Estimation of the furan contamination across the Belgian food chain

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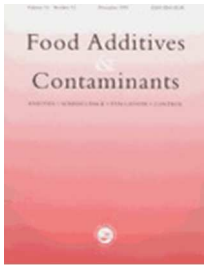

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Abstract

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, but also the consumption frequencies were taken into account in the construction of the sampling plan. Weighting factors such as contamination levels, consumption frequency and 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 µg/kg) with a mean value of 756 µg/kg 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 µg/kg. 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: Furan, Contamination, Food items, Food Chain, Belgium

Introduction

Furan was isolated for the first time in food in the late 70s (Maga, 1979). The first report on its toxicology and carcinogenesis came out fourteen years later (National Toxicology Program, 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 Science (NAS) classified it as a narcotic (NAS, 2000). More recently, furan received an increasing matter of concern since a report about its occurrence in food was

published by the United State Food and Drug Administration (US-FDA) in 2004 (US-FDA, 2004). As a result, national and international food authorities required information about levels in food, human exposure and formation pathways to be gathered (Stadler, 2007). In Europe, the first report published by the European Food Safety Authority (EFSA) contained a compilation of early reported data (EFSA, 2005). Later, EFSA organized the collection and the centralisation of foodstuffs monitoring data from EU Member States in a European database. Since 2009, EFSA published summarised reports on a regular basis (EFSA, 2009; EFSA, 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 et Tsai, 2010). Recently, the production of furan was described in heat-processed food including home-cooked and ready-to-eat items (Crews et al., 2007; Fromberg et al., 2009; Hasnip et al., 2006; Roberts et al., 2008). 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; Owczarek-Fendor et al., 2010b). Significant differences related to origin and brands of products were also reported (Wegener and López-Sánchez, 2010). The authors pointed out clear differences existing 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 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 Food and Agriculture Organisation (FAO) collected data from national surveys to combine them in a European or international database (EFSA, 2009; 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 emphasize or to minimize the role of three selected parameters that we considered as essential. In this context, a pre-requisite condition was to use the same analytical method in order to avoid methods-to-methods analytical biases. In addition, the selected method was sensitive enough to minimize 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 and collaborators (Scholl et al., 2007; Scholl et al., 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. Sample (1 g) was 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, St. Louis, MO, USA) and air-tightly closed. Samples were prepared one by one, as fast as possible, to avoid furan loss by evaporation.

The deuterated standard used for quantification was a 100 pg/µL water solution daily prepared by dilution of d_4-furan. The dilution was carried out in 2 steps: firstly, by addition of 10 µL of d_4-furan in a 20 mL airtight vial full of methanol (picograde, LGC-Promochem, Wesel, Germany); secondly, 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, St. Louis, MO, USA) stock solution used to build up a three levels-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 Vaporization (PTV) injector. The chromatographic separation was achieved on a PoraBond-Q (25m x 0.32mm x 5µm) column (Varian, Palo Alto, CA, USA) at 1.7 mL/min He (99.9997 % purity, Air Products, Allentown, PA, USA) constant flow. The temperature program started at 35°C for 2 min, ramped at 10°C/min to 100°C hold 5 min, followed by a 30°C/min temperature ramp until 260°C hold 6 min. Ions are 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) shall 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 in 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 are matrix-dependant. Temperature was set and kept constant by a Peltier cooling system (CTC Analytics, Zwingen, Switzerland) during extraction. Fibre desorption occurred in the injection port of the PTV kept at 230°C in splitless mode. Finally, fibre was cleaned-up 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 recommendations.

Statistical tests

A Welch's test was used to compare the mean furan concentration from this study with several available studies having 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 3 datasets at 95% 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, *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 as possible information from this limited sampling number, three key parameters were identified and selected to construct the model: contamination levels, food diversity, and consumption frequency as shown in Figure 1. As the Belgian consumption survey (IPH/EPI, 2006) did not include any data for baby food, the model has not been 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.

