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Safe Food For Infants – SAFFI

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SAFFI
Safe Food for Infants in the EU and China

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Introduction to the Special Issue on Safe food for infants: the importance of pursuing integrated approaches to monitor and reduce the risks of biological, chemical, and physical hazards in infant food during the key developmental years☆

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ABSTRACT

Owing to increasing populations and global threats, the integrity and safety of global food chains are at risk. In many countries, simply getting enough to eat can be an issue, with poor quality food often contaminated with hazardous agents, whereas in developed countries the pressure to deliver cheap, affordable food may affect quality and safety. The purpose of this Special issue on Safe food for infants is to emphasize the importance of pursuing integrated approaches to monitor and reduce the risks of biological, chemical, and physical hazards in infant food. A careful integrated approach is proposed to be instrumental in order to minimize the hazards to infant health during the key developmental years and protect children from penalizing nutritional disorders and gastrointestinal diseases.

1. Introduction

The World Food Summit in 1996 defined food security as the situation in which all people, at all times, have physical, social and economic access to sufficient, safe and nutritious food to meet their dietary needs and preferences for leading active and healthy lives.[1]. The concept of food security encompasses both physical and economic access to food that meets people's dietary needs and food preferences.

The European Union has made food security one of the top priorities of its policy agenda. Food security has now become a cross-cutting objective to be integrated into various areas of Community competence, including the Common Agricultural Policy and its rural development pillar, the environment, public health, consumer protection and the completion of the internal market. In response to the food crises of the 1990s, in January 2000 the European Commission published a white paper on food safety which marks an important step in the transformation of European legislation on the subject.[2], in which a legal framework is described which covers the entire food chain - "from farm to table" - according to a global and integrated approach. According to this logic, food safety concerns animal nutrition and health, animal protection and welfare, veterinary controls, animal health measures, phytosanitary controls, food preparation and hygiene. Finally, the white paper of the European Commission indicates the important need to interact permanently with consumers in order to provide adequate information and education on consumption.

Biological, chemical, and physical hazards in infant food owing to national and international control programs, have significantly decreased during recent years.[3] However, despite such decrease the risks

Abbreviations: World Health Organization, (WHO); European Union, (EU); European Food Safety Authority, (EFSA); Food and Agriculture Organization, (FAO); International Standards Organisation, (ISO); Good Manufacturing Practices, (GMP); Hazard Analysis Critical Control Points, (HACCP); International Standards Organisation, (ISO 9000); European Standard, (ES 29000).

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of hazards in infant food remain a global concern.[4] Raising consumer awareness on the consequences of unhealthy food consumption, and a growing attention by the food industry oabout the importance of ensuring protection against contaminants in commercially available products, have limited the risk of food contaminants.[5,6]. However, interventions adopted across the food supply chain to inspect the presence of food contaminants and help to ensure a sustainable supply of nutritious safe food, are currently considered insufficient in providing an extensive and comprehensive protection6. In the economically advanced Western world infant food safety is currently monitored by increasingly strict legal regulations, however, some countries still use banned substances in industrial food production owing to their poor economy and insufficient regulation.[7]. Furthermore, the export and import exchange of commercial, often low-cost, infant food products, may raise serious risks for children's health despite the presence of standard control procedures and techniques, which may be insufficient or inadequate to detect a large variety of contaminants in food products.[7,8].

Owing to increasing populations and global threats, the integrity and safety of global food chains are at risk. In many countries, simply getting enough to eat can be an issue, with poor quality food often contaminated with hazardous agents, whereas in developed countries the pressure to deliver cheap, affordable food may affect quality and safety.[9]. The purpose of this Special issue on Safe food for infants is to emphasize the importance of pursuing integrated approaches to monitor and reduce the risks of biological, chemical, and physical hazards in infant food . A careful integrated approach is in fact instrumental to minimize the hazards to infant health during the key developmental years and protect children from penalizing nutritional disorders and gastrointestinal diseases.

1.1. The European food safety system: a shared responsibility for a safe food management

Today's lifestyles are vastly different than in the past. Lifestyle changes and the increase in single-parent families and working women have led to changes in food preparation and consumption habits. One positive consequence is undoubtedly the rapid advancement of food technology and processing and packaging techniques to help ensure the safety of the food chain, as well as greater convenience of food. However, despite these advances, contamination in the food chain is still possible, due to naturally occurring or accidentally introduced agents or due to improper procedures.Ultimately, the quality and safety of food depends on the efforts of everyone involved in the complex chain of agricultural production, processing, transportation, preparation and consumption. The European Union and the World Health Organization (WHO), consider food safety to be a shared responsibility from field to table. Therefore, maintaining food quality and safety throughout the food chain requires operating procedures to ensure food safety and monitoring systems to ensure that operations are carried out correctly.

1.2. Legal and regulatory frames in the UE

EU food safety procedures cover the entire production chain of food for animal and human consumption. The European Union has adopted comprehensive legislation and outlines the responsibilities of producers and suppliers to help ensure the quality and safety of the food chain. EU regulations are among the strictest in the world. However, in order to make the food regulatory sector more transparent and scientific, a review of the EU regulatory framework was initiated in the late 1990s. In 1997, a new system of scientific advice was developed. In addition to the Scientific Steering Committee, eight new scientific committees were established. In 2002 the European Food Safety Authority (EFSA) was created, an independent body that works in close collaboration with various scientific bodies and institutes of the member states, offering independent scientific advice on all matters that directly or indirectly affect food safety. The body supervises all stages of food production and supply, from the primary sector to distribution to consumers. EFSA also deals with risks linked to the food chain and carries out scientific assessment on any issue that has a direct or indirect effect on the safety of the food supply, including issues related to animal and plant health and welfare.

1.3. Agriculture, transport and food industry

The quality of raw materials is critical to the safety and quality of the final product. Therefore, a systematic approach from field to table is necessary to avoid contamination of food products and to identify potential risks. From the farm or wholesaler, agricultural products are transported to the food industry. This link in the food chain is covered by European legislation on quality standards, hygiene and food safety, which also applies to transport and storage. In fact, the standards of the International Standards Organization (ISO) also include a chapter dedicated to the storage and delivery of food products. In this regard, the Codex Alimentarius was drawn up in 1962 by the World Health Organization (WHO) and the Food and Agriculture Organization (FAO) and includes global recommendations for the protection of foodstuffs as well as issues relating to transport and storage. It is therefore up to the food industry to meet consumer expectations in terms of safety and to comply with legal requirements. Food industries rely on modern quality control systems to ensure the quality and safety of the products they manufacture. The three main systems currently in place include: a) Good Manufacturing Practices (GMP), which include the processing conditions and procedures that, based on long experience, have been proven to deliver consistent quality and safety, b) Hazard Analysis Critical Control Points (HACCP), which has not only complemented but replaced them. While traditional quality assurance programs focused on detecting problems in the finished product, HACCP is a proactive technique that focuses on identifying potential problems and controlling them during the design and production process; c) Quality Assurance Standards, which are developed in accordance with the standards established by the International Standards Organisation (ISO 9000) and the European Standard (ES 29000). They are aimed at ensuring that food industries, catering companies and food industries, catering companies and other companies related to the sector respect and document the procedures established in order to comply with the norms that guarantee adequate food safety. The effectiveness of these programs, which is regularly analyzed by external experts, directly monitors that the adoption of quality assurance procedures at every level, quality management systems are used by the food industry also the collaboration with suppliers (individual farmers and wholesalers of raw materials), transporters and wholesale and retail traders thus ensuring a full sharing of responsibility concerning the entire cycle of food safety at all levels of the production cycle and up to distribution.

1.4. SAFFI: the European commission safe food for infants project

Within the frame of the Horizon 2020 program The EU launched the program safe food for infants (SAFFI). The SAFFI project is one of the 3 projects selected within the framework of the European Horizon 2020 call for projects SFS-37-2019 "Integrated approaches for food safety along the food chain" concerning research and innovation actions. This project, aims to develop an integrated approach to improve the identification, assessment, detection and mitigation of risks linked to microbiological and chemical hazards throughout the food chain in Europe and China SFS-37-2019 "Integrated approaches for food safety along the food chain" concerning research and innovation actions. This project, coordinated by the INRAE QuaPA Quality Research Unit for Animal Products, aims to develop an integrated approach to improve the identification, assessment, detection and mitigation of risks linked to microbiological and chemical hazards throughout the food chain in Europe and China.

1.5. The SAFFI partner centers

Coordinated by the French National Research Institute for Agriculture, Food and Environment (INRAE), SAFFI brings together 14 partners from seven countries across Europe and 6 partners from two Chinese provinces gathering the required expertise in food safety control, infant food production, analytical and data sciences to achieve the project goal. This multi-actor and Sino-European consortium led by INRAE, the Europe's top agricultural research institute and the world's number two centre for the agricultural sciences, involves as full partners five international infant food companies (Friesland Campina, HiPP, YIOTIS, Beingmate, YFFC), two food safety authority institutions (ZAIQ and ANSES who published in 2016 the first total diet study worldwide dedicated to infant), three dynamic European technological SMEs (CremeGlobal, Computomics and BDS) who are specialists in data science for industry decision making, omics data analysis and bio-based technologies, respectively), specialists in infant health and nutrition, EPA-UNEPSA, the Union of National European Pediatric Societies and Association, INRAE Transfert (a company specialized in project management and technology transfer) and several leading European and Chinese academic institutions (Wageningen University, Holland; University of Turin, Italy; Institut de Recerca I Tecnologia Agroalimentaries, Spain; Fraunhofer-Gesellschaft zue Foerderung der Angewandten Forschung E.V., Germany; Zhejiang Academy of Science & Technology for Inspection & Quarantine, China; Jiangsu Academy of Agricultural Sciences, China; Zhejiang Academy of Agricultural Science, China).

2. Conclusions

This EU-China multi-actor consortium of 20 partners involving academia, food safety authorities, infant food companies, pediatric organizations and technological and data-science SMEs contributed to this special issue dedicated to the important topic of ensuring safe food for infants. The fate of nations is determined by what they eat, and all the stakeholders involved are on the front line to contain the risks of food hazards [3,9,10]. They can play a key role if they will actively cooperate and integrate their efforts with governments and local, state, federal, and global public health institutions and agencies, to ensure that infants and children have access to good and safe food [3].

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References

1 *Food And Agriculture Organization Of The United Nations (FAO)*. Report of the World Food Summit; 1996. https://www.fao.org/3/w3548e/w3548e00.htm#adopt05, 13-17 November.
2 European Commission. *White Paper on food safety*; 2022. https://op.europa.eu/en/publication-detail/-/publication/6d4b523b-dad8-4449-b2b4-9fa9b0d6e2be (Accessed, 10, February.
3 Pettoello-Mantovani M, Mestrovic J, Namazova-Baranova Md PhD L, Giardino I, Somekh E, Vural M. Ensuring Safe Food for Infants: The Importance of an Integrated Approach to Monitor and Reduce the Risks of Biological, Chemical, and Physical Hazards. *J Pediatr*. 2021 Feb;229:315–316. e2.
4 Rather IA, Koh WY, Paek WK, Lim J. The sources of chemical contaminants in food and their health implications. *Front Pharmacol*. 2017;8:830.
5 Pettoello-Mantovani M, Ehrich J, Sacco M, Ferrara P, Giardino I, Pop TL. Food insecurity and children's rights to adequate nutrition inEurope. *J Pediatr*. 2018;198:329–330. e1.
6 European Commission. Food safety: overview. https://ec.europa.eu/food/overview_en. (Accessed, 10, February, 2022).
7 European Union. EUR-Lex.Food safety. https://eur-lex.europa.eu/summary/chapter/food_safety.html?root_default=SUM_1_CODED%3D30&locale=en. (Accessed, 10, February, 2022).
8 Ehrich JH, Kerbl R, Pettoello-Mantovani M, Lenton S. Opening the debate on pediatric subspecialties and specialist centers: opportunities for better care or risks of care fragmentation? *J Pediatr*. 2015;167:1177–1178. e2.
9 Hojsak I, Fabiano V, Pop TL, Goulet O, Zuccotti GV, C¸ okugras¸ FC, et al. Guidance on the use of probiotics in clinical practice in children with selected clinical conditions and in specific vulnerable groups. *Acta Paediatr*. 2018;107:927–937.
10 Brillat-Savarin JA. *Physiologie du gout, ou Meditations de gastronomie transcendante. ed*. Philadelphia: Lindsay: A. Sautelet & Cie, 1826. The Physiology of Taste; 1854. Balckstone Ed.

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Safe food for infants: An EU-China project to enhance the control of safety risks raised by microbial and chemical hazards all along the infant food chains☆

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ABSTRACT

The EU-project SAFFI targets food for EU's 15 million and China's 45 million children under the age of three. It aims at developing an integrated approach to enhance the identification, assessment, detection and mitigation of health risks raised by microbial and chemical hazards along EU and China infant food chains.

SAFFI will benchmark the main risks through an extensive hazard identification system based on multiple data sources and a risk ranking procedure. It will also develop procedures to enhance top-down and bottom-up hazard control by combining management options with a panel of technologies for the detection and mitigation of priority hazards. Furthermore, it will explore unexpected contaminants by predictive toxicology and improve risk-based food safety management of biohazards by omics and predictive microbiology. SAFFI will co-develop with and deliver to stakeholders a decision-support system (DSS) to enhance safety control all along the food chain. This DSS will integrate the databases, procedures and methods described above and will be a framework for a generic DSS dedicated to other food.

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This overall methodology will also be implemented in a complementary Chinese side of the project, and exemplified for each side, with four case studies that were selected to cover priority hazards, main ingredients, processes and control steps of the infant food chain. Resulting databases, tools and procedures will be shared, cross-validated, concatenated, benchmarked and finally harmonized for further use in the EU and China.

This EU-China multi-actor consortium of 20 partners involves academia, food safety authorities, infant food companies, a paediatrics association and technological and data-science SMEs.

1. Introduction

Due to the continuously increasing consumer demand for quality and transparency, larger-scale food production, more intensive food trade and increasing urbanisation that shape the food industry, the control of food safety is obviously a priority shared by both the EU and China. Food safety and quality assurance also address the need for trust, transparency and harmonization of practices, which is a prerequisite for the development of efficient domestic and international (EU-China) trade. However, implementing efficient food safety control is complex because of: (i) the variety of products due to the diversity of raw materials, processing, packaging and storage; (ii) the diversity and changes in consumer practices, which may put them at risk in some cases; (iii) the continuous product evolution driven by agri-food innovations, advances in human health knowledge and subsequent regulatory changes; (iv) the different regulatory contexts and health surveillance systems between countries with respect to intensive and/or global trade, resulting in a large set of potential hazards that need to be controlled.

The SAFFI project focuses on foods for infants and young children (which is the correct term according to EU legislation for what is designated as "infant food" in the rest of the text) because from the consumers' perspective this food sector is expected to adhere to the highest safety standards and must be strongly regulated given the vulnerability of its target population. Secondly, the wide international echoes of recent health scandals in this sector[1,2] are illustrations that food safety control is a focus point worldwide, with significant impact when not handled properly. The project is dedicated to the infant (below 3 years old) population. The focus on infant food of the most recent French Total Diet Study[3] was the world's first and demonstrated both the relevance of this issue for European public health authorities and the generic nature of this model; as such, this will allow a large transferability of its results to other food sectors and populations. Moreover, the growth of the infant food sector is particularly strong in China (+78% of the market value between 2010 and 2016). Infant food is also the second biggest food commodity in terms of import, specifically infant formulae from Europe due to its reputation of high quality in Chinese consumers' eyes and their trust in the product. Infant food is the third EU food and drink export product, with €6.6 million exports (+12% in 2017) and China is part of the top 3 export destinations of EU infant food. It is therefore valuable and timely to strengthen EU-China food safety cooperation as highlighted by Vytenis Andriukaitis, the EU Commissioner for Health and Food Safety, and to build an effective and harmonised food safety control system based on the infant food chain as a model.

Through four infant food chains - powdered infant formula, sterilized vegetable mixed with fish/meat, infant cereals and fruit puree- chosen as case studies to cover infant nutrition while encompassing very different hazards, ingredients, processing and control steps, SAFFI targets the control of the main priority hazards (see Table 1) pointed out by the French iTDS[3] and the recent infant food health crises.

The results of the French iTDS[3] and the recent health crises have shown the need to improve the levels of safety control and to identify bottlenecks impairing the effectiveness of the current systems. The aim of the present paper is to exemplify through a presentation of the main axes of the EU-China SAFFI project what research can do to help paediatricians and other actors in the area of infant food safety in order: (i) to have a better insight on microbiological and chemical hazards along the infant food chain; (ii) to identify the main known risks and provide new tools for their identification, detection, assessment and mitigation by both public health authorities and food industry; (iii) to anticipate unknown risks related to chemical contaminants not detected by current monitoring systems; (iv) to prevent public health crises related to foodborne microorganisms by proposing tools for predictive microbiology and risk management based no longer on hazards but on risks; (v) to further share data, practices and critical information in real time to ensure an overall food safety control.

2. Enhancement of food risk assessment

In order to provide paediatricians and other stakeholders with updated and comprehensive knowledge about both possible and priority hazards in infant food, one of the key issues is to refine risk assessment with advances in hazard identification and risk ranking (Fig. 1).

2.1. Hazard identification

Within Quantitative Risk Assessment for both microbiological and chemical hazards, the processes of hazard characterisation, exposure assessment, and risk characterisation are generally carried out following a structured procedure and are well documented. However, the initial part of risk assessment, namely hazard identification, is often not following a structured procedure and is mainly based on expert judgement, and considerations and decisions are not always well documented. Therefore, software prototypes can be developed to structurally identify hazards and rank the risks.[4–6]

In order to be able to evaluate and rank all risks (both chemical and microbiological), it is crucial to start broadly to not miss potential hazards but also being able to select the most relevant ones for further

Table 1
List of priority infant food hazards targeted in SAFFI.

Origin in the Food Chain	Chemical or Microbial Hazard
Production environment	Persistant Organic Pollutants (including PolyChloroDibenzo-Dioxins/Furans (PCDD/Fs), PolyChloroBiphenyls (PCBs))
	Trace elements (As, Pb, Ni)
	Tropane alkaloids
	Mycotoxins
	Microbial pathogens*
	Per- and poly-fluoroalkyl substances (including PerFluoroOctanoic Acid (PFOA), PerFluoroOctane Sulfonate (PFOS))
Agricultural practices	Pesticides
	Pathogens from plants, animals or soils*
Industrial or domestic processes	Process Induced Toxicants (Acrylamide, Furans, HydroxyMethylFurfural (HMF))
	Pathogens from processing plant environment*
	Mineral Oil (Mineral Oil Saturated Hydrocarbons (MOSH), Mineral Oil Aromatic Hydrocarbons (MOAH)),
Packaging	Bisphenols
	Phthalates
	Photoinitiators
Storage	Food contact material migrants (see packaging)
	Pathogens*

*Pathogens include *Salmonella, Cronobacter, Listeria monocytogenes*, spore-formers like *Bacillus cereus* and *Clostridium botulinum*.

risk characterisation. Hazard identification requires the creation of underlying databases by collecting, aggregating, validating and analysing a wide range of available data. In the classical approaches of hazard identification these databases rely mainly on very specific, validated, defined but often limited data.[4] Thanks to the development of data sciences, these databases can now be extended to very broad, generic and not always directly related big data in order not to miss potential hazard. In the project initially the most relevant hazards (both microbiological and chemical) generally in infant foods will be identified. As a next step, a procedure for the selection of the specific hazards from this list will be developed, based on the ingredients, process, and characteristics of the specific food product.

2.2. Risk ranking

The hazard identification will generally result in a long list of hazards, therefore a next step is risk ranking, to then reduce the list of hazards to the most relevant ones in order to carry out for these, further more detailed exposure assessment and hazard characterization.[5] To rank both chemical and microbiological risk, the first possibility is to convert the probability of illness and /or number of cases into Disability Adjust Life Year (DALY); this has been done for instance with arsenic in water[7] and *Listeria monocytogenes* in salmon.[8] However, for some hazards, particularly in the chemical field, it is difficult to go up to the probability of illness and number of cases while performing the risk assessment.

Moreover, consumer perception and political judgements are often also driven by other aspects, and for example will weigh the severity even more than in the DALY and additionally having a fear/fright factor for aspects where the real risk is very low but fear high.[9] Also, in certain cases, the public perception is more pronounced for one situation with 100 related cases than a situation with 1000 disperse cases. DALY's, but also other criteria as suggested by the FAO[9] can be assessed and combined in a multi-criteria decision approach (MCDA). These MCDA have been already used in food safety, for example to select effective health interventions.[10]

3. Enhancement of infant diet quality with innovative food processing technologies

Research can also help paediatricians a.o. by improving the overall quality of infant diet. One way is to propose new tools to enhance the effectiveness of food safety management options to better control the production process of infant food. Another way to improve the quality of infant diet may rely on the assessment of innovative food processing technologies which might advantageously replace classical preservation processes based on thermal treatments.

3.1. Effectiveness of the food management options

Validated hazard control options and risk mitigation strategies must be applied today all along the infant food supply chain, for the currently known hazards as well as for any emerging or new hazard derived from future events and developments. For this purpose, integrated approaches have to be developed enabling to prioritize and to design the most effective solutions combining control measures at critical points of the production process with up-to-date sampling and monitoring strategies along the food chain. This approach shall allow an efficient assurance of the food safety systems at both operational (including HACCP plans) and governmental level.

3.2. Innovative preservation technologies

As in the entire food industry, the infant food sector invests in the development of safer, fresher, healthier and more sustainable products with the implementation of new and emerging technologies like innovative processing and preservation technologies. These new technologies could represent a very attractive alternative to classical thermal treatments, which are known to have negative impacts on several dimensions of the food quality, such as nutritional or sensory properties, but also chemical safety. Pulse combustion drying (PCD) has been proven to efficiently dry chemical and pharmaceutical products, its potential has been also pointed out for some food (e.g. eggs[11]) and it could be promising for the production of dried infant food (powdered infant formula and cereals). The emerging thermal radiofrequency (RD) technology and non-thermal high-pressure processing (HPP) can be targeted for the production of infant food (sterilised and pasteurised). Compared to classical processing and preservation processes based on heat-treatment, RD and HPP should better preserve freshness, nutritional and sensory quality of infant food, while minimising the generation of process induced contaminants, assuring the microbiological safety standards and limiting environmental impact.[12]

The assessment of these innovative processing technologies that can be suspected to be more beneficial for the organoleptic and nutritional properties, more energy-efficient and environmentally-friendly needs to be carried out through the quantification of their impact on the microbiological and chemical relevant hazards for infant food listed in Table 1 (based on ANSES[3]; Bhunia et al.[13]; Mulder et al.[14]; Zwietering et al.[15]). It is necessary to check their preservation capacity towards pathogens in terms of growth, inhibition and inactivation but also to assess their impact on chemical food safety, given their potential influence on the fate of raw material contaminants,[16] on the generation of process-induced toxicants[17] and on the transfer of packaging migrants[13] (Fig. 2).

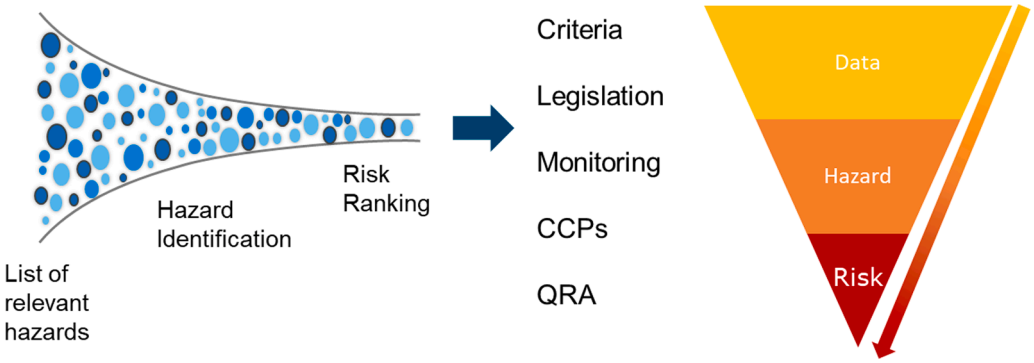

Fig. 1. Stages in hazard identification: initial list of relevant hazards in infant food, identification of hazards in a specific food product and ranking of the risks. Results are relevant to guide further actions in the management of the risks.

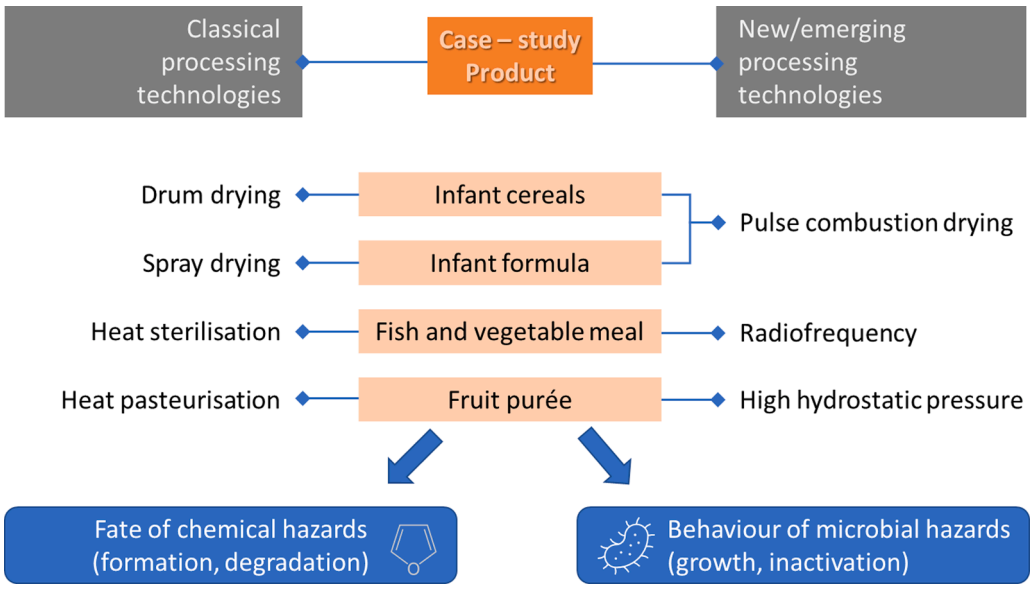

Fig. 2. Infant foods, classical and new/emerging processing technologies affecting the fate of chemical hazards and the behaviour of microbial pathogens.

4. Enhancement of the surveillance of chemical hazards

Research can also help paediatricians through the development of analytical approaches in order to strengthen the surveillance of chemical hazard by both food safety authorities and infant food companies. To this end, two approaches based on both chemo- and bioanalytics can be undertaken. The first approach aims to improve the surveillance of known food chemical hazard while the second one deals with the discovery of unknown and / or unsuspected toxicants.

4.1. Strengthening the surveillance of known chemical hazard

Due to the very low Maximum Limits (ML) of most priority contaminants (e.g. pg/g range for PolyChloroBiphenyls-PCBs), their current detection and monitoring revolves around high-performance validated methods capable of detecting and quantifying key toxic contaminants at targeted ML. However, these methods are often expensive and low-throughput, thus limiting frequency and scope of surveillance by food safety authorities and dissuading routine preventive monitoring by the industry. Two complementary high-throughput, sensitive and cost-effective targeted tools can be developed in order to improve the coverage and the efficiency of non-conformity detection by food safety authorities (top-down approach) and boost self-monitoring by the agri-food industry (bottom-up approach).

The first solution aims to reinforce the top-down surveillance by Food Safety Authorities. Based on chemoanalytics techniques, it consists in cutting the cost and low throughput of up-to-date mass spectrometry based-methods suitable for the detection of known priority contaminants with sample pooling strategy.[18] As shown in Fig. 3, pooling – also known as pool testing, group testing or pooled testing- means combining samples from several individuals or products and conducting one laboratory test on the combined pool of samples to detect the targeted contaminant. The rising interest for this strategy over the past years[19] enabled to clarify its prerequisites in terms of contamination prevalence, analytical cost and sensitivity and it suggests that these application conditions match with the implementation of pooling for the detection of priority chemical hazards in food. The second solution aims to develop the self-monitoring by private companies. Based on bioanalytics, it consists in implementing combinations of bioassays by coupling them to suitable extraction methods to modulate and refine bioassay selectivity.[20–22]

4.2. Discovery of unsuspected/unknown chemical hazard in food

Our knowledge of the chemical universe is very limited and most of the approximately 100,000 industrial chemicals that are in common use

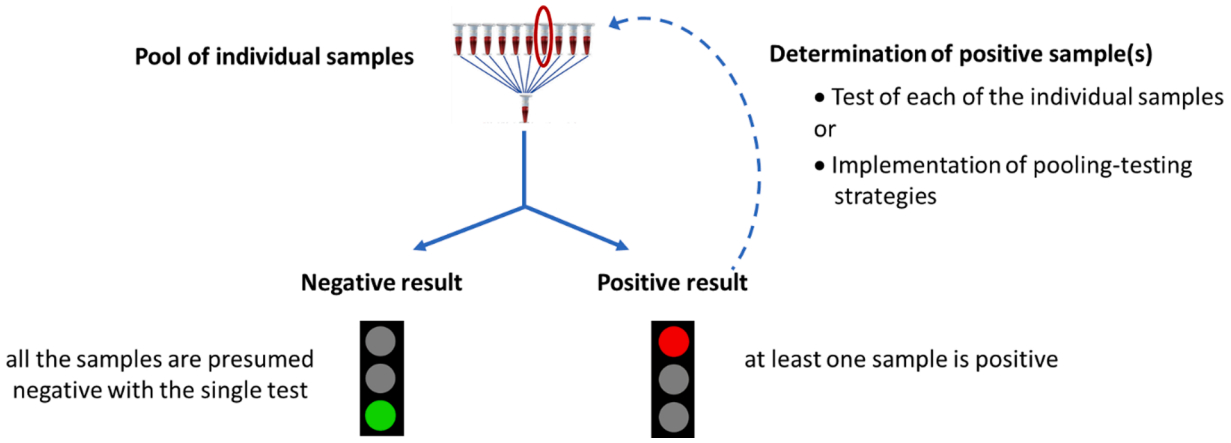

Fig. 3. Principle of the sample pooling strategy for the high-throughput detection of samples contaminated with a targeted chemical contaminant.

have undergone no or only limited safety testing only. This situation will improve significantly due to the REACH legislation, but still the majority of chemicals that surround us will remain untested. Recently, it has been recognized that contamination with unexpected food contaminants, both with known and unknown toxicity and often related to intended or unintended use of contaminated starting products (e.g. dioxin- or PCB-contaminated feed, melamine crisis, process-induced toxicants, packaging migrants) is an issue of concern.[23] Several of these contaminants will be picked up during routine screening in advanced quality control systems, but others (e.g. brominated dioxins, endocrine disruptors) may escape notice. Another complication is the fact that chemicals are present typically in complex mixtures, and that mixture effects need consideration as well. For these reasons, SAFFI will particularly focus on non-targeted methods to explore unsuspected and unknown contaminants. A first option is the non-targeted acquisition mode applied through full scan mass spectrometry analysis which has to be completed with specific filters to highlight chemical contaminants of interest based on 1/ the chemistry of the compounds (particular signatures, e.g. halogens[24]) or 2/ the research of specific patterns with chemometric tools.[25] Non-targeted approaches based only on chemo analytics would lead to limitation due to the complexity of the chemical universe, the lack of data on toxicity of most chemicals and the possibility of cocktail effects. A second option may consist in using bioassays to measure biological activity regardless of chemical structure, or prior knowledge. Bioassays, on the other hand cannot identify the chemical nature of individual compounds and a third option has to be considered. It will combine chemical and biological analytics using their complementary strengths and weaknesses in an effect-directed analysis (EDA)[26] to identify unknown or unexpected chemical hazards. In such a system, effect-based bioassays can best be used for comprehensive screening purposes, while chemical analytics can best be used for identification of chemicals in positive samples.

Despite EDA success in other fields like ecotoxicology, it has been hardly applied for contaminant discovery in food products until recently.[27,28] Recent advances in bioanalytics, chemoanalytics and chemo/bioinformatics could definitely boost this approach and enable the discovery of relevant "emerging" chemical hazards in the coming years.

5. Enhancement of microbial hazard characterization, detection and risk assessment

Research may also help paediatricians by enhancing the microbial safety of infant food through the development of holistic approaches namely omic approaches (Fig. 4). These new omics approaches may enable to refine the prediction of the pathogen behaviour in the food environment by highlighting the characteristics of the microbiota which may restrain or enhance the development or the persistence of pathogens.

Technological advances in DNA sequencing have resulted in a shift in the detection and monitoring of pathogens along food chains. Instead of only isolating pathogens from foods, microbiologists are also interested in capturing the bigger picture in which the pathogen is influenced by both the food environment and the other organisms present.[29] While metataxonomics or amplicon sequencing provides a taxonomic description of the food microbial community, metagenomics or shot-gun sequencing provides an overview of its collective function and metatranscriptomics indicate the genes that are actively transcribed by the community at sampling time.[30] In addition, recent works suggest that volatolomics may provide a promising alternative to more classical metabolomics platform to reveal significant changes in the metabolism of single culture[31] or microbiota.[32] Once integrated, this information provides an overview of microorganism associations and their metabolic and sense/response pathways within the food.[33]

These approaches offer an additional advantage as they are non-targeted and they by-pass the culturing step that may not always recover the targeted microorganism from the food. In infant food, these meta-omics approaches can be used to 1/ detect target foodborne pathogens in foods and their distribution in time and space, 2/ highlight the characteristics of the microbiota which may restrain or enhance persistence of pathogens and 3/ explore the behaviour of the target pathogens in samples or processing conditions that are relevant for food safety and propose predictive models. In parallel, a culture-dependent approach can be applied, coupled to WGS to enhance tracing of microbial hazards throughout the food chain and inferring associations with the microbiota or the environment.[34]

6. Enhancement of infant food safety standards

For raising food safety standards in the EU and China, the SAFFI project aims to develop decision support systems (DSS), recommendations and guidelines which are dedicated to be shared in Europe and China by food safety authorities and infant food companies for the management of safety risks all along the infant food chains.

These integrated approaches consist in collecting, connecting and combining pertinent knowledge and data from 1/ the entire food chain, 2/ the diversity of chemical and microbiological hazards, 3/ the different criteria contributing to risk ranking (including public health impact and perception impact), 4/ different sources of information (knowledge rules, structured databases and holistic data), 5/ different disciplines (risk assessment, food technology, toxicology, residue chemistry, predictive microbiology, paediatrics, data science,

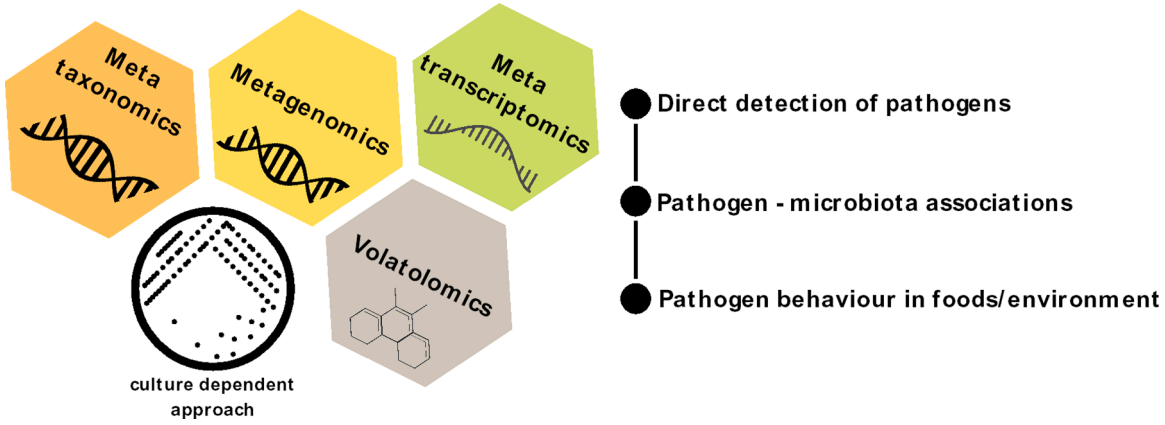

Fig. 4. Microbial communities' studies through omics approaches for infant food microbial safety. Microbial communities can be investigated in a culture independent manner, providing insights on microbial interactions and behaviour under "real" conditions. In parallel, a culture dependent approach may complement and integrate the findings of the omics applied directly in the samples.

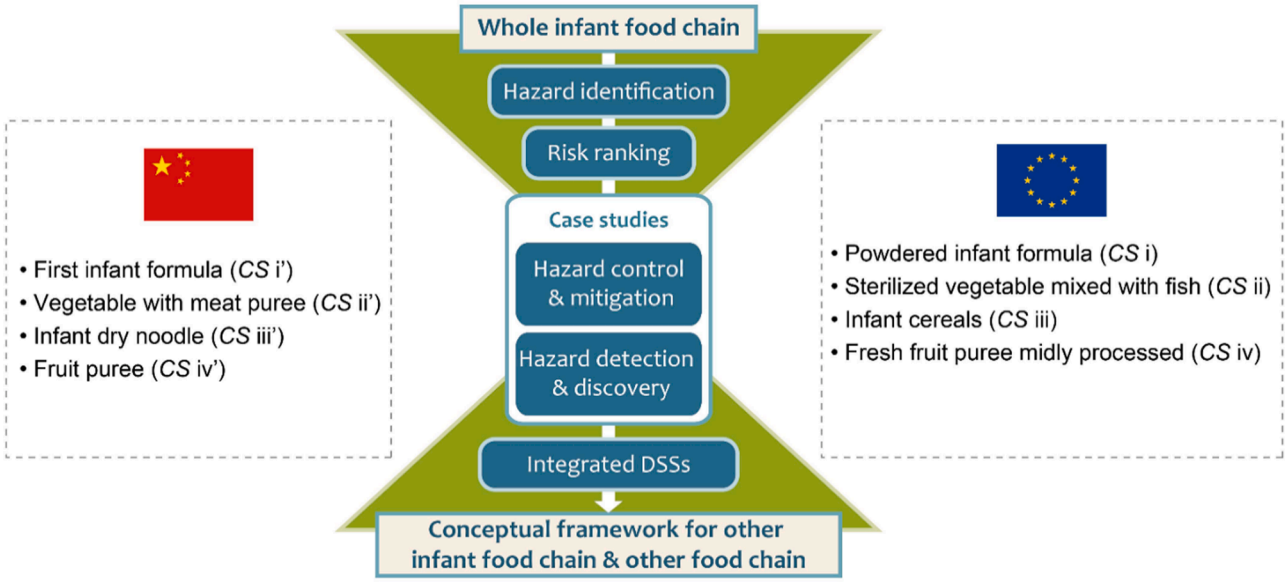

Fig. 5. Scheme of the integrative approach developed by SAFFI.

knowledge engineering), 6/ different criteria (safety, economic, regulatory, perception), 7/ different actors including stakeholders and academia, and 8/ different cultures.

Fig. 5 represents the integrative approach implemented in SAFFI which results in a project organisation that may be illustrated by an hourglass representing the broadness of the scope at the different steps. In the SAFFI strategy, hazard identification and risk ranking have to be as comprehensive as possible and this is the reason why corresponding DSS prototypes have to be developed with a broad scope that of being the whole infant food chain. The hazard identification and risk ranking procedures and related DSS prototypes have to be further applied and tested on a restricted scope to the four infant food chain defined as case studies, for demonstration purposes. The four case studies are first infant formula, sterilized vegetables with fish (or meat puree in China), infant cereals (infant dry noodles in China) and infant fruit puree. These same four case studies can then be used to develop innovative approaches and DSS prototypes for hazard control and mitigation together with a DSS module for detection of both chemical and microbial hazards. The different DSS prototypes or modules that are developed in SAFFI at the different steps of the risk management process are dedicated to be finally integrated to produce a single DSS which will further serve as a conceptual framework for further application to other infant food chains and beyond, to other food chains. Findings, including data, procedures and models will also enable to develop recommendations and guidelines for all stakeholders involved in the food chain.

7. Conclusion

Besides enhancing the current systems by improving targeted detection and assessing innovative preservation technologies, the SAFFI project is implementing several approaches enabling a real paradigm shift compared to practices currently implemented by food safety authorities and infant food companies: These cutting-edge approaches will more particularly focus on (i) enlarging the scope of food safety control to the wide range of unexpected or unknown chemical hazard that may occur in food and may pose a risk on consumers (ii) better predicting the behaviour and final assessment of the risk related to food-borne pathogens, (iii) refining hazard identification and risk-ranking to define the priority hazard to focus on.

The different knowledge, tools, databases, procedures and models collected and developed by the project will be integrated in a user-friendly and upgradable decision support system (DSS) for identification, detection, ranking and control of hazards and risk assessment. This will enable the rapid adoption by the DSS target end-users, that are food safety authorities and infant food companies, and will allow to overcome the complexity and the diversity of food chains. Besides the integration tools provided by data science and knowledge engineering, SAFFI's approach is multi-actor and interdisciplinary.

To reach its scientific, technological, socioeconomic and regulatory objectives, SAFFI's proof of concept will be exemplified on infant food chosen as model food chain. While hazard identification will deal with the infant food chain in its diversity, the case studies will focus on specific infant food products chosen to cover infant nutrition while encompassing very different hazards, ingredients, processing and control steps.

In order to make sure that the outcomes of the project will be adequately demand-driven, food safety authority experts, paediatrics, infant food companies and technological SMEs are involved, besides academia, in the construction, planning, implementation and dissemination of the project.

Finally, in order to create a frame to favour exchanges between the EU and China, SAFFI will set the basis to adapt food safety regulation, knowledge and practices through: 1/ EU-China co-construction of the project, structuration in two SAFFI mirror projects; 2/ training activities; 3/ standard setting, including good control practices; 4/regulation convergence; 5/ joint dissemination events. SAFFI refers to existing European risk assessment authorities (EFSA) and considers tools (e.g. alert system for food and feed (RASFF), animal tracing system (TRACES), European pesticides database) and their Chinese counterparts.

Declaration of Competing Interest

None.

Acknowledgments

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References

1 Jourdan-da Silva N, Fabre L, Robinson E, et al. Ongoing nationwide outbreak of Salmonella Agona associated with internationally distributed infant milk products, France, December 2017. *Euro Surveill*. 2018;23(2):17–00852. https://doi.org/10.2807/1560-7917.ES.2018.23.2.17-00852. bulletin Europeen sur les maladies transmissibles = European communicable disease bulletin.

2 Zhao DT, Sriramesh K. Anatomy of a product safety crisis: fonterra's recall crisis in China. *Asian J Commun*. 2019;29(2):149–163. https://doi.org/10.1080/01292986.2018.1555266.

3 ANSES. *Infant Total Diet Study (iTDS) – Report 1 – ANSES Opinion*. Paris: ANSES; 2016. Request N0 2014-SA-0317 https://www.anses.fr/en/content/infant-total-diet-study-itds.

4 Van Gerwen SJC, De Wit JC, Notermans S, Zwietering MH. An identification procedure for foodborne microbial hazards. *Int J Food Microbiol*. 1997;38:1–15. https://doi.org/10.1016/S0168-1605(97)00077-9.

5 Stella P, Cerf O, Koutsoumanis KP, et al. Ranking the safety of foods: a new tool and its application to composite products. *Trends Food Sci Technol*. 2013;33:124–138. https://doi.org/10.1016/j.tifs.2013.07.005.

6 Monaghan JM, Augustin JC, Bassett J, Betts R, Pourkomailian B, Zwietering MH. Risk assessment or assessment of risk? Developing an evidence based approach for primary producers of leafy vegetables to assess and manage microbial risks. *J Food Prot*. 2017;80:725–733. https://doi.org/10.4315/0362-028X.JFP-16-237.

7 Boué G, Wasiewska LA, Cummins E, et al. Development of a cryptosporidium-arsenic multi-risk assessment model for infant formula prepared with tap water in France. *Food Res Int*. 2018;108:558–570. https://doi.org/10.1016/j.foodres.2018.03.054.

8 Berjia LM, Andersen R, Hoekstra J, Poulsen M, Nauta MJ. Risk-benefit assessment of cold-smoked salmon: microbial risk versus nutritional benefit. *Eur J Food Res Rev*. 2012;2:49–68. https://orbit.dtu.dk/fedora/objects/orbit:115851/datastreams/file_2554009a-c268-4540-9bbd-59e345ec76fa/content.

9 FAO. *Food Safety Risk Management Evidence-Informed Policies and Decisions, Considering Multiple Factors. FAO Guidance Materials, Food Safety and Quality Series 4*. Roma: FAO; 2017. http://www.fao.org/3/i8240en/I8240EN.pdf.

10 Fazil A, Rajic A, Sanchez J, McEwen S. Choices, choices: the application of multi-criteria decision analysis to a food safety decision-making problem. *J Food Prot*. 2008;71:2323–2333. https://doi.org/10.4315/0362-028X-71.11.2323.

11 Wu Z, Yue L, Li J, Mujumdar AS, Rehkopf JA. Pulse combustion spray drying of egg white: energy efficiency and product quality. *Food Bioprocess Technol*. 2015;8:148–157. https://doi.org/10.1007/s11947-014-1384-9.

12 Mújica-Paz H, Valdez-Fragoso A, Tonello Samson C, Welti-Chanes J, Torres JA. High-pressure processing technologies for the pasteurization and sterilization of foods. *Food Bioprocess Technol*. 2011;4:969–985. https://doi.org/10.1007/s11947-011-0543-5.

13 Bhunia K, Sablani SS, Tang J, Rasco B. Migration of chemical compounds from packaging polymers during microwave, conventional heat treatment, and storage. *Compr Rev Food Sci Food Saf*. 2013;12:523–545. https://doi.org/10.1111/1541-4337.12028.

14 Mulder PPJ, De Nijs M, Castellari M, et al. Occurrence of tropane alkaloids in food. *EFSA Support Publ*. 2016;2016(EN-1140):200. https://doi.org/10.2903/sp.efsa.2016.EN-1140.

15 Zwietering MH, Jacxsens L, Membré JM, Nauta M, Peterz M. Relevance of microbial finished product testing in food safety management. *Food Control*. 2016;60:31–43. https://doi.org/10.1016/j.foodcont.2015.07.002.

16 Planche C, Ratel J, Blinet P, et al. Effects of pan cooking on micropollutants in meat. *Food Chem*. 2017;232:395–404. https://doi.org/10.1016/j.foodchem.2017.03.049.

17 Meurillon M, Engel E. Mitigation strategies to reduce the impact of heterocyclic aromatic amines in proteinaceous foods. *Trends Food Sci Technol*. 2016;50:70–84. https://doi.org/10.1016/j.tifs.2016.01.007.

18 Heffernan AL, Aylward LL, Toms LML, Sly PD, Macleod M, Mueller JF. Pooled biological specimens for human biomonitoring of environmental chemicals: opportunities and limitations. *J Expo Sci Environ Epidemiol*. 2014;24:225. https://doi.org/10.1038/jes.2013.76.

19 Mallapaty S. The mathematical strategy that could transform coronavirus testing. *Nature*. 2020;583:504–505. https://doi.org/10.1038/d41586-020-02053-6.

20 Van Vugt-Lussenburg B, Van der Burg B, Besselink H, Brouwer A, Steinberg P. *DR CALUX®, a high-throughput screening assay for the detection of dioxin and dioxin-like compounds in food and feed. High Throughput Screening Methods in Toxicity Testing*. New York: John Wiley and Sons, Inc.; 2013:546–553. edISBN 9781118065631.

21 Pieterse B, Felzel E, Winter R, Van der Burg B, Brouwer A. PAH-CALUX, an optimized bioassay for carcinogenic hazard identification of polycyclic aromatic hydrocarbons (PAHs) as individual compounds and in complex mixtures. *Environ Sci Technol*. 2013;47:11651–11659. https://doi.org/10.1021/es403810w.

22 Van Vugt-Lussenburg BMA, Van der Lee RB, Man HY, et al. Incorporation of metabolic enzymes to improve predictivity of reporter gene assay results for estrogenic and anti-androgenic activity. *Reprod Toxicol*. 2018;75:40–48. https://doi.org/10.1016/j.reprotox.2017.11.005.

23 Van Asselt ED, Marvin HJP, Boon PE, et al. *Chemical and Physical Hazards in the Dairy Chain*. Wageningen, RIKILT Wageningen UR (University & Research Centre); 2016. RIKILT report 2016.003 https://library.wur.nl/WebQuery/wurpubs/fulltext/447318.

24 Léon A, Cariou R, Hutinet S, et al. HaloSeeker 1.0, a user-friendly software to highlight halogenated chemicals in non-targeted high resolution mass spectrometry dataset. *Anal Chem*. 2019;91:3500–3507. https://doi.org/10.1021/acs.analchem.8b05103.

25 Guitton Y, Dervilly-Pinel G, Jandova R, Stead S, Takats Z, Le Bizec B. Rapid evaporative ionization mass spectrometry and chemometrics for high-throughput screening of ractopamine in meat producing animals. *Food Addit Contam*. 2018;35:900–910. https://doi.org/10.1080/19440049.2017.1421778.

26 Brack W, Ait-Aissa S, Burgess RM, et al. Effect-directed analysis supporting monitoring of aquatic environments — an in-depth overview. *Sci Total Environ*. 2016;544:1073–1118. https://doi.org/10.1016/j.scitotenv.2015.11.102.

27 Krüger S, Bergin A, Morlock GE. Effect-directed analysis of ginger (Zingiber officinale) and its food products, and quantification of bioactive compounds via high-performance thin-layer chromatography and mass spectrometry. *Food Chem*. 2018;243:258–268. https://doi.org/10.1016/j.foodchem.2017.09.095.

28 Rosenmai AK, Bengtström L, Taxvig C, et al. An effect-directed strategy for characterizing emerging chemicals in food contact materials made from paper and board. *Food Chem Toxicol*. 2017;106:250–259. https://doi.org/10.1016/j.fct.2017.05.061.

29 Bokulich NA, Lewis ZT, Boundy-Mills KL, Mills DA. A new perspective on microbial landscapes within food production. *Curr Opin Biotechnol*. 2016;37:182–189. https://doi.org/10.1016/j.copbio.2015.12.008.

30 Cocolin L, Ercolini D. Zooming into food-associated microbial consortia: a 'cultural' evolution. *Curr Opin Food Sci*. 2015;2:43–50. https://doi.org/10.1016/j.cofs.2015.01.003.

31 Hidalgo K, Ratel J, Mercier F, Gauriat B, Bouchard P, Engel E. Volatolomics in bacterial ecotoxicology, a novel method for detecting signatures of pesticide exposure? *Front Microbiol*. 2018;9:3113. https://doi.org/10.3389/fmicb.2018.03113.

32 Defois C, Ratel J, Garrait G, et al. Food chemicals disrupt human gut microbiota activity and impact intestinal homeostasis as revealed by *in vitro* systems. *Sci Rep*. 2018;8:11006. https://doi.org/10.1038/s41598-018-29376-9.

33 Cocolin L, Mataragas M, Bourdichon F, et al. Next generation microbiological risk assessment meta-omics: the next need for integration. *Int J Food Microbiol*. 2018;287:10–17. https://doi.org/10.1016/j.ijfoodmicro.2017.11.008.

34 Rantsiou K, Kathariou S, Winkler A, et al. Next generation microbiological risk assessment: opportunities of whole genome sequencing (WGS) for foodborne pathogen surveillance, source tracking and risk assessment. *Int J Food Microbiol*. 2018;287:3–9. https://doi.org/10.1016/j.ijfoodmicro.2017.11.007.

