TDS exposure project: Relevance of the Total Diet Study approach for different groups of substances

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A method to validate the relevance of the Total Diet Study (TDS) approach for different types of substances is described. As a first step, a list of >2800 chemicals classified into eight main groups of relevance for food safety (natural components, environmental contaminants, substances intentionally added to foods, residues, naturally occurring contaminants, process contaminants, contaminants from packaging and food contact materials, other substances) has been established. The appropriateness of the TDS approach for the different substance groups has then been considered with regard to the three essential principles of a TDS: representativeness of the whole diet, pooling of foods and food analyzed as consumed. Four criteria were considered for that purpose (i) the substance has to be present in a significant part of the diet or predominantly present in specific food groups, (ii) a robust analytical method has to be available to determine it in potential contributors to the dietary exposure of the population, and (iii) the dilution impact of pooling and (iv) the impact of everyday food preparation methods on the concentration of the substance are assessed. For most of the substances the TDS approach appeared to be relevant and any precautions to be taken are outlined.

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Abstract

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1. Introduction

A Total Diet Study (TDS) generally consists of selecting, collecting, and analysing commonly consumed food purchased at retail level on the basis of food consumption data to represent a large portion of the typical diet, processing the food as for consumption, pooling the prepared food items into representative food groups, homogenizing the pooled samples, and analysing them for harmful and/or beneficial chemical substances (EFSA, 2011a). From a public health point of view, a TDS can be a valuable and cost-effective complementary approach to food surveillance and monitoring programs to assess the presence of chemical substances in the population diet and to provide reliable data in order to perform risk assessments by estimating dietary exposure. International organisations such as the Food and Agriculture Organization of the United Nations (FAO), the World Health Organization (WHO) and the European Food Safety Authority (EFSA) have supported the TDS approach for several years, and have provided general methodological guidelines (EFSA, 2011a). Nevertheless, none of these documents have proposed a methodology to validate the appropriateness of this approach for assessing exposure to the different types of substances, given that not all substances can be evaluated through a TDS. A TDS follows three essential principles – i.e. (i) representativeness of the whole diet, (ii) pooling of foods, and (iii) food analyzed as consumed – and the applicability of the TDS approach for the different substance groups has to be considered with regard to these criteria.

The aim of this article is to propose a general method to validate the relevance of the TDS approach, and to apply it to different groups of substances, independently on the fact whether they have already been included in a TDS or not. Only chemical substances are dealt with in this work; biological agents have not been considered as their potential effects are mainly linked to acute exposure (TDS aim at assessing chronic and not acute exposures).

This study was carried out in the framework of the European project TDS-EXPOSURE and is a general analysis conducted for whole groups of substances. Some particular cases are sometimes identified, but in general the conclusions are given for groups of chemical agents and may not apply to some specific substances. For example, the TDS approach could be considered as generally not relevant for a group, even if some specific substances of the group may be studied in a TDS, taking particular caution with the method.

2. Materials and methods

2.1. Materials

As a first step, a list of chemicals to be considered in this work has been established based on different data sources: substances for which the EFSA published an opinion or received a mandate for assessment (74 substances or substance classes at the time of the assessment) (EFSA), substances for which the Joint FAO/WHO Expert Committee on Food Additives (JECFA) published an evaluation or a monograph (n = 2412) (JECFA), substances included in the Codex Alimentarius (n = 189) and in the European rapid alert system for food and feed (RAOCH) notifications (n = 103) (European Commission), and substances recorded since 2004 (n > 30) in the International Food Safety Authorities Network (INFOSAN). Due to the large number of existing chemicals it was not possible to be exhaustive, even though all of the most important substances occurring in food and specifically those recognized as being particularly critical in terms of their effects on human health were included. Most importantly, similar principles can be adopted for new sets of substances detected in future studies targeting new/emerging contaminants. The substances selected were divided into eight main groups that can be present in the diet, based on the list of the EFSA/FAO/WHO guidance document (EFSA, 2011a): (i) natural components considered beneficial or essential (e.g. micronutrients such as vitamins, iron, iodine, and selenium), (ii) trace elements and contaminants from the environment (e.g. ‘heavy metals’, polychlorinated biphenyls and dioxins), (iii) chemical substances intentionally added to foods (e.g. preservatives and colours), (iv) chemical residues of substances being deliberately applied at other points in the food production chain (e.g. pesticides and veterinary drug residues), (v) naturally occurring contaminants (e.g. mycotoxins and alkaloids), (vi) contaminants formed during food processing (e.g. polyaromatic hydrocarbons (PAHs), furan and acrylamide), (vii) contaminants transferred from food packaging or food contact materials (e.g. phthalates and bisphenol A), and (viii) other substances that have already been analyzed in one or more TDSs, including radionuclides (American TDS (FDA), Canadian TDS (Health Canada, 2009)), phytoestrogens (UK TDS (FSA), French TDS (ANSES)), or nitrosamines (Canadian TDS), and other chemicals that have never been studied in any TDS but that should be considered in this work, such as flavorings and nanomaterials.

2.2. Methods

The relevance of the use of the TDS approach for the different substance groups has to be considered with regard to three essential principles, i.e. a TDS (i) has to be representative of the whole diet, (ii) is based on pooling of foods, and (iii) involves food analyzed as consumed (EFSA, 2011a). The point is to check if these three principles would not exclude the evaluation of a family of substances. Four criteria have been considered for that purpose. The first criterion is that the substance has to be present in a significant part of the diet or predominantly present in specific and identified food groups. If the substance is present occasionally in a few foods, the TDS approach is not appropriate. The TDS sampling has to cover exposure from the whole diet, i.e. from all the potential food contributors to the exposure. The second criterion is related to the availability of a robust, ideally validated, analytical method to determine with adequately low LODs the chemical in potential food contributors to the exposure of the population. If it is not technically feasible to analyse the substance in all the potential food contributors or this is possible only in a limited portion of the food items (such as in fat for instance, if the substance can be present in the rest of the food), the TDS approach is not relevant. The third criterion consists in checking the dilution impact of pooling (effect of dilution and mixing of samples). If the concentration of the substance will be highly affected by pooling, e.g. because it occurs only in particular foods or is linked to specific geographical regions or is very volatile, so that it will become undetectable in pooled samples, then the TDS approach is not relevant. The fourth and last criterion is related to the possibility to mimic the impact of everyday preparation methods of food, in the household and other places and situations of daily life (e.g. restaurants, cafes, coffee shops) on the concentration of the substance. As foods are analyzed as consumed in a TDS, it is necessary to reproduce as precisely as possible everyday preparation methods, especially if the concentration is highly impacted by them (loss of volatiles, formation of heat-generated substances, pick-up of contaminants from cookware). If these methods are not known, are too variable or cannot be reproduced in the laboratory, the TDS approach may not be appropriate and/or may need supplementation by another study.

These four criteria are summarized in Table 1. For each group of substances dealt with, the four criteria were applied to determine if the TDS approach was generally relevant or not, or relevant only in some specific cases.

3. Results and discussion

3.1. Natural components considered beneficial or essential

Major nutrients, in particular minerals, are present in a large part of the diet. The TDS approach is currently used in several countries to estimate the intake of nutrients, which are then compared to the recommended dietary allowances (Lombardi-Boccia et al., 2003; Lowik et al., 1994; Ockhuizen et al., 1991; Pennington and Schoen, 1996; Turrini and Lombardi-Boccia, 2002; van Dokkum et al., 1989) or to the upper limits (FSANZ, 2008).