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

Secondly, a weighting factor based on already reported contamination levels (US-FDA, 2004; EFSA, 2005) was applied to the number of samples to be analysed in each food group. This weighting factor is within -10 and +5 samples and is 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 like coffee, baby food or crispy food. On the contrary, a -10 factor was applied for items never reported as contaminated (e.g. reported as "not-detected") such as water and fresh eggs. Between these two extreme cases, two additional moderate factors, -5 and 0, were also affected to the remaining food groups and to groups for which no data were available, respectively (Table III).

Thirdly, 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 step of 5) was directly awarded to 3 categories of consumption frequencies: highly (+5 samples), moderate (no change) and little consumed (-5 samples).

Fourthly, 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 having a number of matrices above the cut-off while it remained unchanged below that value.

Fifthly, 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 their own experience in food safety. They provided recommendations to modify the third and fourth weighting factors with the final objective to extract a consensus sampling plan.

Sixthly, to discard any geographical effect, the sampling was spread over the country. As Belgium is divided into 10 provinces, the same number of samples was randomly collected in each province.

At last, to specifically avoid a brand or a food market chain related to a province, the samples were randomly distributed over 7 to 10 food market chains depending upon the availability.

Approximately 100 different markets were visited in the country. Between 5 and 10 items were purchased in triplicate in each market.

Results

According to the authorities' recommendation (EC, 2007), three individual items of each sample were mixed and homogenized. 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 µg/kg 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 CC_β.

Results are summarized in figures 2A and 2B. They 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 µg/kg, were found in coffee. Lower, but nevertheless very high levels close to 100 µg/kg, were found in prepared meat, pasta and rice, baby food and breakfast cereals. Raw meat products, fat, fresh fruits, milk and alcohol groups showed a low mean furan content. Heat-treated foods such as roasted and/or long time-cooked items are characterized by a high furan content (Fromberg et al., 2009; Roberts et al., 2008). 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 3 sub-groups: 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 ± 5 µg/kg. The second group exhibited a much higher mean level (65 ± 57 µg/kg) while the third one is somewhat an intermediate between the first two groups (23 ± 73 µg/kg). 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 of them were selected for a comparison as their data are of the public domain. The present results were then compared to data reported by Switzerland (Reinhard et al., 2004; Zoller et al., 2007) and by EFSA in 2009 (EFSA, 2009). Mean values of the highest contaminated food groups for the three studies are presented in Table II. These results were compared by using the Welch's test with the Bonferroni correction for statistical significance.

For the *Coffee* group, Table II shows that the null hypothesis is neither verified between our study and EFSA, nor between our study and the Swiss work, nor between the 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 one and the EFSA report focus on from raw coffee. Formerly, it was demonstrated by several

groups that the coffee furan content is recipe-dependant (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 our mean value is nearly 2 times 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" sub-categories of the EFSA survey which display very high contamination levels. Only 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 and co-workers (Guenther et al., 2010).

For the *Prepared meat* group, the null hypothesis was verified for the comparison between our survey and EFSA, and between our 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 of values calculated in the two other studies. The Swiss mean value was nearly twice higher than EFSA mean value. One can assume 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 et al., 2010). Other authors suggested that the exact food composition and recipe are geographically related (Merchant et al., 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 our survey to 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 with the composition of the food

item. Firstly, it could be linked to the influence of the local production on the furan content. Secondly, the food group sampling can induce biased results.

For the *Breakfast cereals* group, the null hypothesis is not verified between our study and the Swiss or the EFSA survey. In each case, our contamination level is more than twice higher than the level reported by the Swiss and 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 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 are similar at a 95% confidence interval. It can be explained by the scattering of data in this group (i.e. the standard deviation is equal 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 sub-groups 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, our results are in accordance with the EFSA survey, but in a lesser extend with the Swiss study when using a Welch's test taking into account the Bonferroni correction ($P < 0.017$). For each mean value, except for coffee, the corresponding P is under 0.01. The statistical significance seems to be related to the local products. Therefore, this is one more clue to support that the European survey is "very large", and provides results comparable to local surveys, but that local surveys provide more accurate data, useful to carry 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 to 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 the contamination levels. Baby foods results display a high disparity and can be distributed over 3 groups according to the 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 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. It is also an overall screening of the food chain that can be used for several purposes like risk assessment, identification of the critical items, estimation of the ground level, or identification of some formation critical components.