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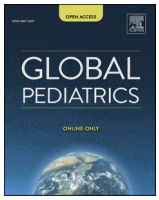

Microbial and chemical hazard identification in infant food chains☆

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ABSTRACT

To ensure foods are safe, food companies, food safety authorities, and governmental agencies work together to improve the control and prevent unwanted food contamination by either biological, chemical or physical agents, namely the hazards. Foodborne illnesses leading to diseases in humans are still frequently reported. To better protect infants and children from foodborne diseases, the SAFFI (Safe Food for Infant) project promoted by the European Union in collaboration with China, will develop an integrative approach to identify, assess, detect and mitigate risks associated with microbial hazards (MHs) and chemical hazards (CHs) in infant foods.

The first stage to tackle this issue was to identify relevant hazards in infant foods. By collecting data from the literature, scientific reports, existing databases, and clinical studies and using these data to compile a list of relevant foodborne hazards. These hazards caused major foodborne outbreaks, frequently contaminated foods, have large public health impacts, and/or are dangerous to young children.

After the initial identification of MHs and CHs in infant foods, we will prioritize the most relevant MHs or CHs present in specific food products and rank the risks associated with these hazards based on the probability of occurrence and severity of each hazard. Standardized and systematic hazard identification (HI) and risk ranking (RR) procedures will be developed and incorporated into HI and RR computational decision support tools that will serve to help food safety agencies, food companies, and risk assessors to identify and rank MHs and CHs in the entire infant food chain in Europe and China.

1. Introduction

Food is the basic nutrient source of life. Globally, a billion tonnes of primary crops are being produced,[1] and in the last two decades, the production of different food commodities has increased about 40–100% (primary crops: 53%, meat: 44%, fruits: 43%, and vegetables: 56%)[2] and may continue to increase in the years coming.[3] This is attributed to the sharp increase in food demand due to the growing population worldwide and the increment in per capita income also facilitates a higher spending power in developed countries. Taking the Netherlands as an example, on average, one kilogram of food is consumed per person per day,[4] and depending on the countries and personal habits, this number may even be higher.

As large amounts of foods are being produced and consumed daily worldwide,[2] and consumer's increasing awareness of the health aspects and product qualities of foods (i.e. nutritional values, presence of chemicals, or biological agents),[5] food safety from farm to fork is of crucial importance. Stimulated by the implementation of food law regulation (Regulation (EC) No 178/2002), there has been a continuing concerted effort from food companies and governmental agencies to monitor the quality and safety of foodstuffs.

1.1. Food contamination and foodborne illnesses

Despite the joined effort, cases of food contamination leading to human illnesses are still being reported frequently.[6,7] These contaminants can either be biological agents like bacteria, viruses, or parasites, or be chemicals like environmental contaminants, processing-induced

Abbreviations: MH, Microbial Hazard; CH, Chemical Hazard; HI, Hazard Identification; RR, Risk Ranking; DSS, Decision Support System.

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compounds, agricultural products, veterinary drug residues, or physical objects like hairs, metals, plastics, glass, insects, or allergens that can trigger abnormal immune responses for a sensitive population.[8] Foods contaminated with biological or chemical agents can lead to more than 200 human diseases, either mild diseases like diarrhea or life-threatening diseases like cancer.[9,10]

Foodborne outbreaks caused by biological or chemical contaminants lead to foodborne illnesses and result in a significant disease burden.[11] Examples of large foodborne outbreaks include: (1) fenugreek sprouts contaminated with Enterohemorrhagic *Escherichia coli* which killed 55 people and made more than 3000 people fell ill in 2011[12]; (2) polony processed meat contaminated with *Listeria monocytogenes* caused 216 deaths and more than 1000 illness cases in 2018 and many other *L. monocytogenes* outbreaks[13]; and (3) a fraud of adulteration of infant milk powder with melamine resulting in at least 294,000 children fell ill, 6 deaths and the hospitalization of 52,000 infants in 2008. After many years, these infants and children still suffer from renal damage (caused by urinary stone formation) and other long-term chronic effects.[14,15]

1.2. Foodborne diseases and estimated burden in children

In 2015, the World Health Organization (WHO) estimated that yearly, every 1 in 10 people fall ill due to consuming contaminated foods, and 420,000 lose their life due to foodborne illnesses.[11] Children under the age of five have relatively a larger burden. Young children have a high risk of foodborne illness and also of more severe effects and account for 1 out of 3 deaths from foodborne diseases.[11] This ratio is high when taking into consideration that children only make up about 10% of the total world population.[16] Therefore, it is important to strengthen food safety and ensure that foods that are fed to people particular infants or children are safe. This can be done by implementing systematic food safety management systems, like good hygiene practice (GHP) and good manufacturing practice (GMP), which are built further to the well-known Hazard Analysis Critical Control Point (HACCP) system.[17,18]

1.3. Food safety management system to ensure safe food

HACCP is a seven-principle science-based safety plan outlined to ensure the control of significant hazards in food businesses.[19] In the context of food safety, a hazard is defined as a biological, chemical, or physical agent in food that has the potential to cause adverse health effects in humans. Risk is the probability and severity of the health effects that consumers face after being exposed to a hazard.[20]

The first principle in HACCP is (1) hazard analysis, which is to collect and evaluate hazards identified in either raw materials, ingredients, environment, process, or foods to decide whether the identified hazards are significant. This is followed by (2) determining the critical control points (CCPs) to have essential measures to control significant hazards. The next step is (3) to establish validated critical limits for control measures set at CCPs and (4) to establish a system to monitor the control of CCPs. Following, when a deviation from the critical limit is observed at a CCP, (5) corrective actions are implemented, and (6) the HACCP plan is validated to confirm a working HACCP system. Lastly, (7) all procedures and records to the HACCP principles and their application are documented.[19]

The HACCP system is widely accepted by governmental agencies, trade organizations, and food companies.[19] It is implemented by food companies to establish the food safety goal, but there are also other systems to monitor food safety from farm to fork, starting from raw material production, procurement and handling, manufacturing, distribution, to consumption at the end. For example at the farm level, good agricultural practices (GAP) are implemented.

In addition, scientific evaluations of adverse effects caused by known biological or chemical hazards in various food commodities are also done to characterize the risk associated with the hazards and to give recommendations to control food safety. This process is called risk assessment and consists of 4 steps;

1 **Hazard identification;** In this step, hazards that may be present in a specific food commodity in given situations, have the potential to survive food processing techniques, and may cause disease in humans are identified.[19,21] This information is either collected based on scientific literature, surveillance/epidemiological data, existing expert knowledge, food analysis databases, or predefined knowledge of known pathogen/food associations,[22] for example, *Salmonella* in eggs.
2 **Hazard characterization;** In this step, the effects caused by hazards present in foods are evaluated, either based on qualitative data or quantitative data.[19,21] The relationship between hazard exposure (route, level, prevalence) and consequences of exposures are derived from foodborne outbreak data associated with illness, physicochemical properties of hazards, hazard infectivity/virulence/ capability to produce toxins, toxicological studies, or human exposure studies[22]. Additionally, the immunological and physical statuses in humans of different age groups are considered, as the same hazard may have different effects for the young/-old/pregnant/immunocompromised populations.
3 **Exposure assessment;** is the stage in which the probability and quantity of hazard intake via foods are evaluated qualitatively or quantitatively, or exposures from other sources if relevant.[19,21] The number of microorganisms or the amount of chemicals/toxins consumed by a population in selected foods is quantified and the exposure to humans is assessed.
4 Lastly in **risk characterization**, based on the outcome of hazard characterization and exposure assessment, the probability of hazard occurrence and the severity of associated/potential adverse health effects caused by the hazard in a given population, including attendant uncertainties are estimated.[19] The risk of a specific hazard in foods is either expressed quantitatively with numerical outputs (e.g., annual illness incidence/100,000 population) or qualitatively with provided evidence and statements to show e.g. presence or absence of hazards.[22]

Notably, steps 2 and 3 can be done in the reverse order or parallel in risk assessment. When biological agents are examined, it is referred to as microbial risk assessment, and when chemicals are evaluated, it is referred to as chemical risk assessment.

2. Monitoring microbial and chemical hazards in foods

In Europe, different parties and local food safety authorities of each member state govern food safety. The European Food Safety Authority (EFSA) conducts scientific evaluations and risk assessments, the European Commission (EC) acts as the risk manager, and the Rapid Alert System for Food and Feed (RASFF) allows rapid sharing of information related to food safety between all member states whenever necessary.[23] RASFF provides notification concerning human health deriving from foods between the member states, EFSA, and the EC. EFSA analyzes the notification contents, conducts risk analysis and assessment, and communicates the scientific evaluation and technical information to the EC and other member states.[23] Each member state takes appropriate management control, reports food safety issues directly to EC, notifies RASFF when a foodborne hazard is encountered and is of international relevance, and provides foodborne outbreak data to the European Centre for Disease Prevention and Control (ECDC).

Annually, these authorities publish monitoring reports on detected foodborne hazards in different food categories, the total number of foodborne outbreaks caused by these hazards, their association with reported human diseases, and public health impacts (e.g. illness, hospitalization, and deaths).[6,24,25] These data are passed on to the International Food Safety Authorities Network (INFOSAN), managed jointly

by WHO and the Food and Agriculture Organization (FAO). INFOSAN integrates the collected information gathered worldwide, conducts their evaluations, and also publishes estimations on the global burden of foodborne diseases and reports on corresponding foodborne hazards. Among all foodborne hazards, microbial hazards (MHs) and chemical hazards (CHs) are the main focuses in food safety.[11,26]

2.1. Microbial hazards

MHs can easily contaminate raw material, food products during processing (via contact surfaces or food handlers), or the end products because they are ubiquitous in the air, water, soil, animal, and humans.[8,27] Pathogenic MHs include bacteria, viruses, toxin-producing molds, and parasites. The difference in their physiologies accounts for aberrations in terms of epidemiology, virulence, and host association.[28,29] Therefore, surveillance and control of these MHs are challenging. While viruses and parasites cannot increase in foods, they are infectious. For some viruses (e.g., norovirus), ingesting just 1–10 virus particles is enough to cause illness in humans,[30,31] especially for young children as their immune systems are not fully developed.[32] For certain pathogenic organisms, a high number of cells need to be ingested to have a relevant probability of disease. However, given the right conditions, the outgrowth of pathogenic bacteria in foods is feasible and certain species can then also produce harmful toxins in the food or upon ingestion that hamper human health.[9] In the current legislation, presence/absence testing is laid down for some MHs, while other MHs are accepted to a certain level.

2.2. Chemical hazards

CHs are small or high molecular weight compounds that can be naturally present in the environment or manmade for specific purposes.[33,34] Their possible impact on human health is based on their physicochemical characteristics, exposure pathways (dermal, oral, or inhalation), and toxicological properties. Depending on their properties, they can contaminate food products at different levels. There are different families of CHs such as substances migrating from food contact materials, persistent organic pollutants (POPs), and naturally occurring substances such as marine biotoxins, mycotoxins, or trace elements and metals. Some chemical hazards (e.g., perfluoroalkyl substances or polychlorinated dibenzodioxins and dibenzofurans (PCDD/F)) can contaminate the soil, biomagnify in the trophic chain and bioaccumulate in the organism after consumption of food.[35,36] In 2001, The Stockholm convention determined some persistent organic pollutants that are closely monitored in Europe.[37] Another category of CHs, heat-induced contaminants such as furan, is produced at significant concentrations during thermal food processing and is associated with DNA damage and cancer.[38]

3. Safe food for infants (SAFFI) in the EU and China

To achieve the goal of reducing foodborne incidents in infants and children in the EU and China, an EU research project funded by Horizon Europe 2020, SAFFI (Safe Food or Infants in the EU and China) has been initiated, and the project group consists of 20 partners from different sectors i.e. academia, food safety authorities, infant food companies, paediatrics, technological and data-science companies.

3.1. SAFFI objective and approaches

The main objective of SAFFI is to develop an integrative approach to identify, assess, detect and mitigate risks associated with MHs and CHs for infants and children (< age of 3) in the EU and China. The first sub-objective is the identification of hazards (similar to the first stage "hazard analysis" of HACCP and the first stage "hazard identification" of risk assessment). The specific ambition behind this project is to establish generic and standard procedures for hazard identification (HI) and risk ranking (RR) within the infant food chain, which will be achieved by the three-steps data-driven approach.

Firstly, data concerning MHs and CHs are collected based on literature, official reports published by governmental agencies, existing databases, clinical studies, and expert knowledge. These data are used to compile a list of relevant MHs and CHs that have the potential to be present in the food chain and cause adverse health effects to young children.

Secondly, the collected data are assembled and stored in structural databases that contain information like survival of MHs at different pH, temperatures, production of harmful toxins, or types of CHs generated during food processing procedures, etc. These specific pieces of information, namely the knowledge rules of the hazards, are prerequisites to understanding their relevant threat in a food product. Concomitantly, procedures for hazard identification are devised, which includes a second step to prioritize the hazards that are relevant for a specific food commodity. For MHs, this HI is based on the knowledge rules of selected organisms, i.e. the prevalence of an organism in a food commodity, survival during/after processing, and its growth opportunity in the specific food product. For CHs, HI is based on the knowledge rules of the hazards, i.e. the relevance in relation to the ingredients, processing conditions, and packaging materials. These devised generic procedures will serve to create a computational hazard identification decision support system (HI-DSS) (Fig. 1).

Lastly, the risk of the prioritized hazards associated with a food commodity will be evaluated, and similarly, procedures for risk ranking will be devised and a computational Risk Ranking Decision Support System (RR-DSS) will be constructed (Fig. 1). The HI-DSS and RR-DSS will be made flexible to easily incorporate newly acquired data in the future. The two DSS systems will be tested and validated with four case studies that represent four types of infant food products (powder infant formula, vegetable puree, infant cereals, and fresh fruit puree). These validated tools with integrated databases, procedures, and methods can be used by food safety agencies, food companies, and risk assessors to facilitate the assessment of either microbial or chemical risks in the infant food chain (see Fig. 1). Moreover, the data-driven approaches developed for infant food chains are also a conceptual, generic framework that can be modified and extrapolated for HI and RR in other food products in the future.

4. Hazard identification for microbial and chemical risks within infant food chains

4.1. Four important sources used for microbial hazards (MHs) identification

MHs in food products, with a special focus on infants and toddlers up to the age of 3 can be derived from public health databases, outbreak data, scientific literature, and expert knowledge. To obtain a list of relevant foodborne MHs in SAFFI, four different sources: (1) Outbreak data; (2) Recalled Foods; (3) public health impact; and (4) Expert knowledge of each MH were considered (see Fig. 2).

4.1.1. Foodborne outbreaks

Firstly, the occurrence and prevalence of MHs causing foodborne outbreaks in Europe were examined based on the data published in the most recent EFSA Zoonoses reports.[6] A total of 24 MHs associated with major foodborne outbreaks in Europe was found.

4.1.2. Recalled foods due to pathogen contamination

Secondly, ~3500 serious alerts on foodborne pathogens, microorganisms, and parasites reported in the period from 1998-2021 in foods were analyzed and causative agents for each alert were manually defined.[25,39] Non-pathogenic organisms were excluded, giving rise to 35 MHs that caused either destruction, retraction, or withdrawal of foods

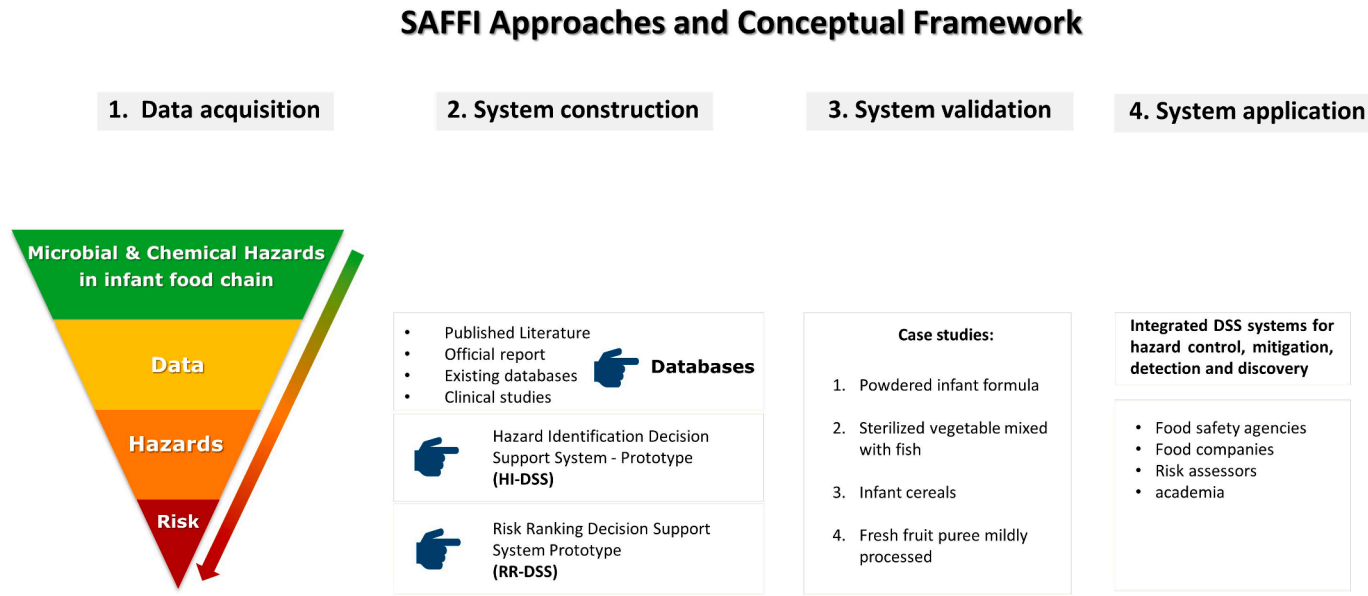

Fig. 1. General approaches of SAFFI and conceptual framework for hazard identification and risk ranking in the entire infant food chain.

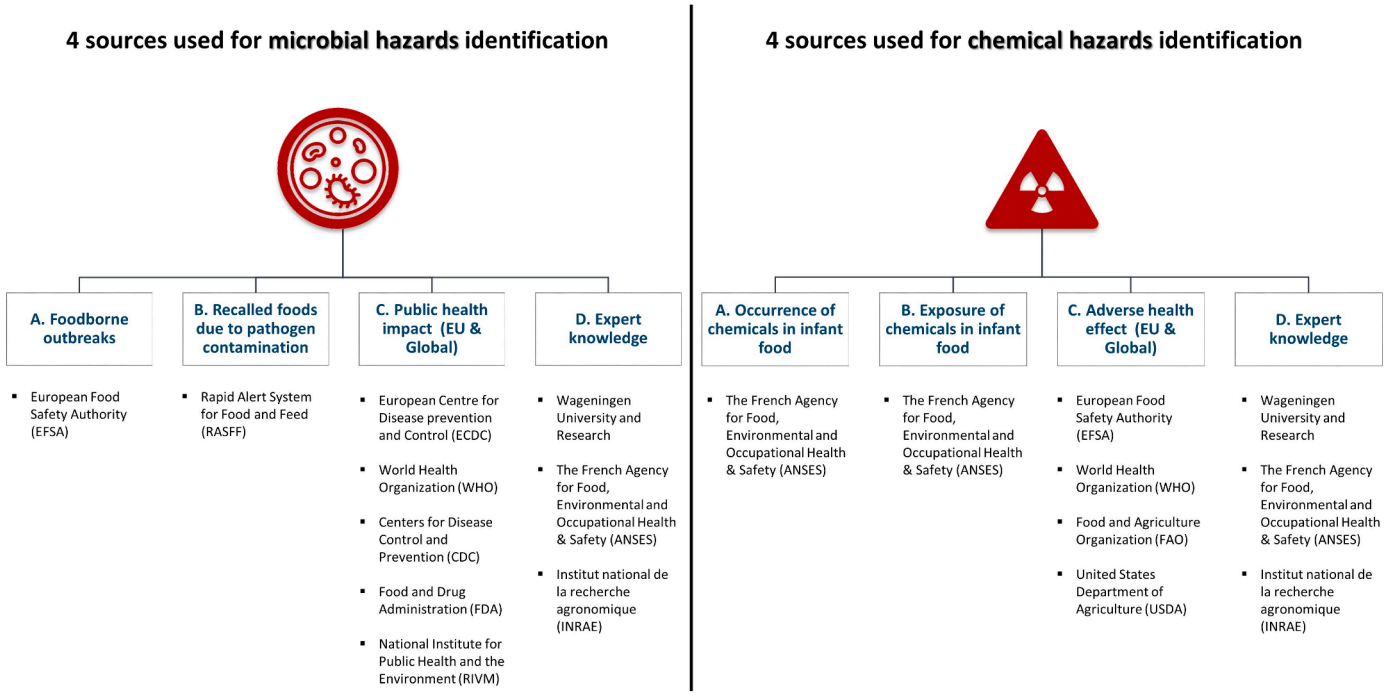

Fig. 2. Four important sources used for microbial and chemical hazards identification.

from the market.

4.1.3. *Public health impact in EU and globally*

Thirdly, foodborne MHs that possessed large health impacts for humans in Europe were listed based on epidemiological reports. ECDC reported 22 foodborne MHs associated with human diseases,[24] the Dutch Institute for Public Health and the Environment (RIVM) showed 14 major MHs with large disease burden,[40] and WHO reported 15 MHs leading to major foodborne illnesses in Europe.[41] Moreover, the global public health impacts of MHs were also determined based on the MH health impact reports in the United States and worldwide. 31 pathogens were causing major foodborne diseases in the United States,[29] among these, 14 were estimated to have the largest overall disease burden.[42] Globally, 28 MHs are the main culprits of foodborne illnesses/death, and WHO also published 20 MHs that caused illnesses in children $<$ 5 years.[32]

4.1.4. *Expert knowledge*

Other than that, some MHs usually do not cause major foodborne outbreaks, and neither are detected frequently in foods and do not have a large public health impact, but can still be a serious threat for infants and children. One such example would be the *Cronobacter* species. Therefore, expert knowledge was also taken as one of the important sources to identify relevant MHs for the young susceptible group.

All MHs identified in the above-mentioned reports were combined, and duplicates were removed. The occurrence of each MH in the reports

was counted. Based on this, a list of 64 MHs that either cause major foodborne outbreaks, have relevant public health impacts, are frequently detected in foodstuffs, and are threatening to infants/children are identified. MHs that were reported in > 1 report were shortlisted, resulting in a total of 32 prioritized MHs, which include 18 bacteria, 6 parasites, 6 viruses, and 2 protozoa (Fig. 3). The identified 32 MHs are the most relevant hazards, after which a further prioritization has to be made by evaluating the food/process association for each of these MHs.

4.2. Four important aspects for chemical hazards (CHs) identification

Dietary exposure assessment reports are a major source for chemical hazard identification. When assessments of a risk associated with chemical hazards in food for a population are required, it is important to have representative contamination data. One of the most efficient methods to obtain this data is conducting a Total Diet Study (TDS). Indeed, contamination data and exposure calculations present in TDS reports give insight into the occurrence and contamination level of CHs in food products as consumed and representative of the whole diet. These reports can be found on food safety agencies' websites or scientific databases such as PubMed or Scopus.

Occurrence, exposure, adverse effects, and expert knowledge were the four important aspects considered to identify chemical hazards (Fig. 2).

4.2.1. Occurrence of chemicals in infant foods

When considering CHs, different types of data are needed. First, occurrence data provide information about the presence and the concentration of CHs in specific food matrices. From birth to the age of three, the diets of infants and toddlers become more and more diverse, going from a milk-based diet to a broader diet by introducing common foods. Infant food can be contaminated at every stage of food production from the raw materials to the consumed products. TDS are internationally recognized as efficient tools to have an overview of the contamination of food prepared as consumed and on which analyses are conducted to measure concentrations of CHs.[43] In 2011, the French Agency for Food, Environmental, and Occupational Health & Safety conducted an infant Total Diet Study (iTDS) with a list of 700 substances or groups of substances.[44] The global detection rate for each hazard listed in the iTDS was used to select the most relevant hazards present in infant foods. To complete the list of chemical hazards, searches in databases such as PubMed and Scopus were conducted to find occurrence data about the presence of some contaminants in infant food. Data from previous or recent assessments from EFSA were retrieved as well.

4.2.2. Exposure to chemicals in infant foods

In the iTDS, dietary exposure was assessed for 500 out of the 700 chemicals or groups of chemical hazards. Dietary exposure values for each age class from 1–4 to 13–36 months are obtained by combining food contamination data, food consumption data, and body weight. Based on these parameters, foods that contributed the most to the exposure were identified for each selected hazard when available. For most of the substances, the risk for infants and toddlers was negligible however for some chemicals such as acrylamide, furan, inorganic arsenic, and lead, the risk could not be excluded.[45,46]

4.2.3. Adverse health effects in the EU and globally

CHs in food can be associated with several adverse health effects. Knowledge of these effects is available from long-term toxicity studies conducted in animals or from epidemiological data. For this project, health-based guidance values (Tolerable Daily or Weekly intake) and toxicological references points (No Observed Adverse Effect Level or Benchmark dose limit) were identified in reports from different safety agencies or expert committees such as EFSA, Joint FAO/WHO Expert Committee on Food Additives (JECFA), Joint FAO/WHO Meeting on Pesticide Residues (JMPR), or WHO.

4.2.4. Expert knowledge

Some CHs were selected for the project even when they were not detected in infant food products in the iTDS, because of the severity of their adverse effects on humans, or when there were concerns in the available literature about the safety of their use. Some chemical hazards for which no health-based guidance value nor toxicological reference point have been established were also included. For example, emerging mycotoxins can be relevant because of the growing impact on climate change and identified effects *in vitro*.[47,48]

Based on the iTDS results and available literature, a list of 101 chemical hazards or groups of chemical hazards from 9 families has been established. One phytoestrogen, 11 substances migrating from food contact materials, 18 trace elements and metals, 28 persistent organic pollutants, 17 pesticide residues, 15 mycotoxins, 6 heat-induced compounds, 2 ionic compounds, and 3 food additives have been selected (Fig. 3).

The 32 MHs and 9 families of CHs were identified to be the most relevant hazards in infant food products, and in the following step,

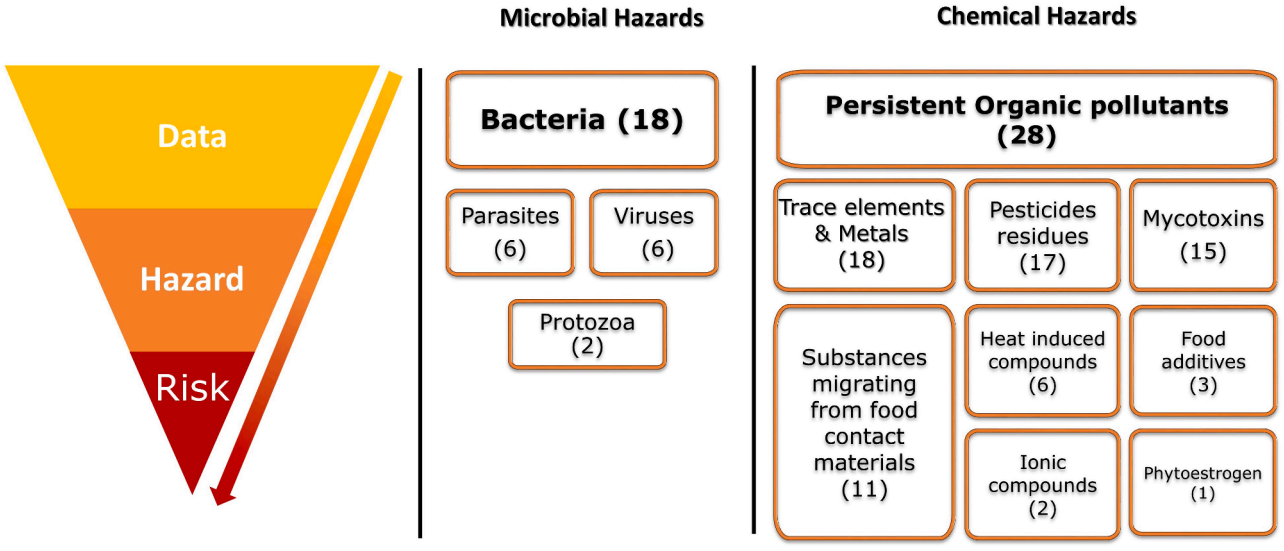

Fig. 3. Relevant microbial and chemical hazards identified in the infant food chain.

hazards will be prioritized for a specific food commodity as described in Section 3.1.

5. Criteria used in risk ranking

Following the initial identification of the most relevant MHs and CHs in infant food products, and subsequently, in specific food commodities, the risk of these hazards will be ranked systematically. For that, the probability of hazard occurrence/exposure and the severity of hazards associated with potential adverse health effects in infants and children will be estimated using selected criteria.

For the risk ranking of MHs, the severity of each MH will be estimated based on the disability adjusted life years (DALY) per case, and the probability of occurrence of each MH will be estimated based on hazard-food characteristics and hazard-food association strength. Hazard-food characteristics are determined using different elements, namely, growth opportunity of a hazard in a given food, processing effect on hazards, recontamination possibility of hazards, post-processing control of hazards, and the effect of meal preparation. Hazard-food association strengths are determined using the evidence of hazard-food alerts, hazard-food outbreaks, hazard-food prevalence, and food consumption data. For the risk ranking of CHs, the severity of each CH will be estimated based on the different toxic effects that a CH possesses, as described in ANSES.[49] The probability/likelihood of occurrence of each CH will be estimated based on the percentage of contribution to the total exposure as well as the corresponding percentage of the health-based guidance value.

6. Conclusion and future perspectives

In conclusion, 32 MHs consisting of bacteria, viruses, parasites, and protozoa and 9 families of CHs consisting of persistent organic pollutants, trace elements and metals, pesticides residues, mycotoxins, substances migrating from food contact materials, heat-induced compounds, phytoestrogen, ionic compounds, and food additives are identified to be the most relevant hazards in the food chain that can be dangerous for young children. In the next step, the identified MHs and CHs are prioritized for specific infant foods. These step-wise procedures will be implemented in the HI-DSS computational tool to identify MHs or CHs present in a specific infant food product. In a following up step, the risks of these prioritized MHs and CHs in infant foods will be evaluated using a structured risk-ranking approach, which takes into account the severity and probability of occurrence of each MH and CH mentioned above. Similarly, procedures for risk ranking will be devised and implemented in an RR-DSS computational tool.

The HI-DSS and RR-DSS tools will be validated using four case studies in following-up works and will be integrated and harmonized with the data obtained from the SAFFI mirror project in China. Together, these tools will be useful for food safety agencies, food companies, and risk assessors to identify and rank MHs and CHs in the entire infant food chain in Europe and China, and improve the control of these MHs and CHs.

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Statement

The corresponding author states on behalf of the co-authors that all Authors have no conflict or competing of interests to declare.

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References

[1] FAO. *World Food and Agriculture - Statistical Yearbook*. Rome, Italy: FAO; 2020. https://doi.org/10.4060/cb1329en, 2020.
[2] FAO. *FAO Statistical Yearbook 2021 - World Food and Agriculture*. World ReliefWeb; 2021. https://reliefweb.int/report/world/fao-statistical-yearbook-2021-world-food-and-agriculture (accessed December 8, 2021).
[3] van Dijk M, Morley T, Rau ML, Saghai Y. A meta-analysis of projected global food demand and population at risk of hunger for the period 2010–2050. *Nat Food*. 2021;2:494–501. https://doi.org/10.1038/s43016-021-00322-9.
[4] Geurts M, van Bakel A, van Rossum C, de Boer E, Ocke M. *Food consumption in the Netherlands and its determinants : background report to what is on our plate?. Safe, Healthy and Sustainable diets in the Netherlands*. Rijksinstituut voor Volksgezondheid en Milieu RIVM; 2017. https://www.rivm.nl/bibliotheek/rapporten/2016-0195.pdf (accessed 21 February 2022).
[5] Pinto VRA, Campos RF de A, Rocha F, et al. Perceived healthiness of foods: a systematic review of qualitative studies. *Future Foods*. 2021;4, 100056. https://doi.org/10.1016/j.fufo.2021.100056.
[6] European Food Safety Authority (EFSA) E. The European Union one health 2019 Zoonoses report. https://www.ecdc.europa.eu/en/publications-data/european-union-one-health-2019-zoonoses-reporthttps://www.efsa.europa.eu/en/efsajournal/pub/6406, 2019 (accessed April 14, 2021).
[7] European Food Safety Authority (EFSA) E. The European Union One Health 2018 Zoonoses Report.https://www.efsa.europa.eu/en/efsajournal/pub/5926, 2019 (accessed April 14, 2021).
[8] Schirone M, Visciano P, Tofalo R, Suzzi G. Editorial: biological hazards in food. *Front Microbiol*. 2017;7:2154. https://doi.org/10.3389/fmicb.2016.02154.
[9] Bintsis T. Foodborne pathogens. *AIMS Microbiol*. 2017;3:529–563. https://doi.org/10.3934/microbiol.2017.3.529.
[10] Mead PS, Slutsker L, Dietz V, et al. Food-related illness and death in the United States. *Emerg Infect Dis*. 1999;5:607–625. https://doi.org/10.3201/eid0505.990502.
[11] WHO. WHO estimates of the global burden of foodborne diseases: foodborne disease burden epidemiology reference group 2007-2015. https://apps.who.int/iris/bitstream/handle/10665/199350/9789241565165_eng.pdf, 2015 (accessed 21 February 2022).
[12] Köckerling E, Karrasch L, Schweitzer A, Razum O, Krause G. Public health research resulting from one of the world's largest outbreaks caused by entero-hemorrhagic *Escherichia coli* in Germany 2011: a review. *Front Public Health*. 2017;5:332. https://doi.org/10.3389/fpubh.2017.00332.
[13] Kaptchouang Tchatchouang CD, Fri J, De Santi M, et al. Listeriosis outbreak in South Africa: a comparative analysis with previously reported cases worldwide. *Microorganisms*. 2020;8:135. https://doi.org/10.3390/microorganisms8010135.
[14] Gossner CME, Schlundt J, Ben Embarek P, et al. The melamine incident: implications for international food and feed safety. *Environ Health Perspect*. 2009;117:1803–1808. https://doi.org/10.1289/ehp.0900949.
[15] Wen JG, Liu XJ, Wang ZM, Li TF, Wahlqvist ML. Melamine-contaminated milk formula and its impact on children. *Asia Pac J Clin Nutr*. 2016;25:697–705. https://doi.org/10.6133/apjcn.072016.01.
[16] United Nations, Department of economic and social affairs, population division. World population prospects 2019, custom data acquired via website. Dep Econ Soc Aff Popul Dyn 2019. https://population.un.org/wpp/DataQuery/, 2019 (accessed December 15, 2021).
[17] Varzakas T, Caballero B, Finglas PM, Toldrá F. *HACCP and ISO22000: risk assessment in conjunction with other food safety tools such as FMEA, Ishikawa diagrams and Pareto*. editors. *Encyclopedia of Food and Health*. Oxford: Academic Press; 2016:295–302. https://doi.org/10.1016/B978-0-12-384947-2.00320-2.
[18] Wallace CA, Mortimore SE, Lelieveld H, Holah J, Gabrić D. *Chapter 3 - HACCP*. editors. *Handbook of hygiene control in the food industry*. 2nd ed. San Diego: Woodhead Publishing; 2016:25–42. https://doi.org/10.1016/B978-0-08-100155-4.00003-0.
[19] Codex Alimentarius Commission. General principles of food hygiene CXC 1-1969. Adopted in 1969, amended in 1999. Revised in 1997, 2003, 2020. Codex Aliment CXC 1-1969 2020. https://www.fao.org/fao-who-codexalimentarius/sh-proxy/fr/?lnk=1&url=https%253A%252F%252Fworkspace.fao.org%252Fsites%252Fcodex%252FStandards%252FCXC%2B1-1969%252FCXC_001e.pdf, 2020 (accessed 18 January 2022).
[20] Food and Agriculture Organization of the United Nations. Definitions for the purposes of the codex alimentarius 2001. https://www.fao.org/3/y2200e/y2200e07.htm#fn4, 2001, (accessed December 15, 2021).
[21] FAO, WHO. Codex alimentarius commission – procedural manual twenty-seventh edition 2019. https://www.fao.org/publications/card/en/c/CA2329EN/, 2019 (accessed 21 February 2022).

[22] Mahoney D, McSweeney PLH, McNamara JP. *Safety and risk mitigation risk assessment in dairy processing - importance of food safety in the dairy processing sector*. editors. *Encyclopedia of Dairy Sciences*. 3rd ed. Oxford: Academic Press; 2022: 776–784. https://doi.org/10.1016/B978-0-12-818766-1.00139-2.
[23] McEvoy JDG. Emerging food safety issues: an EU perspective. *Drug Test Anal*. 2016; 8:511–520. https://doi.org/10.1002/dta.2015.
[24] European Centre for Disease Prevention and Control (ECDC) annual epidemiological reports (AERs) 2014 - 2020. (2020) online report. Available at https://www.ecdc.europa.eu/en/all-topics-z/surveillance-and-disease-data/annual-epidemiological-reports-aers (accessed April 14, 2021).
[25] Rapid alert system for food and feed (RASFF). RASFF annual report 2019. http://op.europa.eu/en/publication-detail/-/publication/2c5c7729-0c31-11eb-bc07-01aa75ed71a1/language-en/format-PDF,2020 (accessed April 14, 2021).
[26] Chammem N, Issaoui M, De Almeida AID, Delgado AM. Food crises and food safety incidents in European Union, United States, and Maghreb Area: current risk communication strategies and new approaches. *J AOAC Int*. 2018;101:923–938. https://doi.org/10.5740/jaoacint.17-0446.
[27] M.H. Zwietering and E.D. van Asselt, Wageningen University, the Netherlands. In Zwietering MH, Straver JM, van Asselt ED, Lelieveld H, Holah J, Gabrić D. *Chapter 4 - the range of microbial risks in food processing**this chapter is an updated version of: the range of microbial risks in food processing*. editors. *Handbook of Hygiene Control in the Food Industry*. 2nd ed. San Diego: Woodhead Publishing; 2016:43–54. https://doi.org/10.1016/B978-0-08-100155-4.00004-2.
[28] Havelaar AH, Haagsma JA, Mangen MJJ, et al. Disease burden of foodborne pathogens in the Netherlands, 2009. *Int J Food Microbiol*. 2012;156:231–238. https://doi.org/10.1016/j.ijfoodmicro.2012.03.029.
[29] Scallan E, Hoekstra RM, Angulo FJ, et al. Foodborne illness acquired in the United States—major pathogens. *Emerg Infect Dis*. 2011;17:7–15. https://doi.org/10.3201/eid1701.P11101.
[30] Food and Drug Administration. *Bad Bug Book, Foodborne Pathogenic Microorganisms and Natural Toxins*. 2nd ed. FDA; 2019. https://www.fda.gov/food/foodborne-pathogens/bad-bug-book-second-edition.
[31] Robilotti E, Deresinski S, Norovirus PBA. *Clin Microbiol Rev*. 2015;28:134–164. https://doi.org/10.1128/CMR.00075-14.
[32] WHO. WHO estimates of the global burden of foodborne diseases. *PLoS Collect*; 2015. https://collections.plos.org/collection/ferg2015/, 2015 (accessed April 13, 2021).
[33] Groh KJ, Geueke B, Martin O, Maffini M, Muncke J. Overview of intentionally used food contact chemicals and their hazards. *Environ Int*. 2021;150, 106225. https://doi.org/10.1016/j.envint.2020.106225.
[34] Md Rahaman S, Md Ramaman M, Mise N, et al. Environmental arsenic exposure and its contribution to human diseases, toxicity mechanism and management. *Environ Pollut*. 2021;289, 117940. https://doi.org/10.1016/j.envpol.2021.117940.
[35] González N, Domingo JL. Polychlorinated dibenzo-p-dioxins and dibenzofurans (PCDD/Fs) in food and human dietary intake: an update of the scientific literature. *Food Chem Toxicol*. 2021;157, 112585. https://doi.org/10.1016/j.fct.2021.112585.
[36] Pasecnaja E, Bartkevics V, Zacs D. Occurrence of selected per- and polyfluorinated alkyl substances (PFASs) in food available on the European market – a review on levels and human exposure assessment. *Chemosphere*. 2022;287, 132378. https://doi.org/10.1016/j.chemosphere.2021.132378.
[37] United Nations Treaty Collection (UNEP) 2001. https://treaties.un.org/pages/ViewDetails.aspx?src=TREATY&mtdsg_no=XXVII-15&chapter=27, 2001 (accessed January 28, 2022).
[38] Javed F, Shahbaz HM, Nawaz A, et al. Formation of furan in baby food products: identification and technical challenges. *Compr Rev Food Sci Food Saf*. 2021;20: 2699–2715. https://doi.org/10.1111/1541-4337.12732.
[39] RASFF. RASFF portal 2021. https://webgate.ec.europa.eu/rasff-window/screen/search 2021, (accessed December 15, 2021).
[40] Lagerweij G, Pijnacker R, Friesema I, Mughini Gras L, Franz E. *Disease burden of food-related pathogens in the Netherlands, 2019*. Rijksinstituut voor Volksgezondheid en Milieu; 2020. https://doi.org/10.21945/RIVM-2020-0117.
[41] WHO Europe. The burden of foodborne diseases in the WHO European Region (2017). https://www.euro.who.int/en/health-topics/disease-prevention/food-safety/publications/2017/the-burden-of-foodborne-diseases-in-the-who-european-region, 2017 (accessed December 15, 2021).
[42] Batz MB, Hoffmann S, Morris JG. Ranking the disease burden of 14 pathogens in food sources in the United States using attribution data from outbreak investigations and expert elicitation. *J Food Prot*. 2012;75:1278–1291. https://doi.org/10.4315/0362-028X.JFP-11-418.
[43] EFSA, FAO. Towards a harmonised total diet study approach: a guidance document. *EFSA J*. 2011;9:2450. https://doi.org/10.2903/j.efsa.2011.2450.
[44] Hulin M, Bemrah N, Nougadère A, Volatier JL, Sirot V, Leblanc JC. Assessment of infant exposure to food chemicals: the French total diet study design. *Food Addit Contam Part A*. 2014;31:1226–1239. https://doi.org/10.1080/19440049.2014.921937.
[45] Sirot V, Traore T, Guérin T, et al. French infant total diet study: exposure to selected trace elements and associated health risks. *Food Chem Toxicol*. 2018;120: 625–633. https://doi.org/10.1016/j.fct.2018.07.062.
[46] Sirot V, Rivière G, Leconte S, et al. French infant total diet study: dietary exposure to heat-induced compounds (acrylamide, furan and polycyclic aromatic hydrocarbons) and associated health risks. *Food Chem Toxicol*. 2019;130:308–316. https://doi.org/10.1016/j.fct.2019.05.009.
[47] Aichinger G, Krüger F, Puntscher H, Preindl K, Warth B, Marko D. Naturally occurring mixtures of Alternaria toxins: anti-estrogenic and genotoxic effects *in vitro*. *Arch Toxicol*. 2019;93:3021–3031. https://doi.org/10.1007/s00204-019-02545-z.
[48] Magan N, Medina A, Aldred D. Possible climate-change effects on mycotoxin contamination of food crops pre-and postharvest. *Plant Pathol*. 2011;60:150–163. https://doi.org/10.1111/j.1365-3059.2010.02412.x.
[49] ANSES opinion collective expert. Méthodologie de hiérarchisation des dangers biologiques et chimiques dans les aliments 2020. https://www.anses.fr/fr/system/files/BIORISK2016SA0153Ra.pdf, 2020, (accessed 18 December 2021).

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Implementation of omics tools for infant food microbial safety☆

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ABSTRACT

Microbial safety of infant food is a priority for food producers, regulatory authorities and consumers. Infant food is destined to a particularly sensitive section of the population that is more susceptible to foodborne diseases if compared to the general population. Preventive approaches are applied during production and commercialization that rely on the principles of Hazard Analysis Critical Control Points and on Good Hygiene Practices to guarantee food safety. Nevertheless, microbial safety hazards in infant foods still pose a public health risk. With the purpose of better understanding why and how pathogenic microorganisms contaminate infant food, an approach that integrates omics methods is here proposed and reviewed. Omics approaches are employed to study the microbial ecology of foods, to investigate the interactions of microorganisms within an ecosystem and to delineate the behavior of microorganisms during food processing.

1. Introduction

Food industry and regulatory authorities strive to provide safe foods to the consumer. When infant food is concerned, there is an inherent increased concern and care in all the steps involved in the production, given the nature and sensitivity of the final consumer. With the term infant food, a diverse group of food commodities is intended, and such diversity has increased in recent years to respond to the desire of consumers in terms of nutritional characteristics, convenience of use, environmental sustainability, ethical aspects of the products. However, the core of infant food is composed of *Infant formulae* and *follow on formulae* and is intended for children below the age of 12 months. The distinction between the two is that infant formulae serve as sole source of nutrition or integrate breast feeding in infants, while follow on formulae are designed and intended to be used during the weaning period, in combination with other foods. A common characteristic of these two types of foods is that they are dehydrated. This characteristic implicates that the products cannot be sterilized and therefore low levels of microorganisms are potentially present. When pathogenic microorganisms are present, a foodborne disease may develop following ingestion of the contaminated product. In fact, infant formulae and to a lesser extend follow-on formulae have been implicated in outbreaks of foodborne disease in infants. Therefore, the microbiological safety of infant food and specifically powdered formulae is of outmost importance to reduce the risk to public health. This review aims at discussing the current status regarding microbial safety of infant food, with particular emphasis, on powdered infant food, and at presenting the approach employed within the SAFFI EU-China project that has as a purpose to further enhance the already high level of safety of infant foods providing knowledge and tools to both industry and regulatory authorities.

2. Characteristics of powdered formulae

The microbiological stability at room temperature, of infant food powders (most commonly milk-based and cereal-based), relies on the low water activity (a_w).[1,2] In fact, in such products a_w is usually in the range of 0.3-0.6 and in any case, it is below 0.8-0.82 that is considered the lowest limit permissive for growth of foodborne pathogens. However, the products are not sterile and may contain low concentrations of microorganisms.[3] The characteristics of the powdered formulae do not allow a processing step that could eliminate/reduce the microbial load of the final product or remove potentially pathogenic microorganisms. Indeed, powdered infant formulae have been involved in sporadic cases

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or outbreaks of foodborne disease, following ingestion of a contaminated product. Epidemiological investigations have highlighted the importance of the procedures of reconstitution of the powdered formula (by water addition and concomitant increase of the a_w) and subsequently, the conditions (temperature, time) of storage of the ready to use, reconstituted product. Water addition leads to a_w conditions favorable for growth of microorganisms. If the product is not immediately consumed or is not stored at refrigeration temperature, then cell proliferation occurs leading to increase in the microbial load. Ingestion of such product represents a high risk for disease development in the consumer.

The infant food formula production process involves some common steps that are: mixing of ingredients (that may have been subjected previously to a microbiocidal treatment), homogenization, drying, packaging. Two types of procedure may be employed for the production: the wet and the dry method. In the wet procedure, the mixing and homogenization take place first, followed by drying and packaging. In the dry procedure, the ingredients are first dried and then mixing and packaging take place. Microorganisms that can be detected in the final product may originate either in the ingredients employed or in the plant production environment. Contamination from the environment needs to be reduced to the minimum since there will be no microbiocidal treatment prior to the packaging and consumption of the product. Thus, Good Hygiene Practices (GHP) during manufacturing become indispensable to guarantee the microbiological safety of the final product. Furthermore, GHP during preparation and use of powdered formulae are equally important, particularly in health care facilities where large quantities of infant food may need to be prepared and possibly stored to satisfy the various needs of neonates (Fig. 1).[4]

3. Pathogens of concern in powdered infant formulae

The large scale of production of infant formula and follow-on formulae, both distributed worldwide, and the relatively low number of infections in infants indicates that the products are normally safe.[5] Nonetheless, issues relating to enteric and foodborne diseases are of particular relevance to paediatricians because the age-specific incidence rates for many of the most commonly reported enteric and foodborne pathogens are highest among infants and young children.[6] Sporadic cases or foodborne outbreaks due to consumption of contaminated infant formula are occasionally reported worldwide.[3] Clear evidence of causality links two pathogens, namely *Cronobacter sakazakii* (formerly *Enterobacter sakazakii*) and *Salmonella enterica* with illness in infants. Infant formula, contaminated with these two microorganisms has been proven epidemiologically and microbiologically to be responsible for infection in infants.

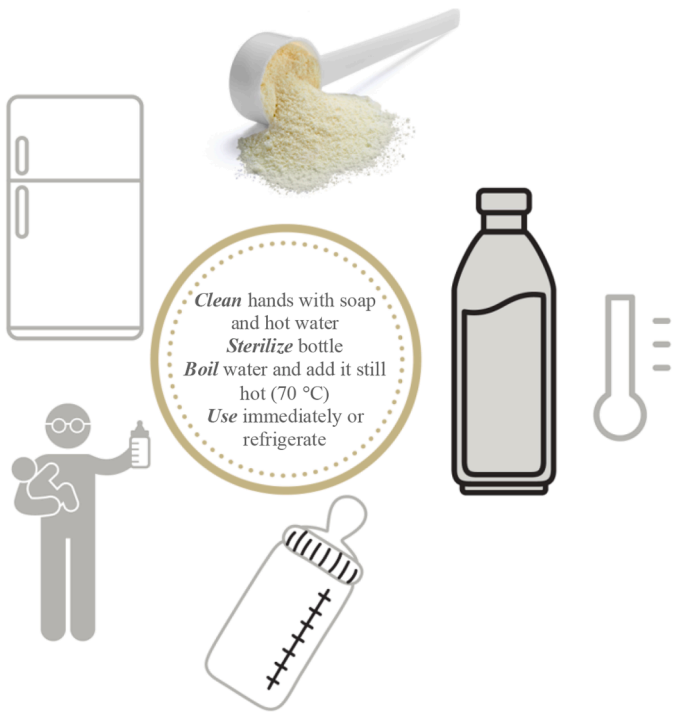

Fig. 1. Basic good hygiene practices for the preparation of infant food from powdered formula (for more information see[4]).

Infections by *C. sakazakii* mainly concern neonates, pre-term, underweight or immunocompromised infants and symptoms range from severe diarrhea to systemic infections, necrotizing enterocolitis, sepsis, meningitis. The mortality rate may be as high as 50% or may lead to serious, long term neurological complications (sequelae) (cdc.gov./cronobacter/technical.html). The habitat of *C. sakazakii* has not yet been identified. It has been consistently reported that contamination takes place from the environment, within the food production plant. For this reason, particular emphasis is placed in the prevention of such contamination through good manufacturing practices and good hygiene practices.

