PROJECT FINAL REPORT

Grant Agreement number: 289108

Project acronym: TDSEXPOSURE

Project title: Total Diet Study Exposure

Funding Scheme: Large collaborative project

Periodic report: **1st** ☐ **2nd** ☐ **3rd** ☑ **4th** ☐

Period covered: from February 2012 to January 2016

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4.1 Final publishable summary report

Executive Summary

The aim of total diet studies (TDS) is to provide realistic data on food contamination and chronic exposure levels to chemicals of relevant populations. The TDS approach limits uncertainties in exposure assessment through improved sampling methods and chemical analysis of foods 'as consumed'. Although several countries have already implemented a TDS, results are not directly comparable because the methods used including data treatment are not the same. The objective of the TDS-Exposure project was to develop a common methodology and to promote TDS in Europe, through the creation of a network of TDS centres and the implementation of pilot studies.
26 beneficiaries from 19 different countries have contributed to the 11 work packages of the project. The project has progressed according to the working plan, and no less than 72 deliverables and 35 milestones have been produced during the 4 years of the project. Populations and substances of interest have been identified and a method to prioritise target substances has been developed. Literature reviews and data analyses of food lists have been completed. A food products database, a protocol for food products collection, and a tool to formulate specific food shopping lists, have also been produced. A report has been written about the effects of cooking methods on target substance concentrations, and recommendations for food sample preparation as well as the best means to capture consumer behaviours have been produced. A glossary defining the vocabulary used in TDS has been created and updated regularly. A TDS centres' network, composed of 20 participants from 11 countries, which participated in TDS-Exposure, and 27 other participants from 19 countries, has been built. Guidelines for implementation of a total quality management system (TQMS) have been produced, and flowcharts and standard operating procedures (SOPs) for TDS have been described. A SWOT analysis (strengths, weaknesses, opportunities, and threats) of food classification and description systems was undertaken to complete the work around quality management. A Wiki describing analytical methods for all target substances has also been developed. The existing food data management system FoodCASE was extended to support the process of a TDS so that TDS data can be managed in the same database as food composition and consumption data. Differences in food consumption pattern amongst populations were examined with a focus on pregnant women. Further magnitude and source of variation in concentration data, e.g. seasonal variations were considered. Uncertainties of TDS data were described and a decision-tree was developed to benefit from combination of Food monitoring and TDS data. In order to harmonize exposure assessment, beneficiaries have been trained to use the Monte Carlo Risk Assessment (MCRA) software, which was adapted for TDS data. Data coming from the different partners was organised to fit the requirements of the system and exposures re-calculated for these partners. A report about food grouping and food classification issues, as well as the influence of sources of variations in relation to TDS, has been published. Based on this, two new functionalities were embedded in the MCRA software; the uncertainty analyses based on summary data combining TDS results with the variation observed in European monitoring programmes and the MCRA TDS risk management tool. Fieldwork for pilot studies organised in five countries (Portugal, Czech Republic, Germany, Iceland and Finland) was achieved, and collection of samples and analyses completed. Reports on the construction of food lists and the development of standardised protocols for sampling, cooking and analysing food in these countries have been distributed. Three summer schools were hosted in Lisbon (Portugal) in 2013, Istanbul (Turkey) in 2014, and Helsinki (Finland) in 2015, respectively, providing a detailed overview of TDS for the delegates. These events included more than 27 lectures given by experts from TDS-Exposure as well as international organisations (e.g. FAO and EFSA). In total, 59 people participated including eight external participants from Cyprus, Ukraine, Italy, Turkey, Finland and Denmark. Five workshops were delivered and eleven individual training exchanges were organised. The website was updated regularly by partners, and four newsletters have been circulated to more than 3000 stakeholders. Stakeholders' workshops were organised in February 2014 and October 2015, and assembled 121 participants in total (41 from partners and 80 external stakeholders, including academia, governments, food and beverages industry and NGO) from Europe as well as the USA, Canada and Columbia. More than 600 flyers about the project have been distributed, and a

twitter account has been created. The immediate impact of TDS-Exposure has been the creation of an information system for TDS and exposure assessment software for food contaminants, which will be useful for risk assessors and risk managers, but also the dissemination of common methodology and quality tools.

Summary description of project context and objectives

Exposure assessment is one of the four steps in food **risk assessment**, which includes: hazard identification, hazard characterisation, exposure assessment and risk characterisation. Exposure assessment in the field of food safety relies on estimating the intake of a substance or a microorganism by the population in a given period of time. It is usually estimated by multiplying food intake data by food contamination data in the deterministic approach or combining probability distributions (food consumption, food concentration corrected or not by uncertainty etc...).

Exposure assessment might be different from one country to another because of the differences in food consumption habits or in food contamination. Furthermore there are significant differences in how consumption and concentration data are generated in various countries as well as differences in how the exposure assessments are performed. Food concentration data produced by food control laboratories can be biased because of targeted sampling changes in approach from deterministic towards probabilistic where appropriate, and different reasons and different potential to collect data and perform exposure assessment. Hence, there is the need to develop a more unified approach to exposure assessments across countries within the EU.

EFSA is responsible for coordinating exposure assessment at the EU level and the necessity to develop accurate exposure assessments in different EU countries is fully recognised.

The aim of **Total Diet Studies** (TDS) is to provide representative and realistic data on the food contamination and the exposure levels of relevant populations. Furthermore, TDS limits uncertainties in exposure assessment through improved sampling methods and by analysing food as consumed.

In order to cover the whole diet with limited costs, TDS uses food sample pooling to limit numbers of foods or food groups to around 200. In dietary surveys, more than 1000 different foods are usually identified and in some countries more than 5000. It is not possible to analyse all these foods separately because costs are prohibitive. Pooling means different foods from the same food group are mixed to create a unique sample. The quantities of the different foods mixed together are related to intake. This pooling method means acute exposure assessment cannot be performed using TDS data without auxiliary information. This is because pooling reduces variation amongst foods and individual contributions cannot be identified. There is no record of food contamination variability because of sample pooling, and acute exposure needs to take into account food contamination variability because the highest values are explained directly by the greatest food contamination. Hence, the main aim of TDS is chronic exposure assessment rather than acute exposure. However, the utility of TDS is very high because chronic exposure assessment has far more relevance. Many more substances are toxic at low dose in the long term compared to the risk of acute exposure.

The second issue is related to the representation of the variability in food exposure. Several intake profiles need to be built to capture the diets of subpopulations. Core foods are chosen depending on the different sub-population to ensure TDS are representative.

Another main interest of TDS is the harmonisation of the methods and their representativeness. TDS methodologies allow assessment of time and country trends because of this harmonisation. However, there are still differences in TDS methodologies amongst countries, which limit not only in the essential comparison of exposure to contaminants between countries for public health purposes but also for research.

In a TDS, foods are analysed after being prepared as usual for consumption. Thus, they might reveal some chemicals, such as nitrosamine or products of Maillard reactions, which are formed during food preparation. On the other hand, foods might not contain certain chemicals originally present in the raw ingredients, which are destroyed during heating or removed during washing and peeling. Thus, the chemicals in the foods analysed in a total diet study are more closely representative of what is

actually ingested on average by consumers rather than what is produced (raw agricultural commodities) or purchased at the retail level.
Unlike most surveillance samples, TDS samples are usually analysed for many different chemicals, which is more cost-effective. This has the additional benefit of facilitating risk-benefit analysis for different chemicals, such as polychlorinated biphenyls, mercury and omega-3 fatty acids in fish and could be also used for studying of exposure to chemical mixtures.

Objective 1: To identify the needs of exposure information that can be fulfilled by TDS

The first objective of TDS-Exposure project was to identify clearly what kind of information TDS studies can provide for exposure and risk assessment. In the past 50 years, TDS have been implemented in different countries. The first TDS was launched in 1961 in the USA and now TDS are used in all parts of the world. For instance, several countries including China, Taiwan, South Korea, Malaysia, Japan, Australia, New Zealand, Cameroon, Lebanon, Canada, United Kingdom, France, Italy, and the Czech Republic have organised TDS in recent years. Traditionally, TDS were used mainly to assess exposure to environmental contaminants such as heavy metals or persistent organic pollutants and nutrients. However, risk assessors also need exposure information for a broad range of substances including migrants from packaging material (e.g. bisphenol A), pesticide residues and substances produced during cooking (e.g. acrylamide or furan). It was necessary to characterise the interest and the limitations of TDS to assess exposure to all of these substances.

Objective 2: Development of TDS in Europe

The second objective of TDS-Exposure was to encourage the development of TDS across Europe and worldwide in order to improve the quality of chemical risk assessments and test the implementation in countries without any TDS. Today, the number of European countries with a TDS is still relatively low. United Kingdom, France, Italy, Spain (region Catalonia) and the Czech Republic are the main countries with TDS. Belgium and the Netherlands reported TDS-like approaches on an ad hoc basis. These studies have provided a lot of exposure information and time trend analysis over the last 20 years. There was a need to encourage other European countries to launch TDS and identify determinants of exposure at the international level as well as means to reduce these exposures, if necessary. In order to implement TDS in Europe there was a need to develop training opportunities such as summer schools for young researchers. Online training could also be developed.
There is also a need to provide assistance to countries without any TDS to implement such studies.
Depending on the substances considered and the level of pooling, the cost of TDS can vary widely but are often more than two million Euros. Therefore, it was not possible for this consortium to develop TDS for all Member States. However, it was possible to implement small-scale TDS limited to specific contaminants in several countries. This small-scale implementation was the best way to determine the fieldwork, and identify and resolve difficulties that could arise in Member States and other European countries.

Objective 3: Harmonisation of TDS

The third objective of TDS-Exposure was to propose a harmonised method for TDS across Europe, test the method and develop a total quality management system (TQMS). The methodologies used in exposure assessment often differ from one country to another for different reasons. Firstly, exposure assessment can differ because different methods are used for related dietary surveys. For instance, 95^{th} or 97.5^{th} percentiles of exposure depend on the number of days of the survey. Secondly, exposure assessment based on TDS can differ from one country to another because different methodologies for sampling and analysing foods are used to estimate food contamination. The lack

of representativeness of the food products collection in reproducing the whole diet can seriously impair the reliability of the results. This means it is crucial to harmonise these two steps. Sampling strategies for food analysis need to use up-to-date information on foods in the market. TDS are based on analysis of food 'as consumed'. This is the reason why there is a need for information on how the foods are stored (e.g. packaging material) and prepared at home or in restaurants in order to be representative of population-wide food habits. The chemical analysis step is also crucial because different analytical methods and their limits of determinations can lead to different results.
Finally, the exposure assessment methodologies and formats needs to be harmonised. Amongst the main issues raised by EFSA were firstly the type of dietary survey used and secondly the statistical models applied to estimate long-term exposure based on short-term dietary surveys. Usually dietary surveys last between one day and seven days when the chronic exposure assessment need to consider months or years of intake. Statistical methods exist to estimate the longer-term intake based on a limited number of survey days, minimally two. There was a need to harmonise these food consumption study methodologies in order to be able to fully compare exposure assessments based on TDS. The most important specifications of TDS studies have been described through the development and implementation of a quality standard framework for TDS centres in Europe. The objective in TDS-Exposure was to develop a TQMS for TDS in Europe, addressing issues including personnel, strategy, design and resources, planning, sampling, sample preparation (including cooking), sample analysis, calculation of exposure doses, risk characterization data evaluation and exchange, and the dissemination and use of results, in order to improve the data quality and comparability amongst countries. Generic flowcharts and SOPs have been developed for every essential TDS process and critical step.

Objective 4: To make a European TDS database available for risk assessors and risk managers

The fourth objective of TDS-Exposure was to build and test a European database of TDS studies useful for risk assessors and risk managers such as EFSA and DG SANCO. Existing TDS results are today published in reports or scientific papers that provide only aggregate results. Risk assessors and risk managers are often not able to access fully the disaggregated TDS results necessary for exposure and risk assessment. The aim of TDS-Exposure was to make available these data via a European database for exposure assessment. Producers and owners of TDS results participated in this project: IFR and FERA for the United Kingdom, Anses for France, NIPH/SZU for the Czech Republic and ISS and INRAN for Italy. This provided an opportunity to build a database, which can be updated in the future when new TDS results become available. Outside EFSA and DG SANCO, researchers will have access to results from this database for their own research activity. Access will not be at the most disaggregated level but at a level enabling easy interpretation of the data.

