CHAPTER 1

Challenges in Chemical Food Contaminants and Residue Analysis

Michel W.F. Nielen and Hans J.P. Marvin

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1. INTRODUCTION

Nowadays food is produced and distributed in a global market leading to stringent legislation and regulation for food quality and safety in order to protect consumers and ensure fair trade. Despite these efforts, food safety incidents occasionally occur and originate from both microbial and chemical contamination. Pesticide and veterinary drug residues, endocrine disruptors, food additives and packaging materials, environmental contaminants (including dioxins and heavy metals) and contaminants of natural origin (including mycotoxins and marine toxins) are of particular concern. As a consequence of the introduction of food commodities containing ingredients produced by modern biotechnology and resulting legal requirements of safety and labeling, a strong additional demand for adequate methods of analysis has occurred. Risk analysis provides a framework for regulatory authorities to protect the consumer from potential food safety hazards and is performed in an iterative manner by food safety managers (regulatory authorities), risk assessors (scientists) and stakeholders (i.e., consumers, industry, non-governmental organizations). The assessment of food safety is a scientific exercise performed by scientists and consists of hazard identification, hazard characterization, exposure assessment and risk characterization. An important prerequisite for performing risk assessment adequately is the presence of data generated by reliable and fit-for-purpose analytical methods to estimate the level of exposure and intake of the consumer to contaminants and residues. Hence, the accuracy of risk assessment will benefit from the availability of comprehensive quantitative monitoring and consumption data. However, cost and time considerations of food safety managers (in regulatory institutions and industry) favour the development and implementation of inexpensive and rapid screening methods having a limited scope and providing qualitative or semi-quantitative “on-off” data only. Global food production practices and the changing climate showed that new unexpected food safety hazards and risks may appear in the food and feed production chain stressing the need for analytical tools capable of early warning for such emerging risks. Some of these potential food safety hazards and methodologies capable of detecting known and unknown emerging contaminants are discussed and related challenges defined. It is argued that monitoring programmes should anticipate these new conspicuous threats. In this context a key role is proposed for bioactivity-based screening concepts and bioactivity-directed identification tools.

The development of both rapid screening methods and comprehensive tools covering as many contaminants as possible including emerging and even unknown contaminants is justified by the different needs from food safety
stakeholders. The rapid screening developments are facing the challenge of multiplex detection in order to extend their scope, the comprehensive methods on the other hand are facing major challenges in generic sample preparation and advanced data evaluation.

1.1 The food chain: A global issue

The food chain as schematically represented by Figure 1 is rather complex and many factors worldwide play a role in the final issue of food quality and safety.

Raw materials for feed and food production come from all over the world with very different local climate, harvesting and storage conditions, all having an impact on the occurrence of microbiological and chemical contaminants such as mycotoxins, pesticide residues, environmental pollution and packaging migrants. The feed producer will mix different raw materials according to its specifications and add additives such as stabilizers but in some cases also medication. Medicated feed production can cause drug residues in non-medicated feed produced at the same facility due to carry-over. Next, the feed is transported to the first consumer level, i.e., the farm animals. The increased awareness of animal welfare might put a stronger demand on feed-related risk-benefit issues. At the farm stage again additives, but also pesticides, veterinary drugs or even illegal hormones might be applied, which residues and/or metabolites can again build up in the food chain. The food industry produces food products and/or food ingredients but they also yield a waste stream which is at least partly recycled and used as feed ingredient. Packaging into smaller

![Figure 1](image_url)
pieces might increase the migration issue of chemicals from the packaging into the food commodity. Following transport the final products come to the retailers where a storage issue might influence the final contamination load. Products being beyond the storage limit date might be recycled and end up in the feed stream again. Finally the consumer buys food that must be stored and prepared for cooking, actions known for their potential introduction of contaminants if hygiene guidelines are not followed. During cooking, food processing contaminants, such as acrylamide, heterocyclic amines and polycyclic hydrocarbons, are introduced but some of the contaminants present might be degraded (or bioactivated) so the real load of dietary intake of contaminants is not so easy to determine. The occurrence of residues from intentionally added chemicals somewhere in the food chain can in theory be avoided, but very much depends on the attitude and behaviour of the actors in the food production chain. Retrospective studies on recent food safety incidents have shown that the human factor (unawareness, fraudulent and illegal actions) plays an important role in the development of food safety incidents [1]. Globalization of food trade, changing climate conditions and agricultural practices, changing food consumption patterns and environmental pollution are all drivers of food safety risks and should be taken into account in systems aimed at identifying emerging food safety hazards and risks. Control of food safety standards, monitoring of contaminants and knowledge about the fate of food contaminants through the entire food chain is needed thus requiring the availability of analysis methods dedicated to the different parts and their actors within the chain.

1.2 Issues according to the Rapid Alert System for Food and Feed

The Rapid Alert System for Food and Feed (RASFF) is primarily a tool for exchange of information between food and feed authorities in the European Union (EU) member states in cases where a risk to human health has been identified and measures have been taken, such as withholding, recalling, seizure or rejection of the products concerned. The European Commission (EC) publishes weekly overviews of RASFF alert and information notifications on its website, and the summarizing annual reports provide an overview of the numbers of notifications and the categories of food products and hazards that they pertained to [2]. These annual reports also highlight conspicuous developments within the particular year. Kleter et al. [3] have explored the utility of notifications filed through RASFF to identify emerging trends in food safety issues. To this end RASFF information and alert notifications published in the four-year period of July 2003–June 2007 amounting to a total of 11,403 notifications were divided into categories and analysed. The breakdown per hazard category is given in Figure 2.

