the individual studies, see Schaarschmidt and Fauhl-Hassek, 2018). The final processing factor was estimated by multiplying the bold factors for (i) the effect of milling (in case of white bread only), (ii) the effect of fermentation plus baking, (iii) the effect of change in moisture, and (iv) the effect by adding other ingredients. The effect of cleaning was not considered since the EU maximum level (ML) for DON in unprocessed wheat also applies to cleaned grain.

The estimation and analysis of processing factors can support risk assessment by pointing out critical steps in feed or food (processing) chains. Besides mycotoxins regulated by law such as DON, also other mycotoxins – including potentially hazardous modified forms – need to be considered. Modified mycotoxins might for example be produced by metabolic activity in plants or in food-producing animals, as well as during food processing. The fate of mycotoxins, with special focus on modified forms, during alkaline cooking of maize for tortilla production was recently reviewed (Schaarschmidt and Fauhl-Hassek, 2019). So far, however, modified and emerging mycotoxins are typically not covered by routine feed/food analysis (see also Subchapter 4.3) and toxicological data are rare or lacking, which has impeded risk assessment that is ongoing in many cases (Berthiller et al., 2013; EFSA CONTAM, 2014). A more detailed analysis of modified mycotoxins, including monitoring during thermal food processing at industrial scale, is one aim of the MyToolBox project (Krska et al., 2016).

Mycotoxins in fruit juice processing chains and the fate of patulin in apple juice production

- **Contamination of fruit supply chain**

In recent years, the world has witnessed several foodborne disease outbreaks related to contamination of fruits and vegetables with pathogenic bacteria and viruses (Callejon et al.,

2015). For fruits, the occurrence of mycotoxins may pose a hitherto underestimated risk on food safety (Van Boxstael et al., 2013). Contamination of fruits with phytopathogenic filamentous fungi or moulds that produce mycotoxins can result in adverse effects on human health through the exposure to these mycotoxins (Bennett and Klich, 2003). The secondary metabolites are produced by fungi under special conditions of temperature, humidity, and pH value. Occurrence of mycotoxins can happen at any phase throughout the fruit supply chain (Sanzani et al., 2016). The fungi can invade or infect the fruits either in the pre-harvest stage in the field or in the post-harvest stage during storage, transport, and processing. The fungal production of mycotoxins in fruits is affected by several factors; some of them regard the fruit itself like the type of fruit and the cultivar, or regard the surrounding conditions like the geographical location of fruit plantation and the climate of the region. The harvest and postharvest conditions like harvest method, handling during transportation, and storage conditions also play an important role (Jackson and Al-Taher, 2008).

- **Fruit juice and the global supply chain**

According to the Centre for the Promotion of Imports from developing countries (CBI), the import of fruit juices from developing countries to the European Union has grown in the last five years in both value and quantity. The total imports of fruit juices to the EU have reached 7.4 million tonnes in the year 2016. Brazil is the largest supplier of fruit juice to the EU. Other countries which export juices to the EU include Vietnam, Costa Rica, Peru, Philippines, Thailand, Argentina, USA, Turkey, and Israel (CBI, 2017a). Germany is the leading country in the world regarding the yearly consumption per capita of fruit juice and fruit nectar with 32 L. The most popular fruit juices in Germany are apple juice and orange juice with 7.6 L per capita of each according to the Association of the German Fruit Juice Industry (VdF) (Vdf, 2017).

- **Occurrence of mycotoxins in fruit juices**

Fruit juices can be contaminated by various types of mycotoxins, among which patulin is the most relevant. Patulin is produced by several fungal species from the genera *Aspergillus, Penicillium*, *Byssochlamys* (Steiman et al., 1989). Patulin is considered a major problem in apple juice and apple-based products, which is illustrated by reports of its occurrence from different parts of the world (Baert et al., 2006; Barreira et al., 2010; de Sylos and Rodriguez-Amaya, 1999; Riteni, 2003; Yurdun et al., 2001; Zaied et al., 2013). However, patulin is also produced in a wide range of other fruit juices including pear, peach, apricot, and mixed fruit juices (Spadaro et al., 2008). It is also known to occur in grape juice and wine (Altmayer et al., 1982).

Some of the *Penicillium* species, which are able to produce patulin, can simultaneously produce the mycotoxin citrinin in apples (Viñas et al., 1993). Ochratoxin A, a mycotoxin produced by several species of *Aspergillus* and *Penicillium,* is considered ubiquitous in food and feed and can occur in fruits as well. It was reported in wines from Swiss retail market for the first time in 1995 (Zimmerli and Dick, 1996). Moreover, fruits can be infected with *Alternaria* species. Consequently, alternariol (AOH) and alternariol methyl ether (AME) were reported to occur in apples and apple juice as well as in other fruit juices (López et al., 2016; Ozcelik et al., 1990; Scott et al., 2006). Furthermore, aflatoxins were reported to occur in orange juice (Varma and Verma, 1987) and several types of fruit juices in Egypt (Abdel-Sater et al., 2001). In addition to the aforementioned mycotoxins, there are individual studies which reported the occurrence of other mycotoxins in fruit juices.

- **Regulations of mycotoxins in fruit juices**

Many *in vitro* studies have suggested that patulin has genotoxic and immune suppressive effects (Al-Hazmi, 2014; Puel et al., 2010; Zhou et al., 2009)**.** Consequently, the Codex Committee on Contaminants in Food set a maximum limit of 50 µg/kg for patulin in non-concentrated apple juice and reconstituted apple juice concentrate in 1995 (CAC, 1995a). In the European Union, maximum limits for the presence of patulin in fruit juices, apple products and certain baby foods were set by Regulation (EC) No 1881/2006. Moreover, ochratoxin A is regulated in grape juice, grape nectar, and grape must (Table 17).

Table 17: Maximum limits for patulin and ochratoxin A in the European Union according to Regulation (EC) No 1881/2006

Foodstuff	Maximum limit [µg/kg]
Patulin	
Fruit juices, concentrated fruit juices as reconstituted and fruit nectars	50
Spirit drinks, cider and other fermented drinks derived from apples or containing apple juice	50
Solid apple products, including apple compote, apple puree intended for direct consumption	25
Apple juice and solid apple products, including apple compote and apple puree, for infants and young children and labelled and sold as such	10
Baby foods other than processed cereal-based foods for infants and young children	10
Ochratoxin A	
Grape juice, concentrated grape juice as reconstituted, grape nectar, grape must and concentrated grape must as reconstituted, intended for direct human consumption	2

In the United States of America, the Food and Drug Administration (FDA) has issued a guidance document setting a maximum level of 50 µg/kg for patulin in apple juice (FDA, 2005). Consistently, patulin is also regulated in apple and apple products in various other countries in the world as shown in Table 18 (Mazumder and Sasmal, 2001).

Table 18: Regulations of patulin in food in various countries

Country	Foodstuff	Maximum limit [µg/kg]
Israel	Apple juice	50
Norway	Apple juice	50
Russia	Bottled/canned/potted fruits & berries & canned vegetables	50
South Africa	All food	50
Switzerland	Fruit juice	50
Uruguay	Fruit juice	50

- **Fate of mycotoxins during juice processing (Patulin in apple juice as an example)**

Apple juice is manufactured through a multi-stage process including different mechanical, physical and chemical treatments of apple fruits and the resulted juice. Ready to drink clear or cloudy apple juice is prepared either from directly processed fruits or by reconstitution of apple juice concentrate to the desired thickness of juice or nectar. Figure 26 shows the general scheme of apple juice processing.

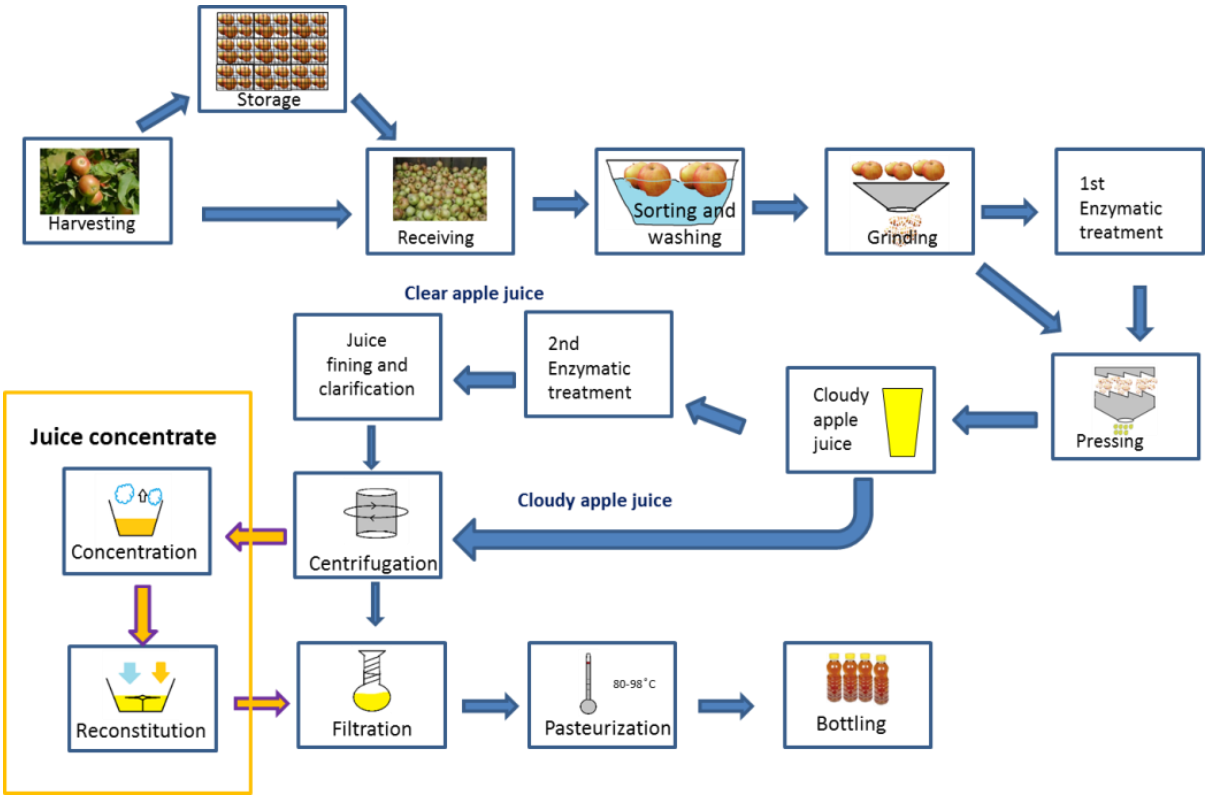


Figure 26: Processing stages of apple juice production

The Codex Alimentarius Commission has recommended Good Agricultural Practices (GAP) and Good Manufacturing Practices (GMP) for the reduction of contamination with patulin in apple juice production (CAC, 2003a). Along with setting criteria for the quality of the fruits selected for processing, these recommendations include measures in the field to improve the production of healthy fruits and harvest measures to avoid the damage of apple fruits. Furthermore, controlling the cleanliness of the containers used for transportation of the fruits, the condition of cold storage, the storage prior to processing, and sanitation of manufacturing equipment are addressed in detail to avoid microbial contamination and fungal growth (CAC, 2003a). Evidently, the amount of patulin in apples used for apple juice production plays an important role on the occurrence of patulin in the final product. Consequently, pre-harvest, harvest, and post-harvest phases are decisive in reducing the amount of patulin in the resulting apple juice. Many studies showed that the selection of cultivars used for apple juice production have a significant effect on patulin levels in the obtained apple juices (Cunha et al., 2014; Watanabe and Ayugase, 2009).

After harvest, the fruits are either stored or transported to the factory. To mitigate fungal growth, apples should be put into storage within 18 hours after harvest (CAC, 2003a). Cold storage can prevent the formation of patulin in apples for up to six weeks; therefore, cooling

and shortening the pre-processing storage is very vital to minimise the amount of patulin in juice (Morales et al., 2007). Furthermore, the atmosphere at storage facilities needs to be controlled (Sant'Ana et al., 2008), i.e. the CO_2/O_2 ratio with low levels of O_2 must be maintained to prevent the accumulation of patulin in fruits (Baert et al., 2007; Paster et al., 1995). Such control measures are not always available in developing countries. However, packaging apples during storage with polyethylene can inhibit the fungal growth of *P. expansum* and consequently reduce the amount of accumulated patulin regardless of the surrounding gaseous conditions (Moodley et al., 2002). Recent studies have suggested the use of biological agents like *Saccharomyces cerevisiae* YE-7 or plant extracts to prevent the production of patulin and to improve the storage integrity (Li et al., 2018; Mahunu et al., 2018; Salas et al., 2012).

During the transportation to the processing plant, measures should be taken to prevent damage of the fruits, which increase the susceptibility to fungal infection (FAO, 2003). A recent study in Belgium has suggested a new effective method based on planning the transport routes and minimising the number and intensity of vibrations during the transportation to prevent the damage of apples (Springael et al., 2018).

After arrival at the processing plant, the apples are either temporarily stored or immediately processed. When cold storage is not possible at the factory, apples are recommended to be processed within 48 hours (FAO 2003). Sorting and washing the fruits as well as trimming the rotten parts from the fruits can reduce the content of patulin effectively (Sydenham et al., 1995). Acar and co-workers found that washing apple fruits with high pressure spray is able to reduce more than 50% of the amount of patulin from apples (Acar et al., 1998). Apple batches with high ratio of damaged or rotten fruits should not be used for juice production (FAO, 2003). After sorting and washing, the apples will be ground to produce apple mash. The fruit mash in turn undergoes pressing and centrifugation to produce cloudy apple juice. This cloudy apple juice will be either centrifuged to obtain the final cloudy apple juice or will be subjected to clarification and fining to obtain clear apple juice. Finally, the pasteurised clear or cloudy apple juice is bottled. For export to foreign markets, the juice is usually concentrated before transport and reconstituted later to the desired formulation of juice or nectar.

Juice processing can be used as an effective way to reduce the content of patulin in the final juice product. Welke and co-workers found that apple processing for juice production can cause overall reduction of patulin content by 95% (Welke et al., 2010). In a previous study, the same authors showed that pasteurisation of apple juice can reduce the patulin content by almost 40% while enzymatic treatment, microfiltration, and concentration processes can result in a mean loss of 28.3, 20.1, and 28.4%, respectively (Welke et al., 2009).

There are contradicting reports about the thermal stability of patulin (Kabak, 2009). While Leggott and co-workers found in their study that there was no effect on patulin content by processing the juice into juice concentrate (Leggott et al., 2000), an inverse correlation between the content of patulin in juice and the temperature applied for concentrating was found by others (Kadakal and Nas, 2003). However, there are many factors which lead to these unexpected differences of the thermal stability of patulin like the equipment (Sant'Ana et al., 2008) and the pH-value of the juice which can affect inversely the thermal stability of patulin (Lovett and Peeler, 1973). Sant'Ana and co-workers emphasised that further studies are needed to understand the controversial impact of pasteurisation on patulin (Sant'Ana et al., 2008), which is considered thermostable in fruit juices of low thiols content (Scott and Somers, 1968).

A different study showed that pressing followed by centrifugation can reduce the patulin concentration in juice by an average of 89% (Bissessur et al., 2001). The same study showed that fining of the cloudy apple juice by using bentonite as a fining agent resulted in an average loss of patulin by 77%. Acar and co-workers compared several treatments to produce

apple juice concentrate and found that conventional clarification using a rotary vacuum precoat filter was more effective than using ultrafiltration for the removal of patulin from apple juice (Acar et al., 1998).

Novel methods like applying irradiation, UV radiation, or inactive yeast were suggested in several studies to eliminate or reduce patulin from apple juice (Assatarakul et al., 2012; Yue et al., 2011; Zegota et al., 1988), but most of these methods have disadvantages, i.e. the quality of the produced juice is negatively affected or the high cost or limited applicability of the procedure (Enjie et al., 2018; Sant'Ana et al., 2008). Therefore, further optimisation of these methods is still needed (Ioi et al., 2017).

After processing, the obtained juice is bottled and marketed for direct fresh consumption or concentrated by evaporation to be marketed as a juice concentrate. Some patulin-producing fungal species are able to survive pasteurisation and produce patulin in packaged apple juice, especially at elevated temperature; therefore, it is very important to maintain a low temperature during the storage of packaged juice (Sant'Ana et al., 2010). During storage, there are several factors which affect the stability of patulin in juice like the presence of light or metal ions or oxygen (Drusch et al., 2007). The same study showed that the effectiveness of adding ascorbic acid to apple juice during filling cannot be considered as a reduction strategy of patulin because of the limited oxygen content in the packaged juice, which will be an obstacle for further degradation of patulin.

At the BfR, a pilot project comprising analytical strategies to investigate the fate of mycotoxins during fruit juice processing was launched in 2016. The project aimed to develop a multimethod for detection and determination of mycotoxins in fruit juices. This method was applied to identify the impact factor of each stage of apple juice processing on four of the most relevant mycotoxins (patulin, ochratoxin A, alternariol, and citrinin). A part of the work was performed in cooperation with Hochschule Geisenheim University. Different fining agents combined with different filtration methods were tested, which are the mostly used for the clarification and fining of apple juice. The fining agents investigated were gelatine combined with silica gel and bentonite, charcoal combined with ultrafiltration, and as a third fining agent protein from different plant sources (peas and potatoes) combined with ultrafiltration. The results of this study showed that mycotoxins behave very differently toward fining agents. Enzymatic treatments, centrifugation, and pasteurisation of the obtained juice had no significant effects on the reduction of the studied mycotoxins. In this investigation, the application of activated charcoal combined with ultrafiltration was the most effective fining agent regarding the reduction of the studied mycotoxins.

Cyanotoxins in blue-green algae-based food supplements

Cyanotoxins are secondary metabolites generated by some genera of cyanobacteria (blue-green algae). One prominent subgroup, the microcystins (MCs) consists of more than 100 representatives with different toxicity. All of them have a monocyclic heptapeptide base structure. Within this group, MC-LR is the most common representative and the most potent hepatotoxin. Nodularin, synthesised by the cyanobacterial species *Nodularia spumigena*, is a cyclic pentapeptide with a chemical structure and toxicity similar to the MCs.

Organic food supplements based on micro algae (*Chlorella* sp.) and cyanobacteria (*Spirulina* sp., *Aphanizomenon flos-aquae*) (Figure 27) are advertised to have health promoting effects. Therefore, the demand for these products has increased. However, especially regarding supplements from cyanobacteria, serious concerns were expressed that contaminations of these products with toxins like MCs, nodularin, or anatoxin-a could cause health risks to the consumers. Nodularin and a number of MCs are reported to be potent hepatotoxins; thus, these compounds should be avoided to enter the food chain and are important to be moni-

tored in blue-green algae food supplements (Liyanage et al., 2016; Testai et al., 2016). Up to now, there are no regulations in Germany and the EU regarding maximum limits for cyanotoxin levels in food supplements.

Figure 27: Food supplements based on blue-green algae in different forms (coated tablet, capsule and powder)

In order to protect consumers from allergic reactions, liver damages, and other negative effects, regular analytical controls of food supplements based on blue-green algae are necessary. To investigate BGAS for MCs and nodularin, an analytical method was optimised regarding high performance liquid chromatography coupled to tandem mass spectrometry detection (LC-MS/MS) at the National Reference Laboratory (NRL) for Marine Biotoxins at BfR (Figure 28) (Kirbes et al., 2017). Characteristic precursor ion to product ion transitions allow for a selective and sensitive analysis.

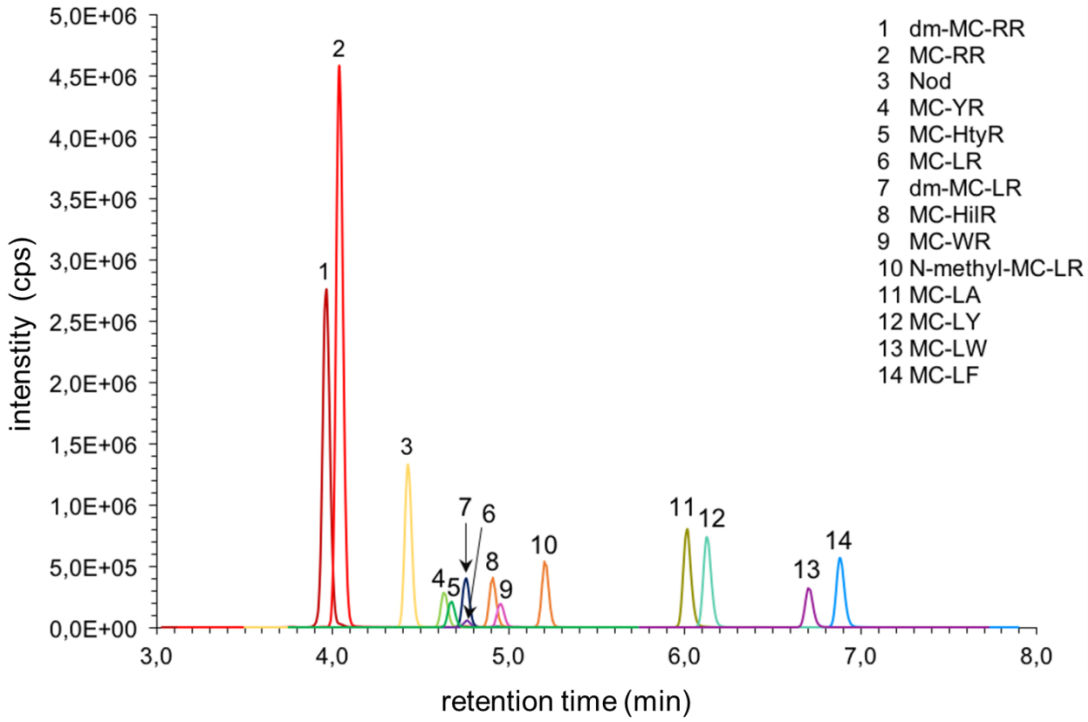


Figure 28: LC-MS/MS chromatogram of a cyanotoxins standard mixture (100 ng/mL)

Furthermore, the sample preparation procedure was improved before the method was in-house validated concerning linearity of the calibration range, recovery rates, matrix effects,

precision, and limits of detection and quantification. Limits of detection ranged from 4–10 µg/kg and limits of quantification from 13–28 µg/kg in *Chlorella* matrix and varied for congeners due to differences in matrix effects. Due to these matrix effects, a matrix-matched calibration is necessary for quantification. Recovery rates were between 49% (MC-LW) and 100% (dm-MC-RR) (Kirbes et al., 2017).

By means of this method, the NRL for Marine Biotoxins at BfR analysed commercial blue-green algae food supplements (*Spirulina*, *Chlorella* and *Aphanizomenon flos-aquae*) in capsules, tablets, and powder form for the cyanotoxins. In 30% of the samples investigated, some MCs were detected or even quantified (Kirbes et al., 2017). The total MC concentration in positive samples ranged from 30 to 660 µg/kg (Figure 29) and, therefore, did not exceed the guidance limit of 1 µg MC-LR_{eq}/g laid down by the Oregon Health Division (Parker et al., 2015). Remarkably, positive samples were mostly products of the blue-green alga *A. flos-aquae*, which is usually harvested from natural sources like the Upper Klamath Lake in Oregon (USA). Compared to blue-green algae grown in monocultures under controlled conditions like spirulina and chlorella subspecies, *A. flos-aquae* is more likely to containcyanotoxins Field conditions regarding climate, nutrients and occurrence of other algae and non-algae species can trigger cyanotoxin production. MC-LR and MC-LA were the most frequently detected toxins (Liyanage et al., 2016; Parker et al., 2015).

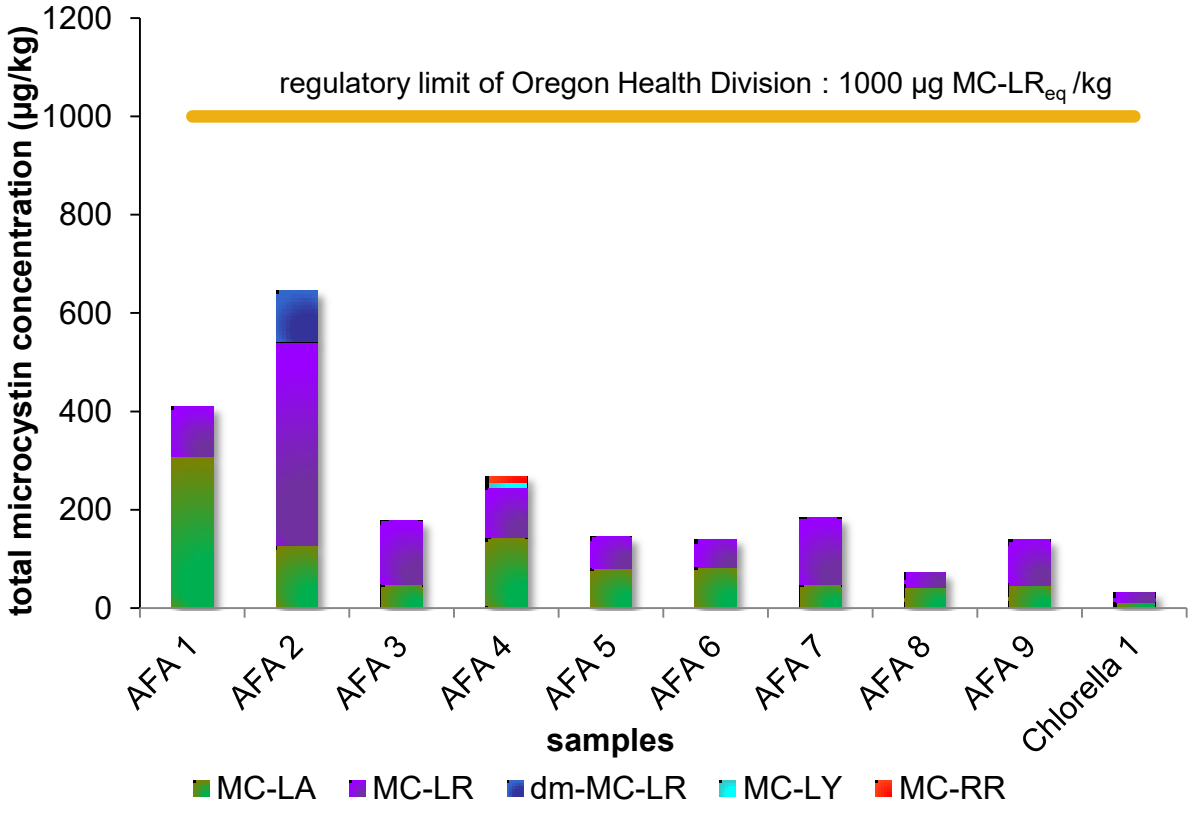


Figure 29: Total microcystin (MC) concentration in MC-positive BGAS samples
The regulatory limit of the Oregon Health Division (1000 µg MC-LReq /kg BGAS product) was not exceeded (Kirbes et al., 2017).

The small scale survey did not raise concern about critical MC levels in BGAS available in German pharmacies and via online trade. However, 30% of the samples contained these compounds, demonstrating the need to monitor cyanotoxins in BGAS. As there are no max-

imum limits set by EU legislation, the food business operators' own control systems need to focus on the monitoring of cyanotoxin levels of the products.

Conclusions

Understanding the fate of natural toxins during processing supports risk assessment by pointing out critical steps in feed and food chains. In cereals, mycotoxins can be produced pre- and postharvest by several fungal species, covering phytopathogenic and commensal fungi. Cereal processing can significantly affect the concentration of mycotoxins, and can lead to the formation of potentially hazardous modified forms. Thus, monitoring of mycotoxins is recommended, but should not be limited to the regulated free forms. More information needs to be acquired particularly on modified mycotoxins as well as on emerging mycotoxins to fill knowledge gaps regarding occurrence in the feed and food chain, fate during processing and potential conversion in food-producing animals, and toxicology.

Most of mycotoxins which occur in fruit and eventually in fruit juices are produced from the fungal species *Penicillium*, *Aspergillus*, and *Alternaria*. Patulin is the most relevant and frequently studied mycotoxin in fruit juices. Regulations were set for its presence in apple juice and apple based products in EU and several parts of the world. Ochratoxin A is regulated in grape juice and it is considered a major occurring mycotoxin in wine and grape based products. Several studies investigated the fate of patulin along apple juice processing. Improper harvest and post-harvest conditions can result in high content of patulin in apple juice. Apple juice processing steps can reduce the content of patulin in apple juice. Further studies are needed to identify the impact factor of each step of apple juice processing on mycotoxins

Occurrence of cyanotoxins in blue-green algae supplements (BGAS) is not a matter of biotransformation during processing but is a natural property of some cyanobacterial species which are directly used as supplements. It can also be caused by contamination of the micro algae or non-toxin-producing cyanobacteria used as food supplements by cyanotoxin-producing bacteria. Aiming at a small scale survey of commercially available BGAS, an LC-MS/MS method was optimised for the determination of 15 cyanotoxins including 14 microcystins (MCs) and nodularin. Despite this complex matrix, limits of detection were below 25 µg/kg for all compounds. Out of 32 analysed samples, 10 contained MCs up to 660 µg/kg total MC concentration, while nodularin was not detected. As a conclusion of this project it can be stated that BGAS should be checked for cyanotoxins, especially before marketing by the producers.

Acknowledgements

Part of the manuscript was prepared in the framework of the MyToolBox project, which has received funding from the European Union's Horizon 2020 research and innovation program under grant agreement no. 678012. The article reflects only the authors' view and the European Commission is not responsible for any use that may be made of the information it contains.

Abbreviations

CAC	Codex Alimentarius Commission
DON	deoxynivalenol
EU	European Union
BGAS	blue-green algae supplements
MC	microcystin

References

Abdel-Sater, M. A., Zohri, A. A., and Ismail, M. A. (2001). Natural contamination of some Egyptian fruit juices and beverages by mycoflora and mycotoxins. *Journal of Food Science and Technology-Mysore, 38*(4), 407-411.

Acar, J., Gokmen, V., and Taydas, E. E. (1998). The effects of processing technology on the patulin content of juice during commercial apple juice concentrate production. *Zeitschrift für Lebensmitteluntersuchung und Forschung A, 207*(4), 328-331. doi:10.1007/s002170050341

Al-Hazmi, M. A. (2014). Patulin in apple juice and its risk assessments on albino mice. *Toxicology and Industrial Health, 30*(6), 534-545. doi:10.1177/0748233712457454

Altmayer, B., Eichhorn, K. W., and Plapp, R. (1982). Analysis of patulin in grape juices and wine. *Zeitschrift für Lebensmitteluntersuchung und Forschung, 175*(3), 172-174. doi:10.1007/Bf01139765

Angel, M., Asya, A., Alaa, B., Alicia, R., and Naresh, M. (2017). Climate change, food security and mycotoxins: Do we know enough? *Fungal Biology Reviews, 31*(3), 143-154. doi:10.1016/j.fbr.2017.04.002

Assatarakul, K., Churey, J. J., Manns, D. C., and Worobo, R. W. (2012). Patulin reduction in apple juice from concentrate by UV radiation and comparison of kinetic degradation models between apple juice and apple cider. *Journal of Food Protection, 75*(4), 717-724. doi:10.4315/0362-028x.Jfp-11-429

Baert, K., De Meulenaer, B., Kamala, A., Kasase, C., and Devlieghere, F. (2006). Occurrence of patulin in organic, conventional, and handcrafted apple juices marketed in Belgium. *Journal of Food Protection, 69*(6), 1371-1378. doi:10.4315/0362-028X-69.6.1371

Baert, K., Devlieghere, F., Flyps, H., Oosterlinck, M., Morshed Ahmed, M., Rajkovic, A., . . . De Meulenaer, B. (2007). Influence of storage conditions of apples on growth and patulin production by *Penicillium expansum*. *International Journal of Food Microbiology, 119*(3), 170-181. doi:10.1016/j.ijfoodmicro.2007.07.061

Barreira, M. J., Alvito, P. C., and Almeida, C. M. M. (2010). Occurrence of patulin in apple-based-foods in Portugal. *Food Chemistry, 121*(3), 653-658. doi:10.1016/j.foodchem.2009.12.085

Bennett, J. W., and Klich, M. (2003). Mycotoxins. *Clinical Microbiology Reviews, 16*(3), 497-516. doi:10.1128/Cmr.16.3.497-516.2003

Berthiller, F., Crews, C., Dall'Asta, C., Saeger, S. D., Haesaert, G., Karlovsky, P., . . . and Stroka, J. (2013). Masked mycotoxins: A review. *Molecular Nutrition & Food Research, 57*(1), 165-186. doi:10.1002/mnfr.201100764

Bissessur, J., Permaul, K., and Odhav, B. (2001). Reduction of patulin during apple juice clarification. *Journal of Food Protection, 64*(8), 1216-1219. doi:10.4315/0362-028x-64.8.1216

BMEL, Bundesministerium für Ernährung und Landwirtschaft. (2005). Leitsätze für Brot und Kleingebäck. In *Deutsches Lebensmittelbuch*.

BMJV, Bundesministerium der Justiz und für Verbraucherschutz. (2010). Verordnung zur Begrenzung von Kontaminanten in Lebensmitteln (Kontaminanten-Verordnung - KmV). http://www.gesetze-im-internet.de/kmv/BJNR028700010.html

Buratti, F. M., Manganelli, M., Vichi, S., Stefanelli, M., Scardala, S., Testai, E., and Funari, E. (2017). Cyanotoxins: producing organisms, occurrence, toxicity, mechanism of action and human health toxicological risk evaluation. *Archives of Toxicology, 91*(3), 1049-1130. doi:10.1007/s00204-016-1913-6

CAC, Codex Alimentarius Commission. (1995a). General standard for contaminants and toxins in food and feed (CODEX STAN 193-1995).

CAC, Codex Alimentarius Commission. (2003a). Code of practice for the prevention and reduction of patulin contamination in apple juice and apple juice ingredients in other beverages (CAC/RCP 50-2003). www.fao.org/input/download/standards/405/CXP_050e.pdf

CAC, Codex Alimentarius Commission. (2017a). General standard for contaminants and toxins in food and feed (CODEX STAN 193-1995, latest amended in 2017). http://www.fao.org/fao-who-codexalimentarius/sh-proxy/en/?lnk=1&url=https%253A%252F%252Fworkspace.fao.org%252Fsites%252Fcodex%252FStandards%252FCODEX%2BSTAN%2B193-1995%252FCXS_193e.pdf

CAC, Codex Alimentarius Commission. (2017b). *Code of practice for the prevention and reduction of mycotoxin contamination in cereals (CAC/RCP 51-2003, latest amended in 2017)*. http://www.fao.org/fao-who-codexalimentarius/sh-proxy/en/?lnk=1&url=https%253A%252F%252Fworkspace.fao.org%252Fsites%252Fcodex%252FStandards%252FCAC%2BRCP%2B51-2003%252FCXC_051e.pdf.

Callejon, R. M., Rodriguez-Naranjo, M. I., Ubeda, C., Hornedo-Ortega, R., Garcia-Parrilla, M. C., and Troncoso, A. M. (2015). Reported foodborne outbreaks due to fresh produce in the United States and European Union: Trends and causes. *Foodborne Pathogens and Disease, 12*(1), 32-38. doi:10.1089/fpd.2014.1821

CBI, Centre for the Promotion of Imports from developing countries. (2017a). Exporting fruit juices to Europe. https://www.cbi.eu/market-information/processed-fruit-vegetables-edible-nuts/fruit-juices/europe/

Cunha, S. C., Faria, M. A., Pereira, V. L., Oliveira, T. M., Lima, A. C., and Pinto, E. (2014). Patulin assessment and fungi identification in organic and conventional fruits and derived products. *Food Control, 44*, 185-190. doi:10.1016/j.foodcont.2014.03.043

de Sylos, C. M., and Rodriguez-Amaya, D. B. (1999). Incidence of patulin in fruits and fruit juices marketed in Campinas, Brazil. *Food Additives and Contaminants, 16*(2), 71-74. doi:10.1080/026520399284226

Drusch, S., Kopka, S., and Kaeding, J. (2007). Stability of patulin in a juice-like aqueous model system in the presence of ascorbic acid. *Food Chemistry, 100*(1), 192-197. doi:10.1016/j.foodchem.2005.09.043

EC, European Commission. (2006a). Commission Recommendation of 17 August 2006 on the presence of deoxynivalenol, zearalenone, ochratoxin A, T-2 and HT-2 and fumonisins in products intended for animal feeding (2006/576/EC). *Official Journal of the European Communities, L 229*, 7-9.

EC, European Commission. (2006b). Commission Recommendation of 17 August 2006 on the prevention and reduction of *Fusarium* toxins in cereals and cereal products (2006/583/EC). *Official Journal of the European Communities, L 234*, 35-40.

EC, European Commission. (2006c). Commission Regulation (EC) No 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs. *Official Journal of the European Communities, L 364*, 5-24.

EC, European Commission. (2017b). Commission Regulation (EC) No 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs. *Official Journal of the European Union, L364*, 5-24.

EFSA CONTAM, European Food Safety Authority's Panel on Contaminants in the Food Chain. (2014). Scientific Opinion on the risks for human and animal health related to the presence of modified forms of certain mycotoxins in food and feed. *EFSA Journal, 12*(12), 3916 (3107 pp.). doi:10.2903/j.efsa.2014.3916

Enjie, D., Jiasheng, W., Xiangyang, L., Xinfeng, W., and Gao, D. (2018). Patulin degradation in apple juice using ozone detoxification equipment and its effects on quality. *Journal of Food Processing and Preservation, 42*(7), e13645. doi:10.1111/jfpp.13645

Escrivá, L., Font, G., Manyes, L., and Berrada, H. (2017). Studies on the presence of mycotoxins in biological samples: An overview. *Toxins, 9*(8), 251. doi:10.3390/toxins9080251

FAO, Food and Agriculture Organization of the United Nations. (2003). Manual on the application of the HACCP system in mycotoxin prevention and control. http://www.fao.org/docrep/005/Y1390E/y1390e00.htm

FDA, U.S. Food and Drug Administration. (2005). CPG Sec.510.150 Apple juice, apple juice concentrates, and apple juice products – Adulteration with patulin. https://www.fda.gov/ICECI/ComplianceManuals/CompliancePolicyGuidanceManual/ucm074427.htm

FDA, U.S. Food and Drug Administration. (2015a). CPG Sec. 683.100 Action levels for aflatoxins in animal feeds. https://www.fda.gov/ICECI/ComplianceManuals/CompliancePolicyGuidanceManual/ucm074703.htm

FDA, U.S. Food and Drug Administration. (2015b). CPG Sec. 555.400 Foods – Adulteration with aflatoxin. https://www.fda.gov/ICECI/ComplianceManuals/CompliancePolicyGuidanceManual/ucm074555.htm

Flores-Flores, M. E., Lizarraga, E., de Cerain, A. L., and González-Peñas, E. (2015). Presence of mycotoxins in animal milk: A review. *Food Control, 53*(163-176). doi:10.1016/j.foodcont.2015.01.020

Ioi, J. D., Zhou, T., Tsao, R., and Marcone, M. F. (2017). Mitigation of patulin in fresh and processed foods and beverages. *Toxins, 9*(5), 157. doi:10.3390/toxins9050157

Jackson, L. S., and Al-Taher, F. (2008). Chapter 4 – Factors affecting mycotoxin production in fruits. In R. Barkai-Golan and N. Paster (Eds.), *Mycotoxins in Fruits and Vegetables* (pp. 75-104). San Diego: Academic Press.

Jestoi, M. (2008). Emerging *Fusarium*-mycotoxins fusaproliferin, beauvericin, enniatins, and moniliformin—A review. *Critical Reviews in Food Science and Nutrition, 48*(1), 21-49. doi:10.1080/10408390601062021

Kabak, B. (2009). The fate of mycotoxins during thermal food processing. *Journal of the Science of Food and Agriculture, 89*(4), 549-554. doi:10.1002/jsfa.3491

Kadakal, Ç., and Nas, S. (2003). Effect of heat treatment and evaporation on patulin and some other properties of apple juice. *Journal of the Science of Food and Agriculture, 83*(9), 987-990. doi:10.1002/jsfa.1339

Kent, N. L., and Evers, A. D. (1994). *Technology of cereals*. Oxford, U.K.: Elsevier Science Ltd.

Kirbes, C., Bodi, D., Kapp, K., Hiller, A., Preiss-Weigert, A., Hamscher, G., and Lahrssen-Wiederholt, M. (2017). *Bestimmung von Microcystinen und Nodularin in Nahrungsergänzungsmitteln*. Paper presented at the 46. Deutscher Lebensmittelchemikertag, Würzburg, 25.–27.09.2017.

Krska, R., de Nijs, M., McNerney, O., Pichler, M., Gilbert, J., Edwards, S., . . . and van Egmond, H. P. (2016). Safe food and feed through an integrated toolbox for mycotoxin management: The MyToolBox approach. *World Mycotoxin Journal, 9*(4), 487-495. doi:10.3920/wmj2016.2136

L'vova, L. S., Kizlenko, O. I., Shul'gina, A. P., Omel'chenko, M. D., and Bystryakova, Z. K. (1998). Distribution of deoxynivalenol in products of processing *Fusarium*-affected soft and hard wheats and barley. *Prikladnaya Biokhimiya i Mikrobiologiya – Applied Biochemistry and Microbiology, 34*(4), 444-449.

Leggott, N. L., Vismer, H. F., Sydenham, E. W., Shephard, G. S., Rheeder, J. P., and Marasas, W. F. O. (2000). Occurrence of patulin in the commercial processing of apple juice. *South African Journal of Science, 96*(5), 241-243.

Li, W., Zhou, T., Wu, T., and Li, X. (2018). *Saccharomyces cerevisiae* YE-7 reduces the risk of apple blue mold disease by inhibiting the fungal incidence and patulin biosynthesis. *Journal of Food Processing and Preservation, 42*(1), e13360. doi:10.1111/jfpp.13360

Liyanage, H. M., Arachchi, D. N. M., Abeysekara, T., and Guneratne, L. (2016). Toxicology of freshwater cyanobacteria. *Journal of Environmental Science and Health Part C-Environmental Carcinogenesis & Ecotoxicology Reviews, 34*(3), 137-168. doi:10.1080/10590501.2016.1193923

López, P., Venema, D., de Rijk, T., de Kok, A., Scholten, J. M., Mol, H. G. J., and de Nijs, M. (2016). Occurrence of *Alternaria* toxins in food products in The Netherlands. *Food Control, 60*, 196-204. doi:10.1016/j.foodcont.2015.07.032

Lovett, J., and Peeler, J. T. (1973). Effect of pH on the thermal destruction kinetics of patulin in aqueous solution. *Journal of Food Science, 38*(6), 1094-1095. doi:10.1111/j.1365-2621.1973.tb02163.x

Mahunu, G. K., Zhang, H., Apaliya, M. T., Yang, Q., Zhang, X., and Zhao, L. (2018). Bamboo leaf flavonoid enhances the control effect of *Pichia caribbica* against *Penicillium expansum* growth and patulin accumulation in apples. *Postharvest Biology and Technology, 141*, 1-7. doi:10.1016/j.postharvbio.2018.03.005

Mazumder, P. M., and Sasmal, D. (2001). Mycotoxins – Limits and regulations. *Ancient Science of Life, 20*(3), 1-19.

Ministério da Saúde. República Federativa do Brasil. (2011). Resolução MS-ANVISA-RDC nº 7, de 18/02/11 DOU de 22/02/11 p.72 – seção 1 nº 37 – Dispõe sobre limites máximos tolerados (LMT) para micotoxinas em alimentos. http://bvsms.saude.gov.br/bvs/saudelegis/anvisa/2011/res0007_18_02_2011_rep.html

Moodley, R. S., Govinden, R., and Odhav, B. (2002). The effect of modified atmospheres and packaging on patulin production in apples. *Journal of Food Protection, 65*(5), 867-871. doi:10.4315/0362-028x-65.5.867

Morales, H., Marin, S., Rovira, A., Ramos, A. J., and Sanchis, V. (2007). Patulin accumulation in apples by *Penicillium expansum* during postharvest stages. *Letters in Applied Microbiology, 44*(1), 30-35. doi:10.1111/j.1472-765X.2006.02035.x

Ozcelik, S., Ozcelik, N., and Beuchat, L. R. (1990). Toxin production by *Alternaria-alternata* in tomatoes and apples stored under various conditions and quantitation of the toxins by high-performance liquid-chromatography. *International Journal of Food Microbiology, 11*(3-4), 187-194. doi:10.1016/0168-1605(90)90011-S

Parker, C. H., Stutts, W. L., and DeGrasse, S. L. (2015). Development and validation of a liquid chromatography-tandem mass spectrometry method for the quantitation of microcystins in blue-green algal dietary supplements. *Journal of Agricultural and Food Chemistry, 63*(47), 10303-10312. doi:10.1021/acs.jafc.5b04292

Paster, N., Huppert, D., and Barkaigolan, R. (1995). Production of patulin by different strains of *Penicillium expansum* in pear and apple cultivars stored at different temperatures and modified atmospheres. *Food Additives and Contaminants, 12*(1), 51-58. doi:10.1080/02652039509374278

Posner, E. S. (2009). Wheat flour milling. In K. Khan and P. R. Shewry (Eds.), *Wheat: Chemistry and Technology* (pp. 119-152). St. Paul, Minnesota: Academic Press.

Puel, O., Galtier, P., and Oswald, I. P. (2010). Biosynthesis and toxicological effects of patulin. *Toxins, 2*(4), 613-631. doi:10.3390/toxins2040613

Raja, R., Hemaiswarya, S., Ganesan, V., and Carvalho, I. S. (2016). Recent developments in therapeutic applications of Cyanobacteria. *Critical Reviews in Microbiology, 42*(3), 394-405. doi:10.3109/1040841X.2014.957640

Riteni, A. (2003). Patulin in Italian commercial apple products. *Journal of Agricultural and Food Chemistry, 51*(20), 6086-6090. doi:10.1021/jf034523c

Rychlik, M., Humpf, H.-U., Marko, D., Dänicke, S., Mally, A., Berthiller, F., . . . and Lorenz, N. (2014). Proposal of a comprehensive definition of modified and other forms of mycotoxins including “masked” mycotoxins. *Mycotoxin Research, 30*(4), 197-205. doi:10.1007/s12550-014-0203-5

Salas, M. P., Reynoso, C. M., Céliz, G., Daz, M., and Resnik, S. L. (2012). Efficacy of flavanones obtained from citrus residues to prevent patulin contamination. *Food Research International, 48*(2), 930-934. doi:10.1016/j.foodres.2012.02.003

Sant'Ana, A. d. S., Rosenthal, A., and de Massaguer, P. R. (2008). The fate of patulin in apple juice processing: A review. *Food Research International, 41*(5), 441-453. doi:10.1016/j.foodres.2008.03.001

Sant'Ana, A. S., Costa Simas, R., Almeida, C. A. A., Cabral, E. C., Rauber, R. H., Mallmann, C. A., . . . Massaguer, P. R. (2010). Influence of package, type of apple juice and temperature on the production of patulin by *Byssochlamys nivea* and *Byssochlamys fulva*. *International Journal of Food Microbiology, 142*(1-2), 156-163. doi:10.1016/j.ijfoodmicro.2010.06.017

Sanzani, S. M., Reverberi, M., and Geisen, R. (2016). Mycotoxins in harvested fruits and vegetables: Insights in producing fungi, biological role, conducive conditions, and tools to manage postharvest contamination. *Postharvest Biology and Technology, 122*, 95-105. doi:10.1016/j.postharvbio.2016.07.003

Schaarschmidt, S., and Fauhl-Hassek, C. (2018). The fate of mycotoxins during the processing of wheat for human consumption. *Comprehensive Reviews in Food Science and Food Safety, 17*(3), 556-593. doi:10.1111/1541-4337.12338

Schaarschmidt, S., and Fauhl-Hassek, C. (2019). Mycotoxins during the processes of nixtamalization and tortilla production. *Toxins, 11*(4), 227. doi:10.3390/toxins11040227

Scott, P. M., Lawrence, G. A., and Lau, B. P. (2006). Analysis of wines, grape juices and cranberry juices for *Alternaria* toxins. *Mycotoxin Research, 22*(2), 142-147. doi:10.1007/BF02956778

Scott, P. M., and Somers, E. (1968). Stability of patulin and penicillic acid in fruit juices and flour. *Journal of Agricultural and Food Chemistry, 16*(3), 483-485. doi:10.1021/jf60157a011

Scudamore, K. A., Hazel, C. M., Patel, S., and Scriven, F. (2009). Deoxynivalenol and other *Fusarium* mycotoxins in bread, cake, and biscuits produced from UK-grown wheat under commercial and pilot scale conditions. *Food Additives & Contaminants: Part A, 26*(8), 1191-1198. doi:10.1080/02652030902919426

Spadaro, D., Garibaldi, A., and Gullino, M. L. (2008). Occurrence of patulin and its dietary intake through pear, peach, and apricot juices in Italy. *Food Additives & Contaminants Part B-Surveillance, 1*(2), 134-139. doi:10.1080/02652030802363790

Springael, J., Paternoster, A., and Braet, J. (2018). Reducing postharvest losses of apples: Optimal transport routing (while minimizing total costs). *Computers and Electronics in Agriculture, 146*, 136-144. doi:10.1016/j.compag.2018.02.007

Steiman, R., Seiglemurandi, F., Sage, L., and Krivobok, S. (1989). Production of patulin by micromycetes. *Mycopathologia, 105*(3), 129-133. doi:10.1007/Bf00437244

Sydenham, E. W., Vismer, H. F., Marasas, W. F. O., Brown, N., Schlechter, M., van der Westhuizen, L., and Rheeder, J. P. (1995). Reduction of patulin in apple juice samples—Influence of initial processing. *Food Control, 6*(4), 195-200. doi:10.1016/0956-7135(95)00020-R

Testai, E., Buratti, F. M., Funari, E., Manganelli, M., Vichi, S., Arnich, N., . . . Sialehaamoa, A. (2016). Review and analysis of occurrence, exposure and toxicity of cyanobacteria toxins in food. *EFSA Supporting Publications, 2016:EN-998*. doi:10.2903/sp.efsa.2016.EN-998

USDA, United States Department of Agriculture. (2011). China's maximum levels for mycotoxins in foods (GB 2761-2012). https://gain.fas.usda.gov/Recent%20GAIN%20Publications/Maximum%20Levels%20of%20Mycotoxins%20in%20Foods_Beijing_China%20-%20Peoples%20Republic%20of_12-29-2014.pdf

Van Boxstael, S., Habib, I., Jacxsens, L., De Vocht, M., Baert, L., Van de Perre, E., . . . Uyttendaele, M. (2013). Food safety issues in fresh produce: Bacterial pathogens, viruses and pesticide residues indicated as major concerns by stakeholders in the fresh produce chain. *Food Control, 32*(1), 190-197. doi:10.1016/j.foodcont.2012.11.038

Varma, S. K., and Verma, R. A. B. (1987). Aflatoxin-B1 production in orange (*Citrus reticulata*) juice by isolates of *Aspergillus flavus* link. *Mycopathologia, 97*(2), 101-104. doi:10.1007/Bf00436846

Vdf, Verband der deutschen Fruchtsaft-Industrie e.V., (2017). In. https://www.fruchtsaft.de/.

Vidal, A., Marín, S., Morales, H., Ramos, A. J., and Sanchis, V. (2014a). The fate of deoxynivalenol and ochratoxin A during the breadmaking process, effects of sourdough use and bran content. *Food and Chemical Toxicology, 68*, 53-60. doi:10.1016/j.fct.2014.03.006

Vidal, A., Morales, H., Sanchis, V., Ramos, A. J., and Marín, S. (2014b). Stability of DON and OTA during the breadmaking process and determination of process and performance criteria. *Food Control, 40*, 234-242. doi:10.1016/j.foodcont.2013.11.044

Viñas, I., Dadon, J., and Sanchis, V. (1993). Citrinin-producing capacity of *Penicillium expansum* strains from apple packinghouses of Lerida (Spain). *International Journal of Food Microbiology, 19*(2), 153-156. doi:10.1016/0168-1605(93)90181-F

Völkel, I., Schröer-Merker, E., and Czerny, C.-P. (2011). The carry-over of mycotoxins in products of animal origin with special regard to its implications for the European food safety legislation. *Food and Nutrition Sciences, 2*(8), 852-867. doi:10.4236/fns.2011.28117

Watanabe, M., and Ayugase, J. (2009). Effects of major apple varieties in japan and the apple fruit components on mycotoxin production by *Penicillium expansum*. *Journal of the Japanese Society for Food Science and Technology-Nippon Shokuhin Kagaku Kogaku Kaishi, 56*(4), 215-222. doi:10.3136/nskkk.56.215

Welke, J. E., Hoeltz, M., Dottori, H. A., and Noll, I. B. (2009). Effect of processing stages of apple juice concentrate on patulin levels. *Food Control, 20*(1), 48-52. doi:10.1016/j.foodcont.2008.02.001

Welke, J. E., Hoeltz, M., Dottori, H. A., and Noll, I. B. (2010). Fungi and patulin in apples and the role of processing on patulin levels in juices: A study on naturally contaminated apples. *Journal of Food Safety, 30*(2), 276-287. doi:10.1111/j.1745-4565.2009.00205.x

Yue, T., Dong, Q., Guo, C., and Worobo, R. W. (2011). Reducing patulin contamination in apple juice by using inactive yeast. *Journal of Food Protection, 74*(1), 149-153. doi:10.4315/0362-028x.Jfp-10-326

Yurdun, T., Omurtag, G. Z., and Ersoy, Ö. (2001). Incidence of patulin in apple juices marketed in Turkey. *Journal of Food Protection, 64*(11), 1851-1853. doi:10.4315/0362-028X-64.11.1851

Zaied, C., Abid, S., Hlel, W., and Bacha, H. (2013). Occurrence of patulin in apple-based-foods largely consumed in Tunisia. *Food Control, 31*(2), 263-267. doi:10.1016/j.foodcont.2012.10.005

Zegota, H., Żegota, A., and Bachmann, S. (1988). Effect of irradiation and storage on patulin disappearance and some chemical-constituents of apple juice concentrate. *Zeitschrift für Lebensmitteluntersuchung und Forschung, 187*(4), 321-324. doi:10.1007/Bf01454421

Zhou, S.-m., Jiang, L.-p., Geng, C.-y., Cao, J., and Zhong, L.-f. (2009). Patulin-induced genotoxicity and modulation of glutathione in HepG2 cells. *Toxicon, 53*(5), 584-586. doi:10.1016/j.toxicon.2009.01.030

Zimmerli, B., and Dick, R. (1996). Ochratoxin A in table wine and grape-juice: Occurrence and risk assessment. *Food Additives and Contaminants, 13*(6), 655-668. doi:10.1080/02652039609374451

4.4 Toxin detection and quantification – regulatory and analytical aspects

Maul, Ronald; Bodi, Dorina, Kemmlein, Sabine; Bahlmann, Arnold

Abstract

Globalization of trade channels is causing great challenges for the safety along the feed and food supply chain. In this context various aspects have to be considered and criteria have to be specified throughout the product chain to ensure a high quality from the raw material to the final consumer product. Special attention has been given to natural contaminants, because due to their widespread occurrence these toxins represent a particular risk at the end of the commodity chain for animals and humans as well. Following the vision to minimize possible health consequences all over the world global measures must be put in place by authorities and producing industry in an equivalent manner. With respect to natural toxins, such measures include e.g. the establishment and harmonization of legal limits, development and standardization of analytical methods and the implementation of performance criteria all of which together unite the basis for a comprehensive control system. This chapter covers a selection of items, that are directly or indirectly linked to each other in order to meet these measures at a national and international level as well. General aspects and challenges regarding the various analytical approaches with a special emphasis on new “emerging” toxins and the corresponding work and interconnections of standardization bodies are also discussed. As such, BfR plays an active role in all these considerations and is entangled in its work to a variety of subjects presented here in this chapter.

Introduction

According to the World Health Organisation (WHO) “*Natural toxins are toxic compounds that are naturally produced by living organisms. These toxins are not harmful to the organisms themselves but they may be toxic to other creatures, including humans, when eaten. These chemical compounds have diverse structures and differ in biological function and toxicity*” (http://www.who.int/news-room/fact-sheets/detail/natural-toxins-in-food). Consequently, natural toxins (or biotoxins as they are also often called) represent an important issue of food and feed safety as well.

The impact of natural toxins on the safety and integrity of food and feed production chains is immense with major economic consequences. Whilst several years ago FAO estimated that about 25 % of the world´s crop production to be contaminated only by toxins belonging to the class of mycotoxins, more recent scientific publications on feed indicated that even more than 70 % might be affected by this compound group at least at low toxin levels (Kovalsky et al., 2016; Schatzmayr and Streit, 2013).

One reason for the increasing frequency of natural toxin detection in food and feed is the constantly evolving sensitivity and selectivity of analytical tools. Thus, according to the actual state of toxicological knowledge, only a small number of these contaminations may be considered as harmful to consumer´s and animal health. Additionally, new “emerging” mycotoxins are coming into focus of the risk assessment bodies and may, if assessed as toxicologically relevant and with levels exceeding critical exposure rates, increase the amount of food and feed that has to be withdrawn from the market as non-compliant products. Besides, various other biotoxins require attention in the global food and feed chain – not only but also due to changing trade routes and consumers’ dietary habits. Plant toxins or marine biotoxins represent two toxin classes of high concern. As for mycotoxins the marine biotoxins surveys confirm the widespread occurrence in sea food commodities at low levels as to be seen from the analysis of mussels from the Adriatic Sea with 37 % samples being tested positive between 2015 and 2018.(Schirone et al., 2018) For plant toxins the natural variety is also large

with respect to chemical structures, their mode of action and their way to enter the food and feed chain. Thus, plant toxins may be inherent metabolites in daily foods such as potatoes, herbs and spices or in herbal preparations or can e.g. be present as contaminants in foods as due to unintentionally co-harvested weeds.(Nijs et al., 2017) From these spotlights on the occurrence of natural toxins it becomes obvious that the challenge to increase food safety is enormous due to the frequency and variability in their occurrence. The changing and expanding global trade chains will also enhance the complexity of toxins present in food and feed on the market. Emerging toxins of fungal, plant or microbial origin as a consequence of changing value chains or processing steps as well as their co-occurrence require a new and careful analytical consideration (Kovalsky et al., 2016). Both, increased numbers and improved analytical power of official controls will be required in the future (Dellafiora and Dall'Asta, 2017). It is worth mentioning that also the frequency and quality of controls in the food and feed producing industry will increase as a consequence of serious economic impacts of the presence of toxins in food and feed. This comprises reputational damage for the distinct producer but also negatively influences the consumer´s trust in food safety in general.

However, the analysis of natural toxins is not only limited to control of incoming goods or surveillance of legal limits but is also required to collect data for exposure and toxicological assessment. Thus, validated analytical methods tailor made for the demand of different purposes are needed. The appropriateness of a method depends on a multitude of factors such as precision and trueness, ease of use, analysis time, cost efficiency or applicability in the field. Particularly, the search for methods that are fast and easy to use is in contradiction with the parameter of accuracy as proper and representative sampling and sample preparation is time consuming however crucial for the analysis of biotoxins that tend to be unevenly distributed throughout the food and feed commodities.

Nonetheless, rapid tests or screening methods with a high throughput while being less accurate than confirmatory methods are useful tools to obtain a first impression of the contamination situation in a commodity. In suspect sample lots a subsequent confirmatory analysis using a confirmatory method, that has gone through a laborious validation protocol, may provide proof for relevant toxin content whilst non suspect samples may be considered as most likely compliant with legal obligations. For the purpose of court proof surveillance of legal limits or the accurate exposure or toxicological assessment thoroughly validated methods are required. All analytical steps need to be reproducible and traceable. A prerequisite is the availability of reliable analytical standards that is often hard to fulfill in the field of natural toxin analysis. Only as soon as reference standards are available a fully validated analytical method comprising sampling, cleanup, measurement and data evaluation can be established according to harmonized and internationally recognized protocols. Data obtained from the application of valid methods may be used for further risk assessment and represent the basis for regulatory action.

In the final stage it must be ensured that regulatory actions are based on reliable scientific findings (Schatzmayr and Streit, 2013). That means analytical method validation and harmonization is directly linked to issues of global trade and food safety delivering the scientific basis for subsequent political management action. Consequently, BfR is engaged at analytical research level, standardisation as well as in harmonization processes at national and international level. This is reflected e.g. by the hosting of various national reference laboratories but also involvement in boards and expert committees of various standardisation bodies. The following sections will highlight some of the most relevant activities of BfR in the field of biotoxins at different levels and put them in the context of global food and feed chains.

Standardisation and harmonisation tools

Globalization of trade is causing major challenges for the safety along the feed and food supply chain. To define minimum quality criteria for food and feed commodities with regard to consumer health protection food and feed standards are an important tool. The following elaboration on BfR partnered standardisation and harmonization activities is not claimed to be exhaustive, but aims to shed spotlights on the ways of how these tools are used in Europe and also worldwide and how the BfR is involved in these activities.

Codex Alimentarius is a collection of internationally recognized standards, guidelines and codes of practice adopted by the Codex Alimentarius Commission (CAC). The CAC is a non-governmental organization founded in 1963 by FAO and WHO, its aim is to protect consumers' health and to ensure fair practices in the food trade. Its work includes provisions in respect of food hygiene, food additives, residues of pesticides and veterinary drugs, contaminants, labelling and presentation, methods of analysis and sampling, and import and export inspection and certification. Under the roof of CAC several committees are working on different subjects including standards for certain food commodities and general ones which apply in a horizontal way over several commodities.

The Codex Alimentarius Committee on Methods of Analysis and Sampling (CCMAS) is a horizontal or general committee dealing with questions on the analysis of ingredients and various undesired substances in all food and feed commodities. Its purpose is to define appropriate criteria for methods of analysis and sampling, to decide on amendment or endorsement of methods proposed to the committee, to develop sampling plans and procedures as well as protocols and guidelines to evaluate food laboratory proficiency and quality assurance systems. As such, CCMAS plays a leading role as standardisation body at global level. Codex standards have become even more important with the establishment of the World Trade Organization (WTO) in 1995, as the WTOs Agreement on the Application of Sanitary and Phyto-Sanitary Measures (SPS) has denominated the FAO/WHO Codex Alimentarius as the relevant standard-setting organization for food safety with specific reference to standards, guidelines and recommendations. Trading partners agree on reasonable requirements on the quality of the traded goods taking legal regulations, national or international food safety standards as the minimum quality requirements into account. It has been shown in several studies, that adoption of food standards increase the trade of commodities affected by these standards (Keiichiro et al., 2015). Codex, as an inter-governmental organization, prepares documents to assist governments in their statutory and regulatory work to protect their citizens from health hazards caused by food consumption (CEN, 2018). The elaboration process of codex standards comprises various steps and involves numerous subsidiary bodies such as the general committees (e.g. CCMAS), commodity and regional coordinating committees as well as national governments (see Figure 30). Particularly, as part of the commenting process input provided by national organisations like the BfR influences the standard´s genesis. While being recommendations for voluntary application by members, Codex standards serve in many cases as a basis for national legislation especially in third countries. As standards are developed and implemented collaboratively by stakeholders and published by non-governmental standardisation bodies, they do not gain legally binding status but can facilitate compliance with legislation (CEN, 2018).

However, at European level, legislation and standards have been interweaving for a long time due to the so called "New Approach" laid down in the Council Resolution No 85/C136/01 on a new approach to technical harmonization and standards. One of its main principles is that *"...national authorities are obliged to recognize that products manufactured in conformity with harmonized standards (or, provisionally, with national standards) are presumed to conform to the 'essential requirements' established by the Directive"(The Council, 1985).* The "New Approach" was established in order to break down technical barriers to trade and, thereby, to reduce uncertainty for economic operators.

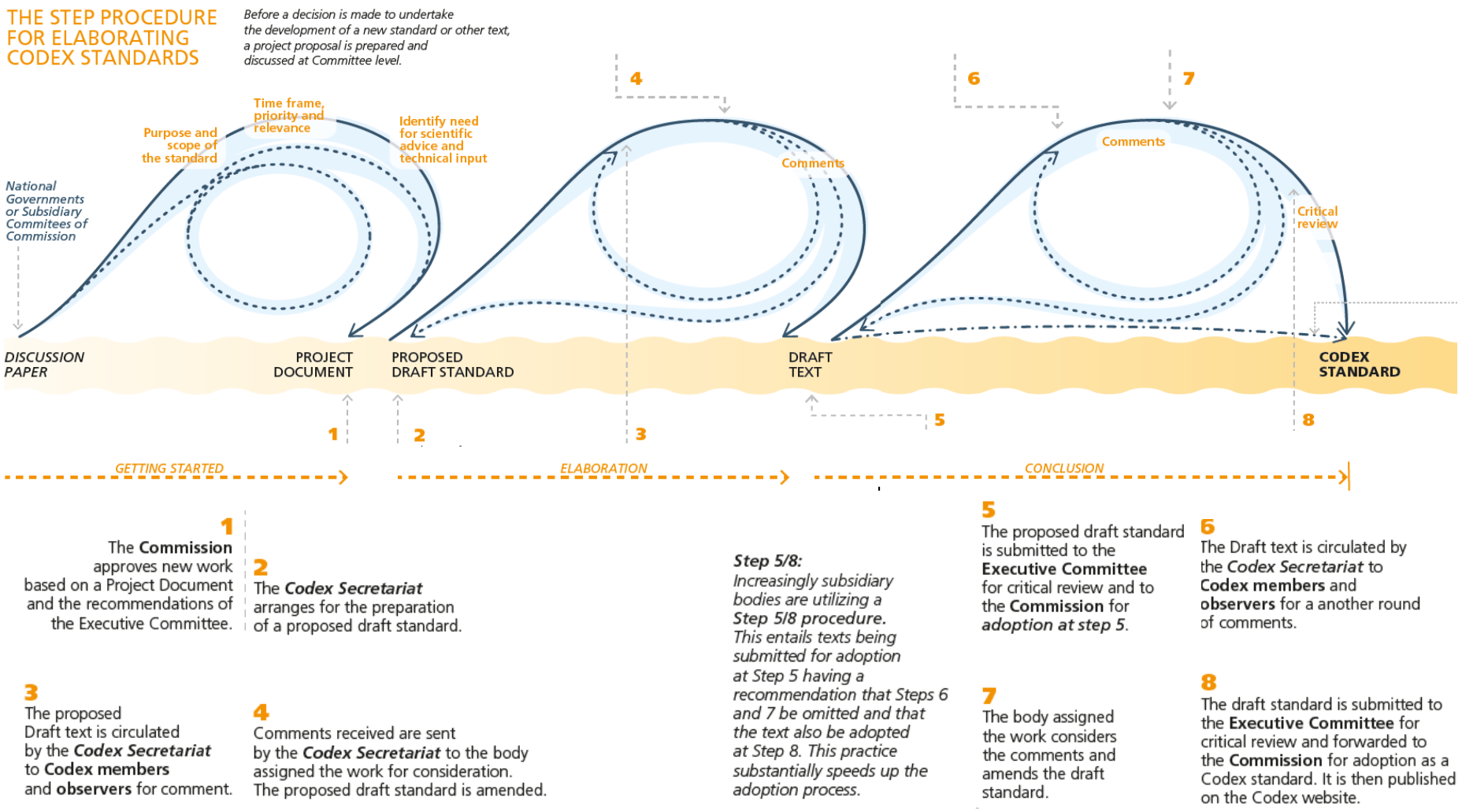


Figure 30: The step procedure for elaborating Codex standards adapted from "understanding codex"
(URL: http://www.fao.org/3/a-i5667e.pdf for more information)

Nevertheless, compliance with regulations and the agreed requirements has to be verified by official control bodies and by own control systems of the food and feed business operators, which involves the use of reliable and internationally accepted analysis and sampling methods. According to Regulation (EU) 2017/625 official controls have to be carried out using *"analytical, testing and diagnostic methods that meet state-of-the-art scientific standards and offer sound, reliable and comparable results across the Union" (Eur. Parliament and The Council, 2017)*.

With the objective to promote the harmonization and improvement of analytical methods within official control of the European Union a network of European and national reference laboratories together with the national official control laboratories was established, whose tasks and obligations are fixed in Regulation (EU) 2017/625. Furthermore, the European Commission is requesting standardisation of certain methods by means of mandates addressed to the European Committee for Standardization (CEN) (CEN, 2018). Standardisation bodies with the same task at international level are e.g. the International Organisation for Standardisation (ISO) and AOAC International. The standardisation bodies do not work simply for themselves by ignoring the activities of the others, in fact there is a continuous flow of interworking, if needed, so selected CEN standards achieve the ISO level e.g. DIN EN ISO 16050:2011-09 “Foodstuffs - Determination of aflatoxin B_1, and the total content of aflatoxins B_1, B_2, G_1 and G_2 in cereals, nuts and derived products - High performance liquid chromatographic method“ or AOAC methods are included in the CCMAS standardisation process (CAC, 2017).

Standardisation of analytical methods has not only become an important task at European or international level in the past but also EU member states such as Germany have a longstanding expertise in standardisation. In Germany, there are e.g. the German Institute for Standardization (DIN), the Federal Office of Consumer Protection and Food Safety (BVL) which publishes the Official Collection of Methods of Analysis and Sampling of the German Food and Feed Act (LFGB, §64) and the Association of German Agricultural Analytic and Research Institutes (VDLUFA), who are traditionally involved in standardisation procedures.

Standardised methods or at least methods, fulfilling criteria according international validation rules or protocols (e.g. of AOAC International (AOAC International, 2016)), are also required to gain reliable occurrence data of harmful substances as a base for exposure and risk assessment. Based on the outcome of risk assessment recommendations for monitoring of harmful substances in feed and food can be derived.

One major issue discussed in different standardisation bodies (e.g. CCMAS, CEN) are advantages and disadvantages of standardisation of methods vs. method performance criteria also with regard to different technical and financial conditions in industrial and developing countries. Less experienced laboratories benefit from method standards as they provide detailed instructions on the conduction of the method. Also it becomes easier to obtain comparable results when the same method is used. Drawbacks of method standardisation are that in some cases validation requirements are not easy to fulfill, like the number of participating laboratories or availability of sample material, and the editorial process up to completion is very laborious. This results in a decelerated process and might hinder analytical progress with regard to costs and efficiency, application of new techniques or extension of a method’s scope to newly available or discovered analytes.

The development of performance criteria needs a certain amount of time as well, taking into account the experience gathered from applying different methods. Afterwards guidance documents describing standardised performance criteria have to go through a Codex evaluation process and can then be used for various analytes and matrices (CAC, 2017e; CEN, 2002). A reasonable evaluation of advantages and disadvantages has to be done, if necessary, for analyte-commodity-combinations individually. Experiences gained on establishing perfor-

mance criteria in e.g. the field of pesticide analyses have to be taken into account for this discussion.

Various documents on performance criteria for the analysis of natural toxins are available, e.g. Commission regulation (EC) No 401/2006 laying down the methods of sampling and analysis for the official control of the levels of mycotoxins in food. This regulation provides performance criteria for confirmatory and semi-quantitative screening methods as specified in Annex II of the document. These requirements shall be met by standardised methods and methods validated in-house as well.

CCMAS decided after four years of work and discussion, to release an information document on criteria approaches for methods which use a 'sum of components' and to amend the CAC Procedural Manual accordingly (CAC, 2017, 2017c). In the frame of these discussions method performance criteria for the determination of biotoxins were proposed in agreement with the CCFFP as a result of the 35th meeting of CCMAS in 2014 (CAC, 2017d).

Furthermore, performance criteria for methods of analysis were already published as a European technical rule in 2010 for single laboratory validated methods of analysis for the determination of mycotoxins in food [CEN/TR 16059:2010]. The European Commission also included the demand for such criteria for mycotoxins in feed in the mandate M/522 to CEN, which is currently being edited (EC, 2013a). Recently, the "Guidance document on identification of mycotoxins in food and feed" (European Commission Directorate General for Health and Food Safety, 2017) was published, which was created and finally edited by the EURL/NRL network. In the near future it is planned, to refine this document and to extend the scope to plant toxins. As the determination of the limits of detection (LOD) and quantification (LOQ) is quite different among the laboratories, which is a particular problem when e.g. evaluating exposure data for risk assessment (Arcella and Gómez-Ruiz, 2018), the Joint Research Centre of the European Commission published the "Guidance Document on the Estimation of LOD and LOQ for Measurements in the Field of Contaminants in Feed and Food" in 2016 (EURL for Polycyclic Aromatic Hydrocarbons et al., 2017).

In the field of marine biotoxins there is still only limited discussion on performance criteria for official control of regulated toxin groups in the EU. Reference methods are defined in the legislation.(EC, 2005c, 2017a) These methods were validated according to internationally recognized protocols and some were also adopted by standardisation bodies like AOAC International. However, performance criteria are part of the reference method for lipophilic marine biotoxins. The LC-MS/MS method to be used for identification and quantification is not prescribed but minimum requirements are laid down in the method protocol (EURL Marine Biotoxins, 2015).

