Fluorinated, Chlorinated and Brominated Contaminants in Fish for Human Consumption

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Chapter 5

Conclusions and future perspectives

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In the last decade, considerable public attention was spent on the safety of our food. A major concern was the presence of environmental contaminants in food. In 1999 the Belgian dioxin crisis (which in fact was a polychlorinated biphenyl (PCB) crisis) showed the world that contaminants might still be present in our food at threatening levels. This crisis showed the vulnerability of our food supply when contaminants accidentally or by means of illegal practices enter the food supply chains. Other dioxin and PCB crises have occurred such as the dioxin contamination of butter and milk related to contaminated citrus pulp (1997-1998) (*1*), dioxin contaminated mozzarella from Campania, Italy (2008) (*2*) and the PCB contamination of Irish pork meat which entered the human food chain (2008). With food chains becoming more complex due to the globalisation of the production and a more intensive transportation of foods, food safety problems have become a global issue. This was clearly demonstrated in the autumn of 2008 when illegal practices of Chinese dairy producers resulted in an extensive melamine contamination (*3,4*). On a Chinese national level the melamine contamination of baby foods resulted in several deaths. In addition, contaminated ingredients were sold worldwide and used in a wide range of foods. As a result, considerable food recall actions were initiated resulting in substantial economic loss and consumer deception.

These crises show that food safety remains an important issue and continuous attention is needed to secure it. A food safety crisis characteristically runs over a relatively short period of time but results in elevated contaminant concentrations in food that approach or sometimes even exceed health safety limits. Some contaminants are continuously present in food. For example, mycotoxins can be produced by e.g. molds and yeasts in vegetable products (e.g. fruits, vegetables, cereals, nuts) during production, storage and transportation. In such cases, continuous monitoring of these products and ingredients is needed. The same holds for the contamination of foods with halogenated contaminants such as DDT, lindane and PCBs. The production of these compounds started before World War II. The production and use have been terminated after adverse effects were discovered. Following this phase out, the release to the environment decreased to a large extent. This is visible from the levels in the environment: long-term trends show a substantial decrease of concentrations (e.g. PCBs, DDT, lindane) in e.g. fish (*5-7*). Exposure to e.g. dioxins and dl-PCBs through food has also decreased (*8*). However, new environmental contaminants were discovered in the last 10-15 years such as brominated flame retardants (BFRs), including polybrominated diphenyl ethers (PBDEs) and hexabromocyclododecane (HBCD) (*9-11*). Furthermore, perfluorinated compounds (PFCs) were discovered as environmental contaminants in the early 2000's (*12-15*). Information on human exposure to these contaminants through food consumption is scarce. In addition, e.g. house dust (BFRs and PFCs) (*16-19*),

drinking water (PFCs) (*20-22*) food cookware materials (PFCs) (*23,24*) and food packaging (PFCs) (*24-26*) were identified as routes that can also contribute to human BFR and PFC exposure.
The risks of exposure to environmental contaminants are evaluated in risk assessment processes. One important aspect of this process is the exposure assessment, which requires information of food consumption as well as the concentration of contaminants in foods. The generation of reliable food contaminant data requires the development of methods being capable to deliver accurate and precise data at the low concentration levels observed in foods.

Analytical method development

In the last decade, considerable developments have taken place on the analysis of halogenated contaminants. The PFCs were discovered as environmental pollutants and they are present in edible fish (*27,28*). Initial methods for fish and other biota relied on ion pair extraction (IPE), followed by detection with liquid chromatography (LC) coupled with electrospray ionisation (ESI) and tandem mass spectrometry (MS/MS) (*29,30*). No clean-up of extracts was applied. In addition, the quality of native and internal standards was very poor (*29*). This led to poor accuracies of the reported data, which was reflected by a poor performance in the 1st world-wide interlaboratory study (ILS). This situation improved largely with (i) the availability of a wide range of high quality native standards, (ii) the availability of many mass labelled standards, especially for important PFCs such as PFOS and PFOA and (iii) the improved knowledge on the physico-chemical behaviour of these compounds, which aided analytical chemists in developing their methods. In the 2nd ILS, large improvements were observed (Figure 5.1). Very precise and comparable results were obtained between laboratories. Even different analytical approaches provided comparable results, which underlines the progress being made in the field. It was concluded that accurate and precise analysis of PFCs in water and fish is feasible if a mass labelled analogue is used for each of the target compounds. The case of PFCs shows that the performance of the analytical community can reach maturity in 5-7 years when chemists are motivated to deliver high quality data and with good instrumentation and the support of suppliers of high quality native and mass labelled standards.
Also in the field of BFRs large developments have taken place. Many laboratories have embarked on the analysis of PBDEs. The analysis may appear similar to the PCBs and, therefore, rather straightforward, but analytical issues such as positive blanks for the major congeners (BDE 47, 99, 100 and 209) negatively affect the accuracy and precision. The analysis of BDE 209 is a challenge in itself with – apart from blank problems – possible de-