The major categories included chemical (44%), mycotoxins (29%) and microbiological hazards (17%), which together accounted for the majority of the notifications (90%). Within the chemical hazard category, contaminants in products from seafood (30%) and spices and condiments (15%) were the most commonly reported. The most frequently reported are allergens (e.g., sulfite and
histamine), heavy metals (e.g., cadmium, mercury and lead), veterinary antibiotics (e.g., the nitrofurans; furazolidone and nitrofurazone, as well as chloramphenicol), dyes (e.g., Sudan 1 and 4) and pesticides (e.g., dimethoate, isophenfos-methyl and omethoate). Aflatoxins account for the majority (93%) of mycotoxins and are mainly (84%) found in nuts, of which (54%) have been imported from Iran. Microbial contaminants include moulds, viruses and bacteria. Bacteria species were most frequently reported of which *Salmonella* and its subspecies were the most numerous (57% of the reports) followed by *Listeria monocytogenes* (16%). It should be noted that the number of reports in the RASFF not necessarily reflects the extent of a specific food safety problem because the nature of the RASFF system implicitly yields a multiplication of a specific finding. Based on warnings from the EU member states authorities in other countries will check suspicious lots which will give an additional RASFF notification. Secondly, cost and time considerations limit the scope of survey and monitoring programs, hence many potential food safety hazards will not be monitored at all or at best be accidentally picked up. The application of more generic screening methods such as bioassays in routine monitoring programs may circumvent this problem and increase the chance of finding new (re)emerging food safety hazards. In general, EC regulators and legislators require the availability of fit-for-purpose analysis methods having a comprehensive contaminant scope in order to provide the data for risk assessment, the establishment of maximum residue limits and the development and execution of monitoring plans. Harmonization of validated methods and interpretation of results is a prerequisite in order to allow their use in data banks and to avoid internal and external trade disputes.
1.3 Issues according to the feed and food industry

According to the General Food Law [4], the producers are responsible for the safety of food and feed they produce, storage, transport, selling (or eventually dispose), tracking and tracing. They should not place unsafe food or feed on the market, and should have a full traceability system for rapid withholding and recalling in case of alerts. Last but not least, unsafe situations should be prevented using critical control points hazard analyses (HACCP) and good manufacturing practices. Because of this responsibility and associated demand to maintain their core product integrity the need of the industry for rapid and inexpensive screening methods has never been greater. It is obvious that unloading an incoming ship or truck carrying raw materials cannot wait for the results from a full comprehensive contaminant analysis; a first screening result should be available in minutes, not days. As a compromise a limited number of key expected contaminants will be rapidly tested by, for example, dipstick-like screening assays, and other contaminant parameters will be checked less frequently. Ideally a rapid screening test would allow the detection of all relevant contaminants and still be inexpensive. But reality is far from that, putting a strong demand for international cooperation on the development of rapid multi-detection methods for chemical contaminants in the food chain.

1.4 The consumer perception

Generally food consumers prefer high quality and intrinsically safe food for a low price, but they are also aware of food-related health and safety concerns. Following the request of the European Food Safety Authority (EFSA) and the EC DG-SANCO a special Eurobarometer was carried out in 2005 aiming at an assessment of how people in the EU perceive risk focusing particularly on food safety [5]: 42% of the EU citizens thought that their health could be damaged by the food they eat or by other consumer goods. When they were asked to freely associate the food-related problems they mentioned most often food poisoning (16%), immediately followed by chemicals including pesticides and toxic substances (14%) and obesity (13%). However, following a reminder of possible risks they expressed widespread concerns having on top of their “worry-scale” pesticide residues in fruit, vegetables or cereals and residues such as antibiotics or hormones in meat. In the mid-range environmental pollutants like mercury or dioxins were considered. Surprisingly EU citizens are less concerned about their personal factors such as food hygiene and food preparation (including preparation induced chemical risks). Asked about regulatory consumer protection 62% agreed that food safety laws are strict, despite some reservations regarding their enforcement. Concerning information sources consumers, physicians and scientists were trusted most while economic actors such as farmers, manufacturers and retailers are the least trusted. According to the same Eurobarometer consumers when they go for shopping indeed go for high quality (42%) and low pricing (40%). Although being based on almost 25,000 interviews (approximately 1,000 per member state) these data should be considered as an
estimation only. Classical chemical residues and contaminant issues were mentioned but, for example, natural contaminants such as toxins were obviously not considered and/or unknown by the interviewers and the respondents. Moreover consumers might show irrational behaviour in answering questions: they tend to underestimate the safety aspect related to their own lifestyle with respect to food hygiene during storage and cooking and to overestimate the external risk of chemical residues. Recently Verbeke et al. [6] discussed why consumers behave as they do with respect to food safety and risk information. Some irrational and inconsistent behaviours were explained based on the nature of the risk and individual psychological processes. Improvement of traceability and labeling, segmented communication approaches and public involvement in risk management decision making might contribute to restore consumer confidence and reduce the gap between risk perception of consumers and experts.

Also the consumer expectations regarding food safety and quality require the availability of fit-for-purpose analysis methods having a comprehensive contaminant scope. Harmonization of methods and interpretation of results is again a prerequisite: consumers tend to trust scientists but they will get much more worried when scientists provide contradictory information!

2. EMERGING CONTAMINANTS

An emerging risk is an issue that in the future may pose a risk to human health, to animals or the environment [7]. In Europe, regulation (EC) No 178/2002 includes the responsibility of identification of such emerging risks [4]. The indication of an emerging risk may relate to a significant exposure to a hazard not recognized earlier or to a new/increased exposure to a known hazard. Some current and potential future emerging risk issues are listed in Table 1.

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
<th>EFSA opinion</th>
</tr>
</thead>
<tbody>
<tr>
<td>BFR</td>
<td>Brominated flame retardants</td>
<td>Yes</td>
</tr>
<tr>
<td>PFOS</td>
<td>Perfluorinated organic substances</td>
<td>Requested</td>
</tr>
<tr>
<td>ER</td>
<td>Endocrine disruptors</td>
<td>No</td>
</tr>
<tr>
<td>Metals</td>
<td>Arsenic, cadmium, lead, mercury, methylmercury, organotin</td>
<td>Yes</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>Pyrrolizidine and ergot toxins</td>
<td>Yes</td>
</tr>
<tr>
<td>Proteins</td>
<td>Abuse of peptide and protein growth promoters</td>
<td>No</td>
</tr>
<tr>
<td>GMO</td>
<td>Gene modification of plants and gene doping of animals</td>
<td>Yes</td>
</tr>
<tr>
<td>Nano</td>
<td>Nanoparticles</td>
<td>No</td>
</tr>
</tbody>
</table>
2.1 Brominated flame retardants

Brominated Flame Retardants (BFRs) such as polybrominated diphenylethers (PBDEs) and hexabromocyclododecane isomers (HBCD) are widespread occurring in the environment and belong to the group of persistent organic pollutants (POPs): BFRs show persistence in the environment like their polychlorinated biphenyls (PCBs) and dioxins/dibenzofurans (PCDDs/PCDFs) analogues. BFRs are suspects for various toxic effects, including endocrine disruption which might be enhanced following in vivo hydroxylation. The EFSA has drafted an opinion on PCBs [8] and advice on relevant compounds in the BFR group. It can be expected that BFRs occur in the food chain accompanied by several other POPs in different concentrations (PCBs, PCDD/PCDF, PAH, chlorinated pesticides).