In addition, our results are consistent with studies already published (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 proof that local surveys induce less variability than international surveys. Precise risk assessments could be better obtained using a local approach especially in order to determine the more risky or exposed population.

In conclusion, the proposed methodology successfully fulfils our requirements that were: combining the results that can be obtained using a screening survey to the results

obtained using an exhaustive methodology with a limited number of samples. Therefore the proposed methodology is a fast and cost-effective methodology useful to carry out a "*pseudo-exhaustive"* contamination assessment across the food chain.

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Table and figure caption

Table I. Groups of food items in the Belgian food chain

Table II. 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 $P < 0.001$.

Legend:
n: number of samples in the group; Mean: mean concentration in $\mu g*kg^{-1}$ or $\mu g*L^{-1}$; SD: Standard Deviation in $\mu g*kg^{-1}$ or $\mu g*L^{-1}$; ND: data unavailable; 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.

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

Legend: for explanation, see the section 3.1. Sampling plan construction

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

Figure 2.
Title: Furan contamination levels across the Belgian food chain
Legend: 2 A and 2 B, all the food chain; 2 C, baby foods (details)

Table I. Groups of food items in the Belgian food chain

(1)	Spirits	(2)	Beer
(3)	Bread	(4)	Breakfast cereals
(5)	Cereal based products	(6)	Cheese
(7)	Coffee	(8)	Cooking fat
(9)	Edible offal	(10)	Egg
(11)	Fish	(12)	Fish based products
(13)	Fresh meat	(14)	Fruit
(15)	Fruit juices	(16)	Goats and rabbits
(17)	Light Soft Drink	(18)	Meat
(19)	Meat based products	(20)	Milk and dairy products
(21)	Other	(22)	Pasta and rice
(23)	Potatoes	(24)	Poultry
(25)	Sauce	(26)	Seafood
(27)	Soft Drink	(28)	Soup and vegetable juices
(29)	Soy based products	(30)	Spreading fat
(31)	Tea	(32)	Vegetarian food
(33)	Vegetables	(34)	Water
(35)	Wine	(36)	Yoghurts and Pudding

Table II. 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 $P < 0.001$.

	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 & Rice	12	43	39	ND	ND	ND	ND	ND	ND
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

Legend:
n: number of samples in the group
Mean: mean concentration in $\mu g*kg^{-1}$ or $\mu g*L^{-1}$
SD: Standard Deviation in $\mu g*kg^{-1}$ or $\mu g*L^{-1}$
ND: data unavailable
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

Table III. 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

Legend: for explanation, see the section 3.1. Sampling plan construction

Number of food group	Number of samples to be analyzed per food group	Weighting factor 1: Reported contamination level	Weighting factor 2: Consumption frequency	Weighting factor 3: Matrix diversity
36	10	-10 to +5	-5 to +5	0 to +5

Geographical distribution:
Random sampling of
50 samples per province

Food chain distribution:
Random sampling over 7 to
11 market chain
(upon availability)

Sampling duration	Sampling	Samples conservation	Samples handling
2 weeks	Cross-sectional survey (triplicate sampling)	-20°C: perishable goods +4°C: long term conservation perishable goods +20°C: imperishable goods	Unpacked and mixed at +4°C (as last as possible)

