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BELGIAN POPULATION EXPOSED TO FURAN : FROM ANALYTICAL DEVELOPMENTS TO RISK ASSESSMENT

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Abstract

Since the acrylamide incident in 2002, food authorities such as EFSA, FDA, FAO and Belgian FFSA paid more attention to the food borne contaminants such as furan, glycidyl esters or nitrosamines. As a consequence, authorities support scientific initiatives to gather information about these toxicants. The thesis was conducted in this framework and was specifically dedicated to the furan issue.

The fundamental concept behind this work on furan was to include analytical developments, to determine its occurrence in Belgian food and to carry out a risk assessment of the Belgian population. The first section was dedicated to the development of a high-sensitive analytical method able to report sub-parts-per-billion (ppb) levels in foodstuffs with the aim to limit the number of unreported results below of the limit of quantification (LOQ). A HS-SPME-GC-MS method has been developed and optimized using the experimental design approach. The developed method has been validated to fulfill the requirements of the European Commission decision regarding the validation of analytical method (2002/657/EC).

The second section was dedicated to the achievement of a contamination survey of the foodstuffs available on the Belgian market with a restricted number of samples (n=496). A specific sampling plan was designed to cover every food matrices with regard to the more consumed and/or contaminated items. The analytical method developed in the first section was applied and 78% of results were reported above LOQ. We concluded to a ubiquitous contamination of our food chain with specially high levels in coffee, roasted and long-time cooking foodstuffs.

The third section was dedicated to the assessment of the risk linked to the furan ingestion by the Belgian population. A methodology involving the estimation of the furan daily intake (by both deterministic and probabilistic approach) and the calculation of the Margin of Exposure (MoE) was applied to 3 sub-populations namely Adults, Children and Toddlers. The adults and children assessments highlighted that almost none have a "High concern risk level" (MoE < 100), that the risk for health tends to be low (median MoE for adults 5486; median MoE for children 5079), and that the risk for children is slightly higher than for adults. For infants, the assessment showed a higher risk (median MoE 817) compared to children and adults. However, this finding has to be tempered by the current limited knowledge of the furan toxicity for toddlers. In addition, the size of the datasets available for this work was low and limited to ready-to-eat baby foods, which are known to be more contaminated than home-made baby food. It gives however the first benchmark for Belgian infants exposed to furan. Thus, developing consumption survey for infants that are harmonized at European level, and basic research on furan toxicity for toddlers are necessary.

Résumé

Depuis 2002 et le scandale médiatique lié à la présence d'acrylamide dans l'alimentation, l'attention des agences sanitaires telles que l'EFSA, la FDA, la FAO et l'AFSCA s'est focalisée sur les contaminants se formant lors de la préparation des denrées alimentaires tels le furane, les esters glycidiques ou les nitrosamines. En conséquence, ces autorités ont encouragé les recherches scientifiques visant à recueillir des informations à propos de ces composés. Cette thèse s'inscrit dans ce mouvement et est dédiée au cas du furane.

Le but de cette thèse est de développer une méthode d'analyse capable de déterminer le niveau de contamination des denrées alimentaires présentes sur le marché belge et finalement d'utiliser ces données pour réaliser une évaluation du risque pour la population belge. La première partie est dédiée au développement d'une méthode d'analyse capable de mesurer des niveaux inférieurs à la part par milliard, et ce dans le but de limiter le nombre de résultats inférieurs aux limites de quantification (LOQ). Une méthode par HS-SPME-GC-MS a été développée à cette fin et optimisée, via la théorie des plans d'expérience. Par la suite, cette méthode a été validée en respectant les recommandations de la décision européenne 2002/657/EC concernant la validation des méthodes d'analyse.

La seconde partie est dédiée à l'évaluation du niveau de contamination des denrées alimentaires présentes sur le marché belge, avec comme limitation un faible nombre d'échantillons (n=496). Un plan d'échantillonnage couvrant toute la chaine alimentaire et tenant compte des fréquences de consommation et des niveaux de contamination a été construit. La méthode d'analyse développée dans la partie précédente a été utilisée pour mesurer les niveaux et nous avons obtenus 78% de résultats supérieurs à la LOQ. Cet évaluation a aussi montré que presque toute la chaine alimentaire est contaminée, et que les aliments rôtis ou ayant de longs temps de cuissons sont les plus contaminés.

La troisième partie est dédiée à l'évaluation du risque lié à l'ingestion du furane par la population belge. Une méthode impliquant l'estimation de la dose ingérée quotidiennement (aussi bien par une approche déterministe que probabiliste) ainsi que le calcul de la Marge d'Exposition (MoE) a été appliqué à 3 sous-populations, soit les adultes, les enfants et les nourrissons. Les évaluations réalisées pour les adultes et les enfants montrent que presque personne ne fait partie du groupe à haut risque (MoE < 100), que le risque pour la santé est faible pour la majorité de la population (médiane de la MoE des adultes : 5486 et des enfants : 5079), et que le risque pour les enfants est très légèrement plus élevé que pour les adultes. En ce qui concerne les nourrissons, l'évaluation montre que le risque est beaucoup plus élevé (médiane de la MoE : 817). Néanmoins, ce résultat doit être nuancé car la toxicité du furane pour les nourrissons est mal connue, la taille des jeux de données est faible, et seul des aliments pour bébés prêts à la consommation, qui sont connus pour être plus contaminés que les aliments préparés à la maison, ont été pris en compte. Il donne cependant une première référence pour l'exposition des nourrissons belges au furane.

Dès lors, la réalisation d'enquêtes de consommation, harmonisées au niveau européen, chez les nourrissons et la recherche fondamentale sur la toxicité furane pour les nourrissons sont nécessaires.

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Finally, I am very thankful to my mother, my sister and all my related for their encouragements, their constant support and to have borne me all over these years.

Thank you

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1. Introduction

1.1. Objectives of the thesis

Since the acrylamide incident in 2002, international food authorities (e.g.: European Food Safety Agency *aka* EFSA, United States Food and Drugs Administration *aka* US-FDA, or Food and Agriculture Organization *aka* FAO) paid more attention to the food borne contaminants such as furan, glycidyl esters, 3-monochloropropane-1,2-diol (3-MCPD) or nitrosamines. In 2004, a report published by the US-FDA highlighted the global occurrence of furan in thermally treated food with a specific focus on canned food, baby food and coffee that appeared to be the most contaminated items (US-FDA, 2005; EFSA, 2005). As a consequence, food authorities decided to launch monitoring programs and to support scientific initiatives to gather information about furan toxicity, food contamination levels, formation pathways, elimination process, daily intake and risk assessment for the human population.

This thesis finds its origin in that proactive movement engaged by the food control authorities and focuses on a lone processing contaminant: the furan (European Recommendation 2007/196/EC). The goal of this study is to develop an analytical methodology to be able to measure the furan occurrence in Belgian food and assessing the risk for the Belgian population.

First is the development of an automated analytical methodology (Chapter 2) especially optimized through the experimental design theory to achieve the highest possible sensitivity and to fulfill the European Commission requirements regarding the validation of analytical methods.

Second is the development of a specific sampling plan (Chapter 3) able to estimate the occurrence of furan through the complete food chain with a restricted number of samples; and in a succeeding time to identify the most critical items.

Third is the achievement of a complete assessment of the risk, from estimation of the daily intake to management proposals, for three Belgian sub-populations (Chapter 4) namely adults, children and toddlers. To achieve that purpose, the estimation of the daily intake only involves Belgian contamination and consumption data, and is performed by two approaches a deterministic and a probabilistic. Additionally, it is also needed to determine which sub-population displays a potential concern for health.

1.2. Identity

Furan or 1,4-epoxy-1,3-butadiene (CAS: 110-00-9) is a five membered heterocyclic aromatic compound belonging to the class of unsaturated cycloether with the general formula: C_4H_4O and the structure displays in the Figure 1.

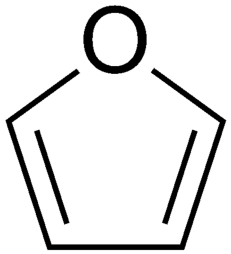

Figure 1 : Furan structure

Furan is a 5 atoms' ring containing 4 carbons and 1 oxygen. In this molecule, each carbon bears one hydrogen atom. The fours carbon atoms form a four centers-four electrons π system connected at both ends to an oxygen atom bearing two π electrons to form an aromatic system. This colorless chemical compound has a low molecular weight (68 g * mol^{-1}) and presents the following physicochemical characteristics:

- Melting point: -86°C
- Boiling point: 31°C
- Density (20°C): 0.936 kg * l^{-1}
- Vapor pressure (20°C): 63.6 kPa
- Water solubility (20°C): 10 g * l^{-1}

Moreover, furan aromaticity induces a molecular flat geometry, and an easy access to the π electrons for chemical reaction purposes.

This chemical was first intentionally synthetized by Heinrich Limprich in 1870 (Limpricht, 1870) through the decarboxylation of furfural ($C_5H_4O_2$; Figure 2), which is an aldehyde substituted furan naturally found in various vegetables (e.g. corncobs, sawdust and oat). Nowadays, furan is industrially synthetized through catalytic oxidation of 1,3-butadiene or catalytic decarboxylation of furfural (Figure 3).

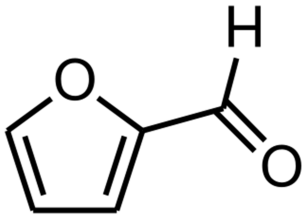

Figure 2 : Furfural structure

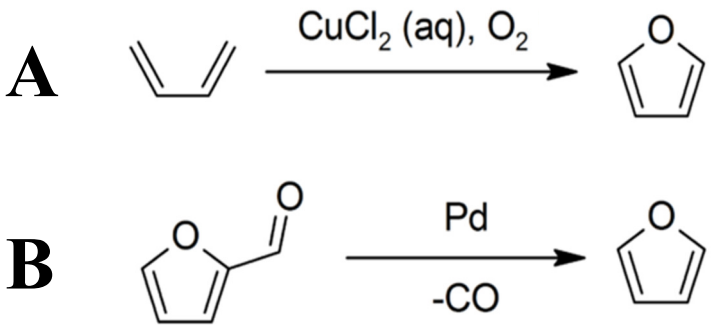

Figure 3 : Industrial synthesis of furan through catalytic oxidation of 1,3-butadiene (**A**) and catalytic decarboxylation of furfural (**B**)

Furan is a building block used in many synthetic pathways in industry and laboratory (NTP, 1993). Here is a non-exhaustive list of its main applications:

- Synthesis of substituted furan such as alkylation, halogenation or sulfonation;
- Synthesis of temperature resistant laminated linear polymers;
- Synthesis of dishwashing detergents without phosphor and/or nitrogen;
- Synthesis of lackers and resins;
- Diene source in pericyclic reactions like Diels-Alder.
- …

Additionally, furan is the base compound of a large number of derivatives compounds referred as ***Furans***. This family contains chemicals with various properties such as reaction solvents (e.g. Tetrahydrofuran), flavor compounds (e.g. furfural), pollutants (e.g. polychlorodibenzofurans), …

In 1979, the presence of furan and its derivatives in heated treated food was highlighted by Maga (Maga, 1979), who put forward their relation with food flavor. For 30 years, the adverse effect of furan was studied (Paragraph 1.3). It led to its classification as narcotic by the American National Academy of Science in 2000 (NAS, 2000) and as

"Possibly Carcinogenic to Humans" (group 2B) by the International Agency for Research on Cancer (IARC) in 1995 (IARC, 1995).

1.3. Toxicology

The toxicology described in this paragraph is limited to the toxicity of furan in acute and chronic timelines. Only chronical toxicity assessment will be studied in the framework of this thesis. The proposed risk assessment focuses on intoxication through daily diet and not punctual incident (acute toxicity). Additionally, it is important to note that to date the toxicity for humans has only been estimated through *in vitro* and rats studies.

1.3.1. Acute toxicity

Furan acute toxicity has been studied for nearly 100 years. First report was from 1925 (Koch et Cahan, 1925) when Koch and Cahan made intravenous injection of furan (over 80 mg * $kg_{BodyWeight}^{-1}$) to dogs until they die by paralysis of the medulla.

Recently, from 80s to 90s, several research groups (Egle and Gochberg, 1979; Terrill et al., 1989; Kedderis et al., 1993; NTP, 1993; McClellan et Henderson, 1995; Kedderis et Held, 1996; Garcia et James, 2000) worked on the determination of lethal doses for 50 percent of an animal population (usually rats, mice or rabbits) through a unique inhalation (LC_{50}: 20 mg * m^3_{air} for rats) or ingestion ($LD_{50:}$ 5.2 mg * $kg_{bodyburden}^{-1}$ for rat). These teams also reported the observed non-lethal adverse effects for acute doses which are namely: analgesia, dilatation of blood vessels, decrease of the blood pressure and respiratory distress. After animal sacrifice, no team observed lesions related to the furan administration within the autopsy.

1.3.2. Chronic toxicity

On the chronic side, the furan toxicity is more complex and is widely studied since the last decade. First report was published by the American National Toxicology Program (NTP) in 1993 who synthetized the current toxicological knowledge (NTP, 1993). This report was based on *in vivo* chronic exposure on rats and highlights hepatic lesions (bile duct hyperplasia, cholangiofibrosis, cytomegaly, degeneration of hepatocytes and nodular hyperplasia of hepatocytes), kidney lesions (tubule dilatation and necrosis of tubule epithelium), thymus atrophy, testicular or ovarian atrophy and carcinogenicity (NTP, 1993).

These findings led the NTP to classify furan as 'Reasonably anticipated to be a human carcinogen'. In 1995, the International Agency for Research on Cancer (IARC) used the NTP information to classify furan in the group 2B, which means 'possibly carcinogenic to humans. Five years later, the American National Academy of Science (NAS) also classified it as a narcotic (NAS, 2000). Afterwards, several independent toxicological studies demonstrated that the furan carcinogenicity was linked to its metabolisation by hepatic enzymes (Kedderis et al., 1993; Chen et al., 1995; Fransson-Steen et al., 1997; Peterson et al., 2000; Peterson et al., 2005; Peterson et al., 2006; Jun et al., 2008; Bakhiya et al. 2010; Hamberger et al., 2010). This metabolic pathway gives more than 10 metabolites and, the latest studies, underlined that furan carcinogenicity is linked to its major primary metabolite, the *cis*-2-butene-1,4-dial (Figure 4; Chen et al., 1995; Peterson et al., 2000; Peterson et al., 2005; Peterson et al., 2006; Lu et al., 2009; Terrell, 2012). This metabolite formed through the hepatic oxidation of furan by cytochrome P-450 is known to induce hepatocellular tumor, mononuclear cell leukemia and cholangiocarcinomas in rats liver through an oxidative stress mechanism (Hickling et al., 2010a,b).

Figure 4 : *cis*-2-butene-1,4-dial structure

On the toxicodynamic side, the low polarity of furan allows to easily cross biological membranes, with the liver as main target. In 1991, Burka and coworkers (Burka et al., 1991) pointed out that about 82 % of furan is eliminated by rats within 24 hours: 40 % by respiration, 22 % in feces and 20 % in urine.

On the quantitative side, US-EPA proposed a Reference Dose for Chronic Oral Exposure (RfD) based on NTP studies (US-EPA Integrated Risk Information System[1]). This dose was calculated according to a 13 weeks rats gavage aimed at causing hepatic lesions. They estimated the Lowest Observed Adverse Effect Level (LOAEL) for rats to be 4 $mg*(kg_{body\ weight}$ and $day)^{-1}$ and the No Observed Adverse Effect Level (NOAEL) was fixed at 2 mg * $(kg_{b.w.}*day)^{-1}$. After the application of precaution factors the US-EPA recommended a $RfD_{chronic\text{-}oral}$ of 1 µg * $(kg_{b.w.}*day)^{-1}$ for humans. Nevertheless, this approach does not fit to

[1] http://www.epa.gov/iris/subst/0056.htm

carcinogenic compounds that have no threshold value. Therefore, a benchmark dose for 10 % extra risk (BMD_{10}) of hepatocellular adenomas and carcinoma and its 95% lower confidence limit ($BMDL_{10}$) was determined: 0.96 mg * $(kg_{b.w.}*day)^{-1}$ (Moser et al., 2009; Benford et al., 2010; Carthew et al., 2010; Williams et al, 2011).

1.4. Formation

Furan can be formed in food through multiple pathways of formation. Early studies highlighted the reduction of amino acids and carbohydrates through the Maillard reactions, as well as the oxidation of the unsaturated fatty acids, triglycerides, carotenoids and ascorbic acid as the major formation pathways (Maga, 1979; Perez and Yayalayan, 2004; Fan et al., 2005; Yayalayan, 2006; Limacher et al., 2007; Varelis et Hucker, 2011). Recent works tried to model the industrial and home cooking using food constituents mixtures, waxy corn starch or real matrix like hazelnut (Senyuva et Gokmen, 2007; Fan et al., 2008; Limacher et al., 2008; Owczarek-Fendor 2010a, 2010b, 2011a, 2011b; Van Lancker et al., 2011; Owczarek-Fendor 2012). They demonstrated that real processes are more complex because they involve several pathways crossing each other, with mixtures of precursors and the need of initiators (e.g.: chemicals, temperature or pH). In this way, Limacher and coworkers (Limacher et al., 2007, 2008) pointed out that the furan formation yield through the carbohydrates degradation is related to the sugar nature (glucose, fructose, arabinose, …) and is enhanced in presence of amino acids. Interactions between the furan precursors such as sugar, proteins, ascorbic acid and lipids (Lancker et al., 2009; Owczarek-Fendor et al., 2011b, 2012) have significant influences on the formation yield (as exposed in the paper presented in the paragraph 1.4.1) as well as pH (variable effect depending upon the nature of the precursor), heating conditions (roasting give higher yields than dry heating) or phosphate content (usually increase the formation yield; Owczarek-Fendor et al., 2010a). Similar conclusions have been reported for the amino acids degradation in presence of carbohydrates (Van Lacker et al., 2011; Owczarek-Fendor et al., 2011a) and for lipids oxidation (Figure 5) in presence of antioxidants and proteins (Owczarek-Fendor et al., 2010b, 2011b).

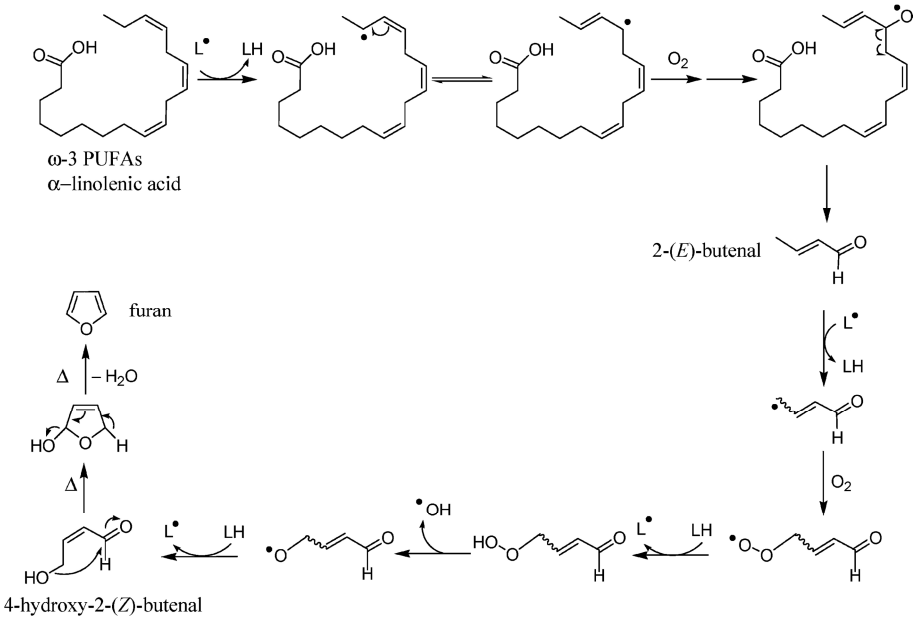

Figure 5 : Formation pathway of furan through the lipid oxidative way (Owczarek-Fendor et al., 2011b

1.4.1. Related paper: Furan formation in food model with regard to the interactions between the precursors

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Furan formation in starch-based model systems containing carbohydrates in combination with proteins, ascorbic acid and lipids

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ABSTRACT

Formation of the "possibly carcinogenic" furan during thermal treatment of a starch-based model food system containing selected sugars alone and in the presence of proteins, ascorbic acid and lipids, respectively, was investigated. The results showed that in starch gels containing various sugars significantly more furan was formed at pH 6 than at pH 4. Moreover, addition of whey proteins enhanced the generation of furan considerably at both pH values tested. In acidic conditions, no significant difference was observed between the amounts of furan found in a starch–carbohydrate–ascorbic acid model system and those formed in a starch-based samples containing only ascorbic acid. Addition of fresh lipids did not affect furan formation. However, when oxidised soybean oil was applied, the generated amounts of furan were higher than expected from the sum of furan found in the separate starch–carbohydrate and starch–lipid samples. Interestingly, the most efficient carbohydrate in furan generation, among the sugars tested, at pH 6, was lactose, especially when heated in the presence of proteins. This is the first report on the generation of furan from lactose.

1. Introduction

Studies performed by the Food and Drug Administration (FDA) in 2004 indicated that furan, classified as "possibly carcinogenic to humans" by International Agency for Research on Cancer (1995), is present in a number of heat treated foods especially when heating was performed in closed containers (US Food and Drug Administration (FDA), 2004). The widespread occurrence of furan in food can be associated with its multiple ways of formation, including degradation of vitamin C, polyunsaturated fatty acids, carbohydrates, either alone or in the presence of proteins (Maillard reaction) and carotenoids as proven by several reports in the literature (Becalski & Seaman, 2005; Crews & Castle, 2007; Fan, 2005a; Limacher, Kerler, Conde-Petit, & Blank, 2007; Limacher, Kerler, Davidek, Schmalzried, & Blank, 2008; Maga, 1979; Perez-Locas & Yaylayan, 2004).

Early studies of Maga (1979) have suggested the thermal degradation of carbohydrates as the primary source of furan. Recently, Limacher et al. (2008) have shown that glucose, fructose, arabinose and erythrose generated around 70–340 μmol furan/mol when investigated under roasting conditions and identified arabinose and erythrose, respectively, as the most and the least effective furan precursor. As in the case of ascorbic acid, furan formation from sugars was less efficient under pressure-cooking conditions than under dry-heat treatment (Limacher et al., 2008). However, ascorbic acid, compared with sugars was the most efficient furan precursor under roasting conditions (Limacher et al., 2007, 2008). Addition of amino acids, such as alanine, threonine and serine to the carbohydrate-containing model systems generally enhanced furan formation under roasting conditions. The most intense effect was observed for glucose (Limacher et al., 2008).

Mechanistic studies, applying the CAMOLA approach, performed by Limacher et al. (2008), have shown that under roasting conditions, furan originates only from the intact carbohydrate skeleton, while under pressure-cooking conditions it is also formed as a result of carbohydrate fragmentation and recombination. Similar studies with labelled precursors under both conditions of heating

Abbreviations: WC starch, waxy corn starch; SBO, soybean oil; AA, ascorbic acid.

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were carried out in Maillard reaction systems as well. The results indicated, that in the presence of amino acids, such as alanine, threonine and serine, furan can also be formed from the reaction of glycoaldehyde, a carbohydrate degradation product, and acetoaldehyde, originating from amino acid degradation (Van Lancker, Adams, Owczarek-Fendor, De Meulenaer, & De Kimpe, 2011). This reaction was favoured mainly under dry roasting conditions (Limacher et al., 2008).

In other CAMOLA experiments (Limacher et al., 2007), sugars were investigated in combination with ascorbic acid under dry-heating conditions. Results for unlabelled ascorbic acid and fully labelled glucose have indicated that in an equimolar mixture of both, furan has been formed, either from ascorbic acid or from glucose, not involving recombination of fragments of glucose or ascorbic acid (no $^{13}C_1$-, $^{13}C_2$- or $^{13}C_3$-furan was generated). However, this study did not exclude furan formation by recombination of ascorbic acid fragments from C-3 to C-6 due to the unavailability of fully labelled ascorbic acid.

Literature data indicated that furan formation from sugars is influenced by changes in pH (Fan, 2005a; Limacher et al., 2008). Limacher et al. (2008) have shown that under acidic conditions (pH 4) very low (<1 μmol/mol) amounts of furan were formed from buffered solutions of glucose, fructose, arabinose, erythrose and of mixtures of glucose with alanine and threonine/seronine, while in the same model systems at pH 7, significantly more furan was generated ranging from 2 to 17 μmol/mol. The enhancing effect of neutral or alkaline pH on furan formation was explained by the fact that at such conditions also carbohydrate fragmentation and enolisation, processes initialising furan formation, are favoured. Fan (2005a), however, observed the enhancing effect of higher pH only for glucose, while in the case of fructose and sucrose, furan formation was significantly reduced at pH 7 compared to pH 3, when all sugars were present in buffered solutions and autoclaved for 25 min at 121 °C.

Limited studies in more realistic food model systems were performed to evaluate the impact of carbohydrate degradation upon heating on furan formation and to define the importance of other components, including furan precursors. Only some studies were carried out with real food samples, such as hazelnuts (Senyuva & Gokmen, 2007), pumpkin puree and carrot juice (Limacher et al., 2007) and orange and apple juice (Fan, 2005b).

Recently, we created a more realistic model food system based on starch, simulating baby foods. This model system was used to investigate furan formation from vitamin C (Owczarek-Fendor et al., 2010a), lipids (Owczarek-Fendor et al., 2010b) and mixtures of lipids, antioxidants and/or proteins (Owczarek-Fendor et al., 2011). The present study was developed to investigate furan formation from sugars in the same model system, as sugars have also been shown to be an efficient source of furan (Fan, 2005a; Limacher et al., 2008; Perez-Locas & Yaylayan, 2004). Also the interaction between sugars and different furan precursors, such as proteins, ascorbic acid and lipids, were investigated in this model system.

2. Materials and methods

2.1. Reagents

Citric acid (monohydrate, 99.5 + %), L-(+)-ascorbic acid (AA) (99 + %) and disodium hydrogen phosphate (dihydrate, 99.5 + %) were purchased from Chem-Lab NV (Zedelgem, Belgium). Furan (99 + %), D_4-furan (98%) and D-(+)-glucose (⩾99.5%) were supplied by Sigma–Aldrich (Steinheim, Germany). D-(−)-fructose (p.a.), D-(+)-sucrose (p.a.) and D-sorbitol (p.a.) were purchased from Acros Organics (Geel, Belgium). D-(+)-lactose (monohydrate, highest purity) was provided by VWR (Leuven, Belgium). Methanol (Picograde®) was supplied by LGC Promochem (Molsheim, France). Milli-Q water was prepared using a Millipore system (Brussels, Belgium). Whey protein isolate (Lacprodan® DI-9224) was provided by Acatris Food Belgium (Londerzeel, Belgium). Cold swelling native waxy corn starch (WC starch) was kindly offered by Cargill (Haubourdin, France). Diacetyl tartaric (acid) ester of monoglyceride (DATEM) was kindly offered by Palsgaard (Juelsminde, Denmark). Soybean oil (SBO) (Leisure brand; 100%) was obtained from a local supermarket.

2.2. Sample preparation

2.2.1. General conditions of sample preparation

Heating experiments were performed using a model food system that mimics a baby food. Therefore, the components of this system and their concentrations were chosen to be similar to baby foods. Generally, the created model system contained 15% (w/w) of cold swelling WC starch and 0.5% (w/w) of monosaccharide equal to 2.78 mmol carbohydrate per 100 g of sample prepared at pH 6 or 4 with a previously described (Owczarek-Fendor et al., 2010a) 0.56 M phosphate–0.44 M citric acid buffer. As in this study, disaccharides and a sugar alcohol were used, besides monosaccharides, their concentration in the model systems were expressed in mmol/100 g sample and were kept constant for all experiments.

Preparation of starch-based samples containing sugars in combination with whey proteins, ascorbic acid or lipids is described in the next sections. The WC starch gel was prepared by slow addition of the starch powder to a buffer (pH 4 or 6) in the appropriate ratio, while mixing intensively and the obtained mixture was homogenised using a dispersing instrument (Ultra-Turrax T 25 digital, IKA, Staufen, Germany) (approx 3 min, 11000 rpm). The same buffer was used to prepare solutions of glucose, fructose, sucrose, lactose and sorbitol at a concentration of 0.278 mmol/ml. The resulting starch gels and carbohydrate/sugar alcohol solutions, prepared at the same pH, were mixed at a 9/1 (w/v) ratio, unless stated otherwise.

Afterwards, similarly as in the previous studies (Owczarek-Fendor et al., 2010a, 2010b, 2011), 15 g of the final starch-based sample were transferred to a headspace vial (HS vials for CTC PAL, 20 ml, clear glass, DIN-crimp neck, Grace Davison Discovery Sciences, Lokeren, Belgium) sealed with a magnetic crimp cap (gold, with Silicone/PTFE liners, Grace Davison Discovery Sciences) and heated for 30 min at 130 °C ± 0.5 °C in an oil bath (deep-fryer, Fritel 2505, Belgium), equipped with a thermometer (Testo 735-2, Ternat, Belgium) and with a stirring mechanism ensuring a homogeneous temperature distribution. One batch of samples consisted of 10 vials held in a metal rack. Immediately after heating, samples were cooled in an ice-water bath for minimum 30 min. Afterwards, the samples were mixed by means of a vortex shaker and they were placed in a cold room at 4 °C where they were prepared for the SPME–GC–MS analysis.

2.2.2. Sugars in combination with proteins

A whey protein (5.5%, w/w)–starch (16.7%, w/w) suspension was prepared in the phosphate-citric acid buffer at pH 4 and 6. Similarly as in our previous studies (Owczarek-Fendor et al., 2010a, 2010b, 2011), whey proteins were used due to their good solubility at the pH values tested. These suspensions were mixed with previously prepared, at the same pH, buffered solutions of glucose, fructose or lactose (0.278 mmol/ml) at a 9/1 (w/v) ratio resulting in a final sample containing 15% (w/w) WC starch, 5% (w/w) proteins and 2.78 mmol carbohydrate/100 g. The final samples were heated (30 min at 130 °C) as described before and analysed for furan content.

2.2.3. Sugars in combination with ascorbic acid

This experiment was performed with a starch-based (15%, w/w) system containing sugars (2.78 mmol of glucose, fructose or lactose/100 g sample) in combination with ascorbic acid (50 mg AA/100 g sample equal to 0.28 mmol AA/100 g) at pH 4 and 6. For this purpose, buffered starch gels (17.65%, w/w) and carbohydrate solutions (0.278 mmol/ml) were prepared as described above. The same buffer was also used to prepare a 56 mM solution of ascorbic acid. Subsequently, the obtained starch gel, carbohydrate and ascorbic acid solutions, prepared at the same pH, were mixed at a 17/2/1 w/v/v ratio. Additionally, starch-based samples containing 0.28 mmol AA/100 g without sugars were prepared as well. The final samples were heated (30 min at 130 °C) as described before and analysed for furan content.

2.2.4. Sugars in combination with lipids

In this experiment furan formation was investigated in starch-based samples (10%, w/w, WC starch) containing carbohydrates (2.78 mmol of glucose, fructose or lactose/100 g) and fresh or oxidised soybean oil (5%, w/w). The starch suspension (17.65%, w/w) and carbohydrate solutions (0.278 mmol carbohydrate/ml) were prepared with phosphate-citric acid buffer at pH 6, as described above. In parallel, fresh and oxidised (150 g of oil incubated for 14 days at 60 °C) soybean oils were used to prepare emulsions. For this purpose, a buffered mixture of oil (15%, w/w) and DATEM (3%, w/w) was homogenised by means of an APV Lab 2000 two-stage homogeniser (APV, An SPX Brand, Erpe-Mere, Belgium) at a total pressure of approximately 250 bar (first stage approx. 200 bar + second stage 50 bar) as described by Owczarek-Fendor et al. (2010b, 2011). The resulting starch gel, carbohydrate solution and oil-in-water emulsion were mixed at a 17/3/10 (w/v/w) ratio by means of a vortex shaker. The final samples were heated (30 min at 130 °C) as described before and analysed for furan content.

The oxidative status of the oils used was defined by the *p*-anisidine value (pAV) measured according to the American Oils Chemists' Society (AOCS) Official Method Cd 18–90 (AOCS, 1989) and by the peroxide value (pV) determined according to the method of Lea and Wheeler (Gray, 1978).

2.3. Furan analysis

2.3.1. Sample preparation

All handlings, such as preparation of the stock, standard and working solutions, sample preparation and headspace SPME–GC–MS analysis have been described in detail before (Owczarek-Fendor et al., 2010a). Briefly, approximately 1 g of cooled and mixed sample was quickly transferred to an analytically weighed vial containing 0.5 ml of Milli-Q water, spiked with 50 μl of D_4-furan working solution in water (approximately 70 pg/μl) and sealed with a crimp cap. This spiked sample was mixed, weighed analytically and analysed directly by headspace SPME–GC–MS.

In parallel, a calibration curve was prepared daily before each analysis by analysing aqueous solutions of furan (1 ml) at exactly-known concentrations ranging approximately from 0.7 to 35 ng/ml in the 20 ml headspace vials spiked with a fixed volume (50 μl) of D_4-furan working solution in water (approximately 70 pg/μl).

2.3.2. SPME–GC–MS analysis

The method for the quantitative determination of furan used in these experiments was described in our previous studies (Owczarek-Fendor et al., 2010a, 2010b). Shortly, the samples were extracted by headspace Solid Phase Microextraction (SPME) followed by GC–MS analysis. The SPME fibre was a CAR-PDMS fibre (75 μm coating phase of Carboxen®-Polydimethylsiloxane) supplied by Supelco (Bornem, Belgium). Extraction of the samples was performed at 16 °C for 15 min. The GC–MS-analyses of the SPME extracts were performed with a Trace GC 2000 (Interscience, Breda, The Netherlands) gas chromatograph coupled to an ion-trap mass spectrometer PolarisQ (Interscience), and equipped with a Varian CP-PoraBOND Q capillary column (25 m × 0.32 mm × 5 μm). The analyses were performed in selective ion monitoring (SIM) mode and the limit of detection (LOD) was 0.18 ng/g. Quantification was based on MS signals at *m/z* 68 for furan and *m/z* 72 for D_4-furan. The following qualifiers were used: *m/z* 39 for furan and *m/z* 42 for D_4-furan.