S. enterica is a well- recognized foodborne pathogen, affecting consumers of all ages and traditionally associated with food products of animal origin. In recent years, it is increasingly being connected to foodborne outbreaks due to consumption of foods of plant origin. Several outbreaks of salmonellosis have been traced to dried milk products and research has shown that failures in the production or presence of *Salmonella* in zones that are difficult to maintain clean were responsible for the contamination.[5] In infants, salmonellosis is manifested as gastroenteritis, but when it is invasive (extra-intestinal) it may lead to serious complications including bacteremia, arthritis, osteomyelitis, fatal meningitis.[7,8]

Other pathogenic microorganisms that may contaminate powder infant formula and are known to cause disease to humans mainly belong to the family *Enterobacteriaceae*. However, in contrast to the two pathogens described above, the epidemiological and microbiological evidences do not support a clear connection between consumption of infant food and infection caused by members of the genera *Klebsiella, Citrobacter, Hafnia.*[3] Similarly, for sporeformers *Bacillus cereus, Clostridium perfringens* and *Clostridium botulinum* no causal relation has been identified. *B. cereus* is a known enteropathogen that causes intoxication, with emesis as the most common symptom or infection, with diarrhea as most common symptom. Intoxication and/or infection disease due to *B. cereus* usually develop sporadically and do not cause outbreaks. Low level contamination of infant formulae is to be expected, mainly due to the heat resistant endospores that persist in the final product.[9,10] *Staphylococcus aureus* and *Listeria monocytogenes* are two additional foodborne pathogens of concern that may cause intoxication and infection respectively in infants but are not considered common contaminants of powdered infant formulae. Nevertheless, *L. monocytogenes* is an ubiquitous microorganism that may potentially contaminate a diverse array of raw materials. In addition, both *L. monocytogenes* and *S. aureus* may be involved in secondary contamination of food products.[11]

4. Food safety management systems and microbiological criteria

In order to satisfy the high food safety standards requested by modern consumers and importantly to protect consumer health from hazards that may be transmitted with food, the food industry employs science-based, preventive approaches to produce and commercialize foodstuffs. The food industry today cannot rely on end-product testing to guarantee food safety. End-product testing is impracticable, considering the amounts of foods produced and the extend of globalization of the food market. More importantly, it is inefficient in identifying food safety breaches that could occur during food production. Therefore, the food industry and competent authorities/regulatory bodies have adopted a

risk-based approach to food safety that essentially follows the principles of Hazard Analysis Critical Control Points (HACCP). Hazards (chemical, biological and physical) that may potentially occur during food production (at any stage of the food chain) are identified and appropriate control measures are put into place to limit or prevent occurrence of the hazard. In parallel, procedures are foreseen that are intended to verify that the HACCP system is working effectively and that the potential hazards are controlled, resulting in a safe product.[12] Intrinsically intertwined with the HACCP system are the Good Hygiene Practices (GHP). These are all the fundamental measures and conditions applied at any step within the food chain to provide safe and suitable food.[13] Microbiological analysis of foodstuffs to verify the efficacy of all the procedures aiming at producing safe foods is advisable. In this context, microbiological criteria that are either used to distinguish safe/unsafe foods or are used to highlight the correct/problematic functioning of the production process have been defined. For foods prepared and commercialized in Europe, EU legislation 1441/2007 defines such criteria (Table 1).

5. Bias of traditional microbiological analysis – bias of cultivation step

Traditional microbiological analyses that rely on the use of culture media have been fundamental in detecting and studying microorganisms in foods. Nevertheless, they present inherent limitations (Fig. 2). Such analytical approach is culture-dependent and by default, may only reveal the presence of microorganisms that at the moment of analysis have the ability to proliferate in synthetic microbiological media. However, under certain conditions, usually conditions of stress, microbial cells may be alive but not able to proliferate. This condition is termed Viable Not Culturable State (VBNC) and it is possible that during food production, microorganisms encounter stressful conditions that prompt the entrance in the VBNC state.[16,17] Also, the composition of the medium, and overall the growth conditions, need to be permissive to growth for a given microorganism. If the conditions are not permissive to growth (for example when an essential nutrient is missing) for a specific microbial group, then the result of the analysis will be a false negative (the microorganism is present in the sample, but is not detected by the method employed). Further, it should be mentioned that when various microbial groups are present in the sample at concentrations that diverge, only the most abundant populations will be detected unless appropriate selective conditions are imposed during the culturing procedure. Evidently, the culture-dependent detection of microorganisms is biased and may result in a distorted view of the microbial ecology of a given sample being analyzed.[18,19]

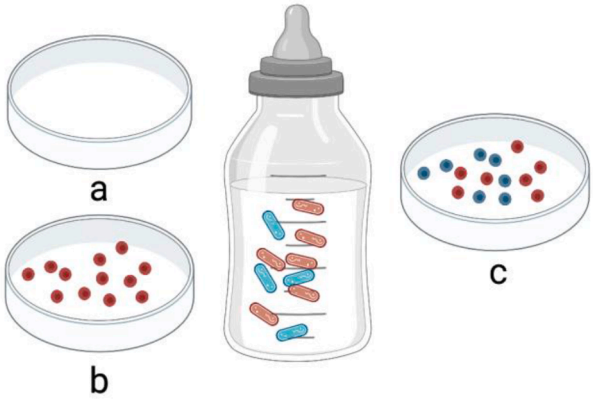

Fig. 2. Possible outcomes of a microbiological analysis, based on cultivation, of a contaminated sample. a. No microorganism detected; (i) the target microorganism was present in the sample in a VBNC state, (ii) prior knowledge regarding the contamination was not informative and the media used not appropriate for the detection of the contaminating microorganisms. b. Detection of part of the microbiota contaminating the sample; a dominant microbial population conceals minor populations. In both cases, the potential safety risk for the consumer is underestimated. c. The result of the analysis reflects the actual contamination of the sample.

When performing a microbiological analysis, the aim is to detect (and/or enumerate) one or more of the microbial groups that are present in a sample. Oftentimes, microbiologists are also interested in expanding their objectives and they pursue a more detailed description of the microbial ecology of the sample by isolating microorganisms and studying them in greater detail to understand their role in a specific ecosystem. This is traditionally performed using pure cultures of microorganisms. More specifically, a microorganism, isolated from the rest of the microbial community, is subjected to various tests with the purpose of obtaining detailed information regarding its behavior. The ability to utilize different substrates (carbon and nitrogen sources), the velocity of growth at different pH, a_w or temperature conditions, the resistance to antimicrobial compounds, tolerance to growth limiting substances are

Table 1
Microbiological criteria in powdered formulae (based on EU regulation 2073/2005, 1441/2007[14,15])

Food Safety Criteria; they define the acceptability of a food product or a batch and they apply for products on the market. Food business operators (FBO) have to comply with them and the testing for these criteria can be used for the verification of the HACCP and GHP procedures. Competent authorities may also sample and test for these criteria in the context of verification of compliance of food business operators.

Food category	Microorganism	n (number of sampling units to be analyzed)	c (number of units that may overcome the limit)	m (limit)	Actions
Dried follow-on formulae	*Salmonella*	30	0	Absence in 25 g	In case of unsatisfactory results, the food product is removed/recalled from the market and the FBO should investigate the reasons that led to the unsatisfactory result, eventually modifying the food safety procedures
Dried infant formulae and dried dietary foods for special medical purposes intended for infants below six months of age	*Cronobacter sakazakii*	30	0	Absence in 10 g	

Process Hygiene Criteria; they indicate the acceptable functioning of the production process and they do not apply to products on the market. Food business operators use such criteria to monitor the production process and employ corrective actions if unsatisfactory results are obtained.

Food category	Microorganism	n (number of sampling units to be analyzed)	c (number of units that may overcome the limit)	m (limit)	Actions
Dried infant formulae and dried dietary foods for special medical purposes intended for infants below six months of age	*Enterobacteriaceae*	10	0	Absence in 10 g	In case of unsatisfactory results, corrective actions need to be taken, including improvement in production hygiene to minimize contamination
Dried follow-on formulae	*Enterobacteriaceae*	5	0	Absence in 10 g	

some examples of physiological tests that are helpful in understanding the behavior of a microorganism. This information is then extrapolated to predict how the same microorganism would behave in a real food. There is however a fundamental difference between the experiments performed *in vitro* and the actual food. The food (at any stage of the food chain) is a complex ecosystem in which biotic and abiotic factors influence each member of the microbial community. Importantly, in the food, the different microbial groups interact in various ways and such interactions eventually affect the population dynamics and the survival or disappearance during food processing. Interactions among microbial groups and with the surrounding environment (i.e. the food or the processing plant) cannot be captured when performing experiments *in vitro* and in pure culture.[20]

Foodborne pathogens, if present in a food usually consist in a minor population of the entire microbiota. For this reason, enrichment approaches are necessarily employed. Through enrichment, an effort is made to increase the concentration of the target microorganism. Usually, this is achieved by using selective agents that should inhibit the competing microbiota. Such approaches are known to differentially influence microorganisms and therefore the outcome of the analysis may be altered when compared to the actual situation in the food at the moment of sampling.[20]

6. Contribution of omics in food microbiology

Already in the late 90's the limitations described above had been recognized through the application of analytical approaches that bypass the cultivation step and rely on detection of genetic material that can be robustly associated with the presence of a given microorganism in a sample. These approaches are collectively termed culture-independent approaches and have been instrumental in the detailed study of the microbial ecology of foods, with particular emphasis on fermented foods.[21] Fingerprinting techniques, particularly the Denaturing Gradient Gel Electrophoresis (DGGE), allowed for the first time the direct analysis of nucleic acids extracted from food samples capturing the complexity of the microbial ecology without the bias of the cultivation step.[22]

The application of DGGE in food analysis paved the way for the use of other culture-independent techniques. The evolution in the DNA sequencing technology and the introduction of Next Generation Sequencing (NGS) in microbiological analysis further improved the detection and description of microbial communities in foods. NGS is a massively parallel sequencing technology that generates millions of sequencing reads. The sequencing reads are then processed and offer taxonomic or functional information (Fig. 3).

The NGS technology may be applied to DNA originating from a food sample to study the microbial communities. In amplicon sequencing or metabarcoding (also metataxonomics), a PCR step precedes the sequencing. A genomic region that is present in all members of the community is amplified in multiple copies and then sequenced. Usually, the target to aplify is chosen based on the taxonomic information it may provide. Commonly, genes that encode for ribosomal RNA molecules are targeted. In this way, potentially all members of the community are represented (amplified) and after sequencing they can be identified (by comparison with available databases). After data analysis, the output is taxonomic information; the composition of the microbial community can be obtained. Amplicon sequencing has been extensively used to study the microbial ecology of foods, particularly the evolution of microbial populations during production of fermented foods but also to explore the microbial spoilage phenomena.[23]

By metagenomics or shot gun sequencing it is possible to obtain information regarding the genomic content of the different members of the microbial community. Therefore, potential functions are predicted.[24] In this case, the DNA extracted from a sample is directly subjected to sequencing. Metagenomics are being explored as a tool to detect and characterize pathogenic microorganisms in foods and food producing environments. Importantly, when using a metagenomic approach, it is

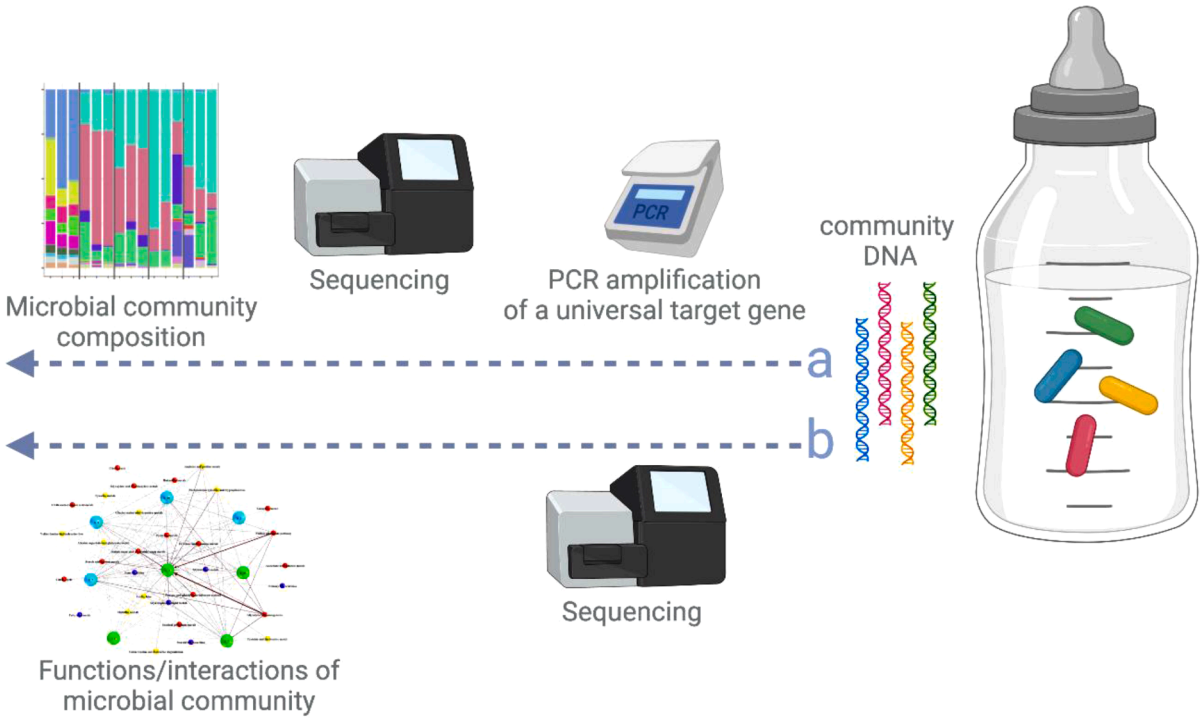

Fig. 3. Next Generation Sequencing applications. *a. Amplicon sequencing* (also referred to as metabarcoding or metataxonomics); total DNA extracted from infant food sample is first subjected to Polymerase Chain Reaction to amplify a target gene, common to all members of the microbial community. The amplification product is then sequenced. The sequence information is used to deduce the composition of the microbial community. *b. Shot gun sequencing or metagenomics*; total DNA extracted from infant food sample is directly sequenced. The sequencing provides information regarding the functioning of the microbial community (for example metabolic potential, virulence potential, antimicrobial resistance). Created with BioRender.com

possible to retrieve information regarding serotype, virulence genes, antimicrobial resistance genes.[24] This type of information is particularly relevant in understanding the potential risk associated with a food sample. From metagenomic sequencing data it is possible to reconstruct the whole genome of microorganisms. This permits to trace a specific biotype across samples that may be related in time or space.[25] For pathogenic microorganisms this could be useful in tracing routes of contamination. Due to the low abundance of pathogenic microorganisms in foods, it may be necessary to perform a short enrichment coupled to deep sequencing. Importantly, metagenomics can be used to observe the dynamics of mixed populations that are present in the food together with the target pathogenic microorganisms during enrichment and optimize the process to maximize its efficiency.[26]

Going beyond the genetic material, it is possible to also analyze total RNA, total proteins or metabolites of a sample. Omics is a term used to encompass the analysis of macromolecules (DNA, RNA, proteins) and metabolites originating from a sample. If the sample being analyzed is a food containing mixed microbial communities, then the macromolecules and metabolites derive from all the microorganisms present in the sample. In this case the term meta-omics is employed. Analysis of molecules such as RNA, proteins and metabolites is of relevance because they provide an overview of the activity of the microorganisms in the sample. Also, they may be present at a concentration above the detection limit even when a microorganism is not prevalent in the sample. Therefore, it may be possible to detect low abundant populations targeting RNA, proteins or metabolites. By combining different omics approaches it is possible not only to detect a target pathogen but also to explore how it interacts with the rest of the microbiota and how it is influenced and behaves based on the environmental conditions that prevail. This information is fundamental since it is known that during food processing microorganisms are subjected to a changing environment and they activate mechanisms to adapt accordingly. Therefore, omics offer the opportunity to move from the determination of the presence of pathogenic microorganisms in foods towards the definition of their behavior.[27] Understanding the microbial behavior under food processing conditions will lead to a refined risk assessment for pathogenic microorganisms in foods[23,28]

In particular, the metabolomics approaches are emerging because they are able to reveal the phenotypic profile of the microbiota. At principle, metabolomics mainly targeted the most abundant metabolites. Nowadays, less abundant compounds with very high informative potential are the focus of increasing interest. Among the large diversity of microbial secondary metabolites, low molecular-weight volatile organic compounds (VOCs) have received growing attention in the past decade. Microbial VOCs (mVOCs) are typically released in a multifarious and dynamic bouquet, essentially originating from the catabolic background, and comprise a majority of low-complexity, rather lipophilic compounds.[29] The volatolome – the VOC profiling of a biological tissue or fluid – has, for instance, already shown its relevance for revealing the exposure of environment,[30] food[31,32] or human consumer[33–35] to chemical hazards.

In order to phenotype complex ecosystems, volatolomics requires the most comprehensive possible profiling of the VOCs released by the different micro-organisms at very variable levels ranging from pg/g food (ppb) to μg/g food (ppm). Today, solid phase micro-extraction (SPME) coupled with gas chromatography and mass spectrometry (GC-MS) is certainly the most frequently used technique in volatolomics. By concentrating the analytes by means of an adsorbent polymer, SPME allows rapid and automated extraction of VOCs. However, because of its limited surface of adsorption, this method suffers from competition phenomena between VOCs related to their adsorption on the polymer.[36] These competition phenomena often limit SPME use to semi-quantitative issues and raise challenges for measurement uncertainties. The latest generations of automated dynamic headspace extraction systems (DHS) might represent a first alternative option that would deserve to be benchmarked with SPME. This technique is also based on VOC trapping on a polymer. Due to its greater adsorption capacity, the implementation of DHS might limit competition phenomena compared with SPME and might then significantly improve the quantification.[37] The static headspace extraction (static HS) might also be a second alternative to SPME since it guarantees a robust VOC quantification. However, the very poor extraction yield requires coupling with the latest generation of mass spectrometers with a very high sensitivity such as the hybrid quadrupole-Orbitrap® high resolution mass spectrometers.[38] Fig. 4 presents an example of a typical workflow commonly implemented in volatolomics studies.

In view to capture the bigger picture in which the pathogens are influenced by both the food environment and the other organisms present,[18] volatolomics may provide a promising alternative to more classical metabolomics platform to reveal significant changes in the metabolism of single culture[30] or microbiota.[34] Depending on the parameters of the food processes, the food microbiome structure could be impacted, thereby creating conditions that could favor activation or inhibition of pathogen growth. A volatolomics-based strategy could be implemented to highlight the characteristics of the microbiota that may restrain or enhance persistence of pathogens. In addition to HACCP approach tracing food pathogens along the entire food chain, the detailed characterization of the food volatolome upstream or at these critical points could thus provide relevant information in order to explore pathogen behavior in samples or processing conditions that are relevant for food safety and propose predictive models to refine microbial risk assessment.

7. The SAFFI approach

The SAFFI project aims to improve infant food microbial safety providing new knowledge to the food industry and competent authorities regarding the prevalence and behavior of pathogens. Omics approaches is the fil rouge in the effort to reach this aim. Two distinct but complementary objectives are being sought.

The first objective is to perform a detailed survey of the microbiota of raw materials, intermediates, final products and importantly of the environment under real production conditions. For this purpose, an intensive sampling campaign has been implemented, covering different seasons throughout the year and focusing on collecting samples that can be correlated (in time and space). Also, relevant metadata are being collected (particularly focusing on physicochemical parameters of the samples). These samples are analyzed with optimized protocols following a traditional, culture dependent approach and a culture independent, omics-based approach. In this way, a comprehensive description of the microbiota will be obtained. The presence/absence of pathogenic microorganisms may be then correlated with particular characteristics of the microbiota in the samples, the distribution in time or space. Further, routes of contamination within the processing plant may be identified. Such type of information is critical in adopting a preventive approach that is based on knowledge and data within a particular production but may also be integrated into a refined risk assessment for infant formulae.

The second objective is to investigate the behavior of *L. monocytogenes*, chosen as a model foodborne pathogen, under *in vitro* conditions that mimic the food production process. In particular, the goal is to delineate the response of the microorganism to various types of stress conditions that are relevant to food production. Omics will be implemented for this purpose as well. Ultimately, biomarkers of adaptation or robustness may be identified that could also have a predictive character. This information will be useful in the exposure assessment step of risk assessment.[28]

8. Conclusions

Infant microbial food safety is of extreme importance. Significant interventions that aim at improving microbial food safety have been

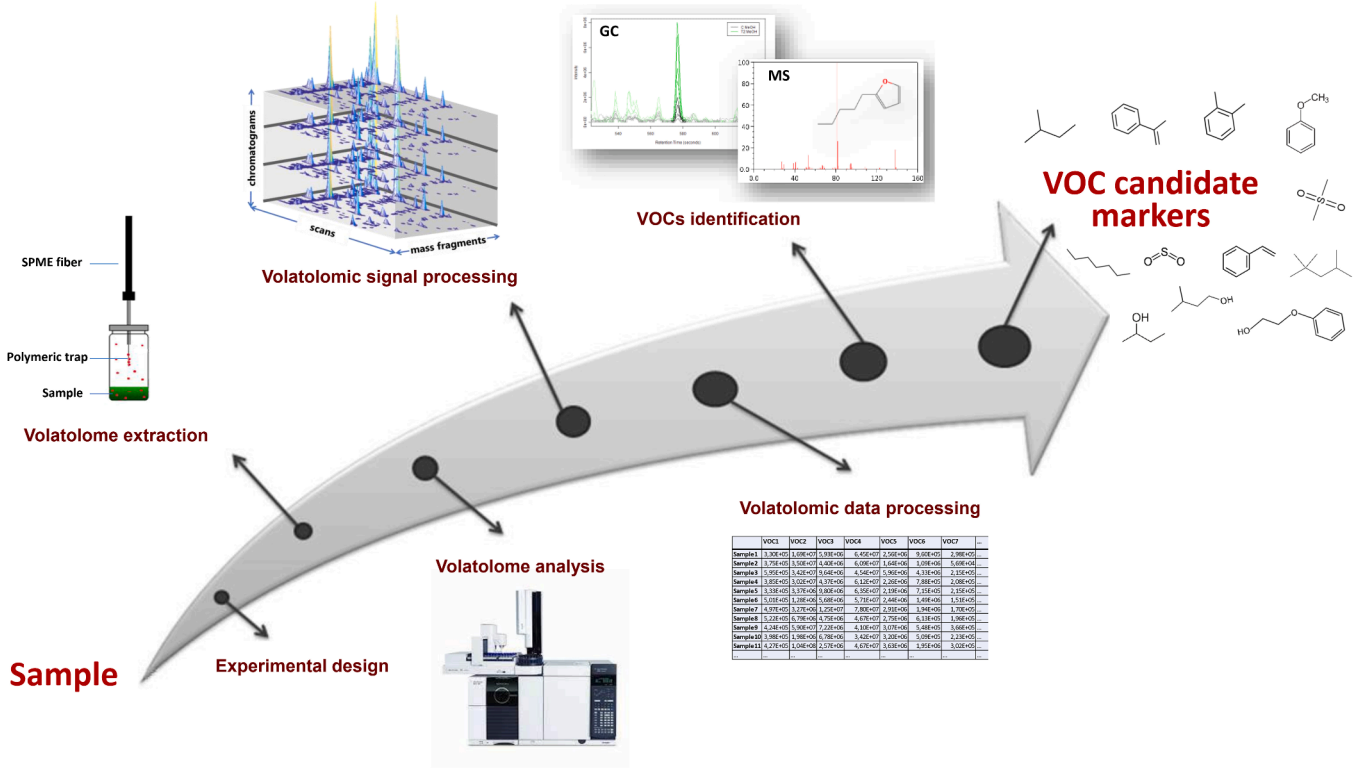

Fig. 4. Typical workflow in volatolomics.

consolidated in the last 20 years. The HACCP approach, GHP, specific guidelines for producers of infant formulae have helped in making this type of products reach a high standard of safety. Nevertheless, contamination by dangerous foodborne pathogens remains a potential threat to public health and outbreaks of disease due to contaminated infant formulae are still occasionally reported. To tackle this safety issue, omics tools are important in identifying contamination routes, highlighting microbial interactions influencing pathogenic microorganisms, understanding their behavior under food production conditions. Generating omics data that could be integrated into risk assessment is the purpose of the SAFFI project with the overarching aim of further improving infant food microbial safety.

Declaration of Competing Interest

The authors declare no conflict of interests.

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References

1 Osaili T, Forsythe S. Desiccation resistance and persistence of Cronobacter species in infant formula. *Int J Food Microbiol*. 2009;136:214–220. https://doi.org/10.1016/j.ijfoodmicro.2009.08.006.
2 Chap J, Jackson P, Siqueira R, et al. International survey of Cronobacter sakazakii and other Cronobacter spp. in follow up formulas and infant foods. *Int J Food Microbiol*. 2009;136:185–188. https://doi.org/10.1016/j.ijfoodmicro.2009.08.005.
3 Fao, Who, Enterobacter sakazakii and other microorganisms in powdered infant formula, (n.d.). 2022.
4 World Health Organization., Food and Agriculture Organization of the United Nations., Safe preparation, storage and handling of powdered infant formular : guidlines., n.d. 2022.
5 Opinion of the Scientific Panel on biological hazards (BIOHAZ) related to the microbiological risks in infant formulae and follow-on formulae. *EFSA J*. 2004;2. https://doi.org/10.2903/j.efsa.2004.113.
6 Sockett PN, Rodgers FG. Enteric and foodborne disease in children: a review of the influence of food- and environment-related risk factors. *Paediatr Child Health*. 2001;6:203–209. https://doi.org/10.1093/PCH/6.4.203.
7 Galanakis E, Bitsori M, Maraki S, Giannakopoulou C, Samonis G, Tselentis Y. Invasive non-typhoidal salmonellosis in immunocompetent infants and children. *Int J Infect Dis*. 2007;11:36–39. https://doi.org/10.1016/j.ijid.2005.09.004.
8 Nelson SJ, Granoff D. Salmonella gastroenteritis in the first three months of life. *Clin Pediatr (Phila)*. 1982;21:709–712. https://doi.org/10.1177/000992288202101201.
9 Risks for public health related to the presence of Bacillus cereus and other Bacillus spp. including Bacillus thuringiensis in foodstuffs. *EFSA J*. 2016;14. https://doi.org/10.2903/j.efsa.2016.4524.
10 Pei X, Yang S, Zhan L, et al. Prevalence of bacillus cereus in powdered infant and powdered follow-up formula in China. *Food Control*. 2018;93:101–105. https://doi.org/10.1016/J.FOODCONT.2018.05.049.
11 Reij MW, den Aantrekker ED. Recontamination as a source of pathogens in processed foods. *Int J Food Microbiol*. 2004;91:1–11. https://doi.org/10.1016/S0168-1605(03)00295-2.
12 General principles of food hygiene, n.d. 2022.
13 Code of hygienic practice for powdered formulae for infants and young children CAC/RCP 66-2008, 2008.
14 COMMISSION REGULATION (EC) No 1441/2007 of 5 December 2007 amending Regulation (EC) No 2073/2005 on microbiological criteria for foodstuffs (Text with EEA relevance), n.d. 2022.
15 Commission regulation (EC) 2073/2005 of 15 November 2005 on microbiological criteria for foodstuffs (Text with EEA relevance), 2005.
16 Ayrapetyan M, Oliver JD. The viable but non-culturable state and its relevance in food safety. *Curr Opin Food Sci*. 2016;8:127–133. https://doi.org/10.1016/j.cofs.2016.04.010.
17 Nai C, Meyer V. From axenic to mixed cultures: technological advances accelerating a paradigm shift in microbiology. *Trends Microbiol*. 2018;26:538–554. https://doi.org/10.1016/j.tim.2017.11.004.
18 Bokulich NA, Lewis ZT, Boundy-Mills K, Mills DA. A new perspective on microbial landscapes within food production. *Curr Opin Biotechnol*. 2016;37:182–189. https://doi.org/10.1016/j.copbio.2015.12.008.
19 Greppi A, Rantsiou K. Methodological advancements in foodborne pathogen determination: from presence to behavior. *Curr Opin Food Sci*. 2016;8:80–88. https://doi.org/10.1016/j.cofs.2016.04.005.
20 Pettengill JB, McAvoy E, White JR, Allard M, Brown E, Ottesen A. Using metagenomic analyses to estimate the consequences of enrichment bias for pathogen detection. *BMC Res. Notes*. 2012;5. https://doi.org/10.1186/1756-0500-5-378.
21 Cocolin L, Alessandria V, Dolci P, Gorra R, Rantsiou K. Culture independent methods to assess the diversity and dynamics of microbiota during food fermentation. *Int J*

Food Microbiol. 2013;167:29–43. https://doi.org/10.1016/j.ijfoodmicro.2013.05.008.
22 Ercolini D. PCR-DGGE fingerprinting: novel strategies for detection of microbes in food. *J Microbiol Methods.* 2004;56:297–314. https://doi.org/10.1016/J.MIMET.2003.11.006.
23 Cocolin L, Mataragas M, Bourdichon F, et al. Next generation microbiological risk assessment meta-omics: the next need for integration. *Int J Food Microbiol.* 2018;287:10–17. https://doi.org/10.1016/j.ijfoodmicro.2017.11.008.
24 Jagadeesan B, Gerner-Smidt P, Allard MW, et al. The use of next generation sequencing for improving food safety: translation into practice. *Food Microbiol.* 2019;79:96–115. https://doi.org/10.1016/J.FM.2018.11.005.
25 Franciosa I, Ferrocino I, Giordano M, Mounier J, Rantsiou K, Cocolin L. Specific metagenomic asset drives the spontaneous fermentation of Italian sausages. *Food Res Int.* 2021;144, 110379. https://doi.org/10.1016/J.FOODRES.2021.110379.
26 Ottesen A, Ramachandran P, Reed E, et al. Enrichment dynamics of Listeria monocytogenes and the associated microbiome from naturally contaminated ice cream linked to a listeriosis outbreak. *BMC Microbiol.* 2016;16:1–11. https://doi.org/10.1186/S12866-016-0894-1/FIGURES/4.
27 Cocolin L, Ercolini D. Zooming into food-associated microbial consortia: a "cultural" evolution. *Curr Opin Food Sci.* 2015;2:43–50. https://doi.org/10.1016/J.COFS.2015.01.003.
28 den Besten HMW, Amézquita A, Bover-Cid S, et al. Next generation of microbiological risk assessment: potential of omics data for exposure assessment. *Int J Food Microbiol.* 2018;287:18–27. https://doi.org/10.1016/J.IJFOODMICRO.2017.10.006.
29 Schulz S, Dickschat JS. Bacterial volatiles: the smell of small organisms. *Nat Prod Rep.* 2007;24:814. https://doi.org/10.1039/b507392h.
30 Hidalgo K, Ratel J, Mercier F, Gauriat B, Bouchard P, Engel E. Volatolomics in bacterial ecotoxicology, a novel method for detecting signatures of pesticide exposure? *Front Microbiol.* 2019;9. https://doi.org/10.3389/fmicb.2018.03113.
31 Ratel J, Planche C, Mercier F, et al. Liver volatolomics to reveal poultry exposure to γ-hexabromocyclododecane (HBCD). *Chemosphere.* 2017;189:634–642. https://doi.org/10.1016/j.chemosphere.2017.09.074.
32 Ratel J, Mercier F, Angénieux M, et al. Identification by volatolomics of hydrocarbon, oxygenated, sulfur and aromatic markers of livestock exposure to α-hexabromocyclododecane. *Food Chem.* 2022;374, 131504. https://doi.org/10.1016/j.foodchem.2021.131504.
33 Defois C, Ratel J, Denis S, et al. Environmental pollutant Benzo[a]Pyrene impacts the volatile metabolome and transcriptome of the human gut microbiota. *Front Microbiol.* 2017;8. https://doi.org/10.3389/fmicb.2017.01562.
34 Defois C, Ratel J, Garrait G, et al. Food chemicals disrupt human gut microbiota activity and impact intestinal homeostasis as revealed by *in vitro* systems. *Sci Rep.* 2018;8:11006. https://doi.org/10.1038/s41598-018-29376-9.
35 Dausset C, Bornes S, Miquel S, et al. Identification of sulfur components enhancing the anti-Candida effect of lactobacillus rhamnosus Lcr35. *Sci Rep.* 2020;10:17074. https://doi.org/10.1038/s41598-020-74027-7.
36 Murray RA. Limitations to the use of solid-phase microextraction for quantitation of mixtures of volatile organic sulfur compounds. *Anal Chem.* 2001;73:1646–1649. https://doi.org/10.1021/ac001176m.
37 Wojnowski W, Majchrzak T, Dymerski T, Gębicki J, Namieśnik J. Dynamic headspace sampling as an initial step for sample preparation in chromatographic analysis. *J AOAC Int.* 2017;100:1599–1606. https://doi.org/10.5740/jaoacint.17-0206.
38 Stettin D, Poulin RX, Pohnert G. Metabolomics Benefits from Orbitrap GC–MS—comparison of low- and high-resolution GC–MS. *Metabolites.* 2020;10:143. https://doi.org/10.3390/metabo10040143.

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Improving infant food safety by avoiding hazards of chemical mixture effects using novel integrated methods based on bioassays and analytical chemistry☆

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ABSTRACT

Humans, including infants are exposed to complex mixtures of anthropogenic chemicals, and food is a major exposure route. Current risk assessment, however, typically does not evaluate mixture toxicity but rather focuses on single chemical exposure scenarios. Nevertheless, there is ample evidence that combined exposures to chemicals is involved in the etiology of major human diseases, and that infants are often more vulnerable than adults. Surprisingly hardly any efficient practical tools and guidelines have been defined to adequately assess mixture effects of food. Evaluation of levels of mixtures of dioxins and related compounds are a notable exception, although also in that area novel insights warrant reevaluation of the relevant compounds to be included in evaluation. Novel approaches are needed, since our knowledge on the toxicity of chemicals is lagging behind and even most of the industrial chemicals that are in common use have undergone no or limited safety testing, while the situation with natural compounds in food is even more challenging. Novel untargeted chemical analytical techniques and quantitative bioanalytical techniques that respond to toxic chemicals independent of prior knowledge on their structure or toxicity can be used to increase the knowledge on chemical mixtures. We discuss the complementarities between these bio- and chemical analytical methods that can be used in an integrated system to improve infant food safety by avoiding hazards of chemical mixture effects.

Introduction

Humans, including infants are exposed to complex mixtures of anthropogenic chemicals, never one at a time. Current risk assessment, however, typically focuses on single chemical exposure scenarios. Exposure to chemical mixtures and their combined effects require better risk assessment and management procedures to protect public health and the environment [1].

In the past, human adverse health issues have been reduced successfully through reduction of exposure to single highly toxic chemicals that posed significant health risk such as ubiquitously used persistent pesticides like DDT, and other persistent organic pollutants (POPs).

Abbreviations: AhR, Aryl hydrocarbon receptor; AOP, Adverse outcome pathway; CALUX, Chemically activated luciferase expression; CP, Chlorinated paraffins; DDT, Dichloordifenyltrichloorethaan; ECHA, European Chemical Agency; ECVAM, European Centre for the Validation of Alternative Methods; EDA, Effect directed analyisis; EDC, Endocrine disrupting compound; EFSA, European Food Safety Authority; EPA, Environmental Protection Agency; FAO, Food and Agriculture Organization (WHO); HRMS, High resolution magnetic sector mass spectrometers; MIE, Molecular initiating event; OECD, Organisation for Economic Cooperation and Development; PBDD, Polybrominated dibenzodioxin; PBDF, Polybrominated dibenzofuran; PCDD, Polychlorinated dibenzodioxin; PCDF, Polychlorinated dibenzofuran; PCB, Polychlorinated biphenyl; PCN, Polychlorinated naphthalene; POP, Persistent organic pollutants; REACH, Registration Evaluation and Authorization of Chemicals; SOP, Standard operating procedure; SVHC, Substance of Very High Concern; TDS, Total diet study; TEF, Toxic equivalency factor; TEQ, Toxic equivalents; TRV, Toxicological reference value; TWI, Tolerable weekly intake; WHO, World Health Organization.

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Today, the focus is shifting to the less obvious effects of pollutants either alone or in mixtures and their influence on more chronic types of toxicities leading to e.g. cancer, disruption of the endocrine system, developmental toxicity, immune- and neurodevelopmental disorders. These relationships are much more difficult to establish since there is no directly visible causal relationship between effect and exposure. Also, exposure in food typically is to mixtures of chemicals, making this analysis even more complicated. Nevertheless, there is ample evidence that combined exposures to chemicals is involved in the etiology of major human diseases.[1–4] Therefore, nowadays, consideration of mixture effects is mentioned in several relevant regulations but surprisingly hardly any efficient practical tools and guidelines have been defined in those regulations to adequately assess mixture effects.[1,5,6]

Food is a major exposure route to chemicals in humans. Food is a complex mixture of chemicals of both natural and synthetic origin.[7] The natural ingredients include nutrients, vitamins, but also natural contaminants and potential toxic natural compounds. For all these compounds toxicity becomes relevant when dosed at a sufficiently high level. Even if individual compounds do not pass a threshold of toxicity, combined doses of compounds that have similar toxicity can lead to adversities.[3,8] Therefore, these combined dose effects need to be assessed. Exposure of infants through food, starting at the earliest age through milk or infant formula is highly relevant because the children's metabolic defenses still need maturation and disturbance of developmental processes can lead to serious health effects.[9] However, our understanding of the effects of early life exposure is limited.[8, 10,11] Efficient methods to measure hazards of chemical mixtures in infant food are still in its infancy and not regularly used. Here we describe novel developments in this important area, and we give examples of advanced methods that have been developed for mixtures of specific compound groups, in particular dioxins and dioxin-like compounds, and the possibilities for their integrated use to comprehensively secure infant food safety.

Presence of chemical mixtures in infant food

Safety assessment of chemicals, such as industrial chemicals and pesticides traditionally focuses on single chemicals only and not to safety issues of chemical mixtures that may occur in relevant exposure scenario's.[6] Food, also infant food, typically is an exposure scenario to highly complex mixtures of chemicals, at concentrations that generally will cause no harm. The basis to make this assumption, however, contains weaknesses. In fact, our knowledge of the extremely complex chemical universe is very limited. Hundreds of thousands of anthropogenic chemicals exist, including their by-products, metabolites and abiotically formed transformation products[1]. Only a very small fraction of these chemicals has undergone safety testing.[6] Even most of the approximately 100,000 industrial chemicals that are in common use have undergone no or limited safety testing only. This situation is improving due to the REACH legislation, but still the vast majority of chemicals that we are exposed to will remain untested or even unknown. This will include industrial chemicals and food processing-derived contaminants, their metabolites and natural chemicals. These natural chemicals include some of the most toxic classes of chemicals that are known, like toxins produced by plants, fungi, and bacteria,[12, 13] but also ones identical to synthetic chemicals like a range of organohalogens.[14] Recently, it has been recognized that unexpected food contaminants, both with known and unknown toxicity and often related to use of contaminated starting products is an issue of concern.[15] Several of these contaminants may be picked up during routine screening in advanced quality control systems, but others may escape notice. Therefore, methods are being developed for non-targeted analysis to assess the presence of unsuspected and unknown contaminants and possible mixture effects.

The impact of early life exposure to toxic chemical mixtures

It has been estimated that approximately 3% of all developmental defects are attributable to exposure to toxic chemicals and physical agents, including environmental factors, and that 25% of all developmental defects may be due to a combination of genetic and environmental factors.[9] This percentage includes all structural or functional abnormalities at birth. This estimate lacks consideration of effects on disease outcomes that are manifest later in life, and also does not cover mixture effects of chemicals. In fact, knowledge in this area is mainly based on animal experiments and human exposure to relatively high dosages of single chemicals, including certain drugs. Little is known about mixture effects leading to either functional anomalies or impact on incidence of disease later in life. However, particularly endocrine systems may be deregulated through developmental exposure to chemical mixtures with consequences on the incidence of disease.[3] These so-called endocrine disrupting compounds (EDCs) almost exclusively are low molecular weight molecules that readily can enter the body and bind to nuclear receptors in cells, thereby disturbing their normal functioning. Main hormonal systems involved are those for the sex steroids and thyroid hormone, but a similar interaction also occurs with the receptor to which dioxins bind, the aryl hydrocarbon receptor (AhR). There still are many gaps in our understanding of this emerging area of research. Since food is a main route of exposure,[8] either via the mother or after birth directly to the developing child, this is an important area to explore using novel approaches to assure optimal infant food safety.

Mechanistic basis of adverse effects related to early life exposure to chemical mixtures

Normal cellular physiology is governed by a range of signaling pathways that secure proper cell growth and differentiation. Disruption of those pathways can lead to diseases, such as cancer and developmental disorders.[9,15] Certain chemicals can interfere with these pathways, often through binding to molecules that are the starting points of the pathway, the so-called molecular initiating events (MIEs). When this occurs sufficiently strong, the pathway can be activated. This not necessarily leads to a toxic, adverse effect, but when stimulation becomes too strong adversity can be a result. Therefore, these physiological pathways are also referred to as adverse outcome pathways (AOPs).[16] Similarly, when different chemicals affect the same pathway, the effect may add up to pass this threshold, leading to adversity. This has for instance been shown to occur with chemicals interacting with sex steroid receptors, leading to combined endocrine system disrupting effects,[3,5] and dioxins.[17,18] Typically, disruption of basic cellular and hormonal pathways can lead to a range of structural and functional defects at birth and disorders later in life. This is because the magnitude and nature of these disorders is dependent on the dosing, but also the timing of exposure, thereby affecting different processes in which the pathway is involved. For example, dioxin's toxic effects are mediated through a single receptor-mediated pathway. Nevertheless, a wide spectrum of structural and functional defects is related to developmental dioxin exposure including cleft palate, hydronephrosis, altered thyroid and immune status, altered neurobehavior at the level of hearing, psychomotor function, and gender-related behaviors, altered cognition, dentition, and development of reproductive organs, and delays in breast development, in addition to altered sex ratios among the exposed offspring.[17,18] The knowledge on these common mechanisms of toxicity has greatly expanded in recent decades, which forms the basis of novel methods to analyze toxicity of mixtures using mechanism-based bioassays.

Mechanism-based bioassays to assess mixture effects of food-derived chemicals

Based on the knowledge on the mode of action of toxicants, modern mechanism (or effect)-based bioassays have been generated. One early well-known example of a mechanism-based bioassay that is used and accepted very frequently is the Ames mutagenesis assay that assesses chemically-induced mutations in bacterial DNA.[19] Using the knowledge of the mechanisms of toxicity of chemicals modern mechanism-based bioassays have been developed covering a wide range of key mechanisms using human cells.[20] This includes assays with a higher predictive value for human genotoxicity than the Ames test.[21] These and other mechanism-based bioassays can be used as alternative methods to assess safety of chemicals and chemical mixtures. More recently, the throughput of analysis has been greatly enhanced using robotics.[22,23] The assays are highly specific and measure interference with distinct toxicity pathways through the CALUX reporter gene technology (Fig. 1).[20] In CALUX assays this interaction with key cellular pathways is made easily measurable through incorporation in a recipient cell line of a so-called reporter gene construct which measures activation of the relevant transcriptional pathway. Activation of that pathway is coupled to expression of the firefly luciferase gene, which leads to an easily measurable product in the mammalian cells (Fig. 1). Many of the assays measure interference with a specific type of nuclear hormone receptors that are frequently targets of pollutants,[20,24] while others focus on assessing influences of chemicals on pathways involved in basic cellular signaling which are for instance relevant for acute toxicity and carcinogenesis.[21]

This panel of mechanism-based assays can be linked to adversities that are important for risk assessment, as established in experimental animals and humans via adverse outcome pathways.[16] The assays have been extensively validated and shown to be predictive of effects in animal studies, as used in current chemical safety legislation. The specificity and sensitivity of the assays is particularly meant to facilitate measurements and interpretation of the results in complex mixtures present in food, feed, water, and a wide range of different environmental- and clinical samples.[20,25,26] Results are quantitative and expressed in toxic equivalents (TEQ) relative to a reference standard of a relevant pathway-activating chemical. Various of the CALUX assays have been used intensively and successfully, initially often for non-regulatory purposes, followed by incorporation in relevant national- and international guidelines.[27-32] This includes the DR CALUX assay that measures activation of the relevant target molecule, the dioxin receptor (AhR). By coupling to a specific workup method, a selection for the most relevant stable ligands is made.

Chemical analytical-based mixture effect assessment as used for dioxin mixtures

The advantage of targeted approaches is that through establishment of the chemical identities, source identification and risk reduction measures are facilitated. In bioanalysis, the contribution of the different chemicals to the TEQ value of chemical mixtures is integrated, while the relative contribution of different chemicals cannot readily be assessed. In chemical analytics the reverse is true since chemical analytics targets exact quantification of single chemicals. However, there are possibilities to estimate mixture effects using chemical analysis, of which the system to analyze dioxins is among the most advanced. Because of their toxicity at extremely low dosages, dioxins are of great concern. Since dioxins are present at significant levels in food, including breast milk and infant formula, measures have been put in place in Europe to reduce intake through this major route of exposure. The approach taken is unique in that the chemical analysis of a range of major congeners is used to estimate their combined biological effect. To do this, the concentration of individual congeners is multiplied by a corresponding toxic equivalency factor (TEF) which expresses its toxicity relative to the most toxic form of dioxins, 2,3,7,8-TCDD. In this way a TEQ value is derived for the respective congener, and by adding the values of the congeners used in this system, the expected sumTEQ value of the mixture is estimated, which, if all relevant congeners are included, would be equivalent to the

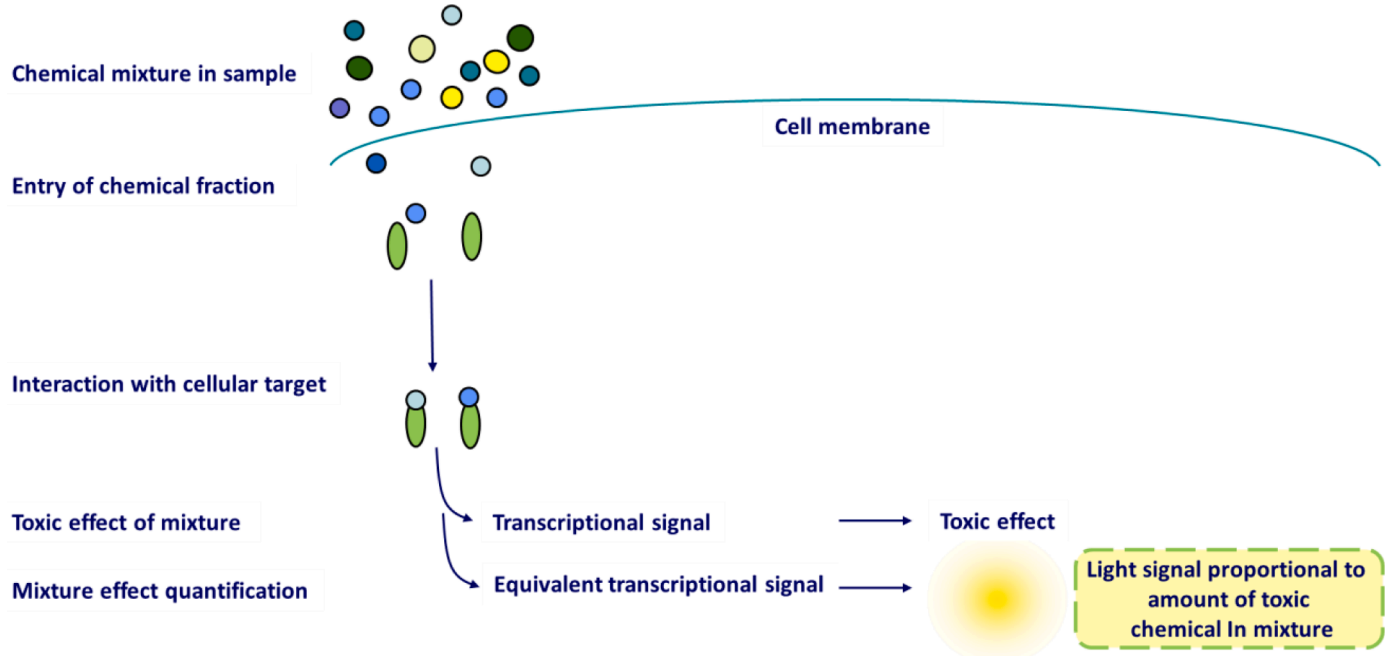

Fig. 1. General principle of a CALUX assay. Exposure of cells to chemicals will lead to a change in gene expression and a consequential change in cellular behavior that is instrumental in the toxic effect of the chemical(s). This response is mediated through a transcriptional response that drives expression of endogenous genes, and as a result the toxic effect. In a CALUX® reporter gene assay this response is modulated in such a way that activation of a signaling pathway is linked to transcription of a stably introduced luciferase gene. Upon addition of a substrate a light signal is generated which is proportional to the amount of bioactive chemical in a sample.

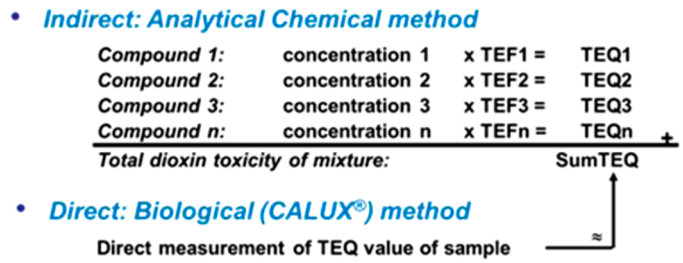

Fig. 2. Determination of the toxicity of mixtures of dioxin-like chemicals. Analytical chemical methods determine compounds of known concern and add up the product of individual concentrations and a relevant toxicity factor, the TEF value. In this way an estimate of the expected biological activity, expressed as sumTEQ, is made. Biological methods, like CALUX measure the SumTEQ through an interaction with the dioxin receptor, and do not rely on prior knowledge on toxicity of individual chemicals.

TEQ measured with a relevant bioassay, like DR CALUX (Fig. 2). Nowadays a range of stable chlorinated dioxins, furans and PCBs are included in this chemical analytical estimating of total dioxin toxicity of a mixture. For mixtures, this approach can be taken if all relevant toxic compounds are known affecting one biological pathway, in this case activation of the dioxin receptor. Since dioxins are among the most studied toxic chemicals with many data on toxicity of individual congeners, this gives confidence that this estimation was correct. However, new data suggest that the current coverage of relevant compounds may not be sufficient (see next section).

Uncertainties in current dioxin-related chemical mixture effect assessment

To reduce uncertainty in the food chain monitoring using chemical analytics one of the major challenges is the choice of chemicals to be monitored while toxicity data on most chemicals are not available.[6] This even applies to one of the most advanced systems of estimating dioxin-related chemical toxicity as present in food. The current system focuses on chemically very stable chlorinated dioxins, furans and PCBs that all can activate the dioxin receptor and are known to cause adverse effects in experimental animals and humans, when dosed at sufficiently high levels. In the light of the missing knowledge on the toxicity of most chemicals it would be surprising that all relevant dioxin receptor-interacting molecules would be known already. Indeed, recent research suggests that there are important omissions in the routinely measured panel of dioxin receptor interacting compounds. As such, various halogenated compounds groups have been identified that pose possible risks that are mediated through dioxin receptor activation, including chlorinated paraffins, polychlorinated naphthalenes, and brominated dioxins and furans.