BfR activities regarding standardisation and harmonisation

It is of major concern for the BfR to implement modern but affordable analytical methods in the field of food and feed control. The coordination of the activities of official laboratories regarding harmonisation and improvement of analytical methods for official food and feed control is one task of the numerous national NRLs. In the field of natural toxins in the food and feed chain the BfR/NRLs are involved in the process of European and national standardisation in various ways. A selection of working groups of DIN, CEN (via the pathway of DIN and CEN also ISO), the Official Collection of Methods of Analysis according to §64 of the German Food and Feed Act (Lebensmittel-, Bedarfsgegenstände- und Futtermittelgesetzbuch, LFGB) and VDLUFA (Figure 31) shows the following list:

- § 64 LFGB-Arbeitsgruppe „Mykotoxine"
- VDLUFA FGr VI Arbeitskreis „Mykotoxine"

- DIN-Normenausschuss Lebensmittel und landwirtschaftliche Produkte (NAL) Arbeitsausschuss (AA) Biotoxine
- DIN NAL AA Futtermittel
- CEN/TC275 Food analysis – horizontal methods (WG 14 Marine Biotoxins)
- Collaboration (2007-2009) with the „Working Group on Marine Biotoxins“ of the Scientific Panel on Contaminants in the Food Chain (EFSA)
- CEN/TC 327/WG 5 Animal feeding stuffs – Methods of sampling and analysis – Natural toxins

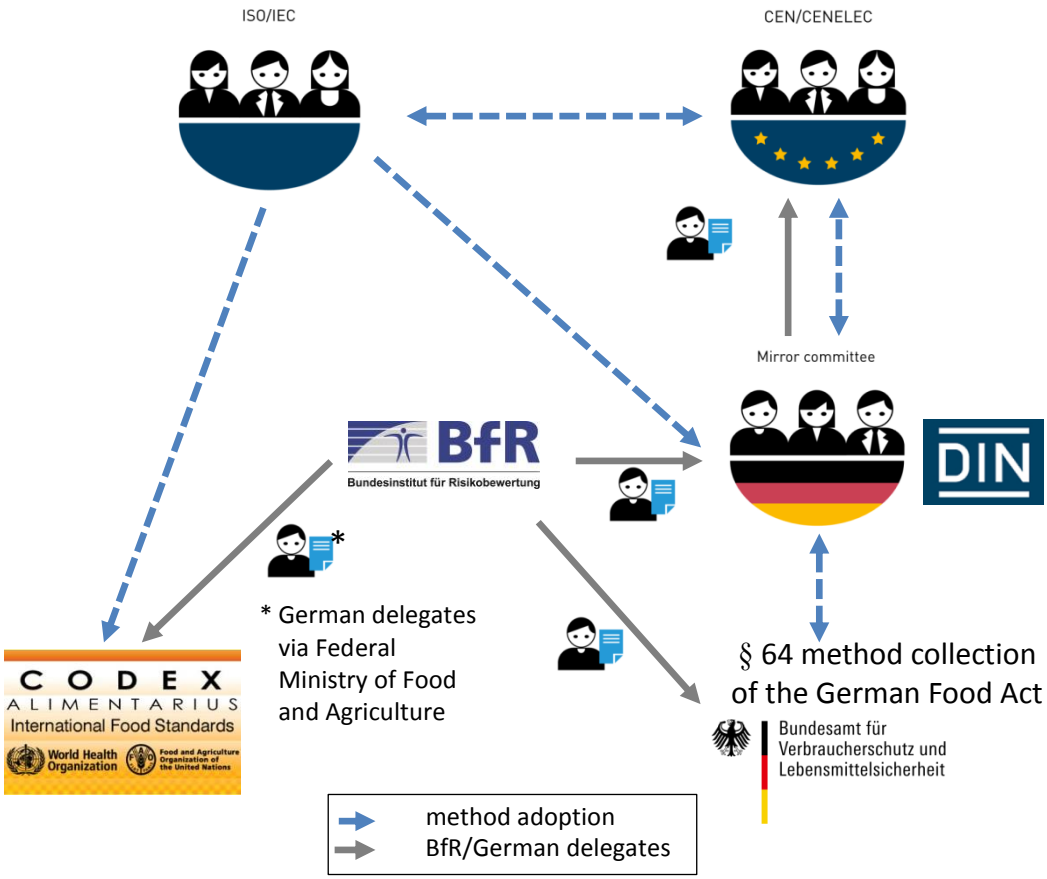


Figure 31: Where BfR participates in standard setting and harmonization initiatives at national and international level.
(Figure modified and partly adapted from https://www.din.de/en/about-standards/din-standards)

Various projects are conducted in the framework of the listed harmonization bodies. Figure 31 images the ways of expert delegation and method adoption between the bodies most relevant for BfR. In the following paragraphs some examples will be described in detail.

The BfR/NRL for Marine Biotoxins has participated in various collaborative studies for the validation of methods becoming EU reference methods for the determination of regulated marine biotoxins. One example is the European standard EN12604 Foodstuffs — Determination of Lipophilic Algal Toxins (DSP-Toxins, Yessotoxins, Azaspiracids, Pectenotoxins) in Shellfish and Shellfish products by LC-MS/MS, which started out as a method validation study conducted by the BfR/NRL for the control of marine biotoxins in cooperation with the official control laboratories. The study was carried out in the framework of the Working Group “Phycotoxins” under the German Food and Feed Code, section 64 (These et al., 2011) and the method was published in the Official Collection of Methods. Afterwards it was submitted to the German and European Committee for Standardization to be accepted as a German

and European standard (DIN/EN16204:2012-08, 2012; EFSA, 2012b). Furthermore, BfR was involved in several projects of the CEN Mandates M/523 EN and M/522 EN for standardisation for methods of analysis in the field of animal nutrition part III including the project lead for "Determination of pyrrolizidine alkaloids in feed materials and compound feed by LC-MS/MS" (EC, 2013a, 2013b). Lead of the project “Determination of T-2 toxin and HT-2 toxin in cereals and cereal products for infants and young children” of the CEN Mandates M/520 in the field of methods of analysis for mycotoxins (DIN EN 16923:2017) was also taken by BfR.

As a member of the working group “Natural Toxins” of the CEN/TC 327 "Animal feeding stuffs - Methods of sampling and analysis" BfR is involved in the development of a criteria approach for methods of analysis for mycotoxins in feed (EC, 2013a).

As no standardised analytical method exists for the determination of *Alternaria* toxins in food or feed, the so far existing exposure data has been gathered by a variety of usually in-house validated methods that often do not cover all of the five *Alternaria* toxins considered relevant. The need for a standardised method for the determination of *Alternaria* toxins in food has been addressed by the European Commission in mandate M/520 and is expected to be issued by CEN/TC 327 WG5 in 2021. For feed, gathering of exposure data is hampered by a lack of a standardised analytical method as well. Thus, BfR and VDLUFA (the Association of German Agricultural Analytic and Research Institutes) collaborate in order to conduct an interlaboratory validation study. Additionally, the method was validated in an international proficiency test on *Alternaria* toxins in tomato juice organized by BfR in 2014 with 16 participants from four European countries.

Further examples for methods that have been developed by the BfR/NRL especially for mycotoxins and are now available as standards are:

- BVL L15.01/02-5:2012-01, Technical rule, German. Untersuchung von Lebensmitteln – Bestimmung von Ergotalkaloiden in Roggen und Weizen – HPLC-Verfahren mit Reinigung an einer basischen Aluminiumoxid-Festphase
- BVL F 0104, Technical rule. Untersuchung von Futtermitteln – Bestimmung von Ergotalkaloiden in Roggen und Weizen – HPLC-Verfahren mit Reinigung an einer basischen Aluminiumoxid-Festphase (Übernahme der amtlichen Methode L 15.01/02-5, Januar 2012, Band I (Lebensmittel) der Amtlichen Sammlung)

A NRL method for the determination of ergot alkaloids in various compound feed by LC-MS/MS was also successfully tested in a collaborative trial with the official control laboratories in 2013, and although it has not been run through a standardisation process, it is most widely used by the official control in Germany. Methods developed at BfR and validated in collaborative trials by BfR for example for the determination of pyrrolizidine alkaloids in tea were used to set up an occurrence database for the risk assessment carried out in BfR. These data were also used by EFSA for their risk assessment of pyrrolizidine alkaloids in food.

To complete the image, BfR is an active member of the German delegation in several Codex committees (CCMAS, CCCF – Codex Committee on Contaminants in Foods, CCFFP - Codex Committee on Fish and Fishery Products), contributing its knowledge and experience in analytical method development, implementation and application in a quality assured environment.

BfR plays an active role in many advisory panels (European Commission, European Food Safety Agency - EFSA), committees (e.g. of Codex Alimentarius) enables the experts to introduce their expertise and ideas at international level. The same applies for the BfRs work in research projects in working groups (e.g. in DIN, CEN and EFSA), aiming at the establish-

ment of food and feed standards regarding not only quality requirements but also at the development of standardised methods to check compliance with established criteria.

Appropriateness of analytical methods

Different sites of trade and production chains require specific analytical methods, i.e. rapid tests or screening methods for initial surveillance in the field or in the case of crisis, validated quantitative methods for official control and legal enforcement, and untargeted comprehensive platforms to identify risks that are unknown at present.

To be applicable as court proof in the field of mycotoxins for analytical methods certain well defined criteria have to be fulfilled. Thus, Commission Regulation (EU) No 401/2006 contains clear rules concerning methods of sampling and analytical performance criteria (concerning e.g. recovery rates and reproducibility to be determined in collaborative trials) (EC, 2006d).

Screening analysis. What the term “screening analysis” in general means is not unambiguously defined. However, for mycotoxin analysis EU regulation (EU) No 519/2014 provides a specific definition: “*Screening method: means method used for selection of those samples with levels of mycotoxins that exceed the screening target concentration (STC), with a given certainty. For the purpose of mycotoxin screening, a certainty of 95 % is considered fit-for-purpose. The result of the screening analysis is either “negative” or “suspect”. Screening methods shall allow a cost-effective high sample-throughput, thus increasing the chance to discover new incidents with high exposure and health risks to consumers. These methods shall be based on bio-analytical, LC-MS or HPLC methods, followed by validation criteria*.” That means screening methods can basically be built on all aforementioned techniques as soon as they are successfully validated according to regulation (EU) No 519/2014. As member of the DIN working group on biotoxins BfR scientists contributed to the standardisation of the CEN multi-method for mycotoxin screening in foodstuffs (prEN 17279:2018).

Confirmatory methods are required to meet the criteria as described in article 34 and annex III of the official control regulation (OCR) (EU) 2017/625. The regulation prescribes which criteria shall be fulfilled for methods applied in official control. Especially for the NRLs allocated at the BfR the initial development of methods compliant with the OCR and regulation (EU) No 519/2014 criteria as well as contribution to such method developments conducted by EURLs has high priority.

Analytical Techniques

Immunosorbent assays for spectrometric or visual detection are a widespread format mainly used for biotoxin analysis primarily used at the early stage of the processing chain. The Enzyme-linked Immunosorbent Assay (ELISA) represents the classical format for rapid determination of toxins feasible within less than one hour. Simple laboratory equipment is required along with a photometric device (plate reader) that can be installed e.g. at trading points or incoming good control labs. Immunosorption as selection criteria leads to a relatively high specificity and the enzyme coupling to enhanced sensitivity. Nevertheless, cross reactivities of target analytes to modified or closely related compounds are a major cause for overestimation of the true toxin content. Other assays based on immunosorbent reaction of the target toxin are on the market that are also designed for toxin screening at incoming good control such as fluorescence polarimetry (FP) (Maragos, 2009). Dipsticks are a simplified version of ELISA allowing for an on-site application as the detection is mostly visualized by colour bars. Thus, semi-quantitative toxin analysis is feasible within less than an hour directly in the field (Lattanzio et al., 2012). The purpose of most immunosorbent assays is not to provide data for legal limit control but to allow for a first on site orientation about the toxin con-

tent of a certain commodity lot. Also aspects of representative sample taking and preparation are often not taken into consideration.

Biosensors, mostly using the surface plasmon resonance (SPR) principle for detection and quantification have gained more attention in fields of application were also ELISA assays have been widely used. Attached to the sensor surface a binding domain specific for the target analyte is linked. In a flow through cell present target structures are captured resulting in a mass dependent signal. Assays developed for fusarium toxins or aflatoxin B1 demonstrated the power and robustness for single or multi-analyte detection (Hossain and Maragos, 2018; Sun et al., 2017). The assay format is relatively new and not yet well established yet. However, it might represent a future technology.

The Mouse Bioassay represents a unique “tool” for detection of acute toxic effects mainly caused by marine biotoxins from seafood. It was used as the official European reference method for the determination of lipophilic marine biotoxins until 2012, when it was replaced by an HPLC-MS/MS method due to animal welfare and also analytical reasons. However, for the detection of paralytic shellfish poisoning toxins it is assigned as the European reference method until 2019 and will then be replaced by an HPLC method standardised by AOAC. The mouse bioassay has its disadvantages when it comes to specificity and toxin quantification (EFSA, 2009a), wherever chemical methods of analyses are available. In case of unknown toxins, or those lacking alternative chemical methods the mouse bioassay is an important screening method.

Targeted analysis by mass spectrometry or optical detection hyphenated to HPLC or GC represents the golden standard for routine food and feed analysis since many years. Before MS and especially tandem MS techniques became sufficiently matured and sensitive mainly HPLC-UV and FLD were the methods of choice. As the necessary instruments are robust affordable and widely available in laboratories similar analysis can be easily carried out worldwide. Hence, still many harmonized standard methods e.g. for routine analysis of mycotoxins are based on optical detection. The drawback of optical detection is obvious. Only UV-active or colored analytes can be detected requiring post column derivatisation for other compounds as in the case of the aflatoxins. Moreover chromatographic separation of analytes and interfering matrix signals is mandatory. Thus, most of these methods are restricted to a small number of analytes and requires time consuming sample pretreatment. Consequently, the desire to quantify multiple toxins in one chromatographic run together with the increasing sensitivity and reliability of MS detection shifted the trend towards targeted multi-analyte HPLC-MS/MS methods that are the mainly used in toxin analysis nowadays (Righetti et al., 2016). The vast majority of recently developed validated reference methods in the field of biotoxin analysis use tandem mass spectrometry. The superior sensitivity of the multiple reaction monitoring (MRM) is widely used in targeted analysis. However, it neglects to detect any of those compounds that are not explicitly on the list of target substances. Targeted analysis is used almost exclusively in official control and legal enforcement.

High resolution mass spectrometry (HRMS) is used in natural toxin analysis since many years. Two instrumental principles are mainly used to achieve the high mass resolution. Either the orbitrap technology using the Fourier transform of the frequency signal caused by the analyte ion or the time-of-flight approach, both leading to comparable results in resolution and sensitivity. The big advantage of HRMS is the possibility to perform screenings simply based on the molecular mass of an analyte derived from the exact mass determined in the MS full scan. Being classically used in metabolomics investigations, HRMS also inherits large benefits for natural toxin analysis related to globalized trade. Untargeted analysis of a sample enables searching for toxin structures related to known compounds (e.g. modified forms) or unexpected contaminations in a novel commodity that might have been missed in targeted analysis (Righetti et al., 2016). For the analysis of emerging toxins that will be dealt with in the following paragraph of the chapter, HRMS offers the possibility to perform a retro-

spective data analysis, that is obviously impossible for targeted methods (León et al., 2016). However, all HRMS techniques were suffering from the higher LOQ and inferior precision compared to targeted triple quadrupole instruments. Recent developments managed to reduce this gap and reached sensitivities that are sufficiently low also for trace analysis. Also in terms of precision and robustness HRMS instruments have improved (Herrero et al., 2014). Consequently, a higher frequency of use can be observed not only in research environment but also in official control laboratories.

Matrix-assisted laser desorption/ionization (MALDI) hyphenated to mass spectrometry enables to map the distribution of a toxin throughout various commodities. However, the relatively low sensitivity and precision is still a drawback. Nevertheless, such applications may provide information on the spatial distribution of the toxin and consequently facilitate the develop technological reduction measures during processing (Hickert et al., 2016). MALDI-MS techniques may rather be considered as tools for optimizing production chains, e.g. for grain pretreatment (also see 4.1.4) than for application in global food chain safety.

In summary, sophisticated HPLC–MS/MS technologies are currently the golden standard methodology for simultaneous multi natural toxin analysis. A combination of the cutting-edge technology with effective sample preparation can provide robust and accurate results in toxin analysis. On the other hand, rapid, field-applicable methods (such as dipsticks and biosensors) are significantly less expensive while still providing acceptable fit for purpose accuracy. These techniques can be applied and adapted for specific requirements along the trade chains (Table 19) (Gamliel et al., 2017).

Table 19: Main types of analysis performed in natural toxin analysis at various stages of the value and processing chain

Type of analysis	Equipment	Quantitative	Field of application
ELISA	Plate reader / photometric device / smartphone	yes / semi	Fast analysis for compliance at goods receipt or in the field
Dipsticks	None / optical device / smartphone	semi	Fast analysis for compliance at goods receipt or in the field
Biosensors	Mainly Surface Plasmon Resonance	yes / semi	Fast analysis for compliance at goods receipt or in the field
Mouse bioassay	Animal facility	semi	(marine) biotoxin control
Targeted analysis by hyphenated chromatography	HPLC-UV or –MS(/MS); GC-FID or –MS(/MS)	yes	Confirmatory analysis at official control or contract laboratories
Non-targeted mass spectrometric analysis	HPLC-HRMS	yes / semi	Confirmatory analysis at official control or contract laboratories as well as unknown screening
Toxin Imaging in matrix	MALDI-MS	semi	Fundamental research and technological development

Particularly, from the producer/stakeholder side the need for rapid tests is emphasized repeatedly as time consuming laboratory analyses (e.g. using HPLC-MS) interfere with a constant and time efficient commodity flow. Several rapid tests are on the market such as ELISA or dipstick tests for aflatoxins, ergot alkaloids or fusarium mycotoxins. However, many of these assays which are particularly easy to use and cost efficient have the drawback that they are at most semi-quantitative. Other more sophisticated but still rapid tests may result in a higher level of accuracy. However, such techniques (e.g. SPR or FP measurements) are more expensive due to the necessary hardware and are less easy to use for untrained per-

sonnel. SPR techniques have been shown to be sufficiently sensitive, however, the proper validation in collaborative trials and thus, fulfillment of regulation (EU) No 519/2014 is still challenging. The final dilemma arises from the fact that on the one hand, producers demand results that are court-proof and on the other hand those results have to provide results very quickly for a low price.

Each methodological approach has its individual strengths and drawbacks and not all techniques can fulfill the central aims of present BfR work. The BfR focus lies on the development, validation and harmonization of validated quantitative methods for official control and legal enforcement as well as untargeted platforms for comprehensive natural toxin screening in different commodities along the food and feed chain. This will be underlined by the following examples selected from recent or ongoing projects.
Amongst a multitude of national reference laboratories the NRL for Mycotoxins and plant toxins is involved in the development of multi-analyte methods

BfR activities in trade chain related method development

- **Pyrrolizidine Alkaloid (PA)**

As mentioned before, BfR was very active in the field of method development for the determination of pyrrolizidine alkaloids in different food commodities and in feed. LC-MS/MS methods published in the past were optimized, extended to newly available compounds and also adapted to be used with little or no changes for the analysis of honey, tea and animal feed. Then again, in case of the method for PA determination in tea the scope the analysed PA compounds was elaborated with respect to their occurrence in tea samples. It was shown 400 available datasets of investigated tea samples that only 21 of the 28 PA included in the method are relevant in different types of tea. Therefore, a recommendation was published to analyse and evaluate only this reduced scope of compounds in tea. Methods for honey and tea were also validated in an collaborative study, while this process is still ongoing in case of the method for the determination of PA in animal feed in the frame of a CEN project under the lead of BfR. The example of the PA dedicated method development demonstrates the challenge of analyzing natural toxins using multi-analyte methods with the constantly changing scope of the methods triggered by identification of new toxins and increasing standard availability. Comprehensive methods are necessary to achieve occurrence data and set an appropriate scope of analytes for method standards for monitoring purposes.

- **Ergot Alkaloids (EA)**

BfR/NRL for mycotoxins in food and feed has long been involved in method development for the determination of EA in cereal-based food and feed. EA are mycotoxins that are found in largest amounts in the fungal species of *Claviceps* genus, most notably *Claviceps purpurea*. The fungus infects the grain ears of various gramineous plants such as wheat, rye, triticale, barley, and millet causing the formation of large discoloured sclerotia (*secal cornutum*) instead of sane grains. These sclerotia contain a variety of potentially toxic substances referred to as EA. Given the current state of knowledge more than 400 plant species are susceptible to infections with *Claviceps purpurea*, including economically important cereals like wheat, rye and triticale (EFSA, 2012b).

To date more than 50 different EAs have been identified (Flieger et al., 1997). In risk assessment, the focus is particularly aimed at ergometrine, ergotamine, ergocornine, ergosine, ergocristine, and ergocryptine and the corresponding isomers ergometrinine, ergotaminine, ergocorninine, ergosinine, ergocristinine und ergocryptinine (EFSA, 2012b).

Over the previous years, methods have been established by the NRL for the analysis of EAs in various cereal products e.g. cereals, bakery products, compound feed etc. As part of standardisation the method for rye and wheat was successfully validated in a collaborative trial for the official compilation of the German Food and Feed Act (BVL, 2012, 2013a; Muller et al., 2009).

In order to expand the scope of the method to more complex matrices e.g. mixed feeding stuffs, the method has been modified. Instead of HPLC-FLD, a detection method, which is completely sufficient for the analyses of EA in cereal flour but is prone to produce false positive findings for complex matrices, the more specific LC-MS/MS was introduced. This method was also successfully validated in a collaborative trial for compound feed. The same method is now in the standardisation process with the focus on bakery products within the framework of the German Food and Feed Act (LFGB).

- **Multimethod for Mycotoxin Analysis in Grains and Legumes**

Several publications confirmed the presence of different mycotoxins or mycotoxin forming fungi in legumes such as peas, beans, chickpea or soy beans that are already well known for grains such as wheat or maize (Garcia et al., 2016; Gutleb et al., 2015; Ramirez et al., 2018; Schollenberger et al., 2007). Nevertheless, unlike for grains hardly any comprehensive occurrence data exist for any of those commodities and consequently, no exposure assessment for humans or livestock is feasible so far.

Particularly, in the light of changing trade chains carrying the potential of longer storage or harvest periods under unfavorable conditions compared to the past when Beans and peas were mainly of local origin, legume based products might become increasingly vulnerable. Following the development of highly sensitive and fast LC-MS/MS instruments, it is becoming more convenient to establish quantification methods for the analysis of various mycotoxins in different commodities.

However, the quantification of a multitude of mycotoxins in only one run poses a significant challenge due to the very different chemical properties of mycotoxins such as patulin, fumonisins or the peptidic enniatins. The impact of the sample matrix on the extractability and ionisation is immense. Nevertheless, standardisation bodies like CEN make strong efforts to establish multi-analyte methods to facilitate mycotoxin analysis for certain toxin groups with similar properties. The pre norm of the screening method “Foodstuffs - Multimethod for the screening of ochratoxin A, aflatoxin B1, deoxynivalenol, zearalenone and fumonisin B1 and B2 in foodstuffs, excluding foods for infants and young children, by HPLC-MS/MS” (prEN 17279:2018) provided the template for an extended quantitative multi-mycotoxin method. A simple acetonitrile/water extraction followed by liquid/liquid partitioning is used for sample preparation. Addition of stable isotope labelled internal standards with the concentration of the STC (i.e. the maximum limit of the respective toxin) allows for the decision whether a sample is compliant or suspect after HPLC-MS/MS analysis.

The method has been validated at the NRL for the quantification of eleven mycotoxins in grains and has additionally been successfully applied to legumes as a more challenging protein and lipid rich matrix. Apart from those analytes regulated by legal limits, the developed method allows for the qualitative detection of a multitude of additional emerging mycotoxins as shown for the group of enniatins or reduced zearalenone metabolites (Figure 32). Also toxins belonging to other classes, e.g. plant toxins, could be included as long as those analytes would be covered by the sample extraction and clean-up steps.

Applying the multi-mycotoxin method to a set of more than 50 pea, bean and lentil samples unveiled detectable toxin amounts in approx. 5 % of the investigated samples. OTA as a typ-

ical indicator for improper storage of was detected in two legume samples at considerable amounts.

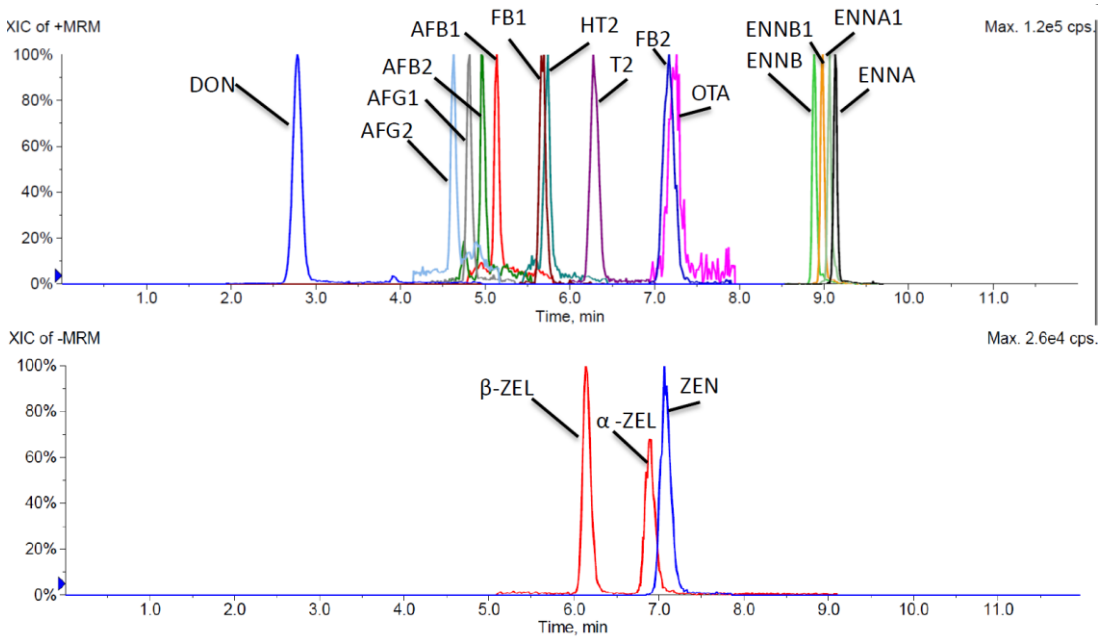


Figure 32: Extracted ion chromatograms from the analysis of a blank wheat flour spiked with aflatoxins, OTA, DON, ZEN, T2-toxin, HT2-toxin, FB_1, FB_2, α-ZEL and β-ZEL, ENNA, ENNA1, ENNB and ENNB1 in the low to medium ppb range

The CEN standardised screening method will allow for the rapid and more cost efficient decision whether a commodity lot is classified as compliant with legal limits or "suspect". The extended quantitative NRL method offers to provide toxin occurrence data in the range of valid legal limits. The screening method is of interest mainly in the food and feed production area to test compliance with contractual demands, however, requires confirmatory analysis for suspect sample lots. Extended to the quantitative NRL method it might gain the status of such a confirmatory or official control method in future. Thus, the application of both, the multi-mycotoxin HPLC-MS/MS analysis methods enabling the simultaneous detection or quantification of the most relevant mycotoxins in legumes can contribute to reach and maintain a higher level of food and feed safety at different stages.

- **Emerging Analytes and Evolving Analyte Patterns as a Consequence of Globalised Trade Chains**

As already mentioned in the section above for the example of legumes, global trade routes are changing and expanding, resulting in greater vulnerability to microbial contamination. The frequency for the occurrence of storage rot often accompanied by mycotoxin contamination may increase with longer or less well controlled trade routes. On the other hand the food basket is diversifying more and more and exotic potentially novel food items enter the European market in increasing numbers. Here it must be expected that the analyte pattern in imported and far traded commodities is different from local products, due to other contamination sources at the place of origin (e.g. other fungi, herbs, or bacteria). So, there is a twofold challenge arising from a more globalized trade for food and feed safety. Firstly, known toxins appearing in commodities where they have traditionally been absent (for example tetrodotoxins in shellfish grown in Europe) and secondly, the toxin presence in novel food items that may either be known toxins or could be toxic compound that have been neglected so far. The latter "group" of toxins is commonly referred to as "emerging" toxins, such as the *Alternaria*

toxins, phomopsins or enniatins. The term emerging toxins also applies for those toxins in products that are recently imported to new markets like ciguatoxins in fish from tropic and subtropical catching areas.

***Alternaria* toxins** – a heterogeneous class of secondary metabolites produced by *Alternaria* fungi presently are one of the most relevant emerging mycotoxin groups. More than 70 metabolites (Figure 33) have been described and some of these have been characterised as mycotoxins. *Alternaria* toxins occur in a variety of commodities, among them cereals, oilseeds, fruits and vegetables (Logrieco et al., 2009). According to a scientific opinion on the risk of *Alternaria* toxins on human and animal health published by the European Food Safety Authority (EFSA), the five *Alternaria* toxins altenuene (ALT), alternariol (AOH), alternariol monomethyl ether (AME), tenuazonic acid (TeA) and tentoxin (TEN) are considered most relevant in this context (EFSA, 2011a). The Standing Committee on Plants, Animals, Food and Feed of the European Commission recommends the monitoring of these five toxins in food and feed since 2012. Since the estimated chronic dietary exposure to AOH, AME and TeA exceeded the relevant TTC value, the Committee identified a need for additional compound-specific toxicity data (Standing Committee on Plants, 2017).

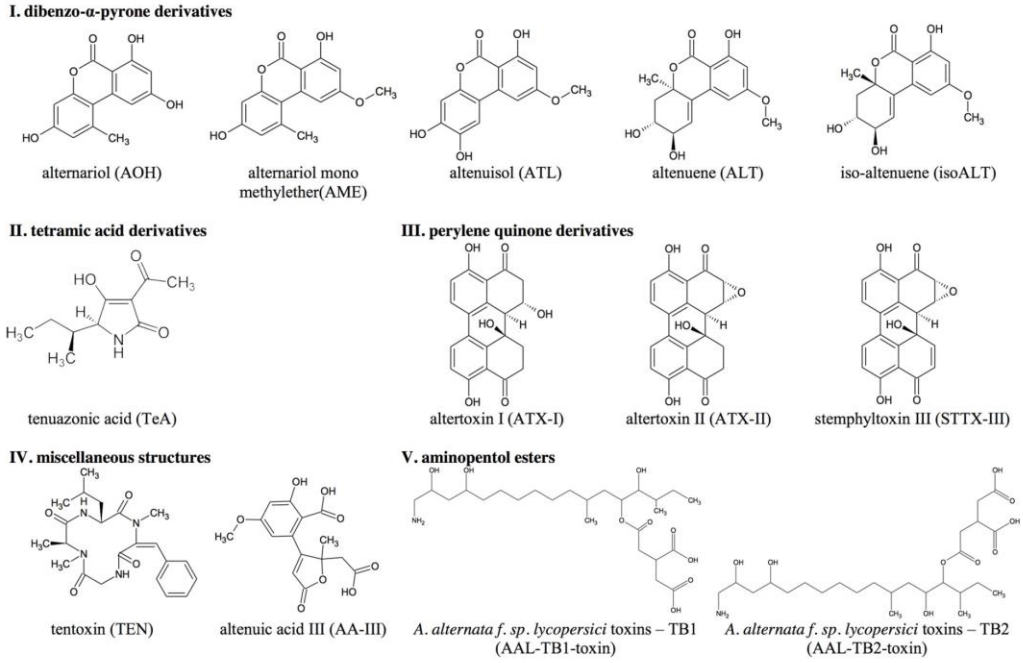


Figure 1. Chemical structures of *Alternaria* toxins (ATs) determined in this study.

Figure 33: Overview on the most abundant *Alternaria* toxins
(Zwickel et al., 2016).

The analytical method initially developed at BfR was further used to assess the exposition of *Alternaria* toxins on humans and animals. Various commodities from the German market were sampled and analysed at BfR. In accordance with previous findings by others (Davide et al., 2016), the highest incidence of *Alternaria* toxins was observed in tomato products (juice, sauce, ketchup, paste, dried tomatoes). 100 % of the 102 sampled tomato products contained at least one *Alternaria* toxin, TeA being the most abundant with levels between 16 and 1100 µg/kg. AOH and AME were also found in many samples. These two toxins were most frequently found in Turkish "Salça" tomato paste samples with 12 out of 12 samples positive and median levels of AOH and AME of 64 and 8 µg/kg, respectively. Contrary to tomato products, all analysed undamaged fresh tomatoes were found to be virtually free of

Alternaria toxins suggesting that the high *Alternaria* toxins levels found in tomato products are caused by insufficient removal of damaged or mouldy fruits. Alternatively, other post-harvest stages such as processing or storage may play a role.

Due to their relevance for human and animal nutrition, cereals were studied extensively at BfR to elucidate the occurrence of *Alternaria* toxins in this commodity. The highest levels of *Alternaria* toxins were found in millet with concentrations of TeA up to 2250 µg/kg at an incidence of 93 % (n=14). Elevated levels of *Alternaria* toxins were also found in rice with up to 1400, 148, 145 and 20 µg/kg of TeA, AOH, AME and TEN, respectively. The levels for other cereals were somewhat lower, still TeA could be detected in levels between 10 and 40 µg/kg in the majority of wheat and maize feed samples. The highest levels of TeA observed in maize feed were as high as 450 µg/kg.

Furthermore, 103 wine and juice samples from the German market were analysed. A total of 68 % of all samples were contaminated with TeA, with concentrations in the range of 1.4-60.0 µg/L (Zwickel et al., 2016). AOH and AME were more prevalent in wine than in fruit juices with levels of up to 7.7 and 1.5 µg/L respectively.

The aforementioned results illustrate the risks associated with changes in food patterns on a global scale. Among the highest levels of *Alternaria* toxins were found in millet, which is relatively novel to the European diet, and Salça, a specialty in Turkish cuisine and widely used within the Turkish minority in Germany. Approximately 2.9 million people of Turkish ancestry live in Germany. It remains a challenge for risk assessment to include the non-uniform diets of migrating populations. Additionally, recent scientific data suggest, that not only the five toxins so far prioritized by EFSA are crucial for *Alternaria* toxin related toxicity. By contrast, substances such as the altertoxins or stemphyltoxins also formed by *Alternaria* fungi appear to be the more potent toxic agents within the toxin spectrum of this fungus (Jarolim et al., 2017). Thus, the *Alternaria* toxins represent the prime example of an emerging toxin group. The entirety of lacking reliable reference standards and standardised methods but also the not yet fully elucidated toxicity and toxin profile additionally varying for the commodities as well as novel food items entering the market causes various challenges to the analytical science, risk assessment and legislation along the entire global trade chain at the same time.
However, similar groups of toxins can be found for the plant toxins (e.g. pyrrolizidine alkaloids) or marine biotoxins (e.g. paralytic shellfish poisoning toxins formed by certain dinoflagellates).

Conclusions

BfR is actively involved in the protection of consumers from risks arising from global food and feed chains in many different ways. With respect to the control and prevention of food contamination for example with natural toxins that may threaten the consumer safety regulatory as well as analytical efforts are undertaken. Some major activities are linked to the contribution to DIN and CEN working groups, development and publication of analytical strategies for pyrrolizidine alkaloid detection or the validation of a multi-mycotoxin screening method. These activities have been highlighted in this section and are also a prerequisite to be prepared to tackle future challenges. BfR constantly diversifies its analytical methodologies that need to be e.g. applicable in the field, as untargeted platforms or confirmatory methods to support food and feed safety control along extended trade chains. The necessary evolution of analytical approaches is not only limited to existing fields of application along the food production chain but also requires the incorporation of new potentially harmful compounds (e.g. emerging toxins) originating from novel raw products or novel production areas. Besides analytical activities, globalized trade demands for a higher level of harmonization and standardisation in method development and validation. Qualified reference laboratories such as the NRL mycotoxins located at the BfR are important institutions for the linkage of the develop-

ment of field applicable rapid tests to validated confirmatory reference methods and should be up to date in terms of analytical sensitivity and comprehensiveness. Thus, the NRL constantly tries to implement modern techniques and up to date standards with regard to future-oriented analytical approaches.

The challenges arising from changing global food and feed chains require a comprehensive approach for the success of future safety assurance. Thus, with regard to the area of biotoxins the interplay of BfR activities in analytical research together with the participation in standard setting bodies at various levels is of particular importance.

References

AOAC International. (2016). Guidelines for Standard Method Performance Requirements (Appendix F). In: AOAC International.

Arcella, D., and Gómez-Ruiz, J. (2018). EFSA Technical Report: Use of cut-off values on the limits of quantification reported in datasets used to estimate dietary exposure to chemical contaminants *EFSA Supporting Publications, 2018:EN-1452*, 11. doi:10.2903/sp.efsa.2018.EN-1452

BVL, Bundesamt für Verbraucherschutz und Lebensmittelsicherheit. (2012). BVL L15.01/02-5:2012-01 Technical rule : Untersuchung von Lebensmitteln - Bestimmung von Ergotalkaloiden in Roggen und Weizen - HPLC-Verfahren mit Reinigung an einer basischen Aluminiumoxid-Festphase. In: Beuth.

BVL, Bundesamt für Verbraucherschutz und Lebensmittelsicherheit. (2013a). BVL F 0104:2013-04 Technical rule : Untersuchung von Futtermitteln - Bestimmung von Ergotalkaloiden in Roggen und Weizen - HPLC-Verfahren mit Reinigung an einer basischen Aluminiumoxid-Festphase In *Untersuchung von Futtermitteln - Bestimmung von Ergotalkaloiden in Roggen und Weizen - HPLC-Verfahren mit Reinigung an einer basischen Aluminiumoxid-Festphase (Übernahme der amtlichen Methode L 15.01/02-5, Januar 2012, Band I (Lebensmittel) der Amtlichen Sammlung)*: Beuth.

CAC, Codex Alimentarius Commission. (2017). Codex Alimentarius Commission Procedural Manual Twenty-sixth edition.

CAC, Codex Alimentarius Commission. (2017c). Information Document on Criteria Aapproaches for Methods which use a 'Sum of Components'. In. Rome.

CAC, Codex Alimentarius Commission. (2017d). Report of the Thirty-Fifth Session of the Codex Committee on Methods of Analysis and Sampling REP14/MAS.

CAC, Codex Alimentarius Commission. (2017e). Guidelines on Performance Criteria for Methods of Analysis for the Determination of Pesticide Residues in Food and Feed (CXG 90-2017). In: FAO/WHO Codex Alimentarius.

CEN, European Committee for Standardization. (2002). EN13804:2002 Foodstuffs: Determination of trace elements — Performance criteria, general considerations and sample preparation. In *Foodstuffs: Determination of trace elements — Performance criteria, general considerations and sample preparation*: Beuth.

CEN, European Committee for Standardization. (2018). New Approach and other Directives. https://www.cen.eu/work/supportLegislation/Directives/Pages/default.aspx

Davide, A., Mari, E., and Angel, G. R. J. (2016). Dietary exposure assessment to Alternaria toxins in the European population. *EFSA Journal, 14*(12), e04654. doi:10.2903/j.efsa.2016.4654

Dellafiora, L., and Dall'Asta, C. (2017). Forthcoming Challenges in Mycotoxins Toxicology Research for Safer Food—A Need for Multi-Omics Approach. *Toxins, 9*(1), 18. doi:10.3390/toxins9010018

DIN/EN16204:2012-08. (2012). Foodstuffs — Determination of Lipophilic Algal Toxins (DSP-Toxins, Yessotoxins, Azaspiracids, Pectenotoxins) in Shellfish and Shellfish products by LC-MS/MS. In: Beuth.

EC, European Commission. (2005c). Commission Regulation (EC) No 2074/2005 of 5 December 2005 laying down implementing measures for certain products under Regulation (EC) No 853/2004 of and for the organisation of official controls under Regulation (EC) No 854/2004. *Official Journal of the European Union, L 338*, 27-59.

EC, European Commission. (2006d). Commission Regulation (EC) No 401/2006 of 23 February 2006 laying down the methods of sampling and analysis for the official control of the levels of mycotoxins in foodstuffs. *Official Journal of the European Union, L 70*, 12–34.

EC, European Commission. (2013a). Mandate for Standardisation addressed to CEN fpr Methods of Analysis in the Field of Animal Nutrition Part II (M/522 EN). ftp://ftp.cencenelec.eu/CEN/WhatWeDo/Fields/Food/M522.pdf

EC, European Commission. (2013b). Mandate for Standardisation addressed to CEN fpr Methods of Analysis in the Field of Animal Nutrition Part III (M/523 EN). ftp://ftp.cencenelec.eu/CEN/WhatWeDo/Fields/Food/M523.pdf

EC, European Commission. (2017a). COMMISSION REGULATION (EU) 2017/1980 amending Annex III to Regulation (EC) No 2074/2005 as regards paralytic shellfish poison (PSP) detection method. *Official Journal of the European Union, L 285*, 8-9.

EFSA, European Food Safety Authority. (2009a). EFSA Panel on Contaminants in the Food Chain (CONTAM): Scientific Opinion of the Panel on Contaminants in the Food Chain on a request from the European Commission on Marine Biotoxins in Shellfish – Summary on regulated marine biotoxins. *The EFSA Journal, 1306*, 23. doi:10.2903/j.efsa.2009.1306

EFSA, European Food Safety Authority. (2011a). EFSA Panel on Contaminants in the Food Chain (CONTAM): Scientific Opinion on the risks for animal and public health related to the presence of Alternaria toxins in feed and food. *EFSA Journal, 9*(10), 97. doi:10.2903/j.efsa.2011.2407

EFSA, European Food Safety Authority. (2012b). EFSA Panel on Contaminants in the Food Chain (CONTAM): Scientific Opinion on ergot alkaloids in food and feed. *EFSA Journal, 10*(7), 158. doi:10.2903/j.efsa.2012.2798

Eur. Parliament, and The Council. (2017). Regulation (EU) 2017/625 of the European Parlament and the Council of 15 March 2017 on official controls and other official activities performed to ensure the application of food and feed law, rules on animal health and welfare, plant health and plant protection products. *Official Journal of the European Union, L95*, 1-142.

EURL for Polycyclic Aromatic Hydrocarbons, EURL for Dioxins and PCBs, EURL for Heavy Metals in Feed and Food, & EURL for Mycotoxins. (2017). *Guidance Document on the Estimation of LOD and LOQ for Measurements in the Field of Contaminants in Feed and Food*. https://ec.europa.eu/food/sites/food/files/safety/docs/cs_contaminants_sampling_guid-doc-lod-loq.pdf

EURL Marine Biotoxins. (2015). EU-Harmonised Standard Operating Procedure for determination of Lipophilic marine biotoxins in molluscs by LC-MS/MS. In: AECOSAN, EURL Marine Biotoxins.

European Commission Directorate General for Health and Food Safety. (2017). *Guidance document on identification of mycotoxins in food and feed - SANTE/12089/2016*. (SANTE/12089/2016). https://ec.europa.eu/food/sites/food/files/safety/docs/cs_contaminants_sampling_guid-doc-ident-mycotoxins.pdf

Flieger, M., Wurst, M., and Shelby, R. (1997). Ergot alkaloids - Sources, structures and analytical methods. *Folia Microbiologica, 42*(1), 3-29.

Gamliel, A., Dehne, H. W., Karlovsky, P., and Fletcher, J. (2017). Detection of Mycotoxins in Food: Applications of Rapid and Reliable Tools in a Biosecurity Context. In M. L. Gullino, J. P. Stack, J. Fletcher, and J. D. Mumford (Eds.), *Practical Tools for Plant and Food Biosecurity: Results from a European Network of Excellence* (pp. 143-162). Cham: Springer International Publishing.

Garcia, L. P., Savi, G. D., Santos, K., and Scussel, V. M. (2016). Fumonisins and fungi in dry soybeans (Glycine Max L.) for human consumption. *Food Additives & Contaminants: Part B, 9*(2), 79-84. doi:10.1080/19393210.2015.1135484

Gutleb, A. C., Caloni, F., Giraud, F., Cortinovis, C., Pizzo, F., Hoffmann, L., . . . and Pasquali, M. (2015). Detection of multiple mycotoxin occurrences in soy animal feed by traditional mycological identification combined with molecular species identification. *Toxicology Reports, 2*, 275-279. doi:10.1016/j.toxrep.2015.01.006

Herrero, P., Cortés-Francisco, N., Borrull, F., Caixach, J., Pocurull, E., and Marcé, R. M. (2014). Comparison of triple quadrupole mass spectrometry and Orbitrap high-resolution mass spectrometry in ultrahigh performance liquid chromatography for the determination of veterinary drugs in sewage: benefits and drawbacks. *Journal of Mass Spectrometry, 49*(7), 585-596. doi:10.1002/jms.3377

Hickert, S., Cramer, B., Letzel, M. C., and Humpf, H.-U. (2016). Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry imaging of ochratoxin A and fumonisins in mold-infected food. *Rapid Communications in Mass Spectrometry, 30*(23), 2508-2516. doi:10.1002/rcm.7733

Hossain, M. Z., and Maragos, C. M. (2018). Gold nanoparticle-enhanced multiplexed imaging surface plasmon resonance (iSPR) detection of Fusarium mycotoxins in wheat. *Biosensors and Bioelectronics, 101*, 245-252. doi:10.1016/j.bios.2017.10.033

Jarolim, K., Del Favero, G., Pahlke, G., Dostal, V., Zimmermann, K., Heiss, E., . . . and Marko, D. (2017). Activation of the Nrf2-ARE pathway by the Alternaria alternata mycotoxins altertoxin I and II. *Archives of Toxicology, 91*(1), 203-216. doi:10.1007/s00204-016-1726-7

Keiichiro, H., Otsuki, T., and Wilson, J. S. (2015). Food safety standards and international trade: The impact on developing countries' export performance. In *Food Safety, Market Organization, Trade and Development* (pp. 151-166).

Kovalsky, P., Kos, G., Nährer, K., Schwab, C., Jenkins, T., Schatzmayr, G., . . . and Krska, R. (2016). Co-Occurrence of Regulated, Masked and Emerging Mycotoxins and Secondary Metabolites in Finished Feed and Maize—An Extensive Survey. *Toxins, 8*(12), 363. doi:10.3390/toxins8120363

Lattanzio, V. M. T., Nivarlet, N., Lippolis, V., Gatta, S. D., Huet, A.-C., Delahaut, P., . . . and Visconti, A. (2012). Multiplex dipstick immunoassay for semi-quantitative determination of Fusarium mycotoxins in cereals. *Analytica Chimica Acta, 718*, 99-108. doi:10.1016/j.aca.2011.12.060

León, N., Pastor, A., and Yusà, V. (2016). Target analysis and retrospective screening of veterinary drugs, ergot alkaloids, plant toxins and other undesirable substances in feed using liquid chromatography–high resolution mass spectrometry. *Talanta, 149*, 43-52. doi:10.1016/j.talanta.2015.11.032

Logrieco, A., Moretti, A., and Solfrizzo, M. (2009). Alternaria toxins and plant diseases: an overview of origin, occurrence and risks. *World Mycotoxin Journal, 2*(2), 129-140. doi:10.3920/Wmj2009.1145

Maragos, C. (2009). Fluorescence Polarization Immunoassay of Mycotoxins: A Review. *Toxins, 1*(2), 196-207. doi:10.3390/toxins1020196

Muller, C., Kemmlein, S., Klaffke, H., Krauthause, W., Preiss-Weigert, A., and Wittkowski, R. (2009). A basic tool for risk assessment: A new method for the analysis of ergot alkaloids in rye and selected rye products. *Molecular Nutrition & Food Research, 53*(4), 500-507. doi:10.1002/mnfr.200800091

Nijs, M. d., Noordam, M. Y., and Mol, H. G. J. (2017). Short inventory of EU legislation on plant toxins in food. *Quality Assurance and Safety of Crops & Foods, 9*(1), 129-139. doi:10.3920/qas2016.0862

Ramirez, M. L., Cendoya, E., Nichea, M. J., Zachetti, V. G. L., and Chulze, S. N. (2018). Impact of toxigenic fungi and mycotoxins in chickpea: a review. *Current Opinion in Food Science, 23*, 32-37. doi:10.1016/j.cofs.2018.05.003

Righetti, L., Paglia, G., Galaverna, G., and Dall'Asta, C. (2016). Recent Advances and Future Challenges in Modified Mycotoxin Analysis: Why HRMS Has Become a Key Instrument in Food Contaminant Research. *Toxins, 8*(12), 361. doi:10.3390/toxins8120361

Schatzmayr, G., and Streit, E. (2013). Global occurrence of mycotoxins in the food and feed chain: facts and figures. *World Mycotoxin Journal, 6*(3), 213-222. doi:10.3920/wmj2013.1572

Schirone, M., Berti, M., Visciano, P., Chiumiento, F., Migliorati, G., Tofalo, R., . . . and Ferri, N. (2018). Determination of Lipophilic Marine Biotoxins in Mussels Harvested from the Adriatic Sea by LC-MS/MS. *Frontiers in Microbiology, 9*(152). doi:10.3389/fmicb.2018.00152

Schollenberger, M., Müller, H. M., Rüfle, M., Terry-Jara, H., Suchy, S., Plank, S., and Drochner, W. (2007). Natural occurrence of Fusarium toxins in soy food marketed in Germany. *International Journal of Food Microbiology, 113*(2), 142-146. doi:10.1016/j.ijfoodmicro.2006.06.022

Standing Committee on Plants, A., Food and Feed,. (2017). Summary Report of the Standing Committee on Plants, Animals, Food and Feed held in Brussels on 08 February 2017 (Section Toxicological Safety of the Food Chain). https://ec.europa.eu/food/sites/food/files/safety/docs/reg-com_toxic_20170208_sum.pdf

Sun, L., Wu, L., and Zhao, Q. (2017). Aptamer based surface plasmon resonance sensor for aflatoxin B1. *Microchimica Acta, 184*(8), 2605-2610. doi:10.1007/s00604-017-2265-5

The Council. (1985). *Council Resolution of 7 May 1985 on a new approach to technical harmonization and standards*. Official Journal of the European Communities. https://publications.europa.eu/en/publication-detail/-/publication/9f7c3a42-449e-4ccc-9a29-5a544003b338/language-en

These, A., Klemm, C., Nausch, I., and Uhlig, S. (2011). Results of a European interlaboratory method validation study for the quantitative determination of lipophilic marine biotoxins in raw and cooked shellfish based on high-performance liquid chromatography-tandem mass spectrometry. Part I: Collaborative study. *Analytical and Bioanalytical Chemistry, 399*(3), 1245-1256. doi:10.1007/s00216-010-4383-3

Zwickel, T., Klaffke, H., Richards, K., and Rychlik, M. (2016). Development of a high performance liquid chromatography tandem mass spectrometry based analysis for the simultaneous

quantification of various Alternaria toxins in wine, vegetable juices and fruit juices. *Journal of Chromatography A, 1455*, 74-85. doi:10.1016/j.chroma.2016.04.066

5 Foodborne pathogens and antimicrobial resistance

Nöckler, Karsten

Introduction

Zoonotic agents in the food chain pose a major challenge for food safety and consumer protection. Foodstuffs traded within the Community or imported from third countries must meet certain quality and safety requirements according to the legislation. Food of animal origin illegally imported into the European Community from third countries pose a particular risk for the introduction of pathogens. This applies in particular to pathogens that are rare or do not exist in the EU Member States and that can lead to trade restrictions if detected in a Member State. In section 5.1 the focus lies on products which are illegally imported, which pathogens were detected in different kinds of food (milk products, meat products) and which regional differences occur. Products from fishing and aquaculture are increasingly being supplied to Europe via various trade chains, especially from Southeast Asia. Seafood is known for its excellent nutritional value, but may also contain potentially pathogenic bacteria such as various *Vibrio* species posing a risk to the consumer. Section 5.2 describes among other things, which pathogenic *Vibrio* species were detected in an EU-wide study on seafood from certain regions. Herbs and spices are the actual classics of the food trade, whose roots go back to the Near East several thousand years ago. Herbs and spices can also contain disease causing microorganisms that are not only heat-resistant but can also persist in dried herbs and spices and might multiply in the prepared dishes. Therefore, section 5.3 deals in particular with hygiene standards in the production and processing of herbs and spices and microbiological testing in order to achieve a high product quality and safety. Foodstuffs on the market can also contain certain pathogens which carry resistance genes making them insensitive to certain antibiotics. Of particular interest here are resistances to antibiotics of the last resort, which are used in emergencies to treat humans because other antibiotics are no longer effective. Using colistin as an example, Section 5.4 shows how resistances can be mediated by certain genes and spread along the food chain involving also horizontal gene transfer (plasmids). Finally, section 5.5 deals with the application of modern methods for the detection and typing of pathogens using Next Generation Sequencing and metagenome studies as examples. It describes which future possibilities are opened up with these methods, for example for the investigation of food-borne outbreaks, the better verification of infection chains and the proof of food authenticity.

Future needs

In order to prevent the illegal import of food which may contain pathogens, information campaigns for travellers should be further developed. Systematic investigations must be carried out across borders to obtain valid data on the occurrence and spread of these pathogens and to identify regions with a higher risk for the occurrence of rare and particularly dangerous pathogens. Monitoring and surveillance programmes on the occurrence of food-borne pathogens, such as enteropathogenic *Vibrio* species in seafood and human diseases caused by them, need to be implemented and combined with a stringent reporting system. For the marketing of certain products from third countries, such as seafood, additional microbiological criteria to improve food safety should be defined where necessary. Both fresh and dried herbs and spices which are very often used in the preparation of food, play an important role as vehicles of pathogens and represent a difficult matrix for routine diagnostics. Therefore, methods for detecting pathogens in herbs and spices need to be continuously developed and optimised. Assessment on the development of antibiotic resistance, e.g. against colistin, requires a holistic approach including human, veterinary and environment-associated aspects according to the One-Health principle. Studies on the development and spread of resistance via horizontal gene transfer must include both commensal and pathogenic microorganisms.

The strategic goal is to use antibiotics specifically to combat diseases, to counteract the development of resistance and to pursue alternative intervention strategies. The new methods of Next Generation Sequencing and the use of metagenome studies are very promising. For the successful application in practice, however, further efforts are necessary, including the standardisation of sequencing and bioinformatic tools, the transfer, storage and online availability of these extensive sequence data as well as the integration of these new methods into a quality assurance system. Evidenced based data are essential for risk assessment and communication strategies need to be adapted to transfer the best scientific knowledge towards decision makers.

5.1 Foodborne pathogens in food confiscated from air passenger luggage

Mayer-Scholl, Anne; Nöckler, Karsten; Appel, Bernd; Richter, Martin; Johne, Annette

Abstract

Foods of animal origin brought illegally from third party countries into the European Community pose a risk for the introduction of diseases. This can lead to animal disease outbreaks with significant economic and social costs and subsequent severe trade restrictions. Further, disease outbreaks in humans due to illegally imported foods of animal origin have been described.

Despite an extensive regulatory framework, substantial volumes of illegal animal products for human consumption continue to enter the European Union (EU) undetected. Passenger baggage is an important route by which illegal products come into a country, as many exotic foods are a traditional part of the diet of many immigrants who live in the EU and the popularity of exotic foods has also increased due to more extensive travelling activities and globalization. Here, projects carried out by the BfR and partner institutions examining volume, geographic origins and the occurrence of foodborne zoonotic bacteria and parasites in such illegally introduced foods are described.

Background

Despite an extensive regulatory framework, substantial volumes of illegal animal products for human consumption continue to enter the European Union (EU) undetected; either as imports brought in by individuals for personal use or larger quantities indicating underlying commercial motivations.

One reason that illegal importation persists is because many exotic foods are a traditional part of the diet of many immigrants who live in the EU. Further, the need for the consumption of exotic foods originates either out of reminiscence or from a religious background (Grabowski et al., 2013). In 2011, nearly 20% of the total German population had a migration background. Most immigrants in Germany from outside the EU originated from Turkey (2.96 M) followed by Russia and Ukraine (1.5 M) and the Balkan countries (0.86 M) (Destatis, 2012). Further, the popularity of exotic foods has also increased in the European population due to more extensive travelling activities and globalization. In 2010, more than 12 M Europeans travelled outside the EU (European Commission, 2014, http://epp.eurostat.ec.europa.eu/portal/page/portal/tourism/data/database).

Outbreaks of exotic animal diseases within the European Community have been caused by virus strains previously not isolated in the Community, including outbreaks of classical swine fever in 1996 and 2000 (Hartnett et al., 2007), a major epidemic of foot-and-mouth disease (FMD) in 2001 (Peiso et al., 2011) and recently, African swine fever (ASF) (Sanchez-Cordon et al., 2018).

These outbreaks can cause significant economic and social costs and lead to severe trade restrictions. Further, disease outbreaks in humans due to illegally imported foods of animal origin have been reported (Noordhuizen et al., 2013). Trichinellosis outbreaks, for example, have been described after travellers visiting family in South East European countries brought homemade sausages to Germany as presents for family and friends (Nockler et al., 2007).

The EU food regulations assure a high level of food safety and consumer protection. But in many third countries animal production, disease surveillance and control, food technology and the hygienic conditions of food processing do not match European standards (Spies,

2008). Therefore, legal food imports into the EU are well monitored for serious risks and alerts are registered through the Rapid Alert System for Food and Feed (RASFF). In contrast, in illegally imported foods neither the origin nor the conditions of food production can be monitored efficiently. The illegal introduction of animal products thus results in an increased risk of importing zoonoses and animal diseases into the EU (POST, 2005).

The majority of the German immigrant population originates from regions where animal diseases such as FMD and ASF are endemic. But also exotic zoonoses such as human brucellosis, which is transmitted through the consumption of raw animal products (e.g unpasteurized milk or cheese (Pappas et al., 2006) occur frequently in such regions. Also, food borne diseases like yersiniosis, campylobacteriosis, salmonellosis and food borne parasites are a major public health problem in many developed and developing countries. Especially countries without strict food safety regulations possess a high prevalence of gastrointestinal diseases. Approximately one third of travellers to less developed areas of the world experience gastrointestinal complaints during their journey (DuPont and Ericsson, 1993).

To mitigate these risks there are strict procedures for the introduction of animal products into the EU. Besides a clear legal framework concerning the commercial import of foodstuff (EC) No 2017/625) the introduction from third party countries by individuals and for personal use is also regulated. Food and food products of animal origin for personal consumption cannot be imported into the EU unless such products fully comply with the Community's commercial import rules. Further, introduction of personal consignments of products of animal origin from third countries which form part of travellers' luggage are also prohibited (EC No 206/2009). Also, the international trade of certain animal taxa (some are used as a food source, i.e. bush meat) is prohibited or regulated for conservation reasons (Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) and (EC) No 338/97).

Related projects at the BfR

Until recently, the amount and characteristics of exotic foods in general and of animal origin introduced illegally into Germany and other European countries by passenger luggage and the potential public health threat associated had not been extensively studied, respectively. In the past ten years a growing number of projects have been initiated studying this problem.

One such project was funded by the EU 7th framework program and termed **PROMISE** — "**PRO**tection of consumers through **Mi**tigation of **S**egregation of **E**xpertise". Between 2012-2014 the PROMISE consortium sampled travellers arriving into EU member states to research the extent of *food* products of animal origin (POAO) entering the EU. The BfR focused on the introduction of illegal foods through the Frankfurt (FRA) and Schönefeld (SXF) Airports.

The focus of the Federal Ministry of Education and Research (BMBF) funded project **SiLe-BAT** (**Si**cherstellung der Futter- und **Le**bensmittelwarenkette bei **b**io- und **a**gro-**t**erroristischen (BAT)-Schadenslagen; https://foodrisklabs.bfr.bund.de/silebat/) was on potential contamination scenarios in the food chain through bio- and agro-terroristic events. The project duration was from 2010 – 2014. One aspect was the identification of world-wide risk areas, from where zoonotic agents could be introduced via the food chain (e.g. global trade, illegally introduced POAO) into Europe including an assessment on pathogen characteristics and potential for misuse. Further, appropriate diagnostic methods for sample preparation and pathogen identification were developed and optimized.

ZooGlow (**Zoo**nosen und Lebensmittelsicherheit entlang **glo**baler **W**arenketten) was a project funded by the BMBF, as part of the 'Research for civil security 2012-2017' framework programme

(http://www.bfr.bund.de/de/zoonosen_und_lebensmittelsicherheit_entlang_globaler_warenketten__zooglow_-193044.html). Here, the impact of global goods trade and passenger transport on the spread and transmission of infectious diseases was assessed. Further, diagnostic methods for pathogen identification were optimized and standardized.

Summary of the results

The results relating to foodborne pathogens in food confiscated from air passenger luggage from all three projects are summarized below.

Approximately 20 million passengers arrive from third party countries at the Frankfurt Airport (FRA) per year (Fraport AG, 2012). Between 2009 and 2011 0.3 % of the passengers (approx. 50,000 per year) were subjected to risk orientated custom controls. On average, 7 % of these passengers carried food items which were confiscated by custom and veterinary officers. The volume of these foods amounted to an average of 6 tons per year (Landesbetrieb Hessisches Landeslabor, 2009, 2010, 2011). Based on the above data and our findings that passengers carried an average of 2 kg of food, this theoretically results in a yearly introduction of 2800 tons of illegal food items into Germany through Frankfurt Airport alone. These data are reflected in the study of Chaber et al., who estimated that 3,287 tons of meat and fish (of which 273 tons were bush meat) are introduced to France per year through Charles de Gaulles Airport (Chaber et al., 2010).

The 296 passengers from whom food items were confiscated arrived in Germany from 35 different departure countries. In Schönefeld (SXF) the passengers originated from 15 different countries, in FRA from 28 countries. 27.7% of all passengers arrived from Turkey, 25.7% from Russia, 8.1% from China, followed by Egypt with 7.1%.

In SXF 38% of the seized foods where brought from Russia, 22% from Egypt and 18% from Turkey. In FRA, 33% of the food stuffs originated from Turkey, 22% from China and 12% from Russia (Figure 34). Overall, 204 kg food was seized on flights from Turkey, 180 kg from Russia, 92 kg from Egypt and 31 kg from flights departing from China.

At SXF a total of 423 kg food of animal origin was confiscated, which amounts to 2.7 kg per passenger (range 0.15-16.3). 276 kg were confiscated at FRA, which corresponds to 2.0 kg per passenger (range 0.5-22). 11% of the passengers carried more than 5 kg food items and the largest individual consignment of food was 22 kg of cheese and butter from a single passenger from Russia.

The amount of meat, sausages, milk products and eggs seized at each airport is described in Table 20. 49 kg of the total confiscated meat could be allocated to whole carcasses (10 x poultry, one lamb, one small piglet), 38 kg to poultry meat and 138 kg to meat of other origin (cattle, small ruminants, pig). Examples of products seized in passenger luggage during these projects can be seen in Figure 35.

Table 20: Number of passengers carrying illegal food of animal origin and number and quantity of food items seized at Frankfurt and Berlin-Schönefeld Airport and included in the analyses

	Passengers	Food				
	No. of passengers	No. of food items seized	Total kg seized			
			meat	meat products	milk products	eggs
SXF	155	350	167	105	151	0
FRA	141	306	58	37	177	4
total	296	656	225	142	328	4

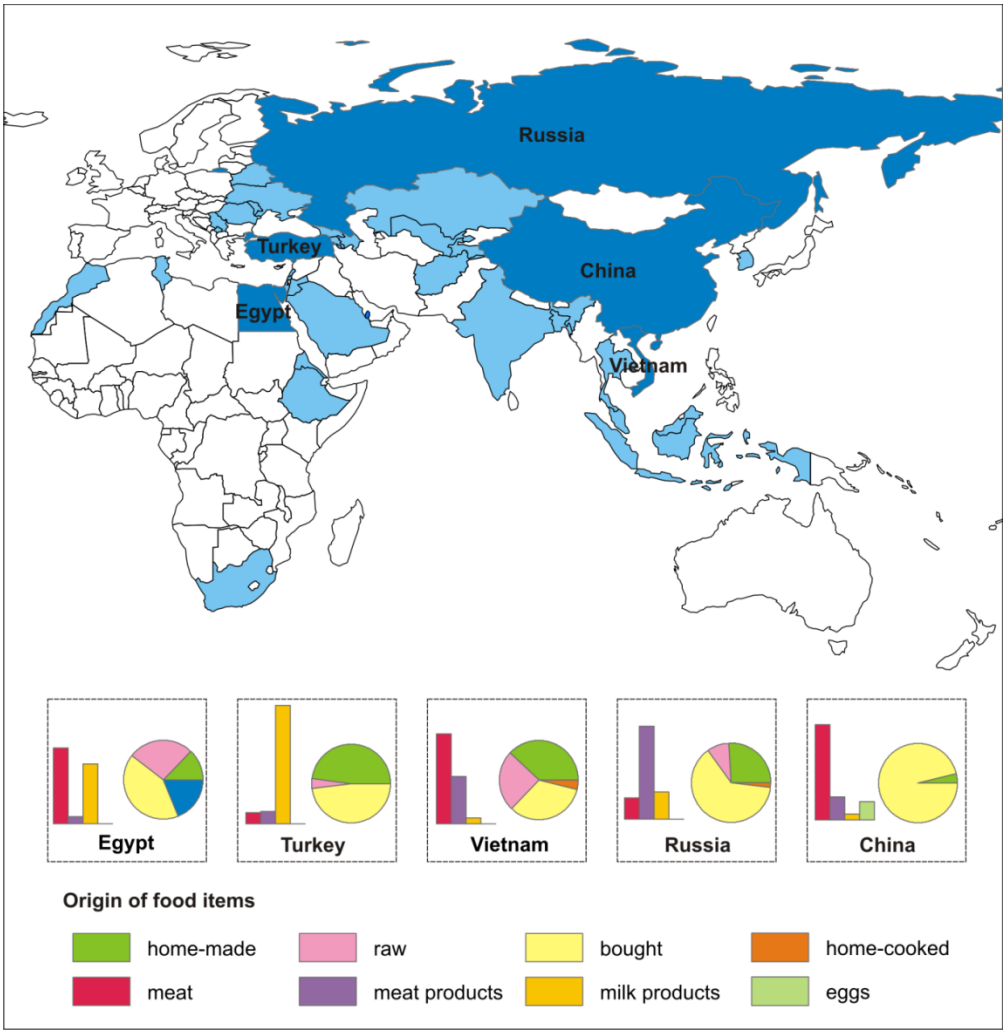


Figure 34: Characterization of food items introduced into Germany
The five departure countries with the largest number of confiscated food items are in dark blue, from the light blue coloured countries food items were introduced on a smaller scale. A: The number of food items tested positive for a foodborne pathogen is described in the colour coded squares for each country. B: The bar charts depict the types of food introduced from the specified countries (in %), the pie charts depict the classification of the foods into degree of food processing (in %).

Figure 35: Examples of food items introduced into Germany
A: meat products from Russia, B: sheep carcass from Tunisia, C: chicken carcass from Azerbaijan, D: raw meat from Vietnam, E: meat products and eggs from China, F: biltong (dried meat) from South-Africa, G: butter from India, H: dried cheese from Usbekistan, I: cheese from Turkey

25% of the meat and meat products revealed increased aerobic mesophilic bacteria counts, which is an indicator of the degree of hygiene and manufacturing conditions.

In total, in 5% of the food items entering Germany via either Frankfurt Airport or Berlin-Schönefeld Airport foodborne pathogens could be detected. From four meat samples originating from Russia *Salmonella enterica* strains were isolated. Two were identified as *S. Infantis* and two as *S. Enteritidis*. In nine food samples *L. monocytogenes* were detected; the *Listeria* species PCR identified in eight samples *L. innocua* and in one sample *L. seeligeri/ L. innocua* sample. 67% of the *L. monocytogenes* positive food items originated from Russia and were isolated from meat (n=1) or meat products (n=5).

Five from seven (70%) of the VTEC positive food samples were cheese, three originating from Turkey, and one each from Egypt and Georgia. Only one isolate of *Yersinia enterocolitica* could be recovered from dried beef from China. The respective isolate was further characterized as a *Y. enterocolitica* of the serogroup O:3. *Brucella* DNA was detected in 3.6% of the samples, *Trichinella* and *Campylobacter* were not detected.

Overall, 42% of the microbiologically positive food items were commercial products, 29% were raw and 29% homemade. The majority of the contaminated products were meat (33%) or meat products (42%), followed by milk products (21%). Foodborne pathogens could be isolated from 5% of the food items from Turkey (number of confiscated food items (n=132), from 10% of the foods from Russia (n=105), from 2% of the Egyptian foods (n=55) and from 6% of the confiscated foods from China (n=51).

Conclusions

Globalization, international trade and the growing flow of goods and people enable pathogens to travel worldwide. International airports serve as bottlenecks for the illegal import of POAO potentially contaminated with animal disease and zoonotic pathogens.

In a variety of studies we could show that autochthonous pathogens such as *Salmonella* spp., *Listeria* spp. and *Yersinia* spp. are introduced into the country via illegal passenger's luggage. In all studies a risk based selection of departure countries from which passengers are controlled was based on the occurrence of animal diseases such as FMD, ASF or avian influenza (AI) and not on the prevalence of foodborne zoonotic diseases. This is the routine procedure at border posts to reduce the risk of introducing animal diseases.

Also, from a public health point of view, a lapse occurs in the veterinary controls of passengers from the Balkan countries which are EU member countries. Approximately 8% of the German immigrant population originates from this region (Destatis, 2012), which is known for, among others, a high *Brucella* and *Trichinella* prevalence and a traditional production of raw milk dairy products (Donev, 2010) and cured sausages (Balescu et al., 2013; Rainova et al., 2018).

Further, a potentially pathway for introducing pathogens into the EU is through bush meat. In our study, the majority of meat products were clearly of food production animal origin, but in some items, the possibility of bush meat could not be excluded. In general, the risk of introduction of bush meat into Germany seems low. The Central and Western African Republics appear to be the main sources of bush meat (Bair-Brake et al., 2013; Chaber et al., 2010). The immigrant population from these regions in Germany was only approx. 100,000 in 2012 (Destatis, 2012).

Due to these study limitations, we could not determine if the risk of infection for the German consumer is greater than through the consumption of food items produced in Germany.

Acknowledgements

We would like to thank the Hessian Ministry for Environment, Energy, Agriculture and Consumer Protection, the Veterinary Border Inspection Post Hessen, the Hessen State Laboratory, the Ministry of Environment, Health and Consumer Protection, Brandenburg and the Veterinary Border Inspection Post, Airport Berlin Schönefeld and the German Customs for their help and cooperation. We are grateful to the veterinarians at the Frankfurt and Berlin Schönefeld airports who made this study possible.

The authors would like to thank the staff of the National Reference Laboratories for Campylobacter, E. coli, Listeria, antibiotic resistance and Salmonella for their support. The work was supported by grants of the German Federal Ministry of Education and Research (SiLeBAT, project No. 13N11202, ZooGloW, project No. 13N12697 by the 7th Framework Programme of the EU (PROMISE, KBBE.2010-2-4-265877).

Abbreviations

EU	European Union
FMD	foot-and-mouth disease
ASF	African swine fever
RASFF	Rapid Alert System for Food and Feed

CITES	Convention on International Trade in Endangered Species of Wild Fauna and Flora
FRA	Frankfurt Airport
SXF	Schönefeld Airport
PROMISE	**PRO**tection of consumers through **Mi**tigation of **S**egregation of **E**xpertise"
SiLeBAT	**Si**cherstellung der Futter- und **Le**bensmittelwarenkette bei **b**io- und **a**gro-**t**erroristischen (BAT)-Schadenslagen;
ZooGlow	**Zoo**nosen und Lebensmittelsicherheit entlang **glo**baler **W**arenketten)

References

Bair-Brake, H., Bell, T., Higgins, A., Bailey, N., Duda, M., Shapiro, S., . . . and Galland, G. (2013). Is That a Rodent in Your Luggage? A Mixed Method Approach to Describe Bushmeat Importation into the United States. *Zoonoses Public Health*. doi:10.1111/zph.12050

Balescu, A., Nemet, C., Zamfir, C., Ispas, D., and Idomir, M. (2013). Identifying risk factors for symptoms of severe trichinellosis--a case study of 143 infected persons in Brasov, Romania 2001-2008. *Vet Parasitol, 194*(2-4), 142-144. doi:10.1016/j.vetpar.2013.01.041

Chaber, A. L., Allebone-Webb, S., Lignereux, Y., Cunningham, A. A., and Rowcliffe, J. M. (2010). The scale of illegal meat importation from Africa to Europe via Paris. *Conservation Letters, 3*(5), 317-323. doi:10.1111/j.1755-263X.2010.00121.x

Destatis. (2012). Bevölkerung und Erwerbstätigkeit, Ausländische Bevölkerung. *Fachserie 1 Reihe 2*.

Donev, D. M. (2010). Brucellosis as priority public health challenge in South Eastern European countries. *Croat Med J, 51*(4), 283-284. doi:10.3325/cmj.2010.51.283

DuPont, H. L., and Ericsson, C. D. (1993). Prevention and treatment of traveler's diarrhea. *N Engl.J Med, 328*(25), 1821-1827. doi:10.1056/NEJM199306243282507

Fraport AG. (2012). Verkehrszahlen, Standort FRA 2009-2011. http://www.fraport.de/de/investor-relations/finanz-und-verkehrszahlen/verkehrszahlen/standort-fra.html

Grabowski, N. T., Klein, G., and Lopez, A. M. (2013). European and German food legislation facing uncommon foodstuffs. *Crit Rev Food Sci Nutr, 53*(8), 787-800. doi:10.1080/10408398.2011.560977

Hartnett, E., Adkin, A., Seaman, M., Cooper, J., Watson, E., Coburn, H., . . . Wooldridge, M. (2007). A quantitative assessment of the risks from illegally imported meat contaminated with foot and mouth disease virus to Great Britain. *Risk Anal, 27*(1), 187-202. doi:10.1111/j.1539-6924.2006.00869.x

Landesbetrieb Hessisches Landeslabor. (2009). *Jahresbericht 2009*. Gießen, Germany: Landesbetrieb Hessisches Landeslabor.