Objective 5: Estimation of dietary intake of chemical substances with TDS

The fifth objective of TDS-Exposure was to develop or adapt existing exposure assessment models to TDS studies and to assess uncertainties. Different simple or more complex models can be applied to assess exposure by using TDS results. The main aim of these models is to estimate variability of exposure between individuals and uncertainties in order to protect the whole population. Exposure varies according to different factors such as age and sex, but also between dietary patterns, countries and regions. Between individuals variability can be estimated through statistics such as high percentiles (95^{th}, 97.5^{th} and 99^{th} percentiles). One constraint in TDS is that foods are pooled before analysis. For this reason, only mean contamination of foods can be estimated directly from the food sample analysis. Usually, a simple deterministic model is used to estimate chronic exposure by

combining food consumption data at the individual level with mean contamination data. Some research has been done regarding the estimation of uncertainties and variability in TDS. Because the sampling and the pooling procedure are known, it is possible to assess uncertainties and to estimate variability in contamination before the pooling of foods.

Description of main S & T results/foregrounds

The main outcomes of the technical WPs are presented below.

WP2 Choice of substances of interest and populations of consumers

Task 2.1. Choice of populations of interest

It was decided to select both sensitive (not because of different eating habits but because they have a specific sensitivity to chemical substances) and more exposed populations (more exposed to one or more chemicals than the general population because of different eating habits for geographical, ethnic/ ethical or medical reasons or because of a highly polluted environment). Four different population groups were identified and sub-groups described: Age – gender groups: sensitive populations stratified by age or gender (infants, pregnant women, elderly…) / Diet type groups: populations having a different diet because of ethical (e.g. vegetarians), religious, ethnic or geographical reasons, or because of specific needs not linked to a disease (e.g. sport) / Geographical, professional or socio-economic status: populations having a different diet or living in a polluted environment (people leaving in coastal areas, contaminated areas…) / Disease or health related groups: populations having a different diet for medical reasons (diabetics, people suffering from hypercholesterolemia…). For each sub-group, a list of substances - for which the sub-group of population is relevant - was drawn up. Specific foods related to these targeted populations were identified to be included in the food baskets, corresponding to foods not consumed or consumed sparingly by the general population, and not selected in the first TDS food list.

Task 2.2. Relevance of TDS approach by type of substance

Firstly, it was decided to have a general list of substances for which the TDS approach should be applied. To do this, different data sources were used: EFSA opinions, JECFA monographies, the International Food Safety Authorities Network (INFOSAN), the Codex Alimentarius and the European rapid alert system for food and feed (RASFF). Four main criteria were defined by the partners to check the relevance of substances in a TDS, with regard to three essential principles of a TDS: representative of the whole diet, pooling of foods, and food analysed 'as consumed'. The objective was to check whether these principles would not exclude or limit evaluation of a substance or family. The four criteria selected were: (i) substances present in a significant part of the whole diet, (ii) an analytical method exists to analyse the substance in all potential food contributors, (iii) there is no or limited impact of pooling on the concentration levels, and (iv) impact of 'as consumed' preparation of food samples can be controlled (ability to reproduce the home-made preparations). This task allowed identification of substances that can be included in a TDS.

Task 2.3. Prioritization of substances: criteria and strategy of selection

The analytical hierarchy process (AHP) method was selected to prioritise substances. This method includes six major steps:

1. Identifying priority problems and targets;
2. Identifying the criteria to be used to compare the various actions;
3. Defining the relative weights for criteria;
4. Making list of alternatives (substances) among which we want to make a prioritization;
5. Evaluating the importance of each alternative (substance) for each criteria;
6. Aggregation of all judgments.

The main criteria and sub-criteria for the prioritisation were identified and four criteria ("level 1") and six sub-criteria ("level 2") were retained. The criteria and sub-criteria were:

1. The "Concern" criterion grouped together different notions such as the health concern, the availability of data on harmful effects in human or animal, and the population, consumer or media concern.

a. Health concern: considered sufficiency of evidence for harmful effects of a substance, based on data on human or animal, but there was no prioritisation of the effects (neurotoxicity, carcinogenicity, hepatotoxicity…);

b. Consumer concern: considered the apprehension or interest of the consumer, population in general and/ or the media, regarding the substance.

2. "Analysis" dealt mainly with the analytical performances of the existing method to analyse the substance, i.e. the analytical limits of the method, and its ability to analyse the metabolites or speciation forms of the substance, if any.

a. Analytical limits: corresponded to the level of left-censorship expected when analysing the substances in the theoretical contributing diet, i.e. the ability to quantify the substances in numerous foods;

b. Speciation forms/metabolites: evaluated the ability of the method to analyse the speciation forms/metabolites, if any, in all foods or in part of the diet

3. "Exposure" dealt with the availability of a health-based guidance value (HBGV) and the availability of previous national or foreign exposure data.

a. HBGV: considered the existence of a reference value and its robustness (based on epidemiology, on data in human or animal…);

b. Exposure refinement: took into account the results of previous studies on exposure assessment (TDS or other type of exposure evaluations), i.e. the availability of exposure data at national or international level, and their conclusion in terms of risk, if any;

4. "Sampling" took into account the theoretical or known distribution of the substance concentration at the geographical or temporal scale, and the possible dilution effect due to the pooling of samples from different geographical or temporal origins.

The relative weight for each criterion was then established on the basis of expert judgement and each substance was allocated a score for each criteria. A final score was then calculated for each substance, which led to a list of priority substances.

WP3 Food sampling: food products collection

Task 3.1 "Collection of ancillary information and data analysis"

The activity aimed to search past (literature) and currently used food lists (EFSA's Comprehensive Database was selected). The purpose was to identify methodologies utilised to implement food product collection in TDS according to the necessary activities, relevant characteristics of past TDS food lists and current food consumption categorisation. A database of food lists by country and age-based population groups, derived using the EFSA comprehensive database, was produced.

Task 3.2 Protocol development

A summary of TDS activities related to the formulation of food shopping list has been produced. The need for a general core-food list, consisting of a list of food categories describing the composition of the universe of foods, was identified. The three identified conceptual steps are 1) characterisation of the food list, 2) formulation of the food shopping list, 3) collection of food products. Each step requires documentation, procedures and tools to be implemented and to obtain outputs for the subsequent one.

Characterizing the TDS food list consists of identifying core foods and risk foods categories to obtain a representative set of composite samples describing a whole reference diet, i.e., the average daily per-capita food intakes; the selection can start from either food consumption data (bottom-up approach) or a pre-harmonised food list (top-down approach) such as the EFSA FoodEx classification system. The selection of core food is related to the target substances and the population to survey taking into account all the relevant information about the country/region where the TDS is to be carried out (geographical strata, seasonality, food consumption data sources, retailing system, recipes, special conditions for sampling, etc.).

The formulation of the food shopping is the result of cross-checks between the selected TDS food list and parameters representing the reference diet. Where, when, which food products should be addressed in building the food shopping list. “Where” means in which geographical area and in which shops; “when” means in which season and on what dates; “which” means a list of either branded or fresh foods to be collected, national or local, and the food categories to include being representative within different food groups (using trade statistics and/or market share data). Retailing systems and purchase habits, as information about food production/food consumption seasonality and other periods of the calendar (e.g., Christmas Eve, Eastern time, etc.), such as information on the food market as market share or trade statistics are to be included in the background “documentation” together with the output from the previous step, i.e., the TDS food list. The sample of shops and the sampling within shops are defined during this step. Specific sampling plans (e.g. for tap water) are also defined.

Food products collection food products are purchased from the shops placed in selected areas according to the established calendar. The amounts of foods to be purchased are defined based on the proportion in the diet, need for subsequent chemical analysis, and numbers of home-treatments necessary to achieve ‘as consumed’ (different eating habits raw vs. cooked and cooking methods for dishes). Procedures for backup, in case of failure or unexpected situations are also defined. A general protocol to be applied in different situations (e.g. food intake data available/ not available; official statistics available/ not available; different relevant parameters by country; population target; substances target) was defined. This tool will provide foods representing the initial set for collection (purchase/ collection of food products belonging to each of the selected food categories) and build the diet in the laboratory (food samples preparation and analysis). Recording and in-putting relevant information about purchased food will create the food shopping list databases that can be used for enhancing the interpretation of the results and understanding differences between studies. The information set can be coded and structured to be used in other tools used in the present projects (e.g., FoodCASE, MCRA).

WP4 Food preparation, composite formation, and chemical analysis

Task 4.1. Food Preparation

A critical review of the extensive literature about the effects of cooking methods on food contaminant concentrations, focussing on the priority contaminants selected in (WP2) but also including all contaminant classes was carried out. Food packaging and food processing - most importantly cooking (i.e. frying, grilling, microwaving, boiling and roasting) - has a significant impact on some contaminant levels. For packaging chemicals (including other food contact articles such as cookware) and for heat-induced contaminants, the link is direct and obvious since packaging (e.g. aluminium, bisphenol A, MOSH) or cooking (e.g. acrylamide, 3-MCPD and its esters, PAHs) are a direct source or cause of the contaminants. For heat-stable contaminants the cooking process has little effect or it might lead to a reduction in contaminant concentrations because lipophillic substances are cooked-out in the fat phase (e.g. dioxins, non-DL PCBs, PBDEs) and element ions (e.g. Hg, Cd, As, Pb, radionuclides) may be removed partly in the cooking water. Finally, if the chemical substances are unstable (e.g. sulphite, aflatoxins, gentamicin) concentrations can be reduced dramatically or their speciation (e.g. inorganic versus organic As and Hg) altered. Two or more of these factors can of course inter-play, to cause concentrations to fall, rise or remain unchanged.

Estimates of contaminant dietary intake using TDS concentration data are only reliable if the foods are packaged and processed according to normal practice prior to eating. This is because food packaging and food processing, especially , have such a significant impact on the occurrence levels of specific contaminants. Other food preparation procedures, such as reconstitution, washing and peeling, must be considered too and practical recommendations on how to ensure that consumer practice is mimicked during preparing of food samples has been produced.

Information on cooking and other food preparation habits can be derived for some countries because these are recorded in food diaries from national food consumption surveys. This source of

information has the advantage of not only identifying food preparation method(s) but also statistical information (based on frequency) on relative importance in population groups of interest. However, surveys are already burdensome for consumers, and there are practical (logistical, financial and compliance) problems in trying to capture ever-more information in this way. Other literature sources of information on consumer habits and practices include recipe books. As the proportion of pre-prepared foods (up to and including complete ready-meals) increases in our national diets and information provided for consumers, by way of food-packaging labels, will become increasingly importance. Labels and recipe books (printed, on-line and other such as television programmes) can provide information on recipes, cooking times, temperatures, etc. but, in isolation, they cannot give information about the relative importance of each food preparation method.

Information with this statistical dimension can be obtained using dedicated consumer survey questionnaires, providing information about the food preparation methods that should be used. It seems likely that internet-based surveys will be used increasingly, where the possible problems of self-selection and representativity could be out-weighed by the larger sample size and statistical power offered.

In conclusion, prior to undertaking a TDS, an evaluation should be conducted for each chemical covered, describing its physical and chemical fate during normal food preparation methods. This evaluation should guide the formation of the food list.

Task 4.2. Composite Formation

Ensuring the homogeneity of TDS composites (also known as pooled food samples) is crucial, but it can also be problematic. Costs can be prohibitive for several portions and averaging-out of concentrations means variability in the analytical method at low concentrations may be large, which limits any conclusions about homogeneity. TDS samples might also be used for several purposes, such as contaminant and for nutrition composition, including future target chemicals not yet decided. For these reasons, it is desirable or even necessary to test for homogeneity using surrogates. For water-soluble substances (e.g. heavy metals, acrylamide), the elemental composition of pooled food composites can be tested because concentrations are high (e.g. sodium, calcium, magnesium) and the analytical method (e.g. acid-digestion followed by ICP-MS) quick and precise. Surrogates for other classes of contaminants are not well established.

Choice of surrogates for homogeneity tests: For a first approximation, the most appropriate surrogates for homogeneity should be linked to the physio-chemical properties of chemical(s) that is/ are targeted in pooled and homogenised samples. Crucially, the surrogate should mimic the polarity of the target chemical, as indicated by solubility in aqueous and fatty media and/or the octanol-water partition coefficient (logPow).