Chlorinated paraffins (CPs), are complex mixtures of hundreds of isomeric groups with varying linear carbon chain length and chlorine number, themselves comprising hundreds of isomers[28,33]. A fraction of the synthesized volumes of CPs is unintentionally released into the environment during the production, use or destruction of products containing them. Due to their lipophilic properties and stability, CPs enter the human food chain through processes like those described for similar halogenated substances like dioxins, furans and PCBs. They can also be accumulated and redistributed from reservoirs related to industrial processing and food preparation, such as in kitchen ovens. In this context, assessing the risk associated with these contaminants in relation to human exposure is a pressing need. Recently, the relevant European Food Safety Agency (EFSA) panel of experts established lowest adverse effect levels for several of these CPs,[33] while some have been classified as persistent organic pollutants (POPs), under the Stockholm Convention (Persistent Organic Pollutants Review Committee 2017) and have been placed on the Candidate List of Substances of Very High Concern (SVHC) under the REACH Regulation. However, the clear lack of toxicological and exposure data previously highlighted limits in the risk assessment associated with dietary exposure to CPs. A key factor explaining the lack of data relates to the challenge of analyzing relevant CP classes that remain despite the recent advances.[34]

Polychlorinated naphthalenes (PCNs) are legacy contaminants gathering 75 congeners. They have been listed by the Stockholm convention, initially for reduction of inadvertent production and ultimately, for elimination. They originate through releases from older electrical equipment, inadvertent contamination in industrial chemicals and from combustion processes such as incineration. Recent advances in measurement techniques have allowed a greater characterization of PCN occurrence, yielding more specific data including individual PCN congener concentrations. Emerging data on food shows widespread occurrence in most commonly consumed foods from different parts of the world. Concurrently, toxicological studies have also allowed a greater insight into the potencies of some congeners, a number of which are known to elicit potent, aryl hydrocarbon receptor (AhR) mediated responses, referred to as dioxin-like toxicity. The dietary pathway is widely recognized as the most likely route to non-occupational human exposure. Overall, the data that are currently available on PCN occurrence in foods suggest a widespread current distribution of these contaminants in foods and food webs. This is remarkable given the time that has elapsed since the unrestricted use of these compounds, and those other commercial chemicals such as PCBs which are known sources of PCNs, ceased (particularly in Western Europe and North America), and underlines the persistence and ubiquity of PCNs. Although the reported contribution is smaller than PCDD/Fs and PCBs, PCN toxicity is likely to add to the cumulative toxicity of other dioxin-like compounds.[35]

Polybrominated dioxins and furans (PBDD/F) are brominated counterparts of the traditionally measured chlorinated compounds. It, however, has been found that polybrominated dioxins and furans have comparable affinity to the human dioxin receptor.[36,37] When using the DR-CALUX bioassay both chlorinated and brominated congeners will contribute to biological activity in a way relevant for human toxicity. Chlorinated dioxins are going down in the diet, but brominated ones are increasing, often as breakdown products of flame retardants. They are found at high levels in children's toys that are made of recycled plastics,[38] and are also entering the food chain.[37] Reported PBDD/F dietary intakes suggest that some population groups, particularly young children, may exceed the revised tolerable weekly intake for dioxin-like contaminants, even for mean consumption estimated with lower bound data. It is evident that the omission of PBDD/Fs from the TEQ scheme results is a significant underestimation of the cumulative toxicity and associated risk arising from this mode of action.[37,38]

Although for several of these novel relevant AhR ligands mass spectrometer (MS)-based analytical methods have been developed already, they have not been incorporated in the international TEF/TEQ-based methodology to assess total dioxin receptor-mediated toxicological burden.[39] Also, very likely novel relevant AhR interacting compounds will be identified in the future, and therefore this methodology will need to be updated regularly. Because of the advances in biological and chemical analytical methods novel, more comprehensive, integrated methods become feasible.

Novel approaches in infant food safety assessment

Food safety assessment is challenging not only due to the lack of knowledge on toxicity of many chemicals, but also because of changes in raw materials, processing, packaging and storage methods and consumer practices, and thus the need to efficiently monitor at critical control points. To do this, methods need to be reviewed and updated using the latest scientific insights and technological developments. The complexity of food and feed samples, together with the low concentrations at which contaminants occur (ppb (ng.g-1) to ppt (pg.g-1)), requires highly sensitive, selective and robust analytical techniques. These requirements need to be reviewed and upgraded, when needed. At the

end of 2018, the EFSA CONTAM expert group carried out a re-evaluation of the Toxicological Reference Value (TRV) for dioxins and dioxin-like PCBs (DL-PCBs) in food. A new Tolerable Weekly Intake (TWI) was proposed, amounting to 2 picograms per kilogram of body weight (pg. kg-1 bw). This TWI is seven times lower than the previous TWI set by the former European Commission's Scientific Committee on Food in 2001. The main reasons for this decrease in level are the availability of new epidemiological and experimental data on the toxicity of these substances in animals, as well as the emergence of more sophisticated modelling techniques to predict the levels of these substances in the human body over time. This provides novel analytical challenges.

For decades, the analysis of dioxins and furans has been performed by GC coupled to high resolution magnetic sector mass spectrometers (HRMS).[40] Recently, tandem mass spectrometry coupled to gas chromatography (GC-MS/MS) has been added in the European Union (EU) legislation as an alternative to HRMS for the confirmatory analysis of dioxins and DL-PCB in food and feed.[41] In this context, innovative ionization techniques have demonstrated increased sensitivity to perform analyses with the required sensitivity and selectivity for this field.[42]

To review the results of current monitoring programs contaminant occurrence data need to be collected and evaluated. Collecting occurrence data for risk assessment purposes relies on the implementation of two distinct strategies. The first one allows gathering occurrence data from routine monitoring programs conducted at the level of a specific country to check compliance of contaminants.[43] This approach has recently been further encouraged through a novel European regulation (Reg 2017/625/EC). An alternative to relying on data from food control systems is the use of the Total Diet Study (TDS) approach. These studies are based on a standardized method as recommended by WHO, FAO and EFSA: steps characterizing a TDS include the selection of foods based on food consumption data to represent as best as possible a typical diet, their preparation to food as consumed and the subsequent pooling of related foods before analysis.[44] Regarding dioxins and furans, the main contributors to the average dietary exposure for most age groups in European countries are fish (in particular oily fish), cheese and cattle meat[13]. In its latest Total Diet Study (TDS) dedicated to children's food, the French risk assessment agency Anses concluded that dietary exposure to dioxins and furans was a cause for concern, recommending to reduce exposures, in particular via everyday food products that contribute strongly to exposure to these molecules in the most exposed children (milk, ultra-fresh dairy products and fish).[45] At the European level, EFSA has recently confirmed the conclusion of previous assessments that dietary exposure to dioxins and dioxin-like PCBs is a health concern. The data collected in Europe indicate that the tolerable intake recently updated by EFSA is exceeded for all age groups. Average and high exposures are respectively 5 to 15 times higher than the new Tolerable Upper Intake Level for adolescents, adults and the elderly. Young children and children under 10 years of age also show a similar exceedance of the TW.[46]

As mentioned above, the analysis of known chemical hazards in complex biological matrices such as food requires sensitive, selective, and robust methods. To achieve the performance levels, the methods are usually targeted, in the sense that they only observe what is being looked for. Targeted methods are by definition selective, they thus do not detect substances that are not considered to be priorities, not suspected to be present in the matrix under consideration or not yet described, e.g. degradation products of known or unknown substances. New strategies which are known as global or non-targeted, have been reported over the last years to seek unknown/emerging exposure substances or unknown degradation products that may be considered as many potential emerging hazards. The recent period has indeed witnessed spectacular advances in chromatography and high-resolution mass spectrometry (HRMS), opening the way to non-targeted full scan fingerprints as a new methodological approach. It combines classical analytical chemistry tools, with sophisticated data analysis.[47] When certain molecular characteristics are targeted, such as the presence of halogens, specific signal processing algorithms can then be implemented to identify emerging POPs-type contaminants.[48] In the future, the development of such global approaches can be increased with the introduction of new analytical techniques which offer a new dimension in addition to chromatography and mass spectrometry for improved analysis of complex mixtures such as food.[49] These advanced novel non-targeted analytical approaches nevertheless remain targeted towards chemical groups with distinct characteristics. By combining with novel bioanalytical tools, additional opportunities for comprehensive, non-targeted chemical safety monitoring possibilities can be obtained.

Integrated analytical approaches to assure infant food safety

Targeted chemical analytics measures the presence of known toxic chemicals specifically, and therefore will not detect relevant unknown ones and their mixture effects. Non-targeted methods can greatly improve the number of chemicals addressed, but still cannot be directly linked to a measure of toxicity. Thus, a link to toxicity assessment is always needed, which can be based on prior knowledge, that is available for a subset of chemicals only. This knowledge is largely based on animal experimentation, but increasingly also on the use of *in vitro* assays. Mixture safety assessment can also make use of these *in vitro* assays, requiring no prior knowledge on safety of chemicals assessed. The net toxic effect of all contaminants in a sample can be measured regardless of their chemical structure and prior knowledge on their toxicity. Although the latter could be regarded as an alternative to current chemical analysis, there are several reasons why a combined system with targeted- and non-targeted chemical analytics will likely be more effective to assure safety of complex mixtures such as infant food. The targeted approaches will allow exact quantification of individual toxicants. which is important in source identification and risk management once a sample is identified with unexpected high bioactivity (Fig. 3). Generation on knowledge on the toxicity of yet uncharacterized "emerging" toxicants can be generated through untargeted chemical analytics or bioanalysis. For the latter, to identify the chemical or chemicals responsible for unexpected bioactivities in sample so-called effect-directed analysis (EDA)[50,51] can be used. In this procedure which involves step-wise fractionation of the chemical mixture, coupled to identification of the fraction with bioactivity leads to purification of the chemical responsible for the bioactivity of interest. When sufficiently pure, the unknown chemical can then be identified using advanced analytics. Another emerging possibility to estimate contribution of unexpected chemicals to mixture effects is to use nontargeted chemical analysis to get a view on additional compounds present in the mixture and link these to existing knowledge on their toxicological properties. Although this approach will be limited due to the limited knowledge on the toxicological properties of chemicals, the introduction of rapid bioanalytical methods and storage of analytical results in databases will increase the possibilities of this approach in the future. If a novel toxicologically relevant compound is identified, it can be added to the range of targeted compounds to be measured (Fig. 3; feedback loop no 2).

It should also be noted that, although the knowledge on toxicity pathways has greatly expanded, some chemical toxicities still are difficult to measure with modern bioanalytics, since no relevant *in vitro* assay has been developed, and thus targeted chemical analytics of those compound groups of special concern is required. If a positive result in a targeted analysis is not matched by a response in a bioassay, this will give a starting point to further improve the bioassay panel (Fig. 3; feedback loop no 1). In this way, an integrated system with chemical and biological analytics can be generated which is much stronger than the individual components.

The property of bioassays not to select chemicals to which they respond can give background issues. Non-specific toxicity to the cells when samples contain compounds that disturb their normal physiological environment, e.g. through strong effects on pH or osmolarity. For

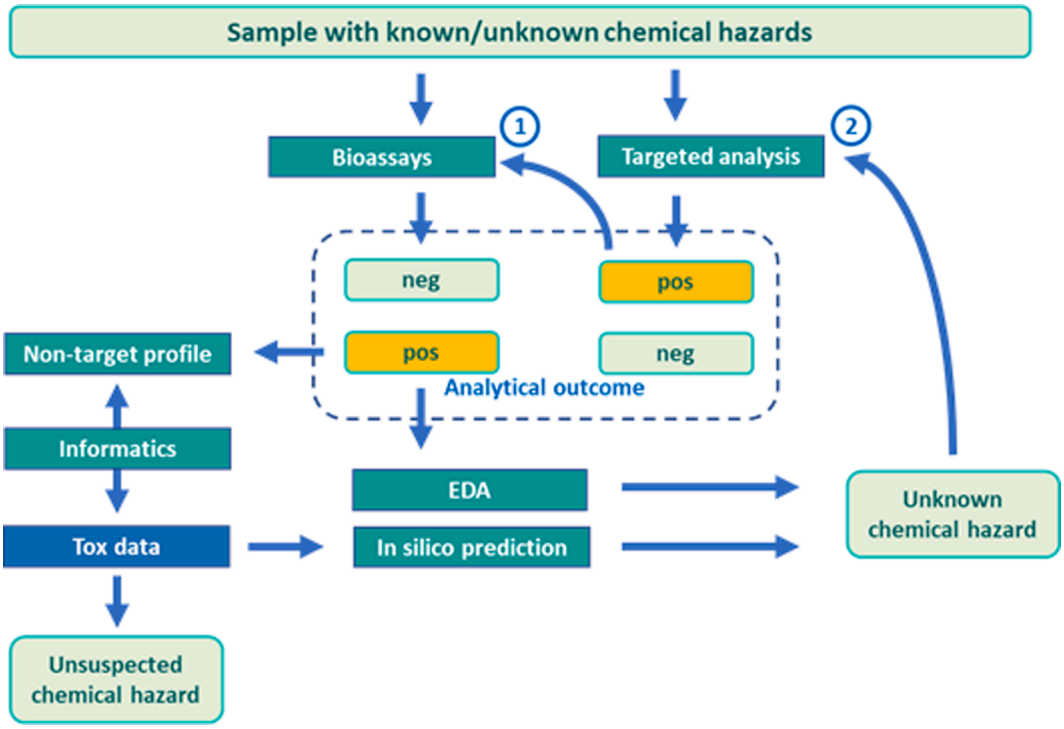

Fig. 3. Integrated analysis of food safety using chemical and biological methods. By using complementary possibilities of bio analytics and chemical analytics an integrated system can be built that can better assure protection against unknown and unexpected contaminants. Through feedback loops 1 and 2, a learing system is generated that will improve bio analytics and chemical analytics, respectively (see text for details).

this, preventive measures can be installed. Generally, a method is needed that extracts toxicologically relevant molecules, leaving behind large molecules like proteins and other irrelevant ones like salts.

In the evaluation of the test results, it should be kept in mind that for all chemicals, also very toxic ones, being alone or in mixtures, a threshold can be defined below which there is no concern. The absence of establishing a threshold for chemical carcinogen has resulted in far too many chemicals being assigned as carcinogens.[52] Also, food contains considerable background levels of natural compounds having toxicological properties, but only when consumed at high levels. This is something to which the human body is adapted, and to which elaborate defense mechanisms are in place that often are much more elaborated than that of short living organisms (Ames and Gold, 2000). Therefore, for any bioassay that is used to assess toxicity it is important to establish a threshold of activity below which there is no concern for adversity in humans. For several CALUX assays this has been defined already for various applications.[25,26,53,54]

The CALUX reporter gene assays have been designed for robustness, sensitivity and specificity and therefore are particularly suitable for analysis of complex mixtures. With the proper conditions in place a wide range of studies have been executed successfully in a wide range of complex and polluted mixtures. In the area of infant safety CALUX bioanalysis for instance has been applied to assess chemical exposure *in utero* (in cord blood samples) or after birth, e.g. through mother's milk, indoor house dust samples and plastic toys.[38,55–59]

In the area of infant food safety, the importance of exposure to dioxins has been studied. Some of the earlier studies have focused on the relationship between dioxin exposure and health outcomes in children. Based on animal studies and human exposure to known chlorinated dioxins developmental dioxin exposure has been linked to a range of disorders, including cancer in all tissues, and endocrine and reproductive effects among the most sensitive ones.[60] The impact of more comprehensive biologically active dioxin mixtures has been studied using the DR CALUX assay. It has been used successfully to assess correlations between developmental exposure to total biologically active stable AhR ligands and some health outcomes and relevant clinical markers. As a result of the limited studies performed to date it was shown that there indeed is a relationship between total dioxin load, hormone action and the ano-genital distance, particularly in boys.[55,61] Further studies are needed to explore the relationship with the suspected wide array of health effects linked to developmental exposure to biological active dioxin-like compounds. This is of particular importance in the light of the newly discovered relevant AhR ligands, including brominated dioxins and furans.[36-38] Since several effects are linked to modulating the effects of the sex steroids, more comprehensive studies should also consider direct interactions of chemical mixtures as present in food with sex steroid receptors. Tools for such studies, including suitable extraction methods have become available recently, and particularly the androgen receptor was found to be suppressed in its activity by chemical mixtures present in mother's milk.[59,62] A relationship of this suppression with possible health outcomes remains to be established.

Conclusions

- Chemical food safety assessment is hampered by the limited knowledge on toxicity of chemicals.
- Both bioanalytical and chemical analytical methods have witnessed huge progress in past decades.
- There are great opportunities to reduce uncertainties in monitoring programs through integrated assessment using recent developments in biological- and chemical analytics.
- Chemist and biologist should increasingly work together to improve the coverage of relevant toxic chemicals stepwise further to be monitored in infant food in an efficient integrated manner, taking advantage of the complementary opportunities of novel developments in chemical- and biological analytics.

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References

1 Drakvik E, Altenburger R, Aoki Y, Backhaus T, Bahadori T, Barouki R, Brack W, Cronin MTD, Demeneix B, Hougaard Bennekou S, van Klaveren J, Kneuer C, Kolossa-Gehring M, Lebret E, Posthuma L, Reiber L, Rider C, Rüegg J, Testa G, van der Burg B, van der Voet H, Warhurst AM, van de Water B, Yamazaki K, Öberg M, Å Bergman. Statement on advancing the assessment of chemical mixtures and their risks for human health and the environment. *Environ Int*. 2019;134, 105267. https://doi.org/10.1016/j.envint.2019.105267.

2 Anderson SE, Meade BJ. Potential health effects associated with dermal exposure to occupational chemicals. *Environ Health Insights*. 2014;8:51–62. 10.4137%2FEHI.S15258.

3 Kortenkamp A. Low dose mixture effects of endocrine disrupters and their implications for regulatory thresholds in chemical risk assessment. *Curr Opin Pharmacol*. 2014;19:105–111. https://doi.org/10.1016/j.coph.2014.08.006.

4 Miller MF, Goodson 3rd WH, Manjili MH, Kleinstreuer N, Bisson WH, Lowe L. Low-dose mixture hypothesis of carcinogenesis workshop: scientific underpinnings and research recommendations. *Environ Health Perspect*. 2017;125:163–169. https://doi.org/10.1016/10.1289/EHP411.

5 Carvalho RN, Arukwe A, Ait-Aissa S, Bado-Nilles A, Balzamo S, Baun A, Belkin S, Blaha L, Brion F, Conti D, Creusot N, Essig Y, Ferrero VE, Flander-Putrle V, Fürhacker M, Grillari-Voglauer R, Hogstrand C, Jonáš A, Kharlyngdoh JB, Loos R, Lundebye AK, Modig C, Olsson PE, Pillai S, Polak N, Potalivo M, Sanchez W, Schifferli A, Schirmer K, Sforzini S, Stürzenbaum SR, Søfteland L, Turk V, Viarengo A, Werner I, Yagur-Kroll S, Zounková R, Lettieri T. Mixtures of chemical pollutants at European legislation safety concentrations: how safe are they? *Toxicol Sci*. 2014;141:218–233. https://doi.org/10.1093/toxsci/kfu118.

6 Kienzler A, Bopp SK, van der Linden S, Berggren E, Worth A. Regulatory assessment of chemical mixtures: requirements, current approaches and future perspectives. *Regul Toxicol Pharmacol*. 2016;80:321–334. https://doi.org/10.1016/j.yrtph.2016.05.020.

7 Ames BN, Profet M, Gold LS. Nature's chemicals and synthetic chemicals: comparative toxicology. *Proc Natl Acad Sci USA*. 1990;87:7782–7786. https://doi.org/10.1073/pnas.87.19.7782.

8 LaKind JS, Lehmann GM, Davis MH, Hines EP, Marchitti SA, Alcala C, Lorber M. Infant dietary exposures to environmental chemicals and infant/child health: a critical assessment of the literature. *Environ Health Perspect*. 2018;126(9):96002. https://doi.org/10.1289/EHP1954. Erratum in: Environ Health Perspect. 2019 May; 127(5):59001.

9 National Research Council (USA). *Scientific Frontiers in Developmental Toxicology and Risk Assessment*. Washington, DC: NAS Press; 2000. https://www.nap.edu/catalog/9871/scientific-frontiers-in-developmental-toxicology-and-risk-assessment.

10 Lehmann GM, LaKind JS, Davis MH, Hines EP, Marchitti SA, Alcala C, Lorber M. Environmental chemicals in breast milk and formula: exposure and risk assessment implications. *Environ Health Perspect*. 2018;126(9):96001. https://doi.org/10.1289/EHP1953.

11 Arnold C. Baby steps forward: recommendations for better understanding environmental chemicals in breast milk and infant formula. *Environ Health Perspect*. 2019;127:64001. https://doi.org/10.1289/EHP4804.

12 Joesten MD, Hogg JL, Castellion ME. *The World of Chemistry: Essentials. Cengage Learning*. Boston MA, USA; 2006.

13 Dolan LC, Matulka RA, Burdock GA. Naturally occurring food toxins. *Toxins (Basel)*. 2010;2:2289–2332. https://doi.org/10.3390/toxins2092289.

14 Gribble GW. The diversity of naturally produced organohalogens. *Chemosphere*. 2003;52:289–297. https://doi.org/10.1016/S0045-6535(03)00207-8.

15 Van Asselt ED, Marvin HJP, Boon PE, Swanenburg M, Zeilmaker M, Mengelers MJB, van der Fels-Klerx HJ. *Chemical and Physical Hazards in the Dairy Chain*. Wageningen, RIKILT Wageningen UR (University & Research centre); 2016. https://library.wur.nl/WebQuery/wurpubs/fulltext/447318. RIKILT report 2016.003.

16 Ankley GT, Bennett RS, Erickson RJ, Hoff DJ, Hornung MW, Johnson RD, Mount DR, Nichols JW, Russom CL, Schmieder PK, Serrrano JA, Tietge JE, Villeneuve DL. Adverse outcome pathways: a conceptual framework to support ecotoxicology research and risk assessment. *Environ Toxicol Chem*. 2010;29:730–741. https://doi.org/10.1002/etc.34.

17 White SS, Birnbaum LS. An overview of the effects of dioxins and dioxin-like compounds on vertebrates, as documented in human and ecological epidemiology. *J Environ Sci Health C Environ Carcinog Ecotoxicol Rev*. 2009;27:197–211. https://doi.org/10.1080/10590500903310047.

18 Yoshioka W, Tohyama C. Mechanisms of developmental toxicity of dioxins and related compounds. *Int J Mol Sci*. 2019;20:617. https://doi.org/10.3390/ijms20030617. Published 2019 Jan 31.

19 Ames BN. Identifying environmental chemicals causing mutations and cancer. *Science*. 1979;204:587–593. https://doi.org/10.1126/science.373122.

20 Van der Burg B, Van der Linden SC, Man HY, Winter R, Jonker L, Van Vugt-Lussenburg B, Brouwer A. *A Panel of Quantitative CALUX® Reporter Gene Assays for Reliable High Throughput Toxicity Screening of Chemicals and Complex Mixtures. In "High Throughput Screening Methods in Toxicity Testing" (P. Steinberg, ed)*. New York: John Wiley and Sons, Inc.; 2013. ISBN 9781118065631.

21 Van der Linden SC, von Bergh A, Van Vugt-Lussenburg B, Jonker L, Brouwer A, Teunis M, Krul C, Van der Burg B. Development of a panel of high throughput reporter gene assays to detect genotoxicity and oxidative stress. *Mutation Res*. 2014;760:23–32. https://doi.org/10.1016/j.mrgentox.2013.09.009.

22 Van der Burg B, Pieterse B, Buist H, et al. A high throughput screening system for predicting chemically-induced endocrine disruption and sex organ deformities. *Reprod Toxicol*. 2015;50:95–103. https://doi.org/10.1016/j.reprotox.2014.11.011.

23 EURL-ECVAM DB-ALM Protocol n° 197: Automated CALUX reporter gene assay procedure. http://cidportal.jrc.ec.europa.eu/ftp/jrc-opendata/EURL-ECVAM/datasets/DBALM/VER3-0/online/DBALM_docs/197_P_Automated%20CALUX.pdf.

24 Sonneveld E, Jansen HJ, Riteco JAC, Brouwer A, Van der Burg B. Development of androgen- and estrogen-responsive bioassays, members of a panel of human cell line-based highly selective steroid responsive bioassays. *Toxicol Sci*. 2005;83:136–148. https://doi.org/10.1093/toxsci/kfi005.

25 Van Vugt-Lussenburg, B., Van der Burg, B., Besselink, H., Brouwer, A. (2013). DR CALUX®: a high-throughput screening assay for the detection of dioxin and dioxin-like compounds in food and feed. In "High Throughput Screening Methods in Toxicity Testing" (P. Steinberg, ed). John Wiley and Sons, Inc. New York. ISBN 9781118065631. Pp 553-546.

26 Besselink H, Brouwer A, Van der Burg B. Validation and regulatory acceptance of bio-based approaches to assure feedstock, water & product quality in a bio-based economy. *Ind Crops Prod*. 2017;106:138–145. https://doi.org/10.1016/j.indcrop.2016.11.026.

27 Commission Regulation (EU) No 252/2012 of 21 March 2012 laying down methods of sampling and analysis for the official control of levels of dioxins, dioxin- like PCBs and non-dioxin-like PCBs in certain foodstuffs and repealing Regulation (EC) No 1883 2006.

28 Commission Regulation (EU) No 278 2012 of 28 March 2012 amending Regulation (EC) No 152/2009 as regards the determination of the levels of dioxins and polychlorinated biphenyls.

29 OECD. *Test No. 458: stably transfected human androgen receptor transcriptional activation assay for detection of androgenic agonist and antagonist activity of chemicals. OECD Guidelines for the Testing of Chemicals, Section 4*. Paris: OECD Publishing; 2020.

30 OECD. *Test No. 455: performance-based test guideline for stably transfected transactivation in vitro assays to detect estrogen receptor agonists and antagonists. OECD Guidelines for the Testing of Chemicals, Section 4*. Paris: OECD Publishing; 2021.

31 ISO 19040-3. *Water Quality — Determination of the Estrogenic Potential of Water and Waste Water — Part 3: In Vitro Human Cell-Based Reporter Gene Assay*. 2022.

32 US-EPA. SW-846. *Test Method 4435: Screening for Dioxin-Like Chemical Activity in Soils and Sediments Using the Calux Bioassay and Toxic Equivalents (TEQs) Determinations*. 2022.

33 European Food Safety Authority (EFSA) panel on contaminants in the food chain (CONTAM). Risk assessment of chlorinated paraffins in feed and food. *EFSA J*. 2020;18. https://doi.org/10.2903/j.efsa.2020.5991.

34 Meziere M, Kratschmer K, Pe Rkons I, et al. Addressing main challenges regarding short- and medium-chain chlorinated paraffin analysis using GC/ECNI-MS and LC/ESI-MS methods. *J Am Soc Mass Spectrom*. 2020;31:1885–1895. https://doi.org/10.1021/jasms.0c00155.

35 Fernandes A, Rose M, Falandysz J. Polychlorinated naphthalenes (PCNs) in food and humans. *Environ Int*. 2017;104:1–13. https://doi.org/10.1016/j.envint.2017.02.015.

36 Budin C, Besselink H, Van Vugt-Lussenburg B, Man HY, Van der Burg B, Brouwer A. Induction of AhR transactivation by PBDD/Fs and PCDD/Fs using a novel human-relevant, high-throughput DRhuman CALUX reporter gene assay. *Chemosphere*. 2020;263:128086–128095. https://doi.org/10.1016/j.chemosphere.2020.128086.

37 Fernandes AR, Falandysz J. Polybrominated dibenzo-p-dioxins and furans (PBDD/Fs): contamination in food, humans and dietary exposure. *Sci Total Environ*. 2021;761, 143191. https://doi.org/10.1016/j.scitotenv.2020.143191.

38 Budin C, Petrlik J, Behnisch P, Besselink H, Van der Burg B, Brouwer A. Detection of high PBDD/Fs levels in children's toys and consumer goods using HR-GCMS and the cell-based DR CALUX® reporter gene assay. *Chemosphere*. 2020;251:126579–126589. https://doi.org/10.1016/j.chemosphere.2020.126579.

39 Van den Berg M, Birnbaum LS, Denison M, De Vito M, Farland W, Feeley M, Fiedler H, Hakansson H, Hanberg A, Haws L, Rose M, Safe S, Schrenk D, Tohyama C, Tritscher A, Tuomisto J, Tysklind M, Walker N, Peterson RE. The 2005 World Health Organization reevaluation of human and Mammalian toxic equivalency factors for dioxins and dioxin-like compounds. *Toxicol Sci*. 2006;93:223–241. https://doi.org/10.1093/toxsci/kfl055.

40 Dervilly-Pinel G, Guerin T, Minvielle B, Travel A, Normand J, Bourin M, Royer E, Dubreil E, Mompelat S, Hommet F, et al. Micropollutants and chemical residues in organic and conventional meat. *Food Chem*. 2017;232:218–228. https://doi.org/10.1016/j.foodchem.2017.04.013.

41 European Commission. Commission Regulation (EU) No 709/2014 of 20 June 2014 amending Regulation (EC) No 152/2009 as regards the determination of the levels of dioxins and polychlorinated biphenyls 2014, No 709/2014 of 20 June 2014 amending Regulation (EC) No 152/2009.

42 Ten Dam G, Pussente IC, Scholl G, Eppe G, Schaechtele A, van Leeuwen S. The performance of atmospheric pressure gas chromatography-tandem mass spectrometry compared to gas chromatography-high resolution mass spectrometry for the analysis of polychlorinated dioxins and polychlorinated biphenyls in food and feed samples. *J Chromatogr A*. 2016;1477:76–90. https://doi.org/10.1016/j.chroma.2016.11.035.

43 Dorne JLCM, Dorne JLCM, Bordajandi LR, Amzal B, Ferrari P, Verger P. Combining analytical techniques, exposure assessment and biological effects for risk assessment of chemicals in food. *TrAC, Trends Anal Chem*. 2009;28:695–707. https://doi.org/10.1016/j.trac.2009.03.008.

44 European Food Safety Authority (EFSA); Food and Agriculture Organization of the United Nations (FAO). Towards a harmonised total diet study approach: a guidance document. EFSA J2011, 9, https://doi.org/10.2903/j.efsa.2011.2450.

45 Hulin M, Sirot V, Vasseur P, et al. Health risk assessment to dioxins, furans and PCBs in young children: The first French evaluation. Food and chemical toxicology : an international journal published for the British Industrial. *Biol Res Assoc*. 2020;139, 111292. https://doi.org/10.1016/j.fct.2020.111292.

46 European Food Safety Authority (EFSA) panel on contaminants in the food chain (CONTAM), Knutsen H, Alexander J, Barregård L, Bignami M, Brüschweiler B, Ceccatelli S, Cottrill B, Dinovi M, Edler L, et al, European Food Safety Authority (EFSA) panel on contaminants in the food chain (CONTAM). Scientific opinion on the risk for animal and human health related to the presence of dioxins and dioxin-like PCBs in feed and food. *EFSA J*. 2018;16. https://doi.org/10.2903/j.efsa.2018.5333.

47 Simonnet-Laprade C, Bayen S, Le Bizec B, Dervilly G. Data analysis strategies for the characterization of chemical contaminant mixtures. Fish as a case study. *Environ Int*. 2021;155, 106610. https://doi.org/10.1016/j.envint.2021.106610.

48 Simonnet-Laprade C, Bayen S, McGoldrick D, et al. Evidence of complementarity between targeted and non-targeted analysis based on liquid and gas-phase chromatography coupled to mass spectrometry for screening halogenated persistent organic pollutants in environmental matrices. *Chemosphere*. 2022;293, 133615. https://doi.org/10.1016/j.chemosphere.2022.133615.

49 Hernandez-Mesa M, Escourrou A, Monteau F, Le Bizec B, Dervilly-Pinel G. Current applications and perspectives of ion mobility spectrometry to answer chemical food safety issues. *Trac-Trends Anal Chem*. 2017;94:39–53. https://doi.org/10.1016/j.trac.2017.07.006.

50 Brack W, Ait-Aissa S, Burgess RM, et al. Effect-directed analysis supporting monitoring of aquatic environments — an in-depth overview. *Sci Total Environ*. 2016;544:1073–1118. https://doi.org/10.1016/j.scitotenv.2015.11.102.

51 Pham Ngoc L, Helmus R, Ehlers AW, Swart K, Besselink H, de Rijke E, Dang Thi Cam H, Brouwer A, van der Burg B. Effect-directed analysis and chemical identification of agonists of peroxisome proliferator-activated receptors in white button mushroom. *Food Funct*. 2021;12(1):133–143. https://doi.org/10.1039/d0fo02071k.

52 Ames BN, Gold LS. Paracelsus to parascience: the environmental cancer distraction. *Mutat Res*. 2000;447:3–13. https://doi.org/10.1016/s0027-5107(99)00194-3.

53 Brand W, de Jongh CM, Van der Linden SC, et al. Trigger values for investigation of hormonal activity in drinking water and its sources using CALUX bioassays. *Environ Int*. 2013;55:109–118. https://doi.org/10.1016/j.envint.2013.02.003.

54 van Tongeren, T., Moxon, T. E., Dent, M. P., Li, H., Carmichael, P. L., & Rietjens, I. (2021). Next generation risk assessment of human exposure to anti-androgens using newly defined comparator compound values. Toxicol in Vitro, 73; 105132. https://doi.org/10.1016/j.tiv.2021.105132.

55 Papadopoulou E, Vafeiadi M, Agramunt S, Mathianaki K, Karakosta P, Spanaki A, Besselink H, Kiviranta H, Rantakokko P, KaterinaSarri Koutis A, Chatzi L, Kogevinas M. Maternal diet, prenatal exposure to dioxins and other persistent organic pollutants and anogenital distance in children. *Sci Total Environ*. 2013;461-462:222–229. https://doi.org/10.1016/j.scitotenv.2013.05.005.

56 Vafeiadi M, Agramunt S, Pedersen M, Besselink H, Chatzi L, Fthenou E, Fleming S, Hardie LJ, Wright J, Knudsen LE, Nielsen JK, Sunyer J, Carreras R, Brunborg G, Gutzkow KB, Nygaard UC, Løvik M, Kyrtopoulos SA, Segerbäck D, Merlo DF, Kleinjans JC, Vrijheid M, Kogevinas M. NewGeneris consortium. In utero exposure to compounds with dioxin-like activity and birth outcomes. *Epidemiology*. 2014;25:215–224. https://doi.org/10.1097/EDE.0000000000000046.

57 Pedersen M, Halldorsson TI, Autrup H, Brouwer A, Besselink H, Loft S, Knudsen LE. Maternal diet and dioxin-like activity, bulky DNA adducts and micronuclei in mother-newborns. *Mutat Res*. 2012;734:12–19. https://doi.org/10.1016/j.mrfmmm.2012.04.006.

58 Suzuki G, Takigami H, Nose K, Takahashi S, Asari M, Sakai S. Dioxin-like and transthyretin-binding compounds in indoor dusts collected from Japan: average daily dose and possible implications for children. *Environ Sci Technol*. 2007;41:1487–1493. https://doi.org/10.1021/es061907l.

59 Collet B, van Vugt-Lussenburg BMA, Swart K, Helmus R, Naderman M, de Rijke E, Eggesbø M, Brouwer A, van der Burg B. Antagonistic activity towards the androgen receptor independent from natural sex hormones in human milk samples from the Norwegian HUMIS cohort. *Environ Int*. 2020;143, 105948. https://doi.org/10.1016/j.envint.2020.105948.

60 Kogevinas M. Human health effects of dioxins: cancer, reproductive and endocrine system effects. *Hum Reprod Update*. 2001;3(7):331–339. https://doi.org/10.1093/humupd/7.3.331.

61 Vafeiadi M, Agramunt S, Papadopoulou E, Besselink H, Mathianaki K, Karakosta P, Spanaki A, Koutis A, Chatzi L, Vrijheid M, Kogevinas M. In utero exposure to dioxins and dioxin-like compounds and anogenital distance in newborns and infants. *Environ Health Perspect*. 2013;121:125–130. https://doi.org/10.1289/ehp.1205221.

62 Collet B, Desalegn A, Swart K, et al. Anti-androgenic compounds in breast milk and cryptorchidism among Norwegian boys in the HUMIS birth cohort. *Sci Total Environ*. 2022;803, 149746. https://doi.org/10.1016/j.scitotenv.2021.149746.

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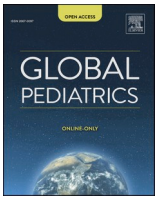

Role of food contact materials in the safety assessment of potentially hazardous substances and in the dietary exposure of infants☆

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ABSTRACT

The largest application area of food contact materials is the packaging of foodstuff, being an essential element in the preservation of the microbiological and sensory quality of the packaged goods. However, chemical compounds present in the packaging material, either intentionally added or non-intentionally present, may migrate into the food during packaging and storage. With a large variety of materials used in the production, food packaging requires safety assessments with respect to the migration of packaging compounds into the packaged goods. The present article deals with the safety assessment of potential migrants from food contact materials, approaches to migration testing and summarizes European food regulatory requirements with special focus on infants' dietary exposure.

Introduction

Food may contact a broad range of different materials or articles during its production, storage, preparation and serving, before the food is finally consumed.

By legal definition of the European Framework Regulation (EC) No 1935/2004,[1] these food contact materials (FCMs) are "a) materials intended to be brought into contact with food, b) materials that are already in contact with food or c) materials that can reasonably be expected to be brought into contact with food or transfer their constituents to the food under normal or foreseeable use".

FCMs comprise of a large variety of materials from glass, paper/cardboard and metals to polymers like polyethylene terephthalate, polypropylene, styrene and silicone or combinations thereof. Although food contact materials and articles have an important function in daily life (e.g. to prolong the shelf life of food, facilitate storage, consumer information), most materials are chemically not fully inert. In contact with food, substances present in these materials, either intentionally added or non-intentionally present, may be released from the food contact material into the food by a process called migration.

Scientifically, migration can be described as the mass transfer from a packaging material into food caused by a concentration gradient between the packaging and the filling good. The extent of the migration process is dependent on several parameters including the intrinsic properties of the food contact material, the physicochemical properties of the migrating substance, the characteristics of the food and the conditions of food contact. Migration takes place until an equilibrium throughout the whole system is reached. The process, following well-known laws of diffusion, can be calculated precisely.

Consequently, consumers of all ages are indirectly exposed to chemicals present in food contact materials via the migration process.

Hence, dietary exposure to potentially hazardous chemicals of various origins has been in the public focus in the past years.[2–5] Although this matter should be monitored closely for all age groups, infants are particularly vulnerable to impairments induced by contaminated foodstuff as they have a higher intake of food per kg body weight compared to adults. Hence, several studies have been performed[6–8] focussing on the exposure of children to food-related contaminants.

In the more recent and comprehensive "Infant Total Diet Study" (iTDS)[8,9] carried out and published by Anses in 2016, the health risks associated with the potential presence of a wide variety of chemical contaminants in food (metal and mineral trace elements, polychlorinated dibenzo-p-dioxins and polychlorinated dibenzofurans (PCDD/Fs), polychlorinated biphenyls (PCBs), perfluoroalkyl acids (PFAAs), brominated flame retardants, mycotoxins, phyto-oestrogens, heat-induced compounds, pesticide residues and food additives) were

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assessed. Out of the nine chemicals or chemical groups of high concern, none of these were likely to originate from materials used for food packaging. Bisphenol A only, a component of widespread use in the fabrication of food contact materials, was considered a substance for which a risk cannot be ruled out. Other chemicals under investigation that originated in food contact materials were deemed as components with "tolerable or acceptable risks" or substances with no feasible risk assessment.

To protect the consumer's health and the quality of the food from FCM-related hazards, binding legal rules and requirements have been established in Europe for over 40 years dating back to the definition of safety requirements of FCMs as early as in the 1976 Council Directive 76/893/EEC.

Any material or article to come into contact (regardless of the material's identity) is subject to the provisions of the European Framework Regulation (EC) No 1935/2004, which establishes the basic principles of safety and risk management concerning food contact materials, providing a harmonised legal EU framework.

The general safety requirements are laid down in Article 3 of the Framework Regulation. According to this, "materials and articles, including active and intelligent materials and articles, shall be manufactured in compliance with good manufacturing practice so that, under normal or foreseeable conditions of use, they do not transfer their constituents to food in quantities which could:

(a) endanger human health; or
(b) bring about an unacceptable change in the composition of the food; or
(c) bring about a deterioration in the organoleptic characteristics thereof."[1]

The Framework Regulation also authorizes the adoption of specific requirements for individual materials listed in Annex 10 of the Regulation in order to further harmonize individual materials at EU level.

The most comprehensive specific EU measure, Commission Regulation (EU) No 10/2011,[10] is in place for materials and articles made of plastics.

Food contact materials composed of plastics or including a plastic layer play a major role in the infant and baby food area with products ranging from plastic bottles or spoons to multilayer laminates for the packaging of cereal-based food and milk powder.

Regulation (EU) No 10/2011, commonly known as the *Plastics Regulation*, sets forth rules on the composition of plastic FCMs and establishes a Union List of substances that are permitted to be used in the manufacture of plastic FCMs. This Regulation also sets out restrictions on the use of these substances and defines rules to determine the compliance of plastic materials and articles made thereof. The Regulation is amended on a regular basis.

As for substances used to manufacture articles intended for young children, tightened rules and conditions may apply: By means of example, 2,2-bis(4-hydroxyphenyl)propane (Bisphenol A) is an authorized monomer used to produce polycarbonate plastics and is also used for coatings for food and beverage cans. However, according to the Plastics Regulation, Bisphenol A is "not to be used for the manufacture of polycarbonate infant feeding bottles" and is "not to be used for the manufacture of polycarbonate drinking cups or bottles which, due to their spill proof characteristics, are intended for infants and young children". Bisphenol A has been in the focus of scientists, toxicologists and legislators for many years due to potential adverse health effects.[11] Back in 2013, France adopted a law banning the use of Bisphenol A for food contact materials for children below the age of three years. Amending the law in 2015, the ban of Bisphenol A was extended to all food contact materials on the French market.[12]

In 2015, the European Food Safety Authority (EFSA) has derived a temporary tolerable daily intake (TDI) of 4 μg per kg body weight per day following an evaluation on potential health risks of Bisphenol A in foods.[13] The TDI is defined as the estimated amount of a substance that can be consumed daily over the entire life without a significant risk to human health.[14] Based on a recent comprehensive re-evaluation, EFSA discusses a tolerable daily intake of 0.04 ng per kg body weight per day, which is 100 000 times lower compared to the previous assessment performed in 2015.[15]

An essential instrument to ensure the safety of plastic materials is the use of migration limits. These limits specify the maximum amount of substances allowed to migrate into foodstuff. Commission Regulation (EU) No 10/2011 establishes these maximum amounts in the form of "Specific Migration Limits" (SMLs). These SMLs are derived by EFSA based on the toxicity data of each specific substance. To ensure the overall quality of the plastic, two criteria have to be met: in the first place, the overall migration into food of all substances together must not exceed the "Overall Migration Limit" (OML) of 10 mg/dm^2 contact surface of the material (or 60 mg/kg food in specific cases). Secondly, the specific migration limit of each constituent present in the material and listed in Annex I of the Plastics Regulation may not be exceeded either.

SMLs of substances intended to be used in articles for young children or infants may be significantly lower than in articles intended to be used for adults: As for the plasticizer ESBO (epoxidized soybean oil) used in PVC materials, the specific migration limit is lowered from 60 mg/kg to 30 mg/kg when applied in PVC gaskets used to seal glass jars containing infant formulae, follow-on formulae, processed cereal based foods or baby foods for infants and young children.

In the context of specific migration (limits), the Plastics Regulation provides detailed migration testing rules. Although migration testing in the food prevails, migration is usually tested using '*simulants*' due to analytical challenges caused by the food matrices. These simulants, as outlined in Table 1, shall mimic the characteristics of real food (due to similar physicochemical properties of food and simulant) and are therefore representative for a certain food category.

The migration testing is done under standardised time and temperature conditions, representative for a certain food packaging application and shall cover the time and temperature of food contact under real-life application.

As an example, for the specific migration testing of a plastic pouch that is intended for the packaging of vegetables in the form of purée for long term storage at room temperature, the migration experiment shall be performed with food simulants C and B (if the pH value of the product is below 4.5) for 10 days at a temperature of 60 °C according to the provisions of Regulation (EU) No 10/2011.

Following the migration contact, specific analytical methods are applied in order to determine the concentration of the migrants in the obtained migration solution.

Depending on their physicochemical properties (e.g. volatility and polarity), the concentrations of the respective analytes in the migration solutions can be quantified using a variety of analytical techniques, ranging from headspace and liquid injection flame ionization gas chromatography/ mass spectrometry (GC-FID/MS) to liquid

Table 1
Assignment of food simulants to food according to Regulation (EU) No 10/2011.

Food simulant	Abbreviation	Food category (examples)
10% Ethanol	Food simulant A	Aqueous food (pH above 4.5)
3% Acetic acid	Food simulant B	Acidic food (pH below 4.5)
20% Ethanol	Food simulant C	Clear drinks, Fruit or vegetable pureé
50% Ethanol	Food simulant D1	Oil in water emulsions (milk products), cloudy drinks
Vegetable oil	Food simulant D2	Fatty food
poly(2,6-diphenyl-p-phenylene oxide)	Food simulant E	Dry food (cereal)

chromatography-mass spectrometry (LC-MS) for organic compounds and atomic absorption spectrometry (AAS) and inductively coupled plasma (ICP) analyses for inorganic compounds.

However, in addition to intentionally added substances (IAS), FCMs made of plastics may contain substances that are not used intentionally and that are not listed as authorized monomers or additives in Commission Regulation (EU) No 10/2011. These substances are commonly referred to as NIAS (non-intentionally added substances) and must also comply with the general safety requirements of Article 3 of the European Framework Regulation 1935/2004.[1] Assessment of these components shall be performed in accordance with internationally recognised scientific principles on risk assessment (Article 19 of Regulation (EU) No 10/2011).

Unlisted substances found in plastic FCMs may include impurities in the starting materials used to make the plastic, reaction intermediates formed during the polymerisation processes, decomposition or reaction products formed during polymerisation, substances formed during thermal processing of package fabrication and chemicals that are applied to the non food contact side (like printing inks) that might be transferred to the food contact side. In such cases, non target screening assays are commonly used that are suitable for detection, identification and quantification of a wide range of potentially migratable chemicals differing in structure, polarity and molecular weight. Such screening analyses[16] include, among others, the analytical techniques outlined in Table 2.

For the assessment of NIAS for which no other basis for evaluation is available, the threshold for the migration of unauthorised substances beyond a functional barrier may be used in accordance with Article 13 of Regulation (EU) No 10/2011. Accordingly, the migration must not exceed a limit of 10 μg/kg (ppb) of food. However, this limit does not apply to substances classified as "mutagenic", "carcinogenic" or "toxic to reproduction" (CMR) according to the criteria set in Regulation (EC) No 1272/2008.

In a conservative approach for risk assessment of genotoxic substances, EFSA has derived a threshold limit of 0.15 μg per kg in food for an adult person, based on a worst-case scenario of a person with a default body weight of 60 kg, a consumption of 1 kg foodstuff per day and taking into account the TTC (Threshold of Toxicological Concern) of 0.0025 μg per kg body weight per day below which the exposure to an unknown contaminant would have negligible consequences for the human health.[17]

For infants, a worst-case scenario based on 5 kg body weight and a consumption of 0.75 kg of food (baby bottle contents such as reconstituted milk formula and water) each day,[18] a much lower threshold limit of 0.017 μg per kg in food can be calculated when taking into account the aforementioned TTC (Threshold of Toxicological Concern) of 0.0025 μg per kg per day.

However, this TTC approach is only intended for substances where no specific toxicological data are available. In the case of substances with an existing toxicological profile, evaluations from other fields of application can be referred to for the risk assessment.[19]

Detection, quantification and regulatory assessment of both intentionally and non-intentionally added substances of high concern and low threshold limits can present a challenging task for both analytical laboratories and supervisory authorities. Analyses of individual components or complex mixtures might require time-consuming and complex analytical techniques such as high resolution gas chromatography mass spectrometry (HR-GC–MS), liquid chromatography Fourier transformation mass spectrometry (LC-FT MS) and two-dimensional gas chromatography mass spectrometry (GCxGC MS).

Hence, integrated approaches to enable the identification, assessment, detection and mitigation of safety risks raised by food contact materials have become increasingly important in recent years.[20–22] At present, safety risks brought about by FCMs are studied extensively within the framework of the SAFFI project,[23] a multinational cooperation to benchmark the main safety risks throughout the food chain of infants' food. With partners from industry and university research centres and funded by the European Union, joint studies such as SAFFI may, in turn, make a valuable contribution to food safety if their results are translated into the legal framework - to distinguish those substances of very high concern from the wide range of substances of minor importance, thus making food contact materials safe in every respect, but in particular for young children and infants.

Table 2
Analytical approaches to determine Specific Migration.

Type of substance	Predominant Analytical Method	Examples
Very volatile organic compounds (b.p. <50 −100 °C)	Headspace, SPME or purge and trap with GC with FID or MS detection	Monomers, residual solvents
Volatile organic compounds (50- 100 °C < b.p. < 250° −300 °C)	Liquid injection (split, splitless, PTV, on-column etc.) with GC with FID or MS detection	Plasticizers, glycols, additives
Semivolatile organic compounds 250–300 °C < b.p. <~400 °C)	Liquid chromatography with diode array, fluorescence or MS detection	Antioxidants, additives, oligomers
Inorganic compounds	Atomic Absorption spectrometry (AAS) or inductively coupled plasma (ICP) with MS detection	Heavy metals

b.p.: boiling point.

Declarations of Competing Interest

None

References

1 Regulation (EC) No 1935/2004, Regulation of the European Parliament and of the Council of 27 October 2004 on materials and articles intended to come into contact with food and repealing directives 80/590/EEC and 89/109/EEC. https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX%3A02004R1935-20210327 (accessed 24 January 2022).
2 Moy GG. Total diet studies Protecting the public from chemical hazards in food. *Ernährungs Umschau*. 2015;62(8):132–139. https://doi.org/10.4455/eu.2015.021. https://de.wikipedia.org/wiki/Total-Diet-Studie (accessed 24 January 2022).
3 What's in your food – the BfR MEAL Study, in: BfR Annual Report 2015. https://www.bfr.bund.de/en/publication/annual_reports-62595.html (accessed 24 January 2022).
4 U.S. Food & Drug Administration, Total Diet Study. https://www.fda.gov/food/science-research-food/total-diet-study (accessed 24 January 2022).
5 27th Australian Total Diet Study. https://www.foodstandards.gov.au/publications/Pages/27th-Australian-Total-Diet-Study.aspx (accessed 24 January 2022).
6 Costopoulou D, Vassiliadou I, Leondiadis L. Infant dietary exposure to dioxins and dioxin-like compounds in Greece. *Food Chem. Toxicol.* 2013;59:316–324.
7 Milićević DR, Milešević J, Gurinović M, et al. Dietary exposure and risk assessment of aflatoxin m1 for children aged 1 to 9 years old in Serbia. *Nutrients*. 2021;13(12):4450. https://doi.org/10.3390/nu13124450.
8 Anses, Infant Total Diet Study (iTDS). https://www.anses.fr/en/content/infant-total-diet-study-itds (accessed 24 January 2022).
9 Hulin M, Bemrah N, Nougadère A, Volatier JL, Sirot V, Leblanc JC. Assessment of infant exposure to food chemicals: the French total diet study design. *Food Addit Contam Part A*. 2014;31(7):1226–1239. https://doi.org/10.1080/19440049.2014.921937.
10 Regulation (EU) No 10/2011, Commission Regulation (EU) No 10/2011 of 14 January 2011 on plastic materials and articles intended to come into contact with food. https://eur-lex.europa.eu/legal-content/EN/TXT/?qid=1642420303340&uri=CELEX%3A32011R0010 (accessed 17 January 2022).
11 Braun JM, Hauser R. Bisphenol A and children's health. *Curr Opin Pediatr*. 2011;23(2):233–239. https://doi.org/10.1097/MOP.0b013e3283445675.
12 Food Packaging Forum, France bans BPA. https://www.foodpackagingforum.org/news/france-bans-bpa (accessed 24 January 2022).
13 EFSA CEF Panel (EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids). Scientific opinion on the risks to public health related to the presence of bisphenol A (BPA) in foodstuffs. *EFSA J*. 2015;13(1):3978. https://doi.org/10.2903/j.efsa.2015.3978.
14 BfR, TDI. https://www.bfr.bund.de/en/a-z_index/tdi-187181.html (accessed 24 January 2022).
15 EFSA, Bisphenol A: EFSA draft opinion proposes lowering the tolerable daily intake. https://www.efsa.europa.eu/en/news/bisphenol-efsa-draft-opinion-proposes-lowering-tolerable-daily-intake (accessed 24 January 2022).
16 Veraart R, Coulier L. Compliance testing of chemical migration from food contact materials. In: Barnes KA, Sinclair R, Watson DH, eds. *Chemical Migration and Food Contact Materials*. Cambridge, UK: Woodhead; 2007:180–202.