Fig. 2 A

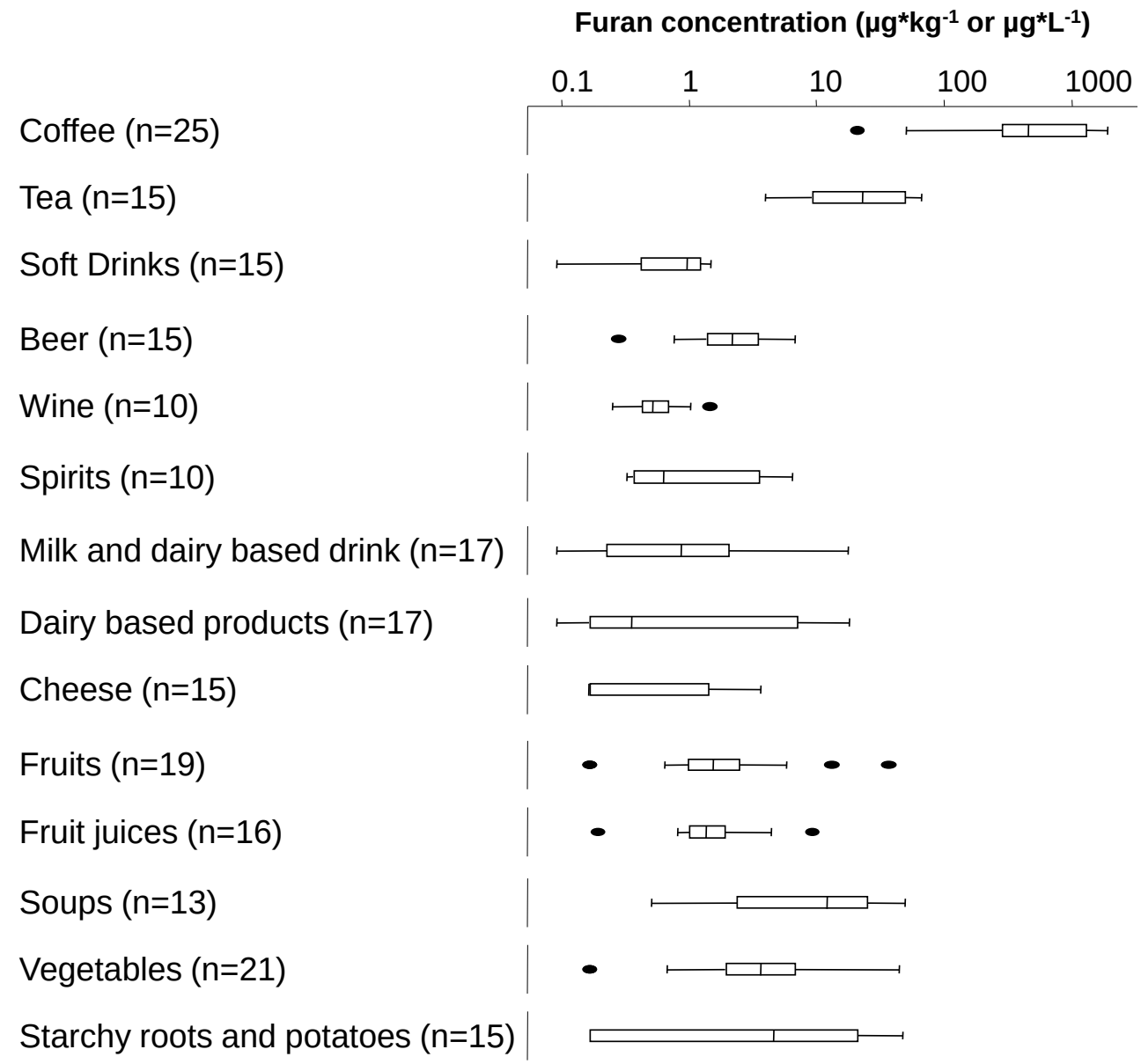

Furan concentration (µg*kg-1 or µg*L-1)
0.1
1
10
100
1000
Coffee (n=25)
Tea (n=15)
Soft Drinks (n=15)
Beer (n=15)
Wine (n=10)
Spirits (n=10)
Milk and dairy based drink (n=17)
Dairy based products (n=17)
Cheese (n=15)
Fruits (n=19)
Fruit juices (n=16)
Soups (n=13)
Vegetables (n=21)
Starchy roots and potatoes (n=15)