2.4. Statistical analysis

The experimental data were analysed by analysis of variance (ANOVA) and significant differences among means from triplicate analysis ($P < 0.05$) were determined by Tukey's multiple range tests using the statistical analysis system, SPSS 16.0 for Windows.

3. Results and discussion

This study is a further elaboration of our previous studies on furan formation from vitamin C, lipids and proteins, and combinations thereof (Owczarek-Fendor et al., 2010a, 2010b, 2011), in a starch-based model system similar to baby food. The present paper focuses on the furan formation as a result of thermal degradation of sugars and/or their interactions with proteins, vitamin C or lipids. In a first series of experiments, the formation of furan was compared after heating of starch-based samples containing a mono-, or disaccharide, or a sugar alcohol, at two pH values, pH 4 and pH 6 (Table 1). The sugar alcohol, sorbitol, was selected for investigation as well since it is used as a sweetener for diabetics and in food canning (Belitz, Grosch, & Schieberle, 2009b). It is also found in some fruits, such as pears, apples and plums (Belitz, Grosch, & Schieberle, 2009a).

From the obtained results it can be noticed that significant amounts of furan were generated during the thermal decomposition of sugars alone, known as caramelization. Caramelization, a non-enzymatic carbohydrate browning reaction, commonly occurs in food processing (Lee & Lee, 1997), and results in the formation of a broad range of non-volatile and volatile compounds including furan and its derivatives (Maga, 1979). The results in Table 1 show that all sugars and the sugar alcohol tested were able to generate furan. For most samples tested, furan formation was higher at pH 6 than at pH 4, probably due to favoured carbohydrate fragmentation and enolisation at higher pH. It should be stressed, however, that the total amounts of furan found in the tested samples originated from both carbohydrate/sugar alcohol and starch matrix degradation (no carbohydrate/sugar alcohol-containing system), which also increased at higher pH. Therefore, the net formation of furan proved to be higher at pH 6 only for glucose, fructose and lactose, while for sucrose, the opposite trend was observed. Furan formation from sorbitol was not influenced by the change in the pH value and it was rather limited (below 1.6 ppb). Regarding sucrose, this result could be explained by an enhanced hydrolysis of this carbohydrate at pH 4 resulting in the formation of glucose and fructose units. Fan (2005a) has also reported significantly higher amounts of furan for sucrose at pH 3 compared to pH 6, however, this author has observed the same trend also for fructose, which was in contrast to the results in Table 1. In his study, increased furan formation upon increasing the pH values was observed only for glucose. On the other hand, Limacher et al. (2008) have found significantly more furan at pH 7 than at pH 4 for all tested sugars (glucose, fructose, arabinose and erythrose).

Table 1
Furan formation in buffered starch-based [15%, w/w (WC) starch] systems containing various carbohydrates/sugar alcohol (2.78 mmol/100 g sample). Samples were heated at 130 °C for 30 min.

pH	Carbohydrate/sugar alcohol	Experimental furan (ppb)[a]	Furan (ppb)[b] (calculated)
4.0	No carbohydrate/sugar alcohol	1.59 ± 0.03 a	0
	Glucose	4.54 ± 0.24 bc	2.95 ± 0.24
	Fructose	8.32 ± 0.84 de	6.73 ± 0.84
	Sucrose	6.44 ± 0.30 cd	4.85 ± 0.30
	Lactose	4.83 ± 0.16 bc	3.24 ± 0.16
	Sorbitol	2.98 ± 0.18 ab	1.39 ± 0.18
6.0	No carbohydrate/sugar alcohol	4.55 ± 0.55 bc	0
	Glucose	10.36 ± 0.70 e	5.81 ± 0.89
	Fructose	16.02 ± 1.38 f	11.47 ± 1.49
	Sucrose	6.43 ± 0.40 cd	1.88 ± 0.68
	Lactose	38.57 ± 1.34 g	34.02 ± 1.45
	Sorbitol	5.32 ± 0.19 c	0.77 ± 0.58

[a] Values (mean ± SD, $n = 3$) with different letters show statistical significance ($\alpha = 0.05$).
[b] Concentrations of furan obtained by reducing the amounts of furan found in carbohydrate/sugar alcohol-containing starch-based samples with those generated in starch-based samples containing no carbohydrate/sugar alcohol.

Regarding the general effect of pH on carbohydrate degradation, it was found that at a higher pH more colour was produced by caramelization processes, induced by both a thermal treatment and γ-radiation (Ajandouz, Tchiakpe, Dalle Ore, Benajiba, & Puigserver, 2001; Oh et al., 2006) and, moreover, a more intense carbohydrate degradation was observed (Ajandouz et al., 2001).

Generally, the most efficient carbohydrate for furan generation at acidic pH was fructose, followed by sucrose and lactose together with glucose, while at pH 6, lactose generated the highest amounts of furan followed by fructose and glucose. Similarly, Limacher et al. (2008), have shown that a ketose (fructose) was a more effective furan precursor than an aldose (glucose) under roasting and pressure-cooking conditions. Surprisingly, in the literature, no attention has been given previously towards furan formation from lactose, which is shown for the first time in the present study to be an important precursor of furan at pH 6. Since the glucose-, fructose- and lactose-containing systems generated the highest amounts of furan at both pH values, they were selected for further experiments.

Furan formation from carbohydrates has been mostly studied in the presence of amino acids or proteins. Such Maillard reaction systems are suggested as a primary source of furan (Maga, 1979), especially under dry-roasting conditions (Limacher et al., 2008). Therefore, whey proteins were added to the starch-based carbohydrate-containing model systems (Table 2).

Table 2
Furan formation in buffered starch-based [15% (w/w) WC starch] systems containing glucose, fructose or lactose (2.78 mmol carbohydrate/100 g sample) in combination with whey proteins (5%, w/w). Samples were heated at 130 °C for 30 min.

Sample composition		Furan (ppb)[b]	
		pH 4	pH 6
Glucose	–[a]	4.54 ± 0.24 a	10.36 ± 0.70 b
	Proteins	31.11 ± 1.09 c	72.71 ± 1.68 d
Fructose	–[a]	8.32 ± 0.84 b	16.02 ± 1.38 e
	Proteins	28.34 ± 2.90 c	75.79 ± 3.96 f
Lactose	–[a]	4.83 ± 0.16 a	38.57 ± 1.34 g
	Proteins	31.52 ± 2.77 c	112.74 ± 3.26 h

[a] Protein-free starch-based samples were studied in a separate experiment (Table 1).
[b] Values (mean ± SD, $n = 3$) with different letters show statistical significance ($\alpha = 0.05$).

The presence of proteins significantly increased the amounts of furan formed at both pH values for all samples. The lactose-protein containing system generated the highest amounts of furan at pH 6, which was quite surprising as disaccharides (lactose or maltose) are considered to be less reactive than monosaccharides (glucose or fructose) in the Maillard reaction (Reineccius, 2006). In contrast to our results, Limacher et al. (2008), have observed a favoring effect of amino acids on furan formation under pressure-cooking conditions only for glucose and not for fructose and the carbohydrate derivative 3-deoxyhexos-2-ulose. Again, the amounts of generated furan were significantly higher at pH 6 than at pH 4. This effect of pH on the furan formation could be related with the effect of pH on the degradation pathway of the Amadori compounds formed in early stages of the Maillard reaction. At lower pH, it involves mainly 1,2-enolisation resulting in the formation of 3-deoxy-1,2-dicarbonyl compounds (3-deoxyosones). At higher pH, it undergoes mainly 2,3-enolisation leading to 1-deoxy-2,3-dicarbonyl compounds (1-deoxyosones) (Nurtsen, 2005). As suggested by Perez-Locas and Yaylayan (2004), the latter pathway, involving the formation of 1-deoxyosone, is the most effective route of furan formation in the Maillard reaction. In general, it has been shown that with increasing pH, the rate of browning, carbohydrate degradation and Maillard reaction also increase (Brands & van Boekel, 2002; Labuza & Baisier, 1992).

In the next series of experiments, the combination of the selected sugars and ascorbic acid was investigated (Table 3). The obtained results show that, even at tenfold lower concentrations, ascorbic acid as such is a much more efficient precursor of furan than the sugars studied at both pH values, except for lactose at pH 6. Moreover, the results indicated that in the starch-based model systems containing sugars and ascorbic acid, significantly more furan was generated as compared to the ascorbic acid-free systems. However, if compared with the starch-ascorbic acid system and heated at the same conditions, no significant differences were observed, except for the fructose and lactose systems at pH 6. In this case, an increase in the furan content was observed, although the amounts of furan were still lower than expected from the sum of both separate systems. Limacher et al. (2007) have also studied furan formation in a binary mixture of ascorbic acid and glucose. In contrast to the results in Table 3, they observed that the amounts of generated furan were significantly lower than in separate ascorbic acid-containing system under pressure-cooking conditions, in particular at pH 4, and under roasting conditions. This phenomenon was attributed to the competing reactions in complex systems disfavouring furan formation. In the present study, however, a different mechanism seems to be involved since, as demonstrated previously (Owczarek-Fendor et al., 2010a), the presence of starch

Table 3
Furan formation in buffered starch-based [15% (w/w) WC starch] systems containing glucose, fructose or lactose (2.78 mmol carbohydrate/100 g sample) in combination with ascorbic acid (0.28 mmol AA/100 g sample). Samples were heated at 130 °C for 30 min.

Sample composition		Furan (ppb)[b]	
		pH 4	pH 6
No carbohydrate	AA	21.62 ± 1.06 c	24.46 ± 1.68 c
Glucose	–[a]	4.54 ± 0.24 a	10.36 ± 0.70 b
	AA	22.68 ± 1.27 c	22.78 ± 1.84 c
Fructose	–[a]	8.32 ± 0.84 b	16.02 ± 1.38 d
	AA	23.65 ± 0.98 c	29.84 ± 2.02 e
Lactose	–[a]	4.83 ± 0.16 a	38.57 ± 1.34 f
	AA	22.99 ± 1.86 c	44.15 ± 1.22 g

[a] Ascorbic acid-free starch-based samples were studied in a separate experiment (Table 1).
[b] Values (mean ± SD, $n = 3$) with different letters show statistical significance ($\alpha = 0.05$).

enhances significantly the formation of furan from ascorbic acid when compared to the buffered solutions.

Again, the most efficient carbohydrate in furan formation was lactose as the highest amounts of furan (44 ppb) were found in the model system containing lactose and ascorbic acid. Surprisingly, no significant difference in furan formation from the vitamin C-containing system at pH 4 and 6 was found, which is in contrast with our previous results indicating a reduction in furan formation from ascorbic acid with increasing pH (Owczarek-Fendor et al., 2010a). In order to elaborate these results, the experiment with vitamin C-containing starch-based samples prepared at pH 4 and 6 was repeated following the same procedure. Similarly, the obtained results (data not shown) were not significantly different ($P > 0.05$). However, the conditions of heating in the present research (130 °C, 30 min) were more intense than in the previous study (120 °C, 20 min) (Owczarek-Fendor et al., 2010a) which might influence these contrasting results. Moreover, it might indicate that the pH at more extreme conditions of heating is less influential on the formation of furan from ascorbic acid. Nevertheless, it seems that the pH has a much more profound influence on furan formation from sugars, in particular from lactose, as compared to ascorbic acid, especially since the amounts of furan formed from the milk carbohydrate at pH 6 were significantly higher than from ascorbic acid at both pH values.

In the final experiment, the impact of the presence of oils, as a source of unsaturated fatty acids, to the starch-based carbohydrate-containing model system, was investigated (Table 4). For this purpose, fresh and oxidised (14 days at 60 °C) soybean oils were selected since it was shown previously (Owczarek-Fendor et al., 2010b), that the oxidised soybean oil, was an efficient furan precursor. The obtained results point out that the amounts of furan found in model system containing both sugars and oils, depends on the oxidative status of the oil used, confirming the previous findings (Owczarek-Fendor et al., 2010b, 2011). From the systems with sugars and fresh oil no significant difference was observed between systems with and without oil, indicating that the presence of oil does not interfere with the furan formation from sugars. In the carbohydrate-containing systems in combination with oxidised soybean oil, however, significantly more furan was formed as compared to both the starch–carbohydrate and starch–lipid samples. Moreover, the generated amounts of furan were higher than expected from the sum of furan found in these separate starch–carbohydrate and starch–lipid samples. Thus, these results clearly indicate the occurrence of a synergistic effect between furan formation from sugars and oxidised oils upon sterilization. It seems unlikely, however, that these phenomena are encountered in practice since such highly oxidised oils are not used in normal food processing.

Table 4
Furan formation in buffered starch-based [10% (w/w) WC starch] systems containing glucose, fructose or lactose (2.78 mmol carbohydrate/100 g sample) in combination with fresh (pV 4.30 ± 0.48 mEq/kg, pAV 1.42 ± 0.02) or oxidised (pV 125.29 ± 0.30 mEq/kg, pAV 16.95 ± 0.15) soybean oil (SBO; 5%, w/w). Samples were heated at 130 °C for 30 min.

Sample composition pH 6		Furan (ppb)[b]
No carbohydrate	SBO fresh	5.31 ± 0.04 a
	SBO oxidised	27.82 ± 0.32 b
Glucose	–[a]	10.36 ± 0.70 c
	SBO fresh	11.64 ± 1.02 c
	SBO oxidised	48.14 ± 1.43 d
Fructose	–[a]	16.02 ± 1.38 e
	SBO fresh	15.99 ± 0.72 e
	SBO oxidised	53.93 ± 0.29 f
Lactose	–[a]	38.57 ± 1.34 g
	SBO fresh	35.95 ± 0.46 g
	SBO oxidised	78.58 ± 0.45 h

[a] Lipid-free starch-based samples were studied in a separate experiment (Table 1).
[b] Values (mean ± SD, $n = 3$) with different letters show statistical significance ($\alpha = 0.05$).

4. Conclusions

In conclusion, it can be stated that, apart from vitamin C and polyunsaturated fatty acids, sugars are also an important source of furan, especially when heated in combination with proteins. Interestingly, the most efficient carbohydrate, concerning furan generation, was lactose at pH 6, a carbohydrate that has not been studied in literature in this respect. Thus, it seems that the presence of lactose in food products can contribute significantly to furan formation. For example, baby foods to which milk carbohydrate is added can be particularly vulnerable in this respect. Moreover, a synergistic effect was observed between sugars and lipids but only when they reached an unrealistically high oxidation degree. Furan formation in carbohydrate-containing systems to which ascorbic acid was added was lower than expected from the sum of both separate systems.

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2. Analytical Procedure

2.1. Purposes

This chapter is devoted to the analytical development of a highly sensitive method to detect and quantify furan in foodstuffs. According to the literature (FDA, 2004; EFSA, 2005), background to high-level content of furan in food varies from sub ng * g^{-1} to above 1 µg * g^{-1} depending on the nature of the food. As a consequence, to achieve our objectives, a robust, specific and highly sensitive analytical method is needed.

According to the furan physicochemical properties and to the literature, the analytical methodology that fit for purpose requires the use of gas chromatography (GC) coupled to mass spectrometry (MS) for the separation and detection; and the Solid Phase MicroExtraction (SPME; Goldman et al., 2005; Ho et al., 2005; Bianchi et al, 2006; Altaki et al., 2007; Jestoi et al., 2009; Kim et al., 2009a; La Pera et al., 2009) or the Headspace Sampling (HS; Reinhard et al., 2004; Belcaski et al., 2005; Belcaski et Seaman, 2005; Senyuva et Gokmen, 2005; Nyman et al., 2006; Crews et al., 2007; Zoller et al., 2007; Morehouse, 2008; Wegener et López, 2010; Bicchi, 2011) for the extraction.

We decided to select the SPME approach because it is a solvent free extraction technique, usually causing less degradation of the GC column and achieving better chromatographic peak shape as few water is transferred (Altaki et al., 2009; Pawliszyn, 2012). SPME is also known as a highly repeatable and reproducible technique, which can be easily automated and integrated to an on-line analytical system.

2.2. Headspace Solid Phase MicroExtraction (SPME)

Headspace Solid Phase Micro Extraction involves the extraction of organic compounds from gaseous samples into the solid phase coating of a silica fiber support. The analytes partition between the gas phase, matrix and coating until equilibrium is reached. The technique is suitable for qualitative and quantitative analysis of volatile compounds. It is appropriate for various families of compounds (Pawliszyn, 2012) such as: Volatile Organic Compound (VOCs, Larroque et al., 2006; Ouyang et Pawliszyn, 2006), Volatile Sulfuric Compound (VSCs, Lestremau et al. 2003), BTEX (Benzene, Toluene, Ethylbenzene and Xylene; Arambarri et al., 2004), Organoleptic molecules (Wright et al., 1986; Koziel et al.,

2006), Pollutants (Menezes Filho et al. 2010; Wang et al., 2011). Automation and integration of this technique is easily achievable (Ouyang et Pawliszyn, 2006; Pawliszyn, 2012) on commercial instruments making the method appropriate for high throughput on-line analytical technique.

Note: the most widely used technique of sampling in SPME, the direct mode (i.e. immersion of the fiber coating into a solution) has not been used here and is not covered in the present dissertation.

In more complex situations, like the extraction of furan from various foodstuffs, the coating of the fiber is usually introduced in the headspace of the vial, above the sample. The principle of headspace SPME is then a competitive equilibrium between the three phases (i.e. the homogeneous matrix, the gas phase or headspace and the fiber coating) of the system (Figure 6). During extraction, analytes equilibrate between all the three phases. The mass balance, of an analyte during the extraction process, should remain constant (Equation 1; Zhang et Pawliszyn, 1993):

$$C_0 V_S = C_f^{\infty} V_f + C_h^{\infty} V_h + C_s^{\infty} V_s$$

Equation 1 : Mass balance of an analyte during the extraction process. C_0 is the initial concentration of the analyte in the matrix; $\boldsymbol{C_f^{\infty}}$, $\boldsymbol{C_h^{\infty}}$ and $\boldsymbol{C_s^{\infty}}$ are the equilibrium concentrations of the analyte in the coating, the headspace and the matrix, respectively; and V_f, V_h, and V_s are the volumes of the coating, the headspace and the matrix, respectively.

The analytes concentrations in the three phases are linked by the chemical equilibrium equations:

$$K_1 = \frac{C_h^{\infty}}{C_s^{\infty}}$$

Equation 2 : Equilibrium constant between the Sample and the Headspace, at equilibrium

$$K_2 = \frac{C_f^{\infty}}{C_h^{\infty}}$$

Equation 3 : Equilibrium constant between the Headspace and the Fiber coating, at equilibrium

$$K = \frac{C_f^{\infty}}{C_s^{\infty}} \Leftrightarrow K = K_1 K_2$$

Equation 4 : Overall equilibrium constant

The mass of analyte absorbed by the coating is given by $n = C_f^{\infty} V_f$ and can be expressed as:

$$n = \frac{K_1 K_2 V_f V_s C_0}{K_1 K_2 V_f + K_1 V_h + V_s} = \frac{K V_f V_s C_0}{K V_f + K_1 V_h + V_s}$$

Equation 5 : Mass of the analyte absorbed by the coating in headspace SPME mode at the equilibrium

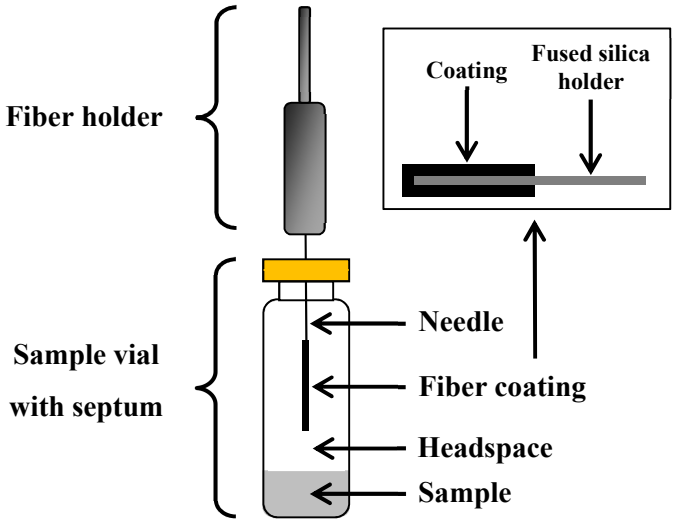

Figure 6 : SPME scheme

According to these equations, as the amount of analytes on the fiber increase, the amount of analytes in the headspace decrease and the amount in the sample decrease to reach back the equilibrium.

The distribution constants (K_1, K_2) are thermodynamics constants that depend on a variety of conditions including temperature, sample matrix characteristics such as pH, salt or ionic strength, and organic component concentration. These parameters need to be optimized to maximize the amount of analytes extracted in the fiber. The partial molar Gibbs free energy variation of the analytes in each phase depend of these parameters. They induce a modification of the transfer energy and therefore of the equilibrium constant. To achieve the highest sensitivity, this value should be as high as possible.

Chemical equilibrium constants are defined for an infinite equilibration time. Therefore, the rate of extraction plays an important role in the analytical development of the method. The kinetic model for SPME developed by Pawliszyn and co-workers is available elsewhere and we refer the reader to related literatures (Pawliszyn, 2012). As a consequence,

extraction time must be optimized as it is possible to reach a "quasi steady-state" which is not the chemical equilibrium but where the amount of the extracted analytes in the fiber is slightly higher. Equation 5 indicates that the use of the headspace above the sample might be an interesting means of accelerating extraction for analytes characterized by high vapor pressure like furan because of the high diffusion coefficients of the analyte in the gas phase. In order to increase transport from matrix into the headspace different options are available such as large sample/headspace interface (e.g. using large diameter vials), well-agitated system, ionic strength increase, and temperature. All these parameters were tested (section 2.5.1.3) using an experimental design approach.

According to the literature, the fiber coating exhibiting the highest efficiency for furan analysis is Carboxen™ (Supelco, Bellefonte USA): a porous carbon polymer with very high specific surface (1200 $m^2 * g^{-1}$) inducing adsorption and absorption phenomena (Bianchi et al., 2006). Other extraction conditions are closely related to each other and were optimized using the experimental design approach. This theory and its results are presented in the section 2.5.

2.3. Gas Chromatography coupled to Mass Spectrometry

As extraction method, SPME does not only extract the target compounds, therefore a separation method is needed before the detection to focalize on target compounds.. To achieve the required specificity and sensitivity (sub ppb level), and according to the physicochemical properties of the furan, gas chromatography coupled to mass spectrometry (GC-MS) was selected as separation and detection technique. Using GC-MS, the quantitative aspect was assured by the isotope dilution (ID) methodology. The next paragraphs give a short description of these techniques and show the results obtained for the furan analysis.

2.3.1. Gas Chromatography

GC is a separation technique based on the volatility of the analytes, their affinity for the column coating, sizes of the column and carrier gas nature. We assume that the GC theory is known and refer the reader to related literature if needed (Grob et Barry, 2004).

When GC is coupled to MS, the carrier gas is usually high purity He (e.g. AirProduct: 99.9996%). H_2 is not used for safety reason with the MS and because chemical reactions can occur in sources and inlet surfaces (Heseltine, 2010).

Analytes were introduce through the injection port in splitless mode set at high temperature (275°C) to achieve a fast and complete desorption of the fiber.

First a non-polar column phase was used: DB-5ms of 30m (Agilent – Santa Clara, CA, USA), but the retention factor (k) of this column for furan was too low (k value between 0 and 1), indicating that furan was eluting with the dead time of the column in classical conditions. In the literature, it was found that a sufficient retention capacity could only be achieved by using cryogenic conditions (Zoller et al., 2007). As a consequence, we decided to select a PLOT (Porous Layer Open Tubular) GC column (styrene/divinylbenzene coating) that was designed to trap small molecules such as permanent gases, light hydrocarbons and volatiles compounds at room temperature. The column selected for furan analysis was a bonded phase CP-PoraBOND Q (Agilent; 25m x 0.32mm x 5µm ;). The Q version is the more versatile PLOT columns type and is suitable for the analysis of apolar to polar volatile compounds. Additionally, this column has a high resistance towards water and can be run at high temperature (until 320°C) if needed.

On the practical side, the analyses were run under a constant flow of 1.7 mL min^{-1} of He with the optimized temperature program displayed in Figure 7 (initial temperature of 35°C for 1 min, increased at 10°C * min^{-1} to 90°C and held for 5 min, then increased at 30°C * min^{-1} to 260°C and finally held for 6 min). This program gave a sufficient capacity of separation between the furan and the interfering substances (Figure 13).

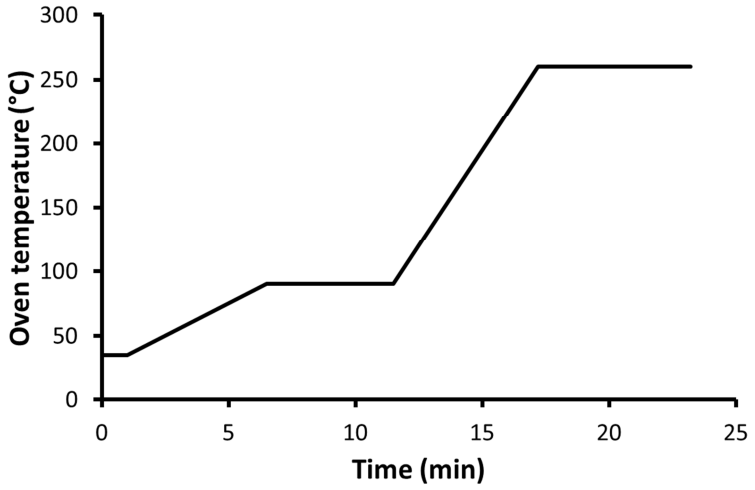

Figure 7 : Optimized temperature program for the furan analysis on a CP-PoraBOND Q (25m x 0.32mm x 5µm)

2.3.2. Mass Spectrometry

In the present dissertation, mass spectrometry (MS) in only used as a highly specific and sensitive detector for a target compound. Therefore, only a short overview of the MS based technique of the ion trap will be displayed before turning to the furan analytical specification.

A mass spectrometer is an instrument used to produce ions in gas phase, separate them according to their mass-to-charge (*m/z*) ratio and counting them. A plot of the relative intensity of each ion related to its *m/z* is obtained and called a mass spectrum (Hoffmann et Stroobant, 2002). This spectrum is directly dependent of the analyzed molecule and of the ionization mode. Mass spectrometers are composed of 5 parts (Figure 8): Sample introduction, Ion production, *m/z* separation, Detection and Data handling and operate under high vacuum.

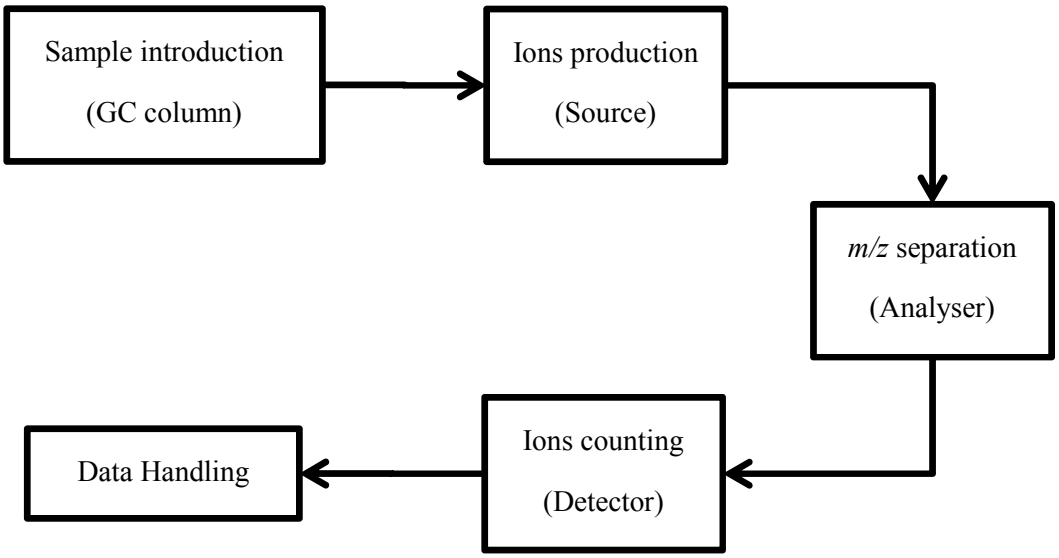

Figure 8 : Schematic principle of mass spectrometry

In the present case, where MS is coupled to a GC, the *Sample introduction* unit is the end of the capillary column. Several different *Ion production* units, called Source, exist. Each of them is used according to the type of target molecules and to the sample introduction system: Electro Spray Ionization (ESI) for liquid chromatography coupling and analyzing semi- to polar molecules until high molecular weight; Atmospheric Pressure Ionization (API) as an alternative to ESI; Matrix Assisted Laser Desorption Ionisation (MALDI) for high molecular weight such as polymers or proteins. When coupled to a GC column, Electronic Ionization (EI) process in gas phase in positive mode is usually used. In EI source, the electrons are produced by heating a wire filament and they are accelerated to 70 eV in the source block. The electrons collide with neutral molecules, transfer their kinetic energy and excite the neutrals above the ionization threshold. The excess of electronic energy is transformed into vibrational energy dissipated by fragmentation. The ionization process follows reproducible cleavage reactions that give rise to fragment ions, providing structural information about the analyte (called *Fingerprint*). The parent ion itself may be absent in the spectrum, if its fragmentation is high.

The *m/z* separation is performed in the Analyzer section of the mass spectrometer. Each type of analyzer (e.g.Time of Flight (ToF); Sector; Quadrupole) has some specification like their *m/z* acquisition range, acquisition speed and mass resolution. An ion trap mass analyzer (Figure 9) from Thermo-Fisher (PolarisQ) has been used for furan analysis.

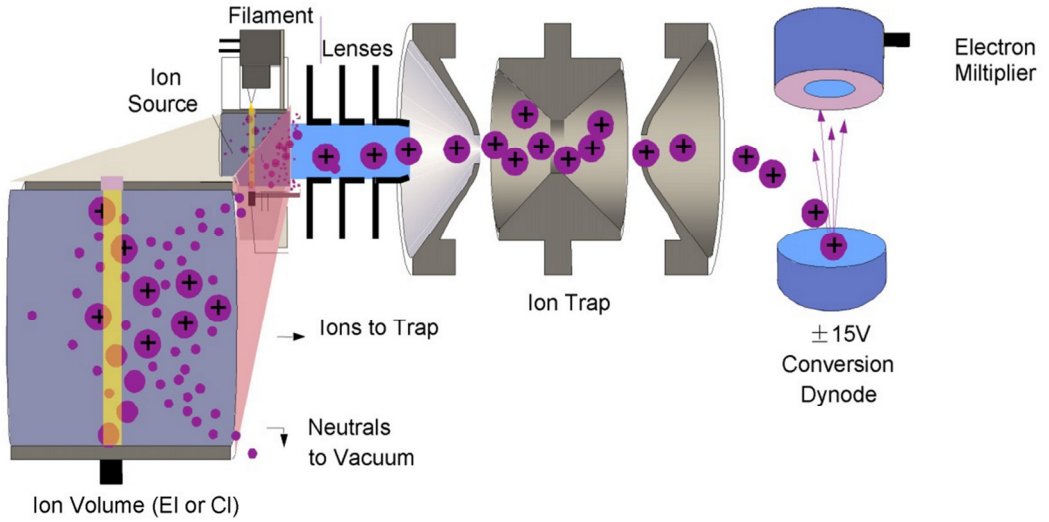

Figure 9 : Scheme of PolarisQ mass spectrometer (with the courtesy of ThermoFisher)

Basically, a quadrupole ion trap consists essentially of three electrodes: two End-cap electrodes and one Ring electrode. For an external ion source (Thermo system), the two end-cap electrodes are not distinguishable and both are characterized with a single hole in the center. The ring electrode is positioned symmetrically between the two end-cap electrodes as shown in Figure 9. A radio frequency (RF) voltage is applied on the ring electrode to trap ions on a stable trajectory. The ions' motion in a quadrupole field can be mathematically described by the solutions of Mathieu second-order differential equations (Mathieu, 1868) leading to the stability diagram. "Stable" ions are ejected from the trap by an axial modulation mode consisting of applying an alternative tension at a specific frequency to the end-cap electrodes. Ions come into resonance and this is reflected by a rapid increase of the amplitude of their movements. Ions are resonantly ejected (process called *Resonant ejection*). The ejected ions hit a conversion dynode to be counted at the detector. Ion trap theory is not the purpose of this dissertation and will not be further explained. A full account of ion trap theory by March et al., 1989; Todd, 1991; March, 1992; are available in the corresponding literature.

The *Detectors* commonly used in mass spectrometry are photo multiplicator and electro multiplier. On the PolarisQ system, a continuous electron multiplier is used (Figure 10). The principle is: ions touch a conversion dynode that proportionally produces electrons, these electrons are multiplied from 10^5 to 10^6 times in the amplifier zone to obtain a recordable signal.

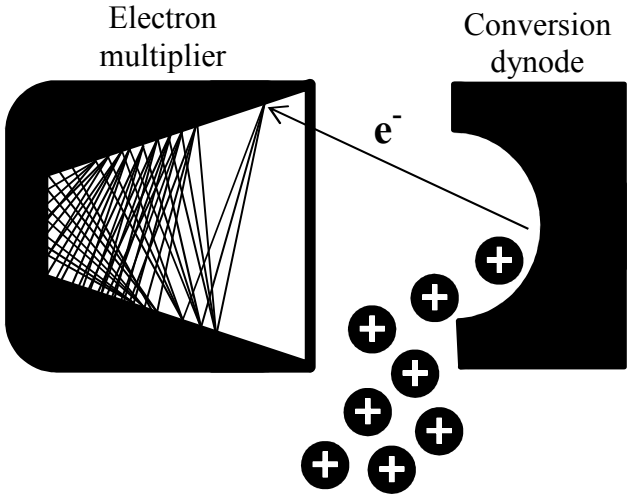

Figure 10 : Scheme of an electron multiplier

The signal from the multiplier is recorded by the *Data handling system* who usually is a computer. Factories data acquisition programs, associate the signal intensity with its respective *m/z* and the GC retention time. A chromatogram called Total Ion Current (TIC) is constructed and can be split in several single mass chromatographic traces (Figure 13).