17 EFSA. Guidance on the use of the threshold of toxicological concern approach in food safety assessment. *EFSA J*. 2019;17(6):5708. https://doi.org/10.2903/j.efsa.2019.5708.
18 EFSA. Scientific opinion on the recent developments in the risk assessment of chemicals in food and their potential impact on the safety assessment of substances used in food contact materials. *EFSA J*. 2016;14(1):4357. https://doi.org/10.2903/j.efsa.2016.4357.
19 EFSA, Threshold of toxicological concern. https://www.efsa.europa.eu/en/topics/topic/threshold-toxicological-concern (accessed 24 January 2022).
20 Lenzner A, Vieth B, Luch A. CMR substances in consumer products: from food contact materials to toys. *Arch Toxicol*. 2018;92:1663–1671.
21 Kato LS, Conte-Junior CA. Safety of plastic food packaging: the challenges about non-intentionally added substances (NIAS) discovery, identification and risk assessment. *Polymers*. 2021;13:2077. https://doi.org/10.3390/polym13132077 (Basel).
22 Tsochatzis ED, Lopes JA, Hoekstra E, Emons H. Development and validation of a multi-analyte GC-MS method for the determination of 84 substances from plastic food contact materials. *Anal Bioanal Chem*. 2020;412:5419–5434. https://doi.org/10.1007/s00216-020-02758-7.
23 SAFFI, Safe food for infants in the EU and China. https://www.saffi.eu/ (accessed 24 January 2022).

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Hazard control through processing and preservation technologies for enhancing the food safety management of infant food chains☆

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ABSTRACT

Food safety of infant foods is of paramount importance due to the high vulnerability of this population. Food business operators guarantee safety of the products they put on the market by implementing control measures that prevent, eliminate, reduce, or keep relevant physical, microbiological and/or chemical hazards to an acceptable level. It is essential that the efficacy of control measures is validated during process design and on-line monitoring and periodic verification activities are implemented during the commercial production. Infant foods are usually processed through conservative thermal treatments that guarantee food safety but usually negatively affect the organoleptic properties, reduce vitamin and nutrient contents. Heat treatments can trigger the formation of process induced contaminants.The EU-SAFFI project aims to set and validate new/emerging processing and preservation technologies (i.e. pulse combustion drying, radiofrequency and high pressure processing) to control key contaminants and pathogens as efficiently as classical technologies and to provide a decision support system to manage food safety in infant food. This article describes how the project is addressing the research to control (i) furan, a key process-induced toxicant in infant food whose formation is induced during thermal preservation processes of foods such as infant formulas and jarred baby foods, (ii) tropane alkaloids, natural contaminants found in agricultural crops due to accidental harvesting of weeds whose presence above the maximum regulated levels have been documented in cereal-based foods for infants and children and (iii) different vegetative and spore forming bacterial pathogens, a group of microbiological hazards with product and technology-specific relevance and resistance.

1. Introduction

Within the food sector, infant foods are particularly relevant for food safety issues because of the high vulnerability of the target population and the wide variety of commodities. Processing and preservation processes are applied by manufacturers to treat different types of infant food.[1] Instead of reacting to foodborne outbreaks, the current regulation requires the food business operators to have a preventive systematic control of the processes implemented, namely the pre-requisite programs and Hazard Analysis and Critical Control Points (HACCP). The HACCP approach provides flexibility with the selection of control measures, enabling the accommodation of changes in food formulation, technology developments and innovations to meet the market and consumer demands. The implementation of HACCP-based programmes is audited by inspection agencies and regulatory authorities and enhances the food safety of produced food and promotes international trade by increasing confidence in food safety system.[2] The HACCP-based approaches focus on hazard control at specific critical control points (CCPs) applied to specific operations within the production process, storage and handling. Food business operators are responsible to obtain

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scientific and technical evidence that specific control measures (alone or combined) are effectively controlling the relevant identified hazards, either of physical, microbiological and/or chemical nature. Within the food safety management systems, control measures aim to prevent, eliminate, reduce, or keep a relevant hazard to an acceptable level that it is not likely to pose a public health risk under normal conditions of distribution and storage.[3,4]

In this context, validation of a control measure acquires a paramount importance and needs to be performed during the design of the process, prior to its rutinary implementation. A validation study aims to provide the evidence that the process and product parameters associated with the control measure, if properly implemented, can control the hazard under a worst-case scenario.[5] Conducting validation studies may be resource intensive. Several complementary approaches can be useful, including (i) the scientific and technical literature, international standards and recognised guidelines, gathering data from previous validation studies and the historical knowledge of the proper performance of the control measure; (ii) scientifically sound experimental approaches based on microbial challenge testing (either with pathogenic microorganisms or qualified surrogates), pilot plant or industrial trials designed and carried out to mimic process conditions and (iii) mathematical models that integrate scientific data on how product and processing factors affect the relevant hazards enabling the assessment on the ability of one or a combination of control measures to achieve the intended food safety outcome. Available data and mathematical models serve of part of the evidence that is being collected in a validation study, and often do not reflect the actual situation good enough, and one will precede with a challenge study to validate the control measure.

Once the validated control measure is implemented, it requires a real-time monitoring during processing of each batch though a planned sequence of measurements of control parameters that confirms that the control measure is operating as intended. In addition to monitoring, periodic verification is needed a posteriori, after the processing, to determine whether the control measure has been operating as intended.[3] Verification activities include reviewing production, maintenance and calibration reports, environmental sampling and product testing, amongst others. Sampling plans and analytical methodologies are frequently described in guidelines and regulations about microbiological criteria and maximum contaminant levels. The relevance of end-product testing as verification procedure depends on the type of product and process.[6]

Within the EU research project funded by Horizon Europe 2020, SAFFI (Safe Food for Infants in the EU and China, https://www.saffi.eu/), focused on foods for infants and children, a work package is currently in progress to develop a prototype of a decision support system for hazard control. The work package also aims to set and validate emerging processing and preservation technologies to control key contaminants and pathogens as efficiently as classical technologies and to set efficient sampling strategies at operational (infant food companies) and governmental (food safety authorities) level to enhance the effectiveness of food safety management options.

2. Infant food and new/emerging processing technologies

Infant food manufacturing involves one or more heating steps of raw materials or intermediate products. The end-products are generally microbiologically shelf stable. Shelf stability is achieved by the low water activity (a_w) of the end-product in the case of powdered infant formula and infant cereals or thanks to the microbial lethality achieved with the heating steps in case of ready-to-eat meals and fruit-based purees. To ensure the inactivation of spoilage and pathogenic microorganisms and enzymes, infant foods are usually processed using conservative time/temperature combinations as thermal treatments. Besides microbial inactivation, heating contributes to soften the raw materials. However, due to the intensive heating, less desirable flavour and colour changes may appear and the vitamin and nutrient contents are reduced.[7] Moreover, intensive thermal treatments trigger the formation of process induced contaminants such as acrylamide, furans, hydroxymethyl furfural (HMF), etc.[8]

The SAFFI project deals with four infant food chains chosen as case studies to cover infant nutrition while encompassing very different hazards (microbial, natural toxins, process contaminants and food packaging migrants), ingredients (of plant and animal origin), processing (including new or emerging technologies) and control steps. The case-studies include: (i) powdered infant formula, (ii) infant cereals, (iii) sterilized vegetables mixed with fish or meat meal and (iv) fruit-based purees. Besides the classical thermal technologies spray and drum drying processes, the pulse combustion drying (PCD) will be explored for case-studies (i) and (ii). The radiofrequency (RF) heating will be investigated as an alternative to classical retort sterilisation, while high pressure processing (HPP) will be the non-thermal alternative for pasteurisation.

2.1. Pulse combustion drying

Spray drying is the most commonly used technology to dry liquid foods, such as infant formula. Spray drying is very intensive on energy use, having a large impact in cost and sustainability. The use of direct heating systems has been eliminated due to the presence of undesirable gases after the fuel combustion, and only low efficient indirect heating is now being used for food processing. Pulse Combustion Drying (PCD) is a new drying technology that uses an engine to produce hot waves of air (3000 waves/minute, at 350–400 °C) that cause a very fast drying of the liquid droplets, resulting in a high-quality dried product, without the gas problems of the traditional direct heating systems.[9,10] Another advantage of PCD is that it is more efficient than indirect heating spray drying for three main reasons. First, PC dryer can handle higher solid loading and viscosity than a conventional spray dryer. High viscosity fluids (such as infant cereals) are not possible to dry by spray drying, and a drum dryer must be used, with potential detrimental effects on final product quality. Secondly, in PCD the heat transfer is very high and drying is completed in a shorter time and at higher temperatures (air temperature between 350 and 500 °C) than classical drying technologies. As a consequence, a higher drying efficiency is achieved with PCD (20% less energy) compared with indirect heating spray drying.[10] Finally, due the smaller size of the equipment and lack of mobile parts inside the drying chamber, PCD requires lower investment and maintenance than classical spray drying equipment. Although the PCD technology is already working under industrial conditions it is not used for infant food production yet. Moreover, there is a need for scientific studies about thermal damage markers, accumulation of process contaminants and the microbial inactivation achieved to substantiate the beneficial potential within the food sector.

2.2. Radiofrequency

Radiofrequency (RF) heating is a technology based on the absorption of electromagnetic waves by a dielectric material, similarly to microwaves in the 10–300 MHz range. When compared to microwaves, RF has a greater penetration into the product and better heating uniformity, minimizing irregular heating or hot spots.[11] Moreover, there is a minimal dirt deposition (less water and cleaning agents are needed for cleaning) due to the removal of hot heat transfer surfaces.[12] RF has a high heating efficiency (>80%) without losses to the surrounding environment.[13] As the heating rate is faster than in conventional heating technologies (such as UHT or retort), nutrient, vitamin and flavour damage is minimized[14] and the organoleptic characteristics of the product can be improved.[15] Different applications of RF for pasteurization can be found in the literature. In liquid or semi-liquid products, applications include orange juice,[16] tomato homogenate[17] and fish soup.[15] Some studies can also be found on the successful application of this technology to infant foods (milk powders) for the inactivation of

Cronobacter sakazakii.[18]

2.3. High pressure processing

HPP is a non-thermal process applied once the product is in its final package to inactivate vegetative forms of pathogenic and spoilage microorganisms.[19] HPP is an alternative to heat processing that provides food safety and extended shelf-life while retaining nutrients and bioactive compounds.[20] Being non-thermal, HPP does not trigger the formation of thermal process contaminants that are formed at high temperature, such as 5-hydroxymethylfurfural (5-HMF).[21] In this sense, a study conducted on commercial heat-treated infant food showed that all tested jams contained 5-HMF, from traces to 72 mg/kg, as well as fruit-based food, whose contents ranged from not detectable to 8 mg/kg.[22] On the contrary, results from SAFFI showed that HPP (600 MPa for 6 min) can be used as non-thermal preservation technology, alternative to heat treatment, in apple and banana-based infant food without inducing the formation of 5-HMF (unpublished data).

It is worth to highlight that consumers perceive HPP as a natural process and more environmental friendly than conventional processes.[23] HPP innovative foods aim to meet consumer demands for minimally processed healthy products with better flavour and fresher appearance compared with the heated ones.[24] HPP of food is an emerging trend in the market of ready-to-eat food, including some infant food products (mainly acidic fruit purees). The current regulation sets microbiological criteria for ready-to-eat infant foods applying zero tolerance against *Listeria monocytogenes*, namely no detection for the pathogen in 10 samples of 25 g (Commission Regulation[25]No 2073/2005 of 15 November 2005 on microbiological criteria for foodstuffs 2005). Several guidance documents published by public health authorities recognise HPP as a technology contributing to the control of this pathogen in ready-to-eat food.[26], [27], [28], [29] HPP is also recognised as a suitable technology to design a control measure to inactivate the pertinent pathogens (e.g. *E. coli* O157:H7, *Salmonella* spp.) in fruit juices.[30]

To develop the case studies listed above, the SAFFI approach consists in the quantification of the fate of key chemicals (degradation, generation, migration) and the behaviour of the identified microbiological hazards (growth or inactivation) along the four infant food chains selected as case studies. Following, few examples on how the research is addressed are described.

2.4. Control of furan, a key process-induced toxicant in infant food

Furan is a highly volatile molecule, which is classified as a possible carcinogen for humans.[31]. The European Food Safety Authority[32] reported a high furan exposure in infants ϕ0.09–0.22 μg/kg.b.w/d) and toddlers 0.05–0.31 μg/kg.b.w/d). For toddlers, most of the exposure is related to consumption of jarred baby foods, fruit juices, milk-based products and cereals-based products; whereas for infants, the major sources of exposure would be infant formula and jarred baby foods. In 2016, ANSES published a report on the total diet for infants and toddlers.[33] In children under 3 years old, dietary exposure to furan was considered to be of concern, especially for breakfast cereals, jarred vegetable with or without meat or fish, placing furan at the top of the list of priority hazard.[34],[35] In the case of infant foods, furan can be formed from amino acids, carotenoids, ascorbic acid, lipids[33],[35] and carbohydrates[36] during thermal preservation processes such as pasteurization or sterilization (Fig.1). management rely on the availability of robust and reliable methods for quantifying furan in infant food in laboratories and monitoring on production lines. It would also require mitigation strategy to control its formation during industrial processes and suitable recommendations to limit child domestic exposure.

Today, furan is generally quantified by solid phase micro-extraction (HS-SPME) coupled with gas chromatography-mass spectrometry (GC–MS).[37] In HS-SPME, a polymer attached to metal rod adsorbs the volatile molecules present in the headspace of the sample, and in particular the furan, which are then quantified by GC–MS. HS-SPME has the advantage of concentrating the analyte, which is very interesting when it is present at trace levels.[33] In counterpart, the competition phenomena between volatile species[36] resulting from the limited adsorption surface of the fibers[37] together with their poor manufacture reproducibility leads to a relatively poor precision of quantification based on this extraction mode. Two alternative options can be considered to overcome this problem. The first one is to circumvent the competition phenomena by using an extraction technique with similar advantages but implementing a fibre with a larger adsorption surface. With this in mind, Dynamic Headspace (DHS,[38]) is now automated and may represent a first valuable alternative to HS-SPME. The second option relies on using static headspace (SHS) extraction. Sampling is done with a syringe inserted at thermodynamic equilibrium between the headspace and the sample. As it does not involve an adsorbent trap, this method is reproducible because there is no competition phenomenon or composition variability. On the other hand, it does not allow the concentration of the analyte in the headspace inducing a significant drop in extraction yield. Presently, this limitation could be compensated by using modern mass spectrometer like Q Exactive-HRMS-Orbitrap®, which are much more sensitive than MS quadrupole.

The three previous analytical options are within the reach of reference laboratories but remain too costly and cumbersome to implement for routine self-monitoring by manufacturers. In this view, a non-targeted approach could be developed to determine compounds that are markers of furan formation, which would be much easier to analyse. By analogy with metabolomics, which consists in studying the variations in the metabolism of a biological organism in response to a stress factor, this new approach might consist in studying the variations in chemical reactions within a matrix in response to different process conditions and might be named procedomics. In the case of thermal preservation processes involved in the generation of furan, it could consist in identifying robust volatile marker of the generation of furan in the volatile fraction of transformed infant products.

Both furan quantification and procedomics would enable to study the effectiveness of furan mitigation by so-called non-thermal and alternative heating processes such as HPP and RF. This include determining the levels of furan generated during these processes, optimizing the influencing parameters and finally, benchmarking mild processes against conventional ones in terms of furan mitigation. It will also rely on setting appropriate recommendations in order to limit domestic exposure to furan via infant food consumption, by identifying hazardous practices by means of surveys on consumption practices (e.g. reheating with water bath, microwave), then determining the levels of furan generated by these practices, assess the risk of exposure in order, *in fine*, to propose the best practices to recommend.

2.5. Control of tropane alkaloids, natural contaminants from weeds

Tropane alkaloids are a class of plant toxins with more than 200 compounds that occur in a wide range of plants in the Solanaceae, Brassicaceae, Convolvulaceae and Moraceae families.[39] Some of these plants, such as potato, aubergine and tomato, are directly consumed by humans, while others can be found in agricultural crops due to accidental harvesting of weeds. For instance, it is well known that seeds of *Datura stramonium* with high levels of tropane alkaloids can be found in cereals such as millet, sorghum, buckwheat, sunflower and linseed.[40]

Most relevant tropane alkaloids are atropine (racemic mix of R- and L-hyoscyamine) and scopolamine. Intoxication by these compounds leads to anticholinergic effects such as dry mouth, blurred vision, muscle spasms, tachycardia, malfunction of the central nervous system, and death in most severe cases.[41] In the EU, the maximum levels of tropane alkaloids in food are set by the Commission Regulation (EC) No 1881/2006[42], namely 1.0 μg/kg atropine or scopolamine in processed cereal-based foods and baby foods for infants and young children, containing millet, sorghum, buckwheat or their derived products.

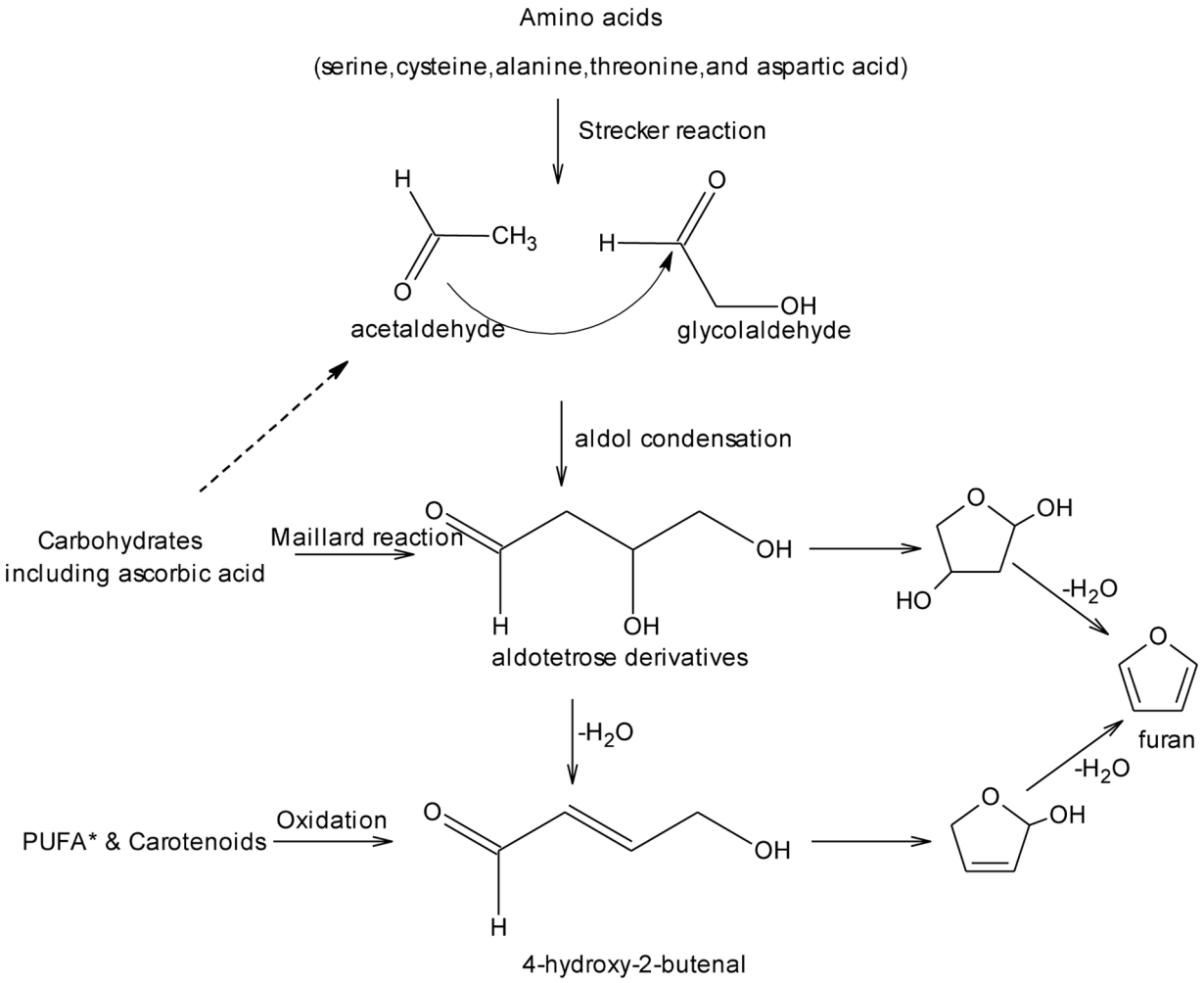

Fig. 1. Different origins of the parent furan formation. *PUFA = polyunsatured fatty acids.

Nevertheless, the presence of tropane alkaloids above these limits has been documented in cereal-based foods for infants and children. According to a survey in the Netherlands in 2011, 2012 and 2014, an average of 4.6, 4.4 and 0.5 μg/kg (respectively) was found in cereal-based food for infants and young children, with maximum levels of 80.8, 57.6 and 3.9 μg/kg, respectively.[43] Another survey found that 20% of cereal-based foods for young children (6–36 months) contained one or more tropane alkaloid, and amongst food groups, the highest mean concentration (130.7 μg/kg) was detected in cereal-based meals for children.[44]

Therefore, it is necessary to update or set up new strategies to control tropane alkaloids in infant food. In this sense, the SAFFI project is working in the assessment of the effect of food processing technologies, such as conventional spray drying or the emerging PCD technology, on the fate of tropane alkaloids in infant cereals. The effect of processing parameters such as pH, temperature and treatment time on the stability of these contaminants will be determined. Furthermore, the project aims to provide sampling, monitoring and analytical strategies to be implemented by infant food companies to establish an accurate and efficient control of these contaminants. A new analytical approach will be proposed, based on existing validated state-of-the-art methods for the determination of tropane alkaloids. These methods include extraction with acidified aqueous-organic solvents, purification steps and chromatographic analysis by LC-MS/MS.[44,45]

2.6. *Effect of infant food processing on bacterial pathogens*

Amongst the microbiological hazard identified in the infant food chains within the SAFFI project activities, both spore forming pathogens (*Bacillus cereus, Clostridium botulinum*) and vegetative bacterial pathogens (entero-haemorrhagic *Escherichia coli, Salmonella* spp. *Cronobacter sakazaki, Listeria monocytogenes*) are of relevance for different type of infant foods. The microbial resistance to heat and other processing technologies depends on several factors,[46,47] including:

- Microbial related factors such as type of microorganism (i.e. spores being much resistant than vegetative cells), though differences at species and strain level may also be considerable. The physiological state of the cells and the conditions to which a microorganism is exposed to prior to any treatment are also of paramount importance, as they may trigger resistance mechanisms and make bacteria more robust towards preservation and processing treatments.
- Product related (i.e. intrinsic) factors such as pH, water activity and specific compounds (natural or intentionally added as part of the product formulation) that may sensitize or protect microbial cells against other stresses.
- Extrinsic factors such as technological parameters associated with the processing and preservation technologies (temperature, pressure, etc.).

All these factors need to be considered when evaluating the efficacy of the control measures when both classical and new/emerging

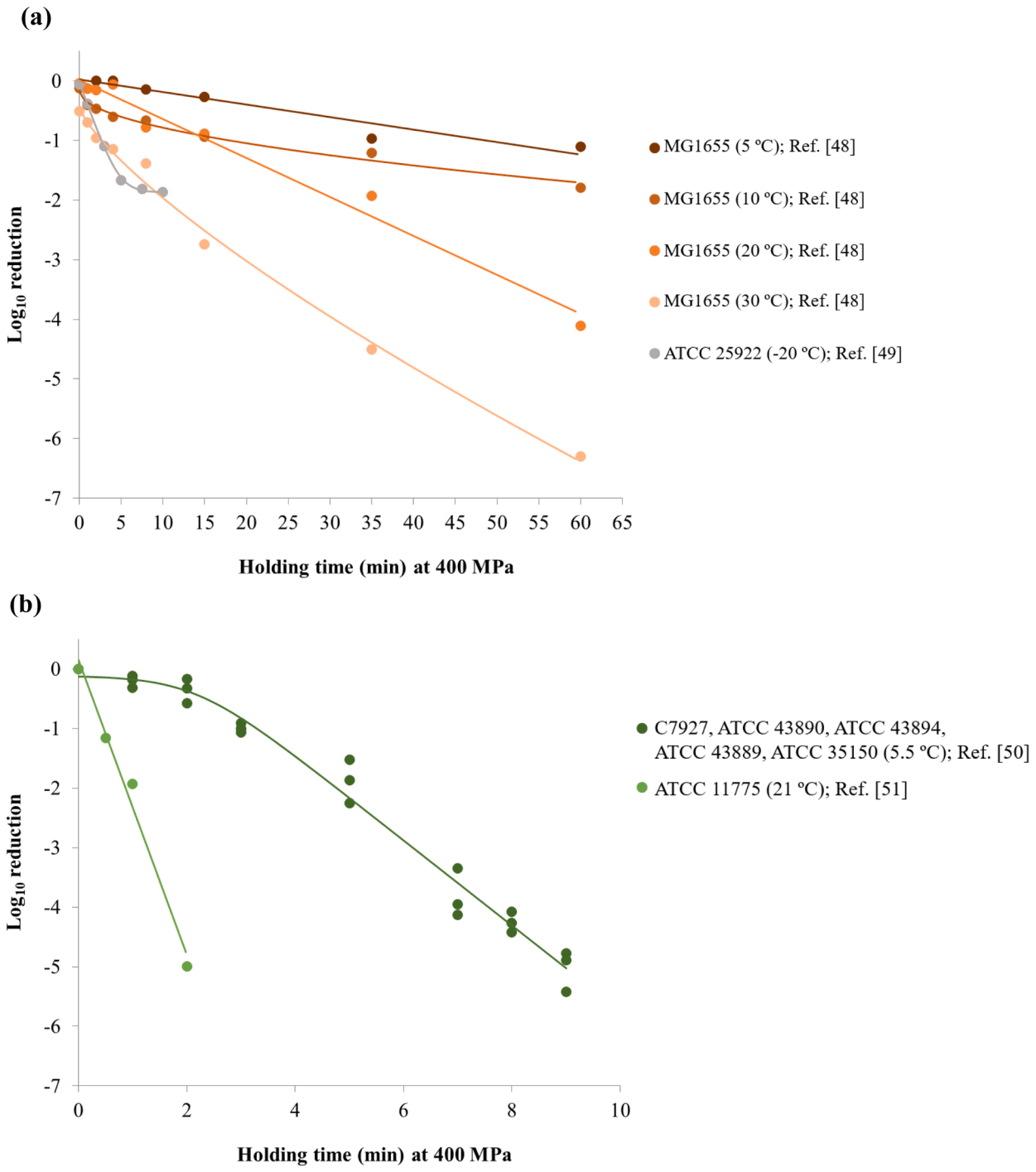

Fig. 2. Inactivation (Log_{10} reduction) of different strains of *E. coli* in apple (a) and carrot (b) juice during high pressure processing at 400 MPa at different temperatures. Data extracted form References[48–51].

technologies are applied. With the aim of developing a prototype of a decision support system (DSS) for hazard control, the SAFFI project aims to collect information available in the scientific literature regarding the behaviour of relevant pathogen associated with specific processing and preservation technologies considered in each case-study. A wide variability of the microbial response is usually recorded when different studies are gathered, which can be related to the differences in the used methods and design of experiments, covering different factors and ranges of conditions. For instance, Fig. 2 shows the inactivation of *E. coli* reported in different scientific articles during HPP of apple and carrot juice, and inactivation kinetics depend on the product, the strain and the temperature.[48–51] Statistical meta-analysis and mathematical modelling strategies can be useful to integrate the results of individual studies and find global estimate of kinetic parameters with their corresponding variability.[52] A meta-analysis can also point to factors that have a significant and main impact on the kinetic parameter.[53] The outputs of meta-analysis are also useful to guide the design of new inactivation experiments and can give a first impression on the efficacy of a processing or preservation treatment. SAFFI will further build on the meta-analyses, and also perform validation studies on pilot plant scale. In these cases, appropriate non-pathogenic microbial surrogates are often used to mimic the behaviour of the target relevant pathogen in the

evaluated process.[5] Also here the meta-analyses can give guidance and can be used as benchmark to evaluate whether the surrogate of choice is a good alternative for the target pathogen. In the SAFFI project, experimental work will also address the occurrence of sublethal injury of pathogens upon product treatment, because sublethally injured cells might recover and growth out in the food product during shelf lifeFig. 1.

This complementary approach, collecting targeted experimental data using a well-designed experimental approach and combine these data with available literature will result in project outcomes with more confidence then when only based on experimental efforts.

Declaration of Competing Interest

Authors declare no conflicts of interest

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References

1 Emenhiser C. Formulation and manufacturing of infant and toddler foods. *Food Technol*. 2013;57(10):26–28.
2 Commission E. Commission notice on the implementation of food safety management systems covering prerequisite programs (PRPs) and procedures based on the HACCP principles, including the facilitation/flexibility of the implementation in certain food businesses (C/2016/4608). *Off J Eur Commun C*. 2016;278:1–32.
3 CAC (Codex Alimentarius Commission). Guidelines for the validation of food safety control measures. *Codex Aliment Comm*. 2008 (CAC/GL 69–2008, amended in 2013). Joint FAO/WHO Food Standards Program.
4 European Commission, European Parliament. Regulation (EC) No 178/2002 of the European Parliament and of the council of 28 January 2002 laying down the general principles and requirements of food law, establishing the European food safety authority and laying down procedures in matters of food safety. *Off J Eur Communit L*. 2002;31:1–24, 2002.
5 Ceylan E, et al. Guidance on validation of lethal control measures for foodborne pathogens in foods. *Compr Rev Food Sci Food Saf*. 2021;20(3):2825–2881. https://doi.org/10.1111/1541-4337.12746. May.
6 Zwietering MH, Jacxsens L, Membré JM, Nauta M, Peterz M. Relevance of microbial finished product testing in food safety management. *Food Control*. 2016;60:31–43. https://doi.org/10.1016/J.FOODCONT.2015.07.002. Feb.
7 Petruzzi L, Campaniello D, Speranza B, Corbo MR, Sinigaglia M, Bevilacqua A. Thermal treatments for fruit and vegetable juices and beverages: a literature overview. *Compr Rev Food Sci Food Saf*. 2017;16(4):668–691. https://doi.org/10.1111/1541-4337.12270. Jul.
8 Bonwick GA, Birch CS. Chapter 1:European regulation of process contaminants in food. *Mitigating Contamination from Food Processing*. 2019:1–16. https://doi.org/10.1039/9781788016438-00001. Pp.
9 Meng X, de Jong W, Kudra T. A state-of-the-art review of pulse combustion: principles, modeling, applications and R&D issues. *Renew Sustain Energy Rev*. 2016;55:73–114. https://doi.org/10.1016/J.RSER.2015.10.110. Mar.
10 Wu Z, Yue L, Li Z, Li J, Mujumdar AS, Rehkopf JA. Pulse combustion spray drying of egg white: energy efficiency and product quality. *Food Bioproc Technol*. 2015;8(1):148–157. https://doi.org/10.1007/S11947-014-1384-9. Jan.
11 Altemimi A, Naji Aziz S, Al-Hilphy ARS, Lakhssassi N, Watson DG, Ibrahim SA. Critical review of radio-frequency (RF) heating applications in food processing. *Food Qual Saf*. 2019;3(2):81–91. https://doi.org/10.1093/fqsafe/fyz002.
12 Calero-Pastor M, Clemente G, Fartdinov D, Bañon S, Muñoz I, Sanjuán N. Upscaling via a prospective LCA: a case study on tomato homogenate using a near-to-market pasteurisation technology. *Sustainability*. 2022;14(3):1716. https://doi.org/10.3390/SU14031716. Feb.
13 Ahmed J, Ramaswamy HS. Microwave pasteurization and sterilization of foods. *Handb Food Preserv*. 2020:713–732. https://doi.org/10.1201/9780429091483-47. Jun.
14 Rosnes JT, Skåra T, Skipnes D. Recent advances in minimal heat processing of fish: effects on microbiological activity and safety. *Food Bioproc Technol*. 2011;4(6):833–848. https://doi.org/10.1007/S11947-011-0517-7/FIGURES/1. Aug.
15 Muñoz I, et al. Comparison of different technologies (conventional thermal processing, radiofrequency heating and high-pressure processing) in combination with thermal solar energy for high quality and sustainable fish soup pasteurization. *Food Bioproc Technol*. 2022. https://doi.org/10.1007/S11947-022-02782-8. Feb.
16 Geveke DJ, Brunkhorst C, Fan X. Radio frequency electric fields processing of orange juice. *Innov Food Sci Emerg Technol*. 2007;8(4):549–554. https://doi.org/10.1016/J.IFSET.2007.04.012. Dec.
17 Abea A, Gou P, Guardia MD, Bañon S, Muñoz I. Combined effect of temperature and oil and salt contents on the variation of dielectric properties of a tomato-based homogenate. *Foods*. 2021;10(12). https://doi.org/10.3390/foods10123124. Dec.
18 Zhang Y, Zhu G, Xie Y, Liu Y. Effects of radio frequency on physicochemical properties of powdered infant formula milk as compared with conventional thermal treatment. *LWT*. 2020;134. https://doi.org/10.1016/J.LWT.2020.110194. Dec.
19 Jofré A, Serra X. Processing of meat products utilizing high pressure. *Food Eng Ser*. 2016:591–623. https://doi.org/10.1007/978-1-4939-3234-4_26.
20 Picouet PA, Hurtado A, Jofré A, Bañon S, Ros JM, Guardia MD. Effects of thermal and high-pressure treatments on the microbiological, nutritional and sensory quality of a multi-fruit smoothie. *Food Bioproc Technol*. 2016;9(7):1219–1232. https://doi.org/10.1007/s11947-016-1705-2.
21 Choudhary A, Kumar V, Kumar S, Majid I, Aggarwal P, Suri S. 5-Hydroxymethylfurfural (HMF) formation, occurrence and potential health concerns: recent developments. *Toxin Rev*. 2021;40(4):545–561. https://doi.org/10.1080/15569543.2020.1756857.
22 Rada-Mendoza M, Olano A, Villamiel M. Determination of hydroxymethylfurfural in commercial jams and in fruit-based infant foods. *Food Chem*. 2002;79(4):513–516. https://doi.org/10.1080/15569543.2020.1756857.
23 Nielsen HB, et al. Consumer perception of the use of high-pressure processing and pulsed electric field technologies in food production. *Appetite*. 2009;52(1):115–126. https://doi.org/10.1016/J.APPET.2008.09.010. Feb.
24 Lawrence I, Jung S. HPP as an innovation tool for healthy foods. *Present Fut High Press Process A Tool Dev Innov Sustain Safe Healthy Foods*. 2020:187–200. https://doi.org/10.1016/B978-0-12-816405-1.00008-X. Jan.
25 Commission Regulation (EC) No 2073/2005 of 15 November 2005 on microbiological criteria for foodstuffs. *Off J Eur Union*; 2005. Available https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=OJ:L, 2005:338:TOC.
26 CAC (Codex Alimentarius Commission), Guidelines on the application of general principles of food hygiene to the control of listeria monocytogenes in foods (CXG 61-2007, annexes II and III adopted in 2009). Joint FAO/WHO Food Standards Program. 2007.
27 FDA (Food and Drug Administration), Control of listeria monocytogenes in ready-to-eat foods: guidance for industry draft guidance, 2017. Accessed: Mar. 15, 2022. [Online]. Available: http://www.regulations.gov.
28 Health Canada, Control measures for listeria monocytogenes in ready-to-eat foods. Requirements for the safe food for Canadians regulations, 2020. https://inspection.canada.ca/preventive-controls/listeria-monocytogenes/eng/1518103693274/1528201904208 (accessed Mar. 14, 2022).
29 EFSA BIOHAZ Panel (EFSA Panel on Biological Hazards). Scientific opinion on the efficacy and safety of high-pressure processing of food. *EFSA J*. 2022;20(3). https://doi.org/10.2903/j.efsa.2022.7128.
30 FDA (Food and Drug Administration), Guidance for industry: juice hazard analysis critical control point hazards and controls guidance, first edition, 2004. https://www.fda.gov/regulatory-information/search-fda-guidance-documents/guidance-industry-juice-hazard-analysis-critical-control-point-hazards-and-controls-guidance-first#v (accessed Mar. 15, 2022).
31 IARC (International Agency for Research on Cancer, Furan (Group 2B) IARC summary & evaluation, 63:393, 1995. https://inchem.org/documents/iarc/vol63/furan.html (accessed Feb. 17, 2022).
32 EFSA (European Food Safety Authority). Update on furan levels in food from monitoring years 2004–2010 and exposure assessment. *EFSA J*. 2011;9(9). https://doi.org/10.2903/j.efsa.2011.2347.
33 ANSES, Infant total diet study (iTDS)-Report 1- ANSES opinion, Request N0 2014-SA-0317, 2016. https://www.anses.fr/en/content/infant-total-diet-study-itds (accessed Oct. 14, 2021).
34 Perez Locas C, Yaylayan VA. Origin and mechanistic pathways of formation of the parent furana food toxicant. *J Agric Food Chem*. 2004;52(22):6830–6836. https://doi.org/10.1021/JF0490403. Nov.
35 Becalski A, Seaman S. Furan precursors in food: a model study and development of a simple headspace method for determination of furan. *J AOAC Int*. 2005;88:102–106 [Online]. Available https://academic.oup.com/jaoac/article/88/1/102/5657559.
36 Oliver-Pozo C, Aparicio-Ruiz R, Romero I, García-González DL. Analysis of volatile markers for virgin olive oil aroma defects by spme-gc/fid: possible sources of incorrect data. *J Agric Food Chem*. 2015;63(48):10477–10483. https://doi.org/10.1021/ACS.JAFC.5B03986/ASSET/IMAGES/ACS.JAFC.5B03986.SOCIAL.JPEG_V03. Dec.
37 Bicchi C, Cordero C, Rubiolo P. A survey on high-concentration-capability headspace sampling techniques in the analysis of flavors and fragrances. *J Chromatogr Sci*. 2004;42(8):402–409. https://doi.org/10.1093/CHROMSCI/42.8.402.
38 Cepeda-Vázquez M, Blumenthal D, Camel V, Rega B. Multivariate optimization of headspace trap for furan and furfural simultaneous determination in sponge cake. *Talanta*. 2017;164:708–715. https://doi.org/10.1016/J.TALANTA.2016.10.073. Mar.
39 Lounasmaa M, Tamminen T. *The tropane alkaloids: chemistry and biology*. In: *The Alkaloids*. 44. New York: Academic Press; 1993.
40 EFSA Panel on Contaminants in the Food Chain (CONTAM). Scientific opinion on tropane alkaloids in food and feed. *EFSA J*. 2013;11(10). https://doi.org/10.2903/J.EFSA.2013.3386. Oct.
41 Kohnen-Johannsen KL, Kayser O. Tropane alkaloids: chemistry, pharmacology, biosynthesis and production. *Molecules*. 2019;24(4):796. https://doi.org/10.3390/MOLECULES24040796, 2019, Vol. 24, Page 796Feb.
42 European Commission, COMMISSION REGULATION (EU) 2016/ 239 - of 19 February 2016 amending Regulation (EC) No 1881/ 2006 as regards maximum levels of tropane alkaloids in certain cereal-based foods for infants and young children, 2016, 10.2903/j.efsa.2013.3386.
43 Mulder PPJ, Pereboom-de Fauw DPKH, Hoogenboom RLAP, de Stoppelaar J, de Nijs M. Tropane and ergot alkaloids in grain-based products for infants and young

children in the Netherlands in 2011–2014. *Food Addit Contam Part B*. 2015;8(4):284–290. https://doi.org/10.1080/19393210.2015.1089947. Oct.
44 P.P.J. Mulder, M. de Nijs, M. Castellari, et al. Occurrence of Tropane Alkaloids in Food. EFSA Supporting Publications, 10.2903/sp.efsa.2016.EN-1140.
45 Gonçalves C, Cubero-Leon E, Stroka J. Determination of tropane alkaloids in cereals, tea and herbal infusions: exploiting proficiency testing data as a basis to derive interlaboratory performance characteristics of an improved LC-MS/MS method. *Food Chem*. 2020;331, 127260. https://doi.org/10.1016/J.FOODCHEM.2020.127260. Nov.
46 den Besten HMW, Wells-Bennik MHJ, Zwietering MH. Natural diversity in heat resistance of bacteria and bacterial spores: impact on food safety and quality. *Annu Rev Food Sci Technol*. 2018;9:383–410. https://doi.org/10.1146/ANNUREV-FOOD-030117-012808. Mar.
47 EFSA BIOHAZ Panel (EFSA Panel on Biological Hazards), Koutsoumanis K, Alvarez-Ordóñez A, Bolton D, et al. Scientific opinion on the efficacy and safety of high-pressure processing of food. *EFSA J*. 2022;20(3):195. https://doi.org/10.2903/j.efsa.2022.7128, 20227128.
48 van Opstal I, Vanmuysen SCM, Wuytack EY, Masschalck B, Michiels CW. Inactivation of Escherichia coli by high hydrostatic pressure at different temperatures in buffer and carrot juice. *Int J Food Microbiol*. 2005;98(2):179–191. https://doi.org/10.1016/j.ijfoodmicro.2004.05.022. Feb.
49 Zhu S, Wang C, Ramaswamy HS, Yu Y. Phase transitions during high pressure treatment of frozen carrot juice and influence on Escherichia coli inactivation. *LWT Food Sci Technol*. 2017;79:119–125. https://doi.org/10.1016/j.lwt.2017.01.022. Jun.
50 Moody A, Marx G, Swanson BG, Bermúdez-Aguirre D. A comprehensive study on the inactivation of Escherichia coli under nonthermal technologies: high hydrostatic pressure, pulsed electric fields and ultrasound. *Food Control*. 2014;37(1):305–314. https://doi.org/10.1016/j.foodcont.2013.09.052. Mar.
51 Usaga J, Acosta Ó, Churey JJ, Padilla-Zakour OI, Worobo RW. Evaluation of high pressure processing (HPP) inactivation of Escherichia coli O157:H7, Salmonella enterica, and Listeria monocytogenes in acid and acidified juices and beverages. *Int J Food Microbiol*. 2021;339. https://doi.org/10.1016/j.ijfoodmicro.2020.109034. Feb.
52 den Besten HMW, Zwietering MH. Meta-analysis for quantitative microbiological risk assessments and benchmarking data. *Trends Food Sci Technol*. May 2012;25(1):34–39. https://doi.org/10.1016/J.TIFS.2011.12.004.
53 van Asselt ED, Zwietering MH. A systematic approach to determine global thermal inactivation parameters for various food pathogens. *Int J Food Microbiol*. 2006;107(1):73–82. https://doi.org/10.1016/j.ijfoodmicro.2005.08.014.

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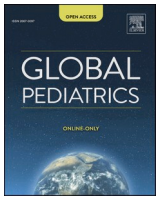

The development of a decision support system for the infant food chain☆

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SUMMARY

In a time where the awareness of food safety and quality increases among the general population, it is vital that consumers are enabled to make informed decisions on risks involving the safety of their food. The SAFFI (Safe Food for Infants in EU and China) project aims to build an integrated decision support system (DSS) for the infant food chain that will enable stakeholders at all levels to make informed decisions regarding infant food. The infant food chain was selected due to its strict regulatory requirements, its vulnerabilities as highlighted by different food safety crises, the economic importance of the infant food sector in the EU and China and the focus on this particular food chain by food safety authorities.

The SAFFI project will incorporate data and models from work packages dealing with hazard identification (HI), hazard detection (HD), hazard control (HC) and risk ranking (RR). The models will be integrated into a user-friendly and upgradeable cloud-based decision support system application. A multi-actor cost-benefit analysis of the project will be carried out, enabling the stakeholders in the project to assess the relevance of implementing the project technologies by integrating food safety, regulatory and economic criteria.

The decision support system will be validated on four specific case studies, and tested on end-users, with the aim of extending this approach to other food chains.

1. Introduction

As the awareness of food safety and quality increases among the general population, it is of paramount importance that consumers are enabled to make better informed decisions regarding the risks they take in their everyday life. In the era of big data, in which the number of research papers on food data has grown nearly 300% every five years since 2010,[18] there has never been a better time to harness this data in such a way to manage the issue of food safety. The SAFFI (Safe Food for Infants in EU and China) project aims to build an integrated decision support system (DSS) for the infant food chain that will enable stakeholders at all levels to make informed decisions regarding infant food. The infant food chain was chosen as the focus of the SAFFI project for several reasons: (1) if the DSS tool can successfully integrate the various elements monitoring the high standards of safety that infant food producers must adhere to given the vulnerability of the population, it can then be expanded to other food chains; (2) the number of high-profile incidents in this supply chain indicate the focus that infant food is given and the susceptibility of this chain to food safety incidents; (3) The most recent French total diet study focused specifically on the infant population, demonstrating the importance of this population to authorities. Finally, the infant food industry is of great importance both in the EU (the sixth most valuable product category exported in 2021[9]) and China, where the growth of the sector has been strong.

The main priorities of SAFFI project are: (i) to have a better insight on microbiological and chemical hazards along the infant food chain; (ii) to identify the main known risks and provide (when needed) new tools for their identification, detection, assessment and mitigation by both public health authorities and food industry; (iii) to anticipate unknown risks related to chemical contaminants not detected by current monitoring systems; (iv) to prevent public health crises related to foodborne microorganisms by proposing tools for predictive microbiology and risk management based no longer on hazards but on risks; (v) to further share data, practices, and critical information in real time to ensure overall food safety control.

The activities based on these priorities will culminate in an

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integrated decision support system. A decision support system can be described as the "*hardware/software that allows a specific decision maker or group of decision makers to deal with a specific set of related problems*".[17] The anticipated decision makers (i.e., the users of the DSS) will range from the preparer of infant food (who, via the DSS, will be enabled to make safer decisions around food use) to infant food companies and food safety authorities, who will be enabled to make appropriate responses when a new product is developed, or a new hazard is identified or suspected in the infant food chain. As part of work package 5, a multi-actor cost-benefit analysis of the decision support system will be carried out. This cost-benefit analysis will enable the different stakeholders in the project to assess the relevance of implementing the project technologies by integrating food safety, regulatory and economic criteria. By assessing this, SAFFI's cost-benefit analysis will enable the project partners to directly assess how the project outcomes will impact the performance and reliability of food safety control all along the infant food chain.

The decision support system will be developed in the form of a beta software programme, and in adherence to the Technology Readiness Levels detailed by the Horizon 2020 programme,[8] will reach a TRL of 4, signifying the technology developed has been validated in a lab. This beta software will be further developed, upon the completion of the SAFFI project, with the goal of producing a commercially ready DSS tool that can be adapted to other food chains and upgraded with new data in the future.

2. Methods

2.1. Decision support system

2.1.1. Data collection

There are seven work packages within the SAFFI project, the first four of which will produce data that forms the basis of the integrated decision support system (DSS) to be developed. These work packages are individually concerned with unique aspects of food safety, including hazard identification (HI), hazard detection (HD), hazard control (HC) and risk ranking (RR). The types of data being generated and collected for use in the integrated DSS are equally unique, ranging from food processing data from pilot experiments to metagenomics data to cost-benefit analysis data. This data, and the subsequent models which it will be incorporated into for the integrated DSS, will be hosted on the SAFFI Data Foundry platform.[5] SAFFI Data Foundry is a cloud based platform developed by Creme Global, Dublin, Ireland, that facilitates the secure collection of datasets, hosts data collection portals and will ultimately host the integrated DSS developed during the SAFFI project. As there is a vast quantity and variety of data being collected it is crucial to the model development process that the data collected is of a high quality and is suitable for integration into a computational model. The data collection within this project needs to be considered on two levels: (1), the data collection process must be managed by Creme Global to ensure that the templates used for data collection and the databases built must be of a suitable standard to be integrated into a computational model, and an integrated DSS; (2) under the Open Research Data Pilot and EU Horizon 2020 project guidelines,[7] project partners are obliged to follow the FAIR principles in making data Findable, Accessible, Interoperable and Reusable. The steps taken to ensure the data produced in this project will meet the FAIR requirements include the use of accepted protocols in recording metadata and labeling datasets, the storage of raw data in a data repository and the agreement of a data sharing agreement (to be established as the project continues), the publication of results in an OpenAccess journal where possible, and the following of OpenAIRE guidelines for online interoperability of results. As part of the SAFFI project, a Data Management Plan has been developed and will be updated and referred to as the project progresses.

2.1.2. Requirements gathering

In order to build models that satisfy the technical requirements of the respective work packages and the overarching technical objectives of the project, the requirements of the model to be developed must be identified. The purpose of the requirements gathering step is to scope out the requirements of the model- including the use cases of the model, the functional requirements / features, the inputs and outputs, possible constraints and interface specifications- and to allow the identification of key data inputs. The key data inputs, as well as the actual data to be incorporated into the model, include the qualitative and quantitative procedures that will be used to evaluate the content of databases, and the mathematical models, algorithms and decision-based models that will be applied to data. To commence the requirements gathering process, surveys will be circulated among the partners in the respective work packages. These surveys will collect, from the partners, descriptions of the proposed models, essential inputs, desired outputs and algorithms underlying the running of the model. To steer the requirements gathering process, close collaboration is required between the model developer and the partners involved in that particular model.

2.1.3. Model development (I)

Data collection templates and databases developed by the SAFFI partners are profiled to ensure that they are suitable for integration into a computational model. Data profiling involves the analysis of the data collected or generated in order to assess the quality of the data, to clean data or to identify gaps, to create metadata, identify dependencies between datasets or to develop schema.[14]

Following this collection and profiling of data, and the collection of model requirements, initial data modeling is performed. This involves the development of conceptual and logical data models[24] using entity relationship diagrams (ERDs) and data-flow diagrams. Entity relationship diagrams are a type of flowchart used to visualize how "entities", in this case datasets and databases, relate to one another in a system, while data-flow diagrams represent the flow of data through a process. Both diagrams will be used in the model development process to position the collected datasets and databases into a database schema that mathematical and decision based models, and software, can interpret and rely on.

The diagrams designed for each model can be used to optimize the model schema for the most efficient performance in terms of speed to run the model and computational load, essentially optimizing the model to allow the least number of calculations to be required for the desired output.

Following the development of conceptual models for each model, and the subsequent optimisation, each proposed model can be evaluated to identify the concept(s) most suitable to progress further in the model development process. Within the SAFFI project, this suitability to progress criteria will involve collaboration between the respective experts in work packages 1 to 4 and the software development experts in work package 5. Aspects to be considered will be the functional requirements of the model, the realistic capabilities within the timeframe of the project, and the limitations of the data that is available to the project.

2.1.4. Model development (II)

When the models to be developed have been selected, more in-depth data profiling and mapping must take place, including a greater inter-dataset analysis and organization of key variables within the datasets. Again, interdependencies will be identified between datasets and even between models, and a logical data model must be designed to visualize the flow of data through the envisaged model. Based on the finalized design of the logical data model, a physical data model will be built. The datasets that had been generated and profiled previously will be adapted and organized to fit the model, this will involve having separate tables that serve as inputs and variables to the underlying algorithms of the model. Data used by the model will be accessible to the user, and editable or updateable as the user requires.