Polar / water-soluble analytes: Intrinsic metals in foodstuffs (e.g. sodium, magnesium, potassium, calcium and manganese) can be used as markers of adequate homogeneity for polar analytes when using ICP-MS analysis, which is a quick, cheap, sensitive and precise option. Alternatively, using a water-soluble, non-toxic and stable dyes, such as fluorescein, can serve the same purpose. Fluorescein and its derivatives are used widely as fluorescent tracers in many applications, including tracking natural water flows of underground streams, water sources and water-courses. Quantitation methods for the fluorescence are very sensitive and cheap.

Non-polar / fat-soluble analytes: Potential fat-soluble markers could be ubiquitous contaminants (e.g. a PCB isomer) or intrinsic substances, such as cholesterol (animal products) or plant sterols (fruit and vegetable products). The choice depends on factors such as speed, cost and precision of the method of analysis in relation to the concentration of surrogates in each food group/ sub-group. Non-polar derivatives of fluorescein or other dye could also be used. Another indicator of homogeneity could simply be fat content, which is described below.

Intermediate-polarity substances: Demonstrating homogeneity of one or both water- and fat-soluble surrogates could cover intermediate-polarity substances.

Fat determination: The fat content of samples can be used to indicate homogeneous distribution of fat-soluble contaminants. Values obtained for total fat are method-dependent. Classical methods are based on continuous extraction of the food in a Soxhlet extractor. The solvent is often petroleum spirit or diethyl ether. Other solvents, such as trichloroethylene or mixed polar and non-polar

solvents including chloroform–methanol, can give a more complete extraction but the use of halogenated solvents is no longer favoured for health and environmental safety reasons. Extraction can be preceded by acid or alkaline hydrolysis to make the fat more available for extraction. The solvent is evaporated subsequently and the extracted residue weighed. As an alternative to a gravimetric determination, the solvent extract can be analysed using gas chromatography (e.g. see methods for determining total fat, saturated, unsaturated and monounsaturated fats in foods using acid hydrolysis and capillary gas chromatography to comply with the requirements of nutrition labelling). The precision of methods, which is most important for TDS, is good (RSD of fat determination is in the range 1 to 5%) and the analytical cost limited.

It has been stated above that, for a first approximation, it can be argued the most appropriate surrogates for homogeneity should be linked to the properties (e.g. log Pow) of the target chemical, However it should be noticed that in a simple two-phase system (A+B, e.g. water and fat) if the sample is mixed sufficiently, such that one phase is homogeneous, then by definition the second phase must also be homogeneous provided there is no gross phase separation on the scale of sub-sampling. In a water-fat mixture (e.g. aqueous and fatty/ oily phase in homogenised meat or fish samples), where metals analysis is used to prove homogeneity in the aqueous phase, it can be assumed the fatty phase is also homogeneously distributed.

As overall conclusions, it is recommended that for a new chemical (new or just new to that TDS laboratory) homogeneity is demonstrated by preparation of composite samples and analysis of replicate portions. If this is not feasible (for the reasons explained above), chemical surrogate(s) in place of the target chemical(s) are to be used. At the same time, the sample preparation process has to be recorded as a SOP, such that following this SOP will in future ensure homogeneity is achieved.

Task 4.3. Chemical Analysis

It is necessary to have clear rules on how laboratory analysis is carried out. This is particularly true of TDS where pooling of samples can give rise to dilution effects and where composite samples may have a more complex character compared with individual foods. This being so, rules for the correct identification of chemicals being measured to determine exposure assessments are especially relevant. As an example, the identification criteria used in Europe for residues analysis by LC-MS/MS in the field of official control evolved from Commission Decision 2002/657/EC, through guidelines of the European Union Reference Laboratories, but with the development of new or enhanced instrumental techniques including, for example, high mass resolution mass spectrometry, with- or – without a secondary ion mobility chamber, further guidance on substance identification rules emerges at regular intervals.

The analytical methods available for TDS were reviewed. One obvious conclusion was simply the huge variety, which was anticipated given the wide range of chemicals of interest in TDS, ranging from nutrients to additives and contaminants, with organics and inorganics, including speciation needing. Given the above, the identification criteria proposed by the analytical laboratory have to reflect properly the state-of-the-art in chemical analysis for each chemical measured in TDS, and should be possibly discussed and agreed by the EuRL and/or the NRL responsible for that chemical sector. This will help ensure a harmonised approach in method performance, helping in turn to ensure comparability of analytical results and the exposure estimates made from them.

Evaluation of analytical method performance as a source of uncertainty in exposure estimates

It is important to consider the nature and origin of analytical measurement uncertainty (MU), and understand and describe how MU carries through into an estimate of consumer exposure in a TDS. If only one food group contributes to consumer exposure then analytical MU contributes directly to exposure with other uncertainties. But, if a number of food composites/ groups contribute to the overall consumer exposure – as should be the case – the effect of MU is attenuated. This is because, if the food groups are analysed in different analytical batches (as is usually the case) and MU is random (uncertainty has a probabilistic basis), MU cancels out. When setting a target value for MU, as a performance criteria, consideration should be given to how many food composites and food groups are expected to contribute to the overall estimate of consumer exposure, and if they are included in just one or in multiple analytical batches. Overall, MU is relatively unimportant in TDS

whereas accuracy (in fact the trueness component of accuracy), along with considerations of LOD and LOQ, are far more important.
Accuracy of analytical determinations is the closeness of an analytical result and the true value. Accuracy refers to a combination of trueness and precision, and actually trueness is the closeness between the analytical result and the true value (ISO 3534-2:2006, clause 3.3.3). Accuracy (and trueness) can only be demonstrated by the analysis of test samples for which the 'true' concentration is known. Ideally, the analytical method used should not have systematic bias. As a practical recommendation, the analytical laboratory should include analysis of Certified Reference Materials or other traceable reference materials. The availability of CRMs and other quality control tools have been summarised. If CRMs are not available or limited in relevance, a laboratory should participate in proficiency testing analysing the target analyte in a closely related food matrix. In such a case, values are not usually 'true' but 'assigned' by consensus.
As fas as LODs and LOQs are concerned, there is a clear need to reduce uncertainty in estimates of exposure brought about by upper- and lower-bound estimates resulting from 'non-detects'. Careful consideration is needed when pooling foods to be sure avoid over-dilution. The target LOD and LOQ can be tuned, depending on the relative importance of the different food groups to the total diet, with the most frequently consumed food groups having the lowest (best) LOD/ LOQs. The tools used in TDS combining concentration data with food consumption data to estimate exposure should be pre-run by inputting LOD/LOQ to calculate their impact on the exposure estimates. The outcome should inform decisions about how much or how little pooling is appropriate, and help specify whether improvements in analytical method performance are needed.
Another option would be to consider the applicability of lower-, mid- or upper-bound estimates. If there are no historical records for a food group or single food items belonging to that group containing the chemical of interest (with sufficient numbers of samples analysed to give confidence) then the concentration for that food group should be set as zero rather than LOD or half the LOD. This decision should be recorded and the impact evaluated by means of an assumptions-uncertainty analysis table.

WP5 Development and implementation of quality standard framework for TDS Centres in Europe

Task 5.1: Development of the overall TQM System for TDS

The EFQM (European Foundation for Quality Management) excellence model can be used by organisations to assess their current 'levels of excellence', and provides users with a set of performance improvement tools for them to achieve and sustain results and excellence. It was chosen as a reference for Total Quality Management implementation because it is a practical, non-prescriptive management framework able to give a holistic view of an organisation and provide an overarching framework. The president of the European Council has supported its use and it supports the UN Global Compact, but it has never been tested in this area of work and, therefore, TDS-Exposure is a pioneer in this aspect. The scope was agreed with WP5 participants and is "to support organisations performing Total Diet Studies (TDS Centres) in their journey towards Excellence through a TQM approach".

The EFQM Excellence Model consists of nine areas/ criteria that are split into two categories: enablers (what the organisation does and how it does it) and results (what the organisation achieves). The model allows comparison of different organisations using a common assessment language. Three TDS-Exposure partners took part in a self-assessment questionnaire based on the EFQM Excellence Model.

Task 5.2 Process design

The TDS-EXPOSURE DoW was reviewed against the WP9 TDS flowchart prepared within WP9, to identify activities performed by the different WPs that are important for process design and SOP development.

Overall framework of TDS processes

17 TDS flowchart steps were identified as follows:

Step 1 – Definition of objectives	Step 10 - Preparation for food collection, sample preparation and analysis
Step 2 – Collection of data and information	Step 11 – Food collection
Step 3 – Allocation of resources	Step 12 – Reception of individual samples at kitchen laboratory
Step 4 – Definition of population groups of interest	Step 13 – Sample preparation at kitchen and analytical laboratory;
Step 5 – Prioritization of substances	Step 14 – Chemical analysis of laboratory samples
Step 6 - TDS food lists and suggestion of specific TDS samples	Step 15 – Exposure assessment
Step 7 – Sampling plan	Step 16 – Risk characterization
Step 8 – Analytical plan	Step 17 – Risk communication and publication of results
Step 9 – Corrective actions	

The processes were identified based on the following strategy:

1. Detailing the process for each step – drawing a flowchart
2. Identifying possible relationships with other steps
3. Defining monitoring indicators
4. Revising/ adding critical control points (CCPs)

SOP development

A framework was designed that identified the TDS process steps, critical control points, related SOPs and guidance documents. This task extends the work completed to describe and document the SOPs identified, namely:

- SOP1 – Preparation for food collection, sample preparation and analysis
- SOP2 – Collection of samples
- SOP3 – Reception of individual samples (at kitchen laboratory)
- SOP4 – Sample preparation (at kitchen laboratory)
- SOP5 – Chemical analysis of TDS samples

In addition, four guidance documents were also identified and are as follows:

- GD01 – Planning a TDS Study
- GD02 – Exposure Assessment
- GD03 – Risk characterisation
- GD04 – Publication/communication of results

Each SOP contains the following structure: scope, objectives, references, acronyms, definitions, responsibilities, procedure and bibliography. By expanding the supporting procedures seen in the TDS flowchart (management, training and database management), quality assurance and quality controls were also included. Areas that could be implemented as part of a quality control process (e.g. random sample checking and homogeneity tests) were highlighted. Any non-specific procedures were identified for future use in compiling country-specific SOPs.

Task 5.3: Mapping food identification and classification systems

The assessment focused on two existing, international food classification and description systems: FoodEx2 and LanguaL. A 'classification system' groups or aggregates foods with similar characteristics whereas a 'description system' seeks to identify foods as precisely as possible. Each system was compared by reviewing published documents and user experience. The report concluded that whilst LanguaL is an extremely detailed description system, which allows users to identify different characteristics of foods, this level of detail is not needed for exposure studies. In comparison, FoodEx2 allows a more basic classification of foods, linked to different food group levels, where required. It was designed specifically for exposure studies in Europe and is still under development, allowing further input in design. LanguaL codes are included in FoodEx2, meaning it is possible to map between the two systems if needed.

Task 5.4: Quality requirements for data generation, collation and interchange

A questionnaire was prepared to define the quality profile of laboratories that could potentially be involved in TDS. Eleven laboratories from 11 countries completed the questionnaire for 22 substances belonging to three substance groups. The questionnaire contained four sets of questions, covering the following aspects: General - containing general questions on laboratory quality profile; Method Performance, e.g. LOD, LOQ, validation, trueness, precision; Internal Quality, e.g. QC checks, Control Charts; and External Quality, e.g. z-scores and participation to PTs/ICTs.

Highest and lowest values for LOD and LOQ, precision (repeatability and reproducibility), trueness and selectivity were reported for all selected chemical substances. Most of the calculated LOS and LOQ values were respectively defined as being equal to 3 and 10- times the standard deviation of the mean of independent blank tests, respectively. The TDS-Exposure questionnaire allowed classification of uncertainty evaluation approaches used by TDS laboratories.

Analytical measurement information has been compiled in an on-line wiki-platform, which will be of value to all collaborators in TDS, not just those involved in analysis. A partnership was set up with the previously developed EuroFIR wiki, which has all the necessary tools and technology available. The platform is based on links to existing information sources. All relevant information regarding analytical measurement of target analytes that are targeted within TDS can be found within the wiki, e.g. official methods of analysis, selected scientific literature, reference material producers, available reference materials, etc.

The analytes of interest and scope of the wiki were defined by the WP2 list of priority substances. Currently, the wiki contains 22 substances; this includes analytes that were analysed by the WP9 pilot study (Mn, Se, Cu and Hg). In addition, relevant information for Pb, As, Cd, radionuclides, dioxins, PCBs, acrylamide, PBDEs and PAHs are also available.