2.3.2.1. Selected Ion Monitoring

In analytical chemistry, the Selected Ion Monitoring (SIM) mode is used to improve the sensitivity towards the target compounds by monitoring only their masses of interest. On the practical side, the analyzer jump from one mass to the other rather than scanning the all mass range. Therefore no acquisition time is wasted by recording non relevant signals. It results in a better signal to noise for target compounds.

To assure the specificity, the ion ratio between the selected ions of a target compounds are measured. These ratios must fulfill a variation criteria related to their relative intensities and define in the legislations. This will be discussed in the 2.6.2.1 section regarding the method validation.

2.3.2.2. Selection of target ions

The mass spectrum of furan in EI+ mode is displayed in the Figure 11 (NIST, 2008). The furan's molecular ion (68 Th) fragments in 2 major ions at *m/z* 29 and 39 Th. The furan specific ionization/fragmentation pathway is shown in the Figure 12.

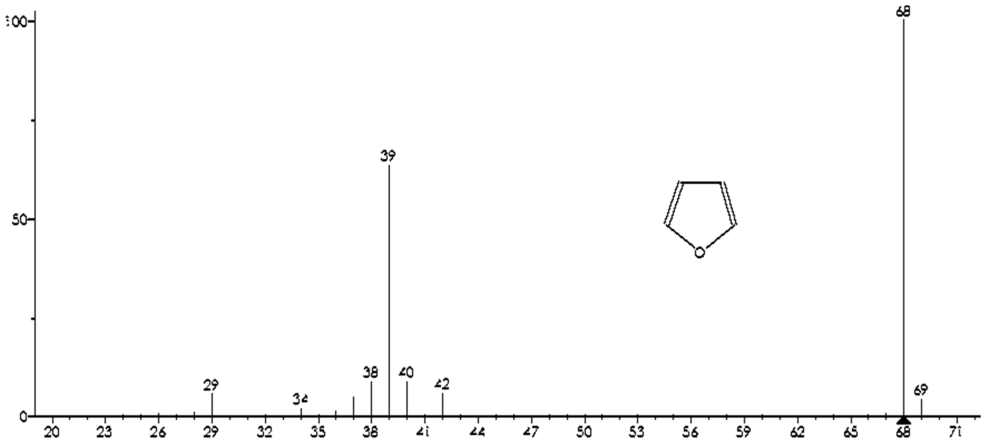

Figure 11 : Mass spectrum of furan in EI+

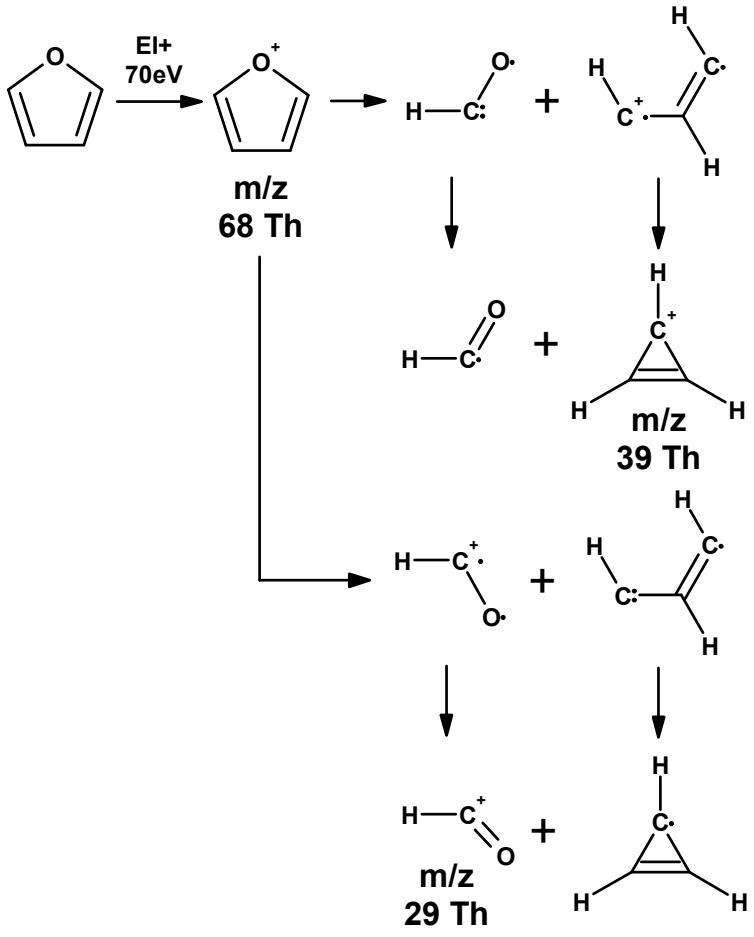

Figure 12 : Ionization / Fragmentation pathway of furan in EI+

Only the 2 last ions (39 and 68 Th) are available when the furan is analyzed with the PolarisQ (i.e. working range from 35 to 1000 Th). Therefore they were selected for the furan analysis and their ion ratio was determined through the calibration curves (2.3.3.1).

2.3.3. Isotope dilution technique

For quantitation, the Isotope Dilution (ID) was used (Hoffmann et Stroobant, 2002; Boyd et al., 2011). It is a specific case of the Standard Addition technique widely used in physicochemical analytical technique such as: gas chromatrography, liquid chromatrography, Infrared spectroscopy or Ultraviolet – Visible Spectroscopy.

The internal standard used for the quantification is a labeled analogue of the target analyte. Labeling is done by substituting at least one of the analyte atom by one of its stable uncommon isotope such as Deuterium (^{2}H or D) or ^{13}Carbon. For the furan analysis, the only commercially available isotopomer is a deuterated analogue where the 4 hydrogen atoms are substituted by 4 deuterium.

The main advantage of isotope dilution is that the physicochemical properties of the analyte and of its isotopomer are the same. If the standard is correctly introduced isotope dilution mass spectrometry should automatically corrects the bias due to the loss of the analyte. As a consequence, standard recovery correction is not any more necessary. Stable isotope dilution is obviously limited to mass spectrometry detection. It is a rather expensive approach but it definitely gives the best accuracy on results. An example of a chromatographic trace for the furan analysis by isotope dilution is given in Figure 13.

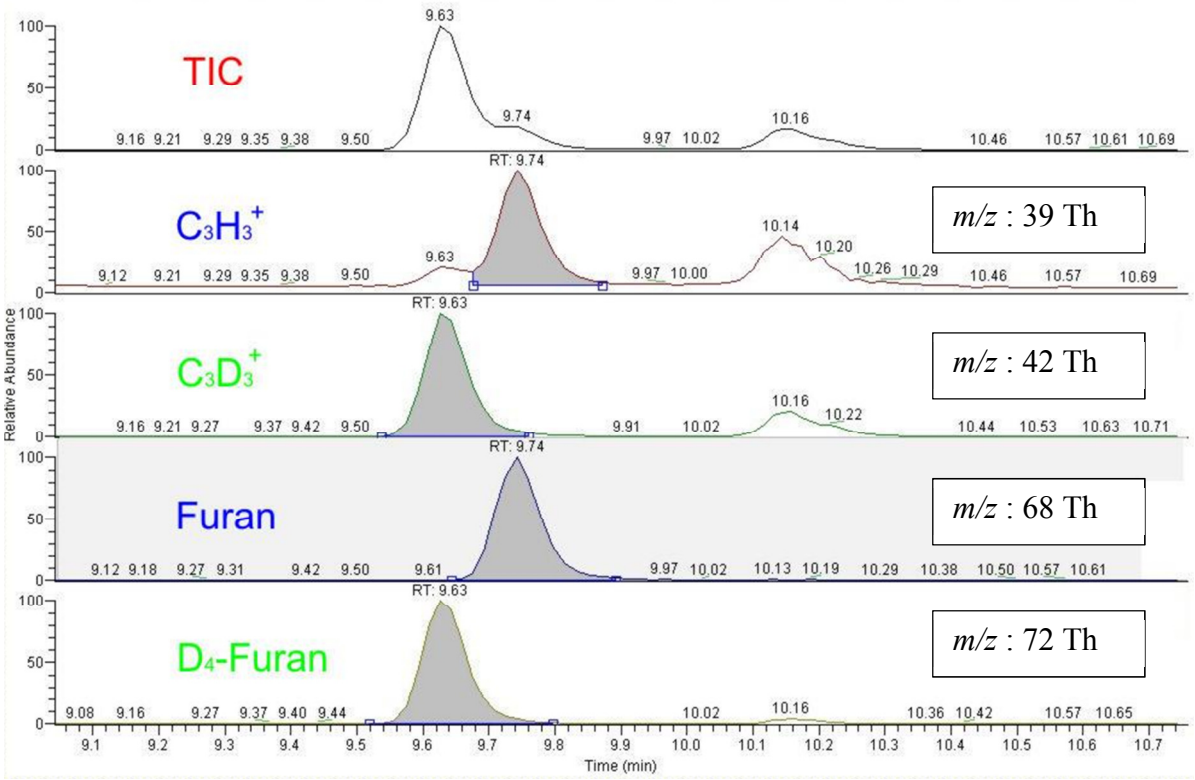

Figure 13 : Chromatographic traces for furan and D_4-furan in SIM mode

2.3.3.1. Calibration with isotope dilution

We just mentioned that the properties of the analyte and its isotopomer are the same, but it is not true at the microscopic scale. The atom substitution by one of its stable isotope changes the molecular ionization efficiency. As a consequence, a correction factor called Relative Response Factor (RRF) need to be applied for quantitative purposes. This factor is calculated over several concentrations either by a regression calibration curve or by the average value of the RRF as explained in the next paragraphs.

2.3.3.1.1. Linear regression

In the linear regression approach, the RRF is the slope of the curve obtained when the area ratio (native over labeled) is plotted over the concentration ratio (Figure 14). This is also the *a* term of the Equation 6.

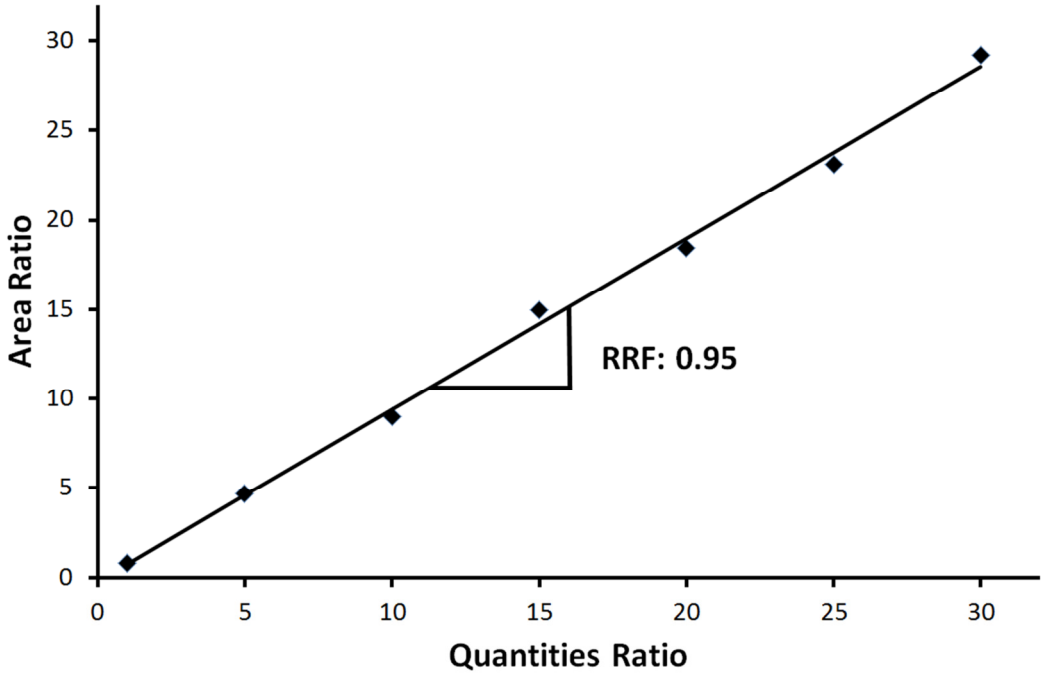

Figure 14 : Graphic representation of the RRF

$$\frac{A_{Nativ}}{A_{Labeled}} = a\frac{Q_{Nativ}}{Q_{Labeled}} + b$$

Equation 6 : Linear regression equation (with $\mathbf{A_i}$: Area and $\mathbf{Q_i}$: Quantity of compound i)

In most cases, the *b* term of the Equation 6 is negligible and only the RRF is used for the concentration calculation. The main limitation of this approach is the bias induced by the higher level calibration point weight. Therefore, weighted calibration curve or Average RRF are often used.

2.3.3.1.2. Average RRF

This approach can only be used if the *b* term of the Equation 6 is negligible. The RRF is calculated for each calibration point by the Equation 7 and the average value is used for the concentration calculation.

$$RRF_i = \frac{Q_{Labeled}A_{Nativ}}{A_{Labeled}Q_{Nativ}}$$

Equation 7 : Average RRF equation (with $\mathbf{A_i}$: Area and $\mathbf{Q_i}$: Quantity of compound *i*)

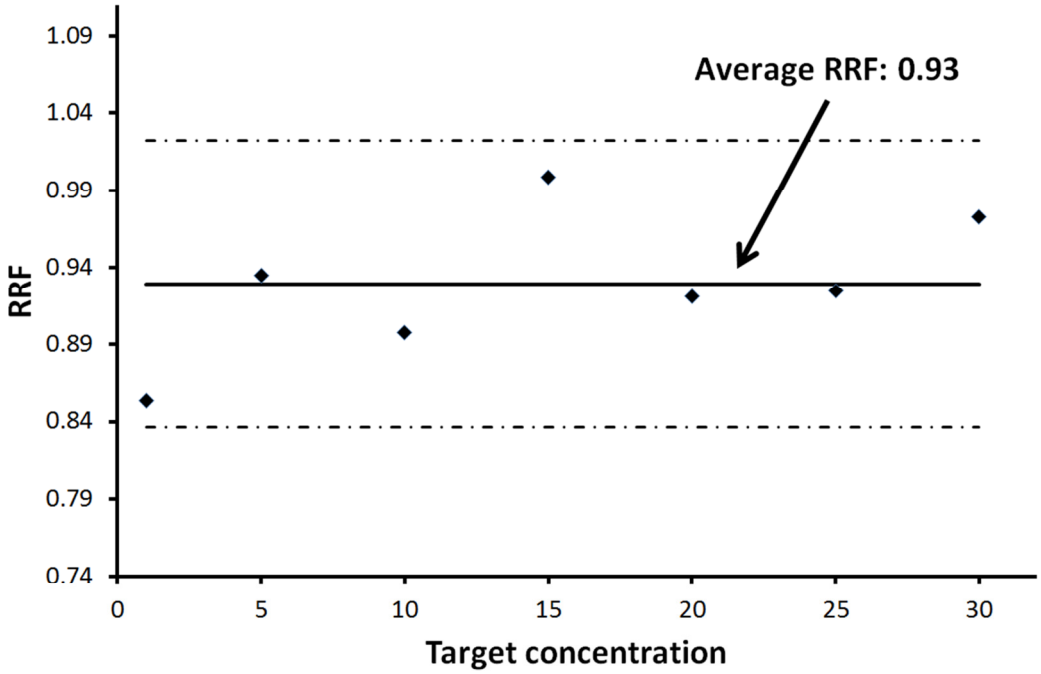

Figure 15 : Average RRF plot

In this approach the overweighting/underweighting issue does not exist as all calibration points have the same weight over the average RRF. Nevertheless, the *b* term must be 0 or negligible to be applicable.

2.3.3.1.3. Results

Here is an example of calibration curve for furan ranging from 0.1 to 100 ng * g^{-1}. Three levels were prepared in triplicates but not injected at once. The three levels were injected at the beginning, in the middle and at the end of the series. Between these calibration injections, real samples were injected. Both, a linear calibration curve (Figure 16) and the average RRF approach (Figure 17) were applied.

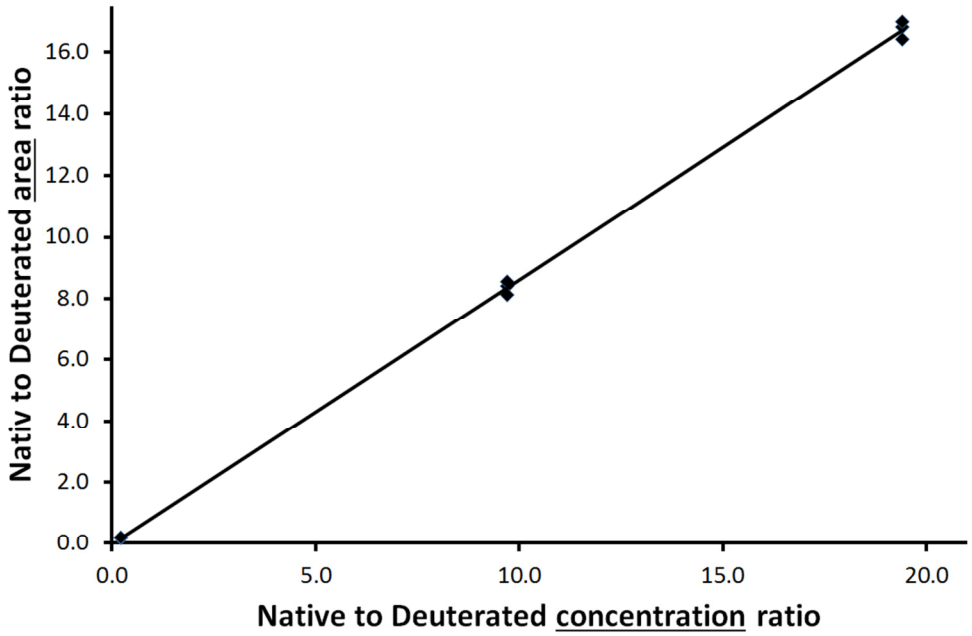

Figure 16 : Furan calibration curve – Linear regression approach

Based on the linear regression approach, the calculated RRF gave 0.862 and the *y*-intercept was 0.005 which is negligible (r^2 was 0.9994).

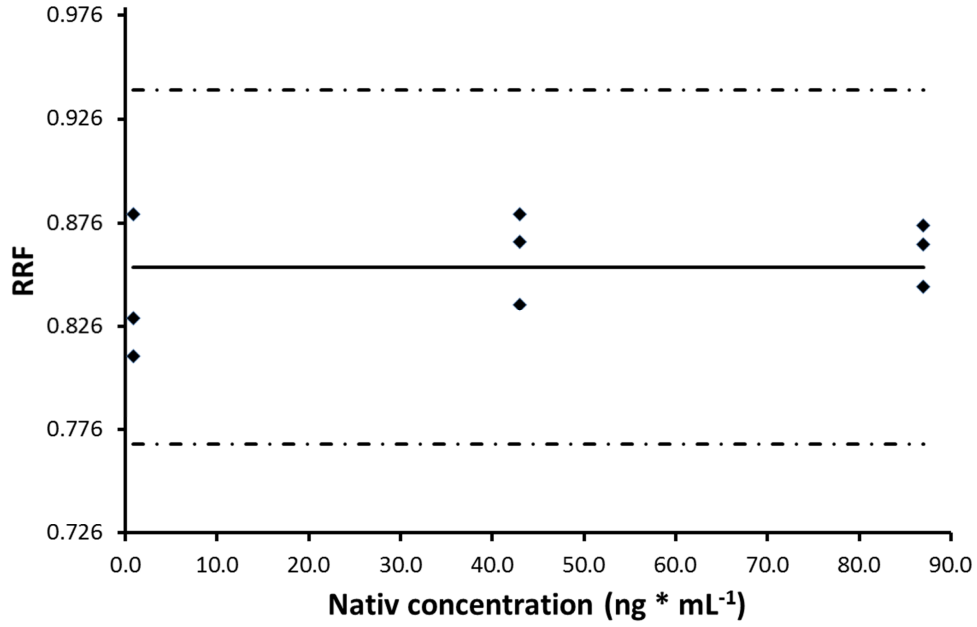

Figure 17 : Furan calibration curve – Average RRF approach

On the average approach, the calculated RRF was 0.855 with a relative standard deviation (RSD) of 2.9% (i.e. <15% which is a general criteria for isotope dilution technique).

The RRFs calculated by these 2 approaches are barely the same. Nevertheless, the linear RRF (0.862) is slightly higher than the average RRF (0.855). Even if both approaches gave similar results, we decided to select the average RRF approach for quantification purpose mainly because it is an unweighted approach.

2.4. Standards and samples preparation

Many papers underlined that handling furan in sample preparation is a critical step due to its high volatility (Reinhard et al., 2004; Goldman et al., 2005; Bianchi et al, 2006; Altaki et al., 2007; Zoller et al., 2007). Therefore, a lot of precaution must be taken to avoid a loss of furan.

The main issue is the fast evaporation of furan and its deuterated analog at room temperature. To solve this problem, different options are available such as working in a thermostatic bath or in a cooled room. In addition, for the standard solutions, it is important to minimize the headspace volume in the vial.

2.4.1. Standards preparation

High purity furan and deuterated analog are commercially available (purity: 99% and 99.9%, respectively). Dilution are necessary to work in the range of the backgrounds levels found in foodstuffs (1-1000 ng * g^{-1} of sample).

For a sample intake of 1 g, a target concentration of 100 pg * μL^{-1} is suitable to reach the calibration curve working range. This can be done in a 2 steps dilution: the first is to reach 500 ng * μL^{-1} by introducing 10 µL of furan in 20 mL of solvent (*aka* stock solution); the second is to get 100 pg * μL^{-1} by introducing 4 µL of stock the solution in 20 mL of solvent (*aka* working solution). In addition, a solvent that can be easily mixed with food matrices is suitable (e.g. water). Unfortunately, the miscibility of furan in water is too low for the first dilution. Thus, methanol was selected for the stock solutions and water for the working solutions. Furthermore, to calculate the solutions concentrations, only weight information (i.e. container, solvent and spike weight) were used as the error on the volume measurement is too high for such huge dilutions. Finally, daily solutions were prepared as recommended when handling volatile compounds.

In practice, a 20 mL weighted vial is completely filled with solvent (no air gap). Next the container is airtightly closed with a rubber septum cap, and furan is added with a 10µL syringe through the septum.

2.4.2. Samples preparation

For the sample preparation, it seems obvious that a limited contact with the atmosphere is required to avoid any losses of furan, but it is not possible in practice. However, the evaporation loss can be reduced by decreasing the furan vapor pressure. The easiest way is to work at low temperature, for instance by working in a 4°C cooled room.

Prior to the food sample intake (± 1g), 1 mL of water is introduced in a headspace vial, which is weighted with its cap and left in the cooled room for 30 minutes. Samples, spike solutions and syringes are also left for the same time in the cooled room. Then, samples are opened, mixed, transferred to vial, spiked with 50 µL of deuterated furan from the working solution and airtightly closed. All these steps must be executed as fast as possible. Finally, vial is kept in a freezer until GC-MS analysis.

2.5. Experimental design

Experimental design is a modern statistical technique used to evaluate the effects of factors on the results of experiments. With this technique, the influence of various factors is studied simultaneously with an experiment design that allows quantifying and comparing their influence but also to separate these influences from the random variations (Govaerts et Leboulengé, 2000; Brereton, 2007; Danzer, 2007). Numerous statistical equation systems have been developed and optimized for specific cases:

- Mixture design for the optimization of tertiary solvent mixture
- Taguchi design to ensure good performance of products processes
- Full factorial design to model the influence over the response of the factors over the working range
- Plackett-Burman design to evaluate the influence of numerous factors with a minimum number of experiments

In comparison with a classical approach, the experimental design gives access to interactions between factors, cut down the number of experiments needed to conclude and reduce the error on the evaluation of the influence of each factor.

The next paragraphs will give a short description of the two designs used for the SPME optimization in the case of furan analysis. In summary, this optimization was carried out in three steps: Screening (Plackett-Burman design), Selection (Pareto principle) and Optimization (Central Composite Design).

2.5.1. Screening design and parameters selection

2.5.1.1. Plackett-Burman theory

Plackett-Burman design was developed in 1946 (Plackett et Burman, 1946) to assess the influence of numerous factors in a minimal number of experiments. It is a statistical matrix equation system used for the screening of factors influences at several levels. Usually, only two levels (the lowest and highest state available) are involved for each factor but more can be used if requested. With two level factors, the number of experiments must be a multiple of four and the minimum number of experiments is given by the Equation 8. This number needs to be adapted to the closer superior multiple of four (i.e.: for three factors, 4 experiments; for 4 factors, 8 experiments). The non-allocated factors are called "*dummies*" and are used to estimate the experimental error. Additionally, several "*Central*" points can be added to improve the design precision.

$$N = m + 1$$

Equation 8 : Minimal number of experiments (**N**) for a given number of factors (**m**). N must be a factor of 4

The system response is given by the Equation 9 and corresponds to the vector of response of the experimental matrix in Table 1.

$$Y = \sum_{x=1}^{N} a_x F_x$$

Equation 9 : Equation of the response for a three factors system (**Y**: overall response; $\mathbf{a_x}$: coefficient of influence of the effect of **x**; $\mathbf{F_x}$: factor value for the experiment **x**)

N	a_1	a_2	a_3	y_x
1	-1	-1	-1	y_1
2	+1	+1	-1	y_2
3	-1	+1	+1	y_3
4	+1	-1	+1	y_4

Table 1 : Plackett-Burman experimental matrix for a three factors system (a_x: coefficient of influence of the effect of **x**; y_x: response of the experiment **x**; **factors values** are normalized to ± **1**)

The coefficient of influence of the effect can be calculated by a matrix (Equation 10) or by an individual way (Equation 11):

$$a = M^{-1}y$$

Equation 10 : Calculation of the vector of coefficients of influence of the effect (**a:** vector of the coefficient of the main effect of the factors; $\mathbf{M^{-1}}$: inverse of the experimental matrix; **y**: vector of response)

$$a_x = \frac{y_x F_x}{N}$$

Equation 11 : Calculation of the coefficient of influence of the effect of the experiment **x** (a_x: coefficient of the main effect of the factor **x**; y_x: response for experiment **x**; F_x: factor value of experiment **x**; **N**: number of experiments)

According to these equations, the higher the absolute value of the coefficient, the greater the influence on the response.

2.5.1.2. Selection principle

True optimization of every factor would require a large number of experiments and is time consuming. Therefore, only few factors are optimized whereas the others are fixed. This selection is carried out in accordance with the Pareto principle: "*For many events, roughly 80% of the effects come from 20% of the causes*" who stated that only 20% of the factors, the most influents, displaying the highest |**a**| value need to be optimized.

2.5.1.3. Results

In the framework of this thesis, the screening design has been carried out on two naturally contaminated matrices: first a liquid with coffee as model; second a viscous matrix with commercial first age carrot baby food as model. We decided that this second

matrix will act as a model for all the other matrices, as solid samples are homogenized and mixed with water.

In the two screening experiments, the selected response is the native furan chromatographic peak area. Five factors were identified as potential influencer:

- Extraction temperature (35 to 80°C)
- Incubation time (0 to 20 min)
- Extraction time (1 to 20 min)
- Stirring (0 to 750 rpm)
- Salt addition (0 to 40%)

Results of the two screening design for these parameters are presented in the next paragraphs.

2.5.1.3.1. Liquid matrices

Results for liquid matrices are summarized in a Pareto plot (Figure 18). This figure displays the value of the coefficient of influence (expressed as a percentage of the overall influence) of each factor.

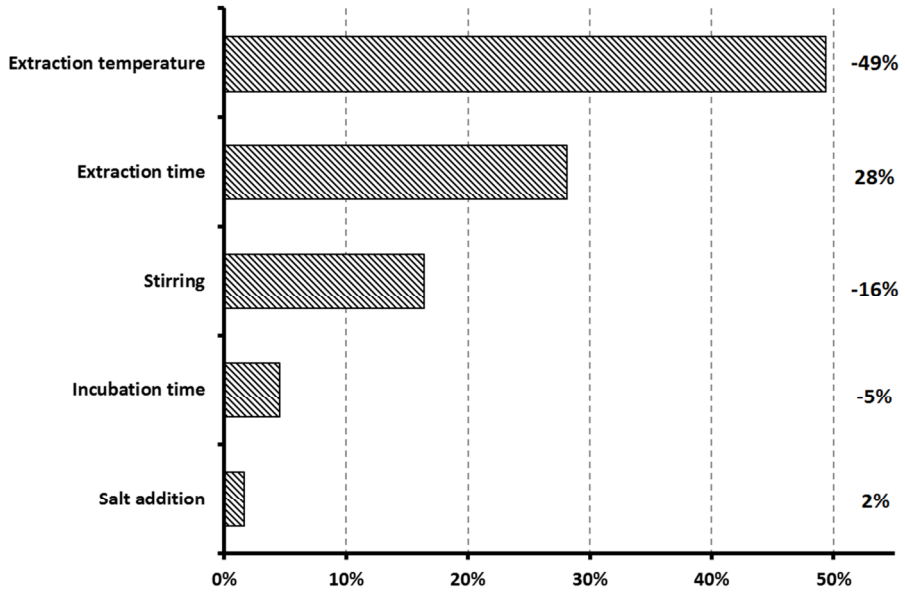

Figure 18 : Pareto plot for liquid matrices

We can conclude and classify the factors influence in a decreasing order as follow: Extraction temperature > Extraction time > Stirring > Incubation time > Salt addition.

According to the Pareto principle, only the 2 most influent factors must be optimized namely, Extraction Temperature and Time. Their optimization is described in the section 2.5.2.2.1. The 3 other factors must be set in a positive influencing way. The presented Pareto plot only highlighted the absolute influence, then a look at the coefficient of influence sign was needed to know if the effect was positive or negative to the response. This analysis showed that the incubation time was not necessary; stirring is not suitable and salt addition has nearly no influence. The established conditions have then been used for every analysis of liquid samples.

2.5.1.3.2. Viscous matrices

Results for viscous matrices are summarized in a Pareto plot (Figure 19)

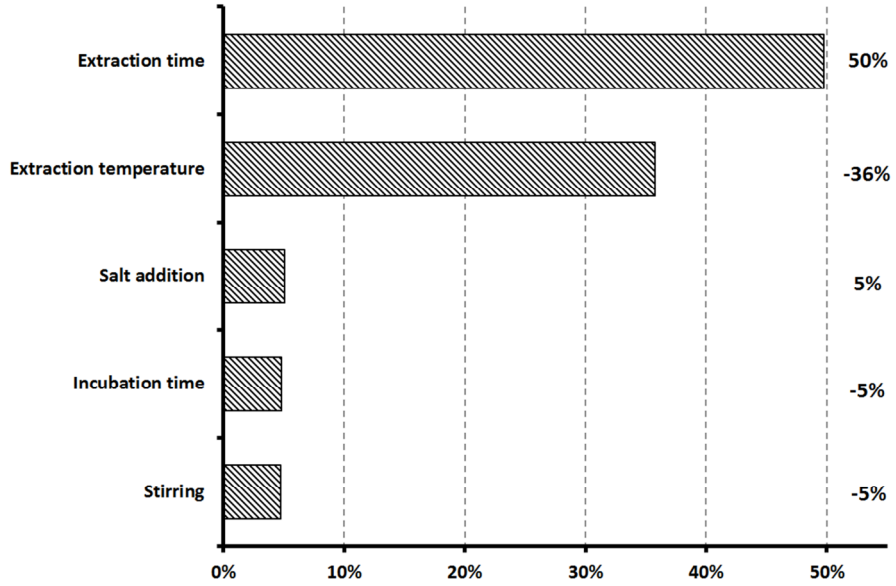

Figure 19 : Pareto plot for viscous matrices

This graph highlighted that the factor influence in a decreasing order can be classified as follow: Extraction time > Extraction temperature > Salt addition > Incubation time > Stirring.

According to the Pareto principle, only Extraction time and Temperature needed to be optimized as for the liquid matrices. Nevertheless, the extraction time is predominant in this case. The optimization of the extraction time and temperature is described in the section 2.5.2.2.2. The 3 other factors were set in a positive influencing way. It means: no incubation time, no stirring and no salt addition as previously observed for

liquid matrices. Once again, the conditions have been used for every analyses of viscous samples.

2.5.2. Optimization design

2.5.2.1. Central Composite Design theory

Central Composite Design (CCD) is one of the equation system dedicated to the optimization of method. It is used to calculate the optimal conditions for each factor in order to obtain the minimum or maximum response.

Classical optimization involves the study of the influence of each factor individually, but it is usefulness because the factors interferences are not taken into account and the "*true*" optimum is often missed. A multivariate technique such as CCD, can reach the "*true*" optimum, because it maps the surface of response and models the interferences between the factors. A complete modelisation of the system is only evaluated by a full factorial design, but it involves 2^n (for 2 factors) number of experiments. Statisticians worked on the reduction of the number of experiments and provided some solution like the one proposed by Box (1954), or by Draper (1969), and CCD corresponds to one of these optimizations.

In CCD approach, the experiments are conducted to cover all the factors working range (Figure 20) with the same precision. It allows mapping the response and finding the optimum. This design includes experiments to evaluate the influence of each individual factor, the influence of each two factors interaction, estimation of the replicate error (repeatability) at the center point and of the lack-of-fit (difference between the total error and the replication error). From a practical point of view, it corresponds to all extreme points **(a)** of the factors working range, several interference points outside of the working range **(b)** and center points replicates **(c)** to calculate the standard deviation.

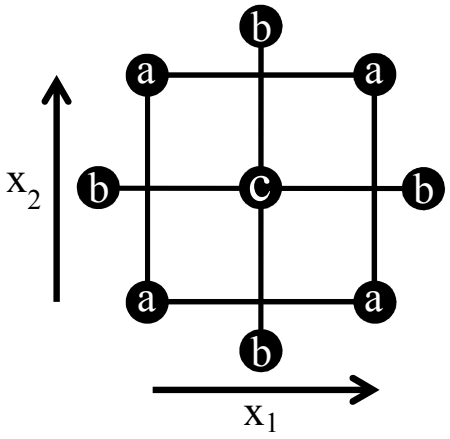

Figure 20 : CCD experiments for 2 factors optimization

2.5.2.2. Results

The CCD protocol has been applied to the two matrices used for the screening experiments: Coffee as liquid samples model and Carrot baby food as the viscous samples model. As previously discussed in Plackett-Bruman design, only Extraction temperature and Time have to be optimized. We concluded that the response decreased when the extraction temperature increased, thus the CCD protocol was applied between 4°C to 30°C (note that 4°C is minimum temperature achievable with our system by Peltier effect). Regarding the extraction time, the response increased when time increased, thus the optimization was carried out from 1 to 30 minutes.