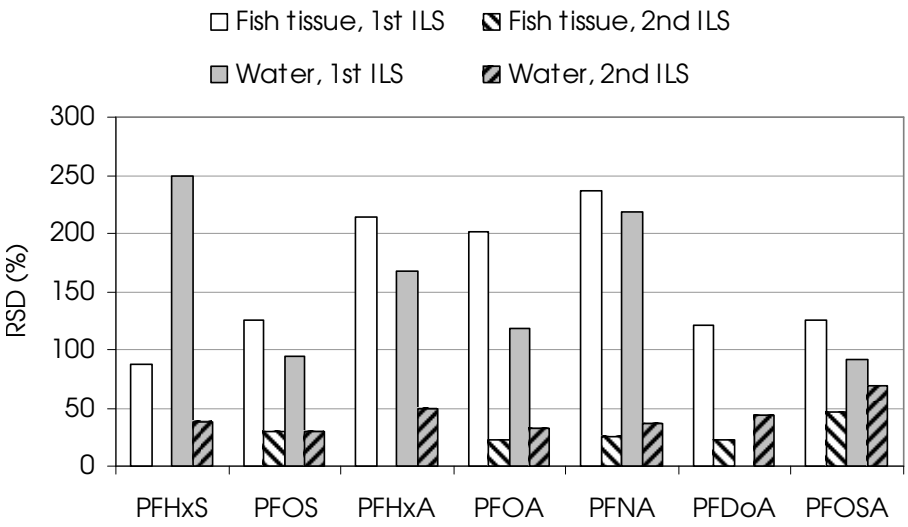

Figure 5.1 Progress being made in two world-wide PFC interlaboratory studies, judged from the relative standard deviations (RSDs) of the submitted pool of data.

gradation in the injector of the gas chromatograph (GC) and on the GC column. To complicate things further, laboratories try to analyse PBDEs, tetrabromobisphenol-A (TBBP-A), the methyl derivative of TBBP-A (me-TBBP-A) and HBCD simultaneously, from extraction to clean-up and finally in a single GC run. This is not the best approach. HBCD degrades in the GC (column and injector), which negatively affects the accuracy. In addition, degradation products interfere with important BDEs like 49 and 99, and this seriously hampers the determination of these important BDE congeners when using GC-ECNI-MS. Analysis of HBCD by LC-MS/MS overcomes these problems and has additional advantages over GC such as the separation of individual diastereomers and the use of mass labelled internal standards. A study in fish samples showed that GC delivered nearly 5-fold higher HBCD values than LC. Although research is not yet conclusive, it is believed that LC-ESI-MS/MS currently delivers highest quality data. GC-electron capture negative ionisation (ECNI)-MS detection limits are still 10-fold lower than in LC-MS/MS, but at the price of delivering inaccurate data, that cannot be considered a great advantage.

For dioxins and dl-PCBs, GC coupled with high-resolution mass spectrometry (HRMS) is the current reference method. Unfortunately, GC-HRMS equipment and maintenance is expensive, and is therefore only applied in a limited number of laboratories. Alternative detection techniques were developed, optimised and in the framework of this study, they were validated. These methods are GC-ion trap (IT)MS/MS, comprehensive multidimensional GC (GCxGC) with electron capture detection (ECD) and the CALUX bioassay. CALUX is simple and straightforward but suffered both from a positive and