Landesbetrieb Hessisches Landeslabor. (2010). *Jahresbericht 2010*. Gießen, Germany: Landesbetrieb Hessisches Landeslabor.

Landesbetrieb Hessisches Landeslabor. (2011). *Jahresbericht 2011*. Gießen, Germany: Landesbetrieb Hessisches Landeslabor.

Nockler, K., Wichmann-Schauer, H., Hiller, P., Muller, A., and Bogner, K. (2007). Trichinellosis outbreak in Bavaria caused by cured sausage from Romania, January 2007. *Euro Surveill, 12*(8), E070823 070822. doi:10.2807/esw.12.34.03254-en

Noordhuizen, J., Surborg, H., and Smulders, F. J. (2013). On the efficacy of current biosecurity measures at EU borders to prevent the transfer of zoonotic and livestock diseases by travellers. *Vet Q, 33*(3), 161-171. doi:10.1080/01652176.2013.826883

Pappas, G., Papadimitriou, P., Akritidis, N., Christou, L., and Tsianos, E. V. (2006). The new global map of human brucellosis. *Lancet Infect Dis, 6*(2), 91-99. doi:10.1016/S1473-3099(06)70382-6

Peiso, O. O., Bronsvoort, B. M., Handel, I. G., and Volkova, V. V. (2011). A review of exotic animal disease in Great Britain and in Scotland specifically between 1938 and 2007. *PLoS One, 6*(7), e22066. doi:10.1371/journal.pone.0022066

POST, Parliamentary Office of Science and Technology (2005). The bushmeat trade. *Postnote*(236).

Rainova, I., Harizanov, R., Kaftandjiev, I., Tsvetkova, N., Mikov, O., and Kaneva, E. (2018). Human Parasitic Diseases in Bulgaria in Between 2013-2014. *Balkan Med J, 35*(1), 61-67. doi:10.4274/balkanmedj.2017.0167

Sanchez-Cordon, P. J., Montoya, M., Reis, A. L., and Dixon, L. K. (2018). African swine fever: A re-emerging viral disease threatening the global pig industry. *Vet J, 233*, 41-48. doi:10.1016/j.tvjl.2017.12.025

Spies, L. A. (2008). Traveler's diarrhea: an update on prevention and treatment. *J Midwifery Womens Health, 53*(3), 251-254. doi:10.1016/j.jmwh.2008.02.009

5.2 Prevalence and Diversity of Pathogenic Bacteria in Seafood – Control of Enteropathogenic *Vibrio* spp. in Retail Food

Strauch, Eckhard; Richter,Martin

Abstract

Fishery and aquaculture products are regarded as food of excellent *nutritional value*, providing high quality protein and a wide variety of vitamins and minerals. However, the presence of potentially pathogenic bacteria in seafood has to be taken into account and provisions to protect consumers are to be made. The most common bacteria in marine ecosystems belong to the species *Vibrio* and the presence of these bacteria is of concern for foods produced in these environments. Vibrios are regularly detected in a variety of seafood with bivalve molluscs carrying frequently high numbers. Foodborne *Vibrio* infections are usually associated with the consumption of raw or undercooked seafood, while sufficient heating before consumption leads to inactivation. Since many retail products are originating from regions endemic for potentially pathogenic *Vibrio* species identifying products with high prevalence of these bacteria remains important. Among *Vibrio* spp., *V. parahaemolyticus, V. cholerae* and *V. vulnificus* are the most important cause of foodborne infections.

Seafood in Germany is imported to a great extent from all over the world. The presence of *Vibrio* spp. isolated from retail originating from different geographical regions and from regional products is examined by official food laboratories. In case of detection of enteropathogenic species *V. parahaemolyticus, V. cholerae* and *V. vulnificus*, isolates are sent to a reference laboratory at the BfR. Here virulence marker distribution of these strains is studied with the aim to distinguish between environmental isolates and potentially pathogenic isolates. Identification of harmful strains may lead to a retraction from retail of the implicated seafood by the competent authorities.

Background

Seafood has a number of health advantages and regular consumption of seafood is regarded as beneficial to the human body. The consumption of seafood has risen only slightly in the EU in the last years (EC, European Commission, Directorate General for Maritime Affairs and Fisheries,, 2017c), but is increasing worldwide more significantly. The utilization of food fish in 2015 was estimated at 20.1 kg per capita annually (FAO, 2016a). While catches of fish are gathered from seas, rivers and lakes, a strong increase in aquaculture production is taking place worldwide. "*Many millennia after terrestrial food production shifted from hunter-gatherer activities to agriculture, aquatic food production has transitioned from being primarily based on capture of wild fish to culture of increasing numbers of farmed species. A milestone was reached in 2014 when the aquaculture sector's contribution to the supply of fish for human consumption overtook that of wild-caught fish for the first time"* (FAO, 2016a).

However, despite its benefits seafood can be contaminated with chemicals, marine toxins, and infectious agents like bacteria, viruses and parasites (Iwamoto et al., 2010) and can pose a risk for the consumer. Seafood includes various types of fish and fish products, shellfish like mollusks and crustaceans and some marine mammals, e.g. whales and seals. Contamination can be derived from the marine environment from which the animals were harvested. The contamination may stem from indigenous sources of the environment or from man-made pollution of the environment. Additionally, contributing factors to contamination may include handling, storage and transportation, as well as cross-contamination with other food in the food chain.
In this chapter we will focus on bacteria of the family *Vibrionaceae* which are worldwide naturally present in marine and estuarine environments. The family contains over 100 described

species, and around a dozen of these are known to cause infections in humans. Infections are initiated from exposure to seawater or consumption of contaminated seafood produce. Because of their common occurrence in aquatic environments vibrio bacteria are easily introduced into the food chain and distribution routes. Foodborne infections are usually associated with the consumption of raw or undercooked seafood, while heating before consumption leads to inactivation. In Germany, products from regional as well as international sources are of concern, as seafood consumed in Germany is imported to a great extent from all over the world (Huehn et al., 2014). Germany's own production including aquaculture and catches contributes to only approximately 20 to 25% of its total seafood consumption for many years now (BLE, 2018). The FAO lists Germany on place six among the top ten importers of fish and fishery products (FAO, 2016a). Depending on the product, the geographical region, as well as the producing country vary remarkably. Marine captures of fish are done by a number of countries e.g. China, Indonesia, USA, Russia, Japan etc. Farmed shrimps and prawn are mainly produced in developing countries, and much of this production enters international trade (FAO 2016). Many seafood products in Germany are originating from regions endemic for pathogenic *Vibrio*, however, numerous other *Vibrio* species are also present in seafood so that identifying products with high prevalence of potentially pathogenic *Vibrio* species is most important. Seafood products are widely consumed all around the world and play a significant role on the economic market. Bacteria of the Vibrio genus can contaminate seafood and thus pose a risk to human health. Species most commonly associated with foodborne infections are *V. parahaemolyticus, V. vulnificus* and *V. cholerae*. These species are responsible for a dramatic increase of seafood-borne infections worldwide (Bonnin-Jusserand et al., 2017).

The family *Vibrionaceae*

Vibrionaceae are ubiquitous Gram-negative bacteria and native inhabitants of marine and estuarine waters, including aquaculture settings. They are a leading cause of seafood-borne bacterial illness. Increasing incidence of *Vibrio* infections in marine animals and humans has been linked to rising seawater temperature due to global warming and the growing global trade of seafood (Baker-Austin et al., 2010).

Whereas several *Vibrio* species are known to cause intestinal and extraintestinal infections, the three species *V. parahaemolyticus, V. cholerae* and *V. vulnificus* are responsible for most foodborne infections (CAC, 2010). Worldwide several data are available about the abundance of *Vibrio* spp. in fish and fishery products. Some studies conducted in Germany on seafood at retail confirm a high prevalence of potentially enteropathogenic *Vibrio* spp. in these commodities (Lhafi and Kuhne, 2007; Messelhäusser et al., 2010; Urmersbach et al., 2014; Vu et al., 2018).

The prevalence of potentially pathogenic Vibrio species is especially high in bivalve molluscs as these organisms feed by filtering the surrounding water. When analyzing blue mussels (*Mytilis edulis*), grown in classified production areas in the German Wadden Sea of the North Sea region, the detected *Vibrio* bacteria mirrored the *Vibrio* population of the corresponding marine environment (Huehn et al., 2014). The most frequently identified species in blue mussels were the environmental species *V. alginolyticus* followed by *V. parahaemolyticus*. Non-O1/non-O139 *V. cholerae* strains were detected in some samples, while *V. vulnificus* was less frequent. In 2013 *Vibrio* spp. were found in all analyzed mussels (100%) and in 2012 in 87% of the analyzed blue mussels (Figure 36). Usually more than one *Vibrio* species was isolated from a single sample and seasonality of *Vibrio* occurrence was observed. While *V. alginolyticus* was present all year, the other three species were found in summer and autumn periods only.

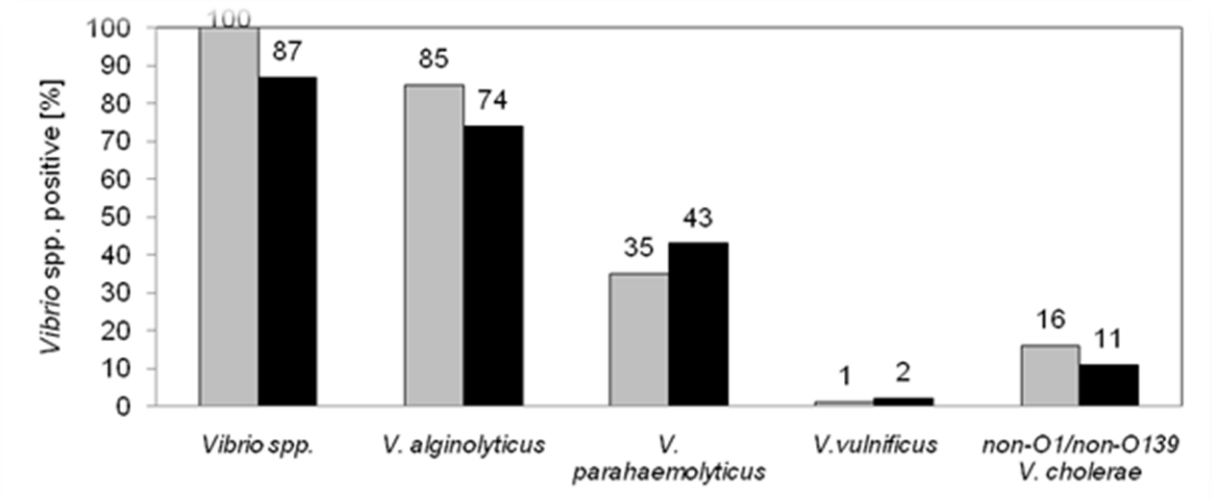


Figure 36: Prevalence of *Vibrio* spp (in %) in Blue Mussels (*Mytilus edulis*) of production areas in the German Wadden Sea in 2012 (n=46 sampling units, grey) and 2013 (n=71 sampling units, black), (Huehn et al. 2014)

- ***Vibrio parahaemolyticus***

V. parahaemolyticus has been recognized as a major cause of seafood-borne gastroenteritis associated with the consumption of raw or undercooked products (FAO and WHO, 2005, 2011). Infections are self-limiting and of moderate severity. The symptoms include diarrhea with abdominal cramps, nausea, vomiting, headache, and low-grade fever and last an average of three days in immunocompetent patients (Nair et al., 2007).

Pathogenic *V. parahaemolyticus* strains are responsible for the majority of seafood-associated infections in the United States, many Asian countries (FAO/WHO 2011) and South America (Velazquez-Roman et al., 2013). Compared to the Asian continent and the USA, *V. parahaemolyticus* infections are rarely reported in Europe. This may be due to a low incidence of illnesses or may be the result of the lack of epidemiological systems for monitoring *Vibrio*-associated illness or *Vibrio* occurrence in seafood (Baker-Austin et al., 2010). Nevertheless several sporadic outbreaks in UK and Spain and single clinical cases in other European countries have been reported (Baker-Austin et al., 2010).

The pathogenicity of *V. parahaemolyticus* is mainly correlated to the possession of genes encoding the thermostable direct hemolysin (TDH) and/or TDH-related hemolysin (TRH) (Nishibuchi and Kaper, 1995; Park et al., 2000). Both epidemiological studies and animal-based studies indicate that at least one type three secretion system (T3SS2) also plays a major role in pathogenicity of *V. parahaemolyticus* (Park et al., 2004). T3SS2 is strongly correlated with the presence of *tdh* and/or *trh* and further subdivided with T3SS2α associated with *tdh* gene on a pathogenicity island and T3SS2β located close to *trh* gene on a different pathogenicity island (Park et al. 2004). Additionally, the *trh* gene is genetically linked to an urease gene cluster associated with a nickel transportation system. However, the majority of *V. parahaemolyticus* strains do not possess the *tdh* and/or *trh* genes and are regarded as environmental strains.

In Germany *tdh* positive *V. parahaemolyticus* strains have been found only in imported seafood so far and their occurence in coastal waters of Germany has never been reported. However, *trh* positive *V. parahaemolyticus* strains are detected in coastal waters (Bechlars et al., 2013). In general, *trh* harbouring strains are detected in a range of about 3 to 5 % at

coastlines of Northern Europe (Ellingsen et al., 2008; Hervio-Heath et al., 2002) with tendancy to rise in coastal areas of France (Robert-Pillot et al., 2004).

- ***Vibrio cholerae***

The Gram-negative bacterium *V. cholerae*, as a species, is part of the normal flora of the aquatic ecosystems worldwide, but many strains of the species can cause severe disease in humans. Strains of *V. cholerae* belonging to the serogroups O1 and O139 are the causative agent of cholera, an epidemic diarrheal disease. Cholera epidemics are essentially confined to third world countries, and occur in cases of inadequate public sanitation systems after natural catastrophes or as a result of political crises. The excretions of diseased people contain high amounts of *V. cholerae* bacteria which are released into waters and contaminate them. In epidemic areas, infection usually occurs through contaminated drinking water or contaminated food that has been in contact with contaminated water (Harris et al., 2012).

Cholera is an acute intestinal infection with a short incubation period of less than one day to five days. Toxigenic *V. cholerae* O1 and O139 strains produce cholera toxin, which causes extensive aqueous diarrhea as an enterotoxin. In the absence of immediate therapy, cholera can quickly lead to severe dehydration and death. *V. cholerae* bacteria colonize the intestinal mucosa and multiply in the intestine. This causes permanent vomiting and diarrhea and the constant loss of water results in internal dehydration of the body and loss of vital minerals. Without treatment, 30-50% of all seriously ill people die within one to six days. The two major virulence factors of toxigenic *V. cholerae* are known to be the cholera toxin (CT) and the toxin-coregulated pilus (TCP). (Zuckerman et al., 2007). These two virulence factors are used as marker for identification of the toxigenic strains.

In Europe, cholera infections are found rarely, and diseased persons have a history of traveling from countries where the disease is endemic. Cholera is endemic in Africa, Asia, South America, and Central America and still a substantial health burden on the affected developing countries (Zuckerman et al., 2007).

Besides the toxigenic O1 and O139 serogroups, *V. cholerae* strains belonging to more than 200 serogroups are widespread in aquatic environments. A number of reports have demonstrated that some strains of these serogroups, collectively referred to as *V. cholerae* non-O1, non-O139, can cause diarrheal diseases, but do not have the ability to cause epidemic outbreaks (Schirmeister et al., 2014). With very few exceptions these strains do not possess the two major virulence factors of toxigenic *V. cholera* and the distinction to toxigenic strains can be made by PCR targeting the *ctx* gene. However, a number of accessory virulence factors which are also present in toxigenic strains are found in non-O1, non-O139 strains (Chatterjee et al., 2009). As it is not yet clear which virulence factors of these bacteria can be made responsible for diarrheal diseases, there is a lack of diagnostic possibilities to distinguish reliably pathogenic non-O1, non-O139 strains from true environmental strains.

- ***Vibrio vulnificus***

V. vulnificus is a potent bacterial pathogen present in coastal waters worldwide and is found preferentially in waters with moderate salinity. It can cause serious wound infections with lethal outcome and is also responsible for death cases caused by consumption of contaminated seafood. In the USA particularly oysters contaminated with *V. vulnificus* have been reported to be responsible for deadly infections (Haq and Dayal, 2005) (FAO and WHO, 2004). The severity of disease is strongly influenced by the health condition of exposed individuals. Immunocompromised individuals and persons with underlying diseases resulting in elevated serum iron levels are especially at high risk. In case of primary septicemia after

consumption of contaminated seafood mortality rates are greater than 50% (Jones and Oliver, 2009). Environmental factors, such as warm water and moderate salinity, are known to favor the multiplication of the pathogen. Therefore, the effect of global warming on sea water temperatures has aroused concerns that infections caused by *V. vulnificus* will increase in numbers (Baker-Austin et al., 2012a). However, despite the frequent occurrence of the pathogen the number of cases reported is relatively low indicating that not all strains of *V. vulnificus* are equally virulent.

Investigations show that the species *V. vulnificus* displays a high degree of intra-species diversity and includes strains with varying virulence potential (Jones and Oliver 2009). Although the majority of strains are virulent in animal models (Thiaville et al., 2011) several investigations showed genetic divergence among strains from clinical and environmental origin. In several studies methods were developed to distinguish clinical (C-type) from environmental (E-type) strains. The potential virlence markers based on variations in the sequence of the small subunit of the 16S rRNA gene, of the virulence correlated gene (*vcg*) (Sanjuan et al., 2009) and the *pilF* gene encoding a protein required for pilus-type IV assembly (Baker-Austin et al., 2012b). Up to now, however, there are no reliable genetic markers to define single strains as environmental strains with no pathogenicity. In contrast, E-type strains have been found in Germany that caused severe and deadly infections (Bier et al., 2013).

In Germany in low salinity marine ecosystems like the Baltic Sea or estuaries of big rivers flowing into the North Sea *V. vulnificus* is present. As these marine environments show an increased warming rate of surface water temperatures, especially in summers, *V. vulnificus* wound infections of humans have increased in years with long warm weather periods after contact with sea water (Baker-Austin et al., 2012a; Huehn et al., 2014). Locally acquired foodborne infections have not yet been reported in Germany, probably as oyster and mussel production does not play a role in the Baltic Sea and river estuaries. Additionally, mandatory notification of suspected *V. vulnificus* infections does not exist in Germany. But investigations on imported seafood have shown that the pathogen is present at low frequency in imported seafood (Vu et al., 2018).

Control of Seafood in Germany

For member states of the European Union the regulation (EC) No. 2073/2005 sets out microbiological criteria for foodstuffs produced and traded in Europe. There are currently no microbiological criteria for *Vibrio* spp. in this regulation so that in the EU there is no legal requirement for *Vibrio* testing of seafood. Nevertheless, in a number of member states, especially those which have an economically relevant production of bivalve molluscs, *Vibrio* testing is carried out. In the RASFF annual report 2016 of the European Commission (EC, European Commission, Health and Food Safety, , 2017d) notifications of pathogenic bacteria are shown that included also notifications of *Vibrio* spp. in crustaceans and products thereof.

In Germany imported and indigenously produced seafood is tested by several official food laboratories and a reference laboratory for *Vibrio* diagnostic is established at the BfR. Depending on the diagnostic capabilities of the laboratories *Vibrio* strains are sent to the reference laboratory for diagnostic confirmation or for characterization of the presence of virulence markers, like *ctx* or *tdh/trh* genes in *V. cholerae* and *V. parahaemolyticus*, respectively.

Methodology for detection of pathogenic *Vibrio*

The detection of the three Vibrio species in food is outlined in a recently renewed ISO Standard (ISO 21872):"*Microbiology of the food chain — Horizontal method for the detection of enteropathogenic Vibrio parahaemolyticus, Vibrio cholerae and Vibrio vulnificus*".

In this standard a horizontal method is specified for the detection of *Vibrio* species including *Vibrio parahaemolyticus, Vibrio cholerae* and *Vibrio vulnificus*. The standard is applicable to products intended for human consumption and for the feeding of animals. Also environmental samples in the area of food production and food handling can be tested with the methodology.

The method requires four successive phases for the detection of potentially enteropathogenic *Vibrio* spp. Recovery of *Vibrio* spp. from foodstuffs is improved by the use of different incubation temperatures depending upon the target species or state of the food matrix. For example, recovery of *V. parahaemolyticus* and *V. cholerae* in fresh products is enhanced by enrichment at 41.5 °C whereas for *V. vulnificus*, and for *V. parahaemolyticus* and *V. cholerae* in deep frozen, dried or salted products, recovery is enhanced by enrichment at 37 °C.

As the three species may be present in small numbers and are often accompanied by a much larger number of other microorganisms belonging to the *Vibrionaceae* family or other bacteria, two successive selective enrichments are carried out to detect the target organisms. For this reason a primary and a secondary enrichment are performed using a liquid selective medium. In the third phase, from the two enrichment cultures inoculation of two solid selective media is performed for isolation and identification of single colonies. These media are Thiosuflate Citrate Bile and Sucrose agar (TCBS) and a second appropriate solid selective medium complementary to the TCBS medium (mostly used Vibrio Chrome Agar). Finally, in the last phase confirmation of the presumptive colonies of *Vibrio parahaemolyticus, Vibrio cholerae* and *Vibrio vulnificus* is done by means of appropriate biochemical and/or polymerase chain (PCR) reaction tests.

In the ISO 21872 new technologies like matrix-assisted-laser-desorption/ionization time-of-flight mass spectrometry (MALDI TOF MS) are not yet included for species confirmation despite its great value for a fast and reliable species identification (Dieckmann et al., 2010). MALDI TOF MS technique will certainly be added to future revisions of the standard.

Conclusion

Vibrio infections are commonly reported in the USA and in many Asian and South American countries. Additionally, there is growing concern that *Vibrio* spp., particularly *V. parahaemolyticus, V. cholerae and V. vulnificus,* may represent an important and growing problem due to climate-driven processes also in Europe. A myriad of epidemiological factors may greatly increase the incidence as well as clinical burden of these pathogens – including rising global consumption and trade of seafood produce coupled to an increase in the number of susceptible individuals consuming seafood produce. It is therefore necessary to establish surveillance systems of non-cholera *Vibrio* infections and to set out microbiological criteria for *Vibrio* contaminants in seafood in Europe.

As neither a requirement for testing of seafood for enteropathogenic *Vibrio* spp. nor microbiological criteria are in the EU legislation, questions arise on what measures should be taken when these bacteria are detected in seafood. Worldwide a number of countries have food safety criteria for *Vibrio* in live bivalve molluscs (LBM) as consumption of mussels/oysters pose a high risk for consumers. LBM often contain high levels of vibrios due to their mode of feeding and are often consumed raw. One of the most critical food commodoties in this con-

text are oysters. The recommendations suggest that certain *Vibrio* level should not be exceeded and in case of higher concentrations the respective food would be unsatisfactory for human consumption. Additionally, in 2010 the Codex Alimentarius published guidelines on the application of general principles of food hygiene to the control of pathogenic Vibrio species in seafood (CAC, 2010).

In Germany the "Deutsche Gesellschaft für Hygiene und Mikrobiologie" (DGHM) gives a recommendation that seafish from warmer geographical regions should be tested for *Vibrio* bacteria. In case of *V. parahaemolytics* or *V. cholerae* contaminations, the ability of toxin production of isolates should be clarified. In Germany decisions are made by local authorities concerning the withdrawal of seafood from the market if a contamination with one of the three enteropathogenic *Vibrio* species is detected. There are no further federal or national regulations.
For a decision to withdraw contaminated seafood from the market, it should be considered if the product will be heated sufficiently before consumption. For ready-to-eat seafood high standards should be in place. Following rules should be applied:

- In case of contamination with toxigenic *V. cholerae* the product has to be withdrawn from the market. This would apply also for any food irrespective if heating of food is provided before consumption.
- In case of *V. vulnificus* contaminations ready-to-eat products should not be allowed to be sold in retail.
- In case of *V. parahaemolyticus* contamination it should be examined if the isolates harbor the *tdh/trh* toxin genes. Such isolates cannot be tolerated in ready-to-eat products.

References

Baker-Austin, C., Lemm, E., Hartnell, R., Lowther, J., Onley, R., Amaro, C., . . . and Lees, D. (2012b). PilF polymorphism-based real-time PCR to distinguish *Vibrio vulnificus* strains of human health relevance. *Food Microbiology, 30*(1), 17-23. doi:10.1016/j.fm.2011.09.002

Baker-Austin, C., Stockley, L., Rangdale, R., and Martinez-Urtaza, J. (2010). Environmental occurrence and clinical impact of *Vibrio vulnificus* and *Vibrio parahaemolyticus*: A European perspective. *Environmental Microbiology Reports, 2*(1), 7-18. doi:10.1111/j.1758-2229.2009.00096.x

Baker-Austin, C., Trinanes, J. A., Taylor, N. G. H., Hartnell, R., Siitonen, A., and Martinez-Urtaza, J. (2012a). Emerging *Vibrio* risk at high latitudes in response to ocean warming. *Nature Clim.Change., 3*(1), 73-77. doi:10.1038/nclimate1628

Bechlars, S., Wustenhagen, D. A., Dragert, K., Dieckmann, R., Strauch, E., and Kubick, S. (2013). Cell-free synthesis of functional thermostable direct hemolysins of *Vibrio parahaemolyticus*. *Toxicon, 76*, 132-142. doi:S0041-0101(13)00362-0 [pii];10.1016/j.toxicon.2013.09.012 [doi]

Bier, N., Bechlars, S., Diescher, S., Klein, F., Hauk, G., Duty, O., . . . and Dieckmann, R. (2013). Genotypic diversity and virulence characteristics of clinical and environmental *Vibrio vulnificus* isolates from the baltic sea region. *Applied and Environmental Microbiology, 79*(12), 3570-3581. doi:10.1128/AEM.00477-13

BLE, Bundesanstalt für Landwirtschaft und Ernährung. (2018). *Selbstversorgungsgrad bei Fisch und Fischerzeugnissen in Deutschland in den Jahren 1980 bis 2017.* https://de.statista.com/statistik/daten/studie/76640/umfrage/selbstversorgungsgrad-bei-fischen-und-fischerzeugnissen-in-deutschland/

Bonnin-Jusserand, M., Copin, S., Le Bris, C., Brauge, T., Gay, M., Brisabois, A., . . . Midelet-Bourdin, G. (2017). Vibrio species involved in seafood-borne outbreaks (*Vibrio cholerae, V. parahaemolyticus* and *V. vulnificus*): Review of microbiological versus recent molecular detection methods in seafood products. *Crit Rev Food Sci Nutr*, 1-14. doi:10.1080/10408398.2017.1384715

CAC, Codex Alimentarius Commission. (2010). *Guidelines on the Application of General Principles of Food Hygiene to the Control of Pathogenic Vibrio Species in Seafood*. (CAC/GL 73-2010). http://www.fao.org/fao-who-codexalimentarius/sh-

proxy/en/?lnk=1&url=https%253A%252F%252Fworkspace.fao.org%252Fsites%252Fcodex%252FStandards%252FCAC%2BGL%2B73-2010%252FCXG_73e.pdf

Chatterjee, S., Ghosh, K., Raychoudhuri, A., Chowdhury, G., Bhattacharya, M. K., Mukhopadhyay, A. K., . . . and Nandy, R. K. (2009). Incidence, Virulence Factors, and Clonality among Clinical Strains of Non-O1, Non-O139 Vibrio cholerae Isolates from Hospitalized Diarrheal Patients in Kolkata, India. *Journal of Clinical Microbiology, 47*(4), 1087. doi:10.1128/JCM.02026-08

Dieckmann, R., Strauch, E., and Alter, T. (2010). Rapid identification and characterization of *Vibrio* species using whole-cell MALDI-TOF mass spectrometry. *Journal of Applied Microbiology, 109*(1), 199-211. doi:10.1111/j.1365-2672.2009.04647.x

EC, European Commission, Directorate General for Maritime Affairs and Fisheries,. (2017c). *EU Consumer Habits Regarding Fishery and Aquaculture Products - Final Report*.

EC, European Commission, Health and Food Safety, . (2017d). *The Rapid Alert System for Food and Feed. 2016 annual report*.

Ellingsen, A. B., Jørgensen, H., Wagley, S., Monshaugen, M., and Rørvik, L. M. (2008). Genetic diversity among Norwegian *Vibrio parahaemolyticus*. *Journal of Applied Microbiology, 105*(6), 2195-2202. doi:10.1111/j.1365-2672.2008.03964.x

FAO, Food and Agriculture Organization of the United Nations. (2016a). *The State of World Fisheries and Aquaculture 2016. Contributing to food security and nutrition for all.* http://www.fao.org/3/a-i5555e.pdf

FAO and WHO, Food and Agriculture Organization of the United Nations and World Health Organization. (2004). *Hazard identification, exposure assessment and hazard characterization of Vibrio spp. in seafood*. http://www.fao.org/3/a-i2225e.pdf

FAO and WHO, Food and Agriculture Organization of the United Nations and World Health Organization. (2005). *Risk Assessment of Vibrio Vulnificus in Raw Oysters. Interpretative Summary and Technical Report No. 9*. http://www.fao.org/3/a-a0253e.pdf

FAO and WHO, Food and Agriculture Organization of the United Nations and World Health Organization. (2011). *Risk assessment of Vibrio parahaemolyticus in seafood: Interpretative summary and Technical report.* . http://www.fao.org/3/a-i2225e.pdf

Haq, S. M., and Dayal, H. H. (2005). Chronic liver disease and consumption of raw oysters: A potentially lethal combination - A review of *Vibrio vulnificus* septicemia. *American Journal of Gastroenterology, 100*(5), 1195-1199. doi:10.1111/j.1572-0241.2005.40814.x

Harris, J. B., LaRocque, R. C., Qadri, F., Ryan, E. T., and Calderwood, S. B. (2012). Cholera. *Lancet, 379*(9835), 2466-2476. doi:10.1016/S0140-6736(12)60436-X

Hervio-Heath, D., Colwell, R. R., Derrien, A., Robert-Pillot, A., Fournier, J. M., and Pommepuy, M. (2002). Occurrence of pathogenic vibrios in coastal areas of France. *J Appl Microbiol, 92*(6), 1123-1135.

Huehn, S., Eichhorn, C., Urmersbach, S., Breidenbach, J., Bechlars, S., Bier, N., . . . and Strauch, E. (2014). Pathogenic vibrios in environmental, seafood and clinical sources in Germany. *International journal of medical microbiology, 304*(7), 843-850. doi:10.1016/j.ijmm.2014.07.010

Iwamoto, M., Ayers, T., Mahon, B. E., and Swerdlow, D. L. (2010). Epidemiology of seafood-associated infections in the United States. *Clinical Microbiology Reviews, 23*(2), 399-411. doi:10.1128/CMR.00059-09

Jones, M. K., and Oliver, J. D. (2009). *Vibrio vulnificus*: disease and pathogenesis. *Infect Immun., 77*(5), 1723-1733. doi:IAI.01046-08 [pii];10.1128/IAI.01046-08 [doi]

Lhafi, S. K., and Kuhne, M. (2007). Occurrence of Vibrio spp. in blue mussels (Mytilus edulis) from the German Wadden Sea. *International Journal of Food Microbiology, 116*(2), 297-300. doi:10.1016/j.ijfoodmicro.2007.01.007

Messelhäusser, U., Colditz, J., Thärigen, D., Kleih, W., Höller, C., and Busch, U. (2010). Detection and differentiation of Vibrio spp. in seafood and fish samples with cultural and molecular methods. *International Journal of Food Microbiology, 142*(3), 360-364. doi:10.1016/j.ijfoodmicro.2010.07.020

Nair, G. B., Ramamurthy, T., Bhattacharya, S. K., Dutta, B., Takeda, Y., and Sack, D. A. (2007). Global dissemination of *Vibrio parahaemolyticus* serotype O3:K6 and its serovariants. *Clinical Microbiology Reviews, 20*(1), 39-48. doi:10.1128/CMR.00025-06

Nishibuchi, M., and Kaper, J. B. (1995). Thermostable direct hemolysin gene of *Vibrio parahaemolyticus*: a virulence gene acquired by a marine bacterium. *Infect Immun, 63*(6), 2093-2099.

Park, K. S., Iida, T., Yamaichi, Y., Oyagi, T., Yamamoto, K., and Honda, T. (2000). Genetic characterization of DNA region containing the *trh* and *ure* genes of *Vibrio parahaemolyticus*. *Infect Immun., 68*(10), 5742-5748. doi:10.1128/iai.68.10.5742-5748.2000

Park, K. S., Ono, T., Rokuda, M., Jang, M. H., Okada, K., Iida, T., and Honda, T. (2004). Functional characterization of two type III secretion systems of *Vibrio parahaemolyticus*. *Infection and Immunity, 72*(11), 6659-6665. doi:10.1128/IAI.72.11.6659-6665.2004

Robert-Pillot, A., Guenole, A., Lesne, J., Delesmont, R., Fournier, J. M., and Quilici, M. L. (2004). Occurrence of the *tdh* and *trh* genes in *Vibrio parahaemolyticus* isolates from waters and raw shellfish collected in two French coastal areas and from seafood imported into France. *International Journal of Food Microbiology, 91*(3), 319-325. doi:10.1016/j.ijfoodmicro.2003.07.006

Sanjuan, E., Fouz, B., Oliver, J. D., and Amaro, C. (2009). Evaluation of genotypic and phenotypic methods to distinguish clinical from environmental Vibrio vulnificus strains. *Appl Environ Microbiol, 75*(6), 1604-1613. doi:10.1128/AEM.01594-08

Schirmeister, F., Dieckmann, R., Bechlars, S., Bier, N., Faruque, S. M., and Strauch, E. (2014). Genetic and phenotypic analysis of *Vibrio cholerae* non-O1, non-O139 isolated from German and Austrian patients. *Eur J Clin Microbiol Infect Dis, 33*(5), 767-778. doi:10.1007/s10096-013-2011-9

Thiaville, P. C., Bourdage, K. L., Wright, A. C., Farrell-Evans, M., Garvan, C. W., and Gulig, P. A. (2011). Genotype is correlated with but does not predict virulence of *Vibrio vulnificus* biotype 1 in subcutaneously inoculated, iron dextran-treated mice. *Infection and Immunity, 79*(3), 1194-1207. doi:10.1128/IAI.01031-10

Urmersbach, S., Alter, T., Koralage, M. S., Sperling, L., Gerdts, G., Messelhausser, U., and Huehn, S. (2014). Population analysis of *Vibrio parahaemolyticus* originating from different geographical regions demonstrates a high genetic diversity. *BMC.Microbiol, 14*(1), 59. doi:10.1186/s12866-015-0565-7.

Velazquez-Roman, J., Leon-Sicairos, N., de, J. H.-D., and Canizalez-Roman, A. (2013). Pandemic *Vibrio parahaemolyticus* O3:K6 on the American continent. *Front Cell Infect Microbiol, 3*, 110. doi:10.3389/fcimb.2013.00110 [doi]

Vu, T. T. T., Alter, T., and Huehn, S. (2018). Prevalence of Vibrio spp. in Retail Seafood in Berlin, Germany. *J Food Prot, 81*(4), 593-597. doi:10.4315/0362-028X.JFP-17-366

Zuckerman, J. N., Rombo, L., and Fisch, A. (2007). The true burden and risk of cholera: implications for prevention and control. *Lancet Infect Dis, 7*(8), 521-530. doi:10.1016/S1473-3099(07)70138-X

5.3 Microbiological safety in the spice and dried culinary herb chains

Frentzel, Hendrik; Mader, Anneluise; Hiller, Petra; Schaarschmidt, Sara

Abstract

The spices and dried culinary herbs chains are probably the most historic example of global food supply chains. In keeping with their global character, they are highly diverse and often complex. Here, an overview is provided on the particular challenges of these food chains, on efforts in standardisation regarding hygienic practices in spice/herb production and product quality/safety, and on potential risks to human health associated with pathogenic and/or toxigenic microbial agents in spices and dried culinary herbs. Although microbial growth is prevented in spices and dried herbs due to the low water activity, some microorganisms are nevertheless able to survive in these products – even for extended periods, as shown by tenacity studies. If spices and herbs contain heat-stable pathogenic microorganisms that are not inactivated by food processing or if contaminated spices/herbs are added to ready-to-eat foods, these microorganisms can proliferate under inappropriate food storage conditions and might cause food-borne diseases. To assess and monitor microbial hazards in spices and herbs, suitable laboratory methods are needed, since plant secondary compounds can impede detection as outlined in this chapter.

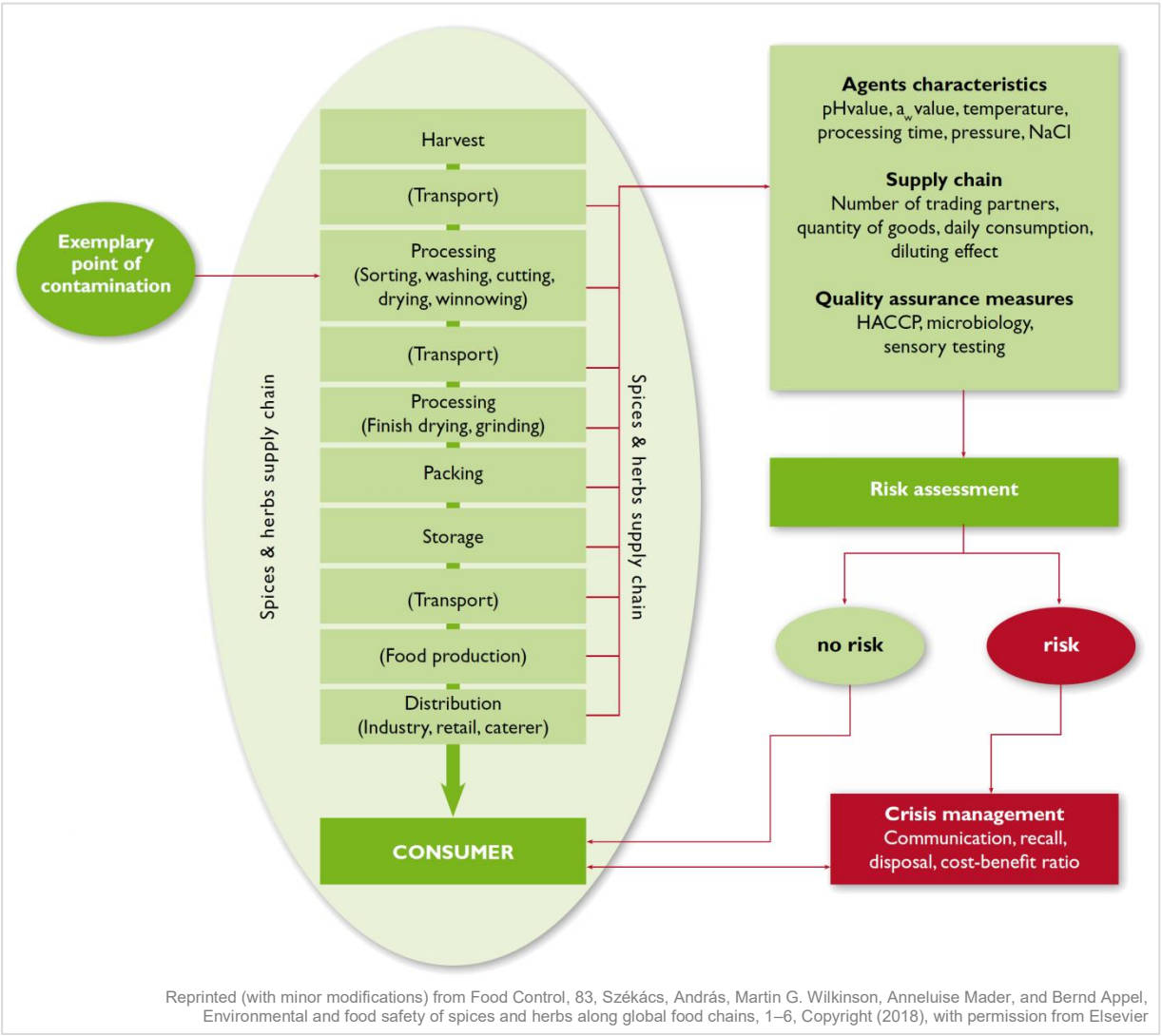
Challenges of the spice and culinary herb chains

Spices and culinary herbs are important food ingredients that are commonly used to flavour, decorate, and/or colour spicy as well as sweet foods. The terms spices and culinary herbs comprise highly diverse products derived from different plant organs of wooden or herbaceous plants from a variety of plant families. There are numerous products known world-wide (for an overview on the most frequently traded spices and dried herbs, see ESA, 2017) and, for a few, the categorisation as “spice” or “herb” might vary regionally. Spices are processed plant parts, which can derive from various organs, such as fruits, seeds, rhizomes, bark, or flowers. Spices are always dry products and are often used in a ground form. Herbs typically consist of leaves or the whole (soft) shoots of herbaceous plants. Herbs are also available in a fresh or frozen state, but often they are used as dried products, which are – like spices – easy to handle and readily available.

The majority of spices and herbs are produced in (sub-)tropical and Mediterranean climates. Their production, processing, and trading chains are diverse (as are the products) and often complex with many actors involved, including small farmers (FAO, 2011). At low moisture, spices and herbs are simple to store and ship and, if unground, many spices can be stored even for years without relevant losses in aroma (Fachverband der Gewürzindustrie, 2018). Thus, the final products often derive from pooled batches of different primary producers, different harvest years, and/or different regions, a situation which impedes backtracing.

The production, processing, and trade chains of spices and culinary herbs are typically vulnerable to microbial contamination (Figure 37). Production often takes place in less developed countries, where proper sanitation and hygiene might be still a challenge. Contaminants can be introduced at several steps along the chains, for example during irrigation, fertilisation, manual handling during harvest and sorting, natural drying in open spaces, and storage and trading in bulk. Although spices and dried herbs are characterised by a low water activity and by a high content of phenolic compounds with potential antimicrobial activity, some pathogenic and/or toxigenic microorganisms are able to survive in these matrices as outlined below. Further, the spices and dried culinary herbs chains are vulnerable to contamination with chemical hazards, and mycotoxins (Reinholds et al., 2016; Reinholds et al.,

2017), and the spice/herb products can be a target of food adultera tions due to their high price (Horn et al., 2018; Reinholds et al., 2015); (see also Chapter 3).



Reprinted (with minor modifications) from Food Control, 83, Székács, András, Martin G. Wilkinson, Anneluise Mader, and Bernd Appel, Environmental and food safety of spices and herbs along global food chains, 1–6, Copyright (2018), with permission from Elsevier

Figure 37: Schematic supply chain of spices and dried culinary herbs with a hypothetical point of contamination and the corresponding hazard identification and risk assessment steps
Microbial contamination can take place at several points along the supply chain and might be caused for example by irrigation with contaminated water or by manual processing steps (e.g. harvest, sorting). If spices/herbs are insufficiently dried, microorganisms might proliferate during storage or transport of these commodities (Székács et al., 2018).

Meeting the need for high-quality spice/herb products can be a challenge – also for the spice industry in the European Union (EU). The EU is one of the world's largest markets for spices and dried culinary herbs. The demand for these products increases year after year and, with it, the corresponding EU imports from developing countries (CBI, 2017b). However, imported foods must meet the product specifications laid down by EU law and must meet at least the same hygiene standards as foods produced within the EU (Eur. Parliament and The Council, 2004). One strategy of EU spice producers/processers for improving transparency and back-tracing and to ensure a high quality and safety of spices and dried culinary herbs is own farming and contract farming in the producing countries. But due to the large number of dif-

ferent products and the high demand, businesses are often dependent on multiple types of supply sources (Schaarschmidt et al., 2016b).

Securing the spices and dried culinary herbs commodity chains in Europe against deliberate, accidental or natural biological and chemical contamination was the major aim of the EU funded project SPICED. This project ran from 2013 to 2016 and was coordinated by the BfR. It addressed the entire chain, from primary production to the consumer, targeting *inter alia* chain analyses and modelling, sampling and monitoring strategies, and improved detection methods (Székács et al., 2018). In the following, representative SPICED results of literature surveys and experimental studies concerning the microbial risks caused by pathogenic and/or toxigenic bacteria in spices and dried culinary herbs are outlined.

Microbial risks and food-borne disease outbreaks associated with spices and dried culinary herbs

Spices and dried culinary herbs are low-moisture foods characterised by a water activity below 0.85. Their low water activity contributes to a long shelf life and has for many years possibly led to the perception that they are not of concern from a microbial food safety perspective. Moreover, spices and dried culinary herbs are minor food ingredients and are, thus, consumed only in low amounts – which also applies to potential contaminations present in these products.

However, spices and dried culinary herbs can act as a vehicle for distributing microbial contaminants. Due to their long shelf life and small application quantity, a single batch is typically disseminated to a large number of end users and dishes. Several microorganisms can be present in spices and dried culinary herbs, including pathogenic and/or toxigenic bacteria. In a comprehensive review, McKee (1995) reported the occurrence of potentially pathogenic microorganisms, such as *Salmonella* spp., *Bacillus cereus*, *Clostridium perfringens*, *Escherichia coli*, *Staphylococcus aureus*, and *Shigella* spp. in various spices and herbs, albeit mostly in low numbers (see also FDA, 2013a). Focusing on *Bacillus cereus* group members, Hariram and Labbé) (2015) demonstrated their frequent occurrence in 247 different samples of U.S. retail spices. Recently, Frentzel and co-workers characterised *Bacillus cereus* group isolates from spices and dried culinary herbs with respect to phylogenetic affiliation and present toxin genes revealing a high prevalence of toxigenic isolates (Frentzel et al., 2018a).

Making use of the Rapid Alert System for Food and Feed (RASFF) as information source, Banach et al. (2016) reported 425 notifications for pathogenic microorganisms within the product category “herbs and spices” (including fresh herbs) between 01/01/2004 and 01/01/2014. Within the 425 notifications, 500 microbial hazards were specified, of which the most frequent was *Salmonella* spp. (369) followed by *Escherichia coli* (61), *Bacillus* spp. (41) and *Clostridium* spp. (7).

When using spices or herbs contaminated with potentially harmful microorganisms as food ingredients, the microbial agents are introduced into foods of higher moisture level, where they might proliferate and/or produce toxins if foods/dishes are not prepared or handled properly. Further, a low infectious dose, as known for *Salmonella* also in certain low-moisture foods, can contribute to a disease outbreak (Lehmacher et al., 1995; Werber et al., 2005).

A number of outbreaks of food-borne illnesses linked to spices or the category ‘spices and dry herbs’ have illustrated the microbiological risk associated with these low-moisture food ingredients (EFSA BIOHAZ, 2013; FAO and WHO, 2014; FDA, 2013a; Van Doren et al., 2013). Further overviews on recent outbreaks associated with ‘spices and herbs’ (potentially including fresh herbs) are also included in studies of EFSA BIOHAZ (2016) and Banach et al. (2016)). Based on outbreak analyses and scenario studies, spices and dried culinary herbs

in combination with *Salmonella* spp. and *Bacillus* spp. were ranked within the top four food–pathogen combinations with regard to the microbial risk related to foods of non-animal origin in the EU (Da Silva Felício et al., 2015; EFSA BIOHAZ, 2013). Among low-moisture foods, spices and dried culinary herbs (including herbal tea) were ranked in the top three concerning microbiological food safety concerns (FAO and WHO, 2014).

A summary of accurately described food-borne outbreaks in Europe from 1973 to 2015 associated with spices/dried culinary herbs is shown in Table 21 (modified according to Mader and Schaarschmidt, 2015). Specified is thereby, that only outbreaks caused by *Salmonell*a spp. (n = 7), *Bacillus* spp. (n = 11), and *Clostridium perfringens* (n = 6) were identified. Most illnesses were caused by *Salmonella* spp. (72.4%), followed by *Bacillus* spp. (23.3%), and *Clostridium perfringens* (4.3%). However, when excluding the *Salmonella* outbreak in Germany in 1993, considering the fact that the number of cases was significantly higher compared to all other outbreaks, the illnesses caused by *Salmonella* spp., *Bacillus* spp., and *Clostridium perfringens* would be 41.6%, 49.3%, and 9.2%, respectively.

It can be assumed that the numbers of outbreaks and cases typically represent only a small proportion of the total, as many patients either do not seek medical care or are not attributed to an outbreak. Further, minor food ingredients, such as spices and herbs, are often not in the focus of outbreak investigations. Disease outbreaks explicitly associated with dried culinary herbs could not be found in the literature, but outbreaks caused by fresh herbs have been reported (Campbell et al., 2001; Pakalniskiene et al., 2009; Pezzoli et al., 2008; Podolak et al., 2010).

To reduce microbial contaminations of spices and dried culinary herbs, particularly with *Salmonella* spp., decontamination procedures are often applied. In the EU, fumigation with ethylene oxide is prohibited and irradiation, which needs to be labelled throughout the chain, is less accepted by the consumer. Thus, steam treatment is the preferred method to reduce the microbial load in spices/dried culinary herbs in the EU. However, precise operation is required to avoid significant losses or changes in aroma and colour by steaming (for example, see Duncan et al., 2017). Further, bacterial spores are typically not (or only less) affected by currently applied decontamination practices. Thus, prevention of contaminations in spices and herbs, particularly by applying good hygienic practices, is crucial (see also Beuchat et al., 2013).

Table 21: Overview on reported food-borne outbreaks in Europe (1973–2015) associated with the consumption of microbial contaminants in spices and dried culinary herbs or foods containing these contaminated ingredients

Condiment implicated	Year	Country	Microbial hazard	Cases	Hospitalisations (deaths)	Reference
Black pepper & garlic salt mix	1975	Finland	*Bacillus cereus*	18	NR (NR)	(Raevuori et al., 1976)
Black pepper (*Piper nigrum*)	1981-82	Norway	*Salmonella* Oranienburg	126	> 25 % (≥ 1)	(Gustavsen and Breen, 1984)
Paprika (*Capsicum annum*)	1993	Germany	*Salmonella* Saintpaul, Rubislaw, and Javiana	1000	NR (NR)	(Lehmacher et al., 1995)
Turmeric (*Curcuma longa*)	1995	England & Wales	*Bacillus subtilis* & *Bacillus pumilus*	2	0 (0)	(Little et al., 2003; Van Doren et al., 2013)
Black pepper (*Piper nigrum*)	1996	England & Wales	*Salmonella* Enteritidis PT4	8	1 (0)	(Little et al., 2003; Van Doren et al., 2013)
Curry powder	2002	England & Wales	*Salmonella* Braenderup	20	1 (0)	(Little et al., 2003; Van Doren et al., 2013)
Spice blend	2007	France	*Bacillus cereus*	146	0 (0)	(EFSA, 2009b; EFSA BIOHAZ, 2013)
Spices and dry herbs (not further specified)	2007	France	*Clostridium perfringens*	19	0 (0)	(EFSA BIOHAZ, 2013)
Spices and dry herbs (not further specified)	2007	Denmark	*Salmonella* Senftenberg	3	0 (0)	(EFSA BIOHAZ, 2013)
Paprika (*Capsicum spp.*)	2009	Denmark	*Bacillus cereus*	48	0 (0)	(FAO and WHO, 2014)
Curry powder	2009	Belgium	*Bacillus cereus*	7	0 (0)	(EFSA, 2011b)
White pepper (*Piper nigrum*)	2010	Denmark	*Bacillus cereus*	112	0 (0)	(EFSA BIOHAZ, 2013)
Cinnamon	2011	Denmark	*Bacillus cereus*	30	0 (0)	(FAO and WHO, 2014)
Turmeric	2011	Finland	*Bacillus cereus*	19	0 (0)	(EFSA BIOHAZ, 2013)
Turmeric	2011	Finland	*Bacillus cereus*	4	0 (0)	(EFSA BIOHAZ, 2013)
Cumin	2011	Finland	*Bacillus cereus*	3	0 (0)	(EFSA BIOHAZ, 2013)
Dried chilies (*Capsicum* spp.)	2011	Denmark	*Clostridium perfringens*	3	0 (0)	(FAO and WHO, 2014)
Red pepper (*Capsicum* spp.)	2011	Denmark	*Clostridium perfringens*	37	0 (0)	(FAO and WHO, 2014)
Black pepper (*Piper nigrum*)	2011	Denmark	*Clostridium perfringens*	19*	0 (0)	(FAO and WHO, 2014)
Black pepper	2011	Denmark	*Bacillus cereus*	52	0 (0)	(EFSA BIOHAZ, 2013)
BBQ spices	2011	Denmark	*Clostridium perfringens*	4	0 (0)	(FAO and WHO, 2014)
Spice blend	2012	Hungary	*Salmonella* Enteritidis	41	6 (0)	(EFSA, 2012c)
Spice mix	2015	Sweden	*Salmonella* Enteritidis	174	NR	(Jernberg et al., 2015)
TOTAL			*Clostridium perfringens* 6 outbreaks	82	0 (0)	
			Bacillus spp. 11 outbreaks	441	0/NR (0/NR)	
			Salmonella spp. 7 outbreaks	1372	≥ 40 (≥ 1)	

*: 2 outbreaks. NR: not reported by the cited study/studies.

Tenacity of microorganisms in spices and dried culinary herbs

The capability of pathogenic microorganisms to persist in food is an important parameter for assessing the risk of food-borne disease emanating from a certain food–pathogen combination. It is widely accepted that low-moisture foods, such as spices and dried culinary herbs, preclude bacterial growth. Nonetheless, they may allow for prolonged survival of pathogens. A way of investigating bacterial survival in food is via tenacity studies. Such studies require an artificial contamination step (spiking), which is crucial for the outcome of experiments. On the one hand, the spiking technique should reflect natural contamination routes. On the other hand, it should allow for a stable initial inoculum.

Dinh Thanh et al. (2017) investigated different direct and indirect spiking techniques and showed their impact on initial cell numbers and long-term survival of *Staphylococcus aureus* in spices and dried culinary herbs. One of the investigated spiking methods, which proved suitable for spiking of condiment matrices, applied sand as a carrier. Thus, in a subsequent study, a sand-carrier spiking method was chosen to determine the tenacity of *Staphylococcus aureus* strains and of spores of *Bacillus cereus* and *Bacillus thuringiensis* strains in different spices and dried culinary herbs (Dinh Thanh et al., 2018). With regard to *Staphylococcus aureus*, significant differences in the survival capacities (expressed as *D* values) were found between strains, and also between matrices. For example, *Staphylococcus aureus* (initial contamination level: 8.1 ± 0.5 $\log_{10}$ cfu/g) declined below the detection limit within five weeks in allspice, but was still detectable in pepper after 20 weeks of storage at room temperature in the dark. In contrast, spores of *Bacillus cereus* and *Bacillus thuringiensis* (initial contamination levels: 5.6 ± 0.2 $\log_{10}$ cfu/g and 6.7 ± 0.1 $\log_{10}$ cfu/g) showed no significant reduction within a storage period of 50 weeks.

Also Lins (2018a) tested several indirect spiking methods using different carrier materials to obtain a dry *Salmonella* Oranienburg inoculum. Finally, agar-agar was used as carrier to spike five different spices and four different dried culinary herbs at a contamination level of about 10^6 cfu/g. Remarkably, in most matrices *Salmonella* Oranienburg remained very stable throughout a storage period of one year at 25 ± 1°C. A significant reduction was observed in allspice and nutmeg samples showing concentrations of around 10^3 cfu/g on day 365. A much more pronounced effect was noted for paprika/chilli with a decline to 10^3 cfu/g already after 101 days and a further reduction to below 10 cfu/g after 188 days, a level which was kept until the end of the study (day 365). The possible underlying mechanisms and the challenges associated with *Salmonella* survival in low-moisture foods were previously outlined (Finn et al., 2013; Podolak et al., 2010).

A high tenacity of pathogenic microorganisms in spices and dried culinary herbs strongly contributes to potential health risks associated with these minor food ingredients.

Efforts in standardisation regarding quality, safety, and traceability of spice and dried culinary herb chains and products

Spices have been valuable trade goods for hundreds of years. Defining minimum product quality criteria is a measure for facilitating global trade. Many public and private standards are available specifically for spices and dried culinary herbs that address physical and chemical product quality as well as chemical safety aspects, such as contaminations with mycotoxins and adulterations with potentially harmful colorants (for an overview, see Schaarschmidt, 2016a). If harmonisation of product or production characteristics covers hygienic aspects, hazard analysis and critical control points (HACCP) guidelines, and/or microbiological criteria it can also support microbiological food safety.

Good practices for the cultivation, pre-processing handling, and processing of spices and culinary herbs, which also address hygienic practices, are made available by several authorities, organisations, and industry associations (for more information, see Schaarschmidt et al., 2016b). For example, already in 1969 the Codex Alimentarius Commission (CAC) established their General Principles of Food Hygiene (CAC/RCP 1-1969), which were most recently revised in 2003 (CAC, 2003b), and in 1995 the Code of Hygienic Practice for Spices and Dried Aromatic Plants (CAC/RCP 42-1995) was adopted (CAC, 1995b). In 2015, the latter was replaced by the Code of Hygienic Practice for Low-Moisture Foods (CAC/RCP 75-2015), which was revised in the following year and recently amended (CAC, 2018).

The CAC also developed a Guide for the Microbiological Quality of Spices and Herbs Used in Processed Meat and Poultry Products (CAC/GL 14-1991) (CAC, 1991). General product specifications for individual spices and dried culinary herbs based on international agreements had been specifically developed by the International Organisation for Standardisation (ISO) (Schaarschmidt, 2016a). Moreover, a new Codex Committee named the Codex Committee on Spices and Culinary Herbs (CX-736) (CAC, 2018) has started its work on elaborating world-wide standards for spices and dried culinary herbs. Regarding microbial contaminations, such general product specifications typically do not contain concrete limits, but claim that the products shall be free from levels that might represent a risk to consumers' health. Also under EU law, specific limits are not laid down for microbial contaminations in spices/herbs (EC, 2005a), but legal limits do exist in several non-member states. Additionally, guidelines from public and private bodies are available. Public and private microbiological standards mainly address *Salmonella*, but also presumptive *Bacillus cereus*, sulphite-reducing *Clostridia*, *Escherichia coli* or coliform bacteria in general, total plate count, and/or mould (and yeast) fungi (Schaarschmidt et al., 2016b). However, heterogenic distribution of microbiological contaminants in low-moisture foods (see also Beuchat et al., 2013) and spice/herb metabolites that interfere with laboratory methods can complicate the detection and quantification of microorganisms in such chains (see below).

Standardisation efforts also address the reporting of data along the food chain. Again, these efforts are complicated by an increasing number and diversity of actors involved. Data reporting is crucial for product tracing (also in case of an incident), which is in return of particular importance in complex and global supply chains. Standardised transfer or exchange of information concerns the parameters and the contents, as well as the manner of reporting. A survey among spice/herb businesses indicated that even handwritten information is still provided (Schaarschmidt et al., 2018). To improve reporting and to harmonise the type of information reported to the next actor, forms are provided by spice/herb associations. Besides information required for product tracing, data on quality and safety testing appears to be frequently requested and reported in the EU (Schaarschmidt et al., 2018).

Challenges in the detection of microbial hazards in spices and herbs

To control and assess the microbiological quality and safety of foods, suitable methods are required for detecting microbial agents. When analysing spices and herbs, however, their high content of volatile oils, which contain terpenes and other phenolic compounds, can interfere with microbiological and molecular biological detection methods.

Plant secondary compounds can impede enumeration and enrichment of microorganisms, depending on the microbial species/strain, the spice/herb, and the dilution factor. For example, Lins (2018a) found a pronounced inhibitory effect on *Salmonella* Oranienburg in 1:10 diluted enrichments of oregano and cinnamon. Also enrichment of *Staphylococcus aureus* (according to DIN EN ISO 6888-3) was found to be impaired by allspice, cinnamon, vanilla, oregano, and thyme (Cabicarová et al., 2016). In contrast, *Staphylococcus aureus* colony counts (DIN EN ISO 6888-1), were not affected within 45 minutes in 1:20 dilutions of paprika,

pepper and oregano (Dinh Thanh et al., 2017). Potential effects on *Bacillus cereus* and *Bacillus thuringiensis* were investigated by Frentzel (2017). A reduction of viable cells was observed in 1:20 dilutions of cinnamon, nutmeg, oregano, pepper, and, although less pronounced, also basil within 45 minutes. In contrast, spore concentrations remained almost unchanged in these dilutions even after incubation at room temperature for 24 h, indicating no inactivation but rather an inhibition of germination and growth. As a consequence, cultural methods applied to condiment matrices may underestimate pathogen concentrations or lead to false negative results.

A way of facing this problem is optimisation of the culturing method e.g. by using higher dilutions, washing of samples, or addition of substances that reduce antimicrobial effects. Another option is the application of non-cultural methods such as polymerase chain reaction (PCR). However, also PCR-based methods can be biased by inhibiting substances from condiment matrices (Focke et al., 2011) and might require some modifications as well.

Aiming at advancement of pathogen detection in spices and dried herbs, Lins (2018b) achieved substantial improvement in the cultural detection of *Salmonella* spp. in cinnamon and oregano based on a modification of ISO 6579. Using higher dilutions (1:20) and addition of 0.5 % (w/v) K_2SO_3 in pre-enrichments, the limit of detection could be lowered when combined with cultural detection, but also in combination with nucleic acid detection based on loop-mediated isothermal amplification (3M™ molecular detection assay *Salmonella*). The effect of washing on the quantitative and qualitative detection of *Staphylococcus aureus* (ISO 6888-1 and 6888-3) in ten different spice and herb matrices was investigated by Cabicarová et al. (2016). Washing of samples had no beneficial effects on the quantitative method (ISO 6888-1), whereas it greatly improved the sensitivity of the qualitative method (ISO 6888-3). This improvement was shown for enrichment-based detection of *Staphylococcus aureus* coupled to plating on Baird-Parker agar, but also coupled to real-time PCR, while the latter was even more sensitive. However, especially in oregano samples, the limit of detection remained high. Therefore, a culture-independent detection method for several food-borne pathogens was investigated in a subsequent study by Minarovičová et al. (2018). By directly extracting DNA from artificially contaminated spices and dried herbs using cetyltrimethylammonium bromide (CTAB), *Staphylococcus aureus*, *Salmonella* enterica, and *Escherichia coli* could be quantified by real-time PCR at detection limits of 10^2 to 10^4 cfu/g. Similarly, Frentzel and co-workers successfully applied CTAB-based DNA extraction combined with real-time PCR to detect and quantify spores of *Bacillus cereus* group species in paprika, pepper, and oregano; detection in allspice was, however, still problematic (Frentzel et al., 2018b).

To summarize, the detection of microbiological agents in spices and herbs is strongly dependent on matrix characteristics. Regarding several problematic matrix–agent combinations, established detection methods require modifications to generate valid results.

Conclusions

Although potential risks to consumers' health associated with microbiological agents in spices and culinary herbs are low compared to foods of animal origin, they should not be underestimated. Toxigenic and pathogenic microorganisms, i.e. *Bacillus cereus* and *Salmonella* spp., were shown to be able to survive in spices and dried herbs for many months, and several outbreaks had been associated with spices contaminated with such agents or with *Clostridium perfringens*. Detection of potentially harmful microorganisms in spice/herb matrices can be a challenge, but has already been successfully optimised for several matrix–agent combinations. In general, many efforts have been taken by different actors, including governmental, industry, and academic bodies, to better protect the global spice and dried culi-

nary herb chains from contaminations and to promote food safety. Such activities also include (international) standards on hygienic practices and product safety.

Acknowledgements

The research was executed in the framework of the EU project SPICED (Grant Agreement: 312631; www.spiced.eu/) with the financial support from the 7th Framework Programme of the EU.

Abbreviations

CAC	Codex Alimentarius Commission
cfu/g	number of colony forming units per gramme
EU	European Union
ESA	European Spice Association
PCR	polymerase chain reaction
RASFF	Rapid Alert System for Food and Feed
w/v	weight per volume

References

Banach, J. L., Stratakou, I., van der Fels-Klerx, H. J., Besten, H. M. W. d., and Zwietering, M. H. (2016). European alerting and monitoring data as inputs for the risk assessment of microbiological and chemical hazards in spices and herbs. *Food Control, 69*, 237-249. doi:10.1016/j.foodcont.2016.04.010

Beuchat, L. R., Komitopoulou, E., Beckers, H., Betts, R. P., Bourdichon, F., Fanning, S., . . . and ter Kuile, B. H. (2013). Low-water activity foods: Increased concern as vehicles of foodborne pathogens. *Journal of Food Protection, 76*(1), 150-172. doi:10.4315/0362-028x.Jfp-12-211

Cabicarová, T., Kaclíková, E., Mader, A., Minarovičová, J., Koreňová, J., and Kuchta, T. (2016). Improvement of the detection sensitivity for *Staphylococcus aureus* in spices and herbs. *Food Analytical Methods, 9*(7), 1980-1984. doi:10.1007/s12161-015-0379-0

CAC, Codex Alimentarius Commission. (1991). Guide for the microbiological quality of spices and herbs used in processed meat and poultry products (CAC/GL 14-1991). www.codexalimentarius.org/download/standards/165/CXG_014e.pdf

CAC, Codex Alimentarius Commission. (1995b). Code of hygienic practice for spices and dried aromatic plants (CAC/RCP 42-1995). http://www.greenfoodec.eu/documents/codeofhygienicpracticeforspicesanddriedaromaticplants.pdf

CAC, Codex Alimentarius Commission. (2003b). General principles of food hygiene (CAC/RCP 1-1969, Revision 2003). www.codexalimentarius.org/download/standards/23/CXP_001e.pdf

CAC, Codex Alimentarius Commission. (2018). Code of hygienic practice for low-moisture foods CAC/RCP 75-2015 (Adopted in 2015. Revision 2016. Amended in 2018). http://www.fao.org/fao-who-codexalimentarius/sh-proxy/pt/?lnk=1&url=https%253A%252F%252Fworkspace.fao.org%252Fsites%252Fcodex%252FStandards%252FCXC%2B75-2015%252FCXC_075e.pdf

Campbell, J. V., Mohle-Boetani, J., Reporter, R., Abbott, S., Farrar, J., Brandl, M., . . . Werner, S. B. (2001). An outbreak of *Salmonella* serotype Thompson associated with fresh cilantro. *Journal of Infectious Diseases, 183*(6), 984-987. doi:10.1086/319254

CBI, Centre for the Promotion of Imports from developing countries. (2017b). Which trends offer opportunities on the European spices and herbs market? https://www.cbi.eu/node/1970/pdf/

Da Silva Felício, M. T., Hald, T., Liebana, E., Allende, A., Hugas, M., Nguyen-The, C., . . . and McLauchlin, J. (2015). Risk ranking of pathogens in ready-to-eat unprocessed foods of non-animal origin (FoNAO) in the EU: Initial evaluation using outbreak data (2007–2011). *International Journal of Food Microbiology, 195*, 9-19. doi:10.1016/j.ijfoodmicro.2014.11.005

Dinh Thanh, M., Frentzel, H., Fetsch, A., Appel, B., and Mader, A. (2017). Impact of spiking techniques on the survival of *Staphylococcus aureus* in artificially contaminated condiments. *Food Control, 73, Part A*, 117-126. doi:10.1016/j.foodcont.2016.10.021

Dinh Thanh, M., Frentzel, H., Fetsch, A., Krause, G., Appel, B., and Mader, A. (2018). Tenacity of *Bacillus cereus* and *Staphylococcus aureus* in dried spices and herbs. *Food Control, 83*, 75-84. doi:10.1016/j.foodcont.2016.12.027

Duncan, S. E., Moberg, K., Amin, K. N., Wright, M., Newkirk, J. J., Ponder, M. A., . . . and Dickson, J. S. (2017). Processes to preserve spice and herb quality and sensory integrity during pathogen inactivation. *Journal of Food Science, 82*(5), 1208-1215. doi:10.1111/1750-3841.13702

EC, European Commission. (2005a). Commission Regulation (EC) No 2073/2005 of 15 November 2005 on microbiological criteria for foodstuffs. *Official Journal of the European Communities, L 338*, 1-26.

EFSA, European Food Safety Authority. (2009b). Community summary report: Food-borne outbreaks in the European Union in 2007. *EFSA Journal, 271*(5), 1-129. doi:10.2903/j.efsa.2009.271r

EFSA, European Food Safety Authority. (2011b). The European Union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2009. *EFSA Journal, 9*(3), 1-378. doi:10.2903/j.efsa.2011.2090

EFSA, European Food Safety Authority. (2012c). Zoonoses monitoring. Hungary—Trends and sources of zoonoses and zoonotic agents in humans, foodstuffs, animals and feedingstuffs in 2012. http://www.efsa.europa.eu/sites/default/files/assets/zoocountryreport12hu.pdf

EFSA BIOHAZ, European Food Safety Authority's Panel on Biological Hazards. (2013). Scientific Opinion on the risk posed by pathogens in food of non-animal origin. Part 1 (outbreak data analysis and risk ranking of food/pathogen combinations). *EFSA Journal, 11*(1), 3025. doi:10.2903/j.efsa.2013.3025

EFSA BIOHAZ, European Food Safety Authority's Panel on Biological Hazards. (2016). Scientific opinion on the risks for public health related to the presence of *Bacillus cereus* and other *Bacillus* spp. including *Bacillus thuringiensis* in foodstuffs. *EFSA Journal, 14*(7), 93. doi:10.2903/j.efsa.2016.4524

ESA, European Spice Association,. (2017). ESA list of culinary herbs and spices. https://www.esa-spices.org/download/esa-list-of-culinary-herbs-and-spices.pdf

Eur. Parliament, and The Council. (2004). Regulation (EC) No 852/2004 of the European Parliament and the Council of 29 April 2004 on the hygiene of foodstuffs. *Official Journal of the European Communities, L 139*, 1-54.

Fachverband der Gewürzindustrie, Association of the German Spice Industry,. (2018). Proper storage and handling of herbs and spices. https://www.gewuerzindustrie.de/en-index-gewuerzindustrie.html/en-verbaucherinformationen-gewuerze-kraeuter/en-verbaucherinformationen-gewuerze-haltbarkeit-und-aufbewahrung-von-gewuerzen-und-kraeutern

FAO, Food and Agriculture Organization of the United Nations. (2011) Spices and herbs for home and market. In*. Diversification booklet number 20*. http://www.fao.org/docrep/015/i2476e/i2476e00.pdf.

FAO and WHO, Food and Agriculture Organization of the United Nations and World Health Organization. (2014) Ranking of low moisture foods in support of microbiological risk management. In*. Preliminary report of FAO/WHO expert consultation on ranking of low moisture foods* (pp. 1-254). http://www.fao.org/tempref/codex/Meetings/CCFH/CCFH46/LMF%20Part%201-3%2030Oct%202014.pdf.

FDA, U.S. Food and Drug Administration. (2013a). Draft risk profile: Pathogens and filth in spices. https://www.fda.gov/downloads/food/foodscienceresearch/risksafetyassessment/ucm367337.pdf

Finn, S., Condell, O., McClure, P., Amezquita, A., and Fanning, S. (2013). Mechanisms of survival, responses, and sources of *Salmonella* in low-moisture environments. *Frontiers in Microbiology, 4*, Article 331. doi:10.3389/fmicb.2013.00331

Focke, F., Haase, I., and Fischer, M. (2011). DNA-based identification of spices: DNA isolation, whole genome amplification, and polymerase chain reaction. *Journal of Agricultural and Food Chemistry, 59*(2), 513-520. doi:10.1021/jf103702s

Frentzel, H. (2017). *Detection, characterization and survival of Bacillus cereus group members in spices and herbs.* (Doctoral Thesis). Freie Universität Berlin

Frentzel, H., Kraushaar, B., Krause, G., Bodi, D., Wichmann-Schauer, H., Appel, B., and Mader, A. (2018a). Phylogenetic and toxinogenic characteristics of *Bacillus cereus* group members isolated from spices and herbs. *Food Control, 83*, 90-98. doi:10.1016/j.foodcont.2016.12.022

Frentzel, H., Thanh, M. D., Krause, G., Appel, B., and Mader, A. (2018b). Quantification and differentiation of *Bacillus cereus* group species in spices and herbs by real-time PCR. *Food Control, 83*, 99-108. doi:10.1016/j.foodcont.2016.11.028

Gustavsen, S., and Breen, O. (1984). Investigation of an outbreak of *Salmonella* Oranienburg infections in Norway, caused by contaminated black pepper. *American Journal of Epidemiology, 119*(5), 806-812. doi:10.1093/oxfordjournals.aje.a113801

Hariram, U., and Labbé, R. (2015). Spore prevalence and toxigenicity of *Bacillus cereus* and *Bacillus thuringiensis* isolates from US retail spices. *Journal of Food Protection, 78*(3), 590-596. doi:10.4315/0362-028x.jfp-14-380

Horn, B., Esslinger, S., Pfister, M., Fauhl-Hassek, C., and Riedl, J. (2018). Non-targeted detection of paprika adulteration using mid-infrared spectroscopy and one-class classification – Is it data preprocessing that makes the performance? *Food Chemistry, 257*, 112-119. doi:10.1016/j.foodchem.2018.03.007

Jernberg, C., Hjertqvist, M., Sundborger, C., Castro, E., Lofdahl, M., Paajarvi, A., . . . and Lof, E. (2015). Outbreak of *Salmonella* Enteritidis phage type 13a infection in Sweden linked to imported dried-vegetable spice mixes, December 2014 to July 2015. *Eurosurveillance, 20*(30), 5-9. doi:10.2807/1560-7917.es2015.20.30.21194

Lehmacher, A., Bockemühl, J., and Aleksic, S. (1995). Nationwide outbreak of human salmonellosis in Germany due to contaminated paprika and paprika-powdered potato chips. *Epidemiology and Infection, 115*(3), 501-511. doi:10.1017/S0950268800058660

Lins, P. (2018a). Antimicrobial activities of spices and herbs against *Salmonella* Oranienburg. *Food Control, 83*, 123-130. doi:10.1016/j.foodcont.2017.05.041

Lins, P. (2018b). Detection of *Salmonella* spp. in spices and herbs. *Food Control, 83*, 61-68. doi:10.1016/j.foodcont.2017.05.040

Little, C. L., Omotoye, R., and Mitchell, R. T. (2003). The microbiological quality of ready-to-eat foods with added spices. *International Journal of Environmental Health Research, 13*(1), 31-42. doi:10.1080/0960312021000063331

Mader, A., and Schaarschmidt, S. (2015). Bakteriologische Sicherheit von Gewürzen – Mikrobiologische Kontaminationen können zu lebensmittelbedingten Krankheitsausbrüchen führen. *Fleischwirtschaft, 95*(11), 42-47.