The complex mixture thus obtained requires analysis methods having a huge separation and identification power. Comprehensive gas chromatography combined with time-of-flight mass spectrometry (GC × GC/TOFMS) is an expensive but comprehensive solution to the analysis of BFRs and related substances in the food chain [9,10]. Contrary to conventional techniques, implementation of a GC × GC/TOFMS multi-analyte/multi-class strategy in food safety control seems feasible, provided that an adequate solution is found for the automated data interpretation challenge related to the very large four-dimensional datasets. Apart from that, the development of bioactivity-based screening approaches remains challenging since they can not only provide an additional screening tool, but even disclose the presence of unknown bioactive pollutants. The DR-CALUX®, an aryl hydrocarbon receptor-based transcription activation bioassay, is being used for dioxin screening in several laboratories [11]. Also a surface plasmon resonance (SPR) biosensor assay for measurement of the thyroid disrupting activity of hydroxylated halogenated aromatic pollutants has been described based on binding with specific human transport proteins [12]. A real comprehensive exposure assessment would integrate the data from these entirely different but highly complementary approaches.

2.2 Perfluorinated organic substances

Perfluorinated compounds (PFCs) or perfluorinated organic substances (PFOS) are used in a wide variety of industrial applications [13]. As a consequence these compounds show a global distribution in the environment [14]. They have been detected not only in environmental samples and fish but also in human blood and liver, and in several wildlife species [15]. PFOS show persistence in the environment and some of them have been related to different carcinogenic actions, for example, perfluorooctanoic acid has been identified as a potent hepatocarcinogen in rodents [16]. Meanwhile PFOS have been recognized by the EFSA and concentration levels, contamination pathways and toxicological potency should be assessed in the food chain.

So far most of the analysis methods are based on liquid chromatography coupled to mass spectrometry or tandem mass spectrometry approaches (LC/MS,
LC/MS/MS) preceded by solid-phase extraction. A key issue is the avoidance of contamination during sampling, storage and analysis: PFOS are everywhere inside the laboratory and its instruments. The specific chemical and physical properties of PFOS hinder the development of rapid screening methods: it is for example unlikely that antibodies can be raised successfully against the PFOS family, no biorecognition-based methods have been reported yet. Still many challenges remain even for the LC/MS approach: the development of simplified sample preparation protocols and harmonization of methods are key issues anyway.

2.3 Endocrine disruptors

Many contaminants occurring in the food chain can be considered as endocrine disruptors: certain pesticides, POPs and metabolites thereof, phytoestrogens (present in fruit and vegetables and soy products), hormones like estradiol endogenously present in cow’s milk and eggs, and residues of illegally applied hormones. Several synthetic endocrine disruptors are actually POPs and suspect for carcinogenity; information related to phytoestrogens is rather contradictory in this respect: both protection and promotion of cancers is claimed in literature [17]. The main classes of phytoestrogens are isoflavones, lignans, coumestans and natural stilbenes, and show structural similarities with potent estrogens. Their consumption by healthy adults may be without risk but the problem might be totally different when exposure occurs at critical stages of development, i.e., at foetal and prepubertal children. Soy-milk-based baby-food is especially relevant to check for adverse effects of phytoestrogens [18]. Cow’s milk on the other hand should be checked for both estradiol and phytoestrogens. For an adequate risk assessment it is crucial to know how much phytoestrogens (or endocrine disruptors in general) are added to the “diet” of vulnerable consumers. Apart from endocrine disruptor analyses in the diet a clear insight into the endogenous estrogen background levels is needed. Recently, new data were presented using sensitive gas chromatography high-resolution mass spectrometry (GC/HRMS) down to the 2 ng/L level in plasma samples [19]. It was shown that the endogenous levels in prepubertal children are much lower than previously thought based on less specific immunoassays; as a result the diet-contribution to the total exposure becomes much more critical and relevant. Within the scope of the EU project BioCop (phyto)estrogen levels have been assessed both in soy and cow’s milk products [20].

Several bioactivity-based approaches are feasible for the screening of endocrine disruptors in the food chain. Apart from the SPR biosensor assay based on binding with specific human transport proteins already mentioned [12], robust transcription activation bioassays are available for estrogens [21]. The performance of the latest generation based on recombinant yeast cells fulfilled all validation and ISO 17025 accreditation requirements and the results obtained compared very well with GC/MS data [22]. Also a highly challenging transcriptomics approach is being explored within BioCop [20]. An MCF7 cell line is exposed to sample extracts. Next the messenger RNA (mRNA) is extracted
from the cells and the cDNA is hybridized on a microarray carrying 47,000 human transcripts. Up and down regulation of specific transcripts was observed which will allow the design of dedicated microarrays having a limited number of transcripts for endocrine disruptor fingerprinting. A major challenge will be to ensure compatibility of real sample extracts with the cells, overall robustness and validation issues.