Well-established methods are available to analyse nutrients in all food contributors, based on e.g. high-performance liquid chromatography (HPLC) for vitamins, inductively coupled plasma optical emission spectroscopy (ICP-OES) or flame atomic absorption spectrometry for macroelements. It is possible to analyse nutrients in all potential food contributors but special care should be taken of labile vitamins because endogenous content could dramatically decrease during the storage and the analytical procedure.

General considerations on the smoothing effect caused by pooling are applicable to nutrients. Pooling of samples in food groups would not highly dilute nutrients levels, but care should be taken in food group composition. When fortified foods and nutritional supplements are considered as individual food categories, pooling should be very carefully arranged, because the concentration of nutrients in this type of food is much higher than the one from...
other foodstuffs. Concentrations of labile vitamins (soluble in water and/or unstable to air, light, heat) may be highly affected by preparation procedures and especially cooking. Cooking may cause an increase in the calcium content of pasta depending on calcium levels in water, whereas the concentrations of other macro-elements are either unchanged or slightly reduced except potassium, which decreases considerably (Cubadda et al., 2009). Overall, home preparation of foods and consumption habits can highly influence the concentration of some nutrients, especially sodium (added salt), lipids (added fats such as butter or oils for cooking), and some other minerals for which spices and herbs added to specific foods/dishes can be major sources. Particular attention should then be paid to the sampling plan and to home preparation conditions and additions in order to ensure the representativeness of the real intake.

To conclude, the TDS approach is relevant for nutrients if the necessary precautions regarding the preparation (culinary treatments) are taken.

3.2. Trace elements and contaminants from the environment

3.2.1. Trace elements

Trace elements (TE) are chemical elements taken up at trace levels from the diet. They include essential (e.g. iron, zinc, copper, iodine, selenium) and non-essential (e.g. mercury, lead, arsenic, cadmium) elements. TE are ubiquitous and their presence in food may derive from natural or anthropogenic sources. Food is the main contributor to consumer exposure (Arnich et al., 2012). As ubiquitous substances, TE have been widely considered in TDSs (Arnich et al., 2012; Food Safety Authority of Ireland, 2011; FSANZ, 2011; Leblanc et al., 2005a; Rose et al., 2010; Saleh et al., 1998; Turconi et al., 2009; van Dokkum et al., 1989; Ysart et al., 1999).

Several techniques are available for the determination of TE in food. Many methods rely on oxidative digestion followed by atomic absorption spectrometry or inductively coupled plasma-based techniques, i.e. optical emission (ICP-OES) and mass spectrometry (ICP-MS) that have both multi-element capabilities. ICP-MS is also very sensitive and has established itself as a robust and high-throughput technique extensively used in TDSs. When it is important to determine an element species with a distinctly higher toxicity (e.g. inorganic arsenic or methyl mercury) this is achieved by sample extraction followed by use of species-specific detection techniques (e.g. HPLC-ICP-MS) (D’Amato et al., 2013).

In general, TE occur in all food groups even though methyl mercury is an element species occurring almost exclusively in one food group (fish and seafood products). Careful pooling of samples into representative food groups and/or subgroups does not result in a significant dilution effect.

Sample preparation (in particular sample homogenization, which involves cutting, grinding or milling) can be a source of contamination by iron, nickel, or chromium (from stainless steel), or other elements such as aluminum (Cubadda et al., 2001), even though similar contamination may occur also during home preparation. Indeed aluminum levels may be increased as a consequence of processing and storage of food in utensils made of this metal (Rose et al., 2010). Similarly, metals such as Pb and Cd can leech from ceramics used for cooking or as tableware. Cooking water plays a major role since it may lower or enrich the inorganic arsenic content of the raw product depending on the relative concentrations in the cooking water and raw food (EFSA, 2009). Pasta cooking with water containing low element concentrations has been shown to significantly reduce the levels of arsenic, cadmium, lead and nickel compared to the uncooked sample (Cubadda et al., 2003). Consequently, careful attention to the cooking methods is needed to reflect the consumer TE exposure for some of them, although TE concentrations are in general not too highly impacted by home preparation (Perello et al., 2008).

It can then be concluded that the TDS approach is relevant for TE.

3.2.2. Dioxins, PCDFs and polychlorinated biphenyls

Polychlorinated dibenzo-p-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs) and polychlorinated biphenyls (PCBs) are chlorinated aromatic compounds. These groups have respectively 75, 135, and 209 congeners. Among the PCDD/Fs, 17 congeners tend to accumulate through the food chain, especially in animal fats and products. Twelve congeners of PCBs are known as dioxin-like PCBs because they share the same mechanism of action with the 17 toxic PCDD/Fs (Sirot et al., 2012b). Food represents the primary source (>90%) of non-occupational exposure to these compounds (EFSA, 2005; Sirot et al., 2012b; Windal et al., 2010). PCDD/Fs and PCBs have already been studied in several TDSs (FAO, 2003; Health Canada, 2009; Hsu et al., 2007; Sasamoto et al., 2006; Sirot et al., 2012b).

Screening methods for PCDD/Fs and dioxin-like PCBs (DL-PCBs) in food may comprise bioassays and gas chromatography–mass spectrometry (GC–MS) methods; confirmatory methods are based on high-resolution GC/high resolution MS (European Commission, 2006). For non-dioxin-like polychlorinated biphenyls (NDL-PCBs) it was proposed to focus the analysis on the six congeners 28, 52, 101, 138, 153 and 158 (EFSA, 2005). Dioxins and PCBs are found in many foods and are not specific to a food item. So there is no reason for which pooling would highly dilute dioxin and dioxin-like PCB levels.

Domingo summarized the results of studies on the effect of cooking processes on the levels of PCDD/Fs and PCBs in foods (Domingo, 2011). The results are very diverse, with some studies observing no sizeable effects and others finding significant reductions or increases. As lipophilic substances, PCDD/Fs and PCBs accumulate in fat and any food preparation method that reduces the fat content (e.g. frying or grilling with subsequent cooked-out fat) or decreases the water content (e.g. baking) can influence the concentration measured. In fact, analytical results for this class

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Table 1

<table>
<thead>
<tr>
<th>Criteria</th>
<th>How to interpret it</th>
</tr>
</thead>
<tbody>
<tr>
<td>Occurrence in a significant part of the diet or predominantly in specific and identified food groups</td>
<td>Is the substance likely to be present in a significant part of the diet? Is it otherwise known to be present predominantly in some specific food groups highly contributing to exposure?</td>
</tr>
<tr>
<td>Analytical method existing to analyze the substance in all potential food contributors</td>
<td>Do we have any robust or validated analytical method to determine the chemical in potential food contributors?</td>
</tr>
<tr>
<td>Impact of pooling (dilution effect and mixing of samples)</td>
<td>Is the presence of the substance likely to occur in many or just a few different foods, with risk to become undetectable in pooled samples? Is the concentration highly impacted by mixing/grinding/crushing of the samples?</td>
</tr>
<tr>
<td>Preparation impact and ability to reproduce the consumers’ habits</td>
<td>Is the concentration highly impacted by the home preparation methods, which can cause loss of volatiles (e.g. furan), formation of heat-generated substances (e.g. acrylamide) pick-up of contaminants from cookware (e.g. melamine, aluminum)? If yes, is it possible to reproduce the relevant consumers’ habits in the laboratory?</td>
</tr>
</tbody>
</table>
of contaminant are often reported on a fat-basis and care is needed to ensure that results are properly related back to the weight of food as consumed. Therefore, it is worth to pay attention to the preparation methods usually applied at home, since it can influence the PCDD/Fs and PCBs concentrations.