2.5.2.2.1. Liquid matrices

The CCD result for liquid matrix is displayed in the Figure 21. This is a 3 dimensions graph displaying the response variation as a function of time and temperature.

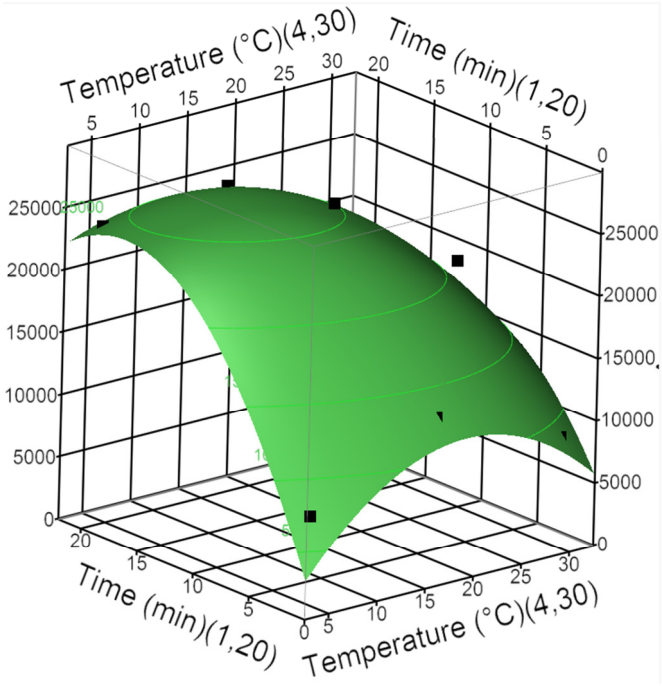

Figure 21 : Surface of response for liquid matrices (JMP Software v6.0)

The graph shows that an optimum can be found in the experimental range. The optimal setting for extraction temperature is 12°C and 15 minutes for the extraction time. The graph puts forward that the response decreases for any time or temperature increases. When the response at room temperature is compared to the response at the optimal temperature, the amount of furan adsorbed on the fiber is increased by roughly a factor 2 (between 50 and 100 % more). There is probably a competition between the furan adsorption and desorption on Carboxen™ fiber. This effect is exalted at higher temperature.

2.5.2.2.2. Viscous matrices

Figure 22 displays the results for the viscous matrices.

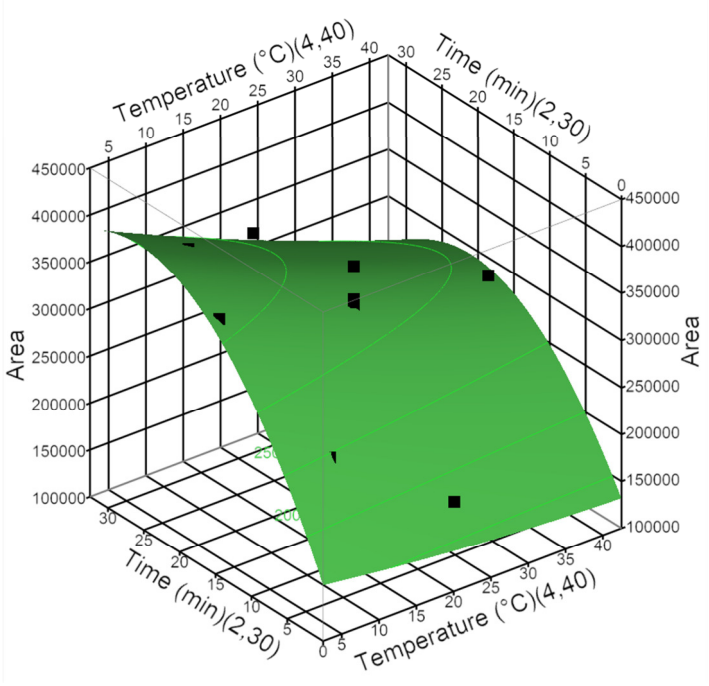

Figure 22 : Surface response for viscous matrices (JMP Software v6.0)

No optimal conditions were found within the experimental range for this matrix. According to the response surface equation, the optimal conditions are reached at 85°C below 0 which is out of our experimental range and obviously wrong because frozen matrices were not experimented. We might expect a trapping of the furan inside the matrix at these conditions. Therefore, the "true optimum" should be within freezing point and 4°C, but our Peltier cooling system was not able to go down below 4°C. As a consequence, optimal conditions within our working range were an extraction temperature of 4°C and an extraction time of 26 minutes. Compared to room temperature, the gain is about 30 %.

2.6. Validation

Validation is an essential step to assess the performances of an analytical protocol and confirm that these performances fit for purpose. According to the ISO 8402:1994, it consists in a series of experiments conducted to establish the performances characteristics and limitations of a protocol. Validation also identifies the critical parameters and the level of confidence of the method.

2.6.1. Protocol

A series of guidelines exist depending on the field of application and on the purposes of the developed analytical protocol: food contaminants, drugs, microbiologicals, … In the present case, furan, as an undesirable substance in food, can be validated according to the European Decision 2002/657/EC establishing the performance of analytical method. According to this Decision, several characteristics need to be determined for quantitative methods, namely Decision limit (CC_α), Detection capability (CC_β), Trueness, Precision, Intermediate precision and Specificity.

All these characteristics were determined for 4 matrices (food, sauces, juices and hot beverage) spiked at 3 levels of interest and but also by taking the advantage of proficiency tests results. At the time of performing the analysis and writing this thesis, no maximum limits for furan in food were laid down by the European legislation. As a consequence, the validation was conducted based on the minimum required performance limits (MRPL) of the analytical method. It means that the experiments need to be carried out near the limits of the method. Thus, the 3 validation levels were: 1.0, 1.5 and 2.0 ng * g^{-1}.

2.6.1.1. Validation parameters

Decision limit (CC_α) and Detection capability (CC_β) were determined in accordance with the ISO 11843 guide. To calculate these parameters, it requires the use of the standard deviation of results at the origin, the standard deviation of the response slopes, an acceptable value of α error (probability of false non-compliant decision) and an acceptable value of β error (false compliant decision). The commonly acceptable value of α error in food contaminants analysis is 1% and of β error is 5%. With these error values and for very low concentrations, CC_α is calculated with Equation 12 and CC_β with Equation 13 (Antignac et al., 2003 ; Cañada-Cañada et al., 2009).

$$CC_\alpha = 2.33 \frac{SD_i}{s}$$

Equation 12 : CC_α determination

$$CC_\beta = \frac{(2.33 + 1.64) SD_i}{s}$$

Equation 13 : CC_β determination

Trueness was expressed by the calculation of the recovery between the fortified and the measured concentrations.

Repeatability was determined by the standard deviation between every 3 replicates at each concentration level.

Intermediate precision by the standard deviation of replicates analysis on several days.

Specificity was checked by measuring the ion ratio for each validation sample and comparing them with the mean ratio of the calibration curve.

Finally, measurement uncertainty (MU), which is not required by the 2002/657/EC, was also determined. It was calculated for every validation level by the top-down approach (ISO; FFSA, 2008). This evaluation involved the measurement of the random error (Equation 14) and systematic error (Equation 15). The resulting expanded uncertainty was then calculated with a coverage factor of 2 by using the Equation 16.

$$u_{Rw} = \frac{s}{m} 100$$

Equation 14 : Random error

$$u_{bias} = \sqrt{\frac{\sum bias^2}{n}}$$

Equation 15 : Systematic error

$$U = k\sqrt{u_{Rw}^2 + u_{bias}^2}$$

Equation 16 : Expanded uncertainty

2.6.2. Results

Validation requirements and results for each matrix are displayed in the next paragraphs. The validation was conducted at 3 levels during 3 days with 6 replicates per level (n=54).

2.6.2.1. Requirements

Currently, there is no legislation mandating levels of performances for the furan analysis. Only a European recommendation regarding the furan monitoring in food is available (European Recommendation 2007/196/EC), and criteria for accepting furan data in the EFSA monitoring exercise[1]. We refer to this document for the limits, intermediate precision and trueness of the method. For the precision, we use the Thompson modification of the Horwitz function (Thompson et al., 2002) and for the specificity, the 2002/657/EC decision criteria. These performances requirements are summarize in Table 2.

Parameter	Goal	Source
CC_α	2 ng * g^{-1}	EFSA (express as LOD)
CC_β	5 ng * g^{-1}	EFSA (express as LOQ)
Repeatability	15 % ($\sim\frac{2}{3}$22%)	Thompson modification of Horwitz (Thompson et al., 2002)
Intermediate precision	20 %	EFSA (RSD)
Trueness	80 %	EFSA (Recovery)
Specificity	± 10 % (reference ion ratio)	2002/657/EC decision

Table 2 : Requirements for the validation parameters

For the specificity parameter, a pre-calculation is needed: the "reference" ion ratio. This value was determined by the calculation of the mean ion ratio for calibration points over time. Monitoring of the ratio for 121 calibration points and real samples is plotted in the Figure 23. In this figure, the plain line is the mean value and the dashed lines are the European tolerance.

[1] http://www.efsa.europa.eu/fr/dataclosed/call/datex061221.htm

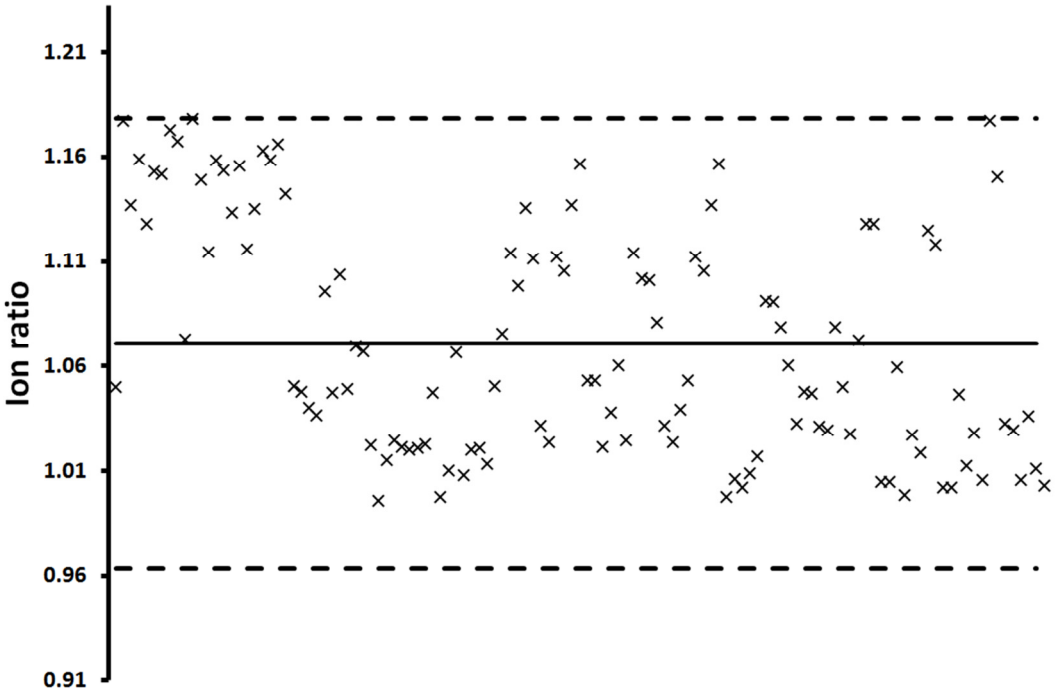

Figure 23 : Monitoring of the ion ratio over 121 calibration points

It highlights that every data points are spread within these tolerance lines and that the calculated reference value is 1.075 with an associated standard deviation of 0.055.

2.6.2.2. Baby food

The validation was carried out on a commercial baby dish: Hachis Parmentier containing mashed baked potatoes, diced meat and sauce. This matrix is naturally contaminated and furan was removed prior to use by constant stirring in a hood until the furan signal is below 1% of the signal recorded for the lowest calibration point.

Figure 24 displays the CC_α and CC_β graphical determination. This is a plot of the calculated concentration over the targeted one. Each line is a linear regression going through the 3 levels of results for one validation day (Table 3). The resulting values are:

- $CC_\alpha = 0.19 \text{ ng} * g^{-1}$
- $CC_\beta = 0.32 \text{ ng} * g^{-1}$

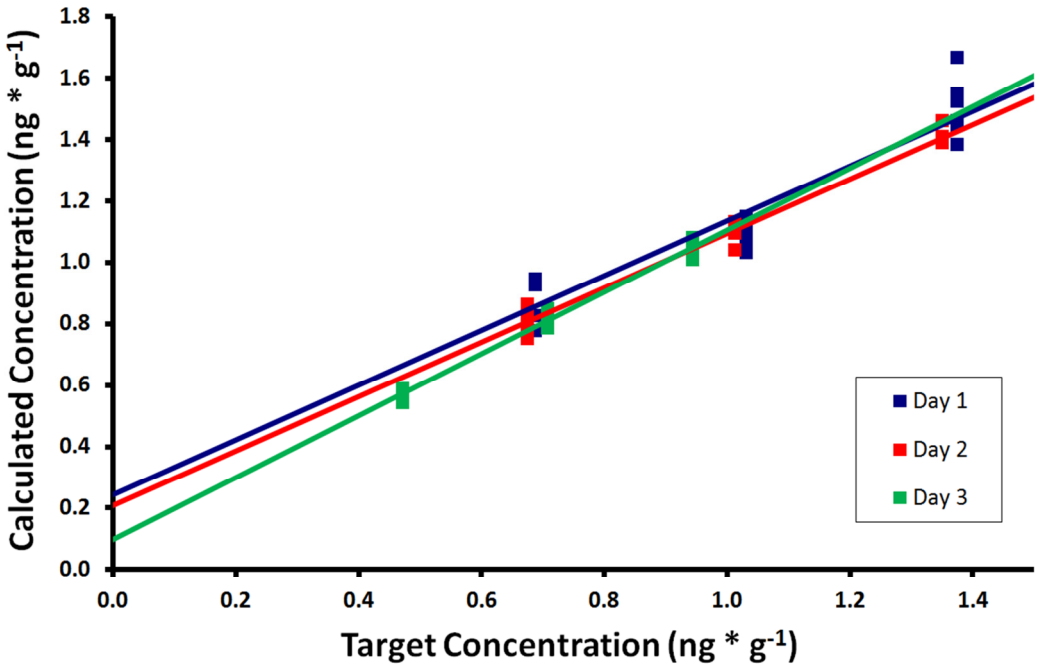

Figure 24 : Determination of CC_α and CC_β in baby food at 3 levels for 3 days

Figure 25 shows the ion ratio for each analysis performed for the validation. In this graph, every result is included within the 2 dashed lines who define the acceptable range (± 10% of the reference value). It means that the specificity criterion is fulfilled.

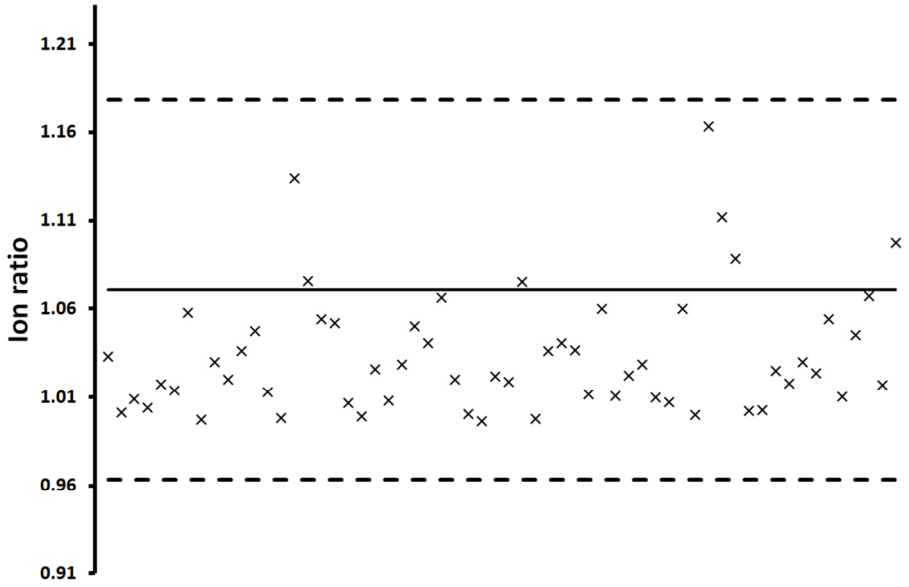

Figure 25 : Ion ratio for the baby food validation

Table 3 presents the values of precisions, recoveries and expanded uncertainties for the 3 validation levels.

Mean target concentration (ng * g^{-1})	Repeatability (RSD, n=6, %)			Intermediate precision (RSD, n=3, %)	Mean recovery (n=18, %)	Expanded uncertainty (k=2; %)
	Day 1	Day 2	Day 3			
0.61	8.1	4.2	2.6	9.6	124	50.1
0.92	3.7	2.8	2.8	4.8	112	24.4
1.22	6.6	1.8	2.2	7.7	110	22.5

Table 3 : Validation parameters values for baby food

These values fulfil the requirements of the section 2.6.2.1. For the precisions, the values are more than 2 times below the requirements, which corresponds to a very stable response over time. The mean recoveries are also acceptable except for the lower level where it is slightly out of acceptable range. In every case, the recoveries highlight a tinny, but acceptable, overestimation of the results as no one is below 100%.

The trueness of the method was also checked on baby food by participating to 2 proficiency tests organised by FAPAS and IRMM. These 2 tests were performed on unfortified matrices, namely parsnip baby food and carrot meal. Results are expressed as recovery value of the consensus concentration and presented in Table 4. These values are acceptable according to our requirements.

Matrix	Consensus concentration (ng * g^{-1})	Measured concentration (ng * g^{-1})	Recovery (%)	z-score (target RSD : 22%)
Parsnip baby soup (FAPAS)	59.6	40.2	81.0	-0.8
Carrot meal (IRMM)	44.2	50.8	115.0	0.7

Table 4 : Results of proficiency tests on baby food

2.6.2.3. Sauces

The validation was carried out on a commercial tomato sauce. This matrix is naturally contaminated and was blanked prior to use by constant stirring in a hood until the furan signal is below 1% of the signal recorded for the lowest calibration point.

Figure 26 displays the CC_α and CC_β graphical determination that have the following values:

- $CC_\alpha = 0.92$ ng * g^{-1}
- $CC_\beta = 1.57$ ng * g^{-1}

This graph also highlights that the experiments of the third level conducted on the third day (green dots) have a lower response and a larger standard deviation compared to the other days. As all the process is automated except for the samples and standards preparation, this discrepancy probably came from a human error during the standards addition spiked at the third level. The impact of these results, not withdrawn, yields to dramatically increase the CC_α and CC_β. However, it still fulfills the CC_α and CC_β requirements: CC_β < 5ng * g^{-1}.

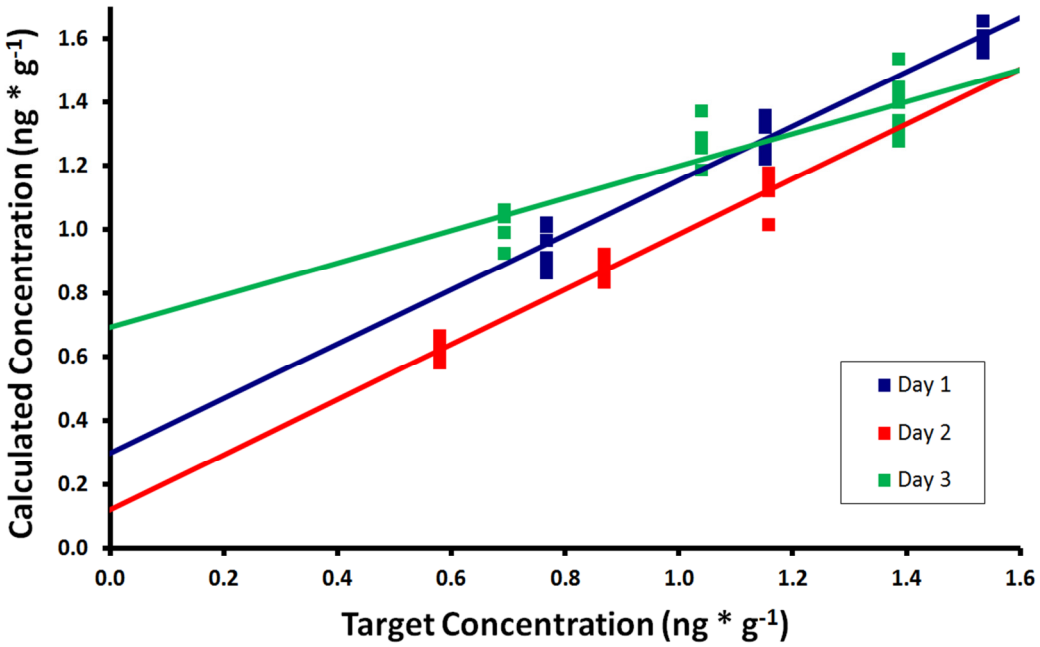

Figure 26 : Determination of CC_α and CC_β in sauces at 3 levels for 3 days

Figure 27 shows the ion ratio for each analysis performed for the validation. As all the validation results are included within the 2 dashed lines defining the acceptable range, the specificity criterion is fulfilled.

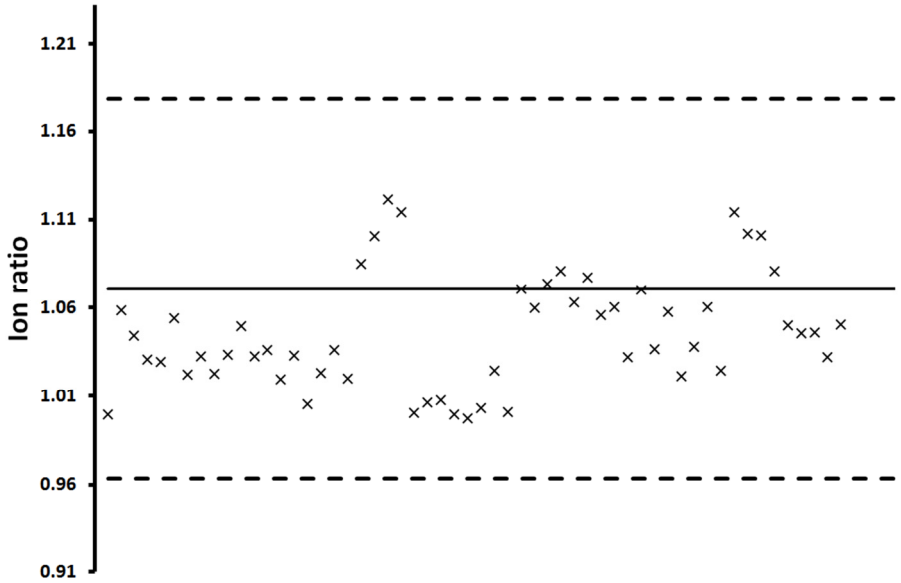

Figure 27 : Ion ratio for the sauces validation

Table 5 presents the values of precisions, recoveries and expanded uncertainties for the 3 validation levels.

Mean target concentration (ng * g^{-1})	Repeatability (RSD, n=6, %)			Intermediate precision (RSD, n=3, %)	Mean recovery (n=18, %)	Expanded uncertainty (k=2; %)
	Day 1	Day 2	Day 3			
0.68	7.0	4.0	5.1	9.1	130	60.9
1.02	4.3	3.8	4.7	6.9	115	32.2
1.37	2.0	5.0	6.3	7.8	106	15.0

Table 5 : Validation parameters values for sauces

Precisions results fulfil the requirements of the section 2.6.2.1 and are low, that corresponds to a very repeatable and reproducible response. On the recovery side, only the second and third levels are in accordance with the requirements. The recoveries also highlight a slight tendency to overestimate the results as they are all above 100%. This tendency growth at low levels and is a lack of linearity. Nevertheless, the first level is below the CC_α and no quantification must occur at this level, so it must be ignored.

2.6.2.4. Juices

The validation was carried out on a homemade orange juice. Contamination of the matrix was checked prior its use, and no furan signal above 1% of the signal recorded for the lowest calibration point was observed.

Figure 28 displays the CC_α and CC_β graphical determination for 3 levels and 3 days. The calculated values are:

- $CC_\alpha = 0.11$ ng * g^{-1}
- $CC_\beta = 0.18$ ng * g^{-1}

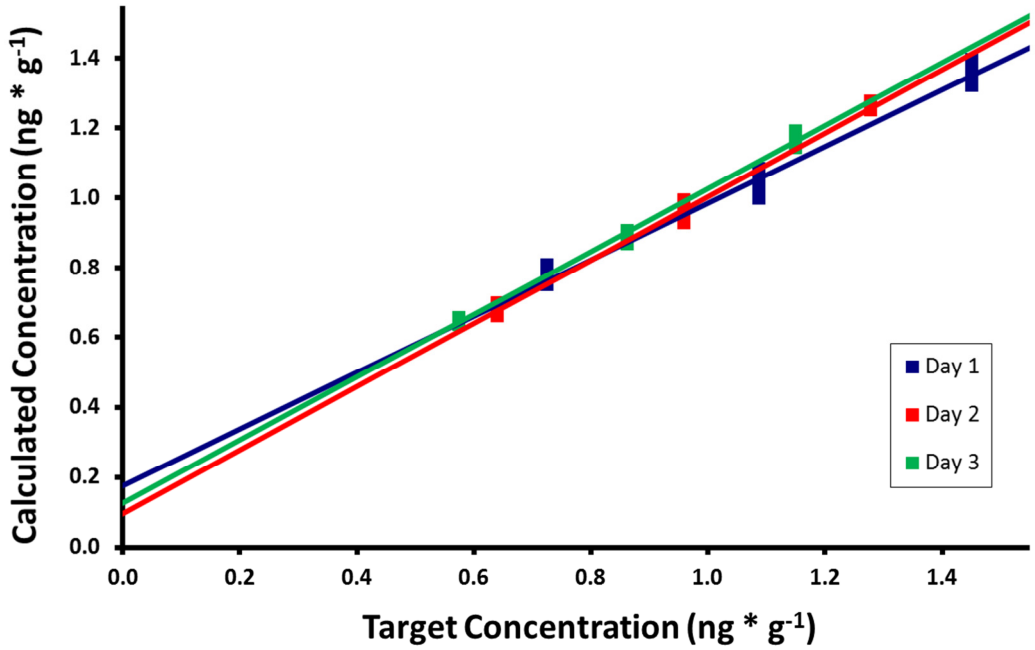

Figure 28 : Determination of CC_α and CC_β in juices at 3 levels for 3 days

Figure 29 shows the ion ratio for each analysis performed for the validation. Every sample fulfills the specificity criterion.

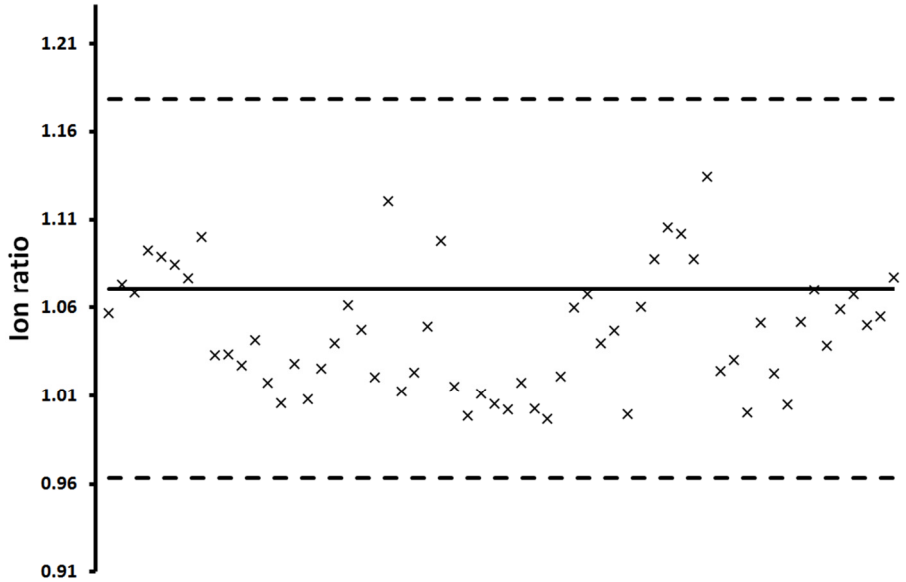

Figure 29 : Ion ratio for the juices validation

Table 6 presents the values of precisions, recoveries and expanded uncertainties for the 3 validation levels.

Mean target concentration (ng * g^{-1})	Repeatability (RSD, n=6, %)			Intermediate precision (RSD, n=3, %)	Mean recovery (n=18, %)	Expanded uncertainty (k=2; %)
	Day 1	Day 2	Day 3			
0.65	2.8	1.9	1.3	3.4	110	20.0
0.97	3.3	2.7	1.3	4.1	95.5	10.5
1.30	1.9	0.7	1.3	2.3	95.8	8.8

Table 6 : Validation parameters values for juices

All parameters at every level fulfil the requirements of the section 2.6.2.1. Precisions values are nearly than 10 times below the requirements, that put in front a very repeatable and reproducible method. The mean recoveries are also excellent as they are within the half of the tolerance and spread over 100 % which corresponds to a non biased dispersion.

2.6.2.5. Hot beverages

The validation was carried out on commercial tea infused in the laboratory. This matrix is naturally few contaminated and was blanked prior to use by constant stirring in a hood until a furan signal below 1% of the signal recorded for the lowest calibration point.

Figure 30 displays the CC_α and CC_β graphical determination over 3 contamination levels for 3 days. The resulting values are:

- $CC_\alpha = 0.60$ ng * g^{-1}
- $CC_\beta = 1.02$ ng * g^{-1}

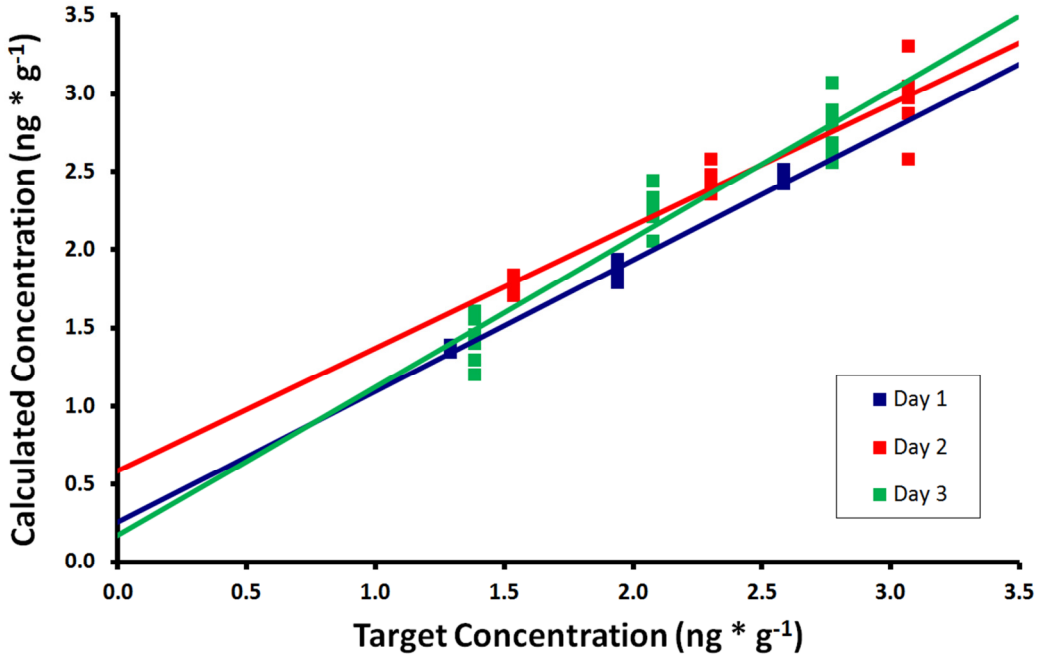

Figure 30 : Determination of CC_α and CC_β in hot beverages at 3 levels for 3 days

Figure 31 shows the ion ratio for each analysis performed for the validation. All results are within the specificity criterion tolerance, but are systematically located below the "reference" ion ratio. This is a consequence of a matrix effect affecting the intensities of the signals of the ions. Nevertheless, as the mean recovery is within the acceptable range (Table 3), only the confirmatory ion (*m/z*:39) seems to be affected.

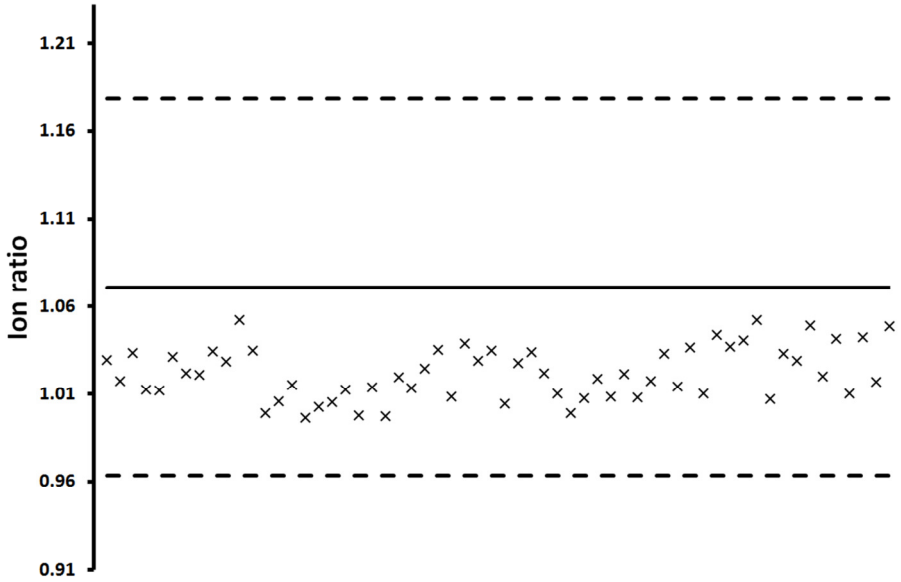

Figure 31 : Ion ratio for the hot beverages validation

Table 3 presents the values of precisions, recoveries and expanded uncertainties for the 3 validation levels.