Task 5.5: Third party recognition/'certification' framework

The aim of the TDS-EXPOSURE quality standard framework was to identify areas in which TDS organisations are working and, thereby, provide a structure for external reviewers to assess all processes and systems in place through a co-operative review. Ultimately, the goal was to provide TDS organisations with an idea of their strengths and weaknesses, how to improve quality systems and harmonisation, and production of high quality data. In addition, benchmarking processes might be identified during review visits. The TDS quality framework differs from the EuroFIR framework because of differences in the work undertaken, e.g. containing a review area for the food consumption study.

The framework is based on the principles of ISO 9001 and, therefore, it was important to test its compatibility with the proposed TDS quality management system based on the EFQM Excellence Model. An external review assessed the current implementation of quality systems in TDS organisations and is compatible with the proposed TQM approach: each area of the framework is linked to the EFQM Excellence Model. Reviews of processes were based on systematic evaluation of requirements associated with a specific activity. After the review meeting, findings were compiled as a confidential, factual report ready for the TDS organisation to comment. Reviews were then revised to take into account comments and clarifications provided by the TDS organisation. Recommendations for improvements to the quality management framework and peer review

process and summaries of the pilot peer reviews were included in the deliverables. A sustainable quality management system for TDS would be beneficial for several reasons: support for training activities; encouraging sharing of useful practices and ideas within a framework; enabling comparison and benchmarking with other TDS organisations; and providing an overview of strengths and weaknesses of TDS and organisational processes.

WP6 Database management, description

Task 6.1 Requirement Gathering for FoodCASE

A multiple steps approach was used to collect software requirements and to gain knowledge about TDS processes. Steps included literature research, interviewing experienced partners and collection of use cases. Use cases are scenarios in which users interact with the system for data input or data retrieval. The final step was to define what information is needed for what entity, in particular what attributes are needed for entities such as food, samples or occurrence data. It was also differentiated what attributes are necessary in which use cases.

Task 6.2 software requirements specification of FoodCASE-Risk

A software requirements specification (SRS) is a description of a software system that should be developed. It lays out functional and non-functional requirements and should include a set of use cases. Based on the output of task 6.1, an SRS for the TDS module of FoodCASE was written and all user masks were described in figures. The SRS includes the following main aspects:

- The system has a client server architecture to allow access from everywhere
- The system is operating system independent
- Storage of TDS data is close to food comp and consumption data and in a similar structure
- A framework supports users to maintain and improve data quality
- User is able to define all thesauri entries such as food categories, campaigns, decision reasons and so on
- The system can import and export data
- Different output files can be generated (reporting)
- Access is control by login credentials and roles

Task 6.3 Implementation of FoodCASE

The development of the TDS module in FoodCASE has been performed based on the final SRS from Task 6.2. The FoodCASE database has been extended to include 39 tables and four views. The four core entities in the TDS database schema are food (TDS food), sample (TDS pool or TDS sample), subsample (TDS Subsample) and occurrence value (occurrence or concentration data value). The TDS module of FoodCASE has the following features:

- Aggregation of consumption data for user-defined groups such as age and gender groups to evaluate foods of interest
- Substance decision tool to evaluate substances of interest
- Mobile application with shopping list, synchronisation to FoodCASE, possibility to add multiple pictures and ability to document information only available in shop
- Status plan to document current status of subsamples which helps to keep track if a subsample was bought, is stored in freezer, is being prepared in kitchen lab or pool was send to laboratory
- Comprehensive value documentation for occurrence data and other entities
- Data export to MCRA and in SSD format (EFSA format)

- Facility to store several studies and to compare shopping list, pools and occurrence data over several studies
- Food linkage to automatically match TDS samples to composition or consumption foods

A demo version of FoodCASE with the TDS module is available online.

Task 6.4: Usability testing and re-testing of FoodCASE

The aim of the usability testing was to improve the TDS module in FoodCASE once users were able to test it. In the first usability workshop the software was presented, tested by participants and feedback was collected in a discussion. The feedback was implemented in FoodCASE and a second usability workshop was organised. This time, participants were given six TDS tasks and a questionnaire was used to collect feedback. FoodCASE was rated to be intuitive and the time needed to perform the tasks as appropriate. Some more improvement feedback was provided and again implemented in FoodCASE.

Task 6.5: Maintenance and support for TDS pilot studies

The goal was to support pilot countries with data preparation for MCRA and to build the pan-European TDS database.

The mapping between the FoodCASE database structure and the MCRA import file structure was defined and was implemented in FoodCASE. Some test data was imported into FoodCASE to test the file interface to MCRA. The automated FoodCASE export to MCRA is helpful for FoodCASE and MCRA users as data preparation is done automatically to save time and increase data quality. Data for the pan-European TDS database consisted of 3 parts for each pilot country: planning data, sampling data and concentration data.

WP9 defined 20 food categories and, for each food category, a separate EXCEL file per country was generated. These 94 EXCEL files in total needed to be imported. The EXCEL files were used during the import to get information about food categories, foods, pools, FoodEx2 codes and pooling decision reasons. The so called collection reports provided information about what, when and where subsamples where bought. Concentration data delivered not only concentration values but also information about laboratory and used methodologies.

Beside data from the 5 pilot countries, data from France (EAT2) and UK (2006) could have been organised and imported. In total 262’758 occurrence values could be imported together with 3’185 TDS foods, 746 TDS samples and 5’855 subsamples. 99.12% of the occurrence values came from the French TDS.

Task 6.6: Research on an advanced data quality framework

Our activities have been focused on exploring new ways of managing data quality in FoodCASE. We wanted to investigate the extent to which data quality can be considered as a data constraint problem by extending and generalising existing constraint models. Constraints are coupled tightly with data quality and mostly embedded in software so that users have no influence or control over them. The first approach introduced the concept of contracts and grouping of constraints that enables a user to define constraints with their importance rating in a contract. The grouping allows to organise constraints into logical units such as data quality dimensions (accuracy, completeness, etc.). Contracts are evaluated in two data quality validation processes. The first is data input validation where user input is evaluated on every input masks. As data can also be imported over automated interfaces, a process of data quality analysis is necessary in which all data in the database is evaluated against contracts. It is possible to define several contracts and they can be valid for single users, for groups of users, for a whole institution or for all users. If no contract is defined by a user, a default contract is taken as a fallback.

The goal of the second approach was to allow programmers to define constraints in their preferred programming language and spread them to all levels of an information system such as database, desktop application or web application. To provide a user with means of constraint specification, we propose a powerful Domain Specific Language (DSL), called UnifiedOCL. Various approaches and technologies of bidirectional transformations between various programming language models were studied. The resulting concept allows transformations from any source programming language into any target programming language such as Java, SQL and so on. This allows programmers to define constraints in their favourite language and to transform them over UnifiedOCL into another language. The second issue that is solved by this approach are non-unique mappings of constraints to data quality dimensions. The issue is that this classification is not always unique so that a constraint can belong to one or several dimensions. The idea in our approach is that users define which constraint belongs to which dimension whereas constraints can belong to multiple dimensions. An external and pluggable data quality analysis tool should then evaluate the constraints and present results in different forms.

Task 6.7: Implementation of the advanced data quality framework

The two approaches described in task 6.6 were implemented for evaluation. As there were some issues, a third approach was implemented in the final version of FoodCASE.

The implementation of the first approach contained an administration part where constraints and their importance ratings can be managed. The administration part also allows loading and export contracts and hence make contracts shareable. Contracts were used in the FoodCASE desktop application to provide data quality feedback on every user input mask and in a data quality analysis tool.

The implementation was evaluated with project partners. 80% of the participants rated the implemented framework as useful. Around 90% of the participants endorsed the concept of assigning important weights to constraints. Almost 50% considered having different contracts for different users and applications of the system useful while no one disagreed. 60% welcomed the opportunity to share and exchange contracts with other users where only around 7% disagreed.

The implementation of the second approach contained a framework to transform a programming language including constraints into another. The framework is a plugin to a certain development environment called Eclipse which is used to implement desktop and web applications in Java.

A so called data quality visualizer uses a file where mappings of constraints to data quality dimension are defined. The visualizer is a standalone application which evaluates constraints and is able to present results for different entities and dimensions.

The constraint definition tool and the visualizer were evaluated with persons having at least basic knowledge in software engineering. The feedback on the tool was very positive in terms of being useful and easy to understand.

The two presented approaches for an advanced data quality framework for FoodCASE proofed to be useful and going into the right direction. However, there were issues why they could not be completely implemented in FoodCASE. The first approach of contracts had a problem with coloring of input fields in desktop applications. The second approach has proofed to be limited with more complex constraints. It was therefore decided to use the existing framework in FoodCASE and extend it with TDS constraints. The set of TDS constraints was evaluated with project partners using a questionnaire and 156 constraints were identified. Some constraints were assessed with completely different importance ratings and implementation in FoodCASE followed the approach of biggest consensus.

WP7 Variation and trends

Task 7.1 Improve the understanding of differences in food consumption in sub-populations of interest and how it influences the establishment of the TDS food list

TDS food lists and national food consumption data were compared for different countries and the contribution of food groups to the overall diet was assessed. Moreover, the consumption frequency of foodstuffs not included in the food lists was also assessed. The aim of this task was to compare age groups as well as men versus women regarding the percentages of total diet that is covered by the defined TDS food list and the ranking of contribution for different food groups to the overall consumption. This work showed that dietary habits differ greatly amongst countries. Therefore, a combination of food lists from other TDS with national food consumption data is not useful. Furthermore, if different sub-populations are considered in a later exposure assessment, levels of aggregation in pooling should be kept low, as differences between subgroups in the contribution of certain foods to overall diet were observed.

The aim of the task was to identify food groups that should also be analysed within TDS with respect to pregnant women. To achieve this aim, dietary patterns of pregnant women from the Norwegian Mother and Child Cohort study were compared with data from the Norwegian Fish and Game study as well as published data from the Norwegian National dietary survey Norkost 3. The conclusion was that a TDS food list should include the main food groups, such as bread, other cereals, milk and yoghurt, fruit and berries, sweet drinks, juice/nectar and drinking water, as pregnant women consumed these in greater amounts. Also, “risk foods”, such as nuts and dried fruits, should be considered. The same is also true for dietary supplements and herbal remedies.

Task 7.2 Overcome the lacking information on variability in food chemical concentration data derived from TDS by learning on variability from secondary data

Food monitoring data from Belgium, Czech Republic, Germany, France and the Netherlands were received for copper (Cu), selenium (Se), manganese (Mn), mercury (Hg), methyl mercury (MeHg) and aflatoxins. For task 7.2, heavy metal data (Cu, Se, Mn, Hg, MeHg) were chosen for statistical description. In total 68877 monitoring data for the metals were provided. Included in the analysis were data with objective and selective sampling strategy, and a minimum sample size of N= 40 with a minimum of 50 % quantified values. For the final analysis, 35808 monitoring data were made available. Foods were categorised according to FoodEx-1 and statistical analysis was conducted separately for each chemical in each country by calculating diverse statistical parameters: min, max, mean, median, percentiles (2.5, 5, 10, 25, 75, 90, 95, 97.5, 99), standard deviation and coefficients of variation (SD/mean, P95/mean, P95/median, P97.5/mean, P97.5/median). Lower bound approach (LB) (data below LOD/LOQ are set to zero) and upper bound approach (UB) (data below LOD/LOQ are set to the corresponding LOD/LOQ) were calculated and compared for each food group.

Based on the description of work (DoW), two chemicals were selected and described in detail; these were Cu and Se from the German food monitoring as these were the most comprehensive dataset available. Additionally, complete analysis and description were carried out for Mn and Hg. The coefficients of variation and the two different approaches to handle non-detects were described and compared, and suspicious results were further analysed. Two further issues arose during this work on the data: (a) the handling of outlying and/or extreme values and (b) the application of a third approach (modified lower bound approach) to deal with non-detects. The German food monitoring authorities have confirmed the outlying values are reliable data and, thus, these values provide information about the distribution of concentration data and should be included in further calculations. The coefficient of variation P95/median is not suitable to describe variation and was excluded from calculations. Different limits of detection from monitoring data influence the coefficients of variation. Simulation studies have shown which approach for handling the non-detects gives the most reliable results.