In summary, the TDS approach is appropriate for PCDD/Fs and PCBs if the necessary precautions are taken regarding the culinary treatment.

### 3.2.3. Brominated compounds

Polybrominated biphenyls (PBB) (EFSA, 2010a), polybrominated biphenyl ethers (PBDE) (EFSA, 2011b), hexabromocyclododecanes (HBCDD) (EFSA, 2011c), tetrabromobisphenol A (TBBPA) and its derivatives (EFSA, 2011d) are widely used brominated flame retardants (BFRs) in plastics, polymers, fibers, and textiles. They are generally persistent and bioaccumulative and, although dust intake and inhalation of indoor air can significantly contribute to the intake for some BFRs, especially in young children, the diet is thought to be the most important source (Covaci et al., 2011; EFSA, 2010a, 2011b, 2011c, 2011d, 2012a). They have been found to occur in several foods with the highest levels in fish and seafood, meat products, milk products, eggs and egg products, and animal and vegetable oils and fats. Levels in samples of plant origin are generally lower. Specific BFRs have been studied in some TDSs (ANSES, 2011b; Health Canada, 2009).

Analytical methods are available to quantify these substances. GC–MS is the preferred method of analysis for BFRs although the use of LC–MS/MS is increasing and is necessary for some of the heat-labile isomers that can inter-convert.

BFRs are mainly found in foods of animal origin. As long as food items of animal and vegetable origin are not combined, there is no reason why pooling would highly dilute BFR levels.

Some studies have addressed the effect of processing on PBDEs (Bayen et al., 2005; Domingo, 2011; Perello et al., 2009; Schecter et al., 2006). Schecter et al. (2006) showed that grilling meat and fish reduced the amount of PBDEs in these foods; similarly Bayen et al. (2005) reported that pan-frying, microwave cooking, boiling and baking of salmon reduced the PBDE concentration. Also Perello et al. (2009) showed decrease of PBDE levels in prepared fish, meat and miscellaneous food products, with the exception of fried potatoes for which a notable increase was observed. Careful attention to the cooking methods is then needed to reflect the consumer exposure.

Summarizing, the TDS approach is appropriate for brominated compounds, but special attention has to be paid to the preparation impact (culinary treatment).

### 3.2.4. Perfluorinated compounds

The diet appears to be the main source of exposure to perfluorinated compound (PFCs). For perfluorooctane sulfonate (PFOS), it was estimated that the contribution of non-food sources was less than 2%, while for perfluorooctanoic acid (PFOA) this could be as high as 50% (Cornelis et al., 2012; EFSA, 2008a; Vestergren et al., 2008). PFOS and PFOA have been detected in meats, fish and seafood, vegetables, fruits, potatoes, drinking water, cereals and rice, dairy products and vegetable oil with the highest concentrations in fish, seafood (PFOA, PFOS), potatoes and eggs (PFOS) (ANSES, 2011b). Other PFCs have only recently been studied (Klenow et al., 2013). Apart from France and Netherlands, also UK and Canada, for instance, have analyzed PFCs in TDSs (FSA, 2006; Tittlemier et al., 2007a).

Liquid chromatography–tandem mass spectrometry (LC–MS/MS) is the preferred method of analysis (ANSES, 2011b; EFSA, 2008a; Pico et al., 2011).

As PFOS and PFOA are found in many foods and are not specific to a food item, there is no reason for which pooling would highly dilute PFOS and PFOA levels.

According to Del Gobbo et al. (2008) all cooking methods (baking, boiling, and frying) reduce the concentrations of PFCs in fish. However, apart from background (environmental) contamination, PFCs may occur in food as a result of migration from food contact materials. In 2009, a Spanish study concluded that it is not sufficiently clear if cooking with non-stick cookware, or packaging some foods, could contribute to a higher human exposure to PFCs (Jogsten et al., 2009). Begley et al. (2005) investigated potential sources of migration from food contact materials and the highest concentrations came from PFCs present as additives and coatings in paper used for packaging and in some cases used in microwave ovens (e.g. popcorn bags, microwaveable ready-meals). It has to be noted that these studies dealt with the USA market and many of these PFCs have been/are being phased out, with substitution of perfluorinated (per = fully substituted) by polyfluorinated (poly = many, but not fully, substituted) compounds in some food packaging applications in Europe. In conclusion, careful attention to the cooking methods including utensils used is needed to reflect the consumer exposure.

In summary, the TDS approach is appropriate for PCFs if the necessary precautions are taken regarding the preparation of food (culinary treatment).

### 3.3. Chemical substances intentionally added to foods (e.g. preservatives and colours)

#### 3.3.1. Food additives

The use of food additives is laid down by the European Regulation EC 1333/2008 which specifies the food groups in which an additive is authorized, and its maximum permitted level. Depending on toxicological issues and the technical need for the substance, while some additives are authorized in all food groups with no maximum limit (like monosodium phosphate or lecithin), some others have very restricted uses (like sodium aluminum phosphate or boric acid). In all cases, additives per se will only be found in processed foods (Commission regulation, 2008) and are not at all ubiquitous compounds. For this reason, a number of food additives will never be selected in TDS. Although not considered as classical substances in TDSs, food additives have already been considered in some existing studies (Bemrah et al., 2012; Food Safety Authority of Ireland, 2011; FSANZ, 2005; Saleh et al., 1998; Van Dokkum et al., 1982).

Food additives belong to various chemical groups and therefore different analytical methods are used for their determination. Sweeteners such as cyclamates may be analyzed by capillary electrophoresis (Bergamo et al., 2011), HPLC (Zygler et al., 2011) or GC (Yu et al., 2012). Food colours are usually determined by spectrophotometry, GC or HPLC (Scotter et al., 2005; Scotter, 2009). Official methods are not always defined for food additives, but robust methods usually exist.

The impact of pooling is substantial for additives in general. Some additives are used only in some specific subgroups and actual occurrence is very variable: some additives, although authorized in a wide range of foods, are not commonly used and usages can be different from one brand to another of the same food type. A classical pooling will lead to inaccurate exposure assessment and will likely underestimate the intake for most additives.

Food preparation should have only a minor impact on additives in general; except for food colours, which can be sensitive to light (e.g. curcuma, chlorophylls, carotenes), pH (e.g. carmines, brilliant black, anthocyanins) or heat (e.g. black PN, betanin) (Emerton, 2008). Some other additives are only used for surface treatment: in that case, the consumer practices (such as peeling) will have a
preponderant impact on the exposure. As the use of food additives in the EU must be declared (mandatory labeling), it is relatively easy to get information on occurrence even if the exact concentration is not provided by labeling. Consequently, it is rather easy to target those food groups and even individual food items and brands that are affected by additive usage and so gain exposure information in ways other than a full TDS.