Mean target concentration (ng * g^{-1})	Repeatability (RSD, n=6, %)			Intermediate precision (RSD, n=3, %)	Mean recovery (n=18, %)	Expanded uncertainty (k=2; %)
	Day 1	Day 2	Day 3			
1.40	1.4	2.9	10.8	12.6	112	27.7
2.11	2.9	3.4	6.4	7.8	109	19.4
2.81	1.6	8.0	6.3	9.9	93.4	17.9

Table 7 : Validation parameters values for hot beverages

As for juices, all parameters fulfil the requirements of the section 2.6.2.1. Only the intermediate precision and mean recovery of the first level are slightly higher, but still in the tolerance. Recoveries results are spread over 100 % that corresponds to non-biased results dispersion.

2.7. Conclusions

As shown by the validation experiments (and summarized in the Table 8), the developed method ID-SPME-GC-MS fulfills all the requirements for the analysis of furan in foodstuffs. The sensitivity we can get by this method is even better than our first objectives ($CC_\beta < 1$ ng * g^{-1}) and also about 5 times lower than the EFSA requirements.

It definitely helped us to conduct the food contamination survey in the best conditions to provide an accurate risk assessment of the Belgian population.

Matrix	CC_α (ng * g^{-1})	CC_β (ng * g^{-1})	Spiked level (ng * g^{-1})	Repeatability (RSD, n=6, %)	Intermediate precision (RSD, n=3, %)	Mean recovery (n=18, %)
Baby Food	0.19	0.32	0.61	2.6 – 8.1	9.6	124
			0.92	2.8 – 3.7	4.8	112
			1.22	1.8 – 6.6	7.7	110
Sauces	0.92	1.57	0.68	4.0 – 7.0	9.1	130
			1.02	3.8 – 4.7	6.9	115
			1.37	2.0 – 6.3	7.8	106
Juices	0.11	0.18	0.65	1.3 – 2.8	3.4	110
			0.97	1.3 – 3.3	4.1	95.5
			1.30	0.7 – 1.9	2.3	95.8
Hot Beverages	0.60	1.02	1.40	1.4 – 10.8	12.6	112
			2.11	2.9 – 6.4	7.8	109
			2.81	1.6 – 8.0	9.9	93.4

Table 8 : Summary of the validation parameters for all matrices

3. Food Contamination Assessment

3.1. Introduction

One of the goals of this thesis is to conduct a risk assessment linked to the furan ingestion through diet for the Belgian population. To achieve this aim a survey of the furan contamination of the food available on the Belgian market is necessary.

A prerequisite to this objective is to develop a high-sensitive analytical method allowing to measure at sub parts-per-billion levels (ng * g^{-1}) in order to limit the number of unreported results. Uncertainty deriving from levels that are non-detectable or also called left-censored data, may impair the capability of drawing conclusions to risk assessment or to regulatory decision making. The headspace SPME GC-MS method developed in the previous chapter meets these objectives by limiting the percentage of non-detectable results.

The approach requires to test large numbers of different food items (matrices selection) but also to keep information on the more contaminated matrices. Testing large numbers of samples of every possible food type individually would be highly resource intensive but also out of financial budget. Our study was then limited to 496 samples. Based on this limited number of samples, we developed a method able to provide the valuable information required to perform an acute risk assessment of the Belgian population exposed to furan.

3.1.1. General purposes of food surveys

Food contamination assessment is a necessary step prior to launch food-monitoring plans by national and international food control authorities. Many examples in different fields such as radionuclides (e.g. ^{137}Cs and ^{90}Sr), pharmaceutical residues (e.g. carbamazepine and primidone), contaminants (e.g. PCDD/Fs and PAHs), pesticides (e.g. DDT and HCB), toxins (e.g. mycotoxins and ciguatera), microbial populations (e.g. *Escherichia coli* and *Staphylococcus aureus*) and many other. Actually, food survey is a powerful tool to assess the evolution of an incident, assess the risk for a population, take decisions or enforce legislations.

3.1.2. Principle

The survey principle is to collect and analyze samples with respect to a dedicated sampling plan. This plan must be designed in a way to collect the desired information, as shown in these practical examples reported in the literature:

- Benzene intake through soft drinks (Medeiros Winci et al., 2012) who only involved soft drinks samples
- PCDD/Fs intake through diet reevaluation (Windal et al., 2010) who only involved critical items samples

As highlighted in these 2 examples, there is not a unique sampling plan design. The sampling design must be constructed to fit for the final objective purpose. Therefore, it must take into account consideration such as sampling frame, food matrix, food origin, importance of the food item and sample preparation, but also the number of samples available or the sampling timeline.

3.2. Sampling plan construction, survey results and discussion

The approach selected has been to divide the food chain into 36 sub-categories (De Vriese et al., 2005) and plan to collect an equivalent number of samples for each of them. Adjustments were made in the number of samples per group to take into account the contamination levels (FDA, 2004; Reinhard et al., 2004; EFSA, 2005; Zoller et al., 2007; Crews et al., 2009; Kim et al., 2009a), the consumption frequencies (De Vriese et al., 2005; Huybrechts et al., 2008a, b) and the food matrix diversity by applying three weighting factors to each of the 36 groups. Next, samples were purchased in different food market chains (and different brands) from a random selection of different locations in Belgium (cities, large and small towns, rural area) over a period of 10 days, as shown in Figure 32. This is intended to take into account of geographical differences and differences in local suppliers. The complete description of the methodology and the results obtained are presented in the paper published in food Additives and Contaminants attached to this chapter.

Figure 32 : Samples collection places

3.2.1. Related paper

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Estimation of furan contamination across the Belgian food chain

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This paper provides an estimate of the furan content of Belgian foods. The objective of the study was to achieve the best food chain coverage with a restricted number of samples ($n = 496$). The geographic distribution, different market chains and labels, and consumption frequencies were taken into account in the construction of the sampling plan. Weighting factors such as contamination levels, consumption frequency and the diversity of food items were applied to set up the model. The very low detection capabilities (CC_β) of the analytical methods used (sub-ppb) allowed reporting of 78.2% of the overall dataset above CC_β and, in particular, 96.7% for the baby food category. The highest furan levels were found in powdered roasted bean coffee (1912 $\mu g\,kg^{-1}$) with a mean of 756 $\mu g\,kg^{-1}$ for this category. Prepared meat, pasta and rice, breakfast cereals, soups, and baby food also showed high mean furan contents ranging from 16 to 43 $\mu g\,kg^{-1}$. Comparisons with contamination surveys carried out in other countries pointed out differences for the same food group and therefore contamination levels are related to the geographical origin of food items.

Keywords: baby food; coffee; meat; processed foods; process contaminants; exposure assessment

Introduction

Furan was isolated for the first time in food in the late 1970s (Maga 1979). The first report on its toxicology and carcinogenesis came out 14 years later (National Toxicology Program (NTP) 1993). In 1995, the International Agency for Research on Cancer (IARC) classified it as 'possibly carcinogen to humans' (group 2B). Five years later, the American National Academy of Sciences (NAS) (2000) classified it as a narcotic. More recently, furan became an increasing matter of concern since a report about its occurrence in food was published by the United States Food and Drug Administration (USFDA) (2005). As a result, national and international food authorities required information about its levels in food, human exposure and the formation pathways to be gathered (Stadler 2007). In Europe, the first report published by the European Food Safety Authority (EFSA) (2005) contained a compilation of early reported data. Later, EFSA organised the collection and centralisation of food-stuffs monitoring data from European Union member states in a European database. Since 2009, EFSA has published summarised reports regularly (EFSA 2009, 2010). In addition, independent and timely studies were also conducted in European countries and in Asia (e.g. Reinhard et al. 2004; Zoller et al. 2007; Crews et al. 2009; Kim et al. 2009; Liu and Tsai 2010). Recently, the production of furan was described in heat-processed food including home-cooked and ready-to-eat items (Hasnip et al. 2006; Crews and Castle 2007; Roberts et al. 2008; Fromberg et al. 2009). Other papers studied the influence of vitamin C or fat oxidation on the generation of furan in a starch-based model (Owczarek-Fendor et al. 2010a, 2010b). Significant differences related to the origin and brands of products were also reported (Wegener and López-Sánchez 2010). These authors pointed out clear differences in the composition and preparation of final products among factories and countries. As a result, country-by-country contamination studies are needed for an accurate estimation of furan levels. Other studies have focused on the toxicity and carcinogenicity of furan in the human diet, as recently reviewed by Bakhiya and Appel (2010). All these studies have

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contributed to fulfil the lack of reliable data needed to conduct an accurate risk assessment (Heppner and Schlatter 2007).

Sampling strategies can follow different approaches. If the study is subjected to economic constraints limiting the number of samples to analyse, then the study can only focus on the most contaminated items (Crews et al. 2009; Wegener and López-Sánchez 2010). On the other hand, large numbers of samples can support exhaustive studies. As an example, EFSA or the Food and Agriculture Organisation (FAO) have collected data from national surveys and combined them in European or international databases (EFSA 2009; Joint FAO/WHO Expert Committee on Food Additives (JECFA) 2010).

In this paper we propose an original approach to carry out, as exhaustively as possible, a contamination assessment of the food chain with a restricted number of samples. The methodology introduces several weighting factors with the idea to emphasise or minimise the role of three selected parameters that were considered as essential. In this context, a prerequisite condition was to use the same analytical method in order to avoid method-to-method analytical biases. In addition, the selected method was sensitive enough to minimise the number of results below the detection capability (CC_β).

Materials and methods

Analytical methodology

The analysis was carried out using the method described and validated by Scholl et al. (2007, 2009). It is a sub-room temperature on-line isotopic dilution–solid-phase microextraction–GC-MS (ID-SPME-GC-MS) methodology. Briefly, samples are mashed and mixed in a cooled room kept at +4°C. A sample (1 g) was then weighed into a tarred 20 ml headspace vial (La-Pha-Pack, Langerwehe, Germany) containing 0.4 g of salt (Sigma-Aldrich, St. Louis, MO, USA) and 1 ml of Milli-Q® water (Millipore, Brussels, Belgium). Rapidly, the sample was spiked with a deuterated-isotopomer (d_4-furan – 98%; Sigma-Aldrich) and closed in an airtight container. Samples were prepared one by one, as quickly as possible, to avoid furan loss by evaporation.

The deuterated standard used for quantification was a 100 pg μl^{-1} water solution prepared daily by dilution of d_4-furan. The dilution was carried out in two steps: first, by addition of 10 µl of d_4-furan in a 20 ml airtight vial full of methanol (picograde; LGC-Promochem, Wesel, Germany); and second, by introducing 4 µl of the first solution in a 20 ml airtight vial full of Milli-Q® water. The same protocol was applied to prepare native furan (purity >99%; Sigma-Aldrich) stock solution used to build up a three-level calibration curve with at least two replicates per level.

The measurement was performed with a PolarisQ ion-trap mass spectrometer (Thermo-Scientific, Waltham, MA, USA) coupled to a Trace GC2000 equipped with a programmable temperature vaporisation (PTV) injector. Chromatographic separation was achieved on a PoraBond-Q (25 m × 0.32 mm × 5 µm) column (Varian, Palo Alto, CA, USA) at 1.7 ml min^{-1} He (99.9997% purity; Air Products, Allentown, PA, USA) constant flow. The temperature programme started at 35°C for 2 min, ramped at 10°C min^{-1} to 100°C, hold for 5 min, followed by a 30°C min^{-1} temperature ramp to 260°C, then hold for 6 min. Ions were produced by a 70 eV positive electron ionisation (EI) source kept at 200°C. The acquisition was recorded in selected-ion monitoring mode (SIM). Ions m/z 68 and 72 were chosen for quantification of furan and d_4-furan, respectively. The relative intensities of both ions of the furan molecule (i.e. m/z 68 and 39) and the d_4-furan (i.e. m/z 72 and 42) should correspond to those of the calibration standard solutions to check the presence of possible interferences.

Furan extraction was carried out with a fully automated sub-room temperature SPME integrated with a Combipal system (CTC Combipal, CTC Analytics, Zwingen, Switzerland). The fibre was a 75 µm Carboxen™-Polydimethylsiloxane coating (Supelco, St. Louis, MO, USA). The extraction time and temperature were matrix dependent. Temperature was set and kept constant by a Peltier cooling system (CTC Analytics) during extraction. Fibre desorption occurred in the injection port of the PTV kept at 230°C in splitless mode. Finally, fibre was cleaned using a side oven maintained at 275°C under He gentle flow.

Samples

Samples were freshly purchased in several markets across Belgium according to a sampling plan described below. Samples were stored at −20°C or at room temperature prior to analysis according to the manufacturer's recommendations.

Statistical tests

Welch's test was used to compare the mean furan concentration from this study with several available studies with unequal variances (Dagnelie 1998). The Bonferroni correction, which is a method used to address the multiple comparisons problem, was also used. This correction is based on the idea that if n hypothesis are involved, each individual hypothesis must be tested at $1/n$ times the significance level to maintain the family-wise error rate. In the present study, we compared together three datasets at a 95%

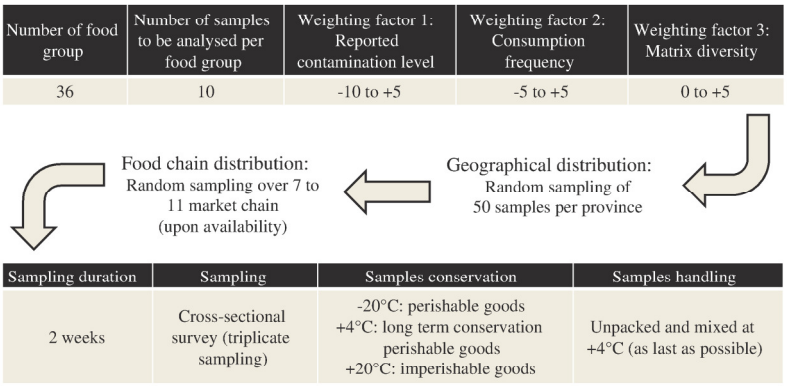

Figure 1. Sampling plan for the estimation of furan contamination across the Belgian food chain.

confidence level. To verify the null hypothesis, the calculated *p*-value must be below or equal to 0.05. However, when using the Bonferroni correction with three comparisons involved, a *p*-value is reduced to 0.017 (Petrie and Watson 2006).

Results and discussion

Sampling plan construction

The number of samples allocated for the furan contamination assessment was limited to 496.

To extract as much information as possible from this limited sampling number, three key parameters were identified and selected to construct the model: contamination levels, food diversity and consumption frequency (Figure 1). As the Belgian consumption survey (IPH/EPI 2006) did not include any data for baby food, the model was not applied to this specific category. The 30 baby food samples were treated separately as a category itself. The 466 remaining samples were distributed over the food chain.

First, the food chain was divided into 36 groups (Table 1), and 10 samples were assigned to each food group without any other consideration.

Second, a weighting factor based on already reported contamination levels (EFSA 2005; USFDA 2005) was applied to the number of samples to be analysed in each food group. This weighting factor was within −10 and +5 samples and was only modified by a 5-samples step (i.e. −10, −5, 0 or +5 samples). For instance, a + 5 factor was selected for items reported to be the most contaminated such as coffee, baby food or crispy food. On the contrary, a − 10 factor was applied for items never reported as being contaminated (e.g. reported as 'not detected') such as water and fresh eggs. Between these two extreme cases, two additional moderate factors were used: −5 and 0, respectively (Table 3).

Table 1. Groups of food items in the Belgian food chain ($N = 36$).

Spirits	Beer
Bread	Breakfast cereals
Cereal based products	Cheese
Coffee	Cooking fat
Edible offal	Egg
Fish	Fish based products
Fresh meat	Fruit
Fruit juices	Goats and rabbits
Light soft drink	Meat
Meat based products	Milk and dairy products
Other	Pasta and rice
Potatoes	Poultry
Sauce	Seafood
Soft drink	Soup and vegetable juices
Soy based products	Spreading fat
Tea	Vegetarian food
Vegetables	Water
Wine	Yoghurts and pudding

Third, frequencies of food consumption were also taken into account in this strategy. Based on the Belgian national dietary survey, a weighting factor between −5 and +5 (by steps of 5) was directly awarded to three categories of consumption frequencies: highly (+5 samples), moderate (no change) and little consumed (−5 samples).

Fourth, a criterion relying on the diversity of food items within a group was also investigated. A cut-off value based on the number of different matrices that could be included within a group was applied. A weighting factor (+5) was computed on groups with a number of matrices above the cut-off, while it remained unchanged below that value.

Fifth, the sampling plan proposal was submitted to a Belgian committee of experts all working in the field of food safety. This committee critically reviewed the proposed weighting factors based on the members' own experience in food safety. They provided recommendations to modify the third and fourth weighting

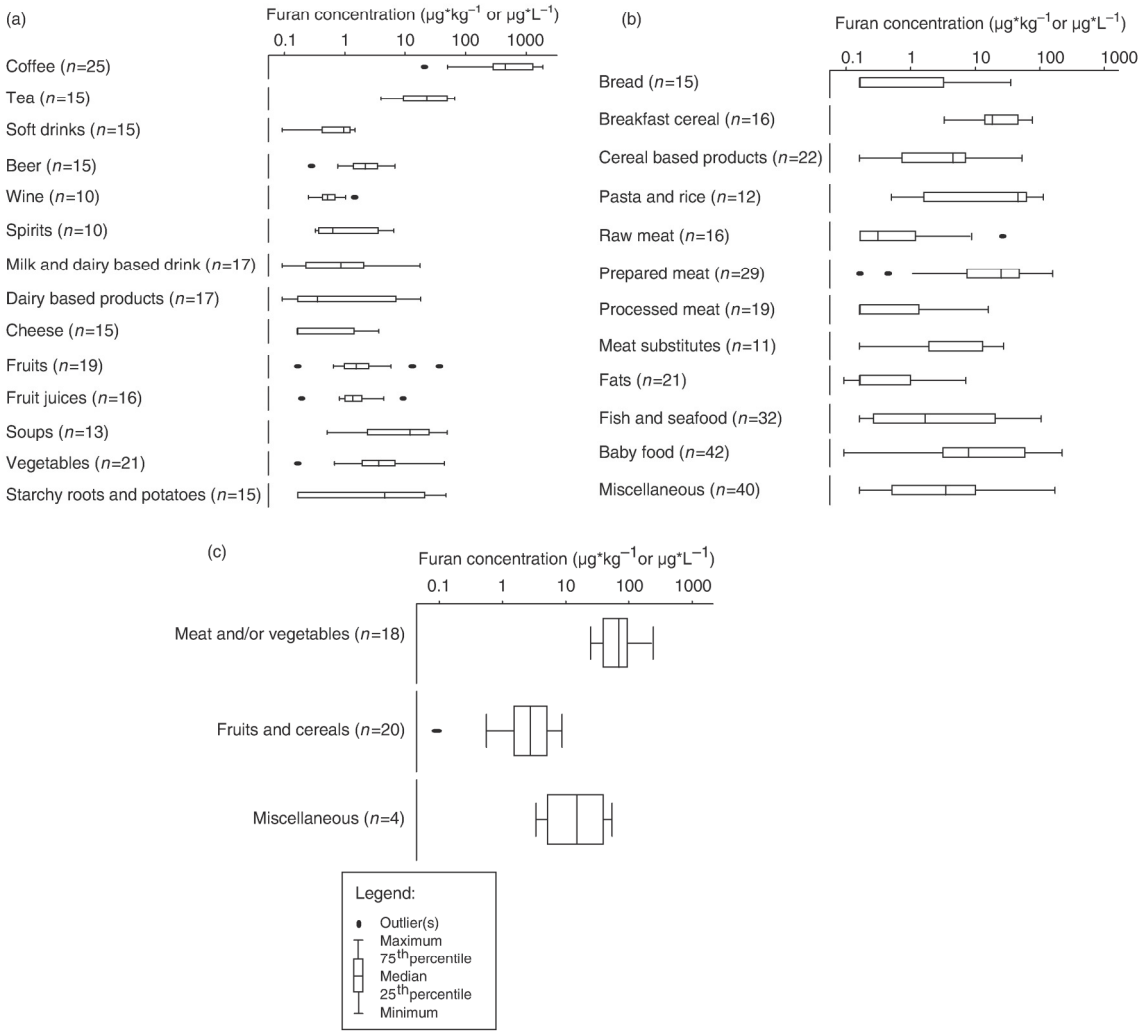

Figure 2. Furan contamination levels across the Belgian food chain: (a, b) all the food chain; and (c) baby foods (details).

factors with the final objective being to extract a consensus sampling plan.

Sixth, to discard any geographical effect, the sampling was spread over the country. As Belgium is divided into ten provinces, the same number of samples was randomly collected in each province.

Lastly, specifically to avoid a brand or a food market chain related to a province, the samples were randomly distributed over seven to 11 food market chains depending upon the availability.

Approximately 100 different markets were visited in the country. Between five and eleven items were purchased in triplicate in each market.

Results

According to the authorities' recommendation (European Commission 2007), three individual items of each sample were mixed and homogenised. A representative aliquot was then sampled for analysis according to the described methodology. The study only focused on raw samples in order to avoid biases from cooking and heating effects. The analytical method was developed to achieve a high sensitivity and a low $\mu g\,kg^{-1}$ detection capability (CC_β). As a result, 78.2% of the overall samples analysed were above CC_β, and in particular 96.7% of baby food samples were above the CC_β.

The results are summarised in Figure 2(a) and 2(b), which show that furan was present in a variety of commercial foods and the levels spanned several orders of magnitude from background levels (sub-ppb) to the highest one (hundreds of ppb). The highest levels, sometimes exceeding $1000\,\mu g\,kg^{-1}$, were found in coffee. Lower but nevertheless very high levels close to $100\,\mu g\,kg^{-1}$ were found in prepared meat, pasta and

Table 2. Comparison of the results of three European surveys for the most contaminated food groups using a two-tailed Welch *t*-test. The limit of significance was defined as $p < 0.017$.

	Belgian survey			EFSA (2009)			Swiss (2004)		
	n	Mean	SD	*n*	Mean	SD	*n*	Mean	SD
Coffee	25	756[b,c]	666	398	1476[c,e]	1292	111	36[b,f]	35
Prepared meat	44	35	38	65	22[d]	28	49	49[a]	44
Pasta and rice	12	43	39	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Baby food	42	35	45	985	25	27	350	28	28
Breakfast cereals	16	31[a,c]	25	99	14[f]	22	11	9[f]	9
Soups	13	16[d]	16	198	24[d]	28	50	39[e,a]	31

Notes: mean = mean concentration in $\mu g\,kg^{-1}$ or $\mu g\,l^{-1}$; n.d., data unavailable; *n*, number of samples in the group; SD, standard deviation, $\mu g\,kg^{-1}$ or $\mu g\,l^{-1}$.
[a]Significantly higher than the EFSA study.
[b]Significantly lower than the EFSA study.
[c]Significantly higher than the Swiss study.
[d]Significantly lower than the Swiss study.
[e]Significantly higher than the present study.
[f]Significantly lower than the present study.

rice, baby food, and breakfast cereals. Raw meat products, fat, fresh fruits, milk and milk products and alcohol groups showed a low mean furan content. Heat-treated foods such as roasted and/or long time-cooked items were characterised by a high furan content (Roberts et al. 2008; Fromberg et al. 2009). For foods that did not follow these cooking recipes, they are mainly classified in the low content category. As expected, the majority of samples below CC_β are gathered in the low levels groups.

In addition, contamination levels are not homogeneous within a food group. They are scattered from low to high concentrations depending either on the food type and/or on the cooking and packaging methodology.

Baby food results are displayed in Figure 2(c). Items are distributed over three subgroups: baby food-containing cereals and fruits, baby food-containing meat and/or vegetables, and other baby food. Low contamination levels were found in the first group with a mean value (with 95% confidence interval) of $3 \pm 5\,\mu g\,kg^{-1}$. The second group exhibited a much higher mean level ($65 \pm 57\,\mu g\,kg^{-1}$); while the third is somewhat an intermediate between the first two groups ($23 \pm 73\,\mu g\,kg^{-1}$). In the present study, the results clearly indicate that the level of furan in baby food is linked to the food composition.

Comparison with previous surveys

Several contamination assessments were conducted around the world. Two were selected for a comparison as their data are in the public domain. The present results were then compared with data reported by Switzerland (Reinhard et al. 2004; Zoller et al. 2007) and by EFSA (2009). Mean values of the highest contaminated food groups for the three studies are presented in Table 2. These results were compared by using Welch's test with Bonferroni correction for statistical significance.

For the Coffee group, Table 2 shows that the null hypothesis is verified neither between the present study and EFSA, nor between the present study and the Swiss work, nor between EFSA and the Swiss study. The result displayed by the Swiss study is several times lower than the results obtained by the other groups. This difference is explained by the applied methodology: the Swiss survey reports results from brewed coffee, whereas the present study and the EFSA report focus on raw coffee. Formerly, it was demonstrated by several groups that coffee furan content is recipe dependent (Kuballa 2007; La Pera et al. 2009; Guenther et al. 2010), and as the same methodology was not applied, these results are not comparable. The alternative hypothesis shows that the mean value is nearly twice lower than the EFSA mean value ($p < 0.0001$). This difference is linked to the results of the 'roasted bean coffee' and of the 'unspecified' subcategories of the EFSA survey which display very high contamination levels. Only a few of them were analysed in the present survey and they did not display such high levels. This seems to be consecutive to the differences linked to the roasting process as previously highlighted by Guenther et al. (2010).

For the Prepared meat group, the null hypothesis was verified for the comparison between the present survey and EFSA, and between the present survey and the Swiss study. Significant differences ($p = 0.0002$) were observed when comparing Swiss and EFSA surveys mean values. The mean value measured in the present study was included within the same range

Table 3. Weighing factors used for the furan sampling across the food chain.

Food groups	Base number of samples	Weighting factor 1: reported contamination level	Weighting factor 2: consumption frequency	Weighting factor 3: food group diversity	Total number of samples
Baby food	30	–	5	10	45
Beer	10	–	–	5	15
Bread	10	–	–	5	15
Breakfast cereals	10	–	–	5	15
Cereal based products	10	–	–	10	20
Cheese	10	–	–	5	15
Coffee	10	5	5	5	25
Cooking fat	10	–	–5	5	10
Edible offal	10	–	–5	–	5
Egg	10	−10	–	–	0
Fish	10	–	−5	5	10
Fish based products	10	–	–	–	10
Fresh meat	10	–	–	–	10
Fruit	10	–	–	10	20
Fruit juices	10	–	–	5	15
Goats and rabbits	10	–	−5	5	10
Light soft drink	10	–	–	–	10
Meat	10	–	–	5	15
Meat based products	10	–	–	5	15
Milk and dairy products	10	−5	–	5	10
Other	10	5	5	5	25
Pasta and rice	10	–	–	5	15
Potatoes	10	–	–	5	15
Poultry	10	–	−5	5	10
Sauce	10	–	–	5	15
Seafood	10	–	–	–	10
Soft drink	10	−5	–	–	5
Soup and vegetable juices	10	–	–	5	15
Soy based products	10	–	–	–	10
Spirits	10	–	−5	5	10
Spreading fat	10	–	–	–	10
Tea	10	–	–	5	15
Vegetarian food	10	–	–	–	10
Vegetables	10	–	–	10	20
Water	10	−10	–	–	0
Wine	10	–	–	–	10
Yoghurts and pudding	10	–	–	5	15

Note: For explanation, see the subsection entitled 'Sampling plan construction'.

of values calculated in the two other studies. The Swiss mean value was nearly twice higher than EFSA mean value. It can be assumed that there is an influence of 'local products' and/or 'local preparation'. Several studies showed that furan concentration is related to the exact composition and preparation recipe of food items (Crews et al. 2007; Roberts et al. 2008; Wegener and López-Sánchez 2010). Other authors suggested that the exact food composition and recipe are geographically related (Merchant and Dehghan 2006; Pennington 2008). This phenomenon presents a higher impact on 'composed' or 'prepared' food items rather than on basic products.

For the Soups group, the null hypothesis was only verified when comparing the present survey with the EFSA study. The comparison between the present study and the Swiss work showed significant differences ($p < 0.0015$), as well as the comparison between EFSA and Reinhard–Zoller mean values. In both cases, the concentration reported by the Swiss survey is more than twice as high. Two hypotheses can be drawn in relation to the composition of the food item. First, it could be linked to the influence of the local production on furan content. Second, the food group sampling can induce biased results.

For the Breakfast cereals group, the null hypothesis is not verified between the present study and the Swiss or the EFSA survey. In each case, the contamination level is more than twice higher than the level reported by the Swiss and the EFSA surveys. Within this group, the major contribution comes from roasted products. Therefore, there are few differences from country

to country. Nevertheless, the null hypothesis was not verified probably because the Swiss and the EFSA surveys also included other types of cereal products that are not roasted, thus presenting lower contamination levels.

For the Baby food group, the null hypothesis is verified for all the comparisons. The mean values of the three surveys were similar at a 95% confidence interval. This can be explained by the scattering of data in this group (i.e. the standard deviation is equal to or higher than its corresponding mean value for each group). As already shown in Figure 2(c), the baby food group is very large and can be divided into three subgroups containing, respectively: meat and/or vegetables, fruits and cereals, and other.

For the Pasta and rice group, the comparison was not possible as this category was not present in the other studies.

In general, the results are in accordance with the EFSA survey, but to a lesser extent compared with the Swiss study when using Welch's test to take into account the Bonferroni correction ($p < 0.017$). For each mean value, except for coffee, the corresponding p-value is less than 0.01. The statistical significance seems to be related to the local products. Therefore, this is one more clue to support the fact that the European survey is 'very large', and it provides results comparable with local surveys, but that local surveys provide more accurate data useful when carrying out more precise risk assessments.

Conclusions

The study shows that almost the whole food chain is contaminated with furan. Roasted foods (such as breakfast cereals or coffee), long-time cooking foods or foods contain sauces (such as prepared meat compared with raw meat) are the most contaminated. Fat, raw meat, milk, alcohol and fresh fruits are the less contaminated items. It suggests that the heat-processing conditions are crucial for contamination levels. Baby food results display a high disparity and can be distributed over three groups according to food composition.

The methodology developed for this assessment is fit for purpose. One can carry out an evaluation of mean levels, ground levels and critical items by using a limited number of samples. Such evaluation usually includes a very high number of samples to be exhaustive or only focuses on the known critical items. The methodology used is useful to determine some information on background levels, mean levels and on the most contaminated items. An overall screening of the food chain can also be used for several purposes, such as risk assessment, identification of the critical items, estimation of the ground level or identification of some formation-critical components.

In addition, the results are consistent with published studies (Reinhard et al. 2004; Zoller et al. 2007; EFSA 2009). However, when comparing data, one should especially be careful with the way of reporting data (furan in coffee). On the other hand, statistical differences could mainly be attributed to the exact food composition, which is linked to the geographical origin of the food item. This tends to prove that local surveys induce less variability than international surveys. Precise risk assessments could be better obtained by using a local approach, especially in order to determine the more risky or exposed population.

In conclusion, the proposed methodology successfully fulfils this study's requirements, which were to combine the results that can be obtained using a screening survey with those obtained by using an exhaustive methodology with a limited number of samples. Therefore the proposed methodology is a fast and cost-effective methodology useful when carrying out a 'pseudo-exhaustive' contamination assessment across the food chain.

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4. Risk Assessment

4.1. Introduction

When considering the impact of the presence of furan in food, an important consideration is the need to determine the risk to public health, which is normally established through a risk assessment. This section examines the means by which dietary exposure to furan may be assessed for the Belgian population. We propose to use a deterministic and a probabilistic approach (section 4.1) to estimate the daily intake of Belgian adults (section 4.2) and children (section 4.3). The exposure to Belgian toddlers was limited to the deterministic approach (section 4.4).

For the adults and children, food items can be considered as similar, but consumption frequencies are different. These frequencies were provided by two consumption surveys: the first Belgian consumption survey (De Vriese et al., 2005) managed by the Institute of Public Health, and the diet survey of preschool children (Huybrechts et al., 2008) organized by the University of Ghent.

For the toddlers, food items and consumption frequencies are completely different and they are changing according to their age. In the present study, we were only able to conduct a preliminary risk assessment due to a lack of consumption data. Indeed, today, there is no Belgian infant diet survey available. We based our risk assessment on data available from Italy (Leclerq et al., 2009). Additionally, contamination data only focuses on ready-to-eat baby foods, which are eaten by a limited portion of the infant population. Finally, the furan toxicity for toddlers is not completely understood as the metabolisation process involves the cytochrome P450, which, in this case, is still in maturation.

4.1.1. Definition

According to the EPA[1], Risk Assessment is: "*The estimation of the nature and probability of adverse health effects related to: microbiology (microbes, bacteria or viruses), chemistry (drugs or pollutants), and other hazards (fire, war or riots)*". In other words, this is: "*The process used to quantify the risk related to a hazard*".

In this definition, two words are pivotal for risk assessment: Risk and Hazard. They need to be correctly defined to avoid any misunderstanding. In the risk assessment framework, the **Risk** is "the probability of the severity of a harmful effect to human health or

[1] EPA Risk Portal : http://epa.gov/riskassessment/basicinformation.htm#arisk

to ecological systems exposed to a hazard" and the **Hazard** is "any physical, chemical, or biological entity that can induce an adverse response". Next to these clarifications, we can turn to the risk assessment methodology used in this study.

Figure 33 : Risk assessment dilemma

4.1.2. Methodology

The risk assessment methodology used in the present study is a 3 steps-cycling process related to the risk namely: Assessment, Management and Communication (Renwick et al., 2004; Huyghebaert A. et Houins G., 2005; Feinberg et al., 2006; Huyghebaert A. et Houins G., 2007; Huyghebaert A. et Houins G., 2008; Figure 34). Every step is defined in the following paragraphs.

First, the **Assessment** itself is a multidisciplinary method as it includes four steps, namely hazard identification, hazard characterization, exposure assessment, and risk characterization who will be defined in the paragraph 4.1.2.1.

Second, the **Management** concerns decisions and actions taken by authorities but also the re-evaluation and the follow-up decisions.

And third, the **Communication** consists in informing the scientific community, managers and the population about the estimated risk and how to deal with it, but also about decisions taken.

As a consequence, risk assessment is a continuous process always going from decision to new decision by re-evaluation of the current situation.