To provide a complete description of samples and explain variability, the Icelandic fish database compiled by MATIS was also used to analyse single units and composite samples for sources of variability. Trace elements (Fe, Se, Hg) and PBCs (sum of PCB7) in fish between (1) data from

individual fish (2a) a composite of 10 fish fillets sorted according length and (2b) a composite of 10 fish fillets taken at random were analysed and compared. Composites from random sampling (such as applied in a TDS), generally, show less variability than variations between individual fish or sorted composites. To capture the extra unit variation present between individual units, it was proposed extra unit variation be simulated by fitting a potential lognormal distribution, as it could be demonstrated for cod. But, as available data were limited for this task, these results need to be confirmed by further calculations.

The extrapolation factors (EFs) SD/mean and P95/mean (derived in task 7.2.4) were tested in statistical simulations for their applicability to:

(i) extrapolate from mean concentrations to high percentiles,

(ii) extrapolate between similar foods,

(iii) extrapolate between different years,

(iv) extrapolate from mean concentrations to high percentiles using single unit data from subtask 7.2.2, to verify the results from the simulation studies.

EFs were applied to simulated or real (single units) pooled TDS means and the extrapolated estimates were compared to the "true" value of the respective food group derived from the Food monitoring (data from the Food monitoring were set as "true" parameters of distribution due to high sample sizes). Deviation (%) and 95% confidence intervals (CIs) were criteria for the quality of extrapolation estimates. Throughout all considered scenarios (i – iii) the EF P95/mean turned out to result in the most robust extrapolated estimates. Compared to SD extrapolation, P95/mean extrapolated results are closer to the "true" value and the CIs are narrower. Further, for some SD extrapolations the CIs did not include the "true" value from the Food monitoring. This may be explained by the fact that the factor SD/mean is more vulnerable to different distributions (e.g. the distribution from one year compared to the distribution from another year). Unexpectedly, extrapolation using single unit data lead to wide deviations from the "true" values, with about 50 % falling outside the expected 95% CI. Therefore the pilot character of the results from WP9 may be explainable for the wide deviations from the "true" distribution, as no regional or seasonal variation could be considered and the availability/use of market share data was limited.

Additionally tested was (a) the impact of the amount of non-detects (NDs) that were included in the calculations of these EFs, (b) the impact of different sample sizes (N) when deriving EFs, and (c) the impact of critical distributions (bimodal or including extreme values) when deriving EFs. These results showed that (a) up to an amount of NDs of 30-40% and more the extrapolated estimates derived from lower bound (LB) and upper bound (UB) approach start to deviate markedly from each other. (b) Regarding the sample size, a number of ≥ 40-60 samples included in the calculation of EFs did not improve the corresponding CIs of extrapolated values considerably. (c) Results for critical distributions showed that the EF P95/mean still performs reasonably well, when derived from critical distributions. Estimates based on SD/mean instead showed high deviations from "true" values with comparably broad CIs.

In conclusion, extrapolation is feasible in each scenario and as distributions are hardly predictable the EF P95/mean is associated with less uncertainty. However, more examples must prove the robustness of these conclusions.

To assess whether seasonal variation in concentration or consumption impacts the exposure level, BfR, C.R.E.A-NUT and ANSES conducted, as a first step, a literature review on seasonal differences (concentration, consumption and exposure, respectively). C.R.E.A-NUT investigated the EFSA Food Comprehensive database to identify studies that were based on repeated 24-h recall and checked whether there time enough between the interviews to include more than one season in the exposure assessment (for the same individual). However, 24-h recalls are often very close (some days/ weeks) and rarely include more than one season. C.R.E.A-NUT also provided a descriptive report on the Italian National Food Consumption Survey INRAN-SCAI 2005-06 showing the most relevant seasonal variations by food groups.

French TDS results were used to compare exposure assessments with and without consideration of seasonal sampling. Chemicals considered were Cu and Mn (pilot studies), As, PCDD/Fs, DL-PCB, sum PCDD/Fs+DL-PCBs, sum 6 iPCBs, and DON and OTA (mycotoxins). After statistical testing confirmed there were no significant differences in the population between seasons, three scenarios considering seasonal sampling in a TDS were tested: (1) Global impact of season on exposure, (2) Impact of seasonal consumption variation, and (3) Impact of seasonal contamination variation (PCDD/Fs, DL-PCBs, sum PCDD/Fs+DL-PCBs, NDL-PCBs, DON and OTA). Results showed significant differences between exposures during warm and cold seasons, for most but not all considered target analytes for both considered population groups (adults and children). Comparison of the scenarios reveals that these differences may (partly) be driven by seasonal differences in concentration (also true for most but not all contaminants). Thus, if a refined exposure assessment is required, seasons have to be considered in the sampling plan. For chemicals with an anticipated seasonal variation pooled samples should be kept separately for each season. In terms of cost reduction, foods with expected low seasonal variation in concentration can be pooled together for different seasons or pooling from one season would be enough.

Task 7.3 Harmonize trend analysis based on TDS

A literature review on trend analysis based on TDS data was carried out. In total, 280 literature sources were screened (scientific articles, reports, book chapters) and examples for trend analysis from the literature were introduced. The feasibility of trend analysis depended on sample size and number of years/ periods available for analysis. Ideally, data are sufficient to apply a (log-) linear regression models. In the Czech TDS data, multiple regression analysis and piecewise linear regression with break-point was applied successfully and tested on exposure doses of Pb and Se. It was concluded that method was sufficient for exposures to Pb but not sufficient for exposures to Se which is probably related to missing consumption and concentration data related to food supplements broadly used by studied population groups. The same Czech TDS data for Pb was used to test how changes in European Maximum Legal Limits will change exposure doses and whether trends found in TDS studies has reflected these real risk management measures. In the specific case of Pb it was demonstrated that decreasing of Pb exposure doses trends is better correlated with deleting of leaded petrol from the EU/CZ market (an environment protection measure) than with any changes in European Maximum Legal Limits for Pb in foods set by the EC.

Task 7.4 Develop an exposure assessment approach combining benefits from TDS and food monitoring data

To develop a combined approach for TDS and food monitoring data a literature review on the objectives, benefits and limitations of both approaches was carried out using PubMed, Web of Science, Scopus and LitDok (internal database, BfR). Titles and abstracts from 663 publications were screened for relevance and finally 153 were classified as relevant. A decision tree and one flow chart were developed from these. The decision tree provides guidance, which data to use (TDS or Food monitoring) in case of a certain research questions according to the specific characteristics of each method. The flow chart describes how TDS and food monitoring can complement each other in terms of food safety.

Task 7.5 Understand remaining uncertainties of exposure assessments based on TDS

There are several uncertainties in each step of a TDS. Generally these can be categorised as avoidable uncertainties (controllable by QM/SOPs) and unavoidable uncertainties (e.g. available food consumption data for compiling the food list). Both have to be considered as far as possible in advance to decrease uncertainties overall or, at least, to ensure they are documented accurately and discussed in the presentation of TDS results. Samples below the limit of detection (<LOD/LOQ) are a significant source of uncertainties in exposure assessments. In a TDS these non-detects (NDs) introduce additional uncertainty in TDS samples (pooled samples).

To address this uncertainty, three approaches were tested: (1) UB and LB methods to bracket the true value; (2) Bayesian inference approach –true value of each of the censored observations is treated as an unknown parameter in the model, and uncertainty due to NDs and sampling

uncertainty are both captured directly within the statistical model; and (3) Bootstrap inference approach. Calculations were carried out using an artificial large dataset (with controlled range of NDs and assuming lognormal distribution) (step 1) and using single unit data from the WP9 pilot studies as realistic examples (step 2). Step 1: (i) using LB and UB alternatives quantified sampling uncertainties by the standard 95% CI, assuming a lognormal distribution and a known standard deviation (SD). However, this method only represents uncertainty about NDs in an informal way. For higher numbers of NDs, the two latter methods are useful. Of these, Bootstrapping is simpler to implement, but associated with additional uncertainty as each ND has to be set to the corresponding limit of reporting (LOR). Instead, the Bayesian approach covers the required uncertainty interval more consistently in cases of high numbers of NDs. However, both are applicable only in cases where there are multiple observed TDS or sub-samples available. Generally, enhancing the number of subsamples reduces uncertainty. Step 2: For all applied methods, unquantified uncertainty remains in practice for the WP9 single unit data (true distributions are not always lognormal and the true σ is unknown). For σ, default factors must be applied that lead to additional uncertainty.

WP8 Exposure Assessment

TDS-Exposure has, among other things sought to build and test a European database for TDS data that is useful for risk assessors and risk managers. Primary producers and owners of TDS results participated in WP8 and they inserted their data into the Monte Carlo Risk Assessment (MCRA) software. They performed several tests described in this section. .
Another objective of TDS-Exposure was to develop or adapt existing exposure assessment models to TDS and assess the impact of variability and uncertainties. Generally, a simple deterministic model is used to estimate chronic exposure by combining food consumption data at the individual level with mean contamination data. Because sampling and the pooling procedures are known, it should be possible to assess uncertainties and estimate variability in contamination before pooling of foods. New functionality was programmed into MCRA.
The WP8 partners used the new functionality and it was applied successfully in WP9. The harmonized approach is described in deliverable 8.3 including user guidance.

Task 8.1 Organising data and performing exposure assessment
All beneficiaries having previously performed TDS in their country were trained to organize their TDS data in a format suitable to perform exposure assessment using the MCRA software. The Netherlands, Belgium, France, Spain, the Czech Republic and the United Kingdom, submitted the harmonised data to a shared folder in the MCRA software. They also contacted responsible persons (e.g. members of EFSA expert group on chemical occurrence data) and asked them to provide monitoring data for lead, acrylamide and mycotoxins in the format of the Standard Sample Description (SSD), which is the standard format in use by EFSA and the Member States to exchange data. An inventory was made to inform the FoodCASE-Risk data structure and how data should be conveyed from FoodCASE-Risk to MCRA. MCRA and FoodCASE-Risk contain similar table structures and queries are implemented in FoodCASE-Risk to retrieve data from the database.

Task 8.2 Describing and harmonizing food grouping
Until now TDS were designed at the national level. At the start of the TDS-Exposure project a national food categorization system was in place linking existing national food consumption data to the analytical data (occurrence data) obtained in the TDS.
In Europe, there is a need for a harmonized TDS approach, which includes a harmonized exposure assessment and a harmonized coding system. Therefore, we checked the feasibility to use a harmonized food coding. Since FoodEx1, and its next version FoodEx2, will most likely become the most accepted and harmonized coding of food for risk assessment, a decision was taken to focus on FoodEx1. A MS Access database was developed to help partners to convert their TDS food items correctly to the food consumption database. This database included a functionality to convert

national codes to FoodEx1 codes. Ambiguity and inconsistencies were encountered when linking national codes to FoodEx1. These were solved with a decision tree facilitating the user and ensuring that the conversion was done in a harmonized way and in line with the requirements of the exposure assessment. Six different countries: Belgium, Czech Republic, France, the Netherlands, Spain and the UK compared the results of exposure assessments for lead and dioxins, using the national coding systems and the FoodEx1 system. Only minor differences were encountered, when comparing the exposure assessment distribtions using either national or the FoodEx1 codes. The results are described in deliverable 8.1.

Task 8.3 Study the influence of sources of variation

In many countries, samples are pooled in a single TDS sample per broad food group. This level of pooling can be a major uncertainty, as information about variability is lost. A TDS might provide reliable exposure estimates when enough samples are taken before pooling, including samples take in different regions and/or different seasons. If the real variation is insufficiently covered in the sampling design of a TDS, the exposure assessment might be uncertain. Therefore we studied the variation in monitoring data as an additional source of information. The monitoring data might better describe the variation, because a larger number of samples were available. The variation in monitoring data of several chemicals were summarised by deriving a coefficient of variation (CV) for individual foods or food sub-types. Different contaminants might have different CVs; for example, mycotoxin concentrations might be explained by seasonal variation and the concentration of lead might be related to local or regional circumstances. The TDS mean values were combined with the CVs of the monitoring data in the MCRA software. The WP8 partners applied the MCRA software and performed two exposure assessments, one with and one without uncertainty analyses.

Apart from sampling uncertainty, other uncertainties are related to the collection of food consumption information; for example, food might be over or under reported. These and many other uncertainties can influence the exposure assessment results. Each country has described their TDS design in terms of how representative sampling has been performed over the years, seasons, and locations within the country. The study on uncertainties and variation is described in deliverable 8.2.