McKee, L. H. (1995). Microbial contamination of spices and herbs: A review. *LWT - Food Science and Technology, 28*(1), 1-11. doi:10.1016/S0023-6438(95)80004-2

Minarovičová, J., Cabicarová, T., Kaclíková, E., Mader, A., Lopašovská, J., Siekel, P., and Kuchta, T. (2018). Culture-independent quantification of pathogenic bacteria in spices and herbs using real-time polymerase chain reaction. *Food Control, 83*, 85-89. doi:10.1016/j.foodcont.2016.12.025

Pakalniskiene, J., Falkenhorst, G., Lisby, M., Madsen, S. B., Olsen, K. E. P., Nielsen, E. M., . . . and Molbak, K. (2009). A foodborne outbreak of enterotoxigenic *E. coli* and *Salmonella* Anatum infection after a high-school dinner in Denmark, November 2006. *Epidemiology and Infection, 137*(3), 396-401. doi:10.1017/S0950268808000484

Pezzoli, L., Elson, R., Little, C. L., Yip, H., Fisher, I., Yishai, R., . . . and Threlfall, J. (2008). Packed with *Salmonella*-investigation of an international outbreak of *Salmonella* Senftenberg infection linked to contamination of prepacked basil in 2007. *Foodborne Pathogens and Disease, 5*(5), 661-668. doi:10.1089/fpd.2008.0103

Podolak, R., Enache, E., Stone, W., Black, D. G., and Elliott, P. H. (2010). Sources and risk factors for contamination, survival, persistence, and heat resistance of *Salmonella* in low-moisture foods. *Journal of Food Protection, 73*(10), 1919-1936. doi:10.4315/0362-028X-73.10.1919

Raevuori, M., Kiutamo, T., Niskanen, A., and Salminen, K. (1976). An outbreak of *Bacillus cereus* food-poisoning in Finland associated with boiled rice. *Journal of Hygiene, 76*(3), 319-327.

Reinholds, I., Bartkevics, V., Silvis, I. C. J., van Ruth, S. M., and Esslinger, S. (2015). Analytical techniques combined with chemometrics for authentication and determination of contaminants in condiments: A review. *Journal of Food Composition and Analysis, 44*, 56-72. doi:10.1016/j.jfca.2015.05.004

Reinholds, I., Pugajeva, I., and Bartkevics, V. (2016). A reliable screening of mycotoxins and pesticide residues in paprika using ultra-high performance liquid chromatography coupled to high resolution Orbitrap mass spectrometry. *Food Control, 60*, 683-689. doi:10.1016/j.foodcont.2015.09.008

Reinholds, I., Pugajeva, I., Bavrins, K., Kuckovska, G., and Bartkevics, V. (2017). Mycotoxins, pesticides and toxic metals in commercial spices and herbs. *Food Additives & Contaminants: Part B, 10*(1), 5-14. doi:10.1080/19393210.2016.1210244

Schaarschmidt, S. (2016a). Public and private standards for dried culinary herbs and spices—Part I: Standards defining the physical and chemical product quality and safety. *Food Control, 70*, 339-349. doi:10.1016/j.foodcont.2016.06.004

Schaarschmidt, S., Spradau, F., Mank, H., Banach, J. L., van der Fels-Klerx, H. J., Hiller, P., . . . and Mader, A. (2016b). Public and private standards for dried culinary herbs and spices—Part II: Production and product standards for ensuring the microbiological safety. *Food Control, 70*, 360-370. doi:10.1016/j.foodcont.2016.06.003

Schaarschmidt, S., Spradau, F., Mank, H., Hiller, P., Appel, B., Bräunig, J., . . . and Mader, A. (2018). Reporting of traceability and food safety data in the culinary herb and spice chains. *Food Control, 83*, 18-27. doi:10.1016/j.foodcont.2016.11.029

Székács, A., Wilkinson, M. G., Mader, A., and Appel, B. (2018). Environmental and food safety of spices and herbs along global food chains. *Food Control, 83*, 1-6. doi:10.1016/j.foodcont.2017.06.033

Van Doren, J. M., Neil, K. P., Parish, M., Gieraltowski, L., Gould, L. H., and Gombas, K. L. (2013). Foodborne illness outbreaks from microbial contaminants in spices, 1973-2010. *Food Microbiology, 36*(2), 456-464. doi:10.1016/j.fm.2013.04.014

Werber, D., Dreesman, J., Feil, F., van Treeck, U., Fell, G., Ethelberg, S., . . . and Ammon, A. (2005). International outbreak of *Salmonella* Oranienburg due to German chocolate. *BMC Infectious Diseases, 5*, 7. doi:10.1186/1471-2334-5-7

5.4 Emergence, distribution and genetic diversity of mcr-1 harboring *Escherichia coli* from livestock and food in Germany

Hammerl, Jens A.,[1]; Irrgang, Alexandra[1]; Shamoun, Dina[1]; Grobbel, Mirjam[1]; Tenhagen, Bernd-Alois[1]; Schmoger, Silvia[1]; Skladnikiewicz-Ziemer, Tanja[1]; Juraschek, Katharina[1]; and Käsbohrer, Annemarie[1,2]*

[1] German Federal Institute for Risk Assessment, Department for Biological Safety, Berlin, Germany

[2] Institute for Veterinary Public Health, University of Veterinary Medicine, Vienna, Austria

Abstract

In 2015, the first plasmid-mediated mobile colistin resistance gene (*mcr-1*) was detected in livestock, food and human beings of China. Thereafter, several studies indicated remarkably high *mcr-1* prevalence for a few countries as well as a global distribution of this gene in different genera of the *Enterobacteriaceae* recovered from the environment, food, livestock, infected patients as well as asymptomatic human carriers. As antibiotic of the last resort, further dissemination of plasmid-mediated mobile colistin resistances might hamper future treatment of severe human infections with multidrug-resistant Gram-negative bacteria.

In this study, we provide an overview on the prevalence, distribution and genetic composition of *mcr-1* carrying *E. coli* isolates from German livestock and food samples recovered between 2010 and 2017. Therefore, commensal *E. coli* isolated within the framework of the German national zoonoses monitoring were further investigated. Out of ~15,000 routinely investigated *E. coli*, more than 700 isolates from the poultry, pig and cattle food chains exhibited a minimum inhibitory concentration (MIC) for colistin of ≥4 mg/l. In ~80% of these isolates the *mcr-1* gene was identified by PCR-based methods. For further typing, whole genome sequencing on twenty-four selected isolates of different sources and periods was performed. Bioinformatic analysis revealed that a broad spectrum of commensal *E. coli* represented by different MLST-, phylo-, and serotypes harbor *mcr-1*. All isolates carried a variety of antimicrobial resistance genes and/or mobile genetic elements (i.e. plasmids). Interestingly, *mcr-1* was detected on at least three different plasmid prototypes, which could not be attributed to a specific period, source, or specific genotype (i.e. MLST).

Current data show that *mcr-1* harboring plasmids are widely spread in Germany, as in several other countries. The spread of *mcr-1* harboring plasmids is probably not associated with clonal spread of specific MLST-, phylo- or serotypes but presumably driven by the susceptibility of the strains for transmissible plasmids and antimicrobial selection pressure.

Background

Control of antimicrobial resistance will be one of humanity's main challenges in the future, as resistance development is a multifaceted phenomenon that affects all societies worldwide (Prestinaci et al., 2015). Nowadays, dissemination of rapidly emerging resistant bacteria is supported by globalization, but mainly reflecting the intensive use of antimicrobials in animals and humans and triggering of resistance development and evolution. The lack of novel antibiotic developments or alternative treatment strategies requires consistent global efforts to minimize the emergence and spread of resistance to the currently available drugs (Al-Tawfiq et al., 2017; Roca et al., 2015).

To preserve the effectiveness of the most critical antimicrobials for human medicine in the future, the World Health Organization (WHO, www.who.int) publish regularly an updated guideline with a strict categorization of antimicrobials currently used in human and veterinary medicine. The WHO recommendations are aimed to force the prudent use of antimicrobial in animals and agriculture for lowering antimicrobial resistance development in the future. In the WHO guideline, antimicrobial substances were assigned to three categories: (A) important, (B) highly important and (C) critically important (divided into the subgroups (C2) high priority and (C1) highest priority critically important antibiotics), based on their impact in the human medicine. Nowadays (5th revision, April 2017), quinolones, 3rd and 4th generation cephalosporins, macrolides and ketolides, glycopeptides and polymyxins are considered as highest priority critically important antimicrobials (C1), because they usually represent the last choice for treatment of serious infections with multidrug-resistant bacteria in human medicine (WHO, 2017).

Recently, polymyxins have become increasingly important. For a long time colistin resistance was supposed to be associated only with variations in products of housekeeping genes involved in the development of the lipopolysaccharide structure in Gram-negative bacteria. In 2015, the first plasmid-mediated mobile colistin resistance gene (*mcr-1*), encoding a phosphoethanolamine transferase, was discovered in livestock, food and humans from China (Liu et al., 2016). Since that, various genera of the *Enterobacteriaceae* were investigated for the presence of *mcr-1* (Sun et al., 2017; Sun et al., 2018) and heterogeneous *mcr-1* detection rates of up to 70% were found. High rates of *mcr-1* carrying isolates were primarily detected in *Escherichia coli* (*E. coli*) and *Salmonella enterica* (*S. enterica*) from livestock, whereas human isolates carried this gene less frequently (Sun et al., 2017). This observation indicates that livestock represents a common reservoir for *mcr-1* carrying isolates and that transmission to humans by direct contact to colonized animals or by ingestion of contaminated foodproducts might take place. In human medicine, the use of colistin is limited to very specific cases of serious infections with multidrug-resistant Gram-negative bacteria on account of a high risk of side effects. However, in veterinary medicine this antimicrobial substance has been extensively used for a long time for disease prevention and treatment, worldwide. Some countries (i.e. China, USA) also used colistin as growth promoter in livestock farming (Sun et al., 2018). This intensive usage may be the main cause for the high prevalence of colistin-resistant isolates among Gram-negative enterobacteria in livestock in these countries. In general, country-specific prevalence rates seem to be strongly associated with the annual colistin sales and usage. To reduce the development and dissemination of colistin resistance, the European Medicines Agency defined a target for reducing the use of colistin in animals by 65% in the next years (EMA, 2016). Future monitoring programs will show if this recommendation will lead to a general decrease of the prevalence of colistin resistance.

The identification of a plasmid-mediated colistin resistance (*mcr-1*) in *Enterobacteriaceae* raised the question about the genetic diversity of plasmid-mediated mobile colistin resistances. Briefly, after *mcr-1* detection, two other *mcr*-genes were successively described. Both genes differ significantly in their amino acid sequence from *mcr-1*, but also encode proteins of the phosphoethanolamine transferase family (Sun et al., 2018). While *mcr-2* was found in pigs and calves from Belgium (Xavier et al., 2016), *mcr-3* was detected in an *E. coli* from a human bloodstream infection of a returning Thailand traveller (Yin et al., 2017). Later on, *mcr-3* genes were also detected in *Salmonella* isolates from Danish patients. Interestingly, some of them could be also associated with a travel history to the Southeast Asia (Litrup et al., 2017). Both genes, *mcr-2* and *mcr-3*, were located on self-transmissible (IncI2, IncX4, IncHI1B, IncHI2A, IncFII and IncFIB) and mobilizable plasmids (ColE), respectively (Sun et al., 2018). Another hallmark in *mcr*-research was the identification of two further phosphoethanolamine transferase genes in *S. enterica* in 2017. Carattoli et al. and Borowiak et al. described the genes *mcr-4* and *mcr-5* in *S. enterica* serovar Typhimurium (Carattoli et al., 2017) and d-tartrate fermenting *S. enterica* serovar Paratyphi B [S. Paratyphi B (dTa+)] (Borowiak et al., 2017), respectively. Similar to *mcr-3*, *mcr-4* and *mcr-5* were also mainly lo-

cated on non-self-transmissible ColE-plasmids, which are either transmissible by helper plasmids and/or mobilizable by a Tn3-type transposon (Borowiak et al., 2017; Carattoli et al., 2017; Hammerl et al., 2018). Based on its genetic difference, a previously reported *mcr*-gene from *Moraxella* was re-classified as *mcr-6* (AbuOun et al., 2018). In 2018, two novel *mcr*-genes were reported in *Klebsiella pneumoniae* (*mcr-7*, *mcr-8*) (Yang et al., 2018). So far, eight different *mcr*-genes, *mcr-1* (Accession no. KP347127) (Liu et al., 2016), *mcr-2* (LT598652) (Xavier et al., 2016), *mcr-3* (KY924928) (Yin et al., 2017), *mcr-4* (MF543359) (Carattoli et al., 2017), *mcr-5* (KY807921) (Borowiak et al., 2017), *mcr-6* (MF176240) (AbuOun et al., 2018), *mcr-7* (MG267386) (Yang et al., 2018) and *mcr-8* (MG736312) (unpublished), coding for proteins of the phosphoethanolamine transferase family have been described to be associated with a variable tolerance of the respective isolate against colistin. Furthermore, sequence databases carry information for several gene variants exhibiting single or a few amino acid deviations. Initial studies on their distribution revealed that all latter described *mcr*-genes were less frequently found than *mcr-1*. Thus, actually *mcr-1* has the highest impact for colistin resistance development worldwide.

To assess the impact of *mcr-1* carrying isolates on public health, the German National Reference Laboratory for Antimicrobial Resistance (NRL-AR) extended its phenotypic monitoring on the occurrence of antimicrobial resistance to a detailed characterization of colistin-resistant *E. coli* isolates from food and livestock. The focus of the present study was the dissection of the genetic basis of *mcr-1* carrying *E. coli* from different sources and time periods.

The mobile colistin resistance gene *mcr-1* is widely disseminated among commensal *E. coli* from German livestock and food samples

The occurrence of *mcr*-genes among commensal *E. coli* isolates from German livestock and food samples recovered within different annual zoonoses monitoring programs between 2010 and 2017 was investigated. Molecular screening was conducted using a conventional multiplex PCR, which was provided by the European Reference Laboratory for Antimicrobial Resistance (EURL-AR) for detection of the plasmid-mediated colistin resistance genes *mcr-1* to *mcr-5*, and their variants (Rebelo et al., 2018). Antimicrobial resistance of more than 15,000 *E. coli* isolates was characterized by broth microdilution according to the CLSI guidelines (M07-A10) between 2010 and 2017. By using EUCASTs (European Committee on Antimicrobial Susceptibility Testing, www.eucast.org) epidemiological cut-offs, more than 700 isolates from various food and animal origins (i.e. poultry, pigs and cattle) exhibited a minimum inhibitory concentration (MIC) for colistin of ≥4 mg/l. Molecular determination of the *mcr*-gene prevalence revealed that the majority of these isolates (>80%) harbored the *mcr-1* gene. Additionally, we sporadically identified some *mcr-4* (~2%) and *mcr-5* (<1%) carrying isolates. Isolates carrying the genes *mcr-2* and *mcr-3* were not detected indicating that up to now these determinants may not be present in colistin-resistant *E. coli* of German livestock. However, as the presence of *mcr-2* and *mcr-3* carrying plasmids was often reported to exhibit only a very slightly increase of the colistin tolerance, the pre-selection of isolates for detailed investigation based on EUCAST epidemiological cut-offs may exclude the *mcr-2*/*mcr-3* carrying isolates. Therefore, we further investigated *E. coli* isolates presenting MICs of 2 mg/l (n=200) and <2 mg/l (n=200). However, we only identified few additional *mcr-1* carrying isolates exhibiting a MIC of 2 mg/l. None of these isolates harbored *mcr-2* or *mcr-3*. Furthermore, in none of the isolates with a MIC of <2 mg/l any *mcr*-determinant could be detected. Our molecular screening indicated that *mcr-1* is by far the most prevalent *mcr*-gene determinant among colistin-resistant *E. coli* isolates from livestock and food in Germany.

Our results showed that *mcr-1* carrying *E. coli* from German livestock and food products are prevalent in different matrices and time periods. Interestingly, we also found many *mcr-1* carrying *E. coli* among isolates before 2015, confirming that this determinant is not a novel phenomenon but has previously not been detected. The detection of *mcr-1* in *E. coli* within

our culture collection dates back to 2010, five years earlier than the first discovery of this gene. This underlines the importance of storing isolates for retrospective analyses and the benefits of extending monitoring to molecular methods for a full recognition of the situation. It would be interesting to analyse historical isolates to date back the origin and the emergence of *mcr-1* in German *E. coli* populations.

mcr-1 carrying *E. coli* are often associated with multiple antimicrobial resistances

To answer the question on the heterogeneity of *mcr-1* carrying *E. coli*, twenty-four isolates from different sources (A, turkey; B, chicken; C, pig) isolated between 2012 and 2015 were selected for detailed characterization of their phenotypic and genotypic properties.

Table 22: Antimicrobial resistance of *mcr-1* carrying *E. coli* isolates used in this study

Isolate	Description	Resistance profile*
2012		
12-AB00025	Turkey, caecum	AMP, CHL, CIP, COL, NAL, SMX, TET, TMP
12-AB00501	Turkey, meat	AMP, CHL, CIP, COL, NAL, SMX, TET, TMP
12-AB00876	Turkey, meat	AMP, CIP, COL, NAL, TET
12-AB01842	Turkey, faeces	AMP, CHL, CIP, COL, GEN, NAL, SMX, TET, TMP
12-AB01861	Turkey, caecum	AMP, CHL, CIP, COL, NAL, SMX, TET, TMP
12-AB02079	Turkey, faeces	AMP, CHL, CIP, COL, NAL, SMX, TET, TMP
2013		
13-AB00012	Chicken, faeces	COL
13-AB00742	Chicken, meat	AMP, COL, FOT, TAZ
13-AB00885	Chicken, caecum	AMP, CHL, CIP, COL, GEN, NAL, SMX, TET, TMP
13-AB01479	Chicken, caecum	AMP, CHL, CIP, COL, GEN, NAL, SMX, TET, TMP
13-AB01693	Chicken, meat	AMP, COL, FOT, SMX, TAZ
2014		
14-AB00001	Chicken, faeces	AMP, CHL, CIP, COL, NAL, SMX, TET, TMP
14-AB00714	Turkey, faeces	AMP, CHL, CIP, COL, NAL, SMX, TET, TMP
14-AB00941	Turkey, caecum	AMP, CHL, CIP, COL, GEN, SMX, TET, TMP
14-AB01030	Turkey, faeces	AMP, CHL, CIP, COL, SMX, TMP
14-AB01041	Turkey, caecum	AMP, CIP, COL, GEN, NAL, SMX, TET, TMP
14-AB01081	Turkey, meat	AMP, CIP, COL, GEN, NAL, TET
14-AB01513	Turkey, meat	AMP, CIP, COL, NAL, SMX, TET, TMP
2015		
15-AB00959	Pig, faeces	AMP, CHL, COL, SMX, TET
15-AB01098	Pig, faeces	AMP, CIP, COL, GEN, NAL, SMX, TET, TMP
15-AB01173	Pig, faeces	CHL, COL, SMX, TET, TMP
15-AB01276	Pig, faeces	AMP, CIP, COL, GEN, NAL, SMX, TET, TMP
15-AB01775	Pig, faeces	AMP, COL, SMX, TET, TMP
15-AB02086	Pig, faeces	AMP, CHL, CIP, COL, NAL, SMX, TET, TMP

* Abbreviations: AMP, ampicillin; CHL, chloramphenicol CIP, ciprofloxacin; COL, colistin; FOT, cefotaxime; NAL, nalidixic acid, SMX, sulfamethoxazole; TAZ, ceftazidime; TET, tetracycline; TMP, trimethoprim; GEN, gentamicin

Comparisons of the antimicrobial resistance profiles of the *mcr-1* carrying *E. coli* showed that almost all isolates exhibited multiple resistances against various classes of antimicrobial substances (Table 22). Beside colistin (COL) the majority of the isolates exhibited also non-wildtype phenotypes for ampicillin (AMP), sulfamethoxazole (SMX) and/or tetracycline (TET).

Interestingly, we also identified two isolates (13-AB00742 and 13-AB01693, Table 22) that both were recovered from chicken meat in 2013, exhibiting MICs above the ECOFFs for cefotaxime (FOT) and ceftazidime (TAZ). Subsequent testing of further beta-lactam antibiot-

ics and carbapenems confirmed Extended-Spectrum Beta-Lactamase (ESBL) production of the isolates.

Based on our data, resistance to colistin is often associated with other important antimicrobial resistances in *E. coli*. The detection of colistin resistance among ESBL isolates is of major concern, as 3rd generation cephalosporins are likewise highest priority critically important antimicrobials and resistance to cephalosporins may be an indication for the use of colistin in medicine.

The *mcr-1* gene is located on a few conserved plasmid prototypes among highly divers *E. coli* isolates

To get an overview on the genetic diversity of *mcr-1* harboring *E. coli*, selected isolates (Table 22) were further characterized using molecular methods. Initial XbaI-PFGE profiling according to the PulseNet protocol (https://www.cdc.gov/pulsenet/pathogens/protocols.html) revealed a high heterogeneity of the isolates. None of them exhibited identical PFGE patterns (Figure 38). Thus, we can exclude that the *mcr-1* carrying isolates included in our analysis reflect the dissemination of a predominant *E. coli* clone via vertical gene transfer.

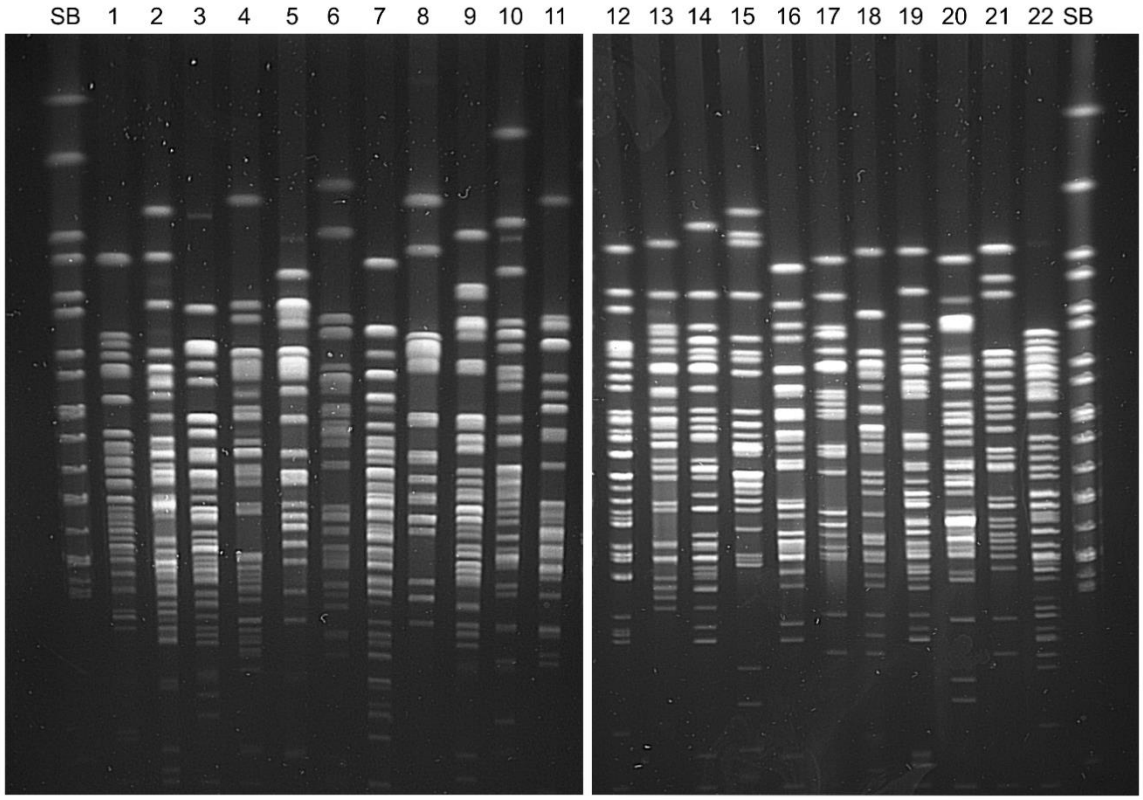


Figure 38: Phylogenetic relationship of *mcr-1* carrying *E. coli* isolates
XbaI-PFGE profiles E. coli isolates used in this study: lane 1: 14-AB00714, lane 2: 14-AB00941, lane 3: 14-AB01030, lane 4: 14-AB01041, lane 5: 14-AB01081, lane 6: 14-AB01513, lane 7: 15-AB00959, lane 8: 15-AB01098, lane 9: 15-AB01173, lane 10: 15-AB01276, lane 11: 15-AB01775, lane 12: 12-AB00501, lane 13: 12-AB00876, lane 14: 12-AB01842, lane 15: 12-AB01861, lane 16: 12-AB02079, lane 17: 13-AB00012, lane 18: 13-AB00742, lane 19: 13-AB00885, lane 20: 13-AB01479, lane 21: 13-AB01693, and lane 22: 14-AB00001. SB: Salmonella Braenderup H9812 XbaI-restriction profile.

As *mcr-1* is with some exceptions a plasmid-mediated mobile colistin resistance gene, further investigations on the genetic basis of this determinant were conducted by S1-PFGE plasmid

profiling (conditions 1-25 s; 17 h, 120°, 6 V/cm) (Borowiak et al., 2017). S1-PFGE revealed that our selection of isolates harbored multiple extrachromosomal elements ranging between 20 and 200 kb (Figure 39). Southern blotting and subsequent DNA-DNA hybridization (Roche Applied Science, Mannheim, Germany) using a digoxigenin-11-dUTP-labelled *mcr-1* PCR probe (PCR DIG Labelling Mix, Roche Applied Science) showed that the coding sequence of the *mcr-1* gene was detected on plasmid bands of different sizes, ranging between 30 and 100 kb. According to their plasmid sizes, three potential plasmid prototypes (A, B, C) were defined (Figure 39).

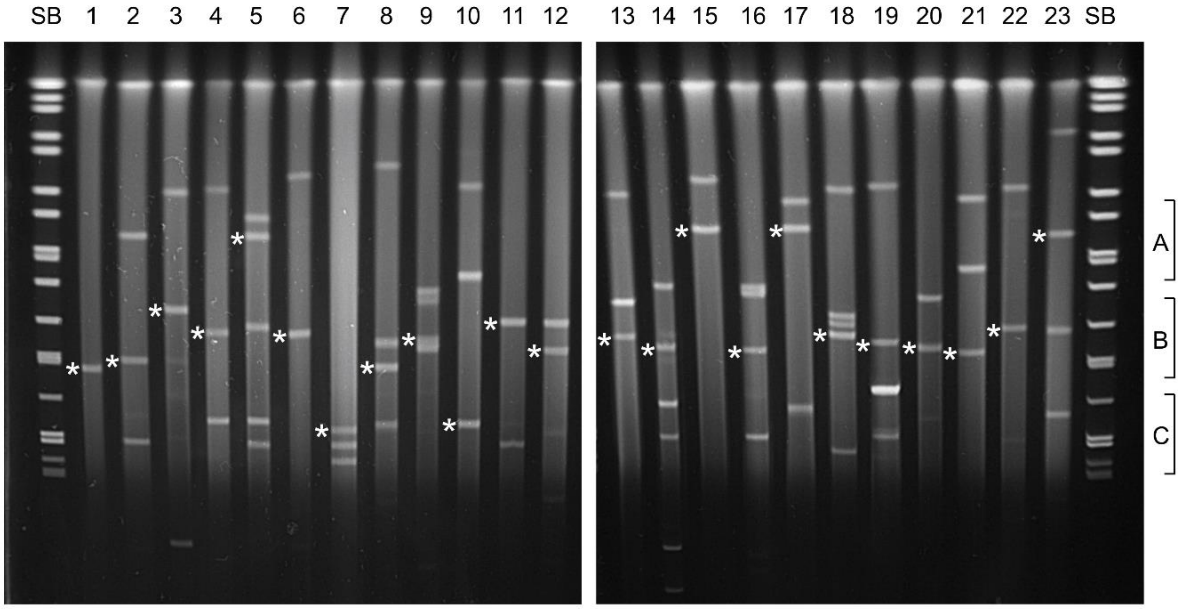


Figure 39: Plasmid profiles of E. coli isolates and determination of the mcr-1 location
S1-nucelase PFGE profiles of E. coli isolates used in this study: lane 1: 13-AB00012, lane 2: 13-AB00742, lane 3: 14-AB01030, lane 4: 14-AB01041, lane 5: 14-AB01513, lane 6: 15-AB00959, lane 7: 15-AB01098, lane 8: 15-AB01173, lane 9: 15-AB01276, lane 10: 15-AB01775, lane 11: 15-AB02086, lane 12: 14-AB01081, lane 13: 12-AB00501, lane 14: 12-AB00876, lane 15: 12-AB01842, lane 16: 12-AB01861, lane 17: 12-AB02079, lane 18: 13-AB00885, lane 19: 13-AB01479, lane 20: 13-AB01693, lane 21: 14-AB00001, lane 22: 14-AB00714, and lane 23: 14-AB00941. SB: Salmonella Braenderup H9812 XbaI-restriction profile. Asterisks indicate the location of the mcr-1 gene determined by Southern blotting and DNA-DNA hybridization using mcr-1 as a probe. According to determined size difference mcr-1 plasmid were attributed to four groups (A to D).

Our investigations showed that the genetic background (especially the chromosome) of *mcr-1* carrying *E. coli* can be highly divers. However, we identified the *mcr-1* determinant on plasmids of a few size ranges. We therefore conclude that *mcr*-carrying plasmids might be transmissible in a wide range of *E. coli* serving as potential host for these mobile elements.

mcr-1 carrying *E. coli* isolates exhibited a highly diverse genetic basis of their chromosomes

Dissection of the *mcr-1* carrying *E. coli* genomes was performed by short read whole genome sequencing (WGS, MiSeq, Illumina, San Diego, USA) as previously described. *de novo* assemblies of WGS data were created using the full SPAdes algorithm of the PATRIC database (Pathosystems Resource Integration Center) (Wattam et al., 2018). Relevant *in silico*-typing results of the genomes are provided in Table 23.

Table 23: Overview on *in silico*-based typing results of *mcr-1* carrying *E. coli* isolates

Isolate*	MLST[A]	Serotype[B]	
	scheme Ec1	O-type	H-type
12-AB00025	ST-428	n.d.	H4
12-AB00501	ST-997	n.d.	H51
12-AB00876	ST-355	O2	H5
12-AB01842	ST-355	O50	H5
12-AB01861	ST-453	O23	H16
12-AB02079	ST-1444	O22	H14
13-AB00012	ST-10	O90	H21
13-AB00742	ST-88	O8	H19
13-AB00885	ST-752	n.d.	H40
13-AB01479	ST-189	O80	H26
13-AB01693	ST-117	O113	H4
14-AB00001	ST-10	n.d.	H40
14-AB00714	ST-1011	O111	H51
14-AB00941	ST-1564	n.d.	H21
14-AB01030	ST-131	O25	H4
14-AB01041	ST-533	n.d.	H31
14-AB01081	ST-533	n.d.	H14
14-AB01513	ST-95	O2	H5
15-AB00959	ST-3628	O2	H32
15-AB01098	ST-1642	O8	H7
15-AB01173	ST-218	O157	H43
15-AB01276	Unknown	O26	H11
15-AB01775	ST-93	O132	H25
15-AB02086	ST-744	O89	H9

* *in silico* analysis was conducted using web-based tools (A) MLST-Finder and (B) SeroType Finder of the Center for Genomic Epidemiology (www.genomicepidemiology.org)

Escherichia coli isolates of our study only slightly differ in their basic genetic setting of genes, coding sequences (CDSs), RNAs (i.e. tRNAs, ncRNA) (data not shown). However, we determined strong differences using different *in silico*-typing tools of the Center for Genomic Epidemiology (CGE, www.genomicepidemiology.org) database. As indicated in Table 23, all analyzed genomes exhibited different multi-locus sequence types (MLST 2.0, https://cge.cbs.dtu.dk/services/MLST/) (Larsen et al., 2012), confirming the diversity of the isolates as observed by XbaI-PFGE. According to their identified MLST-type some of the isolates belong to important clonal complexes that are mainly distributed among food and livestock. WGS assemblies were further used to predict the serotype of the isolates (Serotype Finder 1.1, https://cge.cbs.dtu.dk/services/SerotypeFinder/) (Joensen et al., 2015). However, not for all isolates reliable information on the serotype could be determined. Similar to the MLST-types, serotypes are also highly diverse.

In general, our bioinformatic analysis revealed that a broad spectrum of commensal *E. coli* of different MLST-, phylo-, and serotypes can carry the *mcr-1* determinant. These data confirm that a broad spectrum of *E. coli* isolates may serve as a host or recipient for the acquisition of *mcr-1* carrying plasmids.

mcr-1 carrying plasmids are transmissible and exhibit a diverse genetic basis

The availability of WGS sequencing data supported further *in silico* analyses (CGE, www.genomicepidemiology.org) on the genetic basis of antimicrobial resistance determinants, virulence genes and mobile genetic elements (i.e. prophages, plasmids) that might be involved in the transmission of *mcr-1*. Relevant results of these analyses are summarized in Table 24.

Table 24: Overview on plasmid and chromosomal features associated with colistin resistance of *mcr-1* carrying *E. coli* isolates

Isolate*	PFGE *mcr-1* plasmid prototype	Incompatibility group of *mcr-1* carrying plasmids (contig no., size)	Plasmid transmissibility (*in vivo* filter mating studies)	Chromosomal *pmrA*/*pmrB* mutations associated with colistin resistance
12-AB00025	n.d.	n.d. (N059, 26,761 bp)	+	No
12-AB00501	B	n.d. (N154, 2,940 bp)	+	No
12-AB00876	B	IncX4 (N039, 31,089 bp)	n.d.	No
12-AB01842	A	n.d. (N058, 28,121 bp)	n.d.	No
12-AB01861	B	IncX4 (N078, 21,099 bp)	+	No
12-AB02079	A	n.d. (N027, 63,844 bp)		No
13-AB00012	B	IncX4 (N055, 31,043 bp)	n.d.	No
13-AB00742	B	IncX4 (N047, 31,089 bp)	+	No
13-AB00885	B	n.d. (N106, 8,027 bp)	+	No
13-AB01479	B	n.d. (N223, 2,942 bp)	n.d.	No
13-AB01693	B	no *mcr-1* detection	n.d.	No
14-AB00001	B	n.d. (N012, 98,787 bp)	n.d.	No
14-AB00714	B	n.d. (N183, 2,943 bp)	+	No
14-AB00941	A	n.d. (N058, 26,760 bp)	+	No
14-AB01030	B	n.d. (N153, 2,943 bp)	n.d.	No
14-AB01041	B	n.d. (N100, 13,860 bp)	+	No
14-AB01081	B	no *mcr-1* detection	n.d.	No
14-AB01513	A	n.d. (N101, 6,020 bp)		No
15-AB00959	B	n.d. (N136, 3,674 bp)	+	No
15-AB01098	C	IncX4 (N035, 32,736 bp)	+	No
15-AB01173	B	n.d. (N043, 26,761 bp)	n.d.	No
15-AB01276	B	no *mcr-1* detection	n.d.	No
15-AB01775	C	n.d. (N165, 3,095 bp)	n.d.	No
15-AB02086	B	IncX4 (N051, 32,822 bp)	+	No

* *in silico* analysis was conducted using web-based tools (A) MLST-Finder and (B) SeroType Finder of the Center for Genomic Epidemiology (www.genomicepidemiology.org). Abbreviation: n.d., not detected; +, transmissible

The *mcr-1* gene was detectable in almost all WGS datasets of the studied isolates (Table 24). Only in *E. coli* 13-AB01693, 14-AB01081 and 15-AB01276 no *mcr*-gene could be detected. Re-testing of these isolates via PCR confirmed the presence of *mcr-1*. We therefore conclude that these isolates lost their *mcr-1* plasmid during preparation of the isolates for whole genome sequencing analysis.

Escherichia coli with a confirmed *in silico* detection of the *mcr-1* gene revealed that this resistance determinant is present on only a few different plasmid prototypes. However, based on the prevailing MiSeq-WGS data the *mcr-1* carrying plasmid could not be closed to complete circular genomes due to the presence of complex repetitive sequences and the high content of very similar transposase genes on the individual genomes. Sequence comparison based on *mcr-1* carrying sequence contigs (Table 24) revealed a close relationship to known plasmids of *E. coli*, *S. enterica*, *K. pneumoniae* and *S. flexneri* from the nucleotide sequence databases (i.e. pHNSHP45-2, pSA26-MCR-1, pmcr1_IncX4, pSH111_227, 1205p1, pVT553).

To determine the transferability of the *mcr-1*-carrying plasmids some of the isolates, representing prevalent prototypes of *mcr-1* plasmids, were used in filter mating studies with the sodium azide-resistant *E. coli* strain J53 (Borowiak et al., 2017). Transmission studies indicated that from all tested isolates *mcr-1* carrying plasmids could be transferred to the J53 recipient strain at 37°C (Table 24).

The occurrence of the different plasmid variants could not be attributed to a specific time period, source, or serotype. Our data showed that the spread of *mcr-1* carrying plasmids is probably not associated with different MLST-, phylo- or serotypes but presumably driven by the susceptibility of the strains for the self-transmissible plasmid and antibiotic selection pressure. However, for a reliable prediction of the plasmid diversity, the whole genome sequences of the complete plasmid genomes are necessary. Based on the currently available WGS data only partial sequences of the plasmid can be assessed. It is possible that despite their similar size, plasmids may be highly divers and belong to different types. Thus, further investigations on the plasmid genomes are necessary to assess the impact of the different *mcr-1* carrying plasmids for colistin resistance dissemination.

Discussion

Global spread of plasmid-mediated mobile colistin resistance in food, livestock and the environment represent a meaningful example of the influence of a potential overuse of antimicrobial substances in the animal sector in the past (Sun et al., 2018). Colistin is an antimicrobial substance of the last resort. Its efficacy for treating severe human infection with multidrug-resistant Gram-negative bacteria will be potentially vulnerable in the future (WHO, 2017). However, to develop reliable management strategies for the containment of colistin resistance dissemination, detailed information on its mechanism of action, transmissibility and stability as well as potential bacterial hosts and transmission vectors/vehicles is needed.

Detailed studies on *mcr-1* carrying *Enterobacteriaceae* in China resulted in the assumption that imprudent use of colistin as growth promoter in livestock supported the development and spread of *mcr*-genes. These findings led to a re-evaluation of the colistin usage in this country. Consequently, colistin was completely banned as a growth promoter from the Chinese livestock husbandry (Liu et al., 2016; Sun et al., 2018). Future monitoring of colistin-resistant isolates in different sectors will show if the Chinese management strategy will curtail the problem. However, high prevalence of *mcr-1* carrying isolates is not restricted to China or its neighbouring Asian countries. Nowadays, the prevalence of colistin-resistant *Enterobacteriaceae* in animals, humans and the environment is quite high in some other countries (Sun et al., 2017; Sun et al., 2018). All these countries may also contribute to the distribution of colistin resistant isolates to many other regions or continents via various routes. Therefore, strict regulations and monitoring programs will be needed. Globalization (i.e. travel and trade) is maybe one of the most important drivers for this trend. Currently, almost all countries worldwide are linked by trading goods and international travel. The import of food products of livestock or plant origin enables transfer of pathogens from endemic countries or commensal bacteria to novel ecosystems. The annual amount of legally imported goods, especially food products, constitutes a major challenge for the reliable monitoring of potential microbiological hazards for the food safety agencies. A further challenge are the high amounts of illegally imported goods brought by unconscionable organizations or imprudent travelers, which might be contaminated with potentially hazardous pathogenic agents. Reports show that food products are sometimes important reservoirs for microbiological hazards (Beutlich et al., 2015; Rodriguez-Lazaro et al., 2015). However, up to now quantitative data on their real impact on public health are missing.

The primary origin of *mcr-1* and other mobile colistin resistance genes is unknown. Today, global actions are needed to curtail further development and dissemination. The global aim should be a prudent and limited use of colistin in animals and its global ban for growth promotion purposes. Additionally, widespread prophylactic use needs to be stopped in livestock husbandry. There are some countries with a low to very low prevalence of colistin resistance. These might provide efficient strategies for lowering colistin resistance development and dissemination.

Many further questions have to be answered. One of the questions is the impact of none *mcr-1*-like genes (i.e. *mcr-2* to *mcr-8*) for colistin resistance development. Many reports revealed that in contrast to *mcr-1*, the occurrence of *mcr-2 to 8* seems to be very low. However, as the genetic basis of the *mcr*-genes indicated that these elements are mobile either by plasmid transfer (conjugation, mobilization) or by the action of the transposon/integron, the impact of some *mcr*-genes may shift over time by evolutionary events. In general, many of *mcr*-carrying plasmids bear the potential for a successful transfer of the resistance in a narrow or broad range of potential host bacteria. In this context, further surveillance and research is required to determine further evolutionary changes in the genetic basis or exploration of its mechanistic action to commensal bacteria and pathogens. However, harmonized surveillance of plasmid-mediated colistin resistance is limited by the availability of high throughput detection technologies. Currently, colistin resistance is mainly detected by phenotypic antimicrobial susceptibility testing, which is quite expensive and time-consuming, but does not provide any information on the genetic basis of the resistance. Rapid screening procedures as multiplex PCR-based methods support typing of colistin resistance mechanisms but are not well suited for high throughput routine analysis. In the future, WGS will constitute a powerful tool for real-time surveillance and retrospective in-depth analyses. However, to date the low availability of this technology because of the price for the instrument and the individual costs per isolate hamper its use in the daily routine of diagnostic laboratories.

Conclusion

Our findings indicate that *mcr-1* carrying *E. coli* isolates are widely disseminated in German livestock and food products of animal origin. In general, we suppose that the distribution of *mcr-1* associated colistin resistance is mainly driven by horizontal gene transfer (i.e. plasmid transfer), because of the high degree of diversity among the investigated isolates (i.e. PFGE-pattern, MLST-type, serotype). Overall, three conserved plasmid prototypes were identified to be involved in the *mcr-1* dissemination in this study. Genetic analyses revealed that the location of the resistance gene on a potentially active transposon might further support the dissemination of the resistance determinant to other plasmids or to the chromosome of the bacteria. However, without further data on the diversity of *mcr-1* carrying isolates from human origins the impact of this resistance determinant for public health is still unknown. Further investigations are necessary to reveal the potential missing links of *mcr-1* spread via contact to colonized animals and/or the consumption of contaminated food products. Currently, there is an urgent need for drastic management strategies to curtail colistin resistance development and dissemination.

Acknowledgements

The authors thank Britta Lesniewsky, Katharina Thomas, Silke Jahn, Gaby Carl, Kathrin Oelgeschläger and Anja Schabanowsky for excellent technical assistance. We also appreciate the support of the authorities and the diagnostic laboratories of the German federal states by providing *E. coli* isolates within the German zoonoses monitoring.

The work within this project was financially supported by grants of the German Federal Institute for Risk Assessment (43-001, 43-002 and 1322-648).

Abbreviations

EMA	European Medicines Agency
mcr	mobile colistin resistance

MIC	minimal inhibitory concentration
MLST	multi-locus sequence type
WGS	whole genome sequencing
WHO	World Health Organization

References

AbuOun, M., Stubberfield, E. J., Duggett, N. A., Kirchner, M., Dormer, L., Nunez-Garcia, J., . . . and Anjum, M. F. (2018). *mcr-1* and *mcr-2* (*mcr-6.1*) variant genes identified in *Moraxella* species isolated from pigs in Great Britain from 2014 to 2015. *J Antimicrob Chemother*. doi:10.1093/jac/dky272

Al-Tawfiq, J. A., Laxminarayan, R., and Mendelson, M. (2017). How should we respond to the emergence of plasmid-mediated colistin resistance in humans and animals? *Int J Infect Dis, 54*, 77-84. doi:10.1016/j.ijid.2016.11.415

Beutlich, J., Hammerl, J. A., Appel, B., Nöckler, K., Helmuth, R., Jost, K., . . . and Mayer-Scholl, A. (2015). Characterization of illegal food items and identification of foodborne pathogens brought into the European Union via two major German airports. *International Journal of Food Microbiology, 209*, 13-19. doi:10.1016/j.ijfoodmicro.2014.10.017

Borowiak, M., Fischer, J., Hammerl, J. A., Hendriksen, R. S., Szabo, I., and Malorny, B. (2017). Identification of a novel transposon-associated phosphoethanolamine transferase gene, *mcr-5*, conferring colistin resistance in d-tartrate fermenting *Salmonella enterica* subsp. *enterica* serovar Paratyphi B. *J Antimicrob Chemother, 72*(12), 3317-3324. doi:10.1093/jac/dkx327

Carattoli, A., Villa, L., Feudi, C., Curcio, L., Orsini, S., Luppi, A., . . . and Magistrali, C. F. (2017). Novel plasmid-mediated colistin resistance *mcr-4* gene in *Salmonella* and *Escherichia coli*, Italy 2013, Spain and Belgium, 2015 to 2016. *Euro Surveill, 22*(31). doi:10.2807/1560-7917.ES.2017.22.31.30589

EMA, European Medicines Agency. (2016). Updated advice on the use of colistin products in animals within the European Union: development of resistance and possible impact on human and animal health. *EMA, Available from: http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2016/07/WC500211080.pdf*.

Hammerl, J. A., Borowiak, M., Schmoger, S., Shamoun, D., Grobbel, M., Malorny, B., . . . and Käsbohrer, A. (2018). *mcr-5* and a novel *mcr-5.2* variant in *Escherichia coli* isolates from food and food-producing animals, Germany, 2010 to 2017. *J Antimicrob Chemother, 73*(5), 1433-1435. doi:10.1093/jac/dky020

Joensen, K. G., Tetzschner, A. M., Iguchi, A., Aarestrup, F. M., and Scheutz, F. (2015). Rapid and easy *in silico* serotyping of *Escherichia coli* isolates by use of whole-genome sequencing data. *J Clin Microbiol, 53*(8), 2410-2426. doi:10.1128/JCM.00008-15

Larsen, M. V., Cosentino, S., Rasmussen, S., Friis, C., Hasman, H., Marvig, R. L., . . . and Lund, O. (2012). Multilocus sequence typing of total-genome-sequenced bacteria. *J Clin Microbiol, 50*(4), 1355-1361. doi:10.1128/JCM.06094-11

Litrup, E., Kiil, K., Hammerum, A. M., Roer, L., Nielsen, E. M., and Torpdahl, M. (2017). Plasmid-borne colistin resistance gene *mcr-3* in *Salmonella* isolates from human infections, Denmark, 2009-2017. *Euro Surveill, 22*(31). doi:10.2807/1560-7917.ES.2017.22.31.30587

Liu, Y. Y., Wang, Y., Walsh, T. R., Yi, L. X., Zhang, R., Spencer, J., . . . and Shen, J. (2016). Emergence of plasmid-mediated colistin resistance mechanism MCR-1 in animals and human beings in China: a microbiological and molecular biological study. *Lancet Infect Dis, 16*(2), 161-168. doi:10.1016/S1473-3099(15)00424-7

Prestinaci, F., Pezzotti, P., and Pantosti, A. (2015). Antimicrobial resistance: a global multifaceted phenomenon. *Pathog Glob Health, 109*(7), 309-318. doi:10.1179/2047773215Y.0000000030

Rebelo, A. R., Bortolaia, V., Kjeldgaard, J. S., Pedersen, S. K., Leekitcharoenphon, P., Hansen, I. M., . . . and Hendriksen, R. S. (2018). Multiplex PCR for detection of plasmid-mediated colistin resistance determinants, *mcr-1*, *mcr-2*, *mcr-3*, *mcr-4* and *mcr-5* for surveillance purposes. *Euro Surveill, 23*(6). doi:10.2807/1560-7917.ES.2018.23.6.17-00672

Roca, I., Akova, M., Baquero, F., Carlet, J., Cavaleri, M., Coenen, S., . . . and Vila, J. (2015). The global threat of antimicrobial resistance: science for intervention. *New Microbes New Infect, 6*, 22-29. doi:10.1016/j.nmni.2015.02.007

Rodriguez-Lazaro, D., Ariza-Miguel, J., Diez-Valcarce, M., Stessl, B., Beutlich, J., Fernandez-Natal, I., . . . and Rovira, J. (2015). Identification and molecular characterization of pathogenic bacteria

in foods confiscated from non-EU flights passengers at one Spanish airport. *International Journal of Food Microbiology, 209*, 20-25. doi:10.1016/j.ijfoodmicro.2014.10.016

Sun, J., Zeng, X., Li, X. P., Liao, X. P., Liu, Y. H., and Lin, J. (2017). Plasmid-mediated colistin resistance in animals: current status and future directions. *Anim Health Res Rev, 18*(2), 136-152. doi:10.1017/S1466252317000111

Sun, J., Zhang, H., Liu, Y. H., and Feng, Y. (2018). Towards understanding MCR-like colistin resistance. *Trends Microbiol, 26*(9), 794-808. doi:10.1016/j.tim.2018.02.006

Wattam, A. R., Brettin, T., Davis, J. J., Gerdes, S., Kenyon, R., Machi, D., . . . and Yoo, H. (2018). Assembly, annotation, and comparative genomics in PATRIC, the all bacterial bioinformatics resource center. *Methods Mol Biol, 1704*, 79-101. doi:10.1007/978-1-4939-7463-4_4

WHO, World Health Organization. (2017). WHO list of critically important antimicrobials (CIA). *Available at: http://www.who.int/foodsafety/publications/antimicrobials-fith/en*.

Xavier, B. B., Lammens, C., Ruhal, R., Kumar-Singh, S., Butaye, P., Goossens, H., and Malhotra-Kumar, S. (2016). Identification of a novel plasmid-mediated colistin-resistance gene, *mcr-2*, in *Escherichia coli*, Belgium, June 2016. *Euro Surveill, 21*(27). doi:10.2807/1560-7917.ES.2016.21.27.30280

Yang, Y. Q., Li, Y. X., Lei, C. W., Zhang, A. Y., and Wang, H. N. (2018). Novel plasmid-mediated colistin resistance gene *mcr-7.1* in *Klebsiella pneumoniae*. *J Antimicrob Chemother*. doi:10.1093/jac/dky111

Yin, W., Li, H., Shen, Y., Liu, Z., Wang, S., Shen, Z., . . . and Wang, Y. (2017). Novel plasmid-mediated colistin resistance gene *mcr-3* in *Escherichia coli*. *MBio, 8*(3). doi:10.1128/mBio.00543-17

5.5 New trends for the detection and characterization of foodborne pathogens

Malorny, Burkhard; Borowiak, Maria; Nöckler, Karsten

Abstract

During the last decades a multitude of tools and immunological, biochemical and molecular techniques have been developed for the rapid detection, characterization and typing of foodborne pathogens. The motivation of the scientists behind these efforts is to identify rapidly the germ of infectious diseases in humans and animals and to trace the pathogen back to the source of contamination such as food or feed or the environment. Recently, a number of new technologies in the field came up including the so called 'omics' tools which will have a tremendous impact on food safety and food quality approaches in the future. 'Omics' provide a revolutionary opportunity to greatly enhance our knowledge and understanding how pathogens behave in certain environmental conditions and how they spread through the globalized supply chain. Whereas whole genome sequencing of microorganisms and viruses is increasingly applied in the laboratories, e.g. in case of outbreak studies, many other 'omics' techniques are still in a research phase despite their promising perspectives. We focus here on the major 'omics' concepts including genomics, transcriptomics, proteomics and metabolomics promising effective approaches to make our supply chain safer.

Introduction

Food safety is compromised by foodborne pathogens. The global burden of foodborne diseases is enormous. The WHO estimates that 31 global hazards caused altogether up to 600 million foodborne illnesses and 420.000 deaths in 2010 (WHO, 2015). Due to the rapid globalization of the food trade unsafe food has become a global challenge. Poor food-production processes and food-handling can trigger worldwide public health problems. Recently, a nationwide outbreak of *Salmonella* Agona infections among infants caused 37 cases in France. The contaminated infant milk product was distributed in 66 countries, but luckily only two sporadic cases in Spain and Greece could be linked to the food product (Jourdan-da Silva et al., 2018). The fast recall of the product has prevented the situation from getting worse. Due to the global supply chain a food product consists of ingredients delivered from many different regions in the world, but is locally produced and again worldwide traded. Thus, tracing the microbial hazard of potentially contaminated food and surveillance of the supply chain are challenge for food authorities and need rapid diagnostic tools with a high resolution to detect and characterize suspected pathogens. The microbiology made a quantum leap in research and diagnostic during the past two decades due to the introduction of highly engineered gadgets. Nowadays, data can be generated in high-throughput and high resolution. In this review we would like to give an overview about powerful and potential approaches for the detection and characterization of foodborne pathogens which can be used in the future.

Analytical food safety methodologies in the past and now

The rapid detection, characterization and typing of foodborne pathogens engage scientists since the first observation of bacteria by the Dutch merchant named Anton van Leeuwenhoek in the late 1670s by using self-build microscopes. It took a while until Louis Pasteur, a chemist, postulated the germ theory of disease, proclaiming that microorganisms are the causative agents of infectious diseases. The bacteriologist Robert Koch was able to prove the theory in 1876 when he cultured the germ of anthrax outside of the host. Together with Louis Pasteur, Robert Koch is regarded as the co-founder of the microbiology. The forthcoming improvement of culture-based methods and the ability to isolate and multiply a germ in an artificial medium made it possible for microbiologists to develop new bacterial typing meth-

ods. In the 1930s serotyping based on antigen-antibody reactions was one of the first approaches to differentiate bacteria on species and subspecies level apart from microscopy. Later in the 1950s phage typing schemes, e.g. for staphylococci, have been developed to be even more discriminative (Hood, 1953). These schemes were initially used to trace the source of infections.

The discovery of nucleic acids, the postulation that genetic information is embedded in the DNA and the description of the structure of DNA molecules as double helix by Watson and Crick in the middle of the 1950s founded the molecular biology (Watson and Crick, 1953). The idea for a bacterial strain typing method using nucleic acids of the cells came up by Tenover and colleagues at the beginning of the 1980s and was published as first fingerprinting molecular biology technique applied to distinguish strains in an outbreak investigation (Tenover, 1985). In the late 1980s pulsed-field gel electrophoresis has been developed being a universal typing system for bacteria. The method has been thoroughly standardized worldwide and functioned for two decades as “gold standard” in bacterial typing (Gerner-Smidt et al., 2006). With the advent of DNA sequencing technologies in the middle of the 1970s introduced by Maxam-Gilbert and Sanger it was possible to determine the sequence of base pairs of a DNA fragment. In the late 1980s the polymerase chain reaction was developed. Using this method, a specific piece of DNA is amplified, separated by size in an electric field and visualized by intercalating dyes. The following implication of the polymerase chain reaction as part of the sequencing process significantly improved the applicability of Sanger-sequencing in diagnostics. Many PCR-based detection methods and sequence-based typing approaches for the detection and typing of foodborne pathogens have been developed. One of the most successful sequence-based typing methods is the concept of multilocus sequence typing (MLST). It was initially proposed for the pathogen *Neisseria meningitids* in 1998 (M. C. Maiden, 2006; M. C. J. Maiden et al., 1998). Meanwhile numerous MLST schemes were developed and are applied for hundreds of pathogens (http://pubmlst.org). The technique involves amplification of mostly seven loci of housekeeping gene fragments by PCR followed by DNA sequencing. Subsequently specific DNA sequences are assigned to alleles. A single nucleotide variation at any of these loci defines a different allele and consequently a sequence type (ST). MLST detects changes at DNA level that are not apparent by phenotypic approaches, such as serotyping or Multilocus enzyme electrophoresis (MLEE). Generally, the discriminatory power is comparable or slightly better than known for serotyping. The sequence data generated are portable, non-ambiguous and can be readily compared between laboratories.

Next generation sequencing opens new microbial typing approaches highly useful in illness outbreak detection

The first generation of DNA sequencing, such as the traditional Sanger sequencing, is characterized by low-throughput and non-automatable instruments. The so called 2nd generation or next generation sequencing (NGS) was introduced in 2004 by Roche Life Sciences releasing the 454 sequencer. This sequencing technology was based on pyrosequencing enabling a massive parallelize sequencing process of thousands to millions of reads. The short reads (50-700 bp) are assembled by bioinformatics tools to longer stretches of sequences (contigs) based on their overlapping areas. Meanwhile a number of different next-generation sequencing technologies exist on the market (Quainoo et al., 2017). The most prominent system is the Illumina sequencing by synthesis method producing read lengths up to 2x 300 bp. More recently 3rd generation sequencers are available focusing on long-read sequencing up to 80 kb. The instruments of Pacific Biosciences and Oxford Nanopore use quite different technologies but are able to produce long reads enabling the generation of completely closed genomes (van Dijk et al., 2018).
Next generation sequencing leads microbiology into a new genomic era and currently moves from proof-of-concept studies into routine diagnostics in clinical and veterinary laboratories.

The broad application fields in microbiology, the high resolution and the decreasing costs make next generation sequencing very attractive for diagnostic laboratories. As one of the first application of the NGS technology whole genome sequencing (WGS) of isolates has been implemented in microbial food safety (Allard et al., 2018; Ronholm et al., 2016). WGS provides the possibility for a rapid identification and characterization of food-borne pathogens regardless to the species by following a universal workflow. Due to rapidly declining cost of this technology, WGS can be easily implemented in food safety, public and veterinary health laboratories. The increasing number of submitted genomes of pathogens related to food-borne illnesses in the NCBI Genome database (Figure 40) can be taken as measure for the successful integration of WGS methods in research and routine diagnostics. The applications of WGS in food safety ranges from classical molecular typing of pathogens to the prediction and surveillance of antimicrobial resistance, virulence and pathogenicity, stress, as well as host adaptation by identifying relevant genes to the ascertainment of similarity, relatedness and diversity of foodborne pathogens. Hence, WGS methods and bioinformatical pipelines to analyse WGS data consolidate the numerous different methodologies currently in use in food, veterinary and clinical laboratories including classical serotyping, phenotypical antimicrobial susceptibility testing, 7-gene MLST, pulsed-field gel electrophoresis (PFGE) and multi locus variable-number tandem repeat analysis (MLVA). By allowing the rapid exchange of comparable data between different laboratories on a national and international level, WGS is not only restricted to traceback investigations but has the potential to be used in real-time surveillance of food-borne outbreaks. In outbreak studies the genetic profiles of human isolates from patients are compared with those of suspected foodborne isolates. If these form a so-called cluster, it indicates a close epidemiological link. In connection with the metadata available for each isolate, such as date and place of isolation, origin (animal species, food), producer, etc., further epidemiological investigations with the aim of revealing potential sources of infection are finally possible.

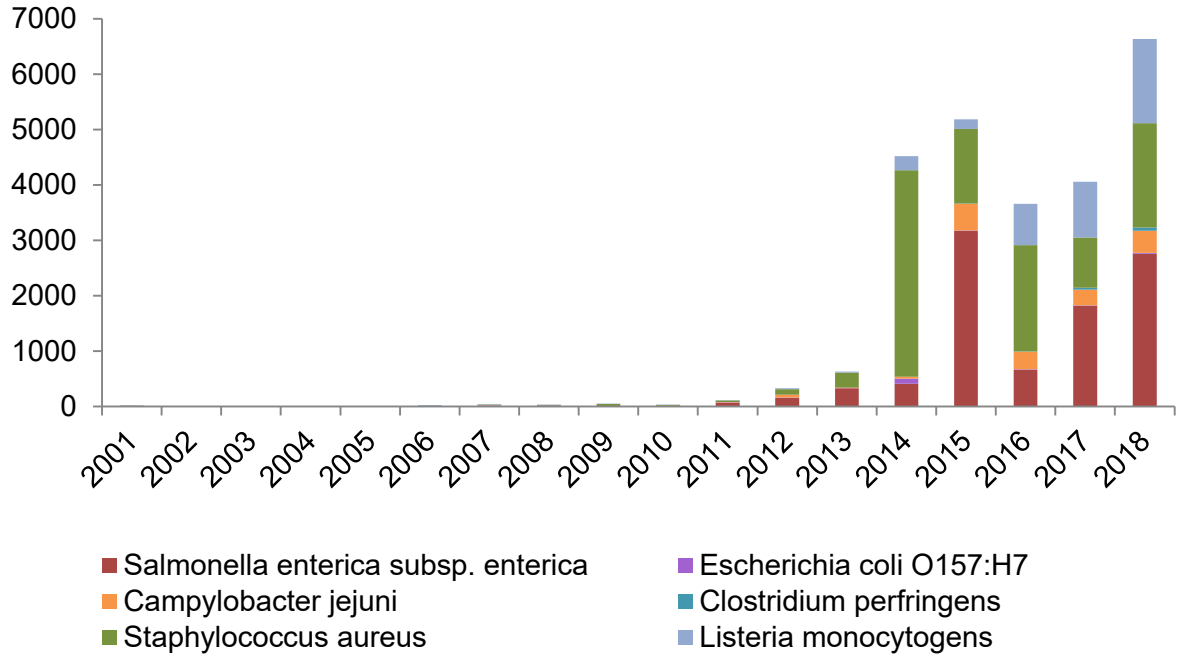


Figure 40: Number of genomes (draft or complete) of selected pathogens associated with food-borne illnesses uploaded to the NCBI Genome database from 2001 to 2018

A number of different microbial sequence typing methods based on WGS data have been developed for the discrimination of foodborne pathogens (Perez-Losada et al., 2017). As one of the most prominent typing scheme the 7-gene MLST concept has been expanded to the core genome (cgMLST) and the complete (whole) genome (wgMLST) which comprises the

allelic diversity identified in hundreds to thousands of genes (Jolley and Maiden, 2014). EnteroBase, a genotyping web-based platform offers various genomic based MLST and nomenclature schemes for selected foodborne pathogens (Alikhan et al., 2018). Raw sequence data submitted to the database are assembled and assigned to alleles and STs at all levels of resolution ranging from 7-gene MLST to wgMLST(https://enterobase.warwick.ac.uk).

The first application of WGS in a foodborne disease outbreak investigation was reported from a nation-wide large outbreak associated with ready-to-eat meat products contaminated with *Listeria monocytogenes* in Canada in 2008 (Gilmour et al., 2010). Three distinct *L. monocytogenes* genetic traits comprising highly related strain types were involved in the outbreak. Furthermore, the microevolution of the outbreak strain could be understood using WGS. The advantage of WGS in real-time outbreak investigation was also exemplified in 2011, during a large outbreak of enterohemorrhagic *Escherichia coli* O104:H4 in first reported in northern Germany causing 4.321 confirmed cases of STEC infection and 852 cases of hemolytic uremic syndrome (HUS) with 54 deaths reported in 14 EU countries, the USA and Canada (Buchholz et al., 2011). Real-time sequencing of the outbreak strain revealed the exceptional virulence markers including adhesive properties typically found in enteroaggregative *E. coli*. A model could be drawn how the strain evolved from a common EHEC O104:H4 progenitor to its highly virulent form (Mellmann et al., 2011). The sequence data was the basis for the development of rapid PCR tests to discriminate further suspected outbreak from non-outbreak strains. Although an isolate from suspicious seeds has never been isolated, the potential of WGS in an ongoing outbreak investigation was demonstrated. Further investigation of the supply chain of the suspicious fenugreek seed and further cases in other European countries led to the conclusion that the seed imported from Egypt were likely the source of the outbreak (EFSA, 2011e).

Whole genome sequencing moves into practice

The *E. coli* O104:H4 outbreak has boosted a paradigm shift in clinical diagnostics and surveillance of microorganisms towards the real-time investigation of outbreaks using WGS worldwide. Many studies have shown the feasibility of WGS as an analysis tool for ongoing outbreaks (Deng et al., 2016). Furthermore, it has become an essential tool for disease surveillance, food monitoring and antimicrobial drug resistance tracing. It provides a one-in-all tool to obtain a maximum of genetic information out of the genome sequence. Due to many advantages including decreased costs, feasibility, scalability, quality, throughput, resolution and precision WGS moves rapidly into practice. The ultimate resolution on genotypic level makes the tool also valuable for short-term evolutional tracing studies of microorganism. Thus, the technique has the potential to be linked not only with datasets from disease cases but also with food delivery data supporting the identification of the source of an outbreak.

There is much effort to establish WGS as a routine typing tool for foodborne pathogens. Existing molecular typing networks such as PulseNet International (http://www.pulsenetinternational.org/) established WGS as a state-of-the-art tool to investigate outbreak clusters (Carleton and Gerner-Smidt, 2016; Nadon et al., 2017). The U.S. Centers for Disease Control and Prevention (CDC) have been conducting WGS in routine surveillance since 2013. Moreover, the GenomeTrakr network was established in the U.S. to provide bacterial sequences of foodborne pathogens isolated from animal, food and feed in a public sequencing database in order to support the colleagues of the U.S. CDC in source tracking of hazards. (Allard et al., 2016). In Europe, Public Health England (PHE) has implemented WGS as routine typing tool for public health surveillance of *Salmonella* infections in April 2015 and traditional serotyping was completely replaced (Ashton et al., 2016). The ECDC and EFSA as coordinating public health and food authorities in Europe developed a strategy to support sequenced-based typing for priority pathogens (EFSA, 2014a). ECDC and EFSA run a joint molecular typing database for the food-borne pathogens *Salmonella*,

Listeria monocytogenes and Shiga-toxin producing *E. coli*. As part of a European Commission request, new concepts are currently reviewed to collect WGS data for surveillance and outbreak investigations in Europe. EFSA and ECDC promote the implementation of WGS by funding smaller WGS projects for characterization of food-borne pathogens and the establishment of WGS in European authorities (Hendriksen et al., 2018). Furthermore, several joint rapid outbreak assessments were conducted to investigate European-wide ongoing or recently finished outbreaks. Globalization of WGS surveillance will result in many advantages. Pathogens will not stop at any border and due to the global supply chain it is worth to build a global genome database for microbial and infectious disease identification. WGS data of isolates can be used to predict the geographic origin of pathogens and significantly improves outbreak investigations. Thus, the Global Microbial Identifier was initiated in 2011 by public health centers and food authorities with the goal to establish a global system to harmonize, aggregate, share, and analyse genome data in real-time (http://www.globalmicrobialidentifier.org/). The approx. 260 experts from 50 countries focus in different working groups on political challenges, storage of sequence and metadata, analytical approaches and quality assurance issues. WHO and FAO are part of the initiative and provide landscape papers and partially training in next-generation sequencing for e.g. low-income countries (FAO, 2016b; WHO, 2018). In order to establish a vital system for the production, sharing and interpretation of WGS data for surveillance and outbreak investigations, worldwide efforts are needed to improve the capacities of sequencing centers and IT infrastructure for storage, sharing and analyses, to provide reliable internet access and speeds, to enhance the skills of researchers (also in low- and middle-income countries), and to gain a common accepted understanding of rules for data sharing.

In Germany, the microbial National Reference Laboratories of the BfR and the National Reference Centre for Salmonellae and other Enterics at RKI have established a close collaboration to apply WGS for outbreak investigations in routine. The laboratories have a common sequence data sharing platform and are able to perform a bundle of bioinformatic analyses. However, more and more public health, food and veterinary authorities of the federal countries will introduce NGS as a routine typing tool. Thus, a strategy for a common database for sharing and analysis of sequence- and metadata between the official authorities involved in outbreak investigations needs to be established. This will lead to faster and more accurate interventions.

The advantage of WGS use in outbreak investigation was impressively demonstrated during a protacted outbreak of invasive listeriosis in Southern Germany with 78 cases and 8 deaths. The outbreak strain belonged to serotype 1/2a and has been firstly reported at the end of 2015 (Ruppitsch et al., 2015). A total of 543 suspicious food isolates sampled from various food matrices and food processing plants have been analysed by PFGE and/or WGS (Kleta et al., 2017). Due to insufficient resolution of PFGE all food isolates with cluster similarity higher than 90% have been applied to WGS. By this approach, in May 2016, a *L. monocytogenes* strain belonging to the cgMLST cluster type 1248 was identified from a smoked pork belly sampled by food inspectors in a Bavarian retail store. Follow-up investigations revealed the same molecular strain type in several other meat products from the production chain of the same company. Recalling of all products from the company finally stopped the outbreak. Altogether, the period of observed human cases assigned to cluster type 1248 ranged from 2012 to 2016.

Metagenomics an approach of undirected analyses

While WGS approaches still require the cultural isolation of the suspicious pathogen from a matrix, metagenomics is a culture-independent application focusing on the community DNA from a sample (Forbes et al., 2017). The motivation of developing metagenomics approaches is to understand the dynamic of microbial communities (e.g. formed by bacteria, viruses

and eukaryotic microbes) in food, clinical, veterinary or environmental samples. For example, the growth and survival of foodborne pathogens depend on the microbial community present in the gut and probiotics secreting antimicrobial compounds can have a protective effect against foodborne pathogens (Arques et al., 2015). Thus, metagenomic sequencing is a promising tool in the context of food safety to get novel insights into microbial community composition within the food and supply chain as well as their mode of action in human guts. The technique and bioinformatics tools are not yet standardisable which makes them not yet applicable in official diagnostic tests for the detection of foodborne pathogens as “one fits all method”. Furthermore, a limitation is still the poor sensitivity due to the omitting of cultivation procedures for multiplication of foodborne pathogens prior to analytical testing and typing. Linked to the lack of cultural multiplication, viable cells are not distinguishable from non-viable cells. Thus, the presence of sequences in a complex sample which can be allocated to foodborne pathogens or other microbial hazards has to be interpreted with caution when comparable data from e.g. culture enrichment experiments are not available.

In general there are two main concepts of generating metagenome sequencing data. The first is focused on the amplification of the 16S ribosomal DNA gene (16S rDNA) or parts thereof which is encoded by all microorganisms. Sequences of 16S rDNA are more or less conserved within a species but differ between species. Thus, a comparison of the sequenced amplification products provides genetic information down to the species level. Since PCR is used as an initial step for specific amplification, the analysis is hampered by amplification bias. Due to the different number of 16S rDNA gene copies in different species quantification of the organisms by reads numbers is not possible because the read number is not necessarily related to the number of cells in a sequenced sample. Beside 16S rDNA sequencing other genetic markers have been described for taxonomic purposes such as *cpn60*, *rpoB* and 23S rDNA (Anthony et al., 2000; Case et al., 2007; Schellenberg et al., 2016). However, they have a similar bias caused by PCR and are not widely used. The second concept of metagenomics includes shotgun sequencing (Figure 41). In this approach a sample DNA is sequenced without any selection or enrichment step. The advantage is that the complete genomes of all microorganisms are sequenced including functional genes such as virulence and resistance determinants allowing the analysis of functional relationships of biomarker molecules. The drawback is that a very large portion of the sequences are mapped unintendedly to the matrix (e.g. plant, animal or human genomes) and needs to be erased from microbial sequences by bioinformatics tools. Only a small proportion of the initially sequenced data remains for the actual analysis. Usually, the large shotgun sequencing datasets require high performance computers for data analysis. Thus, when planning a metagenome project the decision of which approach fits better should be made carefully considering the purpose of the study and available resources.

The study of microbial communities and the detection of certain antimicrobial resistance determinants have the potential to yield information of certain specific habitats or even socioeconomic factors. Recently, Munk et al (2018) quantified and characterized the acquired resistance gene pools of 181 pig and 178 poultry farms from nine European countries using shotgun sequencing metagenomics. The main finding was that the total acquired AMR level correlates with the overall country-specific antimicrobial usage in livestock. Furthermore, countries with comparable AMR usage showed similar resistomes (Munk et al., 2018). Another interesting ongoing study is the global sewage surveillance project coordinated by DTU, Denmark (https://www.compare-europe.eu/Library/Global-Sewage-Surveillance-Project). Wastewater is sampled worldwide from many countries, cities and locations. Metagenomics of the samples will give a better understanding on how antimicrobial resistance (AMR) genes, pathogens and other factors related to human health may spread from healthy animals to (healthy) humans through the environment. The project will serve as a further proof-of-principle for applying metagenomics as a tool for global surveillance of human infectious diseases related to antimicrobial resistance.