In conclusion, for most of the additives, the dilution effect linked with the pooling is high and does not allow to perform a reliable exposure assessment. Nevertheless, for some additives commonly used in a large number of foods (e.g. lecithin) or frequently used in some products for technological reasons (e.g. nitrates in meat product), the approach is relevant.

3.3.2. Flavorings

Flavoring substances are present in a significant part of the diet. They can be intentionally added to the product, but the same substances can also be naturally present in foods (in fruits for example). Unlike additives, they can thereby be present both in processed and unprocessed foodstuffs. According to the 2012 Regulation, the use of flavoring substances is permitted in accordance with good manufacturing practices unless specific restriction is given, in which case they may only be added to definite food categories and under specified conditions of use (Commission Regulation, 2012).

Several flavorings are commonly analyzed with GC–MS (Wang et al., 2008) or HPLC–UV (Schwertner and Rios, 2007).

The majority of the flavors can originate by a large number of different flavoring substances, but also by pools of substances and only some flavors are linked to a specific substance (the character impact compounds). Then, for the majority of flavorings, pooling of products with a same taste will necessarily lead to a dilution of different flavoring substances involved in this taste. Only character impact compounds can be analyzed in pooled samples, but one should keep in mind that detailed consumption data would be needed (flavors for each product consumed) to select the foods to be sampled. Dilution effect can also be important taking into account the fact that industries can use specific flavoring substances for some products and that the consumers are not equally exposed to the products of different brands (due to brand loyalty) (Arcella and Leclercq, 2005).

Many of the flavoring substances (i.e. those associated especially with the aroma dimension of flavor) are volatile (Buttery, 1971; Philippe et al., 2003). Consequently, pooling of products by crushing and milling may lead to loss of substances and then to under-estimation of the actual concentrations, especially for solid foodstuffs. Due to volatility, home preparation such as heating can also impact the concentration; consequently such preparations should be reproduced when preparing samples.

In summary, due to the volatility of the many flavoring substances, but also to the difficulty in identifying in which product a flavoring substance is used (the labeling may declare the presence of flavorings but not specify which chemical is used), the TDS approach does not seem to be the best way to assess the exposure to most of these substances.

3.4. Chemical residues of substances being deliberately applied at other points in the food production chain

3.4.1. Pesticides residues

Pesticides are found in the majority of foods. Vegetables, fruits, cereals, fermented cocoa, coffee, tea, spices and herbs, and products of animal origin (red meat, white meat, fish, milk, cheese, yogurt, cream butter, eggs, honey, etc.) are thought to be the main sources of dietary pesticide exposure (SANCO/12495/2011). Pesticide residues have been analyzed in many TDSs (Caldas et al., 2011; FSANZ, 2011; Gimou et al., 2008; Nougadere et al., 2012; Rawn et al., 2004; Sawaya et al., 2000; USFDA, 2008; Zhou et al., 2012).

The most reliable methods for pesticide residue analysis are MS-based, especially GC–MS/MS and/or LC–MS/MS (Anastassiades et al., 2003; Association of Analytical Communities, 2007; Codex Alimentarius Commission; EN 15622; Lehotay et al., 2005; SANCO/10684/2009; Tomlin, 2000).

Pesticides are applied to many fruits and vegetables at different levels. As long as food items from different origins or species or varieties are not pooled, there is no reason why pooling would highly dilute residue levels. But pooling can decrease the concentrations of some volatile pesticides.

There can be appreciable differences in pesticide levels before and after processing and cooking. Operations such as washing, peeling, blanching and cooking play a role in the reduction of residues (Elkins, 1989). Each operation has an additive effect on the reduction of the pesticides present and the use of so-called processing factors to account for this is well established (Geisman, 1975; Kaushik et al., 2009). These processing factors are substance-specific. For instance, whereas a topical (surface) pesticide sprayed onto fruits can be removed largely by peeling, a systemic pesticide (i.e., distributed throughout the fruit) will be reduced far less. Similarly water-soluble topical pesticides may be reduced if fruits are rinsed with water but lipophilic substances may be not. Consumer practices (particularly washing and peeling) should be carefully reproduced in a TDS to reflect the consumer exposure. Other effects may occur, such as during the baking process the water contained in the food product can entrain pesticide molecules (co-distillation) while heat causes evaporation and degradation (Sharma et al., 2005). Finally, the stability of the substances has also to be taken into account. If the samples have to be frozen for storage pending analysis, concentrations of some pesticides may highly decrease. Sample preparation and storage have to be adapted to the analysis of those pesticides, to keep the approach relevant.

In conclusion, the TDS approach is appropriate only for non-volatile pesticides.

3.4.2. Veterinary drug residues

The presence of veterinary drug residues in foodstuffs results from the use of veterinary medicinal products in food-producing animals. Veterinary drug residues can be present in a significant part of the diet, mainly in products from animal origin (meat, milk, eggs, honey, etc.) but also in water because of environmental contamination. Pharmacologically active substances that are prohibited (e.g. chloramphenicol, furazolidone, metronidazole) or for which MRL are fixed to zero should not be detected in food (Commission Regulation, 2009) and then should not be included in a TDS. Veterinary drug residues have been analyzed in some TDSs as in Canada (Tittlemier et al., 2007b).

There are no specific analytical methods recommended by the European Commission or other European bodies for veterinary drugs residues. Rather, there are detailed performance criteria that any analytical methods must meet. The laboratories generally follow the guidelines of the Commission Decision 2002/657/EC of 12 August 2002 (Commission Regulation, 2002), even though it has to be noted that the performance criteria of the methods used for official control may be inadequate for a TDS where high detection power (i.e., extremely low LODs) is required. The methods are usually based on GC MS/MS or HPLC MS/MS.

According to the European regulation, veterinary drugs can be specific to some animal species only (Commission Regulation, 2009). Consequently, pooling should be planned carefully with separation of products from different species to prevent dilution effects. Veterinary drugs that are commonly used for individual...
and isolated treatment cannot be studied in a TDS because of the dilution effect of pooling. On the contrary, the approach appears to be relevant for those used for mass treatment of animal populations. Nevertheless, usage of veterinary drugs can be different from region to region, and appears difficult to be determined accurately.

Several studies have shown an impact of cooking on veterinary drug residue levels in food products (Rose et al., 1999). The effect of cooking varies according to the physico-chemical properties of the substance. Some residues, such as the furazolidone metabolite 3-amino-2-oxazolidinone, are not impacted by cooking (McCracken and Kennedy, 1997; Steffenak et al., 1994), whereas for some lipophilic compounds with a relatively high octanol/water partition coefficient (Kow) the concentration can decrease with cooking due to lipid loss as for persistent organic pollutants (Bayen et al., 2005; Tittlemier et al., 2007b).

To conclude, the TDS approach is relevant only for residues of commonly used veterinary drugs, with the exception of those that would disappear with cooking, prohibited substances and substances for which MRLs are fixed to zero.

3.5. Naturally occurring toxicants

This group includes different classes of substances such as naturally occurring contaminants, i.e. mycotoxins, compounds directly produced by plants such as alkaloids, glucosides, or steroids, and substances such as phytosterogens that are difficult to be unequivocally classified as healthy or unhealthy. Naturally occurring food toxicants gather hundreds of molecules and only some significant groups are considered hereafter.