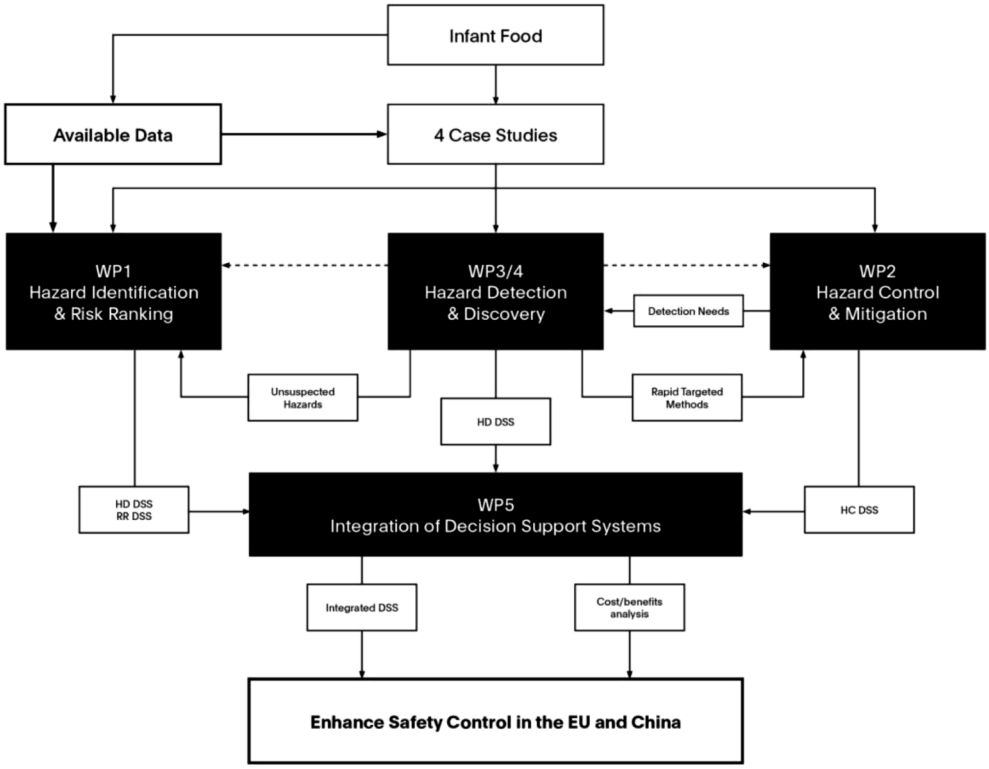

Fig. 1. SAFFI project structure and work package interdependencies.

2.1.5. Testing and deployment

The beta software tool will be tested via scenarios with known results and outputs, in collaboration with the other partners. This testing will involve running the model using inputs from an experiment that has physically been carried out and run, and the outputs that the model generates will be compared to the physical results so as to validate its effectiveness.

The model will be hosted on Data Foundry, a cloud-based software tool that enables the use of complex data science products behind user-friendly interfaces through a web browser. Creme Global's proprietary technology accommodates the processing loads required by the complex data models that will be developed through the SAFFI project. Functionalities of Data Foundry includes account management, data editors, file management systems, data up-loaders and modeling engines. The

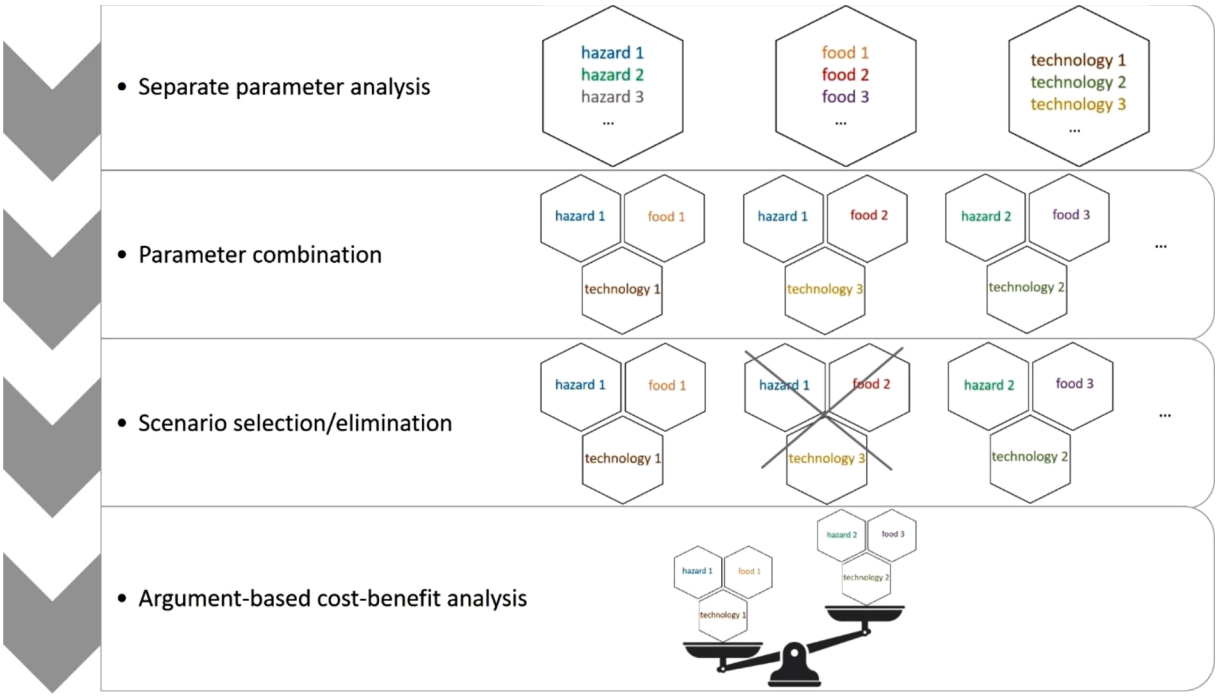

Fig. 2. Steps of cost-benefit analysis.

platform uses Amazon Web Services allowing for a single point of entry that is highly secure. Reproducibility is achieved by keeping copies of the essential data for re-creating scenarios. Collaboration between parties is enabled by the cloud-based nature of the platform, and the provision of shared folders where several users can share assets.

As part of Task 5.5 of the SAFFI project, the beta software will be presented and tested to end-users, who will then be tasked with using the software tool for a period of time. After this time, interviews and surveys will be conducted with the end-user sample who will provide feedback and recommendations from their variety of perspectives. This testing period allows for potential bugs in the software to be identified and for improvements to the software to be suggested. Following feedback from the end-users, an assessment will take place on the potential next steps to make the decision support system software more commercially ready and viable (Fig. 1).

2.2. Cost-benefit analysis

2.2.1. Objectives

Throughout the project and within its different research components, different parameters of food safety in the infant food chain are explored[15,16]). These include the hazards examined, the food products considered, the processing technologies used, the detection tools used for hazard control. Obviously, these parameters are not independent. A given hazard may be prevalent in some foods more than others, and hazards may be unequally impacted by a processing technology, to cite a few examples. Consequently, some scenarios,[10] that is to say, combinations defined by a given hazard in a given food product, undergoing a given processing technology and examined with a given detection tool, will be of salient interest for further discussion in the project. The first objective of cost-benefit analysis is to identify and select such scenarios. The second objective is to draw up a broad-view assessment involving various considerations[21] –from food safety, technical feasibility to nutritional interest, economic impacts, etc. – and various stakeholders[20] for these salient scenarios. The stakeholders involved in infant food safety control include consumers, who should benefit from health issue prevention; professionals of early childhood and healthcare, who play an advisory role with families; the food industry as well as public authorities, concerned about the prevention of health crises, the preservation of public confidence, the availability of efficient and affordable hazard detection tools, the creation of economic opportunities; researchers and industrials of food safety related technologies, etc. Stakeholders may bring different visions and different expectations from the research carried out.[3] The third and final objective is to conclude on the potential of the different scenarios, collectively and for each of the stakeholders and concerns examined.[4]

2.2.2. Methods

The steps of the methodology followed are depicted in Fig. 2.

In the first step, based on the knowledge available through a variety of sources for each parameter separately (hazard, food product, processing technology, detection tool), the pros and cons of focusing on each parameter value (a given contaminant, a given baby food, etc.) are determined and the information is structured and stored in the form of arguments. Argumentation, a reasoning model based on the construction and evaluation of interacting arguments, has been formalized in different disciplines including computer science and artificial intelligence[6,11] and adapted to various uses such as decision making.[2] Its interest in the food sector, together with other system-modeling approaches, has been underlined in several recent reviews.[12,19,1] Developments and argument structuration in the food sector can be consulted e.g. in Thomopoulos et al.[22, 23]

In the second step, all the possible combinations of parameter values are computed. The results are the "scenarios" considered.

Within the scenarios obtained, not all of them make sense. For instance, a processing technology may be irrelevant a given food product. In this case, all the scenarios where the incompatible processing technology and food product were combined can be removed. This is the object of the third step. If too many scenarios still remain, which may impair a thorough study of each of them, a careful selection of scenarios based on the project priorities may be relevant.

Finally, in the fourth step, the same approach as in the first step is applied at the scale of the scenario. To this end, the sets of arguments attached to the parameter values composing the scenario are merged and further completed by additional arguments proper to the combination defining the scenario. These arguments are elicited through multi-stakeholder discussions.

2.2.3. Outcomes

At the end of the fourth step, a so-called "collective attitude" measure can be computed for each of the scenarios considered, allowing the project consortium to compare them. Details on its exact computation can be found in Kurtz & Thomopoulos.[13] This measure can be computed in several modes: (i) either globally, for all stakeholders and concerns brought together, (ii) or modularly, for each stakeholder group or each concern separately. Mode (i) allows the project consortium to highlight the most consensual scenarios to implement and the underlying reasons, provided by the arguments associated with the scenarios. Mode (ii) allows the project consortium to highlight stakeholders or concerns that might most benefit from, or on the contrary be unsatisfied by some scenarios, and the underlying arguments. This is an essential point to anticipate the potential and possible risks of scenarios prior to their implementation.

3. Conclusion

Issues faced in the infant food chain in the EU and China are complex and require equally complex solutions to adequately enhance the reliability and transparency of food safety control. The SAFFI project proposes an integrative approach, essentially bridging the knowledge and data gaps that currently exist by integrating data and knowledge from a diverse range of sources, disciplines, stakeholders and actors. The decision support system, central to this integrative approach, will connect the expertise from disciplines including risk assessment, food technology, predictive toxicology, residue chemistry, predictive microbiology, pediatrics and knowledge engineering, the inputs of stakeholders and end-users and the wealth of food safety knowledge and data developed during the project to modern data science principles, and deliver an upgradeable and user-friendly tool. The proof of concept of this project will be exhibited in the application of the decision support system tool to four case studies in the infant food supply chain, and its success will be measured by the achievement of the scientific, technological, socio-economic, and regulatory objectives.

Declaration of Competing Interest

Authors declare no conflicts of interest.

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References

1 Aceves Lara CA, Athès V, Buche P, Della Valle G, Farines V, Fonseca F, Guillard V, Kansou K, Kristiawan M, Monclus V, Mouret JR, Ndiaye A, Neveu P, Passot S, Pénicaud C, Sablayrolles JM, Salmon JM, Thomopoulos R, Trelea IC. The virtual food system: Innovative models and experiential feedback in technologies for winemaking, the cereals chain, food packaging and eco-designed starter production. *Innov Food Sci Emerg Technol*. 2018;46:54–64. https://doi.org/10.1016/j.ifset.2017.10.006.

2 Amgoud L, Prade H. Using arguments for making and explaining decisions. *Artif Intell*. 2009;173:413–436. https://doi.org/10.1016/j.artint.2008.11.006.
3 Bourguet JR, Thomopoulos R, Mugnier ML, Abécassis J. An artificial intelligence-based approach to deal with argumentation applied to food quality in a public health policy. *Expert Syst Appl*. 2013;40:4539–4546. https://doi.org/10.1016/j.eswa.2013.01.059.
4 Chaib RL, Macombe C, Thomopoulos R. Adaptation of a participatory system-modeling method to the constraints of remote working. In: *Proceedings of the Conference on Complex Systems - France (FRCCS 2021)*; 2021. May 2021. Easychair preprint n°6305, 20 pages https://easychair.org/publications/preprint/l8VF.
5 CremeGlobal, 2021. Data foundry [WWW Document]. URL https://www.cremeglobal.com/data-foundry/.
6 Dung PM. On the acceptability of arguments and its fundamental role in nonmonotonic reasoning, logic programming and n-person games. *Artif Intell*. 1995;77:321–357. https://doi.org/10.1016/0004-3702(94)00041-X.
7 European Commission, 2016. H2020 Programme. Guidelines on FAIR data management in horizon 2020 history of changes 12. Retrieved from: https://ec.europa.eu/research/participants/data/ref/h2020/grants_manual/hi/oa_pilot/h2020-hi-oa-data-mgt_en.pdf.
8 European Commission. 2015 Horizon 2020 - work programme 2014-2015. General Annexes: G. Technology readiness level (TRL https://ec.europa.eu/research/participants/data/ref/h2020/wp/2014_2015/annexes/h2020-wp1415-annex-g-trl_en.pdf.
9 European Commission, 2021. Monitoring EU agri-food trade: developments January-October 2021. https://ec.europa.eu/info/sites/default/files/food-farming-fisheries/trade/documents/monitoring-agri-food-trade_oct2021_en.pdf.
10 Godet, M., 2008. Strategic foresight: use and misuse of scenario building. LIPSOR Working Paper #10, 143 p. Retrieved from: https://prospectivayfuturo.files.wordpress.com/2017/09/godet-strategic-foresight-n10-2009.pdf.
11 Hunter, A., Besnard, P., 2008. Elements of argumentation. Lect. Notes Comput. Sci. (including Subser. Lect. Notes Artif. Intell. Lect. Notes Bioinformatics) 4724 LNAI, 4. 10.1007/978-3-540-75256-1_3.
12 Kansou K, Laurier W, Charalambides MN, Della-Valle G, Djekic I, Feyissa AH, Marra F, Thomopoulos R, Bredeweg B. Food modelling strategies and approaches for knowledge transfer. *Trends Food Sci Technol*. 2022;120:363–373. https://doi.org/10.1016/j.tifs.2022.01.021.
13 Kurtz A, Thomopoulos R. Safety vs. Sustainability concerns of infant food users: French results and European perspectives. *Sustainability*. 2021;13. https://doi.org/10.3390/su131810074.
14 Naumann F. Data profiling revisited. *SIGMOD Rec*. 2013;42:40–49. https://doi.org/10.1145/2590989.2590995.
15 Pettoello-Mantovani M, Mestrovic J, Namazova-Baranova MD, PhD L, Giardino I, Somekh E, Vural M. Ensuring safe food for infants: the importance of an integrated approach to monitor and reduce the risks of biological, chemical, and physical hazards. *J Pediatr*. 2021;229. https://doi.org/10.1016/j.jpeds.2020.10.074, 315-316.e2.
16 SAFFI Project (2020). Available online: https://www.saffi.eu.
17 Sprague RH. A framework for the development of decision support systems. *MIS Q Manag Inf Syst*. 1980;4:1–26.
18 Tao Q, Ding H, Wang H, Cui X. Application research: big data in food industry. *Foods*. 2021;10. https://doi.org/10.3390/foods10092203.
19 Thomopoulos R, Baudrit C, Boukhelifa N, Boutrou R, Buche P, Guichard E, Guillard V, Lutton E, Mirade PS, Ndiaye A, Perrot N, Taillandier F, Thomas-Danguin T, Tonda A. Multi-criteria reverse engineering for food: genesis and ongoing advances. *Food Eng Rev*. 2019;11:44–60. https://doi.org/10.1007/s12393-018-9186-x.
20 Thomopoulos R, Cufi J, Le Breton M. A generic software to support collective decision in food chains and in multi-stakeholder situations. In: *Proceedings of FoodSim 2020*; 2020:201–207, 6–10 September 2020 https://hal.archives-ouvertes.fr/hal-02484363v2/document.
21 Thomopoulos R. A practical application approach to argumentation for multicriteria analysis and decision support. *EURO J Decis Process*. 2018;6:237–255. https://doi.org/10.1007/s40070-018-0087-2.
22 Thomopoulos R, Moulin B, Bedoussac L. Supporting decision for environment-friendly practices in the agri-food sector: When argumentation and system dynamics simulation complete each other. *Int J Agric Environ Inf Syst*. 2018;9:1–21. https://doi.org/10.4018/IJAEIS.2018070101.
23 Thomopoulos R, Salliou N, Abreu C, Cohen V, Fouqueray T. Reduced meat consumption: from multicriteria argument modelling to agent-based social simulation. *Int J Food Stud*. 2021;10:133–149. https://doi.org/10.7455/IJFS/10.1.2021.A1.
24 VisualParadigm, 2022. Data Modeling: Conceptual vs Logical vs Physical Data Model [WWW Document]. Vis. Paradig. Online. URL https://online.visual-paradigm.com/knowledge/visual-modeling/conceptual-vs-logical-vs-physical-data-model#:~:text=The conceptual model is to,implementation of the data model. (accessed 3.1.22).

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Good practices and ethical issues in food safety related research☆

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ABSTRACT

After introducing a historical view of research ethics and the main schools of thought, the paper is structured around two main topics: On the one hand, the protection of the environment surrounding the research experiments conducted, which is a major aspect in food safety related research and includes the staff carrying out the research. On the other hand, collective decision aspects, which are involved in the construction of decision support systems for food safety enhancement. Based on a few examples in food safety related research, the paper reviews the ethical issues considered, the ethical principles applied, and the main measures taken in these cases.

1. Introduction

Birth of research ethics — Historically, the ethical principles of research emerged as a new formalized field, in the tradition of Hippocratic ethics, with the increasing concerns stemming from biomedical research. Their emergence was driven by the need for a balance between the benefits expected from the research conducted, and the risks to be taken, which in some cases led to major scandals [1].

The Nuremberg Code of 1947 first provided formalized safeguards to ensure accordance with ethical principles in research practices [2]. The various versions of the Declaration of Helsinki [3], promulgated by the World Medical Association since 1964, further developed the Nuremberg Code. They introduced in 1975 the need for review and validation of research protocols by an independent committee of ethics. Since these foundation stones of research ethics, numerous guidelines have been defined and specified for various cases and professions. These can be either advisory or have the status of legislations at the international, national or local level. However, codes and laws regulate practices but do not give comprehensive ethical advice. This is where ethical frameworks come into play.

Schools of thought in ethics — Different ethical frameworks have been developed. Among them, consequentialism, deontology-based ethics, and virtue ethics are major approaches [4]. As its name indicates, consequentialism refers to a family of ethical approaches focusing on the consequences or effects of an action, i.e. an action is evaluated with regards to its overall consequences. A classic example of consequentialism is utilitarianism [5] for which an action is deemed morally good if it maximizes the utility of the society. In contrast to that, deontology-based ethics regroups different approaches to ethics that base morality of an action on its compliance with a set of normative rules or duties, regardless of their consequences. An iconic example of that kind of approaches is the categorical imperative introduced by Kant [6], where an action is morally allowed only if it can be elevated as a universal law. Finally, virtue ethics disregard consequences or duties in favor of virtues, i.e. traits of character that are deemed excellent and that need to be nurtured. In that sense, virtue ethics is more interested in how a life should be lived rather than what is the right action in a particular situation —see Vallor [7] for a recent account of virtue ethics.

The answers provided by the different frameworks do not necessarily converge. This raises the issue of how to solve this pluralism in practice, which also opened the way for different conciliation strategies. One of them, known as "principlism", is widely referred to in biomedical ethics. It is based on both deontology and consequentialism, and lies on four principles [8]: respect for autonomy, non-maleficence, beneficence, and

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justice. As far as we are concerned in this paper, the principle of beneficence is the very basis of the research conducted to improve food safety. Indeed, food safety research aims to benefit consumers by preventing health issues of concern, but also the food industry and public authorities, by improving efficiency and reducing costs through the development of efficient high throughput technologies, thus avoiding adverse public health crises and increasing public confidence. As regards non-maleficence, justice and autonomy, these principles will be considered in Sections 2 and 3.

This paper proposes an overview of two ethical aspects which are prevalent in food safety related research, namely: 1) The protection of the environment surrounding the experiments conducted, which includes the research staff carrying out the experiments. 2) The ethics of collective decision, which is implied in the cost-benefit balance of the choices made to enhance food safety, with the involvement of different stakeholders and possibly personal data considerations. These aspects are developed in Sections 2.1 and 3.1, respectively.

2. Protection of the environment surrounding the research experiments conducted

Ethics related to environmental protection and safety concerns research activities that involve the use of elements that may cause harm to the environment, to animals or plants, or to humans, including research staff.

In food safety related research in particular, research labs must be aware of the possible harm to the environment caused by the research and the measures to be taken to mitigate the risks. Practically, they must ensure that appropriate health and safety procedures conforming to the legislation are applied for staff involved in the research.

The principle of non-maleficence is followed here, that is to say, avoiding causing harm is commented in the first example.

2.1. Example 1

Description: a research laboratory carries out microbiological or chemical hazard detection and control, involving the use of potentially infectious or toxic material that might accidentally impact the environment or cause harm to the research staff conducting the experiments.

Ethical issue considered: The kind of ethical issue that arise in relation to microbiological or chemical safety research lies in the risk of environmental health and safety impacts. The eventuality of accidental release of chemicals or pathogenic bacteria in the environment, of accidental contact with humans, has to be anticipated.

In this example, the risk considered primarily goes for the research staff itself —in contrast to research subjects or the general public in other cases. Although researchers may be assumed to have a good understanding of the risks involved, this is not necessarily straightforward when staff with different levels of responsibility, or students, are involved.

Ethical principles applied: This issue is in relation to the precautionary principle. Initially introduced in policies for environmental protection, the precautionary principle has now been much extended. Indeed, according to the European Commission, the principle additionally refers to potentially harmful effects on human, animal or plant health [9]. The principle states that in case an activity introduces a risk of harm, adapted measures should be taken to prevent or limit that harm, even in the absence of a precise assessment of the risk level.

To a lesser extent, and in addition to the precautionary principle, the issue considered is also in relation to the notion of informed consent. This is the most basic requirement originating from the Nuremberg Code [2]. Characterized as the most authoritative set of rules for the protection of human subjects in medical research, the Nuremberg Code has not been entirely adopted as law by any nation, nor as official ethics guidelines by any major medical association [10]. However, its basic requirement of informed consent has been integrated as international law in the International Covenant on Civil and Political Rights [11]. It is also the basis of the International Ethical Guidelines for Biomedical Research Involving Human Subjects, promulgated by the World Health Organization [12].

In reference to this requirement, in case a research activity introduces a risk of harm, the research staff are supposed to have consented to their involvement. Thus, measures to be taken include ensuring that the staff participating has a good understanding of the risk and ability to carry out the research adequately with regard to the risk.

Measures to be taken: legislations and guidelines, defined from the international and national levels until the local level at the scale of the lab, regulate the intake, storage, registration, handling and management of hazardous material. See to this regard. United Nations and WHO's position [13,14]. The protocols required include in particular the training of the staff to good laboratory practices, decontamination and waste management procedures, appropriate human protection equipment (gloves, masks, safety glasses, lab coats), use of biological and chemical hoods. Thus, to summarize, the security measures rely, on the one hand, on informational means, including training and procedure display, and on the other side on physical and chemical barriers to limit the risk of spread.

3. Ethics of collective decision

Addressing societal issues such as public health management through food safety control, involves several stakeholders with different visions of the system, different expectations from the research carried out, and possibly conflicts of interest [15]. Supporting decision-making in such a multi-actor context implies some ethics of decision and relies on the principle of justice in decision-making, since different points of view have to be reconciled [16]. In the case of food safety related research, experts from different disciplines are involved (e.g. food safety, nutrition, food processing), various stakeholders are consulted (e.g. consumers, food companies, public authorities, researchers). In bottom-up hazard control performed by food companies and top-down hazard control performed by food safety authorities, there is a common responsibility and interest in preventing public health problems related to the food chain and a common investment in the food chain safety. Nevertheless, expectations regarding the research carried out may differ. On the move towards modernized hazard control methods, food companies would possibly prioritize, as essential criteria, high-throughput tools and cost-efficiency for self-monitoring in routine use, ease of implementation, and affordable initial investment costs; while on the other hand, for safety authorities, the method capacity to discover unsuspected hazards could be salient.

When choices have to be made, whatever the method used to reconcile viewpoints (e.g. using risk-benefit analysis and multi-criteria decision [17,18], it is based on underlying decision principles. Unfortunately, it is a well-known issue with voting rules (ways of making a decision based on the aggregation of stakeholders' preferences) that none is perfect and each one of them has some defects [19]. Importantly, the choice of the voting rule might impact the decision that is made, a decision that consequently might misrepresent the preferences of the actors. It is thus a matter of justice to acknowledge the bias associated with the decision-making mechanism that is chosen and try to address it. Example 2 addresses these considerations.

3.1. Example 2

Description. A decision support system is designed to analye the costs and benefits of different food safety management strategies (which risks should be high-priority, which technologies should be chosen, etc.) by bringing in the views of the stakeholders concerned.

Ethical issue considered: The issue considered is the risk of providing an unequal representation of the different viewpoints in the decision process.

Ethical principles applied: This issue refers to the principle of justice in research. It is an issue known to the research community, especially in participatory approaches [20,21], that the research process itself induces concerns about: (i) The fair representation of the different groups and stakeholders, offering the opportunity for all viewpoints to be expressed, and avoiding under- or over-representation of certain groups. (ii) The possible influence of the researchers themselves on the decision process, which should be avoided by keeping a neutral posture. These concerns are also shared with other research communities, in particular operational research, which produced a rich literature on the subject [22].

Measures to be taken — Although this issue is inherent to any decision process, adopting a formally well-defined decision methodology, explainable and interpretable, is a way of best addressing the issue. Moreover, providing the possibility to actors to understand and discuss the different aspects of the collective decision, will promote understanding and cohesion between the actors.

In addition to the ethical issues raised by the decision process, another related well-known issue in ethical guidelines is the respect of privacy. With the participation of different stakeholders representative of a range of situations and interest in the society, comes the question of the possible collection of personal data. Example 3 illustrates this issue.

3.2. Example 3

Description. A web survey is launched in order to collect the perceptions of end-users –parents, early childhood professionals, healthcare professionals– concerning the safety of infant food products.

Ethical issue considered: The issue considered is the risk of unconsented collection of personal information, in particular data allowing for the identification of a person, such as names, emails, IP addresses, etc.

Ethical principles applied: The ethical principle involved is the respect of privacy [23]. A recent approach to define privacy is to associate it with the protection of personal information. As it is the case in this example, this definition of privacy relates it to digital concerns such as data protection, at a time where data are valuable goods. This concept of privacy covers, on the one hand, the right to prevent others from obtaining information about oneself; on the other hand, the right to have control on information about oneself that may be registered e.g. on computers. Thus, privacy can be seen as part of the autonomy principle, in the sense that it refers to the right to decide whether and how data originating from oneself are used. With this broad meaning, privacy may not be exclusively restricted to identifiable data, but more generally to information about individuals. Views are however divergent about the scope of privacy [24].

Measures to be taken: The protection of personal data is regulated at the European level by the General Data Protection Regulation and by the Directive (EU) 2016/680 [25]. In the present example, the collection of identifiable data, if unnecessary for the study, can be completely avoided. Technically, this implies in particular the choice of a survey tool that allows the survey designer to block the collection of IP addresses. However, in case the study necessitates the collection of data that can, in some manner, allow one to identify the respondent, then participants should be informed beforehand and provide their consent. But even without the possibility of identifying the respondent, or in the case of anonymized data, good practices suggest to provide adequate information to the participants. Researchers would thus state any significant risks, the purpose of the research, any financial interests and external research funding, the opportunity to ask questions or to change one's mind, all items that directly arise from the Declaration of Helsinki statements.

4. Conclusions

This paper illustrates the challenge of adopting best practices for an ethical research, in the domain of food safety. Even in a restricted domain of research, it can be noted how diverse the issues raised are. Indeed, Section 2 addressed some issues related to the protection of the environment surrounding experimental research on food safety, including the research staff. This is probably the most straightforward aspect of ethics in food safety related research. On the other hand, Section 3 illustrates how food safety related research becomes a societal issue when it comes to decision about food safety management. In this case, very different concerns are raised, in the field of ethics of decision-making and personal data protection.

By highlighting miscellaneous concerns regarding good practices and ethical issues in food safety related research, this paper aims at increasing awareness within academia, industry and other stakeholders, about the variety and complexity of research ethics and its tight imbrication with legislation.

Declaration of Competing Interest

The corresponding author states on behalf of the co-authors that all Authors have no conflict or competing of interests to declare.

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References

[1] Vollmann J, Winau R. Informed consent in human experimentation before the Nuremberg code. *Br Med J (Clin Res Ed)*. 1996;313:1445–1447, 1996.
[2] International Military Tribunals. *Trials of war criminals before the Nüremberg Military Tribunals under Control Council Law No 10*. Washington: US Government Printing Office; 1949:181–182.
[3] World Medical Association, Declaration of Helsinki: ethical principles for research involving human subjects (2008). http://www.wma.net/en/30publications/10policies/b3/index.html (Accessed February 17, 2022).
[4] Wrigley A, Hughes J, Sheehan M, Wilkinson S, Hunter D. *European Commission, Directorate-General for Research and Innovation*. European textbook on ethics in research, Publications Office; 2010. https://data.europa.eu/doi/10.2777/51536 (Accessed February 17, 2022).
[5] Bentham J. *An Introduction to the Principles of Morals and Legislation, Garden City*. Doubleday Ed. London, UK: Originally published by Bensley ED.; 1961, 1789.
[6] Kant I. *Groundwork for the Metaphysics of Morals*. UK: Oxford University Press; 2019.
[7] Vallor S. *Technology and the Virtues: A Philosophical Guide to a Future Worth Wanting*. Oxford University Press; 2016.
[8] Beauchamp TL, Childress JF. *Principles of Biomedical Ethics*. Oxford University Press; 2009.
[9] European Commission. *Communication from the Commission on the Precautionary Principle, COM(2000)1 final*; 2000. https://op.europa.eu/en/publication-detail/-/publication/21676661-a79f-4153-b984-aeb28f07c80a/language-en (Accessed February 17, 2022).
[10] Shuster E. Fifty years later: the significance of the Nuremberg Code. *N Engl J Med*. 1997;337:1436–1440, 1997.
[11] United Nations. *The International Covenant on Civil and Political Rights*. New York: United Nations Office of Public Information; 1966. Suppl. 52. No. 16. Article 7UN document no. A/6316.
[12] Council for International Organizations of Medical Sciences. *International Ethical Guidelines for Biomedical Research Involving Human Subjects*. Geneva; 1993. https://cioms.ch/wp-content/uploads/2016/08/International_Ethical_Guidelines_for_Biomedical_Research_Involving_Human_Subjects.pdf (Accessed February 17, 2022).
[13] United Nations (1999). Transport of Dangerous Goods - Model Regulations. 11th Revised Edition. Geneva. https://unece.org/fileadmin/DAM/trans/danger/publi/unrec/rev21/ST-SG-AC10-1r21e_Vol1_WEB.pdf (Accessed February 17, 2022).
[14] World Health Organization. *Harmonization of Approaches to the Assessment of Risk from the Exposure to Chemicals*. WHO; 1997. https://www.who.int/ipcs/methods/harmonization/areas/final_report.pdf (Accessed February 17, 2022).
[15] Bourguet JR, Thomopoulos R, Mugnier ML, Abécassis J. An Artificial Intelligence-Based Approach to Deal with Argumentation Applied to Food Quality in a Public Health Policy. *Expert Syst Appl*. 2013;(40):4539–4546. https://doi.org/10.1016/j.eswa.2013.01.059, 2013(Accessed February 17, 2022).
[16] Bisquert P, Croitoru M, de Saint-Cyr F, Hecham A. Formalizing Cognitive Acceptance of Arguments: Durum Wheat Selection Interdisciplinary Study. *Minds and Machines*. 2017;27(1):233–252. https://doi.org/10.1007/s11023-017-9424-7 (Accessed February 17, 2022).

[17] Bana E Costa CA. The use of multi-criteria decision analysis to support the search for less conflicting policy options in a multi-actor context: case study. *J Multi-Criteria Decision Anal*. 2001;10(2):111–125, 2001.
[18] Thomopoulos R. A Practical Application Approach to Argumentation for Multicriteria Analysis and Decision Support. *EURO J Decision Processes*. 2018;6(3): 237–255. https://doi.org/10.1007/s40070-018-0087-2, 2018(Accessed February 17, 2022).
[19] Arrow KJ. A Difficulty in the Concept of Social Welfare. *J Polit Econ*. 1950;58(4): 328–346.
[20] Barreteau O, Antona M, d'Aquino P, Aubert S, Boissau S, Bousquet F, Daré W, Etienne M, Le Page C, Mathevet R, Trébuil G, Weber J. Our Companion Modelling Approach. *Journal of Artificial Societies and Social Simulation*. 2003;6(1). http://jasss.soc.surrey.ac.uk/6/2/1.html.
[21] Collectif ComMod. La modélisation comme outil d'accompagnement. *Natures Sci Sociétés*. 2005;13(2):165–168. https://doi.org/10.1051/nss:2005023 (Accessed February 17, 2022).
[22] Ormerod RJ, Ulrich W. Operational research and ethics: A literature review. *Eur J Operational Res*. 2013;228(2):291–307. https://doi.org/10.1016/j.ejor.2012.11.048 (Accessed February 17, 2022).
[23] United Nations (1948). The Universal Declaration of Human Rights. http://www.un.org/Overview/rights.html (Accessed February 17, 2022).
[24] Michael J. *Privacy and human rights: an international and comparative study, with special reference to developments in information technology*. Dartmouth: UNESCO Publishing; 1994. https://digitallibrary.un.org/record/161539?ln=en (Accessed February 17, 2022).
[25] EurLex General Data Protection Regulation and by the Directive (EU) 2016 /680 https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=celex%3A32016L0680 (Accessed February 17, 2022).

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Chemical contaminants in breast milk: a brief critical overview☆

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ABSTRACT

Breast milk is the reference food for the infant both for its content in nutrients, necessary for normal growth and development, and for the presence of biologically active substances that provide protection from infections and a lower susceptibility to several non-communicable diseases typical of adulthood. However, substances that the mother assimilates from the environment, and which can be potentially harmful, can be concentrated in breast milk. In fact, for a long time, breast milk has been considered a reliable biomarker of the environment. The huge increase in the production and use of chemicals that has occurred in recent decades with consequent wide dispersion in the soil, water and air makes it necessary to carefully evaluate the levels of contamination. Based on a synthetic review of current knowledge, it can be confirmed that breast milk is always the first choice. However, various aspects remain to be clarified based on more robust scientific data. This review aims to stimulate further research, managed by multi-disciplinary teams which, with the use of the most modern chemical analysis tools, determine the presence of exogenous chemicals in longitudinal studies during pregnancy and lactation, clarifying their metabolic fate and evaluating them in the 'scope of global exposure (exposome). To this end, the gaps present in the studies conducted so far are also highlighted to make future scientific approaches increasingly robust.

1. Introduction

A recent review has highlighted some significant data on chemical contaminants that have effects of on human health[1].

- in the last decades the industrial production of chemicals has increased significantly and the number estimated varies from 140,000 to over 350,000;
- about 220 billion tons are dispersed into the environment every year;
- these substances are ubiquitous, they spread in the atmosphere, in the soil, in the water and have been found in uninhabited regions, on mountain peaks, at the poles and in the oceans;
- population studies have revealed their presence in various tissues of the human body.

Many of these substances can be hazardous to health even in small doses and the toxicity of many is yet unknown[2].

In USA the presence of 36 environmental chemicals has been tested in children aged 6–18 years through five National Health and Nutrition Examination Survey (NHANES) cycles (2003–2012). Chemicals have

Abbreviations: PAHs, polycyclic aromatic hydrocarbons; BM, breastmilk; DOHaD, developmental origi ns of adult health and disease; FASD, fetal alcohol spectrum disorders; WHO, World Health Organization; EDCs, Endocrine disrupting chemicals; Mg, manganese; Zn, zinc; Mo, molybdenum; Cu, copper; Se, selenium; Pb, lead; Cd, cadmium; Hg, mercury; As, arsenic; MTL, maximum tolerable limits; POPs, Persistent Organic Pollutants; PCBs, polychlorinated biphenyls; BFRs, brominated flame retardants; OCPs, organochlorine pesticides; BPA, bisphenols; PBs, parabens; BPs, benzophenones; MADI, Maximum Acceptable Daily Intake; PFOS, perfluorooctane sulfonic acid; PFOA, perfluorooctanoic acid; PFHxS, perfluorohexane sulfonic acid; CDC, Centers for Disease Control and Prevention; DDTs, dichloro diphenyl dichloro- ethylenes.

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been detected in 93% of sujects and the 5 most represented categories were metals, phenols, pesticides, phthalates, and polycyclic aromatic hydrocarbons (PAHs)[3].

In other words, the development of chemistry has brought considerable benefits to humanity and has favored its development from many points of view, however, one cannot ignore "the other side of the moon" and possible negative effects on human health must be considered also and understood.

The main aim of this overview, since exogenous chemicals are found in breast milk, is to stimulate greater interest in both clinical and basic research to better understand how contaminants may interfere with the positive biological actions of breast milk and the mechanisms that could affect the triad mother-breast milk-child,[4] especially in the long term to avoid any risks to the baby's and mother's health.

2. Advantages of breastfeeding

According to the World Health Organization[5], the American Academy of Pediatrics[6] and the European Society for Pediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN)[7] breastmilk (BM) is the first choice for feeding infants in the first six months of life and in addition to complementary food up to two years of age. This statement was based on several evidence supporting that BM contains macro and micro-nutrients able to guarantee both normal statural-ponderal growth and normal psycho-motor and intellectual development.

Moreover, the bio-active factors contained in BM (oligosaccharides, stem cells, microRNAs, growth factors, hormones, antioxidants, cytokines) are able to perform a wide range of biological actions (still not fully explainable). Actions are both direct and indirect through, for example, changes in the microbiome. These actions are aimed at safeguarding the state of health both in the short and long term.[8].

It is well known in fact that the anti-inflammatory[9], antioxidants[10] and probiotic[11] properties can give to the breast milk an immunological protective effect which, in the poorest Countries, has contributed to the reduction of mortality from infectious diseases in the first years of life[12]. Regarding the long-term effects, there is evidence that BM could help to prevent chronic diseases such as overweight and obesity, hypertension, type 2 diabetes and atopic diseases during adolescence and adulthood[12].

In conclusion, "Breastmilk can be considered a "**live tissue**" which composition varies among women and changes over the course of lactation[13].

It should be remembered also that breastfeeding has benefits for the mother and is associated with less postpartum blood loss and lower risk of breast cancer and cardiovascular disease.

3. Reasons for infant's susceptibility to chemicals

The mammary gland acts as an excretory organ of substances taken by the mother which are transferred into the milk. Since the 1950s BM has been used as a biomonitoring matrix to assess exposure to contaminants in humans, in particular to establish the levels of exposure in the mothers, and pre-natal exposure, and understand transfer of contaminants to the infant through breast feeding[14].

Subsequently, medical research has sought to identify substances in BM that can be harmful for mothers and their infants, however, data at present are scarce.

In the first years of life, infants are particularly sensitive to the possible toxic action of chemicals because of some fundamental differences compared with adults. Infancy is characterized by elevated longitudinl growth, rapid changes in body composition, in numerous metabolic mechanisms and in the various organs. For example, in the first 3 months after birth the brain volume incfeases by over 60% and the brain, lungs, and immune system continue to develop through to the age of 6 years and beyond[15]. Infants eat more food and drink more water per unit of body weight and their respiratory minute ventilation adjusting for weight is greater than in adults[16]. Children's behavior and activity patterns are also much different than that of adults and exposure to contaminents may play an important role[17]. The metabolic processes required for detoxification are immature[18].

Finally, in accordance with the developmental origins of adult health and disease' (DOHaD) hypothesis it has been proven that a contact with different chemicals during pregnancy and the first years of postnatal life can contribute, through epigenetic mechanisms, to favor the onset of metabolic and cardiovascular diseases in later ages[19, 20].

4. Chemical contaminants

4.1. Drugs, therapeutic agents, alcohol, cigarette smoking

In the past years a series of studies have focused on the mother's bad habits (smoking cigarettes, alcohol), on the use of therapeutic drugs and on the problems related with drug addiction.

Briefly, the following pointa summarise current knowledge and indications:

- for the use of therapeutic drugs one can refer to the indications provided by the scientific societies[21].
- the use of illicit.drugs is generally considered a contraindication to breastfeeding[22, 23].
- as far as alcohol is concerned it is is well established that during pregnancy it can harm the unborn baby and be responsible for physical and neurobehavioral damage. These conditions are referred to as fetal alcohol spectrum disorders (FASD).

The possible effects of acohol on breastfed infant is less studied and more controversial. According to some researches, the use of alcohol during lactation can damage neuropsychic development regardless of whether or not there was an exposure in the prenatal period also, and thus should be discouraged[24]. This is also the recommendation of the World Health Organization(WHO)[25]. Other Authors has come to different conclusions after a review of the literature and they argue that there seem to be no risks[26]. Most likely these discrepancies are related with the amount of alcohol consumed the mothers. In the absence of more robust evidence, the Australian guideline recommendation "The evidence does not indicate a safe amount of alcohol that pregnant women and breastfeeding mothers can drink, therefore not drinking is recommended as the safest option"[27] can be supported.

- as far as cigarette smoking is concerned an extensive review of the data collected confirms that smoke is associated with a reduced content of macronutrients, decreased antioxidant properties and altered immune status in human milk. However, there is a need both to deepen the results obtained and above all to place them in a broader context of risk factors[28].

4.2. Metals

Another group of substances worthy of attention is that of metals (some of them also might act as endocrine disrupting chemicals (EDCs) (See next chapter).

Some metals present in trace in BM [(like manganese (Mg), zinc (Zn), molybdenum (Mo), copper (Cu), and selenium (Se)], are necessary for a normal development of the infants.

Others such as lead (Pb), cadmium (Cd), mercury (Hg), and arsenic (As) do not play any biological role, and may cause adverse health effects. Generally, Cd, As and Pb are considered as potential carcinogens and are associated with many diseases affecting cardiovascular and nervous systems and the function of various organs such as liver, kidney, bladder, and bone[29]. Therefore, international organizations such as the WHO have given indications of the maximum tolerable limits (MTL)[30].

In general, the belief has always prevailed that, a part for events (natural and/or industrial disasters), there are no indications for the

suspension of breastfeeding. The widespread diffusion of chemical substances in the environment, the displacement of populations with profound changes in lifestyles and eating habits including eating more prepared food, the industrialization of large areas has stimulated in the last few years, also thanks to more modern chemical analysis skills, the dosage of metals in breast milk in different geographical areas.

The results obtained are heterogeneous[31].

Several studies have focused on one single metal. The determination of lead in breast milk in Moroccan women has, for example, highlighted a mean values of 23.08 μg / L with a very wide range (1.38 - 515.39). Moreover, about 80% of the samples had a Pb content higher than 5 μg / L which is considered the MTL[32].

This wide variability is also found in other Countries of the Mediterranean and other geographical areas with a range between values higher than those in Morocco (31.67 in Saudi Arabia) to values lower than the MTL as in Portugal, Slovakia, and Austria.

Different results have also been reported in the same Country (Italy and Greece in Mediterranean area[32] and in 15 chinese cities[33]. demonstrating the need to carry out, if necessary, a specific biomonitoring in the geographical areas considered to be at greatest environmental risk.

The importance of the place where the mother lives also also turns out to be important in studies that have evaluated the presence in BM of several minerals. In a specific area in Spain, rich in industries and mines, all the mineral elements were present in BM exceeding the MTL with fluctuations from 6% for Cadmium to 60% for Selenium up to a maximum of 92% for Chromium[34].

Higher levels of lead in human milk have been reported in women that live close to industrial or urban areas[35]. The highest levels of arsenic in breast milk were found in a district in West Bengal in India where arsenic in water was above 50 mg/L[36].

However, it should be remembered that various other environmental factors can affect the presence of metals in BM, among these different eating habit. For example, a Norwegian study showed that Hg concentrations were correlated with a high seafood intake as in other Countries (Greece, Italy, Croatia, Slovenia[34],[37]. The European Food Safety Authority recommended that each country should consider its own pattern of fish consumption since 2014[38].

Another example is the correlation between lead levels and the use of lipstick in mothers[39].

4.3. Endocrine Disruptors (EDCs)

Almost 800 chemicals are considered to be Endocrine Disruptors (EDCs) defined as "exogenous substances or mixtures that alters function (s) of the endocrine system and consequently causes adverse health effects in an intact organism, or its progeny, or (sub) populations "[40]. Furthermore, EDCs can cause metabolic disorders and may play an important role in the global epidemic of metabolic diseases. For this reason, for some of these it has also been proposed to use the definition of 'metabolism disrupting chemicals (MDCs)[41].

However, only a small fraction of these chemicals have been tested for their safety or toxicity concern[42].

EDCs are widespread in the environment. They are found in everyday products (toys, personal care products, food and beverages containers, detergents, flame retardants, pesticides, metals). It is therefore not surprising that epidemiological studies have found the presence of EDCs in large populations of adults, children and pregnant women[43, 44] and in the fetus[45]. A wide range of research has shown that EDCs can affect multiple vital mechanisms in the animal world as well as in humans[46, 47] and particularly in the child[48, 49].

The presence of EDCs has also been documented in breast milk.

Among the longest-evaluated substances are Persistent Organic Pollutants (POPs) that include polychlorinated biphenyls (PCBs), brominated flame retardants (BFRs) and organochlorine pesticides (OCPs). These may all persist for many years in the environment and may bio-accumulate in animals and humans (food chain). In addition, to the endocrine effects, especially at the level of the reproductive system, they can cause damage to the central nervous system and be carcinogenic. In 2004, the Stockholm Convention banned their production[50]. In the following years a series of international surveys monitored the presence of POPs in breast milk[51]. Over the years there has been a downward trend in exposure to POPs but, according a recent survey, levels of PCBs have exceeded the toxicologically safe levels in breast-fed infants in all of the reported 51 countries[52].

In addition to POPs, other EDCs may be present in BM. The most studied are reported in Table 1.

In a systematic review that examined 50 scientific publications (over 3000 samples of breast milk) bisphenols (BPA), parabens (PBs), and benzophenones (BPs) were detected in about half of the samples of BM, and this is in agreement with epidemiological data of a wide diffusion in the population. The concentrations of these substances are variable ranging from 0,1 to 3,9 ng/mL of BPA and from 0.5 to>73.5ng/mL of BP-3. This variability can be explained both by methodological differences for the detection, and by the different geographical areas in which the research took place (Asia, America and Europe) and, at least partially, by other several unspecified variables. For instance, higher breast milk concentrations of BPA were observed in multiparous women, in those living in a rural area, and in those with a higher annual household income. Higher concentrations of some PBs were associated with a greater use of plastic food containers or consumption of canned beverages[53].

Although phthalates are one of the categories of chemical substances most produced worldwide and their monoesters were dosed in BM many years ago[54], very few studies have evaluated the presence of both their parent and degradation compounds in breast milk. In a high percentage of samples (up to 100%), however, measurabla amounts of phthalate diesters and / or monoesters have been confirmed and the ingested daily amount recorded was much lower than the Maximum Acceptable Daily Intake (MADI) proposed by European organizations[55].

Per- and polyfluoroalkyl substances (PFASs) represent a group of several thousand substances widely diffused in the environment. Some of these, in particular perfluorooctane sulfonic acid (PFOS), perfluorooctanoic acid (PFOA), and perfluorohexane sulfonic acid (PFHxS) are included in the Stockholm Convention on Persistent Organic Pollutants (POPs). The presence of these substances has been documented in studies carried out in different continents and it is interesting to note that over time their detection rate has decresed but has been replaced by other contaminants which toxicological risk is largely unknown. Finally, the concentrations in milk are lower than those found in maternal blood and in the umbilical cord due to a (protein) mechanism that would hinder the passage into the maternal breast[56].

Table 1
Specific endocrine disruptors and their known main sources.

Endocrine disrupting chemicals	Main sources
Benzophenones	cosmetics sunscreen, food packaging
Bisphenol A (BPA)	polycarbonate for plastic products (drinking bottles, food packaging, toys, medical devices) and epoxy resins (food/beverage containers, electronic devices)
Parabens	food and cosmetic preservatives
Paraffins	flame retardants, metal-cutting fluids, plasticizers and additives in lubricants.
Phthalates	humectants, emollients, or skin penetration enhancers in personal care products. Plasticizers in toys, bags, shoes, cosmetics, food packaging, medical equipment, and building materials.
Per- or polyfluoroalkyl substances (PFAS)	food packaging, cookware, clothing, carpets, fire extinguishers.

4.4. Infant health risks

On the basis of the data reported above, it can therefore be concluded that in a significant percentage of BM samples it is possible to dose chemical substances attributable to general chemical pollution. The point to be addressed is what damage can this cause to human health?

On the basis of current knowledge, there are no definite scientific proven elements that discourage the use of breast milk by weighing advantages and theoretical risks[57].

Therefore, the recommendation that has prevailed is that, beyond events (natural and / or industrial disasters), there is no indication to suspend breastfeeding[58, 59]. The views of the Centers for Disease Control and Prevention (CDC) go in this direction stating that although human milk contamination is a known issue, breastfeeding is recommended and fully endorsed[10]. The same conclusion is reached after an extensive revision of the literature stating that the evaluation of the relationship between the undoubted advantages of breast milk and the possible toxicological disadvantages leads to the conclusion that the former is clearly superior[59].

On the other hand, we cannot forget that some studies report a correlation between EDCs in BM and some clinically evaluable negative consequence in infants like a reduced weight and/or length gain related with BPA[60] or PFAS[61] exposure.

Moreover, many authors agree on the need to deepen the topic with methodologies that take into account the limits of the studies carried out so far[31, 53].

5. Gaps to consider for future research

Some gaps are often found not only in studies that evaluate BM contamination but also in those that more generally take into consideration the possible impact of the environment on human health.

- Many researches are in fact based on the determination of a specific chemical product and they do not evaluate the possible interaction among several categories of chemicals that can act in a synergistic or antagonistic way and the possible presence of substances not foreseen a priori. In real life, one is more likely to come into contact with a mixture of substances. For example, the application of the so-called nontargeted analysis detected the presence 172 halogenated and nonhalogenated cyclic and aromatic compounds in BM and proved that 85% of 40 prioritized contaminants are not typically monitored in breast milk surveys[14].
- To obtain a robust risk assessment it is necessary to assess not only the exposure to the chemicals mixtures but more generally to identify the so-called exposome which on the one hand includes the set of stimuli (environmental and personal) that can come into play and on the other (especially with metabolomics) the ways in which individuals react to such stimuli. It is a difficult task that requires the joint action of specialized teams (epidemiologists, analytical chemists, biologists, biochemists, geneticists and statisticians) but which will allow to pass from simple epidemiological results to the identification of specific biomarkers, to establish a link between the presence in the biological fluids and tissues of various substances and the causality of specific diseases[62].
- There is a need for more longitudinal studies performed from pregnancy (and pre-pregnancy) and to be continued in postnatal life for different reasons: 1) in real life we are continuously in contact with many substances which, even if in small doses, can be harmful even after a distance of time[63]; 2) the presence of a chemical in breast milk can be the expression of a previous accumulation as for Pb which concentration in BM correlates with the amount accumulated in the maternal bones during pregnancy[64]; 3) a single time analysis may not highlight the presence of substances such as BPA which are quickly washed out.

Some other gaps focus more specifically on the features of previous BM studies:

- BM samples must be analyzed repeatedly over time because the concentration of chemicals such as lead, cadmium, aluminium, and arsenic metals has been shown to differ in colostrum, transition milk and mature milk[65]. Many EDCs (polychlorinated biphenyls (PCBs), perfluorooctanoic acid (PFOA), dichloro diphenyl dichloro- ethylenes (DDTs), parabens) are also more present in colostrum[66].
- Comparative studies between cohorts of children who are breast-fed or formula-fed in similar environmental situations would be useful
- Many studies do not specify the type of breast milk tested (pasteurized? fresh?) and whether spot or mixed samples were used. Some substances pass quickly into the milk (BPA) while others have a much slower passage (Pb)
- In general, some reviews of the literature have highlighted a low quality of research[53].