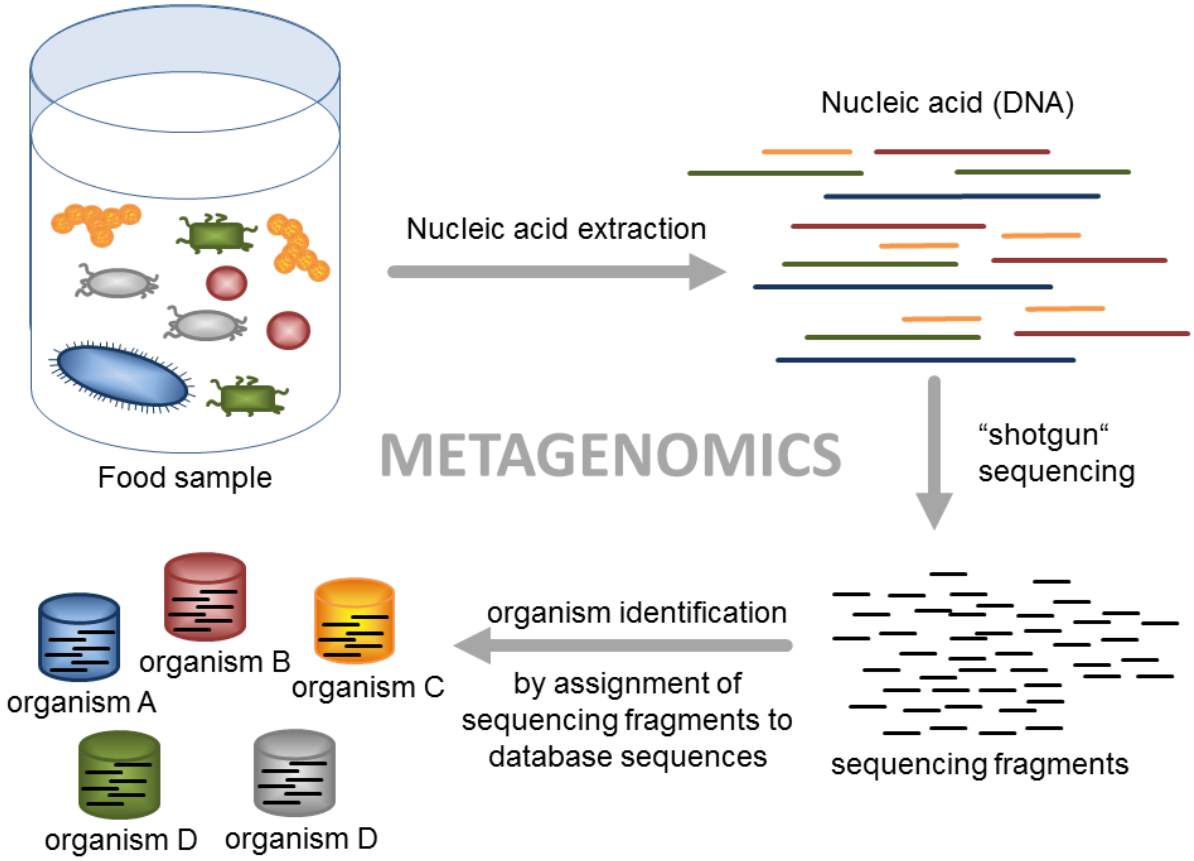


Figure 41: The concept of "shotgun" metagenomics sequencing

In the future, metagenomics in food safety could play a larger role in terms of monitoring the quality of food products and complex food ecosystems. The microbiota at a given time and environment determines the maturity or quality of a food product (Kergourlay et al., 2015). The aim of the application is mainly to understand and optimize the fabrication process of the food product in terms of sensory characteristics, safety and shelf life. As an example, the microbiome from various cheeses has been characterized (Delcenserie et al., 2014; Yeluri Jonnala et al., 2018). It has been shown that the composition of the microbiota is responsible for the flavor compounds of a cheese (Escobar-Zepeda et al., 2016). Another interesting study determined the complex microbiota in beef at slaughter and concurrent storage at 4°C. The authors show that microbial contamination during the handling of the meat can be taken over during refrigerating and cause spoilage (De Filippis et al., 2013). Many studies characterized also fermented food products such as Kefir, a milk or water product fermented with both yeasts and bacteria (Kergourlay et al., 2015; Korsak et al., 2015). Fermentation of food products is widely applied and the composition of microorganisms depends often on the initial compounds used for the fermentation process. Specific starter species added by spices are needed, for example, for formulation of sausages. Those species could be later tracked in the final product (Benson et al., 2014). However, despite the importance of metagenomics for food quality, it is also important to understand the interaction of foodborne pathogens with the environmental microbiota in food in terms of relevant food safety aspects. For example, it has been shown that the growth of *Listeria monocytogenes* can be reduced by certain microbial communities in cheese without influencing the growth of other microbial strains (Imran et al., 2013). Recently, the behavior of *L. monocytogenes* in respect to resident bacteria in food processing environments has been elucidated in a biofilm model (Heir et al., 2018). The results indicate complex multispecies interactions between *L. monocytogenes* and other bacteria. *L. innocua* provided growth inhibition to certain types of *L. monocytogenes*.

A further promising variant of metagenomics is called “metagenomics binning”. It is a bioinformatics technique that enables near-complete microbial genomes to be directly assembled from metagenomics sequence data (Beaulaurier et al., 2018; Tyson et al., 2004). A powerful tool to generate near-complete microbial genomes from mixed samples is also the “Hi-C” method (Burton et al., 2014). Here, DNA molecules are cross-linked in intact cells that are in close proximity. Using this approach recently 913 near-complete bacterial and archaeal individual genomes from Scottish cattle could be drafted (Stewart et al., 2018).

However, despite the importance of metagenomics for food quality applications, microbiota profile of food products, especially those who are locally produced by traditional manufacturing, could also be used to reveal food frauds and indicate the authenticity of a local produced food product. Similar to WGS of foodborne pathogens to identify the source of contamination, a database with standardized metagenomic food profiles is needed to prove food frauds. Those microbial data should be at best combined with plant and animal sequence data derived from a food sample in order to receive a complete molecular fingerprint enabling the discrimination between original and counterfeited manufacturing.

Forthcoming ‘omics’ technologies in food safety

Genomics and metagenomics approaches explore the complexity of microbiota in a clinical, veterinary or environmental sample. In order to understand the fate of undesirable microorganisms, such as pathogens, in the food chain several other ‘omics’ measurements were developed which are based on transcriptomic, proteomic and metabolic profiling of microorganisms (Brul et al., 2012; Zhang et al., 2010). These new techniques are expected to play an increasing role in exploring the complete biological function of pathogens, their survival in hostile ecosystems and their interaction with the hosts (Figure 42). ‘Omics’ approaches are in contrast to traditional methodologies always high-throughput data-, holistic- and top-down-driven and enable scientists to analyse molecules at various levels.

Transcriptomics reflects the global expression of genes to mRNA molecules depending on the environmental factors. The presence of a gene solely does not give information if it is functional and when it is expressed. Characterization of the complete set of RNA transcripts by RNA-sequencing (RNA-seq) provides new opportunities and prospects to understand how pathogens adapt and respond to environmental conditions, e.g. in dry food products or vegetables. Applications of RNA-seq to food and foodborne pathogens are new but increasing in frequency. A number of studies have already elucidated transcriptomics for foodborne pathogens on lettuce, peanut oil or sprouts (Brankatschk et al., 2014; Deng et al., 2012; Fink et al., 2012; Goudeau et al., 2013). For *L. monocytogenes* RNA-seq was applied to explore the transcriptional landscape when growing under vacuum-packaged cold smoked salmon. Results indicated the upregulation of different biosynthesis pathways likely facilitating adaptation to anaerobic conditions and enabling the growth in the presence of the resident microbiota (Tang et al., 2015). The study provides also data for highly-expressed RNA molecules which may be new targets for the development of better detection methods. However, the amount of mRNA quantified does not necessarily correlate with the amount of protein translated from mRNA. Moreover this technique is not able to recognize potential post-translational modification of proteins and the interaction with their environments.

Proteomics reflects the investigation of the entire pool of proteins within a sample or organism and has been termed in analogy with genomics and transcriptomics (James, 1997). Proteins are the next post translational level of RNA transcripts and are large biomolecules with many various vital functions in the cell. While in the past two-dimensional polyacrylamide gel electrophoresis was the most important technique to analyse the composition of cell proteins, today, many different large-scale mass spectrometetry techniques are applied in proteomics. These techniques will enable scientists to analyse the whole size range of proteins and pro-

vide much better detection limits, quantitative information and the accurate mass of the analytes. One well established technology in diagnostic laboratories for the identification of microorganisms including pathogenic bacteria on species level is the Matrix-assisted laser desorption/ionization (MALDI) time-of-flight (TOF) mass spectrometry using whole cells as template (Fagerquist, 2017). The molecules of the cells are ionized and the mass-to-charge (m/z) ratio of the resulting ions is determined. The resulting mass spectra obtained within minutes are compared with those from a database and discriminative peaks are used to classify the species. Another promising mass spectrometric instrument is the Orbitrap ion trap mass analyser used for proteomics (Makarov and Scigelova, 2010). The Orbitrap analyser works as detector for a number of different external accumulation devices such as linear ion trap enabling multiple levels of fragmentation or can be coupled with a chromatographic separation technique in order to analyse complex peptide mixtures. Microbial proteomics is increasingly applied for investigating the behavior of pathogens in food, for example with the aim to control the occurrence of *Bacillus cereus* in milk powder (Alvarenga et al., 2018), to study the adaptation of enterohemorrhagic *E. coli* to temperature and water activity during carcass chilling (King et al., 2016), or to determine the global proteome responses of *Vibrio metschnikovii* under cold stress (Jia et al., 2015).

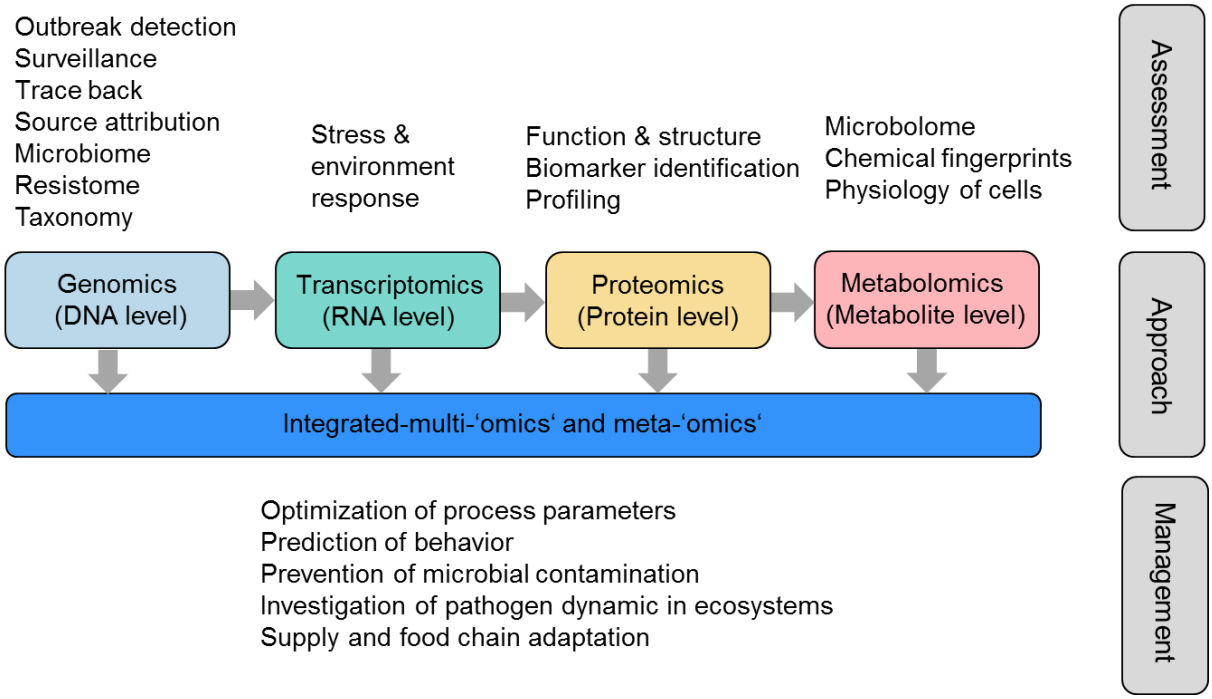


Figure 42: Omics' for detection, identification and characterisation of foodborne pathogens in the food and supply chain: From Assessment to Management

The emerging field of metabolomics focusses on small molecule metabolites (<1.500 Da) which are generated in various chemical processes within a biological sample. The metabolome can provide a fingerprint of the physiology of a biological cell and is the counterpart to the genome, transcriptome and proteome. In 2007, the Human Metabolome Project, which was launched in 2004, completed the first draft of the human metabolome consisting of a database of approximately 2500 metabolites, 1200 drugs and 3500 food components (Wishart et al., 2009; Wishart et al., 2007). The data were compiled from hundreds of mass spectra (MS) and nuclear magnetic resonance (NMR) metabolomics analyses. Other techniques used in metabolomics are gas chromatography time-of-flight (GC-TOF) MS, high-performance liquid chromatography (HPLC) MS or capillary electrophoresis (CE) MS (Villas-Boas et al., 2005). Microbial metabolomics is studied since the 1990s. Especially the human microbial gut and the modification of key health-related functions by the interaction of con-

sumed food and the gut microbiota have been elucidated (Lockett et al., 2016). Effects of microbial metabolites on gastrointestinal bacterial pathogens were investigated on metabolomics level (Vogt et al., 2015). The conclusion of these studies is that microbiota-produced metabolites can affect the growth and virulence of pathogens.

An interesting research tool for generation of microbial metabolic fingerprints is the Biolog Microbial ID System. Direct high-throughput analyses of cellular phenotypes using preconfigured phenotype microplates can continuously monitor the response of a cell in relation to preconfigured substrates (Bochner et al., 2001). Biolog Ecoplates have been used to characterize community level physiological profiles (CLPP) and to elucidate the microbial community structure in the presence of various carbon sources (Oest et al., 2018). Moreover, the survival of foodborne pathogens such *Salmonella* in sprouts has been investigated (Reed et al., 2018). Biolog cell phenotyping results are well suited to be combined with transcriptomic data on single cell level to explore the relation between cell metabolites and RNA transcripts under certain environmental conditions.

The future trend: Integrated multi-‘omics’ approaches

With the advance of technology and data evaluation it is becoming clear that the understanding of the complexity of foodborne pathogens in a certain environment requires integrated multi-‘omics’ data generation on all cell levels (Cocolin et al., 2017; Zhang et al., 2010). Thus, integrated multi-‘omics’ approaches considering each level of information (*DNA, RNA, proteins and* metabolites) and combining the data of the microbiota and the host to understand biological and metabolic pathways will be clearly the trend in infectious biology. Integrated transcriptomics and proteomics is so far the most applied approach comprising many studies with bacterial pathogens (Zhang et al., 2010). A study on the regulation of EHEC virulence shows how a multi-‘omic’ analysis including metabolomics and proteomicis can be used to characterize the role of the aerobic metabolism in the pathogenicity of EHEC infection and to identify new potential therapeutic targets (Kuo et al., 2018). However, multi-‘omics’ data generation will need further development and optimization in order to be used not only in research but also in routine diagnostic laboratories. Especially the development of new user-friendly software and improved statistical tools connecting the results between the different ‘omics’ technologies are needed. Another drawback of the ‘omics’ technologies are the financial constraints. High costs for instruments and consumables do challenge many researchers and hamper the use of the techniques in the laboratories.

Conclusions

Technologies and possibilities for the detection and characterization of foodborne pathogens have evolved dramatically within the past two decades. In contrast to traditional microbiological techniques such as PCR or immunological and biochemical methodologies using simple hand tools and devices, nowadays, mass of data can be generated with new high-throughput instruments at a better resolution, sensitivity and specificity. New next-generation sequence technologies are able to generate unprecedented amount of nucleic acids sequences and provide full genetic information on the entire bacterial genome. Also on proteomics and metabolomics level the technologies have been dramatically improved. The advance of ‘omics’ methodologies and bioinformatics in food safety has just started and applications will increase significantly within the next decade. The technologies are still expensive and are currently rather used in academic research than in routine diagnosis. However, WGS for the characterization of foodborne pathogens has been rapidly established for outbreak investigations and in risk assessment studies in public health and food authorities announcing the new era of genomics in microbiology. We are confident that also other ‘omics’ technologies will be part of the next generation detection and characterization approaches for foodborne

pathogens since they provide a better resolution and understanding of the dynamic and virulence of pathogens in food, animal and human.

Acknowledgements

We would like to thank the German Federal Institute for Risk Assessment for financial support of the next-generation sequencing group, project number 46-002.

Abbreviations

bp	Basepair
CDC	Centers for Disease Control and Prevention
DNA	Deoxyribonucleic acid
DTU	Danish Technical University
ECDC	European Centre for Disease Prevention and Control
EFSA	European Food Safety Authority
EHEC	Entero-haemorrhagic *Escherichia coli*
Kb	Kilobases
HUS	Hemolytic uremic syndrome
MLST	Multilocus sequence typing
MS	Mass spectrometry
MALDI TOF	Matrix-assisted laser desorption/ionization (MALDI) time-of-flight (TOF)
NGS	Next-generation sequencing
NMR	Nuclear Magnetic resonance
PCR	Polymerase Chain Reaction
RNA	Ribonucleic acid
RNA-seq	RNA sequencing
ST	Sequence Type
STEC	Shiga-toxin producing *Escherichia coli*
WHO	World Health Organisation
WGS	Whole genome sequencing

References

Alikhan, N. F., Zhou, Z., Sergeant, M. J., and Achtman, M. (2018). A genomic overview of the population structure of *Salmonella*. *PLoS Genet, 14*(4), e1007261. doi:10.1371/journal.pgen.1007261

Allard, M. W., Bell, R., Ferreira, C. M., Gonzalez-Escalona, N., Hoffmann, M., Muruvanda, T., . . . and Brown, E. W. (2018). Genomics of foodborne pathogens for microbial food safety. *Curr Opin Biotechnol, 49*, 224-229. doi:10.1016/j.copbio.2017.11.002

Allard, M. W., Strain, E., Melka, D., Bunning, K., Musser, S. M., Brown, E. W., and Timme, R. (2016). Practical value of food pathogen traceability through building a whole-genome sequencing network and database. *J Clin Microbiol, 54*(8), 1975-1983. doi:10.1128/JCM.00081-16

Alvarenga, V. O., Brancini, G. T. P., Silva, E. K., da Pia, A. K. R., Campagnollo, F. B., Braga, G. U. L., . . . Sant'Ana, A. S. (2018). Survival variability of 12 strains of *Bacillus cereus* yielded to spray drying of whole milk. *International Journal of Food Microbiology, 286*, 80-89. doi:10.1016/j.ijfoodmicro.2018.07.020

Anthony, R. M., Brown, T. J., and French, G. L. (2000). Rapid diagnosis of bacteremia by universal amplification of 23S ribosomal DNA followed by hybridization to an oligonucleotide array. *J Clin Microbiol, 38*(2), 781-788.

Arques, J. L., Rodriguez, E., Langa, S., Landete, J. M., and Medina, M. (2015). Antimicrobial activity of lactic acid bacteria in dairy products and gut: effect on pathogens. *Biomed Res Int, 2015*, 584183. doi:10.1155/2015/584183

Ashton, P. M., Nair, S., Peters, T. M., Bale, J. A., Powell, D. G., Painset, A., . . . and Group, S. W. G. S. I. (2016). Identification of *Salmonella* for public health surveillance using whole genome sequencing. *PeerJ, 4*, e1752. doi:10.7717/peerj.1752

Beaulaurier, J., Zhu, S., Deikus, G., Mogno, I., Zhang, X. S., Davis-Richardson, A., . . . and Fang, G. (2018). Metagenomic binning and association of plasmids with bacterial host genomes using DNA methylation. *Nat Biotechnol, 36*(1), 61-69. doi:10.1038/nbt.4037

Benson, A. K., David, J. R., Gilbreth, S. E., Smith, G., Nietfeldt, J., Legge, R., . . . and Singh, I. (2014). Microbial successions are associated with changes in chemical profiles of a model refrigerated fresh pork sausage during an 80-day shelf life study. *Appl Environ Microbiol, 80*(17), 5178-5194. doi:10.1128/AEM.00774-14

Bochner, B. R., Gadzinski, P., and Panomitros, E. (2001). Phenotype microarrays for high-throughput phenotypic testing and assay of gene function. *Genome Res, 11*(7), 1246-1255. doi:10.1101/gr.186501

Brankatschk, K., Kamber, T., Pothier, J. F., Duffy, B., and Smits, T. H. (2014). Transcriptional profile of *Salmonella enterica* subsp. *enterica* serovar Weltevreden during alfalfa sprout colonization. *Microb Biotechnol, 7*(6), 528-544. doi:10.1111/1751-7915.12104

Brul, S., Bassett, J., Cook, P., Kathariou, S., McClure, P., Jasti, P. R., and Betts, R. (2012). 'Omics' technologies in quantitative microbial risk assessment. *Trends in Food Sci Technol, 27*(1), 12-24. doi:10.1016/j.tifs.2012.04.004

Buchholz, U., Bernard, H., Werber, D., Bohmer, M. M., Remschmidt, C., Wilking, H., . . . and Kuhne, M. (2011). German outbreak of *Escherichia coli* O104:H4 associated with sprouts. *N Engl J Med, 365*(19), 1763-1770. doi:10.1056/NEJMoa1106482

Burton, J. N., Liachko, I., Dunham, M. J., and Shendure, J. (2014). Species-level deconvolution of metagenome assemblies with Hi-C-based contact probability maps. *G3 (Bethesda), 4*(7), 1339-1346. doi:10.1534/g3.114.011825

Carleton, H., and Gerner-Smidt, P. (2016). Whole-genome sequencing is taking over foodborne disease surveillance. *Microbe Magazine*. doi:10.1128/microbe.11.311.1

Case, R. J., Boucher, Y., Dahllof, I., Holmstrom, C., Doolittle, W. F., and Kjelleberg, S. (2007). Use of 16S rRNA and *rpoB* genes as molecular markers for microbial ecology studies. *Appl Environ Microbiol, 73*(1), 278-288. doi:10.1128/AEM.01177-06

Cocolin, L., Mataragas, M., Bourdichon, F., Doulgeraki, A., Pilet, M. F., Jagadeesan, B., . . . and Phister, T. (2017). Next generation microbiological risk assessment meta-omics: The next need for integration. *International Journal of Food Microbiology*. doi:10.1016/j.ijfoodmicro.2017.11.008

De Filippis, F., La Storia, A., Villani, F., and Ercolini, D. (2013). Exploring the sources of bacterial spoilers in beefsteaks by culture-independent high-throughput sequencing. *PLoS One, 8*(7), e70222. doi:10.1371/journal.pone.0070222

Delcenserie, V., Taminiau, B., Delhalle, L., Nezer, C., Doyen, P., Crevecoeur, S., . . . and Daube, G. (2014). Microbiota characterization of a Belgian protected designation of origin cheese, Herve cheese, using metagenomic analysis. *J Dairy Sci, 97*(10), 6046-6056. doi:10.3168/jds.2014-8225

Deng, X., den Bakker, H. C., and Hendriksen, R. S. (2016). Genomic epidemiology: Whole-genome-sequencing-powered surveillance and outbreak investigation of foodborne bacterial pathogens. *Annu Rev Food Sci Technol, 7*, 353-374. doi:10.1146/annurev-food-041715-033259

Deng, X., Li, Z., and Zhang, W. (2012). Transcriptome sequencing of *Salmonella enterica* serovar Enteritidis under desiccation and starvation stress in peanut oil. *Food Microbiol, 30*(1), 311-315. doi:10.1016/j.fm.2011.11.001

EFSA, European Food Safety Authority. (2011e). Tracing seeds, in particular fenugreek (*Trigonella foenum-graecum*) seeds, in relation to the Shiga toxin-producing *E. coli* (STEC) O104:H4 2011 outbreaks in Germany and France. *EFSA Supporting Publications, 8*(7), 176E. doi:10.2903/sp.efsa.2011.EN-176

EFSA, European Food Safety Authority. (2014a). Use of whole genome sequencing of food-borne pathogens for public health protection. *EFSA Scientific Colloquium 20 Summary Report*. doi:10.2805/66246

Escobar-Zepeda, A., Sanchez-Flores, A., and Quirasco Baruch, M. (2016). Metagenomic analysis of a Mexican ripened cheese reveals a unique complex microbiota. *Food Microbiol, 57*, 116-127. doi:10.1016/j.fm.2016.02.004

Fagerquist, C. K. (2017). Unlocking the proteomic information encoded in MALDI-TOF-MS data used for microbial identification and characterization. *Expert Rev Proteomic, 14*(1), 97-107. doi:10.1080/14789450.2017.1260451

FAO, Food and Agriculture Organization of the United Nations. (2016b). Applications of Whole Genome Sequencing in food safety management.

Fink, R. C., Black, E. P., Hou, Z., Sugawara, M., Sadowsky, M. J., and Diez-Gonzalez, F. (2012). Transcriptional responses of *Escherichia coli* K-12 and O157:H7 associated with lettuce leaves. *Appl Environ Microbiol, 78*(6), 1752-1764. doi:10.1128/AEM.07454-11

Forbes, J. D., Knox, N. C., Ronholm, J., Pagotto, F., and Reimer, A. (2017). Metagenomics: The next culture-independent game changer. *Front Microbiol, 8*, 1069. doi:10.3389/fmicb.2017.01069

Gerner-Smidt, P., Hise, K., Kincaid, J., Hunter, S., Rolando, S., Hyytia-Trees, E., . . . and Pulsenet, T. (2006). PulseNet USA: a five-year update. *Foodborne Pathogens and Disease, 3*(1), 9-19. doi:10.1089/fpd.2006.3.9

Gilmour, M. W., Graham, M., Van Domselaar, G., Tyler, S., Kent, H., Trout-Yakel, K. M., . . . and Nadon, C. (2010). High-throughput genome sequencing of two *Listeria monocytogenes* clinical isolates during a large foodborne outbreak. *BMC Genomics, 11*, 120. doi:10.1186/1471-2164-11-120

Goudeau, D. M., Parker, C. T., Zhou, Y., Sela, S., Kroupitski, Y., and Brandl, M. T. (2013). The *Salmonella* transcriptome in lettuce and cilantro soft rot reveals a niche overlap with the animal host intestine. *Appl Environ Microbiol, 79*(1), 250-262. doi:10.1128/AEM.02290-12

Heir, E., Moretro, T., Simensen, A., and Langsrud, S. (2018). *Listeria monocytogenes* strains show large variations in competitive growth in mixed culture biofilms and suspensions with bacteria from food processing environments. *International Journal of Food Microbiology, 275*, 46-55. doi:10.1016/j.ijfoodmicro.2018.03.026

Hendriksen, R. S., Pedersen, S. K., Leekitcharoenphon, P., Malorny, B., Borowiak, M., Battisti, A., . . . and Cowley, L. (2018). Final report of ENGAGE - Establishing Next Generation sequencing Ability for Genomic analysis in Europe. *EFSA Supporting Publications, 15*(6). doi:10.2903/sp.efsa.2018.EN-1431

Hood, A. M. (1953). Phage typing of *Staphylococcus aureus*. *J of Hyg, 51*(1), 1-15.

Imran, M., Bre, J. M., Gueguen, M., Vernoux, J. P., and Desmasures, N. (2013). Reduced growth of *Listeria monocytogenes* in two model cheese microcosms is not associated with individual microbial strains. *Food Microbiol, 33*(1), 30-39. doi:10.1016/j.fm.2012.08.008

James, P. (1997). Protein identification in the post-genome era: the rapid rise of proteomics. *Q Rev Biophys, 30*(4), 279-331. doi:10.1017/S0033583597003399

Jia, J., Chen, Y., Jiang, Y., Li, Z., Zhao, L., Zhang, J., . . . and Ye, X. (2015). Proteomic analysis of *Vibrio metschnikovii* under cold stress using a quadrupole Orbitrap mass spectrometer. *Res Microbiol, 166*(8), 618-625. doi:10.1016/j.resmic.2015.07.011

Jolley, K. A., and Maiden, M. C. J. (2014). Using multilocus sequence typing to study bacterial variation: prospects in the genomic era. *Future Microbiology, 9*(5), 623-630. doi:10.2217/fmb.14.24

Jourdan-da Silva, N., Fabre, L., Robinson, E., Fournet, N., Nisavanh, A., Bruyand, M., . . . and Le Hello, S. (2018). Ongoing nationwide outbreak of *Salmonella* Agona associated with internationally distributed infant milk products, France, December 2017. *Euro Surveill, 23*(2). doi:10.2807/1560-7917.ES.2018.23.2.17-00852

Kergourlay, G., Taminiau, B., Daube, G., and Champomier Verges, M. C. (2015). Metagenomic insights into the dynamics of microbial communities in food. *International Journal of Food Microbiology, 213*, 31-39. doi:10.1016/j.ijfoodmicro.2015.09.010

King, T., Kocharunchitt, C., Gobius, K., Bowman, J. P., and Ross, T. (2016). Physiological response of *Escherichia coli* O157:H7 Sakai to dynamic changes in temperature and water activity as experienced during carcass chilling. *Mol Cell Proteomics, 15*(11), 3331-3347. doi:10.1074/mcp.M116.063065

Kleta, S., Hammerl, J. A., Dieckmann, R., Malorny, B., Borowiak, M., Halbedel, S., . . .and Al Dahouk, S. (2017). Molecular tracing to find source of protracted invasive listeriosis outbreak, Southern Germany, 2012-2016. *Emerg Infect Dis, 23*(10), 1680-1683. doi:10.3201/eid2310.161623

Korsak, N., Taminiau, B., Leclercq, M., Nezer, C., Crevecoeur, S., Ferauche, C., . . . and Daube, G. (2015). Short communication: Evaluation of the microbiota of kefir samples using metagenetic analysis targeting the 16S and 26S ribosomal DNA fragments. *J Dairy Sci, 98*(6), 3684-3689. doi:10.3168/jds.2014-9065

Kuo, C. J., Wang, S. T., Lin, C. M., Chiu, H. C., Huang, C. R., Lee, D. Y., . . . and Chen, C. S. (2018). A multi-omic analysis reveals the role of fumarate in regulating the virulence of

enterohemorrhagic *Escherichia coli*. *Cell Death Dis, 9*(3), 381. doi:10.1038/s41419-018-0423-2

Lockett, T. J., Bird, A. R., Christophersen, C., Clarke, J. M., Conlon, M. A., and Topping, D. L. (2016). Microbes, Metabolites and Health. In D. J. Beale, K. A. Kouremenos, and E. A. Palombo (Eds.), *Microbial Metabolomics: Applications in Clinical, Environmental, and Industrial Microbiology* (pp. 13-48). Cham: Springer International Publishing.

Maiden, M. C. (2006). Multilocus sequence typing of bacteria. *Annu Rev Microbiol, 60*, 561-588. doi:10.1146/annurev.micro.59.030804.121325

Maiden, M. C. J., Bygraves, J. A., Feil, E., Morelli, G., Russell, J. E., Urwin, R., . . . and Spratt, B. G. (1998). Multilocus sequence typing: A portable approach to the identification of clones within populations of pathogenic microorganisms. *Proc Natl Acad Sci USA, 95*(6), 3140-3145. doi:10.1073/pnas.95.6.3140

Makarov, A., and Scigelova, M. (2010). Coupling liquid chromatography to Orbitrap mass spectrometry. *J Chromatogr A, 1217*(25), 3938-3945. doi:10.1016/j.chroma.2010.02.022

Mellmann, A., Harmsen, D., Cummings, C. A., Zentz, E. B., Leopold, S. R., Rico, A., . . . and Karch, H. (2011). Prospective genomic characterization of the German enterohemorrhagic *Escherichia coli* O104:H4 outbreak by rapid next generation sequencing technology. *PLoS One, 6*(7), e22751. doi:10.1371/journal.pone.0022751

Munk, P., Knudsen, B. E., Lukjacenko, O., Duarte, A. S. R., Van Gompel, L., Luiken, R. E. C., . . . and Aarestrup, F. M. (2018). Abundance and diversity of the faecal resistome in slaughter pigs and broilers in nine European countries. *Nat Microbiol, 3*(8), 898-908. doi:10.1038/s41564-018-0192-9

Nadon, C., Van Walle, I., Gerner-Smidt, P., Campos, J., Chinen, I., Concepcion-Acevedo, J., . . . and Panel, F.-N. E. (2017). PulseNet International: Vision for the implementation of whole genome sequencing (WGS) for global food-borne disease surveillance. *Euro Surveill, 22*(23). doi:10.2807/1560-7917.ES.2017.22.23.30544

Oest, A., Alsaffar, A., Fenner, M., Azzopardi, D., and Tiquia-Arashiro, S. M. (2018). Patterns of change in metabolic capabilities of sediment microbial communities in river and lake ecosystems. *Int J Microbiol, 2018*, 6234931. doi:10.1155/2018/6234931

Perez-Losada, M., Arenas, M., and Castro-Nallar, E. (2017). Microbial sequence typing in the genomic era. *Infect Genet Evol*. doi:10.1016/j.meegid.2017.09.022

Quainoo, S., Coolen, J. P. M., van Hijum, S., Huynen, M. A., Melchers, W. J. G., van Schaik, W., and Wertheim, H. F. L. (2017). Whole-genome sequencing of bacterial pathogens: The future of nosocomial outbreak analysis. *Clinical Microbiology Reviews, 30*(4), 1015-1063. doi:10.1128/CMR.00016-17

Reed, E., Ferreira, C. M., Bell, R., Brown, E. W., and Zheng, J. (2018). Plant-microbe and abiotic factors influencing *Salmonella* survival and growth on alfalfa sprouts and Swiss chard microgreens. *Appl Environ Microbiol, 84*(9). doi:10.1128/AEM.02814-17

Ronholm, J., Nasheri, N., Petronella, N., and Pagotto, F. (2016). Navigating microbiological food safety in the era of whole-genome sequencing. *Clinical Microbiology Reviews, 29*(4), 837-857. doi:10.1128/CMR.00056-16

Ruppitsch, W., Prager, R., Halbedel, S., Hyden, P., Pietzka, A., Huhulescu, S., . . . and Wilking, H. (2015). Ongoing outbreak of invasive listeriosis, Germany, 2012 to 2015. *Euro Surveill, 20*(50). doi:10.2807/1560-7917.ES.2015.20.50.30094

Schellenberg, J. J., Paramel Jayaprakash, T., Withana Gamage, N., Patterson, M. H., Vaneechoutte, M., and Hill, J. E. (2016). *Gardnerella vaginalis* subgroups defined by cpn60 sequencing and sialidase activity in isolates from Canada, Belgium and Kenya. *PLoS One, 11*(1), e0146510. doi:10.1371/journal.pone.0146510

Stewart, R. D., Auffret, M. D., Warr, A., Wiser, A. H., Press, M. O., Langford, K. W., . . . and Watson, M. (2018). Assembly of 913 microbial genomes from metagenomic sequencing of the cow rumen. *Nat Commun, 9*(1), 870. doi:10.1038/s41467-018-03317-6

Tang, S., Orsi, R. H., den Bakker, H. C., Wiedmann, M., Boor, K. J., and Bergholz, T. M. (2015). Transcriptomic analysis of the adaptation of *Listeria monocytogenes* to growth on vacuum-packed cold smoked salmon. *Appl Environ Microbiol, 81*(19), 6812-6824. doi:10.1128/AEM.01752-15

Tenover, F. C. (1985). Plasmid fingerprinting. A tool for bacterial strain identification and surveillance of nosocomial and community-acquired infections. *Clin Lab Med, 5*(3), 413-436.

Tyson, G. W., Chapman, J., Hugenholtz, P., Allen, E. E., Ram, R. J., Richardson, P. M., . . . and Banfield, J. F. (2004). Community structure and metabolism through reconstruction of microbial genomes from the environment. *Nature, 428*(6978), 37-43. doi:10.1038/nature02340

van Dijk, E. L., Jaszczyszyn, Y., Naquin, D., and Thermes, C. (2018). The third revolution in sequencing technology. *Trends Genet*. doi:10.1016/j.tig.2018.05.008

Villas-Boas, S. G., Mas, S., Akesson, M., Smedsgaard, J., and Nielsen, J. (2005). Mass spectrometry in metabolome analysis. *Mass Spectrom Rev, 24*(5), 613-646. doi:10.1002/mas.20032

Vogt, S. L., Pena-Diaz, J., and Finlay, B. B. (2015). Chemical communication in the gut: Effects of microbiota-generated metabolites on gastrointestinal bacterial pathogens. *Anaerobe, 34*, 106-115. doi:10.1016/j.anaerobe.2015.05.002

Watson, J. D., and Crick, F. H. (1953). Molecular structure of nucleic acids; a structure for deoxyribose nucleic acid. *Nature, 171*(4356), 737-738. doi:10.1038/171737a0

WHO, World Health Organization. (2015). WHO estimates of the global burden of foodborne diseases.

WHO, World Health Organization. (2018). Whole genome sequencing for foodborne disease surveillance Landscape paper.

Wishart, D. S., Knox, C., Guo, A. C., Eisner, R., Young, N., Gautam, B., . . . and Forsythe, I. (2009). HMDB: a knowledgebase for the human metabolome. *Nucleic Acids Res, 37*(Database issue), D603-610. doi:10.1093/nar/gkn810

Wishart, D. S., Tzur, D., Knox, C., Eisner, R., Guo, A. C., Young, N., . . . Querengesser, L. (2007). HMDB: the Human Metabolome Database. *Nucleic Acids Res, 35*(Database issue), D521-526. doi:10.1093/nar/gkl923

Yeluri Jonnala, B. R., McSweeney, P. L. H., Sheehan, J. J., and Cotter, P. D. (2018). Sequencing of the cheese microbiome and its relevance to industry. *Front Microbiol, 9*, 1020. doi:10.3389/fmicb.2018.01020

Zhang, W., Li, F., and Nie, L. (2010). Integrating multiple 'omics' analysis for microbial biology: application and methodologies. *Microbiology, 156*(Pt 2), 287-301. doi:10.1099/mic.0.034793-0

6 Exposure to Humans

Greiner, Matthias

Introduction

Human health is one of the central protection goals we have in mind when researching global food supply networks. Human health risk, along with animal health and plant health can even justify protective measures in international trade according to the WTO Agreement on the Application of Sanitary and Phyto-Sanitary Measures (SPS, see section 4.5). Foodborne health risks can be characterized using information about the hazardous properties of biological or chemical agents in food combined with information about the extent to which humans are exposed to the identified hazards via food consumption. Thus, exposure plays a crucial role in determining the risk of human health impairments and is affected by global trade in many ways. The occurrence of biological or chemical agents in food can depend on the area of origin. The globalized food economy leads to an enormous variety of food products and diversity of the origin of food, food components and ingredients. Consumption patterns and food preferences are also a result of globalization to some extent. As a result, an increasing differentiation of consumption and exposure patterns can be observed. This chapter describes the impact of globalized trade on foodborne exposure using selected examples.

The geographical area of origin of food is one of many factors that may contribute to observed variability in presence and concentration of hazardous agents in food on the German market. However, a declaration of the geographical origin is not mandatory for most food categories. In section 6.1, we explore the extent to which the exposure assessment can be refined by considering the area of origin. One particular example of a trade-related emerging health issue is ciguatera fish poisoning in Germany presented in section 6.2. It exemplifies that “exotic” diseases may occur unexpectedly via food supply networks that – in this case – can originate from European or faraway tropical waters. The chapter concludes with an epidemiological study of foodborne zoonotic infections in section 6.3. This work illustrates how intimately human migration is related with international food trade, legal or illegal and that globalization may affect exposure patterns also through human behaviors and food preferences.

Future Needs

This chapter has demonstrated that exposure patterns for food consumers in Germany are strongly impacted by global trade. What future needs can be deduced from our findings? One area for improvement is the use of area of origin information for a refined dietary exposure assessment. It can be concluded that origin-related scenarios should be used in cases where food origin influences the concentration of hazardous substances in food. Comprehensive origin labelling (primary geographic origin) of processed and non-processed food would have a multitude of benefits. It would allow consumers to make informed choices, support outbreak investigations and allow that origin-related substance concentrations being evaluated. The latter would allow a refined and more informative dietary exposure assessment. How could this be achieved in practice? The German Food Monitoring (GFM) should sample relevant food items by food origin and document the primary geographic food origin in every case it is available (mandatory and voluntary food labelling). Geographical information for processing stages should either not be given or clearly labelled as such to avoid confusion with origin information. Origin information should be trustworthy and therefore be subject of controls.

The ciguatera report has shown the importance of information about the epidemiological background. Endemic cases are known to occur in the Canaries and there is disease aware-

ness in the local population as well as in the public health sector. Nowadays, ciguatera outbreaks in Europe occur due to specific global trade networks for both fresh and frozen fish of various species sourced from tropical waters. Some points can be derived from the ciguatera example to illustrate specific issues of health risks along the global food chain. First, there is the aspect of limited awareness of the hazard and the associated disease in importing countries due to the rare and sporadic outbreak pattern in non-endemic areas. The occurrence of the hazard can empirically be linked to ecological factors that are only known on a local scale or by experts. Secondly, there is an incomplete flow of information along the value chain. The ciguatera example also illustrates that even mislabeling of fish species occurs which hinders effective outbreak investigation, risk mitigation and risk assessment. Thirdly, due to its rare nature there are limited laboratory facilities in place to confirm suspect cases and limited public awareness of the diseases. These factors, in turn, may result in an under-estimation of disease incidences. Thus, international co-operation is needed also in this case to secure that globally traded food is safe.

The study on zoonotic disease risk in Berlin migrant population elucidates other knowledge gaps. Migrant populations are understudied due to language barriers. It is noteworthy that national consumption surveys hitherto have systematically excluded non-German speaking populations which results in lack of knowledge for a significant part of the total population. It seems that specific food preferences do occur that are correlated with higher risk for attracting certain zoonotic infections. This situation merits further investigations. It can also be concluded from this study that risk communication should be better tailored towards specific risk subpopulations.

6.1 Food Origin Information in Times of Global Food Supply – Basis for the Refinement of Dietary Exposure Assessment

Fechner, Carolin; Heseker, Helmut[1]; Lindtner, Oliver; Greiner, Matthias

[1] Department of Sports and Health, Paderborn University, Paderborn, North Rhine-Westphalia, Germany

Abstract

Global trade influences food supply and the geographical origin is an important aspect of food safety. Available origin information is limited by food labelling and reporting in German Food Monitoring (GFM). GFM data on different exemplary food items was used to investigate whether information on the origin is available as per the mandatory food labelling to allow a refined exposure assessment. Origin information was compared to Food and Agriculture Organization of the United Nations (FAO) data. We found that the availability of origin data differs markedly among different food categories in GFM. Furthermore, unspecified origins in GFM mask more detailed information. A plausibility check with FAO data was suitable to verify origin data in GFM for agricultural products (tomatoes, pineapples, kiwi fruits), cocoa powder and olive oil, for tomato and apple juice as well as for meat pieces of cattle and pigs. For fish the comparison of country of origin and catching area recorded in GFM was helpful to evaluate the situation. We found that GFM provides valuable origin data to use this information to develop refined exposure scenarios in the case of agricultural products and olive oil. However, origin data was limited for tomato and apple juice as well as for meat pieces of cattle and pigs. Thus, given the limitation of labelled food origin information, alternative data sources are required. Traceability data which is available in crisis situations would be valuable to identify existing delivery channels and to refine dietary exposure assessment.

Introduction

Trade is globalized and food safety is influenced by worldwide food supply and complex delivery (FAO, 2015; Huang, 2004). Food can be derived from different origins which is less transparent because of the increasing complexity (Aruoma, 2006). As an important basis for risk assessment and food safety, dietary exposure assessments are carried out using standard scenarios whereby a so-called brand-loyal consumer is considered to capture conservative assumptions regarding contamination levels (Sarvan et al., 2017). Geographic food origin is not included in standard exposure assessments but, as well as the brands, food origin is of high interest in connection with global trade and the likely impact of geographical origin. Similar considerations on seasonal influences on substance concentration have already been tested for the implementation in total diet studies (Elegbede et al., 2017).

There are several major steps in food supply chains which could increase the complexity (FAO, 2017; Parfitt et al., 2010; Porter and Reay, 2016). Different influences on substances and finally on dietary exposure are possible within supply chains and summarized in a conceptual depiction of potential connections (Figure 43) Local conditions in agricultural primary production could influence substance concentration in foods and dietary exposure as well as factors in transport, storage and further production. The primary origin of a food item can be thought of as a proxy for various factors, such as geographical sourcing, production system or processing that may have an impact on substance levels. Based on this concept, exposure assessment could be extended to origin relations developing refined approaches.

The labelling of the primary geographical origin is an important basis for origin-related dietary exposure assessment. It has been shown that such a refined exposure assessment is possi-

ble (Fechner et al., 2019). However, origin information is only legally required for some, mostly agricultural products but not for processed products (D'Elia et al., 2011; EC, 2011b, 2012, 2013c; Eur. Parliament and The Council, 2000). In this case, origin information may be available based on voluntary declarations. In contrast, explicit information about supply networks is made available to the authorities in crisis situations because food producers involved in the affected supply chain have to share traceability data with competent authorities (Eur. Parliament and The Council, 2002d). This kind of data was exemplarily used to identify supply networks (Buchholz et al., 2011). Traceability of food and food ingredients is based on a mandatory documentation which has to be implemented by business operators to identify their suppliers and the businesses to which their products are supplied (Eur. Parliament and The Council, 2002d). This information has to be documented at all stages of production, processing and distribution but it is not labelled on the food product (Eur. Parliament and The Council, 2002d). Such data collected in the event of a food crisis would in principle be valuable in origin-related exposure assessment, despite the fact that it is not collected for that purpose and captures only a fraction of the market in terms of time window and food categories.

We wanted to explore which kind of origin data is reported in German Food Monitoring (GFM) and whether this database contains enough data on food origin to be used for a refinement of exposure assessment.

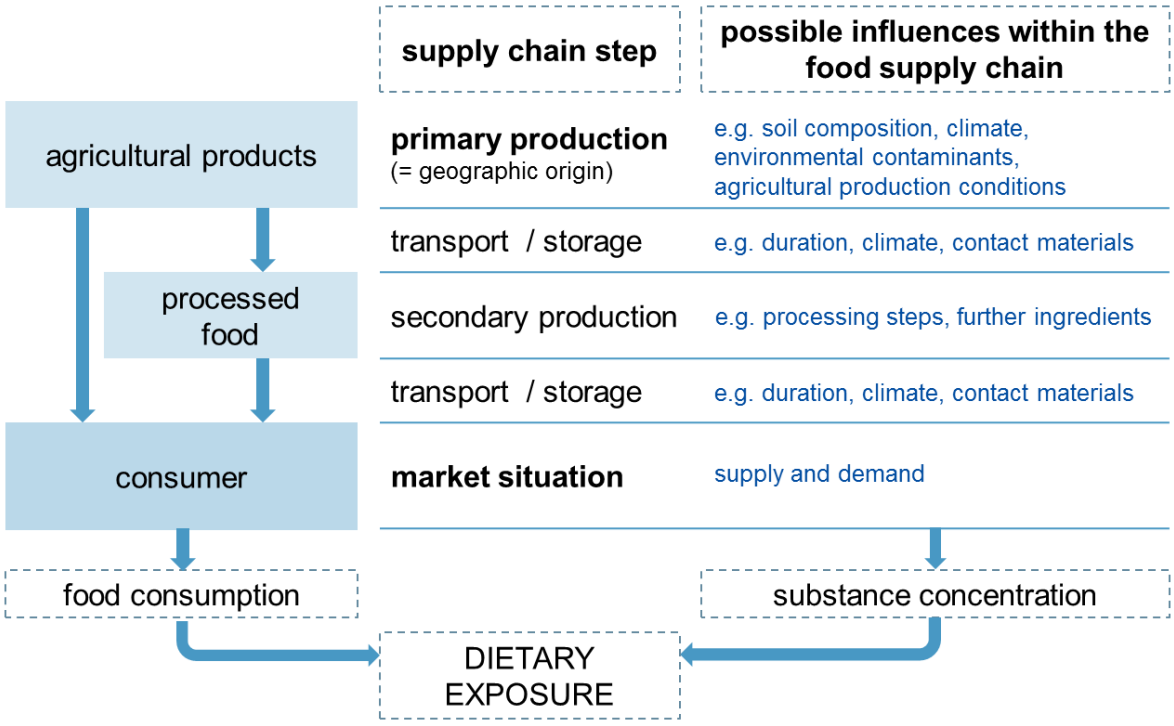


Figure 43: Possible influences within the food supply chain on substance concentration and dietary exposure

Materials and Methods

German Food Monitoring (GFM) data including projects between 2005 and 2015 was used to derive origin information for selected food items, as this data source is important to obtain concentration data for dietary exposure assessment (BVL, 2018b; Lindtner et al., 2013; Sarvan et al., 2017; Sieke et al., 2008). For each available sample of the selected food items, we extracted the country of origin and, in the case of fish also the catching areas as additional provided origin information. For fish, information on country of origin and catching area

was cross tabulated. GFM contains generalised origin information like "without declaration", "unexplained", "unknown foreign country" or "from sea" and also a few undefined codes we summarised as “unspecified origins”. We selected as food items agricultural products, for which it is obligatory to label the country of origin (D'Elia et al., 2011; EC, 2011b), processed products and fish products as examples to study for which general origin information is available for a refined dietary exposure assessment.

To check if the origin declarations in GFM correspond to existing cultivation areas for vegetable agricultural products and processed products thereof, Food and Agriculture Organization of the United Nations (FAO) production data on the yield of appropriate crops was used (FAO, 2018). In the case of meat, FAO production data on stocks of appropriate live animals was used (FAO, 2018). Countries worldwide were checked for existing data in the FAO databases within the monitoring period 2005 – 2015. If a continent instead of a specific country was given in GFM, FAO data was checked for countries within this continent.

Results

For tomatoes, pineapples and kiwi fruits as agricultural products, origin data was available from GFM (Figure 44, Table 25). For tomatoes, the check of FAO data showed 96.5 % (i.e. N = 799) plausible origins in GFM, as the given countries were also listed as cultivation areas in FAO data. No additional specific origins appeared in GFM data which were not part of the FAO database. Most of the tomatoes sampled by GFM on the German market originated from the Netherlands (32.5 %), Spain (24.8 %) and Germany (19.6 %). In contrast to tomatoes, pineapples and kiwi fruits are exotic fruits and only imported, as Germany was not a defined cultivation area in FAO data. For pineapples, the check of GFM using FAO data showed 93.6 % (i.e. N = 335) plausible origins. But for 1.4 % (i.e. N = 5) of the samples Germany, Monaco, Turkey or British pending regions in Europe were named as countries of origin which did not match with FAO data. For kiwi fruits 96.4 % (i.e. N = 238) of the GFM samples had origins which are also shown in FAO data. No additional specific origins appeared in GFM data which were not part of the FAO database. For tomatoes, pineapples and kiwi fruits, unspecified origin information ranged between 3.5 % and 5.0 %.

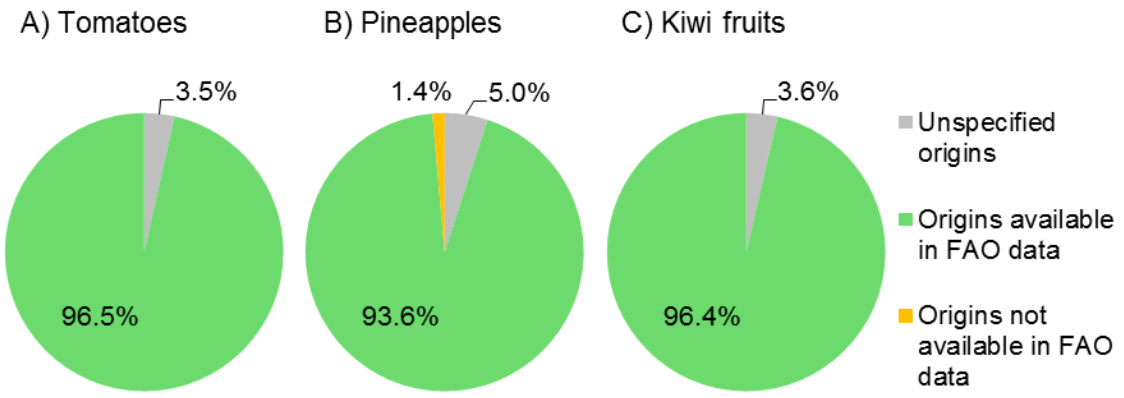


Figure 44: Summarised information on geographical origin for selected agricultural products sampled in the German Food Monitoring (GFM) 2005 – 2015 and checked for plausibility with FAO data

Table 25: Information on geographical food origin for selected agricultural products sampled in the German Food Monitoring (GFM) 2005 – 2015 and checked for plausibility with FAO data

Country of origin	Tomatoes		Pineapples		Kiwi fruits	
	N	%	N	%	N	%
Germany	162 [a]	19.6 [a]	1	0.3	-	-
Belgium	43 [a]	5.2 [a]	-	-	-	-
Bulgaria	1 [a]	0.1 [a]	-	-	-	-
France, incl. Corsica	18 [a]	2.2 [a]	-	-	8 [a]	3.2 [a]
Greece	1 [a]	0.1 [a]	-	-	22 [a]	8.9 [a]
Italy	49 [a]	5.9 [a]	-	-	139 [a]	56.3 [a]
Malta	2 [a]	0.2 [a]	-	-	-	-
Monaco	-	-	1	0.3	-	-
Netherlands	269 [a]	32.5 [a]	-	-	-	-
Portugal	-	-	-	-	1 [a]	0.4 [a]
Spain	205 [a]	24.8 [a]	-	-	2 [a]	0.8 [a]
Turkey	8 [a]	1.0 [a]	1	0.3	-	-
British pending regions in Europe**	-	-	2	0.6	-	-
Africa	-	-	1 [a]	0.3 [a]	-	-
Côte d'Ivoire	-	-	4 [a]	1.1 [a]	-	-
Ghana	-	-	20 [a]	5.6 [a]	-	-
Morocco	22 [a]	2.7 [a]	-	-	-	-
Mauritius	-	-	2 [a]	0.6 [a]	-	-
Cameroon	-	-	3 [a]	0.8 [a]	-	-
South Africa	-	-	5 [a]	1.4 [a]	-	-
Senegal	4 [a]	0.5 [a]	-	-	-	-
Tunisia	2 [a]	0.2 [a]	-	-	-	-
Chile	-	-	-	-	12 [a]	4.9 [a]
Costa Rica	-	-	263 [a]	73.5 [a]	-	-
Ecuador, incl. Galapagos Islands	-	-	13 [a]	3.6 [a]	-	-
Honduras	-	-	8 [a]	2.2 [a]	-	-
Panama	-	-	16 [a]	4.5 [a]	-	-
Israel	13 [a]	1.6 [a]	-	-	-	-
New Zealand	-	-	-	-	54 [a]	21.9 [a]
Unspecified origins*	29	3.5	18	5.0	9	3.6
Total	828	100.0	358	100.0	247	100.0

* "without declaration", "unexplained", "unknown foreign country" summarised
** Gibraltar, Isle of Man, Channel Islands

[a] plausible primary geographical origin because available in FAO data as cultivation area

Also processed products (Figure 45, Table 26,) showed available origin data from GFM. Concerning tomato juice and apple juice, a majority of 70.8 % (i.e. N = 109) and 90.9 % (i.e. N = 731) of the samples in GFM had German origin which was in line with FAO data because apples and tomatoes are cultivated in Germany. Unspecified origin information amounted to 25.3 % (i.e. N = 39) and 7.8 % (i.e. N = 63).

For cocoa powder, GFM data showed only 9.0 % (i.e. N = 18) origins corresponding to FAO data for the production of cocoa beans, 65.2 % (i.e. N = 131) of the declared countries of origin in GFM did not match with FAO data. Furthermore, 25.9 % (i.e. N = 52) of the origins in GFM were unspecific.

For olive oil, 12.5 % (i.e. N = 64) of the samples had German origin which was not in line with FAO information because olives are not cultivated in Germany. For 8.6 % (i.e. N = 44) of the samples, the origin was unspecified, most of the other samples were of Mediterranean origin which was in line with the olive cultivation according to FAO data.

For meat pieces of cattle and pigs, GFM data showed 91.2 % (i.e. N = 416) and 92.1 % (i.e. N = 209) German origin as well as 2.6 % (i.e. N = 12) and 7.0 % (i.e. N = 16) unspecified origins. According to FAO data, all the specific countries stated in GFM were also areas with existing cattle and pig stocks and therefore plausible.

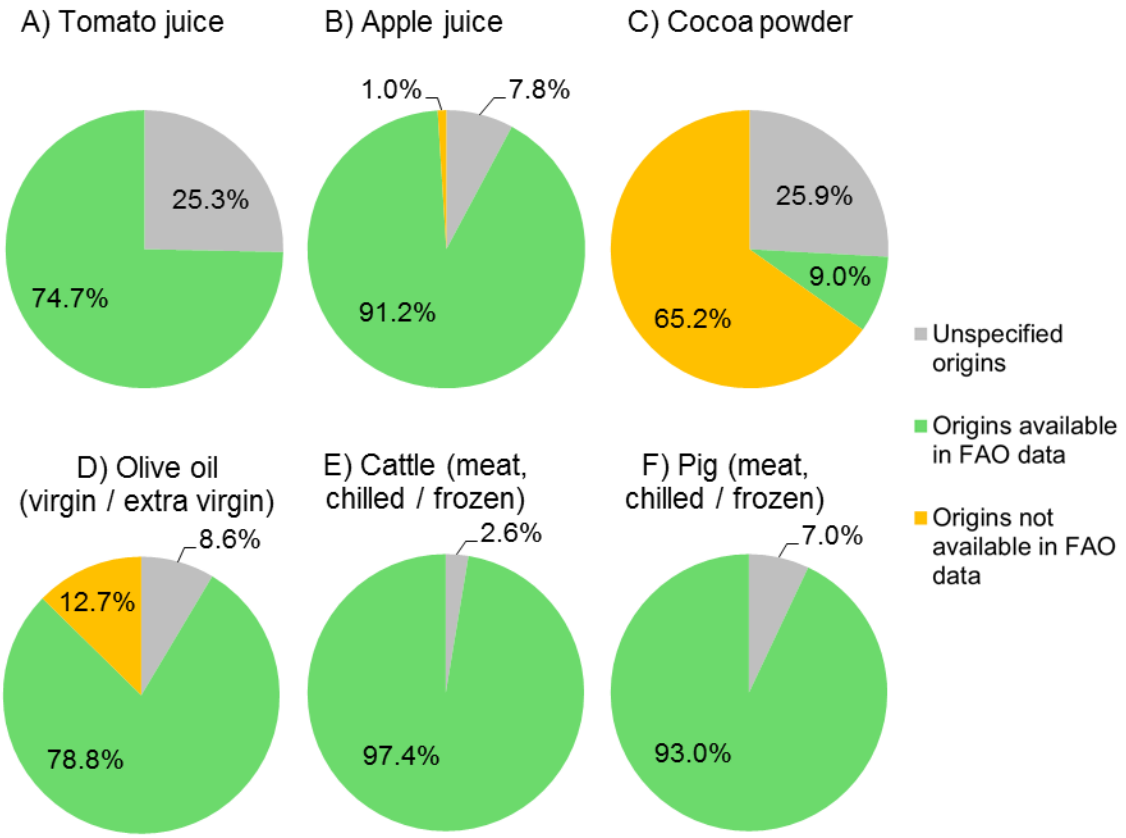


Figure 45: Summarised information on geographical origin for selected processed food products sampled in the German Food Monitoring (GFM) 2005 – 2015 and checked for plausibility with FAO data

Table 26: Information on geographical food origin for selected processed food products sampled in the German Food Monitoring (GFM) 2005 – 2015 and checked for plausibility with FAO data

Country of origin	Food items (N)					
	Tomato juice	Apple juice	Cocoa powder	Olive oil (virgin / extra virgin)	Cattle (meat pieces, chilled / frozen)	Pig (meat pieces, chilled / frozen)
Germany	109 [a]	731 [a]	114	64	416 [a]	209 [a]
EU / Europe	4 [a]	2 [a]	-	59 [a]	-	-
Belgium	-	-	-	-	-	1 [a]
France, incl. Corsica	-	-	1	5 [a]	6 [a]	-
Greece	-	-	-	119 [a]	-	-
Italy	1 [a]	-	1	154 [a]	1 [a]	-
Netherlands	-	-	8	-	1 [a]	1 [a]
Austria	-	8	1	-	1 [a]	-
Poland	-	-	1	-	-	-
Portugal	-	-	-	3 [a]	-	-
Switzerland	-	-	2	-	-	-
Russian Federation	-	-	1	-	-	-
Spain	-	-	1	53 [a]	-	-
Turkey	1 [a]	-	-	6 [a]	-	-
Czech Republic	-	-	-	-	1 [a]	-
United Kingdom	-	-	1	-	-	-
Africa	-	-	11 [a]	-	-	-
Cote d'Ivoire	-	-	1 [a]	-	-	-
Morocco	-	-	-	1 [a]	-	-
Tunisia	-	-	-	3 [a]	-	-
Argentina	-	-	-	-	9 [a]	-
Bolivia	-	-	1 [a]	-	-	-
Brazil	-	-	-	-	6 [a]	-
Chile	-	-	-	-	1 [a]	-
Dominican Republic	-	-	2 [a]	-	-	-
Peru	-	-	2 [a]	-	1 [a]	-
Uruguay	-	-	-	-	1 [a]	-
Georgia	-	-	-	1	-	-
Indonesia, incl. Irian Jaya	-	-	1 [a]	-	-	-
Syria	-	-	-	1 [a]	-	-
Unspecified origins*	39 (25.3 %)	63 (7.8 %)	52 (25.9 %)	44 (8.6 %)	12 (2.6 %)	16 (7.0 %)
Total	154	804	201	513	456	227

* "without declaration", "unexplained", "unknown foreign country" summarised
[a] plausible primary geographical origin because available in FAO data as cultivation area or animal stock area

For fish, country of origin data and information on catching areas was available in GFM. This was considered for Atlantic herring with overall 140 samples Table 27) and tuna filet with overall 173 samples (Table 28). For herring 52.9 % (i.e. N = 74) of the samples originated from European countries or Canada while the cross tabulation showed the connection to the water bodies Baltic Sea, North Sea, Atlantic and the river Elbe (Table 27). The country of origin was unspecified for 22.9 % (i.e. N = 32) and 37.9 % (i.e. N = 53) were unspecified in the catching area, while for 13.6 % (i.e. N = 19) of the samples both applied.

Another situation was found for tuna filet because only 24.9 % (i.e. N = 43) of the GFM samples were from specific summarized geographical regions connected to the Indian Ocean, Atlantic regions and Pacific regions shown by cross tabulation (Table 28). The geographical regions which were sampled are more global than for herring. The country of origin was unspecified for 23.1 % (i.e. N = 40) of the samples and 69.4 % (i.e. N = 120) were unspecified in the catching area, while for 17.3 % (N = 30) of the samples both applied.

Table 27: Information on geographic origin and catching area for Atlantic herring (Clupea harengus) sampled in the German Food Monitoring (GFM) 2005 – 2015

Catching area (N)	Country of origin** (N)									
	DE	DK	IS	NL	NO	PL	GB	CA	Unspecified origins*	Total
Baltic Sea	30	4	-	-	-	-	-	-	1	35
Western Baltic Sea (Kiel Fjord)	3	-	-	-	-	-	-	-	-	3
North Sea	-	4	-	-	-	-	-	-	-	4
Norwegian coast	-	-	-	-	1	-	-	-	-	1
North Atlantic	-	-	-	-	-	-	-	-	1	1
North-west Atlantic (Greenland)	2	1	-	-	1	-	-	-	-	4
North-east Atlantic without Baltic Sea	18	3	-	4	1	-	1	-	11	38
Elbe	1	-	-	-	-	-	-	-	-	1
Unspecified origins*	17	8	1	2	3	2		1	19	53
Total	71	20	1	6	6	2	1	1	32	140

* "without declaration", "unexplained", "unknown foreign country", "from sea" and undefined codes summarised
** according to ISO 3166 ALPHA-2

Table 28: Information on geographic origin and catching area for tuna filet (Thunnus sp.) sampled in the German Food Monitoring (GFM) 2005 – 2015

Catching area (N)	Summarised region of origin (N)					
	Germany	Rest of Europe	South America	Asia	Unspecified origins*	Total
Indian Ocean	14	1	-	10	3	28
Atlantic, not specified	1	-	-	-	2	3
North-east Atlantic without Baltic Sea	1	-	-	-	-	1
Middle-west Atlantic	-	-	1	-	-	1
Middle-east Atlantic	-	1	-	-	-	1
South-west Atlantic	1	-	-	-	-	1
South-east Atlantic	-	-	-	1	-	1
Mediterranean Sea	-	1	-	-	-	1
Pacific, not specified	3	-	-	-	3	6
Central-west Pacific	5	-	-	3	2	10
Unspecified origins*	50	8	1	31	30	120
Total	75	11	2	45	40	173

* "without declaration", "unexplained", "unknown foreign country", "from sea" and undefined codes summarised

Discussion

Origin information in German Food Monitoring and Food and Agriculture Organization of the United Nations data

A check of GFM data for the agricultural commodities tomatoes, pineapples and kiwi fruits with FAO data on crop production helped to evaluate if the given countries of origin are verified as cultivation areas. This is important because the annual GFM reports declare that especially the stated origin Germany does not necessarily correspond to the country of origin but to the place of processing or packaging which was the case e.g. for grapefruits and pineapples in 2013 (BVL, 2013c). In this way, the country of origin is not defined detailed in GFM and other processing or packaging stages could be named. For tomatoes, pineapples and kiwi fruits, more than 90 % of the samples had origin declarations which were in line with FAO data and represented different cultivation areas (Figure 44). Comprehensive and specific origin information was available. This might also be due to the mandatory country of origin labelling for tomatoes, pineapples and kiwi fruits (D'Elia et al., 2011; EC, 2011b, 2013c; Eur. Parliament and The Council, 2013a) which is supported by the results here showing a low percentage of unspecific origins (maximal 5.0 %). Further substance-specific considerations based on origin-related grouping are possible based on the available data in GFM (Fechner et al., 2019).

In comparison to agricultural commodities, for processed food items, several further processing and packaging stages are possible. These stages could be stated as country of origin in GFM, as annual reports declare that especially the stated origin Germany does not necessarily correspond to the primary geographical origin but to a processing or packaging place (BVL, 2013c). In the case of cocoa powder, the comparison of GFM data with the quite specific cultivation areas for cocoa beans stated in FAO data (FAO, 2018) gave important information (Table 26). All the European regions were identified as processing stages and

could be excluded as primary geographical origins. Results show only 9.0 % of the food items had correct origins in GFM corresponding to FAO data. This could be due to the missing mandatory country of origin labelling for cocoa powder as a processed product (Eur. Parliament and The Council, 2011). For cocoa powder, origin information in GFM is not sufficient for further origin-related substance grouping because there is not enough information on primary geographic origins.

For olive oil, the comparison of origin data from GFM with FAO data (FAO, 2018) identified that Germany is not a cultivation area for olives and Mediterranean regions were verified (Table 26). This is supported by a mandatory origin labelling for virgin and extra virgin olive oil which informs about the cultivation region of olives (D'Elia et al., 2011; EC, 2012). But labelling provisions leave some space for unspecific designations like “blend of olive oils of European Union origin” and also more complicated wording concerning cultivation and milling locations (D'Elia et al., 2011; EC, 2012). This could be an explanation for 8.6 % unspecific origins in the results here. For olive oil, the mandatory origin labelling and the exclusion of Germany as cultivation area using FAO data enables the verification of the origin data in GFM. Data of 78.8 % plausible and specific origins could be used for further substance-specific considerations based on origin-related grouping.

For tomato and apple juice as well as for meat pieces of cattle and pigs, Germany is mostly given as origin information (Table 26). As a matter of fact, and consistently reported in FAO data, Germany is a cultivation country for tomatoes and apples and there are also stocks for live cattle and pigs (FAO, 2018). The high reporting of the German origin could result from the missing origin labelling regulations for fruits and vegetables in juices and other processing stages like packaging could be stated as this is not clearly defined in GFM (BVL, 2013c). Existing labelling regulations for the origin of unprocessed beef (Eur. Parliament and The Council, 2000) and pork (Eur. Parliament and The Council, 2011) have been applied since 2000 and 2014 and force producers to state places of birth, rearing and slaughter. This may implicate some uncertainty which information is recorded in GFM as the country of origin is not clearly defined as primary geographic origin and places of birth and rearing could be relevant at the same time (BVL, 2013c). Furthermore, in GFM, food items are generally not sampled with stratification for origin (BVL, 2013b). Therefore, samples of German origin from local producers such as agricultural products or meat might be over-represented. For juices and meat products considered in GFM, the reported origins do not represent many different countries as Germany or unspecific origins are primarily named. Origin information in GFM is not sufficient for further origin-related substance grouping.

For fish, the comparison of the GFM information on countries of origin and catching areas provided some insight into the situation and to see if the data is plausible (Table 27, Table 28). In GFM, the catching area gives information on the living environment of fish and is relevant as primary geographical origin. The country of origin could give an additional hint about the starting point of the supply chain. For tuna filet, it was noticeable that the assignment of Indian Ocean to Europe including Germany, as well as the relation of Pacific regions to Germany include quite a long distance between catching area and country of origin as the waterbody does not border on the landmass. This could be a sign for the reporting of later processing or packaging stages in GFM as country of origin instead of the first country where the fish arrived from sea. However, data is lacking to fully elucidate the supply chain and reporting practice. In the case of tuna filet, origin information in GFM is not sufficient for further origin-related substance grouping because 69.4 % of the catching area was unspecified. On the one hand this could result from labelling regulations for fish because the catching area according to FAO categorization has to be stated for unprocessed fish and could be just voluntarily stated for further fish products like tuna filet (Eur. Parliament and The Council, 2013b). On the other hand the catching area is additional information in GFM and perhaps not documented in every case. For herring, specific data is available which could be used for further substance-specific considerations based on origin-related grouping, as slightly more

than 50 % of the samples had specific declarations in catching area and country of origin. But the reported waterbodies were near each other and therefore it has to be checked if substance-specific origin-related differences appear or if herring migrates constantly within one large waterbody and is caught in different sub-regions.

Origin data from GFM is available for all considered food items but may be only usable for further origin-related considerations for the agricultural products, olive oil and herring. For other case examples, too much data was unspecified or implausible. An additional check of FAO data could identify wrongly reported origins in GFM because they are not listed as cultivation areas. This is only useful under the assumption that the FAO data is comprehensive and error-free and provides insights for food ingredients which are cultivated in specific and limited areas. There remain two main limitations in GFM despite the plausibility check with FAO data. Firstly, the food sampling strategy on the German market does not include representativeness of available origins(BVL, 2013b) and therefore relevant origins could be missing. Secondly, the definition of origin information in GFM is not detailed which makes it able to state processing stages instead of primary origins (BVL, 2013c). Additionally, this is complemented by missing origin information on the packaging especially for processed products because of lacking regulations which could trigger the reporting of other labelled processing stages in GFM. These facts can lead to an over-representation of certain countries of origin or unspecified origins in GFM which complicates further considerations concerning origin-related exposure assessment.

Requirements for origin-related dietary exposure assessment

Standard scenarios for deterministic dietary exposure assessment can be refined by origin-related scenarios calculating with origin-specific grouped concentrations from GFM (Fechner et al., 2019). This is especially relevant if substance concentrations vary significantly by country of origin as has been shown for cadmium in cocoa products, for example (Abt et al., 2018; BfR, 2007) and if there are indications that some consumers prefer food from a specific country of origin. In GFM, various substances in agricultural products or processed food items are analysed and origin information is also partly provided (Sieke et al., 2008). Gathering origin data in addition to other information is time-consuming but could be easier in future with the help of barcodes and electronic support for automated documentation. More complete information on origin would be highly desirable from an exposure assessment point of view. This could be representative data on food origins on the German market, import and export data by the United Nations (UN, 2018) to identify cultivation areas of agricultural products which are supplied to Germany or data on food packaging including origin labelling e.g. by the Global New Product Database of Mintel (Mintel International Group Ltd., 2018). It should be kept in mind that origin information is only a proxy for many individual, typically unobserved causal factors for elevated substance levels. A further refinement of exposure assessment is theoretically possible and advantageous if the respective data is available.

Furthermore, tracing data which is only available in crisis situations (Eur. Parliament and The Council, 2002d) would be of great value to identify existing supply chains and evaluate the stability and composition of trade patterns over longer time periods. There are tools available to display tracing data in crisis situations (Weiser et al., 2016). However, outbreak tracing data is event-based and not able to represent the long-term supply chain situation besides the fact the data is not available for risk assessment in “peace time”. The food items covered in event-based tracing data are also highly selected with regard to known or suspect role as vehicle in an outbreak. Unless tracing data becomes more generally available as part of a transparency policy of the agri-food industry, the use of such data remains conceptual and restricted to research projects. It should be noted that also food consumption data needs to be refined to better capture the individual choices of consumers with regard to food origin.