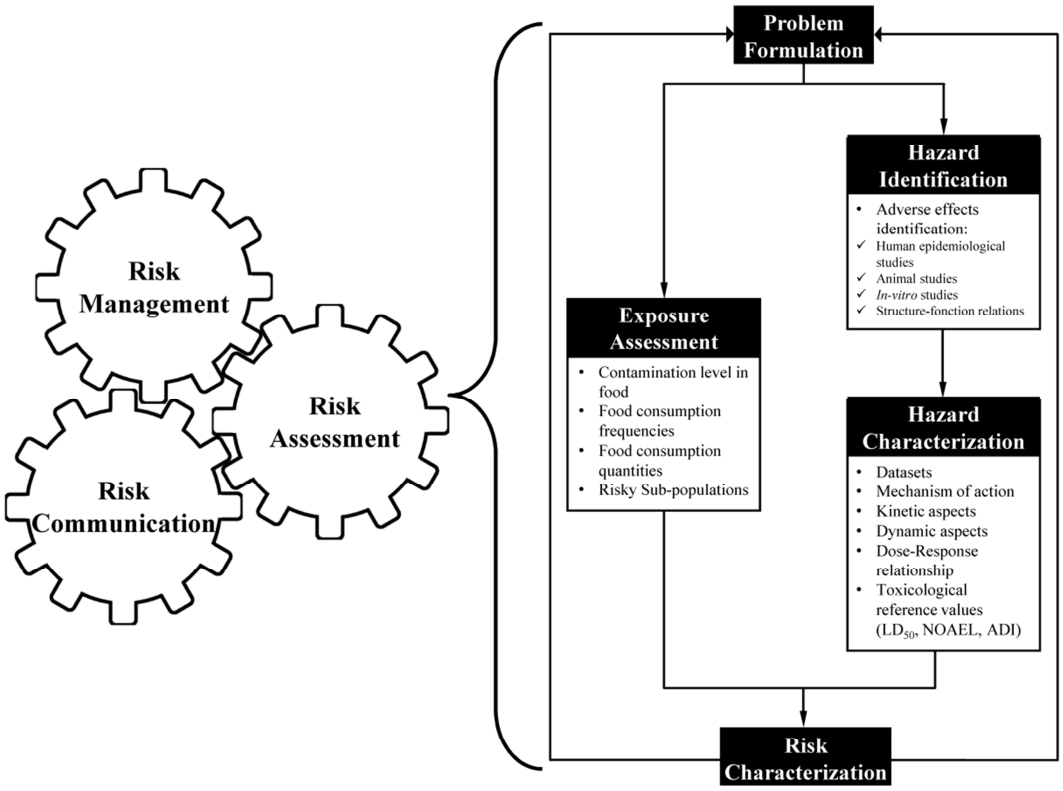

Figure 34 : Risk assessment methodology

4.1.2.1. Assessment

As previously introduced, the **assessment** involves 4 steps:

The first step is the hazard identification, which is the collection of the reported adverse effects of the agent.

The second step is the hazard characterization, which is a toxicological step. First, the toxicological approach namely acute, sub-chronic or chronic needs to be selected. Second, the mode of action (MoA), of metabolisation and of elimination; the dynamic and kinetic aspects; the intake pathways; the Dose-Response relationship; and the toxicological reference values such as LD_{50}, LOAEL or $BMDL_{10}$ need to be defined.

The third step is the exposure assessment, where the toxicant intake by a body is estimated for a given period of time usually one day (EDI), one week (EWI) or one year (EYI). Deterministic or probabilistic datasets of contamination and consumption are combined through equations including factors such as toxicant absorption, metabolisation or excretion to calculate the toxicant intake (Equation 17, Equation 18 and Equation 19). As the

intake is related to a "target" population or sub-population, the exposure assessment is also related to these populations.

$$EDI_{Global} = \sum_{Diet} EDI_{FoodGroup} + \sum_{Other} EDI \; - \sum EDO$$

Equation 17 : Calculation of the global EDI with regard to the intake by the diet and the other pathways (e.g. respiration and/or skin absorption), and to the uptake (e.g. excretion and/or metabolisation)

$$EDI_{FoodGroup} = [Toxicant]_{FoodGroup} \times (relativeDailyConsumption)_{FoodGroup}$$

Equation 18 : Calculation of the daily intake through the diet for each food group

$$relativeDailyConsumption = \frac{DailyConsumption}{BodyBurden}$$

Equation 19 : Calculation of the relative daily consumption

The fourth step is the risk characterization, which is the concluding step. It is a comparison between the results of the exposure assessment and the information of the hazard characterization. Several ways (Feinberg et al., 2006; ILSI, 2009) are available according to the toxicological effect taken into account (acute or chronic; with or without threshold effect). For substances for which thresholds can be identified (e.g. genotoxicity), the classical approach is to compare a toxicological reference dose such as ADI or LD_{50} to the daily intake. In contrast, certain contaminants can have no threshold since it is not possible to define a safe level of exposure. Risk assessment may then be based on a Margin of Exposure (MoE, see Equation 20). The magnitude of the ratio is used to determine the level of risk to health, in the order of 100-10000. A MoE below 100 is a major concern for health, and a MoE above 10000 is a low concern for health.

$$MoE = \frac{BMDL_{10}}{EDI}$$

Equation 20 : MoE calculation for a without threshold MoA

4.1.2.1.1. Deterministic approach

In this approach, the intake is represented by punctual value and is calculated for several categories of the population according to selected cases, e.g. mean, median or worst case scenario. These values are calculated through the Equation 17, Equation 18, Equation 19 and Equation 20 using the equivalent statistical characteristic parameters for contamination, consumption, body burden and excretion.

This is a straightforward tool, which can be applied when few data are available but it only gives results for selected case studies.

4.1.2.1.2. Probabilistic approach

In this approach, the intake is represented by a distribution function (Figure 35) calculated for the overall population. This distribution can be divided into several sub-population functions for analytical purposes.

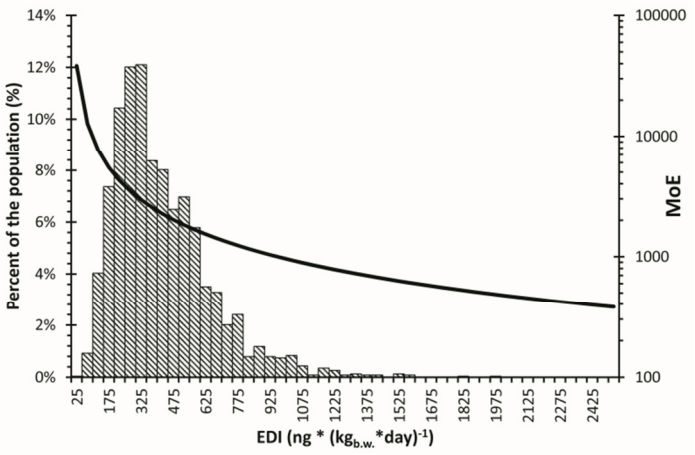

Figure 35 : EDI distribution function

This intake function calculation involves raw contamination and consumption data which are converted into functions of occurrence by using a software such as @Risk (Palisade Corporation, New York, USA). The final intake function is calculated through the 4 equations presented in the section 4.1.2.1 by using simulation such as Monte-Carlo with a hundred of thousands of iterations.

This is a powerful tool, but it's a time consuming method requiring a large set of data to be used.

4.1.2.1.3. Data left censoring

A recurrent problem in the intake calculation is how to deal with the data below the quantification limits (LOQ) of the contamination survey (Figure 36), which is called left censoring.

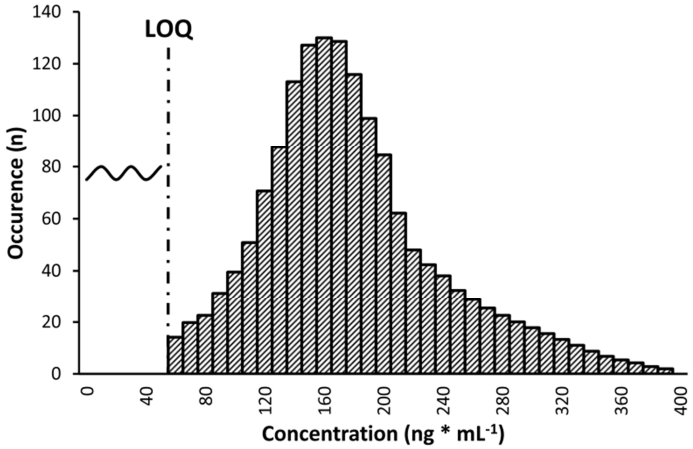

Figure 36 : Left censored data

The WHO recommends three different approaches for the left censoring namely Lower Bound, Middle Bound and Upper Bound (WHO, 2003). In lower bound, the results below the LOQ are replaced by 0. In middle bound, they are replaced by half of the LOQ and in upper bound by the LOQ itself.

In addition, the European Food Safety Authority published a "left censored dietary data management guidelines" (EFSA, 2010), explaining how to handle measurement data below LOQ:

- Characteristic statistical parameters must be directly calculated if there is no data under LOQ
- Middle Bound approach used if less than 60 % of the data are below LOQ
- Lower and Upper Bound approaches used if more than 60 % of the data are below LOQ.

4.2. Risk assessment results for Belgian adults

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Risk assessment of Belgian adults for furan contamination through the food chain

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Risk assessment is an interdisciplinary process used to quantify the risk linked to a hazard. In the present paper it is applied to quantify the risk linked to furan ingestion through the food chain for the Belgian adult population. Two approaches, deterministic and probabilistic, were carried out in parallel. The deterministic method relied on a case study, whereas the probabilistic approach involved statistical distributions of contamination and consumption data to calculate a statistical distribution of the daily intake. First, the deterministic method revealed a low estimated daily intake (EDI) for the average population (380 ng*(kg_{bw}*day)$^{-1}$) and a huge contribution of coffee consumption to the EDI (55%). Increasing or decreasing the daily coffee consumption by one cup can affect the EDI by about 22%. Afterwards, the probabilistic approach showed that the average population has a low EDI (494 ng*(kg_{bw}*day)$^{-1}$), and that high contamination levels were only registered in a small proportion of the population. Finally, a comparison of the $RfD_{chronic\ oral}$ showed that less than 10% of the Belgian population had an EDI above the reference dose proposed by the USEPA; the majority of the population had an EDI 20% below the reference dose. The margin of exposure (MoE) approach indicated that the level of risk related to furan intake through ingestion is low, with a MoE > 10,000 for more than 10% of the population and no result < 100.

Keywords: animal products – meat; bakery products; canned foods; cereals; process contaminants; risk assessment; GC; extraction; exposure assessment

Introduction

Risk assessment is 'The estimation of the nature and probability of adverse health effects related to a hazard, e.g., microbiology, chemistry, fire, riots, etc.' (US Environmental Protection Agency (USEPA) risk assessment; see http://epa.gov/riskassessment/index.htm; also Renwick et al. 2003; Feinberg et al. 2006; WHO 2009). In the present paper, the risk related to furan ingestion throughout the food chain is discussed for the Belgian adult population.

Furan is a low molecular mass toxicant mainly found in food undergoing heat treatment such as canned, jarred or roasted food (Hasnip et al. 2006; Crews and Castle 2007; Roberts et al. 2008; Fromberg et al. 2009; Kim, Lee, Park, et al. 2009). Maga (1979) first reported its occurrence in food in the late 1970s, but its toxicity was only studied since the 1990s (Kedderis et al. 1993; Chen et al. 1995; Peterson et al. 2006; Bakhiya and Appel 2010). Consecutively, the US National Toxicology Program (NTP) published a report on its toxicity (NTP 1993); the US National Academy of Science (NAS) classified it as a narcotic (NAS 2000); and the International Agency for Research on Cancer (IARC) involved it as possibly carcinogenic to humans (group 2B) (http://www.monographs.iarc.fr/eng/classification/classificationsalphaorder.pdf). Nevertheless, furan has only gained interest since 2004, when the USFDA published a report about its wide occurrence in food (USFDA 2005). Since then, national and international food control authorities started gathering information on furan levels, toxicity and risk for the population (Heppner et al. 2007; Stadler and Anklam 2007).

Several contamination assessments have been carried out to date, which led to the implementation of food control plans in some countries (Reinhard et al. 2004; Kim, Lee, Kim, et al. 2009; Liu and Tsai 2010). The main contamination assessment was recently achieved for the European population by the European Food and Safety Authority (EFSA) (2009). It was based on a set of contamination data collected from European control plans, and on several

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independent contamination studies. EFSA combined these contamination data with the results of consumption surveys from several Europeans countries to estimate the furan daily intake. Nevertheless, the risk linked to furan firstly depends on food consumption habits, and secondly on food contamination levels (Feinberg et al. 2006). As consumption habits are related to subpopulations and locations, the present paper describes a risk assessment targeting the Belgian adult population. The estimated daily intake (EDI) was calculated using the deterministic and probabilistic methodologies. Afterwards, the risk was estimated both by comparing it with a toxicological reference dose (the classical way) and also by calculating the margin of exposure (MoE).

Materials and methods

Contamination data

The contamination dataset only included products sold in Belgium, which were analysed by a previously described methodology (Scholl et al. 2009). This assessment was performed using 496 items sampled in the whole country (Scholl et al. 2011). Samples were taken all along the food chain, in several food markets. The sampling plan included weighting factors, such as food consumption and already reported contamination levels, to focus on the main food groups. This methodology was also applied to avoid the bias resulting from a similar product sold in several countries, but for which the preparation and/or composition can be different (Wegener and López-Sánchez 2010).

The assessment relied on a sole methodology to avoid the bias resulting from applying several approaches with different detection capabilities (CC_β) (in a first-approach equivalent to the limit of quantification (LOQ)), repeatability, precisions and expanded uncertainties (Scholl et al. 2009). The procedure used is a solid-phase micro-extraction (SPME) coupled to gas chromatography separation followed by a mass spectrometry detection using the isotopic dilution technique for quantification. The analytical approach is very sensitive and provides a response rate of up to 78.2% above the LOQ.

Consumption data

The Belgian Institute of Public Health provided consumption data. In 2004, De Vriese and co-workers carried out a consumption assessment of the Belgian population (De Vriese et al. 2005). This assessment involved 3200 people of at least 15 years of age from the whole country. The protocol was a recall performed on 2 non-consecutive days combined to a self-administrated questionnaire on food consumption frequency and a final face-to-face interview. This assessment focused not only on consumption habits, but also on socio-economic data of each participant, providing relevant information to study some subpopulations.

Methodology of risk assessment

Hazard identification

Furan is a toxic present in the food chain. In 1993, the NTP published a first report on furan toxicity and carcinogenicity based on *in vivo* studies on rat. This report was used in 1995 by the IARC to classify it in the group 2B, which means 'possibly carcinogenic to humans'. Five years later, the NAS also classified it as a narcotic. Several independent toxicological studies highlighting the carcinogenic effect of its metabolites have been performed since the 1990s (Kedderis et al. 1993; Chen et al. 1995; Peterson et al. 2000, 2005, 2006; Bakhiya and Appel 2010; Hamberger et al. 2010). More recent studies revealed that furan toxicity was linked to its major primary metabolite, the *cis*-2-butene-1,4-dial, which can induce tumours through a genotoxic effect on liver cells (Chen et al. 1995; Peterson et al. 2000, 2005, 2006). This metabolite results from the first hepatic transformation of furan by cytochrome P-450. To date, toxicity for humans has only been extrapolated from *in vitro* and animals studies.

Hazard characterisation

Hazard characterisation corresponds to a dose–response assessment. It is a toxicological step describing the mechanism of action including dynamic and kinetic aspects. The main intake pathway of furan into the body is the food chain. The low polarity of furan allows it to cross biological membranes easily. Studies on rats revealed that about 80% of furan is eliminated within 24 h: 40% by respiration, 22% in faeces and 20% in urine (Burka et al. 1991). The remaining 18% are rapidly metabolised by hepatic enzymes of the cytochrome P-450 into more than ten metabolites (Bakhiya and Appel 2010). Its major primary metabolite, the *cis*-2-butene-1,4-dial resulting from furan oxidation, is known to induce hepatocellular tumour and mononuclear cell leukaemia in rats. Furan is also known to induce cholangiocarcinomas in rat liver through an oxidative stress mechanism (Hickling et al. 2010a, 2010b).

In the late 1980s, the USEPA proposed a reference dose for chronic oral exposure (RfD) based on NTP studies (USEPA Integrated Risk Information System; see http://www.epa.gov/iris/subst/0056.htm). This dose was calculated according to a 13-week study involving rat gavages aiming at inducing hepatic lesions. The

lowest observed adverse effect level (LOAEL) was then estimated as 4 mg*(kg_{bw} and day)$^{-1}$ for rats. The no observed adverse effect level (NOAEL) was fixed at 2 mg*(kg_{bw}*day)$^{-1}$. The application of several precaution factors allowed the USEPA to recommend an $RfD_{chronic\ oral}$ of 1 µg*(kg_{bw}*day)$^{-1}$ for humans.

In addition, based on the NTP and the studies of Moser et al. (2009), a benchmark dose for 10% extra risk (BMD_{10}) of hepatocellular adenomas and carcinoma was established. Subsequently, a 95% lower confidence limit for this benchmark dose ($BMDL_{10}$) for the same mode of action (MoA) was reported to be 0.96 mg*(kg_{bw}*day)$^{-1}$ (Benford et al. 2010; Carthew et al. 2010; Williams et al. 2011).

Exposure assessment

The exposure assessment aims at estimating the daily intake of a toxic (EDI). When available, the EDI may be combined with the daily outtake to estimate the mean absorption.

Furan daily intake throughout the food chain is calculated by applying three equations (see below). The first equation means the global EDI equals the sum of individual EDIs (EDI of each food group). The second equation explains that a food group EDI results from multiplying the specific food group relative consumption by the food group contamination. The third equation shows that the relative daily consumption corresponds to the ratio between the daily consumption and the 'population' weight:

Calculation of global EDI:

$$EDI_{Golbal} = \sum_{FoodGroup} EDI_{FoodGroup} \quad (1)$$

Calculation of EDI:

$$EDI_{FoodGroup} = [Furan]_{FoodGroup} \times (relativeDaily\ Consumption)_{FoodGroup} \quad (2)$$

Estimation of the relative daily consumption:

$$relativeDaily\ Consumption = \frac{DailyConsumption}{Weight} \quad (3)$$

Two approaches were applied to calculate the EDI: deterministic and probabilistic. Both methods are described in the following sections.

Deterministic approach

In the deterministic approach, the EDI was calculated for several categories of the population according to selected cases, e.g. mean or worst case. As furan contamination is directly related to food origin and preparation (Crews and Castle 2007; Wegener and López-Sánchez 2010), only the results of the previously mentioned Belgian contamination assessment were used (Scholl et al. 2011). Three different approaches are recommended by the World Health Organization (WHO) to deal with contamination data below the quantification limits (WHO 2003). These approaches are named lower bound (LB), middle bound (MB) and upper bound (UB), where results below the limit of quantification (LOQ) are respectively replaced by zero, half-LOQ and the LOQ itself. In the present study, as the proportion of non-quantifiable results was very low (<12%), these approaches were expected to provide quite similar results. Consumption depends on the population studied; therefore, only data from the 'First Belgian consumption survey' (De Vriese et al. 2005) were used. Several categories of population were studied for each food group such as the average consumption, the 2.5th, 25th, 50th, 75th and 97.5th consumption percentiles. These categories of population are assessable by two ways depending on how unconsummated items are dealt with. On the one hand, the statistical analysis is performed by considering only the subpopulation consuming the food group; statistical results of consumption are therefore not representative of the whole population. On the other hand, the whole population is included, and the non-consumption of a food item is characterised by a null consumption value. The first approach is usually applied in cases of acute toxicity as is more related to a punctual dose, which is the most appropriate for a worst-case study. The second approach is generally applied in the case of chronic toxicity as it considers the possibility of not consuming a food item. As furan is known to have a chronic toxicity only the second procedure was applied in the present paper.

The real weight of each participant to the food consumption survey was used to estimate the EDI more accurately as it was available.

Probabilistic approach

In this approach, raw (consumption and contamination) data are converted into a function of occurrence (Table 1) and computed using @Risk software (version 5.5; Palisade Corporation, New York, NY, USA). Afterwards, functions are combined through equations (1) to (3) by using a Monte Carlo simulation with 500,000 iterations to obtain a function of furan EDI.

Functions of contamination occurrence are computed in two steps. The function of each food group is first determined by only including contamination data > LOQ (this function is truncated such as no result < LOQ can be drawn). Thereafter, a function

Table 1. List of the distributions used for the probabilistic risk assessment (only distributions for samples > LOQ and for 'consuming' people are presented) according to the @Risk software notations.

Food group	@Risk contamination distribution	@Risk consumption distribution
Vegetables	RiskBetaGeneral (0.21931; 0.22834; 0.8; 30.68)	RiskPearson5 (7.9716; 1688.8; RiskShift (−75.196))
Fruits	RiskPearson5 (0.87353; 1.1588; RiskShift (0.13385))	RiskPearson5 (6.8822; 1997.4; RiskShift (−120.74))
Milk and milky beverages	RiskLognorm (3.3751; 2.9041; RiskShift (−0.5535))	RiskLoglogistic (−54.643; 288.93; 5.0313)
Dessert cream	RiskNormal (7.358; 7.209)	RiskLoglogistic (−16.106; 148.06; 3.764)
Pasta, rice, other grain	RiskExtvalue (22.516; 27.748)	RiskLognorm (203.84; 132.09; RiskShift (−27.127))
Breakfast cereals	RiskLognorm (33.178; 32.292; RiskShift (−1.3011))	RiskPearson5 (8.3866; 564.71; RiskShift (−23.071))
Biscuits	RiskNormal (25.721; 19.913)	RiskInvgauss (49.638; 67.916; RiskShift (−4.3678))
Raw meat	RiskBetaGeneral (0.27138; 0.35639; 0.32; 66.93)	RiskInvgauss (101.66; 226; RiskShift (−11.664))
Processed meat	RiskInvgauss (4.0861; 0.7775; RiskShift (0.17294))	RiskInvgauss (67.906; 106.439; RiskShift (−4.1765))
Fish	RiskExpon (23.148; RiskShift (−0.79903))	RiskBetaGeneral (1.4836; 5.9922; 1.6382; 526.32)
Chocolate, candy bars, paste, etc.	RiskUniform (−0.33556; 8.9656)	RiskPearson5 (3.793; 147.04; RiskShift (−11.33))
Confectioner and non-chocolate	RiskBetaGeneral (0.18312; 0.21331; 0.72; 8.79)	RiskInvgauss (37.276; 33.348; RiskShift (−2.6119))
Cakes	RiskPearson5 (1.2746; 5.7504; RiskShift (1.4743))	RiskInvgauss (48.025; 79.124; RiskShift (−3.4931))
Fruit and vegetable juices	RiskLoglogistic (−0.6429; 2.0314; 5.2505)	RiskLoglogistic (−72.436; 278.71; 3.2801)
Soft drinks	RiskTriang (−0.018347; 0.95; 1.6178)	RiskInvgauss (635.46; 1093.9; RiskShift (−57.616))
Coffee	RiskBetaGeneral (0.4086; 0.47225; 1.13; 106.23)	RiskPearson5 (4.9426; 2648.5; RiskShift (−191.14))
Tea	RiskBetaGeneral (0.29271; 0.27597; 0.37; 2.87)	RiskLognorm (338.95; 321.83; RiskShift (39.603))
Herbal tea	RiskBetaGeneral (0.18842; 0.20477; 0.22; 3.68)	RiskPearson5 (4.2366; 1363.5; RiskShift (−80.915))
Wine	RiskLoglogistic (0.19764; 0.30883; 2.0584)	RiskLognorm (287.29; 189.16; RiskShift (−26.305))
Beer, cider	RiskInvgauss (3.9075; 19.794; RiskShift (−1.2641))	RiskPearson5 (3.0816; 1457; RiskShift (−129.34))
Tomato sauces	RiskLogistic (11.2955; 2.9182)	RiskInvgauss (73.018; 30.148; RiskShift (−0.36396))
Soups	RiskInvgauss (25.542; 10.767; RiskShift (−0.5567))	RiskExtvalue (241.22; 109.2)
Miscellaneous	RiskPearson5 (5.8123; 82.114; RiskShift (−7.3599))	RiskLoglogistic (2.7612; 71.036; 2.4976)
Soya products	RiskExtvalue (1.3338; 1.1449)	RiskInvgauss (135.6; 45.027; RiskShift (−4.31))

dealing with the probability of having data < LOQ is added to the occurrence function determined in the first step. The nature of the second function depends on the approach used to deal with data < LOQ. In the probabilistic method, the three approaches described in the deterministic section (LB, MB and UB) were also applied. A fourth approach named Uniform and involving a random distribution of data < LOQ was used as well because it appears more representative of the reality. Practically, in the first three approaches a discrete function corresponding to, respectively, zero, LOQ/2 and LOQ was used, while a uniform distribution between zero and LOQ was applied in the fourth approach. Four bimodal functions corresponding to the four approaches were calculated for each food group.

The functions of consumption occurrence were also computed in two steps based on raw data of the Belgian consumption survey. First, a function was computed for each food group by including only the consuming subpopulation (functions are also truncated to avoid $a \leq 0$ consumption draw). Secondly, the subpopulation not consuming the food item was calculated. A discrete function set to zero was then

Table 2. Deterministic EDI (ng*(kg_{bw}*day)$^{-1}$) for several contamination approaches.

	Lower bound	Middle bound	Upper bound
First quartile	191	192	193
Median	329	330	331
Mean	379	380	381
Third quartile	500	501	501

proportionally added to the function calculated in the first step. Therefore, the resulting function of consumption occurrence resulting is bimodal like the contamination function.

In this approach, the real population weight was also used and represented by a statistical distribution of the population weight reported in the consumption survey.

Risk characterisation

Two risk-characterisation approaches are presented in this paper: a classical way and a new method. In the first, conclusions were drawn when comparing the EDIs of several proportions of the population with toxicological reference doses. However, as carcinogenic effects have no threshold values, a new methodology called margin of exposure (MoE) was recently developed (Constable and Barlow 2009). In the MoE approach, a level of concern for the risk linked to a mode of action (MoA) is calculated:

MoE calculation for a carcinogenic MoA:

$$MoE = \frac{BMDL_{10}}{EDI} \quad (4)$$

where $BMDL_{10}$ is the 95% lower confidence limit for this benchmark dose for 10% extra risk of hepatocellular adenomas and carcinoma; and EDI is the estimated daily intake.

The risk may be considered as negligible if MoE $>10{,}000$. On the other hand, an MoE < 100 is of major concern. Finally, discussions are needed, according to the involved MoA, for an MoE included in a range between 10,000 and 100 (Constable and Barlow 2009).

Results

Deterministic approach

The results of the deterministic risk assessments are summarised in Table 2 and Figure 1. The three approaches used to deal with values $<$ LOQs gave quite similar results, as shown in Table 2. There is a < 2 ng*(kg_{bw}*day)$^{-1}$ difference between LB and UB for the average population; it is a consequence of the high proportion of results $>$ LOQs ($> 78\%$) in the contamination assessment. In Figure 1, only the MB approach is illustrated for readability.

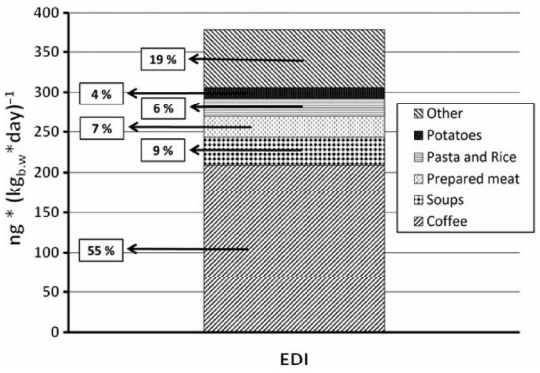

Figure 1. Deterministic EDI for the average population, including the contribution of the most relevant food groups.

Average and median population EDIs are close (380 and 330 ng*(kg_{bw}*day)$^{-1}$, respectively). Therefore, the distribution of the 3200 individual EDIs across the population tends to normality, as illustrated in Figure 2A. Figure 2 also shows that the majority of the population has a low EDI with a mode at 225 ng*(kg_{bw}*day)$^{-1}$ for 9.5% of the studied population. In this approach, the minimal and maximal EDIs were 0.7 and 3843 ng*(kg_{bw}*day)$^{-1}$, respectively.

Only 2.7% of the population presented an EDI above the $RfD_{chronic\ oral}$. The majority of the population has an EDI equivalent to 22% of the $RfD_{chronic\ oral}$, and the average population EDI is equivalent to 38% of the $RfD_{chronic\ oral}$.

Figure 2A also displays the MoE calculation; the proportion of the population at a given MoE is presented in Figure 2C. About 10% of the population presents an MoE $> 10{,}000$ and an MoE > 1500 is observed for $> 90\%$ of the population. The minimal calculated MoE was 404 for 0.02% of the population and no MoE was < 100. Finally, the MoE dispersion mode is 4266 for 9.5% of the population. The main contributors to the EDI are shown in Figure 1. Coffee contributes 55% to the average EDI; the other main groups are soups, prepared meat, pasta and rice, and potatoes, with an overall contribution of 26% to the EDI (ranging individually from 4% to 9%).

Coffee consumption scenario

Coffee was shown to be the major contributor (55%) to the average EDI. This average EDI was achieved after a daily consumption of three cups. The influence of reducing or increasing the daily consumption by one cup was tested in the proposed scenario, as shown in Figure 3. These variations induced a 21%

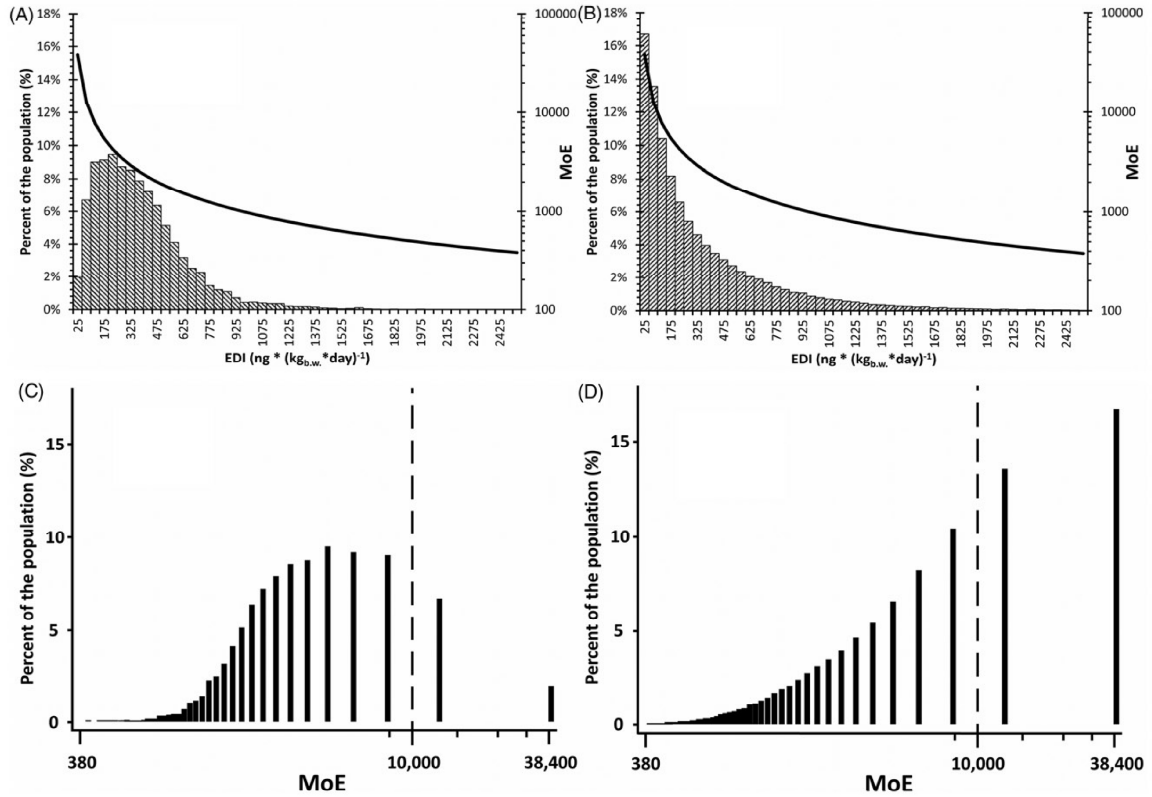

Figure 2. (A) Distribution of the EDI across the population in the deterministic approach, and evolution of the MoE according to the EDI; (B) the same as for (A) but for the probabilistic approach; (C) distribution of the MoE across the population in the deterministic approach; and (D) the same as for (C) for the probabilistic approach.

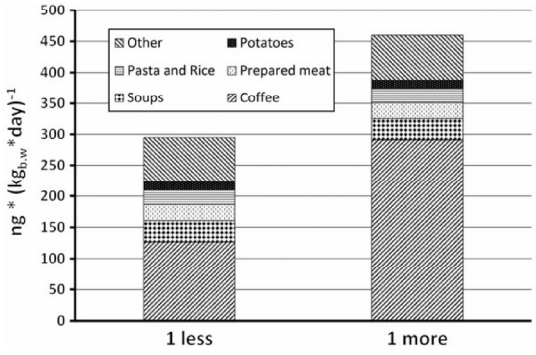

Figure 3. Influence of decreasing or increasing the daily coffee consumption by one cup on the EDI of the average population, including the contribution of the most relevant food groups.

increase (460 ng*(kg$_{bw}$*day)$^{-1}$) and a 23% decrease (294 ng*(kg$_{bw}$*day)$^{-1}$) of the average EDI (380 ng*(kg$_{bw}$*day)$^{-1}$), corresponding to a 12% reduction (or 8% increase) of the contribution to the EDI when simulating both scenarios.

Probabilistic approach

Results of the probabilistic risk assessment are summarised in Table 3 and Figure 2B. Figure 2B displays the distribution of EDIs across the population. A uniform distribution for the results < LOQ and a statistical distribution of the body weights were used to estimate the EDIs.

Differences between the contamination approaches are shown in Table 3. A difference of 6 ng*(kg$_{bw}$*day)$^{-1}$ (from 488 to 494) was highlighted for the average population. Consequently, it was decided to show only the results of the uniform distribution approach in the figures. The median EDI represents 43% (212 ng*(kg$_{bw}$*day)^{1}) of the average EDI and is not a normal distribution as confirmed by Figure 2B. The EDI of the majority of the population is low (23 ng*(kg$_{bw}$*day)$^{-1}$), corresponding to 5% of the average EDI.