2.4 Metal speciation

The total content of the classical heavy metals, lead, cadmium and mercury in foodstuffs and animal feeding stuff, is regulated by EU maximum levels. However, for some trace elements their speciation is very important in terms of food and feed safety and information on the total content only does not give adequate information for correct toxicological risk assessment. For example, in the case of arsenic, the inorganic forms are the most toxic while for mercury, methylmercury is the most toxic form. Seafood is the major dietary source for arsenic and mercury in the European population [23]. For speciation analysis of trace elements the use of either LC or GC with inductively coupled plasma MS (GC- and LC/ICPMS) is currently the state-of-the-art [24,25]. These techniques have been known for a couple of decades but their use is not routine yet, probably due to rather expensive instrumentation, the need for skilled personnel and the lack of standardization, thus clearly defining some of the urgent challenges. Another issue is the lack of rapid and simple field sensors for speciation analysis which address at least partly the toxicity of the specific metal forms. In the past, different microbial biosensors were developed and evaluated in aqueous model systems [26,27]. These transcription activation biosensors contain a reporter gene under metal responsive element(s). Once the cell and the responsive element detect a metal/metalloid the responsive element on the DNA will switch on the transcriptional and translational machinery producing firefly luciferase leading to light emission directly responding to the concentration. These metal biosensors determine intrinsically the bioavailable fraction of the metal, giving an estimation of the toxic potential and complementing the chemical methods where total concentration of the metals is determined. The ability of this type of biosensors to work in real food and feed extracts is a major challenge that still needs to be explored. Some people are hesitating to work with such genetically modified bacterial cell biosensors. To overcome these hesitations luminescent cells immobilised onto a fibre dipstick might be applied to metal and metal speciation analysis [28].

2.5 Alkaloid biotoxins

The EFSA is very interested in alkaloid biotoxins and has requested scientific opinions for ergot alkaloids (EA) in food, and for tropane and pyrrolizidine alkaloids (TA and PA) in feed; an opinion on EA in feed has been completed showing that there is a lack of data on EA patterns in feed materials and on toxic effects [29]. In particular ergotamine and ergocristine are of concern. PA are
widespread and can be found in many plant genera and therefore also in feed, food and herbs, jacobine and lycopsamine being the most abundant. TA, such as atropine and scopolamine, are mainly found in feed as contaminants from Datura species. Plants producing TA have expanded dramatically in parts of Europe and problems are emerging. Data on the sensitivity of animal species towards the alkaloids are incomplete and do not allow the establishment of tolerance levels for individual alkaloids and mixtures thereof; nevertheless the data available so far indicate that adverse effects may occur in animals. The very limited and often incomplete data on tissue distribution and residual concentrations in edible tissues, milk and eggs do not allow an estimation of carry-over rates to food for human consumption. Data on human exposure and sensitivity towards the alkaloids are very incomplete and do not allow the establishment of tolerance levels for individual alkaloids and mixtures thereof.

No harmonized methods are available yet, although different suggestions ranging from ELISA to thin layer chromatography (TLC) and LC/MS/MS can be found in literature [30]. A general issue in the determination of these alkaloids is a lack of reliable analytical standards, (certified) reference materials, proficiency schemes and a lack of harmonized regulation.

2.6 Peptide and protein growth promoters

The use of growth promoters in food producing animals has been banned in many countries since 1988 [31]. Thanks to harmonization efforts most of the EU member states are capable of detecting steroids and β-agonists at the required level, although large differences in specific analyte coverage still exist. Hormone criminality is believed to be linked with sports doping and to occur via international networks. As in sports doping it can be predicted that the abuse will shift from classical growth promoters such as steroids and β-agonists to peptides and proteins when the veterinary control of the former becomes more effective. Bovine and porcine somatotropin (bST and pST), the equivalents of human growth hormone, are 22 kDa proteins and commercially available as recombinant preparations. They are important endocrine factors influencing metabolic and somatogenic processes including growth, immune function, reproduction and lactation. Their species specificity implies low toxicity in humans, apart from potential rare allergic reactions. Major concerns are the observed increased levels of insulin-like growth factor (IGF-1) in milk [32], which are connected to the incidence of cancer [33]. Detection via instrumental LC/MS/MS approaches is feasible [34]. Recently a rapid SPR biosensor immunoassay has been proposed to detect somatotropins in illegal preparations [35]. However, routine sampling and analysis of somatotropins and associated proteins in blood, milk and other relevant matrices is still a major challenge due to the complexity of the endogenous regulation (Figure 3), protein binding and the pulsatile secretion by the pituitary gland. As a result the blood levels show a high intra- and inter-individual variability.

In view of this it is rather unlikely that measurement of a single parameter can provide a validated tool for the enforcement of the ban on somatotropin and
related growth promoters. A huge challenge is envisaged that an assembly of parameters has to be measured followed by appropriate statistics in order to validate its diagnostic value versus untreated control populations.

2.7 Genetically modified organisms (GMO)

Within the framework of safety assessment of food produced by means of modern genetic engineering the comparative analysis of the genetic modified (GM) crop with its unmodified genetic counterpart is an important part [36]. Two approaches are followed to detect intentional or unintentional alteration of the chemical composition of the GMO, i.e., a targeted approach (i.e., investigating defined constituents) and an untargeted approach using profiling technologies to analyse differences in RNA, proteins and metabolites [37]. Targeted analysis of single compounds with focus on important nutrients and critical toxicants has been successfully applied to demonstrate the safety of GM foods. Some criticisms have been expressed on the targeted approach as being biased and focused on known compounds and expected results [38]. This may become increasingly a problem in the second generation of GM crops where the introduction of completely new biosynthetic pathways or modifications in key enzymes in the primary or secondary structure may result in unexpected changes. Non-targeted approaches using profiling technologies based on DNA (e.g., microarray methods), proteins (two-dimensional gel electrophoresis followed by MS and/or metabolites (using e.g., nuclear magnetic resonance (NMR), GC/MS and LC/MS) are nowadays explored for their potential within the food safety assessment of GM food crops.

Figure 3  Endogenous regulation along the somatotropin (growth hormone) axis.
In the EU the release of GMO and GMO-derived ingredients into the environment, and the marketing of GMO-derived food and animal feed is regulated within various specific directives and regulations. The EU regulations 1829/2003 and 1830/2003 concern the premarket safety assessment and the traceability and labeling of food and feed products derived from authorized GMOs establish that food or feed materials containing GMO-derived ingredients above the set threshold of 0.9% must be labeled as such. Implementation and enforcement of this regulation is generally performed by PCR-based methods using GMO-specific DNA probes. These methods must meet a number of agreed quality requirements. On the other hand, the presence of unauthorized GMO is not allowed at all in food and feed. In these cases, qualitative methods, such as DNA microarray methods may in the near future become very useful since these methods allow the detection of many different GMOs and GMO elements in one analysis, albeit their quantitative performance is limited [39]. Major challenges in GMO detection are quantitative aspects, validation and harmonization. Knowledge about the measurement uncertainty at the level of 0.9% is crucial already, and once a minimum required performance level (MRPL) has been assigned to the ban on unauthorized GMO that challenge will become immense.