Task 8.4 Validating and testing the TDS-Exposure assessment tool

New functionality embedded in MCRA

MCRA procedure to test the completeness of the exposure assessment

In a TDS study, food items representing the diet will be bought on the market and will be prepared according to household preparation practices. In an early stage, a shopping list will be designed to ensure that commonly eaten food items will be sampled and analysed. The starting point for preparing such a shopping list is a calculation of the energy intake for the population of interest. The shopping list usually contains food items contributing for 90-95% of the average daily energy intake. In MCRA a module was implemented to diagnose all food items consumed, but not included in the shopping list. The missing food items might be related to an underestimation of the risk and therefore we implemented an option to include the missing information based on read across principles.

Upper and lower bound algorithms in MCRA

Typically, residue concentration datasets contain only a limited number of positive values and a large number of 'non-detects' or 'less-than', i.e. measurements censored at a limit value, which depending on the reporting institution may be a formal limit of detection or a limit of quantification. For the purpose of exposure modelling any such limit will be referred to as the Limit Of Reporting (LOR). We will refer to values' <LOR' as non-detects (NDs). Note that LOR may in principle be different for each measurement, but in practice it is often a constant value for a specific analytical method as applied in a specific laboratory and a specific time period. The exposure assessments were done twice, one assessment assuming that a non-detect is equal to a zero value (lower bound), and one assessment assuming a non-detect is equal to the LOR.

MCRA model to link mean TDS concentration to variation known from surveillance

A disadvantage of pooling is that seasons and regional information is lost. When enough (sub)samples are taken before pooling, we still might consider the TDS as reliable and representative because then the variation is covered in the sampling design. However, when seasonal and/or regional variation is poorly covered in the sampling design, our exposure results are also uncertain.
The potential seasonal and regional variation is studied in WP7 using monitoring data organized in SSD formats as sent to EFSA. WP7 and WP8 derived the mean, standard deviation and coefficient of variation from the monitoring. The information can be entered into a new table of the MCRA input database. The CV and mean values are used in MCRA as a new functionality to estimate the uncertainty.

Risk management tool in MCRA

The MCRA risk prioritization or risk management model is a useful model to study the effect of risk mitigation measures. A risk manager might wish to set residue limits and when these limits are exceeded, food items should be taken from the market by enforcement authorities. Then the foods with potentially high residue levels are removed from the input database for the exposure assessment. MCRA was adjusted to follow the rules of risk mitigation in a pragmatic manner.

Link between database FoodCASE-Risk and exposure assessment software

An important aspect of TDS-Exposure is linking data collection in WP9 to exposure assessment performed by using the MCRA software including the new functionality developed in WP8. To achieve this, WP9 data either input their data direct into MCRA or they can be input to FoodCASE first. FoodCASE-Risk, thereafter, can export all data to MCRA.
FoodCASE-Risk contains all relevant variables to describe the composition of each TDS food sample. Furthermore, data describing the sampling should be stored in the database (e.g., how many sub-samples were taken, regional and/or seasonal variation been considered by sampling at different locations and different seasons).
The new functionality in MCRA was tested and reported in deliverable 8.4.

<u>Task 8.5 Creating a risk-prioritising tool</u>

The risk-prioritisation, also referred to as risk management tool, should address the balance between risk reduction, which can be achieved after risk mitigation measures are taken, and the percentage of food items to be removed as a consequence of the proposed risk mitigation measures.
For risk managers a risk is defined as the exposure assessment results that exceed the toxicological reference values such as the Tolerable/Acceptable Daily Intake (TDI/ADI). The risk management tool was tested by performing two case studies; one on acrylamide and one on methylmercury. The exposure to acrylamide and methylmercury is considered to be too high and the European Commission is currently proposing risk mitigation measures to reduce the exposure to acrylamide and methylmercury.
The limit set for methyl mercury did not affect the exposure distribution using the Czech Republic consumption data. The acrylamide case study showed, that the limits proposed by the Standing Committee of the European Commission, might result in an average decrease in intake of acrylamide of approximately 40%. In the higher percentiles of the exposure contribution the decrease was slightly higher and in the middle part of the exposure distribution the decrease was slightly lower. It was relatively easy to enter and the use the MCRA risk management tool by inserting the value in the relevant input tables. The instructions on how to apply the risk management tool are easy to follow.
Although the 'proof of principle' was successfully applied, some limitations of this work have to be underlined. The statistical approach taken to apply the reduction to limit approach should be seen as a first approach to describe the intervention. A first demonstration of the MCRA TDS risk management tool was provided to the European Commission Standing Committee on 26-11-2015. General speaking the demonstration was well-received by the risk manager communities and the international organisations such as the Codex Alimentarius.

Task 8.6 Comparing old and new improved methodology with biomarkers

The improvement in the exposure assessments needs to be validated against a possible (unknown) true intake. The best way to test this is to compare the correlations between results for exposure assessment and biomarkers studies performed in the same respondents. The Environmental Health Monitoring System in the Czech Republic is a comprehensive system, that includes, among many others fields, both monitoring of human dietary exposure assessment to contaminants from the food chain, the collection of TDS data and human biomonitoring.

Biomarkers are measurements of chemical substances or their metabolites in biological specimens (most frequently urine, blood and breast milk). Human biomonitoring can bring added value for chemical risk assessment in food safety areas (namely exposure assessment) because it integrates exposures from all sources, not only from food. However, this might complicate a good comparison between exposure assessment results, when only the dietary intake is included in the exposure assessment, and biomonitoring is reflecting all routes of exposure.

Lead levels in blood were monitored over many years (1996 – 2009), revealing a significant downward trend over time. Czech biomonitoring data for selenium were available for years 2000 – 2009. At the beginning of this period, a significant increase in selenium concentration in blood (years 2000 - 2003) was observed. Since 2003, the blood selenium levels have been rather steady.

The exposure assessments, using point estimates and MCRA calculations, were compared with the biomarker measurements. Spearman rank correlation coefficients were calculated. The Spearman's correlation coefficient for lead was found to be 0.76 for men and 0.78 for women. For selenium the Spearman rank correlation was 0.14 for man and 0.25 for women. The low correlation observed for selenium, might be due to selenium supplement intake, which were not included in the exposure assessment using Czech monitoring and consumption data.

It is generally known that levels of selected elements in blood are not affected by food intake only, but also by many other factors (e.g. environmental factors, such as air pollution). It is also necessary to take into consideration complex nature of toxicokinetics of the selected compounds in the human body in any exposure assessment.

For optimal comparison of the exposure assessment and biomonitoring data, multifaceted and not only 'monitoring' but also experimental data (external and internal exposure measured on the same subjects) are needed. Then physiologically-based pharmacokinetic / pharmacodynamics (PBPK/PD) models could be used for advanced description of the internal doses. Due to the various limitations and complexity of these models, results for PBPK/PD in this case study were out-of-scope for TDS-Exposure, but a future perspective on how to optimise this approach was discussed. We concluded that exposure assessment using TDS and human biomonitoring is not consistent for selenium, due to potential supplement intake. However, a significant positive relationship between biomonitoring data and exposure assessments using TDS data was found for lead.

WP9 Implementation of TDS methodology on country level – pilot studies

Task 9.1 Development of food list defining food items to be sampled and analysed

All participants developed their country specific TDS food / sample list based on individual data from national consumption surveys containing at least two measurement days (except for Portugal, only one day). Basic discussion about "implementation of TDS" started among project partners with input of information from WP3. All participants collected national consumption data for various population groups (age, gender) as well as additional information needed for construction of a TDS food list. This information was used for initial suggestions for TDS samples composition. Simple comparison of available consumption and supportive data determined our decision to create TDS food lists only for two age-sex population groups (Adults 18-64 and Elderly 65-74 years). In addition during food consumption data collection it was recognised that the format of data considerably differed among countries ("food ingredients data" versus "as consumed data") which required a specific national approach in other tasks of this WP. Collected food consumption data were organised/grouped according to the newly developed hierarchical and descriptive food coding system for risk assessment purposes in EU, called FoodEx2 (EFSA). Staple food items were selected for the national TDS food lists according to transparently described criteria. Composition of TDS pooled samples (individual or mixed) was suggested and two subsequent steps (see Task 9.2) harmonised results among partners to obtain the highest possible level of similarity in TDS sample composition. All steps were documented electronically and detailed results are available online for all project participants including WP6 developers of the new FoodCASE Risk software. This task was exceptionally complicated due to amounts of data used, coding and reorganisation. Practical agreement on how to harmonise results among countries was however, achieved.

Newly developed food coding system FoodEx2 (EFSA) has been recognized as the key element for harmonisation.

Task 9.2 Creating of country specific sample collection protocols

The work on TDS food / sample list has been followed by the creation of the TDS shopping/sampling plan which was completed in four steps: decisions about 1) regionality and seasonality of specific food items involved in TDS samples, 2) TDS sample design and number of subsamples, and 3) design of sampling plan and 4) corrective actions based on feasibility / experience. In step 1, each country participating in the WP9 pilot study decided which of TDS samples should be considered as national or regional, and which TDS samples should be collected repeatedly during a year. When deciding about regionality and seasonality, the characteristics given in Guidance for the use of the TDS approach (EFSA, FAO and WHO, 2011) were followed.

Seasonality was indicated in TDS samples where the level of specific chemical substances varied due to climatic conditions or seasonal supply variations. Sampling was planned repeatedly across different seasons for such samples. In step 2 individual food items for collection were specified. Work was based on national TDS food lists and conclusions adopted during work on Task 9.4 (Composition of TDS Laboratory samples for pilot studies). Templates to be completed for each TDS sample were prepared for partners. It was necessary to provide information about sample characterisation and numbers of food sub-samples in each of TDS sample, selection criteria and detailed information about each food item for purchase (including information about type, brand, place of purchase, quantity needed). Available information on food consumption data, supplementary market share data (available only for some partners) and other sources were used in this work. In step 3, the time schedule for food sample collection throughout the year was prepared. TDS samples planned for analysis in the WP9 pilot studies were allocated to 4 collection campaigns covering one calendar year. Shopping habits (changes in quantity and sources of foods during seasons) of the population and the capacity of the kitchen laboratory were taken into account during design of the schedule. Each country also prepared methods and defined places from where food samples should be collected. The geographical areas for sampling and also types of shops where foods should be

purchased were decided, based on national statistical data. The elaborated sampling plans are country-specific. Each partner prepared an overview of TDS samples, terms and places for the pilot study and described the process of sampling design. In step 4, corrections based on experience from use of the theoretical shopping/ sampling list were completed. Methodology and results achieved within Task 9.3 (kitchen preparation) and Task 9.5 (pre-laboratory sample preparation) were taken into account.

Task 9.3: description of kitchen preparations to be used for food cooking

At the same time when TDS shopping/sampling plan was created, participants started also work on description of standardised kitchen preparations to be used for cooking foods. Two specific approaches were described in the SOPs due to the fact that food consumption data from the partners had two different formats (raw, edible portions and as consumed, edible portions) or mixtures of both. Kitchen preparations used in WP9 pilot studies are based on recipes from national reference culinary books for each country, reflecting consumer cooking practices including methods (grilling, braising, microwaving, etc.), time and practices such as the use of traditional kitchen utensils. Kitchen preparations of the minimum 12 sub-samples that comprise each TDS sample (see Task 9.4) were described for each pilot country in the protocol for standard kitchen food preparation. In this protocol, each TDS sample and sub-sample was identified according to FoodEx2 classifications, and preparation/ cooking operations, temperatures/times, and utensils were specified. Each country decided on the use of salt, seasonings and fat in culinary treatments but most of them followed previously recommended approach (EFSA, FAO and WHO, 2011). Each country also decided sources of water for cooking (e.g. tap, filtered, bottled, place of collection). In principle, use of tap water, salt and fat is as reported in the food consumption surveys. If information was not available, use was as referred in the relevant cookbook or according to common (national) habits.

Task 9.4: composition of TDS laboratory samples for pilot studies

Participants also prepared protocols for composition of each specific TDS sample. These protocols have an essential role in qualitative and quantitative composition of pooled TDS samples.