Last but not least, there is continuous research interest in clarifying the causal factors underlying geographical differences in concentration levels of undesired substances in food.

Conclusions

The German Food Monitoring (GFM) is a comprehensive database providing information on substance concentrations in food for dietary exposure assessment in Germany. Also origin information is recorded. For some food items comprehensive origin data is available and origin-specific exposure scenarios could be performed already. But there are also cases, especially processed food items, where origin data is not sufficient and therefore more origin-specific data should be gathered or alternative approaches to model origin-specific concentrations have to be developed. Information on the food supply chain as available in crisis situations would be helpful to refine dietary exposure assessment.

Acknowledgements

This study uses existing data sources made available by the data owners for free as referenced in the text. The BfR doctoral training programme gives the chance to carry out this research to be used for a PhD thesis.

Abbreviations

GFM	German Food Monitoring
FAO	Food and Agriculture Organization of the United Nations

References

Abt, E., Fong Sam, J., Gray, P., and Robin, L. P. (2018). Cadmium and lead in cocoa powder and chocolate products in the US Market. *Food Additives & Contaminants: Part B Surveillance, 11*(2), 92-102. doi:10.1080/19393210.2017.1420700

Aruoma, O. I. (2006). The impact of food regulation on the food supply chain. *Toxicology, 221*(1), 119-127. doi:10.1016/j.tox.2005.12.024

BfR, Bundesinstitut für Risikobewertung. (2007). BfR schlägt die Einführung eines Höchstgehalts für Cadmium in Schokolade vor. Stellungnahme Nr. 015/2007 des BfR vom 31.01.2007 [Statement]. *15/2007*, 1-18.

Buchholz, U., Bernard, H., Werber, D., Böhmer, M. M., Remschmidt, C., Wilking, H., . . . and Dreesman, J. (2011). German outbreak of Escherichia coli O104: H4 associated with sprouts. *New England Journal of Medicine, 365*(19), 1763-1770. doi:10.1056/NEJMoa1106482

BVL, Bundesamt für Verbraucherschutz und Lebensmittelsicherheit. (2013b). Handbuch Monitoring 2013. 1-1 - 7-6.

BVL, Bundesamt für Verbraucherschutz und Lebensmittelsicherheit. (2013c). *Berichte zur Lebensmittelsicherheit 2013. Monitoring. Gemeinsamer Bericht des Bundes und der Länder* (9.3). https://www.bvl.bund.de/DE/01_Lebensmittel/01_Aufgaben/02_AmtlicheLebensmittelueberwachung/04_Monitoring/lm_monitoring_Berichte_Archiv/lm_monitoring_Berichte_Archiv_node.html

BVL, Bundesamt für Verbraucherschutz und Lebensmittelsicherheit. (2018b). The monitoring programme. https://www.bvl.bund.de/EN/01_Food/_01_tasks/02_OfficialFoodControl/04_LM_Monitoring_en/LM_Monitoring_EN_node.html

D'Elia, G., Alpigiani, I., Bonardi, S., Bacci, C., Lanzoni, E., and Brindani, F. (2011). Food labelling in Europe: mandatory "country of origin" extended to more foods. *Annali della Facoltà di Medicina Veterinaria, Università di Parma, 31*, 65-79.

EC, European Commission. (2011b). Commission Implementing Regulation (EU) No 543/2011 of 7 June 2011 laying down detailed rules for the application of Council Regulation (EC) No 1234/2007 in respect of the fruit and vegetables and processed fruit and vegetables sectors. *Official Journal of the European Union, 157*, 1-163.

EC, European Commission. (2012). Commission Implementing Regulation (EU) No 29/2012 of 13 January 2012 on marketing standards for olive oil. *Official Journal of the European Union, 12*, 14-21.

EC, European Commission. (2013c). Commission Implementing Regulation (EU) No 594/2013 of 21 June 2013 amending Implementing Regulation (EU) No 543/2011 as regards marketing standards in the fruit and vegetables sector and correcting that Implementing Regulation. *Official Journal of the European Union, 170*, 43-59.

Elegbede, C. F., Papadopoulos, A., Kolbaum, A. E., Turrini, A., Mistura, L., Lindtner, O., and Sirot, V. S. (2017). TDS exposure project: How and when to consider seasonality in a total diet study? *Food and Chemical Toxicology, 105*, 119-126. doi:10.1016/j.fct.2017.03.045

Eur. Parliament, and The Council. (2000). Regulation (EC) No 1760/2000 of the European Parliament and of the Council of 17 July 2000 establishing a system for the identification and registration of bovine animals and regarding the labelling of beef and beef products and repealing Council Regulation (EC) No 820/97. *Official Journal of the European Communities, L204*, 1-10.

Eur. Parliament, and The Council. (2002d). Regulation (EC) No 178/2002 of the European Parliament and the council of the European Union of 28 January 2002 laying down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety. *Official Journal of the European Communities, L 31*, 1-24.

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

Eur. Parliament, and The Council. (2013a). Regulation (EU) No 1308/2013 of the European Parliament and of the Council of 17 December 2013 establishing a common organisation of the markets in agricultural products and repealing Council Regulations (EEC) No 922/72, (EEC) No 234/79, (EC) No 1037/2001 and (EC) No 1234/2007. *Official Journal of the European Union, L347*, 671-854.

Eur. Parliament, and The Council. (2013b). Regulation (EU) No 1379/2013 of the European Parliament and of the Council of 11 December 2013 on the common organisation of the markets in fishery and aquaculture products, amending Council Regulations (EC) No 1184/2006 and (EC) No 1224/2009 and repealing Council Regulation (EC) No 104/2000. *Official Journal of the European Union, L354*, 1-21.

FAO, Food and Agriculture Organization of the United Nations. (2015). The State of Agricultural Commodity Markets. Trade and food security: achieving a better balance between national priorities and the collective good. 1-75.

FAO, Food and Agriculture Organization of the United Nations. (2017). The State of Food and Agriculture 2017. Leveraging Food Systems for Inclusive Rural Transformations. 1-160.

FAO, Food and Agriculture Organization of the United Nations. (2018). FAOSTAT. FAOSTAT Retrieved 2018-02-28, from Food and Agriculture Organization of the United Nations. http://www.fao.org/faostat/en/#data

Fechner, C., Greiner, M., Heseker, H., and Lindtner, O. (2019). Refinement of dietary exposure assessment using origin-related scenarios. *Journal of Exposure Science & Environmental Epidemiology*. doi:10.1038/s41370-019-0117-6

Huang, S. (2004). Global trade patterns in fruits and vegetables. *USDA-ERS Agriculture and Trade Report No. WRS-04-06, United States Department of Agriculture*, 1-83.

Lindtner, O., Berg, K., Blume, K., Fiddicke, U., and Heinemeyer, G. (2013). The German approach to estimating dietary exposures using food monitoring data. In G. G. Moy and R. W. Vannoort (Eds.), *Total Diet Studies* (pp. 521-530). New York, US: Springer.

Mintel International Group Ltd. (2018). Global New Product Database (GNPD) (Web page). Global New Product Database (GNPD) Retrieved 2018-05-28, from Mintel International Group Ltd. http://www.gnpd.com/sinatra/home/

Parfitt, J., Barthel, M., and Macnaughton, S. (2010). Food waste within food supply chains: quantification and potential for change to 2050. *Philosophical Transactions of the Royal Society B: Biological Sciences, 365*(1554), 3065-3081. doi:10.1098/rstb.2010.0126

Porter, S. D., and Reay, D. S. (2016). Addressing food supply chain and consumption inefficiencies: potential for climate change mitigation. *Regional Environmental Change, 16*(8), 2279-2290. doi:10.1007/s10113-015-0783-4

Sarvan, I., Bürgelt, M., Lindtner, O., and Greiner, M. (2017). Expositionsschätzung von Stoffen in Lebensmitteln: Die BfR-MEAL-Studie – die erste Total-Diet-Studie in Deutschland. [Dietary exposure assessment of substances in foods: The BfR MEAL study – The first German total diet study]. *Bundesgesundheitsblatt – Gesundheitsforschung – Gesundheitsschutz, 60*(7: Kontaminanten in Lebensmitteln), 689-696. doi:10.1007/s00103-017-2566-1

Sieke, C., Lindtner, O., and Banasiak, U. (2008). Pflanzenschutzmittelrückstände. Nationales Monitoring – Abschätzung der Verbraucherexposition: Teil 1. *Deutsche Lebensmittel-Rundschau, 104*(6), 271-279.

UN, United Nations. (2018). UN Comtrade Database - International Trade Statistics Database (Web page). Retrieved 2018-02-28, from United Nations. https://comtrade.un.org/data

Weiser, A. A., Thöns, C., Filter, M., Falenski, A., Appel, B., and Käsbohrer, A. (2016). FoodChain-Lab: A Trace-Back and Trace-Forward Tool Developed and Applied during Food-Borne Disease Outbreak Investigations in Germany and Europe. *PLoS One, 11*(3), e0151977. doi:10.1371/journal.pone.0151977

6.2 Ciguatera food poisoning in Germany caused by imported tropical fish from tropical areas

Friedemann, Miriam

Abstract

Ciguatera is the most common fish intoxication in tropical areas. Nowadays, ciguatera occurs in non-endemic countries due to increasing imports of tropical fish. The first ciguatera outbreak in Germany occurred in 2012. In total, fresh or frozen snapper fish (Lutjanus) from India, Indonesia and Vietnam caused six outbreaks with 68 cases of ciguatera fish poisoning in 2012 to 2018. Fourteen of the patients were hospitalized, and three of them showed a severe degree of poisoning. Fresh fish was imported via Frankfurt/Main airport. Frozen snappers were imported by ship via ports in Belgium, Denmark, Germany and the Netherlands. Production and distribution chains of imported fresh fish are short, clear and traceable whereas the process of production and distribution of frozen fish is complex and not easy to track. Mislabeling of the fish species and of the origin of the fish was found on various levels of the distribution chain when import, wholesale and retail documents were compared. The series of ciguatera outbreaks in Germany show that preventive measures should be strengthened at all points of the global production chain of the snappers. The manufacturers and exporters in the countries of origin of the fish should consider and communicate the local health risk of ciguatera to importers and wholesalers in non-endemic countries.

Ciguatera – The most common fish intoxication worldwide

Ciguatera is estimated to be the most common fish intoxication with 50,000 to 500,000 annual cases worldwide (Friedman et al., 2017). In high risk areas of the Pacific Islands, where fish is a staple food, incidences may locally reach 150 (e.g. Cook Islands) to 464 (Raivavae Islands) cases per 10,000 inhabitants per year (Chinain et al., 2010a) resulting in nutritional, secondary medical and social problems.

Ciguatera is characterized by a broad spectrum of neurological symptoms in addition to acute gastro-intestinal signs. Cardiovascular symptoms may require emergency medical treatment. Other often observed symptoms are myalgia, arthralgia and hypothermia. More than 400 tropical predatory fish species, e.g. moray eels (Muraenidae), amberjacks (*Seriola* spp.), jacks (*Caranx* spp.), groupers (Serranidae), barracuda (Spyraenidae) and snappers (Lutjanidae) are known to cause ciguatera in humans. But the designation of ciguatera is derived from the indigenous name “cigua” of the marine snail *Cittarium pica*, which is regarded as a delicacy in the Caribbean area since pre-Colombian times. It became endangered locally in recent years, and other marine invertebrates like snails, giant clams, starfish and sea urchins became known as a potential vector for ciguatera (Darius et al., 2018; Silva et al., 2015). In 2014, the protected snail *Tectus niloticus* in French Polynesia caused an outbreak in nine marine tourists which presented cardiovascular and other severe symptoms in addition to typical neurological manifestations.

Ciguatera is widespread and well-known in all tropical coastal areas between latitudes 35°N and 35°S (Friedman et al., 2017), and is newly endemic in some subtropical islands previously not affected. As far as Europe is concerned, endemic ciguatera occurred in the subtropical islands of Madeira (Portugal) and of the Canaries (Spain) since 2004 (Otero et al., 2010; Perez Arellano et al., 2005). Ciguatera food intoxication is rare in countries with temperate climate. First reports about ciguatera in German tourists, mainly in the Caribbean (Dominican Republic and Cuba) but also at the Red Sea, have been published since the early 1990s (Blume et al., 1999; Krause et al., 1994; Ruprecht et al., 2001; Shah et al., 1997; Wjst, 2016). Ciguatera outbreaks aboard ships were first journalized by James Cook during

his sailing journeys of discovery in the South Pacific Ocean in the 18th century (Doherty, 2005) and may be a challenge even in modern shipping and navies (Hagelstein et al., 1991; Schlaich et al., 2012). Ciguatera outbreaks caused by imported tropical fish occurred in Germany annually since 2012 (Friedemann, 2019). Ciguatera remains a huge public health problem in poor endemic coastal areas which are short of food and where fish is the staple food. On the other hand, ciguatera spreads to maritime subtropical areas and to continental, fish importing countries with temperate climate.

Ciguatera intoxications mostly begin with gastrointestinal symptoms like nausea, abdominal pain, vomiting and diarrhea approximately 2 to 12 hours after the consumption of the fish. Some patients feel tingling sensations in and around the mouth during or shortly after the fish meal. In the course of the following 12 to 48 hours, gastrointestinal symptoms alleviate, and neurologic symptoms become prominent. Cardiovascular (bradycardia, hypotension, arrhythmia) symptoms may occur during the acute phase. Patients frequently report disturbances of thermal perception such as shivering, chills, hypothermia or sweating and of severe fatigue (Bagnis et al., 1979; Boucaud-Maitre et al., 2018; Friedemann, 2016; C. Gatti et al., 2008; Mattei et al., 2014).

The spectrum of peripheral and central neurologic symptoms mainly includes paresthesia, pain (arthralgia, myalgia, cephalgia, dentalgia) and dysesthesia (mainly cold allodynia), but also cerebellar syndrome, hallucination, depression, impaired consciousness and cognitive problems (Friedman et al., 2017; Yalachkov et al., 2018). Cold allodynia is the pathognomonic symptom, i.e. the specific and leading symptom of ciguatera. It allows the physicians to diagnose ciguatera when cold allodynia is reported by the patient shortly after the consumption of (sub)tropical fish. The patients feel painful or hot sensations when they come into contact with cold stimuli such as metallic or ceramic objects, cold wind or cold water.

Severe, life-threatening symptoms like bradycardia, respiratory failure, para- and hemiparesis, convulsions, coma may occur after eating large amounts of highly toxic fish species like moray eel as well as after the consumption of fish heads and viscera (Chan, 2016). But death only occurs in about 0.1 % of ciguatera cases (Bagnis et al., 1979).

There is no specific therapy for ciguatera available, but attempts at early treatment may minimize the severity and duration of symptoms ((Friedman et al., 2017). Symptomatic (analgesic, antiemetic, anti-diarrheic, antidepressant, antihistaminic) drugs as well as B-vitamins and calcium channel blockers have shown varying treatment effects. Bradycardia and hypotension should be treated with atropine. Mannitol reduces neuronal edema and should be administered intravenously in sufficiently hydrated patients within the first three days after ingestion of the ciguatoxic marine food products (Friedman et al., 2017; Mullins and Hoffman, 2017). For the treatment of chronic, mostly neurologic symptoms, which may persist several months, and - rarely - years, only a few reports are available (Friedemann, 2016; Friedman et al., 2017; C. M. I. Gatti et al., 2018). Amitriptyline, gabapentin and pregabalin have been used to relieve painful paresthesia and dysesthesia (Brett and Murnion, 2015; Davis and Villar, 1986; Perez et al., 2001).

Ciguatera patients should avoid alcohol, caffeine, (sea- and freshwater) fish, meat (pork, beef, chicken) and nuts as well as increased physical activity, exertion, exposure to sun and wind and other causes of dehydration for several months, because those food items and behavioral factors have been known to trigger the worsening and reappearance of symptoms (Friedemann, 2016; Friedman et al., 2017; C. M. I. Gatti et al., 2018).

Causes of ciguatera – Chemistry of ciguatoxins and pathophysiology

The cause of ciguatera is a broad spectrum of highly potent neurotoxins produced by microalgae of the genera *Gambierdiscus* and *Fukuyoa* (Litaker et al., 2017). Nearly 40 congeners are the result of enzymatic metabolism in the marine food web (Solino and Costa, 2018). Those ciguatoxins (CTX) are chemically large cyclic ladder-shaped polyether compounds of high molecular weight. The commonly encountered Pacific Ciguatoxin 1 or CTX-1B/P-CTX-1 ($C_{60}H_{86}O_{19}$) is very polar and has a molecular weight of 1111.329 g/mol (PubChem, 2019) (Figure 46). Ciguatoxins are classified into several groups due to their number of 13-14 ether rings, different degrees and positions of oxygenation and methylation and differences in both molecular termini.

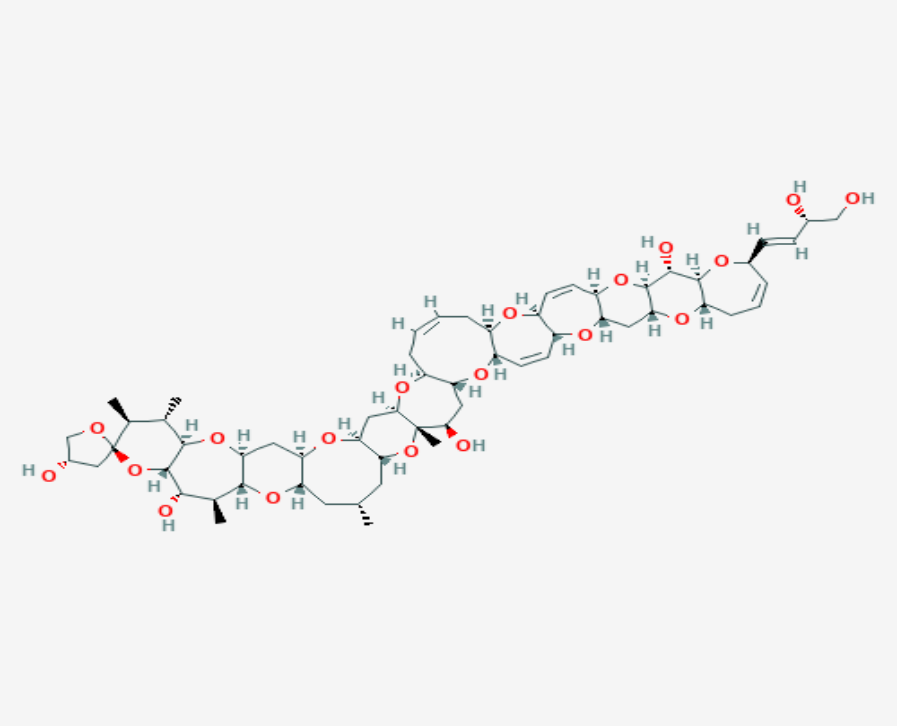


Figure 46: Pacific Ciguatoxin 1 / CTX-1B / P-CTX-1
(PubChem, 2019)

Ciguatoxins originating from the Pacific, Caribbean and Indian Oceans share the main pathomechanism of cell membrane depolarization as a consequence of the activation of voltage gated sodium channels. The P-CTX-1 group contains the most spread, most toxic and most intensively studied congeners (Solino and Costa, 2018). The lowest-observed-adverse-effect level (LOAEL) was calculated to 48.4 pg P-CTX-1 eq per kg bodyweight based on the fish consumption data of affected persons in a series of ciguatera events and corresponding to analytical anamnestic data of the consumed fish (Hossen et al., 2015).

Physically, ciguatoxins are odor- and tasteless, hydrophobic and lipid-soluble, resistant to heat and frost. Ciguatoxic fish flesh is phenotypically and gustatively unchanged, and ciguatoxins cannot be inactivated in marine food products nor eliminated (Lehane and Lewis, 2000; Pearn, 2001).

As there is no screening method for ciguatoxins in fish available, certain rules of fish consumption in endemic areas should be considered. Generally, fish heads and viscera of potentially ciguatoxic fish species as well as highly toxic fish species like moray eels should not

be consumed. People involved in sport fishing people, in particular, should be made aware of current local knowledge about high risk areas and of the fish species that are commonly affected because the concentration and the mixture of ciguatoxins of individual fish may vary even in one fish species as well as regionally, seasonally and annually (Yogi et al., 2011). The positive relationship between man-made (e.g. military actions) and natural (e.g. heavy storms) environmental disturbances has been reported, but is not clear, how much time after risk-increasing environmental conditions the consumption of regional fish should be avoided (Ruff, 1989; Skinner et al., 2011) Finally, potentially ciguatoxic fish species should not be imported from known hotspots (Hardison et al., 2018).

Ciguatera outbreaks in Germany

From 2012 to 2018, a series of ciguatera outbreaks, which occurred annually in Germany, reached a total number of 68 cases. These eight outbreaks were all caused by snappers from India, Indonesia and Vietnam. Case numbers ranged from 1 to 23 affected persons per outbreak. The places where the fish was ingested by the affected persons were seven restaurants and 28 households. Another case was acquired during a tasting and one case was laboratory acquired. Sixty two percent (16/26) of the clusters consisted of single cases or single households.

The age of the patients ranged from 17 years to 80 years, without correlation to the severity. First symptoms occurred at a median of 4 hours postprandial with a range of few minutes to eight hours. Nearly 90 % (43/48) of the patients (with detailed information about symptoms) had at least a moderate course of illness. Three of them, i.e. about 6 %, had a severe degree of poisoning classified according to the Poison Severity Score, a standardized scale for grading the severity of acute poisonings (Persson et al., 1998). Sinus bradycardia < 40 min-1, multiple syncopes, morphine-resistent abdominal cramps and respiratory distress were the classifying symptoms for the severe poisoning degree in these three patients. Patients with a moderate degree of severity presented pronounced gastrointestinal symptoms, paresthesia and dysesthesia, pronounced myalgia and arthralgia, cardiovascular and psychomotoric disorders, felt exhausted and had problems with thermo-regulation. Some of the patients reported the reappearance of neurologic symptoms after the repeated consumption of fish, chicken, fatty food or alcohol (Friedemann, 2019). In the outbreak 2012, nearly half of patients (9/19) had chronic neurologic symptoms for more than 3 months, and seven of them had neurologic symptoms for more than one year (Friedemann, 2016).

Evaluating the ciguatera outbreak series in Germany, the surveillance of ciguatera cases has been included in the joint pilot study of the German Poisoning Centers and the German Federal Institute for Risk Assessment to establish a poisoning monitoring program in Germany since 2017 (BfR, 2017). Poisoning centers play an important role in the diagnostic of ciguatera cases not only in Germany.

Laboratory analysis and prevention

Many methods have been described, but the validation of a reference method remains a critical and urgent issue, in particular due to the lack of certified ciguatoxin standards (Caillaud et al., 2010). This problem may be illustrated by the example of isolation of diminutive amounts of 490 µg, 280 µg and 100 µg of CTX-1, CTX-2 and CTX-3 from 48.3 kg of moray eel viscera in 1991 (Lewis et al. 1991) whereby moray eels and viscera are by themselves the most ciguatoxic items. However, the production of less oxidized precursor toxins in Gambierdiscus cultures (Chinain et al., 2010b) as well as the total synthesis of the most toxic congeners CTX-3C, 51-Hydroxy-CTX-3C and CTX-1B in the early 2000s have been good

precursors for further development of antibodies and analytical methods (Inoue et al., 2006). 2006).
In the USA, a combined approach of the identification of the fish species by DNA barcoding and of the detection and confirmation of ciguatoxins in fish remnants from ciguatera poisoning events is used, in which the in vitro mouse neuroblastoma cell assay (N2a-CBA) proceeds the confirmation and quantification by liquid chromatography tandem-mass spectrometry (LC-MS/MS) (Friedman et al., 2017). Such two-tiered protocol for case and outbreak investigations will be established at the German National Reference Laboratory for Marine Biotoxins. But the analysis of ciguatoxins for the investigations of the outbreaks 2012 to 2017 still had to be carried out in foreign, non-German laboratories. So, CTX-1B, 2,3-dihydroxy-CTX-3C and 51-hydroxy-CTX-3C were found by Prof. Ana Gago-Martinez from the University of Vigo (Spain) to be the causative ciguatoxin congeners in the outbreaks 2012 and 2015 (Friedemann, 2019).

For the prevention of ciguatera outbreaks caused by imported tropical fish, screening for toxicity would be a useful tool, but reliable methods for a broad structural variability of ciguatoxins are still not established. On the other hand, it is not easy to trace ciguatoxic fish in heterogeneously composed export batches from several local fish suppliers. Alternatively, the determination of high-risk fish species at German customs check points has been consolidated. During investigations of ciguatera outbreaks in Germany, the regional food safety laboratories are responsible for the determination of the fish species. It was found that patients in the outbreaks 2012, 2013 and 2017 probably did not buy and eat Red Snapper as they believed when purchasing the fish at retail markets, because DNA-barcoding revealed other snapper species, mostly *Lutjanus bohar*, which is often described as being ciguatoxic (Friedemann 2018). It should be emphasized that all patients must be requested thoroughly, to give detailed information as to where and when they bought the fish, how much fish they ate, and if they kept some leftovers from the fish meal. If possible, all samples should be divided into subsamples for parallel determination of the fish species and ciguatoxin analysis.
A still unsolved problem is the prevention of ciguatera in fish exporting countries. Some endemic agrarian countries, mostly in the Pacific Islands, have established monitoring or surveillance programs (Chinain et al., 2010a). India, Indonesia and Vietnam, the countries, where the ciguatoxic fish that caused the German outbreaks were caught, have not established such programs. In India, public health authorities became active after the huge local ciguatera outbreak in Mangalore in 2016, where more than 200 people - most of them worked in a seafood exporting factory - contracted ciguatera due to the consumption of the cooked heads of large *Lutjanus bohar* (Karunasagar et al., 2018). The establishment and effectiveness of local, regional and global preventive measures should be observed by the fish trading companies involved as well as by the public health authorities of fish-exporting and -importing countries. Management measures including avoidance of exposure to potentially ciguatoxic fish, surveillance and reporting of ciguatera events to public health authorities, and education of consumers and professionals (Friedman et al., 2017) should be assured in endemic and non-endemic countries, and co-operation concerning the risk assessment and risk management of ciguatera is necessary alongside the global product chain.

Global product chains

The model of global product chains illustrates the very complex route(s) of a product on the different steps of production, processing (transformation) and distribution. Multiple companies located in several countries and continents are responsible for the production and distribution of a product (Diercke-Weltatlas, 2018). Those complex product chains comprise rather cross-linking nets for most modern products. The general statements on product chains are applicable for the international fish trade, and will be illustrated here by examples of snapper imports causing a mainly unknown tropical disease in Germany.

The cases of ciguatera intoxications in Germany 2012 to 2017 were adverse effects of the very healthy food item fish which was imported from India, Indonesia and Vietnam. Generally, there is no guarantee for the absence of ciguatoxins in snappers imported from the northern indo-pacific ocean which is an endemic region for ciguatera because detection and analytical methods of CTX are not established in these countries. Otherwise, inadequate performances in the distribution chain, like mislabeling of fish names and catchment areas, were disclosed during the investigation of the German ciguatera outbreaks. A tragic consequence for the affected persons at least of the first outbreak was that they were not adequately attended by German physicians who were not aware of the ciguatera symptoms, and most patients had to suffer from neurologic symptoms for several weeks and up to several months. On the other hand, the outbreak-related case numbers are negligibly small compared with the large quantities of imported snappers. Basically, food sold in Europe must be safe and must not harm health. The importation of tropical fish to European countries, particularly to Germany and France, is a typical example for a costumer-dominated distribution chain which is characterized by an asymmetric relationship between the powers of the exporting and importing companies. The leading import companies of the so-called global north determine the conditions of production in the global south and (of) the further marketing. But both should assume responsibility for the health of their customers. The series of ciguatera outbreaks in Germany shows that preventive measures should be strengthened, and that the corresponding weak-points in the product chains have to be determined. The creation of value should not depend on food fraud including (un)intentional mislabeling. However, this is not fully accepted in global value chains as examples of global trade of snappers can illustrate.

Distribution chains of imported (red) snappers

Snappers, i.e. fish of the family Lutjanidae, are valuable tropical sport and table fish found in the Indian and Pacific Oceans and in warmer areas of the Atlantic Ocean, mainly the Caribbean. The colloquial term red snapper is used for a large variety of similar perch-like red-colored fish. In the USA, only the Northern Red Snapper (*Lutjanus campechanus*) may be called red snapper, and in Germany only the Malabar Blood Snapper (*Lutjanus malabaricus*) Figure 47).

Snappers are caught by local fishermen on coastal and offshore reefs. Usually, reefs colonized with ciguatoxic fish are avoided by fishermen with local knowledge. But economic pressure may drive them to catch fish in unknown or even areas with a known presence of ciguatoxic fish. On the other hand, the conditions of the reefs may change unexpectedly.
When small catches of snappers are being put together to reach the desired size of the export charge, the origin of fish from different local fishing areas is no longer traceable in the mixed batches. According to the Regulation (EU) No 1379/2013, at least the most representative area shall be stated in mixed products, which consist of the same species but originating from a variety of catch areas.

Furthermore, the Regulation (EU) No 1379/2013 requires the unambiguous labelling of the package with the scientific name of the fish species. Therefore, the fish should be sorted by species preferably by the manufacturer on the spot, and mislabeling could be minimized. A side effect of the investigations of the German ciguatera outbreaks 2012 to 2017 was the elucidation of mislabeling of the fish species in three out of six outbreaks. In the international context, “Red Snappers” belong to the most commonly mislabeled fish (Cawthorn et al., 2018; Naaum et al., 2016; Stawitz et al., 2016) (Figure 48).

Figure 47: Correct labeling of Red Snapper (*L. malabaricus*) in a Berlin (Germany) gourmet food store (Foto: Miriam Friedemann, 2019)

In the case of *Lutjanus malabaricus*, the demand to assign fish of the same species to a certain fishery product is not easy to fulfill, because this swarm fish is often associated, e.g. with the Crimson Snapper (*Lutjanus erythropterus*), and even for fish taxonomists it is not easy to differentiate the huge variety of about 350 snapper species (Fishbase, 2018). Nearly impossible is the visual species differentiation of filleted fish (Olmsted, 2016).

In Germany, the sale of fish including snappers (Lutjanidae) is accepted according to the German list of commercial designations (BLE, 2018). For the present, it allows the sale of mixed fishery products containing different species labelled with the family (e.g. Lutjanidae)

or genus (*Lutjanus* spp.) name. This gap is useful for fish industry to cover the trade of mixed snapper batches legally in Germany.

Figure 48: Incorrect labeling of a snapper (*Lutjanus* spp.) at a Berlin (Germany) food fair
(Foto: Miriam Friedemann, 2019)

When fresh tropical fish products are to be exported to Europe, the transport is carried out by air. Accordingly, the lot of 235 kg fresh *Lutjanus* spp., which caused the ciguatera outbreak 2012, was imported at Frankfurt/Main airport in Germany at the end of October 2012. One week later, it was sold with the inadequate designation of *Lutjanus malabaricus* in about 250 retail stores in approximately 200 German cities and in the Czech Republic. Already on the first day of sale, the first ciguatera case occurred in Germany. No cases were reported from the Czech Republic. After the occurrence of further cases in Northern Germany within a week, the remaining fish was recalled by the wholesaler. In total, twenty three affected persons from seven German cities were registered in this outbreak. DNA-barcoding revealed the fish species *Lutjanus bohar* (Two-Spot Red Snapper) and *Lutjanus argentimaculatus* (Mangrove Red Snapper) in samples of this outbreak-related batch, and the European Reference Laboratory of Marine Biotoxins at the University of Vigo (Spain) detected ciguatoxins with the Liquid chromatography-tandem mass spectrometry (LC-MS/MS) method in samples of this batch (Friedemann, 2019).

The batches of frozen snappers, which caused the ciguatera outbreaks in Germany in 2013-2017, reached Europe by ship and were imported via the Netherlands, Belgium and Denmark. Afterwards, the distribution of the frozen fish branched out via multiple intermediaries until reaching retailers and consumers. In November 2015 for example, 373 kg frozen snappers (*Lutjanus* spp.) were imported by the Netherlands and distributed to Belgium, France and Germany in February 2016. Seven ciguatera intoxications occurred in France in June

2016. A further four persons from two households contracted ciguatera in a German city in July 2016 (Friedemann et al., 2017). Genetic typing for the determination of the fish species was not done in this outbreak. The toxicity of fish samples from the causative batch was demonstrated by French authorities with the method of mouse bioassays.

A new and previously unknown distribution chain of snappers exported from India was revealed, when at least seven ciguatera cases occurred in Martinique in July 2017. The frozen snapper filets that caused the outbreak were imported by the French Overseas Department Martinique via continental France and were sold in Martinique by a French hypermarket. Again, in February 2019, a ciguatera outbreak caused by non-local fish, this time imported from Vietnam, occurred in a restaurant in Martinique. The Caribbean island Martinique itself is located in an area endemic for ciguatera. Ciguatera caused by imported fish from another endemic region is not expected there, and may hamper clinical diagnosis, toxin analytics and epidemiological investigations.

The two outbreaks 2017 and 2019 in Martinique and the three German outbreaks 2012, 2016 and 2017 were reported in the Rapid Alert System for Food and Feed (RASFF) of the European Community, where the distribution of (potentially) unhealthy products is documented (EC, 2017). This facilitates the elucidation of ciguatera events as well as the clarification of other food-related outbreaks. Ciguatera outbreaks caused by imported tropical fish are not easy to elucidate because a very few single cases registered in different regions or different countries should be merged via the reference of the common import batch. Furthermore, the RASFF notifications of two ciguatera outbreaks in the Caribbean (Martinique) in 2017 and 2019 brought about by fish from India and Vietnam, revealed surprising distribution chains of imported fish resulting in unexpected clinical courses which were caused by ciguatoxin congeners untypical for this endemic area. For these reasons, every single case of ciguatera poisoning caused by imported tropical fish should be reported to the RASFF.

Undoubtedly, RASFF-information about ciguatoxin contaminations in fish and about urgent RASFF-alert notifications concerning ciguatoxin related food poisoning have increased the awareness of this emerging problem in Europe.

The distribution chains of these three examples of ciguatera outbreaks ended with a recall of the fish remaining on the corresponding market. Generally, the entrepreneurs pursue different strategies to avoid the high costs for the recall of their products. Some refrain from the import of snappers. Others insist on importing snappers according to the wishes of their clients who are asking for Red Snapper. Therefore, it is essential that preventive measures should be implemented in the countries of origin of snappers.

Production and distribution chains of imported fresh fish are short, clear, fast and mostly reproducible because of the rapid spoilage of fresh fish. The production and distribution of frozen fish is complex and not easy to elucidate. Aggravating for the traceability is the fact that most batches of exported snappers constitute mixed catches from different fishing areas representing different hazards concerning the risk of ciguatera. Entrepreneurs and public health authorities of exporting and importing countries should exchange their knowledge and experiences with ciguatera. Mislabeling should be avoided in (global) value chains as well as concerning the protection of health and rights of consumers. Promoting action concerning a more transparent and sustainable trade of snappers at the international level as well as nationally are necessary (Cawthorn et al., 2018).

Conclusions

Conjoined with rising fish exports from tropical areas to highly developed countries, global distribution chains became interweaved, and mislabeling occurs frequently (Cawthorn et al.,

2018). Highly valuable, delicious red snappers represent the most often mislabeled fish species in industrialized countries. Marine food products generally lend themselves to fraud in unethical replacing of a pricier fish by a lower-cost one (Olmsted, 2016). The lack of global harmonization of fish nomenclature complicates the problem of correct fish labelling, even in regions where robust seafood labelling legislation became operative, e.g. in the European Union. The recent series of ciguatera fish poisoning in Germany revealed this labelling problem on different levels of the distribution chains. Beside the consequent implementation of fish labeling legislation are other unsolved problems concerning the prevention of ciguatera in Germany, e.g. the establishment, the enhancement and improvement of the:

- education of fish exporters in the tropics,
- co-operation with local public health authorities,
- laboratory methods for the detection, determination and quantification of ciguatoxins,
- awareness of health care providers.

Recommendations for the reef fish purchasing industry for the prevention of ciguatera, as already established by the FDA (FDA, 2013b), should be developed for Germany and other fish importing European countries.

Abbreviations

BfR	Bundesinstitut für Risikobewertung / German Federal Institute for Risk Assessment /
BLE	Bundesanstalt für Landwirtschaft und Ernährung Federal Office for Agriculture and Food
CTX	Ciguatoxin
DNA	Deoxyribonucleic acid
EU	European Union
FDA	Food and Drug Administration (USA)
LOAEL	Lowest-observed-adverse-effect level
LC-MS/MS	Liquid chromatography tandem-mass spectrometry
RASFF	Rapid Alert System for Food and Feed

References

Bagnis, R., Kuberski, T., and Laugier, S. (1979). Clinical observations on 3,009 cases of ciguatera (fish poisoning) in the South Pacific. *Am J Trop Med Hyg, 28*(6), 1067-1073. doi:10.4269/ajtmh.1979.28.1067

BfR, Bundesinstitut für Risikobewertung. (2017). Pilotstudie zur Etablierung eines Nationalen Monitorings von Vergiftungen. *https://www.bfr.bund.de/de/pilotstudie_zur_etablierung_eines_nationalen_monitorings_von_vergiftungen-202959.html. Accessed 15 January 2019*

BLE, Bundesanstalt für Landwirtschaft und Ernährung. (2018). List of the commercial designations for fishery products and products of aquacultures, accepted in Germany. *https://www.ble.de/SharedDocs/Downloads/DE/Fischerei/Fischwirtschaft/HandelsbezeichnungLatD.pdf?__blob=publicationFile&v=7. Accessed 08 August 2018*.

Blume, C., Rapp, M., Rath, J., Koller, H., Arendt, G., Bach, D., and Grabensee, B. (1999). Ciguatera poisoning. Growing differential diagnostic significance in the age of foreign tourism. *Med Klin (Munich), 94*(1), 45-49.

Boucaud-Maitre, D., Vernoux, J.-P., Pelczar, S., Daudens-Vaysse, E., Aubert, L., Boa, S., . . . and Garnier, R. (2018). Incidence and clinical characteristics of ciguatera fish poisoning in Guadeloupe (French West Indies) between 2013 and 2016: a retrospective cases-series. *Scientific Reports, 8*(1), 3095. doi:10.1038/s41598-018-21373-2

Brett, J., and Murnion, B. (2015). Pregabalin to treat ciguatera fish poisoning. *Clin Toxicol (Phila), 53*(6), 588. doi:10.3109/15563650.2015.1052499

Caillaud, A., de la Iglesia, P., Darius, H. T., Pauillac, S., Aligizaki, K., Fraga, S., . . . and Diogene, J. (2010). Update on methodologies available for ciguatoxin determination: perspectives to confront the onset of ciguatera fish poisoning in Europe. *Mar Drugs, 8*(6), 1838-1907. doi:10.3390/md8061838

Cawthorn, D. M., Baillie, C., and Mariani, S. (2018). Generic names and mislabeling conceal high species diversity in global fisheries markets. *Conservation Letters, 11*(5), e12573. doi:10.1111/conl.12573

Chan, T. Y. (2016). Characteristic features and contributory factors in fatal ciguatera fish poisoning - Implications for prevention and public education. *Am J Trop Med Hyg, 94*(4), 704-709. doi:10.4269/ajtmh.15-0686

Chinain, M., Darius, H. T., Ung, A., Cruchet, P., Wang, Z., Ponton, D., . . . Pauillac, S. (2010b). Growth and toxin production in the ciguatera-causing dinoflagellate Gambierdiscus polynesiensis (Dinophyceae) in culture. *Toxicon, 56*(5), 739-750. doi:10.1016/j.toxicon.2009.06.013

Chinain, M., Darius, H. T., Ung, A., Fouc, M. T., Revel, T., Cruchet, P., . . . and Laurent, D. (2010a). Ciguatera risk management in French Polynesia: the case study of Raivavae Island (Australes Archipelago). *Toxicon, 56*(5), 674-690. doi:10.1016/j.toxicon.2009.05.032

Darius, H. T., Roué, M., Sibat, M., Viallon, J., Gatti, C. M., Vandersea, M. W., . . . and Chinain, M. (2018). *Tectus niloticus* (Tegulidae, Gastropod) as a novel vector of ciguatera poisoning: Detection of pacific ciguatoxins in toxic samples from Nuku Hiva Island (French Polynesia). *Toxins, 10*(1), 2. doi:10.3390/toxins10010002

Davis, R. T., and Villar, L. A. (1986). Symptomatic improvement with amitriptyline in ciguatera fish poisoning. *N Engl J Med, 315*(1), 65.

Diercke-Weltatlas. (2018). Global product chains (using the example of jeans). *https://www.diercke.de/content/globale-warenketten-am-beispiel-jeans-978-3-14-100800-5-271-4-1. Accessed 02 August 2018*.

Doherty, M. J. (2005). Captain Cook on poison fish. *Neurology, 65*(11), 1788-1791. doi:10.1212/01.wnl.0000187128.80551.1b

EC, European Commission. (2017). RASFF portal - notifications list. *https://webgate.ec.europa.eu/rasff-window/portal/?event=notificationsList&StartRow=1. Accessed 03 November 2017*.

FDA, U.S. Food and Drug Administration. (2013b). Guidance for industry: purchasing reef fish species associated with the hazard of ciguatera fish poisoning *http://www.fda.gov/food/guidanceregulation/guidancedocumentsregulatoryinformation/ucm375214.htm. Accessed 31 January 2019*.

Fishbase. (2018). List of nominal species of Lutjanidae (snappers). *https://www.fishbase.de/Nomenclature/NominalSpeciesList.php?Family=Lutjanidae. Accessed 08 August 2018*.

Friedemann, M. (2016). First ciguatera outbreak in Germany in 2012. *Bundesgesundheitsblatt-Gesundheitsforschung-Gesundheitsschutz, 59*(12), 1556-1565. doi:10.1007/s00103-016-2456-y

Friedemann, M. (2019). Ciguatera fish poisoning outbreaks from 2012 to 2017 in Germany caused by snappers from India, Indonesia, and Vietnam. *Journal of Consumer Protection and Food Safety, 14*(1), 71-80. doi:10.1007/s00003-018-1191-8

Friedemann, M., Doberauer, C., Fink, G. R., and Burghaus, L. (2017). [Ciguatera or why you should enquire about fish consumption in cases of cold allodynia]. *Fortschritte der Neurologie-Psychiatrie, 85*(10), 611-615. doi:10.1055/s-0043-118336

Friedman, M. A., Fernandez, M., Backer, L. C., Dickey, R. W., Bernstein, J., Schrank, K., . . . and Fleming, L. E. (2017). An updated review of ciguatera fish poisoning: clinical, epidemiological, environmental, and public health management. *Marine Drugs, 15*(3). doi:10.3390/md15030072

Gatti, C., Oelher, E., and Legrand, A. M. (2008). Severe seafood poisoning in French Polynesia: A retrospective analysis of 129 medical files. *Toxicon, 51*(5), 746-753. doi:10.1016/j.toxicon.2007.11.025

Gatti, C. M. I., Lonati, D., Darius, H. T., Zancan, A., Roue, M., Schicchi, A., . . . Chinain, M. (2018). *Tectus niloticus* (Tegulidae, Gastropod) as a novel vector of ciguatera poisoning: Clinical characterization and follow-up of a mass poisoning event in Nuku Hiva Island (French Polynesia). *Toxins (Basel), 10*(3). doi:10.3390/toxins10030102

Hagelstein, J. G., Jacobs, P., and Vuksanovic, P. M. (1991). Ciguatera - ein Problem in der Schiffahrts- und Touristikmedizin. *Wehrmed Mschr*(5), 226-229.

Hardison, D. R., Holland, W. C., Darius, H. T., Chinain, M., Tester, P. A., Shea, D., . . . and Litaker, R. W. (2018). Investigation of ciguatoxins in invasive lionfish from the greater caribbean region: Implications for fishery development. *PLoS One, 13*(6), e0198358. doi:10.1371/journal.pone.0198358

Hossen, V., Solino, L., Leroy, P., David, E., Velge, P., Dragacci, S., . . . and Diogene, J. (2015). Contribution to the risk characterization of ciguatoxins: LOAEL estimated from eight ciguatera fish poisoning events in Guadeloupe (French West Indies). *Environ Res, 143*(Pt B), 100-108. doi:10.1016/j.envres.2015.09.014

Inoue, M., Miyazaki, K., Ishihara, Y., Tatami, A., Ohnuma, Y., Kawada, Y., . . . and Hirama, M. (2006). Total Synthesis of Ciguatoxin and 51-HydroxyCTX3C. *Journal of the American Chemical Society, 128*(29), 9352-9354. doi:10.1021/ja063041p

Karunasagar, I., Turner, A. D., Maskrey, B., Robertson, A., Shivanagouda-Hosagoudar, S., Rai, P., . . . and Karunasagar, I. (2018). Report of a major outbreak of ciguatera fish poisoning in Mangalore, India. *https://www.icha2018.com/medias/content/files/ICHA_2018_Abstract_Book_v8.pdf. Accessed 15 March 2019*.

Krause, G., Andersch-Borchert, I., and Diesfeld, H. J. (1994). Ciguatera. New cases of fish poisoning in German Caribbean vacationers. *Dtsch Med Wochenschr, 119*(27), 975.

Lehane, L., and Lewis, R. J. (2000). Ciguatera: recent advances but the risk remains. *International Journal of Food Microbiology, 61*(2-3), 91-125. doi:10.1016/s0168-1605(00)00382-2

Litaker, R. W., Holland, W. C., Hardison, D. R., Pisapia, F., Hess, P., Kibler, S. R., and Tester, P. A. (2017). Ciguatoxicity of *Gambierdiscus* and *Fukuyoa* species from the Caribbean and Gulf of Mexico. *PLoS One, 12*(10), e0185776-e0185776. doi:10.1371/journal.pone.0185776

Mattei, C., Vetter, I., Eisenblatter, A., Krock, B., Ebbecke, M., Desel, H., and Zimmermann, K. (2014). Ciguatera fish poisoning: a first epidemic in Germany highlights an increasing risk for European countries. *Toxicon, 91*, 76-83. doi:10.1016/j.toxicon.2014.10.016

Mullins, M. E., and Hoffman, R. S. (2017). Is mannitol the treatment of choice for patients with ciguatera fish poisoning? *Clin Toxicol (Phila), 55*(9), 947-955. doi:10.1080/15563650.2017.1327664

Naaum, A. M., Warner, K., Mariani, S., Hanner, R. H., and Carolin, C. D. (2016). The need for seafood authenticity. In A. P. (Elsevier) (Ed.), *Seafood authenticity and traceability: a DNA-based perspective* (pp. 3-18). Amsterdam, Boston, Heidelberg, London, New York, Oxford, Paris, San Diego, San Francisco, Singapore, Sydney, Tokyo.

Olmsted, L. (2016). Think you bought Red Snapper? Don´t be so sure. *The Wall Street Journal, https://www.wsj.com/articles/think-you-bought-red-snapper-dont-be-so-sure-1470428804. Accessed 24 January 2019*.

Otero, P., Perez, S., Alfonso, A., Vale, C., Rodriguez, P., Gouveia, N. N., . . . and Botana, L. M. (2010). First toxin profile of ciguateric fish in Madeira Arquipelago (Europe). *Anal Chem, 82*(14), 6032-6039. doi:10.1021/ac100516q

Pearn, J. (2001). Neurology of ciguatera. *J Neurol Neurosurg Psychiatry, 70*(1), 4-8. doi:10.1136/jnnp.70.1.4

Perez Arellano, J. L., Luzardo, O. P., Perez Brito, A., Hernandez Cabrera, M., Zumbado, M., Carranza, C., . . . and Boada, L. D. (2005). Ciguatera fish poisoning, Canary Islands. *Emerg Infect Dis, 11*(12), 1981-1982. doi:10.3201/eid1112.050393

Perez, C. M., Vasquez, P. A., and Perret, C. F. (2001). Treatment of ciguatera poisoning with gabapentin. *New England Journal of Medicine, 344*(9), 692-693. doi:10.1056/NEJM200103013440919

Persson, H. E., Sjoberg, G. K., Haines, J. A., and Pronczuk de Garbino, J. (1998). Poisoning severity score. Grading of acute poisoning. *J Toxicol Clin Toxicol, 36*(3), 205-213. doi: 10.3109/15563659809028940

PubChem. (2019). Ciguatoxin-1 (CID=5311333). *https://pubchem.ncbi.nlm.nih.gov/compound/ciguatoxin#section=Top. Accessed 14 January 2019*.

Ruff, T. A. (1989). Ciguatera in the Pacific: a link with military activities. *Lancet, 1*(8631), 201-205. doi:10.1016/s0140-6736(89)91212-9

Ruprecht, K., Rieckmann, P., and Giess, R. (2001). [Ciguatera: clinical relevance of a marine neurotoxin]. *Dtsch Med Wochenschr, 126*(28-29), 812-814. doi:10.1055/s-2001-15703

Schlaich, C., Hagelstein, J. G., Burchard, G. D., and Schmiedel, S. (2012). Outbreak of ciguatera fish poisoning on a cargo ship in the port of Hamburg. *J Travel Med, 19*(4), 238-242. doi:10.1111/j.1708-8305.2012.00619.x

Shah, S., Diesfeld, H. J., and Fleischer, K. (1997). Ciguatera-Fischvergiftung bei Karibik-Reisenden. *Epid Bull, 16*, 111.

Silva, M., Rodriguez, I., Barreiro, A., Kaufmann, M., Neto, A. I., Hassouani, M., . . . and Vasconcelos, V. (2015). First report of ciguatoxins in two starfish species: *Ophidiaster ophidianus* and *Marthasterias glacialis*. *Toxins (Basel), 7*(9), 3740-3757. doi:10.3390/toxins7093740

Skinner, M. P., Brewer, T. D., Johnstone, R., Fleming, L. E., and Lewis, R. J. (2011). Ciguatera fish poisoning in the Pacific Islands (1998 to 2008). *PLoS Negl Trop Dis, 5*(12), e1416. doi:10.1371/journal.pntd.0001416

Solino, L., and Costa, P. R. (2018). Differential toxin profiles of ciguatoxins in marine organisms: Chemistry, fate and global distribution. *Toxicon, 150*, 124-143. doi:10.1016/j.toxicon.2018.05.005

Stawitz, C., Siple, M., Munsch, S., Lee, Q., and Derby, S. R. (2016). Financial and ecological implications of global seafood mislabelling. *Conservation Letters*(November), 10. doi:10.1111/conl.12328

Wjst, M. (2016). [Ciguatera: a multifaceted and puzzling disorder]. *MMW Fortschr Med, 158*(21-22), 76-78. doi:10.1007/s15006-016-9108-x

Yalachkov, Y., Hildner, M., Polomac, N., Jahnke, K., Wagner, M., and Baudrexel, S. (2018). Cytotoxic edema affecting distinct fiber tracts in Ciguatera fish poisoning. *Neurology*, doi:10.1212/WNL.0000000000006789

Yogi, K., Oshiro, N., Inafuku, Y., Hirama, M., and Yasumoto, T. (2011). Detailed LC-MS/MS analysis of ciguatoxins revealing distinct regional and species characteristics in fish and causative alga from the Pacific. *Anal Chem, 83*(23), 8886-8891. doi:10.1021/ac200799j

6.3 Foodborne zoonotic infections in different populations in Berlin

Boone, Idesbald; Hammerl, Jens Andre; Al Dahouk, Sascha

Abstract

Despite considerable measures due to food hygiene legislation, food-borne infectious diseases continue to lead the statistics on notifiable infectious diseases in Germany and are of great economic importance, both for public health and the food industry. Campylobacteriosis is still the most commonly reported bacterial disease followed by salmonellosis, which are mainly caused by contaminated food products of animal origin.

Infections with food-borne zoonoses usually cause acute, self-limiting gastroenteritis, but can also lead to chronic secondary diseases such as irritable bowel syndrome, reactive arthritis, erythema nodosum or the Guillain-Barré syndrome.

Berlin is a multicultural city with a migrant population of ca. 28%. Several populations with a migration background (e.g. Turks, Vietnamese and Russians) may differ in their food habits and exposure risks to food-borne zoonoses. In addition, due to language, socio-cultural and administrative barriers, case numbers of food borne zoonoses in migrant populations are expected to under-ascertained and/or underreported. This requires particular attention with respect to the identification and management of foodborne zoonoses. We described the epidemiological situation of common and rare foodborne zoonoses of public health relevance in Berlin with a focus on migrant populations.

Epidemiology of foodborne zoonoses in Europe, Germany and Berlin

Foodborne zoonoses are infectious diseases which can be transferred naturally from animals to humans through the consumption of contaminated foods, such as meat, milk, dairy products, fish and eggs. Zoonotic diseases are triggered by bacteria and their toxins, viruses, parasites or prions. Zoonoses pose a great risk to public health and can lead to considerable economic losses. Roughly two thirds of all foodborne infections in humans are of zoonotic origin.

The European Food Safety Authority (EFSA) and European Centre for Disease Prevention and Control (ECDC) monitor zoonoses and foodborne disease outbreaks in Europe. In 2014, 236,851 gastrointestinal infections with *Campylobacter* were reported in the European Union (EU) along with 88,715 cases of salmonellosis and 6,625 cases of yersiniosis (EFSA and ECDC, 2015). The reported cases of foodborne zoonoses definitely represent just the tip of the iceberg, since numerous cases are not diagnosed and reported because the patients do not seek medical care or the clinical symptoms are non-specific. The estimated economic damage and follow-on costs for the public health system amount to 2.4 billion euros for *Campylobacter* infections alone (EFSA, 2011d). *Campylobacter* is widespread in livestock, especially poultry and dairy cattle herds, but it is also found in household pets and environmental sources (i.e. water, sand) (Mughini Gras et al., 2012). Salmonellosis has become the second most common gastrointestinal infection caused by bacteria, but the number of reported cases has been decreasing continuously since 2008 due mainly to the introduction of effective control measures in the poultry sector (Regulation EC No. 2160/2003; EC No 517/2011; EC No 200/2012; EC No 584/2008). Despite this, *Salmonella* is still responsible for most foodborne disease outbreaks in the EU, with eggs and egg products being the most common sources of infection. Yersiniosis is the third most common zoonosis in the EU, with *Yersinia enterocolitica* as the most frequently isolated species (EFSA and ECDC, 2015). Consumption of raw or insufficiently cooked pork is regarded as an important risk factor (Rosner et al., 2010). The number of reported salmonellosis and yersiniosis in humans de-

creased from 2008 to 2014, whereas the number of reported cases of campylobacteriosis remained stable on a high level in the EU during the same period.

There are numerous influencing factors which can favour the transmission of foodborne zoonoses (Newell et al., 2010):

1. *Demographic change*: Growing population numbers and overageing result in increasing numbers of individuals who are susceptible to infection.
2. *Liberalisation of world trade and global food supply chains*: Complex international trading and the import of animals and foods of animal origin can lead to the entry of new pathogens or promote the re-emergence of zoonoses that have already been eradicated.
3. *Living and eating habits*: The consumption of raw or insufficiently heated foods, as well as ready-to-eat foods, increases the risk of foodborne infection.

The clinical symptoms of foodborne zoonoses vary greatly on the one hand and are often non-specific on the other. Accordingly, infections with *Salmonella*, *Campylobacter* and *Yersinia*, which are relatively common, all result to the same extent in fever and/or gastrointestinal complaints, whereas other zoonotic diseases produce very different symptom complexes. Sacroiliitis, endocarditis and meningioencephalitis, can occur with brucellosis, for example, infection with a hepatitis E virus can induce icterus, and myalgia can occur together with periorbital edemas with trichinellosis.

Berlin is an international and multicultural city where 28% of the entire population has a migration background (Amt für Statistik Berlin-Brandenburg, 2014). This requires increased attention from general practitioners in primary care, as well as hospital physicians and the public health system in the management of foodborne zoonoses and the care of affected patients. It has to be taken into account that population groups with different migration backgrounds are not evenly distributed across the city that they may differ in their exposure risk to food borne zoonoses, which varies through often traditional eating habits. The occurrence of zoonotic diseases may also be heterogeneously distributed in the various city districts (Figure 49). To what extent a causal connection exists here is the object of the ZooGloW research project (see below). People with a migration background can infect themselves with endemic disease pathogens on trips to their home countries and this also applies to foodborne zoonoses, especially where there are low standards of food hygiene and monitoring. There is of course a comparably higher risk for tourists who adopt the customs and eat the local foods in each country they visit. Travel-associated diseases can be contracted by travelling through endemic areas but, when the pathogen is transmitted via food, it can sometimes only be contracted after the traveller has returned to Germany if contaminated foods of animal origin are illegally imported and consumed. This can lead to smaller disease outbreaks in family groups or circles of acquaintances when foods brought from abroad are shared. Language barriers, socio-cultural differences and administrative obstacles can have the result that, despite sometimes severe clinical symptoms as a consequence of infection with zoonotic pathogens, no medical care is sought, which in turn has the result that fewer cases are recorded in migrant populations.

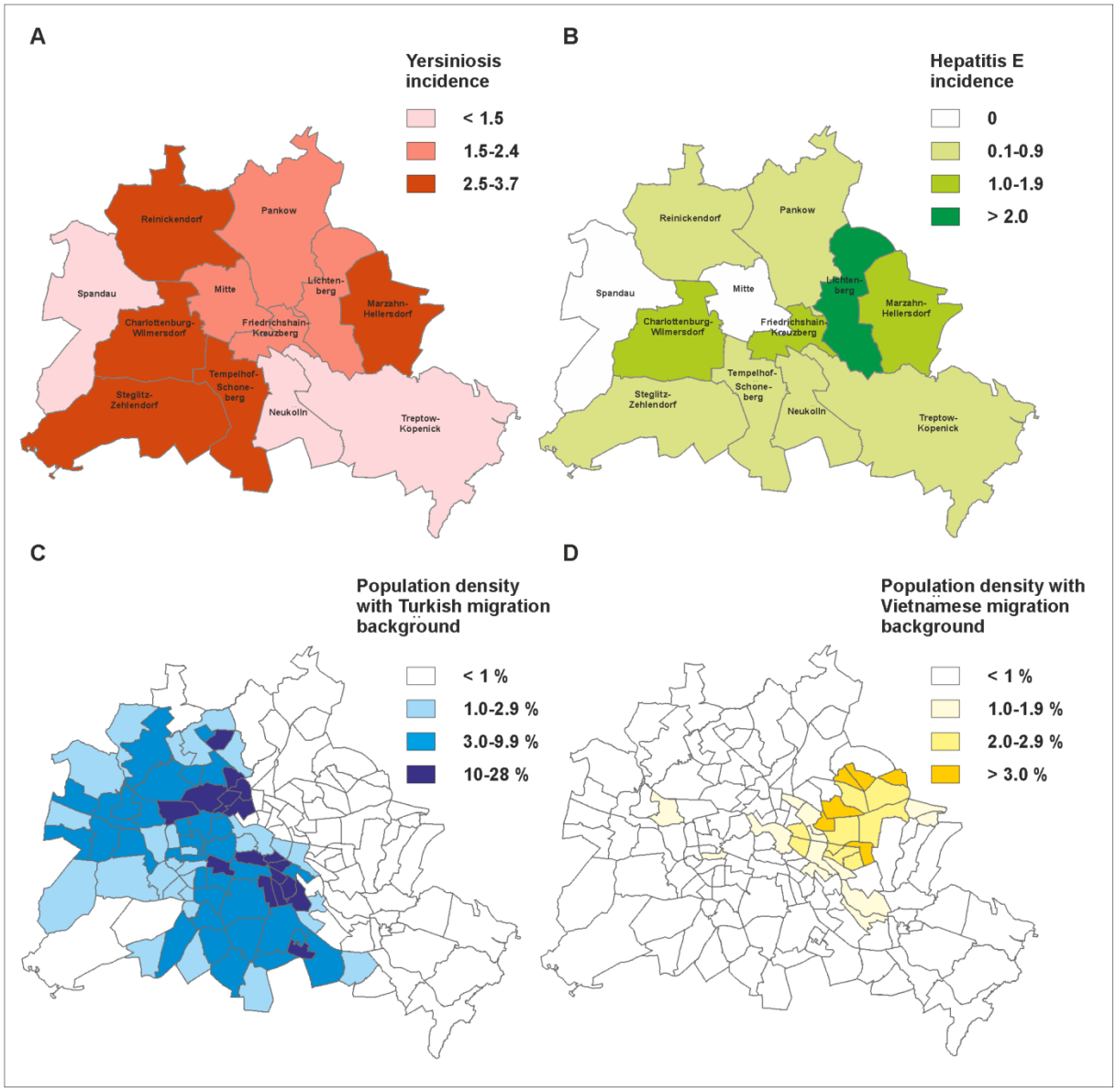


Figure 49: Geographic distribution of yersiniosis (A) und hepatitis E incidence in Berlin in 2014 (reported cases per 100,000 population), Robert Koch-Institute: SurvStat@RKI 2.0, https://survstat.rki.de, request date: 14.05.2015, and populations of Turkish (C) and Vietnamese migration background.

Foodborne infections with rare or atypical pathogens, or known zoonoses with non-specific or unusual symptoms, are often overlooked or only diagnosed at a very late stage in the course of the disease. The incidences of seven typical foodborne zoonoses are comparable in Berlin with those in Germany and Europe (Table 29). The mortality rate is usually below 0.1%. In risk groups, such as YOPI (young, old, pregnant, immunocompromised), the mortality rate can be much higher. Among pregnant women, a hepatitis E infection can lead to lethal fulminant hepatitis in up to 30% of all cases (Perez-Gracia et al., 2017).

Table 29: Selected zoonoses reported in 2014 in Berlin, Germany and the EU with indication of reported cases, incidence, outbreaks and hospitalisation rates

	Berlin[a]		Germany[a]		EU[b]		
Disease	**Cases**	**Incidence[c]**	**Cases**	**Incidence**	**Incidence**	**Outbreaks**	**Hospitalisation rate (%)[d]**
Campylobacteriosis	3,075	89.6	71,017	87.5	71.0	446	30.4
Salmonellosis	640	18.4	16,240	20.0	23.4	1,049	34.4
Yersiniosis	76	2.2	2,498	3.1	1.9	11	44.0
VTEC	86	2.5	1,655	2.0	1.6	41	39.2
Hepatitis E	27	0.8	671	0.8	NA	NA	NA
Brucellosis	6	0.2	47	0.1	0.1	2	66.1
Trichinellosis	0	0	1	0	0.1	17	63.0

[a] Robert Koch-Institut: SurvStat@RKI 2.0, https://survstat.rki.de, Deadline: 27.06.2018
[b] Notified cases in the EU in 2014 (EFSA and ECDC (European Food Safety Authority and European Centre for Disease Prevention and Control) 2015)
[c] Cases per 100000 population
[d] Proportion of confirmed cases for which hospitalisation information was available
NA: not available

Burden of disease of food-borne zoonoses

The effects of foodborne zoonoses on public health and the quality of life of a population can be estimated and compared with the help of the DALY concept. The years of life lost through premature death (YLL) and the years lived with disability (YLD) add up to the disability-adjusted life years (DALY) (Haagsma et al., 2013). Precise estimation of DALY depends on the availability of data (e.g. on incidences, lethality, severity and chronicity of a disease), the quality of the data and the uncertainty of the individual parameters. This means that erroneous and under-diagnosis, as well as gaps in reporting, have to be taken into consideration in the definition of true incidences. Epidemiological studies permit the assumption that the actual number of cases of salmonellosis and campylobacteriosis in Central Europe is ten to twenty times higher than the number of officially reported cases. There are no specific numbers to quantify under-diagnosis of most of these foodborne zoonoses, however. The recording of possible complications of foodborne zoonoses improves the estimation of the burden of disease (see Table 30).

A very high burden of disease due to *Campylobacter* spp. (19.8 DALY/100,000 population) and *Salmonella* spp. (7.7 DALY/100,000 population) was recorded in the Netherlands in 2009, whereas hepatitis E (0.15 DALY/100,000 population) led to a relatively low burden of disease in the overall population (Havelaar et al., 2012).

Table 30: Systemic and organ complications of selected foodborne zoonoses

Zoonosis	Systemic complications and organ manifestations
Campylobacteriosis	Feverish enteritis, postinfectious reactive arthritis, rarely Guillain-Barré syndrome
Salmonellosis	Gastroenteritis, chronic carrier, salmonella sepsis, abscesses, septic arthritis, cholecystitis, endocarditis, meningitis, pericarditis, pneumonia, pyelonephritis, postinfectious arthritis
Yersiniosis	Gastroenteritis, acute lymphadenitis mesenterica ("pseudoappendicitis"), erythema nodosum, postinfectious arthritis
VTEC	Haemolytic-uraemic syndrome, thrombotic-thrombocytopenic purpura (Moschcowitz syndrome)
Brucellosis	Hepatosplenomegaly, spondylitis, sacroiliitis, infectious arthritis, meningoencephalitis, endocarditis, epididymitis
Trichinellosis	Myocarditis with cardiac arrhythmias, encephalitis, bronchopneumonia, sepsis, adrenal insufficiency, psychotic states, coma and cramp attacks
Hepatitis E	Protracted/relapsing course, fulminant hepatitis

Brucellosis: Example of an underestimated foodborne zoonosis in Berlin?

Fourty-seven cases of brucellosis were reported in Germany in 2014, the highest number of reported cases within one year since the introduction of the electronic reporting system in 2001 (RKI, 2015). Six of the cases (13%) had their place of residence in Berlin. *Brucella* infections in Germany are usually caused by the consumption of contaminated raw milk or raw milk cheese. As livestock herds in Germany have been officially free of sheep/goat and cattle brucellosis since 2000, patients diagnosed in Germany usually pick up an infection in endemic areas (e.g. Mediterranean countries) or through the consumption of illegally imported, contaminated foods from these regions. In Germany, the incidence rate of brucellosis in people with a Turkish migration background is significantly higher than in the German reference population (0.3/100,000 population with a Turkish migration background vs. 0.01/100,000 in the German population; incidence rate ratio 29) and roughly half of the reported cases are travel-associated infections with their origins in Turkey (Al Dahouk et al., 2007). General practitioners and hospital physicians in Berlin should therefore observe rare foodborne zoonoses from other countries (such as brucellosis) from a differential diagnostic point of view too, especially if the patient's migration background is known and/or he/she has a positive travel history.

Preventive healthcare through the timely recognition and correct diagnosis of foodborne zoonoses

Foodborne zoonoses require the timely involvement of numerous health management players. The exchange of information between doctors in their own practices or hospitals and medical officers in the public health system is essential, no matter whether they practice human or veterinary medicine and irrespective of whether they are actively involved in the monitoring of public health or animal diseases or have responsibility for food hygiene. This kind of multidisciplinarity is necessary to successfully meet the challenge posed by zoonotic diseases and is reflected in the One Health initiative. General practitioners play a special role in the identification of symptom clusters and thereby the timely detection of outbreaks. Only a frac-

tion of the cases of disease and outbreaks caused by zoonotic pathogens are currently reported. To be able to guarantee that isolated cases are also detected in a timely manner requires not only a high level of attention, but above all sufficiently profound specialised knowledge of the sometimes very non-specific symptoms of foodborne zoonoses and in suspected cases, the choice of the right diagnostic method. In addition to direct detection of the pathogen from stool cultures and the like, molecular diagnostic methods are important for rapid detection as well as subtyping within the scope of traceability examinations.

Zoonoses and food safety along global goods chains (ZooGloW)

Within the BMBF-sponsored joint project ZooGloW (project code number 13N12697), the German Federal Institute for Risk Assessment (BfR) collaborated with Charité Berlin on the investigation of the transmission pathways of zoonotic pathogens. The health risks of different population groups are recorded here along with the possible follow-on complications of foodborne zoonoses.
The preliminary results from an epidemiological cross-sectional study in 1,180 participants recruited between 2014 and 2016 showed differences in the seroprevalence of the examined zoonoses among study participants without a migration background and in populations of Turkish, Russian and Vietnamese origin. For example, it was possible to detect anti-*Yersinia* antibodies much more frequently in Germans (59%) than in Russians and Turks (27-33%). Indications of a lapsed hepatitis E infection were found mostly with study participants with a Vietnamese (30%), but only rarely with a Turkish migration background (12%). It was possible to trace these differences in part to culturally different eating habits or traditional food preparation methods. For example, consumption of raw minced pork was strongly associated with *Yersinia* IgG-antibody seropositivity

Conclusions

To the best of our knowledge this was the first study that investigated the seroprevalence of seven food-borne pathogens in migrant population in Berlin. More detailed studies involving random sampling are required to obtain more precise seroprevalence estimates and to identify risk factors.

Acknowledgements

The study was supported by a grant of the Federal Ministry of Education and Research and was executed within the framework of the project ZooGloW (No. 13N12697).

Abbreviations

DALY	Disability-adjusted life years
ECDC	European Centre for Disease Prevention and Control
EFSA	European Food Safety Authority
VTEC	*verotoxin-producing E. coli*
YLD	years lived with disability
YLL	years of life lost
YOPI	young, old, pregnant, immunocompromised

References

Al Dahouk, S., Neubauer, H., Hensel, A., Schoneberg, I., Nockler, K., Alpers, K., . . . and Jansen, A. (2007). Changing epidemiology of human brucellosis, Germany, 1962-2005. *Emerg Infect Dis, 13*(12), 1895-1900. doi:10.3201/eid1312.070527

Amt für Statistik Berlin-Brandenburg. (2014). *Einwohnerinnen und Einwohner im Land Berlin am 30. Juni 2014, Statistischer Bericht. A I 5 – hj 1 / 14, SB_A01-05-00_2014h01_BE.xlsx*.

EFSA, European Food Safety Authority. (2011d). EFSA Panel on Biological Hazards, Scientific Opinion on Campylobacter in broiler meat production: control options and performance objectives and/or targets at different stages of the food chain. *EFSA Journal, 9*(4), 2105. doi:10.2903/j.efsa.2011.2105

EFSA and ECDC, European Food Safety Authority and European Centre for Disease Prevention and Control. (2015). *The European Union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2014* (1831-4732). https://efsa.onlinelibrary.wiley.com/doi/abs/10.2903/j.efsa.2015.4329

Haagsma, J. A., Polinder, S., Stein, C. E., and Havelaar, A. H. (2013). Systematic review of foodborne burden of disease studies: quality assessment of data and methodology. *International Journal of Food Microbiology, 166*(1), 34-47. doi:10.1016/j.ijfoodmicro.2013.05.029

Havelaar, A. H., Haagsma, J. A., Mangen, M. J., Kemmeren, J. M., Verhoef, L. P., Vijgen, S. M., . . . and van Pelt, W. (2012). Disease burden of foodborne pathogens in the Netherlands, 2009. *International Journal of Food Microbiology, 156*(3), 231-238. doi:10.1016/j.ijfoodmicro.2012.03.029

Mughini Gras, L., Smid, J. H., Wagenaar, J. A., de Boer, A. G., Havelaar, A. H., Friesema, I. H., . . . and van Pelt, W. (2012). Risk factors for campylobacteriosis of chicken, ruminant, and environmental origin: a combined case-control and source attribution analysis. *PLoS One, 7*(8), e42599. doi:10.1371/journal.pone.0042599

Newell, D. G., Koopmans, M., Verhoef, L., Duizer, E., Aidara-Kane, A., Sprong, H., . . . and Kruse, H. (2010). Food-borne diseases - the challenges of 20 years ago still persist while new ones continue to emerge. *International Journal of Food Microbiology, 139 Suppl 1*, S3-15. doi:10.1016/j.ijfoodmicro.2010.01.021

Perez-Gracia, M. T., Suay-Garcia, B., and Mateos-Lindemann, M. L. (2017). Hepatitis E and pregnancy: current state. *Rev Med Virol*. doi:10.1002/rmv.1929

RKI, Robert Koch-Institut. (2015) Infektionsepidemiologisches Jahrbuch meldepflichtiger Krankheiten für 2014. In. Berlin.

Rosner, B. M., Stark, K., and Werber, D. (2010). Epidemiology of reported Yersinia enterocolitica infections in Germany, 2001-2008. *BMC Public Health, 10*, 337. doi:10.1186/1471-2458-10-337

7 Data and Modelling

Greiner, Matthias

Introduction

Researching global supply chains in the context of food and feed risk assessment is an area of extensive data analysis and modelling. One of the overarching research interests in this area is to quantify the extent to which hazardous agents can propagate along global trade routes, to characterize determinants and modulating factors for the underlying processes and to predict adverse consequences. Different levels of spatial resolution need to be considered depending on the scope of the question. National or regional trade statistics for the commodity of interest are at the highest level of aggregation, e.g. annual or monthly import statistics for the European Union. Such data can be useful for estimating trade volumes and volatility and seizing of a potential problem. Explicit information on trade transactions and processing conditions, on the other hand, is non-aggregated data linked to identified commercial enterprises that are part of a given value chain. Such data is required for tracing outbreaks. Environmental conditions such as temperature, humidity and salinity under which products are traded, stored or processed is recorded as non-aggregated data on local scale. Such information is required for predicting concentration changes of a microbiological agent in a product along the value chain until it reaches the consumer's plate. Specialised models are required to address different questions and to capture the complexity of the data at different levels of aggregation. Recent research and development in this area has demonstrated the need for harmonisation in order to allow linking of various pieces of information in a risk assessment.