2.8 Nanoparticles

Application of nanoparticles — particles with one or more dimensions at the nanoscale — in food, medicine and agricultural products is booming and many nanobased products are already on the market [40]. Inherent to their size and surface-to-volume ratio nanoparticles often show a high chemical reactivity. The quantum-size and Coulomb-charging effects of nanoparticles may yield particles with exceptionally electric conductivity or resistance, high capacity for storing or transferring heat or changed solubility properties. Within food technology nanoparticles are used in food conservation, dietary supplements, food additives, packaging materials, functional foods and intelligent food. Some typical examples of application in the food area can be found: bakeries in Western Australia have incorporated nanocapsules containing omega-3 fatty acids in bread, the capsules will open only in the acidic environment of the stomach; the company NutraLease™ utilizes micelles with a diameter of 30 nm to deliver lycopene, beta-carotene, lutein, phytosterols, CoQ10 and omega-3 fatty acids; Unilever is developing low fat ice creams by decreasing the size of the emulsion particles and The Oilfresh Corporation marketed a new nanoceramic product that prevents the oxidation and agglomeration of fats in deep fryers. It is envisaged that within agricultural practices nanoparticles can be used for precision farming, meaning that autonomous nanosensors are applied for real-time monitoring and early warning of plant health issues, and for controlled release of pesticides and herbicides encapsulated in nanomicelles. Some of these developments already come into reality are: Syngenta is using nanoemulsions in its pesticide products and also marketing the microencapsulated product Gutbuster™ that releases its content in the alkaline environment of the insect stomach; and a growth-promoting product PrimoMaxx™ is used to strengthen
the physical structure of turfgrass. The unique properties of nanoparticles make them attractive in the applications mentioned previously but also impose new unforeseen risks, hence making an evaluation of the appropriateness of the current risk assessment protocols and methods necessary. The appropriateness of risk assessment methodologies currently in place to deal with the new properties of the nanoparticles is being addressed by different authorities. Current methods for the identification of nanoparticle hazards are probably adequate but the methods for characterization of the hazard (i.e., establishment of toxicological dose-response relationships) and subsequent exposure assessment need to be adapted. Improvement of current assessment methodology should consider the following aspects: (i) physical parameters such as particle size, size distribution, surface charge, (ii) agglomeration and disagglomeration properties in different environments, (iii) impurities within, and adsorbed species onto the surface and (iv) biological processes involving nanoparticles, including translocation, cellular uptake and toxicological mechanisms.

The analytical challenge for the required monitoring of nanoparticles in the food chain is immense. A vast array of analytical techniques is typically used for the characterization of the physical properties of manufactured nanoparticles: the mean size and its distribution is measured by techniques like photon correlation spectroscopy (PCS), laser diffractometry (LD), light scattering (LS), differential mobility analysis, TOFMS and microscopy, while electrophoresis is typically used to determine the particle charge density [41,42]. The crystalline structure of a nanosuspension can be assessed by differential scanning calorimetry (DSC) [41], polarized optical microscopy and scanning electron microscopy [43]. Electron spectroscopy for chemical analysis (ESCA), X-ray photoelectron spectroscopy (XPS), secondary ions mass spectroscopy (SIMS) and matrix assisted laser desorption ionization MALDI TOFMS are used for surface structure and chemical composition analysis of nanoparticles [42]. It should be stressed that so far the key issue of isolation and sample preparation of nanoparticles from food or feed samples has been hardly addressed. Definitely it will be very difficult to maintain the integrity of the nanoparticle during sample preparation and the subsequent analysis. Apart from that, many of the characterization tools employed and cited previously will not be easily implemented within a routine food control environment.

3. MASKED CONTAMINANTS

A masked contaminant is defined as a contaminant present in such a form that it will escape from rapid screening or instrumental analysis and remain undetected. A contaminant might be conjugated with carbohydrates, sulfates, amino acids, fatty acids or bound to proteins or nucleic acids in the sample of interest. Rapid screening tests such as immuno or receptor assays are based on molecular recognition and will fail recognition when the binding sites of the molecule become less accessible due to modification or steric hindrance elsewhere in the molecule. Chromatographic and mass spectrometric methods
will fail as well due to retention time shifts and changes in m/z. A typical example is the occurrence of glycosylated Fusarium mycotoxins in wheat and maize, as recently shown for deoxynivalenol (DON) and zearalenone by Berthiller et al. [44,45]. In addition to that it was shown that the ratio between free and glycosylated mycotoxins can change during fermentation processes, for example, in beer breweries [46]. It can be assumed that all biotoxins are vulnerable to some degree of conjugation and escape at least partly from detection, unless appropriate modifications to sample preparation schemes are being implemented. Risk assessments and intake data of biotoxins should be reconsidered taking this phenomenon into account, but first of all appropriate analytical methods should become available.

Another example is residues of the nitrofuran antibiotics, the use of which has been prohibited within the EU because of their potentially harmful effects on human health. Former analysis of the parent drugs turned out to be completely inappropriate since they do not persist in edible tissues due to rapid metabolization. The nitrofuran metabolites on the other hand form persisting protein-bound residues which will remain undetected. Conneely et al. [47] described a method for the determination of the total content of nitrofuran metabolites in tissues incorporating an acidic hydrolysis combined with a nitrobenzaldehyde derivatization step. The method was validated for porcine liver at the 5-ng/g level. Subsequent application to poultry had a global impact: high percentages of non-compliant samples were detected and an MRPL was assessed by the EC for harmonization of residue control in importing and exporting countries.

4. UNKNOWN BIOACTIVE CONTAMINANTS

Unknown bioactive contaminants might emerge in the food chain due to climate changes, illegal production methods such as the application of designer steroids and β-agonists in cattle fattening or even because of an act of terrorism. Irrespective of the origin, they will remain undetected and escape from control as long as contaminant monitoring is restricted to a defined list of target substances. At least three different approaches can be distinguished for the detection and mass spectrometric identification of unknown contaminants, a bioactivity-directed, a metabolomics-like and an in silico prediction approach.