Currently, the European LIFE-MILCH project is ongoing, coordinated by the University of Parma (www.lifemilch.eu)[67] to assess the levels of thirteen different EDCs in breastmilk in relation to maternal life- and diet habits and in formula milk studying the relationships with infant growth, body fat distribution and developmentfrom birth up to the age of 12 months.

6. Conclusions

As already underlined in the light of current evidence, breast milk is the reference food for newborns and infants and breastfeeding must be encouraged and supported by private and public initiatives.

The demonstration of the presence in breast milk of a wide range of potentially harmful chemicals, even if not classifiable as risk factors for health, must in any case stimulate a preventive work which is based above all on avoiding, during pregnancy and breastfeeding, the contact with these substances. This means, in particular, to pay attention to the origin of food, its handling and storage, reducing/avoiding the use of personal care products that often contain EDCs, decreasing indoor pollution and reducing contact with the outdoor pollution as much as possible.

Finally, research in this field should be stimulated and privileged, and consistent with current knowledge to better define signals of risks and to clarify the effects including epigenetic mechanisms.

Declaration of Competing Interest

The corresponding author states on behalf of the co-authors that all Authors have no conflict or competing of interests to declare.

References

1 Naidu R, Biswas B, Willett IR, et al. Chemical pollution: A growing peril and potential catastrophic risk to humanity. *Environ Int*. 2021;156, 106616.
2 Futran Fuhrman V, Tal A, Arnon S. Why endocrine disrupting chemicals (EDCs) challenge traditional risk assessment and how to respond. *J Hazard Mater*. 2015;286:589–611.
3 Hendryx M, Luo J. Children's environmental chemical exposures in the USA, NHANES 2003-2012. *Environ Sci Pollut Res Int*. 2018;6:5336–5343.
4 Verduci E, Giannì ML, Vizzari G, et al. The triad mother-breast milk-infant as predictor of future health: a narrative review. *Nutrients*. 2021;13:486–500.
5 WHO. *Protecting, Promoting, and Supporting Breastfeeding in Facilities Providing Maternity and Newborn Services: The Revised Baby-Friendly Hospital Initiative*. Geneva, Switzerland: WHO; 2018. –2019.
6 American Academy of Pediatrics. Breastfeeding and the use of human Milk. *Pediatrics*. 2012;129:e827.
7 Agostoni C, Braegger C, Decsi T, et al. Breast-feeding: a commentary by the ESPGHAN committee on nutrition. *J Pediatr Gastroenterol Nutr*. 2009;49:112–125.
8 Vizzari G, Morniroli D, Ceroni F, et al. Human milk, more than simple nourishment. *Children*. 2021;8:863–877.
9 Kiełbasa A, Gadzała-Kopciuch R, Buszewski B. Cytokines-biogenesis and their role in human breast milk and determination. *Int J Mol Sci*. 2021;12:6238–6255.

10 Lorenzetti S, Plösch T, Teller IC. Antioxidative molecules in human milk and environmental contaminants. *Antioxidants (Basel)*. 2021;4:550–585.
11 Carr LE, Virmani MD, Rosa F, et al. Role of human milk bioactives on infants' gut and immune health. *Front Immunol*. 2021;12, 604080.
12 Victora CG, Bahl R, Barros AJD, et al. Breastfeeding in the 21st century: epidemiology, mechanisms, and lifelong effect. *Lancet*. 2016;387:475–490.
13 Bode L, Raman AS, Murch SH, Rollins NC, Gordon JI. Understanding the mother-breastmilk-infant "triad". *Science*. 2020;6482:1070–1072.
14 Tran CD, Dodder NG, Quintana PJE, et al. Hoh E. Organic contaminants in human breast milk identified by non-targeted analysis. *Chemosphere*. 2020;238, 124677.
15 Perera FP. Multiple threats to child health from fossil fuel combustion: impacts of air pollution and climate change. *Environ Health Perspect*. 2017;2:141–148.
16 Council on Environmental Health. Chemical-management policy: prioritizing children's health. *Pediatrics*. 2011;5:983–990.
17 Hoffman K, Webster TF, Sjödin A, Stapleton HM. Toddler's behavior and its impacts on exposure to polybrominated diphenyl ethers. *J Expo Sci Environ Epidemiol*. 2017;2:193–197.
18 Grijalva J, Vakili K. Neonatal liver physiology. *Semin Pediatr Surg*. 2013;4:185–189.
19 Vaiserman A. Early-life exposure to endocrine disrupting chemicals and later-life health outcomes: an epigenetic bridge? *Aging Dis*. 2014;6:419–429.
20 Barouki R, Melén E, Herceg Z, et al. Epigenetics as a mechanism linking developmental exposures to long-term toxicity. *Environ Int*. 2018;114:77–86.
21 Sachs HC. Committee on drugs. the transfer of drugs and therapeutics into human breast milk: an update on selected topics. *Pediatrics*. 2013;3:e796–e809.
22 Reece-Stremtan S, Marinelli KA. ABM clinical protocol #21: guidelines for breastfeeding and substance use or substance use disorder, revised 2015. *Breastfeed Med*. 2015;3:135–141.
23 Badowski S, Smith G. Cannabis use during pregnancy and postpartum. *Can Fam Physician*. 2020;2:98–103.
24 May PA, Hasken JM, Blankenship J, et al. Breastfeeding and maternal alcohol use: Prevalence and effects on child outcomes and fetal alcohol spectrum disorders. *Reprod Toxicol*. 2016;63:13–21.
25 WHO. *Guidelines for the identification and management of substance use and substance use disorders in pregnancy. In Guideline for the Identification and Management of Substance Use and Substance Use Disorders in Pregnancy*. Geneva, Switzerland: World Health Organization; 2014.
26 Haastrup MB, Pottegård A, Damkier P. Alcohol and breastfeeding. *Basic Clin Pharmacol Toxicol*. 2014;2:168–173.
27 Day CA, Bonomo Y, Burns L, et al. Providing appropriate treatment and care to people with alcohol problems: a summary for key specific populations. *Med J Aust*. 2021;7(Suppl):S21–S27.
28 Macchi M, Bambini L, Franceschini S, Alexa ID, Agostoni C. The effect of tobacco smoking during pregnancy and breastfeeding on human milk composition-a systematic review. *Eur J Clin Nutr*. 2021;5:736–747.
29 Cai LM, Xu ZC, Qi JY, Feng ZZ, Xiang TS. Assessment of exposure to heavy metals and health risks among residents near Tonglushan mine in Hubei. *China. Chemosphere*. 2015;127:127–135.
30 WHO. *Minor and Trace Elements in Breast Milk: Report of a Joint W.H.O*. Geneva, Switzerland: World Health Organization; 1989.
31 Pajewska-Szmyt M, Sinkiewicz-Darol E, Gadzała-Kopciuch R. The impact of environmental pollution on the quality of mother's milk. *Environ Sci Pollut Res Int*. 2019;8:7405–7427.
32 Cherkani-Hassani A, Slaoui M, Ghanname I, et al. Lead concentrations in breast milk of Moroccan nursing mothers and associated factors of exposure: CONTAMILK STUDY. *Environ Toxicol Pharmacol*. 2021;85, 103629.
33 Li T, Tao XW, Zhang SM, Dai YH. The breast milk lead levels among Chinese population. *Environ Sci Pollut Res Int*. 2018;21:20562–20568.
34 Motas M, Jiménez S, Oliva J, Cámara MÁ, Pérez-Cárceles MD. Heavy metals and trace elements in human breast milk from industrial/mining and agricultural zones of Southeastern Spain. *Int J Environ Res Public Health*. 2021;18:9289.
35 Klein LD, Breakey AA, Scelza B, Valeggia C, Jasienska G, Hinde K. Concentrations of trace elements in human milk: Comparisons among women in Argentina, Namibia, Poland, and the United States. *PLoS One*. 2017;8. e 0183367.
36 Rebelo FM, Caldas ED. Arsenic, lead, mercury and cadmium: toxicity, levels in breast milk and the risks for breastfed infants. *Environ Res*. 2016;151:671–688.
37 Vollset M, Iszatt N, Enger Ø, Gjengedal ELF, Eggesbø M. Concentration of mercury, cadmium, and lead in breast milk from Norwegian mothers: Association with dietary habits, amalgam and other factors. *Sci Total Environ*. 2019;677:466–473.
38 EFSA. Scientific opinion on health benefits of seafood (fish and shellfish) consumption in relation to health risks associated with exposure to methylmercury. *EFSA J*. 2014;12(7):3761. https://doi.org/10.2903/j.efsa.2014.3761.
39 Vahidinia A, Samiee F, Faradmal J, Rahmani A, Taravati Javad M, Mercury Leili M. Lead, cadmium, and barium levels in human breast milk and factors affecting their concentrations in Hamadan. *Iran. Biol Trace Elem Res*. 2019;1:32–40.
40 UNEP/WHO. *State of the science of endocrine disrupting chemicals—2012*. Geneva, Switzerland: United Nations Environment Programme/World Health Organization; 2012.
41 Heindel JJ, Blumberg B, Cave M, et al. Vom Saal F. Metabolism disrupting chemicals and metabolic disorders. *Reprod Toxicol*. 2017;68:3–33.
42 Karthikeyan BS, Ravichandran J, Mohanraj K, Vivek-Ananth RP, Samal A. A curated knowledgebase on endocrine disrupting chemicals and their biological systems-level perturbations. *Sci Total Environ*. 2019;692:281–296. Nov 20.
43 Frederiksen H, Jensen TK, Jørgensen N, et al. Human urinary excretion of non-persistent environmental chemicals: an overview of Danish data collected between 2006 and 2012. *Reproduction*. 2014;4:555–565.
44 Woodruff TJ, Zota AR, Schwartz JM. Environmental chemicals in pregnant women in the United States: NHANES 2003-2004. *Environ Health Perspect*. 2011;6:878–885.
45 Street ME, Bernasconi S. Endocrine-disrupting chemicals in human fetal growth. *Int J Mol Sci*. 2020;4:1430.
46 Street ME, Angelini S, Bernasconi S, et al. Current knowledge on endocrine disrupting chemicals (EDCs) from animal biology to humans, from pregnancy to adulthood: highlights from a National Italian meeting. *Int J Mol Sci*. 2018;6:1647.
47 Predieri B, Alves CAD, Iughetti L. New insights on the effects of endocrine-disrupting chemicals on children. *J Pediatr (Rio J)*. 2022. in press.
48 Iughetti L, Lucaccioni L, Street ME, Bernasconi S. Clinical expression of endocrine disruptors in children. *Curr Opin Pediatr*. 2020;4:554–559.
49 Kiess W, Häussler G, Vogel M. Endocrine-disrupting chemicals and child health. *Best Pract Res Clin Endocrinol Metab*. 2021;202(5), 101516.
50 Stockholm Convention, 2018. Protecting human health and the environment frompersistent organic pollutants. http://www.pops.int.
51 Vukavic T, Miloradov MV, Mihajlovic I, Ristivojevic A. Human milk POPs and neonatal risk trend from 1982 to 2009 in the same geographic region in Serbia. *Environ. Int*. 2013;54:45–49.
52 van den Berg M, Kypke K, Kotz A, et al. WHO/UNEP global surveys of PCDDs, PCDFs, PCBs and DDTs in human milk and benefit-risk evaluation of breastfeeding. *Arch Toxicol*. 2017;1:83–96.
53 Iribarne-Durán LM, Peinado FM, Freire C, Castillero-Rosales I, Artacho-Cordón F, Olea N. Concentrations of bisphenols, parabens, and benzophenones in human breast milk: A systematic review and meta-analysis. *Sci Total Environ*. 2022;806(Pt 1), 150437.
54 Latini G, Wittassek M, Del Vecchio A, Presta G, De Felice C, Angerer J. Lactational exposure to phthalates in Southern Italy. *Environ Int*. 2009;2:236–239.
55 Del Bubba M, Ancillotti C, Checchini L, et al. Determination of phthalate diesters and monoesters in human milk and infant formula by fat extraction, size-exclusion chromatography clean-up and gas chromatography-mass spectrometry detection. *J Pharm Biomed Anal*. 2018;30(148):6–16.
56 Liu Y, Li A, Buchanan S, Liu W. Exposure characteristics for congeners, isomers, and enantiomers of perfluoroalkyl substances in mothers and infants. *Environ Int*. 2020;144, 106012.
57 LaKind JS, Lehmann GM, Davis MH, et al. Infant dietary exposures to environmental chemicals and infant/child health: a critical assessment of the literature. *Environ Health Perspect*. 2018;9:96002.
58 Mead. Contaminants in human milk: weighing the risks against the benefits of breastfeeding. *Environ Health Perspect*. 2008;10:A427–A434.
59 Lapillonne A, Bocquet A, Briend A, et al. Pollutants in breast milk: a public health perspective - a commentary of the nutrition committee of the French Society of pediatrics. *J Pediatr Gastroenterol Nutr*. 2021;3:343–346.
60 Jin H, Xie J, Mao L, et al. Bisphenol analogue concentrations in human breast milk and their associations with postnatal infant growth. *Environ Pollut*. 2020;259, 113779.
61 Jin H, Mao L, Xie J, et al. Poly- and perfluoroalkyl substance concentrations in human breast milk and their associations with postnatal infant growth. *Sci Total Environ*. 2020;713, 136417.
62 Sun J, Fang R, Wang H, et al. A review of environmental metabolism disrupting chemicals and effect biomarkers associating disease risks: Where exposomics meets metabolomics. *Environ Int*. 2022;158, 106941.
63 Kalofiri P, Balias G. Tekos F. The EU endocrine disruptors' regulation and the glyphosate controversy. *Toxicol Rep*. 2021;8:1193–1199.
64 Tellez-Rojo MM, Hernandez-Avila M, Gonzalez-Cossio T, et al. Impact of breastfeeding on the mobilization of lead from bone. *Am J Epidemiol*. 2002;155:420–428.
65 Chao HH, Guo CH, Huang CB, et al. Arsenic, cadmium, lead, and aluminium concentrations in human milk at early stages of lactation. *Pediatr Neonatol*. 2014;2:127–134.
66 Park NY, Cho YH, Choi K, et al. Parabens in breast milk and possible sources of exposure among lactating women in Korea. *Environ Pollut*. 2019;255(Pt 2), 113142.
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Overview on child health, nutrition and food hazards during the first thousand days of life☆

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ABSTRACT

This article discusses the issue of food hazards and child health during the first thousand days of life. The aim is to raise the attention of decision makers, healthcare officers and professionals, including pediatricians, pediatric surgeons, obstetricians, nurses, midwives, dieticians and lactation consultants, on the importance of protecting infants and their families during a most critical period for the mother-child binomial. The conclusions emphasize the importance of encouraging the adoption of integrated strategies, useful at establishing adequate preventive efforts and a game-changing perspective shift in order to develop and adopt efficient monitoring strategies and procedures, able to minimize the risks due to hazards in food throughout the first thousand days of life, as a first line of prevention in children's health.

Introduction

Awareness regarding the notion of food safety has raised globally and significantly in recent years, engaging the stakeholders involved in regulating and actively supervising this issue at all levels[1,2,3]. In parallel, the expectations of consumers and advocacy groups have grown for a progressively increased and interventional role of governments, policy-makers, industry, researchers and healthcare professionals in this area of public health. Their demand to the stakeholders in decision making is for addressing food safety issues and developing adequate solutions and actions pointing at further protecting the health of food users.

It is currently an established and commonly accepted notion[4]that food safety is not absolute, and that food safety refers to a "*reasonable certainty that no harm will result from intended uses under the anticipated conditions of consumption*". This definition recognizes that zero tolerance of risks is realistically not feasible for the majority of foods and the majority of safety contexts, including food chains.

An area of food safety particularly sensitive due to its social implications refers to child nutrition, involving in particular all natural and commercial products that are related to the food provided to infants during the first thousand days of life. Therefore, including food consumed by mothers during pregnancy and infant formula, cereal-based product, fruit-based product, vegetable-based product, meat-based product consumed by infants and children during their first two years of life. The need for effective and continuously updated methods of monitoring food safety during the crucial period of the first 1000 days of life, is increasingly considered of paramount importance in public health to protect the mother-child binomial[5]. The monitoring of hazards in foodstuff, covers the infant food chain from the production of primary products (fruits, vegetable and animal-derived raw materials), throughout the consumer's use (process, storage, packaging) [5].

The aim of this article is to raise the attention of decision makers, healthcare officers and professionals, including pediatricians, pediatric surgeons, obstetricians, nurses, midwives, dieticians and lactation consultants, on the importance of protecting infants and their families during a most critical period for the mother-child binomial. In particular, authors encourage the adoption of integrated strategies, useful at establishing adequate preventive efforts and a game-changing perspective shift in order to develop and adopt efficient monitoring strategies

Abbreviations: NCDs, non-communicable diseases; CNS, central nervous system; WHO, World Health Organization; EU, European Union; SAFFI, Safe Food for Infants.

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and procedures able to minimize the risks due to hazards in food during throughout the first thousand days of life, as the first line of prevention in children's health.

The central developmental periods characterizing the first 1000 days

Three distinct periods can be identified during the first thousand days[6]. They include preconception, pregnancy, and infancy, which have been identified as critical in promoting better outcomes in children's lives [3.] Evidence has highlighted the impact that poor parental health and well-being can have on conceptus[7,8,9], prior to and from the time of conception[6,7]. Specific programs and strategies have been developed to ensure that during the preconception period, biomedical, behavioral, and social risks can be identified and modified to protect women's health or pregnancy outcomes through appropriate prevention and management measures, the purpose of which is to address gestational and pediatric adverse illnesses5. Preconception care programs include distinct approaches that ensure adequate nutritional and physiological support for mothers and their developing conceptus to ensure all they need for optimal health. Minimizing toxic exposures and monitoring the risk of any type of hazard that may predispose to adverse outcomes is an additional important element characterizing preconception care programs[9,10]. Preconception is also regarded as an opportunity for mothers to adopt lifestyle changes5. A subset of preconception care for mothers planning additional pregnancies is interconception care, which is provided to women from delivery through the birth of a subsequent child. It addresses the continuity of risks from one pregnancy to the next[9,10].

The importance of safe development during the nine months of pregnancy has been widely recognized for some time[11]. Factors that most affect the health and development of the conceptus during pregnancy include diet, stress, and exposure to environmental toxins[3]. Finally, the importance of supporting parents and infants in the first two years after birth has been emphasized for decades5. Several public health interventions in this area have been developed in many countries and the factors that influence health and development during this period are widely studied. However, the large amount of available data supporting the importance of establishing effective health services during a crucial period for the mother-child pair does not seem to have been effectively converted into comprehensive and integrated programs that allow adequate support for parents and infants during this period [5.]

Nutrition and health during the first 1000 days

An appropriate and adequate nutritional lifestyle in terms of quality and quantity makes it possible to prevent most non-communicable diseases (NCDs), including cardiovascular diseases (such as myocardial infarction, stroke), cancer, chronic respiratory diseases (such as asthma and obstructive pulmonary diseases) and diabetes, which is responsible for approximately 38 million deaths per year worldwide[12,13]. Nutrition during the first 1000 days of life affects not only the child's bodily growth, but also its development and future intellectual abilities. In addition, an increasing number of studies emphasize that nutrients also play a major role in the maturation of the immune system and in the composition of the gut microbiota, now considered a truly metabolically and immunologically active organ[14,15].

Since the critical "1000 days" include not only the first 24 months of a child's life, but also the period of conception and that of pregnancy, attention must therefore be focused on both maternal and child nutrition, leading to a change of mentality in the approach to nutrition strategies for the mother-child pair during this sensitive period of their life. The mother begins to take care of her and her child's lifestyle and nutrition even before birth. Early childhood therefore represents a window of maximum vulnerability but also a great opportunity for the development of the child. In this period, in fact, the body and, in particular, the central nervous system (CNS), are plastic and therefore susceptible to possible epigenetic changes that are able to modify the risk of disease in the long term. To this regard, NCDs are significantly influenced by diet and lifestyle, which are at the same time the main cause and the "easiest" factor on which to intervene to prevent their negative outcomes16.

Educating to a correct lifestyle and a balanced and safe diet since the earliest stages of life means laying the foundations for future health. It is also of fundamental importance to consider that a child is never a small adult and that, even more so, it is not so in the first years of life[16]. Therefore, providing consumers with safe and quality food must be a fundamental objective of the authorities responsible for food safety, in order to ensure that opportunities for adequate and safe growth are provided to infants and children.

Early Child Development is the result of the interaction between individual biological characteristics and the environment in which he or she is born, lives and grows. Therefore, a positive environment must first and foremost ensure adequate nutrition, implement relational processes (within and outside the family unit), ensure equity, opportunities and adequate social and health services to support the mother-child binomial. The characteristics of the theoretical model of this environment have been elaborated by the WHO Knowledge Network for Early Child Development[17,18]. The early stages of life are crucial in order to set up early interventions for development and health status in later ages. Therefore, it is important to develop preventive strategies to ensure expansion of physical, cognitive, psychological and social-emotional skills leading to increased competence, autonomy and independence[19,20].

Pediatricians, caregivers, decision makers and local governments play a crucial role in promoting best practices in the early stages of child development. It is therefore of great preventive importance to identify biological risk factors that characterize this period and that include intrauterine factors (intrauterine growth retardation, inadequate maternal nutrition, maternal infections, use of tobacco and drugs), birth's factors (preterm birth, complications), child nutrition (insufficient breast feeding, caloric and protein malnutrition) and child infections (chronic diarrhea, parasitosis, human immunodeficiency Virus, malaria, micronutrient deficiency) [19,20]

Nutrition in fertile age

A healthy lifestyle characterized by a varied and balanced diet associated with regular physical activity is a determinant of health for women of childbearing age and pregnancy, for future fathers and for the unborn child. In particular, the nutrition of women from childbearing age to pregnancy, represents one of the greatest challenges in public health as it involves not only the health of women, but also that of future generations. In recent years, several studies have shown that there is a close correlation between nutrition and reproductive capacity, both for men and women. Women who decide to plan a pregnancy should receive adequate nutritional counseling from the healthcare personnel in order to identify and correct risk factors and/or behaviors that could favor adverse reproductive outcomes. Hence, the need to create widespread awareness of nutritional issues, so that healthcare professionals can provide initial corrective guidance when necessary. Malnutrition in excess or in defect is associated with intrauterine developmental disorders of the fetus first and then of the newborn and increased risk of NCDs[16]. In reality, about 50% of pregnancies are unplanned and therefore the first contact with the doctor occurs around the 6th week, when fundamental stages in fetal organogenesis have already occurred. Several factors can negatively influence the reproductive outcome, including the pre-pregnancy, Body Mass Index, inadequate dietary pattern and unhealthy lifestyle, such as tobacco and alcohol habits[18].

Nutrition in pregnancy and fetal programming

As in childbearing years, nutrition plays a fundamental role in pregnancy because it is responsible not only for the well-being of the woman herself but also for the well-being of the fetus during intrauterine life, of the newborn, of the child and of the adult in extrauterine

life[21]. In fact, nutrients act as intra and extracellular messengers, capable of influencing the gene expression of the future individual through epigenetic mechanisms and consequently its growth potential and its susceptibility to disease. An inadequate dietary pattern in terms of quantity and quality is therefore able to negatively affect the outcome of pregnancy and the future health of the individual even before conception. Recent studies on a large sample of pregnant women show that even the main obstetrical diseases such as preeclampsia and premature birth have a lower incidence in those patients whose dietary pattern is characterized by a high consumption of fruits, vegetables, whole grains[22]. Conversely, a diet high in saturated fat (butter, animal fat), sugary drinks, and with reduced intake of fruits and vegetables increases the risk of fetal malformations (neural tube defects, congenital heart disease, cleft lip and palate) and adverse pregnancy outcomes[23]. Gestational diabetes and maternal obesity increase the risk that the child in extrauterine life or as an adult will develop the so-called metabolic syndrome, which is characterized by the combination of three or more of the following conditions: abdominal obesity, hypertension, hypertriglyceridemia, low HDL values, and hyperglycemia[24,25]. A varied, healthy and balanced diet is the essential prerequisite to promote a good outcome of pregnancy and lay the foundation for the future well-being of the new individual. Therefore, in pregnancy and lactation are contraindicated restrictive diets or exclusion in order to reduce the risk of nutritional deficiencies[26].

Nutrition of breastfeeding mothers and of the child up to 24 months of age

The nutrition of the breastfeeding woman, as already highlighted for pregnancy and childbearing age, has an important role in the growth and development of the child. Breast milk which is the natural food for the growth and development of the newborn in the first six months of life, can in fact satisfy all its nutritional needs and it is able to provide it with the essential components it needs for an optimal development. Breast milk is a dynamic food, which changes not only according to the age of the baby, but also during the same day and feeding, to adapt to the nutritional needs of the newborn: it is a real biological system[18,27]. Its composition, as well as being influenced by genetic and environmental factors, can also vary according to the mother's diet: it is therefore important that mothers are ensured a safe diet and that the breastfeeding mother follows a healthy diet adequate to the nutritional needs of milk production. This is why it is of fundamental importance to adequately monitor the risks of contamination in food consumed by the mother during the breastfeeding period and of complementary foods that are introduced in the diet of children typically from 6 to 24 months[18,27].

Potential hazards in infant food

Biological, chemical, or physical hazards can be introduced into the food supply at any time during food collection, processing, transportation, preparation, storage, and service. Understanding the hazards associated with each of these steps can significantly reduce the potential for foodborne illness. All can be prevented through an effective food safety management system[28].

Biohazard occurs when food is contaminated with microorganisms. Many microorganisms are beneficial; however, under the right conditions, some can cause foodborne illness[14] . Foodborne illness can be caused by consumption of food or water contaminated with pathogenic microorganisms, which include bacteria and their toxins, fungi, viruses, and parasites[15,29]. Food can be contaminated both at the source as raw material and during food processing, storage, and distribution. Infected or pathogen-carrying individuals and the environment, through food-contact surfaces and structures, can spread microorganisms into raw or processed foods[28].

Food contaminants include environmental contaminants, food processing contaminants, unapproved adulterants, food additives, and migrants from packaging materials[30]. Generally, chemicals used for pest control or for cleaning and sanitizing food contact surfaces and food preparation equipment can contaminate food. Persistent organic pollutants are a common and hazardous group of chemical contaminants that persist in the environment, bioaccumulate through the food web, and pose a risk of causing adverse human health and environmental effects[1,28] (Table 1).

A variety of foreign materials in food products are hazardous to individuals, causing illness or injury. Foreign items may be unintentionally introduced into food products, or naturally occurring items may not be separated along a food processing line and be excluded from consumption[28] (Table 2). Materials normally absent from food products include metal fragments in ground meat, bone chips, pieces of product

Table 1
MOST COMMON PERSISTENT ORGANIC POLLUTANTS (POPs) AND ASSOCIATED CONTAMINATED FOOD AND HEALTH HAZARDS

POPs	CONTAMINATED FOOD	POSSIBLE HAZARDS
Polyaromatic hydrocarbons (PAHs)	Dairy products, Grain, flour and bran, Rice, Fruit and vegetables, Oyster, Water	Mutagenicity/ carcinogenicity, DNA damage, oxidative stress, impaired male fertility, respiratory diseases, cognitive dysfunction among children and cancer (breast cancer)
Organochlorine pesticide (OCPs)	Eggs, Dairy products, Meat and meat products, Rice, Fruit and vegetables, Honey, Oil, Fish, Mussel, Water	Neurological symptoms, endocrine disruption, infertility and fetal malformation, diabetes, cancer (breast cancer, testicular, prostate and kidney cancer), reproductive problems, cardiovascular problems, high blood pressure, glucose intolerance and obesity
Polychlorinated biphenyls (PCBs)	Eggs, Dairy products, Meat and meat products, Rice, Fruit and vegetables, Oil, Fish, Mussel, Water	Endocrine disruption, neurological disorders, liver injury, diabetes, cancer (breast, prostate, testicular, kidney, ovarian and uterine), cardiovascular problems and obesity
Polybrominated diphenyl ethers (PBDEs)	Fish, Mussel,	Reproductive problems, cancer (testicular), diabetes, obesity and cardiovascular problems
Perfluorinated compounds (PFCs/PFOS and PFOA)	Eggs, Fish, Water	Breast cancer
Hexabromocyclododecanes (HBCDs)	Eggs, Oil, Fish,	Endocrine disruption, reproductive problems and behavioral disorders
Polychlorinated naphthalenes (PCNs)	Meat and meat products,	Cancers
Dioxins/furans	Eggs, Dairy products, Meat and meat products, Oil, Fish,	Language delay, disturbances in mental and motor development, cancer, diabetes, endocrine disruption, high blood pressure, glucose intolerance and cardiovascular problems

(From Pettoello-Mantovani et al, J Pediatr. 2021;229:315-316.e2, modified)

Table 2
TYPICAL SOURCES OF PHYSICAL HAZARDS IN FOOD

- **Metal:** fragments from equipment such as splinters, blades, needles, utensils, staples
- **Glass:** light bulbs, glass containers and glass food containers
- **Stones:** incorporated in field crops, such as peas and beans, during harvesting
- **Plastic:** material used for packaging, fragments of utensils used for cleaning equipment
- **Wood:** splinters from wood structures and wooden pallets used to store or transport ingredients or food products
- **Natural components of food:** hard or sharp parts of a food (e.g.: shells in nut products)
- **Metallic contaminants:** Natural and anthropogenic sources of heavy metal contamination include agricultural activities, such as pesticide and herbicide application, contaminated irrigation water, municipal waste used for fertilization and mineral fertilizer containing traces of heavy metals.

(From Pettoello-Mantovani et al, J Pediatr. 2021;229:315-316.e2, modified)

packaging, stones, glass or wood fragments, insects or other dirt, and personal items[31]. In addition, individuals may be exposed to metals and metal compounds as environmental pollutants from industrial or other human activities[31]. Heavy metals such as lead, arsenic, mercury, or cadmium may be considered a potential contaminant. These substances are of concern because of their toxicity[28], especially with long-term intake, because they can accumulate in the body and cause organ damage, especially in susceptible groups, including young children[31].

Coaching families in the practice of healthy nutrition and lifestyles during the first thousand days

Health care professionals, including pediatricians and primary care physicians, are at the forefront of prescribing foods for infants and accompanying families in practicing healthy eating and lifestyles[5,28]. Therefore, it is of central importance for them to be adequately informed about common risks involved in the food chain, such as environmental contamination, process contamination, contamination through packaging, including biological and chemical risk, and misuse5,28. Hence, it is also of paramount importance for this group of stakeholders to be constantly updated and informed about current monitoring programs and new and additional effective procedures and methods that can achieve adequate food safety assessment and monitoring, which are being developed, made available and used by government and formal authorities to safeguard their communities[5,28].

Monitoring food safety in economically advanced countries. Europe

The food industry in economically advanced areas of the world, particularly Europe, has long been interested in the issue of food chain safety, and over time has developed adequate means for internal monitoring of safety processes[5,28]. However, the international market is expanding and the European Union is overexposed to imports of non-EU products that often lack adequate safety procedures in the countries where imported baby food is produced, as well as serious uncertainty about quality assurance of the various steps involved in the food chain in non-EU countries (i.e., the cold chain and its logistics). In order to properly address this issues, the EU Commission has launched the Safe food for infants (SAFFI) project, within the frame of the Horizon 2020 program[32]. The scope of this EU project is to develop adequate monitoring systems and assist competent authorities and industry to further advance their control procedures and formulate appropriate decisions in this sensitive area of public health, while contributing strongly to further ensure the safety of the population and its perception of being adequately cared for. Establishing adequate technical measures useful to advance the monitoring of baby food safety, will further protect the European food industry from economic and reputational damage to the sector, possibly caused by health incidents due to the difficulty of adequately supervising the safety chain of imported baby food. .

Conclusions

As emphasized by recent reports, nutrition is a desperately neglected aspect of maternal, newborn, and child health, and the reasons for this neglect are understandable but not excusable[33,34]. According to UNICEF, more than 200 million children living in both economically advanced and poor countries worldwide do not reach their developmental potential in the first 5 years of life because of poverty, inadequate nutrition, insufficient health services and psychosocial care.

Available scientific evidence confirms that the first 1,000 days of life are critical for proper physical and mental development and many preventive and/or curative interventions implemented early in this window of time lead to positive short, medium and long-term health outcomes for the individual and the community[33,34].

Due to population growth and global threats, the integrity and security of global food chains are at risk. In many countries, simply having enough to eat can be a problem, with poor quality food often contaminated with dangerous agents, while in developed countries the pressure to provide cheap and accessible food can affect quality and safety[5,16].

The fate of nations is determined by what they eat [35], and pediatricians are on the front lines of containing the risks of food hazards[7,17]. They can play a key role if they actively collaborate and integrate their efforts with governments and local, state, federal, and global public health institutions and agencies to ensure that infants and children have access to nutritionally adequate diets and safe food[5,16]

Statement

The corresponding author states on behalf of the co-authors that all Authors have no conflict or competing of interests to declare.

References

1 Rather IA, Koh WY, Paek WK, Lim J. The sources of chemical contaminants in food and their health implications. *Front Pharmacol*. 2017;8:830.
2 Pettoello-Mantovani M, Ehrich J, Sacco M, Ferrara P, Giardino I, Pop TL. Food insecurity and children's rights to adequate nutrition in Europe. *J Pediatr*. 2018;198, 329-30.e1.
3 European Commission. *Food safety: overview*; 2022. https://ec.europa.eu/food/overview_en.
4 Bedair M, Glenn KC. Evaluation of the use of untargeted metabolomics in the safety assessment of genetically modified crops. *Metabolomics*. 2020;16:111.
5 Indrio F, Dargenio VN, Marchese F, Giardino I, Vural M, Carrasco-Sanz A, Pietrobelli A, Pettoello-Mantovani M. The importance of strengthening mother and child health services during the first 1,000 days of life: foundation of optimum health, growth and development across the lifespan. *J.Pediatrics*. March 2022. First online.
6 Moore T, Arefadib N, Deery A, West S. The first thousand days: an evidence paper. Analysis and policy observatory. *Report*; 2017. https://apo.org.au/node/108431.
7 Wilcox AJ, Baird DD, Weinberg CR. Time of implantation of the conceptus and loss of pregnancy. *N Engl J Med*. 1999;340:1796–1799.
8 Chavatte-Palmer P, Vialard F, Tarrade A, Dupont C, Duranthon V, Lévy R. DOHaD and pre- or peri-conceptional programming. *Médécine Sci*. 2016;32(1):57–65.
9 Genuis SJ, Genuis RA. Preconception care: a new standard of care within maternal health services. *BioMed Researc*; 2016. https://www.hindawi.com/journals/bmri/2016/6150976/.
10 Ratcliffe SD, Rosener SE, Frayne DJ. Preconception care. Eds.. In: Paulman PM, Taylor RB, Paulman AA, Nasir LS, eds. *Family Medicine: Principles and Practice (7th. Ed.)*. Geneva, Switzerland: Springer International; 2017:127–139
11 Kim HW, Kim DH, Lee HY, Lee YJ, Ahn HY. Adult perceptions of healthy pregnancy: a focus-group study. *Int J Environ Res Public Health*. 2020;17(7):2460. Apr 3.
12 Goldhaber Michael H. The attention economy and the net. *First Monday*. 1997;2(4). http://www.firstmonday.org/issues/issue2_4/goldhaber.
13 Essential Nutrition Action: mainstreaming nutrition through the life-course. https://apps.who.int/iris/bitstream/handle/10665/326261/9789241515856-eng.pdf.
14 Hojsak I, Fabiano V, Pop TL, Goulet O, Zuccotti GV, Çokuğraş FC, Pettoello-Mantovani M, Kolaček S. Guidance on the use of probiotics in clinical practice in children with selected clinical conditions and in specific vulnerable groups. *Acta Paediatr*. 2018;107(6):927–937. Jun.
15 Fabiano V, Indrio F, Verduci E, Calcaterra V, Pop TL, Mari A. et al. Term Infant Formulas Influencing Gut Microbiota: An Overview. *Nutrients*. 2021;13(12):4200. Published 2021 Nov 23.
16 Pop TL, Namazova-Baranova L, Mestrovic J, Pettoello-Mantovani M. The role of healthy lifestyle promotion, counseling, and follow-up in noncommunicable diseases prevention. *J Pediatr*; 2019 Nov 15. http://www.epa-unepsa.org/sites/default/files/LibroDef.pdf.

17 Maćkowiak B, Matějka F, Wiederholt M. European Central Bank. Frankfurt. Working paper series. Rational inattention: a review. https://www.ecb.europa.eu/pub/pdf/scpwps/ecb.wp2570~a3979fbfa5.en.pdf (Accessed, 04 May, 2022).
18 Maggi S, Irwin LG, Siddiqi A, Poureslami I, Hertzman E, Hertzman C. 6-9. Knowledge network for early childhood development. Analytic and strategic review paper: International perspectives on early child development. Vancouver. *Human Early Lear Partn*. 2005.
19 Victora CG, Adair L, Fall C. Maternal and child undernutrition study group. Maternal and child undernutrition: consequences for adult health and human capital. *Lancet*. 2008;371:340–357.
20 Bhutta ZA, Ahmed T, Black RE, et al. What works? Interventions for maternal and child under-nutrition and survival. *Lancet*. 2008;371:417–440.
21 Calkins K, Devaskar SU. Fetal origins of adult disease. *Curr Probl Pediatr Adolesc Health Care*. 2011;41(6):158–176. https://doi.org/10.1016/j.cppeds.2011.01.001.
22 Englund-Ögge L, Brantsæter AL, Sengpiel V, Haugen M, Birgisdottir BE, Myhre R, Meltzer HM, Jacobsson B. Maternal dietary patterns and preterm delivery: results from large prospective cohort study. *BMJ*. 2014 Mar 4;348:g1446.
23 Vujkovic M, de Vries JH, Dohle GR, et al. Associations between dietary patterns and semen quality in men undergoing IVF/ICSI treatment. *Hum Reproduct*. 2009;24(6):1304–1312.
24 Felipe Lobelo F. Fetal programming and risk of metabolic syndrome: Prevention efforts for high-risk populations. *Pediatrics*. 2005;116(2):519–520.
25 Boney CM, Verma A, Tucker R, Vohr BR. Metabolic syndrome in childhood: association with birth weight, maternal obesity, and gestational diabetes mellitus. *Pediatrics*. 2005;115(3):e290–e296. Mar.
26 Ferrara P, Corsello G, Quattrocchi E, Dell'Aquila L, Ehrich J, Giardino I, Pettoello-Mantovani M. Caring for infants and children following alternative dietary patterns. *J Pediatr*. 2017;187. Aug339-340.e1.
27 World Health Organization. Infant and young child feeding. https://www.who.int/news-room/fact-sheets/detail/infant-and-young-child-feeding (Accessed, 05 May, 2022).
28 Pettoello-Mantovani M, Mestrovic J, Namazova-Baranova Md PhD L, Giardino I, Somekh E, Vural M. Ensuring safe food for infants: the importance of an integrated approach to monitor and reduce the risks of biological, chemical, and physical hazards. *J Pediatr*. 2021;229. Feb315-316.e2.
29 Pettoello Mantovani M, Guandalini S, Ecuba P, Corvino C, di Martino L. Lactose malabsorption in children with symptomatic Giardia lamblia infection: feasibility of yogurt supplementation. *J Pediatr Gastroenterol Nutr*. 1989;9:295–300.
30 Silano M, Vincentini O, Luciani A, Felli C, Caserta S, Esposito S. Early tissue transglutaminase-mediated response underlies K562(S)-cell gliadin-dependent agglutination. *Pediatr Res*. 2012;71:532–538.
31 Raia PK, Leeb SS, Zhangc M, Tsangd YF, Kime KH. Heavy metals in food crops: health risks, fate, mechanisms, and management. *Environ Int*. 2019;125:365–385.
32 SAFFI. Taking microbial and chemical hazards off baby's menu. https://www.saffi.eu (Accessed, 04 May, 2022).
33 Black RE, Victora CG, Walker SP, et al. Maternal and child nutrition study group. Maternal and child undernutrition and overweight in low-income and middle-income countries. *Lancet*. 2013;382:427–451.
34 Horton R. Maternal and child undernutrition: an urgent opportunity. *Lancet*. 2008;371:179.
35 ed. Brillat-Savarin JA. Physiologie du gout, ou Meditations de gastronomi transcendante. Cie, 1826. In: Sautelet A, ed. *The Physiology of Taste*. Philadelphia: Lindsay & Balckstone Ed; 1854

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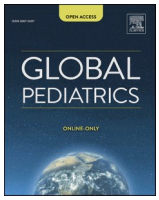

Food safety and public health within the frame of the EU legislation☆

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ABSTRACT

The purpose of this article is to examine some of the issues related to qualitative food safety within the framework of European Union legislation. The development of a multidimensional regulatory system at European level, which has necessarily included international sources, regulations and European Union laws, also relating to national and regional legislations, has rendered finding a balance between the legitimate interests of food producers and consumers problematic. In recent years, the ethical dimension of food has progressively developed, which has led to a greater attention to the way food is produced and consumed, while respecting health protection, food quality and European and international trade dynamics. On the consumer side, however, there has been a growing awareness of the possible risks linked to food and an attention to the issues of food safety. Such awareness is intensified by the use of certain technologies in the food sector. Consumers are increasingly looking to buy commercial products capable of minimizing damage to their health. Consumers consciousness has also influenced the industry, which has increasingly felt the need to pay greater attention to the entire production cycle, thus encouraging production carried out by following the correct methods of supply, processing, up to the final stages of packaging, storage, processing and distribution, in accordance with the "Good Agricultural Practices" (GAP). In conclusion, the important monitoring path of product traceability that has led to a significant increase in the commitment to EU legislative supervision, risk assessment and review of the substances used in food production.

1. Introduction

The various food incidents that took place during the late 1990s draw attention to the need to establish general principles and requirements concerning food and feed law at Union level. In response, the European Commission developed a comprehensive and integrated approach to food safety, 'from farm to fork'[1,2], primarily set out in its White Paper on Food Safety. The approach covers all sectors of the food chain, including feed production, primary production, food processing, storage, transport and retail sale[3].

In 2002, the European Parliament and the Council adopted Regulation (EC) No 178/2002 laying down the general principles and requirements of food law (hereinafter, the "General Food Law Regulation")[3] The General Food Law Regulation is the foundation of food and feed law. It sets out an overarching and coherent framework for the development of food and feed legislation both at Union and national levels. To this end, it lays down general principles, requirements and procedures that underpin decision making in matters of food and feed safety, covering all stages of food and feed production and distribution. The European Parliament also took an important step forward by developing the European Food Safety Authority (EFSA)[4], an independent agency responsible for scientific advice and support. Currently, the General Food Law Regulation ensures a high level of protection of human life and consumers' interests in relation to food, while safeguarding the effective functioning of the internal market[2].

This article seeks to analyze some of the issues related to qualitative

Abbreviations: Good Agricultural Practices", (GAP); Food and Agriculture Organization, (FAO); European Food Safety Authority, (EFSA); Treaty on the European Union, (TEU); Treaty on the Functioning of the European Union, (TFEU); Safety food for infants, (SAFFI); Hazard Analysis and Critical Control Point, (HACCP); Rapid Exchange of Information System, (RAPEX).

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food safety within the framework of EU legislation. Indeed, the development of a multidimensional framework at European level has rendered finding a balance between the rightful interests of food producers and those of consumers complicated. In order to achieve a reasonable balance between the interests of the involved stakeholders, maximum cooperation of the countries involved is deemed essential, especially at EU level, but also between nations, with trade agreements concerning the marketing of food, in order to reduce forms of protectionism and implement the free trade of food products both in the European Union and internationally. Finally, it is necessary to consider the great push of technological innovation in the field of food production, which will have to be increasingly controlled in order to avoid abuses and violations of legislative rules, considering, however, the importance of changes in dietary habits and food traditions[5], without giving in to the often extreme or manipulative positions represented and proposed by pressure groups and opinions.

1.1. Food security. General concepts

According to legal doctrine, the notion of "food safety", includes the concept of both "quantitative food safety", aimed at solving hunger related problems and forms of inequality, and "qualitative food safety", which meets the needs of the market and its marketing of products and involves issues related to health protection[3,5,6]. In less economically advanced societies, the problem relating to food quantity is prevalent, while in economically advanced societies, food quality issues represent a factor of primary importance. According to this conceptual approach, food therefore presents numerous risks to people's health. In fact, as a result of the globalization of markets and the continuous technological progress, there has been a growing push to the development of so-called "unconventional" foods, produced at a lower cost and often with the aim of replacing the "traditional" ones[6].

New knowledge and technological innovations have diversified food products, but at the same time have increased the risk of consuming the same products[2]. Moreover, in industrial countries there is an exploitation of natural resources and a situation of uncertainty in research methodologies and quality control that makes it difficult to predict, verify and quantify the consequences arising from the proper or improper use of these technologies in food production[7]. In addition, the presence of areas with high environmental impact within national territory as well as the presence of contaminants can determine an additional risk for consumers[7]. It is also important to emphasize that the harmful effects of the use of modern technologies can occur after a long time, following continuous exposure to substances or ingestion of food, which even in small doses could damage the health of the individual in the short, medium or long term.

In recent years, an ethical dimension of food has progressively developed, which has led to a greater attention to the way food is produced and consumed, while respecting health protection, food quality and European and international trade dynamics[8]. On the consumer side, however, there has been a growing awareness of the possible risks linked to food and an attention to the issues of food safety for the consumer, who is increasingly looking to buy commercial products capable of minimizing damage to health and a strong fear of new forms of intervention of technological development in this sector. This greater awareness has also influenced the industry, which has increasingly felt the need to pay more attention to the entire production cycle, thus encouraging the development of production carried out according to Good Agricultural Practices, following the correct methods of supply, processing, up to the final stages of packaging, storage, processing and distribution. Therefore, with an important monitoring path of product traceability[8] that has led to a significant increase in the commitment to supervision, risk assessment and review of the substances used.

In order to obtain a reasonable balance between the interests of the stakeholders involved, maximum cooperation of the countries involved is required, particularly, at EU level, but also between nations with trade agreements concerning food marketing, in order to reduce forms of protectionism and implement the free trade of food products both within the European Union and internationally[9]. Finally, it is necessary to consider the significant push of technological innovation in the field of food production, which will need to be increasingly controlled in order to avoid abuses and violations of legislative rules, while also taking into account the importance of changes in dietary habits and food traditions[10], without succumbing to the frequently extreme or manipulative positions represented and proposed by pressure groups and opinions[10].

1.2. Free circulation of products, food safety and food legislation in the European Union

The main problems to be dealt with in relation to food safety concern the different application of legislation on product safety from one Member State to another. In this regard, legislative requirements related to goods are complex for economic operators, who have to deal with different legislative acts to be applied to a food product. In addition, further inconsistencies have emerged in product legislation, such as the use of different terminologies to describe concepts common to European legislation[11,12]. A further issue concerns the presence of conflicting interests and behaviors of the subjects involved, between the protection of the free movement of food products in the European market and the protection of health[12].

The free movement of goods within the European legislation is one of the founding factors of the single market and represents the core of the establishment of the European Union. Since the 1970s, European Union legislation has guaranteed uniform protection of the consumer, the environment and energy resources through the free movement of goods within the Union. To this end, an integrated strategy has been developed in order to ensure a high level of health protection through consistent measures and adequate controls[2]. In this context, Union action related to health is auxiliary to the action of Member States, therefore the European Union has played a coordinating role, unlike the European harmonization policies implemented in the agricultural sector[1]. Thus, the European action aimed at protecting the right to health, on the one hand, has given rise to direct measures through the provision of secondary legislation and, on the other hand, has determined the adoption of soft law policy documents[13], without, however, ignoring the needs of individual states. Therefore, with reference to consumers, European policy has supported and integrated national policies to protect food safety and health. In this regard, an important element is represented by the EU Charter of Fundamental Human Rights, which, while recognizing in Article 16 the freedom to conduct a business, in accordance with EU law and national laws and practices, protects other interests which primarily include health. In this regard, Article 35 of the Charter indicates that a high level of protection of human health shall be ensured in the definition and implementation of all Union policies and activities, and Article 38 states that *"Union policies shall ensure a high level of consumer protection"*[3].

The Treaty on the European Union (TEU) states that health is protected in the same way as business and consumers[14]. On this matter, Article 3 of the TEU provides that the European Union must work for the sustainable development of Europe. Therefore, based both on balanced economic growth and price stability and on the awareness that these economic processes are based on a highly competitive social market economy aiming at full employment and social progress, constructed on a high level of protection and improvement of the quality of the environment. Article 6 of the Treaty on the Functioning of the European Union (TFEU) states that the European Union supports, coordinates and supplements the action of Member States both in protecting and improving human health. In particular, Article 168 of the TFEU provides in the first paragraph that in the implementation of the policies and activities of the European Union, a high level of human health protection must be guaranteed, through the prevention of diseases and illnesses and the elimination of sources of danger. According to European legislation,

therefore, the action of the Union must complement national policies and is aimed at improving public health, preventing diseases and eliminating sources of danger to physical and mental health. This action includes the fight against major scourges such as pandemics, promoting research into their causes, their spread and their prevention. Nevertheless, legislation in this area goes further and includes the important activity of health information and education, as well as surveillance, alerting and combating serious cross-border health threats.

1.3. Consumer protection

A large and important legislative chapter of the European Union is dedicated to consumer protection through Article 169 of the TFEU[14]. With this provision, the Union is committed to protecting health, safety and economic interests of consumers as well as promoting their right to information, education and organization to safeguard their interests. In particular, the European Union, through its legislation and regulations, pursues the objective of guaranteeing citizen participation in the single market through greater protection in the purchase of goods and services. Therefore, this explicit involvement of consumers implies the definition of an evolving regulatory framework. That is, one that is capable of identifying the tools and filling the gaps in existing norms and practices in Europe and through a process of education, information and awareness, pursuing the objective of creating an environment in which consumers can choose the best offers for products and services.

Moreover, Article 191 of TFEU[14] completes the European regulatory framework by providing for the precautionary principle to protect not only the environment, but also health. This is a general principle codified at Union level, which compels the competent authorities (including local authorities) to adopt appropriate measures in order to prevent potential risks to public health, safety and the environment (including foodstuffs), by means of providing, in advance, protection of the application of the principle of prevention, in the absence of the verification of a causal link between the harmful event and the resulting prejudicial effects. The application of the precautionary principle, in the case of a situation in which the potentially dangerous effects of a product or of a process have not been identified and in which the preliminary scientific evaluation has not made it possible to determine a potential risk with sufficient certainty, has made it possible to prevent the distribution or to withdraw dangerous food products from the market, thus letting the protection of the right to health or of the environment prevail over economic interests[14]. This principle must be certainly coordinated with those of free competition, freedom of establishment and freedom to provide services provided for by the TFEU.