negative bias and limited precision. GCxGC-ECD was precise and accurate but suffered from a time-consuming manual integration of peaks, especially at the low concentrations relevant for food. GC-ITMS/MS showed a very good performance and has the potential to become a true alternative to the current reference method GC-High Resolution MS (HRMS), provided utmost attention is paid to the maintenance (source cleaning, etc.). Commission Regulation 1883/2006 specifies performance criteria for screening methods, and all three methods meet these requirements. Apart from the final analysis, sample extraction and clean-up are time consuming as well and therefore add substantially to the costs. Integrated sample extraction and clean-up methods were developed based on accelerated solvent extraction (ASE) and combined with within-cell clean-up and fractionation of the extracts using silica to reduce sample clean-up time (*31*,*32*). The results for a herring sample were lower to those obtained by classical extraction and clean-up, showing that further development of this approach is needed. When estimating the costs per analysis, it is clear that labour costs make up half, or even more of the total costs per sample. These costs are mostly related to laborious extraction and clean-up processes and the largest improvements can be obtained there. For that reason, further efforts are needed to integrate extraction and clean-up methods, thereby resulting in a lower price per sample. Also, instrumental developments (e.g. sensitivity, specificity, and accuracy) are ongoing, and it is expected that in the next decade low cost alternatives to GC-HRMS will become available that deliver the accuracy and precision needed for this complicated analysis. On the other hand the prices of GC-HRMS instruments are dropping and these instruments will come within reach of more laboratories. Either way, it is expected that the capacity on the analysis of dioxins and dl-PCBs in food will increase considerably, which will support a stronger monitoring network globally.

Contaminant concentrations, exposure and risks

Fish is the predominant contributor to the exposure of halogenated contaminants in several European diets. However, it was not fully understood to what extent this also holds for Dutch citizens. It was also not clear which fish contributes predominantly to the Dutch exposure and to what extent newly farmed species contribute. Finally, emerging contaminants like PBDEs, HBCD and PFCs had not been investigated before and no knowledge was available on the exposure of Dutch citizens to these compounds. This study has filled several of these knowledge gaps as we have used the aforementioned analytical methods for analysis of contaminants in a wide range of fish commonly consumed in The Netherlands. The resulting data was used as input for exposure calculations.

Many of the investigated contaminants were present in the wild and farmed fish species. PFOS was present in many samples, and to a lesser extent also

the longer chain acids (perfluoroundecanoic acid (PFUnA), perfluorododecanoic acid (PFDoA), perfluorotridecanoic acid (PFTrA). PFOA and other shorter chain PFCs were only found in a few samples. This is caused by high water-solubility of these compounds, which makes them hardly bioaccumulate. α-HBCD is the predominant BFR present in fish. The PBDE profiles are dominated by the BDEs 47, 49, 99 and 100. However, in farmed shrimp and pangasius BDE 209 was the dominant contributor. It is not clear if this is caused by a different accumulation mechanism or by contamination during harvesting, processing and packaging. PCBs and OCPs were also present in all samples, with CB 153 as the dominant congener in all samples. This shows again how persistent these compounds are and how long it takes before they disappear from our environment and food, even after a complete ban on their production and use. Several OCPs were found in the samples (DDTs, drins, HCHs, HCB etc.), but only HCB and the DDTs (p,p'-DDT, p,p'-DDD and p,p'-DDE) were found in the majority of the samples. Dioxins and dl-PCBs were also present in all samples. When comparing contaminant concentrations in wild and farmed fish, shellfish and shrimp samples, they (generally) decrease in the following order sum 7 indicator PCB > PFOS ≈ sum 3 DDTs > sum 8 PBDEs > HCB ≈ HBCD >> total-TEQ (the sum of 2,3,7,8-tetrachloro-*p*-dioxin equivalents (TEQ)). When expressing the results per fish species, these lipophilic contaminants decreased in the following order: wild eel >> farmed eel > herring ≈ salmon > mussel ≈ mackerel > others. For PFOS, the situation was slightly different: wild eel > herring > plaice ≈ wild shrimp > sole ≈ mackerel > other fish, underpinning the different bioaccumulation process of PFOS. Highest total-TEQ concentrations were found in wild eel from the Dutch river deltas (e.g. New Merwede and Hollands Diep). Eel from these locations exceeded the EC maximum limit (ML) of 12 pg TEQ/g ww. Very low concentrations were observed in farmed fish species such as the recently introduced tilapia and pangasius, and in farmed shrimp (~0.2 pg TEQ/g ww, upperbound). These concentration differences (high in wild eel, low in pangasius, tilapia and farmed shrimp) were also found for indicator PCBs, PBDEs, HBCD, DDTs and HCBs. Eel is a carnivorous species, requiring large amounts of animal proteins. Eel is a lipid-rich fish and accumulates large amounts of organic contaminants when its habitat (and therefore also the prey animals) is polluted. These contaminants are stored in the lipids. Pangasius and tilapia feed on a higher proportion of vegetable proteins and lipids and these are less contaminated, resulting in much lower contaminant concentrations. The differences between concentrations of the lowest contaminated fish (pangasius, tilapia) and the highest contaminated samples (eel) were large, spanning several orders of magnitude: 250-fold (total-TEQ, upperbound) and 50,000-fold (indicator PCBs).