4.1 Bioactivity-directed identification of emerging unknown contaminants

Ideally, the sample should be extracted and purified in such a way to maintain all relevant contaminant classes of interest, the first challenge being clearly in the field of generic sample preparation development. The sample extract is analysed for bioactivity using a suitable assay (whole cell-, receptor-, immuno-, microbiological inhibition assay, etc.). When the sample has been screened suspect in one of these assays and the cause cannot be identified using the
prescribed confirmatory analysis method targeted for the presence of known contaminants, then possibly an emerging unknown contaminant is present in the sample. Next the unknown can be identified using the generic experimental set-up shown in Figure 4. In short, the suspect sample is fractionated by gradient LC and narrow fractions are collected in duplicate using a parallel 96-well plate set-up. One plate is re-analysed for bioactivity using the original bioassay yielding a bioactivity chromatogram or “biogram”. The duplicate well number(s) of the suspect positions in the biogram are subjected to full-scan LC/TOFMS or GC/TOFMS techniques in order to perform chemical identification of the bioactive unknown, preferably using accurate mass measurement and isotope fitting which allows elemental composition assessment of the molecular ion and its collision-induced dissociation fragments. This approach has been demonstrated for cell-, immuno- and SPR biosensor assays and applied successfully to the identification of β-agonists in feed, hormones in urine and antibiotics in chicken muscle [48–50]. A key issue in this approach is the biocompatibility between the LC fraction and the subsequent bioactivity measurement. In this respect we have good experience with wells filled with a mixture of 2μL dimethylsulfoxide (DMSO) and 50μL water as a keeper solvent prior to the fractionation. The
water–acetonitrile eluate from the LC gradient yields a large number of filled wells which are simply evaporated overnight in a fume hood, leaving the substances of interest in a tiny volume of water/DMSO. Of course, an LC fractionation is also directly compatible with atmospheric pressure ionization MS. That option requires only one well plate for fraction collection and bioactivity assessment and the suspect well number is then simply correlated with the retention time in the parallel MS system [51].

4.2 Metabolomics approach to the identification of emerging unknown contaminants

Another sophisticated means of identification of unknown contaminants is based on a metabolomics-like approach. Again the sample should be extracted and purified in such a way to maintain all relevant contaminant classes of interest. Next the sample, still being a highly complex mixture, is analysed by a high-resolution chromatographic technique such as ultra performance liquid chromatography (UPLC), GC or even comprehensive GC × GC, combined with a sensitive full-scan MS technique such as TOF, ion trap or FT Orbitrap. The data from the sample replicates are aligned in the retention time domain and compared with the data from a set of reference sample replicates. Finally uni- or multi-variate statistics are applied in order to assess the significant differences between the suspect sample and the regular reference situation. By using appropriate data analysis software contaminants could be retrieved automatically from an oily preparation, drinking water and grass samples [52].

Successful application of this approach requires the availability of a clean, stable and highly reproducible chromatography system, including reproducibility in solvent and column impurities. Moreover, the reference situation is crucial, requiring a more or less reproducible sample matrix background. For homogeneous samples such as drinking water this can be relatively easily achieved but an adequate reference for inherently inhomogeneous samples having a fluctuating composition such as feed will be very difficult to obtain. Last but not least, intelligent data analysis software is required which can automatically correct for small changes in retention time and/or mass accuracy and is capable of keeping the underlying raw data accessible for retrospective analysis, reprocessing and evaluation in its original software format. Note that a GC × GC/TOFMS analysis creates a challenging four-dimensional dataset. Ignoring all these factors will yield many irrelevant data from system impurities, bulk composition changes, etc., and probably not identify the unknown emerging contaminant.

4.3 In silico prediction approach to the identification of unknowns

Starting from a chemical core structure of a specific contaminant or contaminant class and having knowledge about reactivity at specific atom positions in that core structure, one can predict in silico the chemical structure of unknown contaminants, metabolites and emerging risks. On the basis of such a structure
physicochemical parameters such as solubility, polarity, acidity can be estimated as well, thus providing a basis for the prediction of chromatographic and mass spectrometric behaviour of the unknown. Finally, GC- or LC/MS techniques can be used to perform a targeted search on the presence of the predicted unknown in samples of interest. Recently we demonstrated the feasibility of this approach by a UPLC/TOFMS search for in silico predicted metabolites and designer modifications of glucocorticosteroids in urine [53]. Even the potential modifications can be automated: we modified a commercially available software package for metabolite finding in such a way that all kinds of chemical modifications were automatically searched for in the TOFMS dataset yielding a listing of both known compounds already present in a user library and unexpected options caused by a combination of different reactions relative to the core structure, all supported by elemental compositions thanks to the accurate mass measurement.

5. EMERGING TECHNOLOGIES

5.1 Omics

Genomics, proteomics and metabolomics ("omics") are extremely valuable tools in studying biological processes, in bio- and disease marker discovery, in drug discovery, in nutrition and toxicology. Generally, cells, plants or animals are exposed and divided into a treated and an untreated group. Following the experiment both groups are analysed at the mRNA, the protein or the metabolite level using DNA microarrays, 2D-gel electrophoresis plus MALDI/TOFMS or LC/MS, NMR plus GC- or LC/MS, respectively. Next the differential regulation of thousands of targets is assessed using appropriate statistics. Usually both experimental groups are well-defined and identical, except for the treatment, and the dose of exposure is relatively high as compared to levels normally encountered in food contaminant and residue analysis. On the other hand, biomarkers thus obtained might be used for the development of dedicated screening assays based on PCR, tailored DNA microarrays, receptor or immunoassays. Assuming that such screening assays will be developed at least two scenarios can be distinguished: (i) exposure of a standard cell system or organism to the food sample extract of interest followed by isolation of mRNA, proteins or metabolites from the cells and analysis using the developed dedicated screening assay and (ii) isolation of mRNA, proteins or metabolites directly from biofluids in the food sample, usually restricted to farm animals and crops. A major challenge would be to obtain, purify and maintain the integrity of a representative isolate. But most important of all will be the validation of the biomarker targets versus their natural background variability in food, feed and biofluid matrices: real-life is quite different from standard cells or organisms! The on-going European project on new technologies to screen multiple chemical contaminants in food, acronym BioCop, has taken this challenge and is studying both transcriptomics and proteomics for chemical food contaminant analysis [20]. An MCF-7 standard cell line is used and exposed with food sample extracts for
phytoestrogen and mycotoxin analysis, next the extracted mRNA from the cells is analysed by tailored DNA microarrays. In the proteomics topic of BioCop a multiplex SPR biosensor immunoassay is being developed to analyse protein biomarkers in blood of bovines for the screening of steroid abuse in cattle fattening.