3.5.1. Mycotoxins

Mycotoxins (e.g. aflatoxins, ochratoxin A, patulin, fumonisins, deoxynivalenol, zearalenone, T-2, HT-2) are naturally occurring chemicals produced by certain molds which can grow on a variety of crops and are found in cereals, nuts, spices, dried fruits, apple juice, wine and coffee. Mycotoxins have already been considered in some TDSs, such as those of Czech Republic, France, Australia, Canada, Spain (ANSES, 2011b; FSANZ, 2011; Leblanc et al., 2005b; Tam et al., 2011; Urieta et al., 1996).

Most mycotoxins are toxic in very low concentrations and require sensitive and reliable methods for their detection. They have miscellaneous structures and thus different methods have to be used for their determination. Antibody-based immunoaffinity columns for cleanup have been intensively investigated since they offer high recoveries of the analyte (Turner et al., 2009). Rapid screening tests are available for detection of mycotoxins (e.g. commercial test kits based on immunoassays or enzyme-linked immunosorbent assays) but for accurate and quantitative determination HPLC or LC–MS/MS are commonly used. Multi-analysis of mycotoxins by LC–MS/MS, which allows determining different mycotoxins in many foodstuffs with the same extraction method, has also been set up in recent years and is going to be developed further. GC-based methods are not widely used due to the necessity of derivatization.

As long as food items from different origins (animal/vegetal) are not combined, there is no reason why pooling would dilute mycotoxin levels more than other environmental contaminants.

Most mycotoxins are chemically stable and tend to survive storage and processing, even when cooked at relatively high temperatures such as those reached during baking of bread or breakfast cereal production (Turner et al., 2009). In general, mycotoxins are very resistant to heat and other production processes (Alberts et al., 1990; Deshpande, 2002; El-Banna et al., 1983; Hazel and Patel, 2004; Jackson et al., 1997; Jackson and Bullerman, 1999; JECFA, 2001; Schollenberger et al., 2007; Schothorst and van Egmond, 2004; Scott et al., 1983; Scott, 1984; Scudamore et al., 2003).

It can thus be concluded that the TDS approach is appropriate for mycotoxins.

3.5.2. Phytosterogens

Phytosterogens are a group of non-steroidal polyphenolic plant metabolites that present close structure to 17ß-oestradiol. Phytoestrogens have been quantified in a large number of foods, including cereal-based products, milk, vegetables, pulses, fruits, and eggs (ANSES, 2011b; Boker et al., 2002; Kuhnle et al., 2009a). Nevertheless, the soy-based products appear to be the highest contributors, especially for isoflavone and lignan intake. Apart from TDS-like studies with analysis of phytosterogens in a limited number of food groups, such as fruits (Liggins et al., 2000a) or vegetables (Liggins et al., 2000b), to our knowledge, the French TDS was the only one that has included phytosterogens (ANSES, 2011b).

To detect phytosterogens in food matrices, multi-residue methods can be used such as LC–MS/MS with negative electrospray ionization. Nevertheless, a high variability of results for different samples of the same food may be found for some matrices. This can be due to interactions between phytosterogens and the matrix (in particular in plant products) leading to poor extraction reproducibility.

Except for soy-based products, phytosterogens are found in many foods and are not specific to a food item. Pooling would not highly dilute phytosterogens levels, if soy-based products are not pooled with other products (for instance soy-based beverage or “soy milk” with other beverages). However, environmental factors, varieties, harvesting and processing can impact the phytosterogens concentrations (Kuhnle et al., 2009b) and pooling can be done with caution to avoid dilution effect. Since vegetarians are likely to have higher than average consumption of soy-based products and grains, the TDS approach can be supplemented by targeted duplicate diet studies based on wit diets from vegetarians (Clarke et al., 2003).

Coward et al. (1998) showed that the total isoflavone concentration was not modified under normal cooking conditions (baking or frying at 190 °C). Other data suggested that isoflavone concentration could be reduced up to 30% by cooking in tofu (Grun et al., 2001) and by boiling and freezing in soybean seeds (Simonne et al., 2000). Some cooking methods could then affect the phytosterogens concentrations in foods, nevertheless, phytosterogens remain quantifiable after cooking.

To conclude, the TDS approach is appropriate for phytosterogens if care is taken in pooling food with special reference to soy-based products and when the analytical method has proven to be robust for the selected matrices.

3.5.3. Alkaloids

Alkaloids are a group of hundreds of chemical compounds containing one or more nitrogen atoms, usually in a heterocyclic ring. They can be produced by plants, bacteria, fungi and animals. Depending on the alkaloid, it can be found in numerous foods. The extraction and analytical method for alkaloid detection depends on the nature of the compound or family. For instance, HPLC–FLD and HPLC–MS/MS are the only two analytical methods allowing the unambiguous identification and quantification of the major ergot alkaloids in food and feed (EFSA, 2012b). Concerning pyrrolizidine alkaloids, only methods with MS detection provide the prerequisites to analyze them at trace levels in food (EFSA, 2011e).

Insofar as each alkaloid occurs in very specific plants, then foodstuffs or food groups, pooling will decrease concentrations significantly. For instance, EFSA suggested for opium alkaloids in poppy
seeds that mixing seeds from different origins could have a size-
able effect on the occurrence (EFSA, 2011f).

The alkaloid content may vary with the processing of food. For instance, it has been shown that the alkaloid concentration of poppy seed samples could be reduced by washing, soaking, and heat treatments using high temperatures (EFSA, 2011f). Moreover, most studies on baking/pancake preparation with ergot alkaloids contaminated flours or flour-based products showed a time dependent reduction of concentrations in food (EFSA, 2011e).

Mainly due to pooling effect that tends to decrease their concentrations, the TDS approach does not seem to be the best way to assess the exposure to most of these substances.

3.6. Contaminants formed during food processing

3.6.1. Polycyclic aromatic hydrocarbons

Exposure to polycyclic aromatic hydrocarbons (PAHs) occurs through many routes (including air inhalation, smoking, water, food, and skin contact), but for non-smokers the major route of exposure is the consumption of food, mainly from cereal products, seafood, vegetables, vegetable oils and traditionally smoked foods (EFSA, 2008b). PAHs have already been considered in some TDSs (ANSES, 2011a; Food Safety Authority of Ireland, 2011; FSA, 2002; FSANZ, 2008).

The two main sufficiently sensitive analytical techniques for determining PAHs in foods are HPLC-FLD and GC–MS.

Since food preparation (specifically smoking, frying, barbequing or grilling) can highly influence the PAH concentration in certain foods (e.g. fish and meat) (Abnet, 2007; Domingo, 2011; Perello et al., 2009), pooling of these food items prepared in different ways (e.g. boiled fish pooled with smoked fish) can influence the PAH concentration. It is advisable to analyze some prepared foods separately. In other cases, where the source of contamination by PAHs is likely to be environmental (e.g. cereals, seafood) then broader food groups could be pooled.

To conclude, the TDS approach is appropriate for PAHs if the necessary precautions are taken regarding the impact of preparation (culinary treatment).

3.6.2. Furan

Furan can be generated during food processing of heat-treating foods, such as canned foods and jarred foods, because they are in sealed containers receiving a high thermal load (EFSA, 2010c, 2011g). Therefore furan can be found in numerous types of foods. It is possible to analyze furan in different foodstuffs. There is no specific recommendation from the European Commission to analyze furan in monitoring programs, but EFSA recommends using static headspace extraction-GC–MS and head space/solid phase micro-extraction-GC–MS.