The protocols specify rules about how food items should be combined in pooled TDS samples, which are sent to laboratories for homogenization and analysis. Consumption data were of primary importance in determining the composition of TDS samples. Also, market share data were needed to select the most commonly consumed brands. The situation regarding selected TDS samples was different amongst participating countries. Consumption of foods differs and the importance of regional and seasonal parameters differs considerably (compare conditions and dietary habit differences e.g. in Finland – Portugal – Iceland). Therefore, it was important to harmonise procedures for preparation of TDS samples. The number of food sub-samples pooled in a TDS sample was discussed and decided in the protocol. It was concluded that each TDS sample should be composed of at least 12 sub-samples, based on previous statistical considerations (see details in D9.3). Rules for selection of these sub-samples were also crucial for harmonisation. TDS samples can be composed of one or different types of food. In the individual food approach, sub-samples belong to the same food (e.g. different varieties of apple). In the mixed food approach, on the other hand, sub-samples are composed of different food types, e.g. apples, oranges and bananas. EFSA states the mixed food approach might be appropriate for screening purposes, but pooling individual foods allows for the more robust estimation of mean concentrations. The French TDS model for combining sub-samples was selected as a basis for pooling of sub-samples under the individual food approach for practical reasons (cost of sampling, feasibility). This model states the weight of each sub-sample should be equal. If the number of sub-samples is 12, the weight of each sub-sample represents 8.33% of the total weight. Decisions about the combination of sub-samples for various types (mixed approach), varieties and brands for some specific TDS samples was based on available information, primarily quantities consumed.

Task 9.5: Pre-laboratory sample preparation

Pre-laboratory treatments of TDS samples aim to achieve homogeneous samples and, thus, achieve more reliable analytical results. Testing of homogeneity for pooled TDS samples in advance was important to estimate and assure sufficiency of pre-homogenisation in the pilot study. Since the participating countries had different types of equipment available for pre-homogenisation of pooled samples, homogeneity had to be tested by selected TDS laboratory in each country. The procedure for testing homogeneity was harmonised across WP9 countries. The homogeneity of TDS samples before chemical analyses for previously agreed model chemical substances (total Hg, Cu, Mn, Se) was tested in each pilot laboratory. Approximately 10% of the total numbers of TDS samples were selected for testing. Problematic samples in terms of preparation and homogenisation (e.g. foods composed from soft and hard particles) were preferred. Specific testing protocol and software utilities were elaborated based on the testing methodology described for proficiency testing of chemical laboratories (FAPAS). Food items making up the TDS samples tested were purchased based on the shopping lists, and kitchen preparations and pre-homogenisation were performed following the procedures previously defined. Following the protocol, each pooled and homogenised TDS sample was randomly divided into 12 analytical sub-samples and sent to the TDS laboratory. Each aliquot was re-homogenised and sampled in duplicate, resulting in 24 samples for analysis. The order of sample analysis was also randomised. All replicate samples were analysed in one continuous run, where possible. After analysis of the elements, the results were tested using a statistical tool released by NIPH/SZU for all partner countries. Results exceeding the laboratory LOQ were taken into consideration. In addition, the laboratory LOQ was required to be less than the predicted "target LOQ" (for 30% of Tolerable Daily Intake limit). Possible analytical outliers were first detected using Cochran's variance test and rejected before being tested for homogeneity. Homogeneity was evaluated by comparing estimates of between-sample variance with permitted quantity using the test for acceptable between-sample variance. As a target standard deviation, either the laboratory target standard deviation or calculated Horwitz standard deviation was used. Methodology 'how to set max values of analytical LOQs for every TDS sample' and chemical substance was also suggested in task 9.5, based on expected upper level exposure dose estimate when all values would be under LOQs and expected theoretical range of exposure values for 95-99 percentiles (upper tile) of consumers. The equipment used for pre-laboratory treatment of TDS samples, and packing and storage of pooled, homogenised samples was also reviewed in the protocol.

Task 9.6: One year pilot study

A TDS field study was performed in 5 partner countries (CZ-SZU, DE-BfR, FI-EVIRA, IS-MATIS, PT-INSA) during one year to cover all seasons. Due to limited time, personal and financial capacity, only food groups with expected substantial contributions to dietary exposure of target analytes (Cu, Hg, Mn) were selected as mandatory for participating countries. All countries with exception of Germany did also analyse selenium. Based on available data, the following 6 FoodEx2 food groups were determined as mandatory for all pilot countries: Grains and grain based products, Meat and meat products, Fish and seafood, Fruit and fruit products, Legumes, nuts, oilseeds and spices, Milk and dairy products. Partner countries analysed also some other food groups but this work was not obligatory. Therefore this additional work has been financially supported from national resources.

Each partner trained and used a team of TDS sample operators to ensure high quality of work described in tailored national TDS SOPs. Typically, food samples were collected in at least two regions – one represented national and one regional area for sampling. Almost all partners used own TDS "kitchen" which is a laboratory designed to standard culinary treatment of food. Only one partner used external TDS "kitchen". Practical experience and recommendation for future work is to use own facility because of operational advantages improving a quality of work. All partners also used TDS laboratories accredited according to the ISO/CEN 17025. Data describing TDS samples, including visual information (pictures, bar codes), and also laboratory results (for total Hg, Mn, Cu and Se) were collected in files shared with WP6 to be used for development and testing of newly developed software "FoodCASE-Risk".

Task 9.7 Calculation of exposure doses based on results from pilot studies and food consumption databases

The first step to allow calculation of exposure doses with TDS analytical results and national food individual consumption data was to develop "TDS tailored calculation software" based on the RIVM MCRA software (Monte Carlo Risk Assessment). A new version of this software was developed by RIVM (NL) to work with a specific format of TDS data. A more detailed description is available in WP8 deliverable 8.3. All partners started by organising their individual food consumption and laboratory data into prescribed formats (MS Access DB) for the MCRA 8.1 (TDS option) calculation purposes and becoming more familiar with this software. Specific training of national experts from participating countries followed this initial phase. RIVM facilitated work with national TDS data "how to use MCRA 8.1 (TDS option)" for calculation of exposure doses that was essential for the finalization of the task. Every partner performed calculations exposure doses for mandatory chemical substances (Cu, Hg, Mn, Se) and obligatory food groups which were extended from 6 to 9 Level 1 FoodEx2 groups because all partners had the same TDS concentration data also for 3 other not-obligatory groups (Composite dishes, Eggs and eggs products, Starchy roots or tubers and products). Work was coordinated by the NIPH/SZU to produce formally similar exposure data for comparison. Work with data was not easy because consolidation of complex TDS databases of food consumption and concentration data took longer time than expected in plans (complexity of TDS tasks is very high and needs much trained experts to produce reliable results).

First of all, initial parameters for harmonized calculations of exposure doses for selected chemicals were established for all partners by coordinator. All partners calculated exposure doses for these population groups: Adults + Elderly together (age 18-74, M+F); Adults versus Elderly; Male versus Female. Calculations were performed for all 9 shared food groups but also selectively for total Hg only for group Fish and seafood. All partners tested to usage of two calculation models (OIM and LNN; INSA only OIM due to 1 day FCD). Based on result of statistical evaluation of calculations by Q/Q plots, OIM model has been preferred for production of comparable results. Uncertainty has been tested only for FCD. All partners produced standardized Excel tables where exposure doses were described as a population group mean and specific percentiles for 50 90 95 99; percentage for upper tail exposures - 97,5. Each calculation was completed by description of the staple food sources for exposure doses. These sources were compared among partners and observed differences were discussed. Estimated exposure doses were compared with official health based guidance values (EFSA or other international organizations like JECFA FAO/WHO or US EPA) to characterize health risk. These first complex results were presented by partners during WP9 meeting organized in BfR, Berlin, September 2015. Each partner summarized and commented results from all pilot studies: BfR (DE) presented results for Cu, MATIS (IS) presented results for total Hg/MeHg, EVIRA (FI) presented results for Mn, INSA (PT) presented results for Se, and NIPH/SZU (CZ) presented selected results for total Hg/MeHg and Se only for food group "Fish and seafood".

Organizational conclusion from this complex exercise is clear. It is much more effective to have specialized national/regional TDS team of experts (e.g. TDS "centre") than to use "not specialized and less trained staff".

Task 9.8 Finalization of results and publishing

Finalization of results

All partners continued with "modelling" of exposure doses for various sub-population groups, genders, and/or specific food groups to understand relationships between food consumption rates and observed intakes of selected chemical substances. Main food sources were described and discussed. It must be clearly stated that calculated exposure doses do not represent "national exposure doses" because the pilot studies were organized only as a TDS feasibility study. Therefore the full risk characterization is not possible with the project results. The basic objective of pilot studies was to test procedures and new TDS tools (e.g. software) and compare partial results amongst partners (exposure doses and principal drivers of exposure). This should serve for better understanding of the key TDS principles and its potential before any investment into the full national TDSs based on developed and tested harmonized principles.

Publications

During the last project time period the deliverable D9.4 “Scientific paper on the practices used for development of standardized protocols based on D9.3” was finalized and submitted to the journal Food, Additives and Contaminants: Part A, in February 2016 (Name: “Development of harmonized food and sample lists for Total Diet Studies in five European countries”).

The first outline of the last WP9 deliverable D9.6 “Scientific paper with key results from the TDS pilot studies based on report D 9.5” has been prepared. (Name: “Harmonization of procedures for Total Diet Studies: a pilot study in five European countries (TDS-Exposure project)”). It is intended also for the journal Food, Additives and Contaminants: Part A.

Partners involved in pilot studies agreed to publish details of harmonized TDS methodology used in particular countries and exposure assessment results specifically for model chemical substances and or food groups (e.g. total Hg/MeHg in Fish and sea food) as a part of work after finishing of this project because many technical details needs specific publications (these are not obligatory deliverables).

Task 9.9 Quality management practices

The Task was aimed at designing a quality management system for the implementation of WP9 “TDS methodology on country level- pilot studies of TDS-Exposure”. The Deliverable 9.1 “Report on generic SOPs-mandatory/recommended requirements, acceptance criteria and tolerance limits” describes all details. Theoretical background is described in documents prepared within WP5. A general procedure documents identified TDS process steps, critical control points and related SOPs, and Guidance Documents. These general procedure documents were followed by a summary of the content of the main quality documents, and in the end by outline of their mandatory/recommended requirements, acceptance criteria and tolerance limits. Requirements for the determination of the 4 model substances (total Hg, Mn, Cu and Se) selected to be analyzed in TDS samples were considered.

Some of the outputs of other TDS-Exposure WPs were integrated in the SOPs and developed guidance documents.

This work was also the starting point for WP5 quality documents for application by “TDS Centres” for future TDSs and particularly identified Critical Control Points were the basis for the development of the quality control and quality assurance activities delivered within D5.4 “Report including list of SOPs required and quality control and quality assurance activities”.

Procedures

It was recommended that each TDS pilot country will have a QMS in place consisting among other elements of a description of the TDS process with a flowchart and related SOPs. In order to harmonize the TDS process among countries a generic flowchart was developed. Documents describe:

Planning a Total Diet Study

- Definition of objectives (step 1)
- Collection of data and information (step 2)
- Allocation of resources to all TDS steps (step 3)
- Definition of population groups of interest (step 4)
- Selection of chemical substances (step 5)
- TDS Sample/Food List Preparation (step 6)
- Sampling Plan (step 7)
- Analytical Plan (step 8)
- Corrective actions (step 9)

Executing a Total Diet Study

- Preparation for food collection, sample preparation and analysis (step 10)
- Collection of samples (step 11)
- Reception of individual samples (at kitchen laboratory) (step 12)
- Sample preparation (at kitchen and analytical laboratory) (step 13)
- Chemical Analysis of Laboratory samples (step 14)
- Exposure assessment (step 15)
- Risk Characterization (step 16)
- Risk Communication and Publication of results (step 17)

Potential impact and main dissemination activities and exploitation results

The immediate impact of TDS-Exposure has been the creation of an information system for TDS and exposure assessment software for food contaminants, which will be useful for risk assessors and risk managers for existing EU TDSs. The majority of organisations currently responsible for EU TDS (CZ, FR, IE, IT, UK, ES, NL etc.) were engaged in TDS-Exposure, and one work package was entirely dedicated to building a resource (FoodCASE-Risk) to meet users' needs and managing existing data. To support accurate estimation of exposure, TDS-Exposure has improved and adapted MCRA software. MCRA includes new functionalities to address uncertainties in the TDS approach and estimate the impact of mitigation measures set by risk managers in cases where exposure levels exceed acceptable daily intakes. Practical instructions on how to use MCRA from the perspective of a harmonised approach are provided. This will impact and facilitate future decisions to be made by the Codex Alimentarius and the European risk managers based on TDS data.