In this chapter we explain that graph theory and network analysis provides a sound theoretical foundation for describing and modelling trade networks (section 7.1). A series of software tools is presented that support the assessment of transfer of chemical and microbiological hazards along the value chain (section 7.2). Applications range from transfer models to toxicokinetic models and the use of blockchain technology. Software for tracing foodborne outbreaks, FoodChain-Lab (FCL), is described in section 7.3. FCL has been successfully used in the clarification of several large outbreaks in Europe. The needs for more efficient data collection using the open-source platform Epi-Lab is described in section 7.4. The approach is tailored to risk assessment needs but also enables other stakeholders to obtain an insight into the required data, which may lead to a better sharing of data in the future. The Risk Assessment Modelling and Knowledge Integration Platform (RAKIP) is described in section 7.5. This international project aims at further harmonization of models in the area of quantitative microbiological risk assessment with outreach to other areas of risk assessment.

Future Needs

The availability of data and suitable models for data analysis is the prerequisite for any progress in adapting risk assessment approaches to the reality of global supply networks. A lot has been achieved already and many promising approaches are on their way – examples are shown in this chapter. Further breakthroughs in digitalization in terms of technology, legislation and societal acceptance will dramatically increase the range of possibilities and will have an impact on risk assessment practices where today only proof-of-concepts can be provided.

Keeping track of the growing complexity of the food supply networks calls for effective communication and data exchange strategies which can also involve public-private partnership. More (publicly) available data sources and more reliable data sets are needed for increasing accuracy and granularity of models. We know that information about the structure and dy-

namics of the network can be incomplete, obsolete, missing, false or distorted. As a result, conclusions from network analysis will be flawed unless corrective adjustments can be made. It is therefore imperative to demonstrate that the strategies derived from network analysis are based on reliable descriptions of reality. On a technical level, harmonized data collection is required to facilitate national and international cooperation. Adequate information technology infrastructures must be available to cope with the big data properties and high performance computing methods such as machine learning, artificial intelligence and blockchain.

The needs described above apply in particular also to risk assessment and management tasks in an outbreak situation. One big challenge here is the time factor. In crisis situations communication, data exchange, analysis, assessment and interpretation have to happen in real time. FoodChain-Lab (FCL) has been designed to meet these criteria and has already been applied successfully. It should be further maintained and promoted. The current RASFF system can be a valuable source of information. Artificial intelligence approaches should be developed to extract information presented in unstructured format (e.g. free text in RASFF notifications). Technical solutions need to be secure, reproducible and auditable. The smooth exchange of data and information among different sectors (between risk management and risk assessment, between state level and federal level, between food safety, public health, animal health, etc.) can be a key success factor for outbreak investigations. These aspects should be considered in the planning of crisis trainings on national and international level.

Platforms for sharing epidemiological information such as the Epi-Lab project should be developed that can serve as a reference for national and international food safety risk assessment. This entails high demands in terms of data quality and functionalities such as data plausibility checks, visualization tools, and interfaces with other software tools (e.g., FoodChain-Lab). It should be noted that the quality of data uploaded or linked to such a data platform first and foremost depends on the properties of the original data. Some of the original data may not be collected for the purpose of food safety risk assessment or outbreak investigations. Thus, ensuring data quality for these purposes inevitably requires communication as much as technical efforts.

A plethora of mathematical models exist in the area of quantitative microbiological risk assessment. The RAKIP initiative will facilitate the organization, storage, retrieval, exchange, interpretation, and application of such models along with the associated data. It will also contribute to more harmonized terminologies and concepts in the application areas and advance the definition of metadata for model documentation. On a practical level, RAKIP will support the deployment of available open source software libraries through its converter tools and web-based model repository which enables the user to share, search and execute models which are compliant with the defined standards.

7.1 Network analysis for food supply networks

Selhorst, Thomas

Abstract

Supply chains have been undergoing a major transformation for years, affecting many of their characteristics: the number of chain links and their geographical distribution is increasing and we are seeing a general increase in interdependence. This is expressed in the fact that we are no longer talking about supply chains but about global supply networks. This expansion and densification make trade networks more complex and more difficult to overlook.

In this section, network analysis is presented as an essential tool for maintaining an overview of complex networks and their dynamics. The methods have proven their efficiency in many areas and are used at the BfR for specific tasks in connection with ensuring food and feed safety.

Introduction

This section is about answering the question whether application of graph theory along with its methods originally developed in the field of social network analysis, are suited to generate supplementary knowledge about the state of food networks so that we can use this knowledge to secure or even improve food quality. Furthermore, the question is examined whether the results of a quantitative characterization of food networks and their nodes and edges can be incorporated into a quantitative risk assessment process. Answers to these questions were actually given in 2011, when the methods of network analysis were successfully used to clarify the EHEC outbreak in Germany (Appel, 2011) (Figure 50).

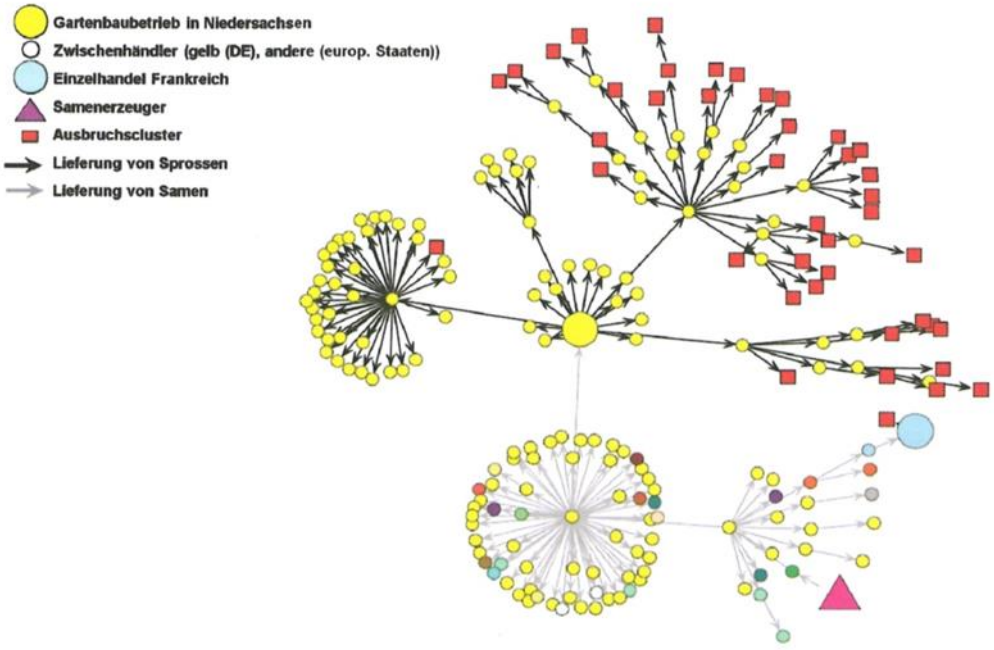


Figure 50: Combined network view of all relevant fenugreek and sprout deliveries
Supplier network at EHEC -O104:H4 outbreak 2011, combined forward and backward tracking: Here you can see all companies that have come into contact with the suspicious batch of seeds or produced sprouts. (created with R-package 'network': Butts, 2008, https://statnet.org).

The production of food is done in food supply networks that are different from supply chains: a production chain is a linear sequence of production units for the production of a product or service. In production networks, linearity has been abolished and the production units are connected in many ways (Figure 50). We will illustrate the difference later in this text. “[Food] Supply networks are emergent, stable patterns in the relationships among specialized economic and public agents that are involved in manifold coordinated activities which cumulate in providing end users with a product [(food)] or service [, i.e. monitoring, surveillance of food products]. The relationships are of many kinds but four are essential in food supply networks in Europe.

1. exchange of goods or services and of money,
2. transport of goods,
3. exchange of information about the food product’s quality including traceability information,
4. exchange of meta information for coordination the first three relationships (Mueller, 2007).

The development from a rigid supply chain to a dynamic network is necessary because networks are expected to allow swift adaption to a changing environment changes (Borgatti and Li, 2009), where the term "environment" refers here to everything related to the production of foodstuffs. Adaption includes both, short-term responses to acute food quality deficiencies and alignment to medium- and long-term changes in consumer demands for food quality.

To provide the end user with high quality food products, supply networks need some concerted coordination efforts by their agents. Agents in a supply network should be concerned with three coordination questions (Lambert and Cooper, 2000):

1. Who are the key supply chain members with whom to link processes?
2. What processes should be linked with each of these supply chain members?
3. What level of integration and management should be applied to each process link?

Food supply networks may involve many agents, especially when we think of global supply networks. As a consequence, productive supply network relationships are unlikely to come about easily when the agents of a potential supply network do not share a common perception of their positions in the network. Supply networks are, however, mental constructs and there may be as many perceptions of a given network as there are minds perceiving it. Like a shared map that allows two travelers to meet at some agreed upon point, a shared model of the supply network showing agents and the linkages among them, greatly facilitates managers’ task to define their position in the network, to identify agents with whom to link processes, and to define the nature of the links to specific agents (Mueller, 2007).

Graph theory is the artistry with which this map can be crafted. Furthermore, the application of graph theory methods is expected to inform network’s actors in a way to better fulfil their coordinating tasks aiming at the networks developed in the desired direction.

Representation of a supply network

General definitions of the term network range from “a network is made up of nodes and ties that conned these nodes” (Kim et al., 2011), to “a […] network model includes five components: a set of actors (nodes), a collection of links representing the relationships between ordered pairs of actors, a sociograph consisting of nodes and directed or undirected links between the nodes, an adjacency matrix, which has a many rows and columns and rows as there are actors and where the elements of the matrix encode the relationships between the actors, and a characteristics matrix, which has as many rows as there are actors and as

many columns as there are attributes of interest." (Mueller, 2007). Building blocks of a virtual representation of a supply network are the nodes (actors) and the connection between nodes (Figure 51).

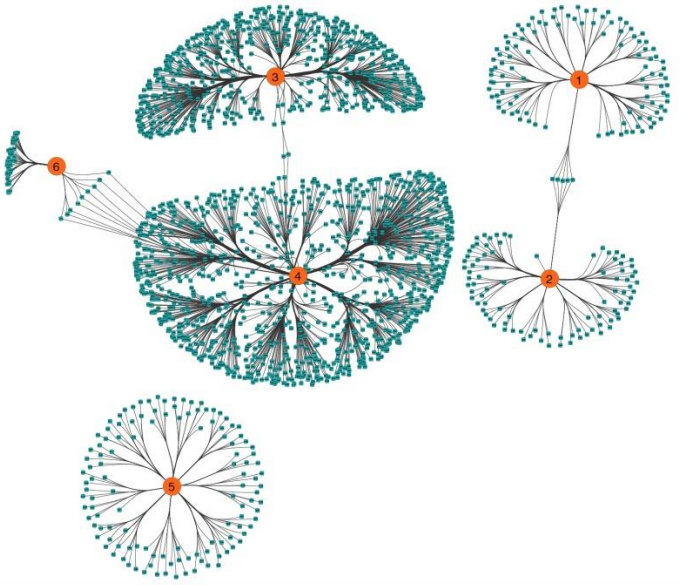


Figure 51: Trade contact between actors of the food supply chain pork
Red nodes represent slaughter houses and nodes colored in green represents group of farms delivering pigs to slaughter houses.

Nodes / Actors

In a supply chain networks, the actors can be e.g. producers, transporters, retailers, and other parties such as laboratories, food safety authorities, or state authorities. We may be dealing with different groups of actors or even just one group. The number of groups to which actors belong is not strictly defined, but naturally always depends on the task to be answered with the application of methods of network analysis. It is therefore quite conceivable that even every single consumer might be considered as an actor in the network. Actors can be part of only one or multiple layers of the network (Clemente et al., 2015).

If we want to quantify the importance / significance of nodes this cannot be derived from nodes properties but only from their interaction with other nodes (actors) in the network. The significance of the nodes can only be determined with the help of network analysis which focusses on the interactions in a network. Whether node importance correlates with the properties of the nodes can only be determined after node importance has been quantified.

Links

The connections between a pair of actors in a network can represent everything that is exchanged between actors in the network (Lentz et al., 2011; Liljeros et al., 2003). In supply networks this is of course the flow of materials and goods and the flow of money and information. However, a link can also indicate the relationships between actors based on particularly good or bad experiences between actors or financial partnerships (Appel, 2011; Schulz et al., 2017). Since goods and money have a sender and a recipient, the links are directed in this case. But we can also imagine undirected connections like in the case of friendship / partnership.

The connection between a pair of nodes can of course contain further information, which can be nicely explained by the flow of goods if a link contains additional information about the type and quantity of goods traded, and information on the time of trading. Again, the amount of information depends on the question to be answered by application of network analysis methods.

Perception and the Graphical network representation

In order to recognize the own and also the positions of other actors in the network, graphic representations of the network are used. That is difficult, because we have not yet agreed on the term position! When we think of food chains in the classical sense, we think of production plants and these plants are geographically located. But let us be careful, because graphical representations of networks focus on a different understanding of the term position, which must be derived from relationships with other actors. Therefore, the geographical position of the actors is not used in the graphical representation of networks because it disturbs and hinders the perception process. Rather, the actors are placed in a graphical representation in such a way that one has an unobstructed view of the network of relationships.

Under certain conditions, for example, we want to derive the centrality (e.g. importance) of individual actors within the network of relationships. If the result is to be displayed graphically, then it would make sense to place the important companies in the middle of the image and the less important ones at the edge of the image, even if the geographical position would result in a different position. Nevertheless, the geographical positioning of nodes on a map may be useful if the geographical representation is of particular interest. However, whether or not the graph conveys any perceivable information depends on the structure of the network, i.e. the data at hand.

But here, too, as already mentioned above, there is no single graphical network representation to be used; rather, the representation depends on the question. In addition, new forms of graphic representation are continuously being developed that are suitable for specific requirements.

Data requirements

Above it was stated that the virtual representation of a supply network consists of the adjacency matrix, and the matrix for the attributes of the actors. It was indicated that the adjacency matrix is a square matrix with dimension n x n, where n is the number of actors. The attribute matrix has the dimension n x a, where a is the number of attributes per actor. If there are m different relationship types between the actors, then the data volume corresponds to $N=n(n+mn)$. The data volume increases in square terms with the number of players.

For large networks (e.g. global food chains), the volume of data needed to set up a network representation corresponds to the definition of Big Data.

But other aspects of Big Data also need to be taken into account, and here the aspects of Validity and Velocity are dealt with. It is indisputable that the data, in particular the information on the actors and the links between the actors must be complete and valid in order to obtain a correct representation of the network. The completeness is not always given today, since the information about the interactions is collected from different actors of the network itself or outside the network, and access to this information may be restricted and the quality of the data received with regard to completeness and accuracy cannot always be checked. Here approaches of the blockchain can possibly help to guarantee the validity (Tian, 2016).

The speed at which information is provided (Velocity) can also influence the validity of the network model. For example, data on trade in livestock between farms has been used for several years to establish and study these trade networks. The data used to create these networks have to be entered manually into the system by farm managers within a specified period of 7 days. Own investigations have shown that this period can be exceeded. Data delivery is not only delayed, but asymmetric, which can lead to a distorted, fuzzy network model. Furthermore, networks continue to develop and are created in order to be able to adapt quickly to changing environmental conditions. Data over networks can be outdated and invalid, so network models are also outdated and/or invalid to describe the current state of a supply chain.

Network metrics

There are many metrics to characterize networks and it would be far below the scope of this publication to present all of them. We limit ourselves to those with which we can determine which nodes are in the center and at the periphery of the network, further we want to be able to determine the diameter of the network.

Centrality parameters are measures used to quantify the centrality of actors or links or other entities belonging to a network. The centrality parameters presented here all originate from the social sciences and were all developed for the social sciences. That is why the name of the method as a whole is Social Network Analysis (SNA). Supply chains may contain social aspects, but the term SNA does not really fit for supply chains and the meaning of the centrality parameters must also be adapted to supply chains. Some important centrality parameters are presented below. At the same time, it is shown how they can be interpreted when applied to supply networks (Borgatti and Li, 2009).

The identification of the key actors in a social network is one of the primary uses of network analysis (Lambert and Cooper, 2000). Centrality reflects the relative importance of a node in a network. Important node-level centrality metrics are: degree centrality, closeness centrality and betweenness centrality.

The *degree centrality* is the most straightforward centrality measure. The degree centrality for an actor is the fraction of actors it is directly connected to. The more links an actor has, the more central it is. A high degree centrality points to “where the action is” (Wasserman and Faust, 1994) in a network. When an actor is connected to a large number of other nodes, the actor has high degree centrality. Due to its greater connectedness with other actors, an actor with high degree centrality would necessarily be more visible in the network (Lambert and Cooper, 2000). Considering a contractual of information network, degree centrality quantifies the influential extend of the actor in comparison to the other actors. In this case degree centrality describes the extent to which an actor has an impact on the operational decisions or the strategic of general behavior of other actors in a supply network (Marsden, 2002). In contrast, nodes with low degree centrality are considered peripheral in a network. If a node is completely isolated (i.e. degree centrality 0), then removing this node from the network would not be recognized by the rest of the network.

The *closeness centrality* of a network actor is the sum of its distance to all other nodes in the graph or in the case that the graph is not connected to all other nodes in the connected component containing that actor. The calculation of the closeness centrality includes the calculation of the shortest paths from the actor to all other reachable actors, which is a CPU intensive task when the number of actors in the network becomes large.

The closeness centrality is a good example to demonstrate that the selection of centrality measures depends and the research question and, as exemplified here, even on the network

type. In directed networks the shortest path from actor a(i) to actor a(j) may differ from the shortest path form actor a(j) to a(i). As pointed out (Kim et al., 2011), this does not make physical sense in the case of supply network. But, if we focus on information, closeness centrality quantifies the extent to which a firm has freedom from the controlling action of others in terms of accessing information. The actor given a high closeness centrality has greater autonomy in accessing, collecting various information with greater autonomy.

The *betweenness centrality* of a network actor *a* is the fraction of all-pairs shortest paths that pass through *a*. The betweenness can be viewed as indicating how much gatekeeping an actor has for the other actors. Gatekeeping occurs because the actor is located on the shortest path between actors and is able to control or at least record the flow between the actors.

Application of network analysis tools

We will now apply methods of network analysis to answer a simple question: How do supply chains differ from supply networks in terms of selected centrality metrics? Both networks consist of 5 actors. The supply chain is linear, and the supply network is highly interconnected (Figure 52).

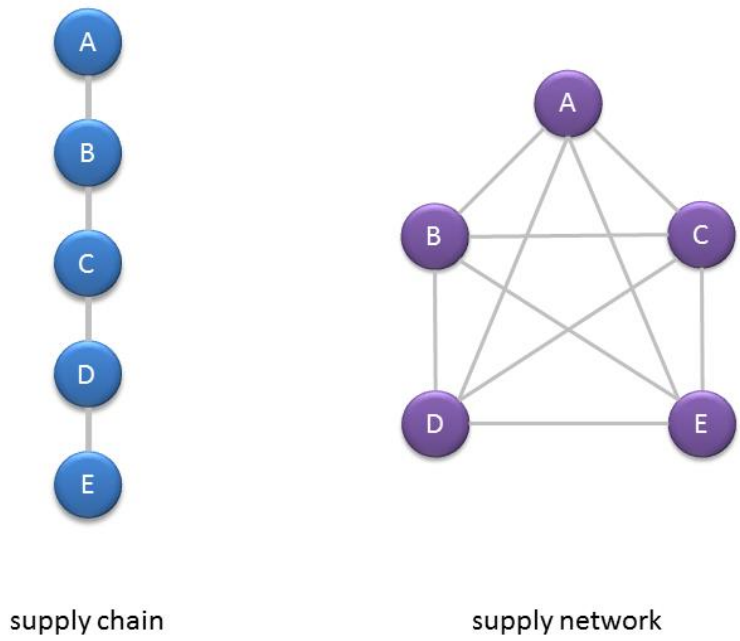


Figure 52: Graphical sketch of a supply chain and a supply network

The calculation of node centralities and network characteristics is done with the software tool NetworkX (Hagberg et al., 2008). NetworkX is a Python package for the creation, manipulation, and study of the structure, dynamics, and functions of complex networks. NetworkX provides tools for the study of the structure and dynamics of social, biological, and infrastructure networks; a standard programming interface and graph implementation that is suitable for many applications; a rapid development environment for collaborative, multidisciplinary projects; an interface to existing numerical algorithms and code written in C, C++, and FORTRAN; and the ability to painlessly work with large nonstandard data sets.

With NetworkX you can load and store networks in standard and nonstandard data formats, generate many types of random and classic networks, analyze network structure, build network models, design new network algorithms, draw networks, and much more.

The audience for NetworkX includes mathematicians, physicists, biologists, computer scientists, and social scientists. NetworkX contains almost all known current algorithms for the analysis of networks and is continuously expanded and updated.

Figure 52 describes the transition from a historical supply chain to a supply network. We intuitively understand that the production network is much more interconnected than the production chain. This feeling can be quantified, both at the network level and at the node level. Here we use the metrics introduced above (Table 31).

Table 31: Network metrics

Network	Center	Diameter	Periphery
Supply chain	C	4	A,E
Supply network	A, B, C, D, E	1	A, B, C, D, E

The observed densification of the relationships is reflected in the metrics. While one can still find actors in the retail chain who are in the center (node C) or at the edge (nodes A and E) of the chain, the actors in the network are all in the center. The center and the edge even collapse. The network is highly compact, and the diameter shrinks from 4 to 1. At the same time differences between the nodes in terms of the selected metrics disappear, all nodes in the network are synchronized (Table 32).

Table 32: Node centralities

Network	Degree	Closeness	Betweenness
Supply chain	A:1, B:2, C:2, D:2, E:1	A:2.5, B:1.75, C:1.5, D:1.75, E:2.5	A:0, B:0.5, C:0.66, D:0.5, E:0
Supply network	A:4, B:4, C:4, D:4, E:4	A:1, B:1, C:1, D:1, E:1	A:0, B:0, C:0, D:0, E:0

The compaction and synchronization of the chain is also reflected here. The different visibility of the nodes in the chain is changed to a greater and equal visibility for all actors in the network. The proximity of the players among each other is reduced to a minimum, all players in the network are direct neighbors. In the chain, the nodes were further apart on average and differed from each other. Gatekeeping can no longer take place in the trading network, since there are no nodes between the trade connections that could fulfill this task (betweenness centrality = 0 for all nodes).

Valuation and outlook

Methods for analysing food supply networks have the potential to support decision-makers in improving food quality and safety, as they can be used to identify key players in the food supply networks. The own position within the food supply network can also be optimized in

order to achieve certain goals. In order to fully exploit this potential, however, the data with which virtual networks are set up must be available. And this is exactly the problem: the methods are available, but often the data is missing or incomplete, and it becomes particularly hazardous when we incorrectly assume that the data represents a correct reflection of reality.

In the following chapters special tools are introduced with the help of which food supply networks can be managed in certain situations.

References

Appel, B. B., Gaby-Fleur; Greiner, Matthias; Lahrssen-Wiederholt, Monika; Hensel, Andreas. (2011). EHEC-Ausbruch 2011. Aufklärung des Ausbruchs entlang der Lebensmittelkette *BfR Wissenschaft, 04/2011*.

Borgatti, S., and Li, X. (2009). *On Social Network Analysis in a Supply Chain Context* (Vol. 45).

Clemente, F., Nasuelli, P., and Baggio, R. (2015). *Formal network analysis of a food supply chain system: a case study for the Italian agro-food chains* (Vol. 6).

Hagberg, A., Swart, P., and S Chult, D. (2008). *Exploring Network Structure, Dynamics, and Function Using NetworkX.* Paper presented at the 7th Python in Science Conference, Pasadena, USA.

Kim, Y., Choi, T. Y., Yan, T., and Dooley, K. (2011). Structural investigation of supply networks: A social network analysis approach. *Journal of Operations Management, 29*(3), 194-211. doi:10.1016/j.jom.2010.11.001

Lambert, D. M., and Cooper, M. C. (2000). Issues in Supply Chain Management. *Industrial Marketing Management, 29*(1), 65-83. doi:10.1016/S0019-8501(99)00113-3

Lentz, H. H. K., Konschake, M., Teske, K., Kasper, M., Rother, B., Carmanns, R., . . . and Selhorst, T. (2011). Trade communities and their spatial patterns in the German pork production network. *Preventive Veterinary Medicine, 98*(2), 176-181. doi:10.1016/j.prevetmed.2010.10.011

Liljeros, F., Edling, C. R., and Amaral, L. A. N. (2003). Sexual networks: implications for the transmission of sexually transmitted infections. *Microbes and Infection, 5*(2), 189-196. doi:10.1016/S1286-4579(02)00058-8

Marsden, P. V. (2002). Egocentric and sociocentric measures of network centrality. *Social Networks, 24*(4), 407-422. doi:10.1016/S0378-8733(02)00016-3

Mueller, R. A. E. B., Doreen; Seidel-Lass Linda. (2007). Supply Chains And Social Network Analysis.

Schulz, J., Boklund, A., Halasa, T. H. B., Toft, N., and Lentz, H. H. K. (2017). Network analysis of pig movements: Loyalty patterns and contact chains of different holding types in Denmark. *PLoS One, 12*(6), e0179915. doi:10.1371/journal.pone.0179915

Tian, F. (2016). *An agri-food supply chain traceability system for China based on RFID and blockchain technology.* Paper presented at the 13th International Conference on Service Systems and Service Management (ICSSSM).

Wasserman, S., and Faust, K. (1994). Social network analysis: methods and applications. doi:10.1017/CBO9780511815478

7.2 Applications of supply chain network modeling for feed and food safety

Fuhrmann, Marcel; Numata, Jorge; Bulik, Sascha; Filter, Matthias

Abstract

Risk assessment of food and feed safety becomes more and more complex in a globalized world, as information on trade and international levels of food and feed safety needs to be included. Supply chain network models are therefore versatile tools to support risk assessment. Modelling supply chains based on scarcely available data sets is a challenging task and demands innovative approaches at different scales. We present several projects currently in development at BfR to address these challenges.

The Knowledge Lab Toolkit is a supply chain modelling framework that enables easy access to a repository of supply chain models for both modelers and risk assessors. It supports the creation, storage and continuous update of supply chain models and includes algorithms for identification of food-borne disease outbreaks sources and simulation. Another project is FeedChainCheck, which will become a resource to support specifically the German feed safety domain by modelling the animal feed supply chain containing algorithms to discover critical points and tracing sources regarding chemical and biochemical agents such as dioxins, PFAS, mycotoxins and pyrrolizidine alkaloids. FeedChainCheck will allow to link to models that allow to predict the transfer of contaminants from feed into foods of animal origin via individual farm animals. Such toxicokinetic models are developed to predict the accumulation and depuration of contaminants in edible tissues (blood, fat, muscle, liver and kidney) and products (e.g. milk, eggs) as a consequence of contaminations in the feed and drinking water. Further projects involve distributed ledger technologies (DLT) such as Blockchains, which promise to change logistics as we know it. Blockchain technology may be used in feed and food supply chains to prevent fraud and enable completely digital backtracing. For risk assessment, including biological and chemical analytical data into the Blockchain would allow for very novel applications. Finally, FoodProcess-Lab is an open-source software framework that allows to performing simulations on the effect of food processing conditions on microbial hazards (e.g. during production steps in slaughter houses or dairy factories). Its intention is to help the food- and feed industry to monitor microbial development in production processes and to help public authorities to assess risks.

Introduction

In this chapter we will present additional software tools currently under development by BfR that support the integration of knowledge (i.e. data and models) on supply and processing chains into risk assessment for food and feed safety. In recent years a variety of data, experimental results and mathematical models have been generated. However, its reusability for risk assessment purposes is curtailed due to a deficit in transparency caused by lacking standards for harmonized knowledge representation and annotation.

Therefore, the usability of existing resources for risk assessment is impeded as risk assessors may not be able to access (or even reproduce) the required knowledge in an efficient and/or effective manner. To allow transparent and efficient access to relevant information, BfR develops new software instruments and generic knowledge representation schemata.
In the first section of this chapter we introduce the Knowledge Lab Toolkit (KLT) that allows to describe and design a supply chain network model with a modular and flexible approach. The purpose of this toolkit is to give risk assessors easy access to existing supply chain networks and at the same time provide modelers the required tool to describe their network model with all necessary metadata.

The second section will introduce FeedChainCheck that is designed to become a knowledge base to support risk assessments in the German feed sector. This toolkit will model the animal feed supply chain and allow for discovering critical points and tracing sources regarding chemical and biochemical contaminants, such as dioxins, PFAS, mycotoxins and pyrrolizidine alkaloids. It is also linked to models describing the transfer of contaminants within individual farm animals. Such toxicokinetic models can simulate the transfer rates and are based on experimental results. In a third section we discuss how Blockchain technology may be used in feed and food supply chains in the future to prevent fraud and enable completely digital backtracing. The inclusion of analytical data on food products into a Blockchain would allow for very novel risk assessment applications. However, Blockchains also have downsides and vulnerabilities that cast doubts about their actual usefulness.

In the final section we present FoodProcess-Lab, which allows model-based simulations for food processing operations.

Food Supply Chain Modelling *(Fuhrmann, Marcel; Filter, Matthias)*

Mathematical models and model-based simulations are getting more and more relevant for scientific research in general as well as for food or feed safety risk assessments. In case of supply chain network modelling the type, content and nature of models is very heterogeneous and models are stored in various resources (e.g. publications, code repositories, information repositories or sometimes are only available from authors themselves). There are models that cover transport, supply & demand, production & distribution, time and spatial factors as well as a wide range of mathematical algorithms that lead to empirical or mechanistic supply chain models.

It can be assumed that each of the models published in scientific literature is functional and worthwhile in their own respect. Unfortunately, it is very difficult to apply or adapt such models when it comes to risk assessments for a specific risk assessment scenario. The sheer amount of available models with different range of applications, multiple data sources and nontransparent provision of metadata makes it difficult for risk assessors to work in an efficient and/or effective way. Risk assessors are therefore interested in having a resource that provide scientifically validated models ready to use, in a way that they can also make a fast and accurate decision on which model to apply for the given risk assessment task. Unfortunately, such a resource does not exist so far, as in many cases transparency and proper annotations are missing, e.g. missing information on the range of application of a specific model.

To address this issue BfR is currently developing a new software tool, called Knowledge Lab Toolkit (KLT). KLT is a set of tools and services that serve as easy access point for risk assessors, as it provides an overview on readily available models with sufficiently clear annotations. Furthermore KLT will contain a variety of tools for network model generation, analysis, and model based simulations. KLT will support the provisioning of necessary metadata, e.g. detailed information on the range of application of the model. It also supports modelers that prefer to develop their model in a specific scripting language, as it provides easy import options for these kind of models. KLT will also check for completeness of the entered metadata according to the community standard Food Safety Knowledge-Markup Language (FSK-ML). In short, FSK-ML is a file format definition for script based risk assessment models allows to store all necessary information in one file, like the original raw data set, scripts for visualization, metadata and reference publications. KLT will take into account that modelers want to provide interested third parties with their respective network models and all necessary information to make use of it with least effort possible. To meet all of these constraints, KLT has been designed in a modular fashion, to be as flexible as possible and be able to incorporate a great variety of network models (if not all).

To accomplish that KLT defines certain concepts: First of all, a supply chain network is defined as a directed graph with nodes and edges (details see chapter 7.1). In supply chain networks each node must have the following properties:

- an actor (e.g. producer, consumer, food processor, etc.),
- a food item / product (e.g. eggs, vegetables, slaughter products, etc.) and
- a location and time where the food item was handled by said actor.

An edge in terms of a supply chain network model has properties like the amount of product being transported, and optionally it may contain possible transport or conversion losses within the supply chain (e.g. water loss in conversion process from milk to cheese). Another example for possible use for edges of a network would be an estimate about the uncertainty of this information. This could lead to Bayesian Network analyses and therefore help with certain analysis tools like foodborne disease source identification models (Abigail L. Horn, 2018). These properties of supply chain networks within the framework of the KLT, we will call Network Data Dimension (NDD).

In order to develop a flexible and modular tool, we store information on each data source and how it will be handled and used. We also allow to process data in a modular way give an end user type–specific front end guiding end users to tools and services relevant to them. As KLT terminologies are both flexible and well defined, they contribute to build a piece of technology that benefits modelers and risk assessors alike.

In the following the KLT concept will be illustrated in more detail. With respect to “data objects” - within KLT each “data object” will contain information on the following properties:

- the data file itself,
- necessary metadata and
- information about the data format and how to read it.

“Data objects” that meet these simple constraints can be used as basis for the generation of network models in KLT.

KLT also defines “Raw data object” (RDO). This is a subset of all data objects. Therefore it consists of a raw data set, the corresponding metadata and the information about the format of the data for the KLT to read in.

The metadata, like reliability or date and location of acquisition will be stored using the FSK-ML format specification.

However, initial raw data sets might not meet the aforementioned NDD in the first place. As an example, the available raw data might be provided on a NUTS-1 level (french: *“nomenclature d'unités territoriales statistiques”*, geocode standard for referencing the nations of the European Union), but the modelled supply chain network model is supposed to address NUTS-3 level (in case of Germany that would be the 402 “Landkreise”) questions. This conflict may be engaged if, for example, additional information exists about how many people work in the specific food industry domain in each NUTS-3 level region and a model is constructed to process the available raw data to fit the predefined network structure or NDD.

KLT then defines “Network data object” (NDO). The data set is defined to fit the NDD and is derived from a RDO via a Data processing routine (DPR). The data set of the NDO consists of information about the nodes and the edges in the network. It is bundled together via the Pajek File Format (Batagelj, 2014). Necessary metadata for the NDO is information about how the DPRs are applied or in our already defined terminology how the NDO is derived from the RDO. This will be accomplished by the introduction of a *Provenance* report.

The interface between RDO and NDO is the DPR, which is in fact a data object on its own. Intrinsically it is a script (e.g. R or Python) that transforms a RDO into an NDO. Since the RDO does not necessarily fit the NDD, a script has to be developed that processes the raw data set into a format that fits the predefined network structure. The metadata for a DPR that is necessary to follow the transformation script is provided as before in the FSK-ML format.

Now that we defined all building bricks of data objects that might occur in the KLT, we need to define the grout that binds everything together. In our case this role is filled by the data processes that might be applied to our data objects.

One important feature is the process of creating, editing and querying certain data objects. This is true for all mentioned data objects and here may all necessary information be added to the data object in question. Considering NDO in case of a very complex network model, a user might want to look at certain aspects of the network. A property filter is implemented, that filters according to actor category, product, location and/or time. Other features include import files to the users work flow and export it to other tools like the Spatio-Temporal Epidemiologic Modeler (STEM) (http://www.eclipse.org/stem/) or the Food Chain Lab (FCL) (see chapter 7.3). Another useful feature is the visualization of the network which is sometimes the key to understand certain aspects of it. Furthermore, KLT's analysis tools help with what can´t been observed directly. For example, the identification of the source of a foodborne disease outbreak (Abigail L. Horn, 2018), identifying the impact of a contaminated item in the food chain aka forward tracing, and analysis of resilience factors in a supply chain network will be provided.

KLT will also allow integrating existing national network models into a more global one. For instance, it is possible to incorporate a network model of Germanys supply chain into a network model that addresses the supply chain on a European level.

KLT has been designed to serve the need of different user groups.

The first user type is the risk assessor. He or she is usually only interested in querying the RDOs and NDOs, but rarely wants to edit or create them. Another field of interest would the analysis, the filtering and the visualization of said NDOs.

Another user type is the modeler. He or she is equipped with much more functionalities from KLT. The Modeler needs to be able to create new and edit existing RDOs and NDOs. Moreover, he or she might want to create, edit and apply DPRs for processing the data. Furthermore, he or she will be able to analyze, filter and visualize said NDOs in the same way as the risk assessor user group can.

A further type of user is the curator. As a curator of KLT, he or she is responsible of the up-to-dateness of the raw data sets. Therefore, the curator needs to be able to create new and edit or query existing RDOs.

KLT will enable risk assessors to be more effective and efficient when it comes to assessing the supply chain network in their respective field of interest. KLT has been implemented as an extension to the open source data analytics platform KNIME (Konstanz Information Miner software, https://www.knime.com) (see the conceptual design in Figure 53).

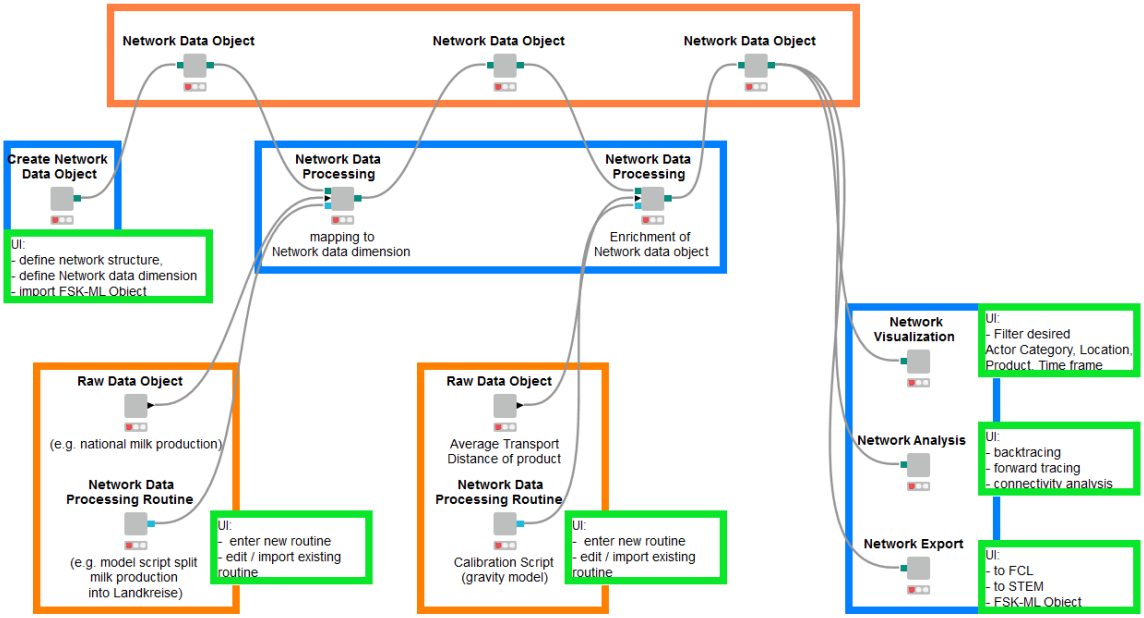


Figure 53: Conceptual KNIME workflow, graphical interpretation of the presented concept of Knowledge Lab Toolkit
The necessary information about the structure of the network model are introduced in the "Create Network Data Object" node. Here the Network data dimension is defined. Next step is the incorporation of raw data, which needs to be preprocessed to fit the Network data dimension. The Network data object accumulates information. After all raw data has been put in, missing information is modelled (e.g. by a gravity model), enriching the network data object further. After this process is finished, the completed network model can be visualized, analyzed or exported to other existing frameworks.

FeedChainCheck: Supply chain modelling for the feed chain *(Numata, Jorge)*

FeedChainCheck is a knowledge base under development to support German feed safety in an era of globalized supply chains. Its aim is discovering critical points and tracing sources in the animal feed supply chain regarding chemical and biochemical contaminants. In contrast to the current practice of ad-hoc recreation of supply networks from paper or digital invoices released under the intense pressure of contamination events or crises, FeedChainCheck aims to proactively map those networks in advance for the most important animal feed supply chains. The feed supply chain includes for example the "stations" cultivation and import of raw materials; processing; transportation/logistics; and consumption at animal producing facilities (see Figure 54). The FeedChainCheck project assesses and predicts critical breaking points and vulnerabilities in the supply chain of animal feed. Compared to food manufacturing facilities, the knowledge available on feed manufacturing plants and their logistics is comparatively more detailed. There is work in progress to classify feed plants according to the risks they could pose, although not all plants and not all risks have been registered yet. Additionally, the tool FeedChainCheck will provide means to depict mixing and processing of feed. Within the scope of FeedChainCheck, there are basically three different kinds of risks to be assessed:

- chemical contamination: undesirable substances in the feed chain (e.g. dioxins, poly and perfluoroalkyl substances)
- biochemical and biological contamination: e.g. spoilage of feed (storage and transport)
- feed logistics security: ensuring continuous supply of feeding needs of the primary production of foods of animal origin under disturbed logistic conditions.

The most straightforward application in case of a contamination event is in back tracing of e.g. chemical and biochemical contaminants or biological agents in the feed chain. Another application scenario related to feed security is the simulation of effects and management options for the minimization of catastrophe damage (for example, rerouting of supply logistics after storm damage). A further one is the minimization of disruption of supply chains after a contamination event, with the purpose of minimizing disruption to logistic routes stopped to avoid spread of contamination and without bringing the whole product's supply chain to a halt.

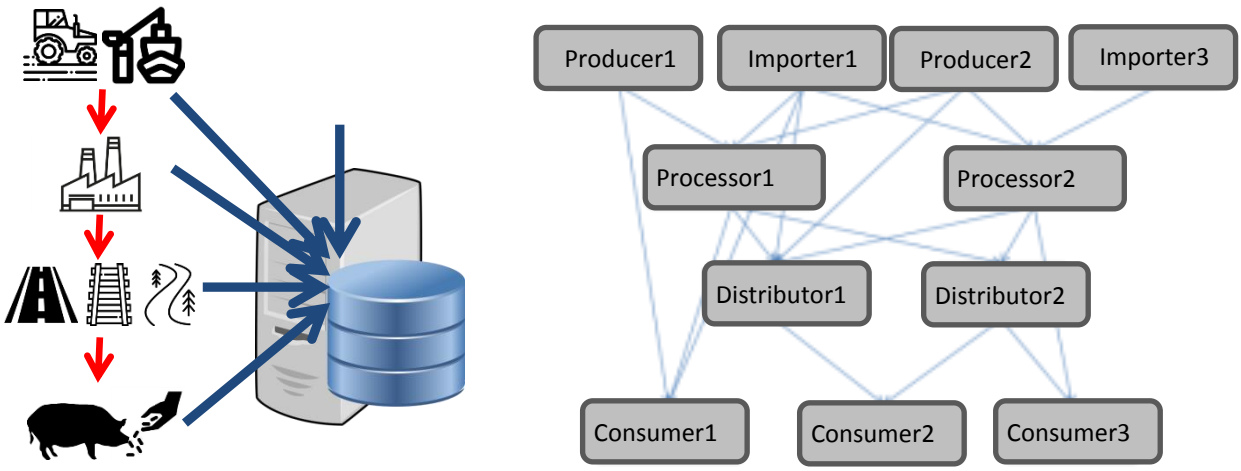


Figure 54: The FeedChainCheck knowledge base takes as its input market and supply chain information on the "stations": cultivation and import of raw materials; processing; transportation/logistics; and consumption by animal producing facilities
Image attributions: Icon images, freely distributable with attribution: Server by Ahkâm https://www.freeiconspng.com/img/3700 / Port: Icon made by Smashicons from www.flaticon.com / Waterway: Icon made by Eucalyp from www.flaticon.com / Rest of icons: Icon made by Freepik from www.flaticon.com

- **Modeling the transfer of chemical and biochemical contaminants in the feed and food chain**

In the context of global supply chains and risk assessment, toxicokinetic models allow us to represent the farm animal as a link in the chain regarding its ability to transfer chemical and biochemical contaminants from feed to foods of animal origin. Farm animals may upconcentrate or depurate the contaminants present in the feed. The transfer of agents from feed to food is a function of the substance(s), the animal metabolism and the food of animal origin in question. Because feed to food transfer of agents involves complex biochemical and biophysical mechanisms, it is not possible as of now to accurately predict the transfer of a specific feed agent to a specific food of animal origin without experiments and solely from first principles. Although great strides are being made in the in-silico, in-vitro and ex-vivo prediction of contaminant transfer, it is still usually necessary to perform in-vivo experiments with each contaminant and each target animal species. We thus often resort to a combination of in-vivo experiments followed by computer modeling using the experimental data. The toxicokinetic model extrapolates the results to cases different from the experiment.

A toxicokinetic model is a mathematical representation of the absorption, distribution, metabolism and excretion of a toxic substance in the body of an organism. It answers the question: what does the organism do to a toxin (e.g. accumulate it to a certain degree), as opposed to what the toxin does to the organism (toxicodynamics; e.g. cause cancer). Toxicokinetics studies in essence the same set of phenomena as pharmacokinetics, but focusing on undesirable substances and toxic contaminants, as opposed to pharmaceutical compounds.

Some relevant contaminants of global feed supply chains that may be transferred in relevant quantities into foods of animal origin include heavy metals, polychlorinated dibenzo(p)dioxins and furans (PCDD/Fs, colloquially called “dioxins”), polychlorinated biphenyls (PCBs), poly- and perfluoroalkyl substances (PFAS), pesticides, mycotoxins, pyrrolizidine alkaloids and marine biotoxins. For more information, see the chapter 4.1.3 on “Transfer of chemical agents and natural toxins along the food chain of animal-derived products”. Toxicokinetic models allow the quantitative estimation of the expected transfer from feed to food. A typical use case is, knowing the concentrations of a contaminant in feed, the time frame and amounts of the contaminated lot and asking what concentrations are expected in the milk, eggs or meat produced. In some cases, the point in the life cycle of the animal is also a relevant factor (e.g. for growing pigs).

After a toxicokinetic model is established based on the experimental data, it is usually published in a scientific journal as a series of equations and parameters in a form that is not directly useful for non-mathematicians. Nevertheless, there is a wide interest among other members of the academic, regulatory and commercial community in estimating the transfer of contaminants from feed to foods of animal origin. This is why the BfR takes the extra step of programming toxicokinetic computer tools with easy-to-use graphical interfaces (Siemen, 2017). Two examples are RITOPS and PERCOW.

RITOPS (Risk tool for estimation of PFAA concentration in swine) is a computer tool developed for modeling the toxicokinetics of seven PFAS in fattening pigs. The aim of the tool is to predict the accumulation and depuration of PFAS in edible tissues (blood, fat, muscle, liver and kidney) as a consequence of PFAS in the feed and drinking water. The tool has been optimized for use by non-modelers/non-mathematicians and is intended to aid food safety authorities. Using an intuitive graphical interface, the user can set variables in order to determine animal growth and toxicological exposure. Calibration is based on data from transfer experiments performed by the BfR (Numata, 2014). The results are graphically shown as thin lines. This does not imply that there is no uncertainty in the prediction, just that the uncertainty is currently not being quantified. See Figure 55 for an example of a use case of RITOPS.

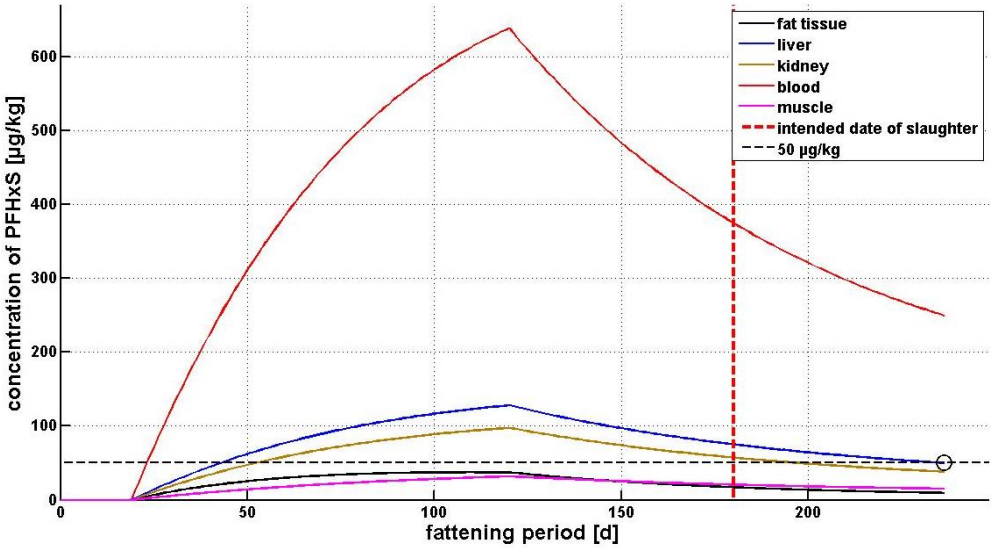


Figure 55: Example for a typical output generated by RITOPS: Time course of perfluorohexanesulfonic acid (PFHxS) concentration in fat, liver, kidney, blood plasma and muscle
The planned date of slaughter (180 days after weaning) is marked with a vertical red dashed line. This plot shows a pig that received PFHxS contaminated feed from day 20 to day 120 after weaning. Although the PFHxS concentration in the feed is constant at 20 µg/kg over time, the amount of the daily feed intake increases (as usual for fattening pigs), resulting in an increasing daily absolute dose. At the same time, the gain in body weight results in a dilution. After the contaminated feeding stops at day 120, a depuration occurs. As an example, the input uses a (fictional) legal maximum level of PFHxS in the liver of 50 µg/kg (black horizontal dashed line). The tool estimated a violation of that maximum level at the intended age of slaughter and automatically prolonged the simulation time until that maximum level is reached (here day 236, marked by a black circle). In this case, the model is predicting non-marketable livers by the planned slaughter date.

PERCOW (Perfluoroalkyl Acids in Cow's Milk Calculator) is a separate software tool for performing kinetics estimations for perfluorooctane sulfonic acid (PFOS) in cow's milk. It is based on model of van Asselt (Asselt, 2013) featuring milk as an additional pathway of excretion for PFAS, in addition to the urinal pathway also available to pigs.

- **Blockchain in supply chain management for feed and food safety**

A Blockchain is a distributed ledger technology (DLT), or a decentralized database. It consists of linked batches of transactions or database entries called blocks. Identical copies of this database are stored in a decentralized manner. The original Blockchain is the heart of the popular and controversial cryptocurrency Bitcoin. The transactions are cryptographically signed and linked into chains. The authenticity and the order of the transactions is very hard to falsify and impossible by changing just one copy of the ledger. Bitcoin proved that it's possible to use Blockchain to build a trustworthy online database that operates outside the control of any one company or organization. The main advantage of the Blockchain technology is the added layer of trust between actors it conveys.

The most straightforward and obvious application of Blockchain to food and feed supply chains is the prevention of food fraud. Furthermore, during a safety incident (such as when a chemically or biologically contaminated batch is discovered or a food poisoning outbreak occurs), it would be easy to go back and forward trace it in a digital fashion. This is much more efficient than the current method of manually reassembling the supply chain from digitized paper invoices. Blockchains need not be totally public. The database can be distributed only among trusted actors, including private producers, government regulators and institutes, analysis laboratories, retailers and external auditors.

Blockchains can go beyond being static repositories of information. A Blockchain can perform agreed-upon actions in the form of smart contracts, otherwise called self-executing contracts, saving all partners potential conflicts. In the case of a contamination event, many steps including the delivery of information to risk assessors and risk managers as well as the execution of intervention measures can be automated using smart contracts.

Blockchain has potentially revolutionary applications in supply-chain management since it allows for complete traceability for anyone with access to the database. For risk assessment, including biological and chemical analytical data (quality assurance laboratory results) into the database allows for very novel applications. For instance, it would be possible to perform a virtual total diet study by tracing back dietary preferences of sub-population groups to the contaminants present in the raw materials, simulating process and cooking effects and averaging over a window of time. Blockchain can also be very disruptive to current models of distribution. In particular, these databases might create a lot more direct relationships between suppliers and users, eliminating many middlemen and changing the landscape of the supply networks as we know them today.

In an article in Harvard Business Review (Iansiti, 2017), Blockchain is praised, not as an immediately revolutionary technology (like the smartphone), but as a technological foundation (like the TCP/IP protocol from 1972). Innovations such as email, Skype or Amazon did not come immediately with the introduction of distributed, non-centralized networks based on a small packet basis (TCP/IP), but were definitely made possible by this breakthrough. Ultimately, it took more than 30 years for TCP/IP to move through all phases - individual application, localized use, substitution and transformation - and to reshape the economy. Although the timeframe for Blockchain is unclear, it will probably take years before a transformation takes place. One important reason is that Blockchain is simultaneously trying to transform many of the fundamentals of today's transaction management, organization and validation layers. This requires major social, legal and political changes that will not happen overnight. At the same time, the current implementations of Blockchain are vulnerable to manipulations such as the 51% attack (where someone controls the majority of the miners of a Blockchain and becomes its sole ruler) or plain old hacking, which may undermine what is the major advantage of blockchains: trust that doesn’t depend on a central authority.

The supporting voices of hype and the detracting voices of naysayers are equally loud. Nevertheless, the BfR has decided to devote some resources to explore the applications of Blockchain to global supply chains in the form of a joint project proposal with a German University and cooperations with other German public research institutions.

Process models, Transfer models *(Bulik, Sascha)*

Foodborne illnesses exert damage to public health (Belaya et al., 2012; Quinlan, 2013). In order to support the protection of consumers, risks related to food products have to be assessed. Food production is a complex multi-step process where different kinds of hazards can be introduced and modified at different positions of the production chain.

Experts create informed assessment of risks, with estimates for unknown and not understood parts of the processes involved. This gets even harder when the product to be assessed is a product of a chain of processes where each part of this chain can introduce new hazards. These experts utilize in addition to data obtained from studies and primary research their long-standing experience for estimates and also for the judgment of risk severity of the processes involved. This personal factor makes the reasoning for a risks assessment hard to document and sometimes incomprehensible. For more complex processes in supply chains several experts from different fields have to pool their proficiencies and cope with different

evaluation schemes. For these complex processes model based risk assessments can profitably be utilized at least for parts of the process chain.

The usage of computational models provides several benefits. Mathematical implementation demands a precise description of the modeled process what eliminates ambiguity sometimes inherent in verbal description. This promotes direct and accurate communication between cooperating experts. The experience that published models are often not reproducible due to lack of or inaccurate documentation led to the development of model description standards as for example SBML (Hucka et al., 2003) but also to the development of standards for the annotation of data and metadata e.g. MIRIAM, RAKIP (Le Novere et al., 2005; Plaza-Rodriguez et al., 2018). Usage of these standards enables reusability of the models and independence from the specific development environment. The development of standards for the annotation of specific simulations to enable the automatized reproduction of model results is still in development but standards as SEDML (Waltemath et al., 2011a; Waltemath et al., 2011b) are emerging. A further benefit of computational models is the integration and balancing of sometimes contradictory data from diverging sources even though this often provides severe challenges to the model developer. Insufficiently understood parts can be identified and closed in this process. Models offer the opportunity to describe single elements or modules of the processes and to interconnect these parts. This reductive approach enables the description of complex processes without the need for a complete comprehension. Furthermore, depending on the nature of the model, simulations provide quantitative results that support risk assessment. In addition to these descriptive traits of the model, they are also predictive i.e. they can be applied to situations that have not been or cannot be accessed by experiment and yield results. The reliability of the predictions depends on model assumption and model completeness and has to be critically evaluated. For example the potential effects of actions for reduction of risk can be evaluated.

Opposing the beneficial rewards of computational models is the effort that has to be spent to develop a reliable tool supporting the risk assessment.

Several QMRAs have been developed with vast effort for specific hazards in specific food products. As an example, for the assessment of Salmonella in pork meat the EFSA developed an extensive model (EFSA BIOHAZ, 2010; A. Hill et al., 2010) over several years. Exhaustive data research and the utilization of expert panels have been necessary for the development of this complex model with country specific parameters for different member states of the EU. The model consists of modules from farm over transport, slaughter and cutting to preparation and consumption including a dose response model to assess the risk of illness, which also have been published in recent years (A. A. Hill et al., 2016; Simons et al., 2016; Swart et al., 2016a; Swart et al., 2016b) and a graphical user interface front-end tool was also developed and is provided by EFSA where a number of parameter variations for this model can be simulated. However, only parts of this valuable model are really specific for Salmonella and if a risk assessor wants to apply the model to a different scenario or to extent it, he has to re-implement the published model or enter collaboration with the original developers. Also it would be desirable that the gathered data specific for different member states of the EU would be assessable even if they are not included in the published version of the QMRA.

In brief the usability of the models by non-experts and the reusability of the models for different questions is hampered by the way the models are provided, stored and annotated despite the high quality of the models themselves. In parallel to the model development activity there is a row of databases established supporting model development, storage and reusability and tools that are intended for supporting non-expert modelers in the usage of models for simulation of microbial hazards in food products. Some examples for useful tools are: i) Combase (Baranyi and Tamplin, 2004), a database with quantified microbial responses to the food environment and predictive models for growth and inactivation of microorganisms in

food matrixes; ii) GroPIN (URL: www.aua.gr/psomas/gropin), a model collection and implementation as Microsoft Excel plugin; iii) MicroHibro (González et al., 2019) a model repository and simulation tool for single step and multistep processes; iv) FDA iRisk (FDA/CFSAN, 2017) a web-based integrated system for analyzing data on microbial and chemical hazards in food to the resulting health burden on the population. In addition, there is a suite of tools developed at the BfR (URL: https://foodrisklabs.bfr.bund.de/frl) comprising i) OpenFSMR (URL: https://foodrisklabs.bfr.bund.de/openfsmr/) a search engine for predictive microbial models, ii) PMM-Lab (Weiser et al., 2012), a KNIME (URL: www.knime.org) extension for fitting experimental data with predictive microbial models and prediction of growth and decay of microorganisms and iii) FSK-Lab (de Alba Aparicio et al., 2018), a KNIME extension to wrap, annotate and execute models. To complement this we have developed FoodProcess-Lab (https://foodrisklabs.bfr.bund.de/foodprocess-lab_de/). This KNIME extension provides a set of tools that support the creation of a risk assessment in which multi-level food manufacturing processes are considered. It is planned to extend the currently existing software that i.e. experimental data, annotations, metadata and models, from food technology, microbiology, epidemiology, statistics, nutrition, veterinary and human medicine and other fields can be incorporated.

As a first step towards this objective a web based graphical user interface for the definition of food product itself (e.g. ingredients and additives), processes (e.g. mixing, separating, packaging or heating) and conditions (e.g. temperature, pH) and a xml-scheme (xsd) for communication with other tools as KNIME extensions or storage in a database was created (see Figure 56).

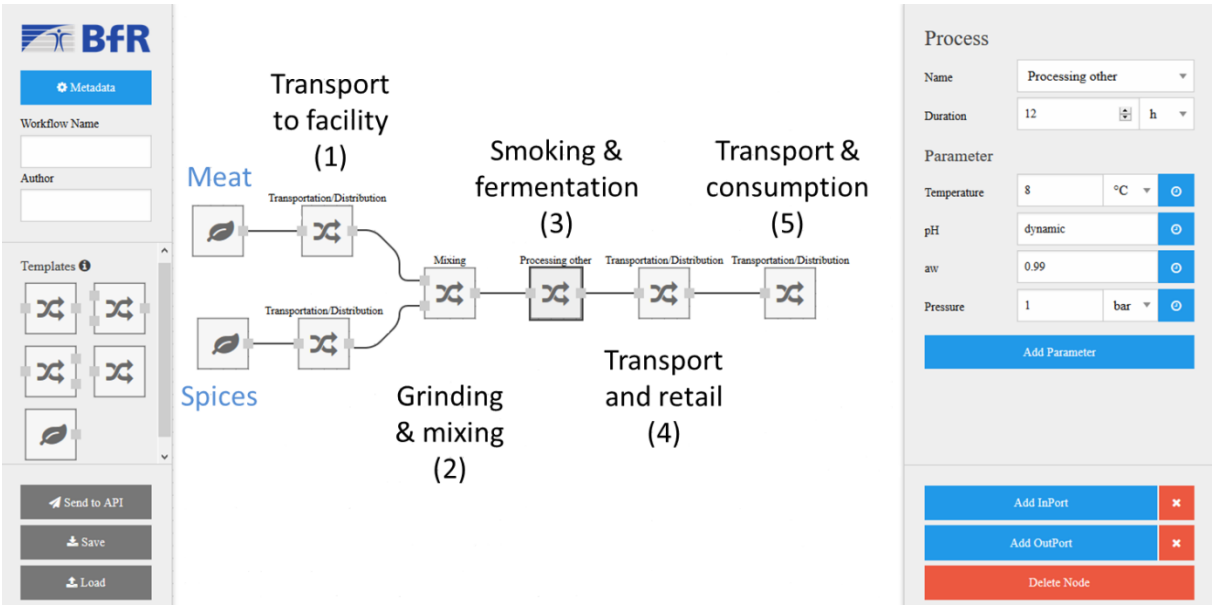


Figure 56: Schematic implementation of food production process in a web-based application
In the given example two basic food items (meat and spices) get transported to a processing facility(1). Here (2), the ingredients get ground and combined into a single entity (sausage meat). The processing (smoking and maturation/fermentation) is depicted in a single processing step (3) with one input and one output. Afterward the product gets transported to the retail stores (4) and the final process in this example is transportation to the customer (5). The left hand panel provides basic nodes that can be arranged and connected via drag and drop. Additionally, basic information can be provided and the workflow can be saved to disc. The panel on the right hand site allows a specification of certain conditions of each individual node including the number of input and output ports.

The information provided in the web-based tool on a specific process chain can be interlinked in FoodProcess-Lab with a hazard. The hazard can either be self-defined or be select-

ed from a database. The independence of the definition of the food product (matrix) from the hazard allows for reusability of the information on the process chain. Subsequently, predictive models (e.g. on growth, degradation or inactivation) of the hazard can be described or selected. Here, it is not necessary to create or implement all models by the user himself since import from model repositories like the PMM-Lab model database is already supported.

FoodProcess-Lab provides the opportunity to perform model based simulations for a given process chain and linked PMM-Lab models. In the future it will be extended to support also models that are compliant to the FSK-ML standard. This will also allow to describe and simulate susceptibility of (sub-)populations to specific hazards, as this is a typical question for QMRA. This includes the option to define specific properties of the target population, e.g. a typical consumption behavior, or dose-response models.

Concluding, FoodProcess-Lab has been designed to support efficient collection and re-use of knowledge relevant to QMRA. For that, FoodProcess-Lab was designed as a modular framework extending the open source KNIME Analytics platform. FoodProcess-Lab also supports harmonized information exchange formats that are currently established in the risk assessment domain (PMM-ML and FSK-ML). FoodProcess-Lab is capable of executing model-based simulations via its integration with the PMM-Lab and FSK-Lab KNIME extensions. The intuitive graphical user interface (GUI) supports documentation, transparency and reproducibility of all simulation results.

Conclusions

In this chapter we have presented a set of innovative tools for food and feed safety risk assessment that are currently in development at the BfR.

The concept of the Knowledge Lab Toolkit was introduced as a systematic and modular approach to implement transparent and reusable network models for food safety risk assessment. The main advantage of this tool is that it creates a “bridge” between scientists that create and share network models and end users like risk assessors (and managers) that want to apply or adapt these models for specific risk assessment questions. This toolkit supports the high expectations of such end-users in respect to accessibility, transparency and reproducibility of models.

FeedChainCheck was presented as a toolkit to assess and predict critical breaking points and vulnerabilities in the supply chain of animal feed. Applications may be back tracing of chemical and biochemical contaminants in the feed chain after a contamination event, the simulation of effects and management options for the minimization of losses due to incidents or resilience assessments of logistic routes and strategies of how to minimize spread of contamination along transport routes.

FoodProcess-Lab can be applied to describe and model food processing steps or chains. It’s modular design concept allows to create graphically any risk scenarios relevant in food safety risk assessment. Simulations carried out in FoodProcess-Lab provide quantitative results and can make use of existing knowledge from food safety model repositories or own databases. FoodProcess-Lab stores systematically all information relevant for the simulation including those metadata required for future re-use by other users.

These tools and resources are and will be available as open source community projects. Cooperation and joint development linking to resources developed by other risk assessment authorities are therefore important objectives leading in the long run to improved knowledge exchange and improved efficiency in risk assessment or outbreak investigation tasks.

Abbreviations

Combase	Online tool for quantitative food microbiology
DPR	Data processing routine
DLT	Distributed ledger technology
EFSA	European Food Safety Agency
EU	European Union
FCL	Food Chain Lab
FDA iRisk	system for analyzing data concerning microbial and chemical hazards in food
FSK-Lab	Food Safety Knowledge - Lab
FSK-ML	Food Safety Knowledge-Markup Language
KNIME	Konstanz Information Miner software
KLT	Knowledge Lab Toolkit
MicroHibro	Software tool for predictive microbiology and microbial risk assessment
MIRIAM	Minimal Information Required in the Annotation of Risk Assessment Models
NUTS	Geocode standard for referencing the nations of the European Union
NDD	Network Data Dimension
NDO	Network data object
OpenFSMR	Open Food Safety Model Repository
PCDD/Fs	Polychlorinated dibenzo(p)dioxins and furans (colloquially called “dioxins”)
PERCOW	Perfluoroalkyl Acids in Cow's Milk Calculator
PFAS	Poly- and perfluoroalkyl Substances
PMM-Lab	Predicitve Microbial Modeling - Lab
QMRA	Quantitative microbial risk assessment
RAKIP	Risk Assessment Modelling and Knowledge Integration Platform
RITOPS	Risk tool for estimation of PFAA concentration in swine
RDO	Raw data object
SBML	Systems Biology Markup Language
SEDML	Simulation Experiment Description Markup Language
STEM	Spatio-Temporal Epidemiologic Modeler

References

Abigail L. Horn, H. F. (2018). Locating the Source of Large-scale Diffusion of Foodborne Contamination. *arXiv, 1805* (03137v1). doi:arXiv:1805.03137 [physics.soc-ph]

Asselt, E. D. v., Janine Kowalczyk, Jan C. H. van Eijkeren, Marco J. Zeilmaker, Susan Ehlers, Peter Fürst, Monika Lahrssen-Wiederholt, and Huberdina Johanna van der Fels-Klerx. (2013). Transfer of perfluorooctane sulfonic acid (PFOS) from contaminated feed to dairy milk. *Food Chem., 141*(1489-1495). doi:10.1016/j.foodchem.2013.04.035

Baranyi, J., and Tamplin, M. L. (2004). ComBase: a common database on microbial responses to food environments. *J Food Prot, 67*(9), 1967-1971. doi:10.4315/0362-028x-67.9.1967

Batagelj, V. M., Andrej (2014). Pajek. *Encyclopedia of Social Network Analysis and Mining*. doi:10.1007/978-1-4614-6170-8_310

Belaya, V., Hansen, H., and Pinior, B. (2012). *Measuring The Costs Of Foodborne Diseases: A Review And Classification Of The Literature*. https://ideas.repec.org/p/ags/gewi12/138195.html

de Alba Aparicio, M., Buschhardt, T., Swaid, A., Valentin, L., Mesa-Varona, O., Günther, T., . . . and Filter, M. (2018). FSK-Lab – An open source food safety model integration tool. *Microbial Risk Analysis, 10*, 13-19. doi:10.1016/j.mran.2018.09.001

EFSA BIOHAZ, European Food Safety Authority's Panel on Biological Hazards. (2010). Scientific Opinion on a Quantitative Microbiological Risk Assessment of Salmonella in slaughter and breeder pigs. *EFSA Journal, 8*(4), 1547. doi:10.2903/j.efsa.2010.1547

FDA/CFSAN, Food and Drug Administration Center for Food Safety and Applied Nutrition , Joint Institute for Food Safety and Applied Nutrition (JIFSAN) and Risk Sciences International (RSI),. (2017). FDA-iRISK® version 4.0. https://irisk.foodrisk.org/

González, S. C., Possas, A., Carrasco, E., Valero, A., Bolívar, A., Posada-Izquierdo, G. D., . . . and Pérez-Rodríguez, F. (2019). ‘MicroHibro’: A software tool for predictive microbiology and

microbial risk assessment in foods. *International Journal of Food Microbiology, 290*, 226-236. doi:10.1016/j.ijfoodmicro.2018.10.007

Hill, A., Simons, R., Ramnial, V., Tennant, J., Denman, S., Cheney, T., . . . and Hald, T. (2010). Quantitative Microbiological Risk Assessment on Salmonella in Slaughter and Breeder pigs: Final Report. *EFSA Supporting Publications, 7*(4), 46E. doi:10.2903/sp.efsa.2010.EN-46

Hill, A. A., Simons, R. R. L., Kelly, L., and Snary, E. L. (2016). A Farm Transmission Model for Salmonella in Pigs, Applicable to EU Member States. *Risk Analysis, 36*(3), 461-481. doi:10.1111/risa.12356

Hucka, M., Finney, A., Sauro, H. M., Bolouri, H., Doyle, J. C., Kitano, H., . . . and Forum, S. (2003). The systems biology markup language (SBML): a medium for representation and exchange of biochemical network models. *Bioinformatics, 19*(4), 524-531. doi:10.1093/bioinformatics/btg015

Iansiti, M., and Karim R. Lakhani. (2017). The Truth About Blockchain. *Harvard Business Review, Jan/Feb 2017*.

Le Novere, N., Finney, A., Hucka, M., Bhalla, U. S., Campagne, F., Collado-Vides, J., . . . and Wanner, B. L. (2005). Minimum information requested in the annotation of biochemical models (MIRIAM). *Nat Biotechnol, 23*(12), 1509-1515. doi:10.1038/nbt1156

Numata, J., Janine Kowalczyk, Julian Adolphs, Susan Ehlers, Helmut Schafft, Peter Fürst, Christine Müller-Graf, Monika Lahrssen-Wiederholt, and Matthias Greiner. (2014). Toxicokinetics of Seven Perfluoroalkyl Sulfonic and Carboxylic Acids in Pigs Fed a Contaminated Diet. *J. Agric. Food Chem, 62* (28), 6861-6870. doi:10.1021/jf405827u

Plaza-Rodriguez, C., Haberbeck, L. U., Desvignes, V., Dalgaard, P., Sanaa, M., Nauta, M., . . . and Guillier, L. (2018). Towards transparent and consistent exchange of knowledge for improved microbiological food safety. *Current Opinion in Food Science, 19*, 129-137. doi:10.1016/j.cofs.2017.12.002

Quinlan, J. J. (2013). Foodborne illness incidence rates and food safety risks for populations of low socioeconomic status and minority race/ethnicity: a review of the literature. *Int J Environ Res Public Health, 10*(8), 3634-3652. doi:10.3390/ijerph10083634

Siemen, H., Helmut Schafft, Christine Müller-Graf, Matthias Greiner, Monika Lahrssen-Wiederholt, and Jorge Numata. (2017). Risk tools for ready-to-use modeling of PFAS transfer from contaminated feed into foods of animal origin *Organohalog. Compd., 79*, 512-515.

Simons, R. R. L., Hill, A. A., Swart, A., Kelly, L., and Snary, E. L. (2016). A Transport and Lairage Model for Salmonella Transmission Between Pigs Applicable to EU Member States. *Risk Analysis, 36*(3), 482-497. doi:10.1111/risa.12390

Swart, A. N., Evers, E. G., Simons, R. L. L., and Swanenburg, M. (2016a). Modeling of Salmonella Contamination in the Pig Slaughterhouse. *Risk Analysis, 36*(3), 498-515. doi:10.1111/risa.12514

Swart, A. N., van Leusden, F., and Nauta, M. J. (2016b). A QMRA Model for Salmonella in Pork Products During Preparation and Consumption. *Risk Analysis, 36*(3), 516-530. doi:10.1111/risa.12522

Waltemath, D., Adams, R., Beard, D. A., Bergmann, F. T., Bhalla, U. S., Britten, R., . . . and Le Novere, N. (2011a). Minimum Information About a Simulation Experiment (MIASE). *Plos Computational Biology, 7*(4). doi:10.1371/journal.pcbi.1001122, ARTN e1001122

Waltemath, D., Adams, R., Bergmann, F. T., Hucka, M., Kolpakov, F., Miller, A. K., . . . and Le Novere, N. (2011b). Reproducible computational biology experiments with SED-ML - The Simulation Experiment Description Markup Language. *Bmc Systems Biology, 5*. doi:10.1186/1752-0509-5-198, Artn 198

Weiser, A. A., Filter, M., Falenski, A., Brandt, J., Kasbohrer, A., and Appel, B. (2012). An Open-Source Community Resource for Creating, Collecting, Sharing and Applying Predictive Microbial Models (PMM-Lab). *Future Security, 318*, 462-465.

7.3 FoodChain-Lab: an innovative tool to increase food safety through supply chain analyses

Gottschald, Marion; Falenski, Alexander; Rügen, Marco; Thöns, Christian; Käsbohrer, Annemarie; Appel, Bernd; Mosbach-Schulz, Olaf, Weiser, Armin A.*

** European Food Safety Authority (EFSA), Assessment and Methodological Support Unit, Via Carlo Magno 1A, 43126 Parma, Italy*

Abstract

In a more and more globalised world, foodborne disease outbreak investigations and food tracing are becoming increasingly complex. Moreover, since 2002 traceability of food is legally required in the European Union. Hence, there was a strong demand for simple and fast analysis tools that would enable the responsible authorities to quickly and reliably identify the source of a contaminated food and thereby solve foodborne outbreaks. For this purpose the German Federal Institute for Risk Assessment (BfR) developed the tool FoodChain-Lab (FCL).

FoodChain-Lab is a free and open-source software. It provides trace back and forward analyses for food items along food supply chains, *e.g.* in case of foodborne disease outbreak investigations. FCL provides integrated data management, validation, enrichment and visualisation features as well as interactive analysis and reasoning methods.