Furan can be found in different products and is not specific to one product. Nevertheless, furan is highly volatile and pooling tends to decrease levels.

Because of its volatility, furan concentrations are likely to decrease with domestic food preparation. In its 2010 report, EFSA reviewed different studies showing that levels of furan decrease with microwave heating or heating in a saucepan. Taking this point into account, re-heating (that could be a common domestic practice) will also probably reduce the concentrations. Serving foods into plates or serving coffee into cups and waiting before drinking also appeared to decrease furan concentrations (Goldmann et al., 2005; Kim et al., 2009; Zoller et al., 2007). Then, furan concentrations and human dietary exposure depend not only from the food consumed and the preparation methods that can be taken into account, but also from the consumption habits, the preparation methods and or the way the prepared product is consumed.

To conclude, the TDS approach does not seem appropriate for furan because of its volatility and in that pooling/homogenizing tends to decrease its concentration. Preparation and consumption habits are the main factors determining the concentration for furan (both the formation during heating but also the loss by volitization), and it appears difficult to recreate representative home conditions.

3.6.3. Acrylamide

Acrylamide is a contaminant that may be formed in foods, particularly plant-based foods rich in carbohydrate, during cooking, frying, baking or roasting, at temperatures of 120 °C or higher (FAO/WHO, 2002). Acrylamide can be found in several food items such as French fries, potato chips, biscuits, coffee (FAO/WHO, 2002; JECFA, 2011; Sirot et al., 2012a). Acrylamide has been analyzed as part of chemicals studied in several TDSs (Food Safety Authority of Ireland, 2011; FSA, 2005; Sirot et al., 2012a; USFDA, 2006).

Several analytical techniques are available for the analysis of acrylamide in food. The methods of choice are based on LC–MS/MS, also applied in validation studies (Wenzl et al., 2008). Other analytical techniques include GC with electron capture detection (GC/ECD), GC–MS and GC–MS/MS, LC with high resolution mass spectrometry (LC–HRMS), and diode array detection (Eberhart et al., 2005; Tekkeli et al., 2012; Wenzl et al., 2003; Zhang et al., 2006). Acrylamide is not significantly volatile. LC-based methods enable its determination as such, whereas GC based methods entail derivatization of acrylamide prior to further analysis.

As higher levels of acrylamide can be found in starchy food when submitted to heat-based treatments (i.e. >120 °C), a pooling effect resulting in acrylamide dilution likely results from food grouping. Further sub-grouping may be necessary in order to accurately estimate exposure. For instance, potato chips should be analyzed separately from baked/boiled/microwaved potatoes and potato products.

Acrylamide formation is affected by the heating process, heating duration and storage of raw materials. Home preparation and processing habits may significantly contribute to the uncertainties of a TDS approach. Consumers toast bread, roast and fry potatoes, etc., to different degrees depending on preference and the effect of acrylamide concentrations can be dramatic. Storage of potatoes below 6 °C is known to cause increased levels of reducing sugars and consequently of acrylamide (EFSA, 2011h). Moreover, the levels of acrylamide in roasted coffee and cocoa powder significantly decrease during storage (JECFA, 2011). Sample storage (especially when dealing with potatoes) should be carefully monitored before analysis.

Different preparation practices based on national and regional habits (e.g. American coffee or espresso) may also considerably affect acrylamide concentration (Sirot et al., 2012a). Another source of variability may come from the use of the raising agent ammonium bicarbonate that has been found to increase the potential for acrylamide formation due to the ammonium component. Samples baked with ammonium bicarbonate show higher acrylamide levels than samples baked with the sodium or potassium bicarbonates (Levine and Smith, 2005). This should be considered when reproducing domestic preparation.

The TDS approach is relevant for acrylamide, with the understanding that the preparation impact is very high and depends also on the type of food used (e.g. storage conditions, presence of ammonium bicarbonate) and that pooling of samples should be made with special attention.

3.6.4. 3-Monochloropropane-1,2-diol and related compounds

Chlorinated propanols, such as 3-monochloropropane-1,2-diol (3-MCPD), are formed when chloride ions react with triglycerides
in foods under a variety of conditions, including food processing, cooking, and storage. Relatively recently glycidol, a group 2A carcinogen, has been shown to be associated with 3-MCPD in foods. Esters of 2- and 3-MCPD and glycidol esters have been lately recognized as important contaminants of processed edible oils used as foods or food ingredients. Even though 3-MCPD in its free state is usually associated to acid-hydrolyzed vegetable proteins and soy sauce, it can be found in a great variety of foodstuffs (Baer et al., 2010). MCPD esters and glycidol esters typically occur in refined vegetable oils but can be found in cereals, bread, milk and milk products, frying oils and mixtures, animal fats and oils and composite processed foods.

Analytical methods for the determination of free MCPDs in potential food contributors are based on GC–MS and require the use of isotopically labeled standards. 3-MCPD can be formed or lost during the process of extraction if strict protocols are not followed. Determination of esters may be direct, by means of LC–MS, LC–MS/MS, LC–TOF MS, or indirect, after transesterification to free 3-MCPD and 2-MCPD, by means of GC–MS.

The effect of pooling may be substantial, since it may dilute the compounds to levels which are not analytically measurable. Refined oil containing MCPD esters is a common ingredient of infant formulae and infant follow-on formulae, thus exposure assessment of this vulnerable population might require a specific sampling and pooling scheme.

The effect of domestic cooking procedures on the level 3-MCPD has been addressed in a study entailing 23 foods, comprising stock cubes, gravies, a cake mix, batters, breads, cheese and meats, which were subjected to a range of cooking procedures including grilling, toasting and microwaving (Crews et al., 2001). Grilling and toasting produced substantial increases in the 3-MCPD content of bread and of most cheeses. Microwave cooking produced elevated 3-MCPD levels in some cheeses. Frying batters gave mixed results.

The remaining foods showed little or no discernible increase on cooking.

The TDS approach appears to be relevant for 3-MCPD and related compounds, even though only certain food groups contribute to exposure and pooling might require some adaptation for this group of substances. Cooking practices have also to be taken into account.

3.7. Contaminants transferred from food packaging or food contact materials

3.7.1. Melamine

According to the EFSA (EFSA, 2010b), melamine is found in many foods and is not specific to a food item.

Following the incidents of adulterated food and feed, several analytical methods for the determination of melamine in various matrices have been developed. As a result, reliable extraction and sample clean-up techniques are available for most food types including foods high in protein, fat and carbohydrates. The most sensitive and selective analytical method to measure melamine and its structural analogs is LC–MS/MS (EFSA, 2010b).

The main source of melamine in the diet is migration from can coatings with melamine-based resins (Bradley et al., 2011) and migration from melamine-formaldehyde plastics that are used in cups, plates and the like (Bradley et al., 2005, 2010). Melamine is a very stable molecule which is not affected by heat treatment. On the other hand, the migration process is strongly dependent on the contact conditions used and it is necessary to know the consumers’ practices regarding the household use of melamine-formaldehyde plastics to be able to mimic them in the laboratory.

To conclude, the TDS approach does seem appropriate for melamine, when it is feasible to take into account the different types of food packaging in sampling and the cooking practices (use of melamine utensils).