1.4. Control over the trade of food products within the European Union

Article 36 of the TFEU[14] states that quantitative restrictions on imports and measures having equivalent effect (Article 34 TFEU) and quantitative restrictions on exports and measures having equivalent effect (Article 35 TFEU) may be enforced on imports, exports and goods in transit on grounds of public morality, public order, public security, health protection, life or animal protection or plant preservation. However, it is also indicated that such prohibitions or restrictions must not constitute a means of arbitrary discrimination or a disguised restriction on trade between Member States[26]. In this regard, the Member State, before importing animals or products of animal origin from a country of the European Union, may carry out controls of a non-discriminatory nature. In particular, Article 10 of Regulation No. 1235/2008 of the European Commission[15] provides that a list must be drawn up of the inspection bodies and authorities recognized for the purposes of equivalence that are competent for carrying out controls and issuing certificates in third and public countries. The general requirement for the export of foodstuffs is compliance with the food hygiene regulations in force in the exporting country, and the basic requirement in exporting countries is compliance with the regulations of the European Commission[15]. Therefore, it is indispensable for the producer to guarantee the traceability of food products from their origin to the consumer's table. European legislation foresees that in situations of potential danger for the consumer in the production of a food product, it is necessary to apply procedures capable of identifying the product placed on the market and withdrawing it even when it has been exported to other countries. The safety of exported products requires the observance of conditions of reciprocity with third countries, and initiatives related to this requirement allow for the specific objective of strengthening and improving product safety through effective market surveillance throughout the EU.

1.5. Food safety in the European legal system

The regulatory evolution of food safety involves a plurality of players and provides for action plans with different procedures. Initially, the legislation on the safety of products marketed within the European Community[16] (Directive n. 92/59/EEC, and the subsequent Directive n. 2001/95/EC, on industrial products, including foodstuffs) required operators to place on the market products that were safe for human health[16]. However, this regulation evolved in the nineties, and following numerous episodes that involved the public health of the European population, (such as food contaminations and environmental disasters) the community regulation of production and marketing of food and feed was modified. In particular, according to a food strategy pursued by the European Union, both a legislation on the safety of food products and animal feeds and a regulation based on scientific evidences have been foreseen as a legislative support to the formal deliberations and control acts[3]. Therefore, European legislation provides for the control of food at all stages of the food production process, from production, processing, transport, distribution to supply to the consumer[3]. In this context, Union law also specifically provides for preventive and subsequent protection in favor of the consumer, notwithstanding the promotion of free trade of food products. The European Union, however, also protects possible obstacles to trade, disparities in safety standards in Member States and possible distortions caused by competition in the internal market. With regard to the consumer and the protection of his or her health, the precautionary principle provides for safety requirements for every product placed on the market and intended for consumption and establishes that a food product is considered safe when it does not present any risk, or presents reduced and acceptable risks[2,3,4]. The efforts of the European Commission are therefore directed to food safety and to the development of advanced and scientifically useful procedures to minimize the risks of food contamination, starting from foods produced for infants, as it is well evidenced by the project Safety food for infants (SAFFI) financed by the European Commission in the framework of the Horizion 2020 program to monitor risks[6,17].

Regulation No. 178/2002 of the European Parliament and of the European Council of January 28, 2002[18] establishing *"the general principles and requirements of food law"* and laying down *"procedures in the field of food safety*" represented a milestone in European Union food legislation. In fact, this regulation aims to harmonize the free movement of food with the principles of food safety, inspired to the search of a high level of protection of health and animals and to the control of the movement of food and feed along the entire chain of agricultural products, following the principle of protection of food from farm to table[2,18]. Although complex and articulated in a variety of norms that need to be balanced and integrated, the European legislative framework provides that each of its provisions is applied to the extent that there are no specific provisions with the same objective that regulate the safety of the products in question3,18. In particular, the provision of the principles of food legislation in Regulation No. 178 of 2002[18] has allowed for the issuance of further regulations, directives and decisions governing various aspects of food safety. In this regard, when there is specific legislation, it applies to aspects or categories of risks not foreseen by general legislation18. For example, in the case of legislation relating to

genetically modified organisms, the integrating norms refer to various legislative passages[19] that include Regulation (EC) No. 1829/2003, Regulation (EC) No. 1830/2003, Regulation (EC) No. 1333/2008 of the European Parliament and of the Council[19], which established a Union list of food additives, and finally[20], Directives No. 2008/60/EC, No. 2008/84/EC, 2008/128/EC and 2009/10/EC, concerning specific purity requirements for food additives[20]. Therefore, legislation does not always help to clarify the rules to be followed in the field of food safety, whereas simpler and clearer legislative norms would be necessary to apply to such an important area of interest for public health as food safety and its monitoring.

While at the European level, there are various areas of intervention and coordination in the field of research and regulation, at national level there is a sector of discipline and collaboration between operators in the food chain. For example, regarding the attribution of competences, Regulation No. 178 of 2002[21] has appointed the legislative functions to Community institutions. On the other hand, it is foreseen that the Commission, the government bodies of the Member States and the national and European Union authorities with their respective committees and bodies carry out a co-administrative action for the purpose of achieving food safety which seems however not to be always effective. In this regard, Article 23 of Regulation No. 178 of 2002, for example, entrusts the European Food Safety Authority with a different role and different tasks, such as the creation of a system of networks between organizations, and assigns responsibility for the functioning of these structures, thus delegating responsibilities and in some way removing the possibility of a closer, direct and effective control[21].

1.6. The relationship between European and national legislations

The relationship between European and national legislation requires that the principle of coordination be applied. Therefore, the above-mentioned European legislation is flanked by the regulations of individual Member States, in order to prevent risks to the health and safety of the consumer. However, as far as food safety is concerned, European legislation has not excluded the regulatory intervention of Member States. Each Member State is required to organize its own system in accordance with the European coordinated system of food safety[3]. Therefore, the current food safety discipline can be found in the coordination between the European system and those of the individual Member States and between the latter and any local legislation. Although it can be further improved, we can consider the European food legislation as complete, since it regulates the actions of the operators involved and it contextualizes and differentiates, through a general legislation applicable to food and feed and a special legislation in those areas where it is necessary, a more specific consumer protection, which includes food hygiene, the use of pesticides, food supplements, colorants, antibiotics, vitamins, minerals and similar substances3.

1.7. Risk management: traceability requirement

Consumer protection is concerned with preventing harm from the circulation of foods that are hazardous to health. This preventive function is identifiable in the regulations that prohibit the marketing of foods that are either harmful to anyone, or to individuals who require specific protection against the intake of certain foods[22]. Aforementioned Regulation No. 178 of 200221 constitutes the foundation in the food sector of a high level of protection of the health and interests of consumers and at the same time of the functioning of the internal market. In addition, the analysis of hazards and critical points in the system of production and distribution of food products is regulated by technical rules taken from the Hazard Analysis and Critical Control Point ("HACCP") system. According to the existing Regulation[22], there is an obligation for producers and distributors of food products to place on the market products that comply with predetermined safety requirements, which include risk analysis (Art. 6), the precautionary principle (Art. 7), protection of consumer interests (Art. 8), transparency in the development of food law (Art. 9), consumer information (Art. 10), safety obligations for food business operators (Art. 11-20), establishment of the European Safety Authority (Art. 22-49), and procedures related to food emergency situations (Art. 50-57)[22,23].

1.8. Definition of food according to European legislation

Article 2 of Regulation (EC) No. 178 of 2002[18] indicates that food is any processed, partially processed or unprocessed substance or product intended to be ingested by humans. However, the rule does not solve the question of the different designations provided in Member States. Therefore, the Court of Justice and part of the doctrine have applied the criterion of mutual recognition, which attributes equivalence to national rules of production and presentation of foodstuffs in intra-community trade[18]. In addition, it is of fundamental importance to distinguish human foodstuffs (food) from medicinal products, which according to Directive 2001/83/EC are products with a therapeutic effect and those which, despite not having such effects, are presented as such[18,24].

A further differentiation and definition concerns feed, which according to Article 3 of the regulation are any substances or products, processed, partially processed or unprocessed, intended for oral nutrition of food. The difference concerns the definition of feed, in which nutrition is foreseen, and of food, which foresees ingestion. However, part of the doctrine does not attribute any legal relevance to this difference, since food and feed, however, are treated in the same way and with the same regulatory provisions.

The current food legislation[3] provides for specific responsibilities and safety obligations to protect the health of the consumer. In fact, the food or feed business operator is defined in Article 3 as the natural or legal person who is responsible for ensuring compliance with the provisions of the legislation in the food or feed business under his control. In particular, producers and distributors have the obligation to place safe products on the market in compliance with food legislation at the stages of production, processing, transport, storage, custody and final distribution. However, there are questions of interpretation, since the food safety obligations provided for by regulations No. 178 of 2002, No. 852 of 2004 and No. 853 of 2004, are different for the various operators in the food chain[18].

For what concerns food safety requirements, risk analysis is a general principle of food legislation for the protection of consumer health. It is characterized by the principle of decision-making, which is divided into different areas, assigned to the responsibility of different subjects. With regard to risk analysis, the European legislator distinguishes between risk assessment, which is based on scientific evidence and must be carried out in an independent, objective and transparent manner, and risk management, which must take into account the results of risk assessment, in particular the opinions of the Food Safety Authority, as well as any additional elements, if relevant but not specifically mentioned, and the precautionary principle.

1.9. Definition of foods that pose a risk to consumer health

Regulation (EC) 178/2002[18] defines the possible risks as a function of the probability and severity of an adverse health effect resulting from the presence of a hazard. In addition, the hazard or hazardous element is defined as the chemical or physical biological agent contained in a food or feed or condition in which a food or feed is found capable of causing an adverse health effect. Thus, food is considered to be unsafe when it is injurious to health or unfit for human consumption according to the probable immediate and/or short-term and/or long-term effects of food on the health of a person consuming it and that of their descendants due to probable toxic or cumulative effects of a food. The same Regulation (No. 178 of 2002) establishes that any food which is considered at risk under the definition set out in the Regulation cannot be placed on the market.

In addition, the notion of food at risk is foreseen by the Regulation in the categories of food harmful to health and food unfit for human consumption of European legislation[4,20]. It provides that the safety of the food is assessed according to the normal conditions of use of the food at each stage of production, processing and distribution and according to the information shown on the label or other information related to the harmful effects resulting from the food[4].

1.10. European legislative principles for risk assessment of foodstuffs

European Union legislation provides that the risk is identified by evaluating the probability and severity of the harmful effect of the food or feed on health, resulting from the presence of a hazard. The risk assessment is carried out through a scientifically based procedure, which evaluates the exposure to the hazard and the risk, the probability and the severity of the harmful effect on health. This control is carried out by the European Food Safety Authority, which collects communications from Member States or national authorities, consumers, food businesses, the academic community and those interested in food safety[25].

After the risk assessment, the European Commission establishes the procedures for a correct risk management according to the precautionary principle and a careful evaluation of the available information and of the possible harmful effects on health, through the analysis between the alternatives of intervention and the adoption of restrictive measures and appropriate preventive and control choices to protect health[25].

Finally, the European Union foresees the important step of risk communication, through the exchange of information and opinions between managers, consumers, food companies and other interested parties, regarding the elements of danger and the risks detected. In order to facilitate coordination between businesses and the competent authorities of Member States, the European Union has set up the Rapid Exchange of Information System (RAPEX), which is the European Union's rapid alert system for unsafe consumer products and consumer protection[26]. In addition, rapid notifications are an additional tool for assessing possible risks. In order to notify, in real time, direct or indirect risks to health deriving from the consumption of food or feed, the Rapid Alert System of the European Union (RASFF)[27] has been established. This newly created alert system is a form of network in which the European Commission, the EFSA (Food Safety Authority)[25] and the Member States of the Union participate. The activity of the EU alert system includes the withdrawal of products considered dangerous to human or animal health[25].

Regulation No. 178 of 200218 also provides for additional safety obligations, such as the traceability obligation[18], which was developed for the control of beef, in relation to the emergency related to the spread in Europe of Creutzfeldt-Jakob disease, commonly referred to as "mad cow disease". Regulation No. 178/2002 has provided for this obligation of traceability for professional operators in various sectors, as a tool of food safety, in order to proceed with "withdrawals" aimed at informing consumers or those responsible for controls. According to the approach defined as one step back, one step forward it is therefore necessary to set up control systems and procedures in order to identify who supplied what and the companies to whom the products were supplied. The traceability provided for in Regulation No. 178/2002 concerns the flow of raw materials and components within the production process of an individual food business. This regulation facilitates the identification of the operator who is obliged to comply with the regulatory provisions for the protection of the safety of the food product and the obligation to communicate any dangerous situation to consumers or to those responsible for withdrawing it from the market. In particular, the traceability system foreseen by the regulations in question allows for the identification of the person responsible for the danger produced and the damage caused[66] and, with reference to food imported from third countries, foresees the possibility of adopting, for the protection of public health, animal health and the environment, appropriate emergency measures at Union level for food and feed imported from a third country, should the risk not be adequately dealt with by measures adopted by Member States[18].

Conclusions

In the context of the European Union, there is a clear intention of the legislator to balance the interests of food producers with the interests of consumers with the aim of guaranteeing healthy and safe food to people, through the regulation of individual production phases and the behavior of individual operators involved in the food production sector. All this through the use of control mechanisms and an information network capable of involving individual Member States in the implementation of this food safety.

In recent years there has been a significant effort to update legislation that reflects the growing sensitivity of the European Parliament and the Commission toward the issue of Food Safety. In this regard, it is important to point out that the original Regulations No. 854/2004 and No. 882/2004 have been replaced by the subsequent EU Regulation No. 625/2017. Finally, after 15 years also EU Regulation No. 382/2021[28] has updated the (EC) Regulation No. 852/2004. The new regulation now incorporates the update of the Codex Alimentarius guidelines published in September 2020[29] on both Food Hygiene and the new Policy for the Prevention and Management of Allergenic Cross-Contact. In the new regulations, the basic prerequisites are in fact updated and new requirements for the reduction of food waste are introduced. The fundamental concepts of Food Safety Culture are therefore introduced in the European legislative framework, demonstrating an attention to public health issues that may have been lacking in the early years of the legislative life of the Union, thus contributing to further bring the European population closer to its institutions, increasingly perceived as an element of guarantee of their rights, including the fundamental right to health.

Statement

The corresponding author states on behalf of the co-authors that all Authors have no conflict or competing of interests to declare.

References

1 European Union. From Farm to fork. Controlling the safety of agri food chan. Laws and regulations. 2020, https://ec.europa.eu/food/system/files/2016-12/fs_infograph_from-farm-to-fork_en.pdf (Accessed, 12 February, 2022).
2 European Commission. White Paper on food safety. 2020, https://op.europa.eu/en/publication-detail/-/publication/6d4b523b-dad8-4449-b2b4-9fa9b0d6e2be. (Accessed, 10, February, 2022).
3 European Union. Eur-Lex. European Union Laws, 2020. https://eur-lex.europa.eu/legal-content/EN/ALL/?uri=CELEX:32002R0178 (Accessed, 12 February, 2022).
4 European Union. European Food Safety Authority (EFSA). 2020, https://www.efsa.europa.eu/en (Accessed, 12 February, 2022).
5 Pettoello-Mantovani M, Ehrich J, Sacco M, Ferrara P, Giardino I. Pop food insecurity and children's rights to adequate nutrition in Europe. TL. *J Pediatr*. 2018;198:329–330.
6 Pettoello-Mantovani M, Mestrovic J, Namazova-Baranova L, Giardino I, Somekh E, Vural M. Ensuring safe food for infants: the importance of an integrated approach to monitor and reduce the risks of biological, chemical, and physical hazards. *J Pediatr*. 2021;229:315–316. Febe2.
7 European Union. Transparency and sustainability of the EU risk assessment in the food chain. 2020, https://ec.europa.eu/food/horizontal-topics/general-food-law/transparency-and-sustainability-eu-risk-assessment-food-chain_en (Accessed, 12 February, 2022).
8 Fusco G, Coluccia B, De Leo F. Effect of Trade Openness on food security in the EU: a dynamic panel analysis. *Int J Environ Res Public Health*. 2020;17(12):4311. https://doi.org/10.3390/ijerph17124311. PMID: 32560272; PMCID: PMC7344940.
9 Mo C. Product traceability requirements in the european union: an overview. 2020, https://www.compliancegate.com/product-traceability-requirements-european-union/ (Accessed, 12 February, 2022).
10 Ferrara P, Corsello G, Quattrocchi E, Dell'Aquila L, Ehrich J, Giardino I. Pettoello-Mantovani M. Caring for Infants and Children Following Alternative Dietary Patterns. *J Pediatr*. 2017;187:339–340.
11 van der Meulen B. *EU Food Law Handbook*. Wageningen Academic Publisher; 2014. European Institute for Food Law series, Volume 9.
12 El Gemayel M. Food quality in the european union: does the consumer have free choice? *Eur J Risk Regulation*. 2021:1–18. https://doi.org/10.1017/err.2021.39.

13 Wendler F. The European Food Safety Authority as a Source of „Soft Law“: Towards more Effective and Legitimate EU food safety governance?CONNEX Workshop: „Soft Governance and the Private Sector: The EU and Global Experience. Darmstadt, 1-3 November 2005. http://www.mzes.uni-mannheim.de/projekte/typo3/site/fileadmin/research%20groups/6/Papers_Soft%20Mode/Wendler.pdf (Accessed, 12 February, 2022).
14 European Union. Eur-Lex. The treaty on the functioning of the european union. 2020, https://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=CELEX:12012E/TXT:en:PDF (Accessed, 12 February, 2022).
15 Eco.Lex. Commission Regulation (EC) No. 1235/2008 laying down detailed rules for implementation of Council Regulation (EC) No. 834/2007 as regards the arrangements for imports of organic products from third countries. 2020, https://www.ecolex.org/details/legislation/commission-regulation-ec-no-12352008-laying-down-detailed-rules-for-implementation-of-council-regulation-ec-no-8342007-as-regards-the-arrangements-for-imports-of-organic-products-from-third-countries-lex-faoc086651/#:~:text=(EC)%20No.-,1235%2F2008%20laying%20down%20detailed%20rules%20for%20implementation,Council%20Regulation%20(EC)%20No.&text=This%20Regulation%20lays%20down%20detailed,and%20labelling%20of%20organic%20products. (Accessed, 12 February, 2022).
16 United Kingdom. Council Directive 92/59/EEC of 29 June 1992 on general product safety. 2020, https://www.legislation.gov.uk/eudr/1992/59/adopted# (Accessed, 12 February, 2022).
17 Safe Food for Infants in the EU and China, 2020. www.saffi.eu. (Accessed, 12 February, 2022).
18 INFO Mea Treaties. Regulation (EC) No. 178/2002 of the European Parliament and of the Council 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. 2020, https://www.informea.org/en/legislation/regulation-ec-no-1782002-european-parliament-and-council-laying-down-general-principles#:~:text=(EC)%20No.-,178%2F2002%20of%20the%20European%20Parliament%20and%20of%20the%20Council,in%20matters%20of%20food%20safety. (Accessed, 12 February, 2022).
19 Official Journal of the European Union. Regulation (Ec) No 1830/2003 Of The European Parliament And Of The Council of 22 September 2003 concerning the traceability and labelling of genetically modified organisms and the traceability of food and feed products produced from genetically modorganisms and amending Directive 2001 /18/EC. https://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2003:268:0024:0028:EN:PDF (Accessed, 12 February, 2022).
20 Food Standards Agency. Food Standards Agency Consultation. Enforcement of European Parliament and Council Regulations on Additives and Enzymes. Laws and Regulations collection. 2020, https://www.foodlaw.rdg.ac.uk/pdf/uk-09016-draft-additive-enforcement-regs.pdf (Accessed, 12 February, 2022).
21 Eur.Lex Regulation (Ec) No 178/2002 Of The European Parliament And Of The Council. 28 January 2002. General principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety. 2020, https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:02002R0178-20140630&rid=1 (Accessed, 12 February, 2022).
22 European Commission. Consumer rights directive. https://ec.europa.eu/info/law/law-topic/consumer-protection-law/consumer-contract-law/consumer-rights-directive_en (Accessed, 12 February, 2022).
23 European Commission. Review of EU consumer law. 2020, https://ec.europa.eu/info/law/law-topic/consumer-protection-law/review-eu-consumer-law_en (Accessed, 12 February, 2022).
24 European Union. Directive 2001/83/Ec of The European Parliament and of the Council of 6 November 2001 on the Community code relating to medicinal products for human use, 2020. https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:02001L0083-20121116&qid=1472567249742&from=EN (Accessed, 12 February, 2022).
25 European Food Safety Authority. 2020, https://www.efsa.europa.eu/en (Accessed, 12 February, 2022).
26 European Commission. About European Rapid Alert system for dangerous products (RAPEX), 2020. https://joinup.ec.europa.eu/collection/rapex/about (Accessed, 12 February, 2022).
27 Food Standards Scotland. The Rapid Alert System for Food and Feed (RASFF), 2020. https://www.foodstandards.gov.scot/consumers/food-crime/report-a-problem/european-commission-food-alerts-rapid-alert-system-for-food-and-feed-rasff#:~:text=The%20Rapid%20Alert%20System%20for,contact%20materials%20and%20animal%20feed. (Accessed, 12 February, 2022).
28 Daenen M. New EU Regulation Enforces A Food Safety Culture For Food Operators. 2020, https://diversey.com/en/blog/new-eu-regulation-enforces-food-safety-culture-food-operators#:~:text=New%20EU%20Regulation%20Enforces%20A%20Food%20Safety%20Culture%20For%20Food%20Operators,-AUTHOR&text=The%20European%20Union%20(EU)%20recently,of%20appropriate%20Food%20Safety%20culture. (Accessed, 12 February, 2022).
29 Food and Agriculture organization of the United Nations. Codex Alimentarius Commission: 24-26 September 2020 and 12 October 2020. https://www.fao.org/news/story/en/item/1308515/icode/ (Accessed, 12 February, 2022).

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Overview on Endocrine disruptors in food and their effects on infant's health☆

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ABSTRACT

Endocrine disruptors (EDs) are natural or synthetic chemicals that cause changes in the body's hormonal and homeostatic systems. Children, especially developing fetuses and infants, are more likely to be affected by these chemicals than adults. Intake through food is one of the primary pathways for EDs to enter the body. While EDs can be found naturally in some foods, synthetic EDs primarily contaminate food, including breast milk and water. Although safe doses have been reported for many EDs, this issue may be controversial because of the low-dose effects and non-monotonic dose responses of EDs. Because of their epigenetic effects, their effects may occur in subsequent generations that are not directly exposed. Some EDs are persistently present in the environment. These chemicals are transported through water and air currents, as well as migratory animals and enter the food chain even if the chemical is banned or not produced in that specific area.

In this article, we aim to provide information on EDs by emphasizing those found in food and their effects especially on the health of children including developing fetuses. Some suggestions have also been given to reduce the danger due to EDs.

1. Introduction

Endocrine disruptors (EDs) are substances that can be natural or synthetic, which can cause changes in the hormonal and homeostatic systems of the organisms exposed to their action. Some EDs are thought to mimic natural steroid hormones and interact with their receptors as analogues or antagonists due to the presence of a phenolic moiety. Thus, they can act as estrogens, androgens, and antiandrogens. They could also act as thyroid hormone receptor agonists and antagonists.

Phytoestrogens are one of the main natural EDs found in our food. Synthetic endocrine disruptors include chemicals used as industrial lubricants/solvents and their byproducts:Some examples include plastics [bisphenol A (BPA), pharmaceutical agents [diethylstilbestrol (DES)], dioxins, fungicides (vinclozolin), pesticides [methoxychlor, chlorpyrifos, dichlorodiphenyltrichloroethane (DDT)], polybrominated biphenyls (PBB), plasticizers (phthalates), polychlorinated biphenyls (PCBs)][1].

The developmental age at which exposure to an endocrine disruptor occurs is critical. In the case of exposure to a presumptively "safe" dose during a life stage such as the intrauterine period, when there is no endogenous hormonal exposure, the potential effects of exposures even at very low doses should be considered[2]. In addition, there is evidence indicating that very low doses of EDs might be more effective than higher doses, and nonmonotonic dose responses are not uncommon findings when EDs are studied[3].

Endocrine disruptors are taken into the body mainly in three ways: inhalation, ingestion, and dermal contact[4]. Some EDs are not metabolized and remain in high levels in the environment for a long time; they are called persistent organic pollutants (POPs). Thus, EDs that were banned even decades ago can be found in human and animal bodies. On the other hand, some can change into compounds that are even more

Abbreviations: AGEs, Advanced glycation end products; BPA, bisphenol A; DES, diethylstilbestrol; DDT, dichlorodiphenyltrichloroethane; DEHP, Di-etylhexyl Phthalate; DINP, Di-isononyl Phthalate; DIDP, Di-isodecyl Phthalate; DOHaD, Developmental Origin of Health and Disease; EDs, Endocrine disruptors; EU, European Union; PBBs, polybrominated biphenyls; PCBs, polychlorinated biphenyls; PE, polyethylene; PET, polyethylene terephthalate; POPs, persistant organic pollutants; PVC, polyvinyl chloride; TNC, transnonachlor; WTO, World Trade Organization.

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toxic and can be detected at distances from where they were produced or released. These chemicals are transported through water and air currents, as well as migratory animals and enter the food chain. Others, such as BPA, do not remain in the environment for very long but are quite widespread in their use[1].

The age of exposure to an ED might be associated with different outcomes. During specific periods of development, exposure to environmental chemicals, drugs, altered nutrition, infections, or stress may cause functional changes in tissues, whereas the same effects may not be seen at other stages of life.

Nowadays, it is well known that changes that occur in the early years of life can pave the way for disease in later stages of life[5]. The term "the fetal basis of adult disease" has been used to describe observations of the maternal environment, the egg, and the external environment and identifies an individual's propensity to develop a disorder later in life[6]. Thus, the fact that EDs can be effective at critical periods of development could coincide with the concepts of fetal basis of adult disease and Developmental Origin of Health and Disease (DOHaD)[7].

Another important factor to be considered about EDs is the latency from exposure, which represents the time it takes for an endocrine disruptor to show its effect. The effect is more likely to be achieved when exposure occurs at a younger age, as survival will be longer.

Individuals may be exposed to several EDs in the same time interval, and although the individual components at doses considered safe have no effect on physiology or homeostasis, they cause synergistic effects when taken together, which is called the "cocktail effect." This is caused by enhancement of ligand binding affinity and recruitment of transcriptional coactivators. For example, transnonachlor (TNC) (a banned organochlorine pesticide) and 17α-ethinyl estradiol can bind to the same nuclear receptor (pregnane X receptor-PXR) at the same time with up to 100-fold higher affinity than the individual compounds. This synergistic activation leads to changes in the regulation of several physiological functions, whereas the separate effects of the individual components would be negligible[8].

Another interesting point about EDs is that there is not necessarily a linear relationship between dose and endocrine disruptor effect; a U-shaped or inverted U-shaped curve can be seen. Therefore, low doses may also cause more potent effects than higher doses[1,3].

The effects of EDs can be seen in subsequent generations by being transmitted through changes in factors that regulate gene expression through their epigenetic effects (transgenerational effect). Three main mechanisms of epigenetic regulation have been described are DNA methylation, posttranslational modifications of histones, and through noncoding RNAs. Alteration of any of these epigenetic regulators in germ cells is associated with increased disease risk[9].

2. Endocrine disruptors and children's health

Endocrine disruptors are of special importance to pediatricians because children, especially young children, are different from adults in many ways. Their rates of organ development are variable, they have greater absorption from the skin when adjusted for body weight, longer life expectancy, and lower cytochrome p450 enzyme activities. Children consume more daily calories, fluids, and oxygen per body weight, are exposed to breast milk, dairy products, and fat-soluble endocrine disruptors, put everything in their mouths, and crawl on the floor.

Endocrine disruptors, as the name suggests, affect the hormonal system in many different ways: They interact with hormone receptors by activating or antagonizing them, alter hormone receptor expression or signal transduction in hormone-sensitive cells, induce epigenetic changes in hormone-sensitive or hormone-producing cells, alter the synthesis, metabolism, or clearance of hormones, and alter the transport of hormones across cell membranes[10].

2.1. Risks posed by EDs to children's health

EDs could have several effects on different body functions; these effects seem to be increasing as the results of extensive studies are published. For now, these effects can be listed as follows:

Breast cancer (both prenatal and pubertal effects could be responsible), prostate cancer, diabetes, obesity, thyroid disease, puberty disorders, reproductive system disorders, infertility, weakened immune system, neurological and behavioral changes[11].

The observation of these diverse effects has led to the need for regulations regarding endocrine disruptors. REACH (EC 1907/2006) aims to improve the protection of the environment and human health by better and earlier identifying the intrinsic properties of chemicals through the four processes: Registration, Evaluation, Authorization, and Restriction of Chemicals.

2.2. Chemicals with endocrine disrupting properties found in food

- **Phytoestrogens**

Phytoestrogens are the main natural endocrine disruptors that can be found in human and animal food. Genistein is a phytoestrogen found naturally in soybeans. In infants fed soy formula, urinary concentrations of genistein have been found to be about 500 times higher than those fed cow formula. This substance binds to estrogen receptors and also has goitrogenic activities. Evidence also shows an association between feeding soy infant formula and autoimmune thyroid disease[12,13].

- **Phthalates (PAEs)**

Phthalates are widely used as major plasticizers in industry. These substances are used to improve the extensibility and elasticity of polymers, such as polyvinyl chloride (PVC), polyethylene (PE), and polyethylene terephthalate (PET).

Phthalates are a global human health concern. Di-ethylhexyl phthalate (DEHP) is considered "toxic to reproduction" in the European Union (EU) and a "priority hazardous substance" under the EU Water Framework Directive. The widespread use of phthalates in food packaging causes "putative food toxicity" due to the migration of these substances into food.

According to a recent EU risk assessment, more information is still needed on the risk in infants fed DEHP-contaminated breast milk. To minimize the health risk, DEHP has been replaced by two substances that are not considered hazardous under REACH: Di-isononyl phthalate (DINP) and Di-isodecyl phthalate (DIDP)[14].

The World Trade Organization (WTO) announced an EU proposal to change the restriction of three phthalates (DEHP, DBP, and BBP) in March 2018 under entry 51 of Annex XVII of REACH (Safeguard 54/18). In January 2019, the number of restricted phthalates was expanded from three (DEHP, DBP, and BBP) to four (DEHP, DBP, BBP, and DIBP). The EU has set specific migration limits in food and beverage contact plastics for five phthalates, namely DBP, DEHP, BBP, DINP and DIDP. DIBP is not permitted in food contact materials. DBP and DEHP are only allowed in plasticizers in repeated-use materials and nonfat food contact articles. DINP, DIDP and BBP may only be used in plasticizers in repeated-use and single-use materials and articles in contact with nonfatty foods (except infant formulae and follow-on formulae as defined by Directive 2006/141/EC or processed cereal-based foods and baby foods as defined by Directive 2006/125/EC)[14].

Di-ethylhexyl phthalate is the most abundant phthalate in water in PET bottles, followed by DBP and DIBP, and their levels increase when stored at high temperatures. Because 0.5 L PET bottles have a higher surface area/volume ratio, PAE concentration was found to be the highest in 0.5 L bottles compared to larger bottles. PAE levels increase even more when the bottles are stored in sunlight or when hot water is placed in these bottles. Soft drinks are more likely to be contaminated

with PAEs than mineral water due to their higher acidity. Also, longer duration of contamination leads to higher levels.

As for dairy products, PAE contamination can occur during all stages of production. On the other hand, raw milk has also been found to be contaminated with DIBP and DEHP due to contaminated feed. The mechanical milking process with PVC pipes is also considered important for contamination. Cooling tanks may lead to further increases in DEHP levels. In Belgian farms, the highest levels of DEHP were found in creamers while the lowest levels were in light milk. Replacement of DEHP with other types of plasticizers has led to a decrease in DEHP levels in European cow's milk, while outside Europe (Canada and South Korea), DEHP levels in milk are still high. During the pasteurization process, DEHP content has been reported to increase most likely due to tubes and sealants that are DEHP-containing contact materials[14].

Prenatal exposure to DEHP

There are studies showing the effects of DEHPs as a result of in-utero exposure. Barakat et al.[15-18] conducted many animal studies on this topic. The results of these studies showed that male mice prenatally exposed to DEHP had increased germ cell apoptosis, oligo/azoospermia, and degenerated seminiferous tubules, i.e., induces premature reproductive senescence. This effect has been reported to be dose dependent[15]. In addition, it has been shown that these reproductive effects are passed on to subsequent generations through epigenetic modification of germ cells[16]: In experimental animals, when a pregnant female is exposed to an endocrine disruptor, one might expect that the first generation would be directly affected, and the second generation would be affected because germ cells of the first generation would also be affected. However, Barakat et al.[18] reported that in the third generation (those not directly exposed to DEHP), fertility and reproduction were also affected as a surprising finding suggesting epigeneticity.

Another effect of prenatal DEHP exposure in mice has been reported as impaired recognition memory and elevated anxiety behavior. These effects have been attributed to neurodegeneration due to inflammation and oxidative damage in the hippocampus, as well as decreased testosterone levels and androgen receptor expression in the brain[17].

- **Advanced glycation end products (AGEs)**

The Maillard reaction is a chemical reaction that occurs during the processing or cooking of foods at high temperature: As a result of the glycation of proteins in foods, AGEs appear; they are found in various foods such as bread, cheese, processed meats, cookies, and peanut butter. AGEs have been associated with the pathophysiology of several diseases, such as type 2 diabetes mellitus, polycystic ovary syndrome, and allergies. When the mother is exposed to exogenous AGEs, these glycated proteins are passed to the baby through lactation. Kutlu T.[19] described that infants may receive up to 15 kU/kg of AGEs directly from breast milk, which could increase to 76 kU/kg by the age of 6 months. Infants may also be exposed to exogenous AGEs from the diet through formula feeding. Dry heating of milk readily increases dietary AGEs in infant formula compared with normal cow's milk products. CML (Nε-carboxymethyl-lysine) is the major glycotoxin in infant formula and is found 7 to 12 times less in goat milk formula than in cow's milk products[20].

- **Persistant organic pollutants (POPs)**

The development of industry has brought with it environmental pollution. POPs are man-made chemicals that are lipophilic, highly resistant to degradation, and concentrate in living organisms (bioaccumulation). Animals and humans in the food chain not only absorb these chemicals, but also spread them as they travel. As a result, they can be found miles away from the original source. Exposure to POPs can cause many health disorders, such as impairment of neurodevelopment, reproductive and immune function, as well as disruption of endocrine system function. The severity of these effects can vary depending on the developmental period during exposure to POPs[21,22].

In May 2001, the Stockholm Convention on Persistent Organic Pollutants took place in Sweden with the participation of more than 90 countries. The goal was to protect the environment and human health from POPs, and 12 POPs (the dirty dozen) were chosen to be eliminated and/or reduced: aldrin, chlordane, DDT (dichlorodiphenyltrichloroethane), dieldrin, endrin, heptachlor, mirex, toxaphene, PCBs, hexachlorobenzene, dibenzodioxins, and dibenzofurans[23].

- **Pesticides**

Pesticides can pollute soil, water, grass, and flora. In addition to killing insects or weeds, pesticides may be poisonous to other creatures such as birds, fish, beneficial insects, and non-target vegetation. Exposure of males to pesticides may affect sex hormones, sperm (morphology, concentration, and motility), and semen quality[24].

In a retrospective study conducted in Belgium, which later became a pioneering study indicating the effect of pesticides on the timing of puberty, it was determined that 28% of the 145 cases treated with the diagnosis of precocious puberty were patients who had migrated from developing countries. When pesticide levels were checked in these cases, p,p'DDE (a derivative of the organochlorine pesticide DDT) was found in immigrants with precocious puberty, whereas this chemical could not be detected in most native Belgian cases. This suggested that the mechanism of precocious puberty might involve previous exposure of endocrine disruptors[25]. Other studies have also shown the effect of prenatal pesticide exposure on early menarche and early breast development[26,27].

Dichlorodiphenyltrichloroethane had been banned in the early 1970s. However, the effects of DDT have recently been demonstrated in granddaughters whose grandmothers were exposed to the pesticide DDT. These granddaughters had higher rates of obesity and early menstrual periods. These may increase the granddaughters' risk for breast cancer, as well as high blood pressure, diabetes, and other cardiometabolic diseases[28].

Measures can be taken on an individual basis to limit pesticide exposure, such as washing, peeling, and eating organic food. In particular, some fruits and vegetables that absorb high levels of pesticides (strawberries, cherries, apples, grapes, potatoes, peppers, spinach) could be purchased organic[29].

It is well known that the pre-harvest interval, i.e., the time between the last application and harvest, is important when dealing with pesticides. For example, for strawberries, many types of insecticides are widely used, as this soil-grown fruit is the target of many insects, A recent study showed that with respect to different pre-harvest intervals, the amount of thiacloprid residues showed variability[30]. In another study, residual levels of fosthiazate, a widely used nematicide, was measured in tomatoes and cherry tomatoes, and the 3-week preharvest interval was determined to be safe to consume[31]. For fungicide-treated pomegranates, the half-life of fluopyram and tebuconazole varied by pomegranate part. Pre-harvest intervals for combined tebuconazole and fluopyram treatment were 47 to 59 days in pomegranate fruits but 158 to 173 days in leaves[32].

Herbicides are widely used in dicot crops, and residues are of great concern. A recent study showed that vegetables, especially those with a short growing season could be easily contaminated with the aryloxyphenoxy-propionate herbicides (except propaquizafop). Vegetables treated with fluazifop and lettuce and cauliflower treated with quizalofop are declared unsuitable for infant feeding[33].

- **Parabens**

Parabens are p-hydroxybenzoic acid esters commonly used in cosmetics, food, and pharmaceuticals as preservatives[34]. They increase

adipogenesis and reduce basal lipolysis in white adipose tissue, while attenuating adrenergic stimulation of lipolysis in brown adipose tissue[35]. Thus, parabens are obesogenic endocrine disruptors present in foods. Estimated daily intake values of total parabens were reported as 307, 273, 470, 879, and 940 ng/kg body weight/day for adults, adolescents, children, toddlers and infants, respectively, and among the 8 food categories (beverages, fruits, vegetables, dairy products, fats and oils, cereals, fish and shellfish, and meat), the highest amount of parabens was found in cereals[34].

- **Bisphenol A (BPA)**

Bisphenol A is a synthetic organic compound that is used to make polycarbonate plastics. It is commonly found in food and beverage packaging, medical devices, and dental materials. It can contaminate air, soil, food and beverages. Especially in children, 99% of BPA exposure occurs through the diet, with beverages and canned food being the main sources.

Bisphenol A, as an endocrine disruptor, exhibits hormone-like properties and causes hormone-dependent cancers and has negative effects on reproduction and immune regulation. It can bind to estrogen, androgen, and thyroid hormone receptors.

Acidic or basic conditions and heating increase the migration of BPA from plastics into foods and beverages. Thus, heating foods in plastic packages or bottles increases BPA exposure. In addition, the release of BPA from materials is increased by contact with sodium chloride or vegetable oils, making canned food a major source.

Therefore, to avoid the harmful effects of BPA, the consumption of plastic materials should be restricted and the use of BPA-free products should be promoted[36]. At the governmental level, restrictions should be lifted in the use of BPA and also for other alternatives, such as bisphenol S (BPS) and bisphenol F. At the individual level, BPA- and BPS-free bottles should be purchased or bottles containing the number 7 inside the recycling symbol should be avoided, and fresh foods should be consumed instead of canned foods[29].

3. Foodstuff exposed to the risk of contamination by endocrine disruptors

- **Eggs**

In a recent study conducted in Turkey, endocrine disruptors were analyzed in three types of eggs (battery, free-range, and organic)[37]. The results of the study showed that PAEs were the most abundant in battery eggs, while total DDT concentrations, although low, were highest in free-range eggs, although DDT was banned in Turkey more than 35 years ago. The eggs were found to be contaminated with more than one chemical; however, all were within the acceptable risk limit. There was no difference between the eggs in terms of PCB and PBDE concentrations; however, PBDEs have never been produced and PCBs are banned in Turkey[3]. This result supports that these chemicals are found in the environment despite restrictions and bans. From the authors' point of view, the term of acceptable limits should be stated very carefully, since it is well known that when it comes to EDs even very low levels can produce effects[3].

- **Breast milk**

The World Health Organization recommends breastfeeding until 2 years of age, and breastfeeding is undoubtedly the accepted mode of infant feeding for the first six months; however, infants can also be exposed to endocrine disruptors through breast milk. Highly lipid-soluble chemicals are concentrated in the mammary gland in lactating women. Thus, especially breast milk with a high fat content carries a higher risk. In addition, the degree of exposure is influenced by maternal age, duration of lactation, and number of pregnancies. The most prevalent chemicals in breast milk are: heavy metals, pesticides, organochlorine cyclodienes, semivolatile organohalogens, dioxins, furans, DDT, parabens, octylphenols, bisphenols, and other organic compounds including PAEs[4]. In a study conducted in Germany, 15 phthalates were measured in 78 breast milk samples. Of these, DEHP, DnBP, and DiBP levels were measured at significantly higher concentrations, whereas other phthalates were found in only a few of the samples or were not detectable in any of the samples. However, it was stated that the elevated values were still well below the recommended tolerable daily intake[38]. Again, the debatable question of acceptable or tolerable limits from the authors' point of view might come into the picture for breast milk as well. On the other hand, these chemicals have been shown to be at much higher levels in infant formula, and it was concluded in the study by Fromme et al.[38] that exposure through breast milk does not pose a significant health risk to infants.

- **Drinking water**

Drinking water is one of the main ways people can be exposed to endocrine disruptors. Brazilian researchers evaluated 15 articles published in the last decade to study chemical contamination in Brazilian drinking water. Of the 77 parameters studied in groundwater, surface, and rainwater sources, 10 parameters exceeded health limits for potability. In particular, 17 α-ethinylestradiol, widely used in contraceptive pills, exceeded 52,549 times the proposed guideline value. Its presence in water is thought to be due to contamination of the urine of women taking these drugs, and indirect exposure of pregnant women and thus the developing fetus to this chemical is a major concern. The authors concluded that instead of removing these chemicals from water, it would make more sense to prevent their contamination by reducing pesticide use and improving wastewater treatment[39].

4. Conclusions

Endocrine disruptors are mostly synthetic molecules introduced into our lives in an attempt to make living more convenient and easy. However, they have brought with them their own health risks. Children, especially developing fetuses and infants, are more likely to be affected than adults. Studies over time show that the health risks they cause are not only for people today but also for future generations. The use of some EDs has been banned and restricted. However, industry tries to compensate for the restricted chemicals by producing new molecules that in turn could also cause problems. Studies have been done to find out the maximum acceptable levels of these chemicals; however, the atypical dose-response curves of some EDs make determining these levels difficult and questionable from the authors' perspective, especially when fetuses and developing children are affected. One of the most important steps to reduce the health effects of EDs is to increase awareness of the risks in the general population. On the other hand, the many issues related to EDs require the involvement of scientists from different disciplines. Thus, an international multidisciplinary council of scientists must work together to reduce or eliminate health risks related to EDs now and in the future.

Declaration of Competing Interest

The authors declare no conflict of interests.

References

1 Diamanti-Kandarakis E, Bourguignon J-P, Giudice LC, Hauser R, Prins GS, Soto AM, et al. Endocrine-disrupting chemicals: an Endocrine Society scientific statement. *Endocr Rev*. 2009 Jun;30(4):293–342.
2 Zoeller RT, Brown TR, Doan LL, Gore AC, Skakkebaek NE, Soto AM, et al. Endocrine-disrupting chemicals and public health protection: a statement of principles from The Endocrine Society. *Endocrinology*. 2012 Sep;153(9):4097–4110.

3 Vandenberg LN, Colborn T, Hayes TB, Heindel JJ, Jacobs DRJ, Lee D-H, et al. Hormones and endocrine-disrupting chemicals: low-dose effects and nonmonotonic dose responses. *Endocr Rev*. 2012 Jun;33(3):378–455.
4 Picone S, Paolillo P. Chemical contaminants in breast milk. *Early Hum Dev*. 2013 Oct 1;89:S117–S118.
5 Heindel JJ, Vandenberg LN. Developmental origins of health and disease: a paradigm for understanding disease cause and prevention. *Curr Opin Pediatr*. 2015 Apr;27(2):248–253.
6 Barker DJP. The developmental origins of adult disease. Vol. 18, European journal of epidemiology. Netherlands; 2003. p. 733–6.
7 Birnbaum LS, Miller MF. Prenatal Programming and Toxicity (PPTOX) Introduction. *Endocrinology*. 2015 Oct;156(10):3405–3407.
8 Delfosse V, Huet T, Harrus D, Granell M, Bourguet M, Gardia-Parège C, et al. Mechanistic insights into the synergistic activation of the RXR-PXR heterodimer by endocrine disruptor mixtures. *Proc Natl Acad Sci U S A*. 2021 Jan;118(1).
9 Xin F, Susiarjo M, Bartolomei MS. Multigenerational and transgenerational effects of endocrine disrupting chemicals: A role for altered epigenetic regulation? *Semin Cell Dev Biol*. 2015 Jul;43:66–75.
10 La Merrill MA, Vandenberg LN, Smith MT, Goodson W, Browne P, Patisaul HB, et al. Consensus on the key characteristics of endocrine-disrupting chemicals as a basis for hazard identification. *Nat Rev Endocrinol*. 2020 Jan;16(1):45–57.
11 Schug T, Janesick A, Blumberg B, Heindel J. Endocrine Disrupting Chemicals and Disease Susceptibility. *J Steroid Biochem Mol Biol*. 2011 Aug 27;127:204–215.
12 Doerge DR, Sheehan DM. Goitrogenic and estrogenic activity of soy isoflavones. *Environ Health Perspect*. 2002 Jun;110(Suppl 3):349–353. Suppl 3.
13 Fort P, Moses N, Fasano M, Goldberg T, Lifshitz F. Breast and soy-formula feedings in early infancy and the prevalence of autoimmune thyroid disease in children. *J Am Coll Nutr*. 1990 Apr;9(2):164–167.
14 Giuliani A, Zuccarini M, Cichelli A, Khan H, Reale M. Critical Review on the Presence of Phthalates in Food and Evidence of Their Biological Impact. *Int J Environ Res Public Health*. 2020 Aug;17(16).
15 Barakat R, Lin P-CP, Rattan S, Brehm E, Canisso IF, Abosalum ME, et al. Prenatal Exposure to DEHP Induces Premature Reproductive Senescence in Male Mice. *Toxicol Sci*. 2017 Mar;156(1):96–108.
16 Barakat R, Seymore T, Lin P-CP, Park CJ, Ko CJ. Prenatal exposure to an environmentally relevant phthalate mixture disrupts testicular steroidogenesis in adult male mice. *Environ Res*. 2019 May;172:194–201.
17 Barakat R, Lin P-C, Park CJ, Best-Popescu C, Bakry HH, Abosalem ME, et al. Prenatal exposure to DEHP induces neuronal degeneration and neurobehavioral abnormalities in adult male mice. *Toxicol Sci*. 2018 Aug;164(2):439–452.
18 Barakat R, Lin P-C, Park CJ, Zeineldin M, Zhou S, Rattan S, et al. Germline-dependent transmission of male reproductive traits induced by an endocrine disruptor, di-2-ethylhexyl phthalate, in future generations. *Sci Rep*. 2020 Mar;10(1):5705.
19 Kutlu T. Dietary glycotoxins and infant formulas. Turk Pediatr Ars. 2016 Dec;51(4):179–85.
20 Prosser CG, Carpenter EA, Hodgkinson AJ. N(ε)-carboxymethyllysine in nutritional milk formulas for infants. *Food Chem*. 2019 Feb;274:886–890.
21 Damstra T. Potential effects of certain persistent organic pollutants and endocrine disrupting chemicals on the health of children. *J Toxicol Clin Toxicol*. 2002;40(4):457–465.
22 Pettoello-Mantovani M, Mestrovic J, Namazova-Baranova Md PhD L, Giardino I, Somekh E, Vural M. Ensuring Safe Food for Infants: The Importance of an Integrated Approach to Monitor and Reduce the Risks of Biological, Chemical, and Physical Hazards. Vol. 229. *The Journal of pediatrics. United States*. 2021:315–316. e2.
23 Bilcke C. The Stockholm Convention on Persistent Organic Pollutants. *Rev Eur Community Int Environ Law*. 2003 Feb 13;11:328–342.
24 Swan SH, Kruse RL, Liu F, Barr DB, Drobnis EZ, Redmon JB, et al. Semen quality in relation to biomarkers of pesticide exposure. *Environ Health Perspect*. 2003 Sep;111(12):1478–1484.
25 Krstevska-Konstantinova M, Charlier C, Craen M, Du Caju M, Heinrichs C, de Beaufort C, et al. Sexual precocity after immigration from developing countries to Belgium: evidence of previous exposure to organochlorine pesticides. *Hum Reprod*. 2001 May;16(5):1020–1026.
26 Vasiliu O, Muttineni J, Karmaus W. In utero exposure to organochlorines and age at menarche. *Hum Reprod*. 2004 Jul;19(7):1506–1512.
27 Wohlfahrt-Veje C, Andersen HR, Schmidt IM, Aksglaede L, Sørensen K, Juul A, et al. Early breast development in girls after prenatal exposure to non-persistent pesticides. *Int J Androl*. 2012 Jun;35(3):273–282.
28 Cirillo PM, La Merrill MA, Krigbaum NY, Cohn BA. Grandmaternal perinatal serum DDT in relation to granddaughter early menarche and adult obesity: three generations in the child health and development studies cohort. *Cancer Epidemiol Biomarkers Prev a Publ Am Assoc Cancer Res cosponsored by Am Soc Prev Oncol*. 2021 Aug;30(8):1480–1488.
29 Sicker Trasande L. *Fatter, Poorer: The Urgent Threat of Hormone-Disrupting Chemicals to Our Health and Future . . . and What We Can Do About It*. Boston, New York: Houghton Mifflin Harcourt; 2019.
30 Rahman MM, Oh YG, Lee D-G, Moon J-K, Shim J-H, Cho IK. Establishment of import tolerance for the insecticide thiacloprid in strawberry. *Biomed Chromatogr*. 2021 May;35(5):e5057.
31 Lin S, Zhou Y, Wu J, Zhang Z, Cheng D. Dissipation and residue of fosthiazate in tomato and cherry tomato and a risk assessment of dietary intake. *Environ Sci Pollut Res Int*. 2022 Feb;29(6):9248–9256.
32 Yogendraiah Matadha N, Mohapatra S. Siddamallaiah L. Distribution of fluopyram and tebuconazole in pomegranate tissues and their risk assessment. *Food Chem*. 2021 Oct;358, 129909.
33 Jursík M, Hamouzová K, Hajšlová J. Dynamics of the Degradation of Acetyl-CoA Carboxylase Herbicides in Vegetables. *Foods (Basel, Switzerland)*. 2021 Feb;10(2).
34 Liao C, Liu F, Kannan K. Occurrence of and dietary exposure to parabens in foodstuffs from the United States. *Environ Sci Technol*. 2013 Apr;47(8):3918–3925.
35 Elmore SE, Cano-Sancho G, La, Merrill MA. Disruption of normal adipocyte development and function by methyl- and propyl- paraben exposure. *Toxicol Lett*. 2020 Nov;334:27–35.
36 Cimmino I, Fiory F, Perruolo G, Miele C, Beguinot F, Formisano P, et al. Potential Mechanisms of Bisphenol A (BPA) Contributing to Human Disease. *Int J Mol Sci*. 2020 Aug;21(16).
37 Kuzukiran O, Yurdakok-Dikmen B, Sevin S, Sireli UT, Iplikcioglu-Cil G, Filazi A. Determination of selected endocrine disruptors in organic, free-range, and battery-produced hen eggs and risk assessment. *Environ Sci Pollut Res Int*. 2018 Dec;25(35):35376–35386.
38 Fromme H, Gruber L, Seckin E, Raab U, Zimmermann S, Kiranoglu M, et al. Phthalates and their metabolites in breast milk–results from the Bavarian Monitoring of Breast Milk (BAMBI). *Environ Int*. 2011 May;37(4):715–722.
39 Zini LB, Gutterres M. Chemical contaminants in Brazilian drinking water: a systematic review. *J Water Health*. 2021 Jun;19(3):351–369.


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