These contaminants found in fish result in exposure of consumers. In order to assess the exposure, detailed data on fish consumption and fish sales (farmed shrimp, pangasius and tilapia) were combined with detailed contaminant

data. The consumption data was used as 'average consumer', meaning that the results describe the mean Dutch exposure from fish consumption. High-consumers (e.g. sports-fisherman that consume own caught fish) were not considered. The consumption of fish by Dutch consumers (mean) decreases in the following order: Gadidae (e.g. cod, haddock, fish fingers, kibbeling) > herring > salmon > mussels = plaice > other fish and shellfish. The consumption of other fish is much lower than these species. When looking at the dietary exposure to the total of contaminants investigated the order changes (Figure 5.2). Herring predominates (41%), followed by salmon (21%) and Gadidae (14%) (total 76%). The other fish species together contribute 24%. The contribution of pangasius, tilapia and farmed shrimp is less than 1%. This order only slightly changes when looking at individual contaminant groups rather than at the totals. These data are relevant for other European countries also. The newly farmed species are introduced in other countries as well and will become part of our diet. In addition, herring, salmon and cod are also popular species for other countries (e.g. Scandinavia) and will probably contribute substantially to the exposure in those countries as well.

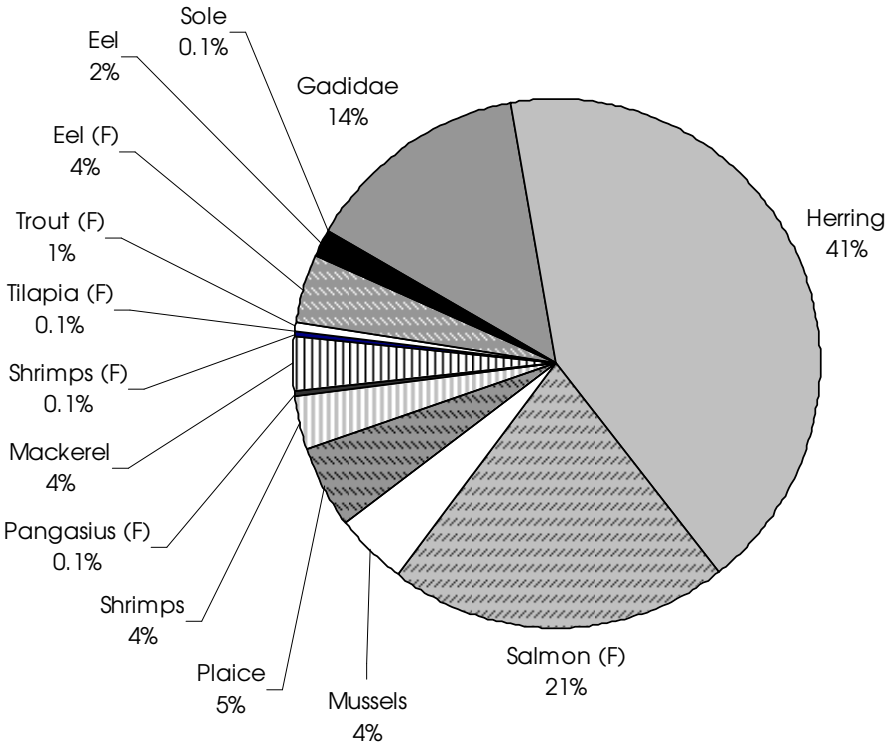

Figure 5.2 Contribution of different fish species to the dietary exposure of the sum of investigated contaminants. Farmed species are indicated by F between brackets.

The absolute exposure per contaminant group decreases in the following order: ∑indicator-PCBs (1.1 ng/kg bw per day) > PFOS (1.0 ng/kg bw per day) > ∑3DDTs (0.45 ng/kg bw per day) > ∑8PBDEs (0.27 ng/kg bw per day) > HCB (0.09 ng/kg bw per day) ≈ α-HBCD (0.06 ng/kg bw per day) >> dioxins and dl-PCBs (0.26 pg TEQ/kg bw per day). The exposure for high fish consumers is not considered, but may be substantially higher. The margin of safety (MOS) is smallest for dioxins and dl-PCBs, as there is only an 8-fold difference between the fish-related exposure and the WHO tolerable daily intake of 2 pg/kg bw per day). When also dairy, pork and other food items would be taken into account, this margin becomes even smaller. The MOS for the other contaminants are larger (e.g. 150 for PFOS, 2,400 for HCB and $3.7x10^7$ for HBCD). The MOS for PFOS as derived by EFSA was much lower (MOS of 3), which was caused by the fact that (i) EFSA used a PFOS concentration which was the average of all European data available, (ii) EFSA used a high estimate of the fish consumption (consumers only) and regarded the sum of fish without specifying per species. This resulted in a very conservative exposure estimate. In the present study, it is shown that with fish-specific consumption data and fish-specific PFOS contamination data, the exposure is much lower. This information will be helpful to risk assessors and policy makers when setting their priorities on the human exposure to PFCs.