An EDI below the RfD$_{chronic\ oral}$ was reported for 91.9% of the population. The average EDI and the EDI of the majority of the population represented about 49% and 0.2% of the RfD$_{chronic\ oral}$, respectively.

Table 3. Probabilistic EDI (ng*(kg_{bw}*day)$^{-1}$) for several contamination approaches.

	Lower bound	Middle bound	Upper bound	Uniform
Mode	25	23	27	23
First quartile	80	80	82	81
Median	211	212	213	212
Mean	494	494	488	494
Third quartile	492	493	493	493
Proportion of results > RfD_{oral} (%)	8.1	8.1	8.1	8.1

MoEs related to specific EDIs are displayed in Figure 2B; the proportion of the population relative to an MoE is shown in Figure 2D. A MoE > 10,000 was reported for 30% of the population. Seventeen per cent of the population presented a MoE = 38,400 (distribution mode), and 90% of the population had an MoE > 1164. The calculated MoE decreased to 0.1 for a maximum estimated EDI of 9.2 mg*(kgbw*day)$^{-1}$ (<0.0002% of the population), while only 0.03% of the population displayed an MoE < 380.

Discussion

Consumption data

As previously mentioned, it was decided to use Belgian datasets with the objective to avoid bias linked to consumption habits observed in other countries. Several consumption surveys have been carried out to date in Belgium, but only the Belgian study achieved in 2004 included the whole adult population. This survey was representative of the Belgian population as it included 3200 people (out of a 10.4 million global population registered in 2004) from the whole country, and homogeneously distributed according to gender, age, education level, working field, etc. Nevertheless, data are outdated as the survey was performed 7 years ago. However, consumption habits have probably not evolved a lot since the end of the survey. Thus, one can assume that differences between current and reported consumption habits are minor. The ideal situation would have been to work on a freshly updated survey involving a constant review of data, but such a tool is not yet available.

Contamination data

The contamination dataset was provided by a survey focusing on local products in order to avoid any bias linked to different preparations and compositions of the same product sold in other countries. In addition, the study relied on a highly sensitive analytical methodology specifically designed to reach low quantification limits. There are two beneficial consequences. Firstly, a high proportion of data > LOQ (up to 78%), characterised by small differences between LB and UB, were observed. Secondly, inter-laboratory analytical biases resulting from applying different methodologies (different LOQs, expanded uncertainties, etc.) were eliminated.

The representativeness of this study comes from its design. It was constructed to avoid a geographical or branding side-effect. The dataset is not outdated as it was compiled only 2 years ago. But as already mentioned for the consumption study, regular updates are needed to avoid biases related to a modified production and/or distribution scheme. An on-going survey based on a constant re-evaluation through a food control plan would be suitable.

Deterministic versus probabilistic approach

Deterministic and probabilistic approaches should not be seen as different but rather as complementary methods.

The comparison of average EDIs only displayed few differences. However, comparing EDI distributions and medians or modes displayed important differences, especially for the low daily intake. Furthermore, the EDI distribution is narrower and the EDI increases faster in the probabilistic approach compared with the deterministic approach. This is a consequence of a difference of contamination data management. In the deterministic approach, only the average contamination value of each food group was taken into account, whereas in the probabilistic approach a distribution of values (including zero and very high levels) was used. Therefore, average EDIs and extreme values are biased in the deterministic and in the probabilistic approaches respectively.

MoE distributions are very different, but led to similar conclusions. The majority of the population presented a high MoE and a > 10,000 MoE was reported for a meaningful percentage of people. Differences were, however, observed for extreme values: the minimal MoE was 404 in the deterministic approach, while it decreased to 0.1 in the probabilistic method. Such a low MoE results from very high consumption and contamination values, only encountered in a statistical distribution. Therefore, such a combination is very unlikely and should be ignored.

The same differences were observed in the $RfD_{chronic\ oral}$ approach: the EDI of the majority of the population corresponded to < 50% of the $RfD_{chronic\ oral}$ and few a per cent had an EDI > $RfD_{chronic\ oral}$. The probabilistic methodology tended to display a higher risk than the deterministic approach. As already observed for the MoE, it finds its

explanation in the unlikely extreme EDI values and biased average values in the probabilistic and deterministic approaches, respectively. Consequently, most results tended to display a low risk level for furan intake. The real situation should fit between both approaches.

Coffee consumption scenario

Coffee consumption was shown to be a critical parameter regarding its impact on furan daily intake. Several straightforward or basic recommendations such as a reduction of the daily consumption, a thorough mixing of coffee before drinking or even a coffee percolation allows a reduction in the exposure (Kuballa 2007).

Conclusion

The present study showed that the majority of the population presents a low daily intake; only extreme consumers are really at risk. The major contamination is due to coffee consumption (one of the most contaminated items) and changing consumption habits can strongly impact the EDI.

One must be cautious regarding the conclusions drawn from the risk characterisation. If it is commonly accepted that an MoE $>10{,}000$ corresponds to a low risk level, and that a MoE < 100 means a high risk; there is no consensus for the results included between 10,000 and 100. In the present survey, 10–30% of the population are exposed to a low risk level. Nevertheless, the risk level is not defined for the remaining 70–90%. The majority of the population displays a high MoE and therefore a low risk for the selected MoA (hepatocellular adenomas and carcinomas). Similar conclusions are drawn when using the classical way (a comparison between EDI and $RfD_{\text{chronic oral}}$).

Finally, the present study only focused on the adult Belgian population, and a question remains about the furan exposure of subpopulations at risk, such as babies and children. Carrying out a risk assessment targeting these subpopulations would be relevant, but it remains a great challenge as few data are available on their consumption habits.

Acknowledgments

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4.3. Risk assessment results for Belgian children

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Risk assessment for furan contamination through the food chain in Belgian children

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Young, old, pregnant and immuno-compromised persons are of great concern for risk assessors as they represent the sub-populations most at risk. The present paper focuses on risk assessment linked to furan exposure in children. Only the Belgian population was considered because individual contamination and consumption data that are required for accurate risk assessment were available for Belgian children only. Two risk assessment approaches, the so-called deterministic and probabilistic, were applied and the results were compared for the estimation of daily intake. A significant difference between the average Estimated Daily Intake (EDI) was underlined between the deterministic (419 ng kg^{-1} body weight (bw) day^{-1}) and the probabilistic (583 ng kg^{-1} bw day^{-1}) approaches, which results from the mathematical treatment of the null consumption and contamination data. The risk was characterised by two ways: (1) the classical approach by comparison of the EDI to a reference dose ($RfD_{chronic\text{-}oral}$) and (2) the most recent approach, namely the Margin of Exposure (MoE) approach. Both reached similar conclusions: the risk level is not of a major concern, but is neither negligible. In the first approach, only 2.7 or 6.6% (respectively in the deterministic and in the probabilistic way) of the studied population presented an EDI above the $RfD_{chronic\text{-}oral}$. In the second approach, the percentage of children displaying a MoE above 10,000 and below 100 is 3–0% and 20–0.01% in the deterministic and probabilistic modes, respectively. In addition, children were compared to adults and significant differences between the contamination patterns were highlighted. While major contamination was linked to coffee consumption in adults (55%), no item predominantly contributed to the contamination in children. The most important were soups (19%), dairy products (17%), pasta and rice (11%), fruit and potatoes (9% each).

Keywords: chromatography, GC/MS; risk assessment; process contaminants; processed foods; coffee

Introduction

Furan is a low molecular weight food toxicant first reported in food in the late 70s by Maga (1979). Since the year 2000, a series of reports and papers described its widespread occurrence in food (USFDA 2004; Hasnip et al. 2006; Crews and Castle 2007; Roberts et al. 2008; Fromberg et al. 2009; Kim et al. 2009). Therefore, national and international food control authorities gathered information on furan levels in food, its toxicity and also the risk for the population (Hepner et al. 2007; Stadler 2007).

Furan generation in foodstuffs involves a heating source such as cooking or canning (Hasnip et al. 2006; Crews et al. 2007; Roberts et al. 2008; Fromberg et al. 2009) and the presence of a combination of precursors, such as carbohydrates, proteins, ascorbic acid or lipids (Owczarek-Fendor et al. 2010a,b, 2011, 2012). Therefore, the furan amount is dependant of the food composition and of the exact recipe (Wegener and López-Sánchez 2010).

Furan toxicity was only studied since the 90s (Kedderis et al. 1993; NTP 1993; Chen et al. 1995; Peterson et al. 2000, 2005, 2006; Bakhiya et al. 2010; Hamberger et al. 2010) and was further classified as a narcotic by the American National Academy of Science (NAS 2000), and as "possibly carcinogenic to humans" (group 2B) by the International Agency for Research on Cancer (IARC 1995).

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The present paper reports the first risk assessment carried out on furan contamination through the food ingestion by Belgian children. Risk assessment is the process used to quantify the risk linked to a hazard (Feinberg et al. 2006; WHO/IPCS 2009). It is an interdisciplinary process involving several steps and sub-steps described by Feinberg and co-workers and also by the World Health Organization (WHO). Children are a sub-population of great concern for risk assessment as they belong to the young, old, pregnant and immuno-compromised persons (YOPIs) group.

Previous research demonstrated that contamination data, consumption data and risk assessment results were geographically dependent; therefore, the geographical origin of raw data, such as consumption and contamination data, is of major importance (Wegener and Lopez-Sanchez 2010; Scholl, Humblet, et al. 2012). Consequently, this study aimed at investigating furan exposure among Belgian children using local consumption and contamination data.

The population EDIs were estimated by two methodologies: the deterministic approach, involving a case-study, and the probabilistic analysis relying on statistical modelling. The risk was characterised firstly by a classic methodology that consists in comparing the EDI to a toxicological reference dose ($RfD_{chronic\text{-}oral}$) and, secondly, by a recent methodology that consists in calculating the Margin of Exposure (MoE).

Finally, a comparison between the Belgian children and adult populations (Scholl, Humblet, et al. 2012) was conducted to examine differences between childhood and adult furan exposure due to ingestion.

Materials and methods

Contamination data

The contamination dataset considered in the present study only involves products sold on the Belgian market, which were analysed as sold by a unique methodology (Scholl, Humblet, et al. 2012).

Briefly, 496 samples were collected across the whole country. Samples were spread over the food chain, the food markets, the producers and the geographical localisation according to a precise sampling plan. This plan was constructed by applying a series of weighting factors to take into account the previous parameters, but also the food consumption and the already reported contamination levels (Scholl, Scippo, et al. 2012).

The applied analytical methodology is a Solid Phase Micro-Extraction (SPME), coupled to GC/MS detection using the isotopic dilution (ID) for absolute quantification (Scholl et al. 2009). The method was validated according to the 2002/657/EC recommendation, which includes the calculation of the detection capabilities (CC_β) (i.e. that can be understood as a limit of quantification (LOQ) in a first approach). These limits are low (less than $1\,\mu g\,kg^{-1}$ product) inducing a rate of samples with a concentration above the LOQ is high: 78.2%.

Consumption data

Consumption data were provided by the Department of Public Health of the Ghent University. Between 2002 and 2003, Huybrechts and collaborators carried out a dietary survey on children aged from 2.5 to 6.5 years old (Huybrechts, Matthys, Bellemans, et al. 2008; Huybrechts, Matthys, Vereecken, et al. 2008). In total, 696 parents and/or caregivers completed a dietary record during three consecutive days in which they wrote down all foods and drinks (including quantities) consumed by the child. In addition, a separate questionnaire was used to collect socio-demographic and lifestyle information. Weight and height of the children were reported by the parents.

Risk assessment methodology

Hazard identification

Since the 1990s, a number of toxicological studies have been performed on furan (Kedderis et al. 1993; NTP 1993; Chen et al. 1995; Peterson et al. 2000, 2005, 2006; Bakhiya et al. 2010; Hamberger et al. 2010). Several of them highlighted the liver carcinogenic potential of furan through one of its major primary metabolites, the *cis*-2-butene-1,4-dial, which results from the first hepatic transformation of furan by cytochrome P-450. As a consequence, furan was classified by the International Agency for Research on Cancer (IARC 1995) in the group 2B, which means "possibly carcinogenic for humans".

Hazard characterisation

Food consumption is the most predominant route for human exposure to furan as its low polarity allows it to easily cross biological membranes. Nevertheless, Burka and collaborators reported that 82% of furan was eliminated within 24 h in rats (Burka et al. 1991). The remaining 18% were metabolised in liver into more than 10 metabolites by cytochrome P-450 enzymes (Bakhiya et al. 2010).

It has been shown that furan and its metabolites can induce cholangiocarcinomas, hepatocellular tumour and mononuclear cell leukaemia in rats (Hickling, Hitchcock, Chipman, et al. 2010; Hickling, Hitchcock, Oreffo, et al. 2010).

During the 1980s, a Reference Dose for Chronic Oral Exposure ($RfD_{chronic\text{-}oral}$) based on a 13 weeks-rat

gavage study was established by the US-EPA.[1] After applying several precaution factors, the $RfD_{chronic\text{-}oral}$ resulted to be $1\,\mu g\,kg^{-1}\,bw\,day^{-1}$. Later, a consensus established a benchmark dose for 10% extra risk (BMD_{10}) of hepatocellular carcinoma (Moser et al. 2009; Benford et al. 2010; Carthew et al. 2010; Williams et al. 2011). Its 95% lower confidence limit ($BMDL_{10}$), which needs to be used in the MoE, was estimated at $0.96\,mg\,kg^{-1}\,bw\,day^{-1}$.

Exposure assessment

The furan daily intake throughout the food chain is calculated in three steps. First, the food group relative consumption is calculated by establishing the ratio between the daily consumption and the reported weight (Equation (1)). Secondly, the EDIs related to food groups ($EDI_{FoodGroup}$) are determined by the product of the food group relative consumption by the food group contamination (Equation (2)). Thirdly, the global EDI corresponds to the sum of EDIs calculated for each food group (Equation (3)).

Estimation of the relative daily consumption:

$$\text{Relative daily consumption} = \frac{\text{Daily consumption}}{\text{Weight}} \tag{1}$$

Calculation of EDI:

$$EDI_{FoodGroup} = [\text{Furan}]_{FoodGroup} \times (\text{Relative Daily Consumption})_{FoodGroup} \tag{2}$$

Calculation of global EDI:

$$EDI_{Global} = \sum_{FoodGroup} EDI_{FoodGroup} \tag{3}$$

The following sections describe the two approaches used for estimating the EDI, namely deterministic and probabilistic.

Deterministic approach. The deterministic approach involves case studies splitting the population of the consumption assessment (Huybrechts, Matthys, Bellemans, et al. 2008; Huybrechts, Matthys, Vereecken, et al. 2008) into ranges: 2.5th, 25th, 50th, 75th and 97.5th percentiles.

Regarding contamination data (Scholl, Humblet, et al. 2012), three approaches are recommended by the WHO (WHO 2003), namely Lower Bound (LB), Middle Bound (MB) and Upper Bound (UB), for which the results below the LOQs are replaced by 0, half-LOQ and the LOQ value, respectively. Nevertheless, according to the European Food Safety Authority (EFSA) left censored dietary data management guidelines (EFSA 2010), only the MB results should be presented as more than 60% of the data are above the LOQs.

Concerning the body weight, individual child data (estimated by the parents/caregivers) was used to calculate individual EDI.

Probabilistic approach. In this approach, raw consumption, contamination and weight data are converted in function of occurrence (Table 1) by using the @Risk software (version 5.5; Palisade Corporation, New York, USA). Afterwards, these functions are combined through Equations (1)–(3) using a Monte-Carlo simulation with 500,000 iterations to obtain a function of the furan EDI distribution.

For the contamination data below the LOQs, four approaches were used. The three first ones are similar to the deterministic approach, and are modelled using a discrete function. The fourth method, called Uniform, applies a uniform function between 0 and the LOQ to model the results below the LOQ.

Risk characterisation

In the present paper, two approaches were used to characterise the risk. The first consisted in comparing the EDI to a low risk toxicological reference value such as the $RfD_{chronic\text{-}oral}$. The second method, called Margin of Exposure (MoE), is the level of concern for the risk linked to a Mode of Action (MoA) and is calculated by Equation (4) below (Constable et al. 2009):

MoE calculation for a carcinogenic MoA

$$MoE = \frac{BMDL_{10}}{EDI} \tag{4}$$

In this approach, it is commonly admitted that the risk for a selected MoA is negligible if the MoE is above 10,000 and is a major concern if below 100, while discussion is necessary if it falls between both values (Constable et al. 2009).

Results

Deterministic approach

The results of the deterministic approach are summarised in Table 2. At first sight, the average EDI resulting from applying the LB ($417\,ng\,kg^{-1}\,bw\,day^{-1}$), the MB ($419\,ng\,kg^{-1}\,bw\,day^{-1}$) and the UB approach ($421\,ng\,kg^{-1}\,bw\,day^{-1}$) were very close. The small difference ($4\,ng\,kg^{-1}\,bw\,day^{-1}$) is a consequence of the high number of results above the LOQs (78.2%). As previously mentioned, only the results expressed in MB are displayed for discussion (EFSA 2010).

The average EDI and the relative proportion of major contributors are depicted in Figure 1. These

Table 1. List of the distributions used for the probabilistic risk assessment (only the distributions for the samples with a result above the LOQ and for "consuming" people are presented) according to the @Risk software notations.

Food group	@Risk Contamination distribution	@Risk Consumption distribution
Fruits	RiskPearson5(0.87353;1.1588;RiskShift(0.13385))	RiskInvgauss(206.46;868.3;RiskShift(−44.174))
Milk and milk beverages	RiskLognorm(3.3751;2.9041;RiskShift(−0.5535))	RiskInvgauss(334.52;850.61;RiskShift(−4.3687))
Dessert cream	RiskNormal(7.358;7.209)	RiskPearson5(15.379;3567.8;RiskShift(−100.73))
Pasta, rice, other Grain	RiskExtvalue(22.516;27.748)	RiskExtvalue(67.758;43.257)
Breakfast cereals	RiskLognorm(33.178;32.292;RiskShift(−1.3011))	RiskLoglogistic(−3.4739;34.336;4.0572)
Biscuits	RiskNormal(25.721;19.913)	RiskLoglogistic(−0.37929;24.611;2.7989)
Raw meat	RiskBetaGeneral(0.27138;0.35639;0.32;66.93)	RiskGamma(2.1135;30.286;RiskShift(1.1379))
Processed meat	RiskInvgauss(4.0861;0.7775;RiskShift(0.17294))	RiskPearson5(4.3188;164.43;RiskShift(−8.7831))
Fish	RiskExpon(23.148;RiskShift(−0.79903))	RiskPearson5(14.618;2049.7;RiskShift(−68.183))
Chocolate, candy bars, etc.	RiskUniform(−0.33556;8.9656)	RiskInvgauss(34.908;47.024;RiskShift(−1.9272))
Confectioner and non-chocolate	RiskBetaGeneral(0.18312;0.21331;0.72;8.79)	RiskInvgauss(61.08;111.53;RiskShift(−5.7136))
Cakes	RiskPearson5(1.2746;5.7504;RiskShift(1.4743))	RiskBetaGeneral(3.9781;83.985;7.0004;1376.2)
Fruit and vegetable juices	RiskLoglogistic(−0.6429;2.0314;5.2505)	RiskPearson5(5.9816;1961.9;RiskShift(−97.476))
Soft drinks	RiskTriang(−0.018347;0.95;1.6178)	RiskPearson5(4.2316;1093;RiskShift(−61.411))
Coffee	RiskBetaGeneral(0.4086;0.47225;1.13;106.23)	RiskLoglogistic(−12.156;110.02;2.5288)
Tea	RiskBetaGeneral(0.29271;0.27597;0.37;2.87)	RiskLognorm(172.36;103.26;RiskShift(23.675))
Herbal tea	RiskBetaGeneral(0.18842;0.20477;0.22;3.68)	RiskExtvalue(144.96;79.283)
Wine	RiskLoglogistic(0.19764;0.30883;2.0584)	RiskInvgauss(11.244;10.024;RiskShift(1.4374))
Beer, cider	RiskInvgauss(3.9075;19.794;RiskShift(−1.2641))	RiskBetaGeneral(0.23403;0.35597;6;200)
Tomato sauces	RiskLogistic(11.2955;2.9182)	RiskTriang(2.1389;11;168.62)
Soups	RiskInvgauss(25.542;10.767;RiskShift(−0.5567))	RiskExtvalue(154.553;78.072)
Miscellaneous	RiskPearson5(5.8123;82.114;RiskShift(−7.3599))	RiskLoglogistic(−4.1004;52.716;2.74)
Soy products	RiskExtvalue(1.3338;1.1449)	RiskPearson5(6.6052;2567.6;RiskShift(−145.91))

Table 2. Deterministic EDI (ng kg^{-1} bw day^{-1}) for several contamination approaches.

	Lower bound	Middle bound	Upper bound
First quartile	253	255	257
Median	364	366	367
Mean	417	419	421
Third quartile	532	534	537
95th Percentile	865	869	871

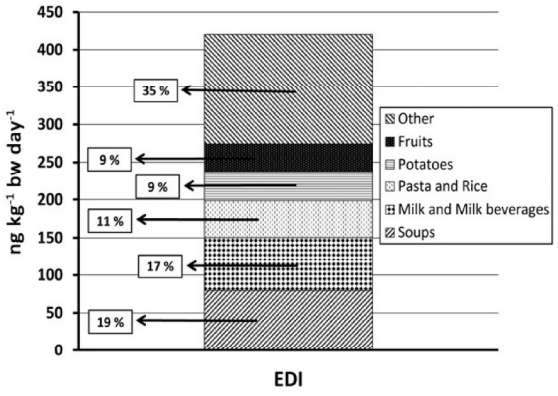

Figure 1. Estimated daily intake (EDI) of furan based on a deterministic approach for the child population average, including the contribution of the most relevant food groups.

groups are, respectively, in decreasing order: soups (19.0%), milk and milk beverage (16.8%), pasta and rice (11.4%), potatoes (9.4%) and fruits (8.6%). Other food groups individually contributed by less than 5% and were gathered in the group named "other" (34.8%).

Figure 2(A) illustrates the distribution of children's EDIs ($N = 1847$ EDIs). It shows an asymmetrical distribution shape slightly tailed. This distribution spans 42–1975 ng kg^{-1} bw day^{-1} with a mode at 250 ng kg^{-1} bw day^{-1}. The discrepancies between the mode (250 ng kg^{-1} bw day^{-1}), the median (366 ng kg^{-1} bw day^{-1}) and the mean (419 ng kg^{-1} bw day^{-1}) EDI reflects the asymmetrical scattering of the data (Table 2).

Regarding the risk characterisation, only 2.7% of children displayed an EDI above the $RfD_{chronic\text{-}oral}$. The trends in MoE is represented by the thick line in Figure 2(A), and the distribution of MoEs among the children is shown in Figure 2(C); approximately 1% of the population has a MoE above 10,000. The MoE

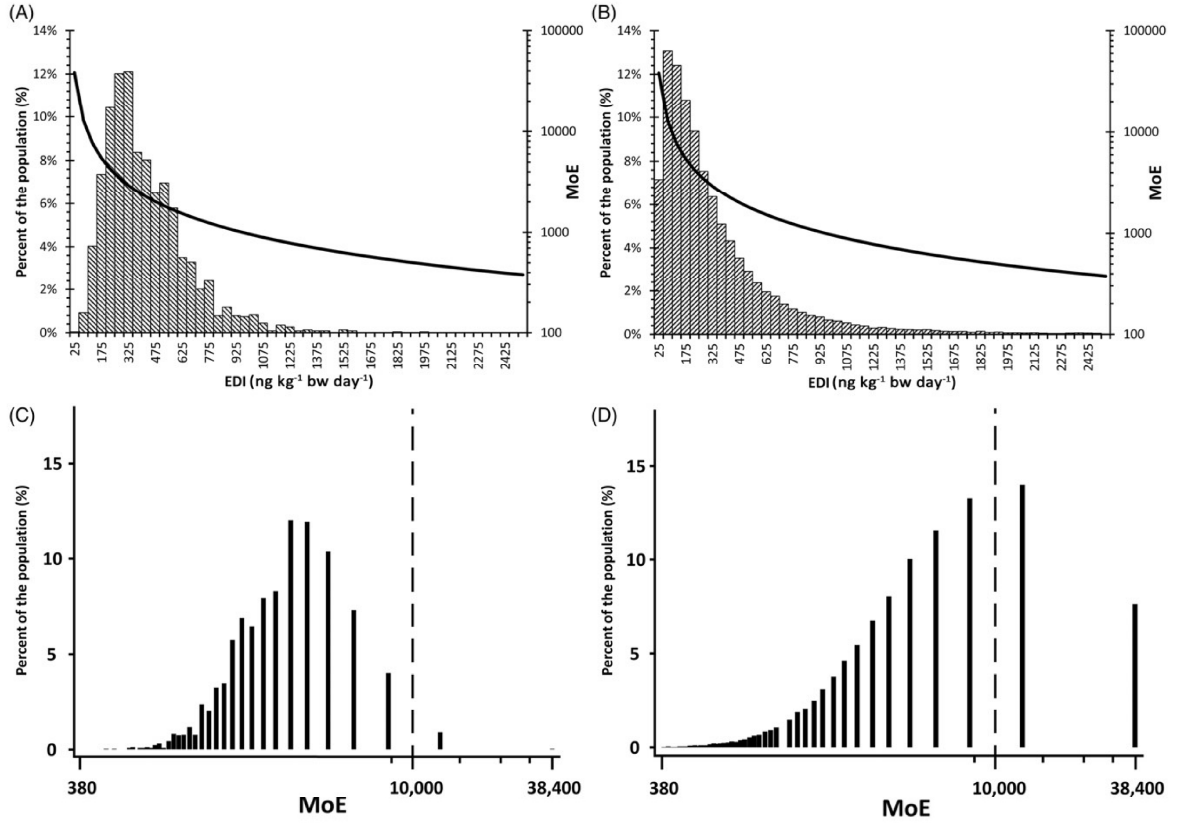

Figure 2. (A) Distribution of the estimated daily intake (EDI) of furan among the population in the deterministic approach and evolution of the MoE according to the EDI. (B) Same as A but for the probabilistic approach. (C) Distribution of the furan MoE furan among the population in the deterministic approach. (D) Same as C for the probabilistic approach. The thick line represents the margin of exposure (MoE) for a given estimated daily intake (EDI).

distribution mode is 2954 for 12.1% of children, and a MoE above 1400 was recorded for almost 90% of children. No child presented a MoE below 100 (minimal calculated MoE: 486 for 0.04% of the population).

Probabilistic approach

Results of the probabilistic approach are summarised in Table 3. As for the deterministic methodology, the children average EDI calculated thanks to the different contamination approaches are very similar (from 582 to 585 ng kg^{-1} bw day^{-1}). Therefore, only the results of the Uniform approach will be considered.

Table 3 and Figure 2(B) show a great difference between the mode (82 ng kg^{-1} bw day^{-1}), the median (238 ng kg^{-1} bw day^{-1}) and the mean (583 ng kg^{-1} bw day^{-1}) EDIs, indicating a pronounced asymmetrical tailed distribution. The calculated EDI ranged from 0.1 to 4,637,792 ng kg^{-1} bw day^{-1}.

Table 3. Probabilistic EDI (ng kg^{-1} bw day^{-1}) for several contamination approaches.

	Lower bound	Middle bound	Upper bound	Uniform
Mode	61	70	77	82
First quartile	118	120	122	120
Median	234	237	240	238
Mean	582	585	583	583
Third quartile	444	448	448	447
95th Percentile	1156	1153	1155	1154
Proportion of results > $RfD_{chronic\text{-}oral}$ (%)	6.6	6.6	6.6	6.6

Regarding risk characterisation, only 6.6% of children displayed an EDI above the $RfD_{chronic\text{-}oral}$. The trends in MoE is represented by the thick line in Figure 2(B), while the distribution among children is shown in Figure 2(D). One can point out that around 20% of children have a MoE above 10,000; the MoE

distribution mode is 12,800 for nearly 14% of children, and more than 90% of the population display a MoE above 1,300. Furthermore, less than 0.01% of children have a MoE below 100, the minimal MoE being 0.2 (EDI of 4.6 mg kg^{-1} bw day^{-1}), for less than 0.0002% of the children population.

Discussion

Consumption data

The consumption dataset available does not include the whole Belgian population, but is limited to its Flemish sub-population (6,000,000 registered in 2003, the year of consumption data), which represents about 60% of the Belgian population (10,350,000 in 2003) located in the Northern part of the country. Representativeness can be questioned but the consumption survey carried out on adults did not display any disparity between the two Belgian sub-populations (De Vriese et al. 2005). Therefore, these results can reasonably be extended to the whole Belgian population.

The consumption study was conducted in 2003 and could be considered as obsolete. However, consumption habits evolve slowly and 8 years in this timescale should have a limited impact on up-to-date practices (Duquesne et al. 2006a, b). Finally, this study was very well documented and recorded information such as children's socio-demographics and anthropometrics.

Contamination data

Contamination results were obtained using a unique analytical methodology and carried out in a single laboratory to avoid inter-laboratory bias (Scholl, Scippo, et al. 2012). This method was especially developed and validated to reduce the LOQs and to provide a high rate of results above the LOQ (up to 78%). The high percentage increased the quality of the dataset and explains the small differences observed between the different contamination approaches.

The sampling plan was designed to cover as much as possible the food chain but also the country (Scholl, Humblet, et al. 2012). Only samples available on the Belgian food market were considered in this study to focus on Belgian-based products only.

The cooking effect was not involved in the contamination level measurements. It leads to an overestimation of contamination by not taking it into account (Roberts et al. 2008). It would be very interesting to precisely quantify the cooking effect, but it depends on food type and preparation recipe. Unfortunately, it cannot be normalised.

The complete dataset was compiled in 2009 and is therefore representative of the currently available food. Nevertheless, a continuous re-evaluation through a food control plan is suitable for the future.

Deterministic versus probabilistic approach

The two approaches should not be seen as different ways to obtain the same result, but rather as complementary.

Figure 2(A) and (B), like Tables 2 and 3, display a disparity of EDI distribution, especially on the left part of the graphs (small EDI values), whereas the two methodologies present similar shapes close to the mean EDI. The probabilistic approach highlights a narrower EDI distribution characterised by a higher tailing.

A Welch's test, for unequal variances at 95th confidence limit (Petrie and Watson 2006), performed between the average population EDIs, and indicates a statistically significant difference between the two approaches. Significant differences were also observed when Welch's test was extended to the distribution modes and to the median EDIs. These differences come from data handling. In the deterministic approach, only average contamination levels were considered for calculation, whereas distributions from 0 to high levels were involved in the probabilistic method. Consequently, the calculated EDIs are biased to average values when using the deterministic approach, while they are weighted by extreme values when using the probabilistic method. Accordingly, the true EDI should fall within these two approaches.

In the RfD approach, only a low percentage (~7%) of children presented an EDI above the $RfD_{chronic\text{-}oral}$. In addition, the majority of children display an EDI below 50% of the $RfD_{chronic\ oral}$. The risk linked to furan ingestion seems higher in the probabilistic approach than in the deterministic method.

As it can be seen in Figure 2(C) and (D), the MoE distributions show very different shapes. However, the majority of the population has a high MoE (respective median and distribution mode are: 5079 and 12,800 in the probabilistic approach; 2757 and 2954 in the deterministic approach), a finding that suggests a low risk linked to the furan exposure for the Belgian children especially in the probabilistic approach. A non-negligible percentage of the children (20%) display a MoE above 10,000 (stands for very low risk) in the probabilistic approach, whereas few (1%) have such a high MoE in the deterministic. The minimal MoE values were also found to be very different (i.e. 486 for deterministic and 0.2 for probabilistic) when comparing the two approaches. The probabilistic minimal calculated MoE value is extremely low and unrealistic as it stands for an excessive worst-case scenario, which is unlikely. Therefore, the risk displays by these two approaches does not indicate a major health concern (MoE < 100). Nevertheless, results highlight a low risk

Table 4. Comparison between the adult and child EDI ($ng\,kg^{-1}\,bw\,day^{-1}$).

	Deterministic (Middle bound)		Probabilistic (Uniform)	
	Child	Adult	Child	Adult
Mode	250	225	82	23
First quartile	255	192	120	81
Median	366	330	238	212
Mean	419	380	583	494
Third quartile	534	501	447	493

level for a great portion of the population in the probabilistic approach, whereas they highlight an intermediate health concern in the deterministic approach. Consequently, the risk induced by furan ingestion among the Belgian children is not a major health concern, but this risk is not negligible with regard to the median population.

Comparison with the Belgian adult population

The results obtained in the present study were compared to a previously published risk assessment dedicated to the Belgian adult population (Scholl, Humblet, et al. 2012) and are summarised in Table 4 while Figure 3 shows the distributions.

The EDI distribution pattern of adults and children are compared in Figure 3(A) for the deterministic approach and in Figure 3(B) for the probabilistic method. The results presented here (Table 4) are consistent with the previously published data for adults with, however, a systematically higher EDI for children. A Wilcoxon rank sum test (Petrie and Watson 2006) was applied to compare the EDI distributions between adults and children. At a 95% confidence level, the *p*-value was below 0.0001, which highlights a statistical difference between adult and children distributions.

In the MoE approach, risk patterns are compared between the deterministic (Figure 3C) and probabilistic (Figure 3D) methods. A higher percentage of adults (10% for adults versus 1% for children in the deterministic approach; 31% versus 20% in the probabilistic approach) has a MoE above 10,000, due to higher EDI levels for children. Even if the risk associated to furan ingestion is not very high for both population studied, it seems slightly higher for Belgian children than for to Belgian adults (respective MoE median and distribution mode in the probabilistic approach: 5079 and 12,800 for children; 5486 and 38,400 for adults).

The contributions of the major food groups to the mean EDI for adults and children is shown in a radar plot as depicted in Figure 4. The patterns are different with a very high contribution of coffee for adults (55%) whereas it is obviously close to zero for children (3%). The same trends are observed for the prepared meat group, which contributes by 7% in adults and only <1% in children. The opposite is observed for groups like soups, milk and milk beverages, fruits, potatoes with, respectively 19, 17, 9 and 9% for children and 2, 2, 3 and 4% for adults. Only the "pasta and rice" group is of great importance for both populations, contributing by 11% for children and 6% for adults.