FCL was successfully applied in several large national and EU-wide foodborne outbreaks and is involved in a number of projects and cooperations.

To make tracing more efficient and ready for the challenges of the changing patterns in global food production and trade, we need more effective communication, data assessment and data exchange strategies as well as powerful tracing tools.

Background

Foodborne diseases create a high burden to society, with high costs of illness and high loss of quality-adjusted life years (Hoffmann et al., 2012). Furthermore, food safety incidents put considerable economic strains on food businesses amongst others due to withdrawals and recalls of affected food items or loss of reputation, potentially with subsequent general loss of business (Gadiel, 2010). This situation is aggravated by changing patterns in the global food production and supply chain networks resulting in more and more complex delivery chains – on the one hand promoting a fast and thorough spread of contaminated food items but also making foodborne outbreak investigations and food tracing increasingly complex (Ercsey-Ravasz et al., 2012). Moreover, traceability of food is legally required in the European Union by the Regulation (EC) No 178/2002 since 2002 (Eur. Parliament and The Council, 2002d). The regulation states that traceability of food and feed must be established at all stages of production, processing and distribution. Food and feed business operators further need to have knowledge on their direct suppliers and their direct customers (“one step back, one step forward” approach) and they need to have systems and procedures to transmit the information to the competent authorities on demand.

Both, globalised trade and legal requirement of traceability, underline the importance of simple and fast analysis tools that would enable the responsible authorities to easily analyse food (and feed) supply chains, quickly and reliably identify the source of a contaminated food and thereby solve foodborne outbreaks efficiently. For this purpose, and driven by the large

EHEC/HUS outbreak in Germany in 2011, the German Federal Institute for Risk Assessment (BfR) developed the tool FoodChain-Lab (FCL) (Armin A Weiser et al., 2013; Armin A. Weiser et al., 2016).

Technical and analytical features of FoodChain-Lab

FoodChain-Lab is a free and open-source software. It provides trace-back and -forward analyses for food items along food supply chains, *e.g.* in case of foodborne disease outbreak investigations.

FoodChain-Lab software

The software can be downloaded from https://foodrisklabs.bfr.bund.de/foodchain-lab/. The website also offers tutorials and a fictitious norovirus scenario to learn how to use FCL.

FCL provides integrated data management, validation, enrichment and visualisation features as well as interactive analysis and reasoning methods. For each product (food item or ingredient) and each station (*e.g.* food producer, restaurant) scores are computed to estimate the likelihood that the product/station is related to a contamination event (*e.g.* causing an outbreak). The software also allows running simulations based on geographical parameters or cross contamination events in food production facilities. A detailed description of the features can be found in Weiser *et al.* (Armin A. Weiser et al., 2016). As FCL is an extension of the free Konstanz Information Miner software (KNIME) for interactive data analysis, tracing data can be analysed with lots of pre- and post-processing functionalities additional to FCL.

There are numerous interfaces to exchange tracing data between FCL and other systems. First, there are the **standard FCL Excel templates**. The easiest way to exchange tracing data for all other systems is to create interfaces to those templates. The FCL templates are available in four languages: German, English, Spanish and Hungarian. Different types of templates are available for different purposes: 1) a template for a step-by-step data collection for each station separately, 2) an all-in-one template that enables to collect delivery data and delivery relations for all stations at once. The latter may be of benefit for data collection from unstructured systems like RASFF notifications and was applied during the fipronil case. So far two other systems are supported by FCL: **Commodity Online Services** (COS), a tracing data collection system used in North Rhine-Westphalia, and **ALB templates**, a German data format and template that was developed by an administrative German working group. The ALB templates are similar to the template system of FCL.

Successful applications in recent outbreaks and other incidents

During the last years, FCL was successfully applied in several settings. The software was developed in the course of a large EHEC/HUS outbreak caused by Shiga toxin-producing *Escherichia coli* O104:H4 in Germany in 2011. Using FCL, sprouted seeds from a Lower Saxonian horticultural farm could be identified as vehicle of the pathogen and a specific lot of Egyptian fenugreek seeds could be determined as the probable source of the contamination (Armin A Weiser et al., 2013). The largest German foodborne outbreak – a norovirus gastroenteritis – took place in 2012 and the software could successfully identify a single lot of Chinese frozen strawberries as the source of the outbreak in a retrospective analysis (Bernard et al., 2014; Task Force „Lebensmittel- und Futtermittelsicherheit“, 2012). Another milestone was the successful application of FCL by the EFSA during a large and prolonged multi-country hepatitis A outbreak in 2013 and 2014 which was associated with mixed frozen berries. European-wide trace-back analyses could not identify one single source causing the contamination. However, Bulgarian blackberries and Polish redcurrants were the most common components of the mixed berries lots associated with the outbreak and are therefore the most likely cause (EFSA, 2014b). In 2016, Public Health England and the Food Standards Agency investigated an outbreak of Shiga toxin-producing *Escherichia coli* O157:H7 in the

UK associated with contaminated salad leaves. They used FCL templates to assess supply chain data and analysed them with FCL (Gobin et al., 2018; Inns et al., 2018). FCL also helped to map sheep movements to clarify the zoonotic source of the outbreak strain that was found in the salad leaves (Mikhail et al., 2018). In the fipronil case in 2017, the software was used to map the forward trace of eggs and egg products that were contaminated or potentially contaminated with fipronil helping to identify knowledge gaps in the trace of the eggs. FCL also was applied to identify Sudanese sesame seeds as well as an irregularity in the processing of sesame seeds to paste at a Greek manufacturer as the source of a multi-country *Salmonella enterica* outbreak in 2016 and 2017 investigated by EFSA and ECDC (EFSA and ECDC, 2017) (Figure 57). In the course of the outbreak, two food items were tested positive for the outbreak strain. On the one hand, a sesame paste that was produced using Sudanese sesame seeds was tested positive. The seeds arrived at the Greek manufacturer in November 2015 and were used to produce sesame paste in March 2016. The production came to an irregular halt and the paste was temporarily stored in plastic tanks whose sanitation conditions could not be clarified. On the other hand, hulled and pasteurised sesame seeds produced from Nigerian seeds by the same Greek manufacturer were tested positive. The Nigerian seeds arrived at the Greek manufacturer in September 2016 and were amongst others processed to hulled pasteurised seeds from October 2016 to January 2017. In November 2016, one lot of the hulled pasteurised seeds was tested Salmonella positive by a German wholesaler and distribution of this lot was stopped. In conclusion, the Nigerian seeds arrived at the Greek manufacturer after the production of the contaminated sesame paste that caused the outbreak. Hence, they could not be the source of the outbreak but might be subject to cross-contamination from the sesame paste production described above. As both isolates had the identical serovar which appeared for the first time and due to the big distance between Nigeria and Sudan, an independent contamination of seeds from both origins seemed unlikely. The Greek manufacturer was the only common link between Nigerian seeds and sesame paste from Sudanese seeds indicating a cross-contamination at this place (EFSA and ECDC, 2017).

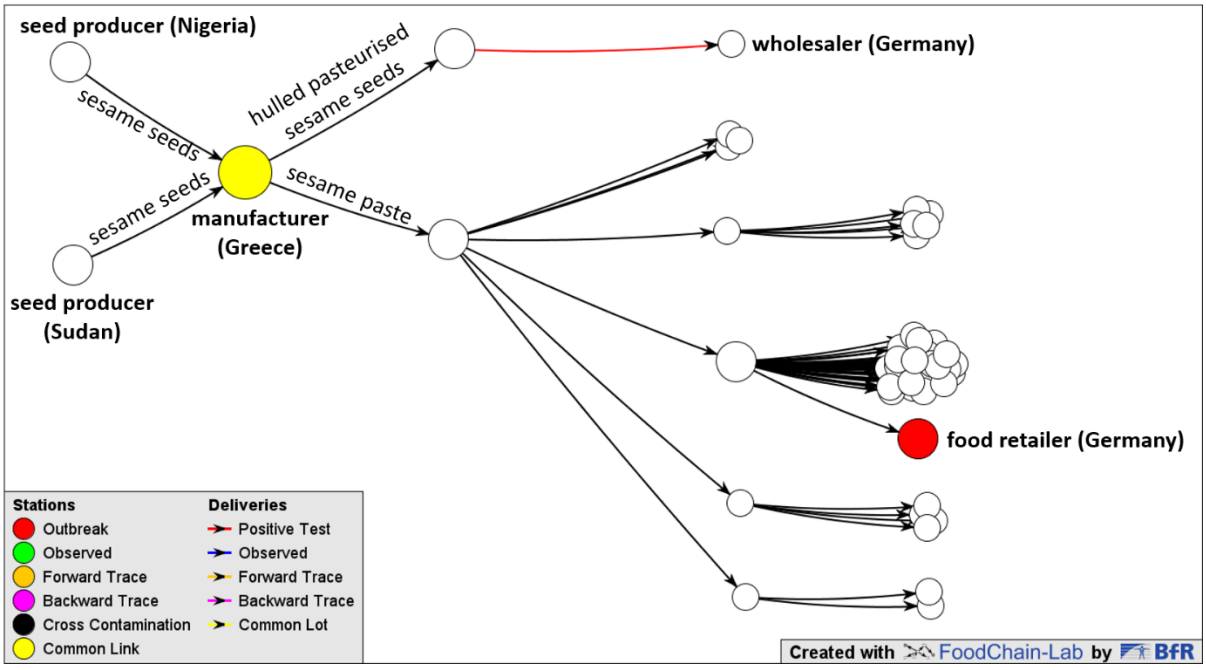


Figure 57: FoodChain-Lab mapping the delivery network of sesame seeds and sesame paste during a multi-country Salmonella enterica outbreak in 2016 and 2017
The red circle represents the retailer from which the human cases arose which were caused by contaminated sesame paste produced from Sudanese sesame seeds. The red arrow represents the hulled and pasteurised sesame seeds tested positive for the outbreak strain. The yellow circle represents the common link between the positively tested isolates.

In a German EHEC/HUS outbreak with sorbitol-fermenting *Escherichia coli* O157:H- in 2016 and 2017, FCL was used to follow the trace of minced meat and boiled sausages. Due to a limited quality of the underlying tracing data, a common source of the outbreak could not be identified. However, there are no new cases and the outbreak appears to be over. Further, FCL and its interface to the tracing system COS was successfully tested during a field study simulating a fictitious foodborne outbreak. In addition, the Hungarian National Food Chain Safety Office (NÉBIH) applied FCL for mapping pig and pork meat transportation routes to identify high risk entry points of African swine fever to Hungary. Most recently, trace-forward analyses were conducted with FCL in the course of a listeriosis outbreak in Austria to find out whether the distribution of food items tested positive for *Listeria monocytogenes* maps to the distribution of listeriosis cases.

FCL support team

In the framework of the EFSA-BfR cooperation, the FCL team provides support for EU member states in terms of planning and conducting tracing analysis during current outbreaks. We also support authorities in applying FoodChain-Lab or conduct the analyses, if required. Please contact us via email (foodrisklabs@bfr.bund.de) or telephone (+49 30 18412 4444).

Project involvements

Past projects

During the **SiLeBAT** project (2010-2014), which dealt with securing the feed and food supply chain in the event of biological and agro-terrorist (BAT) damage scenarios, FoodChain-Lab was developed as a tool for authorities. The aim was to quickly and systematically analyse supply chains to identify food items contaminated with a pathogen in case of crises. The decision to develop the software was influenced by the large EHEC and norovirus outbreaks in 2011 and 2012 during which FCL was able to identify the contaminated lots of the food items which caused the outbreaks.

BfR also had projects with the North Rhine-Westphalian State Agency for Nature, Environment and Consumer Protection (LANUV) on the basis of several **LANUV-BfR cooperations**. In the first project, BfR developed an interface to connect FCL to the Commodity Online Services (COS) - a tracing system developed by LANUV - to facilitate data visualisation and analysis. COS is mainly a well-curated database comprising all companies residing in North Rhine-Westphalia. The interface between COS and FCL is working in both ways: Tracing data is submitted from COS to FCL, the supply chain network is visualised and analysed via FCL and the results are sent back from FCL to COS. The interface is a web-service provided by BfR and the data exchange is realised with a well-defined XML format that is recognised by both systems.

The COS-FCL interface was tested in a real-world setting during the fipronil case in 2017. Supply chain data was collected from RASFF notifications and introduced into COS by LANUV. Data could successfully be transferred to FCL and the results could be exported back to COS via the interface.

In 2017, LANUV organised a field study together with BfR. The study addressed the tracing of suspicious food items along supply chains during a fictitious foodborne outbreak to test a new data assessment template for supply chain data as well as an interface to connect FCL with COS. Different stakeholders (pizzeria; retailer; wholesaler; slaughter, meat cutting and processing plant; industrial bakery) were interviewed on how they manage to trace their products. The companies were asked to fill the new template and to rate its applicability. The companies were also asked to express their needs to allow easy and fast tracing data assessment in case of an outbreak. After each visit at a company, exemplary data assessed with the new template were loaded into COS and transferred to FCL to visualise supply

chains. The study allowed gaining valuable insights into the tracing reality and the different tracing challenges each company has to face at different levels in the supply chain. Useful feedback concerning an adapted data assessment template could be gathered e.g. concerning missing parameters, renaming parameters or adding Excel sheets according to the needs of the companies. However, in general, the companies were mainly satisfied with the template. In addition, they expressed the need for data entry options other than Excel.

Current projects

In 2016, The **EFSA-BfR Framework Partnership Agreement** on "Risk Assessment Tools for the Safety of Global Food and Feed Supply Chains" (GP/EFSA/AMU/2016/01) started. The project area 1 ("Mapping and analysing global food and feed supply chains") is focused on the analysis of supply chains with the aim to enhance the communication and exchange on research projects, priorities and training opportunities. Tasks are centred around FCL and include further development of FCL, applying it in foodborne disease outbreaks, supporting member states of the EU in tracing tasks during foodborne disease outbreaks and propagating its use by conducting training workshops as well as establishing an international network of tracing experts.

FCL is also involved in the **One Health European Joint Programme** (EJP) which is a consortium of 40 partners from 19 European countries, headed by the French Agency for Food, Environmental and Occupational Health & Safety (ANSES). Two sub-projects are in the scope of FCL: COHESIVE (One Health structure for signalling and risk-assessment of emerging threats across Europe) and NOVA (Novel approaches for the design and evaluation of cost-effective surveillance across the food chain). The tasks in the framework of EJP are to develop a browser-based user portal including user management and central data management for FCL to facilitate data exchange. Moreover, further analysis features should be implemented in the FCL desktop and web version. This includes the integration of whole genome sequencing data, network analyses and tools to model the spread of contaminated food items.

In addition, the **LANUV-BfR cooperation** is ongoing in several current and future projects, such as the development of a browser-based data entry mask for delivery data and a second field study.

Discussion and outlook

FCL is a powerful software tool to collect, visualise and analyse supply chain data which could help authorities to identify a common source of contamination during foodborne outbreaks. However, additional enhancements of the tool are needed to *e.g.* improve the automated analysis of networks and to evaluate whether the result of the analysis is more than background noise or the normal market situation. Furthermore, it is important to develop new interfaces to data systems (curated master databases for company data, merchandise management systems) to facilitate fast data assessment and increased data quality.

One milestone in the history of FCL is the development of the FCL web application (Figure 58). The FCL web app is available everywhere and does not need to be installed locally. It offers an easy and intuitive user interface, visualisation and analysis tools as well as additional automated layout styles. A JSON-based bi-directional data

FoodChain-Lab web app

The beta version of the new FCL web application (Fig. 58) is ready to be explored at https://foodrisklabs.bfr.bund.de/fcl-web-app/. It is not recommended for productive use at this stage but you can test it and help making the FCL web app even better. Please email us about your experience with the app (foodrisklabs@bfr.bund.de).

exchange between the desktop and the web app version of FCL allows using the advantages of both systems. Although browser-based, datasets remain completely on the client side to ensure data protection. However, the future is centralised data management. One of the most important goals is to develop a well-protected user portal, which would allow for a central data collection system as well. This system is expected to simplify data collection and dramatically increase data quality. Another essential aim is the development of new visualisation features. On the one hand an automated hierarchical layout will be implemented to visualise delivery networks according to the type of business in a farm-to-fork manner. On the other hand features to easily customise and simplify delivery network views will be developed to emphasise relevant information, for example to publish networks in reports or in EFSA-ECDC Rapid Outbreak Assessments. Such an automated layout could be similar to a Microsoft Visio design and maybe even editable in Visio or directly online.

In addition to the central data management system and new visualisation possibilities more features from the desktop FCL version will be made available in the web app to successively replace the desktop version.

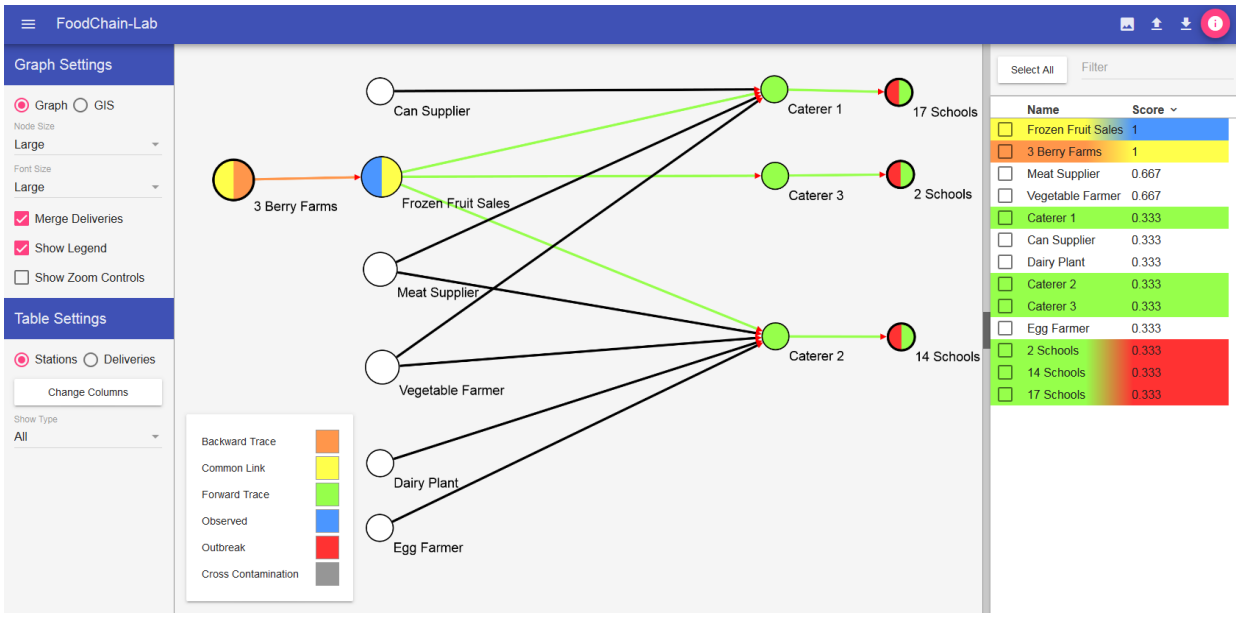


Figure 58: FoodChain-Lab web application mapping the supply chain of a fictitious outbreak

FCL will also be applied to visualise delivery networks obtained from ESFA activities on RASFF information. In this project, delivery data will be extracted from RASFF notifications and their attachments by means of systematic data extraction methodology. Data will be written into a database that can be accessed by FCL to visualise the information and to create automated reports for different purposes. There are also efforts to use artificial intelligence to automatically extract delivery data from RASFF.

FoodChain-Lab workshops

BfR regularly conducts free trainings on the FCL software for . FCL workshops are intensive practical courses to convey expert knowledge on the use of FCL for tracing contaminated foods in foodborne outbreak events. The first part of the workshop is a basic training in FCL with live demonstrations, interactive tutorials and the application of FCL in a fictional outbreak scenario. The second part is an advanced training in which the participants present their institutional tracing practice and share supply chain data. The advanced session is very valuable as it offers the opportunity to work on tracing issues relevant for one or more of the participating countries and thereby getting a deeper knowledge of FCL.
Recent and upcoming workshops can be found on https://foodrisklabs.bfr.bund.de/ events/.
If you are interested in hosting a free FoodChain-Lab training, please contact foodrisklabs@bfr.bund.de.

Since forward tracing seems to become increasingly important, models will be used to associate the distribution of cases and the distribution of (contaminated) products. This might help to strengthen epidemiological evidence in outbreaks where a connection to a specific causative food item is weak or missing.

Trade is accelerating, becomes more and more complex and the network of countries trading globally grows. To make tracing more efficient and ready for these challenges we need more effective communication, data assessment and data exchange strategies as well as powerful tracing tools. Although recent large food crises pointed towards these necessities, they are not yet in place. The RASFF system is used to communicate food and feed safety issues between the EU member states. It is used for tracing as well but since this was not the initial intention of RASFF notifications, the quality of tracing data within RASFF is low. Delivery documents are attached as scanned and in part hand-written documents which are often barely legible and not machine-readable at all. The electronic RASFF Excel templates are only occasionally used to exchange delivery data. RASFF also struggles with unstructured and missing information. Improvements in RASFF are required, for example by implementing a standardised data structure and by pushing towards machine-readable data exchange formats. Moreover, we need harmonised data structures, digital data collection and, if possible, the use of interfaces to data systems to allow for a faster data exchange on member states level. As thorough tracing requires accurate and detailed datasets, digital data assessment tools that allow on-site data validation are desirable as well. Extracting tracing data from sources like RASFF is time-consuming and labour-intensive. Artificial intelligence approaches could help to be more efficient. Furthermore, improved communication strategies between food safety and public as well as animal health authorities are required to clarify each other's information needs, share expertise and synchronise the approaches to solve foodborne crises quickly. Regular interdisciplinary crisis trainings on EU and on member states level would encourage the exchange between the disciplines and would help to be prepared for future crises.

Acknowledgement

BfR wishes to thank the German Federal Ministry of Education and Research (SiLeBAT, *No. 13N11202*), the European *Food Safety* Authority (EFSA-BfR Framework Partnership Agreement), the European Commission (One Health European Joint Programme) and the North Rhine-Westphalia State Agency for Nature, Environment and Consumer Protection (LANUV-BfR cooperations) for funding and supporting the development of FoodChain-Lab and projects centred around the software. We also would like to especially thank Matthias Filter for actively pushing forward the development of FoodChain-Lab during its first years of development.

Abbreviations

ALB	Working group "food, utensils, wine and cosmetics" belonging to the working committee 'consumer protection' of the federal states of Germany (Arbeitsgruppe "Lebensmittel, Bedarfsgegenstände, Wein und Kosmetika" der Länderarbeitsgemeinschaft Verbraucherschutz)
ANSES	French Agency for Food, Environmental and Occupational Health & Safety (Agence nationale de sécurité sanitaire de l'alimentation, de l'environnement et du travail)
BfR	German Federal Institute for Risk Assessment (Bundesinstitut für Risikobewertung)

BVL	Federal Office of Consumer Protection and Food Safety of Germany (Bundesamt für Verbraucherschutz und Lebensmittelsicherheit)
COS	Commodity Online Services
EFSA	European Food Safety Authority
EHEC	Enterohaemorrhagic *Escherichia coli*
EJP	European Joint Programme
EU	European Union
FCL	FoodChain-Lab
HUS	Haemolytic-uraemic syndrome
KNIME	Konstanz Information Miner
LANUV	Agency for Nature, Environment and Consumer Protection of North Rhine-Westphalia (Landesamt für Natur, Umwelt und Verbraucherschutz)
NÉBIH	National Food Chain Safety Office of Hungary (Nemzeti Élelmiszerlánc-biztonsági Hivatal)
NRW	North Rhine-Westphalia
RASFF	Rapid Alert System for Food and Feed

References

Bernard, H., Faber, M., Wilking, H., Haller, S., Hohle, M., Schielke, A., . . . and Werber, D. (2014). Large multistate outbreak of norovirus gastroenteritis associated with frozen strawberries, Germany, 2012. *Euro Surveill, 19*(8), 20719. doi:10.2807/1560-7917.es2014.19.8.20719

EFSA, European Food Safety Authority. (2014b). Tracing of food items in connection to the multinational hepatitis A virus outbreak in Europe. *EFSA Journal, 12*(9), 3821. doi:10.2903/j.efsa.2014.3821

EFSA and ECDC, European Food Safety Authority and European Centre for Disease Prevention and Control. (2017). Multi-country outbreak of new Salmonella enterica 11:z41:e,n,z15 infections associated with sesame seeds. *EFSA Supporting Publications, 14*(6), 1256E. doi:10.2903/sp.efsa.2017.EN-1256

Ercsey-Ravasz, M., Toroczkai, Z., Lakner, Z., and Baranyi, J. (2012). Complexity of the International Agro-Food Trade Network and Its Impact on Food Safety. *PLoS One, 7*(5), e37810. doi:10.1371/journal.pone.0037810

Eur. Parliament, and The Council. (2002d). Regulation (EC) No 178/2002 of the European Parliament and the council of the European Union of 28 January 2002 laying down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety. *Official Journal of the European Communities, L 31*, 1-24.

Gadiel, D. (2010). The economic cost of foodborne disease in New Zealand. *Prepared for: New Zealand Food Safety Authority, November 2010*.

Gobin, M., Hawker, J., Cleary, P., Inns, T., Gardiner, D., Mikhail, A., . . . and Oliver, I. (2018). National outbreak of Shiga toxin-producing Escherichia coli O157:H7 linked to mixed salad leaves, United Kingdom, 2016. *Euro Surveill, 23*(18). doi:10.2807/1560-7917.Es.2018.23.18.17-00197

Hoffmann, S., Batz, M. B., and Morris, J. G. J. (2012). Annual cost of illness and quality-adjusted life year losses in the United States due to 14 foodborne pathogens. *J Food Prot, 75*(7), 1292-1302. doi:10.4315/0362-028x.Jfp-11-417

Inns, T., Cleary, P., Bundle, N., Foulkes, S., Sharp, A., Utsi, L., . . . and Decraene, V. (2018). Novel application of the matched case-control design to compare food supply chains during an Escherichia coli O157 outbreak, United Kingdom, 2016. *Euro Surveill, 23*(18). doi:10.2807/1560-7917.Es.2018.23.18.17-00195

Mikhail, A. F. W., Jenkins, C., Dallman, T. J., Inns, T., Douglas, A., Carrion Martin, A. I., . . . and Hawker, J. (2018). An outbreak of Shiga toxin-producing Escherichia coli O157:H7 associated with contaminated salad leaves: epidemiological, genomic and food trace back investigations. *Epidemiology and Infection, 146*(2), 187-196. doi:10.1017/s0950268817002874

Task Force „Lebensmittel- und Futtermittelsicherheit“. (2012). Tätigkeitsbericht der Task Force „Lebensmittel- und Futtermittelsicherheit“ bei der lebensmittelseitigen Aufklärung des Gastroenteritis - Ausbruchsgeschehens.

https://www.bvl.bund.de/SharedDocs/Downloads/01_Lebensmittel/Krisenmanagement/Task_Force_Gastroenteritis_Bericht.pdf?__blob=publicationFile&v=4

Weiser, A. A., Gross, S., Schielke, A., Wigger, J.-F., Ernert, A., Adolphs, J., . . . and Greiner, M. (2013). Trace-back and trace-forward tools developed ad hoc and used during the STEC O104:H4 outbreak 2011 in Germany and generic concepts for future outbreak situations. *Foodborne Pathogens and Disease, 10*(3), 263-269. doi:10.1089/fpd.2012.1296

Weiser, A. A., Thöns, C., Filter, M., Falenski, A., Appel, B., and Käsbohrer, A. (2016). FoodChain-Lab: A Trace-Back and Trace-Forward Tool Developed and Applied during Food-Borne Disease Outbreak Investigations in Germany and Europe. *PLoS One, 11*(3), e0151977. doi:10.1371/journal.pone.0151977

7.4 Epi-Lab - going the next step. Data collection, analysis and visualization within one platform

Weiser, Armin A.; Falenski, Alexander; Lewicki, Birgit; Tölle, Dominic

Data is the ground for all kind of analysis and modeling. And the quality of data determines the value of its outcomes. This counts for all scientific disciplines and therefore for investigations of the supply chain as well. Truth to be told is that the collection of data is unbeloved, troublesome and error-prone.

Epi-Lab aims to become a platform guaranteeing for high data quality allowing for epidemiological data analysis and modelling of supply chains. The platform will provide results and visualizations to a restricted community and depending on the underlying data optional also to everyone interested via web services.

Epi-Lab is developed as an open-source browser-based software solution. It is deployed on a server provided, hosted and administered by BfR.

In the starting phase Epi-Lab relies on use cases and scenarios for BfR purposes. The source code is open and may be used by international stakeholders for their purposes as well.

Background

The main focus of this project is the integration of epidemiological data from heterogeneous sources and internal data and analyses into a web-based portal. This needs to be done by implementing validation, visualization, specific analysis and automatic reporting for the sake of risk assessment as well as linking to already available supply chain tools.

The specific use case for the initial development is as follows. The BfR hosts several NRLs for different bacteria and viruses. Isolates are physically sent to the BfR regularly by the laboratories of the German Länder originating from different points of the supply chain, i.e. samples taken from food or animal matrices. The underlying metadata and the results produced by the NRLs are stored in the internal LIMS of the BfR.

One of the sources of data quality issues is the transmission of the metadata by different providers which is done by email. This transmission is indirect which means that the sender of the data does not get immediate plausibility feedback for that data. This is one of the main reasons for bad data quality, which is true not only for the specific use case described here. And this issue can be overcome by direct feedback, e.g. by a web portal that immediately checks for plausibility.

Of course, finally, the main benefit of good data quality is to be able to be more precise in analyzing global supply chains and to suggest meaningful measurements to be undertaken, e.g. for the purpose of drug law amendments concerning antibiotics.

There are many other use cases besides the above mentioned concrete one, where such a portal will be useful, e.g. for the purposes of data collection and analyses in food-borne related disease outbreaks (FoodRisk-Labs Team, 2019a; Weiser et al., 2016) – which is one the next development steps.

Results

The web-based portal allowing for data exchange with relevant stakeholders was developed in a modern software framework, i.e. Angular (Google, 2019) for the client side and Node.js (Node.js Foundation, 2019) for the server side. The development language was chosen to be Typescript (Microsoft, 2019). The portal is accessible by all modern browsers – this excludes the Internet Explorer and old versions of Firefox, Chrome and Safari. The reason for the exclusion is to avoid security issues in the communication between client and server. Details on the requirements can be found in the wiki of the portal (FoodRisk-Labs Team, 2019b).

The software is open-source licensed under the MIT-license (Open Source Initiative, 2019). The source code as well as discussions and the wiki are located on GitHub.

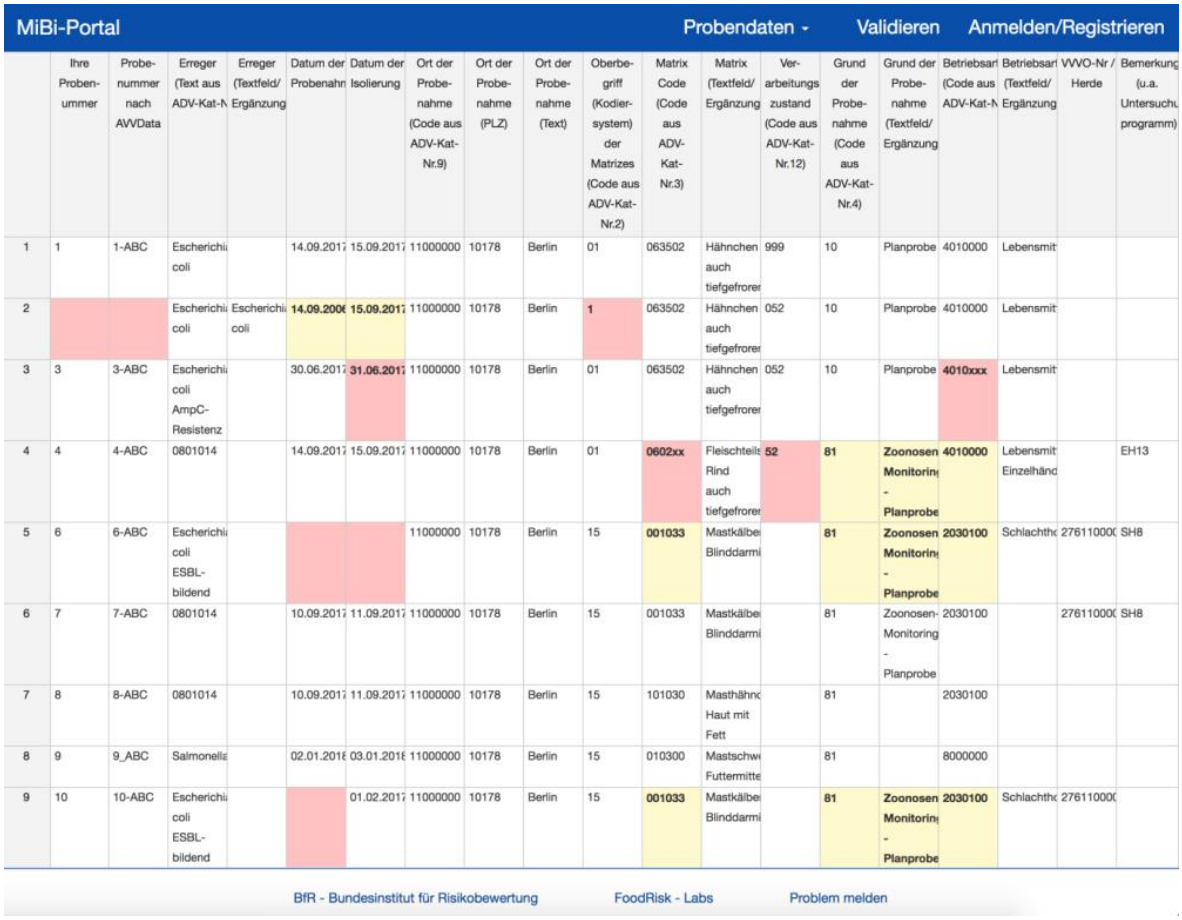
A first instance of the project is available as MiBi-portal (FoodRisk-Labs Team, 2019c) and is actually in use for the purpose of data collection for the microbiological NRLs at the BfR. The portal provides a FAQ for urgent questions; however, the usage was kept simple and self-explaining.

The MiBi-portal allows for immediate plausibility checks of sent data and therewith for immediate feedback to the data provider (Figure 59). The data provider has different choices for data transmission:

1. direct online typing into the web interface
2. upload of a well-defined Excel sheet
3. direct transmission via REST interface as well-defined JSON file

The detailed needed formats as well as the plausibility checks undertaken for validation can be found in the wiki of the portal.

Additionally, the portal has a secure protected area comprising of high security standards in which all sensitive data is collected and stored.



	Ihre Proben-ummer	Probe-nummer nach AVVData	Erreger (Text aus ADV-Kat-N	Erreger (Textfeld/ Ergänzung	Datum der Probenahn	Datum der Isolierung	Ort der Probe-nahme (Code aus ADV-Kat-Nr.9)	Ort der Probe-nahme (PLZ)	Ort der Probe-nahme (Text)	Oberbe-griff (Kodier-system) der Matrizes (Code aus ADV-Kat-Nr.2)	Matrix Code (Code aus ADV-Kat-Nr.3)	Matrix (Textfeld/ Ergänzung	Ver-arbeitungs zustand (Code aus ADV-Kat-Nr.12)	Grund der Probe-nahme (Code aus ADV-Kat-Nr.4)	Grund der Probe-nahme (Textfeld/ Ergänzung	Betriebsart (Code aus ADV-Kat-N	Betriebsart (Textfeld/ Ergänzung	VVVO-Nr / Herde	Bemerkung (u.a. Untersuchu programm)
1	1	1-ABC	Escherichia coli		14.09.2017	15.09.2017	11000000	10178	Berlin	01	063502	Hähnchen auch tiefgefroren	999	10	Planprobe	4010000	Lebensmit		
2			Escherichia coli	Escherichia coli	14.09.2006	15.09.2017	11000000	10178	Berlin	1	063502	Hähnchen auch tiefgefroren	052	10	Planprobe	4010000	Lebensmit		
3	3	3-ABC	Escherichia coli AmpC-Resistenz		30.06.2017	31.06.2017	11000000	10178	Berlin	01	063502	Hähnchen auch tiefgefroren	052	10	Planprobe	4010xxx	Lebensmit		
4	4	4-ABC	0801014		14.09.2017	15.09.2017	11000000	10178	Berlin	01	0602xx	Fleischteils Rind auch tiefgefroren	52	81	Zoonosen Monitoring - Planprobe	4010000	Lebensmit Einzelhänd		EH13
5	6	6-ABC	Escherichia coli ESBL-bildend				11000000	10178	Berlin	15	001033	Mastkälber Blinddarmi		81	Zoonosen Monitoring - Planprobe	2030100	Schlachth	276110000	SH8
6	7	7-ABC	0801014		10.09.2017	11.09.2017	11000000	10178	Berlin	15	001033	Mastkälber Blinddarmi		81	Zoonosen-Monitoring - Planprobe	2030100		276110000	SH8
7	8	8-ABC	0801014		10.09.2017	11.09.2017	11000000	10178	Berlin	15	101030	Masthähnc Haut mit Fett		81		2030100			
8	9	9_ABC	Salmonella		02.01.2018	03.01.2018	11000000	10178	Berlin	15	010300	Mastschwe Futtermitte		81		8000000			
9	10	10-ABC	Escherichia coli ESBL-bildend			01.02.2017	11000000	10178	Berlin	15	001033	Mastkälber Blinddarmi		81	Zoonosen Monitoring - Planprobe	2030100	Schlachth	276110000	

Figure 59: Visual Validation – view on the underlying plausibility checks

Conclusions

The portal at its current stage has great value. It guarantees for high data quality, it consumes less human resources for data processing; it eases data exchange in an efficient and secure way. By now, it is in use by some of the stakeholders and will be used by all of them medium-term.

An additional value of the portal is the ability to collect huge amounts of decentral stored validated sampling data along a complete supply chain at once automatically via the REST interface. This would be an easy task by requesting all nationally or internationally affiliated labs to send their sample information belonging to a certain pathogen, supply chain and given time period of interest. This would allow for fully automated data analyses within seconds.

References

FoodRisk-Labs Team. (2019a). FoodChain-Lab. https://foodrisklabs.bfr.bund.de/foodchain-lab
FoodRisk-Labs Team. (2019b). MiBi-Portal Wiki. https://github.com/SiLeBAT/mibi-portal-resources/wiki/Browser-requirements
FoodRisk-Labs Team. (2019c). MiBi Portal. https://opensource.org/licenses/MIT
Google. (2019). Angular. https://angular.io
Microsoft. (2019). Typescript. https://www.typescriptlang.org
Node.js Foundation. (2019). Node.js. https://nodejs.org
Open Source Initiative. (2019). The MIT License. https://opensource.org/licenses/MIT
Weiser, A. A., Thöns, C., Filter, M., Falenski, A., Appel, B., and Käsbohrer, A. (2016). FoodChain-Lab: A Trace-Back and Trace-Forward Tool Developed and Applied during Food-Borne Disease

Outbreak Investigations in Germany and Europe. *PLoS One, 11*(3), e0151977. doi:10.1371/journal.pone.0151977

7.5 Knowledge Plattform RAKIP

Filter, Matthias; Plaza Rodríguez, Carolina; Ungaretti Haberbeck, Leticia[1], Swaid, Ahmad; Georgiadis, Stylianos[1]; Valentin, Lars; Günther, Taras; Buschhardt, Tasja; de Alba Aparicio, Miguel; Mesa Varona, Octavio; Desvignes, Virginie[2]; Guillier, Laurent[2]; Nauta, Maarten[1]

[1] Technical University of Denmark (DTU), National Food Institute (DTU Food), Kemitorvet, Buildings 201-204 on Lyngby Campus, DK-2800 Kgs. Lyngby, Denmark

[2] French Agency for Food, Environmental and Occupational Health & Safety (ANSES), 14 rue Pierre et Marie Curie, 94701 Maisons-Alfort Cedex, France

Abstract

Quantitative microbial risk assessment and predictive microbiology modelling are gaining increasing relevance to food safety professionals and public authorities in times of global food supply chains. In these research domains, great efforts have been undertaken to create a rich variety of data, models and software tools supporting risk assessors, food quality experts and researchers. However, there is a lack of solutions that enables efficient dissemination and exploitation of existing knowledge to the whole food safety community.
In this context the Risk Assessment Modelling and Knowledge Integration Platform (RAKIP) project was created as a joint ANSES, BfR and DTU Food effort, aiming at creating open resources that support the exchange of models relevant for food safety and risk assessment between existing and future software tools and databases in a transparent and consistent way. Critical steps in this project were

- the development of a harmonized conceptual description of modelling activities in the domain,
- the development of generic metadata schema for risk assessment models including controlled vocabularies,
- the development of a specification for an open information exchange format (FSK-ML)

In addition, BfR developed a new open source software resource (FSK-Lab) that also serves as technical basis for the first web-based model repository that allows to share, search and execute FSK-ML compliant models online (URL: https://foodrisklabs.bfr.bund.de/rakip-web-portal/). In this way RAKIP supports the whole risk assessment community in sharing and deploying their mathematical models.

Background

Experimental data and mathematical models (knowledge) generated by the food safety community are of high relevance for risk assessment efforts by governmental agencies and private companies. Currently it is difficult to get a quick overview of the available knowledge and it is even harder to get quick access to the relevant data and models. The creation of publicly available knowledge repositories would promote knowledge sharing between all stakeholders in this area. However, to achieve efficient knowledge exchange there is the need to development harmonized information exchange formats and open source software code libraries, as suggested by Plaza-Rodriguez *et al* (Plaza-Rodriguez et al., 2015). This would also facilitate transparency and quality control of scientific research results and could serve as foundation for efficient machine-to-machine communication in the future.

A specific challenge within the food safety risk assessment community is the need to support legacy models which have been developed in the past in different software tools or in differ-

ent scripting languages like e.g. R, Python or Matlab. Currently no stakeholder has sufficient personal resources to re-implement all these legacy models into software independent information exchange formats. Therefore the RAKIP project developed a harmonized markup language that does not require to “re-implement” legacy models, but allows to create harmonized description around the original model code (see Figure 60).

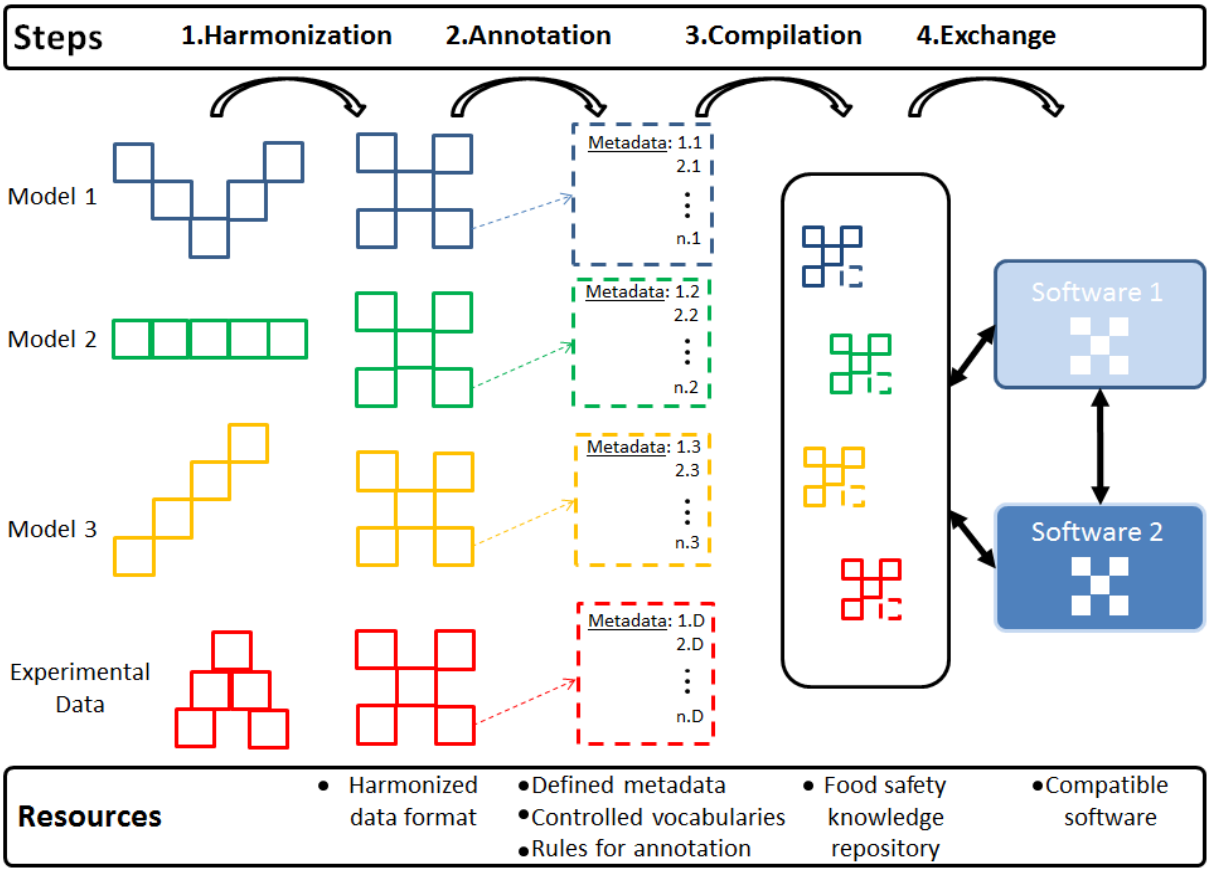


Figure 60: Steps and resources needed to reach transparent and consistent integration and exchange of knowledge in the food safety community
Source: (Plaza-Rodriguez et al., 2018)

The proposed strategy does however not only support legacy models, it could also be adopted for any new model (and data) that is about to be published e.g. in a peer-reviewed journal. The adoption of that standardized information exchange format in peer-reviewed publications would allow straightforward comparison, re-use and extension of published models, supporting effective progress within the risk assessment and predictive microbial (PM) modelling domains.

A prerequisite for the establishment of such harmonized information exchange format is the harmonization of terminologies, e.g. on modelling processes or information objects that should be covered by annotation metadata (see Figure 61).

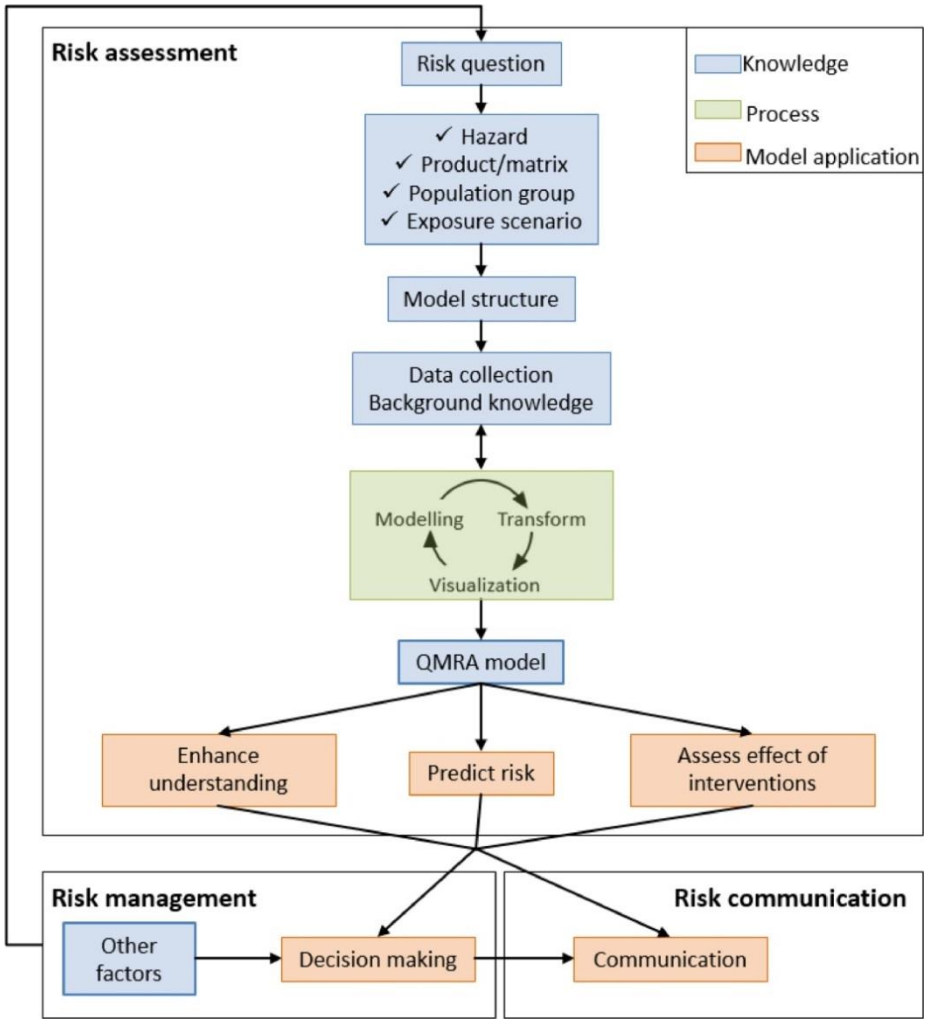


Figure 61: The risk analysis process and the positioning of a quantitative microbial risk assessment (QMRA) modelling process
Source: (Haberbeck et al., 2018)

Proper definition of annotation metadata concepts is a prerequisite for correct understanding of what a model is about and for correctly interpreting model-based results. The establishment of a set of harmonized metadata fields helps avoiding misunderstandings and improves the quality and efficiency of knowledge exchange (Haberbeck et al., 2018). Such harmonized metadata concepts also support search & filter functions in model repositories.

Harmonization resources

- **Food Safety Knowledge Metadata**

Models and data need to be annotated in a harmonized way. For this, the RAKIP community defined a structured metadata schema, called “Metadata Master Table”. This schema describes concepts relevant for annotating data or models in the risk assessment and predictive microbial modelling domains. For each metadata concept it has also been defined if this concept is considered mandatory or not and what cardinality each metadata concept has.
Within the Metadata Master Table there are several sheets. The “Generic Metadata Schema” sheet contains a comprehensive list of metadata concepts that allows to describe in detail all type of models or data. Apart from the “Generic Metadata Schema” there are dedicated

sheets for specific model / data classes, using relevant subsets of the “Generic Metadata Schema”.

The most up-to-date “Metadata Master Table” can be accessed via: https://goo.gl/PE4ysP

- **Community driven Controlled Vocabularies**

To support harmonized annotation of food safety knowledge, RAKIP established an online resource with controlled vocabularies / term catalogues for those metadata concepts in the “Metadata Master Table” that are not free text. Some of these term lists are specific for the different model classes. The controlled vocabularies are based on the terms used by other sources like ontologies, standards and tools (Standard Sample Description ver. 2.0 (SSD2), FOODON, MIME, PMM-Lab, OpenFSMR, Bibliographic Ontology Specification, *etc.*)

The lists of proposed controlled vocabularies for food safety knowledge annotation can be accessed via: https://goo.gl/wbFoZU

- **Glossary**

The terms describing the steps and entities in the risk assessment model generation process including PM and QMRA modelling have been discussed within the RAKIP project until consensus was reached. All these terms have been detailed in an online glossary that can be freely accessed.

The proposed glossary with terms describing the risk assessment model generation process can be accessed via: https://goo.gl/b4ADho

- **Proposals for improving RAKIP Harmonization Resources**

The new RAKIP harmonization resources need to be improved and updated by the community on a regular basis, for example if new model types, such as chemical risk assessments, should be covered. Any proposal for improving those resources can be made via an online form. So far, the RAKIP partners are the curators of these resources.

The online form for making proposals for improving RAKIP Harmonization Resources can be accessed via: https://goo.gl/QR57Df. *In addition there is also an online resource that provide information on the evolution of these resources and which software resource supports which harmonization resource versions*: https://goo.gl/9g7zUF

- **Food Safety Knowledge Markup Language (FSK-ML)**

The terms, concepts and metadata schema are an important foundation for the creation of the first domain-specific open information exchange format called “Food Safety Knowledge Markup Language (***FSK-ML***)” (URL: https://foodrisklabs.bfr.bund.de/fsk-ml-food-safety-knowledge-markup-language/). The FSK-ML specification document is a guidance document for software developers that explicitly specifies the structure and content of files containing all model-related information (***FSKX-file***) to be generated, exchanged and read-in by software tools in the future. Similar markup languages are used in other scientific domains like the Systems Biology Markup Language (SBML) (URL: http://sbml.org/Main_Page). FSK-ML can also serve as a foundation for future model repositories, as it guarantees that all relevant

information on models and data can be exchanged within one single file. This also simplifies “communication” between different software tools and other popular information repositories, as e.g. Zenodo (URL: www.zenodo.org). With the information exchange format FSK-ML it will also be guaranteed that metadata stay linked to the correct model or data set. A specific feature of FSK-ML is that it supports the exchange of models that are provided in specific script-based programming languages (e.g. R, Matlab and Python).

RAKIP model repository

The RAKIP Model Repository (see Figure 62) is a web-based repository for data and models that can be freely assessed by any user. It currently contains mainly microbiological risk assessment models and model modules provided by the RAKIP partners in the FSK-ML format. All users are able to search, filter or download data / models from the RAKIP portal free of charge. Registered users will be allowed to submit own models into the repository and to execute simulations online with user-defined parameters for those models available within the repository. The RAKIP Model Repository will be maintained and extended by the RAKIP partners in a collaborative fashion. This includes an internal model curation and quality control process that is based on the so called MIRARAM guidelines developed with financial support from the European Food Safety Authority (EFSA). The repository can be accessed via this link: https://foodrisklabs.bfr.bund.de/rakip-model-repository-web-services/

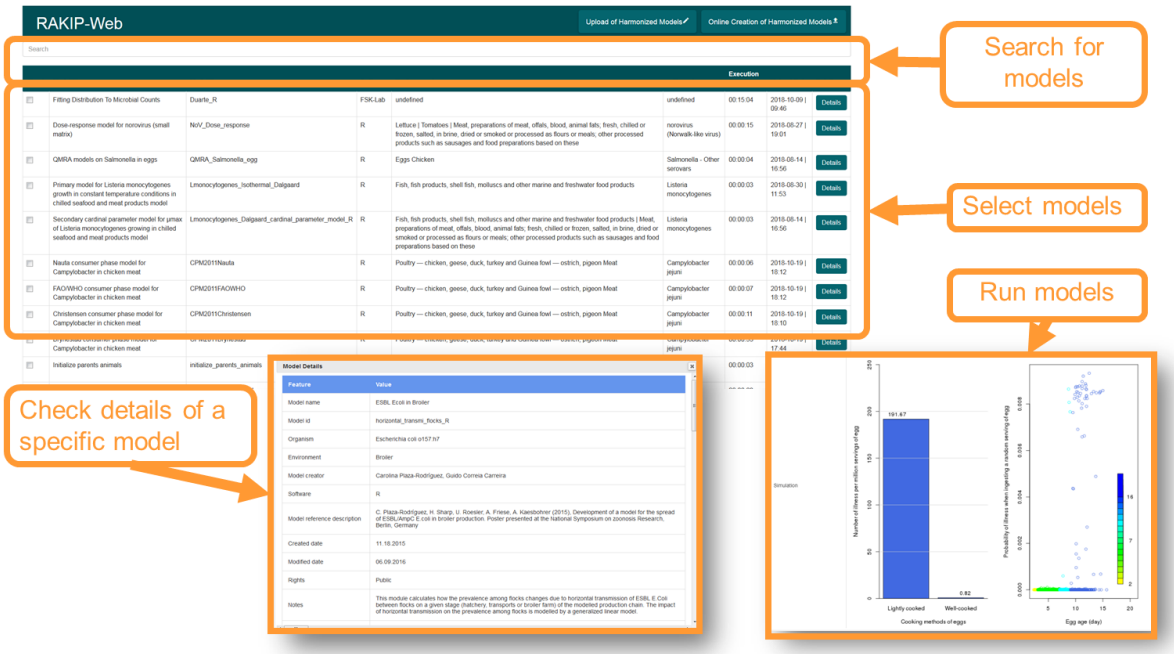


Figure 62: General overview of the user interface and core functionalities of the RAKIP model repository

FSK-Lab

FSK-Lab is a free, modular, open-source toolkit for the generation, annotation, execution and combination of script based models (de Alba Aparicio et al., 2018). FSK-Lab extends the open source data analytics platform KNIME and provides a set of new software features as so-called nodes. Through the connection and combination of nodes into a data processing workflow users can perform desired data processing, modelling or simulation tasks. Thus FSK-Lab can be characterized as a graphical modelling toolbox- see Figure 63.

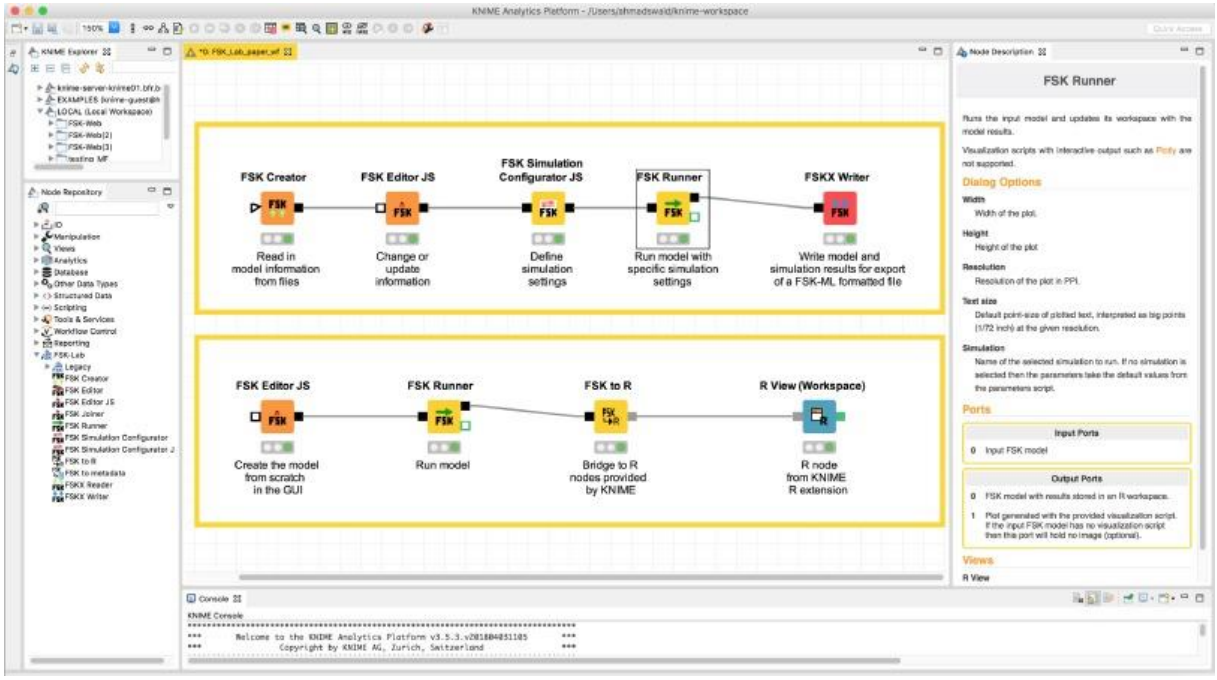


Figure 63: A screenshot of the FSK-Lab Graphical User Interface (GUI)
Source: (de Alba Aparicio et al., 2018)

A specific feature of FSK-Lab is, that it is designed to support the creation, modification, combination and execution of risk assessment models from different programming languages. Currently, FSK-Lab supports specifically models written in R or Python scripting language, but other programming languages and stand-alone software tools will be supported in the future. FSK-Lab also allows to import and export models in the FSK-ML information exchange format.

Discussion

Risk assessment is a scientific process comprising of knowledge integration, knowledge evaluation and knowledge generation steps. Therefore it is an intrinsic motivation of risk assessment authorities to promote and develop resources that facilitate efficient knowledge exchange within the scientific community.

During the first phase of the RAKIP project (Figure 64) new community resources were established that support knowledge integration and exchange within the Quantitative Microbial Risk Assessment (QMRA) domain. These resources facilitate the exchange of data and models in a harmonized file format, e.g. via the RAKIP Web Portal.

Figure 64: The RAKIP project work programme

Nonetheless, in the future it will be necessary to further strengthen and maintain free and open resources that support information exchange in the food safety risk assessment domain. To reach this, the following tasks are important to address:

- To extend and maintain web-based ***infrastructures*** for organization, storage, retrieval, exchange, interpretation, and application of risk assessment models (and data)
- To extend and maintain ***harmonized controlled vocabularies*** for terms and concepts relevant for risk assessment modelling
- To develop further ***open source software libraries and converter tools*** and to directly support the enhancement of existing tools / databases / resources facilitating the adoption of the proposed harmonized information exchange format FSK-ML
- To ***engage with the global food safety risk assessment community*** and create broad support and compliance

An effort along these objectives is the establishment of the RAKIP Initiative. The RAKIP Initiative will serve as an umbrella for all agencies that want to create synergistic actions taking up the efforts initiated by ANSES, BfR and DTU Food.

Acknowledgement

The work presented in this paper has been partly supported by the EFSA-BfR Framework Partnership Agreement GP/EFSA/AMU/2016/01, by funds of the Federal Ministry of Food and Agriculture (BMEL) for the project "FoodAuthent" (No. 2816502914) based on a decision of the Parliament of the Federal Republic of Germany via the Federal Office for Agriculture and Food (BLE) under the innovation support programme, as well as by the German Federal Institute for Risk Assessment (BfR), the National Food Institute (DTU Food) from the Technical University of Denmark (DTU) and the French Agency for Food, Environmental and Occupational Health & Safety (ANSES).

References

de Alba Aparicio, M., Buschhardt, T., Swaid, A., Valentin, L., Mesa-Varona, O., Günther, T., . . . and Filter, M. (2018). FSK-Lab – An open source food safety model integration tool. *Microbial Risk Analysis, 10*, 13-19. doi:10.1016/j.mran.2018.09.001

Haberbeck, L. U., Plaza-Rodríguez, C., Desvignes, V., Dalgaard, P., Sanaa, M., Guillier, L., . . . Filter, M. (2018). Harmonized terms, concepts and metadata for microbiological risk assessment models: The basis for knowledge integration and exchange. *Microbial Risk Analysis, 10*, 3-12. doi:10.1016/j.mran.2018.06.001

Plaza-Rodriguez, C., Haberbeck, L. U., Desvignes, V., Dalgaard, P., Sanaa, M., Nauta, M., . . . Guillier, L. (2018). Towards transparent and consistent exchange of knowledge for improved microbiological food safety. *Current Opinion in Food Science, 19*, 129-137. doi:10.1016/j.cofs.2017.12.002

Plaza-Rodriguez, C., Thoens, C., Falenski, A., Weiser, A. A., Appel, B., Kaesbohrer, A., and Filter, M. (2015). A strategy to establish Food Safety Model Repositories. *International Journal of Food Microbiology, 204*, 81-90. doi:10.1016/j.ijfoodmicro.2015.03.010

8 Figures

Figure 1: Feed material consumption in the EU and Germany in 2015/16 by category in percent of total feed consumption 12

Figure 2: Percentage of different feed categories of the total EU feed import 13

Figure 3: Advantageous efficiency of samples analysed for bottleneck control on the left compared to monitoring on the right 17

Figure 4: Percentage of food and feed samples of non-animal origin imported from third countries (random sampling of low health risk products for bottleneck control) reported non-compliant due to exceeding maximum levels of pesticide residues and resulting number of RASFF notifications. 18

Figure 5: Use of cereals a) on a global scale and b) in the EU (Schmid and Goldhofer, 2017) 20

Figure 6: Production volume of grain maize in the European Union in 2017 23

Figure 7: Export volume of agricultural goods over the last 10 years 27

Figure 8: Import volume of agri goods over the last 10 years 28

Figure 9: Value (in billion EUR) of food goods exported from Germany in the last 10 years 32

Figure 10: Value (in billion EUR) of food goods imported to Germany in the last 10 years 34

Figure 11: Value (in million EUR) of food goods exported to Russia in the last 10 years 36

Figure 12: Value (in million EUR) of food goods imported from Turkey to Germany in the last 10 years 37

Figure 13: Imported volume of hazelnuts without shell, fresh or dried from TR to Germany during the last 10 years, expressed in 1000 tons and value in EUR 1 million 38

Figure 14: World fleet by principle vessel type in 2017 42

Figure 15: Container port volumes in 2016, percentage shares 43

Figure 16: Container port volumes in million TEU at selected container terminals in 2016 44

Figure 17: Bio- and agroterroristic research initiatives coordinated or conducted by BfR since 2007 55

Figure 18: Food Fraud cases in the Administrative Assistance and Cooperation (AAC) System in 2015, 2016 and 2017 (EC, 2018) 65

Figure 19: Current traceability approaches in connection with analytical methods for food and feed authentication 74

Figure 20: Systematic of analytical approaches for food and feed authentication 75

Figure 21: International, national and in-house research projects of BfR on food and feed authentication in the context of global supply chains 78

Figure 22 Estimated annual release of PFCAs from fluoropolymer production sites in the United States (US), Western Europe and Japan (purple) as well as in China, Russia, Poland and India (orange) 87

Figure 23 Scheme of the fate of PFAS through product life-cycle, their emissions and entering into the food chain (Adapted from Figure 1, Figure S2 and Figure S9 of Wang et al. 2014 (Wang et al., 2014) and Figure 1 of Wang et al. 2017 (Wang et al., 2017)) 88

Figure 24: Transformation of gambiertoxins to ciguatoxins through the food web 96

Figure 25: Estimation of the DON processing factor in the production of wheat bread and comparison with legal obligations in the EU 104

Figure 26: Processing stages of apple juice production 107

Figure 27: Food supplements based on blue-green algae in different forms (coated tablet, capsule and powder) 110

Figure 28: LC-MS/MS chromatogram of a cyanotoxins standard mixture (100 ng/mL) 110

Figure 29: Total microcystin (MC) concentration in MC-positive BGAS samples 111

Figure 30: The step procedure for elaborating Codex standards adapted from “understanding codex” 121

Figure 31: Where BfR participates in standard setting and harmonization initiatives at national and international level. 124

Figure 32: Extracted ion chromatograms from the analysis of a blank wheat flour spiked with aflatoxins, OTA, DON, ZEN, T2-toxin, HT2-toxin, FB_1, FB_2, α-ZEL and β-ZEL, ENNA, ENNA1, ENNB and ENNB1 in the low to medium ppb range 131

Figure 33: Overview on the most abundant *Alternaria* toxins (Zwickel et al., 2016). 132

Figure 34: Characterization of food items introduced into Germany 144

Figure 35: Examples of food items introduced into Germany 145

Figure 36: Prevalence of *Vibrio* spp (in %) in Blue Mussels (*Mytilus edulis*) of production areas in the German Wadden Sea in 2012 (n=46 sampling units, grey) and 2013 (n=71 sampling units, black), (Huehn et al. 2014) 151

Figure 37: Schematic supply chain of spices and dried culinary herbs with a hypothetical point of contamination and the corresponding hazard identification and risk assessment steps 160

Figure 38: Phylogenetic relationship of *mcr-1* carrying *E. coli* isolates 175

Figure 39: Plasmid profiles of E. coli isolates and determination of the mcr-1 location 176

Figure 40: Number of genomes (draft or complete) of selected pathogens associated with food-borne illnesses uploaded to the NCBI Genome database from 2001 to 2018 185

Figure 41: The concept of “shotgun” metagenomics sequencing 189

Figure 42: Omics‘ for detection, identification and characterisation of foodborne pathogens in the food and supply chain: From Assessment to Management 191

Figure 43: Possible influences within the food supply chain on substance concentration and dietary exposure 202

Figure 44: Summarised information on geographical origin for selected agricultural products sampled in the German Food Monitoring (GFM) 2005 – 2015 and checked for plausibility with FAO data 203

Figure 45: Summarised information on geographical origin for selected processed food products sampled in the German Food Monitoring (GFM) 2005 – 2015 and checked for plausibility with FAO data 205
Figure 46: Pacific Ciguatoxin 1 / CTX-1B / P-CTX-1 216
Figure 47: Correct labeling of Red Snapper (*L. malabaricus*) in a Berlin (Germany) gourmet food store (Foto: Miriam Friedemann, 2019) 220
Figure 48: Incorrect labeling of a snapper (*Lutjanus* spp.) at a Berlin (Germany) food fair 221
Figure 49: Geographic distribution of yersiniosis (A) und hepatitis E incidence in Berlin in 2014 229
Figure 50: Combined network view of all relevant fenugreek and sprout deliveries 237
Figure 51: Trade contact between actors of the food supply chain pork 239
Figure 52: Graphical sketch of a supply chain and a supply network 242
Figure 53: Conceptual KNIME workflow, graphical interpretation of the presented concept of Knowledge Lab Toolkit 249
Figure 54: The FeedChainCheck knowledge base takes as its input market and supply chain information on the “stations”: cultivation and import of raw materials; processing; transportation/logistics; and consumption by animal producing facilities 250
Figure 55: Example for a typical output generated by RITOPS: Time course of perfluorohexanesulfonic acid (PFHxS) concentration in fat, liver, kidney, blood plasma and muscle 252
Figure 56: Schematic implementation of food production process in a web-based application 255
Figure 57: FoodChain-Lab mapping the delivery network of sesame seeds and sesame paste during a multi-country Salmonella enterica outbreak in 2016 and 2017 261
Figure 58: FoodChain-Lab web application mapping the supply chain of a fictitious outbreak 264
Figure 59: Visual Validation – view on the underlying plausibility checks 270
Figure 60: Steps and resources needed to reach transparent and consistent integration and exchange of knowledge in the food safety community 273
Figure 61: The risk analysis process and the positioning of a quantitative microbial risk assessment (QMRA) modelling process 274
Figure 62: General overview of the user interface and core functionalities of the RAKIP model repository 276
Figure 63: A screenshot of the FSK-Lab Graphical User Interface (GUI) 277
Figure 64: The RAKIP project work programme 278

9 Tables

Table 1: Feed (a) and total (b) import volume of different materials into the EU and the respective main countries of origin and EU trading partners 13

Table 2: World production and trade of cereals, soy beans and protein meals in 2017/2018 14

Table 3: Origin of feed materials in the compound feed industry (adapted from (Schubert, 2012)) 14

Table 4: Overview on production units and transport capacities in the feed chain of Germany 15

Table 5: Composition of yellow dent corn kernels from seven American midwest hybrids 20

Table 6: Crude protein and metabolisable energy of cereal grains as relative values compared to maize 21

Table 7: Maize production in selected states worldwide in 2016/2017 21

Table 8: Production volume of grain maize in the European Union in 2017 (EU DG AGRI, 2018a) and import and export volume in 2017/18 (EU DG AGRI, 2018c) 22

Table 9: Germany's most important trading partners in agri-food exports by year and trade value in billion EUR 30

Table 10: Germany's most important trading partners in agri-food imports by year and trade value in billion EUR 31

Table 11: Germany`s top seller (6 digit code) in the top category`s for export for the year 2017 33

Table 12: Germany`s top seller (6 digit code) in the top category`s for import for the year 2017 35

Table 13: International seaborne trade, selected years 41

Table 14: World fleet by principle vessel type, 1980-2017 42

Table 15: Container throughput in the port of Hamburg in 1,000 TEU 44

Table 16: Different types of Food Fraud, their explanation and examples 63

Table 17: Maximum limits for patulin and ochratoxin A in the European Union according to Regulation (EC) No 1881/2006 106

Table 18: Regulations of patulin in food in various countries 106

Table 19: Main types of analysis performed in natural toxin analysis at various stages of the value and processing chain 128

Table 20: Number of passengers carrying illegal food of animal origin and number and quantity of food items seized at Frankfurt and Berlin-Schönefeld Airport and included in the analyses 144

Table 21: Overview on reported food-borne outbreaks in Europe (1973–2015) associated with the consumption of microbial contaminants in spices and dried culinary herbs or foods containing these contaminated ingredients 163

Table 22: Antimicrobial resistance of *mcr-1* carrying *E. coli* isolates used in this study 174

Table 23: Overview on *in silico*-based typing results of *mcr-1* carrying *E. coli* isolates 177

Table 24: Overview on plasmid and chromosomal features associated with colistin resistance of *mcr-1* carrying *E. coli* isolates 178

Table 25 Information on geographical food origin for selected agricultural products sampled in the German Food Monitoring (GFM) 2005 – 2015 and checked for plausibility with FAO data 204

Table 26: Information on geographical food origin for selected processed food products sampled in the German Food Monitoring (GFM) 2005 – 2015 and checked for plausibility with FAO data 206

Table 27: Information on geographic origin and catching area for Atlantic herring (Clupea harengus) sampled in the German Food Monitoring (GFM) 2005 – 2015 207

Table 28: Information on geographic origin and catching area for tuna filet (Thunnus sp.) sampled in the German Food Monitoring (GFM) 2005 – 2015 208

Table 29: Selected zoonoses reported in 2014 in Berlin, Germany and the EU with indication of reported cases, incidence, outbreaks and hospitalisation rates 230

Table 30: Systemic and organ complications of selected foodborne zoonoses 231

Table 31: Network metrics 243

Table 32: Node centralities 243