Fig. 2 B

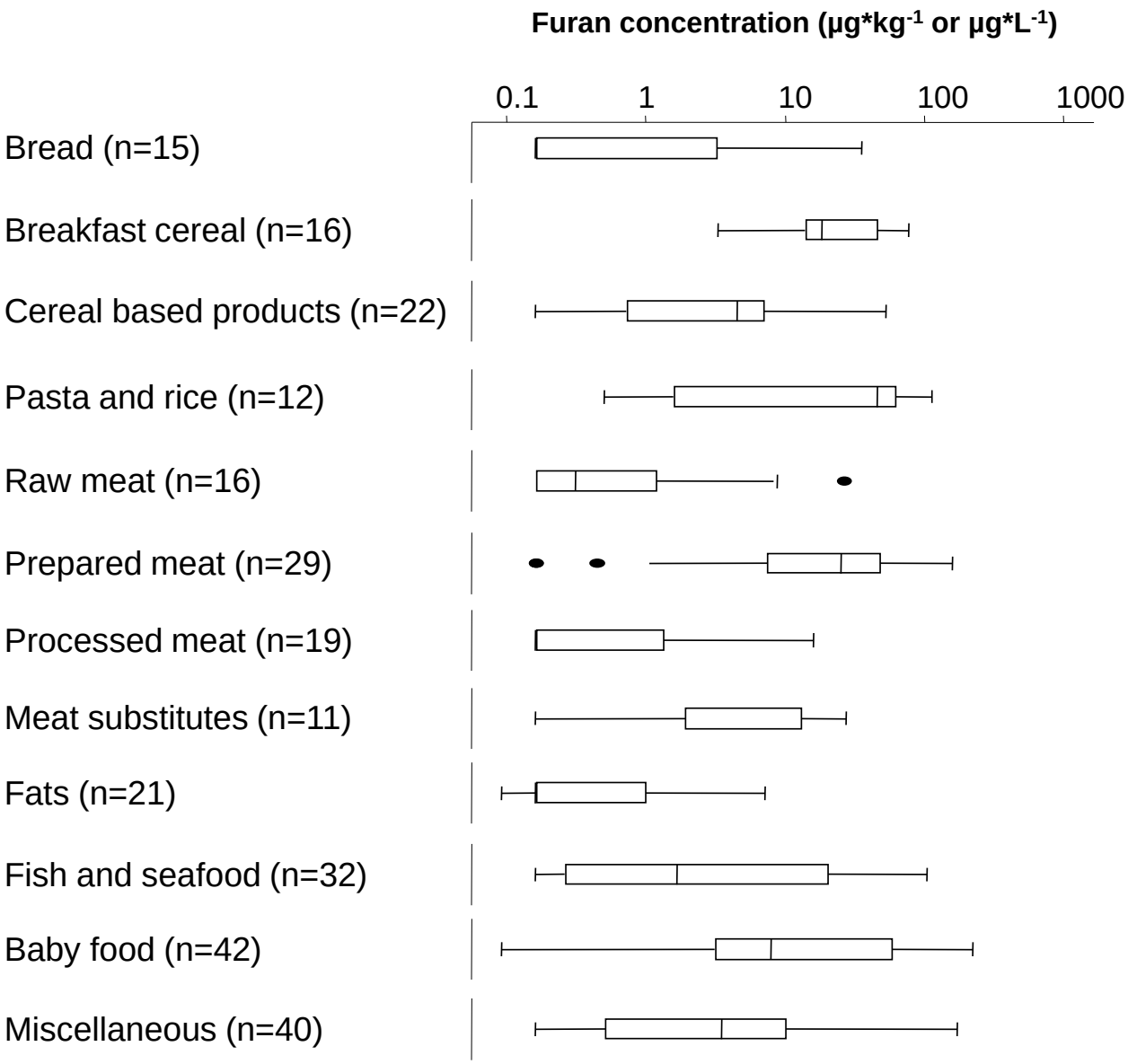
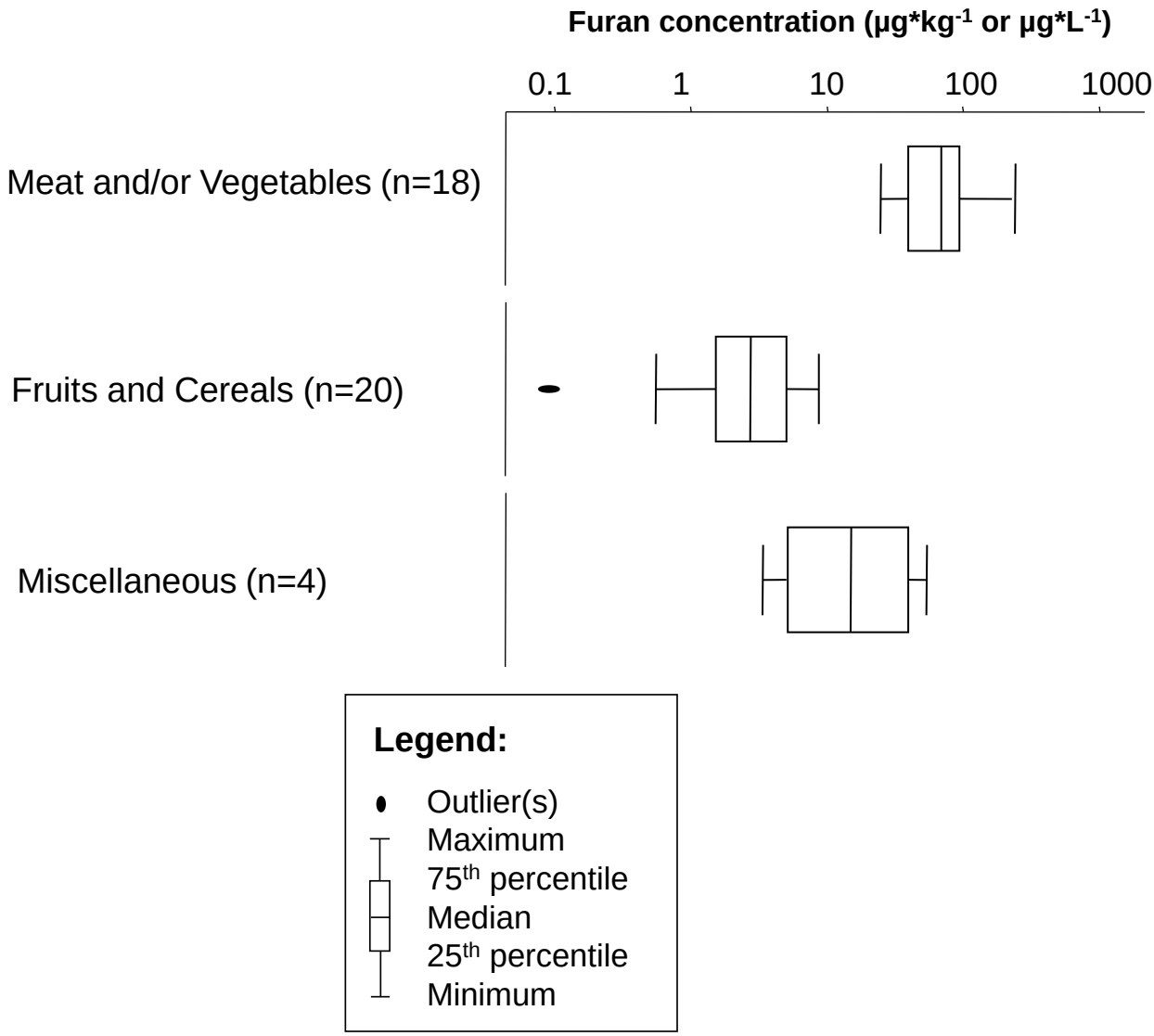

Furan concentration (µg*kg-1 or µg*L-1)
0.1
1
10
100
1000
Bread (n=15)
Breakfast cereal (n=16)
Cereal based products (n=22)
Pasta and rice (n=12)
Raw meat (n=16)
Prepared meat (n=29)
Processed meat (n=19)
Meat substitutes (n=11)
Fats (n=21)
Fish and seafood (n=32)
Baby food (n=42)
Miscellaneous (n=40)

Fig. 2 C


Furan concentration (µg*kg-1 or µg*L-1)
0.1
1
10
100
1000
Meat and/or Vegetables (n=18)
Fruits and Cereals (n=20)
Miscellaneous (n=4)
Legend:
Outlier(s)
Maximum
75th percentile
Median
25th percentile
Minimum