Management options

First, reducing the consumption of the main contributing food groups (soups, milk and milk beverages, pasta and rice, potatoes and fruits) is considered as a possibility to reduce children exposure to the furan. Nevertheless, when comparing to quantities recommended by the National Childhood Office,[2] and as concluded by Huybrechts, Matthys, Vereecken, et al. (2008) when compared to the Flemish dietary recommendations, the quantities consumed by the majority of children are already too low. In consequence, such a recommendation will decrease the furan EDI, but also the vitamin and nutriment intake and is, therefore, not suitable.

Secondly, tips for cooking could be easily implemented. According to Roberts et al. (2008), avoiding cooking in closed vessels, and thoroughly mixing food while heating can induce a decrease in furan content of up to 80%. This management option is more realistic and can be easily achieved by public advice.

Finally, Guenther et al. (2010) described the importance of the food processing conditions which could be optimised in a way to decrease the furan contamination levels. However, it could not be done without reducing the product quality and/or safety (Guenther et al. 2010; Stadler 2011).

Conclusions

In this study, the EDI values calculated for children are pretty low, with a distribution mode corresponding to 8% of the $RfD_{chronic\text{-}oral,}$ and a median corresponding to 24% of the $RfD_{chronic\text{-}oral}$ in the probabilistic approach. In contrast, the average population EDI is about half of this reference dose. Only 6.6% of the children (extreme consumers) have an EDI above the $RfD_{chronic\text{-}oral}$. Regarding the MoE in the probabilistic approach, 20% of children have a 10,000, less than 0.01% under 100, while the majority is above 1000.

Even if mean and median EDIs significantly differ between the deterministic and probabilistic approaches, similar conclusions can be drawn. The

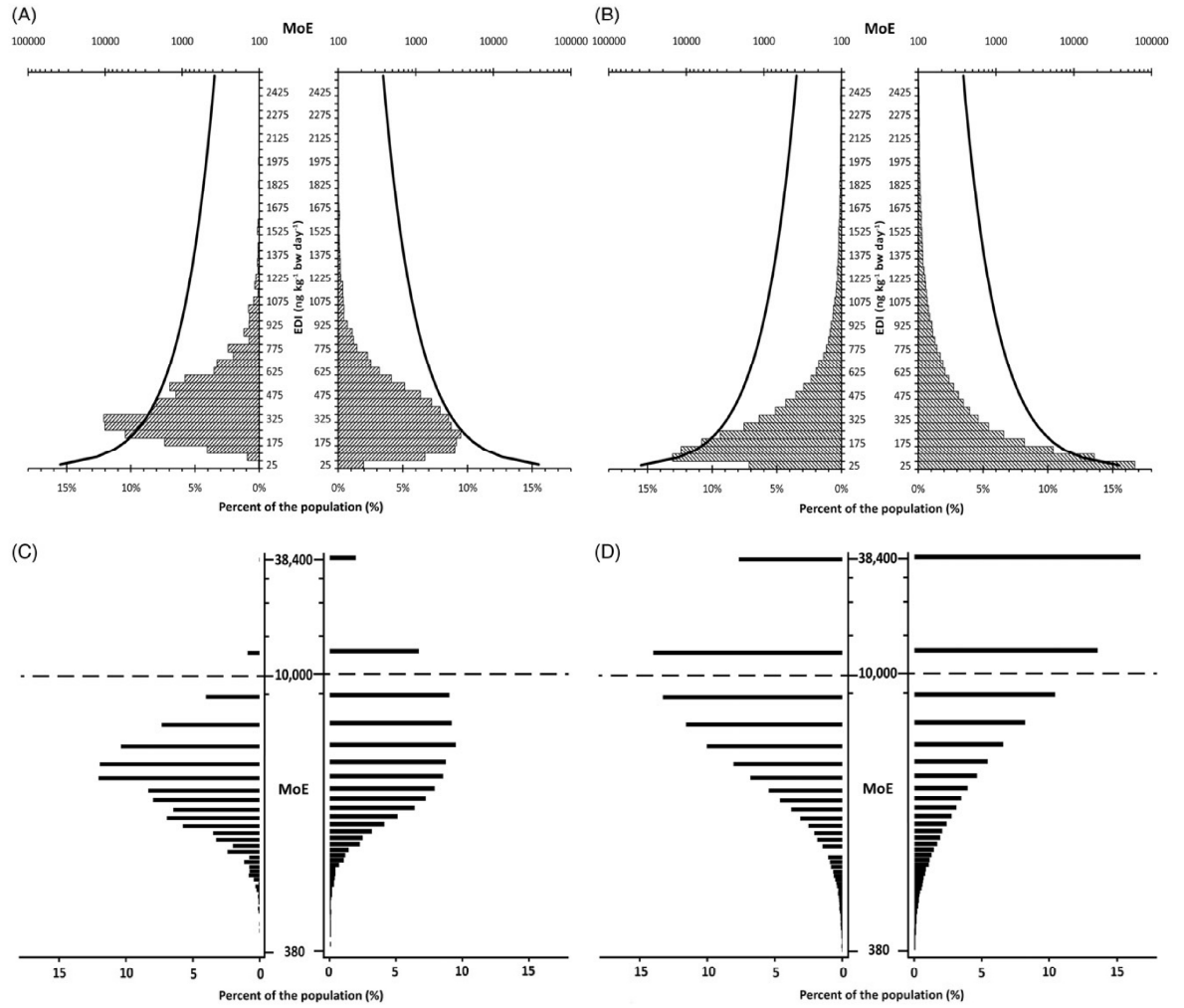

Figure 3. Comparison between the distribution of the EDI for the child (left) and adult (right) Belgian populations: (A) deterministic EDI and (B) probabilistic EDI. Comparison between the distribution of the MoE for the child (left) and adult (right) Belgian populations: (C) deterministic MoE and (D) probabilistic MoE. The thick line is the margin of exposure (MoE) for a given estimated daily intake (EDI). On each graph, the children are displayed on the left and the adults on the right.

risk linked to furan ingestion by Belgian children through diet is an intermediate health concern (median MoE: 5079 in probabilistic and 2757 in deterministic). One should note that the risk is more important when calculated in the deterministic way than using the probabilistic approach.

This study also put forward that the main contributors to furan intake are the food groups containing high levels of vitamins and essential nutriments. The beneficial effects on health of such nutrients have to be put in the balance. Therefore, it is advisable to ask consumers to change their food preparation habits rather than to advice them to reduce such food consumption.

A comparison between Belgian children and adults shows a significant difference between their EDI distributions whereas their shapes are similar; children tend to be exposed to a higher risk. It also shows a completely different EDI contribution pattern with several important contributors for children (soups, milk and milk beverages, fruits, potatoes as well as pasta and rice) and a single main contributor (coffee) for adults.

To conclude, the risk linked to furan ingestion is not a priority health concern for Belgian children as no MoE values <100 were found. The risk linked to its ingestion is however not negligible as a high percentage (99% in deterministic and 80% in probabilistic

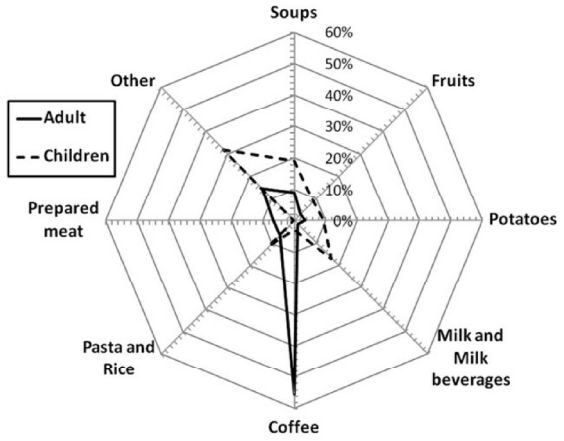

Figure 4. Radar plot comparing the main contributors to adult and child estimated daily intake (EDI) of furan. Adult main contributors are displayed with dashed lines and children main contributors as dotted lines.

approach) of the children display a MoE within 100 and 10,000 with pretty low median values. In addition, comparison to the adult risk level indicates a slightly higher risk for children than for adults. Consequently, as a difference between children and adults was highlighted, it could be interesting to compare these sub-populations with younger children, such as toddlers and babies.

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Notes

1. US-EPA Integrated Risk Information System (http://www.epa.gov/iris/subst/0056.htm).
2. ONE: http://www.one.be/

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4.4. Preliminary risk assessment results for toddlers

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Preliminary assessment of the risk linked to furan ingestion by babies consuming only ready-to-eat food

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The risk linked to furan ingestion has been assessed in previous papers for Belgian adults and children. The present paper focuses on infants consuming only ready-to-eat baby food. As there is no Belgian baby dietary database, the furan exposure assessment was carried out by using an Italian infant consumption database and Belgian contamination data. The estimated daily intake (EDI) was calculated according to a deterministic methodology. It involved 42 commercially available ready-to-eat baby food and 36 baby consumption records. The mean EDI was 1460 ng*$(kg_{bw}$*day$)^{-1}$ which is 3.8 times higher than the 381 ng*$(kg_{bw}$*day$)^{-1}$ reported for Belgian adults, and 3.5 times higher than the 419 ng*$(kg_{bw}$*day$)^{-1}$ measured for Belgian children. To assess and characterise the risk for babies' exposure, the margin of exposure (MoE) was calculated. It highlighted that 74% of infants have a MoE < 1000, with a minimum of 140. However, these are only preliminary results as they were calculated from a very small dataset and the infant cytochrome P450 activity is significantly different compared with the adult's. Therefore, the risk linked to furan ingestion by babies should be assessed in a different manner. To this end, additional data regarding a baby diet as well as a better understanding of furan toxicity for babies are needed to characterise more accurately the risk for infants.

Keywords: furan; baby; risk assessment; food chain; deterministic approach; margin of exposure (MoE)

Introduction

Furan is a low molecular mass food toxicant first reported by Maga (1979). Since that time, several papers have reported its occurrence in food, especially in baby food (US-Food and Drug Administration 2005; Hasnip et al. 2006; Crews & Castle 2007; Roberts et al. 2008; Fromberg et al. 2009; Kim, Lee, Kim, et al. 2009; Arisseto et al. 2010; Scholl, Scippo, et al. 2012). Within the same period of time, a series of papers described the furan formation pathway in food, and suggested baby food was highly contaminated (European Food Safety Authority 2009; Arisseto et al. 2010; Owczarek-Fendor, De Meulenaer, Scholl, Adams, Van Lancker, Yogendrarajah, Eppe, et al. 2010; Owczarek-Fendor, De Meulenaer, Scholl, Adams, Van Lancker, Yogendrarajah, Uytterhoeven, et al. 2010; Wegener & López-Sánchez 2010; Owczarek-Fendor et al. 2011, 2012). As a consequence, complementary information on the risk linked to furan ingestion was required (Heppner & Schlatter 2007; Stadler & Anklam 2007).

Since the 1990s, furan toxicity has been studied through *in vitro* and *in vivo* protocols (Kedderis et al. 1993; National Toxicology Program 1993; Chen et al. 1995; National Academy of Sciences 2000; Peterson et al. 2000; Peterson et al. 2005, 2006; Bakhiya & Appel 2010; Hamberger et al. 2010); it was classified in group 2B as "possibly carcinogenic to humans" by the International Agency for Research on Cancer (1995).

The present paper is the third part of a furan risk assessment study that has focused on the overall population: adults (Scholl, Humblet, et al. 2012), children (Scholl, Huybrechts, et al. 2012) and now babies. The risk assessment methodology was adapted from Feinberg and co-workers' protocol (Feinberg et al. 2006). It includes the following four steps: hazard identification, hazard characterisation, exposure assessment and risk characterisation. The application of this protocol to furan was extensively discussed in the previous publications (Scholl, Humblet, et al. 2012; Scholl, Huybrechts, et al. 2012). In this work, the baby food sampling and analyses were performed in Belgium (Scholl, Scippo, et al. 2012). However, facing the lack of true infant consumption data in Belgium, the risk assessment was carried out using an Italian database recently published by Leclercq et al. (2009). This approach can be seen as acceptable for a preliminary study, but Belgian infant dietary records will be needed to conduct a complete risk assessment. Due to the limited size of available

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databases, only a deterministic approach was applied to assess furan exposure of infants. Finally, in the context of risk characterisation, the calculation of the margin of exposure (MoE) was performed.

Materials and methods

Contamination data

The contamination dataset used in the present study involved 42 ready-to-eat baby food sold on the Belgian market. A specific predetermined sampling plan was implemented and a single analytical methodology was applied to samples as purchased, both previously described (Scholl, Scippo, et al. 2012). The percentage of results exceeding the LOQs was particularly elevated (i.e. 96.7%).

Consumption data

Consumption data were provided by the Italian National Research Institute for Food and Nutrition (INRAN). The dataset came from a cross-sectional survey conducted on the Italian population: INRAN-SCAI 2005–06 (Leclercq et al. 2009). In this study, the diet of 36 infants aged from 1 month to 2 years was recorded during 3 consecutive days by the parents and caregivers under the control of trained fieldworkers. Socio-demographic and anthropometric information was also recorded at the same time.

Risk assessment

The four-step methodology (hazard identification, hazard characterisation, exposure assessment and risk characterisation) developed by Feinberg et al. (2006) was applied in the present risk assessment. A full description of these four steps is available in a previous paper (Scholl, Humblet, et al. 2012).

Briefly, a series of toxicological studies were performed on furan (Kedderis et al. 1993; National Toxicology Program 1993; Chen et al. 1995; Peterson et al. 2000, 2005, 2006; Bakhiya & Appel 2010; Hamberger et al. 2010) which highlighted its potential liver carcinogenic effect through the first hepatic transformation of furan by cytochrome P450 (Moro et al. 2012). Consequently, the 95% lower confidence limit of the benchmark dose for 10% extra risk ($BMDL_{10}$) of hepatocellular adenomas and carcinoma was established at 0.96 mg*(kg_{bw}*day)$^{-1}$ (Moser et al. 2009; Benford et al. 2010; Carthew et al. 2010; Williams et al. 2011).

The estimation of the furan daily intake (EDI) is calculated with Equations (1)–(3):

Equation (1): Estimation of the relative daily consumption:

$$\text{Relative Daily Consumption} = \frac{\text{Daily Consumption}}{\text{Weight}}$$

Equation (2): Calculation of EDI:

$$\text{EDI}_{\text{Food Group}} = [\text{Furan}]_{\text{FoodGroup}} \times (\text{Relative Daily Consumption})_{\text{FoodGroup}}$$

Equation (3): Calculation of global EDI:

$$\text{EDI}_{\text{Global}} = \sum_{\text{FoodGroup}} \text{EDI}_{\text{FoodGroup}}$$

These equations were applied for a deterministic approach of the daily intake.

The risk was characterised by the calculation of MoE, as shown in Equation (4) (Constable & Barlow 2009):

Equation (4): MoE calculation for a carcinogenic mode of action (MoA):

$$\text{MoE} = \frac{\text{BMDL10}}{\text{EDI}}$$

Results

The EDI of each infant was calculated by using his/her respective diet and body weight, and the mean contamination level of baby foods spread over six groups: beverages (7% of the meal), cereals (11%; baby biscuit as bread soup biscuit), fruits (13%), vegetables and/or meat (17%), milk and dairy products (51%), and miscellaneous (1%) (Table 1). The EDIs and MoEs of several population ranges are summarised in Table 2. The EDIs ranged from 141 to 3442 ng*(kg_{bw}*day)$^{-1}$, with a mean EDI relatively close to the median EDI (1461 versus 1174 ng*(kg_{bw}*day)$^{-1}$). The distribution of daily intakes across the infant population was normal, as depicted in Figure 1A and assessed by a Shapiro–Wilk test (p = 0.46; Shapiro & Wilk 1965; Petrie & Watson 2006).

The contribution of the different food groups to the mean EDI is presented in Figure 1B. The major contribution comes from the vegetable and/or meat group (69.4%), which is also the most contaminated baby food group (Scholl, Scippo, et al. 2012). Milk and fruits represent

Table 1. Belgian contamination data expressed as ng*(g or ml)$^{-1}$.

Food item	Minimum	Mean	Maximum
Cereals (N = 8)	1.1	10.9	53.5
Fruits (N = 8)	0.5	13.6	92.6
Vegetables and/or meat (N = 17)	24.9	72.3	224.4
Beverages (N = 5)	0.2	3.1	7.5
Milk and milk products (N = 2)	2.1	3.2	4.3
Miscellaneous (N = 2)	7.5	18.4	29.4

Table 2. Babies' estimated daily intake (EDI) (ng*(kg$_{bw}$*day)$^{-1}$) and margin of exposure (MOE) for several population ranges.

Parameter	EDI	MoE
Minimum	141	279
2.5th percentile	242	346
25th percentile	829	664
Median	1174	817
Mean	1461	1045
75th percentile	1723	1020
97.5th percentile	2797	4164
Maximum	3442	6811

12.2% and 10.3% of the mean EDI, respectively. Other food groups contribute by less than 10%.

Regarding the risk characterisation, MoE ranged from 6811 to 279, and 74% of infants displayed an MoE < 1000 (Figure 1C).

Discussion

Consumption data

The consumption dataset used in the present study is a small part of a well-documented Italian survey involving the overall population from 1 month to 97 years and 8 months old (Leclerq et al. 2009). These relatively new data were recorded by carers but under the supervision of trained fieldworkers.

However, the number of recorded data here are too small (n = 36) and the age distribution is too large (1 month to 2 years old) for a sound statistical evaluation. Therefore, this dataset only provides an overview of preliminary results. A Belgian true consumption dataset involving hundreds of infants would be highly suitable to improve the accuracy of future risk assessment.

Contamination data

Contamination results were obtained after applying only one analytical methodology carried out in a single laboratory in order to avoid inter-laboratory bias (Scholl, Scippo, et al. 2012). The solid phase microextraction coupled to a gas chromatography separation and a mass spectrometry detection (SPME-GC-MS) method was especially developed and validated to lower the LOQs and to provide a high rate of contamination results above the LOQ (up to 96.7%). The sampling plan covered any kind of commercially available ready-to-eat baby foods and was compiled only 3 years ago.

Nevertheless, a larger database involving home-made baby food items, of which the consumption is twice as important as ready-to-eat baby food in Belgium (Lenaers et al. 2002), is suitable to better illustrate the real context. In addition, a continuous re-evaluation through a national food control plan would be useful to keep the database up to date.

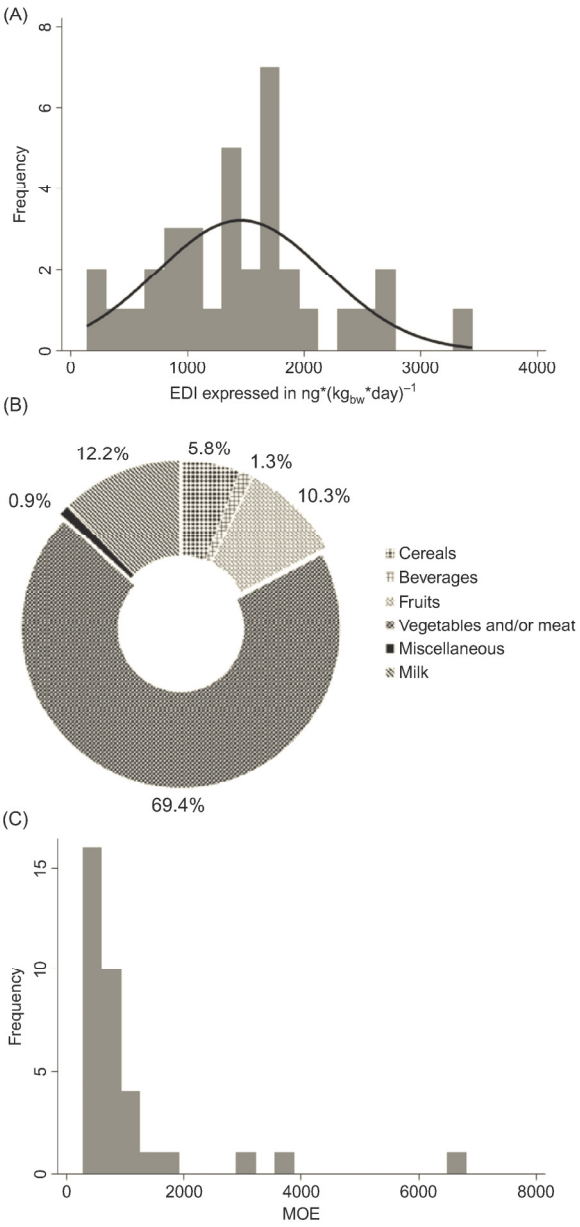

Figure 1. (A) Distribution for infants of estimated daily intakes (EDIs) for furan contamination after ingestion; (B) contribution of the different food groups to the infant mean furan EDI; and (C) distribution of the estimated margin of exposure (MOE) for furan contamination after ingestion.

Risk characterisation

The risk linked to furan ingestion by babies appears higher compared with both Belgian adult and child populations. Firstly, the mean EDI for infants (1461 ng*(kg$_{bw}$*day)$^{-1}$) was included in the range (230–1770 ng*(kg$_{bw}$*day)$^{-1}$)

estimated by Minorczyk et al. (2011) in Poland. Secondly, the mean EDI for infants is 3.8 times above the mean EDI reported for adults (380 ng*(kg_{bw}*day)$^{-1}$), and 3.5 times above the mean EDI reported for children (419 ng*(kg_{bw}*day)$^{-1}$) in Belgium. Thirdly, 74% of infants display an MoE < 1000 versus less than 5% for children and adults in Belgium.

In addition, the present results are about five times higher than those already published by European Food Safety Authority (2011). This difference can mainly be explained by the contamination food dataset where only ready-to-eat baby food was selected here, whereas home-made and ready-to-eat baby food were taken into account in the European study. It would then be very interesting to investigate how consumption habits could influence the infant daily intake.

Furthermore, the present mean EDI is also 1.8 times higher than for Brazilian infants (820 ng*(kg_{bw}*day)$^{-1}$) (Arisseto et al. 2010). Both studies involve only ready-to-eat baby food to assess furan contamination, and the sizes of databases are quite similar (42 samples for Belgium versus 31 for Brazil). The main differences are linked to the origin of data, the size of the dietary database (136 babies for Brazil), and the age disparity of babies (6–11 months for Brazil). The last two dissimilarities are mainly responsible for the EDI differences. The preliminary results are probably biased by the use of a too small dietary database and a too high age range.

In addition, the risk characterisation for babies should take into account the consideration given by Alcorn and McNamara (2003). They pointed out that the cytochrome P450 activity of infants less than 3 months old is negligible (as liver maturation is not finalised yet), while between 3 months and 3 years old it is 1.5–2 times higher than that for the adults. It means that the formation rate of *cis*-2-butene-1,4-dial through furan metabolism by hepatic enzymes is different. The use of the $BMDL_{10}$ calculated for adults should be preferably refined for infants, even if the safety factors taken into account for this estimation should also concern the more risky subpopulation.

Finally, the combination of Italian dietary records with Belgian contamination data also brings a bias.

Conclusions

The present study highlighted a higher EDI (mean = 1461 ng*(kg_{bw}*day)$^{-1}$) for babies' furan ingestion through commercially available ready-to-eat baby food than for adults. It also showed that the major contributor to daily intake is the most contaminated food group (meat and/or vegetables; 69%). In addition, other major contributors are highly consumed food groups such as milk (12%) and fruits (10%).

The results showed that infants display a higher risk of furan ingestion through their diet compared with children and adults. The conclusion drawn here is confirmed by the publications of other research groups, but the daily intake and the risk level for infants calculated here are much higher, taking into account that a Belgian infant dietary assessment is not yet available, yielding to a significant bias and a lack of accuracy of the present assessment. A second limitation is the current knowledge of furan toxicity for babies. Thus, further investigations in the Belgian infant diet as well as in the cytochrome P450 activity for infants are required to refine babies' exposure to furan.

The furan risk assessment for Belgian babies presented here can be considered as a pilot study since the assessment should be refined (1) by introducing home-prepared baby food, reported as less contaminated (Roberts et al. 2008; Kim, Lee, Park, et al. 2009) and more consumed by Belgian infants (Lenaers et al. 2002); (2) by increasing the size of the datasets, especially for individual consumption data; (3) by scaling down the age disparity within the dietary assessment; and (4) by integrating and combining all these data with new knowledge on furan toxicity in infants, once available.

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5. Conclusions

Our journey in the furan country finally comes to its end. Food scientists would tell that contaminants investigation is a never-ending story, but there is a time where we must sit and look back to our achievements before beginning a new journey. Thus, here comes the time to conclude this thesis work, and to give some perspectives and advices.

This thesis finds its origin in the proactive movement engaged by the food control authorities towards the food borne contaminants. It focused on a lone processing contaminant: the furan. This work presented results from analytical development to furan risk assessment for the Belgian population with a particular regard to the children sub-population.

The first aim of my thesis was to develop a high-sensitive analytical method able to report sub ppb levels in foodstuffs. The headspace SPME GC-MS method presented in the chapter 2 meets this goal and fulfills the requirements of the European Commission decision regarding to the validation of analytical method (2002/657/EC). Additionally, this is a highly integrated and automatable method able to achieve high throughput (± 40 samples per day). The method was successfully used in the investigation of the furan formation pathways (Owczarek-Fendor et al., 2010a, b, 2011a, b, 2012) requiring a fully automated approach. To complement this advanced method, the high sensitivity achieved enabled to report more than 78% of results above LOQ when performing the Belgian food contamination survey. It has definitely refined our risk assessment.

The second aim of my thesis was to assess the furan contamination level of the products available on the Belgian market with a limited number of samples. To achieve this goal, a dedicated sampling plan taking into account the already reported contamination levels, the food items consumption frequencies and the matrix diversity was designed. This survey highlighted a nearly ubiquitous contamination of the food chain and pointed out that the roasted and long-time cooking food were the most contaminated items, whereas the fat, raw meat, milk, alcohol and fresh fruits were the less contaminated. It also underlined that the developed sampling plan was fit for purpose as we obtained results for the complete food chain. But, an unanswered question remained: what is the contamination level after the home preparation of the food? Finally, this evaluation was a snapshot of the 2009 contamination state of the Belgian food chain. And nowadays, the Belgian FFSA annual control plan involves the monitoring of the furan most contaminated food matrices (e.g. coffee, baby foods and sauces). This annual control plan allows the authorities to monitor the time evolution of the furan contamination for the critical food items. Nevertheless, a periodic complete

reevaluation (e.g. every 5 or 7 years) is suitable to keep the furan contamination knowledge up to date.

The final aim of my thesis was to assess the risk linked to the furan ingestion by the Belgian population. The methodology described in the chapter 4 was applied to 3 sub-populations namely Adults, Children and Toddlers.

The Adults and the Children risk assessments only involved Belgian consumption and contamination datasets. They highlighted a "No concern risk level" for a significant portion of the population (MoE > 10000 for 31 % of adults and 20 % of children) and a "High concern risk level" for almost none (MoE < 100 for 0.03% of adults and 0.01% of children). The major part of the adults and children population displayed a MoE between 10000 and 100 with median MoE for adults and children at 5486 and 5079, respectively (mode MoE for adults and children are 38400 and 12800, respectively). Mode and median values tend to indicate a low concern for health through dietary exposure to furan at population level. In addition, these assessments showed the difference between the major contributors to the daily furan intake for adults and children. For adults, coffee is the major contributor and it contributes to more than 55% of the EDI. In such case, different risk management options would have to be considered. These might include providing consumer advice through the food control authorities, e.g. about vigorously mixing their coffee before drinking or advising consumers on the maximum number of cups of coffee that should be drunk per day or per week. Regarding the children, there are 5 major contributors: soups, milk and dairy products, pasta and rice, fruits and potatoes contributing to 19, 17, 11, 9 and 9% of the EDI, respectively. In comparison with the adults, the same food groups only contribute to 11% of the EDI.

In the case of infants, a Belgian food consumption survey is unfortunately not yet available; the only available data were from an Italian survey (Leclerq et al., 2009). The low median MoE for toddlers did indicate a concern for health through ready-to-eat infants' formulae exposure (i.e. MoE ranges from 279 to 6811 with a median at 817, with 74% of the toddlers displaying a MoE beneath 1000). Additionally, the infants' formulae containing vegetables and/or meat are the major contributors with 69% of the EDI. However, these results must be taken with great care for three reasons. First, the sets of data were limited with only 42 food items and 36 infants' consumption habits. Therefore, a Belgian toddlers' consumption survey should be performed to improve the accuracy of the forthcoming infants'

risk assessments. This survey should include recording of the evolution of the consumption habits from birth to at least three years old of hundreds of toddlers. Second, the cytochrome P450 is not mature in toddlers and the toxicity of furan should differ from adults. As a consequence, we would need more study on the furan toxicity for toddlers to refine the risk assessment. Third, only ready-to-eat infants' formulae were taken into account in the present assessment. Roberts and coworkers (Roberts et al., 2008) showed that they are more contaminated than home-made baby food while the consumption of home-made baby food by Belgian infants is twice higher than the consumption of ready-to-eat baby food (Lenears et al., 2002). As a consequence, it sounds necessary to include home-made baby foods in a toddlers risk assessment to construct a better model of the real baby diet.

The access to the consumption data, especially for infants, is the major pitfall in the achievement of a risk assessment. First, many consumption surveys only include specific information (such as nutriments or energy intake) or sub-population data (e.g. ethnic or social). Second, carrying out an exhaustive survey takes long time; therefore they are not often updated. But, the consumption habits change over time and surveys can be outdated. Third, a better cooperation between consumption data owners and risk assessors is needed. The data access can be refused for senseless reasons such as the lack of interest of the subject for the survey organizer or the membership to an institution.

Finally, results of this thesis were used by the Belgian Federal Public Service of Health, Food Chain Safety and Environment as support to set up actions, and in improvement of their annual control plan. Besides, these results were the Belgian contribution to the evaluation of the risk at a European level.

6. Abbreviations & Acronyms

- α error: probability of false non-compliant decision
- AA: Ascorbic Acid
- ADI: Admissible Daily Intake
- AFSCA: Agence Fédérale pour la Sécurité de la Chaine Alimentaire
- *aka*: also known as
- ANOVA: ANalysis Of VAriance
- AOCS: American Oil Chemists' Society
- API: Atmospheric Pressure Ionization
- aq: aquous
- β error: probability of false compliant decision
- b.b.: body burden
- Belgian FPS: Belgian Federal Public Service
- BMD_{10}: Benchmark Dose for a 10% extra risk
- $BMDL_{10}$: 95 % confidence Limit for the Benchmark Dose for a 10% extra risk
- BPA: Bisphenol A
- BTEX: Benzene, Toluene, Ethylbenzene and Xylenes
- b.w.: body weight
- CAMOLA: CArbon MOdule LAbeling
- CAR: Carboxen®
- CART: Center of Analytical Research and Technology
- CAS Registry Number: Chemical Abstracts Service Registry Number
- CC_a: Decision Limit
- CC_b: Detection Capability
- CCD: Central Composite Design
- DDT: DichloroDiphénylTrichloroéthane
- EC: European Commission
- EDI: Estimated Daily Intake
- EDO: Estimated Daily Outake
- EFSA: European Food Safety Agency
- e.g.: exempli gratia
- EI: Electronic Ionization
- EPA: Environmental Protection Agency

- ESI: ElectroSpray Ionization
- EWI: Estimated Weekly intake
- EYI: Estimated Yearly intake
- FAO: Food and Agriculture Organization
- FDA: Food Drug Administration
- FERA: The Food and Environment Research Agency
- FFSA: Federal Food Safety Agency
- GC: Gas Chromatography
- GEMS: Global Environment Monitoring System
- GMO: Genetically Modified Organism
- HCB: HexaChloroBenzene
- HS: HeadSpace
- IARC: International Agency for Research on Cancer
- ID: Isotopic Dilution
- i.e.: id est
- INRAN: Istituto Nazionale di Ricerca per gli Alimenti e la Nutrizione
- IPCS: International Program on Chemical Safety
- IPH: Institute of Public Health
- ILSI: International Life Sciences Institute
- IRIS: Integrated Risk Assessment System
- IRMM: Institute of Reference Materials and Measurements
- ISO: International Organization of Standardization
- JECFA: Joint FAO/WHO Expert Committee on Food Additives
- k: coverage factor
- LB: Lower Bound
- LC_{50}: Lethal Concentration for 50% of the population
- LD_{50}: Lethal Dose for 50% of the population
- LOAEL: Lowest Observed Adverse Effect Level
- LOD: Limit of Detection
- LOQ: Limit of Quantification
- *m/z*: mass to charge ratio
- MALDI: Matrix Assisted Laser Desorption Ionisation

- MB: Middel Bound
- MCPD: monochloropropane
- MoA: Mode of Action
- MoE: Margin of Exposure
- MS: Mass Spectrometry
- MSLab: Mass Spectrometry Laboratroy
- MU: Measurement Uncertainty
- NAS: National Academy of Science
- NIST: National Institute of Standard and Technology
- NOAEL: No Observed Adverse Effect Level
- NTP: National Toxicology Program
- ONE: Office National de l'Enfance
- PAHs: Polycyclic Aromatic Hydrocarbons
- PB: Plackett-Burman
- PCDD/Fs: PolyChloroDibenzoDioxines et Furanes
- PDMS: PolyDiMethylSiloxane
- PLOT: Porous Layer Open Tubular
- ppb: part per billion
- ppm: part per million
- PTFE: PolyTetraFluoroEthylene
- PTV: Programmable Temperature Vaporisation
- Quad: Quadrupole
- RA: Risk Assessment
- RF: Radio frequency
- RfD: Toxicological Reference Dose
- RRF: Relative Response Factor
- RSD: Relative Standard Deviation
- SBO: SoyBean Oil
- SD: Standard Deviation
- SPME: Solid Phase MicroExtraction
- SIM: Selected Ion Monitoring
- TIC: Total Ion Current

- ToF: Time of Flight mass spectrometer
- U: Expanded uncertainty
- UB: Upper Bound
- ULg: University of Liège
- UREAR: Research Unit in Epidemiology and Risk Assessment applied to the veterinary sciences
- USA: United States of America
- US-EPA: United States Environmental Protection Agency
- US-FDA: United States Food and Drugs Administration
- VOC: Volatile Organic Compound
- VSC: Volatile Sulfuric Compound
- WC: waxy corn starch
- WHO: World Health Organisation
- YOPIs: Young, Old, Pregnant and Immuno-compromised persons

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