3.7.2. Mineral oil hydrocarbons

Mineral oil hydrocarbons (MOH), or mineral oil products, are hydrocarbons containing 10 to about 50 carbon atoms. MOH have been divided into two main types, mineral oil saturated hydrocarbons (MOSH) and mineral oil aromatic hydrocarbons (MOAH). MOSH are present at different levels in nearly all foods.

Currently, the most efficient methods for analysis of MOSH and MOAH in food and feed comprise extraction followed by pre-separation by HPLC coupled to GC with FID. Comprehensive GCxGC-FID enables a rough separation and quantification of paraffins and naphthenes in the MOSH fraction, but it is of limited practicality for routine analysis. Contamination with polyolefin oligomeric saturated hydrocarbons (POSH), e.g. from plastic bags, heat sealable layers or adhesives, may interfere with MOSH analysis. Analytical capacity to distinguish the different MOAH subclasses in food is limited. For this purpose, GCxGC appears to be the most effective method (EFSA, 2012c). Given the complexity of the mixtures involved, the analytical results have a higher than normal degree of uncertainty which is mainly due by the fact that it is not possible to resolve MOH mixtures into individual components for quantification. However, methods based on gas chromatography (GC) are reliable to quantify the concentration of total MOSH and MOAH fractions.

According to the EFSA opinion (EFSA, 2012c), MOH is found in many foods and is not specific to a food item. So there is no reason for which pooling would highly dilute MOH levels.

The effect of home preparation on the MOSH concentration in foods will depend on the source of the contamination and the type of food affected. MOH are lipophilic substances that are not especially volatile and they are rather stable. In that regard, if they arise as environmental contaminants then they are analogous to the persistent organic pollutants (POPs) and they will tend to follow the fat phase during cooking etc., as described in earlier sections. On the other hand, if they originate by migration from polluted paperboard, they can affect foods that do not have a high fat content and their behavior during cooking can be unpredictable.

To conclude, the TDS approach is appropriate for MOH if the necessary precautions are taken regarding culinary treatment.

3.7.3. Bisphenol A

Diet is thought to be the main source of bisphenol A (BPA) exposure, although recent studies suggest that also non-food sources could be of importance (Geens et al., 2011). The presence of BPA has been observed in canned food, including a large variety of foods including drinks, fruits, and vegetables (FAO/WHO, 2011a).

According to the FAO/WHO joint expert meeting report, the most reliable methods for BPA are MS-based, for example GC–MS or GC–MS/MS (FAO/WHO, 2011b).

If samples of drinks or foods packed in different packaging materials (e.g. canned drinks, drinks in PET bottles, drinks in glass bottles) are pooled, it is likely that the concentration of BPA turns out to be very low since BPA is mainly found in canned foods and drinks (Geens et al., 2010; Sungur et al., 2014). The other main source of exposure is by migration from polycarbonate plastics as used in food processing equipment and in household items such as cups, plates and especially baby bottles for milk, formula and juices (Ehlert et al., 2008; Kubwabo et al., 2009). The later application for baby bottles has been banned in Europe since 2011.

Goodson (Goodson et al., 2004) concluded that there were no appreciable differences in the BPA level before and after cooking or heating. On the other hand, the migration process is strongly dependent on the contact conditions used.
To conclude, the TDS approach does seem appropriate for BPA, when it is feasible to take into account the different types of food packaging in sampling.

3.7.4. Phthalates

Phthalates have been detected in numerous foods, i.e. bread, cereals, oils and fats, nuts, meat products, poultry, fish and fish products, cheeses, frozen fries, vegetables, fruits, sugar, candy, infant food and formulae (COT-UK, 2011; Fierens et al., 2012a). The share of food versus other sources of exposure in the total intake of phthalates is highly dependent on the congener. Wormuth (Wormuth et al., 2006) estimated that food is the most important source of phthalates for DEHP, DiBP, DnBP (all age/consumer groups), BBP (children and adults) and DIDP (teens and adults).

Detection by GC–MS or LC–MS/MS is preferred (COT-UK, 2011). Phthalates can be ubiquitous in the laboratory environment and can be especially problematic in the food preparation area of a TDS.

Phthalates are found in many foods and are not specific to a food item. So there is no reason for which pooling would highly dilute phthalate levels.

As aromatic esters, phthalates are rather stable to heat and hydrolysis and consequently they are moderately persistent. The limited information about the preparation impact shows that processing food at home generally declined phthalate concentrations (Fierens et al., 2012b; Ishida, 1993). The main loss mechanism is via fat loss during cooking. Therefore, it is worth to pay attention to the preparation methods usually applied at home.

To conclude, the TDS approach is appropriate for phthalates, providing that scrupulous efforts are directed at minimizing background contamination during homogenization and pooling.

3.8. Other classes

3.8.1. Radionuclides

Background levels of radionuclides in foods vary and are dependent on several factors, including the type of food and the geographic region where the food has been produced. The concentration of natural radionuclides varies because of differences in background levels related to soil, climate and agricultural conditions (UNESAR, 2010). Common radionuclides in food are $^{40}$K, $^{226}$Ra along with the series of uranium, thorium and their associated progeny ($^{238}$U, $^{234}$U, $^{230}$Th, $^{226}$Ra, $^{210}$Pb, $^{210}$Po, $^{223}$Ra, $^{222}$Th, $^{228}$Th).

In case of nuclear leaks radioisotopes generated in nuclear installations (e.g. $^{89}$Sr, $^{90}$Sr, $^{103}$Ru, $^{106}$Ru, $^{131}$I, $^{129}$I, $^{232}$U, $^{235}$U, $^{239}$Pu, $^{240}$Pu, $^{134}$Cs, $^{137}$Cs, $^{103}$Ce) can enter and spread in the food chain and then be found in a significant part of the diet. Radionuclides have been considered in a limited number of TDSs (FSA, 2004; Health Canada, 2009; Sugiyama et al., 2008; USFDA, 2008).

Qualitative and quantitative information on radionuclides can be obtained by alpha or gamma spectroscopy and total beta counting (Anderson and Cunningham, 2005; Jha et al., 2012). Instrumental neutron activation analysis and, in certain cases, ICP-MS can also be used.

In general terms, radionuclides are not specific for a product so that pooling of samples in food groups would not highly dilute radionuclide levels. Long storage and processing times will reduce the activity contents of short-lived radionuclides in foodstuffs (Green, 2001; IAEA, 2010) such as $^{131}$I. Therefore radioactive decay has to be kept in mind in planning TDSs dealing with short-lived radionuclides.

Data on the behavior of radionuclides during food preparation are scarce with the exceptions of radioisotopes of caesium, strontium and iodine. It is generally believed that normal practices used in the preparation, cooking or processing of food can significantly reduce radionuclide concentration in food; the extent of this reduction depends on the radionuclide, the type of foodstuff and the method of processing (Green, 2001; Long et al., 1995; Noordijk and Quinault, 1992). Apart from reduction by radioactive decay, the fate of radionuclides depends entirely on their chemical characteristics and so useful parallels can be drawn from other trace elements. Studies usually show that preparation and cooking of food reduces the concentration of radionuclides (Bengtsson, 1992; Green and Wilkins, 1995; Green, 2001; Lotfi et al., 1990a, 1990b).

The TDS approach is relevant for radionuclides following release of radioactivity in the environment (e.g. nuclear leaks) or in naturally highly contaminated areas. Radioactive decay has to be kept in mind in planning TDSs dealing with radionuclides with (relatively) short half-lives.