To summarise, the present unique and detailed study created a large database on contaminant levels in many wild fish species that are important in the Dutch diet (herring, salmon, Gadidae) and in newly farmed fish species like tilapia and pangasius. This dataset allowed the determination of the relative contribution of different contaminants and different fish species to the exposure of Dutch citizens and provided the following answers: (i) the exposure is dominated by the indicator PCBs and by PFOS; (ii) herring and salmon are the predominant contributors whereas contribution from farmed shrimp, pangasius and tilapia in negligible, (iii) the exposure to dioxins and dl-PCBs (as WHO-TEQ) is lowest but due to the high toxicity of these compounds, the margins of safety are smallest, and (iv) detailed PFOS data provided a more accurate exposure estimate than the recently calculated exposure by EFSA, resulting in a substantially larger margin of safety.

Future perspectives

Analytical methods

After the ban of PCBs, history repeated itself with e.g. PBDEs, and several undesired (and adverse) side-effects were found again such as widespread environmental pollution and accumulation in aquatic and human food chains (*33*,*34*). Due to measures agreed in the Stockholm Convention, the use and emissions of the current POPs were successfully decreased. The process

of assessing potential bioaccumulation, persistency, adverse effects and long range transportation in the framework of the Stockholm convention continues. PBDEs, HBCD and PFOS were proposed for inclusion in the convention. In addition, the EU REACH program (Registration, Evaluation, Authorisation and restriction of Chemicals) is initiated for registration and evaluation (and ultimately authorisation) of chemicals in the EU (http://echa.europa.eu/). One of the aims is to prevent the emission of chemicals that can do harm to humans, animals and the environment and as such, REACH intends to prevent that e.g. the history of PCBs and PBDEs repeats again with other chemicals. Detailed testing programs should be undertaken and completed with satisfactory results before allowing a chemical to be used (in products) on the European market. However, because of the growing global population, the use and presence of chemicals in our daily life will increase. Chemicals will be emitted to the environment and may end up in human food chains. Safety aspects of these chemicals and their doses are important with regard to a safe environment and safe food and feed. Analytical chemistry will continue to play an important role in food safety research. Continuous development of methods is needed for (i) identification of unknown chemicals that enter the food chain, (ii) very sensitive detection of contaminants at the parts-per-trillion and parts-per-quadrillion range and (iii) development of methods that allow rapid and high-throughput analysis of food and feed. Developments of versatile instruments continue. Mass spectrometry, once a hardly available technique, has become more robust and affordable, and is nowadays routinely applied for identification and quantification (e.g. quadrupole and ion trap instruments and to a certain degree also time-of-flight instruments). With extraction and clean-up of samples, the emphasis will be on minimizing sample handling and increasing speed and high throughput. Further integration of extraction and clean-up will take place (e.g. by accelerated solvent extraction (ASE) and within-cell or in-line clean-up). Improved instruments will enable detection at the required low levels and reduce the influence of matrix constituents. This helps to deliver accurate data to scientists, policy makers and food industry, and food safety will benefit from this.

Bioanalytical methods are increasingly used in food safety research. They have been applied on organohalogenated contaminants such as the dioxin receptor (DR)-CALUX (*35-37*) and immunoassays using surface plasmon resonance (SPR) techniques for the detection of dioxins and dl-PCBs in food (*38*).