Implementation of the harmonized TDS methodology led to modifications of ongoing longitudinal national TDS programme in the Czech Republic and new TDS programme in Germany. It has been signalized that the project key conclusions and recommendations (like harmonized SOPs, sampling plans, and exposure calculations by the new version of software MCRA 8.1) will be implemented in coming TDS in these countries.

TDS-Exposure has established a European TDS Centres Network including experts in the implementation of TDS methodology for countries currently without TDS. With 26 participants in 19 countries, including four countries outside the EU, TDS-Exposure benefited from participation of teams managing national TDS as well as invited experts from WHO and FAO, which have provided TDS training for more than 20 years. Together with harmonisation of TDS methods, these outputs will enable better understanding of food safety determinants, improve food safety, and help set priorities in public health policies across Europe in the long term. It will also allow monitoring of trends and verification of subsequent behaviour.

Similarly, for public health, the expected impact is better knowledge of contaminant exposure from the diet amongst populations and, as a consequence, improved protection of Europeans through regulations and other means. For EU Member States with TDS, benchmarking of exposure to food contaminants will focus efforts on the most important public health topics. For food/ agriculture producers, the European food industry, and food distribution and service sectors, the expected impacts are information about high priority food contaminants and foods contributing to population exposure, which will facilitate efficient allocation of chemical food safety budgets and better targeted risk management (e.g., Hazards Analysis of Critical Control Point).

For consumer associations and non-governmental organisations, TDS-Exposure has contributed to delivery of transparent public information on chemical food risks, helping to ensure only foods with a favourable risk assessment, measured using harmonised approaches, are available for consumption throughout the European Union, especially cross-border. For scientists, TDS-Exposure tools and resources offer a unique opportunity to test hypotheses, and integrate results with food and health research. For instance, TDS-Exposure food contamination data can be used in epidemiological studies whilst differences in exposure amongst countries can be used in epidemiological geographical studies. Economists can also use TDS-Exposure outputs in cost-benefit analyses because they provide information applicable to risk and benefit assessment.

The main dissemination and training activities carried out during the four years of the TDS-Exposure project were:

Establishment of a list of stakeholders

A priority stakeholder group was identified and stakeholder distribution list was compiled, and updated on a regular basis. This list consists of stakeholders from the most important scientific areas for TDS, i.e. monitoring of food contamination, risk assessment, risk managers and decision makers, authorities, health, agricultural and environmental sectors as well as the food and beverage industry. All partners of the consortium contributed to the compilation of the stakeholder list, which includes representatives from Europe, USA, Canada, Australia, Asia, India and Africa.

Project website

The TDS-Exposure website (www.tds-exposure.eu) was launched on April 30th 2012 and has been updated continuously since its inception. The goal of the website was to be a powerful dissemination tool and an interface between the project and the external community (stakeholders) interested in total diet studies and exposure evaluation as well as an internal project communication tool. The project website consists of a public and a private platform (restricted to registered participants). The public pages include content such as background information, objectives, descriptions of the work packages (WPs), a list of participants, contact details, and the design manual. In addition, publicly available outcomes from the project can be accessed at the website e.g. e-newsletters, presentations from the stakeholder workshops, public deliverables, links to open access publication. In total the website has had more than 14600 sessions by 8525 users. Visitors are from (top 10) USA (no partners), DE, UK, FR, India (no partners), Brazil (no partners), IT, PT, BE, SP.

The website will be maintained and updated by EuroFIR AISBL for at least two years from 31st of January 2016. After this, key information will be retained on a microsite where access to the publicly available outcomes will continue to be supported, e.g. public deliverables, links to open access publications, access to FoodCASE-Risk and the e-learning module.

e-newsletter

Four TDS-Exposure e-newsletters have been published and distributed annually to more than 3000 stakeholders globally. In addition, the project has been presented in other e-newsletters that are distributed by the partner organisations to their contact network.

TDS-Wiki

Although analytical measurements are only a small aspect of the TDS approach, the quality of analytical data, which are used for intake and risk assessment, is essential. The availability of sound analytical methods together with sufficient quality assurance tools is, therefore, important. The TDS-Exposure wiki presents this information for a limited number of priority analytes. The wiki adds value for all participants in TDS, not just those engaged in analytical measurements.

The information is presented via an on-line database/wiki-platform. It is based on links to existing information resources (official methods of analysis, scientific literature, reference material producers, proficiency testing schemes, etc.). Compounds listed in the wiki are based on those contaminants and residues identified as a priority by TDS-Exposure. Currently, this list covers compounds of relevance only within Europe. The wiki includes details on appropriate analytical methods for each contaminant, including reference methods and other available methods (key steps in each method; might be restricted due to copyright), (certified) reference materials, and details on appropriate proficiency testing schemes (PT-schemes). Other information, such as legislative requirements (both national and European), could also be included.

Currently, the University of Helsinki hosts the wiki

(https://wiki.helsinki.fi/pages/viewpage.action?pageId=117213443). The aim is to transfer it to EuroFIR AISBL, which will facilitate cross-referencing with the existing databases and would enhance dissemination via the website. The wiki shares the same IT-platform as the EuroFIR Exchange Platform, making these links technically feasible. Although the wiki is currently only accessible to TDS-Exposure partners, it will be made freely available at a later stage, probably at the same time as the website will be consolidated into a microsite. Information in the wiki serves as a knowledge base on the analytical state-of-the-art of chemicals that will be investigated in future TDS.

Information material

To ensure visibility and recognition of TDS-Exposure, the first communication activity was the creation of a project logo and background picture for any media (website, PowerPoint template, etc.). The final versions were distributed to all project partners and they were encouraged to use them; these are also available to download from the intranet and the official TDS-Exposure design manual is available from the website (public pages). During the project, a leaflet and poster were developed for dissemination to stakeholders and the general public. Both described the objectives, approach and methods as well as the expected outcome of the project. Partners distributed TDS-Exposure flyers (available in EN, FR, DE, PT, ES, IT, SE, FI) throughout Europe. This information material will remain available for download via the website.

Presentations at scientific conferences and to the general public

Partners have presented the TDS-Exposure project at numerous occasions during the lifetime of the project. The project has most commonly been presented at scientific conferences and to policy makers in the area of public health. However, it has also been presented at fairs and events targeting general public e.g. science café and open day for the public at partner organisations.

Stakeholder Workshops

Two stakeholder events were organised during the lifetime of the project to ensure the TDS-Exposure outputs met the needs of stakeholders The first TDS-Exposure stakeholder workshop *"Better data – Better decisions"* was successfully held in Brussels (BE), February 5th 2014. Its purpose was to inform a targeted group of stakeholders about progress and obtain feedback regarding needs and consult with respect to steps that could be taken to ensure project relevance. Towards the end of the project, the second TDS-Exposure stakeholder workshop *"A Harmonised Approach to European Total Diet Studies"* was also held in Brussels (BE), 8th October 2015. The aim of this second workshop was to share TDS-Exposure activities and, in particular, the outcomes of the project with all relevant stakeholders.

Publication guidelines

To assure consent and agreement regarding dissemination of project results from TDS-Exposure, publication guidelines were prepared by EuroFIR and distributed to all partners. These guidelines were intended to support scientific publication of all types whilst ensuring fairness for all beneficiaries and the quality of publications. In addition, the guidelines gave detailed information about what to do before and after dissemination.

Scientific publications (or other publications)

To date, six peer-reviewed papers have been published and at least 15 papers are planned, based on TDS-Exposure results. Further, two MSc thesis based on the work done in TDS-Exposure were

submitted and published by the relevant Universities and one PhD thesis will be submitted based on the project results.

Workshops

A programme of specialised workshops was developed from September 2013. The workshops were free-to-attend for employees and (graduate) students of TDS-Exposure Beneficiary, provided the TDS-Exposure Beneficiaries were willing to pay travel and accommodation costs. However, these specialised workshops were also open to external participants, including other EU-funded projects, provided they were: self-financing / EU27, Candidate Country (Croatia [HR], Former Yugoslav Republic of Macedonia [MK], Iceland [IS], Montenegro [ME], Serbia [RS] and Turkey [TR]) / International cooperation partner countries, which are classified as low-, mower-middle- or upper-middle-income countries not including those associated with FP7 or entities established in Hong Kong, Macao or Taiwan / Resident of a country or region without existing TDS (country/ regions with established TDS will be considered if they are new to an established TDS).

Five workshops were organised during the project: ***Workshop 1: Food collection, preparation and analysis in Total Diet Studies (Monday 28th-Tuesday 29th April 2014, FERA - UK*)** addressed several aspects of total diet study methods, which the TDS-Exposure beneficiaries had expressed a need for further in-depth information, namely food collection, preparation (e.g. cooking), sample preparation (e.g. pooling) and analysis (e.g. ICP-MS); ***Workshop 2: Food coding: LanguaL and FoodEx2 in food and dietary assessment studies (Wednesday, 30th April 2014, FERA - UK)*** addressed one specific issue the TDS-Exposure beneficiaries had expressed a need for, namely food coding, and in particular the software most commonly used for this purpose, namely LanguaL and FoodEx. This workshop also provided training for iFAAM (GA no. 312147); ***Workshop3: Food composition for nutrient and exposure assessment (Monday, 23rd-Wednesday 25th March 2015 - Science 14 Atrium, Brussels - BE)*** was delivered as part of the annual EuroFIR AISBL Food Forum; **Workshop 5: Managing TDS Data with FoodCASE and Export TDS Data (13th February 2015, ETHZ - CH)** helped likely users of the FoodCASE-Risk module to become familiarly with FoodCASE and export of data from this software to MCRA for risk assessment; and **Workshop 6: Advanced users of FoodCASE (27th March 2015 - Science 14 Atrium, Brussels - BE)** continued to address Sub-task 10.1.1 (FoodCASE-Risk documentation and training materials) and was also delivered as part of the annual EuroFIR AISBL Food Forum on 27th March 2015 in partnership with Premotec GmbH (CH).

Training exchanges

From previous experience, individual training activities (face-to-face practical learning) are important for knowledge-transfer and provide added value recognised by the European Commission. Eleven individual training exchanges were arranged during the project.

Summer schools

Three summer schools were hosted in Lisbon (Portugal) in 2013, Istanbul (Turkey) in 2014, and Helsinki (Finland) in 2015, respectively, providing a detailed overview of TDS for the delegates.

The programmes consisted of five elements: (1) basic foundations of total diet studies, (2) design and planning, (3) sample preparation and analysis, (4) exposure assessment and publication, and (5) quality management, each including aspects of theoretical and practical learning. Basic foundations of total diet studies introduced TDS and planning as well as application of food consumption data in dietary assessment of contaminants; Design and planning explained development of a food list, criteria for selecting chemical substances and populations of interest, sampling plans and collection of foods for TDS; Sample preparation and analysis looked at the culinary preparation of foods (as

eaten) and a range of analytical issues, and how food databases are created and managed, and food information coded accurately; Exposure assessment and publication explored data management using FoodCASE, exposure and risk assessment using MCRA, and how data should be processed and used appropriately; and Quality management described quality control approaches and documentation standards.

The objectives of each Summer School were to introduce total diet studies, generally, and dietary exposure to contaminants, or more specifically to: (1) Explore scientific and technical knowledge underpinning TDS for exposure assessment, (2) Provide insight into methods and approaches, and the quality of data and (3) Enable students to apply this knowledge in their expert field (e.g. public health, food technology, research).

These events included more than 27 lectures given by experts from TDS-EXPOSURE as well as international organisations (e.g. FAO and EFSA). In total, 59 people participated including eight external participants from Cyprus, Ukraine, Italy, Turkey, Finland and Denmark.

e-Learning

The TDS-Exposure e-learning module was developed to help those engaged in and/ or using results from total diet studies (TDS) to understand where data come from, their strengths and limitations, and the use of TDS data in risk assessment. It is intended only as an introduction to TDS since this approach for determining exposure to contaminants is complex, and proper use of the data requires an intimate understanding of both the theory and practice at the national level. Currently, the e-learning can be accessed via http://www.topshare.com/apps/tdsexposure/html/index.html, but it will be moved to the TDS-Exposure website http://tds-exposure.eu once testing is completed. The module will remain accessible via the website, or the microsite, for as long as members of the European TDS Network, established by TDS-Exposure, feel it is relevant and sufficiently up-to-date.

O**ther activities**

Results produced over the four years have also been presented in media e.g. national press and radio in several European countries as well as social media via a Twitter account (@TDS_Exposure). In addition, the project has been presented in a news item at the home page of the majority of the 26 partner organisations that participated in TDS-Exposure.