5.2 Bioassays

The acceptance and application of whole-cell bioassays in routine chemical food contaminant analysis is progressing slowly but steadily. Typical examples are the DR-CALUX® bioassay and hormone reporter gene bioassay. The principle of such assays is illustrated in Figure 5: Genetically modified cells are exposed to the sample extract containing the contaminant(s). The cells are modified in such a way that they express the receptor protein of interest, in this example the aryl hydrocarbon (Ah) receptor which binds to dioxins and PCBs. Upon binding the receptor–ligand complex travels into the cell nucleus and binds onto the modified DNA at specific responsive elements which act as a switch for the activation of a gene encoding for the production of a marker protein such as luciferase or green fluorescent protein (GFP); as a result the cells will generate light upon exposure, even at trace levels [54]. Cell assays can be performed in parallel in 96-well plates and require hardly any reagents. Only the suspect samples will require confirmatory analysis using expensive instrumental analysis methods such as GC/HRMS in the case of dioxins. A key issue in the application to food and feed samples is the stability of the cells. In general, mammalian cells seem to be more vulnerable to cell toxicity caused by matrix components and require a more stringent sample clean-up in contaminant and residue analysis. Yeast cells on the other hand are inherently more robust, allowing application to

![Figure 5](image_url)  
*Schematic representation of the mechanism of the DR-CALUX®. Reproduced and kindly provided by T.F.H. Bovee [54].*
residue analysis of estrogens in feed and urine samples following a simple solid-phase extraction step [54].

5.3 (Bio)nanotechnology

The nanosciences intend to deliver radical new products and processes at nanoscale dimensions by manipulation of surfaces and macromolecular assemblies. In 2005, Bruce et al. [55] reviewed the role of nanotechnology in food analysis but no real-life application of (bio)nanotechnology in chemical food contaminant analysis was presented. Since then the situation is not so much different. However, it should be noted that any SPR biosensor instrument already used in chemical food contaminant analysis is actually an example of bionanotechnology since the biointeraction is measured within a distance of 150 nanometre of the functionalized surface and the flow cells of the microfluidic system are typically in the order of some tens of nanolitres. For example, by applying SPR, Farré et al. [56] showed part-per-trillion sensitivity for the pesticide atrazine in natural water samples and Haasnoot et al. [57] showed the SPR determination of sulfonamide antibiotics in broiler serum and plasma as a prediction tool for residue levels in edible tissues. Another option is the use of localized surface plasmons (LSPs) sustained by functionalized silver or gold nanoparticles: a highly specific, label-less immunosensor has been constructed for the residue analysis of the anabolic steroid stanozolol down to the nanomolar range [58]. The nanoscale format of SPR can also be coupled successfully to nanoLC/TOFMS as demonstrated for the residue analysis of the antibiotic enrofloxacin in chicken muscle [59]. Gold nanoparticles can also be applied to enhance the performance of immunochromatographic strip tests (“dipsticks”) as shown by Tanaka et al. [60]. A special application of nanoparticles in the detection of chemical food contaminants will be the use of quantum dots (QDs). QDs are semiconducting crystals of a few nanometres and have unique photophysical properties such as size-tunable luminescence spectra, high quantum yields, broad absorption and narrow emission wavelengths. As a result they will be increasingly used as fluorescent labels in, for example, immunoassays [61], also in chemical food contaminant and residue analysis. Apart from detection, nanomaterials might be used in sample preparation as well. Zhao et al. [62] used carbon nanotubes as a solid-phase extraction adsorbent for the GC/MS/MS analysis of barbiturate drug residues in pork at sub-ppb levels.

5.4 Ambient mass spectrometry

In 2004 a novel ambient ionization technology, called Desorption ElectroSpray Ionization (DESI), was proposed by the Cooks group at Purdue University [63,64]. For the first time, surfaces can be probed and analysed by MS under real native ambient conditions, without any sample preparation or matrix addition. The typical lay-out of a DESI source is shown in Figure 6. In short, a pneumatically assisted electrospray is used to produce charged microdroplets.
and gas-phase solvent ions that are directed onto the sample at a surface. The ionization process closely resembles conventional ESI-MS; however, the sample is not present in the solvent nor ionized during the electrospray process, and is therefore less vulnerable to ionization suppression caused by the presence of salts and other interfering matrix components. Instead, ionization of the sample molecules takes place at or above the surface as a result of proton transfer, electron transfer, or ion transfer by gas-phase ions [65]. Reagents can be added to the electrospray solvent in order to tune the selectivity and the sensitivity of the DESI process [66]. That versatility is a great advantage over the direct analysis in real time (DART) ionization alternative in which helium or nitrogen is used as a reaction gas [67]. Depending on the capabilities of the MS, targeted (selected ion mode), untargeted (full-scan mode) or accurate mass analysis is possible, yielding for example, new biomarkers from differential metabolomics with the aid of principal component analysis (PCA) [68]. The ion transfer line to the atmospheric inlet of the MS can be extended and integrated with the electrospray nozzle, thus creating a probe for in situ sensing of any native or cut surface. Widely variable substrates such as paper, metal, Teflon, human skin, animal organs such as pancreas, liver and brain, plant parts such as stems, seeds, flowers and roots, glass, leather, bricks, polymers (Nylon, latex, PDMS) and silica gel have been sampled successfully. Measurements take on average 3 s but high-throughput analysis (3 samples/s) with a moving belt has also been described [69]. Automated chemical imaging of surfaces is possible. Depending on the dimensions of the electrospray tip and the distance to the surface, spot sizes will vary from $2 \times 2 \text{mm}$ down to $50 \times 50 \mu\text{m}$. In terms of analytes, the technique is more versatile than standard ESI-MS, as also highly non-polar molecules such as carotenoids, steroids, terpenes and even volatiles can be analysed. The sensitivity of the technique lies in the low picogram or high

**Figure 6** Schematic view of a DESI source.
femtogram range. DESI-MS is also believed to be quantitative but we have some reservations in that respect. So far, the applicability of DESI technology has been demonstrated in forensics (detection of explosives, of drug residues on banknotes, Ecstasy), pharmaceutical [69–71], plant sciences \( \textit{in vivo} \) analysis of plant alkaloids [72], and clinical analysis, diagnosis of cancerous tissue [73] and metabolomics [68].