Examples of other biosensors based on antibody binding are multi-analyte ELISA and lateral flow assays. These type of sensors can be used on broad range of food contaminants, proteins, allergens whenever a suitable immunoassay is developed (e.g. (*39,40*). The method offers sensitivity, specificity, speed (high degree of automation) and multi-analyte detection in complex analytical matrices. Drawbacks of bioanalytical tools are the lack of

identification and cross reactivity with other compounds and matrix constituents and, therefore, the higher degree of uncertainty associated with the test result (as compared to chemical analytical techniques). For that reason, they are often applied as screening techniques and complemented with a chemo analytical confirmatory technique (e.g. GC-HRMS).
The increasing number of halogenated (and non-halogenated) industrial contaminants that are being found in our environment may further stimulate bioassay analyses. Effect-directed analysis (EDA) combines bioassays with chemical analysis for compound identification. Extracts that show a specific response in a bioassay are fractionated and the compounds responsible for the bioassay response in a fraction are identified by chemical analytical analysis. This approach combines the advanced analytical methods described above with toxicological information (*41,42*). The EDA approach has hardly been applied in food safety testing, but certainly provides a powerful approach for the identification of unknown biologically active contaminants. Bioassays such as the DR-, estrogen receptor (ER)- (*43,44*), androgen receptor (AR)-CALUX (*45*) can be used for that purpose. This approach was successfully applied for the identification of endocrine disrupting potency of chlorinated contaminants in fish which were responsible for estrogenic activity (*46,47*).

Human exposure
The exposure part of the present study was focussed on fish only. Several other pathways, such as exposure from other foods (e.g. diary, cereals, meat) were not taken into account. For lipophilic compounds, food is the most significant pathway, with dairy, fish and meat being predominant contributors (*8,48*). This may be different for more water soluble PFCs such as perfluorbutane sulfonate and perfluorbutanoic acid. For water-soluble PFCs, drinking water (*20-22*), beverages and milk (*49*) may be relevant contributors. In addition, uptake of these contaminants by plants from groundwater or contaminated agricultural land (by sludge) may be a relevant source as well (*50*). PFCs were detected in vegetable products (*51,52*) and there is a need to determine the mechanisms behind the plant uptake.
Apart from dietary exposure, exposure from air and dust also contributes to the overall human exposure to e.g. PBDEs, HBCD and PFCs (*16-18,53,54*). To evaluate this, one should analyse representative samples from each of the pathways. This would provide in-depth insights, allowing a total-exposure assessment. Obviously, this is a time consuming task. On the other hand the analysis of human samples (e.g. blood, plasma) provides an integrated descriptor of exposure from all pathways. This approach has some benefits: (i) absorption through membranes (e.g. dermal and intestines) has been accounted for, (ii) the distribution over body tissues can be estimated by relating it to animal experiments and toxicokinetic models, and (iii) possible

degradation in the human body can be determined by analysis of metabolites in blood or excreted body fluids (e.g. urine).
PFOSA is shown to be a precursor of PFOS. Biodegradation occurs in fish livers and liver microsomes, thereby contributing to the PFOS body burden (*55*). Several PFOSA type compounds exist with methyl, ethyl or even more complex groups covalently bound to the nitrogen atom (e.g. N-MeFOSA, N-EtFOSA) to which we are exposed. N-substituted PFOSAs can also biodegrade to PFOS (*56*). The same holds for fluorotelomer alcohols and polyfluoroalkyl phosphate surfactants (PAPs) which showed to biodegrade to PFOA in vivo (*57*). PAPs are applied in food packaging and this is presumably a relevant source of PFOA after ingestion. These precursors may result in a continued exposure to their stable degradation products (e.g.. PFOS and PFOA). On the other hand, Haug et al. recently demonstrated in a time-trend study in human serum samples from Norwegian people (1976-2006) that PFOS and PFOA concentrations increased and reached a plateau in the mid 1990s. From 2001 onwards, the concentrations were decreasing. Longer chain acids (perfluornonanoic acid and perfluordecanoic acid) did not decrease, suggesting different sources or/and longer half-lives (*58*). Clearly, the potential risks of PFCs in relation to exposure are not completely understood yet.
Apart from the contaminant classes studied in this thesis, there are several others that were discovered in recent years as environmental contaminants that (may) contaminate foods. These include phosphorus flame retardants (*59-61*), siloxane compounds (*62-64*), and nano particles (*65*). Concerning the latter, the EFSA recently published an opinion on the nano particles in food and feed (*65*). EFSA recommends that research is needed to address the multitude of uncertainties in this field. Specific recommendations include (i) the investigation of the interaction and stability of nano particles in food and feed, in the gastro-intestinal tract and in biological tissues, (ii) the development and validation of routine methods to detect, characterise and quantify nano particles in food contact materials, food and feed, and (iii) the development, improvement and validation of test methodologies to assess toxicity of nano particles (including reliability and relevance of test methods). This calls for further development of analytical approaches that help to address these questions.

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