3.8.2. Nanomaterials

Nanomaterials are natural, incidental or manufactured nano-objects with one or more external dimensions in the size range 1–100 nm approximately (the term nanoparticle is used when the three external dimension are in the nanoscale). Manufactured nano-sized materials are increasingly used in a variety of consumer products and their use in food applications is expected to increase as well. In addition, some approved food additives have been found to contain a nano-sized fraction and are extremely relevant for exposure assessment. Nanomaterials have never been investigated in a TDS.

The TDS approach is currently not appropriate for nanomaterials because of the absence of validated analytical methods capable of detecting nano-sized particles in such complex matrices as food (Linsinger et al., 2013). However, it must be noted that progress has been made in the last years and new analytical techniques have been developed such as single particle ICP-MS and asymmetrical flow field flow fractionation, either coupled with optical detectors and/or ICP-MS (AF4-ICP-MS), which now complement established imaging techniques based on electron microscopy (Calzolai et al., 2012). European collaborative research projects aiming at developing analytical methods for detection and characterization of nanomaterials in food and interlaboratory collaborative trials are ongoing.

3.8.3. Nitrosamines

Nitrosamines are chemical substances produced from nitrites and secondary amines. They can be ingested through foods (e.g. beer, smoked fish, processed meat) but also endogenously formed in the acidic conditions of human stomach by nitrosation of the amine groups of certain proteins. Nitrosamines have been studied by the Canadian TDS (Health Canada, 2009) and are planned to be analyzed in the current French infant TDS.

N-nitrosamines can be analyzed by GC–MS after having been isolated by a steam distillation method (Li et al., 2012). Capillary GC-thermal energy analysis has also been used (Xu et al., 1991). In order to determine nitrosamines in drinking water, the US-EPA recommends using solid phase extraction and capillary column GC with large volume injection and chemical ionization tandem MS (US-EPA, 2004).

Nitrosamines can be found in different foods (Health Canada, 2009; Jakzyn et al., 2004; Tricker and Preussmann, 1991; Webb and Gough, 1980) and are not specific for a limited number of food items. Pooling would not highly dilute nitrosamines levels.

Frying has been shown to increase the content of nitrosamines in some foods (Li et al., 2012). Moreover, microwave cooking has been shown to significantly lower levels of volatile nitrosamines (Österdahl and Alriksson, 1990). Therefore, nitrosamines concentrations in foods highly depend on the preparation and cooking methods. Careful attention for the cooking methods is therefore needed to reflect consumer exposure.
To conclude, the TDS approach is appropriate for nitrosamines if the necessary precautions are taken regarding the cooking impact. The summary of the assessments of the relevance of the TDS approach for all the substances so far discussed is presented in Table 2.

### Table 2

<table>
<thead>
<tr>
<th>Substance group</th>
<th>Substances</th>
<th>Relevance of the TDS approach</th>
<th>Special attention or reason of exclusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nutrients</td>
<td>Nutrients</td>
<td>Yes</td>
<td>–</td>
</tr>
<tr>
<td>Environmental chemicals</td>
<td>Trace elements</td>
<td>Yes</td>
<td>Preparation impact (culinary treatment)</td>
</tr>
<tr>
<td></td>
<td>Dioxins, PCDFs, PCBs, brominated and perfluorinated compounds</td>
<td>Yes</td>
<td>Preparation impact (culinary treatment)</td>
</tr>
<tr>
<td>Chemical substances intentionally added to foods</td>
<td>Foods additives</td>
<td>Yes</td>
<td>Only for additives commonly used in a large number of foods or ever used in some products for technological reasons</td>
</tr>
<tr>
<td>Chemical residues of substances being deliberately applied at other points in the food production chain</td>
<td>Flavorings</td>
<td>No</td>
<td>Volatility</td>
</tr>
<tr>
<td></td>
<td>Pesticide residues</td>
<td>Yes</td>
<td>Pooling impact</td>
</tr>
<tr>
<td>Contaminants formed during food processing</td>
<td>PAHs</td>
<td>Yes</td>
<td>Preparation impact (culinary treatment)</td>
</tr>
<tr>
<td></td>
<td>Furan</td>
<td>No</td>
<td>Volatility due to the preparation impact</td>
</tr>
<tr>
<td></td>
<td>Acrylamide</td>
<td>Yes</td>
<td>Preparation (culinary treatment) and pooling impact</td>
</tr>
<tr>
<td></td>
<td>3-MCPD and related compounds</td>
<td>Yes</td>
<td>Pooling impact</td>
</tr>
<tr>
<td>Naturally occurring contaminants</td>
<td>Mycotoxins</td>
<td>Yes</td>
<td>Pooling impact: avoid pooling of soy-based products with other products</td>
</tr>
<tr>
<td></td>
<td>Phytoestrogens</td>
<td>Yes</td>
<td></td>
</tr>
<tr>
<td>Contaminants transferred from food packaging or food contact materials</td>
<td>Alkaldoids</td>
<td>No</td>
<td>Volatility impact (culinary treatment)</td>
</tr>
<tr>
<td></td>
<td>Melamine, MOSH, bisphenol A, phthalates</td>
<td>Yes</td>
<td>Preparation impact (culinary treatment) (phthalates, MOSH, melamine)</td>
</tr>
<tr>
<td>Others</td>
<td>Radioionucleides</td>
<td>Yes</td>
<td>Radioactive decay (in planning TDS for short-lived radionucleides)</td>
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<tr>
<td></td>
<td>Nanoparticles</td>
<td>No</td>
<td>No validated analytical method</td>
</tr>
<tr>
<td></td>
<td>Nitrosamines</td>
<td>Yes</td>
<td>Cooking impact</td>
</tr>
</tbody>
</table>

To conclude, the TDS approach is appropriate for nitrosamines if the necessary precautions are taken regarding the cooking impact.

The summary of the assessments of the relevance of the TDS approach for all the substances so far discussed is presented in Table 2.

### 4. Conclusions

A list of substances for which the TDS approach is applicable has been established on the basis of relevant scientific literature and expert judgment according to the availability of analytical methods and according to the three essential principles of a TDS (EFSA, 2011a), i.e. (i) representativeness of the whole diet, (ii) pooling of foods, and (iii) food analyzed as consumed. The availability of a health-based guidance value was discussed, but it was not used here as a selection criterion. Health-based guidance values are used to characterize risks, which is of course an important outcome of a TDS but it is not the only objective. A TDS provides occurrence and exposure data that can be compared with international data or assessed as trends even if no validated health-based guidance value for the substance under study is available.

For most of the substances dealt with in the present work, the approach was considered relevant. This list includes nutrients, environmental contaminants, some food additives, pesticide and veterinary drug residues, some contaminants formed during food processing, some naturally occurring contaminants, contaminants transferred from food packaging or food materials, radionuclides and nitrosamines. Nevertheless, the conclusions are drawn for each general group, and the inclusion in a TDS of a specific substance has to be analyzed case by case considering the properties of the substance and taking into account the national situation. Moreover, for almost all those substance groups, even if the approach is relevant, special attention has to be paid to the impact of preparation and/or pooling, when establishing a sampling plan for a TDS.

### Conflict of Interest

The authors declare that there are no conflicts of interest.

### Transparency Document

The Transparency document associated with this article can be found in the online version.

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