The option of direct surface analysis of plant alkaloids, pesticide residues and natural toxins will be very attractive for chemical food contaminant analysis. For complex, mixtures the combination of TLC and DESI can be considered, as recently demonstrated for alkaloid dietary supplements [74].

6. VALIDATION AND QA/QC CHALLENGES

The validation requirements for contaminants and residue analysis in the food chain are laid down in different international regulations. Some contaminant plus sample matrix groups are using the concept of normalized and harmonized reference methods, in other situations the requirements are limited to method validation and data interpretation issues. In any case, the number of available certified reference materials and interlaboratory studies is far too low, mainly due to financial limitations and other priorities. Two issues are particularly challenging: (i) the validation of biochemical or biological rapid screening methods and (ii) the validation of generic comprehensive instrumental methods.

6.1 Biochemical or biological rapid screening assays

Validations for classical instrumental chemical analysis methods are relatively straightforward, but the introduction of (multiplex) biochemical or biological rapid screening assays requires a new fit-for-purpose approach. Typically the data output is generating qualitative or semi-quantitative results, while the scope of bioactive analytes covered might be rather diverse in terms of physicochemical parameters. As a pragmatic solution we applied the following validation procedure to the validation of a whole-cell estrogen bioassay: first, five different bioactive estrogen representatives were selected ranging from polar to apolar chemistries assuming that both known and unknown estrogens would have physicochemical characteristics within that range. Then, representative sample matrices, in this case calf urine and three different types of feed matrix, and acceptable spiking levels were defined based on current legislation and/or expected sensitivity of the bioassay for that particular compound. For each sample matrix both unspiked and samples spiked with the representative individual estrogen were analysed. By doing so the unspiked samples were always screened compliant while the spiked samples were always screened suspect non-compliant at the levels chosen, allowing validation as a qualitative screening method and assessment of \( CC_a \) and \( CC_b \) values according to 2002/657/EC; even ISO 17025 accreditations could be obtained [75,76]. Moreover,
as shown in Figure 7 routine operation of the validated bioassay showed that the non-compliant control sample was always screened above the $CC_\alpha$ decision limit and declared non-compliant while the compliant blank was only screened two times false non-compliant over a two-year period, despite the inherent signal variability of such a bioassay [22]. In other words, even a biological assay can provide consistent results as an on/off screening tool in residue and contaminant control.

**6.2 Comprehensive instrumental analysis methods**

Thanks to the introduction of highly sensitive ion trap and TOF mass analysers in GC/MS and LC/MS full-scan positive and negative ion data of the residues and contaminants in a particular sample of interest can be easily obtained, provided that the analytes are effectively extracted and survived the clean-up step, the injection, chromatographic separation and ionization. Then the sky is the limit and hundreds of residues and contaminants can be searched for. QA/QC considerations will require the co-analysis of control samples, and then particularly the preparation of a non-compliant control sample having a known concentration of all analytes of interest will be a huge task. The co-analysis of such a control sample will be crucial for the assessment of false compliant results, especially because in the end automated raw data processing and evaluation will be the only option to handle the screening for hundreds of residues and contaminants. It should be noted that state-of-the-art raw data processing and evaluation software will function quite well in academic standards but most often fail in real samples at residue or contaminant level.

![Figure 7](image)

**Figure 7** Long-term performance of a validated estrogen bioassay for blank and estradiol-containing control samples [22].
Considering validation of the total analysis including data processing it might be questioned whether the immense efforts of a full validation for all these hundreds of analytes in all sample matrices of interest is really required. In analogy with Section 6.1 a set of a limited number of representatives might be chosen for an initial in-house validation covering a range of physicochemical analyte characteristics and/or contaminant (sub)groups. Ideally, the choice of these representatives should be validated by performing at least a within-day replicate analysis experiment of blanks and control samples containing all analytes of interest. Additional contaminants of interest might be added to the control sample during routine operation thus providing a continuous extension of the scope of the method.

7. CONCLUSIONS AND FUTURE TRENDS

The current use of routine monitoring systems for a limited number of chemical food contaminants and residues in food industry and food control laboratories does not mean in any way that the challenges we are facing are less, they simply evolved under the influence of globalization, new food technologies, climate change, etc. Not surprisingly different stakeholders have different wishes for improvements in chemical food contaminant and residue analysis: in industry priority will be at the implementation of rapid and inexpensive methods having a scope for those substances which are of paramount importance to their specific core product integrity. Recognized challenges are further increase of speed and reduction of costs, and extension of the scope. Despite their primary responsibility for food safety as laid down in the General Food Law, it cannot be reasonably expected from the food industry that they take care of any emerging known or unknown risk, which might pop up somewhere in the food chain. On the other hand, some of the emerging issues raised in this chapter will cause a problem sooner or later, requiring the availability of analysis methods having a comprehensive scope in order to provide an early warning to the food safety authorities and generate the data for risk assessment. We believe that bioactivity-based screening methods and full-scan accurate mass spectrometric identification methods are highly complementary technologies and both essential in this respect. It is recognized that still major efforts should be put to generic sample preparation and automated data processing and evaluation before such comprehensive tools can be routinely applied in a real-life environment. Current EU monitoring plans are restricted to a limited list of target contaminants and residues in a limited number of samples. Also current validation requirements are typically developed for target analysis. The advocated more comprehensive scope of analysis does not fit easily into such policies. It is recommended that at least a part of the associated efforts and resources is moved towards the actual application of comprehensive methods for known and unknown emerging food contaminants.
ACKNOWLEDGEMENTS

Partners in EU-framework projects SAFEFOODS, BioCop (www.biocop.org) and CONF/IDENCE (www.confidence.org), and colleagues at RIKILT-Institute of Food Safety are acknowledged for their stimulating contributions.

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